Report on August 2001 sampling of invertebrate assemblages at mound springs of the Three Springs area.

Produced for the Western Australian Threatened Species and Communities Unit, CALM

by

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INTRODUCTION

Initial sampling of aquatic invertebrates at the mound springs near the town of Three Springs was undertaken by Sheila Hamilton-Brown (CALM Species and Communities Branch) and Adrian Pinder (CALM Science Division) in March 2001. Five mound springs were sampled and 88 species of invertebrates identified in total (56 if those found in a dam excavated from a former mound spring were excluded). In a report on this sampling it was concluded that "While most of these species are common and widely distributed, there is a small subset of species that appear to be rare or absent in other types of aquatic habitat in the [northern] wheatbelt and a few may even be uncommon in the broader south-west" and that "These springs may represent a northern outlier for the distribution of several species otherwise known only from the higher rainfall south-west". However, because the springs were sampled during early autumn when water levels were likely to have been at their lowest, and some springs were dry and therefore not sampled, it was recommended that the original five springs plus others should be re-sampled in late winter/early spring, with the aims of:

1. Producing a more complete list of invertebrates utilising these spring habitats over the year.
2. Collecting additional specimens of some taxa which could not be identified from the limited or immature material collected in this survey (such as the water mites, tubificid worms, ?*Candopsis* and Darwinulidae sp. 715).
3. Assessing whether those invertebrates of particular interest (as discussed above) occur across a greater proportion of these springs than is apparent to date.

This recommendation was accepted and in late August 2001, Sheila and Adrian re-sampled four of the original five springs (leaving out the highly degraded site TST06) and sampled two additional sites. An interim report (Pinder 2002) was produced after a portion of the samples had been sorted and identified because funds then available did not allow all samples to be processed. The remaining samples have now been sorted and this report is an update of Pinder (2002).

METHODS

Extent of August sampling

Fourteen samples were collected from six mound springs (TST01, TST03, TST05, TST20, TST10 and TST13). Of these, the first four were also sampled in March 2001 and the last two were sampled for the first time.

Table 1. Site names and sampling times. TST codes established by Sheila Hamilton-Brown differ from those on the WATCU Threatened Ecological Communities Database as follows:

TST site code	WATSCU occurrence (MSTS code)	Number of samples March 2001	Number of samples August 2001
1	1	1 (combined from two excavated holes)	3
3	5	1 (combined from two excavated holes)	3
5	2	1	3
6	11	1	0
10	14	0	1
13 (=TST 'Big')	13	0	2
20 (=TST A)	12	1	2

In addition to these samples collected from the mound springs, we also collected a sample from the dam associated with TST06 in March and one from the stream arising from TST01 in August. The same stream was later sampled further downstream by Pinder *et al.* (2004).

Plate 1 shows some of the mound springs and habitats sampled.

Sampling methods

Unless otherwise stated, all samples were collected by filling plastic containers (250 ml or 2000 ml) with water and passing this water through a net with a mesh size of 50 µm. Samples were preserved in the field and sorted in the laboratory.

TST01. Sample 1 was collected using both a 50 µm mesh net and a 250 µm mesh net from a large flooded area with open coloured water amongst melaleucas on the southern edge of the mound. Specimens from these two samples were picked separately in the

