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Microrefuges from drying for invertebrates in a seasonal wetland

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Summary

1. Seasonally intermittent freshwater environments show large temporal changes in area and environmental conditions (which may be harsh). We investigated whether microhabitats that retain moisture could provide a refuge during drying in a seasonal wetland.
2. We investigated occupancy by invertebrates of three potential microrefuge types: surface depressions, shallow cracks and deeper fissures in the sediment of a wetland in Western Australia. Our aims were to determine whether the assemblages occupying these microrefuges differed and whether they changed as the wetland dried and reflooded.
3. Ten microrefuges of each type were sampled for invertebrates, sediment and temperature during each of three hydrological phases: the damp phase (surface water absent but sediment moist), the dry phase (groundwater at its lowest level) and the reflooded phase (surface water present). Sediment samples taken from each microrefuge in the damp and dry phases were reflooded in the laboratory to reveal species aestivating or present as eggs, and sediment organic matter content was measured.
4. Sediment organic matter content did not change between wetland phases. The invertebrate assemblage in the microrefuges showed almost complete species turnover between phases. Invertebrate composition differed between microrefuges, and temperature in the deeper fissures was on average $> 10\text{ }^{\circ}\text{C}$ lower than in surface depressions and shallow cracks.
5. Microcrustaceans and gastropods survived the drier months as resting stages in the microrefuges and either emerged or hatched from eggs upon reflooding. Several species, including isopods and caddisflies, were collected only from cracks and fissures as the wetland dried. During drying, a high diversity of carnivorous species was observed in the microrefuges.
6. Sediment microrefuges apparently underpin resistance to drought by invertebrates in South Lake. Those species that depend on sediment fissures to survive may be threatened by declining the groundwater table in the region. Changes to wetland hydrology and other human activities that affect wetland sediment or vegetation will affect the range of microrefuges available to invertebrates to survive drying, and may thus alter wetland biodiversity.

Introduction

Loss of water is the most deleterious factor influencing aquatic animals and although it occurs annually in seasonal wetlands, its effect on individual species and their survival is poorly understood (Sim *et al.*, 2013). Seasonally intermittent wetlands in regions with a Mediterranean climate show large temporal changes in habitat area and conditions, ranging from extensive surface water in the winter, to a dry landscape and harsh conditions in summer. During summer drying, freshwater animals are increasingly exposed to ultraviolet light, high temperature, large fluctuations in salinity, pH and oxygen, and the rapid disappearance of surface water (Alekseev, DeStasio & Gilbert, 2007). Increased temperature may exceed physiological tolerances and lead to high mortality or local extinction (Maxted *et al.*, 2005). Temperature influences the duration of egg incubation, hatching success, duration of hatching and the induction and termination of resting stages (Oliver, 1971; Alekseev *et al.*, 2007), as well as minimum size at pupation, sex ratios in insects, increased metabolic rate (Terblanche *et al.*, 2005), changes in food availability and food web structure (Rosenzweig *et al.*, 2008). Given the range of physical changes that occur during drying, invertebrates that live in intermittent aquatic environments often show adaptive traits.

Species may show several types of response to adverse conditions: life history adjustments (altered phenology), morphological, behavioural and physiological responses (Lytle & Poff, 2004; Robson *et al.*, 2011). Some crustaceans produce drought resistant eggs or cysts (Datry *et al.*, 2012; Stubbington & Datry, 2013). Other groups, such as many large predators (e.g. Dytiscidae), show behavioural responses including dispersal by flight to wetter areas (Boulton and Lake, 2008), while other species may move into refuges when drying occurs (Robson *et al.*, 2011). Refuges support populations of species not able to live in the surrounding environment due to the effects of disturbance; a refuge is also a source of colonists for the surrounding environment once the disturbance has passed (Robson *et al.*, 2013). Many types of refuge have been identified in flowing waters (e.g. Lancaster, 2000; Chester & Robson, 2011; Robson *et al.*, 2013; Stubbington & Datry, 2013), but relatively few in standing waters, aside from studies of wetland seed and egg banks (e.g. Brock *et al.*, 2003; Jenkins & Boulton, 2007; Tuckett *et al.*, 2010).

Refuges may be large, such as perennial waterbodies, or they may be smaller habitats and occur within waterbodies (Stubbington & Datry, 2013). These smaller refuges were described as microhabitat sources of colonists by Stubbington & Datry (2013) and are termed microrefuges here. Microrefuges include cracks in sediments, damp areas beneath algal mats (Strandine, 1941), stones (Chester & Robson, 2011) or leaf litter and areas with high

humidity such as crayfish burrows (Stubbington & Datry, 2013 and references therein), all of which may occur in wetlands.

Sediment characteristics will partially determine the types and frequency of microrefuges in wetlands. For example, the organic matter content of the sediment can affect drying rates, because organic matter can hold up to 20 times its mass in water and improves water retention (Reddy & DeLaune, 2008). Sediment moisture content has been shown to be a major determinant of seedbank viability (Stubbington & Datry, 2013). Therefore, patches of wetland sediment with higher organic matter content may hold moisture for longer periods, increasing the capacity for aquatic organisms to survive dry summer conditions. Small differences in organic matter content between microrefuges could mean that certain areas within a wetland are more likely than others to provide refuges during summer.

We chose three potential microrefuge types: surface depressions, shallow cracks and deeper fissures in wetland sediment, to investigate invertebrate occupancy, including resting stages. These potential microrefuges were the main water-retaining physical features found on the wetland bed in open water areas; no other refuge types were apparent. The hypotheses were that invertebrate assemblages occupying these microrefuge types would differ because of differences in temperature and level of inundation; and that assemblages would change over time as the wetland dried and reflooded. We expected that assemblages in the deep fissures would gain species during the dry phase because the deeper fissures would provide a cooler microenvironment for invertebrates.

