Resurrection ecology of invertebrates in temporary wetlands in the Cape Floristic region: effects of urbanisation and fire

Michelle Blanckenberg

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Department of Biological Sciences, University of Cape Town, Rondebosch, 7701, South Africa

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Supervisors: Mr Musa Mlambo[1] and Dr Cecile Reed[2]

[1] *Department of Freshwater Invertebrates, Albany Museum, Somerset Street, Grahamstown, 9139, South Africa*

[2] *Department of Biological Sciences, University of Cape Town, Private Bag X3, Rondebosch, Cape Town 7701, South Africa*

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Abstract

Despite their importance to regional biodiversity, temporary wetlands and their invertebrate communities are generally understudied and under-conserved. Resurrection ecology is used to study the communities present in the dry phase of temporary wetlands to gain a better understanding of the functioning and health of these systems. The hatching success of invertebrate propagules in dried soil sediments, collected from temporary wetlands in Cape Sand Fynbos regions of Cape Town, were investigated in 2016. Soil samples were collected from conserved and urban sites (during May and June 2016) using a standard soil auger method and complemented with monthly aquatic phase sampling using standard sweep net methods (during September and October 2016). The effects of fire were tested, on hatching success by staging vegetation fires over the collected soil samples for five temporary wetland areas in the same region. Mann-Whitney U tests were used to test differences in abundances between treatments, whereas for comparison of taxon richness, Shannon diversity and Pielou's evenness linear mixed effect models (LMER) were used. Non-metric multidimensional scaling (nMDS) plots were used to graphically visualize the community composition between the different treatments, with 'adonis' analysis used to test their statistical significance. Results found a total of 18 invertebrate taxa hatched, while a total of 27 taxa were identified from the wet phase. Results from hatching assays showed no significant differences in diversity patterns of invertebrates between conserved and urban areas in the dry phase. However, wetlands that were in the conserved area supported higher invertebrate diversity during the wet phase, although not significant (lmer, $Z = -1.75$, $p = 0.08$). These results suggest that human activity (alien vegetation, littering and polluting) in the area did not impact the ability of invertebrate propagules to emerge, but did slightly affect the natural aquatic phase. Fire had a significantly negative impact on invertebrate diversity measures (lmer, $p < 0.001$) and community composition (adonis, $F = 24.494$, $p = 0.001$) during hatching assays. Management of unpredicted and uncontrolled fire in urban areas is essential to ensure future protection of critical biodiversity areas created by these temporary wetlands. More research should focus on the impact of increasing fire frequency and intensity on these systems to understand the management challenges in a changing climate.

Keywords: Macroinvertebrate assemblages, Cape Sand Fynbos, hatching assays, fire ecology, invertebrate conservation

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Introduction

Temporary (ephemeral) wetlands are small, shallow, isolated depressions that become inundated after sustained rainfall events and can hold water for a few days, weeks or months before completely drying up (Harmse & Le Grange 1994, Brinson & Malvárez 2002, Williams 2005, Ellery *et al*. 2009). These freshwater aquatic systems are a common feature of the south-Western Cape (Silberbauer & King 1991, De Roeck *et al*. 2007, Mlambo *et al*. 2011, Oberlander *et al*. 2014) and southern Africa in general (Allan *et al*. 1995, Goudie & Wells 1995). They are differentiated from permanent wetlands on a hydrological and biotic level (Zacharias *et al*. 2007, Schael *et al*. 2015). For example these wetlands usually occur where annual evaporation rates are more than annual rainfall causing wetlands to dry up outside of peak rainfall months (Ellery *et al*. 2009, Day *et al*. 2010). Globally, temporary wetlands provide habitats for unique communities of biodiversity and, disproportionately to their size, contribute a large portion to regional biodiversity (Oertli *et al*. 2002, Martinez-Sanz *et al*. 2012). Both plant and passive animal communities need to be adapted to wet and dry periods resulting in often unique and specialised communities (Leibowitz 2003, Meyer *et al*. 2003, Day *et al*. 2010). As a result of their isolated nature and generally high levels of biodiversity, temporary wetlands have been proposed as useful and excellent field-laboratories to study ecology, evolution, and conservation biology (Blaustein & Schwartz 2001, de Meester *et al*. 2005, Williams 2005). Temporary wetlands exclude species which cannot respond or cope with periods of flooding followed by desiccation (Brinson & Malvárez 2002, Urban 2004). Given their periodic drying up, organisms endemic to these systems (which are unable to migrate) rely on desiccation resistant or dormant propagules (e.g. eggs, cysts, seeds, spores, etc.) to survive the dry phase (Williams 1985, Day *et* *al*. 2010, Strachan *et al*. 2015). Understanding conditions that lead to hatching success of these propagules is an important science that is not well studied in South Africa.

Resurrection ecology

The apparent lack of surface flow between temporary wetlands has given rise to the view that they are hydrologically 'isolated' (Leibowitz 2003). Species in these wetlands are affected by the physically isolated nature of these systems (Leibowitz 2003, Schael *et al*. 2015). Organisms in temporary wetlands have been generally categorized into two dispersal strategies: passive and active dispersers (Wiggins *et al*. 1980; Wissinger 1999). Active dispersers can disperse out of the habitat prior to desiccation or have a dispersal life stage. Active dispersers are able to deal with periodic desiccation by only entering the wetland system during periods of favourable conditions (Urban 2004). However, in order for passive dispersing populations to survive in temporary wetlands without relying on new individuals entering the community through dispersal, species require adaptations for dealing with periodic drying up (Urban 2004). Passive dispersers require vectors for dispersal among communities and survive drought with diapause adaptations (e.g. production of 'resting eggs') (Williams 1985, Day *et al*. 2010, Strachan *et al*. 2015).

The evolution of 'resting eggs' acts as a mechanisms for desiccation resistance in order to persist during dry phases (Strachan *et al*. 2015). Eggs or cysts remain dormant within the top layers of soil and re-establish once conditions become favourable (i.e. during wet phase inundation). Understanding the composition and hatching success of these propagules can be used to determine past and future effects of physical and biological conditions of temporary wetlands (Mlambo *et al*. 2011). Furthermore the propagules provide important reservoirs determining community and population structures from season to season (Day *et al*. 2010, Mlambo *et al*. 2011). The presence of species with dormant stages in the dry phase will determine system functioning in the following wet phase (Day *et al*. 2010). Hatching assays determine the community composition during the dry phase, and their likely succession patterns in the wet phase (Mlambo *et al*. 2011). This ensures a comprehensive knowledge of species abundance and occurrence is established despite high species turnover during wet phases (Mlambo *et al*. 2011, Strachan *et al*. 2015).

Temporary wetland fauna are dominated by macroinvertebrate and microcrustacean species (Day *et al*. 2010). Macroinvertebrates are defined as taxa ≥ 1 mm in size and visible to the naked eye, while microcrustaceans are defined as any invertebrate > 80 µm in size and usually include Copepod, Ostracod or Cladocera (Bird & Day 2016). Wetland invertebrates are considered important because of their potential role as biomonitoring tools as well as forming the base of many food webs (Bowd *et al*. 2005, Bird *et al*. 2013b, van den Broeck *et al*. 2015). Temporary wetland invertebrate species predominantly use 'resting eggs' to survive desiccation as these are mostly passive dispersing species (Day *et al*. 2010). The ability to survive both wet and dry phases has resulted in fairly unique invertebrate communities developing within temporary wetlands, worldwide (Leibowitz 2003, Meyer *et al*. 2003, Day *et al*. 2010, Mlambo *et al*. 2011). Studying the abundance and composition of smaller invertebrate groups is essential to understanding the functioning and ecology of temporary wetlands as they persist in the wet and dry phase of a wetland (de Meester *et al.* 2005, Day *et al*. 2010). However, despite the unique invertebrate biodiversity, there has been little research into the structuring of invertebrate communities within temporary wetlands, especially across both wet and dry phases. Previous studies (Zacharias *et al*. 2007, Bagella *et al.* 2010, Mlambo *et al*. 2011, Bird & Day 2016) have investigated invertebrate abundances and occurrence during wet months only, but this provides only a snapshot of these communities. Species turnover during the wet phase can be very high, so analysis of both wet and dry phases is needed to get a full picture of temporary wetland biodiversity (Day *et al*. 2010, Mlambo *et al.* 2011). Sediment analysis focusing on 'resting eggs' enables one to use hatching experiments to study temporary wetlands sampled in the dry season (de Roeck *et al*. 2007, Day *et al*. 2010, Pinceel *et al*. 2013, Henri *et al.* 2014, Liefferink *et al*. 2014). Wetland health and functioning can therefore be determined despite desiccation and apparent decreases in species presence (Ferreira *et al*. 2012, Henri *et al*. 2014).

Hatching experiments can follow either an isolation or non-isolation method. Isolation methods aim to separate resting eggs from the sediment in which they are found to hatch within an Aachener Daphnien medium (ADaM) known as the Onbé-Marcus method (Onbé 1978, Marcus 1990, Liefferink *et al.* 2014). The isolation method has been described as more cost-effective and time-effective when looking at zooplankton communities however mortality rates are higher than non-isolation methods (Liefferink *et al*. 2014). Non-isolation methods simply observe hatching from sediment after inundation with distilled water (Liefferink *et al*. 2014). While hatching rates are lower than isolation methods, this method is more appropriate when dealing with invertebrates emerging from dormant phases compared to zooplankton (Liefferink *et al*. 2014). Increased food availability allows emerging invertebrates to survive longer making identification more likely (Liefferink *et al*. 2014). Several studies have shown that hatching from resting eggs can be highly dependent on environmental cues (Bagella *et al*. 2010, Pinceel *et al*. 2013, Henri *et al.* 2014, Liefferink *et al*. 2014). The two hatching methods highlight how resting eggs can react to the medium in which they are inundated. This again was supported by Henri *et al*. (2014) who showed that branchiopod eggs were significantly impacted by the salinity of the medium in which the sediment was inundated. These hatching experiments highlighted the impact of Acid Mine Drainage (AMD) exposure on the resurrection of temporary wetlands (Henri *et al.* 2014). Both the initial hatching and recovery of branchiopod species was negatively affected by wetlands exposed to AMD (Henri *et al.* 2014). A different set of hatching experiments showed that light was an important cue for resting egg hatching for crustacean species (i.e. the fairy shrimp *Branchipodopsis wolfi*) (Pinceel *et al*. 2013). A single continuous pulse of light resulted in higher hatching rather than several short pulses of light when working in a controlled lab environment (Pinceel *et al*. 2013). This was likely a result of natural pigmentation variation in the eggs of the fairy shrimp resulting in variations in hatching depending on variations in light (Pinceel *et al*. 2013).

Urban ecology

The environment (water chemistry, light, etc.) found within a temporary wetland will impact the resurrection of invertebrates from resting eggs in the sediment (Pinceel *et al*. 2013, Henri *et al.* 2014, Liefferink *et al*. 2014). The health and functioning of a temporary wetland, and therefore its environment, is largely dependent on landscape scale processes (i.e. movement of water and nutrient loads) (Mitch & Gosselink 2000, Leibowitz & Nadeau 2003, Schael *et al*. 2015). Anthropogenic activity impacting on these processes will therefore impact wetland health and functioning (Hollis 1990, Schael *et al*. 2015). These systems, like many others, are under threat from increasing human activity/development (urbanisation) and climate change, coupled with this many temporary wetlands in urbanised areas fall outside the conserved areas network making them vulnerable to these activities (Hollis 1990, Schael *et al*. 2015, City of Cape Town 2016). Harmse & Le Grange (1994) showed that increased human activity (mainly recreational) and therefore pollutants (i.e. litter) can significantly influence the hydro-chemistry of temporary wetlands during inundation. Nitrate concentration, ammonia and pH all increased in areas where human activity, in the immediate vicinity of a wetland, increased (Harmse & Le Grange 1994). The study suggested that pollution of temporary wetland water occurred in the immediate surroundings of the wetland (Harmse & Le Grange 1994). The impact of high nitrate and ammonia levels was suggested to affect bird populations negatively as well as the 'pan environment' (Harmse & Le Grange 1994).

Increasing urbanisation is a common feature of low-lying regions as a result of increasing human populations, and has led to many species declines (McKinney 2006, Johnson *et al*. 2013, Bird & Day 2016). In the Western Cape province of South Africa, temporary wetlands are common within the low-lying Cape Sand Fynbos vegetation type (Day *et al*. 2010, Mlambo *et al*. 2011). The Cape Flats region of Cape Town is an example of the lack of protection for temporary wetlands in the Western Cape (Figure 1). The influx of people into the Cape Flats urban areas suggests that natural areas containing temporary wetlands are at greater risk of being impacted by human activity (Harmse & Le Grange 1994). Investigating the impacts of urbanisation on invertebrate communities within a matrix of conserved versus urbanised land uses is an important aspect for the conservation and management of temporary wetlands (Zacharias *et al*. 2007). Cape Sand Fynbos has drastically reduced in recent years due to population increases and urbanisation (Rebelo *et al*. 2006). Invertebrate communities within poorly conserved temporary wetlands are likely to be particularly vulnerable to these changes (Bird & Day 2016). Bird & Day (2016) assessed the effects of habitat transformation on wetland communities in the wet phase within the Kenilworth Racecourse Conservation Area (KRCA), suggesting that invertebrates are directly responsive to changes in habitat. Urbanisation has been linked to a 60% reduction in taxon richness over 4 years in 201 wetlands across Colorada, USA (a rapidly developing area in the US) (Johnson *et al.* 2013). The study specifically recorded a 33% decrease in aquatic insects as a result of changes in abiotic factors such as increased nutrients as well as biotic factors such as introduced fish species (Johnson *et al*. 2013). These factors are all associated with increased urbanisation within areas surrounding, therefore indirectly impacting, these wetlands (Johnson *et al.* 2013). This was observed in many permanent wetlands across the United States of America which was thought to be more robust than temporary systems (Johnson *et al*. 2013). The impacts of urbanisation are likely to be elevated in these closed temporary wetland systems as a result of local processes (Cottenie 2005, Johnson *et al*. 2013). Metacommunity theory suggests that systems with species with passive dispersal mechanisms, as those seen in the Cape Sand Fynbos temporary wetlands in Cape Town, will be greatly affected by local processes as opposed to regional processes (Urban 2004, Cottenie 2005). This suggests that differences could potentially be detected between relatively close systems, depending on the changes in habitat transformation (Johnson *et al*. 2013). Comparing the invertebrate communities between two relatively close and environmentally similar sites, which have varying degrees of urbanisation could help determine whether these local processes are influencing changes in species compositions in temporary wetlands (Urban 2004).

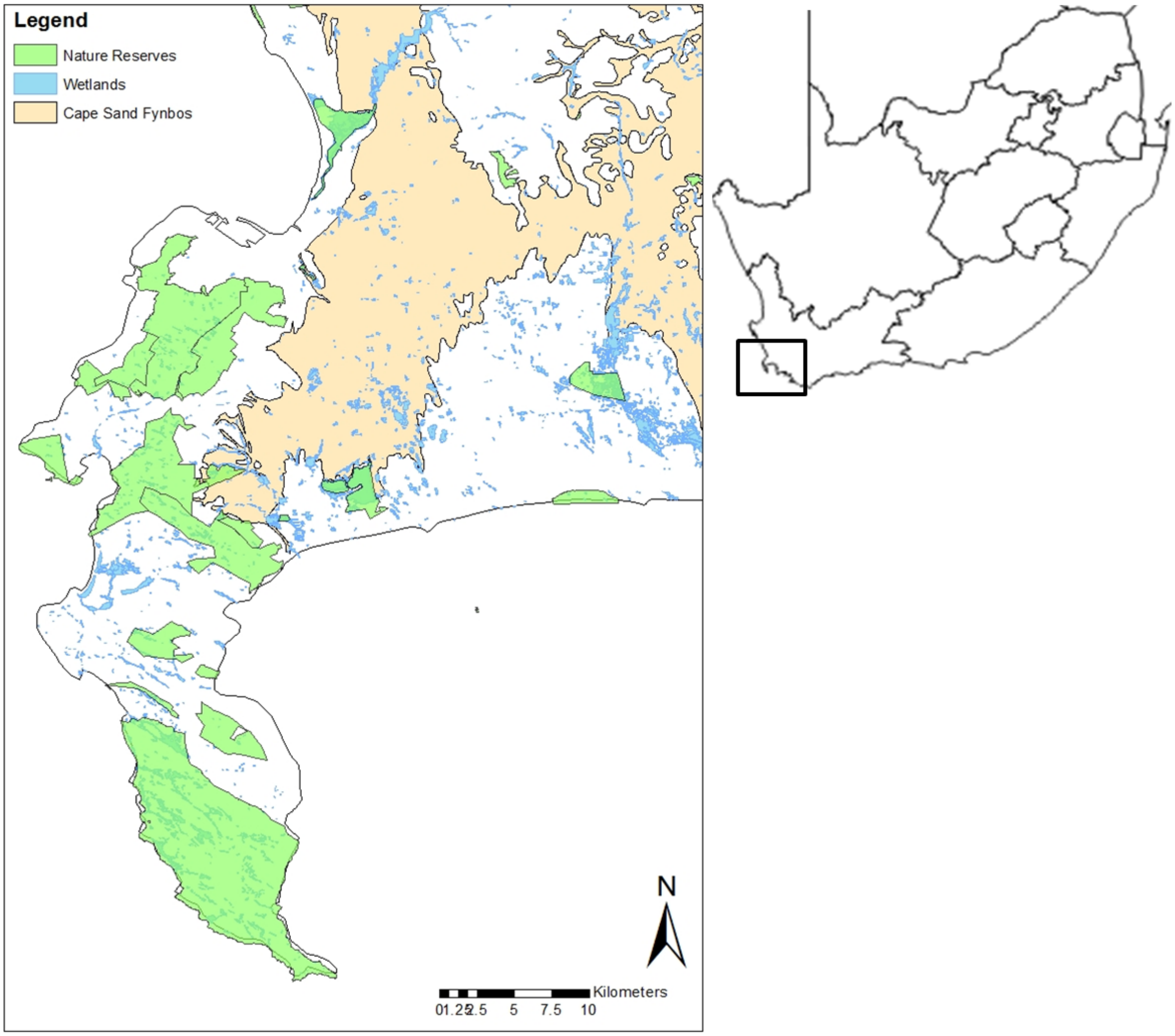

Figure 1: Temporary and permanent wetlands located within the Cape Peninsula region of the Western Cape, South Africa, in relation to formal nature reserves and areas of Cape Sand Fynbos (Created in ArcGIS).

Fire ecology

Fire is also a crucial feature in the life of Fynbos systems of the Western Cape in South Africa and many plant species have fire-dependent germination traits (Keeley & Fotheringham 2000, van Wilgen 2009, Schael *et al*. 2015). The natural fire regime in Fynbos systems is between 10-15 years, however recently fires have become more frequent within the system largely due to anthropogenic activities (van Wilgen 2009, van Wilgen *et al*. 2011, Janion-Scheepers *et al*. 2016) The dependence of Fynbos on fire suggests Cape Sand Fynbos temporary wetland fauna and flora have evolved with fire as a dominate feature (Schael *et al*. 2015, Janion-Scheepers *et al*. 2016). It is suggested that fire would have effects on vegetation, physio-chemical properties of the soil and water, as well as impact carbon and nitrogen cycles within the sediments (Zacharias *et al*. 2007, Schael *et al.* 2015). However, there is little research testing the impact of fire on fauna and flora communities or the post-fire recovery of temporary wetlands (Schael *et al*. 2015). Most fire research has looked at common soil species (i.e. Collembola) or the impacts on amphibian populations and plant propagules within the northern hemisphere (Russel *et al*. 1999, Pilliod *et al*. 2003, Malmström 2012, Janion-Scheepers *et al*. 2016). Kotze (2013) identified that fire management within a wetland is context dependent, as the response of a wetland to fire depends on the climatic and hydrological characteristics of the wetland. This suggests it is difficult to create a blanket fire management plan for wetlands (permanent and temporary), and research into the specific responses of wetlands to fire is vital for their management (Kotze 2013). Understanding how temporary wetlands burn is important for understanding the responses of fauna and flora to fire. There is little research into how temporary wetland in the Cape Flats region burn, however on the ground reports suggest most of the wetland basin does burn, as during dry months soil and vegetation is relatively dry. The mat forming grasses in some of the wetlands may form a protective layer for the soil species within the basin.