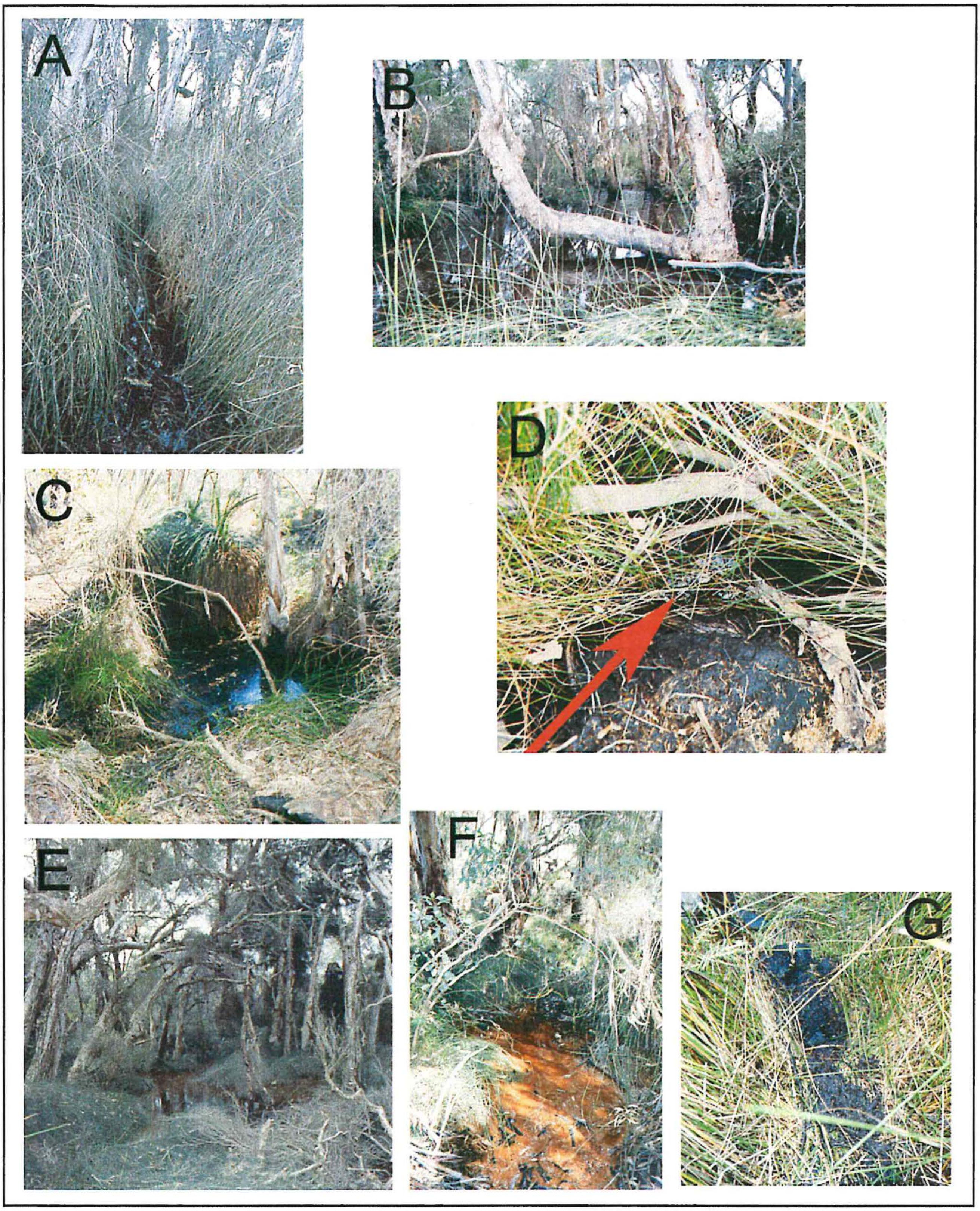

Plate 1. Habitats sampled for aquatic invertebrates. A, inundated sedges at TST01; B, flooded Melaleuca at TST01; C, small pool at TST03; D, natural hole at base of clump of sedges at TST05; E, Pool at TST10; F, Creek with iron floc on sediment at TST13; G, Hole dug into peaty soil at TST20.

lab but combined prior to identification. Sample 2 was collected from a small shallow flooded channel within *Baumea* just north of sample 1 and sample 3 was collected from a small hole dug into another area of *Baumea*. A water sample was taken from sampling point 1.

TST03. Sample 1 was collected from a small pool of water with large amounts of leaf litter and soft organic sediment. Sample 2 and 3 were collected from an inundated stand of *Baumea vaginalis*. Sample 2 was taken by scooping water from a shallow muddy vegetated area on the edge of the *Baumea* while sample 3 was taken using a sweep net from within an area of deep open water in the middle of the *Baumea*.

TST05. Sample 1 was from a shallow flooded tract of sedges close to the northern entrance, off the track between this spring and TST01 while sample 2 was taken from a small natural hole with open water.

TST10. Two samples collected with a 50 µm mesh sweep net in small areas of open water and pooled in the field.

TST13. Sample 1 was from a trickle flowing into a pool while sample 2 was from the pool itself, both collected using a 50 µm sweep net.

