Report on the October 2008 sampling of the Aquatic Invertebrate Assemblages of Mound Springs of the Three Springs Area Threatened Ecological Community

Prepared for Species and Communities Branch, Nature Conservation Division, Department of Environment and Conservation

Adrian Pinder and Anna Leung
Science Division, Department of Environment and Conservation, Western Australia

August 2010

INTRODUCTION

A series of 30 springs at the headwaters of some upper tributaries of the Arrowsmith River, located about 30km west of the town of Three Springs, were listed as a Threatened Ecological Community in 2002 (Assemblages of the Organic Mound Springs of the Three Springs Area). A key element of the nomination was the invertebrate communities that include a unique combination of groundwater species, species more typical of the higher rainfall south-west and species believed to be regionally restricted and/or uncommon (Pinder and Pennifold 2001; Pinder *et al*. 2006). An interim recovery plan for these springs (Rees and Broun 2005) recommended ongoing survey and monitoring and a monitoring plan was produced by Pinder *et al*. (2009). In August 2008 these springs were sampled as part of the Significant Species and Communities component and the Inland Aquatic Integrity component of the State-wide Resource Condition Project. A review of the character and biodiversity values of these springs is being produced for the Inland Aquatic Integrity project (DEC in prep). The primary aim of the new sampling was to determine if there had been changes to the water chemistry and the richness and composition of the invertebrate assemblages.

METHODS

Sampling

Five of the mound springs were sampled in October 2008, all of which were sampled in August 2001 and four of which were sampled in March 2001 (Table 1). Methods used were generally the same as in 2001 and are outlined in Pinder *et al*. (2009). The actual habitats available to sample at a spring vary between visits, depending on surface and subsurface moisture and discharge, so it is not always possible to sample the same habitats or locations within a particular spring. Figure 1 shows the location of the five sites sampled in 2008.

The mound springs were given TST (**T**hree **S**prings **T**umulus spring) codes by the authors of the reports on the 2001 sampling events, whereas they were given MSTS (**M**ound **S**prings of the **T**hree **S**prings area) codes in DEC's Threatened Ecological Community database. Table 1 has both sets of codes, but the MSTS codes are otherwise used throughout this report.

Table 1. Site names and sampling effort per spring. TST codes are as used in the DEC wetlands research database and in previous reports. MSTS codes are as used in the DEC Species and Communities Branch TEC database.

Species and Communities occurrence code (MSTS code)	TST site code	Number of samples March 2001	Number of samples August 2001	Number of samples October 2008
1	1	1 (combined from two excavated holes)	3	3
5	3	1 (combined from two excavated holes and flooded leaf-filled hollow)	3	2
2	5	1	2	1
11	6	1	0	0
14	10	0	1	0
13	13 (=TST 'Big')	0	2	2
12	20 (=TST 'A')	1	2	1

Figure 1. Satellite image showing sites sampled in October 2008.

MSTS1 (=TST001)

Habitat	Sampled in Mar 2001 (sample #)	Sampled in Aug 2001 (sample #)	Sampled in Oct 2008 (sample #)
Open water pool	No	Yes (1)	Yes (1)
Interstitial water from excavated hole in sedges	Yes (1)	Yes (3)	Yes (3)
Free water amongst flooded sedges	No	Yes (2)	Yes (2)

2008 sample 1: 50 µm and 250 µm mesh sweep net samples from open water. Samples combined in lab.

2008 sample 2: 50 µm sample from water in flooded vegetation.

2008 sample 2: Sample scooped from excavated hole and passed through 50 µm mesh net.

Water sample from same site as invertebrate sample 1.

MSTS2 (=TST005)

Habitat	Sampled in Mar 2001 (sample #)	Sampled in Aug 2001 (sample #)	Sampled in Oct 2008 (sample #)
Interstitial water from excavated hole	Yes (1)	No	No
Mud and water from	No	Yes (1)	Yes (1)

flooded sedges			
Small natural pool	No	Yes (2)	No

2008 sample 1: Mud and water from flooded sedges passed through a 50 µm mesh net.

Water sample from same site.

MSTS5 (=TST003)

Habitat	Sampled in Mar 2001 (sample #)	Sampled in Aug 2001 (sample #)	Sampled in Oct 2008 (sample #)
Small flooded pool on west side of mound	Yes but combined with 1	Yes (1)	Yes (1)
Small flooded pool on north side of mound	No	No	Yes (2)
Inundated Baumea (?on south-east side)	No	Yes (2 and 3)	No
Composite of several habitats including excavated hole and inundated leaf litter	Yes (1)	No	No

2008 sample 1: 50um and 250um sample from open water in pool 1

2008 sample 2: 50um and 250um sample from open water in pool 2

Water sample from pool 1.

MSTS11 (= TST006): Not sampled in Oct 2008

MSTS12 (= TST020)

Habitat	Sampled in Mar 2001 (sample #)	Sampled in Aug 2001 (sample #)	Sampled in Oct 2008 (sample #)
Small flooded pool on south-west side of mound	Yes (1)	Yes (1)	Yes (1)
Interstitial water from excavated hole	Yes (combined with 1)	Yes (2)	No (not enough interstitial water)

2008 sample 1: 50 µm mesh net used.

Water sample from same site.

MSTS13 (=TST013)

Habitat	Sampled in Mar	Sampled in Aug	Sampled in Oct 2008

	2001 (sample #)	2001 (sample #)	(sample #)
Water trickling from mound in to small pool	No	Yes (1)	Yes (1)
Small pool		Yes (2)	Yes (2)

2008 sample 1: 50 µm mesh net used.

2008 sample 2: 50 µm mesh net used.

Water sample from pool.

Data analysis

Primer v6.1.11 (Primer-E 2008) was used to perform non-metric multidimensional scaling ordinations, using the Bray-Curtis similarity measure, 100 re-starts and Kruskal fit scheme 1. A permanova analysis of differences in community composition between years was also performed using Primer and Permanova+ v1.0.1 (Primer-E 2008).

RESULTS

Water chemistry

Table 2 contains all water chemistry data collected for these springs over the three sampling rounds. In 2008 pH varied between 5.59 (at MSTS5) and 7.68 (at MSTS1), just outside the range of 6.1 to 7.19 recorded in 2001. Field measured electrical conductivity/salinity and laboratory measured total dissolved solids are all about the same as recorded in the same springs in 2001. Most of the sampled springs are fresh (TDS < 1 g/L), with the exceptions of MSTS14 which was fresh but with slightly higher salinity (1.7 g/L) in August 2001 and MSTS13 which was brackish in August 2001 (~ 5.5 g/L) and marginally fresh (1.98 g/L) in October 2008.

Total persulphate nitrogen and phosphorus is measured from an unfiltered water sample so includes dissolved forms (e.g. nitrates, ammonia), organic forms (proteins etc.), forms bound to (or within) particulates and suspended solids and that assimilated within planktonic biota. This gives an indication of the total amount of nutrients within the water column, including in living tissues. Filterable nitrogen and phosphorus is measured from a filtered water sample so excludes N and P incorporated within particulates, suspended solids and organisms (i.e. just dissolved and organic chemical forms). On some occasions filterable nitrogen has been measured as two forms (nitrate/nitrite and ammonia) but this then excludes dissolved organic nitrogen. In monitoring programs these are often used together since uptake and release of nutrients by organisms affects the concentrations of dissolved nutrients.