Methods

Study site

South Lake (32°06.227'S, 115°49.125'E), in suburban Perth, Western Australia (with a Mediterranean climate) has a maximum area of 31.5 ha and is a seasonally inundated groundwater-dependent basin, protected within the Beeliar Regional Park (Hill *et al.*, 1996). It is surrounded by native vegetation, has extensive beds of emergent macrophytes and does not receive street drainage. South Lake and its sediment are therefore relatively undisturbed by urbanisation and bed modification. The lake has a faunal assemblage similar to other natural wetlands on the Swan Coastal Plain. The invertebrate fauna of these wetlands is well known and further descriptions may be found in Davis & Christidis (1999), Horwitz *et al.* (2009) and Sim *et al.* (2013).

Water regime

Maximum wetland depth usually occurs in spring (September-October) and the dry phase in autumn (March-April) (Davis & Froend, 1999). After the surface water disappears from South Lake (during summer and autumn), the water table drops between 0.1 – 1 m below the sediment surface. As the water level drops, the sediment cracks and tessellates, creating shallow cracks and deeper fissures that may connect to the groundwater table (Fig. 1). During the damp phase (March 2012), the water in a drying pool had a temperature of 27.1 °C, conductivity of 5.49 mS cm⁻¹ and pH of 8.21. In the reflooded phase (May 2012), when the wetland had started to refill, water temperature was 19.2 °C, conductivity was 1.90 mS cm⁻¹ and pH was 8.43, these recordings were from a single point.

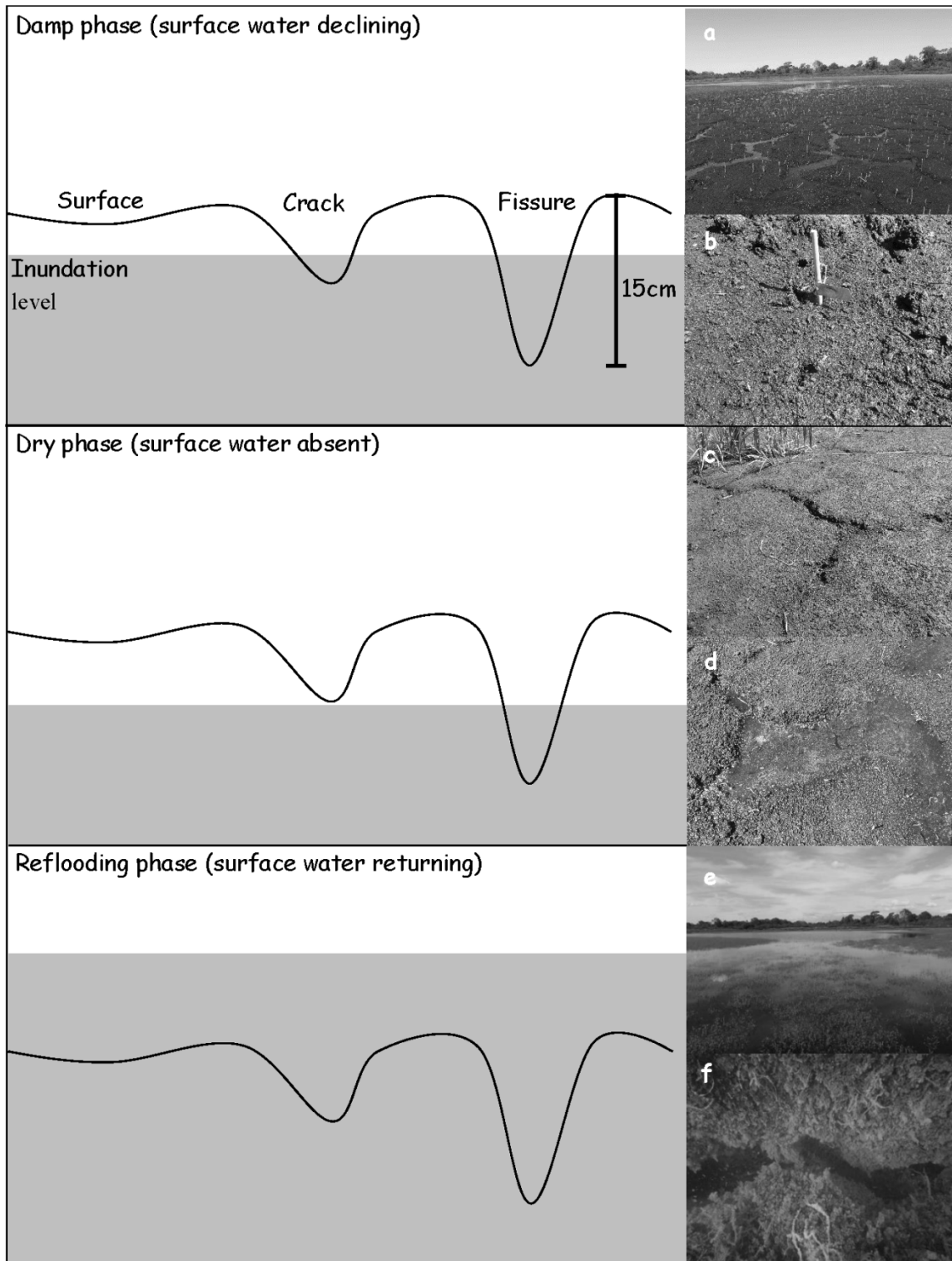


Figure 1. Microrefuges and the inundation level at the three sampling times. In the damp phase, South Lake drying out and water levels have dropped: (a) only the cracks and fissures are inundated; (b) a dry surface microrefuge. During the dry phase, the water table has dropped below the surface: (c) crack microrefuges and (d) a fissure still containing water. In the reflooded phase: (e) South Lake starting to reflood and (f) a submerged fissure.

