

**Habitat preference, environmental tolerance
and population viability of *Westralunio carteri*
Iredale 1934, a threatened freshwater mussel of
south-western Australia**



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**This thesis is presented for the Doctor of
Philosophy**

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Australia**



DECLARATION

I declare that this thesis is my own original account of my research and contains as its main content, work which has not previously been submitted for a degree at any tertiary education institution and as author, I reserve the right of copyright under the *Copyright Act 1968*, Commonwealth of Australia.

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Abstract

Freshwater mussels can play major roles in aquatic ecosystems by improving water quality through filter feeding, providing shelter to other animals, and enhancing the connection between benthic and pelagic systems through burrowing, depositing nutrients and releasing biological waste. Carter's freshwater mussel, *Westralunio carteri*, is the only freshwater mussel species in south-western Australia and is endemic to the region. The range of *W. carteri* has declined by 49% over the past 50 years, resulting in its classification as Vulnerable by both international and Australian conservation regulatory bodies. While recent studies have clarified many aspects of the biology of *W. carteri*, there remains a number of knowledge gaps that are hindering the development of an effective conservation management plan. This study addresses a number of these knowledge gaps, including the population structure and microhabitat preference of extant populations; and the tolerance of mussels to increasing salinity and water emersion, which are believed to be the key ongoing environmental stressors to the species.

Living individuals of *W. carteri* were found in 31 of 47 sampled sites (19 of 30 sampled rivers) and most of these populations had evidence of recent (within the last 10 years) recruitment. Density ranged from 2-169 individuals m⁻², with a mean over all sampled sites of 29.5 individuals m⁻². Mussels were strongly aggregated in the habitat (variance:mean ratio = 5.9, Lloyd's Patchiness Index = 3.6). The abundance of *W. carteri* was positively associated with fine substrate grain size, closeness to the river bank, and the presence and height of debris above the surface of the stream bed, suggesting that burrowing difficulty and protection from water flow are important habitat requirements for the species.

Secondary salinisation has been primarily responsible for the dramatic recent decline in the range of *W. carteri* and remains a major threat to the survival of the species. Laboratory assessment of tolerance to acute increases in salinity in adult mussels from two populations found LC₅₀ values of 5.87-5.96 gL⁻¹, substantially greater than values found in a previous study. Mussels were able to acclimatise to gradual increases in salinity, with gradual LC₅₀ values ranging from 6.43-6.45 gL⁻¹. Furthermore, survival times increased with a slower rate of increasing salinity. Future research should determine both the salinity tolerance of glochidia and juvenile mussels

and the non-lethal impacts of increased salinity on mussel fitness, so that the impacts of rising salinity levels in the rivers of south-western Australia can be more accurately predicted. The salinity tolerance of teleost hosts for the glochidia also warrants attention, as the decline in host abundance or presence may also impact mussel viability.

South-western Australia has undergone dramatic declines in rainfall and river flow since the mid-1970s and all climate models project this drying trend to continue. In two recent cases of mass mortality in populations of *W. carteri* associated with drying rivers, larger shells were significantly over-represented in the cohort of dead mussels. Field and laboratory experiments suggested that mussels respond to water emersion by tracking the receding water levels and burrowing into the substrate. There was no relationship between mussel size (shell length) and horizontal movement, but smaller mussels burrowed earlier and were more likely to be completely buried in the substrate. Smaller mussels were also more likely to be removed through predation or scavenging after death. In laboratory experiments, mussels were rapidly killed by water emersion in the open, but shading and burrowing into the substrate increased the tolerance of mussels to emersion, allowing them to survive for at least 60 days out of water. Finer substrate allowed for greater depth of burrowing and increased survival rate, independent of size. These findings have a number of implications: (1) projected future reductions in water flow are likely to increase the mortality rate in *W. carteri*; (2) mortality risk may be greater for larger, older, more fecund mussels, producing a disproportionate decrease in recruitment; (3) factors that affect burrowing ability, such as presence of appropriate substrate, will have a synergistic effect on mortality rate; (4) the adverse effects of drying rivers may be at least partially averted by increasing riparian shading.

The ongoing global decline of ecologically important but less charismatic aquatic fauna is of great concern and can only be halted by increased research, monitoring and management attention. It is hoped the findings of this thesis, along with related research on the ecology of *W. carteri*, will help the plight of the species and the unique ecosystems upon which it relies.

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Chapter 1: Literature review

1.1 Introduction

Freshwater mussels (Mollusca: Bivalvia: Unionida) play a significant role as ‘ecological generalists’ in freshwater ecosystems (Vaughn & Hakenkamp, 2001; Strayer, 2008; Bolotov *et al.*, 2014; Vaughn, 2018). Firstly, they can benefit aquatic communities by filter feeding particulate matter out of the water column (Vaughn *et al.*, 2008; Vaughn, 2018), thereby increasing light penetration and in turn influencing the productivity of aquatic ecosystems (Welker & Walz, 1998; Strayer *et al.*, 1999; Caraco *et al.*, 2006). Their presence further improves water quality by reducing the overall nutrient level (Howard & Cuffey, 2006; Chowdhury *et al.*, 2016), which may decrease the likelihood of detrimental algal blooms and eutrophication (Welker & Walz, 1998; Pigneur *et al.*, 2014). Freshwater mussels are also key couplers, enhancing the connection of benthic and pelagic systems through activities such as burrowing through the sediment and releasing biological waste into the water column (Vaughn & Hakenkamp, 2001; Strayer, 2014), and in both life and death, their shells may be employed as shelters and refuges by other aquatic organisms (Gutiérrez *et al.*, 2003). Along with benefiting humans through enhancing water quality, they can be of direct economic value as a source of mother-of-pearl and food (Ray *et al.*, 2011; FAO, 2014).

Unionida is an ancient and very speciose group, inhabiting freshwater environments in all continents except Antarctica (Cummings & Bogan, 2006; Bogan, 2008; Bogan & Roe, 2008). It is also one of the most threatened animal groups in the world, with recent dramatic declines in species diversity, geographic ranges, and population sizes attributable largely to anthropogenic impacts (Bogan & Roe, 2008; Lopes-Lima *et al.*, 2018). Most knowledge of the diversity, abundance and conservation status of freshwater mussels has come from studies in the Northern Hemisphere, particularly North America and Europe. While there have been some recent studies of freshwater mussel diversity in Africa (Graf & Cummings, 2007b; Graf & Cummings, 2009; Graf & Cummings, 2011; Sousa *et al.*, 2016), Australia (Brainwood *et al.*, 2008b; Walker *et al.*, 2014; Graf *et al.*, 2015) and South America (Santos *et al.*, 2013; Pereira *et al.*, 2014), the conservation challenges facing this group in the Southern Hemisphere are largely

under-appreciated. In this chapter, the taxonomic diversity, conservation status and threatening processes for Southern Hemisphere Unionida are reviewed, relating the findings to the amount of research effort on the group in this part of the world, and then discuss in more detail the current knowledge of unionids in Australia.

1.2 Global taxonomic diversity and biogeography

There are approximately 1,200 species of freshwater bivalves, mostly in the orders Unionida (freshwater mussels) and Venerida (freshwater clams, quagga mussel, zebra mussel) (Lopes-Lima *et al.*, 2018). Members of the order Unionida are characterised by a larval stage that is parasitic on fish hosts. This review focuses on the order Unionida.

Globally, Unionida is one of the most geographically widespread and diverse animal orders in the world (Graf & Cummings, 2007a; Graf, 2013; Lopes-Lima *et al.*, 2018). There are about 900 species of freshwater mussels, which are generally categorised into two Suborders: the Unionidina with the Superfamilies Unionoidea (Families Margaritiferidae and Unionidae) and Mulleroidea (Families Mulleriidae, Etheridae and Iridinidae), and the Hyriidina with the Superfamily Hyrioidea (Family Hyriidae) (Carter *et al.*, 2011; Graf, 2013; Lopes-Lima *et al.*, 2018).

The freshwater mussel fauna of the Southern Hemisphere is represented by 334 species from five families (Figure 1.1), compared to 568 species from three families in the Northern Hemisphere (Figure 1.1) (Lopes-Lima *et al.* (2018). Although no species of Margaritiferidae are found in the Southern Hemisphere, the region includes all species of Mulleriidae, Hyriidae and Iridinidae and three of four Etheriidae species.

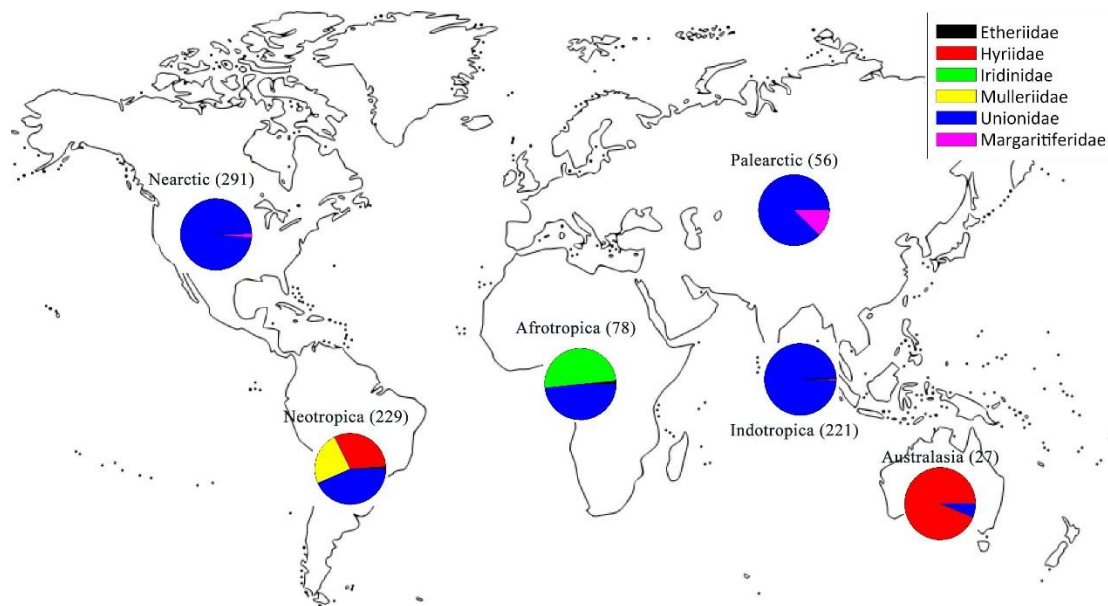


Figure 1.1. Global diversity of Unionida by ecoregion (number of species in parentheses). Southern Hemisphere ecoregions: Neotropica - South America and the middle-south of Central America; Afrotropica - Sub-Saharan Africa; Australasia - Australia, New Zealand, New Guinea and associated islands. Northern Hemisphere ecoregions: *Indotropica – India, South and Southeast Asia; Nearctica – North America; Palearctica – Europe, Middle East and North Asia. *Principally Northern Hemisphere, although parts of Southeast Asia, including Indonesia lie below the equator. Data from Lopes-Lima *et al.* (2018); ecoregions adapted from Graf and Cummings (2007a).

The 334 species in the Southern Hemisphere are distributed discontinuously over three major ecoregions (Figure 1.1). Approximately 69% of these species, from four families (Etheriidae, Hyriidae, Unionidae and Mulleriidae), are found in Neotropica (Graf, 2013; Lopes-Lima *et al.*, 2018), 23% from three families (Etheriidae, Iridinidae and Unionoidae) inhabit sub-Saharan Africa (Graf & Cummings, 2011) and 8% from two families (Hyriidae and Unionoidae) are found in Australasia (Marshall *et al.*, 2014; Walker *et al.*, 2014).

The mussel fauna of Neotropica consists mostly of Unionidae (42%), Hyriidae (31%) and Mulleriidae (14%); including all the members of family Mulleriidae in the world. The Unionidae are found principally in Mesoamerica (Central America) and Amazonas-Orinoco, where they form a group largely endemic to the American tropics and distinct from the Unionidae in North America (Graf & Cummings, 2007a). The

South American fauna is dominated by two families of Gondwanan origin, Hyriidae and Mulleriidae; with an additional two species of Etheriidae, *Bartlettia stefanensis* and *Acostaea rivolii*. The most species-rich hydrographic areas in Neotropica are the south and southeast Atlantic regions and the Amazon River (Pereira *et al.*, 2014).

The freshwater mussel fauna of sub-Saharan Africa is distinct from that of Northern Africa and relatively better documented than that of any other continent in the Southern Hemisphere (Graf & Cummings, 2011). Approximately 51% of all species found in this region belong to the Iridinidae and 48% to the Unionidae, with one widespread species of Etheriidae (*Etheria elliptica*). All Iridinidae are endemic to Africa, but Unionidae are found throughout the world. Graf and Foighil (2000) considered African Unionidae to be a non-monophyletic group derived from multiple invasions from the Northern Hemisphere, although more recent phylogenetic analyses cast some doubt on this interpretation (Bolotov *et al.*, 2017; Lopes-Lima *et al.*, 2017). The areas of sub-Saharan Africa with the greatest species richness are the Lake Victoria Basin and Upper Nile River (Graf & Cummings, 2011).

All except two of the freshwater mussel species found in Australasia are in the Hyriidea, with two representatives of the Unionidae (*Haasodonata fannyae* and *H. vanheurni*) in New Guinea. These species may be derived from an invasion of the Unionidae from the Indotropica region (Bolotov *et al.*, 2017), although there is still some doubt regarding their phylogenetic affinities (Graf & Cummings, 2007a). While Australasian diversity is relatively low, the fauna is highly endemic and phylogenetically distinct, a consequence of isolation following the breakup of Gondwana (Walker *et al.*, 2014). The greatest diversity of species is in the eastern and northern coastal regions of the Australian continent, New Guinea and the Solomon Islands (Williams, 1980; Walker *et al.*, 2001; Walker *et al.*, 2014).

1.3 Conservation status of freshwater mussels

Of the estimated 895 species of extant (and recently extinct) freshwater mussels in the world, 533 had been evaluated against the Red List criteria of the International Union for the Conservation of Nature (IUCN) as of the 1st Dec 2016. Assessment of

conservation status on the IUCN Red List is based on information about population size, generation length, rate of population decline, extent of occurrence and area of occupancy, if population data are unavailable. Only 115 species from the Southern Hemisphere have been evaluated, a disproportionately low percentage (35%) compared with the Northern Hemisphere (418 species, 72%). The assessed rate (64 species, 19%) is even lower if “Data Deficient” species are excluded. The rate of evaluation is particularly poor in Neotropica (12%) and Australasia (33%) (Figure 1.2). The freshwater mussel fauna of sub-Saharan Africa has been comprehensively evaluated (100% of described species); as a consequence of the recent, thorough reviews of the distribution and abundance of African mussels in several studies (Marijnissen *et al.*, 2009; Graf & Cummings, 2011; Nel *et al.*, 2012; Akele *et al.*, 2015a; Akele *et al.*, 2015b).

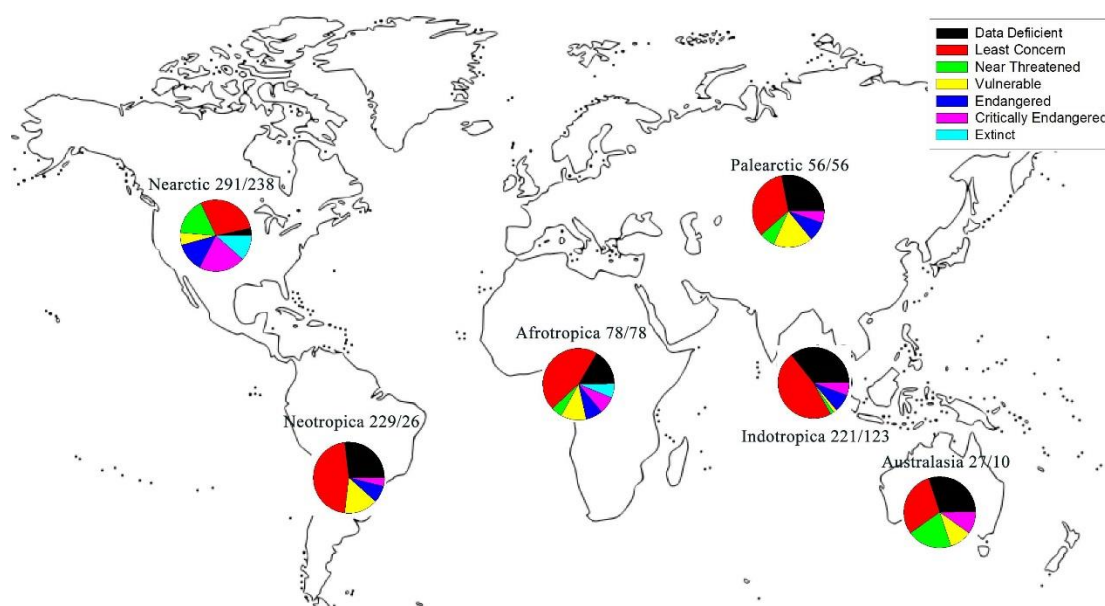


Figure 1.2. IUCN (2016) Red List status of Unionida by ecoregion (total number of species/number of species IUCN listed). Data Deficient species included. Checked on Dec 1st 2016.

Of the 533 assessed species of freshwater mussels in the IUCN database, 32 (6%) are Extinct and 162 (30%) have been categorised as threatened (Critically Endangered, Endangered or Vulnerable), with a further 52 (10%) listed as Near Threatened (IUCN, 2016). This is a stark demonstration of the catastrophic global decline in both species richness and overall abundance of freshwater mussels, when compared with other

animal groups. For example, the comparable proportions in different threat categories for all assessed animals are 1.2% Extinct, 20% threatened and 6% Near Threatened, while for all assessed aquatic animals they are 0.5% Extinct, 17% threatened and 5% Near Threatened (IUCN, 2016). This disproportionate decline in mussel populations may be a consequence of life history traits that increase their vulnerability to environmental changes: adult mussels are usually relatively sedentary, with limited ability to disperse away from in-stream disturbances; and are also extremely long-lived with delayed reproduction, which constrains their ability to both recover demographically and respond adaptively to disturbances (Haag, 2012).

Five freshwater mussel species in the Southern Hemisphere (*Germainaia geayi*, *Germainaia geayi*, *Unio cariei*, *Unio madagascariensis* and *Unio malgachensis* from sub-Saharan Africa) have been categorised as Extinct (3% of assessed species), with 30 species (26%) threatened and six species (5%) Near Threatened, while 23 species (20%) are Data Deficient (Figure 1.2). The proportion of species categorised as imperiled is greater in the Northern Hemisphere fauna; 27 species (6%) extinct, 132 species (31%) threatened and 46 species (11%) Near Threatened, with 68 species (16%) Data Deficient (Figure 1.2). If Indotropica is excluded, the discrepancy in relative proportions is much greater; 27 species (9%) extinct, 113 species (38%) threatened and 44 species (15%) Near Threatened, with only 24 species (8%) Data Deficient (Figure 1.2).

Of the 110 non-extinct species in the Southern Hemisphere that have been assessed, the populations of *Chambardia bourguignati* (Afrotropica), *Chambardia wahlbergi* (Afrotropica), *Coelatura gabonensis* (Afrotropica), *Coelatura hauttecoeuri* (Afrotropica), *Cucumerunio novaehollandiae* (Australasia), *Nitia monceti* (Afrotropica), *Nitia mutelaeformis* (Afrotropica) and *Nitia teretiuscula* (Afrotropica) are classified as stable. The population trend of all other assessed species in the Southern Hemisphere is either declining or is yet to be determined.

Government legislation with respect to threatened species varies widely throughout the Southern Hemisphere. Australia has national and state level acts on the conservation of threatened species, including Bivalvia (IUCN, 2016), while the New Zealand

government has included sensitive invertebrates in natural conservation protection areas (Grainger *et al.*, 2014). In sub-Saharan Africa, although the conservation status of a large proportion of freshwater mussels has been assessed for the IUCN Red List, there is little legislative recognition and protection at the national level (IUCN, 2016). Similarly, only four South America countries, Brazil, Colombia, Paraguay and Uruguay have published lists of threatened fauna, including freshwater molluscs (Pereira *et al.*, 2014).

1.4 Global threatening processes for freshwater mussels

Finding a comprehensive predictive model for the factors limiting the distribution and abundance of freshwater mussels has proved elusive (Townsend, 1980; Gosling, 2003; Strayer, 2008). A wide range of potentially limiting environmental factors have been identified. These include biotic variables, such as food, fish hosts, predators, competitors and pathogens; and abiotic variables, such as substrate stability, presence of refuges, water flow, stream connectivity, nutrients and other water quality parameters (Table 1.1). The importance of these factors varies spatially and temporally, not only among different species of mussels, but also among different life stages of the same species.

Anthropogenic activities, such as habitat destruction, habitat degradation and water quality deterioration, may cause declines in mussel populations because of their effects on environmental factors that influence distribution and abundance (Poole & Downing, 2004; Bogan, 2008; Jones & Byrne, 2010). For example, dams directly destroy habitat and reduce population connectivity (Strayer, 2008), agricultural or urban pollutants may be toxic or affect water quality in other ways (Young, 2005; Ma, 2016) and human harvesting for food or fashion increases predation pressure (Fuller, 1974; Strayer, 2008). As most Unionida are very long-lived, the impact of anthropogenic activities which affect fertility or the survival of larval or juvenile mussels may not appear immediately (Dame, 1996; Strayer, 2008).

For those species of mussels categorised as Threatened or Near Threatened in the IUCN Red List, the number of identified threats by ecoregion are provided in Table 1.2.

Pollution from urban sources represents the greatest threat for freshwater mussels in the Southern Hemisphere (making up 21% of identified threats). Despite being largely unquantified, urban pollution is of particular concern in sub-Saharan Africa. For example, mussels in Lake Victoria are suffering through ecosystem change due to increasing urbanisation and industrialisation (Hecky *et al.*, 2010; Downing *et al.*, 2012). Agricultural pollution, such as the waste from livestock, and overload of pesticides and fertilisers, is also important throughout southern ecoregions (14% of identified threats), as are habitat degradation as a result of river regulation and sedimentation (7% and 11.5%, respectively), particularly in Africa (e.g. *Etheria elliptica*, Van Damme & Seddon, 2017) and Australia (e.g. *Hyridella narracanensis*, Klunzinger *et al.*, 2014a)

Table 1.1. Factors determining the abundance and distribution of freshwater mussels.

Factor	Effects	References
Biotic		
Abundance and accessibility of fish hosts	Limits recruitment and dispersal	Strayer (2008), Klunzinger <i>et al.</i> (2015)
Availability of food	Reduces growth and reproduction	Fuller (1974); Cummins (1975); Gosling (2003)
Predation	Reduces survival	Fuller (1974); Gosling (2003); Chattopadhyay (2011)
Competition	Reduces survival, growth and reproduction	Gosling (2003); Howard and Cuffey (2006)
Infection by pathogens and parasites	Reduces survival, growth and reproduction	Fuller (1974); Strayer (2008); Chattopadhyay (2011)
Habitat disturbance	Human activities that reduce the suitability of habitat	Fuller (1974); Poole and Downing (2004)
Abiotic		
Physical features of water channel (width, depth, slope, etc)	Reduces juvenile settlement	Neves and Widlak (1987); Layzer and Madison (1995)
Substrate type	Reduces juvenile settlement, limits burrowing activity	Morales <i>et al.</i> (2006)
Current flow	Reduces juvenile settlement and food availability	Steuer <i>et al.</i> (2008); Maloney <i>et al.</i> (2012)
River drying	Reduces survival	Vaughn <i>et al.</i> (2015); Nakano (2018)
Artificial barriers (dams, river regulation)	Limits dispersal, may reduce habitat suitability	Watters (1996); Strayer (2008); Jones and Byrne (2010)
Water quality (temperature, dissolved oxygen, pH, turbidity, salinity, BOD, nutrients, calcium)	Reduces survival (directly toxic) or growth, limits food availability	Watters (1999); Gosling (2003); Augspurger <i>et al.</i> (2003)
Pollutants (heavy metals, biocides, general waste)	Reduces survival (directly toxic), growth or reproduction	Fuller (1974); Milam <i>et al.</i> (2005); Zieritz <i>et al.</i> (2016)

Table 1.2. Number of species of Unionida species (with % in total number of assessed species) for which different threatening processes have been identified in the IUCN Red List (IUCN, 2016). Note that one species may be affected by multiple threats. Asterisks denote Southern Hemisphere ecoregions.

Ecoregion	Agricultural pollution	Alien species	Climate change	Over-fishing	River regulation	Sedimentation	Urban Pollution	Unknown	No threat
Afrotropica*	11 (2.06%)	2 (0.38%)	7 (1.31%)	5 (0.94%)	5 (0.94%)	11 (2.06%)	21 (3.94%)	37 (6.94%)	9 (1.69%)
Australasia*	1 (0.19%)	1 (0.19%)	6 (1.13%)	2 (0.38%)	0 (0%)	2 (0.38%)	0 (0%)	2 (0.38%)	0 (0%)
Neotropica*	4 (0.75%)	0 (0%)	1 (0.19%)	0 (0%)	3 (0.56%)	0 (0%)	3 (0.56%)	15 (2.81%)	4 (0.75%)
Indotropica	32 (6.00%)	0 (0%)	29 (5.44%)	3 (0.56%)	10 (1.88%)	13 (2.44%)	25 (4.69%)	50 (9.38%)	19 (3.56%)
Nearctica	55 (10.32%)	12 (2.25%)	54 (10.13%)	5 (0.94%)	4 (0.75%)	22 (4.13%)	41 (7.69%)	106 (19.89%)	31 (5.82%)
Palearctica	22 (4.13%)	4 (0.75%)	17 (3.19%)	3 (0.56%)	9 (1.69%)	0 (0%)	23 (4.32%)	22 (4.13%)	1 (0.19%)
Global	125 (23.45%)	19 (3.56%)	114 (21.39%)	18 (3.38%)	31 (5.82%)	48 (9.01%)	113 (21.20%)	232 (43.53%)	64 (12.01%)

Overfishing, while only representing 6% of identified threats over all southern ecoregions, is of concern for a number of species in South America and Africa. For example, in Neotropica, harvesting of *Castalia ambigua* and *Prisodon syrmatophorus* for button manufacture is believed to be a major threat (Beasley, 2001). The African mussels *Brazzaea anceyi*, *Chambardia wahlbergi* and *Coelatura horei* are also heavily harvested for button manufacture and other purposes (Marijnissen *et al.*, 2009; Graf *et al.*, 2011). Additionally, the African species *Chambardia petersi*, *Chambardia wissmanni*, *Coelatura hypsiprymna*, *Grandidieria burtoni*, *Pleiodon spekii*, *Pseudospatha tanganyicensis* may be affected by harvesting of their fish hosts (Marijnissen *et al.*, 2009).

Alien species are of minor importance (3% of identified threats), usually as competitors or predators of native mussels, but also as predators or competitors of host fishes. For instance, in South America, *Anodontites tenebricosa* has been observed to be affected by competition with the alien mussel, *Limnoperna fortunei*, from Asia (Bergonci *et al.*, 2009; Darrigran *et al.*, 2011). In Sub-Saharan Africa, *Coelatura hypsiprymna* is adversely influenced by the invaded exotic fishes (Kaunda *et al.*, 2010). In Australia, Klunzinger *et al.* (2012b) found that the alien fish species *Carassius auratus* and *Geophagus brasiliensis* were not competent hosts for *Westralunio carteri*, and suggested that this could reduce recruitment success in some populations.

Climate change has been identified as a disproportionately large threat to Southern Hemisphere mussels (12% of identified threats, compared to 3% for Northern Hemisphere species). This is presumably a consequence of strongly seasonal streamflow in these ecoregions leading to regular cycles of flooding and drying and the reliance of freshwater communities on dry-season refuge pools (Magoulick & Kobza, 2003). Decreased streamflow and increasing temperatures as a result of climate change will affect the size, number and connectivity of these refuges, with major implications for populations of mussels and other freshwater fauna (Beatty *et al.*, 2017). Increasing

drought stress as a result of climate change has been identified as an important threat to several unionid species such as *Cucumerunio novaehollandiae*, *Hyridella glenelgensis* and *Westralunio carteri* in Australia (Walker *et al.*, 2014) and *Coelatura hypsiprymna*, *Mutela ovata* and *Unio foucauldianus* in Africa (Kaunda *et al.*, 2010; Van Damme & Ghamizi, 2010).