Nitrogen and phosphorus concentrations are generally low in these springs, although there was an increase in the concentration of total nitrogen between August 2001 and October 2008 at all sampled springs except for MSTS1. Most measurements of total nitrogen have

been between about 300 and 1000 ug/L but much higher values were recorded at MSTS12 and MSTS13 in October 2008 (6300 and 3100 ug/L respectively). For MSTS12 most of the nitrogen was in non-dissolved form so the high concentration was largely due to suspended particulates or planktonic organisms, although plankton abundance was low in the pool from which the water sample was taken and the turbidity value of 93 NTU does not indicate excessive suspended sediment. For MSTS13 about half of the nitrogen was in dissolved forms. These two springs also had much higher total phosphorus concentrations in 2008 (170 ug/L and 190 ug/L respectively) than in 2001. As for nitrogen, most of the phosphorus in MSTS12 sample was in non-dissolved forms whereas at MSTS13 all of the phosphorus was in dissolved form (although this result is unusual and may reflect an error in sample processing, analysis or reporting). MSTS14 also had very high nitrogen and phosphorus concentrations in August 2001 (5200 and 300 ug/L respectively) but this site was not sampled in 2008.

The cause of the particularly high nutrient concentrations in some springs is not clear. The spring complex is set within an agricultural landscape and direct (via drift) or indirect (via groundwater) contamination is a possibility. However, periodically high nutrient concentrations may also result from complex absorption and release processes associated with flooding and drying of wetland soils. Most of a wetland's nutrient load is usually bound within sediments, but can be periodically released into the water column. Also, water samples have not always been taken from the same locations (due to habitat availability) within the springs, and this may be a source of variation in the data. Further monitoring will be required to determine if nutrient concentrations are increasing or just fluctuating as a result of natural processes. In any case, there is no evidence of increased algal growth, probably because the springs are highly shaded.

Invertebrates

Species richness and diversity

Seventy four species of aquatic invertebrate were identified from the five springs sampled in October 2008 (Appendix 1). This is higher than the total richness for Mar 2001 (59 species from 4 springs) and only slightly lower than richness obtained in Aug 2001 (80 species from 6 springs). Twenty two of the species collected in 2008 were not recorded in 2001, bringing the total list to 124 species (excluding those only recorded in the excavated MSTS11 [=TST006] in March 2001).

Pinder and Pennifold (2002) and Pinder and Stratford (2006) highlighted some invertebrate species as being of special interest and contributing to the unique composition of the invertebrate communities of these springs. Many of these were collected again in 2008. These are phreodrilid oligochaetes (not identified in this survey due to lack of mature specimens), syncarid crustaceans, darwinulid and candonid ostracods, the mosquito *Culiseta atra*, harpacticoid copepods sp. 2 and sp. 6, orthoclad chironomid sp. I, the dragonflies *Archaeosynthemis occidentalis* and *Archiargiolestes pusillus* and caddisfly *Notoperata tenax*. Some additional species can be added to this list, as follows.

Candonid ostracod sp. 2. Candonid ostracods have been recorded from four of the mound springs. Previously collected specimens have all been identified as *Candona* sp. or *Candonopsis tenuis*, although it is recognised that the generic identity of the *Candona* is

uncertain. Two candonid species were collected from TST001 in 2008. One of these is probably the same '*Candona*' as collected in the past, but one specimen clearly belongs to another genus. As there is only a single specimen of the latter it has not been dissected, but will be sent to an ostracod taxonomist for identification.

Orthocladiinae sp. Q. This chironomid appears to be rare but widespread in the south-west, with the two previous DEC records being from a freshwater farm dam near Tardun (northern Wheatbelt) and a perched sedge swamp near Kojonup (Ngopitchup Swamp). There are likely to be other records of this species collected by other research groups but under a different name if published.

Table 2. All water chemistry data for the three sampling occasions (excluding the highly modified MSTS11)

	MSTS1 (TST001)			MSTS5 (TST003)			MSTS2 (TST005)			MSTS14 (TST010)	MSTS13 (TST013)		MSTS12 (TST020)		
	Mar-01	Aug-01	Oct-08	Mar-01	Aug-01	Oct-08	Mar-01	Aug-01	Oct-08	Aug-01	Aug-01	Oct-08	Mar-01	Aug-01	Oct-08
pH (measured in field)	6.72	6.1	7.68	6.27	6.2	5.59	6.62	6	6.32	6.2	6.5	6.66	7.19	6.8	6.61
electrical Conductivity (EC) (uS/cm)	1687	1348	1357	1430	2160	1227	1216	1409	1295	3530	8020	3650	808	775	762
temperature (^{0}C)	-	11.1	16.4	-	14.8	8.5	-	16.2	14.2	14.1	12.8	15	-	10.7	19.1
salinity (g/L) converted from EC in meter	0.6	0.4	0.7	0.5	0.9	0.63	0.3	0.5	0.66	1.7	5.6 #	1.98	0.1	0.1	0.39
turbidity (NTU)	30	25	0.9	700	0.4	20	800	0.5	2.3	52	69	87	4.5	0.4	93
colour (TCU)	46	150	25	8	120	14	66	210	190	41	71	17	150	62	250
total dissolved solids (g/L)	0.88	0.7	0.75	0.88	1.2	0.76	0.76	0.9	0.7	0.7	1.7	2.1	0.47	0.4	0.42
alkalinity (mg/L)	30	33	25	1	45	30	15	30	30	<2	45	55	38	30	20
hardness (mg/L)	140	99	93	110	130	-	100	95	-	140	240	-	61	42	-
iron (mg/L)	20	3.8	-	12	2.9	-	35	0.3	-	0.1	2.9	-	0.09	<0.05	-
silica (mg/L)	45	51	52	162	78	49	21	45	44	36	70	78	35	29	41
sodium (mg/L)	283	213	201	191	360	191	223	282	210	198	551	589	140	131	117
calcium (mg/L)	6	3	3	4	4	-	7	7	3	7	7	-	4	2	-
magnesium (mg/L)	30	22	20.7	24	29	19.7	21	21	14.4	29	53	71	12	9	5.9
potassium (mg/L)	19	17	15.2	16	23	14.6	21	16	13	11	32	34.5	11	9	11.2
chloride (mg/L)	470	330	355	320	540	329	350	420	344	320	880	1060	200	180	192
bicarbonate (mg/L)	37	40	31	<2	55	37	18	37	37	<2	55		46	37	24
carbonate (mg/L)	<2	<2	<1	<2	<2	<1	<2	<2	<1	<2	<2	<1	<2	<2	<1
sulphate (mg/L)	23	31	35.1	162	46	35.4	61	46	35.1	76	83	110	43	43	25.1
filterable reactive phosphorus (µg/L)	10	10	<10	10	10	10	10	30	10	10	10	190?	10	10	20
total persulphate phosphorus (µg/L)	10	30	5	-	20	90	-	10	20	300	20	190	10	70 *	170
ammonia nitrogen (µg/L)	70	40	-	250	40	-	230	10	-	30	10	-	50	40	-
nitrate/nitrite (µg/L)	40	60	<10	10	40	-	40	20	-	20	30	-	30	20	-
filterable nitrogen (µg/L)	-	-	180	-	-	140	-	-	150	-	-	1400	-	-	870
total persulphate nitrogen (µg/L)	510	990	320	-	680	900	-	70	290	5200	490	3100	920	930	6300