TST20. Sample 1 was from a small pool amongst sedges and shrubs while sample 2 was from a hole dug into moist soil.

Sorting and identification

Sorting was done by Jane McRae, Nadine Guthrie and Leah Stratford and identifications were by Jane McRae and Leah Stratford, other than ostracods (Stuart Halse and Koen Martens) and oligochaetes (Adrian Pinder).

Curation

Specimens are held in the laboratory of Dr. Stuart Halse at the Department of Conservation and Land Management's Wildlife Research Centre, Woodvale.

Data analysis

A classification of Salinity Action Plan and TST (August) wetland invertebrate communities was produced using the flexible UPGMA clustering routine based on Bray Curtis similarity within PATN 3.04. The association matrix showed a moderate positive skew reflecting generally low similarity between communities in the dataset but re-estimating associations (in an older DOS version of PATN) produced a bi-modal association matrix and a very similar classification. An ordination and scatter plot was produced using the SSH routine in PATN 3.04.

RESULTS

Water chemistry

All springs were freshwater in August (Table 2), with hardly lower total dissolved solids than when sampled in March. Salinity at these sites is not higher than a range of other good condition wetlands in the northern agricultural zone, including some claypans, other springs, swamps and creeks. Most sites were slightly more acidic than when sampled in March, less turbid and, with the exception of TST20, more highly coloured. The lower turbidity undoubtedly reflects the greater presence of open surface water, from which clean water, rather than cloudy interstitial water, could be obtained. Colour is within the range expected for vegetated swamps in the region.

Invertebrates

Excluding invertebrates caught in the stream flowing off TST01, eighty taxa were picked from the samples collected in August 2001 (Appendix 1), including 52 that were not recorded during the first sampling round (March 2001). This brings the total count for both seasons (again excluding the stream plus the excavated dam near TST06) to 103 taxa. Of these, 49 were recorded from just one spring, with between 8 and 44% (average 24%) of the fauna of any single spring being unique. Only 18 taxa (17%) occurred at more than three springs and none occurred at all springs. There is thus considerable heterogeneity in invertebrate composition between sites, indicating that there is probably not a consistent assemblage type that occurs across the springs. This may reflect the differing array of habitats present between springs, though a component of the observed heterogeneity may be a result of the small size of the samples compared to the area of the springs. TST01 appears to have a richer invertebrate fauna than the other springs and TST01, 03 and 05, which are geographically close to one another, are the richest sites, even when the two sampling occasions are considered separately.

Fifteen additional species were collected only from the stream arising from TST001, all of which are common in the region. None of the species of particular interest were present in the stream.

The August sampling increased richness at the four re-sampled sites by between 36% and 233%, showing that the additional sampling provided a more complete picture of community composition at these wetlands. The number of species collected only

Table 2. Water chemistry at Three Springs Tumulus Springs August 2001

	TST01	TST03	TST05	TST10	TST13	TST20
pH (measured in field)	6.1	6.2	6.0	6.2	6.5	6.8
E Conductivity (µS/cm)	1348	2160	1409	3530	8020	775
temperatrure (°C)	11.1	14.8	16.2	14.1	12.8	10.7
salinity (g/L) converted from EC in meter	0.4	0.9	0.5	1.7	-	0.1
oxygen (%)	11.7	6	-	-	-	-
turbidity (NTU)	25	0.4	0.5	52	69	0.4
colour (TCU)	150	120	210	41	71	62
total Dissolved Solids (g/L)	0.7	1.2	0.9	0.7	1.7	0.4
alkalinity (mg/L)	33	45	30	<2	45	30
hardness (mg/L)	99	130	95	140	240	42
Iron (mg/L)	3.8	2.9	0.3	0.1	2.9	<0.05
silica (mg/L)	51	78	45	36	70	29
sodium (mg/L (% meq))	213	360	282	198	551	131
calcium (mg/L (% meq))	3	4	3	7	7	2
magnesium (mg/L (% meq))	22	29	21	29	53	9
potassium (mg/L (% meq))	17	23	16	11	32	9
chloride (mg/L (% meq))	330	540	420	320	880	180
bicarbonate (mg/L (% meq))	40	55	37	<2	55	37
carbonate (mg/L (% meq))	<2	<2	<2	<2	<2	<2
sulphate (mg/L (% meq))	31	46	46	76	83	43
nitrate (mg/L (% meq))	0.1	0.0	0.0	0.0	0.0	0.0
soluble reactive phosphorus (µg/L)	10	10	10	30	10	10
total persulphate phosphorus (µg/L)	30	20	10	300	20	930
ammonia nitrogen (µg/L)	40	40	10	30	10	40
nitrate/nitrite (µg/L)	60	40	20	20	30	20
total persulphate nitrogen (µg/L)	990	680	70	5200	490	930