A recent study on springtail (Collembola) abundance and diversity post fire showed that there was dramatic declines in abundance immediately post fire but steady recovery for three years post fire (Janion-Scheepers *et al*. 2016). This shows the potential impact of fire on invertebrates occurring within the soil (Janion-Scheepers *et al*. 2016). Fire is particularly important or threatening during the dry phase of these wetlands as fuel loads within the wetland is high and soils relatively dry (Zacharias *et al*. 2007). The communities present within the egg banks in the soil are likely to be impacted by fire occurring during the dry phase (Keeley & Fotheringham 2000, Zacharias *et al.* 2007). This will impact on the invertebrate community composition in the wet phase depending on how fire impacts different species (Day *et al*. 2010). Burning can accelerate the mineralisation of organic matter providing a valuable supply of nutrients for invertebrate species emerging during the wet phase (Keeley & Fotheringham 2000). However, de Szalay & Resh (1997) showed that while burning can increase dominant macroinvertebrate and microcrustacean densities, some species reacted negatively to elevated soil temperatures. Dormant cysts and eggs in the first few centimetres of sediments may be more vulnerable to elevated temperatures than those found further down (de Szalay & Resh 1997, Zacharias *et al*. 2007). Most studies (Russel *et al*. 1999, Pilliod *et al*. 2003, Malmström 2012, Janion-Scheepers *et al*. 2016) have largely looked at once off fire events or impacts of preventing fire (i.e. use of toxic fire retardants), however, none have focused on temporary wetlands specifically. Little research has investigated the potential impacts of fires at varying intensities and frequencies, and few studies have looked at impacts across the faunal community. Assessing the effects of fire on invertebrate hatching over a range of spatially diverse temporary wetlands, through fire treated hatching experiments, will provide essential information on the role of fire within these systems. Understanding the changes in hatching success after burning could help provide valuable information on fire management and ideal burning regimes for conserved areas containing temporary wetlands.

Significance of the study

Globally temporary wetlands are threatened by increasing urbanisation, agriculture, pollution and invasion species (Zacharias *et al*. 2007, Calhoun *et al*. 2016). The unique physical and ecological characteristics of temporary wetlands (i.e. small, cryptic nature and predominantly passive dispersing biota) make these systems particularly vulnerable to external threats, especially human activity (Zacharias *et al*. 2007). Several studies (Bird 2012, Batzer *et al.* 2004, Batzer 2013) show how temporary wetlands can be considered relatively resilient systems. This resilience is attributed to the generalist nature of most of the species present (particularly invertebrate species), and the frequent exposure to environmental fluctuations. Batzer *et al.* (2004) found that macroinvertebrate found in wetlands across Minnesota had no significant response to changing environmental variables. However, these wetlands represented relatively pristine old-growth forest. The decline of temporary wetlands in urban areas has not been extensively measured, largely due to their small, inconspicuous nature however, several areas have reported increasing degradation of these systems (Zacharias *et al*. 2007). The complex interactions between invertebrates and environmental variables make predicting the response of wetlands to disturbance increasingly difficult, and suggests these impacts are highly context specific making extensive research on a range of temporary wetland systems vital (Batzer 2013).

Temporary wetland classification is often difficult as both abiotic and biotic factors need to be considered over extended temporal scales (Ollis *et al.* 2015). Some wetlands only support flora and fauna that are observed for short periods of time but are essential for accurate classification (Day *et al*. 2010). These wetlands are known as “cryptic”, and are difficult to identify outside of wet phases and therefore difficult to study (Day *et al.* 2010). Conservation of these systems requires basic understanding of abiotic and biotic features to understand the health and functioning of temporary wetlands (Schael *et al*. 2015). More complex management questions can be considered once these baselines are created, for example the potential impacts of climate change on these systems (Schael *et al*. 2015). South African climate is predicted to have decreasing precipitation with more extreme and erratic rainfall events, while temperatures are likely to increase across the country (Bernstein *et al*. 2008, Mitchell 2013, Dallas & Rivers-Moore 2014). This will affect ground and surface water flow impacting hydrological characteristics of temporary wetlands (Erwin 2009, Junk *et al*. 2013). The timing and length of dry phases for these systems could change impacting on the hatching of dormant eggs and cysts depending on which hydrological cues individual species may rely on (i.e. length of inundation, successional wetting events) as well as impact the connectivity of these wetlands (Erwin 2009, Bagella *et al*. 2010, Johnson *et al*. 2010, Junk *et al*. 2013, Schael *et al*. 2015). However, it could be argued that the adaptations of the invertebrates found in temporary wetlands reflect fluctuation lifestyles suggesting subtle climate changes may not affect these invertebrates as much as other systems which do not experience extreme yearly shifts (Batzer *et al.* 2004). Investigating the response of temporary wetland invertebrates to drought and fire will improve our understanding of the potential impacts, or lack thereof, of climate change on temporary wetlands.

Temporary wetlands found in the Western Cape face similar threats seen impacting temporary wetlands worldwide and despite their vulnerability and importance, temporary wetlands are relatively understudied in South Africa as an extensive survey of wet and dry phase biota has not been explored (Schael *et al*. 2015). Currently biodiversity assessments by the City of Cape Town for the Cape Peninsula area identified temporary wetland areas on the cape flats (Cape Sand Fynbos region) as critical biodiversity areas (City of Cape Town 2016). However, these areas do not fall within the conserved areas network of the region (City of Cape Town 2016). Lack of research on invertebrate communities in temporary wetlands and their responses to various external threats (i.e. urbanisation and fire) creates knowledge gaps within the understanding of the basic functioning of these systems. Filling these gaps is crucial for full classification of temporary wetlands so that adequate management and conservation is achieved.

The unique vegetation of the Western Cape Province, South Africa has resulted in a high degree of endemism (Wishart & Day 2002). Cape Sand Fynbos temporary wetlands in particular provide habitat for rare and endemic amphibian and invertebrate species (De Villiers 2004, Day *et al*. 2010). Temporary wetland habitats host a range of these rare and endemic species, more so than permanent wetlands found in the same areas (Day *et al*. 2010). Wetlands within the Kenilworth Racecourse Conservation Area (KRCA), for example, support one of the last robust populations of the rare and endemic microfrog (*Microbatrachella capensis*) (De Villiers 2004). The temporary wetland habitats are essential for the conservation of these endemic species. Although KRCA supports unique wetland ecosystems, baseline knowledge of the invertebrate community throughout the year is unknown. Wetland invertebrates act as an important food source for microfrog populations, as well as providing important information about the health of the system. Understanding the structure of wetland invertebrate communities is essential for adequate management of these conservation areas. Furthermore describing the potential impact of fire on these communities will highlight the role fire can play in managing reserves like KRCA. The small, isolated nature of these wetlands makes them particularly vulnerable to habitat transformation and disturbance (Mitchell 2013). Understanding the differences between conserved and urban wetlands is essential for future conservation planning. The pressure from increasing drought and extreme weather conditions may add stress to wetlands already impacted by anthropogenic activity (Erwin 2009, Junk *et al*. 2013). In the Cape Sand Fynbos region fire is also likely to be impacted by climate change. Increased intensity and frequency of fire can impact the post-fire recovery of invertebrate communities that have evolved in a system that has a typically low fire interval (Janion-Scheepers *et al*. 2016).

Aims and Objectives

The study will describe temporary wetland invertebrate communities for dry and wet phases in Kenilworth Racecourse Conservation Area (KRCA) and Ottery public park space, as well as dry phase descriptions in Milnerton Racecourse Conservation Area (MRCA), Youngsfield Army Base and Rondevlei Nature Reserve. The study has three main objectives. Firstly, to assess the abundance and occurrence of invertebrates in eleven temporary wetlands (six within KRCA and five within the Ottery Green Belt) within Cape Sand Fynbos regions in the south-western Cape. To do so, hatching experiments were used to determine dry phase abundances and occurrence, complemented with wet phase abundances and occurrence from monthly water column sampling (Mlambo *et al*. 2011, Bird & Day 2016). Taken together, this will give a year-round assessment of invertebrate occurrence and abundance successions. Second, the possible invertebrate differences between conserved and urban wetlands will be described. To do so five urban wetlands distributed across the Ottery public park (urban) will be compared with the five conserved wetlands in the KRCA, during both dry and wet phases. Abundance, taxon richness, evenness and invertebrate compositions were compared between sites. We predict that urban areas will likely have higher nutrients loads but increased pollution and disturbance impacting on species richness and hatching success. It is hypothesised that urban areas will have higher hatching success, but decreased species richness. Third, possible effects of fire on invertebrate abundances and occurrence within temporary wetlands will further be described. To do so invertebrate occurrence and abundance from soil sediment samples which have been exposed to controlled fire will be compared to samples which remain unburnt.

Samples will represent five sites across the Cape Sand Fynbos region to ensure a wide geographic range is incorporated into the analysis. We predict recently burnt areas will likely have higher nutrient loads but increased soil temperatures will impact on species richness and hatching success. We hypothesise recently burnt areas will have higher hatching success, but have decreased species richness.

Methods

Study site

A total of sixteen temporary wetlands were sampled across five areas in the Cape Flats region of the greater, Cape Town metropolitan area (Figure 2). All these wetlands fell within the Cape Sand Fynbos vegetation type, a critically endangered habitat (Rebelo *et al*. 2006). The hydrological regime of temporary wetlands in these areas is such that they are usually inundated in late June to late November in good rainfall years, predominantly groundwater fed with a significant portion of water also received from precipitation (Bird & Day 2016). The inundation period for the individual wetlands varied considerably between the different areas, and the conditions between the dry and wet phase were substantially different (Figure 3). These wetlands are considered hydrologically isolated as there are no inlets or outlets connecting them to each other or any other body of open water (Bird & Day 2016). Although they fall within the Cape Sand Fynbos vegetation type, the temporary wetlands were dominated by slightly different plant communities (Gehrke *et al*. 2011). For example, a matt forming grass (*Isolepus rubicunda*) was more dominant in KRCA, while an invasive grass (*Pennisetum clandestinum*) dominates in Youngsfield Army Base (Bird & Day 2016). Bird *et al*. (2013a) studying these systems, reported a negative impact of dominant vegetation on the wetland’s physio-chemistry. The variety of

Isolepus rubicunda found in KRCA, which is unusual in the way it forms thick layers of matt (Prof Muthama Muasya, *pers comm*.), is often associated with populations of the rare and endemic microfrog (*Microbatrachella capensis*) (Burger *et al*. 2004).

Figure 2: Location of five areas within Cape Town, Western Cape where temporary wetlands were sampled (Source: Google Maps 2017).

Figure 3: Temporary wetland from Kenilworth Racecourse Conservation Area (taken in 2016) showing how wetlands remain dry during summer months (left), only becoming inundated during wet winter months (right).

To assess the effects of urbanisation ten wetlands were studied; five each from Kenilworth Racecourse Conservation Area (KRCA) and Ottery Public Park space (Figure 4). Wetlands within the KRCA were shielded from urbanisation pressures, as the area is a formal conserved area relatively devoid of contemporary urbanisation pressures. KRCA has been identified as an area that supports numerous wetlands scattered across the site which support a number of endangered populations (Gehrke *et al*. 2011, Bird & Day 2016). On the other hand the Ottery greenbelt is currently used as a public area with several walking paths and is frequently disturbed by residents of the surrounding areas, thus representing an area with urbanisation impacts. High levels of litter are frequently observed. The close proximity of these two areas to each other (~2 km) allowed for the assumption that most environmental variables, such as local climate, vegetation type and hydro-morphometry, will be similar and consistent between the sites. These areas therefore were used for a basic comparison of temporary wetland invertebrate communities in a conserved area versus an urban area suffering from urbanisation impacts (e.g. alien vegetation and pollution).

Figure 4: Location of Kenilworth race course and Ottery greenbelt within Cape Town (top) and the spatial distribution of temporary wetlands assessed within the Kenilworth Race Course Conservation Area (bottom left) and the Ottery public space (bottom right) (Source: Google Maps 2017).

To assess the effects of fire, two wetlands from five sites were sampled, namely KRCA, Ottery Public Park, Milnerton Racecourse Conservation Area (MRCA), the Youngsfield Army Base and Rondevlei Nature Reserve. This allowed for a spatially diverse assessment of the invertebrate responses to fire in the Cape Flats portion of the Cape Floristic region. In each site, the soil sediment for each wetland was equally divided, with one portion subjected to burning simulation and the other not manipulated, i.e. left as a control. The management of fires within these areas varied between the sites. KRCA, MRCA, Youngsfield Army base and Rondevlei

Nature Reserve actively managed burning as an act to facilitate fynbos growth as these areas sat within varying levels of protection. The Ottery Public Park however experience accidental fire which was harder to manage and regulate. The details of burning and the varying fire management techniques are described in Appendix I for each area sampled. While data on fire events for Ottery Public Park and Youngsfield Army Base could not be obtained (lack of records) we assumed that fire was erratic and frequent for Ottery, and infrequent at the Army Base. This is based off verbal communication with residents in the surrounding areas.

Data collection

Environmental variables

Basic physio-chemical measurements were also recorded namely pH, dissolved oxygen, conductivity and temperature for each wetland. Further physio-chemical variables were not recorded due to limited time and resources allocated to the project. All measurements were taken at a consistent time (9 - 11 am) each day of sampling, during a period no longer than two hours as suggested by Bird *et al*. (2013a). These measures reflect conditions during early October when the wetlands have been inundated for approximately two months.

Soil collection

Soil sampling took place between May and June 2016 during the dry phase of these wetlands and invertebrate sampling took place during September and October during the wet phase. To determine abundance and occurrence of dormant invertebrate species during the dry phase of temporary wetlands, soil sediment cores were taken when the wetlands were dry (Table 1). A standard soil auger with a sharpened iron cutting edge was used to core the first 10 – 15 cm of sediment in a wetland (Henri *et al*. 2014). This ensured that all dormant eggs within the soil were captured as 80% of species present in the soil are found within the top layers (first 15 cm). Nine soil sediment cores were taken in each wetland covering all the microhabitats and positions. Each wetland was divided into three sections (right edge, middle, left edge), with three samples per section combined together. At each sample site the GPS position, date and description of surrounds were recorded (Table 2). Each sample bag was stored open in a cool, temperature controlled room for approximately two months so that soil was completely dry before hatching assays began. Only soils from Kenilworth and Ottery were used for urbanisation experiments, while soils from all five sites (Kenilworth, Ottery, Youngsfield Army Base, Milnerton and Rondevlei) were used for fire experiments.

Table 1: Details of sampling time, number of wetlands sampled and wetland position for each temporary wetland area during 2016.

Site	**Date sampled**		**Number of wetlands sampled**		**Wetland ID (*Name and GPS position*)**
	Dry phase	*Wet phase*	*Dry phase*	*Wet phase*	
Kenilworth Racecourse	27/05/2016 12/06/2016	14/09/2016 26/10/2016	5	4	KRCW 1 -33°59'35"S,18°29'02"E KRCW 2 - 33° 59' 39"S 18° 29' 00"E KRCW 3a - 33° 59' 46"S 18° 29' 03"E KRCW 3b - 33° 59' 46"S 18° 29' 05"E KRCW 4 - 33°59'49"S 18°28'57"E KRCW 5 - 33°59'55"S 18°29'00"E
Ottery greenbelt	28/05/2016 12/06/2016	21/09/2016	5	4	OTT 1 – 34˚00.892'S 18˚30.479'E OTT 2 – 34˚00'56"S 18˚30'35"E OTT 3 34˚00'49"S 18˚30'41"E OTT 4 – 34˚00.803'S 18˚30.465'E OTT 5 – 35˚00.771'S 18˚30.405'E
Milnerton Racecourse	31/05/2016	-	2	-	MIL 1 - 33˚51'22"S 18˚30'44"E MIL 2 - 33˚51'25"S 18˚30'38"E
Youngsfield Army Base	31/05/2016	-	2	-	AB 1 - 34˚00'11"S 18˚29'20"E AB 2 - 34˚00'36"S 18˚29'24"E
Rondevlei Nature Reserve	31/05/2016	-	2	-	RON 1 - 34˚03'25"S 18˚30'12"E RON 2 - 34˚03'36"S 18˚29'41"E

Wet phase invertebrate sampling

Aquatic invertebrates were collected during periods of wetland inundation (Table 2). Sampling protocol followed methods established by Rundle *et al*. (2002) and modified by Bird & Day (2016). Invertebrates were sampled using a standard square-frame sweep net with a 235 mm mouth and 80 µm mesh. Wetlands were divided into three biotopes, areas with predominantly submerged vegetation, areas with predominantly emergent vegetation and open water (Bird & Day 2016). Each biotope was sampled three times in up to three separate areas of a wetland (dependent on size and biotope availability) to produce a total sampling effort of 27 sweeps per wetlands (Bird & Day 2016). Each biotope sample consists of pooled samples from nine individual sweeps. This ensures at least 60 – 80% of total invertebrate species will be consistently sampled (Rundle *et al*. 2002, Bird & Day 2016). Sweeps followed standard protocol for sampling temporary and permanent wetlands as indicated by several authors (Rundle *et al.* 2002, Bird & Day 2016). Each sweep consists of dragging the net down at a 45° angle until nearly touching the bottom, and pulling the net back up at a similar angle to create a sweep arc roughly one metre in length. Each biotope sample was then preserved and stored separately.

Samples were fixed on site in buffered 10% formalin which was replaced with 70% ethanol after 24 - 48 hours so that long-term preservation can occur. Macroinvertebrate and microcrustaceans were identified and counted using the sub-sampling procedure outlined by Bird & Day (2016). Large rare macroinvertebrates and microcrustacean taxa (large, easily visible specimens represented by <10 individuals per sample) were removed first by scanning the whole sample for five minutes. The sample was then emptied into a tray and divided into grid square cells of equal size which were randomly sampled to pick out ca. 200 macroinvertebrate organisms (recommended sub-sample size for wetlands as shown by King & Richardson, 2002).

Macroinvertebrate abundance was extrapolated by multiplying whole sample estimates by cell number to standardise final numbers. After macroinvertebrates were removed the sample was filled with 70% ethanol for microcrustacean identification.