* = wrongly entered as 930 µg/L in Pinder *et al*. (2006)

= estimated from conductivity

***Culex* (*Neoculex*) *latus*.** This rare mosquito is a south-western Australian species that primarily inhabits swamps. During DEC projects we have recorded it only from far south-west wetlands and The Department of Health (DoH) and The University of Western Australia's Arbovirus Research Laboratory (ARL) have no records of this species north of Perth (Cheryl Johanson, ARL pers. comm.). This species was recorded from the degraded MSTS11 in March 2001 and from MSTS1 in October 2008. However, the single specimen from MSTS1 was damaged, so the identification is tentative at this stage.

***Coquilletidia* nr *linealis*.** A common mosquito in south-western Australia, but apparently rare north of Perth. DoH/ARL has a few records of this species from near Watheroo (Cheryl Johanson, ARL pers. comm.) and DEC has a record from a freshwater lake near Dowerin. This species was collected from MSTS1 and MSTS55 in August 2001 and from MSTS5 in October 2008.

Table 3 lists all of those species that particularly contribute to the unique invertebrate community composition of these springs. Taxonomic impediments limit understanding of the conservation significance of some of these species. However, the 2008 sampling confirms that these springs have an unusually high number of species that are rare (or at least rarely encountered) in the region or broader south-west and which would be unlikely to occur in this combination elsewhere. Almost of these are south-western Australian endemics.

It should be noted that the record of *Setodes* caddisflies from MSTS2 in March 2001 is almost certainly a mis-identification. The specimens were very juvenile and therefore not identifiable. It is extremely unlikely that these are *Setodes*.

Figure 2 shows the number of invertebrate species collected from each spring and all springs combined for the three sampling occasions. Patterns over time are not consistent across the springs, but samples collected in October 2008 were generally as rich as those collected in August 2001 with most variation being within expected ranges of natural variation. There is certainly no indication of a general decline in richness. The largest differences between sampling events appear to be related to the diversity of habitats available for sampling. For example, at MSTS2 richness was much greater in August 2001 (when both flooded sedges and a small open pool were sampled) than in either March 2001 or October 2008 (when sampling was restricted to interstitial water or flooded sedges respectively). Similarly, at MSTS12 the lower number of species collected in March 2001 probably reflects the smaller area of open water present in that season compared to August 2001 and October 2008.

Table 3. Invertebrate species that are rare (at least in the northern agricultural region) and which significantly contribute to the uniqueness of the mound springs invertebrate assemblages.

Group	Species of special interest occurring in the mound springs	Comments
Oligochaeta	*Antarctodrilus horwitzi*	Geographic outlier from core range - otherwise known only from far south-west.
	Phreodrilid WA3	Geographic outlier from core range - otherwise known only from far south-west or Lesmurdie Falls.
	Tubificidae WA28 (ex *Pristina* sp. 1)	Of unknown identity due to lack of mature specimens, but very similar worms found in two groundwater samples from the Pilbara, and from Jimperding Brook near Toodyay.
Water mite	*Austrotrombella* water mites	Rare, known only from a few northern agricultural zone springs/spring fed streams. Only other species from South Australia.
Syncarids	Syncarida/Bathynellidae	Groundwater species - identity uncertain.
Copepods	Harpacticoida sp. 2	Uncommon, few records in south-west swamps, Yerina Spring (Hutt catchment) and one (possible) record from a permanent Pilbara river pool.
	Canthocamptidae sp. 6	Three springs mound springs plus one record from a dammed granite rock pool in the mid-West.
	Microcyclops sp. S1	Known only from Three Springs mound springs to date - but some taxonomic uncertainty.
Ostracods	Darwinulid ostracods	Groundwater species - identity uncertain.
	Candonid ostracod sp. 1	Groundwater species - identity uncertain.
	Candonid ostracod sp. 2	Groundwater species - identity uncertain.
Chironomids	Pentaneurini sp. F	Known only from Three Springs mound springs to date - may have been found elsewhere by other research groups
	Genus "woodminer"	Geographic outlier from core range - otherwise known only from higher rainfall south-west.
	Orthocladiinae sp. I	Only known from springs in northern agricultural region (Three Springs mound springs and from 3 springs/spring fed creeks in Hutt Catchment) and from lakes swamps near south-coast/Muir-Unicup.
	Orthocladiinae sp. Q	Apparently rare - DEC records from Ngopitchup Swamp (nr Kattanning), reservoir near Tardun and MSTS5.
Mosquitoes	*Culiseta atra*	Common in south-west in suitable habitat (tannin stained water with decaying leaves) but in northern agricultural region known only from Three Springs mound springs and from Yarder Gully (Hutt catchment – Quinlan *et al.* 2009).
	Culex (Neoculex latus)	An apparently rare species. MSTS populations geographic outliers from core range – higher rainfall south-west in swamps.
	Coquilletidia nr *linealis*	A common south-west species, but apparently rarer north and east of Perth.
Dragonfly	*Archaeosynthemis occidentalis*	Geographic outlier from core range - higher rainfall south-west in boggy streams, seepages and swamps. Recently collected in a spring in the Hutt river catchment (Quinlan *et al.* 2009).
Damselfly	*Archiargiolestes pusillus*	Uncommon north of Perth - fairly common in higher rainfall south-west in boggy streams, seepages and swamps, also occurred in two Carnarvon Basin pools, Cockleshell Gully (SAP survey) and Feast Soak in the Hutt Catchment (Quinlan *et al*. 2009). A record from a brackish site in Meckering (DEC data) is probably a mis-identification.
Caddisfly	*Notoperata tenax*	Geographic outlier from core range - otherwise known only from higher rainfall south-west streams and swamps.

Figure 3 shows the richness of those species that are particularly rare or which are biogeographic outliers and which especially contribute to the unique nature of the assemblages (listed in Table 3). As for the whole assemblages, there is no consistent trend in the richness of these species over time at individual springs, but there is some indication that changes in richness represent varied sampling effort at some sites. MSTS1 consistently had the highest number of these species and total richness across the springs was higher in October 2008 than in either of the 2001 sampling occasions.