Sampling and laboratory procedure

The three microrefuge types were: surface depressions (on the surface of the sediment where water may pool during drying), shallow cracks (in soft sediment and of restricted depth, approximately < 8cm and with a firm bottom) and deep fissures (in the substratum, approximately > 15cm deep, which had very loose sediment at the bottom sometimes mixed with filaments of iron bacteria, suggesting possible connection to the deeper groundwater). During the damp phase (March 2012), 90 microrefuges (30 of each type) were marked on the bed of South Lake (Fig. 1). Ten microrefuges of each type were randomly sampled at each of the three sampling times, chosen to represent particular stages in the wetting and drying cycle: the damp phase (early autumn, late March), the dry phase (mid-autumn, April) and the reflooded phase (late autumn, May) 2012. Different microrefuges were sampled on each occasion, so that no microrefuge was sampled twice.

In the damp phase, daytime air temperatures were high (31.3 °C, mean daily maximum, Jandakot Airport, BOM, 2013) and the wetland sediment remained damp. The water table had dropped just below the sediment surface and water filled the cracks and fissures (Fig. 1). In the dry phase, air temperatures and day length had declined (26.0 °C, mean daily maximum, Jandakot Airport, BOM, 2013) and the water table had dropped so that only the fissures still contained standing water (Fig. 1). The day following the dry phase sampling, rainfall was recorded nearby (28 mm Jandakot Airport; BOM, 2013) and the wetland began to refill. By the sampling date during the reflooded phase a further 97 mm of rain had fallen (BOM, 2013) and air temperatures and day length were at their lowest (22.7 °C mean daily maximum, Jandakot Airport, BOM, 2013). Fissures, cracks and surface depressions were all underwater (Fig. 1).

Data loggers (HOBO pendant temp/light, 64k UA-002-064; Onset Computer Corporation, Bourne, MA, USA) were used to record temperature in the different microrefuge types. Loggers were randomly placed in three of each of the refuge types: in depressions, cracks and fissures. Loggers in fissures were suspended 10cm below the surface on monofilament line, while others were pegged directly to the substratum at the deepest point for that microhabitat. Temperature was logged every half an hour from the 23 March 2012 to the 8 May 2012 during the period when the wetland dried out and then refilled.

Different refuge types and inundation phases necessitated somewhat different collection methods, but each sample comprised an equivalent sampled area (0.2 m length of crack, fissure, depression), amount of sediment (\approx 500 g) and volume of water (\approx 2 L, damp and reflooded phases). Invertebrates were collected from the free water in cracks and crevices

(Fig. 1) during the damp phase using a net (250 μm mesh, approx. 30 second sweep). In the dry phase, sediment was collected using a trowel. In the reflooded phase when the microrefuges were submerged (Fig. 1), a hand pump and hose was used to collect water from within microrefuges and passed through a 125 μm sieve; sediment was collected using a trowel. Sediment was placed in thick plastic bags and transported back to the laboratory for preservation in 70% ethanol. Invertebrates were removed from all samples, counted and identified to the lowest possible taxonomic level.

The organic content of the sediment was determined by loss on ignition, for damp and dry phase samples only. Samples could not be collected effectively in the reflooded phase when the lake was inundated. Subsamples of sediment (10g) from each invertebrate sample were heated at 500 °C for 6 hours to burn off the organic matter and then reweighed (nearest mg) to determine the loss.

Invertebrate resting stages

To separate the invertebrates that were active in the wetland from those which hatched out from the egg bank or emerged after aestivation, a 200g subsample was taken from each of the dry sediment samples prior to preservation of the remaining sample. The subsample were placed in 1 L jars and inundated with 400 ml of distilled water to permit hatching of eggs/cysts in the sediment or emergence of aestivating individuals. On day three, the water was decanted from the jars and passed through a 125 μm sieve and the captured material preserved. The jars were refilled with distilled water and allowed to sit in the laboratory until day 20, whereupon the water was again decanted, sieved and preserved, and the sediment also preserved. Invertebrates were counted and identified to the lowest possible taxonomic level.

Data analysis

Sampling was randomized, with different, interspersed microrefuges sampled independently at each time. The design was therefore fully factorial. The organic content of sediment in each microrefuge for damp and dry phases was analysed using a two factor analysis of variance (ANOVA) (time, two levels, fixed; microrefuge type, three levels, fixed) in SPSS (version 21, IBM Corp. 2012), normality and homogeneity of variance assumptions were met without transformation. Using logger data, mean maximum daily temperatures were calculated for each phase (damp: 27 March – 4 April; dry: 13 April – 27 April; reflooded: 28 April – 5 May). As a measure of daily variation in temperature in each microrefuge type,

squared differences were calculated for 12 hour lags (i.e. intervals with the highest negative correlation) for each logger, within each phase; square-roots of the averages of these were then analysed in three single factor ANOVAs (microrefuge type, three levels).

Patterns in invertebrate assemblage composition were similar for both abundance and presence/absence data so only the latter are presented here. Multivariate analyses were conducted using the PRIMER package (Clarke & Warwick, 2001). Ordination plots (nonmetric Multi-dimensional Scaling, nMDS) based on a Bray-Curtis similarity matrix were used to display differences between inundation phase and microrefuges (default settings were used). A two factor analysis of similarity (ANOSIM) (inundation phase, three levels; microrefuge type, three levels) was used to determine whether assemblage composition differed between inundation phases and microrefuges (Clarke, 1993). However, the effect of inundation phase was so large that differences between microrefuge types were then analysed separately in three, single factor (microrefuge type, three levels) ANOSIMs, one for each phase. SIMPER analyses were used to identify the taxa associated with differences between sampling times, and associated with each microrefuge type (Clarke & Warwick, 2001). For the invertebrate resting stages samples, a two factor (sampling time, two levels, day three and twenty; microrefuge type, three levels) ANOSIM was used to determine whether the composition of taxa present as resting stages differed among microrefuge types.