1.5 Global research effort on freshwater mussels

To determine research effort for Unionida, a search of the Web of Science for primary research publications prior to July 2016, using the keywords “freshwater mussel”, “Unionoida” and “Unionida” with genera and species names was organised. Research effort was unevenly distributed across ecoregions, with only 6.6% of all published articles concerning Southern Hemisphere species (Figure 1.3). Lopes-Lima *et al.* (2014) also found that research effort for Unionida did not necessarily correspond to hotspots of biodiversity, but was instead concentrated in North America, Europe and Russia. Relative to species richness, research effort in Southern Hemisphere ecoregions is proportionally greatest in Australasia and smallest in sub-Saharan Africa (Figure 1.3). There are likely to be many reasons for this disparity. Research infrastructure has traditionally been well developed in Australia and New Zealand, and research history may exert a positive effect on subsequent research effort, stimulating increased work in the same region

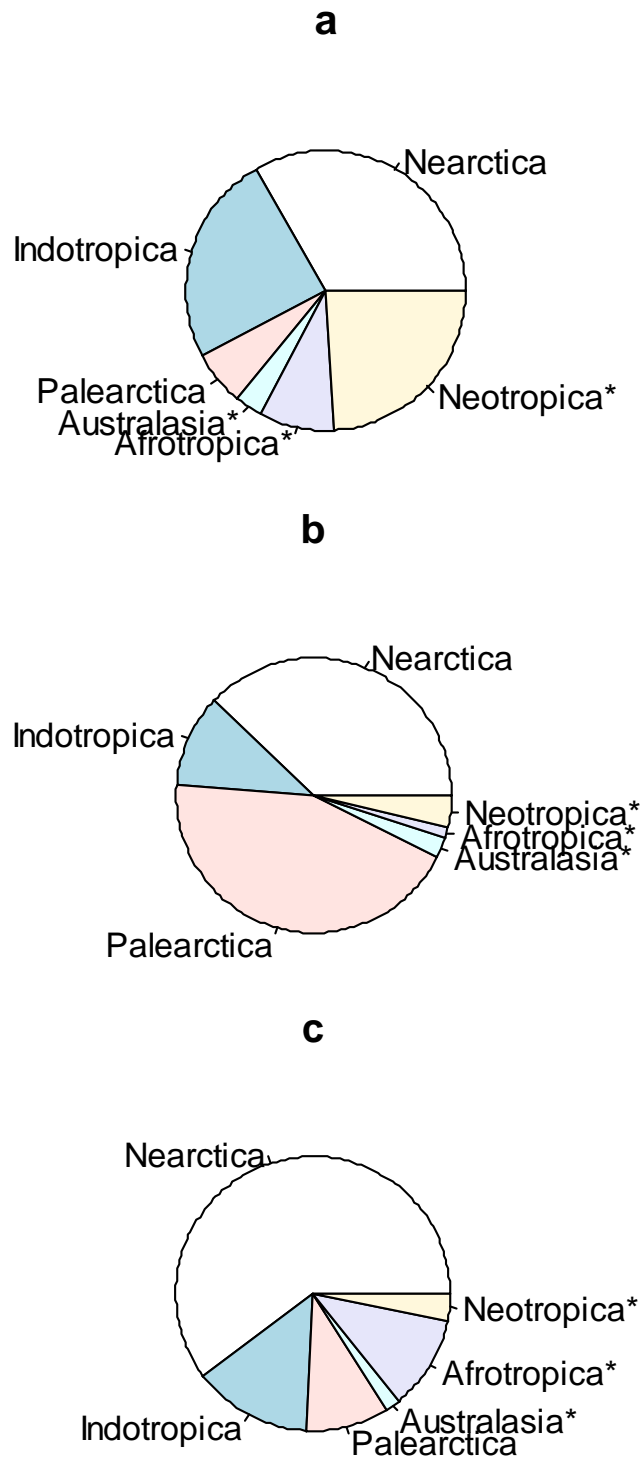


Figure 1.3. Proportion of (a) total published research articles, (b) described Unionida species and (c) Unionida species categorised as threatened (NT/VU/EN/CR) in the IUCN Red List by ecoregions. Asterisks denote ecoregions in the Southern Hemisphere.

There was no relationship between research effort and the number of threatened species in an ecoregion (Spearman's rank correlation; $\rho = 0.31$, $P = 0.54$), which is partly a reflection of the very small sample size (six ecoregions), but also a consequence of the recent, comprehensive assessment of the conservation status of mussels in sub-Saharan Africa. When this ecoregion is removed from the analysis, there is a strong correlation between research effort and number of threatened species in the IUCN red list (Spearman's rank correlation; $\rho = 0.90$, $P = 0.04$). The success of conservation actions depends on evidence-based knowledge for the species or habitat concerned (Sutherland *et al.*, 2004) and a number of studies, particularly with bird species, have found a positive association between threat status of a species and research effort (Brooks *et al.*, 2008; de Lima *et al.*, 2011). This is usually taken to indicate that the IUCN Red List drives investment in conservation research, but at a global scale, the causal pathways may be more complex than this. We might expect regions that have historically had greater research effort in a taxonomic group to be more likely to have the documented demographic and ecological data required for assessment of conservation status and therefore more likely to have a greater number of species categorised as threatened.

Since the middle of the 20th Century, there has been a steady increase in the number of published papers on unionids in Southern Hemisphere ecoregions (Figure 1.4). The types of information required for species conservation include phylogenetic and taxonomic descriptions, ecological function and values, current and historical distribution, population size and demographic attributes, habitat requirements, and identification of threats (IUCN/SSC, 2008). Research on Southern Hemisphere unionids has traditionally been dominated by taxonomic studies, although in recent years there has been an increasing number of publications in the field of ecology/conservation biology (Figure 1.4), mirroring a global trend (Lopes-Lima *et al.*, 2014). This increase in ecological studies has been greatest in Australasia (Figure 1.5). As well as the historical contingencies referred to earlier, this may also be a reflection of the relatively low species richness in Australasia; providing the opportunity for more

intensive biological studies. By contrast, the opportunities for intensive research effort on African species are more constrained, although there have been a number of recent ecological studies in this region (Akele *et al.*, 2015a; Ouedraogo *et al.*, 2015). Neotropica, like Australasia, has also had a dramatic increase in ecological studies on freshwater mussels since the 1980's (Pereira *et al.*, 2014).

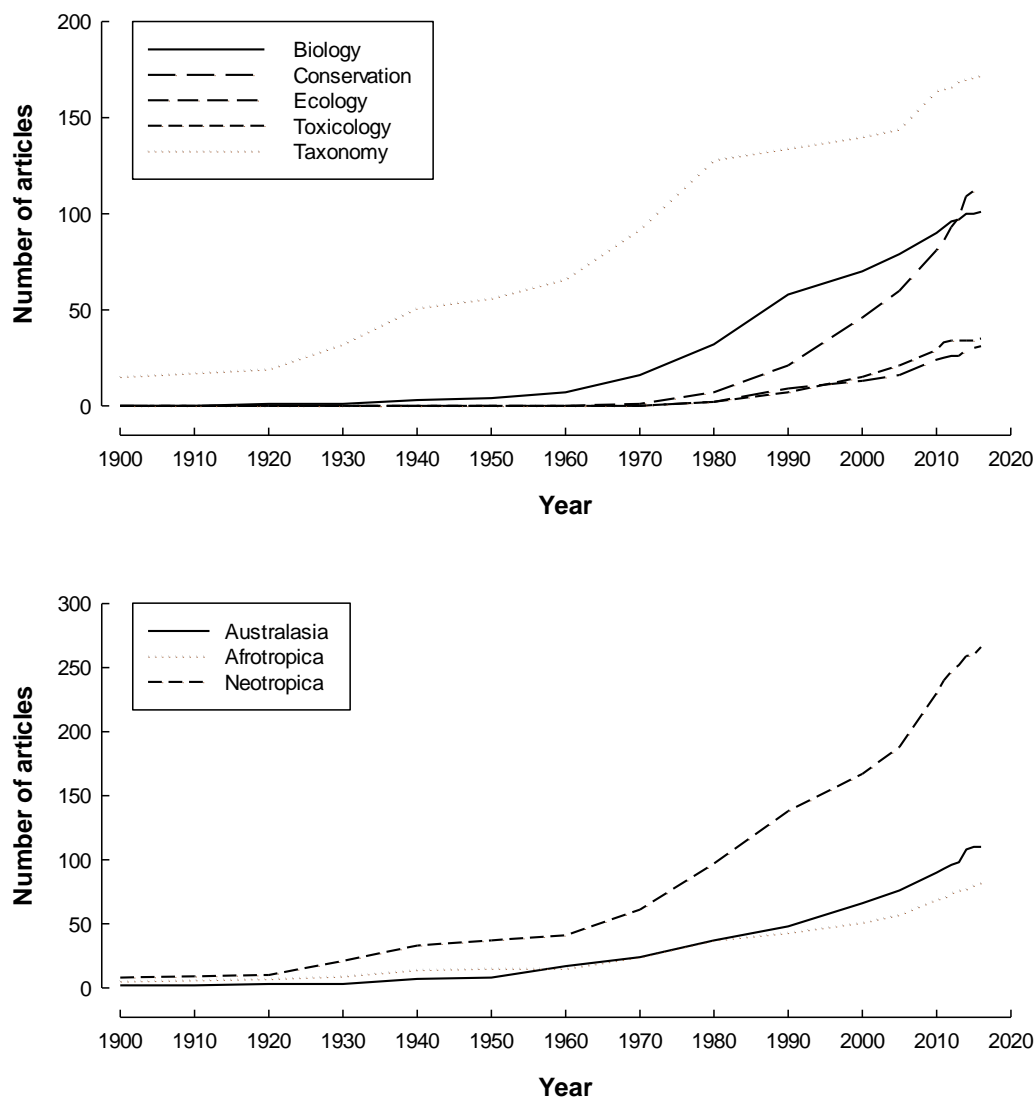


Figure 1.4. Cumulative number of articles on Southern Hemisphere Unionida over time, broken down by (a) discipline and (b) ecoregion.

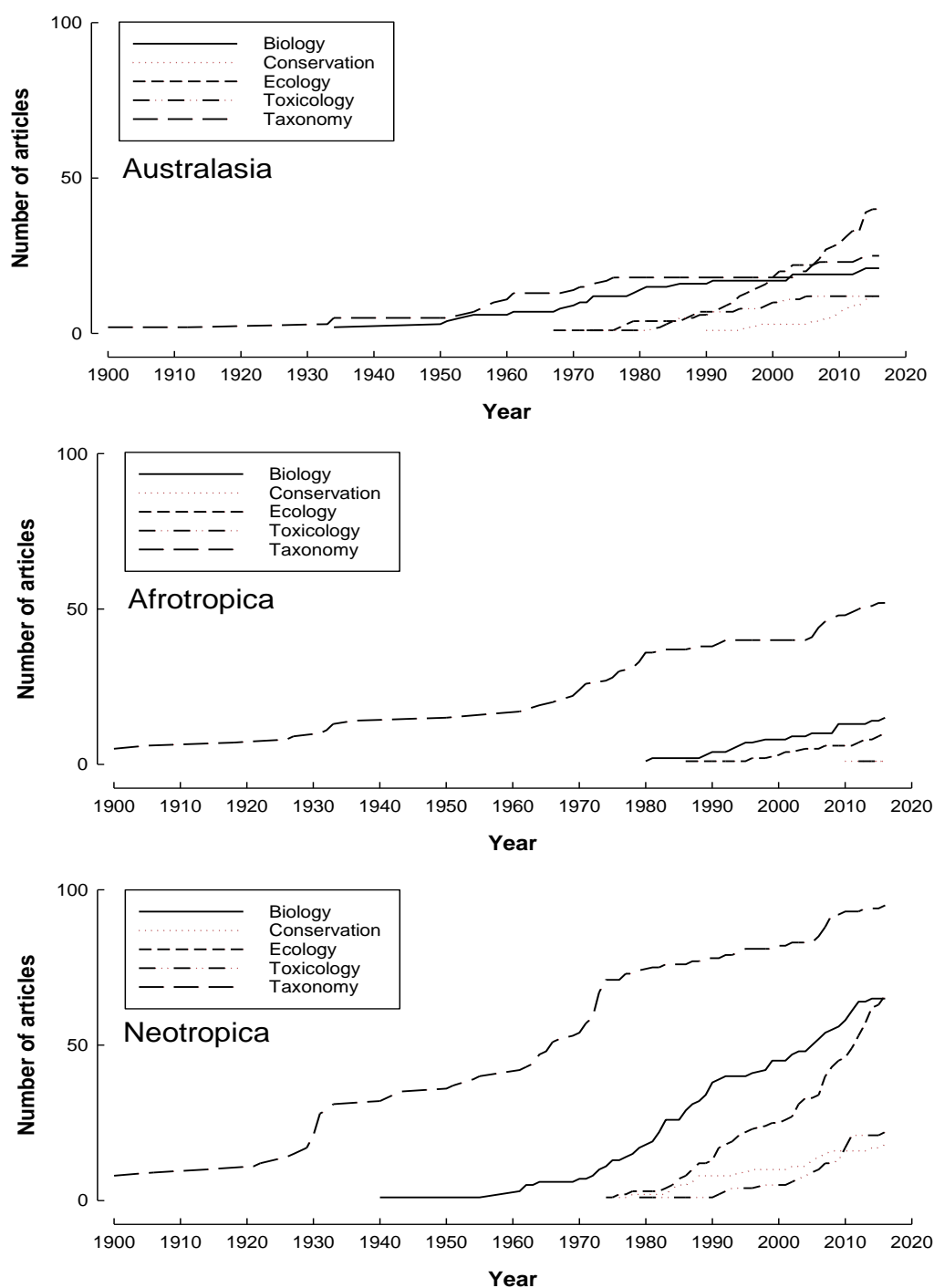


Figure 1.5. Cumulative number of articles on Southern Hemisphere Unionida over time, broken down by discipline for each ecoregion: (a) Australasia; (b) Afrotropica; (c) Neotropica.

1.6 The importance of freshwater mussel conservation

Freshwater is essential for life, including human life, but freshwater resources are under increasing pressure throughout the world. Only 0.3% of global freshwater resources (0.007% of all water resources) are currently available for human society (Vitto, 2001), and the quantity and quality of these resources are continually being reduced by population growth, and industrial and agricultural pollution (Vitto, 2001). Protecting freshwater resources is an urgent environmental problem.

Freshwater mussels improve water quality directly, through biofiltration, and indirectly, by acting bioindicators. There is a growing interest in using the filtering capacity of mussels to treat water for human use. For example, it has been estimated that mussels in a 480 km reach of the Mississippi River filter approximately 53 million m⁻³ day⁻¹ (Newton *et al.*, 2011). As well as removing phytoplankton, mussels may also remove disease organisms and contaminants such as pharmaceuticals and herbicides from water supplies (Vaughn, 2018). In addition to improving water quality through filtration, mussels can act as environmental sentinels, because they bioaccumulate particles, allowing measurement of stressor levels in their soft tissues (Sorey and Edward, 1989).

1.7 Australian Hyriidae

Nine genera and 32 described species of unionids, most in the family Hyriidae but two in Unionidae, are found in the Australasian ecoregion (McMichael & Hiscock, 1958; Walker *et al.*, 2014). Eighteen of the 32 species, from six genera, are found in Australia (Table 1.3). Recent genetic studies have identified a number of cryptic species in the genus *Velesunio* (Baker *et al.*, 2004; Hughes *et al.*, 2004), and it is likely that the current taxonomy underestimates the true species diversity of Australian unionids.

Seven Australian unionid species appear in the IUCN Red List: *Hyridella glenelgensis*

is listed Critically Endangered, *Westralunio carteri* as Vulnerable, and *Hyridella narracanensis* and *Velesunio moretonicus* as Near Threatened; while *Cucumerunio novaehollandiae* is listed as Least Concern, and *Alathyria jacksoni* and *Hyridella narracanensis* as Data Deficient.

Table 1.3. Australasian species of Hyriidae (Walker *et al.*, 2014).

Species	Distribution	Species	Distribution
<i>Alathyria condola</i>	Australia	<i>Hyridella narracanensis</i>	Australia
<i>Alathyria jacksoni</i>	Australia	<i>Hyridella guppyi</i>	New Guinea, Solomon Islands
<i>Alathyria pertexta</i>	Australia, New Guinea	<i>Lortiella froggatti</i>	Australia
<i>Alathyria profuga</i>	Australia	<i>Lortiella opertanea</i>	Australia
<i>Cucumerunio novaehollandiae</i>	Australia	<i>Lortiella rugata</i>	Australia
<i>Cucumerunio websteri</i>	New Zealand	<i>Microdontia anodontaeformis</i>	New Guinea
<i>Echyridella lucasi</i>	New Zealand	<i>Velesunio ambiguus</i>	Australia
<i>Echyridella menziesii</i>	New Zealand	<i>Velesunio angasi</i>	Australia
<i>Echyridella onekaka</i>	New Zealand	<i>Velesunio moretonicus</i>	Australia
<i>Haasodonta fannyae</i>	New Guinea	<i>Velesunio sentaniensis</i>	New Guinea
<i>Haasodonta vanheurni</i>	New Guinea	<i>Velesunio wilsonii</i>	Australia, New Guinea
<i>Hyridella aucklandica</i>	New Zealand	<i>Virgus beccarianus</i>	New Guinea
<i>Hyridella australis</i>	Australia	<i>Westralunio albertisi</i>	New Guinea
<i>Hyridella depressa</i>	Australia	<i>Westralunio carteri</i>	Australia
<i>Hyridella drapeta</i>	Australia	<i>Westralunio flyensis</i>	New Guinea
<i>Hyridella glenelgensis</i>	Australia		
<i>Hyridella misoolensis</i>	New Guinea		

1.8 Carter's freshwater mussel, *Westralunio carteri*

1.8.1 Distribution and biology

Westralunio carteri Iredale, 1934, commonly named Carter's freshwater mussel, is the only unionid found in south-western Australia and is endemic to this region (Klunzinger

et al., 2012a; Klunzinger & Walker, 2014). This species is also the sole representative of *Westralunio* in Australia, although two other members of the genus occur in New Guinea. The current distribution of *W. carteri* includes freshwater streams, rivers, reservoirs and lakes within ~100 km of the coast of south-western Australia, from Gingin Brook north of Perth to the Waychinicup River on the south coast (Figure 1.6).

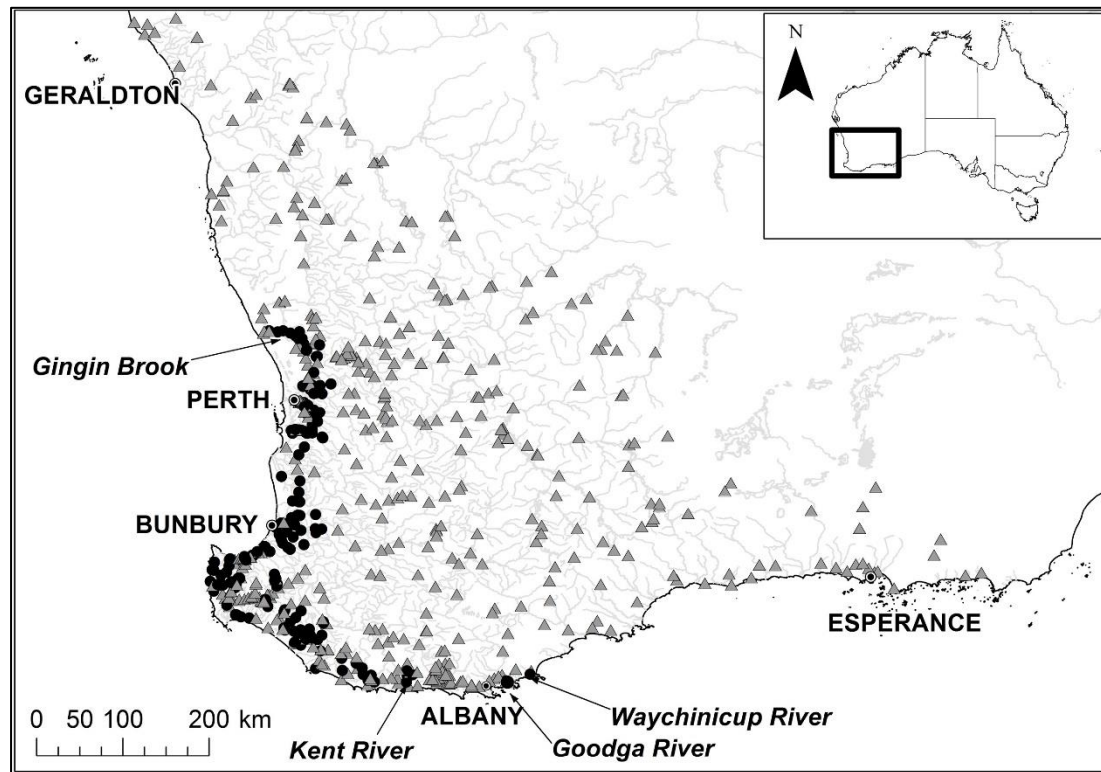


Figure 1.6. Current distribution of *Westralunio carteri*. Black circles indicate mussel presence and grey triangles indicate mussel absence, as determined from survey data. After Klunzinger *et al.* (2015).

Westralunio carteri is a long-lived, slow growing species, with maximum age of at least 52 years, a mean growth constant of 0.134 and age to maturity of 4-6 years (Klunzinger *et al.*, 2014b). This suite of life history traits is similar to other slow-growing freshwater mussel groups, such as the family Margaritiferidae and the tribes Amblemini, Pleurobemini, and Quadrulini in the family Unionidae, and is often considered an adaptation for stable aquatic habitats (Haag, 2012).

The species is dioecious, with occasional hermaphroditism (Klunzinger, 2012). Spawning is highly synchronised in late winter (July/August) and glochidia, which are morphologically distinct from those of other species of Hyriidae, are released in thick, mucus strands in late spring (September/November) (Klunzinger, 2012; Klunzinger *et al.*, 2013). *Westralunio carteri* is a host generalist and glochidia have been found on seven native and four alien fish species, although prevalence and intensity of infection differs among host species and is greatest on native, benthic fishes (Klunzinger *et al.*, 2012b). Glochidia transformation and release of juvenile mussels occurs in 20-30 days (Klunzinger *et al.*, 2012b).

1.8.2 Conservation status

The first report of population declines in *W. carteri* was by Kendrick (1976), who reported the disappearance of mussels from the Avon River, presumably as a result of secondary salinisation. The species was nominated for addition to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, following a river health monitoring program in the late 1990s (Halse *et al.*, 2002; Pinder *et al.*, 2004), which resulted in its listing as Vulnerable (IUCN, 1996). In 2011, however, this listing was changed to Least Concern (Köhler, 2011).

Klunzinger *et al.* (2015) compared the current distribution of *W. carteri* with historical museum records, and estimated a decline of 49% in extent of occurrence over the last 50 years (less than three generations of the species) (Figure 1.7). Species distribution

modelling suggested that this decline was principally caused by secondary salinisation, a consequence of land clearing in the south-western Australian Wheatbelt, although distribution was also limited by the decreasing water permanency and increasing nitrogen pollution in rivers and streams (Klunzinger *et al.*, 2015). As a result of this study, the species was relisted as Vulnerable by the IUCN in 2014; and was also listed as Vulnerable under the Western Australian *Wildlife Conservation Act 1950* in 2014 and under the Commonwealth of Australia *Environment Protection and Biodiversity Conservation Act 1999* in 2018.

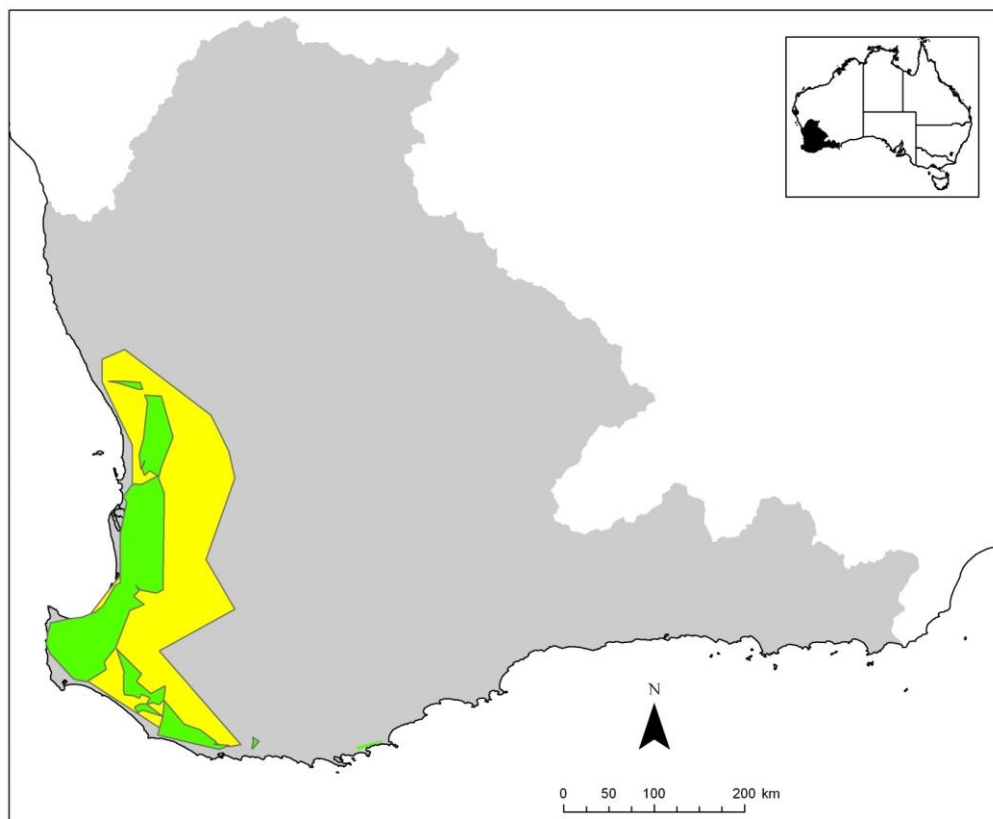


Figure 1.7. Historic (yellow) and current (green) Extent of Occurrence of *Westralunio carteri* within the South West Coast Drainage Division (shaded grey). Adapted from Klunzinger (2012).

Despite the recognition of the threatened status of *W. carteri* by international and Australian agencies, there are no conservation management or threat abatement plans in place for the species. Theoretically, some degree of protection could be afforded to populations that are found in terrestrial conservation reserves, but such reserves are not as effective for protecting aquatic biota as they are for terrestrial biota (Moilanen *et al.*, 2008; Herbert *et al.*, 2010). Conservation management for *W. carteri* is hindered by a lack of knowledge of key aspects of the biology of the species, such as the density and age structure of extant populations, microhabitat preferences and tolerance to key threatening processes. The aim of the current study is to address some of these key knowledge gaps.

1.9 Aims, hypothesis and structure of thesis

The general aim of this project is to address key knowledge gaps relating to the biology and ecology of *Westralunio carteri* for the purpose of developing conservation management plans for the species.

Chapter 2 investigates the density, age structure, fine scale distribution and microhabitat preference of populations of *W. carteri* within the known range of the species. It is hypothesised that, given the recent decline in range and the life history traits of the species, that extant populations would have limited recruitment. Many mussel species have an aggregated distribution and this is tested for *W. carteri*, using indices of the mean and variance among quadrat samples. It is hypothesised that the species has an aggregated distribution and that this can be explained, at least in part, by preferences for particular stream microhabitats.

Secondary salinisation and reduced water flow as a result of climate change have been identified as the major threats to the survival of *W. carteri* (Klunzinger *et al.*, 2015). In **Chapter 3**, the tolerance of the species to acute and gradual increases in water salinity

under controlled laboratory conditions is examined. These both represent realistic scenarios in the wild; salinity is gradually increasing in most rivers in south-western Australia, while in many rivers there is also a regular, seasonal spike in salinity caused by the flow of water from salinised upper catchments with the onset of winter rains. It is hypothesised that salinity tolerance will be greater if mussels are permitted to acclimatise to more gradual increases in salinity, as found, for example in the zebra mussel *Dreissena polymorpha* (Order Veneroida) (Kilgour *et al.*, 1994).