Hatching experiments

Urban ecology

Hatching assays closely followed the standard methods outline elsewhere (Day *et al*. 2010, Liefferink *et al*. 2014). All hatching experiments took place in a temperature and light controlled room (12° C ± 1° C with a 12 hr light/12 hr dark light cycle) within the Department of Biological Sciences, University of Cape Town approximately two months after the soil was collected. Urbanisation experiment comprised of 5 - 6 replicates of soil for each individual wetland (total of 10 wetlands, 5 from Kenilworth and 5 from Ottery). Fire experiments comprised of 2 replicates of burned and 2 replicates of unburned soil for each wetland (total of 10 wetlands) In each case 1 cm of soil was placed in identical 2 litre plastic containers (30 cm by 20 cm) and filled with 4 cm of deionised water, sufficient to completely inundate the soil. Total numbers of invertebrate hatchlings were recorded every 24 hours per replicate by individually removing invertebrates every day with a wide-opening 2 ml pipette. This process was repeated until no hatchings were observed for 4 consecutive days (minimum of 26 days and maximum of 36 days). The live hatchlings that were removed during these experiments were placed in different containers containing inundated soil (non-wetland soil) to allow them to grow until they could be accurately identified. This aids with species level identification as in early larval stages invertebrate identification is increasingly difficult. Invertebrates within these growing containers were fed by adding yeast to the containers every 72 hours to avoid starvation and death of invertebrates.

The first set of hatching experiments took place over 26 days focusing on soil samples collected at Kenilworth (conserved) and Ottery (urban) to determine if any differences in diversity, abundance and hatching success. After the first wetting period, the soil was dried out by pouring off excess water and leaving soil to dry naturally for several days. Once the soil was completely dried, a second wetting occurred to determine if any viable dormant eggs required multiple wettings before hatching. Again the total hatchings per day per site, and species were recorded. The second set of experiments lasted 36 days, as no invertebrate hatched for 4 consecutive days after day 32.

Fire ecology

The combined sediment of nine auger samples for each wetland was divided into two equal samples for hatching experiments. One half of the samples were burned, in a controlled fire following the procedure outlined by Ripley *et al*. (2015). One hundred grams per sample (0.002 m^2) of dry hay (< 10% moisture) was used as fuel, as an average representative fuel load for Fynbos systems. This reflected the approximate amount of dry litter or dead material (< 6 mm) found within a Fynbos system (van Wilgen & Richardson 1985, van Wilgen *et al*. 1990, Scholes *et al*. 1996). The hay was ignited along one side in a wind, speed and direction, controlled environment to allow for an even burn lasting approximately 5 minutes (Ripley *et al*. 2015). Once burned the soil was placed in 2 litre plastic containers and left for one week before inundation. The second half of the samples remained unchanged acting as a control sample. Inundation and hatching experiment procedure remained the same as previously described for urban ecology hatching assays. Total hatchings per day per site and species compositions were recorded over 46 days. A second wetting did not occur due to limited time and resources for the study. After all experiments were terminated, individuals from growing containers were removed and preserved in ethanol to be identified at a later stage.

Identification of invertebrates

Microcrustacean identification followed the subsampling technique outlined by McCallum (1979) and Bird & Day (2016). Samples were drained of their ethanol and filled with tap water in a glass beaker, which was then homogenised by blowing bubbles into the beaker for 5 seconds through a 5 mm pipette. Once homogenised, 1 ml subsamples were removed using a pipette and placed in a Bogrow tray so that individuals can be enumerated under a dissecting microscope (Leica BS-3300, Magnification 7× ～ 46×). This was repeated until ca. 200 individuals were removed (approximately 20 ml). As suggested by Bird & Day (2016) the full range of taxa likely to be found should be seen within a 200 organism sub-sample size.

Macroinvertebrates and microcrustaceans were identified to the lowest possible taxonomic level (either a genus or species level where possible) using the 'Guides to Freshwater Invertebrates of Southern Africa' (see http://www2.wrc.org.za/Pages/DisplayItem.aspx?ItemID=7141&FromURL=%2FPages%2FAllKH.aspx%3F) and 'Freshwater Life' (Griffiths *et al*. 2015). Misidentification of individuals in early life stages is common therefore all identifications were made at adult life stage so to avoid misidentification. Ostracod, dipteran, and coleopteran individuals can only be identified to genus or family level. Specialised taxonomists were used to identify specific problematic taxa (e.g. Collembola and Coleoptera) as these taxa require certain expertise. A full list of macroinvertebrate and microcrustacean taxa recorded for each environment is presented in Appendix II.

Statistical analysis

Number of species hatching per day was compared between treatment (conserved vs. urban and burned vs. unburned) and significance was determined using a Mann-Whitney U test (Wilcox function) using R. Species accumulation curves, based on the number of new hatchlings per day, were determined using R (RStudio v0.98.507) software (using the 'plyr', 'vegan' and 'reshape2' packages) (Wickham 2007, Wickham 2011, R Core Team 2015, Oksanen *et al*. 2016). Liner mixed-effect models (LMER) and a post-hoc Tukey test using RStudio ('vegan' and 'lme4' packages) were used to test if there were significant difference in species richness and abundance between the different treatments (i.e. conserved vs. urban or burned vs. unburned), as well as between wettings assays and hatching phase (Bates *et al.* 2015, R Core Team 2015, Oksanen *et al*. 2016). Three univariate measures were used to test these differences; taxon richness (S), Shannon's diversity index (H') and Pielou's index of evenness (J'). Site replicates were used as a random effect variable to account for replication within wetlands. Significance level was set *a priori* at $\alpha = 0.05$, however was adjusted to account for multiple comparisons using Holm's sequential Bonferroni correction (Holm 1979).

Non-metric multidimensional scaling (nMDS) was used to graphically represent compositional differences between invertebrate communities between treatments and between hatching phase (referring to when a species hatched during a wetting) using the Bray-Curtis similarity coefficient in R (RStudio v0.98.507) (using the 'car', 'vegan' and 'shape' packages) (Fox & Weisberg 2011, Soetaert 2014, R Core Team 2015, Oksanen *et al*. 2016). Grouping within hatching phase (week at which a species may emerge from the soil after inundation) was also tested to determine if specific species hatched early (week one and two), intermediate (week three and four) or late (week five, six and seven) during the entire hatching period. nMDS plots allowed for a visual representation of the different treatments or representative wetland environments (i.e. burnt vs. unburnt, conserved vs. urban), if any, influencing invertebrate assemblages. Species abundance was square root transformed to account for overly abundant species for multivariate analysis only. Significant groupings were determined using a PERMANOVA using the 'Adonis' function in R (RStudio v0.98.507) (using the 'labdsv', 'vegan' and 'betapart' packages) (R Core Team 2015, Oksanen *et al*. 2016, Roberts 2016, Baselga *et al*. 2017). Wet phase univariate and multivariate data was analysed as described above for dry phase data. Here both the treatment (urban vs. conserved) and time (October vs. September) were analysed to see if there were any significant changes in species richness, abundance and composition between the sites over a period of two months.

Results

A total of 45 taxa were identified from both wet and dry phases across all temporary wetlands sampled (Appendix II). Of the total number of taxa collected, 19 were collected during dry phase hatching assays. Three taxa were exclusively collected in the dry phase and not collected during wet phase sampling, namely: *Dugesia* sp. (Turbellaria), *Mesamphisopus* sp. (Isopoda) and *Philonthus* sp. (Coleoptera). A total of 27 taxa were collected during wet phase sampling in the conserved (KRCA) site, while only 14 taxa were collected in the urban (Ottery) site. There were 21 taxa that were exclusive to the wet phase. However the conserved (KRCA) site saw unique a taxon (i.e. Amphipoda, *Paramelitidae*, *Paramelita pinnicarnis*) collected in the wet phase, which is a known endemic to Kenilworth Racecourse temporary wetlands (Prof

Charlie Griffiths, *pers comm.).* All figures and tables referred to are found at the end of the results section. A full species list is provided in Appendix II.

Environmental variables

Water chemistry measurements were only collected once-off, during October 2016, for wetlands within KRCA due to drought conditions causing the other wetland sites to dry before measurements could be taken. Measurements for KRCA were averaged across five temporary wetlands during October 2016. Average conductivity (µs) was measured as 2.3 (± 2.2 SD), average temperature (˚C) was 26.9 (± 4.5 SD), average pH was recorded as 5.9 (± 1.0 SD) and average dissolved oxygen (%) 31.9 (± 4.2 SD).

Hatching experiments

Urban ecology

A total of 18 different invertebrate taxa and one protozoan species (Kingdom: Protozoa, *Paramecium* sp.) hatched out during urban hatching assays. In the conserved sites (KRCA) 15 taxa emerged, while the urban sites (Ottery) only 11 different taxa emerged (Table 2) (Appendix II). Hatching assays showed that average number of hatchings per day (abundance, referred to as hatching success) was not significantly different between the different sites in either the 1st or 2nd wetting assay (Table 2, Figure 5 and 6). Nevertheless, hatching success was slightly higher in the urban-influenced site (Ottery), which was urban, compared to the conserved site (KRCA) for both the 1st and 2nd wetting assays (Figure 5 and 6). Hatching occurred immediately during the 1st wetting assay (Figure 5), while for the 2nd wetting assay hatching only started on the fourth day (Figure 6) for both conserved (KRCA) and urban (Ottery) sites. Also hatching success declined considerably earlier (day 12) in the 1st wetting assay (Figure 5) compared to the 2nd wetting assay (day 18) (Figure 6) for both conserved (KRCA) and urban (Ottery) sites. Species accumulation curves for the 1st wetting assay showed those urban sites plateaued first while for conserved sites were still increasing (Figure 7A). On the other hand, both sites levelled off at around the same time during the 2nd wetting assay (Figure 7B).

Table 2: Mann-Whitney U test results showing differences in average number of invertebrate individuals hatching for temporary wetlands in Conserved (Kenilworth) and Urban (Ottery) areas in Cape Sand Fynbos vegetation in the Western Cape, South Africa (2016), for two separate wetting assays. Significant differences marked with an asterix.

Wetting	**Days of Inundation**	**Average hatching abundance**	
		W	P
First	26	105	0.30
Second	36	177	0.64

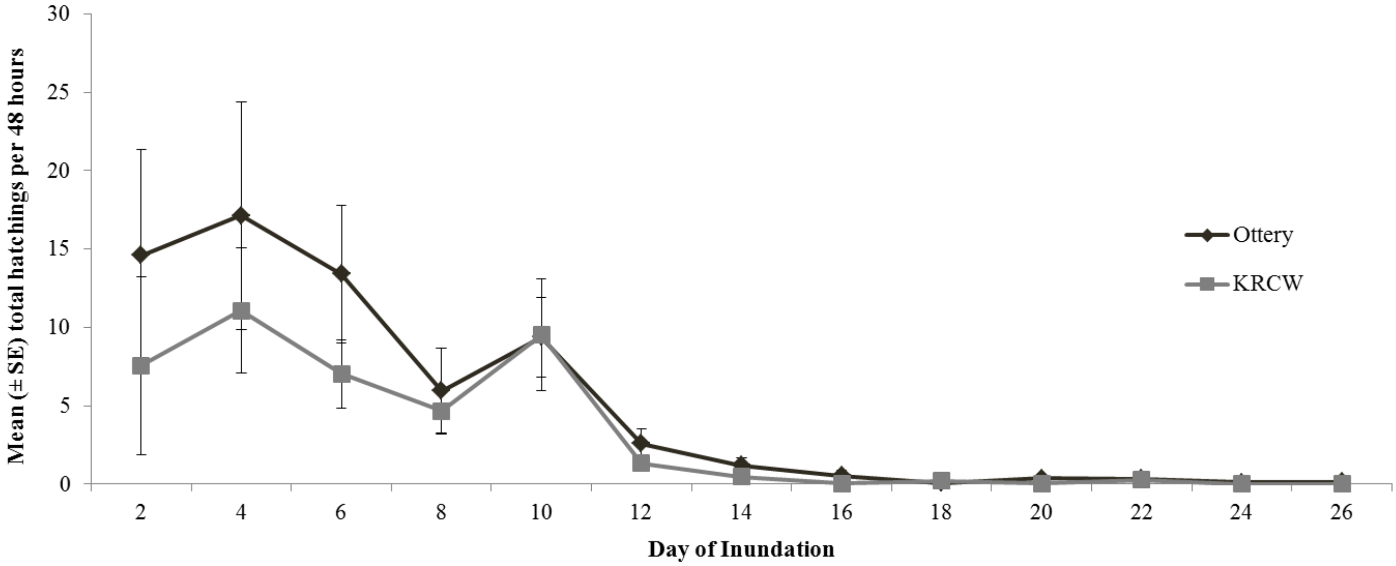

Figure 5: Average number of daily invertebrate hatchings between the Conserved (KRCA) and Urban (Ottery) sites during the first wetting assay.

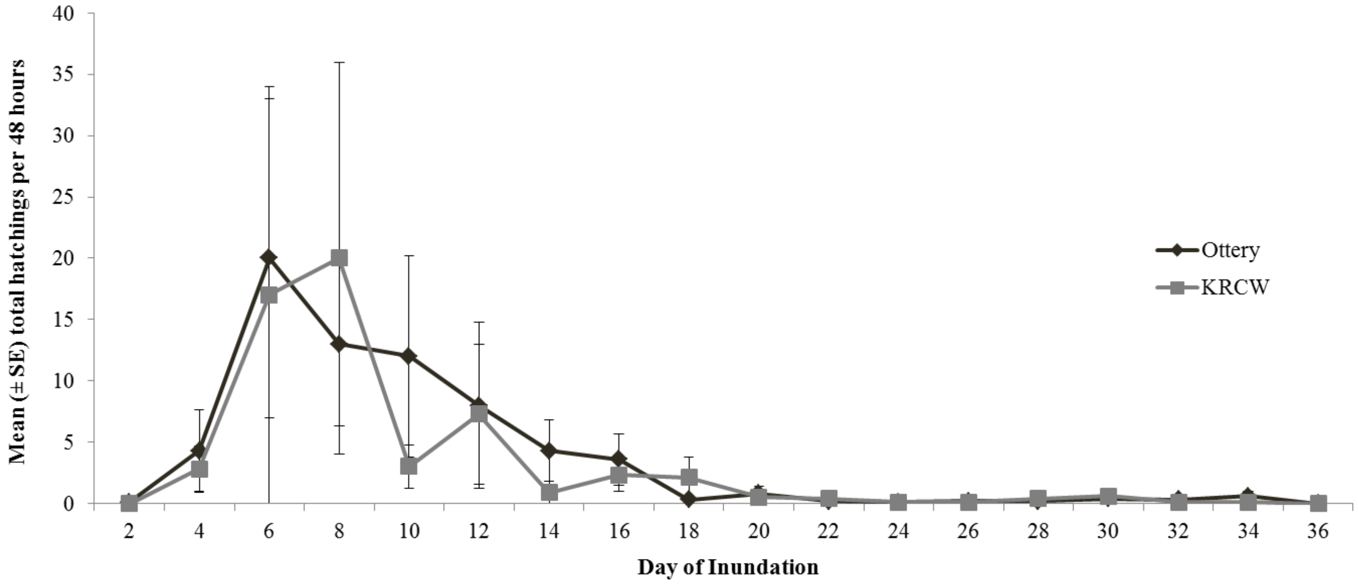

Figure 6: Average number of daily invertebrate hatchings between the Conserved (KRCA) and Urban (Ottery) sites during the second wetting assay.

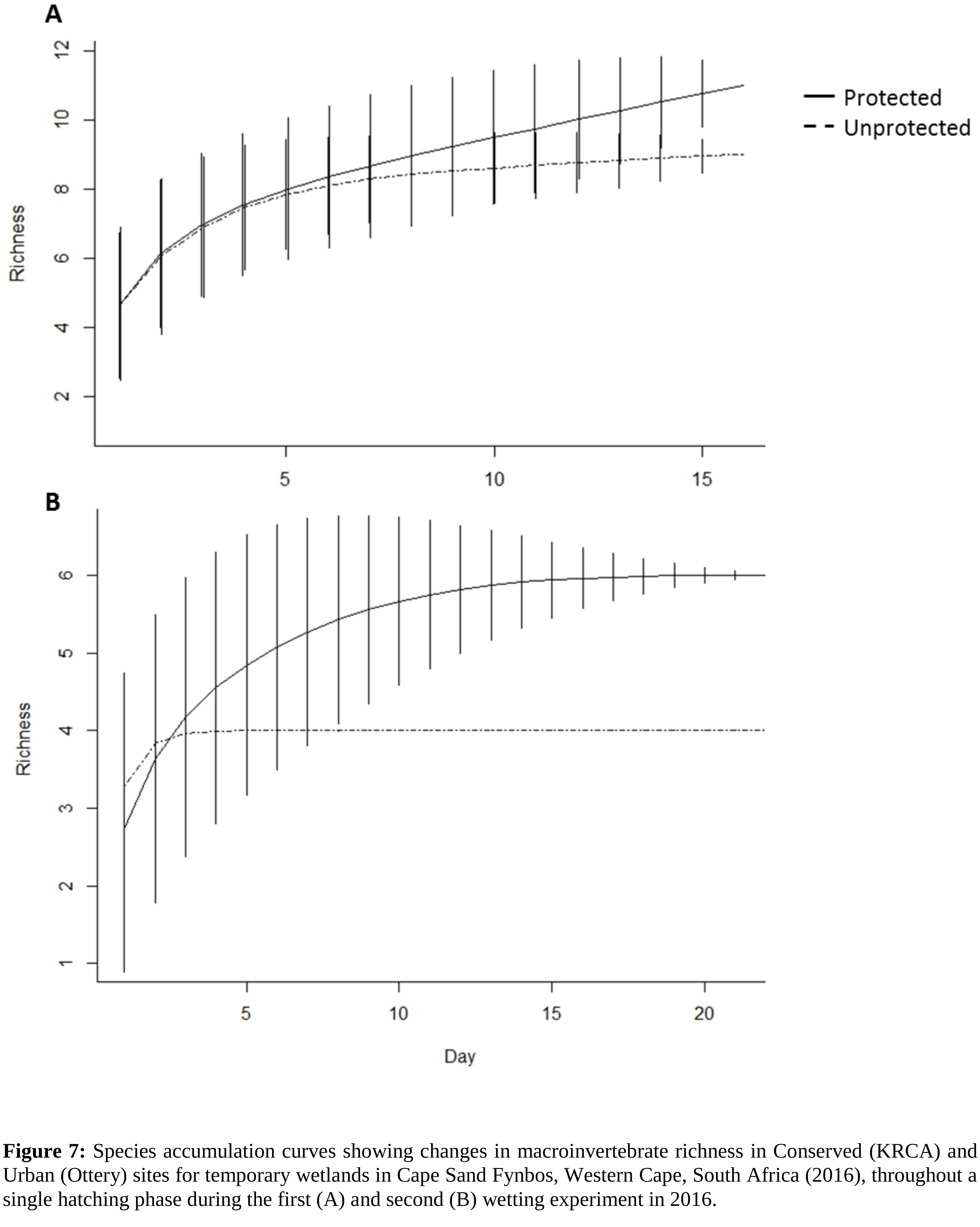

Figure 7: Species accumulation curves showing changes in macroinvertebrate richness in Conserved (KRCA) and Urban (Ottery) sites for temporary wetlands in Cape Sand Fynbos, Western Cape, South Africa (2016), throughout a single hatching phase during the first (A) and second (B) wetting experiment in 2016.