Results

Sediment composition and temperature in microrefuges

There was no difference in the organic content of the sediment between times (range of means: 37.4 – 38.2%; $F_{1, 54} = 3.24$, $P = 0.08$), or microrefuges (range of means: 38.4 – 40.9%; $F_{2, 54} = 1.57$, $P = 0.22$). Generally, loggers in surface depressions and shallow cracks recorded lower temperature maxima after inundation (average 27.6 °C), than before (Fig. 2a). In all phases of inundation, fissures had significantly lower daily temperature maxima ($F_{2, 4} = 18.8 - 20.1$, $P < 0.005$), ranging from 19.7 – 20.8 °C, than the other microrefuges, which did not differ. Light intensity (lux) records (not presented) confirmed that there was complete darkness inside fissures. Surface depressions and cracks were similarly exposed to solar radiation, with maxima at 37.3 – 42.1 °C during drying (Fig. 2a) However, one logger in a crack recorded lower daily maxima at all times (26.5 – 28.4 °C).

Daily variation in temperature (twelve-hour temperature differences) followed the same pattern, but variation in fissures was very low compared with the other refuge types, with average fluctuations of < 2 °C over 12 h during the dry season, compared with 13 – 16

°C at the surface or in shallow cracks (Fig. 2b). When the wetland surface was dry, temperature variation in shallow cracks and surface depressions varied from 6.5 – 16.6 °C, but after inundation temperature was less variable, being 6.2 – 10.3 °C. Moisture content in the sediment beneath surface depressions and cracks exceeded 50% at all times because interstitial water was present, although the sediment surface was dry.

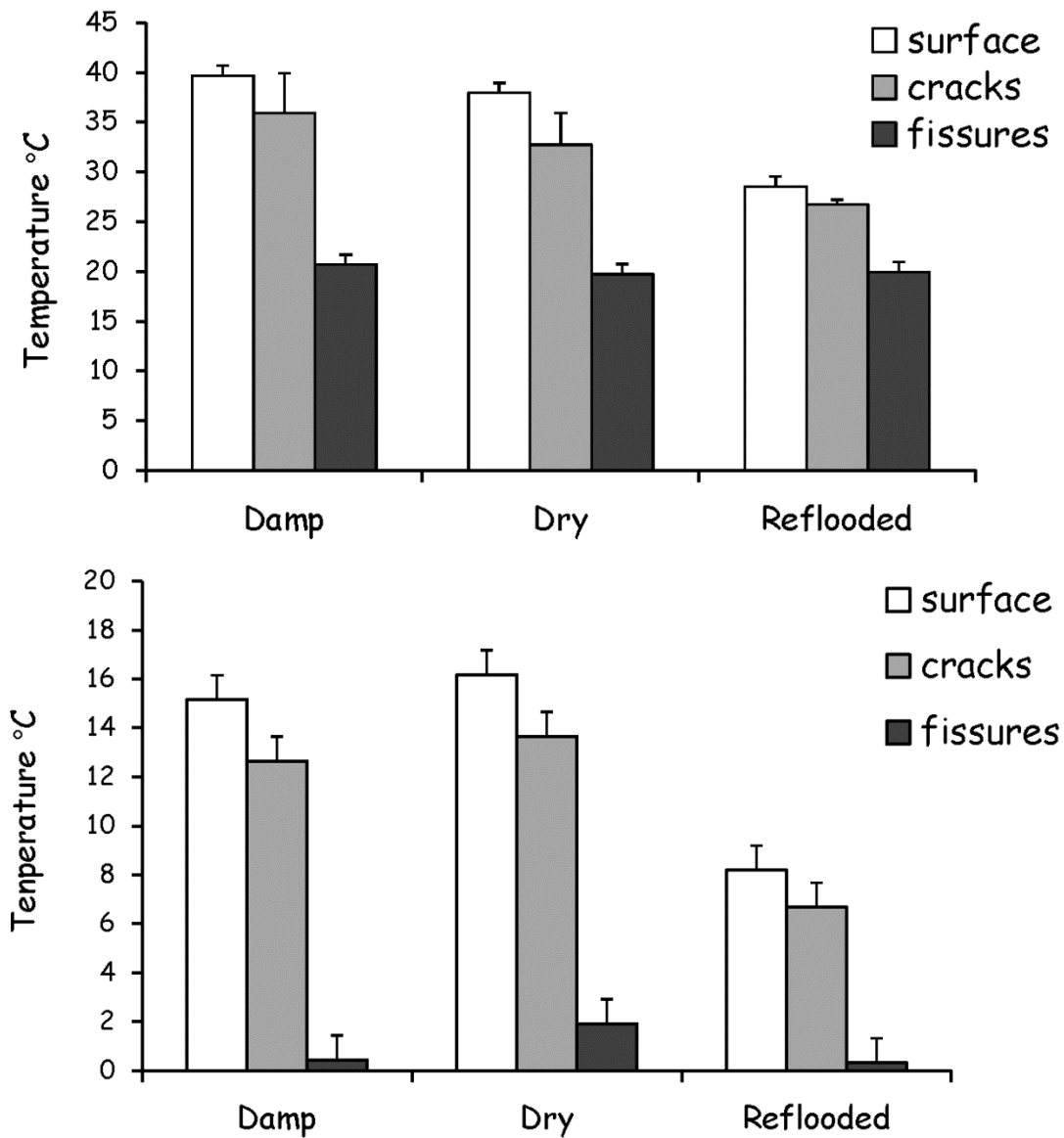


Figure 2. Temperature logger data within each microhabitat, $n = 3$, for each inundation phase: a) mean maximum daily temperature (+1 SE); b) mean 12-hour fluctuation (+1 SE). Temperatures within microrefuges differed at each phase: damp ($F_{2, 8} = 18.77$, $P = 0.002$), dry ($F_{2, 8} = 24.43$, $P = 0.001$) and reflooded ($F_{2, 8} = 20.15$, $P = 0.002$).