Chapter 4 combines field observations on drought-related mortality in populations of *W. carteri*, with experimental studies of behaviour and tolerance to water emersion. It is hypothesised that mussels can reduce their risk of mortality during water emersion by moving to track receding water levels or by burrowing into the substrate, and that the size-related mortality observed in the field can be explained by ontogenetic differences in horizontal and vertical patterns of movement.

Chapter 5 provides a summary of the results from the previous three chapters and discusses their implications for the future viability and conservation management of *W. carteri*, with suggestions for further studies.

Chapter 2: Population structure and microhabitat preference of the threatened freshwater mussel, *Westralunio carteri*, in south-western Australia

2.1 Introduction

Freshwater mussels (Class Bivalvia, Order Unionida) represent an ancient and widely separated lineage dating back to the Cambrian period, and are characterised by the presence of two hinged valves and a requirement for freshwater habitats (Fang, 2006; Bogan, 2008; Levine *et al.*, 2011). Almost 1,000 species of freshwater mussels exist, and are found across all continents with the exception of Antarctica (Graf, 2013; Graf & Cummings, 2014). Freshwater mussels play an important functional role in freshwater ecosystems, and are often recognised as keystone species because they improve water quality and enhance connectivity between benthic and pelagic zones (Strayer, 2008; Mackie & Claudi, 2009). Over the last century, there has been a dramatic global decline in both species richness and overall abundance of freshwater mussels, resulting in Unionida being recognised as one of the most endangered faunal groups in the world (Lydeard *et al.*, 2004; Badiu *et al.*, 2011; IUCN, 2016).

The global decline of freshwater mussel populations is likely due, in part, to their life-history characteristics (Galbraith *et al.*, 2010). Most freshwater mussels have an obligate parasitic stage as larvae, making them vulnerable to co-extinction with their fish hosts, while adult mussels are relatively sedentary, increasing their vulnerability to environmental disturbances. Mussels are also often long-lived (decades to up to 100+ years, for example in *Margaritifera margaritifera*), with delayed reproduction and long generation times; this not only limits their capacity to recover from population declines, but also means that adult populations can persist after juvenile recruitment has ceased.

Such relict populations, consisting solely of adult mussels, have been reported for a number of species (e.g. Hanson & Locke, 2001; Österling *et al.*, 2008; Strayer & Malcom, 2012) and thus may represent an extinction debt.

Understanding the factors that determine the distribution and abundance of freshwater mussels is essential to their conservation, as this knowledge underpins the development of environmental targets to guide river and catchment management (Quinlan *et al.*, 2015a). A wide range of factors, operating at local habitat, river reach and catchment scales, have been identified as determinants of mussel distribution and abundance (Strayer, 2008; Haag, 2012). In particular, a number of studies have emphasised the importance of microhabitat characteristics, such as substrate type, hydrology, in-stream debris and macrophyte cover for mussels (e.g. Strayer, 1993; Hastie *et al.*, 2000b; Box *et al.*, 2002; McRae *et al.*, 2004; Cyr *et al.*, 2012).

Carter's freshwater mussel, *Westralunio carteri* Iredale 1934, is the only Unionida known from south-western Australia, a recognised biodiversity hotspot (Myers *et al.*, 2000), and is the sole representative of the genus *Westralunio* present on the Australian continent (Klunzinger *et al.*, 2012a). A combination of secondary salinisation, reduced water flow and nitrogen pollution has led to a 49% reduction in extent of occurrence of *W. carteri* over the past 50 years (Klunzinger *et al.*, 2015), resulting in its classification as Vulnerable by the IUCN (Klunzinger & Walker, 2014), and both the Western Australian and Australian governments.

While recent studies have clarified many aspects of the biology of *W. carteri*, including larval release and morphology (Klunzinger *et al.*, 2010); host fish species (Klunzinger *et al.*, 2012a); and growth rate, longevity and age at maturity (Klunzinger *et al.*, 2012a), there are still a number of knowledge gaps that are hindering the development of effective conservation management plans. In particular, although the current range of *W. carteri* was comprehensively mapped by Klunzinger *et al.* (2015), this was based

almost entirely on presence/absence data; the density, age structure and recruitment status of most extant populations is not known. In addition, while some key water quality variables (salinity, water permanency and nitrogen level) limiting the range of the species have been identified (Klunzinger *et al.*, 2015), there is no information on microhabitat requirements. The aim of the current study was to address these knowledge gaps by surveying a number of extant populations of *W. carteri* to estimate: (1) mussel density; (2) pattern of spatial distribution; (3) size and age structure; and (4) the relationship between mussel abundance and microhabitat features.

2.2 Methods

2.2.1 Study area

Mussel populations were sampled from 47 sites from 13 river basins in the Southwestern Province (former South West Coast Drainage Division, SWCDD), one of 12 drainage divisions in Australia. The Southwestern Province encompasses a land mass of ~326, 000 km² and 19 river basins. The region has a characteristic Mediterranean climate with hot dry summers and cool wet winters (Pen, 1999). *Westralunio carteri* has an estimated extent of occurrence of 22, 600 km² (Klunzinger *et al.*, 2012a). Sampling sites for the current study were chosen from throughout the known distribution of the species, and all had recently recorded occurrence data (Klunzinger *et al.*, 2015). Sampling occurred between February and June in 2016 and 2017 (i.e. autumn to early winter) when the water level is low. The location of each site was recorded using a Garmin® Etrex30 GPS handset.

2.2.2 Mussel sampling

At each site, three to five transects were selected across the width of the channel, depending on channel accessibility, and 10 random quadrats (each 625 cm²) were taken at each transect. For each transect, surveys occurred in a downstream to upstream direction for a distance of up to 5 m of stream. Quadrat sampling is the preferred method

for estimating dispersion and density of mussels in wadeable streams (Obermeyer, 1998; Strayer & Smith, 2003). A range of quadrat sizes are typically used, depending on mussel density; which for this study was based on a quadrat size using preliminary, unpublished estimates of density in *W. carteri* populations of up to 40 mussels per m². At this density, and using quadrats of size 625 cm², 15 quadrat samples would be required to estimate mean density with a precision of 20% (Downing & Downing, 1992). Mussels in each quadrat were searched and collected by hand, measured for maximum shell length (ML) (McMichael & Hiscock, 1958) to the nearest 0.1 mm and then promptly returned to their original location. For some sites, sediments were sieved by hand to search for juvenile mussels.

2.2.3 Microhabitat variables

A range of habitat variables were selected that are known to influence microhabitat selection in other species of freshwater mussels (see habitat survey sheet in Figures 2.7, 2.8, Supplementary Information). For each quadrat the following characteristics were recorded: distance from quadrat centre to the riverbank (Neves & Widlak, 1987; Morales *et al.*, 2006); water depth (James, 1985; Annie *et al.*, 2013); riverbed slope (James, 1985; Strayer, 1999); current velocity (Smith & Meyer, 2010; Quinlan *et al.*, 2015b); substrate grain size (McRae *et al.*, 2004; Brainwood *et al.*, 2008a); debris type (wood, leaves or artificial) (Smith & Meyer, 2010; Niraula *et al.*, 2015), height and percentage cover; percentage cover of total vegetation; percentage cover of macrophytes (Brainwood *et al.*, 2008a); and percentage cover of algae (Table 2.1). Distance to riverbank and water depth were measured with a tape measure to the nearest 5 cm. Riverbed slope was measured using an EmpireTM Magnetic POLYCAST[®] protractor to the nearest 1 degree. Substrate grain size was calculated using the particle size index of Strayer (1999). Briefly, the percentage cover of 10 sediment types (silt, clay, earth, sand, coarse sand, peat, grave/pebble, cobble, boulder, and rock) following Raven *et al.* (1998) was calculated, and each sediment type was assigned a value from 1 to 10 in order of increasing grain size, and a weighted average calculated for each

quadrat. Debris height was measured with a hard ruler to the nearest 0.5 cm. All percentage cover values (i.e. sediment type, debris, total vegetation, macrophytes and algae) were estimated by visual inspection to the nearest 5%.

Table 2.1. Variables measured for modelling the microhabitat preference of *Westralunio carteri*.

Variable Name	Type	Unit	Mean \pm SE	Range
Distance to riverbank	Continuous	m	1.0 \pm 0.04	0-4.7
Water depth *	Continuous	m	0.26 \pm 0.01	0.02-0.92
Riverbed slope	Continuous	°	8.1 \pm 0.31	0-49
Current velocity	Continuous	ms ⁻¹	0.05 \pm 0.01	0-1.00
Substrate grain size	Continuous	particle size index	3.0 \pm 0.1	0-11.1
Debris type	Categorical	wood, leaf, artificial		
Debris height	Continuous	cm	3.4 \pm 0.3	0-116
Debris cover	Continuous	%	38.3 \pm 1.6	0-100
Vegetation cover	Continuous	%	35.5 \pm 1.7	0-100
Macrophyte cover *	Continuous	%	13.8 \pm 1.3	0-100
Algae cover *	Continuous	%	19.9 \pm 1.5	0-100

* Variables removed in the final model

2.2.4 Data analysis

Shell length-frequency data were plotted for all populations. Klunzinger *et al.* (2014b) validated shell ring annuli for *W. carteri*, but these cannot be measured reliably on living animals (i.e. without sectioning the shell). To estimate age of mussels the age at length curves calculated by Klunzinger *et al.* (2014b) for five populations of *W. carteri* were used. Growth rate varies among populations, so for each population in this study two age estimates are reported; a high estimate based on the slowest growth rate and a low estimate based on the fastest growth rate reported by Klunzinger *et al.* (2014b).

Although the reproductive biology of *W. carteri* has not been intensively studied, Klunzinger (2012) reported the seasonality of gamete production in a number of populations; the youngest mussels in which gametes were found were 5 years old, corresponding to a mean ML of 31.5 mm (Klunzinger *et al.*, 2014b); mussels less than 31.5 mm in length were therefore considered to be juveniles. As juvenile mussels are likely to be under-represented in samples, the proportion of animals <10 years old (corresponding to a mean ML of approximately 54 mm; (Klunzinger *et al.*, 2014b) was used as an indication of recent recruitment (Österling *et al.*, 2010; Strayer & Malcom, 2012). Differences in this recruitment index among populations were assessed with a χ^2 test.

Mussel density was estimated for each population from both quadrat data and transect data and expressed as number of mussels m⁻². Mean and median density were calculated over all populations, with 95% confidence intervals around the mean from 1,000 bootstrap replications. Numerous indices have been proposed to describe the dispersion of individuals in a population (Hulbert, 1990; Bez, 2000; Reiczigel *et al.*, 2005). Quadrat data was used to estimate the variance to mean ratio (s^2/\bar{x}), and the Lloyd (1967) index (Lloyd, 1967) of patchiness $IP = 1 + s^2/\bar{x} - 1/\bar{x}$, both of which take a value of 1 for a random distribution, less than 1 for a distribution which is underdispersed (even) and greater than 1 for a distribution which is overdispersed (aggregated). Ninety five percent confidence intervals for each statistic were calculated using 1,000 bootstrap replications, and the significance of the departure of IP from 1 was also assessed by a χ^2 test, as recommended by Young and Young (1998).

Microhabitat preference was analysed using a generalised linear model in R (R Development Core Team, 2016), with number of mussels per quadrat (referred to here as abundance) as the response and microhabitat variables as predictors. Prior to analysis, correlations among microhabitat variables were examined and significant correlations were found between distance to riverbank and water depth, and percentage vegetation

cover with both percentage macrophyte cover and percentage algae cover (Table 2.2). To reduce collinearity among predictors, and to minimise over-fitting during model construction, water depth, macrophyte cover and algae cover were removed from the variable set. This left eight, largely independent microhabitat predictors (all pairwise correlations < 0.3 , Table 2.2).

Table 2.2. Correlations among microenvironmental variables. BD: Distance to riverbank, WD: Water depth, BS: Riverbed slope, DH: Debris height, DC: Debris cover, FV: Flow velocity, SG: Substrate grain size, VC: Vegetation cover, MC: Macrophyte cover, AC: Algae cover. Significant correlations ($p < 0.05$) in **bold**.

	WD	BS	DH	DC	FV	SG	VC	MC	AC
BD	0.436	-0.290	0.013	-0.070	0.058	0.103	0.026	0.085	0.019
WD		-0.184	-0.055	-0.130	-0.031	0.223	-0.063	0.145	-0.171
BS			-0.020	-0.038	-0.132	-0.046	-0.043	-0.141	0.003
DH				0.268	0.122	-0.084	-0.052	-0.086	-0.041
DC					-0.176	-0.243	-0.054	-0.220	0.062
FV						0.035	-0.140	0.054	-0.188
SG							0.081	0.148	0.003
VC								0.540	0.673
MC									-0.122

Because mussels were aggregated in space (see Results), mussel distribution was modelled as a negative binomial, using the R package MASS (Ripley *et al.*, 2018). Because there was a large number of quadrats which contained no mussels, a zero-inflated negative binomial model was examined using the R package pscl (Jackman, 2017). Neither model showed evidence of overdispersion (ratio of residual deviance to residual degrees of freedom less than two, and dispersion parameter close to one in both cases). The Vuong test for model selection gave inconclusive results (and in any case may not be appropriate as a test of zero-inflation; (Wilson, 2015), and there was little

difference between the models in normal QQ plots, or in AIC or BIC values (see Supplementary Information, Figure 2.9 and Table 2.6). Zero-inflated models assume that excess zeros are generated by a separate process from count data and are therefore modelled separately. Because this analysis was restricted to localities that contained mussel populations on a reach scale (i.e. there was never a zero probability of mussels being present in a quadrat in the selected reach), the negative binomial model was more appropriate and is presented here. The results from the zero-inflated model are presented as Supplementary Information (Table 2.7).

A multi-model inference approach was employed to determine the relative importance of microhabitat variables in explaining mussel distribution. First, a global model was fit using all eight non-correlated predictors. The global model was used to generate a set of all possible models using the R package MuMIn (Bartoń, 2018). Models were ranked by their AIC value and model averaging performed across all models within four AIC values of the best model. The importance of each variable was measured as the proportional occurrence of that variable across all models within the top ranked set.

To investigate whether spatial autocorrelation among predictor variables might be influencing the results, the analyses were repeated separately for catchments on the west coast (i.e. those flowing into the Indian Ocean) and the south coast (those flowing into the Southern Ocean; see Figure 2.1). To investigate whether microhabitat variables affected mussels of different ages differently, ML (assuming a Gaussian distribution) was modelled with the same set of predictors and using the same approach as described above for the GLM analysis.

2.3 Results

A total of 1080 mussels were found and measured from 31 sites in 10 river basins (Figure 2.1). No mussels were found in 15 sites, despite them having been recorded as

present in these localities by Klunzinger *et al.* (2015), based on occurrence records from 1992-2012.

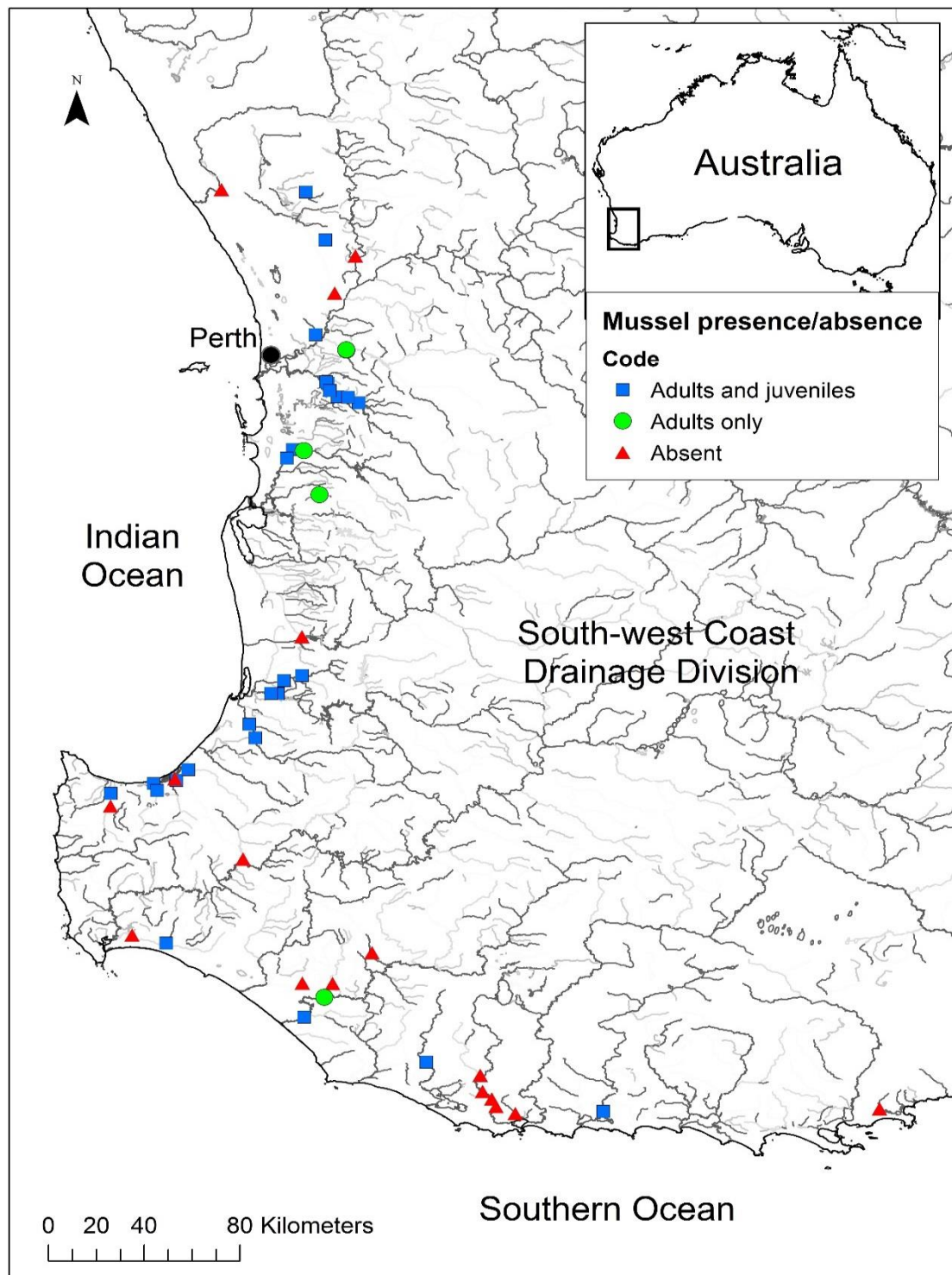
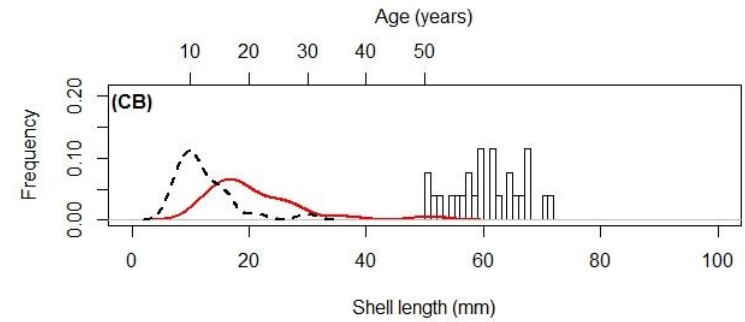
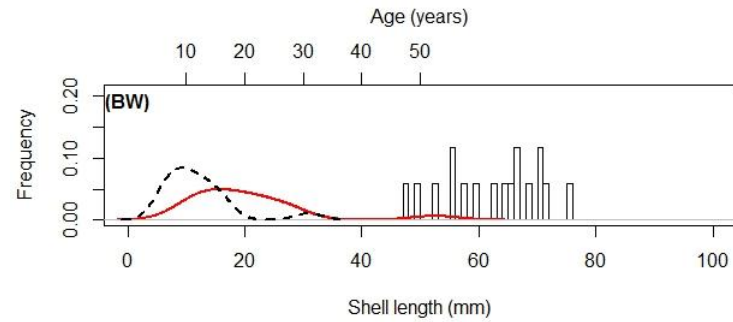
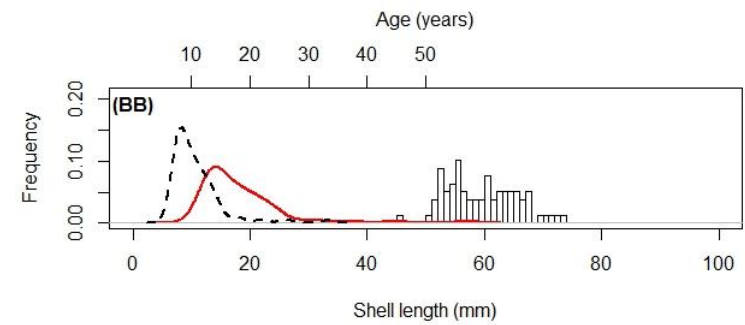
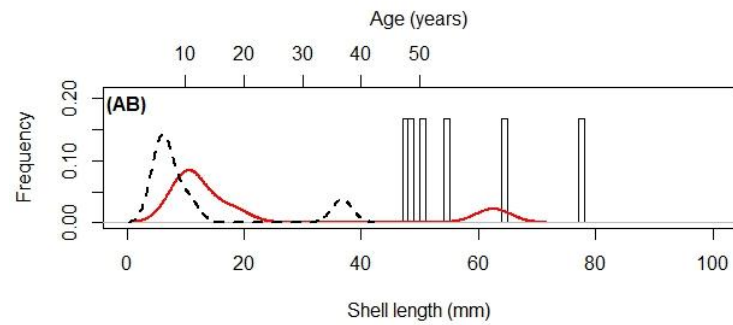
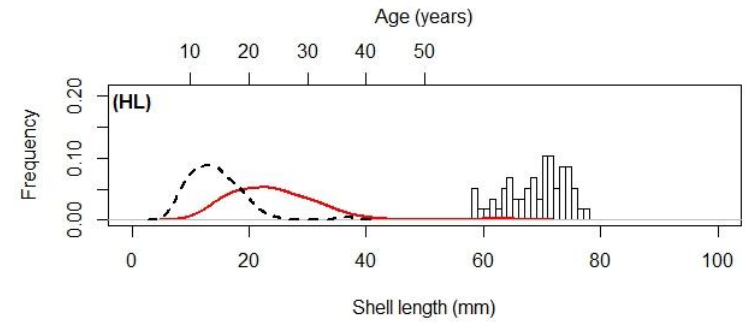
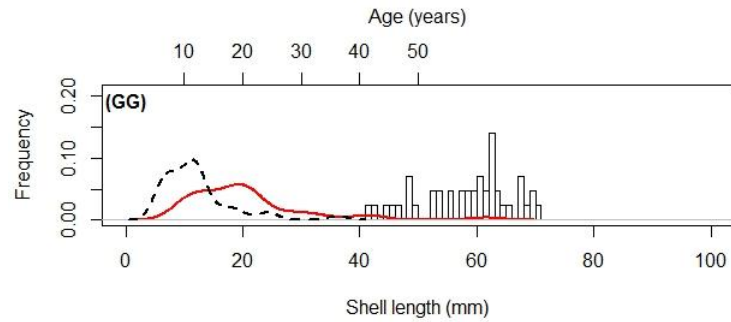
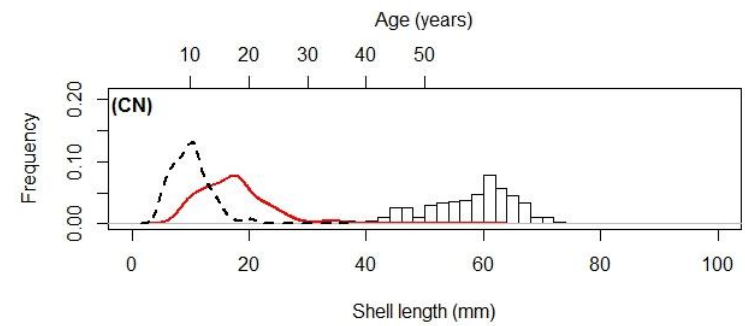
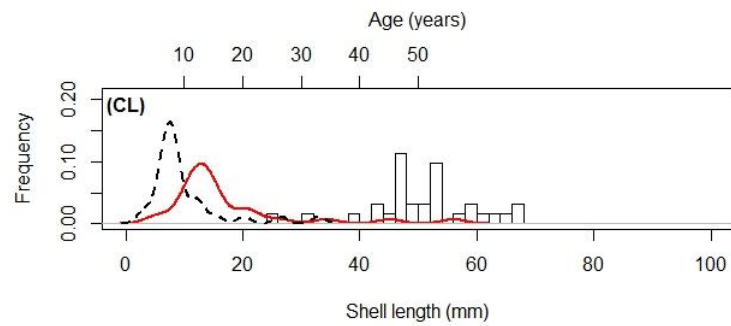


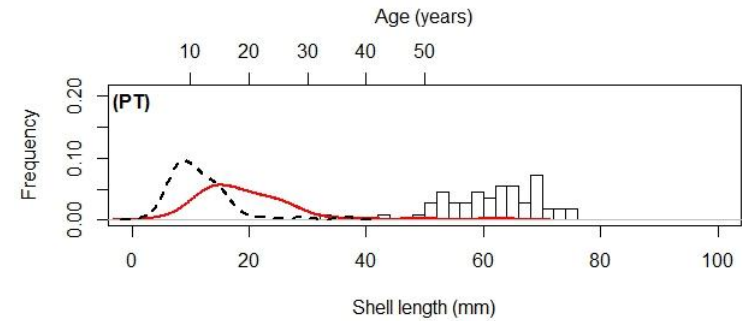
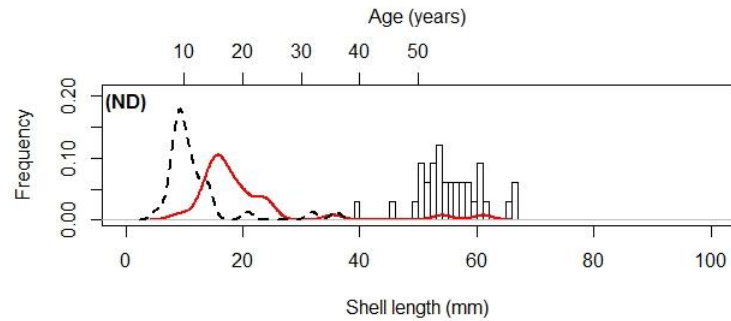
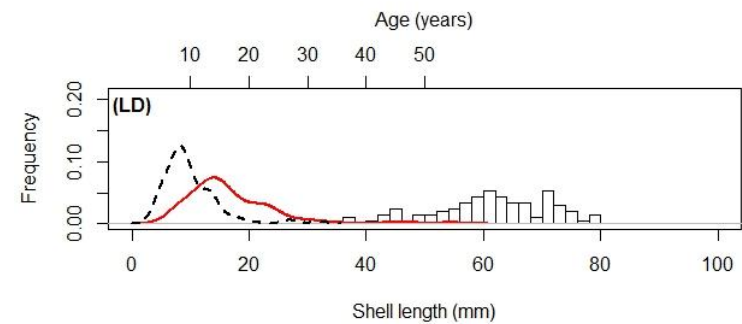
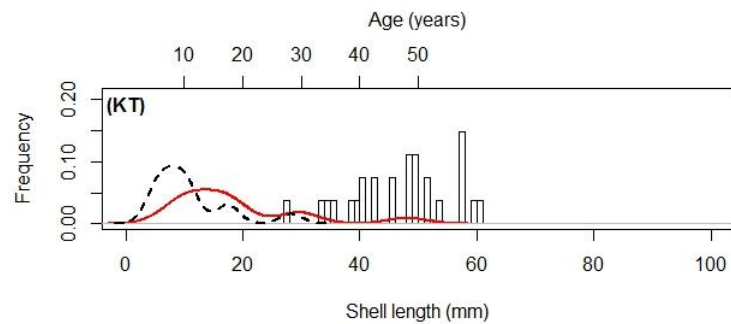
Figure 2.1. Sampling localities and survey findings. Young mussels refer to those with a maximum shell length < 54 mm, corresponding to an age of approximately 10 years (see Methods).