in one season was consistently high (72-87%) for the four resampled sites and more species were collected in August than in March for three of the four sites. The latter was probably because those three sites (TST01, 05 and 20) were much wetter in August than in March whereas this was not as true for TST03 (which was fairly wet in March). Surprisingly, the large area of open water at TST01, which was not present in March, did not contribute a particularly large number of species to richness of this site in August and most of the species that did occur in the pool also occurred in the habitats with little or no open at this spring and/or other springs. Likewise, the sample from the open water pools of TST10 had only one species (a very common and widespread ostracod) not otherwise found in the habitats with little open water at other springs. These results suggest that the fauna of the springs is largely one capable of inhabiting small inundated holes, small areas of inundated dense vegetation or saturated soils. This is in contrast to the Kimberley mound springs reported on by Halse (2001) where presence of open water positively affected the diversity of surface water macroinvertebrates found.

In a cluster analysis of invertebrate communities from all 65 natural freshwater sites from the Salinity Action Plan survey (SAP) (Pinder et al., 2004) plus the six mound springs, the latter all grouped together separate from the SAP sites (Appendix 2). Comparison of SAP and mound spring data showed that there was a particular assemblage of species that were largely restricted to the mound springs and a small group of SAP sites (mostly sedge swamps – group 8 in Appendix 2) located in the Lake Muir and south coast areas (to Mount LeGrand east of Esperance). The reason that the mound springs did not group with these SAP sites (or any SAP sites) is probably that the mound springs lacked a large number of species that were very common at SAP sites. An ordination (albeit with high stress – 0.23) suggested that the TST sites were not well separated from SAP sites in ordination space and did not all group close together. Figure 1 shows axes 1 v 2 of the ordination, with the TST sites, sites of group 8 from

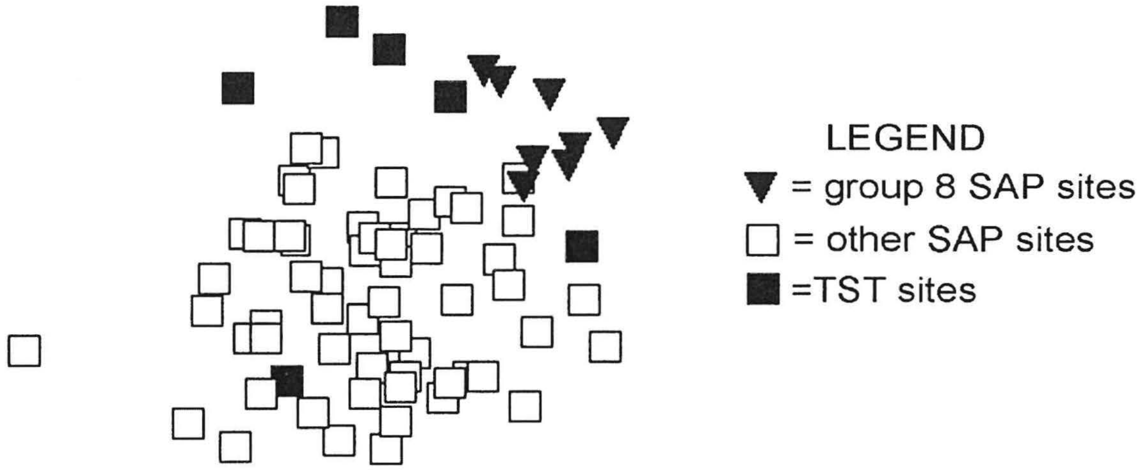

Fig. 1. Scatter plot of axes 1 v 2 of ordination of SAP and TST sites.

the dendrogram and other SAP sites shown with different symbols.