Invertebrate dynamics through the inundation cycle

Sixty-four taxa were recorded in samples from the three microrefuges during this study from a total of 97 129 individuals collected (Appendix 1). The fauna comprised cnidarians (*Hydra*), annelids, arthropods and a mollusc (*Physa acuta*). All were found in the refuges during the study (Table 1), but crustaceans dominated the sediment reflooded in the laboratory (Table 2). The almost complete turnover of the invertebrate assemblage between sampling times was the largest source of variation in species composition at South Lake (Global $R = 0.918$, $P < 0.001$, Fig. 3a). Sediment organic matter did not differ between inundation phases or microrefuges, so it probably had little effect on species turnover. Instead, the rapid species turnover, which occurred over an eight week period, coincided with marked changes in water level and temperature. Similarly, pairwise tests showed that the largest differences in composition were between the reflooded phase and the other phases (dry cf. reflooded: $R = 0.981$, $P < 0.001$; damp cf. reflooded samples: $R = 0.981$, $P < 0.001$). The difference in assemblage composition between damp and dry samples was not as large ($R = 0.782$, $P < 0.001$). This is because some invertebrates present in the reflooded phase were absent at the two earlier phases (Table 1), or were present only as dormant stages in the sediment, as shown by the composition of the resting stage samples (see below).

Species composition during the reflooded phase was dominated by invertebrates that emerged from the dry sediment, mostly microcrustaceans such as ostracods, cladocerans and copepods as well as gastropods (*P. acuta*) that were aestivating during the previous phase. The dominant insect taxa present in the microrefuges in the damp and dry phases were carnivores preying and scavenging upon stranded and dying invertebrates (Table 1). Mostly, the predators were rapidly completing their aquatic life stage to emerge as terrestrial adults before the wetland refilled. For example, we observed stratiomyid larvae foraging in surface depressions, preying on stranded and dying invertebrates. These insects, along with hydrophilid beetle larvae, were completing their larval stage. Some of these carnivores, such as the noterid and dytiscid beetles, were already adults, able to fly from place to place to feed.

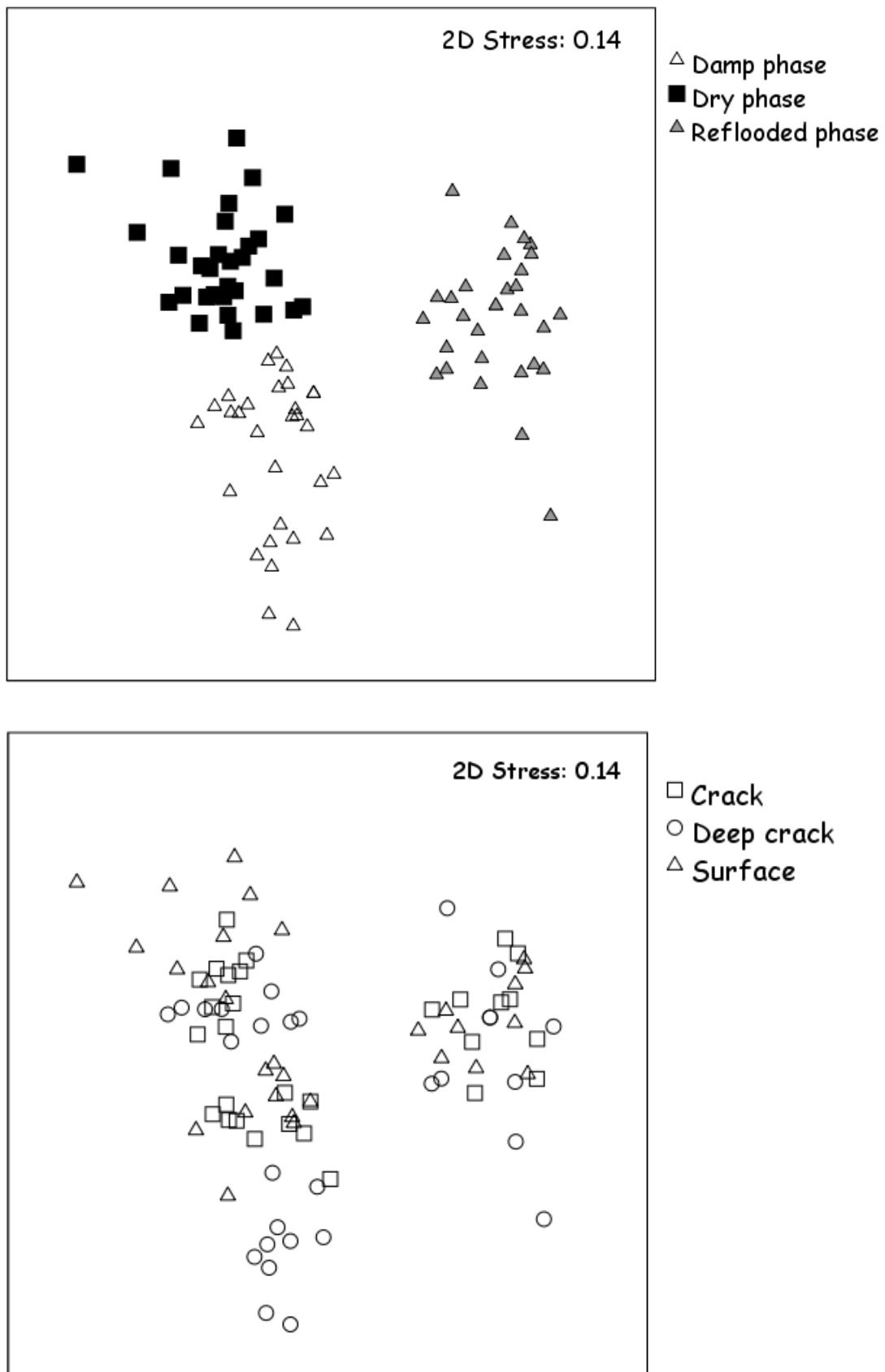


Figure 3. NMDS ordination plot of invertebrate presence/absence data (a) labelled by sampling time and (b) labelled by microrefuges.

Table 1. Dominant invertebrate taxa collected for each time and microrefuge, identified through SIMPER analysis. Taxa listed comprised approximately 90% of the differences between the microrefuges and times. Taxa are listed in descending order of dominance. Life history phase: larvae (L), adult (A) and pupae (P).