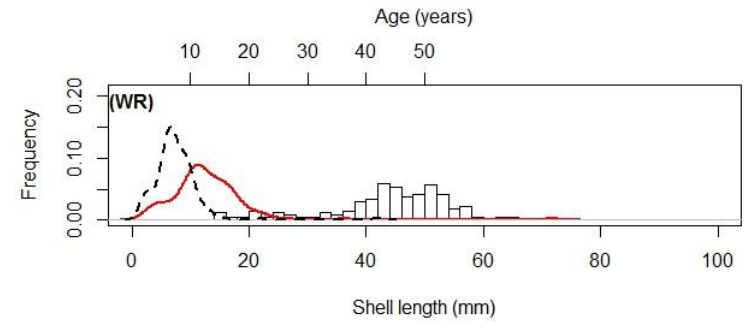
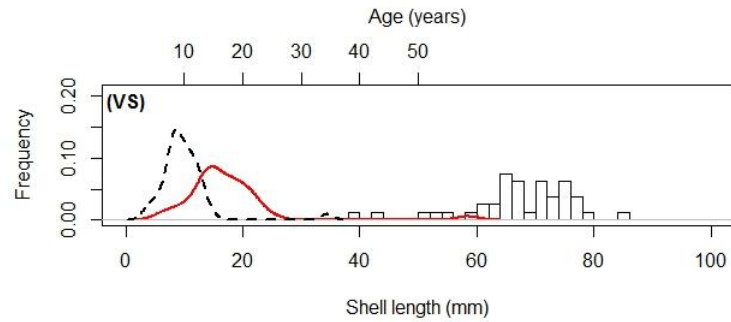
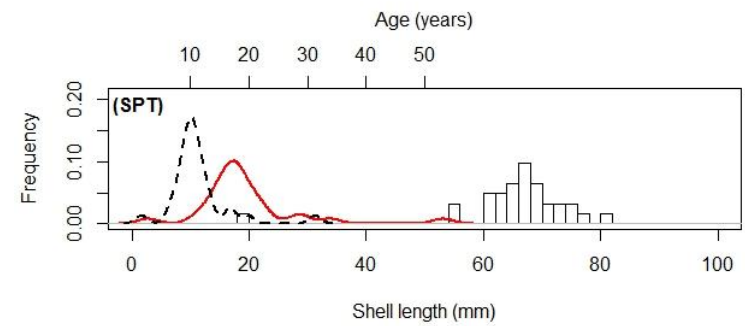
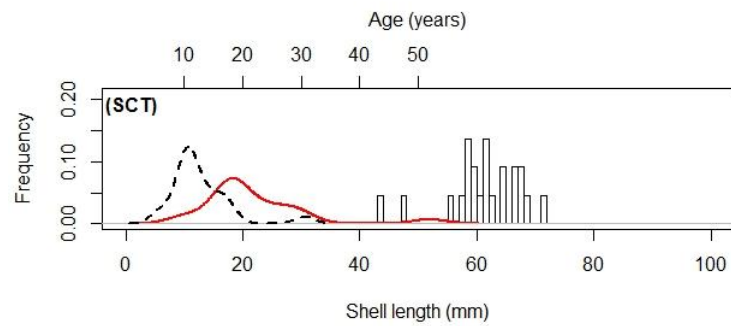
2.3.1 Population structure

For analysis of population structure, some adjacent sites in the same river were combined, giving a total of 17 sampled populations. Over all populations, ML of sampled mussels ranged from 15.0 mm to 95.6 mm (mean = 60.4 ± 0.4 mm). Using size at age estimates from Klunzinger *et al.* (2014b), this corresponds to an age range of approximately 1.5-71.6 years, with the majority of mussels aged between 10 and 20 years. The size distribution was generally similar across all populations (Figure 2.2). Juvenile mussels (ML < 31.5 mm) were rare and found in only four populations. However, all populations showed some evidence of recent recruitment (i.e. mussels aged 10 years or less). Recruitment index (proportion of mussels ≤ 10 years) varied from 0.07 – 0.57 (mean = 0.29 ± 0.03), with no significant difference in the ratio of young: old mussels among populations ($\chi^2_{16} = 1.69$, P = 1).









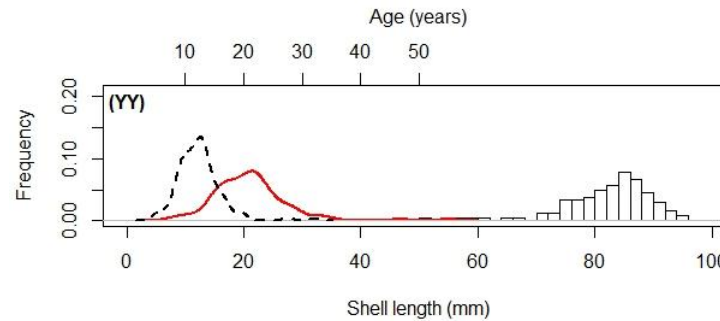


Figure 2.2. Distribution (proportion) of shell lengths (bars) and ages (dashed line - lower estimate; solid line – upper estimate) of mussels from all sampled populations. AB – Abba River, BB – Bennett Brook, BW – Brunswick River, CN – Canning River, CB – Carburnup River, CL – Collie River, GG – Gingin Brook, HL – Helena River, KT – Kent River, LD – Ludlow River, ND – North Dandalup River, PT – Preston River, SCT – Scott River, SPT – Serpentine River, VS – Vasse River, WR – Warren River, YY – Yalyal Brook.

2.3.2 Density and dispersion

The number of mussels per quadrat over all sites ranged from 0 to 32, with a mean of 1.8 (95% CI 1.6 – 2.2). Almost half (46%) of the quadrats contained no mussels. Estimates of mussel density over all populations on a per quadrat and per transect basis are shown in Table 2.3.

Table 2.3. Range, mean and median density (number of mussels m⁻²) of *Westralunio carteri* over 31 different sites, estimated from quadrat and transect samples.

Parameter	Min	Max	Mean (95% CI)	Median
Quadrat density (all)	0	512	29.5 (25.9-34.5)	16
Transect density (all)	2	169	28.7 (22.6–37.9)	23

Westralunio carteri had an aggregated dispersion within populations, approximating a negative binomial with $k = 0.47$ (Figure 2.3; observed values not significantly different from expected by χ^2 test, $p = 0.72$). The variance: mean ratio = 5.9 (95% CI 4.4-8.4) and LPI = 3.6 (95% CI 3.0 - 4.7) (χ^2 test of departure from randomness, $p < 0.001$). The dispersion remained aggregated even when quadrats without mussels were removed from the analysis (variance: mean ratio = 4.3, 95% CI 3.0-6.5; LPI = 2.0, 95% CI 1.6-2.5, χ^2 test $p < 0.001$).

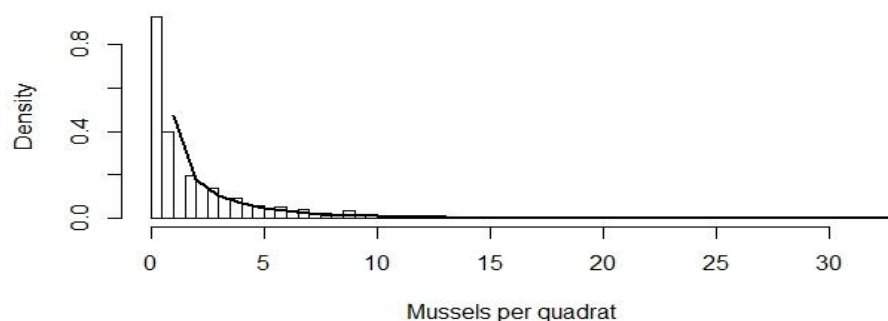


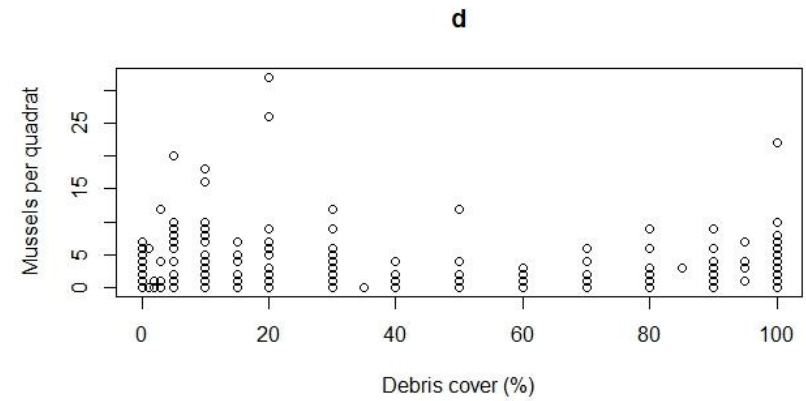
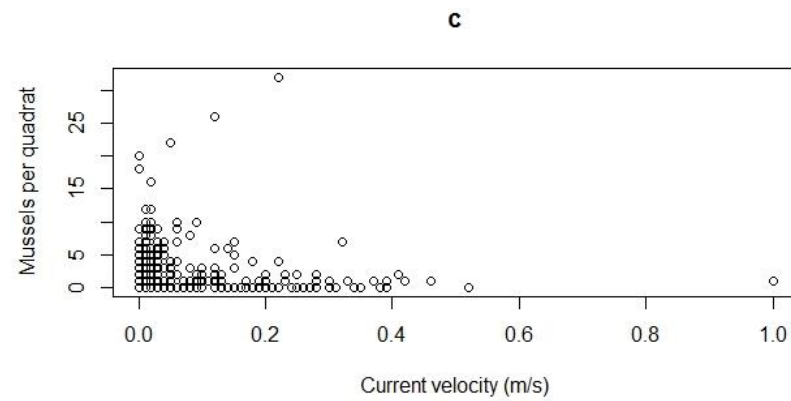
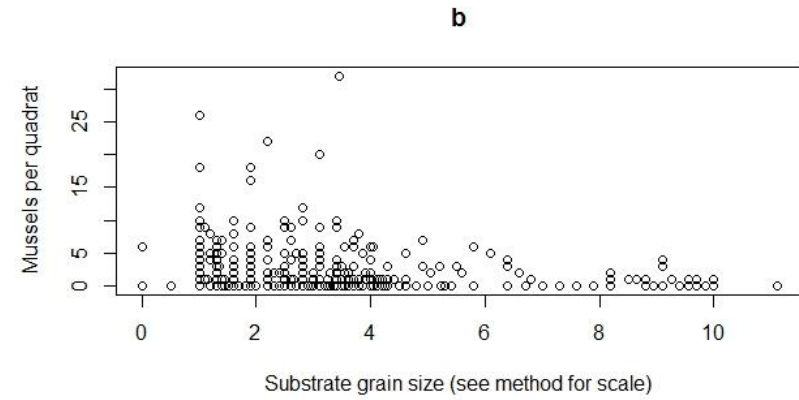
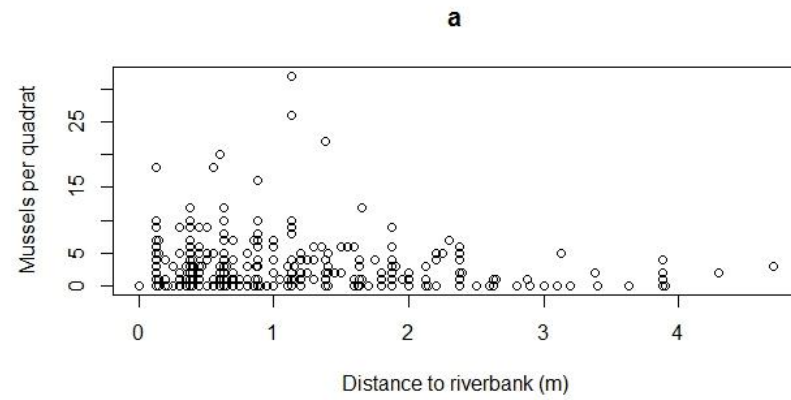
Figure 2.3. Observed distribution of mussel densities (calculated on a per quadrat basis) with fitted negative binomial line.

2.3.3 Microhabitat preference

The global GLM for mussel abundance, using all seven predictor variables, had a residual deviance of 530.9 on 538 degrees of freedom and a dispersion parameter of 0.57, indicating that no correction was necessary for overdispersion. Model averaging suggested that substrate grain size, distance to the riverbank, current velocity and the percentage cover of debris in the quadrat were the most important predictors of mussel abundance (Table 2.4). Mussel abundance was greater closer to the riverbank, and in quadrats with low current flow, finer substrate, less debris and debris of a greater height (Figure 2.4). When west and south coast regions were analysed separately, the same trends in predictor variables were found (see Supplementary Information, Tables 2.8 and 2.9).

Table 2.4. Association between microhabitat variables and abundance of *W. carteri* in quadrat samples. Variables are ranked by relative importance (probability of selection in AIC best-fit model), with coefficient estimates and standard errors from model averaging. Significant predictors ($p < 0.05$) are shown in **bold**; those near significance ($p < 0.1$) are *italicised*.

Microhabitat variable	Importance	Est. Coef. (\pm s. e.)	Z Value	Pr ($> z $)
<i>Distance to riverbank</i>	<i>1.00</i>	<i>-0.155 (± 0.087)</i>	<i>-1.780</i>	<i>0.075</i>
Substrate grain size	1.00	-0.182 (± 0.035)	-5.182	<0.001
<i>Current velocity</i>	<i>0.78</i>	<i>-1.390 (± 0.811)</i>	<i>-1.714</i>	<i>0.087</i>
Debris cover	0.64	-0.006 (± 0.002)	-2.460	0.014
Riverbed slope	0.29	0.011 (± 0.009)	1.129	0.259
Debris type-leaf	0.15	0.235 (± 0.157)	1.497	0.134
Debris type-wood	0.06	0.104 (± 0.157)	0.661	0.508
Debris height	0.11	0.025 (± 0.009)	2.813	0.005
Vegetation cover (%)	0.06	0.0003 (± 0.0017)	0.224	0.823



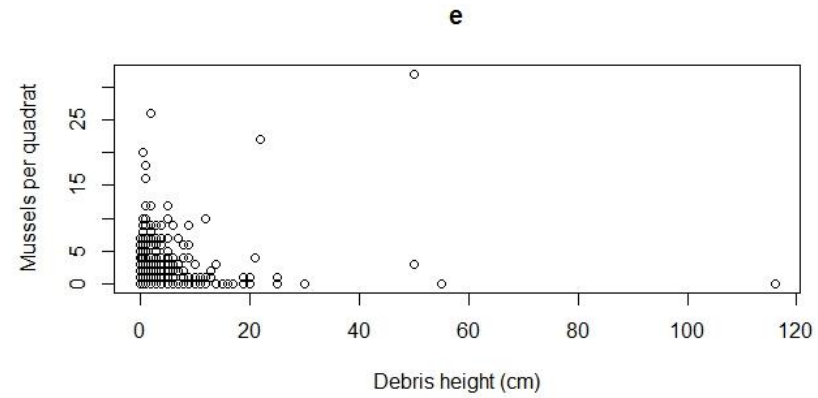


Figure 2.4. Relationships between mussel abundance and microhabitat variables identified as important in the GLM analysis: (a) Distance to riverbank; (b) Substrate grain size; (c) Current velocity; (d) Debris cover; (e) Debris height.

The variable with most influence on mussel abundance was substrate grain size. Mean substrate grain size was significantly less in quadrats which contained mussels than in those quadrats in which mussels were not found (Figure 2.5; $t_{549} = 4.62$, $p < 0.0001$). Figure 2.6 shows the distribution of grain size scores for those quadrats which contained mussels; mussels were most abundant on silt and sand substrates.

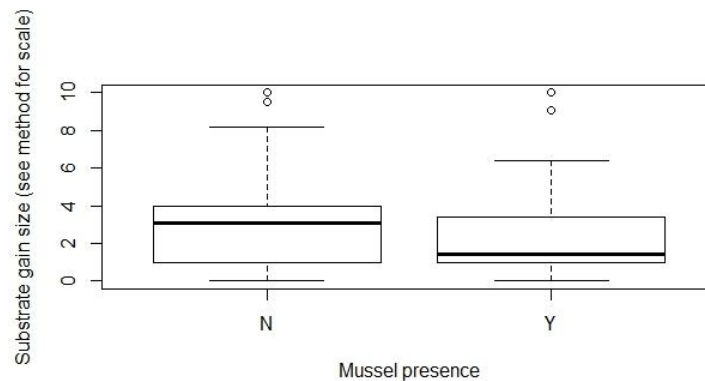


Figure 2.5. Substrate grain size score for quadrats with (Y) and without (N) mussels.

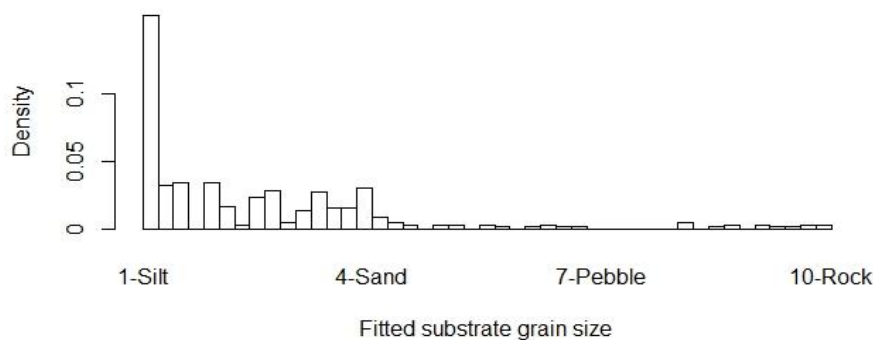


Figure 2.6. Distribution of substrate grain size scores for all quadrats in which mussels were found.

The global general linear model for ML, using all seven predictor variables, had a residual deviance of 531 on 539 degrees of freedom and a dispersion parameter of 0.56, indicating that no correction was necessary for overdispersion. Model averaging

suggested that distance to the riverbank, current velocity, and the type, height and percentage cover of debris in the quadrat were the most important predictors of mussel size (Table 2.6). Mussel size was negatively correlated with distance to the riverbank and percentage cover of vegetation, and positively correlated with the presence and height of organic (as opposed to artificial) debris.

Table 2.5. Association between microhabitat variables and maximum shell length of *W. carteri* in quadrat samples. Variables are ranked by relative importance (probability of selection in AIC best-fit model), with coefficient estimates and standard errors from model averaging. Significant predictors ($p < 0.05$) are shown in **bold**.

Microhabitat variable	Importance	Est. Coef. (\pm s. e.)	t Value	Pr ($> z $)
Distance to riverbank	1.00	-2.886 (± 0.414)	-6.966	<0.001
Current velocity	1.00	17.668 (± 5.355)	3.300	0.001
Debris type-leaf	1.00	3.000 (± 0.812)	3.695	<0.001
Debris type-wood	1.00	3.300 (± 0.821)	4.018	<0.001
Debris height	1.00	0.111 (± 0.023)	4.902	<0.001
Vegetation cover	1.00	-0.068 (± 0.009)	-7.756	<0.001
Debris cover	0.58	-0.018 (± 0.011)	-1.637	0.102
Substrate grain size	0.54	-0.331 (± 0.221)	-1.496	0.135
Riverbed slope	0.27	-0.013 (± 0.052)	-0.257	0.797

2.4 Discussion

Westralunio carteri, the only freshwater mussel species in south-western Australia, has undergone a dramatic range decline in the last 50 years (Klunzinger *et al.*, 2015). Although the current distribution has been mapped and the principal environmental threats identified (Klunzinger *et al.*, 2015), this is the first study to investigate the age structure, density, dispersion and microhabitat preference of remaining extant populations of the species. These findings have important implications for the

conservation management of this threatened species.

2.4.1 Population structure

Mussels were found in only 64% of examined sites, despite the sample sites being chosen from a database of recent (1992-2012) occurrence records. This is not necessarily an indication of recent extinction of these populations; occurrence records provided no indication of population size, so it may be that mussel density was very low and that transect sampling did not locate them. Nevertheless, given the current listing of the species as Vulnerable, the absence of mussels in this study is of concern and there is a need to revisit these sites for more intensive study.

An encouraging finding from this study was evidence of recent recruitment in almost all of the populations where mussels were sampled, in contradiction to the initial hypothesis that recruitment would be limited. Although sediments in each quadrat were sieved by hand to locate mussels, this was unlikely to detect juvenile mussels (ML < 31.5 mm) and these were therefore under-represented in the samples (only 5 juvenile mussels over all populations). This is not surprising because juvenile freshwater mussels are often difficult to detect by standard hand sampling techniques (Strayer & Smith, 2003). This is partly due to their small size, but also to a propensity to burrow deeper into the substrate (Hastie *et al.*, 2000a), and in many cases they have different habitat requirements to adult mussels (Neves & Widlak, 1987; Morales *et al.*, 2006). In addition, most freshwater mussel species are long-lived and the juvenile phase represents a only a very small component of the lifespan, so they are expected to have a relatively low abundance when hand sampling in most populations (Strayer & Smith, 2003).

The proportion of young mussels (those with ML < 54 mm, approximately equivalent to < 10 years of age) may represent a better measure of recruitment success. By this measure, all except two populations appeared to be actively recruiting, although there

were substantial differences among populations, with between 7 and 57% of the population in this age bracket. Studies of the age structure of freshwater mussel populations are relatively rare, but where they have been carried out, the distributions are frequently right-skewed, which might reflect unionid longevity and higher mortality rates in juvenile mussels (Österling *et al.*, 2010; Strayer & Malcom, 2012). Juvenile mortality and recruitment failure has been associated with a number of abiotic and biotic factors in other studies, including high water flow (Myers-Kinzie, 1998), sensitivity to pollutants (Augspurger *et al.*, 2003), increased turbidity and sedimentation (Österling *et al.*, 2010), and nitrogen enrichment of the sediment (Strayer & Malcom, 2012).

The maximum age recorded by Klunzinger *et al.* (2014b) in five populations of *W. carteri* was 52 years, in a mussel with a shell length 78.6 mm. The maximum shell length found in the current study was 95.6 mm; although the precise age of this individual was not determined, using the range of age at length estimates of Klunzinger *et al.* (2014b), this corresponds to an age range of 42 – 72 years. *Westralunio carteri* is a relatively slow-growing, long-lived species, compared to other Australian hyriids (Walker *et al.*, 2001), although maximum age and growth rates are similar to other species of freshwater mussels in the families Unionidae and Margaritiferidae (Haag & Rypel, 2011). This has raised concerns about the health of existing populations of *W. carteri* because of their unknown age structure (Klunzinger *et al.*, 2014b). Evidence from the current study of active recruitment has alleviated these concerns to some extent, although the possible absence of mussels from a number of populations previously identified by Klunzinger *et al.* (2015) indicates that there is not room for complacency about the ongoing conservation of this species.

2.4.2 Density and dispersion

The mean density across the 31 sites from which *W. carteri* sampled in this study was approximately 29 mussels m⁻², when estimated at both quadrat and transect scales.

Previous studies have found great variability in the density of freshwater mussels, both within and among species, with estimates ranging from much less than one to several hundred per square metre (Strayer *et al.*, 1981; James, 1985; Downing & Downing, 1992; Hastie *et al.*, 2000a; Zigler *et al.*, 2008; Annie *et al.*, 2013). There have been only limited estimates of population densities among Australian Hyriidae: Humphrey (1984) found a maximum density of 18.2 m⁻² for *Velesunio angasi* in Magela Creek, Northern Territory; Walker (1981) reported densities of 1.2 m⁻² for *Alathyria jacksoni* and 3.7 m⁻² for *Velesunio ambiguus* in the Murray River, South Australia; and Brainwood *et al.* (2008b) found a mean density of 50 mussels m⁻² in mixed species assemblages (*V. ambiguus*, *Hyridella australis* and *H. depressa*) in the Hawkesbury-Nepean River in New South Wales. The relatively high density of *W. carteri* may be at least partially explained by a lack of interspecific competition, as it is the only freshwater mussel species in south-western Australia.

Westralunio carteri had a strongly aggregated dispersion within populations. This is not uncommon in freshwater mussels (Neves & Widlak, 1987; Downing & Downing, 1992; Downing *et al.*, 1993; Hastie *et al.*, 2000a), although a random dispersion has been reported in some species (Cyr *et al.*, 2012) and patterns of dispersion may differ among populations of the same species and temporally in the same population (Downing & Downing, 1992; Perles *et al.*, 2003; Zieritz *et al.*, 2014). There are a number of (not mutually exclusive) explanations for aggregation in freshwater mussel populations. Aggregation on the stream bed might be simply a by-product of the aggregation of glochidia among fish hosts, which has been previously reported for *W. carteri* (Klunzinger *et al.*, 2012b) and is an almost universal feature of macroparasite/host relationships; or of random spatial variation in the survival of juvenile mussels, once they detach from the host (Hansen & Larsen, 2005; Verween *et al.*, 2007; Terui & Miyazaki, 2015). Alternatively, aggregation may be an adaptation, for example to enhance reproductive success; Downing *et al.* (1993) found a strong relationship between aggregation and fertility in *Elliptio complanata* and aggregation has been

found to increase during spawning periods in a number of species (Amyot & Downing, 1998; Zieritz *et al.*, 2014). Aggregation may also arise if juvenile or adult mussels exhibit microhabitat preferences and preferred microhabitats are spatially discontinuous (Perles *et al.*, 2003).

2.4.3 Microhabitat preference

The distribution of freshwater mussel species is likely to be influenced by a number of different factors operating over a range of ecological scales (Strayer, 2008). Klunzinger *et al.* (2015) identified water salinity, habitat permanence and nitrogen level as important reach-scale determinants of the presence of *W. carteri*. In this study, a number of habitat features were found to be associated with mussel abundance at a local scale, including: substrate grain size, the amount of organic debris, debris height and distance to the riverbank. More mussels were found on fine sediments, in the proximity of tall debris and close to the riverbank. It should be noted, however, that the absence of “good” environmental characteristics that predict mussel density does not preclude the presence of *Westralunio carteri*, with individuals also occasionally found in both steep and rocky riverbeds, as has been shown in other species across the world (Brainwood *et al.*, 2008a; Smith & Meyer, 2010).

A number of previous studies have found substrate grain size to be an important predictor of mussel abundance, although in contrast to the results in this study, most have reported an apparent preference for coarser substrates (James, 1985; Huehner, 1987; Neves & Widlak, 1987; Hastie *et al.*, 2000b; Johnson & Brown, 2000; McRae *et al.*, 2004; Brainwood *et al.*, 2008a; Quinlan *et al.*, 2015b). These studies have generally been in large rivers with permanent water flow, where stream bed stability, which is greater at larger particle sizes, is an important requirement for benthic organisms. Stream bed scouring associated with flood events can be a major source of mortality for freshwater mussels (Vannote & Minshall, 1982; Hastie *et al.*, 2001; Morales *et al.*, 2006). However, south-western Australia, like many other areas of the world with arid,

semi-arid or Mediterranean climates, is characterised by relatively small, temporary river systems, which cease to flow in the dry season and are often reduced to a number of disconnected refuge pools. Flooding events are rare and benthic stability may not be a major habitat requirement in these systems.

A few previous studies have also found mussel abundance to be associated with finer sediments. For example, Michaelson and Neves (1995) found that *Alasmidonta heterodon* (the Endangered dwarf wedgemussel) preferred fine to medium and coarse sediments, and Box *et al.* (2002) recorded an association of *Villosa lienosa* and proportion of fine sediments in the substrate. Finer substrates, such as silt, may contain more organic matter as a food source for filter feeding mussels (Wentworth, 1922; Lara & Parada, 2009), although silt is also considered to be detrimental to feeding and respiratory activity in mussels (Roper & Hickey, 1995; Box & Mossa, 1999). A more likely explanation for a preference for finer substrates may be that they enhance burrowing efficiency (Lewis & Riebel, 1984; Morales *et al.*, 2006). Burrowing may provide protection against drought (Amyot & Downing, 1997; Gough *et al.*, 2012), which would be an important adaptation in regions with temporary rivers, such as south-western Australia, where river drying is a regular seasonal occurrence. The effect of borrowing on survival during drying in *W. carteri* is investigated in more detail in Chapter 4.