Taxon richness (number of unique groups) from dry phase hatching experiments was not significantly different between the conserved (KRCA) and urban (Ottery Public Park) sites during the 1st wetting assay (Table 3 and Figure 8A). Shannon Diversity and Pielou's Evenness were also not significantly different between the two sites (Table 3 and Figure 8). These patterns were reflected in the 2nd wetting assays for both the conserved and urban sites (Table 3 and Figure 9). Taxon richness was not significantly different between the 1st and 2nd wettings assays for the conserved sites in the dry phase (Table 3), however was significantly different in the urban sites (Table 3). The conserved sites had significantly higher diversity and evenness in the 1st wetting assay compared to the 2nd (lmer, -0.81 (± 0.22), Z = -3.61, p = 0.0002 and lmer, -0.37 (± 0.12), Z = -2.94, p = 0.003 respectively) (Table 3). This pattern was reflected in the urban sites where diversity and evenness were significantly higher in the 1st wetting assay compared to the 2nd wetting assay (lmer, -0.59 (± 0.18), Z = -3.24, p = 0.001 and lmer, -0.26 (± 0.11), Z = -2.26, p = 0.02 respectively) (Table 3).

Table 3: LMER results showing differences in taxon richness, Shannon-Weiner diversity and Pielou's Evenness for temporary wetlands in Conserved (Kenilworth) and Urban (Ottery) areas in Cape Sand Fynbos vegetation in the Western Cape, South Africa (2016), over different seasonal phases. Significant differences marked with an asterix. Dry phase = conserved versus urban hatching assays, wetting assay = 1st versus 2nd wetting assay, wet phase month = September versus October in conserved wetlands.

Predictor	**n**	**Taxon Richness**			**Shannon Diversity (H')**			**Pielou's Evenness (J')**		
		Estimate (± SE)	Z	P	Estimate (± SE)	Z	P	Estimate (± SE)	Z	P
1st wetting assay	11	0.50 (± 0.63)	0.78	0.43	0.02 (± 0.17)	0.15	0.88	-0.07 (± 0.14)	-0.54	0.58
2nd wetting assay	11	0.48 (± 0.61)	0.76	0.48	0.04 (± 0.19)	0.17	0.86	-0.06 (± 0.12)	-0.50	0.61
1st vs 2nd wetting (conserved)	12	-1.33 (± 1.02)	-1.30	0.19	-0.81 (± 0.22)	-3.61	0.0002*	-0.37 (± 0.12)	-2.94	0.003*
1st vs 2nd wetting (urban)	10	-1.80 (± 0.37)	-4.81	< 0.001*	-0.59 (± 0.18)	-3.24	0.001*	-0.26 (± 0.11)	-2.26	0.02*
Wet Phase: Treatment (conserved vs. urban)	13	-3.25 (± 1.76)	-1.83	0.06	-0.43 (± 0.24)	-1.74	0.08	-0.07 (± 0.08)	-0.98	0.32
Wet phase: Month (conserved)	5	-1.25 (± 1.76)	-0.70	0.48	-0.21 (± 0.24)	-0.86	0.39	-0.006 (± 0.03)	-0.21	0.83
Wet vs. Dry (conserved)	18	7.49 (± 0.99)	7.35	< 0.001*	1.08 (± 0.22)	4.91	< 0.001*	0.15 (± 0.11)	1.34	0.17
Wet vs. Dry (urban)	15	3.25 (± 0.82)	3.94	< 0.001*	0.52 (± 0.25)	2.07	0.03*	0.11 (± 0.13)	0.90	0.36

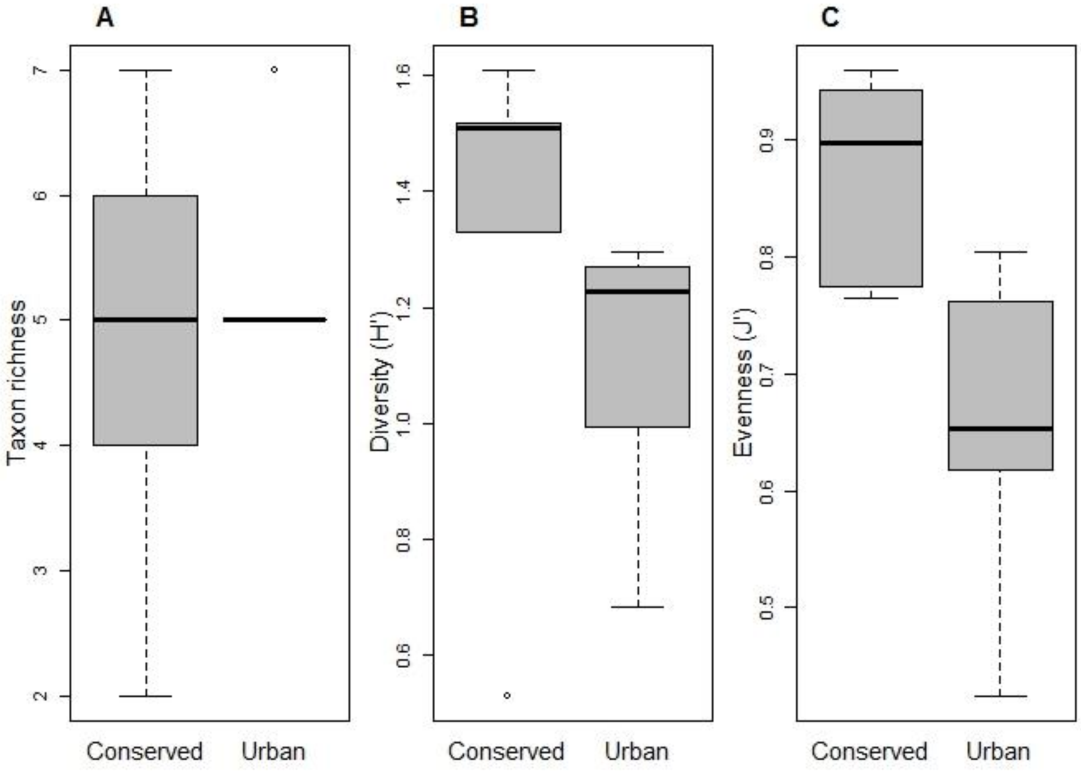

Figure 8: Taxon richness (A), Shannon-Weiner diversity (B) and Pielou's Evenness (C) of invertebrates hatched from dried soil sediments of temporary wetlands in the Cape flats region in the 1st wetting assay. Boxplots represent median values with interquartile range. Whiskers represent maximum and minimum values.

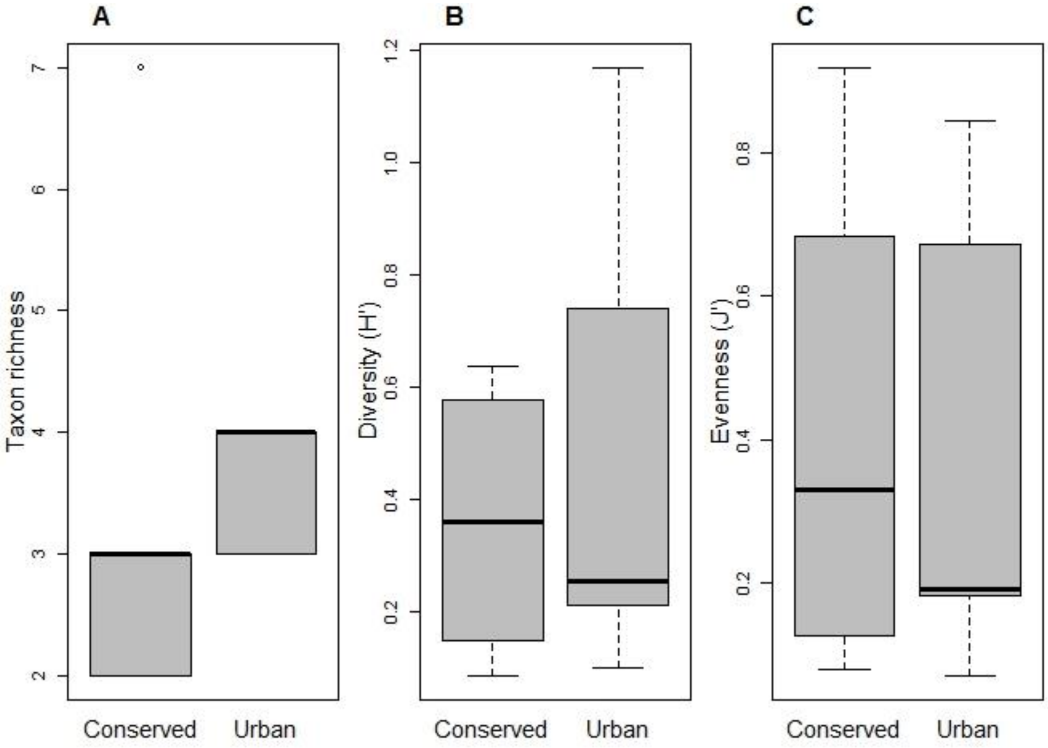

Figure 9: Taxon richness (A), Shannon-Weiner diversity (B) and Pielou's Evenness (C) of invertebrates hatched from dried soil sediments of temporary wetlands in the Cape flats region in the 2nd wetting assay. Boxplots represent median values with interquartile range. Whiskers represent maximum and minimum values.

Even though not statistically significant, conserved sites had higher taxon richness (lmer, $Z = -1.83$, $p = 0.06$) and diversity (lmer, $Z = -1.75$, $p = 0.08$) compared to the urban sites during the wet phase (Figure 10). Taxon richness was significantly higher in the wet phase compared to dry phase for both the conserved (lmer, $Z = 7.53$, $p < 0.001$) and urban sites (lmer, $Z = 3.94$, $p < 0.001$). Diversity mirrored this pattern, while evenness showed no difference for both the conserved and urban sites (Table 3).

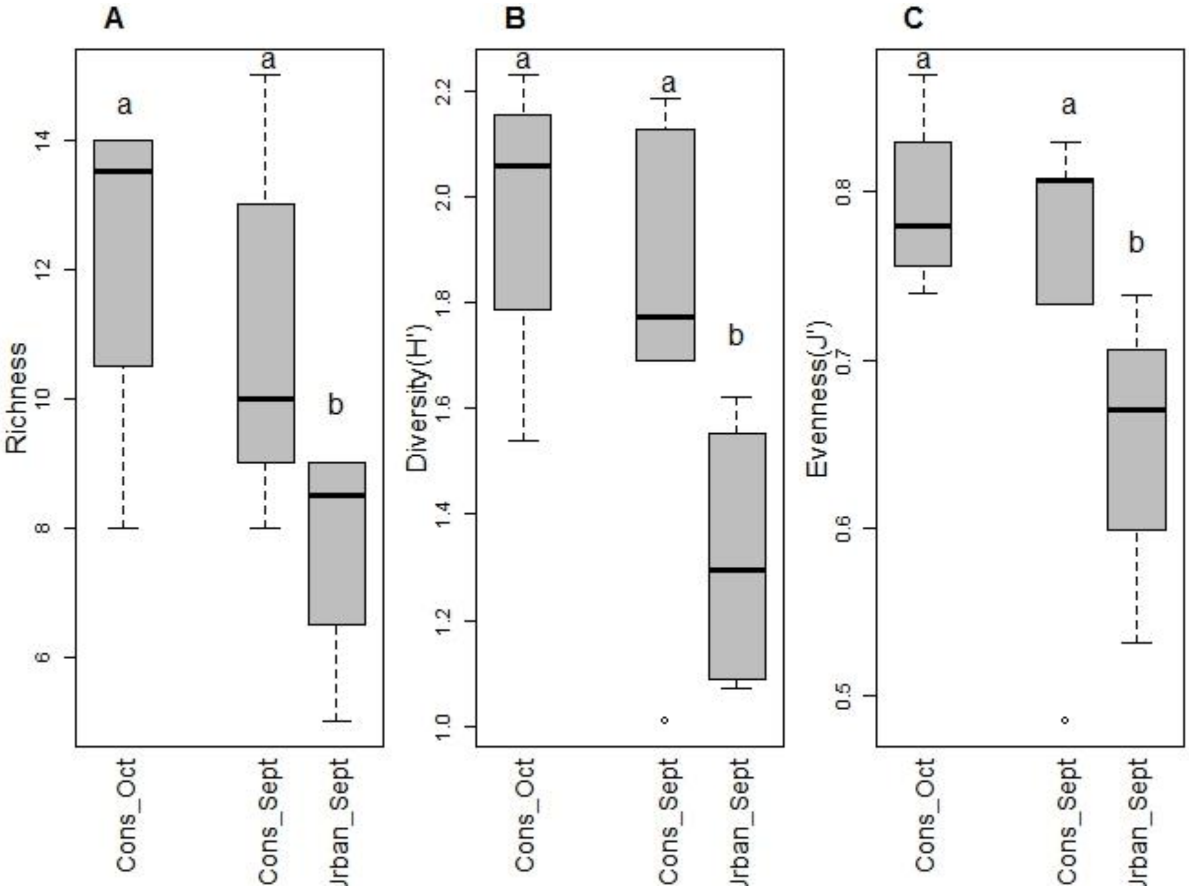

Figure 10: Taxon richness (A), Shannon-Weiner diversity (B) and Pielou's Evenness (C) of invertebrates collected during the wet phase of temporary wetlands in the Cape flats region. Cons_Oct denotes conserved sites sampled in October, cons_sept is Conserved sites sampled in September and Urban_sept is urban sites sampled in September. Boxplots represent median values with interquartile range. Whiskers represent maximum and minimum values. Sites sharing a letter do not differ significantly (Tukey's test, $P < 0.05$).

Table 4: LMER results showing differences in taxon richness, Shannon-Weiner diversity and Pielou's Evenness for temporary wetlands in Conserved (Kenilworth) and Urban (Ottery) areas in Cape Sand Fynbos vegetation in the Western Cape, South Africa (2016), during the dry phase, comparing different successional hatching times. Significant differences marked with an asterix. E – I = Early phase – Intermediate phase, E – L = Early phase – Late pahse, and L – I = Late phase – Intermediate Phase.

Predictor	**n**	**Taxon Richness**			**Shannon Diversity (H')**			**Pielou's Evenness (J')**		
		Estimate (± SE)	Z	P	Estimate (± SE)	Z	P	Estimate (± SE)	Z	P
Conserved 1st										
E – I	18	-1.16 (± 0.71)	-1.62	0.31	-0.31 (± 0.24)	-1.29	0.58	-0.22 (± 0.19)	-1.16	0.73
E – L		-2.32 (± 0.81)	-2.84	0.01*	-0.79 (± 0.27)	-2.84	0.01*	-0.48 (± 0.22)	-2.19	0.08
L - I		-1.15 (± 0.81)	-1.41	0.47	-0.47 (± 0.27)	-1.71	0.25	-0.26 (± 0.22)	-1.18	0.70
Conserved 2nd										
E – I	18	0.52 (± 0.50)	1.04	0.89	0.23 (± 0.29)	0.79	1.00	0.13 (± 0.25)	0.51	1.00
E – L		0.80 (± 0.51)	1.56	0.35	0.45 (± 0.30)	1.48	0.41	0.33 (± 0.26)	1.26	0.62
L - I		0.27 (± 0.50)	0.55	1.00	0.22 (± 0.29)	0.75	1.00	0.20 (± 0.25)	0.80	1.00
Urban 1st										
E – I	15	-0.80 (± 0.87)	-0.91	1.00	-0.06 (± 0.20)	-0.32	1.00	0.15 (± 0.18)	0.83	1.00
E – L		-2.89 (± 1.01)	-2.84	0.01*	-0.72 (± 0.24)	-3.02	0.007*	-0.35 (± 0.21)	-1.67	0.28
L - I		-2.09 (± 1.01)	-2.05	0.12	-0.66 (± 0.24)	-2.74	0.01*	-0.50 (± 0.21)	-2.39	0.05*
Urban 2nd										
E – I	15	1.60 (± 0.45)	3.52	0.001*	0.35 (± 0.19)	1.85	0.19	0.24 (± 0.17)	1.38	0.50
E – L		0.60 (± 0.45)	1.32	0.56	0.46 (± 0.19)	2.40	0.04*	0.57 (± 0.27)	3.28	0.003*
L - I		-1.00 (± 0.45)	-2.20	0.08	0.10 (± 0.19)	0.55	1.00	0.33 (± 0.17)	1.90	0.17

In terms of hatching phases in the conserved sites, taxon richness and diversity were significantly higher in the early vs late phases during the 1st wetting assay (Table 4 and Figure 11). Evenness was, however, not significantly different between any of the hatching phases. During the 2nd wetting assay no diversity measure showed significant differences (Figure 12). For urban sites, taxon richness and diversity were significant higher in the early phase compared to the late phase during the 1st wetting assay (lmer, Z = -2.84, p = 0.01 and lmer, Z = -3.02, p = 0.007 respectively) (Figure 11). Also, intermediate and late phases were significantly different in terms of diversity (lmer, Z = -2.74, p = 0.01) and richness (lmer, Z = -2.39, p = 0.05). For the 2nd wetting, taxon richness was significantly lower in the early phase compared to the intermediate phase for urban sites (lmer, Z = 3.52, p = 0.001) (Figure 13). Richness was not significantly different between early and late phases and intermediate and late phase for urban sites in the 2nd wetting assay (Table 4 and Figure 14). Diversity and evenness was only significantly different between early and late phases for urban sites in the 2nd wetting assay (lmer, Z = 2.40, p = 0.04 and lmer, Z = 3.28, p = 0.003 respectively).

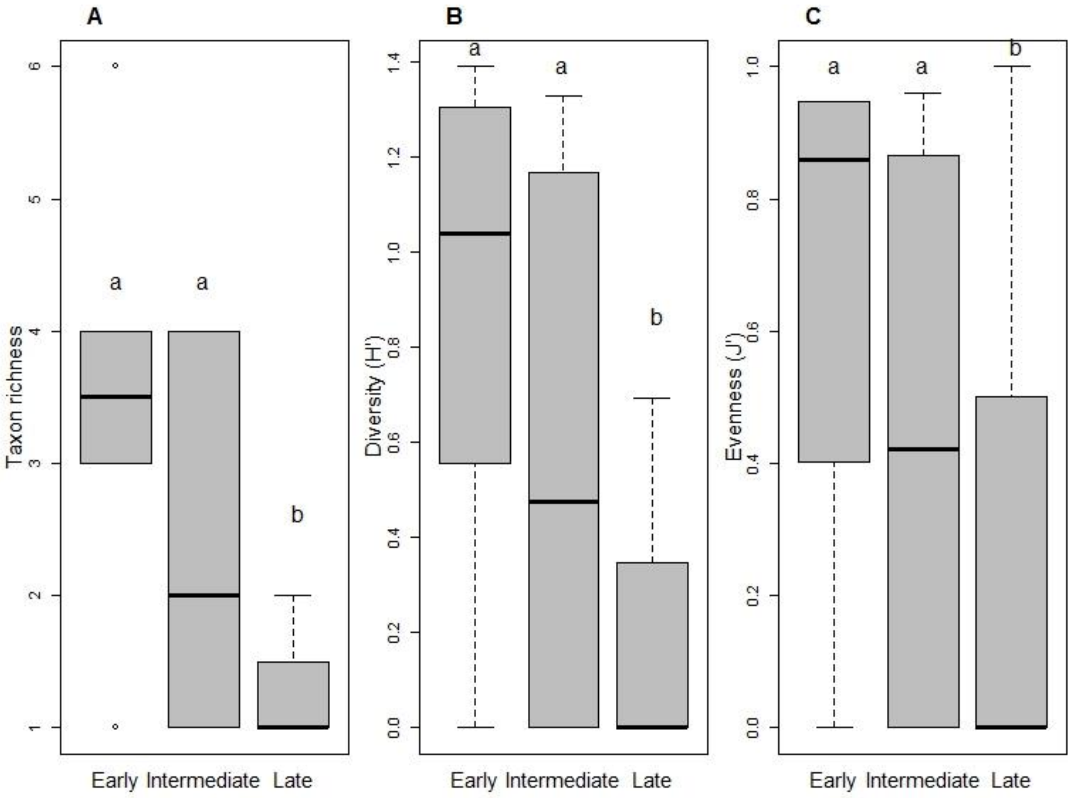

Figure 11: Temporal variation of taxon richness (A), Shannon-Weiner diversity (B) and Pielou's Evenness (C) of invertebrates hatched from dried soil sediments of temporary wetlands in the Cape flats region based on the 1st wetting assay of conserved sites. Boxplots represent median values with interquartile range. Whiskers represent maximum and minimum values. Sites sharing a letter do not differ significantly (Tukey's test, $P < 0.05$).