Water regime phase	Microrefuge	Dominant invertebrate taxa
Damp phase	Surface	Oligochaeta, <i>P. acuta</i> , Dolichopodidae (L), <i>M. ambiguosa</i> , Stratiomyidae (L), <i>Cyprretta</i> sp., <i>D. spinosa</i> .
	Cracks	<i>P. acuta</i> , Dolichopodidae (L), Stratiomyidae (L), <i>M. ambiguosa</i> , <i>Cyprretta</i> sp., Oligochaeta, <i>D. spinosa</i> .
	Fissures	<i>P. acuta</i> , <i>Cyprretta</i> sp., <i>T. australis</i> , <i>D. spinosa</i> , Oligochaeta, <i>M. ambiguosa</i> , <i>P. palustris</i> , <i>Paranisops</i> sp. (A), small damselfly (L), small dragonfly (L), Dytiscidae sp. (A) Orthocladiinae (L).
Dry phase	Surface	Ceratopogonidae (L), Noteridae (A), <i>P. acuta</i> , dipteran (P), Hydrophilidae (L), <i>D. spinosa</i> , Dolichopodidae (L), Orthocladiinae (L), <i>Cyprretta</i> sp.
	Cracks	<i>P. acuta</i> , Dolichopodidae (L), Ceratopogonidae (L), Orthocladiinae (L), Noteridae (A), <i>Cyprretta</i> sp., <i>D. spinosa</i> , <i>M. ambiguosa</i> , dipteran (P), Forcipomyiinae.
	Fissures	<i>Cyprretta</i> sp., Noteridae (A), <i>D. spinosa</i> , Ceratopogonidae (L), <i>M. ambiguosa</i> , Dolichopodidae (L), <i>P. acuta</i> , Forcipomyiinae, Orthocladiinae (L).
Reflooded phase	Surface	Oligochaeta, <i>P. acuta</i> , Ilyocryptidae sp., <i>D. spinosa</i> , Chydoridae spp., <i>B. australis</i> , <i>C. novaezelandiae</i> .
	Cracks	Oligochaeta, <i>P. acuta</i> , Ilyocryptidae sp., <i>C. novaezelandiae</i> , <i>B. australis</i> , <i>Cyprretta</i> sp., <i>D. spinosa</i> .
	Fissures	Oligochaeta, Ilyocryptidae sp., <i>P. acuta</i> , <i>Cyprretta</i> sp., <i>C. novaezelandiae</i> .

Table 2. Dominant taxa in the invertebrate resting stages samples (hatching experiment) for each time and microrefuge identified through SIMPER analysis. Taxa listed comprised approximately 90% of the differences between the microrefuges and times. Taxa are listed in descending order of dominance.

Time period	Microhabitat	Dominant invertebrate taxa
Three days	Surface	Collembola, <i>C. funebris</i> , <i>D. spinosa</i>
	Cracks	Collembola, <i>D. spinosa</i> , <i>C. funebris</i> , Oligochaeta
	Fissures	Collembola, <i>C. novaezelandiae</i> , <i>D. spinosa</i> , <i>C. funebris</i> , Oligochaeta
Twenty days	Surface	Collembola, Ilyocryptidae sp., <i>C. funebris</i>
	Cracks	Collembola, Calanoida, <i>P. acuta</i> , Ilyocryptidae sp., Oligochaeta
	Fissures	Ilyocryptidae sp., Collembola, <i>C. funebris</i> , Calanoida, Oligochaeta

Microrefuges for invertebrates

Assemblage composition also differed between microrefuges (Global $R = 0.217$, $P < 0.001$, Fig. 3b), but the microrefuges were more similar to each other at the same time than they were to the same microrefuge type at different times. Consequently, assemblages among microrefuges were analysed for each phase separately. In the damp phase when the wetland had just dried at the surface, assemblage composition differed between microrefuges (Global $R = 0.373$, $P < 0.001$), because the assemblage in the deeper fissures differing from that in the cracks ($R = 0.549$, $P = 0.002$) and the surface depressions ($R = 0.637$, $P = 0.001$). Shallow crack and surface assemblages did not differ ($R = -0.011$, $P = 0.51$, Fig. 3b). Surface depressions and cracks were characterised by the presence of the gastropod *P. acuta*, ostracod species and a variety of dipteran larvae including scavenging Stratiomyidae. The deeper fissures differed owing to the presence of isopods (*Paramphisopus palustris*), early instar odonates and backswimmers (*Paranisops* sp.) (Table 1).

In the dry phase when only the deeper fissures contained free water, there was no difference between assemblages in the microrefuges (Global $R = 0.08$, $P = 0.4$). There were fewer species of ostracod present and several types of dipteran larvae were common, along with adult Noteridae beetles (Table 1).

Oligochaetes, *P. acuta* and ilyocryptids (cladocerans) were abundant in all three microrefuges during the re-flooded phase (Table 1) but assemblage composition differed among the microrefuges (Global $R = 0.198$, $P < 0.001$) due to the different distribution patterns of three ostracod species. *Cypretta* sp. was only found in the fissures, whereas

Diacypris spinosa and *Bennelongia australis* were found in the depressions ($R = 0.438$, $P < 0.001$). All three species were found in the crack microrefuge, so it did not differ from either the surface or fissures (Table 1).

Some species, although not found in large numbers, showed interesting distribution patterns (Table 1). The shredding caddisfly *Triplectides australis* was recorded in the fissures during the damp phase, but were less frequent in the dry phase when they were present in the shallow cracks as well as the fissures. By the reflooded phase, they were largely absent. Similarly, carnivorous Ceratopogonidae larvae were found occasionally in the damp phase in the surface depressions and shallow cracks and in the dry phase were found frequently in all microrefuges. Adult noterid beetles were found in some samples from all microrefuges in the damp phase. In the dry phase, they were often found in samples from the fissures, but were absent by the reflooded phase. The isopod *P. palustris* was prevalent in the fissures and also occurred in low numbers in the surface and cracks in the damp phase, but in the dry phase were found only in the fissures.