Of the species that were flagged as being of particular interest by Pinder and Pennifold (2001) (2 chironomids, groundwater crustaceans, two copepods, *Archaeosynthemis macrostigma occidentalis* and *Culiseta* mosquitoes) most were collected again during the August sampling as follows:

- The chironomid Orthocladiinae 'genus woodminer' was collected at TST20 in March and at both this site and TST01 in August. It is common in the higher rainfall parts of the south-west (Don Edward, UWA retired Pers. comm.) but has not otherwise been recorded in the Wheatbelt. The other chironomid of note, Pentaneurini sp. F, was collected at TST01, TST03 and TST05 in March and at these same three springs again in August. It has not been collected elsewhere by the CALM wetlands research group. Cranston (2000) lists a similar species (his Pentaneurini sp. E) from acidic wetlands in south-west WA and possibly also from NSW, but the Three Springs specimens are sufficiently different to suspect that they are a separate species. Another Orthocladiinae, species I, was recorded at both TST01 and TST20 in August but at only three sites during the SAP survey. The latter were all near the south coast, so this another species whose mound spring population may represent a northern outlier.

- *Culiseta* mosquitoes were collected at only TST01 in March but at all sites except TST10 in August so it is common within these springs. The specimens collected in August were more mature than those collected in March and all have been identified as *Culiseta atra*. The single specimen of *Culiseta* sp. 1 collected in March looks like early instar specimens collected with mature *Culiseta atra* in August, so it is almost certainly a juvenile of the same species. Another specimen collected in March and identified as *Culiseta* sp. 2 is most likely also a juvenile of *C. atra*. *Culiseta atra* is common in suitable habitats (coloured, still waters with decaying leaf litter) only in the wetter south-west (>600mm average annual rainfall according to Liehne 1991) and was not recorded from the wheatbelt during the SAP survey.

- The copepod Harpacticoida sp. 2 was recorded at TST01 and TST03 in March 2001 and recorded again at TST01 in August. This species is otherwise known from a swamp on the eastern slopes of the Darling Range (Darkin Swamp) and two sites in the far south-west (Lake Noobijub and Lake Pleasant View) (CALM, unpublished data). Another harpacticoid copepod, Canthocamptidae sp. 6, was collected only once in March and otherwise collected only once in the SAP survey (on a granite outcrop north-east of Perenjori. It was not recollected in August. Two of the cyclopoid copepods collected in August were identified as being of interest. *Microcyclops* sp. S1 is sufficiently different from the widespread *Microcyclops varicans* to flag it as something of interest. Although this may be a new undescribed species, only one specimen was collected, at TST05 in the flooded sedges, and further specimens are required to confirm its status. Other cyclopoids were similar to the widespread *Metacyclops* sp. 434, but the genus is in need of revision. It is uncertain at this stage whether the mound spring specimens belong to a variable "sp. 434" or some more narrowly defined separate taxon. These were collected from TST01, 03, 05 and 13.

- The dragonfly *Archaeosynthemis macrostigmata occidentalis*, collected at TST01 and TST05 in March was collected in TST01 and TST10 in August. It is common in streams along the Darling Range and higher rainfall parts of the south-west (Sutcliffe 2003), but the only other record north of Ellenbrook is from Yerina Spring, north of Hutt Lagoon (Pinder et al. 2004). A model relating occurrence of this species to environmental attributes of sites (calculated before the mound spring and SAP records were available), suggested that high rainfall and slope were positively associated with its occurrence, with optimum rainfall being 1400mm. The presence of permanent water at spring sites seems to enable this species to exist in drier areas than it mostly inhabits.

- One of the putative groundwater elements in the fauna collected in March was the darwinulid ostracods. The identity of these was uncertain at the time and they were called Darwinulidae sp. 715 as an interim measure (Pinder and Pennifold 2002). Koen Martens (visiting CALM from the Institut Royal des Sciences Naturelles de Belgique) suggests that those are juvenile specimens that cannot be given a species name. However, he has identified the darwinulids collected in August as the cosmopolitan *Penthesilenula brasiliensis*. The juveniles collected in March may be the same species. The other groundwater element, bathynellid crustaceans, were not collected in August.

The following invertebrates, not recorded in March, are also noteworthy.