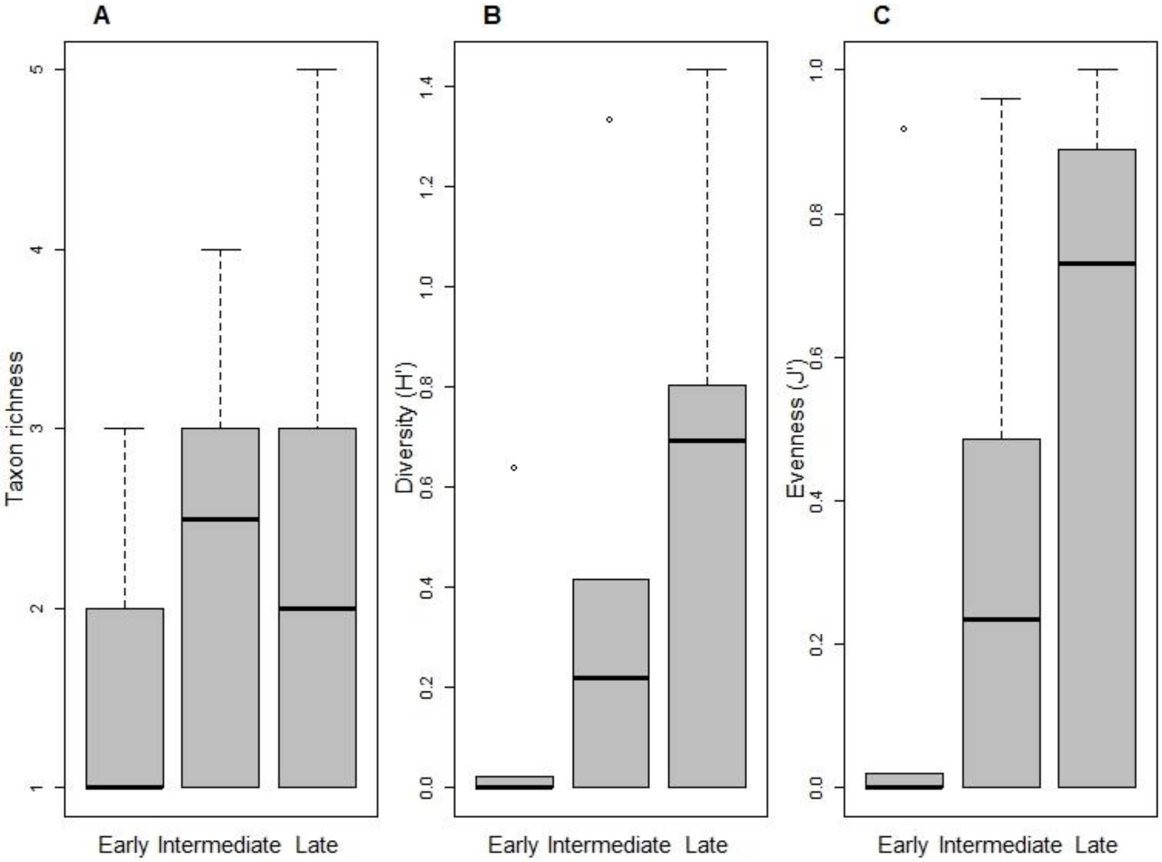

Figure 12: Temporal variation of taxon richness (A), Shannon-Weiner diversity (B) and Pielou's Evenness (C) of invertebrates hatched from dried soil sediments of temporary wetlands in the Cape flats region based on the 2nd wetting assay of conserved sites. Boxplots represent median values with interquartile range. Whiskers represent maximum and minimum values.

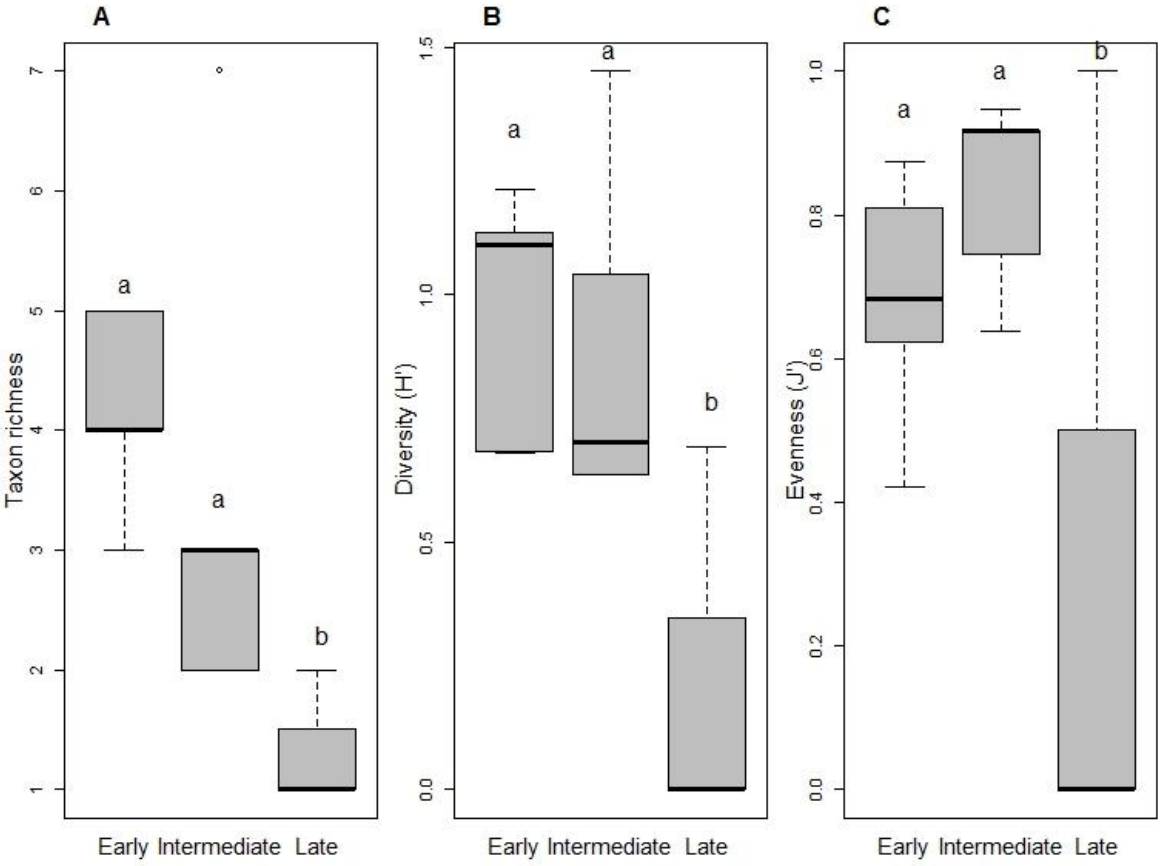

Figure 13: Temporal variation of taxon richness (A), Shannon-Weiner diversity (B) and Pielou's Evenness (C) of invertebrates hatched, first hatching assay, from dried soil sediments of temporary wetlands in the Cape flats region based on the 1st wetting assay of urban sites. Boxplots represent median values with interquartile range. Whiskers represent maximum and minimum values. Sites sharing a letter do not differ significantly (Tukey's test, $P < 0.05$).

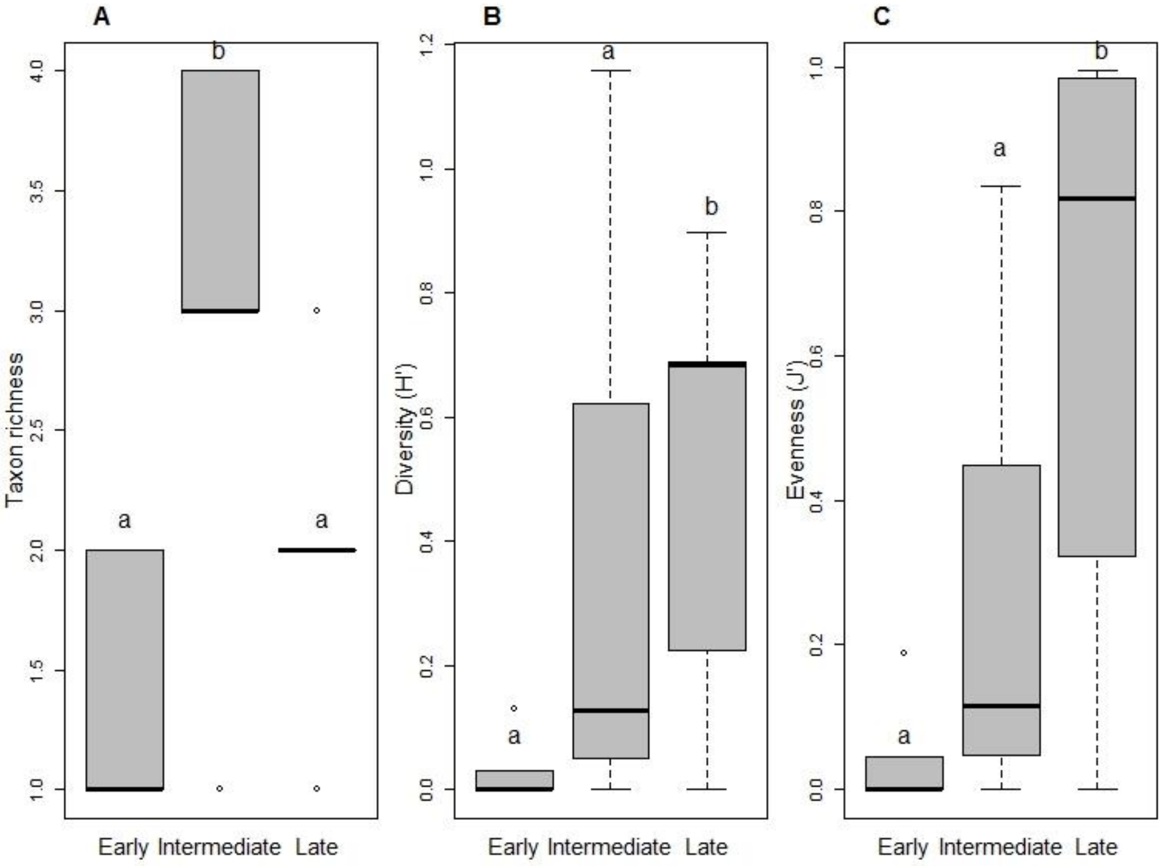

Figure 14: Temporal variation of taxon richness (A), Shannon-Weiner diversity (B) and Pielou's Evenness (C) of invertebrates hatched from dried soil sediments of temporary wetlands in the Cape flats region based the 2nd wetting assay of urban sites. Boxplots represent median values with interquartile range. Whiskers represent maximum and minimum values. Sites sharing a letter do not differ significantly (Tukey's test, $P < 0.05$).

For community composition analyses different hatching assays treatments did not have a clear differentiation between conserved and urban sites (Figure 15A). There was, however, a clear differentiation in invertebrate community composition between 1st and 2nd wetting assays ('adonis', $F = 13.34$, $p < 0.001$). For both treatments and wettings there was significant grouping of taxa at different stages of the hatching succession, i.e. early, intermediate and late (Figure 15B and Table 5). Taxa that hatched within the first two weeks of inundation (Collembola, *Paramecium*, Hydracarina) were considered early phase hatchings, taxa which hatched within week three and four were intermediate phase (Cladoceran and Copepods) and week five, six and seven were considered late phase (Ostracods). Late phase taxa (i.e. Ostracods) were clearly differentiated from early and intermediate taxa. The 2^{nd} wetting assay was dominated by mostly intermediate phase taxa (Figure 15B). A full list of taxa for each hatching phase is provided in Appendix III.

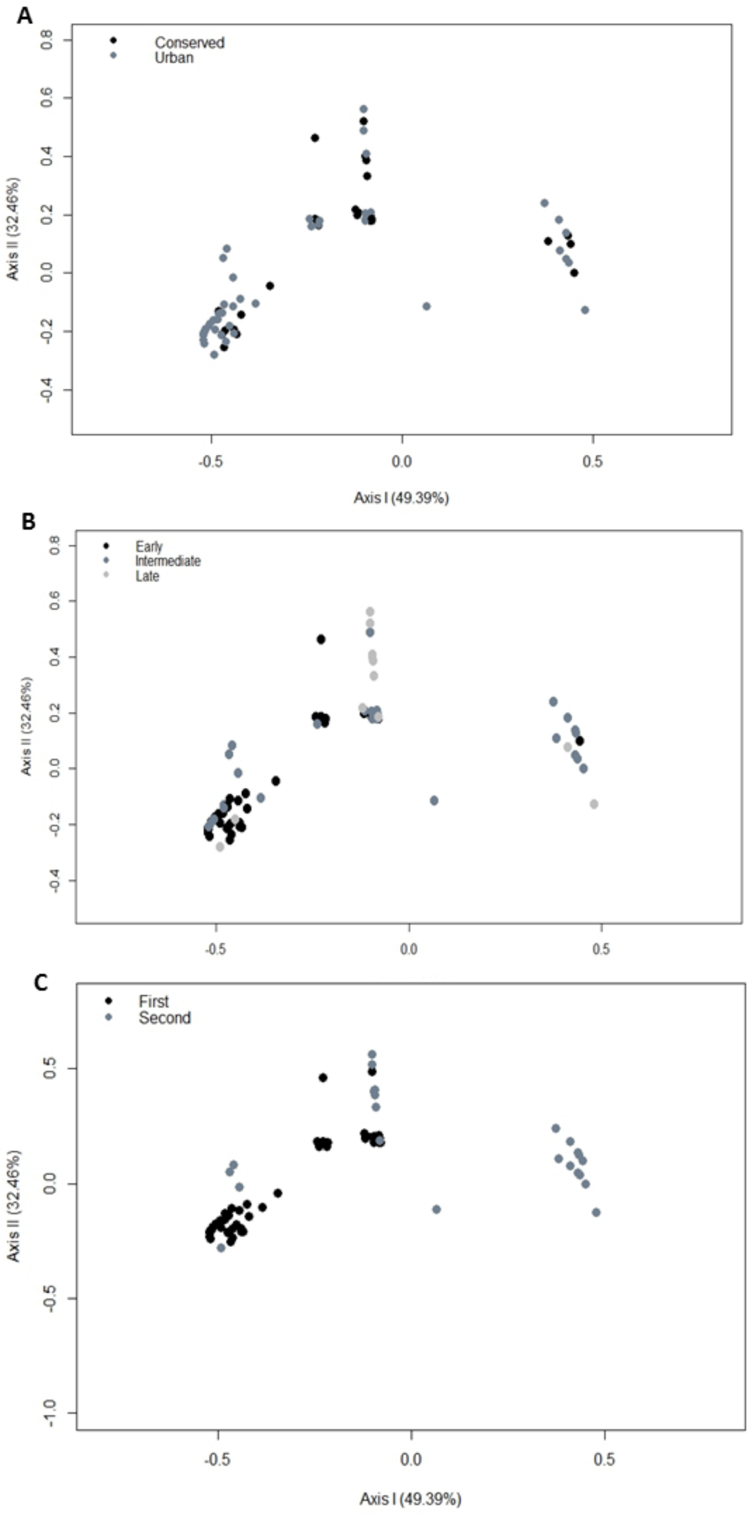

Figure 15: Non-metric multidimensional scaling (nMDS) plot showing the species association over three hatching phase comparing treatment (A, where the grey ellipse = Urban and the black ellipse = Conserved) and wetting (B, where the grey ellipse = 1^{st} wetting and the black ellipse = 2^{nd} wetting).

There was significant differentiation between invertebrate assemblages between conserved and urban sites in the wet phase (‘adonis’, F = 2.54, $p < 0.001$) (Figure 16 and Table 5). There was also no significant differentiation between the September and October samples within the conserved site (Figure 16 and Table 5).

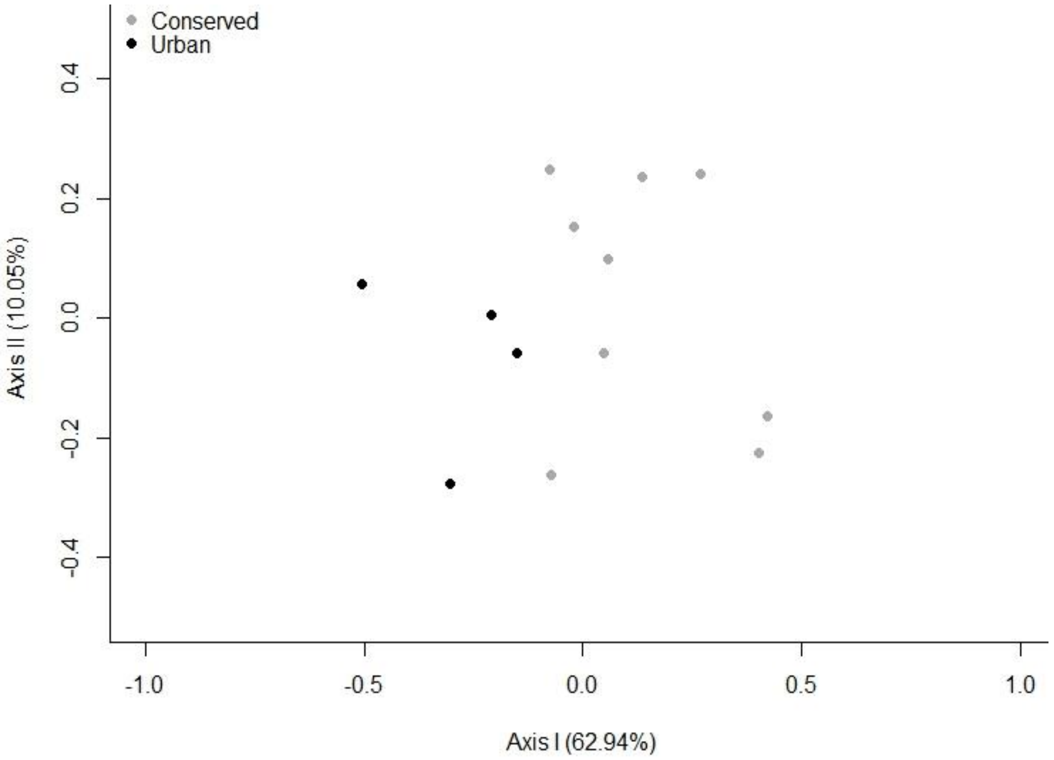

Figure 16: Non-metric multidimensional scaling (nMDS) plot showing the species association between Conserved (grey point) and Urban (black point) sites over two months (triangle = September, circle = October) in the wet seasonal phase of selected temporary wetlands in 2016.

Table 5: PERMANOVA and Adonis results for nMDS plots comparing significant groupings between conserved and urban sites during both wet and dry seasonal phases. Significant differences are marked with an asterix.