In addition to the association with finer substrates, suggesting a preference for ease of burrowing over stream bed stability, avoidance of rapid water flow also appears to be another important microhabitat requirement for *W. carteri*. Mussel abundance was greater close to the riverbank, where flow velocity is usually lower, and in areas with tall woody debris, which would provide protection against high flow. Recent studies have identified a range of hydraulic variables as important predictors of mussel density (Steuer *et al.*, 2008; Maloney *et al.*, 2012), and mussel abundance is often greatest in flow refuges, such as channel banks and border areas, amongst root-mats and the

downstream side of boulders, logs and large woody debris (Neves & Widlak, 1987; Strayer, 1999; Howard & Cuffey, 2003; Zigler *et al.*, 2008). This apparent preference for flow refuges is usually considered an adaptation to prevent mussels being swept away (Strayer, 1999; Maloney *et al.*, 2012), although areas of low flow may also provide a better food source for filter feeders such as mussels (Morales *et al.*, 2006) and proximity to river banks may be a hypoxia-avoidance strategy, with deeper habitats more likely to contain reduced dissolved-oxygen saturations, as a result of water column stratification (Quinlan *et al.*, 2015b). In the current study, untested evidence suggested that mussels were present further from the river bank in flat, relatively shallow channel beds, which is consistent with the strategy of reducing the risk of hypoxic exposure, although the data were not adequate to properly test this hypothesis. In addition to providing protection in high flow conditions, organic debris may reduce sedimentation (Hastie *et al.*, 2000b; Quinlan *et al.*, 2015b).

A similar pattern of association between mussel abundance and microhabitat variables in different age classes of *W. carteri* was found in the current study, although there was a tendency for smaller mussels to be more associated with areas of low current flow and greater vegetation cover. This may reflect a greater tolerance of high flow conditions in older, larger mussels (Strayer, 2008) or a greater density of fine organic matter, more suitable as a food source for small mussels, in flow refuges (Neves & Widlak, 1987). Although some previous studies on other species of mussels have found juvenile and adult mussels to occupy distinct microhabitats (Walker, 1981; Brainwood *et al.*, 2008a), other studies, like this one, have found similar microhabitat requirements across age classes (Hastie *et al.*, 2000b; Annie *et al.*, 2013).

Associations between mussel abundance and microhabitat features may arise through a random distribution on settling, followed by differential mortality in habitats of different suitability, or from active site selection by mussels. Although freshwater mussels are often regarded as relatively sessile organisms, movement rates of up to 60

cm per day have been recorded, with rates varying seasonally and between species (Amyot & Downing, 1997; Amyot & Downing, 1998; Schwalb & Pusch, 2007; Gough *et al.*, 2012; Daniel & Brown, 2014). This suggests that active site selection is possible and, indeed, this has been demonstrated in laboratory experiments for several mussel species (Huehner, 1987; Michaelson & Neves, 1995). During the current study, extensive tracks left by *W. carteri* on stream beds were frequently observed and these may be related to microhabitat selection, although this hypothesis has not yet been tested.

2.5 Conclusions

This investigation is the first to examine the population structure, density, dispersion and microhabitat preference of the freshwater mussel, *W. carteri*. Mussels were found in 31 of 47 sites, and almost all of these populations had some evidence of recent (within the last 10 years) recruitment. Density was relatively high (approximately 29 mussels m^{-2}) and mussels were strongly aggregated. The abundance of *W. carteri* was positively associated with fine substrate grain size, closeness to the river bank, and the presence and height of debris on the stream bed. This suggests that substrate burrowability and protection from fast water flow are important habitat requirements for the species. The use of habitat variables characterised specifically for the Australian environment as used here, rather than those based upon European and North American studies, will enable better understanding of the factors influencing the fine-scale distribution of these important aquatic organisms.

2.6 Supplementary Information

Table 2.6. The AIC and BIC value for Generalised liner model (GLMs.NB) Model and Zero-Inflated Model (ZINB) with negative binomial distribution.

Model	AIC value	Df in AIC	BIC Value	Df in BIC
GLMs NB	1944.033	13	2000.085	13
ZINB	1909.683	21	2000.229	21

Table 2.7. Association between microhabitat variables and abundance of *W. carteri* in quadrat samples (ZINB Analysis). Variables are ranked by relative importance (probability of selection in AIC best-fit model), with coefficient estimates and standard errors from model averaging. Significant predictors ($p < 0.05$) are shown in **bold**.

Microhabitat variable	Importance	Est. Coef. (\pm s. e.)	Z Value	Pr ($> z $)
Distance to riverbank	1.00	-0.103 (\pm 0.098)	-1.060	0.289
Substrate grain size	1.00	-0.167 (\pm0.036)	-4.616	<0.001
Current velocity	0.78	-1.840 (\pm0.831)	-2.214	0.027
Debris cover	0.64	-0.004 (\pm0.002)	-2.082	0.037
Riverbed slope	0.29	0.016 (\pm 0.010)	1.547	0.122
Debris type-leaf	Not applicable for non-numerical variables			
Debris type-wood	Not applicable for non-numerical variables			
Debris height	0.11	0.039 (\pm0.016)	2.340	0.019
Vegetation cover (%)	0.06	0.001 (\pm 0.0016)	-0.630	0.529

Table 2.8. Association between microhabitat variables and abundance of *W. carteri* in quadrat samples in the West Coast Subdivision. Variables are ranked by relative importance (probability of selection in AIC best-fit model), with coefficient estimates and standard errors from model averaging. Significant predictors ($p < 0.05$) are shown in **bold**; those near significance ($p < 0.1$) are *italicised*.

Microhabitat variable	Importance	Est. Coef. (\pm s. e.)	Z Value	Pr ($> z $)
Distance to riverbank	1.00	-0.276 (± 0.105)	- 2.636	0.008
Substrate grain size	1.00	-0.138 (± 0.036)	-3.801	<0.001
<i>Current velocity</i>	<i>0.60</i>	<i>-1.637 (± 0.950)</i>	<i>-1.723</i>	<i>0.085</i>
Debris type-wood	0.57	0.104 (± 0.157)	0.661	0.508
Riverbed slope	0.40	0.006 (± 0.010)	0.661	0.508
<i>Debris cover</i>	<i>0.32</i>	<i>-0.005 (± 0.002)</i>	<i>-1.953</i>	<i>0.051</i>
Debris type-leaf	0.31	0.235 (± 0.157)	1.497	0.134
Vegetation cover (%)	0.16	-0.0002 (± 0.0018)	-0.124	0.901
Debris height	0.15	0.002 (± 0.008)	0.307	0.759

Table 2.9. Association between microhabitat variables and abundance of *W. carteri* in quadrat samples in the South Coast Subdivision. Variables are ranked by relative importance (probability of selection in AIC best-fit model), with coefficient estimates and standard errors from model averaging. Significant predictors ($p < 0.05$) are shown in **bold**; those near significance ($p < 0.1$) are *italicised*.

Microhabitat variable	Importance	Est. Coef. (\pm s. e.)	Z Value	Pr ($> z $)
Debris height	0.78	0.086 (± 0.041)	2.081	0.037
<i>Current velocity</i>	<i>0.74</i>	<i>- 5.046 (± 2.66)</i>	<i>-1.896</i>	<i>0.058</i>
Substrate grain size	0.72	-0. 346 (± 0.131)	-2.652	0.008
<i>Debris cover</i>	<i>0.62</i>	<i>-0.012 (± 0.007)</i>	<i>-1.794</i>	<i>0.073</i>
Vegetation cover (%)	0.59	-0.017 (± 0.006)	2.659	0.008
Riverbed slope	0.33	0.037 (± 0.029)	1.303	0.192
Debris type-wood	0.25	0.580 (± 0.393)	-1.474	0.140
Debris type-leaf	0.24	-0.551 (± 0.382)	-1.444	0.149
Distance to riverbank	0.17	-0.188 (± 0.084)	2.223	0.026

Mussel Survey Form

Date		Suburb		River	
GPS	S	E	Site	Transect	Left Mid-Channel Right
Channel		Left Bank		Right Bank	
Width	Full	Bank Face			Type Cont
	Water	Top Tree			Type Cont
Channel	Valley Form	*Tick box below	Hight	m	Hight
	Sky Cover%		Type of Cover		m
Transect ID		Selected Quadrats			

Data Type Instruction:

Grid ID: Transect ID + Quadrat No.

Sediment (Code-Score): silt (SI-1), clay (CL-2), earth (EA-3), sand (SA-4), coarse sand (CS-5), peat (PE-6), grave/pebble (G/P-7), cobble (CO-8), boulder (BO-9), rock (RO-10), artificial (AR-11), not visible (NV-0/?)

Water plant (Veg): None/Not Visible (N/NV), Liverworts/Moses/Lichens (LML), Emergent Broad-leaved Herbs (EBH), Emergent Reeds/Sedges/Rushes/Grasses/Horsetails (EGR), Floating-leaved/rooted (FLR), Free-floating (FFL), Amphibious (AMP), Submerged Broad-leaved (SBL), Submerged Liner-leaved (SLL), Submerged Fine-leaved (SFL), Filamentous Algae (FAL).

Debris: Wood (WD), Leaf (LF), Rubbish (RB), Other Interesting Objectives (Indicated)

Bank Face: Bryophytes (BR), Short Creeping Herbs/Grass (SC), Tall Herbs/Grass (TH), Scrub/Shrubs/Bush (SB), Saplings/Trees (TR)

BANKTOP AND BANKFACE VEGETATION STRUCTURE To be assessed within a 10m wide transect (SECTION F)			
bare	B	bare earth/rock etc.	vegetation types
uniform	U	predominantly one type (no scrub or trees)	bryophytes short/creeping herbs or grasses
simple	S	two or three vegetation types	tall herbs/grasses scrub or shrubs
complex	C	four or more types	saplings and trees

Top Tree: None (NO), Isolated/Scattered (IS), Regularly Spaced/Single (RS), Occasional Clumps (OC), Semi-continuous (SC), Continuous (CS).

Current velocity: Approximately 10 cm below water surface.

Valley (tick one box only)

Form:

☐ shallow vee

☐ concave/bowl

☐ deep vee

☐ asymmetrical valley

☐ gorge

☐ U-shape valley

☐ no obvious valley sides

Distinct flat valley bottom? No ☐ Yes ☐

Natural terraces? No ☐ Yes ☐

Sky (Canopy) Cover: Trees (TR), High Bank (HB), Bridge (BR), Building (BD), Other (indicate)

Left
 Water Flow →
 Right

Note:

Figure 2.7. Mussel habitat survey data sheet (front)

Grid ID		Depth	cm	Trashline Height	cm	Trash-Bank Distance	m
Slope	°	Debris Type		D-Height	cm	Cover	% Flow V m/s
Sediment	1.	2.	3.	Veg	1.	2.	3. Note:
Cover	%	%	%	Cover	%	%	%

Grid ID		Depth	cm	Trashline Height	cm	Trash-Bank Distance	m
Slope	°	Debris Type		D-Height	cm	Cover	% Flow V m/s
Sediment	1.	2.	3.	Veg	1.	2.	3. Note:
Cover	%	%	%	Cover	%	%	%

Grid ID		Depth	cm	Trashline Height	cm	Trash-Bank Distance	m
Slope	°	Debris Type		D-Height	cm	Cover	% Flow V m/s
Sediment	1.	2.	3.	Veg	1.	2.	3. Note:
Cover	%	%	%	Cover	%	%	%

Grid ID		Depth	cm	Trashline Height	cm	Trash-Bank Distance	m
Slope	°	Debris Type		D-Height	cm	Cover	% Flow V m/s
Sediment	1.	2.	3.	Veg	1.	2.	3. Note:
Cover	%	%	%	Cover	%	%	%

Grid ID		Depth	cm	Trashline Height	cm	Trash-Bank Distance	m
Slope	°	Debris Type		D-Height	cm	Cover	% Flow V m/s
Sediment	1.	2.	3.	Veg	1.	2.	3. Note:
Cover	%	%	%	Cover	%	%	%

Grid ID		Depth	cm	Trashline Height	cm	Trash-Bank Distance	m
Slope	°	Debris Type		D-Height	cm	Cover	% Flow V m/s
Sediment	1.	2.	3.	Veg	1.	2.	3. Note:
Cover	%	%	%	Cover	%	%	%

Grid ID		Depth	cm	Trashline Height	cm	Trash-Bank Distance	m
Slope	°	Debris Type		D-Height	cm	Cover	% Flow V m/s
Sediment	1.	2.	3.	Veg	1.	2.	3. Note:
Cover	%	%	%	Cover	%	%	%

Grid ID		Depth	cm	Trashline Height	cm	Trash-Bank Distance	m
Slope	°	Debris Type		D-Height	cm	Cover	% Flow V m/s
Sediment	1.	2.	3.	Veg	1.	2.	3. Note:
Cover	%	%	%	Cover	%	%	%

Grid ID		Depth	cm	Trashline Height	cm	Trash-Bank Distance	m
Slope	°	Debris Type		D-Height	cm	Cover	% Flow V m/s
Sediment	1.	2.	3.	Veg	1.	2.	3. Note:
Cover	%	%	%	Cover	%	%	%

Grid ID		Depth	cm	Trashline Height	cm	Trash-Bank Distance	m
Slope	°	Debris Type		D-Height	cm	Cover	% Flow V m/s
Sediment	1.	2.	3.	Veg	1.	2.	3. Note:
Cover	%	%	%	Cover	%	%	%

Surveyor: _____ Recorder: _____

Figure 2.8. Mussel habitat survey data sheet (back)

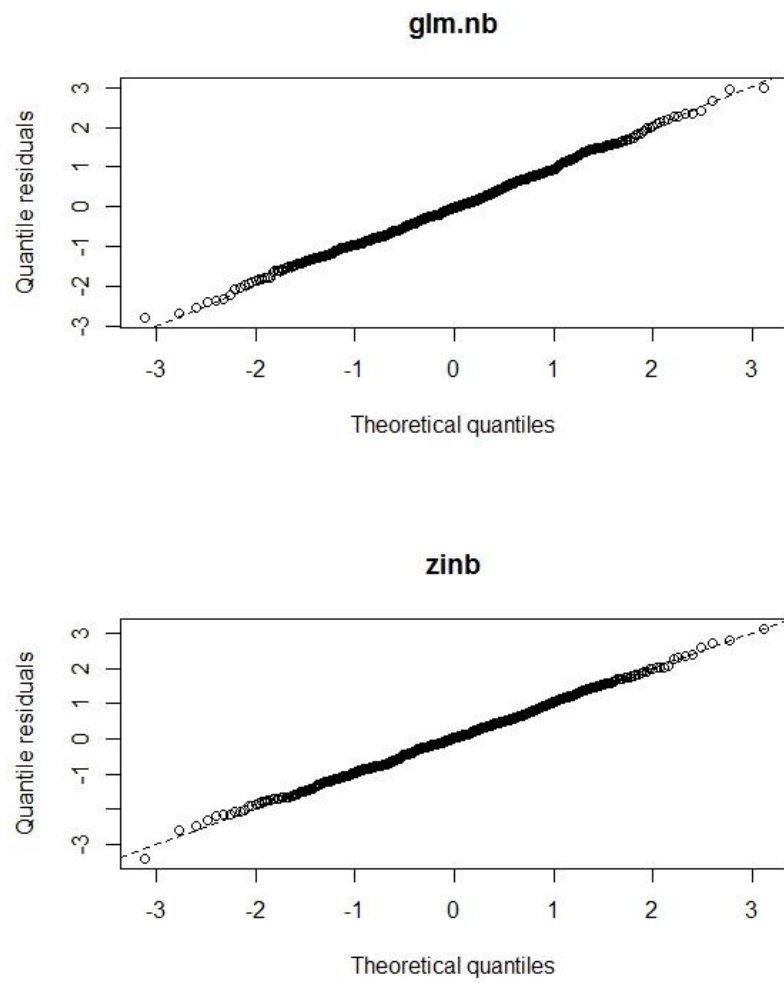


Figure 2.9. QQ plots for negative binomial generalised linear model (glm.nb) and zero-inflated negative binomial model(zinb)

Chapter 3: The tolerance of the threatened freshwater mussel, *Westralunio carteri*, to acute and gradual increases in salinity

3.1 Introduction

Freshwater is an essential natural resource for all terrestrial organisms, including people. Freshwater ecosystems are also very biodiverse; they occupy less than 1% of the Earth's surface, yet they harbour more than 10% of all animal species (Stendera *et al.*, 2012). This unfortunate confluence of exceptional biodiversity and importance to human society means that freshwater ecosystems are much more threatened, and experiencing greater declines in biodiversity, than even the most impacted terrestrial and marine ecosystems (Vörösmarty *et al.*, 2010; Naiman & Dudgeon, 2011).

Amongst the many threats to freshwater environments, secondary salinisation (anthropogenic increases in the concentration of dissolved inorganic ions) is one of the most widespread and significant (Millennium Ecosystem Assessment, 2005; Cañedo-Argüelles *et al.*, 2013). There are many causes of secondary salinisation, including mining activity, industrial discharge and de-icing of roads, but the most important are irrigation (irrigation salinity) and clearing of natural vegetation (dryland salinity). Irrigation and dryland salinity affect more than 160 Mha of land in arid and semi-arid regions of the world, including large parts of Africa, central and South America, the Middle East, central Asia and Australia (Williams, 1999; Cañedo-Argüelles *et al.*, 2013).

Unionid mussels are generally considered to be highly sensitive to elevated salinity because they maintain a low internal salt concentration compared to other animals and their filter feeding life style exposes them to high levels of contaminants, such as increased salt levels (Blakeslee *et al.*, 2013; Castillo *et al.*, 2018). Most published studies on salinity tolerance, however, rely on distribution data or field studies of

increasing salinity, rather than controlled laboratory experiments. For example, Verbrugge *et al.* (2012) recorded maximum salinities of 0.5-3 mgL⁻¹ for five unionid species (*Anodonta anatina*, *A. cygnea*, *Unio crassus*, *U. pictorum* and *U. tumidus*) in the river Rhine in the Netherlands; Ertan and Ali Serhan (2014) found 100% mortality in *Unio crassus* when exposed in cages to a salinity of 4.5 gL⁻¹ in a Turkish stream; and Patnode *et al.* (2015) found 98% mortality in *Epioblasma torulosa rangiana* exposed to discharge from a brine facility (water salinity between 2.1 and 6.8 mgL⁻¹) in the Allegheny River, Pennsylvania. Blakeslee *et al.* (2013) tested the response of *Elliptio complanata* to acute salinity increases in the laboratory and found 100% mortality at 4 gL⁻¹. Walker *et al.* (2001) proposed from field distributions that most Australian unionids are restricted to salinities less than 3 gL⁻¹. However, Dunlop *et al.* (2008) reported an acute salinity LC₅₀ of more than 8 gL⁻¹ for *Cucumerio* sp. from north-eastern Australia in laboratory studies, which suggests a much broader range of salinity tolerances in Australian species. Aquatic organisms in Australia, which have evolved in a region with extensive natural (primary) salinisation are expected to have evolved a higher salinity tolerance than similar species in historically less-saline areas, such as Europe and North America (Kefford *et al.*, 2012; Castillo *et al.*, 2018).

South-western Australia, a recognised biodiversity hotspot (Myers *et al.*, 2000), is particularly affected by dryland salinity. The replacement of large areas of perennial native vegetation with annual crops and pastures has reduced evapotranspiration; thereby increasing groundwater recharge and mobilising salts stored in the soil (George, 1992). As a consequence, approximately 1 M ha of agricultural land in south-western Australia is currently salt-affected (Pannell & Ewing, 2006). Salinisation of streams, rivers and wetlands occurs by the direct seepage of saline groundwater, by salt deposited on agricultural land being washed into drainages and by the accidental or deliberate discharge of saline groundwater from engineering processes used to repair salinised land (Ghassemi *et al.*, 1995). Because of secondary salinisation, less than 50% of flow in the largest 30 rivers in south-western Australia can be classified as fresh, the

majority being brackish or saline (Mayer *et al.*, 2005; Ruprecht *et al.*, 2014).

Freshwater mussels are often regarded as keystone species because of their contribution to ecosystem functioning (Gutiérrez *et al.*, 2003; Geist, 2010), but they are also among the most endangered faunal groups in the world (Lydeard *et al.*, 2004; Badiu *et al.*, 2011; IUCN, 2016). Carter's freshwater mussel, *Westralunio carteri*, is the only freshwater mussel of the south-western Australia and is endemic to the region (Walker *et al.*, 2014). The extent of occurrence of *W. carteri* has declined by 49% in the last 50 years and the species is now categorised as Vulnerable on the IUCN Red List and the Western Australian Wildlife Conservation Act 1950. Klunzinger *et al.* (2015) identified secondary salinisation as both a major cause of this decline and a significant ongoing threat for the species.

Freshwater ecosystems in south-western Australia can experience both acute and gradual increases in salinity during the year. As with many other arid and semi-arid parts of the world, river flow often ceases in the dry season and isolated pools provide ecological refuges, which gradually increase in salinity due to evaporation (Miyazono *et al.*, 2015; Beatty *et al.*, 2017). During the wet season, salinity often rises sharply in the main channel of the river following high rainfall events, due to increased water flow from the upper, salinised parts of the catchment (Mayer *et al.*, 2005; Beatty *et al.*, 2011). Knowledge of both the acute and gradual salinity tolerance of *W. carteri* is therefore necessary to improve our understanding of the threat posed by secondary salinisation and to guide future conservation actions. Klunzinger *et al.* (2015) found LC_{50} in acute salinity tolerance experiments to vary between 1.6 and 3.0 gL⁻¹, with some evidence that mussels from populations experiencing higher salinity had greater tolerance. This suggests that mussels may be able to acclimate to increasing salinity, but gradual salinity tolerance was not measured in these experiments.

The current study aimed to extend the work of Klunzinger *et al.* (2015) by determining

the tolerance of mussel populations to both acute and gradual increases in salinity. It was hypothesised that gradual salinity tolerance would be greater than acute salinity tolerance, and that the more gradual the change, the greater the tolerance of mussels to increasing salinity.

3.2 Methods

3.2.1 Mussel sampling

Live *W. carteri* with shell lengths from 50 to 94 mm were collected by hand from two localities; Canning River (32.0666°S, 116.0000°E) and Yalyal Brook (31.5025°S, 115.9965°E). Both rivers are classified as fresh; at the Canning River site, salinity ranged from 0.26~0.64 gL⁻¹ (mean = 0.38 ± 0.120 gL⁻¹) and at the Yalyal Brook site from 0.23~0.75 gL⁻¹ (mean = 0.48 ± 0.106 gL⁻¹) (Western Australian Department of Water, 2014). Mussels were transported to the laboratory in aerated river water and acclimated in aquaria with aerated freshwater (salinity < 0.2 gL⁻¹) for two days before salinity tolerance experiments commenced.

3.2.2 Salinity tolerance experiments

All experiments were conducted at room temperature (18-25 °C) in aerated 9 L aquaria with four mussels from each population in each aquarium and four replicate aquaria for each treatment. All aquaria were continuously aerated and mussels were fed with approximately 0.01 g crushed dry algae (*Chlorella* sp.) fortnightly. Water was changed fortnightly or when a mortality event occurred.

Acute and gradual salinity tolerance experiments were conducted separately. Salinity treatment solutions were prepared by dissolving lake salt (WA Salt Supply Inc.) in dechlorinated tap water. Lake salt is derived from saline groundwater, which is responsible for secondary salinisation of waterways in south-western Australia. The ionic composition of groundwater and salinised rivers is typically similar to that of sea

water, except for lower concentrations of potassium (Partridge *et al.*, 2008). During the experiments, salinity concentrations were determined using an Oakton™ PCD650 portable water quality meter. Salinity levels in all aquaria were monitored daily, and adjusted if necessary to maintain the desired target concentration.

For the acute tolerance experiment, mussels from each population were randomly assigned to one of five salinity levels: control ($<0.2 \text{ gL}^{-1}$), 5, 6, 7 and 8 gL^{-1} . Mussels were taken from their acclimatisation aquaria, added directly to experimental aquaria and observed daily over a period of 30 days. Mussels were removed when dead, which was indicated by gaping valves that did not close when prodded.

For the gradual tolerance experiment, two rates of increase (0.15 gL^{-1} per day and 0.5 gL^{-1} per day, approximately 3%~10% of the LC_{05}) and three salinity concentration endpoints (5.5 , 6.5 and 7.5 gL^{-1} approximately 110%~150% of the LC_{05}) were used, plus one control group (salinity maintained at $<0.2 \text{ gL}^{-1}$ throughout); giving seven different treatments for each population. Mussels from each population were moved from their acclimatisation aquaria and randomly assigned to experimental treatments, all of which started at the control salinity ($<0.2 \text{ gL}^{-1}$). To reduce the time in which mussels were kept under experimental conditions, salinity in all treatments (except the control) was initially increased at $0.5 \text{ gL}^{-1}\text{day}^{-1}$ until reaching 5 gL^{-1} (approximately equal to the LC_{50} observed in the acute salinity tolerance experiment). Mussels were then acclimated at this salinity for 7 days before commencing the experiment. As described for the acute tolerance experiment, mussels were observed daily and mortalities removed.

3.2.3 Data analysis

All data analyses were conducted in JMP 10 (SAS Institute Inc., Carey, NC, USA) and R (R Development Core Team, 2013). In both experiments, all mussels kept at control salinities ($<0.2 \text{ gL}^{-1}$) survived for the full 30 days. These groups were not used in

analyses to compare mortality rates or survival times among treatments, but they were used in the calculation of LC₅₀ and LC₉₅ values.

To examine the effects of salinity level, mussel population, rate of salinity increase (for the gradual tolerance experiment) and their interactions on mussel mortality generalised linear mixed models (GLMMs) were fitted, with replicate aquaria nested within treatments as random effects, using the R package lme4 (Bates *et al.*, 2017). Mortality was modelled as a binomial variable with a logit link function.

To examine the effects of salinity level, mussel population, rate of salinity increase (for the gradual tolerance experiment) and their interactions on time to death, parametric survival models were fitted using JMP 10. Significance was determined by comparing the log likelihood of models with and without each effect. Akaike Information Criterion (AIC) values were used to choose among Weibull, lognormal, exponential, Fréchet, and loglogistic survival distributions for each analysis.

Bootstrap logistic regression analysis was used to determine the LC₅₀ and LC₉₅ values, with 95% confidence intervals (Beatty *et al.*, 2011; Klunzinger *et al.*, 2015), for each population of mussels in each experiment. Regression curves were generated from bootstrapping 1000 random samples, using the R package boot (Canty & Ripley, 2017).

3.3 Results

3.3.1 Tolerance to acute salinity change

Over the 30 days of the experiment, all mussels from both populations survived at a salinity of 5 gL⁻¹, whereas all individuals in salinities of 7 and 8 gL⁻¹ were found to be deceased at some stage over the duration of the trial. At a salinity of 6 gL⁻¹, 6% of mussels from the Canning River and 31% of mussels from Yalyal Brook survived (Figure 3.1). The difference in 30 day survival rate among salinity treatments was

significant ($\chi^2_3 = 106.6$, $P < 0.0001$), but there was no significant difference between populations ($\chi^2_1 = 0$, $P = 1$) and no significant interaction between population and salinity treatment ($\chi^2_3 = 0$, $P = 1$).

A Fréchet distribution provided the AIC best fit survival model. Survival times differed significantly among salinity treatments ($\chi^2_3 = 194.4$, $P < 0.0001$), but not populations ($\chi^2_1 = 0$, $P < 1$), although there was a significant interaction between population and salinity treatment ($\chi^2_3 = 35.7$, $P < 0.0001$). For all three salinity treatments at which mortalities occurred, mussels from Yalyal Brook survived for longer than those from Canning River; this was especially marked at 8 gL^{-1} , where all Canning River mussels died between 10 and 14 days (mean = 11.9 ± 0.3 days), while the mussels from Yalyal Brook died between 21 and 30 days (mean = 26.6 ± 0.8 days) (Figure 3.1).