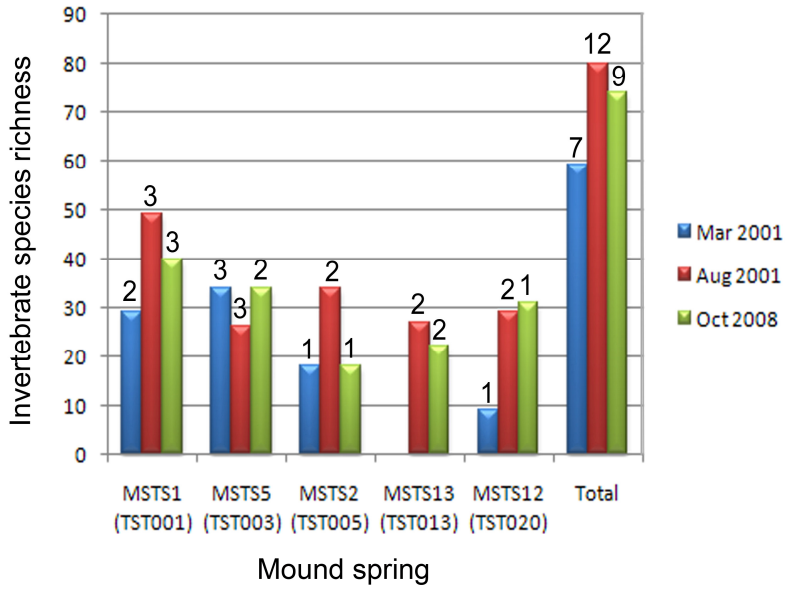

Figure 2. Richness of aquatic invertebrate communities present in samples from mound springs in the three sampling occasions. Only those springs sampled in 2008 included. Numbers above bars indicate number of samples collected. The stream flowing out of MSTS1 sampled in March 2001 was excluded.

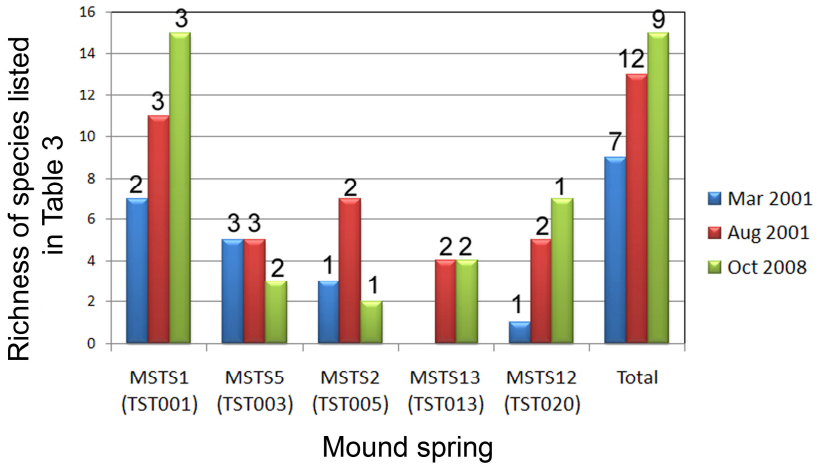

Figure 3. Richness of species listed in Table 3 in samples from mound springs in the three sampling occasions. Only those springs sampled in 2008 included. Numbers above bars indicate number of samples collected. The stream flowing out of MSTS1 sampled in March 2001 was excluded. Last column is for all springs combined.

Figure 4 shows the richness of all invertebrates in three habitats within individual springs in August 2001 and October 2008. Samples from different habitats collected in March 2001 were combined prior to sampling so cannot be displayed in this way. Invertebrate richness in open water habitats was higher in 2008 than in 2001, whereas richness in interstitial habitats was about the same in both years and in sedges richness was lower in 2008. These results should be viewed with caution given the low sample sizes for some of the habitats.

Cumulative richness of invertebrate assemblages within the five springs that have been sampled three times is shown in Figure 5. The total number of species recorded from each spring is still increasing with additional sampling, linearly for MSTS1 and MSTS12. This suggests that additional sampling will continue to increase the number of species recorded from each spring. The continuing increase in total richness at individual springs could be due to non-detection of species on a single visit or to immigration from other springs or other wetlands. Non-detection of species could reflect limited sampling or to presence of only propagules or juvenile life stages of in earlier sampling. Immigration is likely for some insects and phoretic water mites. As the number of species recorded from individual springs increases we get a better understanding of the differences in community composition between springs. Additional sampling is revealing that the springs are more similar in the species they support over time than is suggested by single sampling events (Figure 6). While this is entirely expected, the rate of increase in similarity between springs is declining with each sampling event, suggesting that there may be some real differences in composition between springs that are not artifacts of limited sampling.

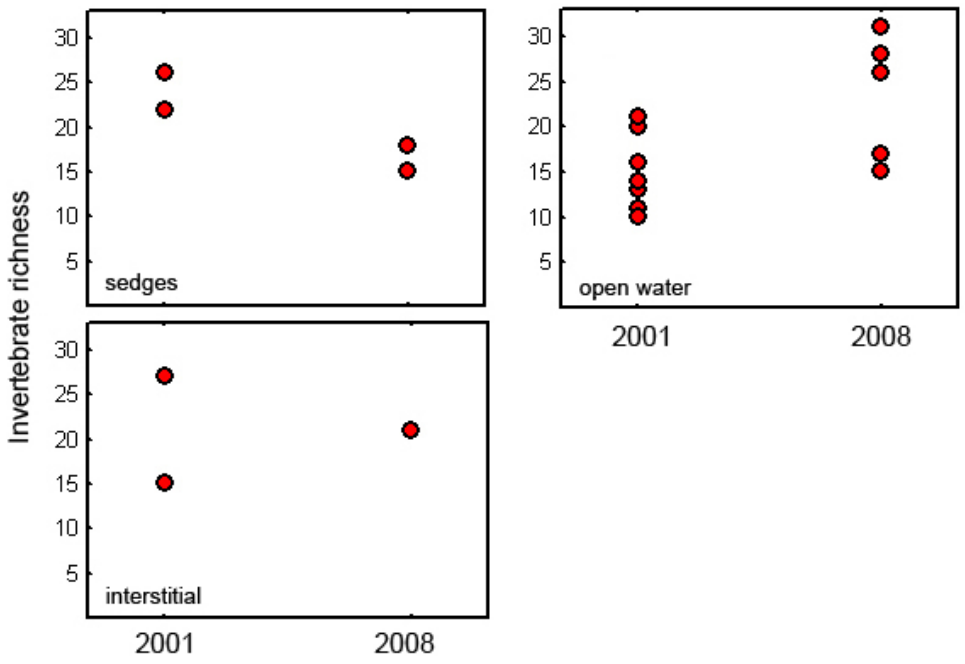

Figure 4. Richness of invertebrates in August 2001 and October 2008 in three habitats (sedges, interstitial water and open water).