There were changes in the dominant taxa that emerged from resting stages in the sediment between days 3 and 20 (Global $R = 0.162$, $P = 0.001$, Table 2). Collembola was the most abundant taxon at all times and microrefuges except for the deeper fissures on day 20. At day three, ostracods emerged from aestivation, dominating the samples. After 20 d, cladocerans and copepods had hatched from the egg bank and the gastropod *P. acuta* and the ostracod *Cypridopsis funebris* had emerged from aestivation (Table 2). Microrefuge types also differed (Global $R = 0.458$, $P = 0.001$): composition differed between surface depressions and crack samples ($R = 0.184$, $P = 0.001$), surface and fissure samples ($R = 0.171$, $P = 0.002$) and crack and fissure samples ($R = 0.135$, $P = 0.009$). Fewer species emerged from surface depressions than from cracks or fissures and *C. novaezealandiae* only emerged from fissure samples.

Discussion

Microrefuge characteristics

There was a large difference between both the mean temperature and range of temperatures in the microrefuges. Fissures provided a thermal refuge for invertebrates during the hottest and driest periods and more generally, a refuge for invertebrates intolerant of emersion. The cooler and very consistent temperatures in the deeper fissures arose because they are in darkness, but also due to upwelling groundwater, indicated by the presence of iron bacteria in the bottom of the fissures where deeper hypoxic water was upwelling (Emerson *et*

al., 2010). Importantly, the fissures in the lake bed persist through the year and do not completely close up, potentially creating a continuous link to the groundwater. Access to groundwater appears to be important for invertebrates without a terrestrial or desiccation resistant life stage, such as isopods (e.g. *P. palustris*) or amphipods, to take refuge from drying (Gouws & Stewart, 2007). Also, the temperature differences between the microrefuges persisted even when the whole lake bed was inundated, suggesting that animals may have access to lower temperature microhabitats during hot weather even in inundated wetlands. There may also be places outside fissures with more moderate temperature fluctuations, perhaps with significant shading provided by small-scale topography (Sim *et al.*, 2013).

Patterns of microrefuge use

Although we did not directly measure invertebrate movement, some of the patterns observed here are consistent with movement between microrefuges. For example, the ostracod *Mytilocypris ambigua* was found in all microrefuges in the damp phase but in the dry phase was present only in cracks and fissures, suggesting that individuals were moving into the cooler refuges, avoiding the surface depressions. Some larger crustaceans, such as isopods, burrow down towards the groundwater table when surface waters disappear (Williams & Hynes, 1976). The isopod *P. palustris* was prevalent in the surface depressions and shallow cracks in the damp phase, but in the dry phase they were only found in the deeper fissures, which are both the last and first place inundated. It appears probable that these isopods were actively using deeper fissures as a microrefuge during wetland drying. Some microcrustaceans such as ostracods can withstand drying by entering a state of quiescence, where they close their carapace and metabolic activity is lowered (Delorme, 1991). Emergence has been observed in sediment during damp periods but mostly occurs after refilling (Aguilar-Alberola & Mesquita-Jones, 2011) as observed here. With immature stages already in the dry sediment, these ostracods may have a developmental head start when the water returns (Horne, 1993).

Several insect taxa showed a distribution pattern consistent with a larval stage present during the drying phase, but absent by the time reflooding occurred, because they had emerged as flying adults. For example, predatory dolichopodid larvae were present at the surface and in shallow cracks in the damp and dry phases, but were not found in any of the microrefuges in the reflooded phase. Similarly, ceratopogonid larvae were found in all three microrefuge types in the dry phase only, preying upon the other macroinvertebrates. Orthocladiinae were present in the fissures in the damp phase, while in the dry phase they

were found in all of the microrefuges and in the reflooded phase were rare. The availability of stranded invertebrates as prey for predatory coleopteran and dipteran larvae makes the drying phase particularly suitable for these taxa by assisting them to complete their larval life stage (Boulton & Lake, 1992).

Some of the insects with carnivorous larvae that consume stranded and dying invertebrates on the sediment surface were uncommon. Predatory noterid beetle adults were present in all microrefuges in the damp phase, but were more common during the dry phase. As these beetles were absent when the wetland refilled, they were probably preying upon invertebrates trapped in the microrefuges. The predatory larvae of the Forcipomyiinae occurred only in the cracks and fissures in the dry phase. This dipteran sub-family is semi-terrestrial (Gooderham & Tsyrlin, 2002) and when water is scarce they complete their life cycle rapidly.

Predators such as odonates that inhabit temporary waterbodies may grow rapidly (Watson, Theischinger & Abbey, 1991; De Block, McPeck & Stoks, 2008) although deteriorating conditions in drying waterbodies may limit the growth rate of some species (De Block & Stoks, 2005). In the present study, small newly hatched damselflies and dragonflies were found frequently only in cool, wet fissures during the damp phase; no odonates were found after this time. This suggests either that larvae of these species grew quickly and emerged in the four weeks before the next samples were taken, or that they were consumed by predators (Morin, 1984). Some potential predators of juvenile odonates such as adult *Paranisops* and diving beetles (Dytiscidae) were also only found in the fissures during the damp phase and may have consumed the odonate larvae.

Some non-predatory insects also apparently moved into the fissure microrefuges in the dry phase. For example, larvae of the caddisfly *T. australis* were present in the surface depressions and shallow cracks in the damp phase but in the dry phase were mostly found in deeper fissures and were absent from surface depressions. Larvae probably pupated in the fissures and emerged prior to reflooding (e.g. Jannot, 2009), explaining their absence from reflooding samples. Oviposition may be triggered by rain (St Clair, 1993) and may occur during reflooding.