Phase	Predictors	Assemblage composition (response matrices)	
		F	P
Dry	Treatment	1.25	0.28
	Wetting	13.34	0.001*
	Hatching Phase	9.82	0.001*
Wet	Treatment	2.54	0.001*
	Month	1.35	0.19

Fire ecology

A total of 15 invertebrate taxa hatched during fire hatching assays, along with one protozoan species (Kingdom: Protozoa, *Paramecium* sp.) (Appendix IV). The unburned samples had 16 taxa hatch, while the burned samples had only 6 different taxa. There were no unique taxa emerging in the burned samples that did not emerge in the unburned samples. Unburned and burned samples did not have significantly different hatchings per day (Table 6). However, there was a slight increase in hatchings in the unburned samples compared to the burned samples in the first 10 days. After the 14th day hatching abundance increased in burned samples compared to the unburned samples (Figure 17). Increases in hatchings in the burned samples from day 16 appear to be the result of a single *Paramecium* species. Hatching success declined at similar times (day 26) for both burned and unburned samples (Figure 17). Species accumulation curves show that both burned and unburned sample curves did not plateau during the inundation period for fire hatching assays (Figure 18). There is still a slight increase after 25 days (for burned samples) and 30 days (for unburned samples) (Figure 18) which continues until the experiment was terminated (46 days).

Table 6: Mann-Whitney U test results between burned and unburned samples in average number of individuals hatching from temporary wetlands in Cape Sand Fynbos vegetation in the Western Cape, South Africa (2016). Data was testing with *Paramecium* sp. abundance and without.

	Days of Inundation	**Average hatching abundance**	
		W	P
With *Paramecium*	44	307	0.13
Without *Paramecium*	44	211	0.47

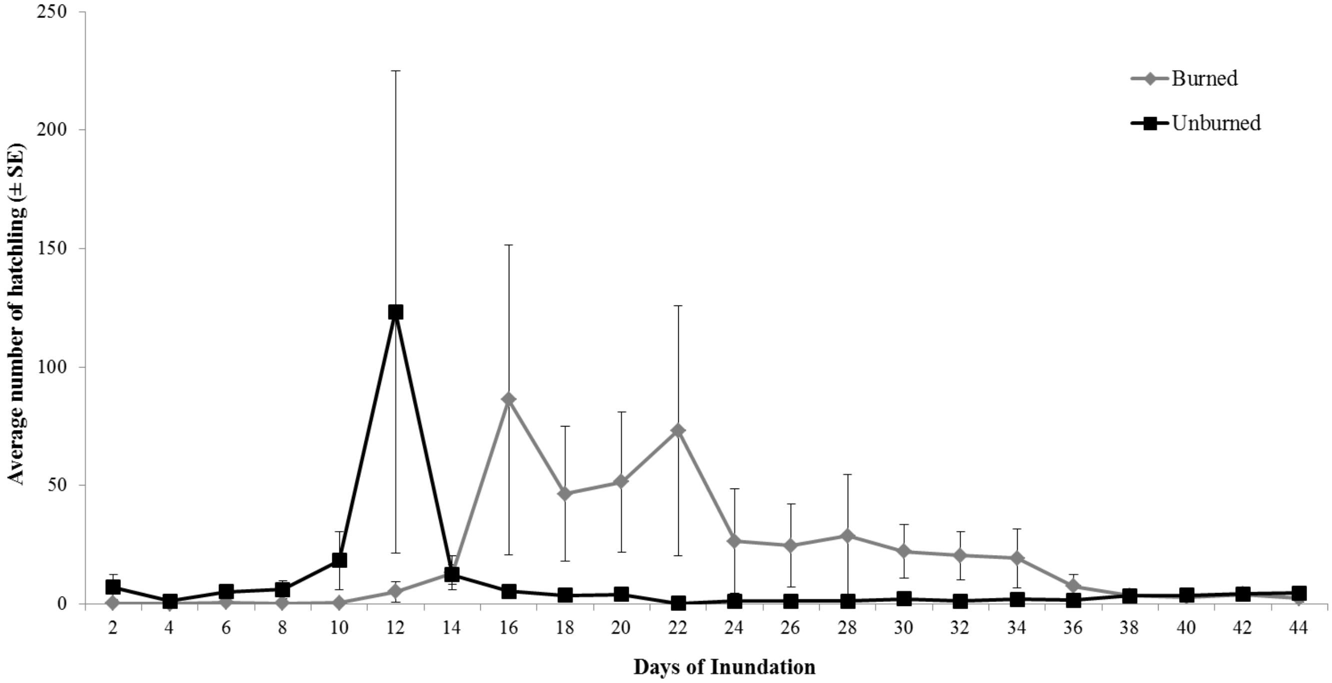

Figure 17: Average number of daily invertebrate hatchings between burned and unburned samples takin from five temporary wetland areas in the Cape Sand Fynbos region, Western Cape in 2016.

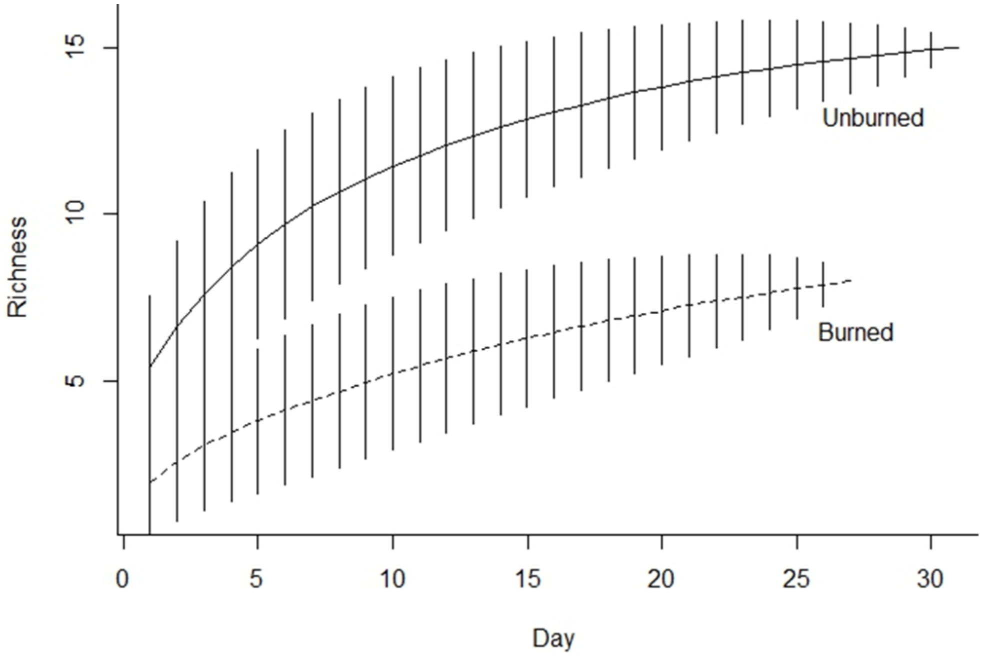

Figure 18: Species accumulation curve showing the number of species hatching over a single hatching period comparing burned and unburned treatments.

Taxon richness significantly decreased in burned samples compared to unburned samples (lmer, 3.15 (± 0.46), Z = 6.81, p = < 0.001) (Table 7 and Figure 19). Similar patterns were observed for diversity (H’) (lmer, 0.71 (± 0.13), Z = 5.44, p = < 0.001) and evenness (J’) (lmer, 0.42 (± 0.09), Z = 4.75, p = < 0.001), as well (Table 7 and Figure 19). While the range of taxa was similar between burned and unburned sites the total number of taxa found in the unburned sites was almost double those found in the burned site (Figure 19). Burned sites were dominated by a single abundant species for the majority of the hatching time, while unburned sites had a wider range of unique taxa emerging (Table 7).

Table 7: LMER results showing differences in taxon richness, Shannon-Weiner diversity and Pielou's Evenness for temporary wetlands in Cape Sand Fynbos vegetation in the Western Cape, South Africa (2016), comparing burned and unburned samples. Significant differences marked with an asterix.

Predictor	**n**	**Taxon Richness**			**Shannon Diversity (H')**			**Pielou's Evenness (J')**		
		Estimate (± SE)	Z	P	Estimate (± SE)	Z	P	Estimate (± SE)	Z	P
Burned vs. Unburned (dry phase)	40	3.15 (± 0.46)	6.81	< 0.001*	0.71 (± 0.13)	5.44	< 0.001*	0.42 (± 0.09)	4.75	< 0.001*

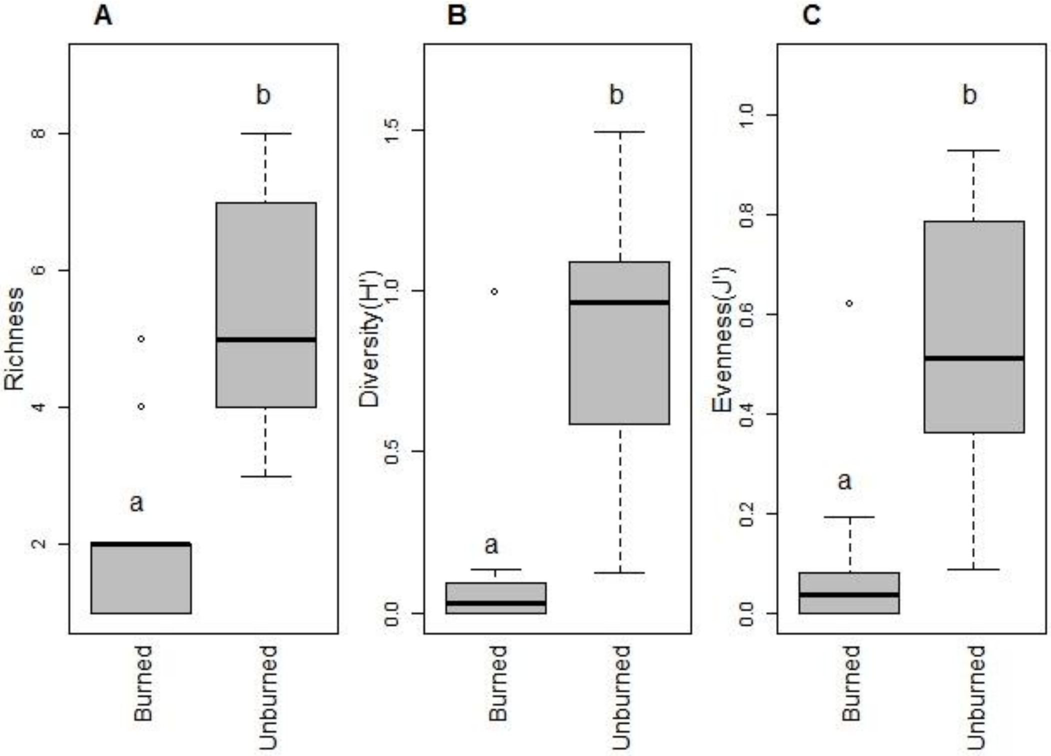

Figure 19: Taxon richness (A), Shannon-Weiner diversity (B), and Pielou's Evenness (C) of invertebrates hatched from dried soil sediments of temporary wetlands in the Cape Sand Fynbos region. Boxplots represent median values with interquartile range. Whiskers represent maximum and minimum values. Sites sharing a letter do not differ significantly (Tukey's test, P < 005).

Taxon richness was only significantly higher in the early phase compared to the late phase for unburned samples (lmer, -1.40 (± 0.45), Z = -3.10, p = 0.005) (Table 8 and Figure 20A). There was no significant difference in taxon richness between early and intermediate phases and intermediate and late phases for unburned samples (Table 8 and Figure 20A). There was no significant difference in diversity and richness between any hatching phase for unburned samples (Table 8 and Figure 20). Burned samples were not significantly different between any hatching phases for taxon richness, diversity and evenness (Table 8 and Figure 21).

Table 8: LMER results showing differences in taxon richness, Shannon-Weiner diversity and Pielou's Evenness for temporary wetlands in Cape Sand Fynbos vegetation in the Western Cape, South Africa (2016), comparing different successional hatching times in burned and unburned samples. Significant differences marked with an asterix. E – I = Early phase – Intermediate phase, E – L = Early phase – Late phase, and L – I = Late phase – Intermediate Phase.

Predictor	**n**	**Taxon Richness**			**Shannon Diversity (H')**			**Pielou's Evenness (J')**		
		Estimate (± SE)	Z	P	Estimate (± SE)	Z	P	Estimate (± SE)	Z	P
Unburned										
E – I	20	-0.47 (± 0.46)	-1.01	0.93	0.04 (± 0.21)	0.21	1.00	0.06 (± 0.17)	0.36	1.00
E – L		-1.40 (± 0.45)	-3.10	0.005*	-0.18 (± 0.20)	-0.87	1.00	-0.01 (± 0.16)	-0.08	1.00
L - I		-0.92 (± 0.46)	-1.98	0.14	-0.22 (± 0.21)	-1.06	0.85	-0.07 (± 0.17)	-0.44	1.00
Burned										
E – I	20	-0.54 (± 0.42)	-1.28	0.59	-0.27 (± 0.18)	-1.49	0.40	-0.17 (± 0.18)	-0.91	1.00
E – L		-0.72 (± 0.40)	-1.78	0.22	-0.29 (± 0.17)	-1.69	0.27	-0.20 (± 0.17)	-1.13	0.77
L - I		-0.17 (± 0.38)	-0.45	1.00	-0.02 (± 0.16)	-0.12	1.00	-0.02 (± 0.17)	-0.17	1.00

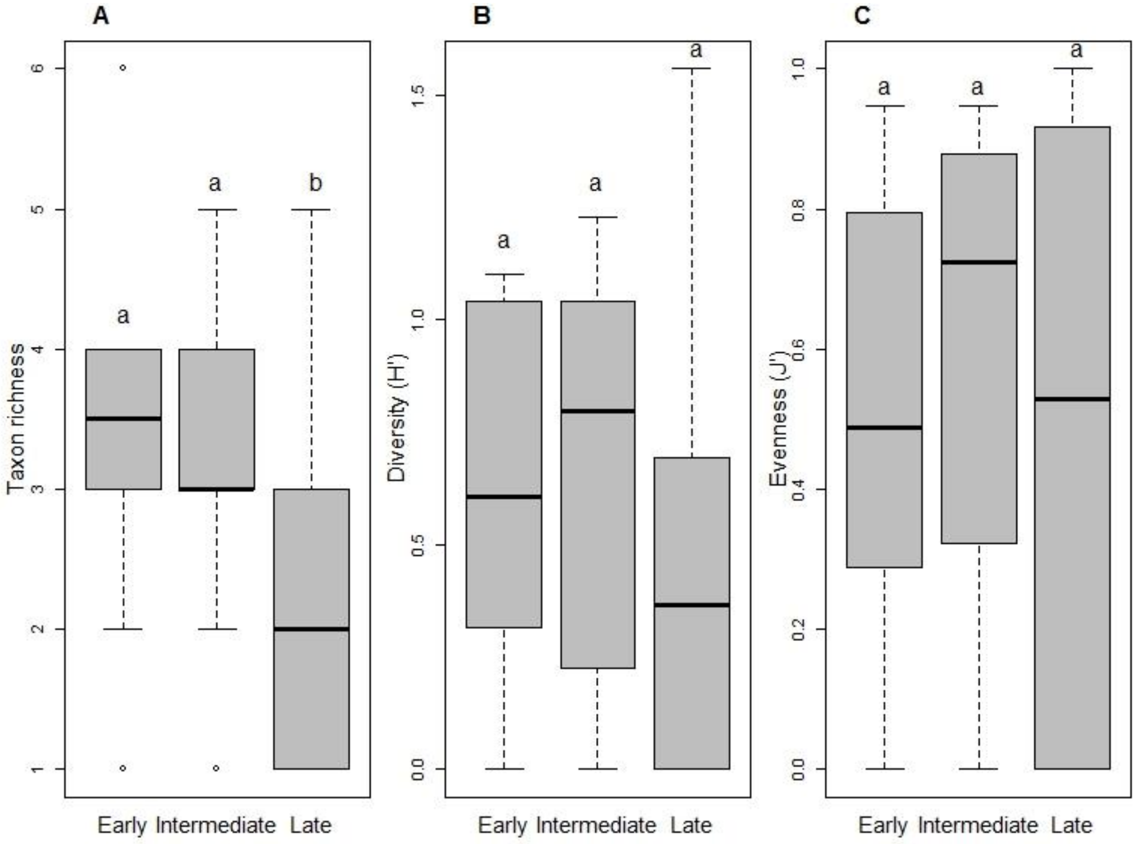

Figure 20: Taxon richness (A), Shannon-Weiner diversity (B), and Pielou's Evenness (C) of invertebrates hatched from dried soil sediments of temporary wetlands in the Cape Sand Fynbos region. Boxplots represent median values with interquartile range. Whiskers represent maximum and minimum values. Sites sharing a letter do not differ significantly (Tukey's test, P < 005).

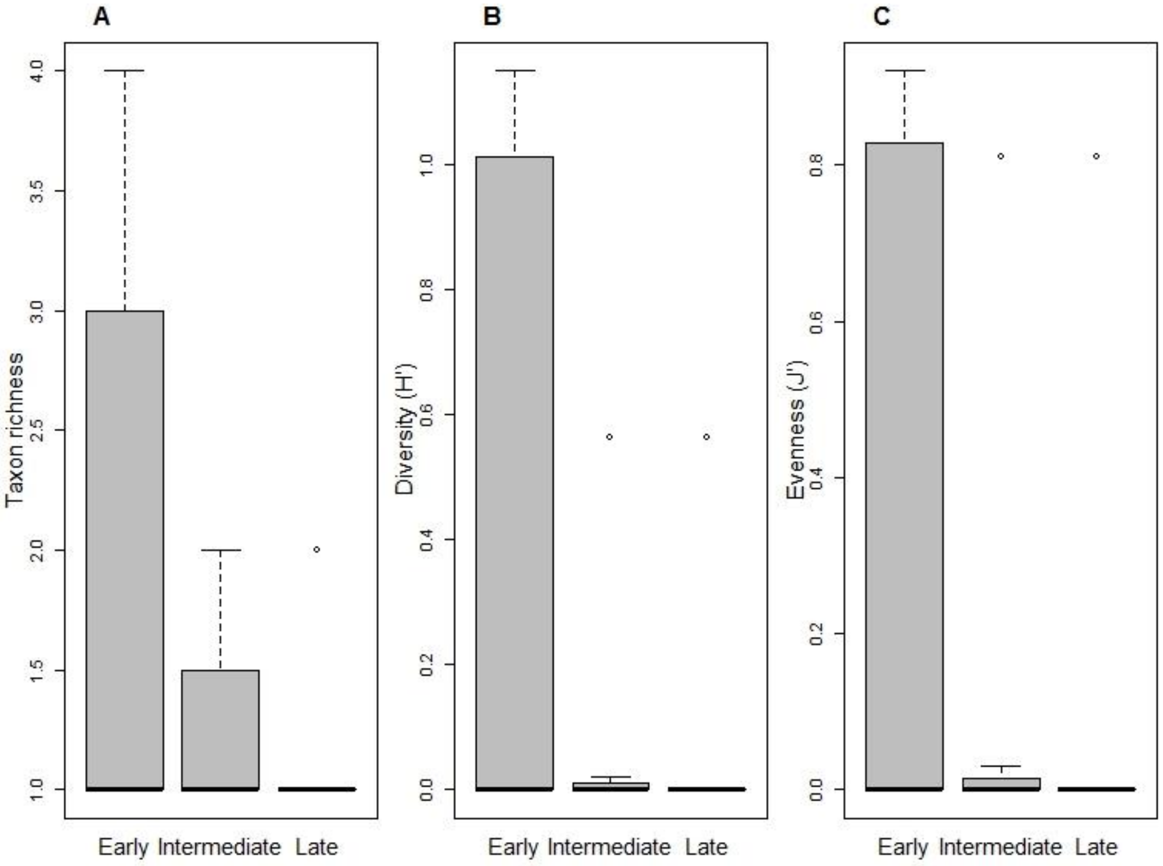

Figure 21: Taxon richness (A), Shannon-Weiner diversity (B), and Pielou's Evenness (C) of invertebrates hatched from dried soil sediments of temporary wetlands in the Cape Sand Fynbos region, comparing successional hatching phases in burned samples in 2016. Boxplots represent median values with interquartile range. Whiskers represent maximum and minimum values.