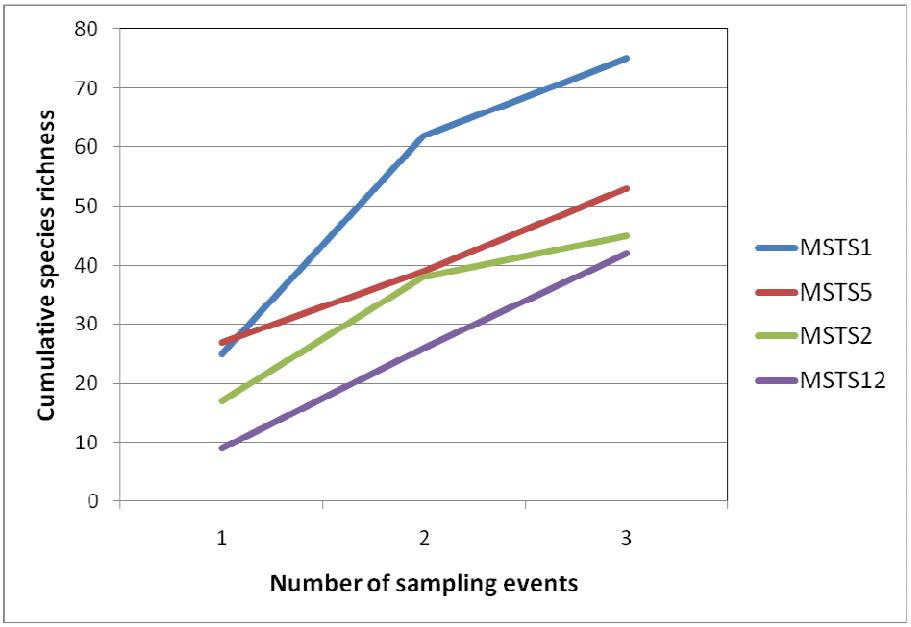

Figure 5. The cumulative number of species recorded at five springs over the three sampling events (March 2001, August 2001 and October 2008). The stream flowing from MSTS1 is excluded.

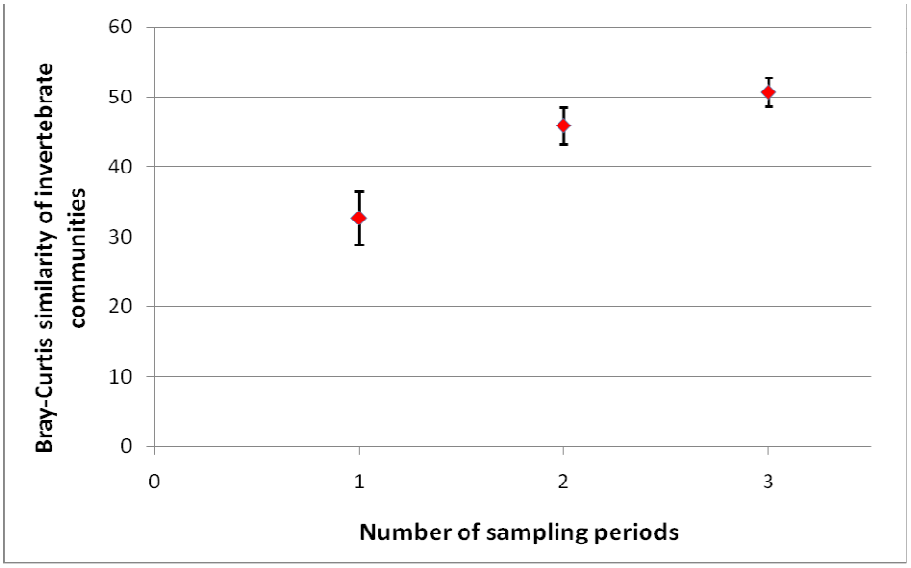

Figure 6. Changes in the similarity of cumulative invertebrate assemblage composition between springs with increasing sampling effort (number of sampling periods).

Community composition

An ordination of samples collected in August 2001 and October 2008 is shown in Figure 7. There is little indication in these graphs of consistent differences in community composition between habitats, although some habitats are poorly represented. Although the 2001 and 2008 samples are indicated by different colours on this plot, for most habitats there are too

few samples to make a meaningful comparison between years. The only habitat type sampled more than three times in both years is 'open water'. A separate ordination of just the open water samples shows little separation of samples collected in 2001 from those collected in 2008 (Figure 8) and a permanova analysis suggested no significant difference in community composition in this habitat between years (pseudo-F = 1.14, d.f. = 1,9 and p = 0.32).

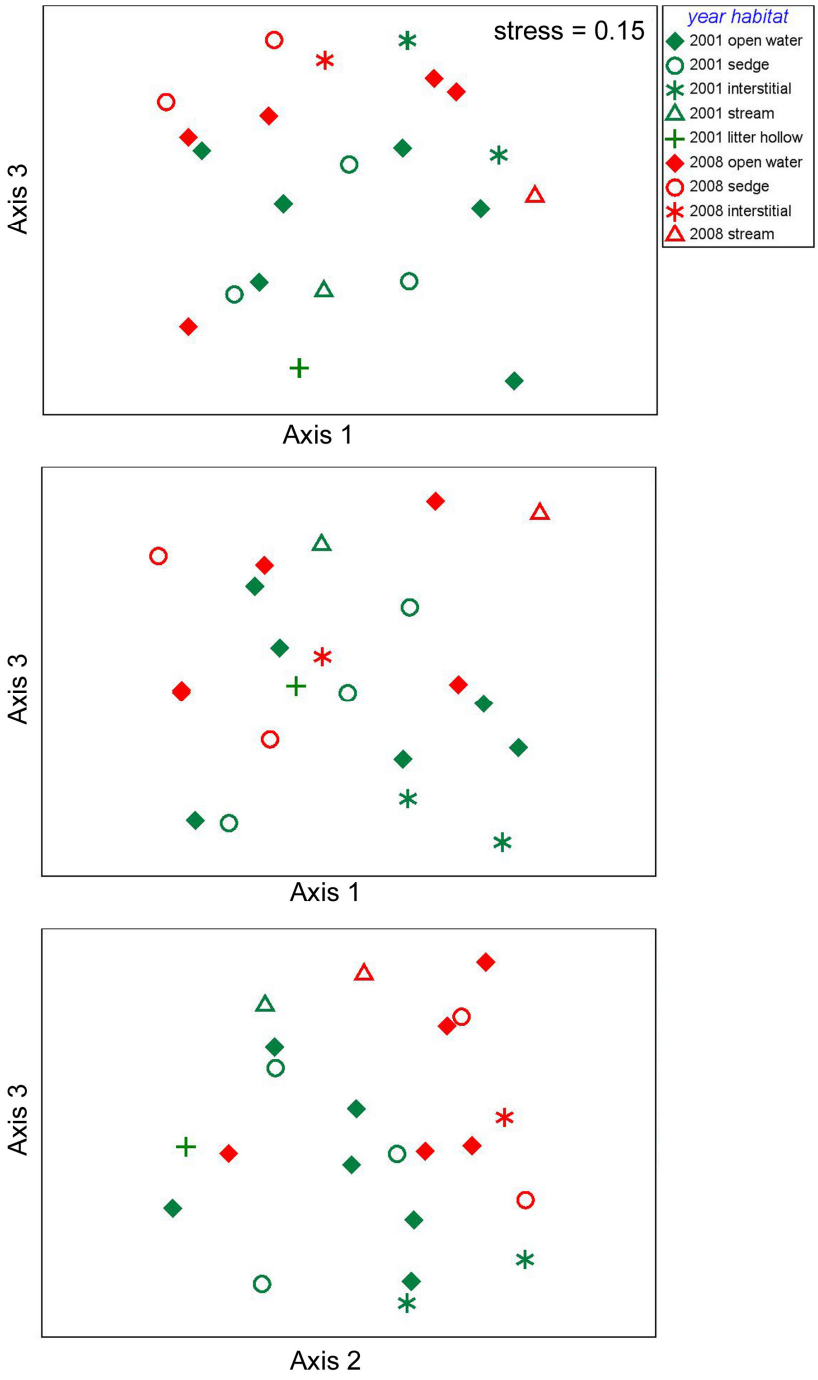

Figure 7. Three dimensional non-metric multidimensional scaling ordination of invertebrate samples collected in August 2001 and October 2008. Green symbols are for August 2001 samples

while red symbols are for October 2008 samples. Different habitats are represented by different symbols. The stream flowing out of MSTS1 is excluded.