- The oligochaete 'Phreodrilid WA3' is an undescribed species listed in Pinder and Brinkhurst (1997) and Pinder (2003) that is common in the Warren region, but has otherwise only been collected from seepages at Lesmurdie Falls near Perth and from a bore on the Perth Basin south of Gingin. The record from TST13 is thus a range extension for this species. The other phreodrilid, recorded at three of the springs is *Antarctodrilus horwitzi*. All previous records of this species are from between Denmark and Pemberton (Pinder, 2002). Phreodrilids are rare in the lower rainfall parts of the south-west, other than on granite outcrops and subterranean and spring habitats. Only four of the 26 freshwater Salinity Action Plan sites in the northern wheatbelt had phreodrilids and none of these records were of WA3 or *horwitzi*.

- The *Austrotrombella* water mite from two mound springs is an undescribed species of a genus otherwise known only from South Australia (Mark Harvey, WA Museum, pers. comm.) and has been collected from only two other localities, both streams on the Geraldton Sandplains sampled during the SAP survey.

- The damselfly *Archiargiolestes pusillus* prefers permanent water, or at least rivers drying to pools. This species is common in the forested south-west south of the Avon River (Sutcliffe 2003) but was collected from only one of 26 freshwater SAP sites (Pinder *et al.* 2004), five out of about 45 northern wheatbelt AUSRIVAS sites sampled by Kay *et al.* (2001) and two river pools in the Carnarvon Basin (Halse et al. 2000). In this study it was collected at four of the seven mound springs.

- The caddisfly from three of the mound springs is similar to *Notoperata tenax*. The Australian authority on leptocerid caddisflies, Ros St Clair (Victorian EPA) examined specimens for the interim report (Pinder 2002) but was uncertain whether these specimens were conspecific with *tenax*. There are only minor morphological differences between *N. tenax* larvae described by St. Clair (2002) and those mound spring specimens. Ros suggested that examination of adults might help. *Notoperata tenax* is common in the higher rainfall south-west but has rarely been recorded north of Dirk Brook in the Darling Range south-east of Perth (St Clair 2002, Sutcliffe 2003). It was collected from two of 45 northern Wheatbelt sites during the AUSRIVAS survey but was not collected in the central and northern Wheatbelt during the SAP survey (Pinder, 2004). New specimens identified since the interim report will be sent to Ros St Clair shortly.

SUMMARY

The August sampling achieved the three aims outlined in the introduction: 1) a more complete species list has been produced, almost doubling the number of species known from the mound springs, 2) the identity of some taxa has been resolved as a result of collecting more material and 3) some species, including those that make the mound spring assemblages distinct, have been shown to occur at a larger number of mound springs. The additional sampling has revealed further species that are otherwise uncommon in the northern agricultural zone or elsewhere outside of the higher rainfall south-west. For these species, the mound springs appear to provide a perenially moist refuge in a generally dry region. Being outliers, mound spring populations of some of these species may represent genetic diversity not well represented elsewhere.

The additional sampling has further distinguished the Three Springs mound springs as having diverse aquatic invertebrate communities, including an unusual combination of groundwater species, species more typical of the higher rainfall south-west and species that may be regionally restricted and uncommon (e.g. the *Austrotrombella* water mite and some copepods). Few other wetlands in the agricultural zone would have invertebrate communities with such a large number of notable elements.

ACKNOWLEDGEMENTS

Thanks to Sheila Hamilton-Brown for assisting with the field work and initiating the study and to Rosemarie Rees for assistance with information relating to the springs. Stuart Halse and Koen Martens identified the ostracods and Nadine Guthrie sorted some of the samples.