Invertebrate assemblages were significantly different between burned and unburned sites when looking at treatment and hatching phase (Figure 22 and Table 9). There was no differentiation between sites in either the burned or unburned treatments. Burned sites were dominated by *Paramecium* sp. and reflect a subset of the unburned site communities which had an R value of 0.061 therefore non-separable (Figure 22 and Table 9). The differentiation between burned and unburned communities however, was significant (Adonis, R = 0.061, F = 24.494, p = 0.001) (Table 9). As with hatching assays looking at urban ecology hatching phase (early, intermediate and late) predicted significant differentiation in invertebrate assemblages (Adonis, R = 0.114, F = 4.865, p = 0.003) (Table 9). Late phase taxa (i.e. Ostracods) were separate from early and intermediate phase taxa for both unburned and burned sites (Figure 22). A full list of taxa for each hatching phase is provided in Appendix III.

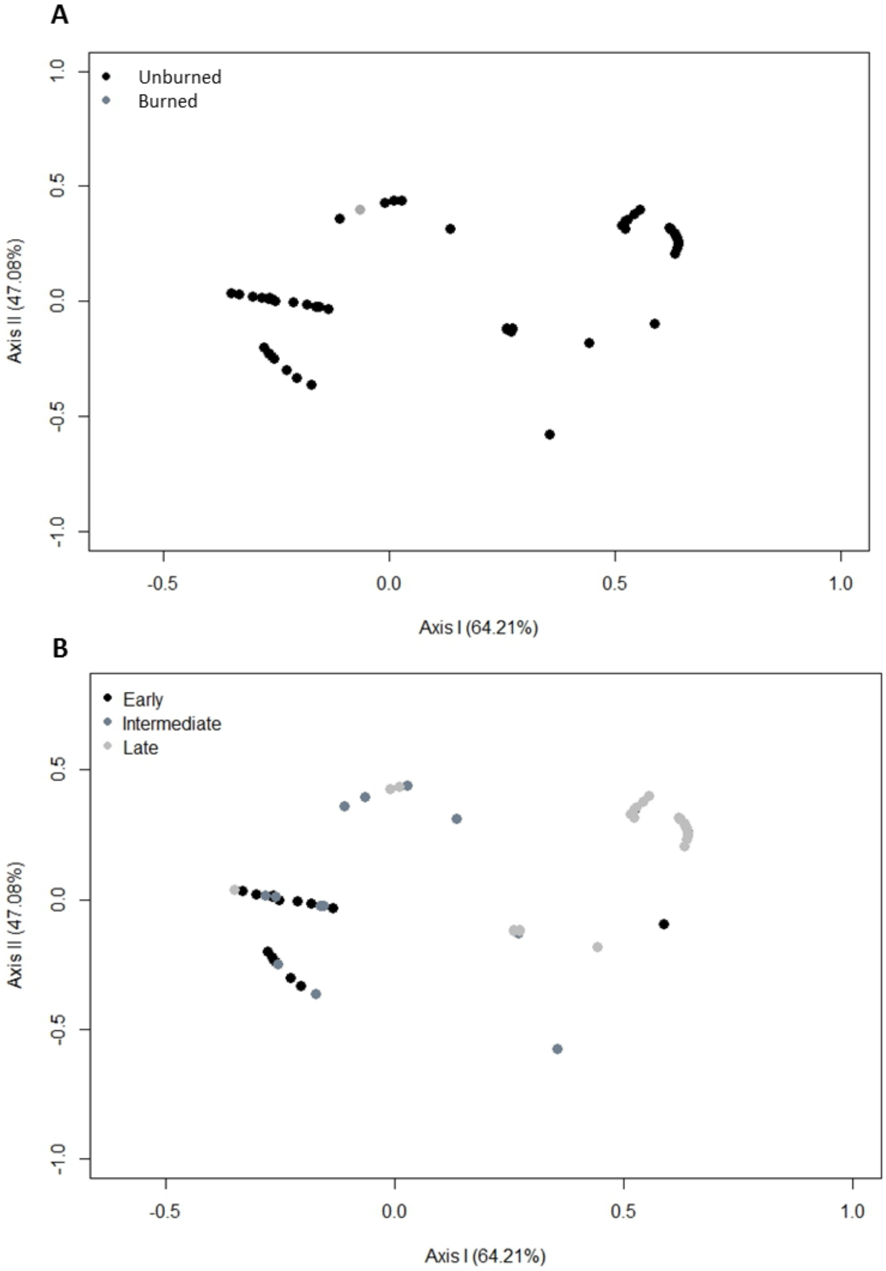

Figure 22: Non-metric multidimensional scaling (nMDS) plot showing differences between burned and unburned sites (A) and the taxa associations over three hatching phases (B) in the dry seasonal phase of selected temporary wetlands in the Cape Sand Fynbos region, Western Cape, during 2016 .

Table 9: PERMANOVA and Adonis results for nMDS plots comparing significant groupings between burned and unburned sites during the dry seasonal phases. Significant differences are marked with an asterix.

Predictors	**Assemblage composition (response matrices)**		
	R	F	P
Treatment	0.06	24.49	0.001*
Phase	0.11	4.86	0.003*

Discussion

Invertebrate diversity

Of the 45 taxa found in this study, from across all 5 temporary wetlands sites, a relatively large portion (18 taxa) was present within hatching assays. This shows 40% of temporary wetland invertebrate biodiversity present during the wet phase are emerging from the soil. This highlights the important role of dormant or resting eggs in contributing to the biotic diversity of temporary wetlands in the Cape Sand Fynbos region (Brendonck *et al*.1998, Williams 1985, Dietz-Brantley *et al*. 2002, Henri *et al*. 2014). The majority of taxa present during hatching events were collembola, large branchiopods and microcrustaceans. This is consistent with previous studies which suggest newly inundated wetlands have few insects and more microcrustaceans compared to ‘mature’ wetlands (Schael *et al*. 2015). Crustaceans are the most well represented group in the dry phase, while both insects and crustaceans are equally well represented in the wet phase (Boix *et al*. 2001, Mlambo *et al*. 2011, Bird & Day 2016).

Invertebrate communities in temporary wetlands are known to exhibit high levels of succession, with early “pioneers” radically different from the climax communities (Boix *et al*. 2001). Hatching assays in this study confirmed this pattern (see Table 6). Typically more insect species emerged in the early phases (i.e. Collembola and Coleoptera), while most microcrustacean taxa emerged in the intermediate and late phases (Boix *et al.* 2001, Bird & Day 2016). The successional emergence of taxa suggests that different taxa have different environmental cues indicating favourable conditions (Brendonck *et al*. 1998).

Extreme weather events are likely to increase in the Western Cape in the coming year as a result of climate change (Coetzee *et al*. 2009, Hoffman *et al*. 2009). Drought and increased fire during this dry/wet transition is likely to impact species compositions of wetlands. For example erratic rains and drought can significantly reduce the inundation period of wetlands. Late phase emerging taxa (i.e. Ostracoda) will be impacted as wetlands are no longer inundated for long enough for a significant portion of the population to emerge. It is however, important to note that succession in hatching assays were observed under lab conditions. The length of these hatching phases may change under natural conditions, however one can assume that the pattern holds.

Paramecium (Kingdom: Protozoa), a likely important food source for emerging invertebrates, were found in very high numbers during hatching assays. A study on ciliate diversity and abundance in temporary ponds in America showed that abundance reduced when predators (copepods, ostracods, rotifers and other crustaceans) were experimentally introduced into a system (Gilbert & Jack 1993, Andrushchyshyn & Magnusson 2003). This was a consistent result for the present study across treatment and site as in all treatments ciliate density peaked early in a hatching event, followed by a decrease or plateau when predator emergence increased. This was consistent with previous research on a range of ciliates including *Paramecium* sp. (Andrushchyshyn & Magnusson 2003). Where predators species where not present (i.e. burned samples from fire hatching assays) ciliate abundance was higher and dominant compared to other samples. This supports the idea that abundance will increase without predators present in the system (Gilbert & Jack 1993, Andrushchyshyn & Magnusson 2003).

Amphipods (*Paramelita pinnicornis*) (Figure 23) were only found in KRCA during the wet phase and nowhere else. This was consistent with the results of previous studies (Mlambo *et al*. 2011, Bird & Day 2016). This finding is particularly interesting due to the Gondwanan origins of the species. The restricted range of this endemic species highlights the potential variability of biodiversity between geographically close temporary wetlands. KRCA appears to be an important area for the conservation of this species. The failure of this species to hatch out from the soil sediments and its no-occurrence in Ottery park and other neighbouring areas, which have fairly similar environmental conditions as KRCA, is concerning.

Figure 23: An endemic Amphipod species (*Paramelita pinnicornis*) found within a few temporary wetlands in Kenilworth Racecourse Conservation Area, Western Cape (Photo courtesy of Prof Charles Griffith).

The emergence of a Rove Beetle (*Philonthus* sp.) (Figure 24), a typically terrestrial insect, from soil cores after periods of drying followed by inundation was perplexing. These insects emerged during the 1st wetting assays typically occurring in the early hatching phase (within the first two weeks of inundation). Identification to specific species level was not possible as this group is particularly difficult to identify, however there are likely three different species present in these samples (Prof Mike Picker *pers comm*.). More research is certainly needed to understand their relationship with temporary wetlands.

Figure 24: A typically terrestrial Coleoptera species known as the Rove Beetle (*Philonthus* sp.) found during hatching assays in both the conserved and urban sites (Photo courtesy of Assoc. Prof Mike Piker).

Urban ecology

The hypothesis, that effects of urbanisation (see Harmse & Le Grange 1994) will lead to higher abundances but lower diversity in urban-influenced versus conserved sites during the dry phase, was not supported. Despite differences in human pressures, diversity patterns of invertebrates hatching out from soil sediments between the conserved (KRCA) and urban (Ottery) sites were largely similar in both the 1st and 2nd wetting assays. However, the community composition of the wet phase samples revealed significant differences between these sites. High levels of habitat transformation and human activity, similar to the conditions in Ottery site, have been shown to result in poor physio-chemistry and changes in species composition (Bird *et al*. 2013a, Bird & Day 2016) which support the findings of this study.

Interestingly, significant differences in diversity and evenness were observed between the 1st and 2nd wetting assays for conserved sites, while urban sites saw significant differences in all measures of diversity. This suggests that community structure is significantly influenced by the number of wettings that occur before extended inundation. Species are likely to respond to successional wetting and drying as an environmental cue to emerge which supports the theory that resting stage invertebrates are heavily influenced by environmental conditions (Brock *et al*. 2005). Drought could severely impact the community structure of temporary wetlands in the wet phase, as decreases in the number of wettings before full inundation will influence which dry phase species emerge and will be present in the wet phase. The richness and diversity of temporary wetlands in conserved and urban sites was significantly higher in the wet phase compared to the dry, however this may shift depending on the number of wettings before full inundation. Significant differences in diversity patterns and community compositions between the 1st and 2nd wetting assays is instructive, suggesting that different processes might be at play here, like bet-hedging based on the eggs-bank (Blaustein & Schwartz 2001, Henri *et al*. 2014). Hatching success of invertebrate propagules is known to be heavily influenced by environmental conditions (Brock *et al*. 2005). Understanding which species emerge after a certain number of wettings and the mechanisms that govern this is vital for understanding the structure of invertebrate propagules and has bearing on the wet phase dynamics as well.

The overall invertebrate community emerging from soil sediment was similar at both sites (conserved vs urban) potentially suggesting that changes in community composition seen in the wet phase are a result of external environmental factors (likely habitat transformation) (Bird *et al.* 2013a). This could be the result of different responses for actively dispersing species (Wissinger 1999). Their ability to control dispersion suggests these species can choose environmentally suitable temporary wetlands. Temporary wetlands found in more urban sites may be undesirable (Harmse & Le Grange 1994, Johnson *et al.* 2013) compared to wetlands in conserved sites. This could explain why there were increased insect species (active dispersers) in the conserved sites during the wet phase compared to predominantly microcrustacean species in the urban sites.

Fire ecology

Previous fire ecology studies, which used hatching experiments, have focused on the post-fire recovery of communities several months or years after burning (Janion-Scheepers *et al.* 2016), or simply examined the impact of fire-retardant stress on invertebrate communities (Angeler *et al.* 2005). These studies however were not specific to temporary wetlands rather a range of sediment sites. The present study investigated hatching success of invertebrate propagules in the soil sediment from temporary wetlands and found that burned soil samples had significantly lower diversity patterns compared to unburned ones. Given that fire is an important stimulant (Keeley & Fotheringham 2000) or management tool (van Wilgen *et al.* 2010, 2011) in the Fynbos biome, the negative effect it caused on the hatching success is striking, providing one more additional layer of evidence for the need of proper and adaptive management of fire (see van Wilgen *et al.* 2010). This raises interesting questions about adaptive capacity of invertebrate propagules (i.e. egg-bank replenishment and resilience), given that fire occurrence is frequent in this part of this biome.

Previous studies have suggested various anostracan species are fairly resistant to fire at the resting stages of temporary wetlands (Wells *et al*. 1997, Bright *et al*. 2016). However, no anostracan species were found within burned samples for this study, suggesting that either anostracan resting eggs where not present in the soil, or that Fynbos species are less resistant to fire as compared to grassland or shrubland species in the Mediterranean (Wells *et al*. 1997). Although fire hatching assays continued for over 40 days, which was longer than urban hatching assays, both burned and unburned sample accumulation curves remained slightly inclined at experiment termination unlike the conserved and urban species accumulation curves. This suggests the full range of species richness was not entirely reached. Fire hatching assays were terminated once four consecutive days saw no new hatchings.

The effect of fire on the composition of dry phase invertebrate communities was significant. Previous studies (Wells *et al*. 1997, Clegg *et al*. 1999, Bright *et al*. 2016) have suggested that fire causes physio-chemical changes in the soil (and therefore the water after inundation) that tend towards extremes impacting richness and diversity as well as composition. Grassland temporary wetlands showed no significant difference in diversity or richness between burned and unburned hatching assays (Bright *et al*. 2016). Grasslands have higher fire frequency relative to Fynbos which could account for the difference in invertebrate responses. Grassland temporary wetland invertebrates have evolved with regular fire present in the system resulting in little to no impacts on community structure (Higgins 1984, Bright *et al*. 2016). Fynbos species are likely to have adapted to fire intervals between 10 - 20 years (van Wilgen *et al*. 2010, Janion-Scheepers *et al*. 2016). The difference in frequency could account for the varying responses of species evolving in different regions and habitat types having different responses to fire. Fire intensity is also relatively low in grassland habitats making heat transfers to soil low (Wells *et al.*

1997). This may account for differences in wetland invertebrates as fuel loads are higher in Fynbos habitat due to longer between fire periods compared to grasslands (Bright *et al*. 2016). A higher fuel load potentially improves heat transfer to the soil compared to grasslands having a greater impact of species that may seem resistant to fire in grassland temporary wetlands (Wells *et al*. 1997). An important aspect of future research needs to look at how the vegetation may impact how a wetland burns. For example mat forming grasses may have a more protective role for soil invertebrates compared to wetlands without this grass, while soil moisture content may change depending on the season and climate. The patchiness, intensity and length of a burn should be manipulated to determine these impacts. The scope of this study limited this aspect, therefore creating baseline data on how soil invertebrates in wetlands responds to fire creates a basin for further investigation.

The presence of selected species common throughout temporary wetland systems after burning suggested these species are either more robust than others or have developed mechanisms to cope with fire within the system. The ability of a species to cope with fire relates to the ability of the egg or cyst stage to survive increases in soil temperature or the adaptation by adults to deposit eggs at varying depths in the soil (Lavens & Sorgeloos 1987, Wells *et al*. 1997, Bright *et al*. 2016, Janion-Scheepers *et al*. 2016). It is not clear which mechanisms are responsible for the resistance or resilience to fire (Bright *et al*. 2016, Janion-Scheeper *et al*. 2016). Previous studies on springtails (Figure 25) explain the mechanisms used by adult forms (i.e. moving deeper into soil to avoid fire) however, in this study collembola species emerging from soil were from resting eggs or cysts (Janion-Scheeper *et al*. 2016). The fire resistance mechanisms for these early developmental stages are understudied.

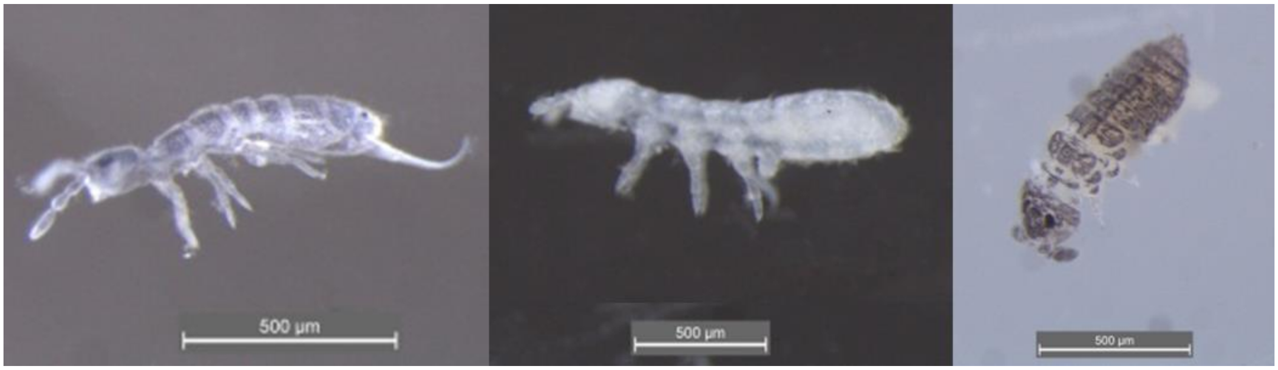

Figure 25: Three different genera of Collembola hatched from the wetland soil sediments, *Isotomid* (left), *Onychiurid* (middle), *Hypogastrurid* (right) (Photos courtesy of Dr Charlene Janion-Scheepers).

Bright *et al*. (2016) found that sediment sampled below the top layer of soil contained invertebrates that readily hatched after fire treatments. Previous research has suggested the crypobiotic nature of crustacean resting eggs is an underlying mechanism for resistance to a variety of environmental stressors including heat pulses aimed to counter desiccation but would be helpful in dealing with fire events (Lavens & Sorgeloos 1987, Wells *et al*. 1997, Bright *et al*. 2016). Recovery over subsequent years post fire is likely to rely on the quantity and quality of remaining organic matter, climate and availability of refugia (Schneider & Frost 1996, Strachan *et al*. 2014, Janion-Scheepers *et al*. 2016).