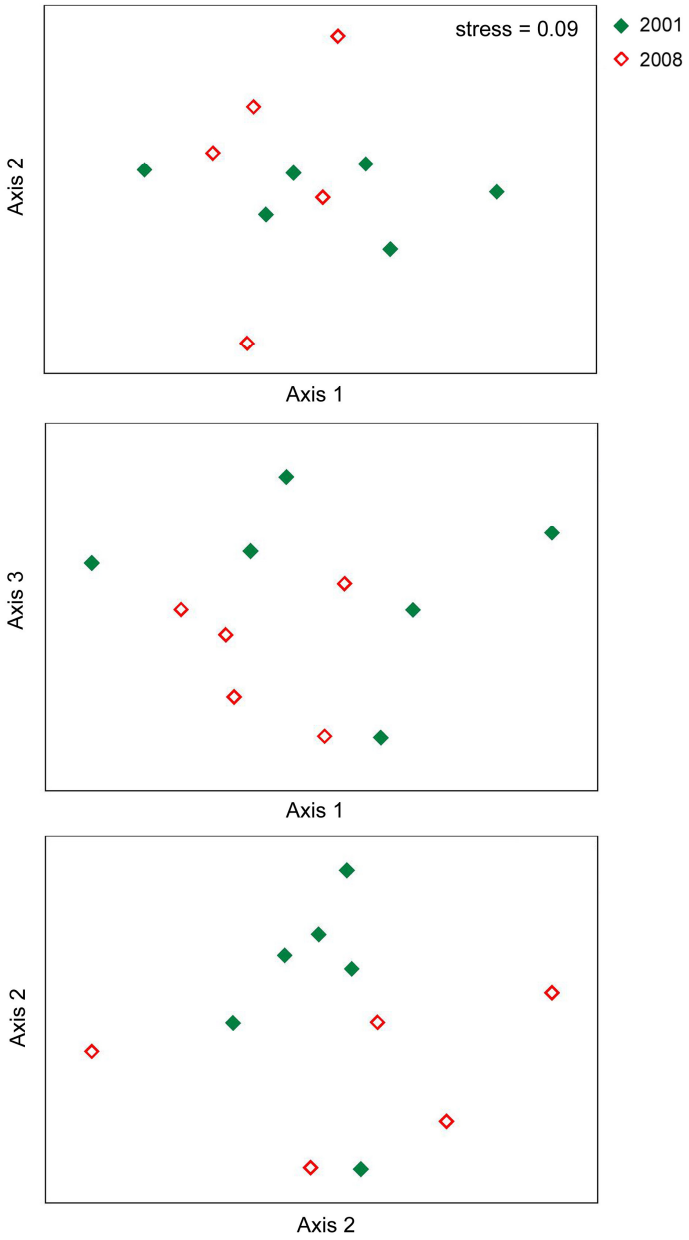

Figure 8. Three dimensional non-metric multidimensional scaling ordination of invertebrate samples collected from open water non-flowing habitats in August 2001 and October 2008.

DISCUSSION

Invertebrate sampling

A monitoring protocol for these springs was produced by Pinder *et al*. (2009). The objectives of the protocol are to:

- determine if there are significant changes in water chemistry;
- determine if there are changes to the known diversity in invertebrate taxa; and
- determine if there are likely to be linkages between changes in hydrology and changes in biota, should such changes occur.

Invertebrate sampling in 2008 followed the methods used in 2001 and listed in Pinder *et al*. (2009). However, these methods were designed to investigate the composition of the aquatic invertebrate assemblages, rather than monitor invertebrate diversity over time. The two objectives are not necessarily incompatible, but the original intent of the sampling should be kept in mind. Sampling effort at the springs has varied over time, partly reflecting variability in extent of aquatic habitats present, which is determined by rainfall and groundwater discharge. While sampling will always be affected by type and amount of habitat present there is some scope for making sampling more standardised within habitats.

There are five main types of aquatic habitats present on the mound springs and all should be sampled where they are present at a particular spring when visited. These habitats are**:**

1. Interstitial water in waterlogged soil
2. Inundated sedges
3. Open water in ponds (can be very small areas)
4. Water in litter-filled hollows
5. Flowing water (e.g. the creek flowing from MSTS1 or the creek within MSTS13)

The following sampling procedures are a refinement of the protocols provided in Pinder *et al*. (2009). In particular, we have suggested more quantitative techniques while not diverging significantly from what has been done in the past.

Interstitial water. A fixed volume of waterlogged soil should be collected, followed by removal of a fixed volume of any interstitial water that fills the hole. The soil should be removed using a corer or quadrat, with soil removed to a fixed depth in either case. These samples should remove about 1000 cm^3, with a fixed volume of water (we suggest two litres) then removed from the hole as it fills. The water should be passed through a 50 µm mesh sieve and the contents of the sieve should be preserved together with the soil. Two or three of these samples should be taken.

Inundated sedges. Where water depth is sufficient, a 50 µm mesh sweep net can be used to sample the water column (aiming to keep the sample free of sediment by only gently moving the net through clean water) and a 250 µm mesh net then used to collect a separate sample of stirred-up sediment. Sampling should occur over an area of about two linear metres where possible. Where water depth is too shallow or where the sedges are too dense for the use of a sweep net, a wide-mounted container should be used to sample the clean surface water and stirred-up sediment (again separately) over

an equivalent area (where possible) and the water or sediment passed through the appropriate net. These samples should be preserved separately.

Open or flowing water. Areas of open or flowing water can be sampled using a 50 µm mesh sweep net to sample the water column (aiming to keep the sample free of sediment by only gently moving the net through clean water) and then a 250 µm mesh net to sample stirred-up sediment, leaf litter and vegetation. It is difficult to set a fixed distance for this habitat as large samples in a small pool will be an unacceptable disturbance, but small samples may underestimate diversity in a larger pool. We suggest each sample should be taken over a distance of one metre, with the number of samples determined by pool size, to a maximum of five samples. MSTS1 and MSTS5 occasionally have pools large enough for 5 x 1 metre samples, but most other springs have only small pools where a single sample should be taken.

Water within litter-filled hollows. These areas are usually too small to sample with nets. If there is sufficient clear surface water this can be gently sampled using a 50um mesh sweep net or a container to scoop water into the net. A sample of the litter and underlying sediment should then be taken (perhaps 1 litre – may need to be scooped by hand or a container). Amount of litter removed should be small compared to the size of the habitat to minimise disturbance: a litre should suffice.

While the above procedures will help to standardise sampling, some flexibility is still needed as habitats will not always easily fit into these categories.