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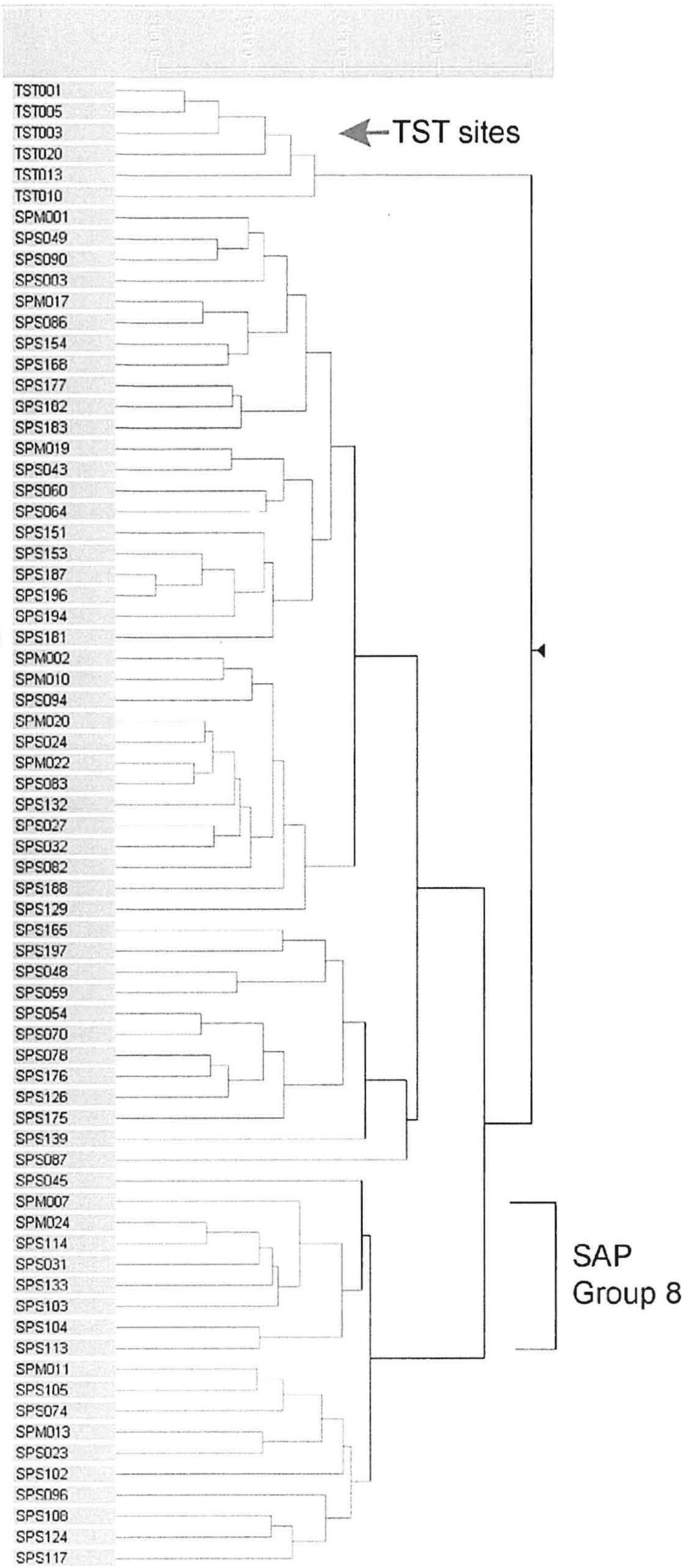
Appendix 1. Invertebrates collected during August 2001 sampling of The Three Springs mound springs