A significant portion of taxa present in the unburned sites were however, not present in the burned sites. The recovery of missing communities, however, is evident in previous studies (Janion-Scheepers *et al*. 2016) as seen with Fynbos springtail populations which appeared to recover two or three years post fire. Dispersal of invertebrates between isolated wetlands occurs frequently enough to allow for recolonization after disturbance (Cohen & Shurin 2003, Green *et al*. 2008, Rogers 2014, Incagnone *et al*. 2015). Internal and external dispersal by various vectors (i.e. waterfowl) is a likely mechanism for both invertebrate and plant propagules (Figuerola & Green 2002, Green *et al*. 2008, Rogers 2014). Cohen & Shurin (2003) highlighted the importance of wind as dispersal mechanism. Wind could disperse invertebrate resting stages either via movement of water in the wet phase or from soil in the dry phase (Incagnone *et al*. 2015).

The significance of different dispersal mechanisms is likely to depend on the characteristics of the environment, as well as the properties of propagules being dispersed (Vanschoenwinkel *et al*. 2008, Incagnone *et al*. 2015). For example, wind as a physical vector of dispersal is most effective across shorter distances however size of resting stages of invertebrates could cause variation in dispersal distance (Incagnone *et al*. 2015). Furthermore human activity (i.e. movement of livestock, walking within wetlands when dry, etc.) has been shown to play a minor role in the dispersal of resting stage invertebrates and plants (Incagnone *et al*. 2015). Determining how recolonization of temporary wetlands occurs after fire events is an important step towards understanding the functioning of these systems, improving ability to adequately conserve them.

Future conservation and management

Temporary wetlands are known to function as small natural features which have a disproportionately high importance relative to their size (Calhoun *et al*. 2016, Hunter 2016). The temporary wetlands within the Cape Sand Fynbos region face increasing threats and despite several invertebrate taxa being identified as rare and endemic to these systems (i.e. Amphipod, *Paramelita pinnicarnis*) the impacts of these threats have been poorly documented (Mlambo *et al*. 2011, Bird & Day 2016). The management of these wetlands requires both local and landscape scale interventions which makes policy making difficult (Calhoun *et al*. 2016).

The ecological differences that occur between the wet and dry phases within just one year is a major challenge to making holistic management plans. Understanding the biotic elements, and therefore the functioning, of these systems during the different phases, as well as the transition between these phases, provides an important baseline for the conservation of temporary wetlands. Hulsmans *et al*. (2006) found that during hatching experiments, focusing on the Makgadikgadi Pans, insignificant hatchings occurred after 13 days of inundation and argued this was likely the result of eggs being unviable from this point. The present study however, suggests that hatching could occur up to and beyond 46 days of inundation, with significant differences in the taxa emerging depending on the length of inundation. Without the full hatching period several species (i.e. Ostracods) will not be present within the wet phase community at the same levels expected for these systems. Understanding the hatching cycle of temporary wetland invertebrates within their unique environmental context is essential for conservation.

Further investigation is required to investigate the differences in physio-chemical characteristics between conserved and urban sites. The 2016 drought limited the abiotic comparison between KRCA and Ottery however, it would be interesting to look at the role pH and humic substance play in the differences in invertebrate community structure during the wet phase. Bird & Day (2016) suggested that habitat transformation had a significant effect on invertebrate communities during the wet phase which was reflected in the present study. Changes in vegetation, pH and humic substances were a consistent response to habitat transformation (Bird *et al*. 2013a and 2013b). The present study assumes that Ottery could act as a continuation of the transformation gradient sampled within the Bird & Day (2016) study. This suggests that pH and humic substances will significantly change in urban compared to conserved impacting invertebrate community structure during inundation periods. Further measurements at a fine temporal scale for both conserved (KRCA) and urban (Ottery) sites are needed to provide evidence for this probable pattern.

An important aspect of future research for temporary wetland systems is the impact of climate change on hydroperiod characteristics of temporary wetlands. Decreases in precipitation and increases in temperature predicted for the Western Cape suggests drought will become more intense and more frequent within the Cape Sand Fynbos region (Coetzee *et al*. 2009, Hoffman *et al*. 2009). Drought will likely impact on the duration of the dry/wet phase changeover within temporary wetlands. Understanding how short hydroperiods will impact invertebrate community structure will help determine how biodiversity shifts from year to year. As dry phase invertebrate species act as a reservoir for ~ 40% of the wet phase communities, changes in the dry phase could determine community structures in the wet phase. This could have knock-on effects for subsequent wet seasons, impacting populations that rely on these invertebrates as a major food source. However, the ability for these community to adapt to regular environmental perturbations (as a result of the nature of temporary wetland hydrology) suggests that small incremental changes in climate may not have as greater impact as other climate sensitive systems (Batzer *et al.* 2004, Batzer 2013). The degree of impact as a result of climate change will change depending on the specific characteristics of the wetland (geographic, climatic and hydrological) (Batzer 2013).

The results in the present study suggested that fire had a significant impact on dry phase invertebrate communities of temporary wetlands. Fire is an important aspect of the Fynbos biome and frequently used as a management tool for conserved areas within this habitat (Schael *et al*. 2015, Janoion-Scheepers *et al*. 2016). Understanding the implications of fire for temporary wetland invertebrates during the dry phase is essential for management of wet phase communities. Decreases in dry phase taxa during inundation may be the result of fire within the previous dry phase. Conservation of animal vectors that help disperse resting stages of invertebrates is essential for the recovery of the communities post fire (Green *et al*. 2008). This is an example of how conservation of temporary wetlands is dependent on landscape scale management (i.e. waterfowl conservation). Large conserved areas that incorporate multiple temporary wetlands and the matrix between, for instance KRCA, are ideal for a more holistic approach to wetland management. Controlled fire management within reserves like KRCA and MRCA should continue to maintain a fire interval of 10 - 15 years mimicking the natural fire regime of Fynbos habitats. However, in areas with no formal protection (i.e. Ottery) effective management of wildfires is key for the conservation of the temporary wetland invertebrate communities. Currently fire within the Ottery public park area is unmanaged and relatively frequent compared to natural Fynbos fire regimes. The difference in fire regimes between conserved and urban sites may be a contributing factor explaining the differences in wet phase communities. Further research into aspects of fire ecology such as the effects of varying fire frequency (multiple burn events) and intensity (varying fuel loads) would provide insight into these differences. Further fire ecology research would also provide valuable insight into how communities respond to increasing fire events expected in the Western Cape as a result of climate change.

A major challenge to the future conservation of temporary wetlands is the lack of public awareness and understanding of these systems (Calhoun *et al*. 2016). Unlike permanent wetlands, temporary wetlands are often overlooked by the general public. This is largely due to their inconspicuous nature, small size and limited protection and lack of education about the different characteristics and functioning of wetlands (i.e. permanent versus temporary) (Calhoun *et al*. 2016). Future conservation of temporary wetlands is dependent on public awareness especially in areas where wetlands are not formally conserved (i.e. Ottery Public Park). Community driven conservation has merit within the urban context and buy-in from surrounding communities can improve aspects of conservation like fire management and waterfowl conservation.

Limitations of study

It is important to note, however, that several limitations to this study make it challenging to pinpoint the precise impact of urbanisation and fire on the Cape Flats temporary wetland systems. There was little time and resources available for this study causing the spatial and temporal scope to be limited. While the fire aspect of the study attempted to incorporate temporary wetlands from a wider geographic range across the Cape Flats region, the urbanisation aspect of the study could only investigate two temporary wetland sites. This suggests that any urbanisation impacts recorded in this study are specific to this limited region of the Cape Flats (Kenilworth and Ottery). Batzer (2013) states that macroinvertebrates' responses to change in wetlands are context specific, therefore any conclusions drawn from this study shouldn't be scaled up to temporary wetlands in general. However, this study does provide interesting insights into the broad range of responses temporary wetland invertebrates will have to urbanisation and fire. This provides valuable information for the future management of these systems, specifically for the organisations conserving Kenilworth Racecourse temporary wetlands (namely Kenilworth Racecourse Conservation Area).

Furthermore the limited time available to the project and the drought resulted in wet phase sampling being poorly represented, both spatially and temporally. This again resulted in information that is mostly context specific to Kenilworth and Ottery in 2016. A major limitation to the study was the lack of environmental data available to make comparisons between Kenilworth and Ottery (i.e. pH, temperature, conductivity). The combination of limited resources and drought resulted in no environmental data being recorded for Ottery. Additional information on the nutrient load in each wetland over the wet phase would have also greatly benefitted the study and one would be able to single out the likely causes for the difference in species compositions in the wet phase between Kenilworth and Ottery. Again lack of resources available to the project limited the amount of information collected on abiotic factors for each wetland and site.

Conclusion

The temporary wetlands found within Cape Sand Fynbos vegetation are highlighted as important environments for unique (and in some instances endemic) invertebrate taxa (Mlambo *et al*. 2011, Bird & Day 2016). Temporary wetland invertebrate communities, while relatively robust, can be significantly influenced by destructive external environmental conditions (i.e. human activity and fire) (Day *et al*. 2010, Schael *et al*. 2015). Changes in the physio-chemical conditions of the temporary wetland environment (either through urbanisation or fire) impacts the richness, diversity, evenness and community composition of invertebrates. The impact of urbanisation in this study is limited to the wet phase invertebrate communities, decreasing richness and diversity compared to relatively protected environments. However changes in wet phase communities can impact on the invertebrate species available to contribute to the dry phase sediment egg bank. This could have a significant influence on the community structure in subsequent years.

Severe weather events are likely to have a lasting impact on temporary wetland invertebrates. Fire significantly negatively impacts the diversity and composition of emerging invertebrates from temporary wetland sediments in this study. The frequency and intensity of these fires will impact the ability for these communities to recover. Temporary wetlands in the Fynbos biome have likely evolved to a fire interval of 10-15 years (typical for Fynbos systems), suggesting the invertebrate species are adapted to fire at this frequency. Recovery has been observed (i.e. Springtail recovery post fire) for these populations, however increasing fire frequency could significantly impact this post fire recovery period. Climate change, as well as increasing instances of manmade fires has decreased the average time between fires within Fynbos systems. Climate change is further likely to increase the intensity of these fires due to decreased precipitation and increased temperatures (Batllori *et al.* 2013).

Alternately drought will also have a significant impact on the structuring of temporary wetland invertebrate communities from dry to wet phases. The number of initial wettings occurring before full wet phase inundation has been shown to impact on the richness and diversity of invertebrates emerging from dry phase sediment samples. Different taxa have different environmental cues that trigger hatching (Bagella *et al*. 2010), one of which could be the number of wettings before full inundation. Increased instances of drought, decreased precipitation and higher ambient temperatures can cause the number of wettings to decrease. This suggests certain taxa, that require more than one wetting in order to emerge, will not be present in the wet phase invertebrate communities. Dry phase taxa contribute ~ 40% of the wet phase invertebrate community, suggesting changes in the taxa emerging from the dry phase will have a significant impact on the taxa present in the wet phase.

While many of the threats to invertebrate communities in temporary wetlands are difficult to avoid (i.e. impacts of fire and climate change), management of these impacts is important and possible. Previous studies have shown that these communities can and are likely to recover after major disturbances (Janion-Scheepers *et al*. 2016). Facilitating this post disturbance recovery is essential to the management of temporary wetlands (Schneider & Frost 1996). For example ensuring that important water fowl species are conserved in temporary wetland areas will allow for sustained dispersal of passive invertebrate species. Furthermore decreasing the impacts of human activity (i.e. alien vegetation management and public awareness) will decrease the impacts of urbanisation on these systems. The cryptic nature of temporary wetlands during the dry season creates a major challenge for conservation and management especially in an urban setting. Education and public awareness are essential for the continued management and conservation of temporary wetlands.

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Appendix I

Description of fire management regimes used for each area sampled within the Cape Sand Fynbos region, Western Cape, South Africa.

Area	Fire management	Frequency	Most recent burn (year)	Description of management tool	Accidental fire (incidence/frequency)
Kenilworth Racecourse	Controlled ecological burns	Every 10-15 years	Wetland 1, 2, 3 & 4 – 2015 Wetland 5 - 2015	All fires post 2012 occur in Feb/March (wetland dry phase) and last 24 hours, ca. 4-6 hours active burning and the rest is post fire operations	Wetland 5 wildfire - 2013
Milnerton Racecourse	Controlled ecological burns	Every 12-15 years	Block 1 – 2008 Block 2 – 2010 Block 3 - 2012	Veld is assessed by botanist to determine best time (12 or 15 years) for burn	Accidental fire - 2015
Rondevlei Nature Reserve	Controlled ecological burns	~10 year interval	Seasonal wetlands - 1993 & 1994 Whole area - 2015	Seasonal wetlands undergo controlled burns however arson and fire from unknown cause fairly regular in the area and occasional affect wetlands	Arson (1998 & 2001) Flares from larger fires (1999 & 2000)

Appendix II

Full species list for invertebrates found throughout all five temporary wetland areas. Differences in species composition between Kenilworth Racecourse Conservation Area (KRCA) and Ottery public park area are highlighted. Species found only during hatching assay experiments are indicated with a single asterix, while species with two asterix were found in both wet and dry phase sampling. Species with no asterix were found only during wet phase sampling.

Class	Order	Family	Taxon	KRCA	Ottery
Arachinida	Hydracarina**			x	x
Branchipoda	Anostraca	*Streptocephalidae*	*Streptocephalus* sp.*	x	
	Cladocera	*Chydoridae*	*Pseudochydorus* (Probably *P. gr. Globosus)*	x	x
		Daphniidae	*Ceriodaphnia*	x	x
			Daphnia barbata	x	
			*Daphnia pulex***	x	x
			Daphnia laevis	x	
			*Daphnia longispina**	x	x
			*Megafenestra aurita**	x	
			Simocephelus exspinosus	x	
		Macrothricidae	*Echinisca* sp.	x	x
		Moinidae	*Moina* sp.	x	x
Clitellata	Oligocheata	*Tubificidae**		x	x
Copepoda	Calanoida	*Lovenula*	*Lovenula simplex***	x	
		Paradiaptomus	*Paradiatomus lamellatus*	x	
	Cyclopoida	*Cyclopidae**		x	x
Collembola	Entomobryomorpha	*Isotomidae**		x	x
	Poduromorpha	*Onychiuridae*	*Deuteraphorura* sp.*	x	x
		Hypogastruridae	*Hypogastrura* sp.*	x	x
Eurotatoria	Ploima	*Brachionidae*		x	x
Gastropoda		*Physidae*	*Physa acuta***	x	x
		Planorbidae	*Ceratophallus* sp.	x	
Insecta	Coleoptera	*Dytiscidae*	*Cybister* sp.	x	x
		*Gyrinidae***		x	

Appendix II (cont.)

Full species list for invertebrates found throughout all five temporary wetland areas. Differences in species composition between Kenilworth Racecourse Conservation Area (KRCA) and Ottery public park area are highlighted. Species found only during hatching assay experiments are indicated with a single asterix, while species with two asterix were found in both wet and dry phase sampling. Species with no asterix were found only during wet phase sampling.

Class	**Order**	**Family**	**Taxon**	**KRCA**	**Ottery**
		Superfamily: *Hydrophiloidae*		x	x
		Hydraenidae		x	x
		Hydrophilidae		x	x
		Hydrochidae	*Hydrocus* sp.	x	
		Spercheidae	*Spercheus* sp.	x	
		Staphylinidae	*Philonthus* sp.*	x	x
	Diptera	*Chironomidae***		x	x
		Culicidae *(*Subfamily: *Culicinae)*	*Culex* sp.	x	x
	Ephemeroptera	*Baetidae*	*Cloeon* sp.	x	x
	Hemiptera	*Belostomatidae*	*Appasus* sp.	x	
		Corixidae		x	x
		Gerridae	*Gerris* sp.	x	
		Notonectidae	*Notonecta* sp.	x	
		Pleidae	*Plea* sp.	x	
	Odonata - Anisoptera	*Aeshnidae*		x	
		Coenagrionidae		x	
		Gomphidae		x	
Malacostraca	Amphipoda	*Paramelitidae*	*Paramelita pinnicarnis*	x	
	Isopoda		*Mesamphisopus* sp.**		x
Ologohymenophorea	Peniculida	*Parameciidae*	*Paramecium* sp.*	x	x
Ostracoda	Podocopida	*Cyprididae**		x	x
Turbellaria	Tricladida	*Dugesiidae*	*Dugesia* sp.*	x	x

Appendix III

List of specific invertebrate taxa found at various stages (number of weeks) of inundation during hatching assays comparing dry phase community compositions in temporary wetlands for various treatments.

Treatment	**Hatching Phase**		
	Early (1-2 weeks)	***Intermediate (3-4 weeks)***	***Late (5-7 weeks)***
Conserved (KRCA)	Collembola, Hydracarina, Oligocheata, Cladocera, Anostraca, Paramecium, *Philonthus*	Collembola, Hydracarina, Cladocera, Anostraca, Paramecium, Ostracod	Collembola, Hydracarina, Oligochaete, Copepod, Ostracod, Paramecium
Urban (Ottery)	Paramecium, Collembola, Hydracarina, Oligocheata, *Philonthus*, Chironomid	Collembola, Paramecium, Hydracarina, Oligocheata, *Philonthus*, Cladocera, Copepod, Ostracod	Paramecium, Collembola, Ostracod, Cladocera
Burned	Paramecium, Isopod, Ostracod, Collembola, Hydracarina	Paramecium, Ostracod	Paramecium, Ostracod, Cladocera
Unburned	Paramecium, Isopod, Ostracod, Collembola, Hydracarina, Cladocera, Anostraca, Oligocheata	Paramecium, Collembola, Cladocera, Anostraca, Planaria, Ostracod	Paramecium, Ostracod, Cladocera, Planaria, Rotifer, Copepod

Appendix IV

Full species list for invertebrates found throughout all five temporary wetland areas during fire hatching assays. Differences in species composition between Kenilworth Racecourse Conservation Area (KRCA), Ottery public park, Milnerton Racecourse Conservation Area (MRCA), Youngsfield Army Base and Rondevlei Nature Reserve area are highlighted.

Class	**Order**	**Family**	**Taxon**	**KRCA**	**Ottery**	**MRCA**	**Youngsfield**	**Rondevlei**
Arachinida	Hydracarina			x	x			
Branchipoda	Anostraca	*Streptocephalidae*	*Streptocephalus* sp.	x				x
	Cladocera	*Daphniidae*	*Daphnia pulex*	x	x	x	x	x
			Daphnia longispina		x	x	x	
			Megafenestra aurita	x		x	x	
Clitellata	Oligocheata	*Tubificidae*		x			x	
Copepoda	Calanoida	*Lovenula*	*Lovenula simplex*					x
	Cyclopoida	*Cyclopidae*		x				
Collembola	Entomobryomorpha	*Isotomidae*		x	x	x	x	
	Poduromorpha	*Onychiuridae*	*Deuteraphorura* sp.	x	x	x	x	x
		Hypogastruridae	*Hypogastrura* sp.	x	x	x	x	x
Gastropoda		*Physidae*	*Physa acuta*	x				
Malacostraca	Isopoda		*Mesamphisopus* sp.		x			x
Ologohymenophorea	Peniculida	*Parameciidae*	*Paramecium* sp.	x	x	x	x	x
Ostracoda	Podocopida	*Cyprididae*		x	x	x	x	x
Turbellaria	Tricladida	*Dugesiidae*	*Dugesia* sp.	x		x	x	