Preservation of samples. Samples collected with a 50 µm mesh net or filtered through a 50 µm mesh sieve are best preserved in buffered formalin as this is better for preserving copepods and rotifers. However, formalin is highly toxic and requires careful handling and transport. It should not be used while on the mound spring in case of spillage. An alternative is to use 100% ethanol in the field and to replace this with fresh 100% in the lab as soon as practical. Providing that there is minimal water in the sample upon preservation this should suffice until sorting. Samples collected with a 250 µm mesh net can be preserved in 100% ethanol.

Data analysis and sampling adequacy

Temporal patterns in invertebrate richness could be analysed at two scales: the individual spring and across multiple springs (i.e. at the spring complex level). Statistical analysis of the former would require much more intensive and consistent sampling within a spring than is currently employed, with replicate samples taken from each habitat of interest. It is likely that the number of replicates that would be required would be so high that sampling itself could become a disturbance, which is undesirable. In any case, replication is not always possible. Analysis at the level of the entire spring complex could be achieved either by summing richness across habitats within a spring or by analysing each habitat type separately. In either case, the same habitats would need to be sampled on each occasion where possible. To give sufficient power to an analysis it is also likely that a greater number of springs would have to be sampled. We have not attempted an analysis of required sampling effort because of the small number of springs sampled to date and the differences in habitats sampled in the different springs.

Formal statistical analysis (and a sampling design with power to allow it) is frequently considered desirable in a monitoring program. However, for some habitats, especially those limited in size and occurrence (as TECs tend to be), such an approach is not always feasible. The sampling program to date has been sufficient to determine the overall composition and conservation significance of the invertebrate assemblages, although the total number of species collected at the springs can be expected to grow with more sampling (as per Figure 5). Sampling has also been sufficient to confirm that the distinctive elements of the fauna present in 2001 (Table 3) were still present in 2008, allowing for the fact that detection of all of these on any one date is unrealistic. We could also demonstrate (at least graphically) that total richness across the springs was as high in 2008 as it was in August 2001 and that there is no obvious pattern of declining richness. The current sampling effort is adequate for at least this level of assessment, although more rigour in the sampling methods, as described above, would give greater confidence.

For invertebrates, we recommend continuing the current monitoring program but with greater effort to be more consistent with both the habitats sampled and the amount of sampling, both within and between springs. We also recommend increasing the amount of replication at the spring level by sampling some additional springs. Invertebrate sampling frequency should be at least every 2 or 3 years.

Non-biological monitoring

An alternative or supplementary (and less expensive) approach to monitoring would be to monitor aquatic habitats rather than (or in addition to) the invertebrates. This could involve a combination of soil moisture and groundwater monitoring, water chemistry and photo-point monitoring points. We also recommend that the extent of surface water and water logged habitats be documented on each visit.

Water chemistry variables have been measured at all springs sampled for invertebrates. We suggest that only some of these need to be monitored. Of the nutrient variables, at least total persulphate and total filterable N and P should be measured. Electrical conductivity and pH are also important, but the other variables are of less importance and concentrations of individual ions (Na^+, Cl^- etc.) need not be monitored. They were initially measured to characterise the ionic composition of the water but this is unlikely to change unless the springs become saline and then it is the salinity that would be the problem not the ionic composition.

Conclusions

Nitrogen concentrations in water samples were higher in 2008 than in 2001 for most springs and a couple of springs had particularly high nitrogen and phosphorus concentrations. It is not clear whether the higher nutrient concentrations are a result of natural biogeochemical processes in the springs or anthropogenic enrichment from surrounding agricultural land. This is something to monitor closely. Total invertebrate richness in 2008 appears to have been as high as it was in 2001. There was no consistent change in species richness between 2001 and 2008 at individual springs, with some springs having lower richness than in 2001 and others having richness as high or higher than in 2001, the differences partly related to sampling effort. The total number of regionally rare and/or restricted species collected was

higher in 2008 than in 2001, so this component of the fauna, which gives the assemblages their unique composition and conservation significance, appears not to have declined. For most habitats there were too few samples to analyse differences in community composition between years, but for the habitat with most replicates, open water, there was no difference in community composition between 2001 and 2008. Such general observations are probably all that can be expected with the current sampling program, but the amount of sampling required to provide statistical rigour would be prohibitively expensive and/or would be a disturbance in itself. Nonetheless, some improvements in consistency of sampling can easily be made and these will allow more confident interpretation of results.

ACKNOWLEDGEMENTS

Field work was undertaken by the authors with the assistance of Glen Daniel and Stephen Kern (DEC). David Cale identified some of the copepods. The field and laboratory components of this work were funded by the Inland Aquatic Integrity and the Significant Species and Communities components, respectively, of the State-wide Resource Condition Monitoring Project, an Australian government initiative.

REFERENCES

DEC (in prep.). *Resource Condition Report for a Significant Western Australian Wetland: Organic mound Springs of Nebroo Nature Reserve.* Department of Environment and Conservation, Perth, Western Australia.

Pinder, A.M. and Pennifold, M.G. (2002). *A Survey of the Aquatic Invertebrates of Some Organic Mound Springs in the Shire of Three Springs, Western Australia.* A report prepared for the Western Australian Threatened Species and Communities Unit, Department of Conservation and Land Management, Western Australia.

Pinder, A., Clarke, V. and Harding, C. (2009). Monitoring of the Invertebrate Assemblages of Mound Springs of the Three Springs Area Threatened Ecological Community. Version 1.0 (August 2009). Prepared for Significant Native Species and Ecological Communities – Resource Condition Monitoring Project.

Pinder, A.M., Stratford, L. and McRae, J.M. (2006). *Report on August 2001 sampling of invertebrate assemblages at mound springs of the Three Springs area*. Department of Environment and Conservation, Perth.

Pinder, A.M., Halse, S.A., McRae, J.M. and Shiel, R.J. (2004). Aquatic invertebrate assemblages of wetlands and rivers in the Wheatbelt region of Western Australia. *Records of the Western Australian Museum* **67**: 7-37.

Primer-E Ltd (2008). Primer 6 (v.6.1.11) and Permanova+ 1.0.1. Primer-E Ltd. Plymouth, United Kingdom.

Rees, R., and Broun, G. (2005) *Assemblages of Organic Mound Springs of the Three Springs Area: Interim Recovery Plan 2005 -2010*. Interim Recovery Plan 196. Prepared by the

Western Australian Threatened Species and Communities Unit (WATSCU) for the Department of Conservation and Land Management, Perth, Australia.

Quinlan, K., Pinder, A.M. and Cale, D. (2009). *Assessment of the aquatic fauna conservation values of wetlands in the Hutt River/Hutt Lagoon catchments*. Department of Environment and Conservation, Perth.