		TST001				TST003			TST005		TST10	TST013		TST20	
Identification		1	2	3	stream	1	2	3	1	2	1	1	2	1	2
Protozoa	*Arcella* sp.		1								1				
	Centropyxis sp.		1	1							1			1	
	Nebela sp.		1												
	Nebela sp. 1			1											
	Nebela sp. 2													1	
Flatworms	Turbellaria	1										1			
	Microturbellaria				1										
Nematodes	Nematoda				1	1			1			1	1	1	1
Tardigrades	Tardigrada								1						
Rotifers	*Testudinella patina*				1										
	Brachionus angularis				1										
	Colurella uncinata				1										
	Euchlanis dilatata				1										
	Lecane bulla				1										
	Lecane ludwigii				1										
Oligochaetes	Phreodrilidae			1											
	Phreodrilidae WA3												1		
	Antarctodrilus horwitzi	1						1	1		1	1	1		
	Tubificidae without hair chaetae	1							1	1			1		
	Pristina longiseta	1	1	1				1	1			1		1	
	Enchytraeidae		1						1					1	
Water mites	*Austrotrombella* sp.	1							1						
	Rutacarus sp.			1											
	Arrenurus (*Truncaturus*) sp.	1		1			1			1					
	Oribatida	1							1						
	Mesostigmata	1			1			1	1						
	Oribatida group 1		1	1		1							1	1	
	Oribatida group 4							1							
	Oribatida group 5		1			1	1				1				1
Ostracods	*Limnocythere mowbrayensis*				1				1						
	Penthesilenula brasiliensis			1										1	1
	Candonopsis tenuis			1							1				
	Candonopsis sp.			1											
	Alboa worooa				1							1			
	Bennelongia barangaroo				1										
	Sarscypridopsis aculeata										1				
Copepods	*Microcyclops varicans*				1										
	Microcyclops sp. S1								1						
	Metacyclops nr sp. 434	1					1		1			1			
	Mesocyclops brooksi	1			1										
	Paracyclops chiltoni					1									
	Paracyclops ?chiltoni											1			
	Paracyclops sp 1 (nr *timmsi*)	1	1						1			1			
	Cyclopoida			1										1	
	Australocamptus sp. 5								1			1			
	Harpacticoida sp. 2		1												
Amhipods	*Austrochiltonia subtenuis*				1										
Beetles	*Allodessus bistrigatus*									1					
	Liodessus inornatus		1				1	1		1		1			
	Platynectes decempunctatus decempunctatus													1	
	Platynectes sp.				1										
	Rhantus sp.	1				1									
	Bidessini		1									1			
	Helochares/Enochrus mastersi larvae							1							
	Staphylinidae												1		
	Scirtidae sp.	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Limnichidae sp. 4	1							1						
	Limnichidae sp. 5								1	1					
	Brentidae				1				1						
Mosquitoes	*Aedes ratcliffei*	1				1									
	Culiseta atra	1	1			1	1	1	1	1		1		1	
	Coquillettidia linealis		1					1							

Appendix 1 (cont.)

Identification		TST001				TST003			TST005		TST10	TST013		TST20	
		1	2	3	stream	1	2	3	1	2	1	1	2	1	2
Biting midges	*Bezzia* sp. 1		1		1							1		1	
	Culicoides sp.										1	1		1	1
	Monohelea sp.													1	
	Monohelea sp. 4			1					1						1
	Forcypomyia sp.			1								1			
	Dasyhelea sp.								1					1	1
	Ceratopogonidae														1
Black Flies	*Simulium ornatipes*				1										
March Flies	Tabanidae			1					1						
Other Flies	Stratiomyidae														1
	Tipulidae			1											
	Dolichopodidae sp. A		1						1		1				
	Dolichopodidae sp. B												1		
	Ephydridae			1											1
Non-biting midges	*Procladius paludicola*			1											
	Ablabesmyia notabilis											1			
	Paramerina levidensis	1	1	1	1			1		1	1			1	
	Larsia albiceps		1	1						1				1	1
	Pentaneurini sp. F			1				1		1					
	Corynoneura sp. (V49)				1										
	Thienemanniella sp. (V19)				1										
	Compterosmittia ? sp. A												1		
	Gymnometriocnemus sp. A														1
	Orthocladiinae					1									
	Orthocladiinae sp. G												1		
	Orthocladiinae sp. I			1											1
	Orthocladiinae 'woodminer'			1										1	
	Tanytarsus sp. G													1	
	Tanytarsus nr *bispinosus*								1	1					
	Tanytarsus sp. I			1											
	Chironomus aff. *alternans* (V24)	1	1			1		1	1		1	1			
	Polypedilum nubifer											1			
	Polypedilum nr. *convexum*		1	1				1			1			1	1
Lepidoptera	Lepidoptera (non-pyralid) sp. 3	1				1		1							
Dragonflies and Damselflies	*Austrolestes analis*				1										
	Austrolestes annulosus				1										
	Archiargiolestes pusillus	1	1				1			1		1			
	Archaeosynthemis occidentalis			1							1				
	Hemicordulia tau							1							
	Epiproctophora (=Anisoptera)													1	
Caddisflies	*Hellyethira* sp.			1											
	Notoperata tenax		1	1					1	1					1
Water Bugs	*Microvelia* sp.							1							



Appendix 2. Dendrogram from cluster analysis of Salinity Action Plan sites and TST sites