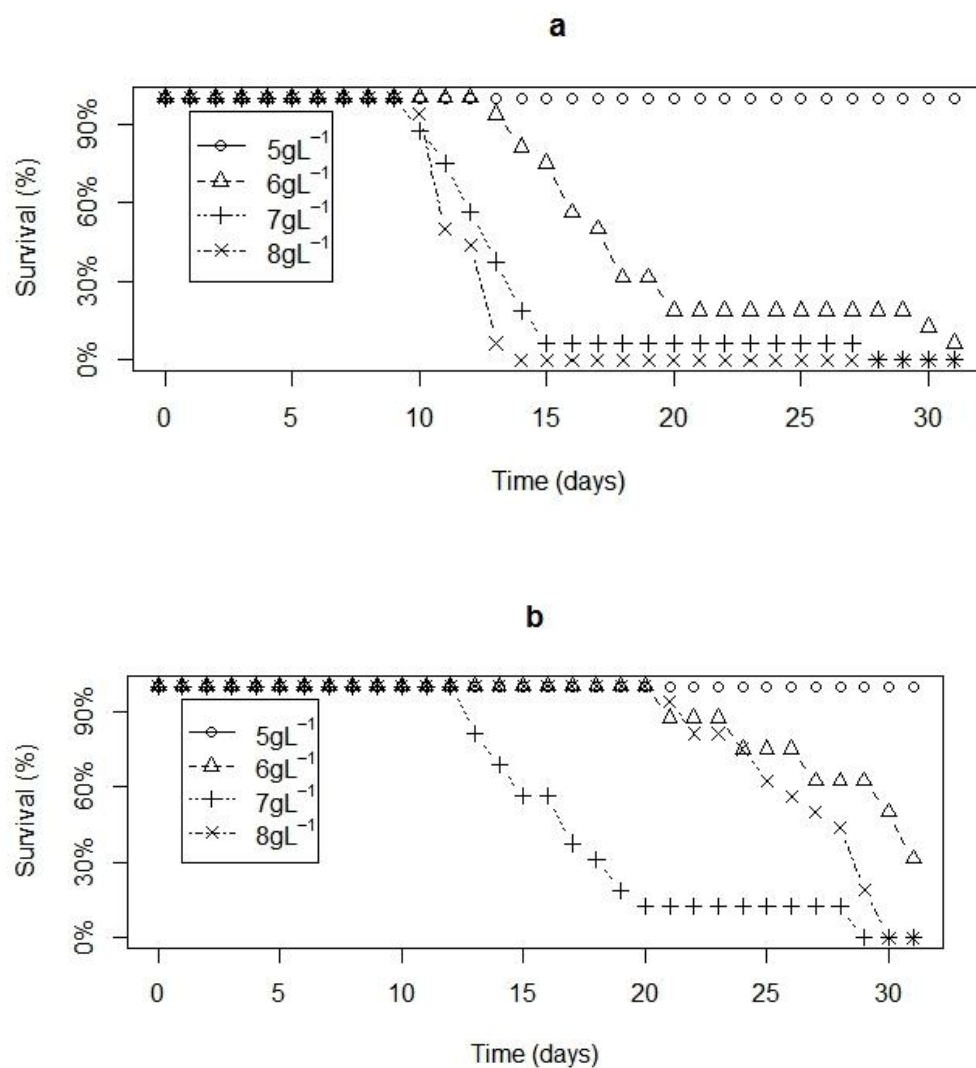


Figure 3.1. Daily survival rates during acute salinity tolerance trials for *Westralunio carteri* from (a) Canning River and (b) Yalyal Brook. Percentage of mussels surviving over 30 days shown for four salinity treatments (5, 6, 7 and 8 gL⁻¹). All mussels kept under control conditions (<2 gL⁻¹) survived for the full 30 days.

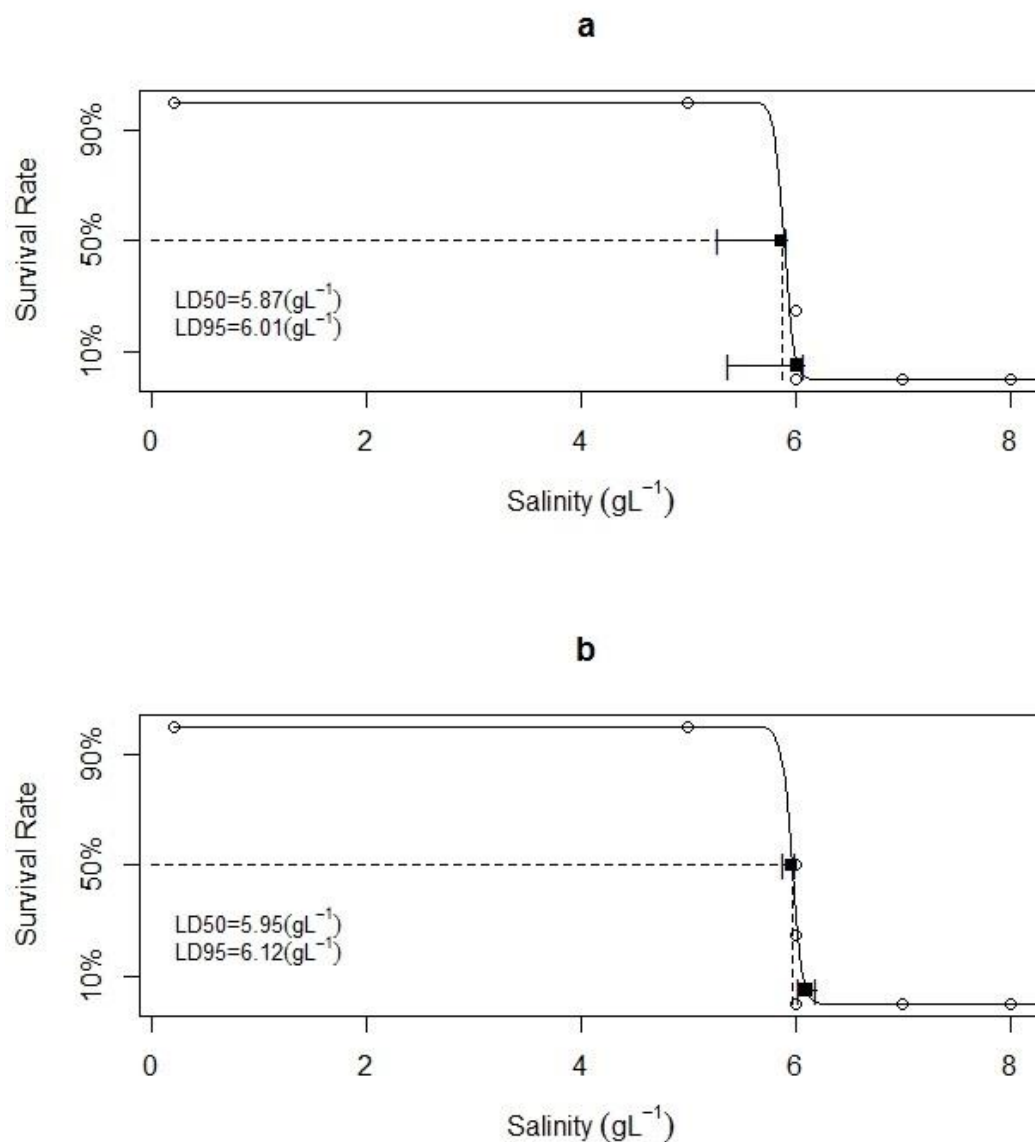


Figure 3.2. Logistic regression curves (with 95% confidence interval) for percentage survival at salinities between 0.2 and 8 gL⁻¹, for *Westralunio carteri* from (a) Canning River and (b) Yalyal Brook. Vertical lines show salinities for 50% and 95% mortality.

In the logistic regression curves (Figure 3.2), the LC50 value for mussels from the Canning River was 5.87 gL⁻¹ (95% CI: 5.28-5.93 gL⁻¹) and LC95 was 6.01 gL⁻¹ (95% CI: 5.38-6.09 gL⁻¹). The LC50 value for mussels from Yalyal Brook was 5.96 gL⁻¹ (95% CI: 5.89-6.01 gL⁻¹) and the LC95 was 6.10 gL⁻¹ (95% CI: 6.03-6.19 gL⁻¹).

3.3.2 Tolerance to gradual salinity change

Over the 30 days of the experiment, all mussels from both populations survived at the salinity end point of 5.5 gL⁻¹, for both low (0.15 gL⁻¹per day) and high (0.5 gL⁻¹per day) rates of increase. Mussels from the Canning River that were exposed to 6.5 and 7.5 gL⁻¹ salinity end points had cumulative mortalities of 93.8% and 100% when salinity increased at the low rate, and 100% at the high rate of increase. Mussels from Yalyal Brook that were exposed to 6.5 and 7.5 gL⁻¹ salinity end points had cumulative mortalities of 87.5% and 100% when the salinity increased at the low rate, and 93.8% and 100% at the high rate (Figure 3.3). The difference in 30 day survival rate among salinity treatments was significant ($\chi^2_3 = 226.8$, $P < 0.0001$), but there were no other significant main effects (population or rate of salinity increase), two-way interactions, or three-way interactions ($\chi^2 \approx 0$, $P = 1$ in all cases).

A loglogistic distribution provided the AIC best fit survival model. Survival times differed significantly between salinity endpoints ($\chi^2_3 = 350.7$, $P < 0.0001$), but not between populations or rate of salinity increase ($\chi^2_1 = 0$, $P < 1$ in both cases). There was, however, a significant interaction between salinity end point and rate of increase ($\chi^2_2 = 10.6$, $P = 0.01$). No other two-way or three-way interactions were significant. For all three treatments at which mortalities occurred, survival time was typically greater at the lower rate of salinity increase, although this was more marked at lower salinity end points (Figure 3.3).

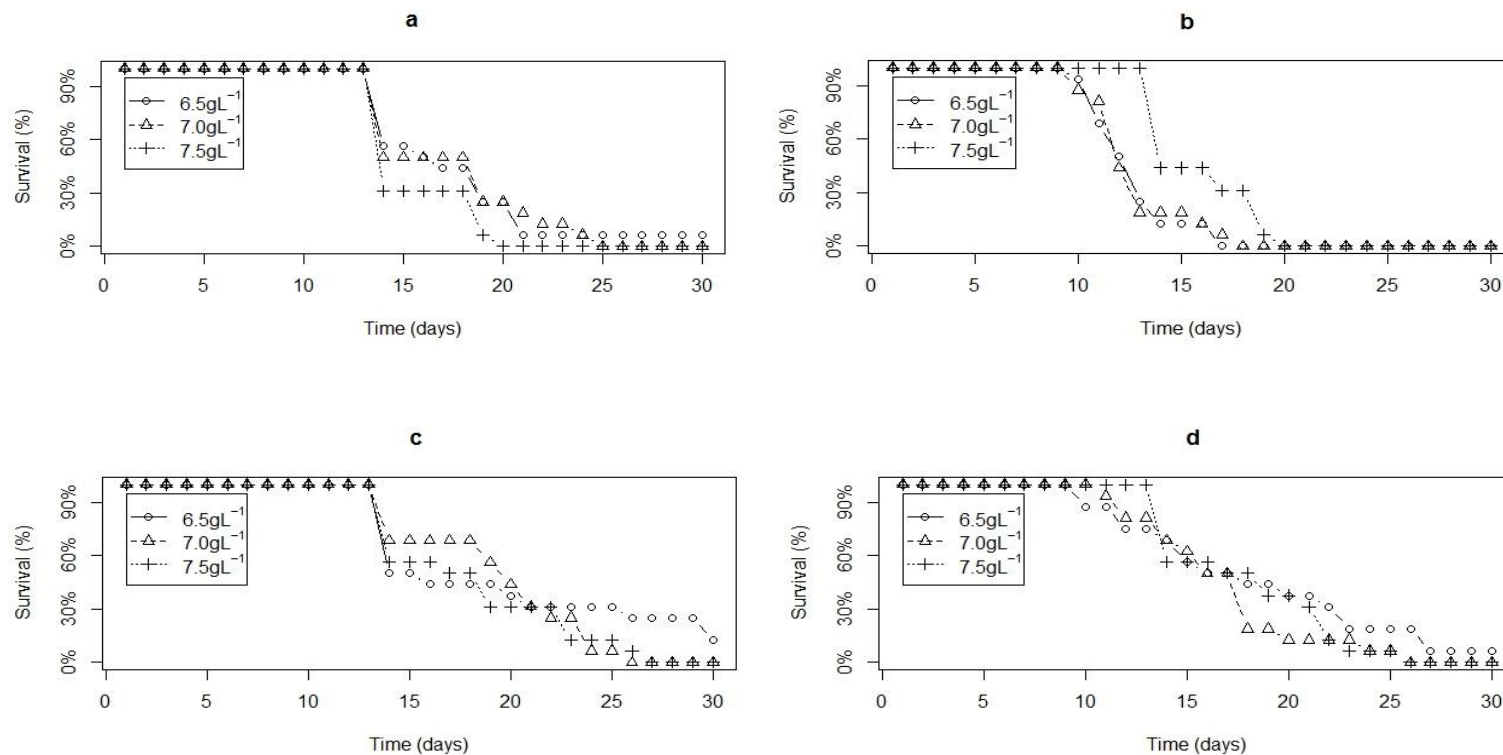


Figure 3.3. Daily survival rates during gradual salinity tolerance trials for mussels from Canning River and Yalyal Brook at low and high salinity increasing rates. Percentage of mussels surviving over 30 days shown for three salinity treatments (5.5 , 6.5 and 7.5 gL^{-1}) and two rates of increase (low - 0.15 gL^{-1} per day, and high - 0.5 gL^{-1} per day). (a) Canning river mussels, low rate; (b) Canning River mussels, high rate; (c) Yalyal Brook mussels, low rate; (d) Yalyal Brook mussels, high rate. All mussels kept under control conditions ($<2 \text{ gL}^{-1}$) survived for the full 30 days.

From the logistic regression curves (Figure 3.4), LC_{50} and LC_{95} values for Canning River mussels were 6.43 gL^{-1} (95% CI: $5.76\text{-}6.47 \text{ gL}^{-1}$) and 6.51 gL^{-1} (95% CI: $5.86\text{-}6.57 \text{ gL}^{-1}$) at the low rate of salinity increase, and 6.01 gL^{-1} (95% CI: $5.72\text{-}6.43 \text{ gL}^{-1}$) and 6.06 gL^{-1} (95% CI: $5.82\text{-}6.53 \text{ gL}^{-1}$) at the high rate. For mussels from Yalyal Brook the LC_{50} and LC_{95} values were 6.45 gL^{-1} (95% CI: $6.00\text{-}6.48 \text{ gL}^{-1}$) and 6.52 gL^{-1} (95% CI: $6.10\text{-}6.59 \text{ gL}^{-1}$) at the low rate of salinity increase and 6.43 gL^{-1} (95% CI: $5.78\text{-}6.47 \text{ gL}^{-1}$) and 6.51 gL^{-1} (95% CI: $5.88\text{-}6.57 \text{ gL}^{-1}$) at the high rate.

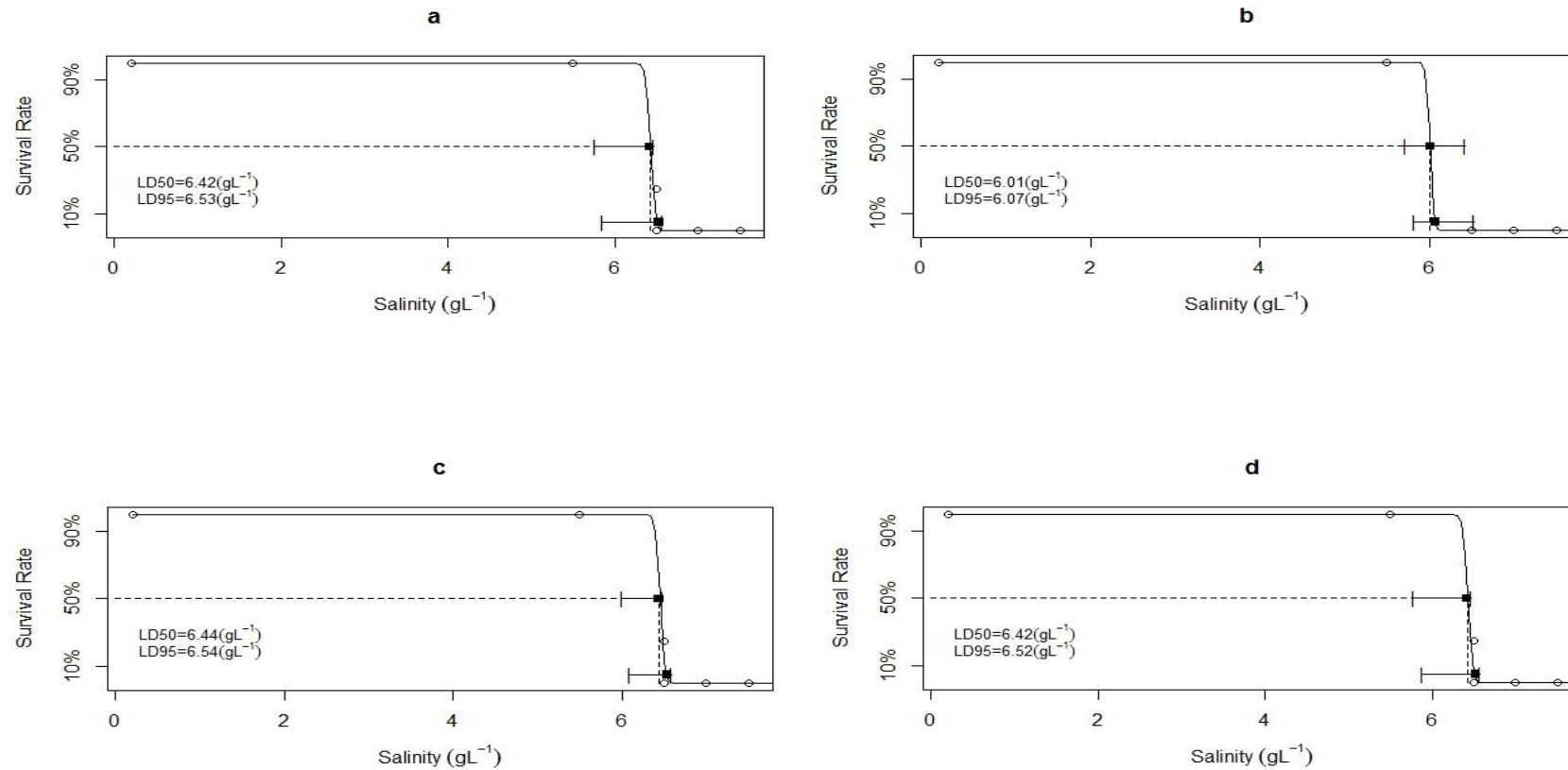


Figure 3.4. Logistic regression curves (with 95% confidence interval) for percentage survival at salinities between 0.2 and 7.5 gL^{-1} , for mussels from Canning River and Yalyal Brook at two rates of increase (low 0.15 gL^{-1} per day, and high 0.5 gL^{-1} per day). (a) Canning mussels, low rate; (b) Canning mussels, high rate; (c) Yalyal mussels, low rate; (d) Yalyal mussels, high rate. Vertical lines show salinities for 50% mortality.

3.4 Discussion

There were three major findings from this study. First, *W. carteri* had a higher tolerance to acute increases in salinity than previously reported by Klunzinger *et al.* (2015) for this species. Second, they had a higher tolerance to gradual increases in salinity than to acute increases, providing the first evidence for short-term acclimatisation to increasing salinity in *W. carteri*. Third, there was some evidence for differences in salinity tolerance between different populations of *W. carteri*, which may result from differences in acclimatisation history (i.e. different salinities experienced during the lifetime of the mussels used in the experiment) or provide evidence of genetic changes (adaptation) in response to increasing salinity. These findings have implications for the conservation of *W. carteri* in the face of increasing secondary salinisation in south-western Australia, where salinity in some rivers has been recorded as reaching more than 30 gL⁻¹ (Beatty *et al.*, 2011), and for other freshwater mussels throughout arid and semi-arid regions of the world, where salinisation is becoming an increasingly important issue (Williams, 1999; Cañedo-Argüelles *et al.*, 2013).

3.4.1 Tolerance to acute salinity change

Klunzinger *et al.* (2015) found LC₅₀ values of 1.62–3.04 gL⁻¹ for *W. carteri* in response to an acute increase in salinity, compared to the values of 5.87–5.96 gL⁻¹ found in the current study. This is unlikely to be due to physiological or genetic differences resulting from exposure to different salinity levels; mussels from one locality (Yalyal Brook) were used in both studies and there were no major changes in salinity at this site in the time period between the two studies. Most experimental conditions, including the source of salt, were the same; the main way in which the two studies differed was that mussels in the current study were fed regularly throughout this study, whereas those in the study by Klunzinger *et al.* (2015) were not. Lack of feed may have added an additional stress to mussels in the earlier study, which reduced their salinity tolerance (Lavy *et al.*, 1997; Rodríguez *et al.*, 2005; Hashemi *et al.*, 2008). Preliminary

investigations provide some evidence that mussels fed on crushed dry algae (as in this study) survive for longer in the laboratory than unfed mussels (Supplementary Information, Figure 3.5).

Knowledge of the acute salinity tolerance of *W. carteri* is important, because in many south-western Australian rivers, freshwater organisms in the lower catchment are exposed to sudden increases in salinity when winter rains bring pulses of saline water from the salinised upper catchment and adult *W. carteri* have limited movement capacity. For example, in the Blackwood River, the longest river in the region, salinity in the main channel of the lower catchment may increase from less than 1 mgL⁻¹ to 4.5 mgL⁻¹ with the first flows in winter (Beatty *et al.*, 2009). It is also important to understand the timing of mortality, because salinity may rapidly decrease as continued rainfall contributes proportionally more to river flow (Beatty *et al.*, 2009). Even with acute increases in salinity to 8 gL⁻¹, mussels did not begin to die for at least 10 days, which in many cases may provide enough time to survive salinity spikes in the field. Sylvester *et al.* (2013) found that the golden mussel *Limnoperla fortunei* (Order Mytiloida) had a much greater tolerance to acute increases in salinity when salinity was allowed to fluctuate between high and low levels over a period of approximately 13 hours, than when salinity was held constant after the change. This was hypothesised to be due to valve closure in response to increasing salinity, as this has been shown to prevent exposure to harmful environmental conditions in bivalves (Jørgensen *et al.*, 1990).

3.4.2 Tolerance to gradual salinity change

There was a substantial increase in LC₅₀ for *W. carteri* when salinity was increased gradually rather than acutely; from 5.87 to 6.43 gL⁻¹ in mussels from Canning River, and from 5.96 to 6.45 gL⁻¹ in mussels from Yalyal Brook. This appears to be the first report of acclimatisation to increasing salinity in unionids, although it has been reported

previously in other freshwater bivalves, such as *L. fortunei* (Deaton *et al.*, 1989) and the zebra mussel *Dreissena polymorpha* (Order Veneroida) (Kilgour *et al.*, 1994).

During this study, the rate of increase in salinity did not affect mortality rate, but it did influence survival time, with longer survival in mussels exposed to a lower rate of increase. This may reflect the opportunity for a wider range of physiological responses with more gradual environmental changes. For example, immediate responses to environmental stressors such as an increased requirement for energy to support homeostasis are often met from limited energy stores, whereas over a longer timescales, changes in gene expression can be employed (Peck, 2011). At this stage, however, the physiological mechanisms underlying acclimatisation to increasing salinity in *W. carteri* are unclear and should be the subject of future research.

The rivers of south-western Australia, like those of many other arid and semi-arid areas of the world have ephemeral and intermittent streamflow. Freshwater communities in such temporary rivers are structured by seasonal cycles of flooding and drying, with isolated pools or waterholes providing ecological refuges when streamflow ceases (Beatty *et al.*, 2017). Freshwater mussels are likely to be vital components of refuge pool communities because their filter feeding activities improve water quality and help to maintain freshwater biodiversity (Vaughn & Spooner, 2006; Aldridge *et al.*, 2007; Haag, 2013; Vaughn, 2018). Salinity in these pools gradually increases over the dry season as the pool size shrinks because of evaporation. Acclimatisation to increasing salinity is likely to be important to the survival of *W. carteri* in summer refuge pools and therefore the effects of salinity on this species has direct implications for the broader ecosystem. Moreover, the functional importance of mussels in refuge pools is likely to increase because of climate change. South-western Australia has undergone a 50% reduction in median streamflow over the last 30 years, with a further 25% reduction expected by 2030 (Suppiah *et al.*, 2007). Decreased streamflow will affect the number, size and connectivity of refuges, with concomitant increases in organism

density and decreases in water quality (Davis *et al.*, 2013; Jaeger *et al.*, 2014; Beatty *et al.*, 2017).

3.4.3 Potential for adaptation to increasing salinity

Field surveys indicate that populations of *W. carteri* are rarely found in localities with mean salinity concentrations greater than 1.62 gL⁻¹ (Klunzinger *et al.*, 2015), which is substantially less than the salinity tolerances that were found in the laboratory. This is not consistent with findings from other studies on macroinvertebrates, which have generally found a close correspondence between laboratory-derived LC₅₀ values to acute salinity change with the maximum salinity with which a species has been collected in the field (Kefford *et al.*, 2004; Verbrugge *et al.*, 2012). These previous studies have not included unionids, however, and there is evidence that the early life stages of freshwater mussels (glochidia and juveniles) are particularly sensitive to salt and other chemical contaminants (Gillis, 2011; Blakeslee *et al.*, 2013; Nogueira *et al.*, 2015; Patnode *et al.*, 2015). Furthermore, high salinity may have non-lethal physiological or behavioural effects on mussels, decreasing individual and population fitness even without direct mortality. For example, adult *E. complanata* had permanently reduced metabolic rate when exposed to salinity levels below the measured LC₅₀ value (Blakeslee *et al.*, 2013), while the attachment rate for glochidia of *E. complanata* and *A. anatina* was reduced upon exposure to non-lethal salinity (Blakeslee *et al.*, 2013; Beggel & Geist, 2015).

Predictions for future river salinity in south-western Australia are influenced by catchment-specific characteristics such as topography, geology and proportion of cleared land, but reduced rainfall in the region is expected to produce a general trend of higher river salt levels (Morgan *et al.*, 2003; Mayer *et al.*, 2005), which suggests that a further reduction in the range of *W. carteri* may be inevitable, pushing this threatened species to the brink of extinction. Given the limited dispersal capability of freshwater mussels (except in the very short parasitic phase), populations of *W. carteri* can only

respond to increasing salinity by acclimatisation or adaptation. This study has demonstrated that *W. carteri* has some capacity for physiological acclimatisation, and has also found slight differences among populations in salinity tolerance, which may suggest that sufficient genetic variation exists for adaptation to increasing salinity levels.

Klunzinger *et al.* (2015) studied the acute salinity tolerance of mussels from two rivers with different mean salinity levels and reported a higher LC₅₀ in mussels from the river with the greater salinity. However, because salinity tolerance was measured in separate experiments for these two populations, they were unable to provide conclusive evidence of a difference. The current study provided a direct comparison in salinity tolerance between mussels from different populations and, while no significant differences in mortality rate were found, there were significant differences in survival times when mussels were exposed to acute (but not gradual) increases in salinity. Survival times were longer in mussels from the population with greater mean salinity. The difference in mean salinity levels in the two rivers from which mussels were obtained in the current study was relatively small, suggesting that there may be greater variation in salinity tolerance among other populations from throughout the range of *W. carteri* compared to the modest differences found here. This is an area deserving of further study.

3.5 Conclusions

Secondary salinisation has been primarily responsible for the dramatic recent decline in the range of *W. carteri* and remains a major threat to the survival of the species (Klunzinger *et al.*, 2015). This study provides some degree of optimism, as it revealed an upward re-assessment of the acute salinity tolerance of adult mussels, demonstrated acclimatisation to a gradual change in salinity, and provided further evidence for differences in salinity tolerance among populations, which may suggest a capacity for long-term adaptation. Nevertheless, the threat to the species remains severe. There is an urgent need to better understand both the salinity tolerance of glochidia and juvenile

mussels and the non-lethal impacts of increased salinity on mussel fitness, so that environmental managers can more accurately predict the impacts of rising salinity levels in the rivers of south-western Australia, whilst also considering that the loss of their teleost host to secondary salinisation may be another factor in the decline of this endangered species.