					MSTS1 (TST001)			MSTS5 (TST003)		MSTS2 (TST005)	MSTS13 (TST013)		MSTS12 (TST020)
				sample	1	2	3	1	2	1	1	2	1
Major group	**Order**	**Family**	**Code**	**Lowest level of identification**	open water pool	flooded sedges	mud + interstitial	open water pool 1	open water pool 2	flooded sedges	open water pool	stream	small open water pool
Protozoans	Arcellinida	Arcellinidae	BP010199	*Arcella* sp.		1				1	1	1	
		Centropyxidae	BP020199	*Centropyxis* sp.				1	1	1			1
		Difflugiidae	BP030199	*Difflugia* sp.				1		1			1
		Nebelidae	BP040199	*Nebela* sp.						1			1
Turbellarians	-	-	IF999999	Turbellaria									1
Nematodes	-	-	II999999	Nematoda			1	1	1		1	1	1
Rotifera	Ploimida	Lecanidae	JP090110	*Lecane bulla*	1			1					
			JP090129	*Lecane hamata*		1							
Oligochaetes	Tubificida	Phreodrilidae	LO0399A1	Phreodrilid with dissimilar ventral chaetae			1				1	1	1
			LO0399A2	Phreodrilid with similar ventral chaetae									1
		Naididae	LO049999	Naididae (ex Tubificidae)					1			1	1
			LO050507	*Pristina leidyi/longiseta*		1	1	1		1	1	1	1
		Enchytraeidae	LO089999	Enchytraeidae				1	1			1	1
Water mites	Acariformes	Oxidae	MM090302	*Oxus orientalis*				1					
		-	MM999999	Acariformes						1			
		-	MM9999A1	Oribatida	1					1		1	1
		-	MM9999A6	Trombidioidea							1		
	Parasitiformes	-	MM9999A2	Mesostigmata							1	1	1
Crustaceans	Copepoda	Cyclopidae	OJ310703	*Mesocyclops brooksi*	1	1	1	1	1	1			
			OJ310799	*Mesocyclops* sp. (juveniles)							1		
			OJ311701	*Merideicyclops baylyi*					1				
			OJ3102A2	*Metacyclops* nr sp. 434		1							
			OJ3111A1	Paracyclops sp. 1				1				1	
			OJ3111A0	*Paracyclops ?chiltoni*			1	1					
		Diosaccidae	OJ6999B0	Harpacticoida sp. 2	1								
		Canthocamptidae	OJ6199A1	Canthocamptidae sp. 6?			1						
			OJ999999	Harpactoicoida sp. (juveniles)	1						1	1	
	Cladocera	Macrothricidae	OG060201	*Macrothrix breviseta*	1								1
	Ostracoda	Darwinulidae	OH059999	Darwinulidae			1						1
		Candonidae	OH071099	*Candonidae* sp. TST1 (smooth shell) = *Candona* sp.			1				1	1	
			OH0799A2	Candonidae sp. TST2 (stepped shell)			1						
		Cyprididae	OH080101	*Alboa worooa*							1	1	
			OH080599	*Cypretta* sp.	1								
		Cypridopsidae	OH090101	*Sarscypridopsis aculeata*									1

					MSTS1 (TST001)			MSTS5 (TST003)		MSTS2 (TST005)	MSTS13 (TST013)		MSTS12 (TST020)
				sample	1	2	3	1	2	1	1	2	1
Major group	**Order**	**Family**	**Code**	**Lowest level of identification**	open water pool	flooded sedges	mud + interstitial	open water pool 1	open water pool 2	flooded sedges	open water pool	stream	small open water pool
	Syncarida	-	ON999999	Syncarida									1
Insects	Coleoptera	Dytiscidae	QC091205	*Limbodessus inornatus* (ex *Liodessus*)	1				1	1			
			QC092301	*Rhantus suturalis*					1				
			QC099999	*Allodessus bistrigatus*	1								
			QC0999A6	Bidessini larvae		1		1				1	1
		Hydrophilidae	QC111203	*Helochares tenuistriatus*				1					
			QC119999	Hydrophilidae (larva)		1							
		Staphylinidae	QC189999	Staphylinidae									1
		Scirtidae	QC209999	Scirtidae sp.	1		1	1	1	1			1
	Diptera	Tipulidae	QD0199A4	Tipulidae type E (SAP)									1
			QD0199A5	Tipulidae type F (SAP)									1
		Culicidae	QD070105	*Anopheles atratipes*	1	1				1			1
			QD070506	*Aedes ratcliffei*	1			1	1				
			QD070601	*Culiseta atra*	1	1	1	1	1	1			
			QD070704	*Culex globocoxitus*	1	1		1					
			QD070707	*Culex latus* ?	1								
			QD070801	*Coquillettidia nr linealis*				1					
		Ceratopogonidae	QD0919A3	*Monohelea* sp. 4 (SAP)						1			
			QD090899	*Culicoides* sp.			1						
		Tabanidae	QD239999	Tabanidae						1			1
		Dolichopodidae	QD369999	Dolichopodidae								1	1
		Muscidae	QD899999	Muscidae							1	1	
		Chironomidae	QDAE0803	Procladius P1	1	1	1	1					
			QDAE1201	*Paramerina levidensis*	1	1	1	1		1	1		1
			QDAE99A6	*Pentaneurini sp. F*	1		1						
			QDAF0699	*Corynoneura* sp.						1			
			QDAF1202	*Paralimnophyes pullulus*	1			1					1
			QDAF19A0	*Limnophyes vestitis* (ex *Compterosmittia* sp. A)						1			
			QDAF99A7	Orthocladiinae sp. I (SAP)			1					1	1
			QDAF99B6	Orthocladiinae sp. Q (SAP)					1				
			QDAH0410	*Tanytarsus fuscithorax/semibarbitarsus*	1			1					
			QDAH04B9	*Tanytarsus* nr bispinosus	1			1					1
			QDAI04A0	*Chironomus* aff. *alternans* (V24) (CB)	1	1		1	1		1		
			QDAI0804	*Polypedilum nubifer*								1	

					MSTS1 (TST001)			MSTS5 (TST003)		MSTS2 (TST005)	MSTS13 (TST013)		MSTS12 (TST020)
				sample	1	2	3	1	2	1	1	2	1
Major group	**Order**	**Family**	**Code**	**Lowest level of identification**	open water pool	flooded sedges	mud + interstitial	open water pool 1	open water pool 2	flooded sedges	open water pool	stream	small open water pool
			QDAI08A2	*Polypedilum convexum*	1		1	1			1		1
	Hemiptera	Mesoveliidae	QH520199	*Mesovelia* sp. (juvenile)					1				
		Veliidae	QH560103	*Microvelia* (*Austromicrovelia*) *peramoena*		1		1					
			QH560101	*Microvelia* (*Pacificovelia*) *oceanica*	1								
			QH569999	Veliidae (juvenile)			1						
		Gelastocoridae	QH640199	*Nerthra* sp.					1				
	Odonata	Megapodagrionidae	QO070401	*Archiargiolestes pusillus*	1	1	1						1
		Aeshnidae	QO120201	*Adversaeshna brevistyla* (ex *Aeshna*)				1					
		Libellulidae	QO171602	*Orthetrum villosovittatum villosovittatum*				1					
		Synthemistidae	QO230101	*Archaeosynthemis occidentalis*	1		1						1
	Trichoptera	Leptoceridae	QT250605	*Notoperata tenax*			1			1	1		
				Richness per sample	26	15	21	28	15	18	15	17	31
				Richness per spring	40			34		18	22		31