The role of resting stages in the sediment

Resting stages in wetland sediments have previously been identified as a major source of recolonists when inundation occurs (Brock *et al.*, 2003; Jenkins & Boulton, 2007; Tuckett *et al.*, 2010), especially the microcrustacea. Ehippia (a thickened section of the carapace that

protects the eggs of Cladocera; Williams, 1988) were present in every microrefuge during the damp and dry phases but were less common in samples taken in the reflooded phase. This correlated with the appearance of cladocerans in both the 20 day resting stage samples and the reflooded microrefuges. Although these cladocerans (Ilyocryptidae) were found in the field only in the reflooded phase, they were present in all microrefuges, indicating that they had survived the dry period as desiccation-resistant eggs. Some ephippia were still present in the reflooded samples and had not hatched. This may have been because they were buried and did not receive their hatch cue (Alekseev *et al.*, 2007), or because of a form of cohort splitting that relies on prolonged diapause (Robson *et al.*, 2011). Similarly, invertebrate assemblages emerging from dry phase sediment inundated in the laboratory differed between three and 20 day periods of inundation, because of different hatching times among taxa (Paltridge *et al.*, 1997; Brock *et al.*, 2003).

Two ostracod species, *Candonocypris novaezelandiae* and *Bennelongia australis* (both from the family Cyprididae) were also found commonly only during the reflooded phase. Some ostracods in temporary waters use a form of diapause, where development of the young does not follow immediately after deposition of the eggs and may be delayed for up to several years if conditions are unfavourable (Williams, 1988). We observed hatching of *C. novaezelandiae* eggs in the resting stage samples although, oddly, *B. australis* was not also found. Possibly, *B. australis* eggs were not laid in the microrefuges we sampled or we did not replicate the necessary hatching cue. Four other ostracod species (*Cyprretta* sp., *M. ambigua*, *D. spinosa*, *C. funebris* (all Cyprididae)) closed their carapace and aestivated in response to drying and then emerged from dormancy when reflooded. Thus, ostracod species from the family Cyprididae present in South Lake showed two alternative strategies for surviving drying.

The role of wetland microrefuges in a drying climate

Most taxa present during the drying phase were more common in shallow cracks and/or deeper fissures than in surface depressions. Recent studies of other seasonal wetlands on the Swan Coastal Plain show that the climatic drying is causing shorter hydroperiods in seasonal wetlands and that some are no longer inundated by groundwater (Sim *et al.*, 2013). Although Sim *et al.* (2013) did not observe significant aridification of this fauna, some taxa are at risk from declining groundwater tables. Gouws & Stewart (2007) identified the endemic isopod *P. palustris* as at risk from groundwater decline, given its poor dispersal capacity, dependence on aestivation and high genetic diversity within the region. Our results

support the proposition that *P. palustris* depends on connections to the groundwater, because we observed it only within deep fissures when surface water was absent from South Lake. This species and others with similar traits are likely to be negatively affected by further regional drying.

A few studies have now described the mechanisms by which stream and river fauna survive drying, focussing on the interaction between species traits and potential refuge habitat (e.g. Sheldon *et al.*, 2010; Chester & Robson, 2011; Stubbington & Datry, 2013). It is well known that some wetland invertebrates aestivate or produce eggs resistant to desiccation in response to the loss of surface water (Brock *et al.*, 2003; Jenkins & Boulton, 2007). However, other responses to drying by wetland invertebrates are less well known, as are the interactions between species responses and environmental conditions in wetlands (Sim *et al.*, 2013). This study shows that small-scale dynamics occur between microhabitats and invertebrates in wetlands, just as they do in streams. Furthermore, these dynamics occur over a relatively short time period (eight weeks). Further studies of wetland microhabitats are needed to understand the role of different types of sediment and vegetation on the ability of species to resist desiccation, and to identify microrefuges in wetlands. Knowledge of the types and distribution of microrefuges used by wetland species is necessary to manage wetland biodiversity across mosaics of intermittent and perennial wetlands (Sim *et al.*, 2013). Changes to water regimes along with human activities that affect the structure of sediment or vegetation in wetlands will affect the range of microrefuges available to invertebrates during annual drying, and may thereby alter wetland biodiversity.

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Appendix 1. Species list of invertebrates found in this study (adult (A), juvenile (J), larvae (L)).

Cnidaria

Hydra

Oligochaeta

Mollusca

Physa acuta

Ferrissia sp.

Arthropoda, Arachnida

Pionidae sp.

Piona cumberlandensis

Oribatida sp. 1 (A, J)

Unknown mite

Arthropoda, Crustacea, Copepoda

Calanoida

Cyclopoida

Harpacticoida

Arthropoda, Crustacea, Cladocera

Chydoridae,

Ilyocryptidae,

Arthropoda, Crustacea, Ostracoda

Bennelongia australis

Candonocypris novaezelandiae

Cypridopsis funebris,

Cyprretta sp.

Diacypris spinosa

Mytilocypris ambigua

Ilyodromus sp.

Arthropoda, Crustacea, Amphipoda

Austrochiltonia subtenuis

Arthropoda, Crustacea, Isopoda

Paramphisopus palustris

Arthropoda, Collembola

Arthropoda, Insecta, Coleoptera

Dytiscidae sp. (A)

Dytiscidae sp. (L),

Berosus sp. (A),

Berosus sp. (L),

Noteridae sp. (A)

Hydrophilidae sp. (L)

Limnichidae (A)

Haliplidae sp. (L)

Unknown Beetle (A)

Arthropoda, Insecta, Diptera

Chironomidae

Orthoclaadiinae (L)

Chironominae (L)

Culicidae

Culex (L)

Ceratopogonidae

sp. 1 (L)

sp. 2 (L)

Forcipomyiinae sp. (L)

Dolichopodidae sp. (L)

Stratiomyidae sp. (L)

Syrphidae sp. (L)

Unidentified Dipteran (L)

Arthropoda, Insecta, Hemiptera

Corixidae

sp. 1 (J)

Micronecta robusta (A, J)

Sigara sp. (A)

Notonectidae

sp. 1 (J)

Paranisops sp. (A)

Gerridae sp. (A)

Nepidae sp. (A)

Arthropoda, Insecta, Odonata

Anisoptera (L)

Zygoptera (L)

Arthropoda, Insecta, Trichoptera

Leptoceridae

Notalina sp. (L)

Triplectides australis (L)