3.6 Supplementary Information

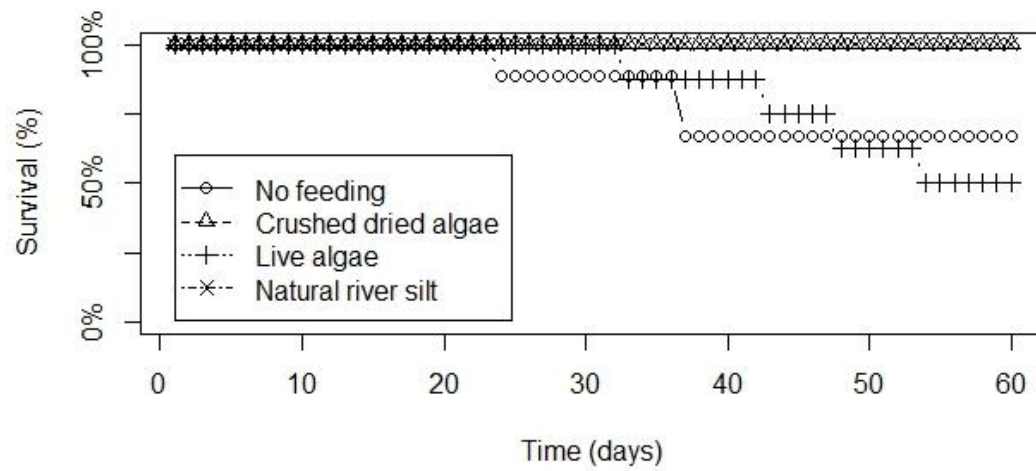


Figure 3.5. Survival time of mussels from the Canning River when maintained for 60 days with no feeding, fed crushed, dried algae, live algae and natural river silt

Chapter 4: Drought tolerance and burrowing behaviour in the freshwater mussel, *Westralunio carteri*: implications for survival in a drying climate

4.1 Introduction

Freshwater environments are extremely biodiverse, but this biodiversity appears to be declining at a faster rate than among terrestrial and marine ecosystems, largely because of anthropogenic impacts (Dudgeon *et al.*, 2006; Vörösmarty *et al.*, 2010). One of the most important of these impacts is climate change, which affects freshwater ecosystems directly, through increased air and water temperature and altered flow regimes, and indirectly, by exacerbating the impacts of water extraction, habitat degradation and invasive species (Dudgeon *et al.*, 2006; Tisseuil *et al.*, 2012; van Vliet *et al.*, 2013).

Reduced water flow is one of the most pervasive, yet under-appreciated, effects of climate change on freshwater ecosystems in arid, semi-arid and Mediterranean climatic regions. Over the last 50 years, streamflow has decreased by up to 30% across large areas of Australia, southern Europe, the Middle East, western and southern Africa, south-east Asia, western North America and South America (Milliman *et al.*, 2008), and projections from climate change models suggest that these decreases will continue into the future (Jiménez-Cisneros *et al.*, 2014; Schewe *et al.*, 2014). These areas of the world all have strongly seasonal rainfall and are characterised by rivers with ephemeral and intermittent streamflow (temporary rivers). Freshwater communities in temporary rivers are structured by seasonal cycles of flooding and drying, with isolated pools or waterholes providing ecological refuges when streamflow ceases (Magoulick & Kobza, 2003; Larned *et al.*, 2010). Decreased streamflow as a result of climate change will affect the size, number and connectivity of ecological refuges, with major impacts on freshwater biota (Davis *et al.*, 2013; Jaeger *et al.*, 2014).

Unionid mussels are important functional components of freshwater ecosystems (Vaughn, 2018), but are among the most imperilled faunal groups in the world (Lydeard *et al.*, 2004; Bogan, 2008; Lopes-Lima *et al.*, 2018). Mussels are particularly at risk from reduced streamflow because, except for a brief larval phase in which they are parasitic on fishes, they are largely sedentary and have limited ability to respond to loss of flow by migration into refuge pools. A number of studies have reported mass mortalities and severe population declines in freshwater mussel species following prolonged droughts (Golladay *et al.*, 2004; Haag & Warren, 2008; Galbraith *et al.*, 2010; Walters & Ford, 2013; Vaughn *et al.*, 2015).

Although unionids are generally much more resistant to water emersion than other aquatic molluscs, such as gastropods (Collas *et al.*, 2014), physiological tolerance may vary widely among mussel species (Gough *et al.*, 2012; Nakano, 2018). Mussel species may also differ in their behavioural responses to emersion. Gough *et al.* (2012) described two different behavioural strategies in three mussel species responding to drought conditions in Opintlocco Creek, Alabama; horizontal movement to track receding water levels and vertical movement (burrowing). These strategies appeared to be related to the desiccation tolerance of the species; species that burrowed had a greater tolerance than those that tracked receding water (Gough *et al.*, 2012).

Carter's freshwater mussel, *Westralunio carteri* Iredale 1934, is the only Unionida in south-western Australia and the sole representative of *Westralunio* on the Australian continent (Klunzinger *et al.*, 2012a). A combination of increasing salinity, reduced water flow and nitrate-related pollution has led to a 49% decline in the range of the species over the past fifty years, resulting in its classification as Vulnerable by both international and national regulatory bodies (Klunzinger & Walker, 2014; Klunzinger *et al.*, 2015). Since the mid-1970s, south-western Australia has undergone a 16% reduction in annual rainfall, corresponding to 50% reduction in median annual

streamflow (Suppiah *et al.*, 2007; Petrone *et al.*, 2010; Silberstein *et al.*, 2012). Global climatic models predict this drying trend to continue, with median reductions of an additional 8% in annual rainfall and 25% in annual stream flow expected by 2030 (Suppiah *et al.*, 2007; Silberstein *et al.*, 2012). Without active intervention, this is likely to lead to further substantial reductions in range and population size of *W. carteri*.

The aim of this study was to document recent observations of drought-related mortality in *W. carteri*, determine the physiological tolerance of the species to emersion, and investigate behavioural strategies which may increase survival. This information will be important for predicting those populations most at risk of extinction as stream flow decreases, and for informing potential conservation management strategies which may decrease this risk.

4.2 Materials and Methods

4.2.1 Field observations

Dead mussels were found in two sites in south-western Australia, apparently as a result of water emersion (Figure 4. 1). In February, 2011 a 64 m² area within the Collie River with unseasonably low water levels (33°18'5``S, 115°48'59``E) that included both an exposed sandbar (36 m²) and adjacent submerged habitat (28 m²), was surveyed for mussels. All exposed and submerged mussels within the area were collected, measured for maximum length (ML; McMichael & Hiscock, 1958) and recorded as alive or dead; live mussels reacted to handling by closing their shells tightly and dead mussels were either agape or floated when placed in water. The difference in ML between living and dead mussels was examined by a t-test.

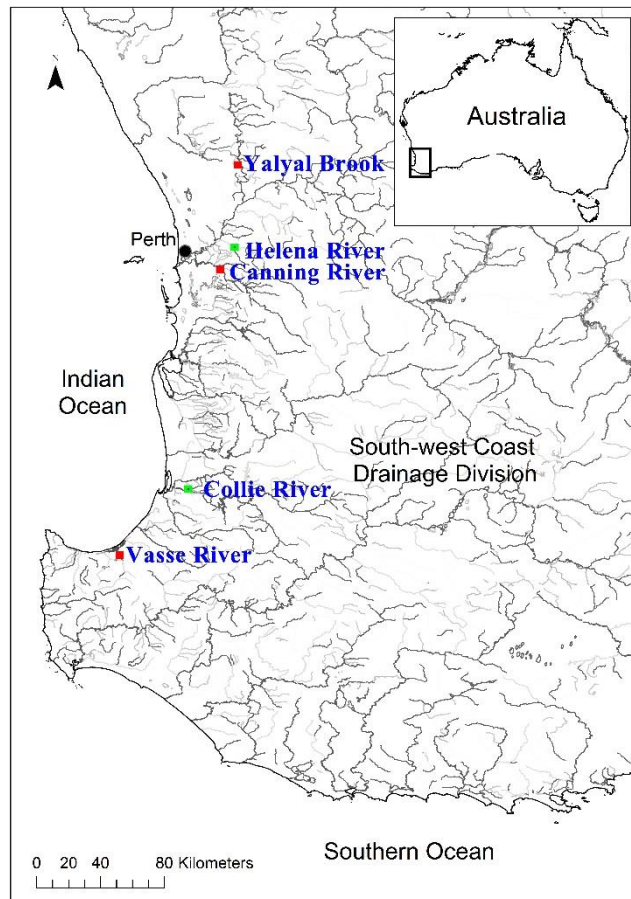


Figure 4.1. South-west Coast Drainage Division, showing sites from which mussels were sampled. Field work sites: Collie River and Helena River (green); Mussels sampling sites for laboratory experiment: Canning River, Vasse River and Yalyal Brook (red)

In the Lower Helena Reservoir, a dam on the Helena River ($31^{\circ}56'24''\text{S}$, $116^{\circ}4'12''\text{E}$), much of the water body was drained in April-May 2011, to allow work on a scour valve. Following draining, mussels were collected from 60 1 m^2 quadrats, placed randomly in an area of approximately 600 m^2 on the exposed bank and from the same number of quadrats, placed randomly in a 400 m^2 submerged area within the dam, immediately adjacent to the exposed bank. Mussels were measured for ML and the difference in ML between living and dead mussels was examined by a t-test, as described above.

4.2.2 Field experiments

In February, 2012, a field study was conducted to determine movement patterns and predation of *W. carteri* on the sandbar in the Collie River. One hundred and ninety two mussels were collected from an area approximately 200 m downstream from the exposed sand bar described previously. The mussels were measured for ML and fixed with small (8 x 4 mm) individually numbered polyethylene shellfish tags (Hallprint Pty Ltd, Hindmarsh Valley, SA 5211).

Movement patterns: Ninety five tagged mussels were released from five marking pegs, which were spaced one metre apart and oriented parallel to and 28 cm from the water's edge on the sandbar. After three days and again after six days, the area was searched for marked mussels and the direction of movement (towards or away from the water), the horizontal distance travelled from their respective release peg (to the nearest cm), and whether the mussels had burrowed or not, was recorded. The relationship between ML and horizontal distance moved was examined by Spearman's rank correlation, and the relationship between ML and propensity to burrow by logistic regression.

Predation: Following the movement experiment described above, 97 tagged mussels were frozen overnight and then placed along a 10 m line in the middle of the exposed sandbar. An infrared, motion-detecting game camera (Reconyx Hyperfire PC800) was mounted to star pickets 100 mm off the ground and oriented so that it faced the exposed frozen mussels. The sandbar was searched carefully and all remaining mussels recorded after three days and six days. Mussels which had disappeared were recorded as "predated". The relationship between ML and predation was examined by logistic regression.

4.2.3 Emersion experiments

Two separate experiments were conducted to examine the survival of mussels during water emersion, the first using 116 mussels collected from the Vasse River

(33°41'12.9"S, 115°21'52.7"E), and the second using 56 mussels collected from each of the Canning River (32°04'12.5"S, 116°00'22.0"E) and Yalyal Brook (31°30'09.0"S, 115°59'47.4"E) (see Figure 1). All of these sites contain dense populations of mussels with active recruitment (see Chapter 2). In each experiment, mussels were collected in the field, transported to the laboratory in river water, held 48 hours in laboratory tanks, and then measured for ML, individually tagged and randomly assigned to replicate 12 L plastic containers in the experimental treatments.

Experiment 1 had two treatments, one with three levels and the other with two levels, with four replicate containers each with four mussels in each treatment combination, giving a total of 24 replicates and 96 mussels. The treatments were: presence of substrate (no substrate, dry sand, moist sand); and exposure to the sun (fully exposed, shaded). The sand for the substrate was washed river sand, either dried for one week (mean moisture content over replicates $2.0\% \pm \text{SD } 0.4\%$) or moistened with river water (mean moisture content $10.5\% \pm \text{SD } 2.1\%$). No further water was added, and by the end of the experiment the moisture content in all treatments was similar (mean over all replicates $0.9\% \pm \text{SD } 0.6\%$). Sand was placed to a height of 150 mm in the containers, which was of an adequate depth to enable all mussels to burrow. Fully exposed treatments were placed in the open in full sun and shaded treatments in an adjacent area and fully protected from the sun by a tiled roof. The experiment was conducted over 62 days in autumn 2015. The mean maximum daily temperature over the period of the experiment was 28.5°C and the mean minimum temperature was 16.7°C . Mussels were examined daily for mortality (indicated by gaping valves). Twenty mussels were maintained as controls in river water in laboratory tanks, without feeding (as the mussels did not open their shells in the experimental treatments without water), for the duration of the experiment.

All control mussels survived. To examine the effects of substrate and exposure on mussel mortality, a generalised linear mixed model (GLMM) was fitted, initially with

replicate containers nested within treatment combinations as a random effect, using the R package lme4 (Bates *et al.*, 2017). Because very little variance was explained by replicate, it was removed from the final model, which had only the fixed effects of substrate, exposure and their interaction, with ML as a covariate. Mortality was modelled as a binomial variable with a logit link function. To examine the effects of substrate, exposure and their interaction on time to death a parametric survival model was fitted, with ML as a covariate, using JMP 10. Significance was determined by comparing the log likelihood of models with and without each effect. Akaike Information Criterion (AIC) values were used to choose among Weibull, lognormal, exponential, Fréchet, and loglogistic survival distributions for each analysis.

In experiment 2, all mussels were fully exposed to the sun, with two different treatments: population (Canning River, Yalyal Brook); and presence of substrate (no substrate, 20.7% \pm SD 1.3% moisture river sand, 61.1% \pm SD 2.7% moisture river silt). Each level of substrate had four replicate containers, with four mussels from each population in each replicate, giving a total of 12 replicates and 96 mussels. River sand was obtained from Yalyal Brook and silt from Canning River, and placed to a height of 150 mm in the containers. The experiment was conducted over 83 days in autumn 2017. The mean maximum temperature over the period of the experiment was 23.8°C and the mean minimum temperature was 14.9°C. Mussels were examined daily for mortality (indicated by gaping valves). Sixteen mussels were maintained as controls in river water in laboratory tanks, without feeding, for the duration of the experiment.

All control mussels survived and all mussels exposed to the sun died. To examine the effects of population, substrate and their interaction on time to death a parametric survival model, with ML as a covariate, using JMP 10 was fitted. Significance was determined by comparing the log likelihood of models with and without each effect. Akaike Information Criterion (AIC) values were used to choose among Weibull, lognormal, exponential, Fréchet, and loglogistic survival distributions for each analysis.

4.2.4 Burrowing experiments

Two experiments were conducted to examine the burrowing behaviour of mussels and the effect of burrowing on mussel survival during emersion. In the first experiment, 50 mussels from the Vasse River were measured for ML, individually tagged and randomly assigned to one of five replicate 12 L plastic containers containing moist river sand (mean moisture content $11.4\% \pm \text{SD } 1.4\%$) to a height of 150 mm. The experimental containers were then placed in full sun for 7 days, after which mussel mortality (indicated by gaping valves), whether the mussel had begun to burrow (yes/no) and whether the mussel was completely buried (yes/no) were recorded. The experiment was conducted in autumn 2015, with a mean maximum temperature over the experimental period of 29.3°C and a mean minimum temperature of 17.8°C . The relationships between shell length and either burrowing behaviour or mortality were examined by logistic regression, while the relationships between burrowing behaviour and mortality were examined using a Fisher exact test.

In the second experiment, 10 mussels from each of the Canning River and Yalyal Brook were individually tagged, measured for ML and placed in the centre of one of two identical 250 L containers, with shade at one end and water at the other end (Figure 4.2). One container was filled with moist river sand (collected from Yalyal Brook) and the other with moist silt (collected from Canning River) to a depth of 150 mm, and both were placed in full sun. After 48 hours, the direction of horizontal movement, the horizontal distance moved to the nearest cm, whether burrowing occurred (yes/no) and the depth of burrowing relative to length (measured as the ratio of the distance from the surface to the end of the burrowed shell: ML) was recorded for each mussel. A general linear model was used to examine the effect of substrate type, population, and the interaction of substrate and population on depth of burrowing. Residuals from this analysis did not differ significantly from a normal distribution (Shapiro-Wilks test, $W = 0.98$, $P = 0.22$).

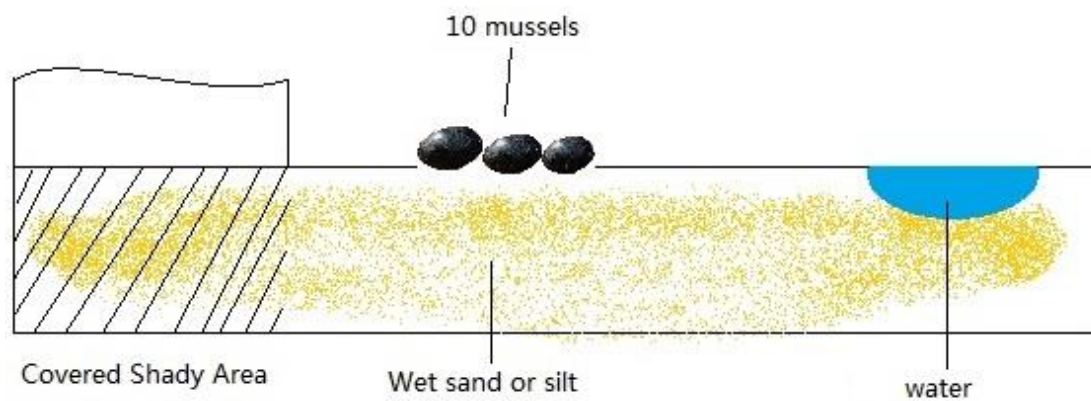


Figure 4.2. Diagrammatic representation of the setup for the second burrowing experiment.

4.3 Results

4.3.1 Field observations

In the Collie River, 106 mussels were found on the exposed sandbar and all were dead, while 175 mussels were found in the submerged area, with three dead and 172 living. The ML of exposed mussels was significantly greater than that of submerged mussels ($57.9 \pm \text{SE } 0.7$ mm compared to 52.6 ± 0.6 mm; $t_{279} = 5.6$, $P < 0.0001$).

A total of 366 mussels were found on the exposed bank of the Lower Helena Reservoir; 93 of these (25.4%) were dead. There was no difference in ML of living and dead mussels on the bank (70.8 ± 1.0 mm compared to 70.4 ± 0.6 mm; $t_{364} = 0.31$, $P = 0.76$). One hundred and eighty two mussels were sampled from the submerged area of the reservoir and all were living. The ML of exposed mussels (living and dead) was significantly greater than that of submerged mussels (70.6 ± 0.5 mm compared to 62.1 ± 0.7 mm; $t_{546} = 9.74$, $P < 0.0001$).

4.3.2 Field experiments

Movement patterns: All 98 mussels were recaptured after three days, and 88 mussels

were recaptured after 6 days. After three days, most mussels (97.5%) had moved horizontally (all in the direction of the retreating river water, with the distance moved ranging between 5 and 85 cm (mean = 21.1 ± 1.3 cm). There was no relationship between ML and distance moved ($\rho = 0.09$, $P = 0.38$). Fifty two percent of mussels had begun to burrow and there was an inverse relationship between ML and propensity to burrow ($\chi^2_1 = 15.50$, $P < 0.0001$; mean ML of mussels that had begun to burrow = 53.6 ± 1.3 mm, compared to 61.3 ± 1.4 mm for those that had not begun to burrow). After six days, little further horizontal movement had occurred (mean = 19.1 ± 1.5 cm for those mussels that were recaptured) and, as before, there was no relationship between ML and distance moved ($\rho = 0.18$, $P = 0.10$). More mussels had begun to burrow (86.4%) and there was no longer any relationship between ML and propensity to burrow ($\chi^2_1 = 0.26$, $P = 0.61$).

Predation: Twenty six (27%) of the 97 mussels had disappeared (were “predated”) after three days, and 40 (41%) after 6 days. The data from the game camera revealed that purple swamp hens (*Porophyrrio porophyrrio*), Australian wood ducks (*Chenonetta jubata*) and what appears to be a black rat (*Rattus rattus*) visited the site, although it is possible that other fauna also triggered the camera but were not recorded, as several blank photographs were taken. There was no relationship between ML and likelihood of being predated after 3 days ($\chi^2_1 = 0.1$, $P = 0.83$), but after six days, there was a significant inverse relationship ($\chi^2_1 = 7.7$, $P = 0.006$). The mean length of ‘surviving’ shells after 6 days was 59.2 ± 1.3 mm, compared to 53.0 ± 1.6 mm for mussels that had disappeared.

4.3.3 Emersion experiments

Experiment 1: Over the 62 days of the experiment, 41% of mussels died, with 75% of these deaths occurring when the mussels were exposed to full sun (Figure 4.3). There was a significant difference in 62 day survival rate among substrate types ($\chi^2_2 = 20.8$, $P < 0.0001$) and exposure levels ($\chi^2_1 = 40.5$, $P < 0.0001$), but no significant interaction

between substrate and exposure treatments ($\chi^2_2 = 0$, $P = 1$). There was also a significant effect of ML ($\chi^2_1 = 8.1$, $P < 0.004$), due to a positive relationship between ML and survival in mussels in the shade (logistic regression, ($\chi^2_1 = 9.5$, $P = 0.002$), although this was not evident in mussels exposed to full sun (logistic regression, ($\chi^2_1 = 0.1$, $P = 0.87$).

A Weibull distribution provided the AIC best fit survival model. Survival times differed significantly among substrate types ($\chi^2_2 = 32.1$, $P < 0.0001$), exposure levels ($\chi^2_1 = 85.6$, $P < 0.0001$) and the interaction of these treatments ($\chi^2_2 = 13.2$, $P = 0.001$). Mussels on moist sand survived for longer than on dry sand, in both the sun and the shade, whereas mussels on dry sand survived longer than those without any substrate in the sun, this was reversed in the shade (Figure 4.3). ML also had a significant effect on survival time ($\chi^2_1 = 6.0$, $P = 0.01$), due to a positive relationship between ML and survival in mussels in the shade (linear regression, $F_{1,46} = 6.7$, $P = 0.01$), although this was not evident in mussels exposed to full sun (linear regression, $F_{1,45} = 0.1$, $P = 0.75$).

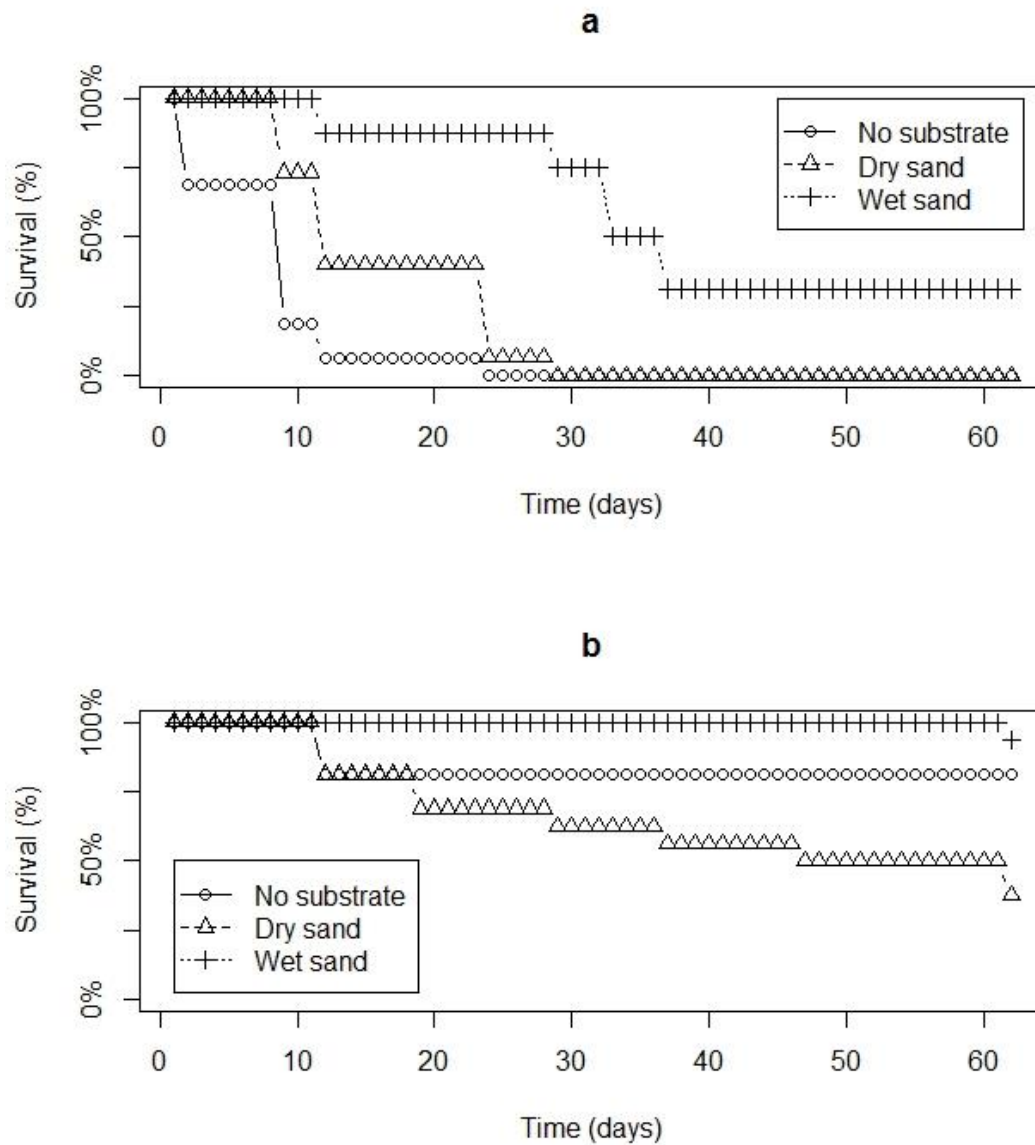


Figure 4.3. Daily survival rates of mussels from the Vasse River when subjected to water emersion with different substrate types, under conditions of (a) full exposure to the sun, and (b) full shade.

Experiment 2: All exposed mussels died, with survival times ranging between two and 24 days (Figure 4.4). A loglogistic distribution provided the AIC best fit survival model. Survival times differed significantly among substrate types ($\chi^2_2 = 94.4$, $P < 0.0001$), but not populations ($\chi^2_1 = 0.1$, $P = 0.91$), although there was a significant interaction

between population and substrate ($\chi^2_2 = 16.4$, $P = 0.003$). In both populations, mussels survived for longer in silt than in sand, with the shortest survival times with no added substrate, but the difference in survival time between mussels on sand and those without added substrate was much more marked for mussels from Yalyal Brook than those from Canning River (Figure 4.4).

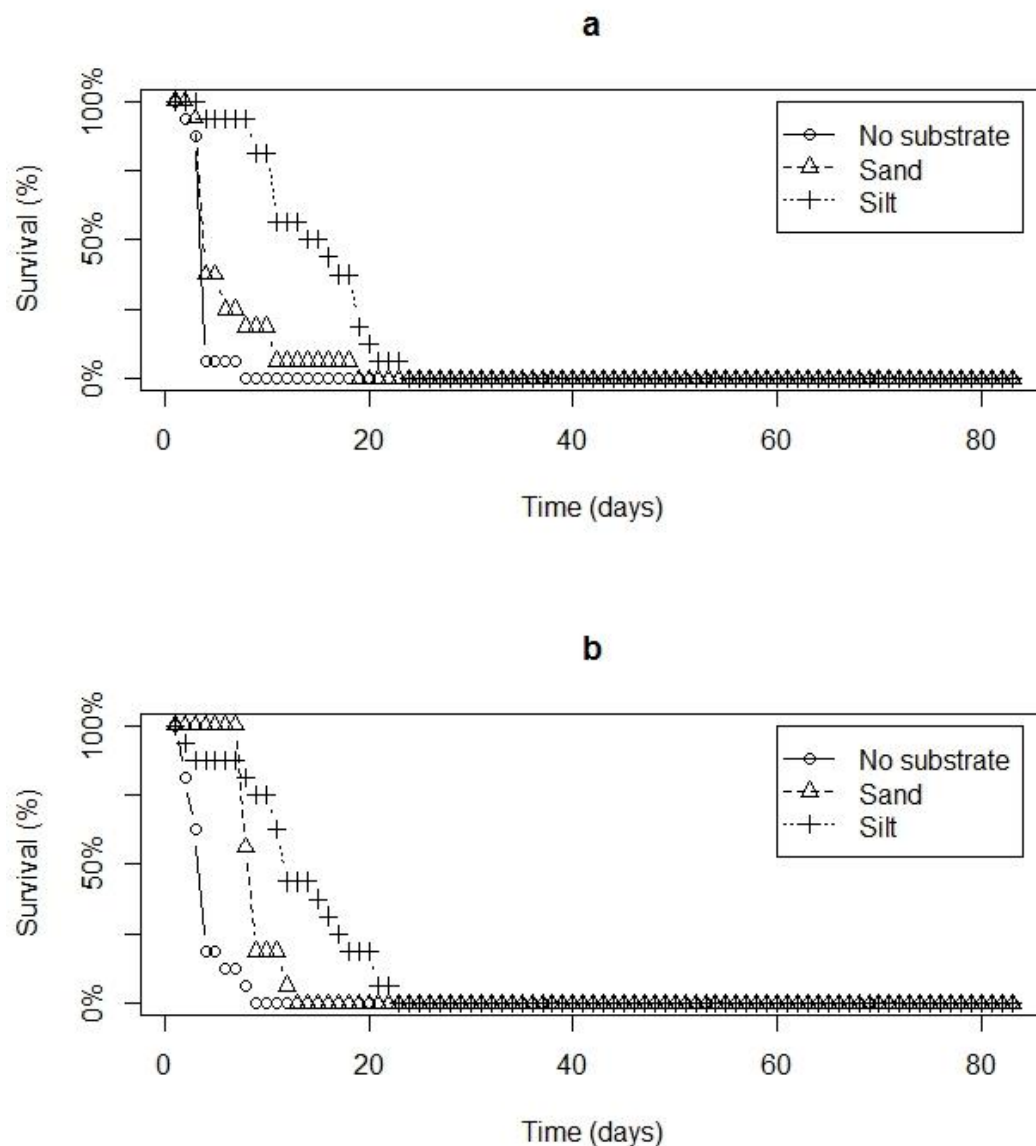


Figure 4.4. Daily survival rates of mussels from (a) Canning River and (b) Yalyal Brook when subjected to water emersion, with full exposure to the sun, on different substrate types.

4.3.4 Burrowing experiments

Experiment 1: Forty six (92%) of mussels survived the seven day exposure period. Thirty two mussels (64%) burrowed into the moist sand substrate and 17 (53%) of these were completely buried. There was no effect of ML on propensity to burrow ($\chi^2_1 = 0.1$, $P = 0.73$), but smaller mussels were more likely to be completely buried ($\chi^2_1 = 10.4$, $P = 0.001$). The mean length of mussels that were completely buried was 51.1 ± 2.7 mm, compared to 65.9 ± 2.1 mm for those not completely buried. The probability of survival was not influenced by shell size ($\chi^2_1 = 1.8$, $P = 0.18$) or by whether mussels were completely buried (Fisher exact test, $P = 0.29$), but mussels which had begun to burrow to any extent were more likely to survive than those which did not burrow (Fisher exact test, $P = 0.01$).

Experiment 2: Mussels exhibited no horizontal movement, but all except one burrowed into the substrate. Fifty percent of mussels burrowed at least half of their shell into the substrate (median depth of burrowing ratio over all treatments = 0.51), although only one mussel was completely buried. Depth of burrowing relative to ML was significantly affected by substrate type ($F_{1,60} = 8.1$, $P = 0.006$), but not by population ($F_{1,60} = 2.0$, $P = 0.16$) or the interaction of population and substrate ($F_{1,60} = 1.6$, $P = 0.21$). Mussels burrowed more deeply into silt (mean depth of burrowing ratio = 0.57 ± 0.04) than into sand (0.42 ± 0.04).

4.4 Discussion

This study has provided the first evidence of the effect of water emersion on *W. carteri*. In two separate locations, a large number of dead mussels were found stranded above the waterline as the water level dropped. Laboratory experiments confirmed that water emersion can rapidly kill mussels, although survival was enhanced by shading and the provision of substrate. Burrowing appears to be the major behavioural response to water

emersion.

4.4.1 Mortality during emersion

A large number of dead mussels were found as a result of a rapid, natural decline in water levels in the Collie River, which left a shallow sand bar exposed, and following the deliberate draining of a section of the Lower Helena Reservoir. Mussel mortality following drought has been previously recorded in a number of studies in the USA, with population declines often as high as 60-100% (Gagnon *et al.*, 2004; Golladay *et al.*, 2004; Haag & Warren, 2008; Walters & Ford, 2013; Vaughn *et al.*, 2015). Mortality is usually ascribed to the direct physiological effects of emersion, although Walters and Ford (2013) also observed increased predation on *Potamilus amphichaenus* that were exposed during low flow in the Sabine River in Texas. Evidence of predatory mammals and birds removing frozen *W. carteri* was found in the current study, and signs of predation, such as scratch, bite and peck marks, have also been recorded on empty shells exposed on the river bank (A.J. Lymbery, unpublished data). Very few previous studies have reported predation of Australian Hyriidae, although Vestjens (1973) found that Australian White Ibis (*Threskiornis molucca*) fed on *Velesunio ambiguus*.

Desiccation trials indicated that *W. carteri* could survive water emersion for at least 62 days at a mean maximum temperature of 28.5°C, and additional observational data (unpublished) has demonstrated survival up to 83 days in shaded conditions. Although desiccation trials for freshwater mussels are rare, this is a comparatively long survival duration. Nakano (2018), for example, found maximum survival times ranging from 3.0-8.5 days for six species from Japan (*Nodularia douglasiae biwae*, *Pronodularia japonensis*, *Obovalis omissis*, *Lanceolaria grayii*, *Inversidens brandtii* and *Sinanodonta japonica*) maintained at 25°C and 71% relative humidity. Gough *et al.* (2012) found maximum survival times at 25°C of 1.5 weeks for *Pyganodon grandis*, 6.4 weeks for *Lampsilis straminea* and 35.5 weeks for *Unio tetrasmus* from south-eastern USA and classified these species as intolerant, semi-tolerant and tolerant

of drought conditions.

Water emersion causes direct mortality in freshwater mussels primarily through desiccation of soft tissues and associated respiratory failure (Byrne & McMahon, 1994; Nakano, 2018). Species that can close their valves tightly may minimise the rate of water loss, but this requires anaerobic respiration, which is inefficient and produces toxic waste products (Collas *et al.*, 2014) or specific adaptations such as a reduction in resting metabolic rate or respiration through an exposed, mucus-sealed mantle margin (Golladay *et al.*, 2004). Where living *W. carteri* have been found in dry river beds, valves have always been tightly closed and sealed with a mucus plug, as also described for this species by Storey and Edward (1989). Changes in metabolic rate or respiratory function have not yet been investigated but are worthy of further study, given the physiological capacity of the species to survive long periods of water emersion.

Survival times for *W. carteri* were enhanced by shading and, when in full sun, by the ability to burrow into the substrate. When burrowing, survival times were greater with increased moisture content and finer sediment particle size, which allowed for deeper burrowing (see below). Other studies have also reported that mussel survival in drought conditions is enhanced by the presence of shade, by utilising moist microhabitats created by large, woody debris and by burrowing into moist sediments (Gagnon *et al.*, 2004; Golladay *et al.*, 2004).

4.4.2 Behavioural responses to emersion

Although freshwater mussels have traditionally been viewed as sessile organisms, there is now abundant evidence that they may undertake substantial movements, both horizontally, across the surface of the sediment and vertically, by burrowing into the sediment (Uryu *et al.*, 1996; Amyot & Downing, 1998; Schwalb & Pusch, 2007; Negishi *et al.*, 2011). Movement patterns may vary substantially, both within species (Amyot & Downing, 1997) and among species (Allen & Vaughn, 2009; Daniel &

Brown, 2014). Gough *et al.* (2012) identified different behavioural strategies in three species of freshwater mussels from Opintolocco Creek, Alabama when exposed to drought conditions: *Pyganodon grandis*, which has a low physiological tolerance to emersion, moved horizontally to track receding water levels; *Lampsilis straminea*, which is semi-tolerant of emersion, initially tracked receding water and then burrowed vertically; and *Unio tetrasmus*, with a high physiological tolerance of emersion, showed little horizontal movement and immediately burrowed. Newton *et al.* (2015) also found a divergence in behavioural strategies in two mussel species during water drawdown in the Upper Mississippi River; *Lampsilis cardium* moved horizontally into deeper water, whereas *Amblema plicata* responded by vertical burrowing into the substrate.

Westralunio carteri appears to follow a tracking, then burrow strategy. Field experiments showed that most mussels released on a sandbank next to a stream initially moved horizontally towards the water, but then began to burrow, and within seven days more than 85% had burrowed into the substrate. In the second laboratory experiment, mussels burrowed immediately rather than moving towards the water, but this may reflect the artificial nature of the experiment, where mussels were placed next to a small pool rather than the river, as in the field experiment. The mean horizontal distance moved in the field was 21.1 cm (maximum distance 85 cm) in three days, with little further movement between three and six days. This level of movement falls into the “intermediate” category of Daniel and Brown (2014), who found three categories of in-stream movement rates in nine common mussel species in Louisiana: ≤ 5 cm/day; < 10 cm/day; and 30-60 cm/day. Gough *et al.* (2012) recorded mean movements of approximately 500 cm over 21 days for *P. grandis* and *L. straminea* in a dry stream transect; with much lower movements in transects that retained water.

Burrowing is an important behavioural response to emersion for many mussel species and is also believed to be used to escape high water flows and predation, although little

is known about the proximate cues that initiate burrowing (Archambault *et al.*, 2014; Newton *et al.*, 2015). In drought conditions, burrowing may reduce the rate of desiccation as stream sediments typically show a gradient of increasing moisture level and decreasing temperature with increasing depth (Archambault *et al.*, 2014; Newton *et al.*, 2015). In the current study, the burrowing depth of *W. carteri* was inversely related to substrate particle size, with mussels able to burrow deeper in silt than in sand. Survival times were also greater in silt than in sand, and this may explain, at least in part, the apparent microhabitat preference of *W. carteri* for finer substrates (see Chapter 2).

4.4.3 Mortality and mussel size

A feature of the natural mortality events observed in *W. carteri* was an over-representation of larger, older mussels on the exposed banks, compared to adjacent submerged habitat. There are a number of possible explanations for this finding. First, smaller mussels may be better able to track receding water levels and therefore are less likely to be stranded; there was no evidence, however, of any relationship between mussel size (ML) and amount of horizontal movement in field experiments, which mirrors findings by Schwalb and Pusch (2007) on three freshwater mussel species (*Unio tumidus*, *U. pictorum* and *Anodonta anatina*) in Germany. Second, smaller mussels may be more likely to burrow beneath the surface and therefore not be discovered when searching the exposed bank; in field experiments smaller individuals began burrowing before larger individuals of *W. carteri*, and in laboratory experiments there was an inverse relationship between ML and likelihood of complete burial beneath the sediment (moist sand). Schwalb and Pusch (2007) and Annie *et al.* (2013) also reported that smaller individuals of *U. tumidus*, *U. pictorum* and *A. anatina* were more likely to be found in deeper sediments. Third, smaller mussels may be more likely to be removed by predators and scavengers, as found when frozen mussels were left on an exposed sandbar.

It seems likely, then, that the over-representation of larger mussels in drought-associated mortalities of *W. carteri* can be explained by a combination of deeper burial into the sediment by smaller mussels, and size-biased removal by predators and scavengers of those mussels that remain exposed. If mussels have an increased chance of survival when buried beneath the sediment, then larger, older mussels will have a greater mortality rate during droughts. This may accelerate the impact on population viability as droughts become longer and more frequent in a drying climate, as mussel fecundity is directly related to the mussel age, which is positively related to the shell size (Klunzinger *et al.*, 2012b; Klunzinger *et al.*, 2014b). However, although there is some evidence that mussels which burrowed more deeply had longer survival times, there was no indication that complete burial enhanced survival.

4.4.4 Implications for conservation

Klunzinger *et al.* (2015) identified the absence of perennial water as an important factor limiting the current distribution of *W. carteri*. Nevertheless, the current study has demonstrated that mussels can survive water emersion for substantial periods of time (at least two months), with survival enhanced by the presence of shade and by burrowing into the substrate. Burrowing is presumably an adaptation to the temporary nature of streamflow in south-western Australia. Although mussels may be able to survive away from refuge pools for up to two months, this may not be sufficient to prevent major population declines in the future, as streamflow in south-western Australia is predicted to be severely impacted by climate change.

South-western Australia has experienced a substantial decline in rainfall since 1950, with a disproportionately larger decline in streamflows (Suppiah *et al.*, 2007; Petrone *et al.*, 2010; Silberstein *et al.*, 2012). This trend is expected to continue under all global climatic models (Suppiah *et al.*, 2007; Silberstein *et al.*, 2012), leading to longer no-flow periods and a decrease in the number and size of dry-season refuge pools in the region (Beatty *et al.*, 2017). In addition, climate change is expected to increase the

frequency of length of droughts throughout most of Australia, including the south-west (Mpelasoka *et al.*, 2008; IPCC, 2012). Based on the results of the current study, I expect that this will lead to an increase in the number and severity of mass mortality events for freshwater mussels as a result of prolonged water emersion.

4.5 Conclusions

Westralunio carteri has undergone a 49% reduction in range in the last 50 years, and with global climatic models predicting an increasingly warmer and dryer climate in south-western Australia, reduced water flow presents a substantial extinction risk. Two recent cases of mass mortality in populations of *W. carteri* appear to be associated with drying rivers. In both cases, larger shells were significantly over-represented in the cohort of dead mussels than the surviving population. Field and laboratory experiments suggested that mussels respond to water emersion by initially tracking receding water levels, then burrowing into the substrate. There was no relationship between mussel size (shell length) and horizontal movement, but smaller mussels burrowed earlier and were more likely to be completely buried in the substrate. Shading and burrowing into the substrate increased the tolerance of mussels to emersion, allowing them to survive for at least 60 days out of water. Finer substrate allowed for greater depth of burrowing and increased survival rate, independent of size. These findings have a number of implications: (1) predicted future reductions in water flow are likely to increase the mortality rate in *W. carteri*; (2) mortality risk may be greater for larger, older, more fecund mussels, producing a disproportionate decrease in recruitment; (3) factors which affect burrowing ability, such as presence of appropriate substrate, will have a synergistic effect on mortality rate; (4) the adverse effects of drying rivers may be at least partially averted by increasing riparian shading.

Chapter 5: General discussion

5.1 Introduction

Freshwater mussels are widespread and functionally important components of aquatic ecosystems throughout the world. They are also one of the aquatic faunal groups most threatened by anthropogenic activities (Lopes-Lima *et al.*, 2018). Although the conservation status of mussels in the Northern Hemisphere has been relatively well documented (Lopes-Lima *et al.*, 2014), Southern Hemisphere species are under-represented in threatened species lists, such as the IUCN Red List. This appears to be related to both the number of species from the Southern Hemisphere presented for assessment, and to the adequacy of data for accurate determination of conservation status. Research effort on freshwater mussels, as measured by scientific publications, is unevenly distributed across the globe, mirroring the trend in conservation status. This may indicate that, on a global scale, research effort is driving conservation priorities in freshwater mussels, rather than the reverse. This is not an unexpected finding, but it does highlight the need for a greater research focus on mussels outside of the traditional areas of study in the Northern Hemisphere.

In recent years, there has been a dramatic increase in research on the ecology and conservation biology of freshwater mussels in a number of countries throughout Australasia and South America, although much is still unknown about freshwater mussels in other areas of the Southern Hemisphere, particularly sub-Saharan Africa. Such research is important, not just to provide a contemporary baseline to monitor population trends and identify and prioritise species in need of protection, but also to identify preferred habitats and threatening processes, which is essential if effective conservation plans are to be devised and implemented.

In this thesis, a number of features of the biology of *Westralunio carteri*, the only freshwater mussel species in south-western Australia, have been examined. The range of *W. carteri* has declined by more than 49% in the past 50 years (Klunzinger *et al.*, 2015) and active intervention may be required to prevent extinction. The broad aim of my research was to address a number of knowledge gaps which were hindering the development of conservation management plans. More specifically, in Chapters 2-4 I: (a) estimated the density, dispersion, population structure and habitat preference of a number of extant populations of *W. carteri*; (b) determined the tolerance of adult mussels to acute and gradual increases in salinity; and (c) investigated the response of mussels to water emersion.

5.2 Population structure and microhabitat preference

Klunzinger *et al.* (2015) used presence/absence data to map the current distribution of *W. carteri*, related this distribution to water quality variables obtained from river monitoring stations, and compared the current distribution with historical museum records. My study has extended this work by revisiting 47 sites that were reported as containing mussels in surveys between 1992 and 2012 (Klunzinger *et al.*, 2015), and estimating population density, dispersion and age (size) structure. Mussels could not be found at 15 of these sites (32%); this may indicate that population declines are still occurring, although it is possible that mussels were present, but at very low densities. I recommend that these sites be re-surveyed, using techniques designed for detecting low-density mussel populations (e.g. Smith *et al.*, 2003), and a long-term monitoring program implemented to directly inform an adaptive management plan.

Density varied widely among sites where mussels were found, with a mean of approximately 29 mussels m⁻², which is relatively high compared to other unionid species studied in Australia (Walker, 1981; Humphrey, 1984) and overseas (e.g. Strayer *et al.*, 1981; Downing & Downing, 1992; Hastie *et al.*, 2000a; Annie *et al.*, 2013). Most

of these populations also had evidence of recent recruitment, as shown by the presence of mussels < 10 years old (shell length < 54 mm). As *W. carteri* has a lifespan of at least 52 years (Klunzinger *et al.*, 2014b), populations should be monitored regularly for size/age distribution to ensure that they are not relicts, composed only of adult mussels, as has been recorded for other species (Hanson & Locke, 2001; Österling *et al.*, 2008; Strayer & Malcom, 2012).

As is common in freshwater mussels, *W. carteri* had a highly aggregated dispersion. It is still unclear whether such aggregation is an adaptation, for example to increase reproductive success (Downing *et al.*, 1993; Zieritz *et al.*, 2014) or a by-product of the over-dispersion of glochidia among fish hosts or of active micro-habitat selection by transformed mussels (Perles *et al.*, 2003). This is a topic that requires further investigation, because if aggregation is an adaptation, this would suggest that populations need to maintain a minimum density to ensure long-term survival. However, regardless of the ultimate cause and potential benefits of aggregation, it may also have adverse consequences, leading to mass mortalities if populations are exposed to spatially discontinuous environmental events, such as sedimentation or river drying.

Knowledge of the preferred habitat of threatened freshwater mussel species is important to guide river and catchment management and restoration (Quinlan *et al.*, 2015a) and to identify suitable release sites for captive breeding and translocation schemes (Moorkens, 2018). A number of micro-habitat factors were associated with the abundance of *W. carteri* in quadrat samples. Of primary importance was substrate grain size, with mussels more abundant on finer sediments. This contrasts with a preference for coarser substrates in most other unionid species that have been studied in Australia (James, 1985; Brainwood *et al.*, 2008a) and other parts of the world (McRae *et al.*, 2004; Quinlan *et al.*, 2015b). I hypothesise that the apparent preference for finer substrates in *W. carteri*, which allow for more efficient burrowing, may be an adaptation to the seasonal cessation of flow and drying in the temporary rivers of south-western Australia.

In addition to a greater abundance on fine sediments, mussels were also found more frequently close to the riverbank and in areas with tall, woody debris. This may suggest an avoidance of rapid water flow, as has been found in other mussel species (Neves & Widlak, 1987; Howard & Cuffey, 2003; Zigler *et al.*, 2008), although proximity to the riverbank may also indicate a preference for higher oxygen-saturation levels (Quinlan *et al.*, 2015b). Further studies in other seasons are required to investigate these apparent micro-habitat preferences and also to investigate other environmental factors that may influence mussel survival and function, as, despite the correlation of mussel abundance with riverbanks and fine sediment, individuals were occasionally found in steep and rocky riverbeds, with a thin layer of substrate.

5.3 Salinity tolerance of *Westralunio carteri*

Klunzinger *et al.* (2015) identified salinity as the most important predictor of the difference in distribution between current and historical records of the occurrence of *W. carteri*, and found an acute salinity tolerance (LC₅₀) for adult mussels of 1.6–3.0 g L⁻¹ in laboratory tests. A substantially higher tolerance to acute increases in salinity was detected in the current study, with LC₅₀ values of 5.87–5.96 g L⁻¹. The only major difference in experimental conditions here and that of Klunzinger *et al.* (2015) was that mussels were fed regularly in the current study, which may have increased their ability to cope with environmental stress. Salinity tolerance was increased (to an LC₅₀ of 6.43–6.45 g L⁻¹) when salinity was increased gradually, rather than acutely, with a longer survival time when the rate of increase in salinity was lower. This was not an unexpected finding, but it is the first time that acclimatisation to increasing salinity has been demonstrated in unionids. The salinity tolerance of *W. carteri* is greater than that found in other unionid species in Europe and North America (e.g. Verbrugge *et al.*, 2012; Blakeslee *et al.*, 2013; Patnode *et al.*, 2015), which may reflect the high levels of natural salinity in Australian waterways.

The ability of mussels to acclimatise to gradual increases in salinity, and their ability to survive even large, acute increases of salinity (up to 8 g L⁻¹) for a substantial time (10-30 days) before dying, helps to predict how mussel populations will be affected by sudden, seasonal pulses of saline water and by the gradual drying of refuge pools over summer/autumn. Also, a substantial variation in survival times was observed among individual mussels when exposed to acute and gradual increases in salinity, which suggests a capacity for adaptation to increasing salinity. This is further supported by the significant differences in survival times among populations. This potential for adaptation should be investigated further. In the current project, the number of mussels from each population that could be used for salinity tolerance tests was restricted due to the threatened status of the species. However, given the current findings, a strong case can now be made to sample mussels from a number of rivers that differ in salinity profiles in order to test their salinity tolerance. It is also important to extend the current study by examining the salinity tolerance of glochidia and juvenile mussels, which other studies have found to be more sensitive to salinity than adult mussels (Gillis, 2011; Blakeslee *et al.*, 2013; Nogueira *et al.*, 2015).

5.4 Drought tolerance of *Westralunio carteri*

Klunzinger *et al.* (2015) found that, after salinity, lack of perennial water flow was the environmental factor most limiting the distribution of *W. carteri*. In this study, mussels which were exposed to full sun without a substrate into which they could burrow, died within 10 days. Survival times were enhanced, however, by shading or, when in full sun, by burrowing into the substrate. Under these conditions, *W. carteri* could survive water emersion for at least 62 days, which is similar to survival times recorded for drought-tolerant unionid species such as *Unio merus tetralasmus* (Gough *et al.*, 2012).

Burrowing into the substrate is a behavioural strategy that has been observed in other

mussel species in response to drought (Gough *et al.*, 2012; Newton *et al.*, 2015). In my study, mussels were able to burrow more deeply into finer sediments and this was associated with longer survival times, which reinforces the hypothesis from micro-habitat studies that *W. carteri* may show a preference for finer sediments as an adaptation to survive drought conditions. This has important implications when prioritising populations for conservation management under projected changes associated with climate change.

South-western Australia has experienced a substantial decline in rainfall since 1950, with a disproportionately larger decline in streamflows (Suppiah *et al.*, 2007; Petrone *et al.*, 2010; Silberstein *et al.*, 2012). This trend is expected to continue under all global climatic models (Suppiah *et al.*, 2007; Silberstein *et al.*, 2012), leading to longer no-flow periods and a decrease in the number and size of dry-season refuge pools in the region (Beatty *et al.*, 2017). In addition, climate change is expected to increase the frequency and length of droughts throughout most of Australia, including the south-west (Mpelasoka *et al.*, 2008; IPCC, 2012). Based on the results of the current study, I expect that this will lead to an increase in the number and severity of mass mortality events for freshwater mussels as a result of prolonged water emersion. One way in which this could be mitigated is through increased strategic riparian replanting at a catchment scale, which would have the additional benefit of reducing water temperatures in dry-season refuge pools (Davies, 2010).

5.5 Every species deserves a chance

Professor Zhongli Ding said in a media interview at the 2009 United Nations Climate Change Conference (COP 15 Copenhagen): “The earth does not need humans to save it, we are saving ourselves”.

It is a tenant of conservation biology that those species which are affected by human

activities should be protected. The philosophy behind this is simple; protecting these species protects ecosystem services and therefore human existence (Cardinale *et al.*, 2012). Recent research has, in general, found a positive relationship between biodiversity and ecosystem function (Tilman *et al.*, 2014) and resilience (Oliver *et al.*, 2015). In a natural ecosystem, each species has its own role and function, and disruption at any one trophic level may have cascading consequences throughout the food web (Barnes *et al.*, 2018).

The astounding biodiversity that we see in the world today is the outcome of approximately 3.5 billion years of evolution. Evolution by the process of natural selection is a fair race with only one condition: survival of the fittest. As the principle that “all men are created equal” in the *United States Declaration of Independence* is widely accepted by human society, it is also self-evident that all species are created equal by nature with certain rights; namely that they are entitled to participate in the evolutionary race.

These rights have been subverted by human activities. Natural forests are currently being destroyed at the fastest rate in human history (Runyan & D'Odorico, 2016); freshwater has been extracted and polluted on all continents (Meybeck *et al.*, 1989; Wheal, 1991); the atmosphere is being polluted both chemically and physically (Akimoto, 2003; IPCC, 2014); species are being transported far from their native ranges (Lowe *et al.*, 2000); and, as a consequence, global biodiversity is rapidly declining (Pimm *et al.*, 1995; Butchart *et al.*, 2010).

Every species deserves a chance to be fairly assessed for its conservation priority. Unfortunately, in the distribution of limited resources for conservation activities, animal charisma is more important than conservation status or functional importance in an ecosystem (Colléony *et al.*, 2017) and there is a considerable bias against freshwater species towards terrestrial and charismatic fauna (Darwall *et al.*, 2011). Unionid

mussels, despite their endangered status and vital functional roles in freshwater ecosystems, are small, plain, unobtrusive and easily overlooked as a species. Such is the case with *W. carteri*, the only freshwater mussel in south-western Australia. The range of this species has declined dramatically in the past 50 years and, as a result, it has only very recently been listed as Vulnerable on Western Australian, national (Threatened Species Scientific Committee, 2018) and international (Klunzinger and Walker, 2014) threatened species list. Predicted rates of salinisation and flow restriction indicate that further population declines are inevitable (Klunzinger *et al.*, 2015). Despite this, there have been no active attempts to develop a conservation management plan and the plight of the species has been largely ignored by government agencies and the general public.

I hope that the work in this thesis can contribute to the conservation of *W. carteri* and help shine more light on the plight of unionids more generally. Preventing the extinction of this unique keystone species will require a holistic conservation management strategy, incorporating the selection of priority populations, habitat protection, mitigation of threatening processes and possibly captive breeding. Additionally, it is always better to protect the ecosystem/community than one or two species, however, as part of broader conservation planning, targeting key species such as *W. carteri*, can help enhance the conservation of the overall ecosystem. As a result of my work, and that of my colleagues who have worked before me, we now understand the basic life cycle of this species, host range for the larval stage (Klunzinger *et al.*, 2013), longevity, age at sexual maturity, population density and structure, microhabitat preferences, tolerance to acute and gradual salinity, and tolerance to water emersion. This information provides a sound basis to protect this species for the benefit of our waterways now and into the future. We must ensure that this information is used for the benefit of conserving the species and not simply stored away. It is now time to get to work.

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