

Field studies into the biology and conservation requirements of *Engaewa* species in the South West and Warren DEC regions.

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SUMMARY.

1. This report presents the findings of survey and monitoring work undertaken throughout 2006-2007. Although this is the final report an additional report will be presented to DEC early 2009 once the results of further systematic studies are known. Mention will be made in this report of populations that appear to require taxonomic clarification and any revisions to species descriptions and/or distributions resulting from on-going morphological and molecular studies and any further survey work will be presented in that report.
2. Surveying and monitoring was conducted in the Warren and South-West DEC regions incorporating some 67 sites. Of these sites 45 contained previously unknown/unconfirmed *Engaewa* populations, including 1 of the critically endangered *E. pseudoreducta*, 9 of the endangered *E. reducta* and 7 of the vulnerable *E. walpolea* (conservation status is listed as per IUCN criteria). It is possible that some of these populations are in fact linked but at this stage it is difficult to assess the degree of connectivity of populations. It is hoped that the on-going molecular studies may help gain a better understanding of gene flow within and between different swamps and creeks.
3. Recording of habitat characteristics relating to vegetation, soil and water characteristics and (potentially) related behavioural characteristics for each species are discussed in this report. The data and analyses presented in this report are preliminary with further detail to appear in the additional report that will be presented to DEC early 2009. Although it is difficult to make any conclusive statements at this time it appears that a combination of soil type, landform and vegetation may be the best indicator of likely occupation by specific species. These three characteristics appear to be related and it is difficult to determine which (if any) are causal and not just correlated. Despite this *Engaewa* species appear able to adapt to sub-optimum habitat and it may be the influence of water table levels that ultimately dictates presence/absence of these crayfish.
4. *Engaewa* appear capable of burrowing in to virtually any soil type, however, there appears to be a general trend where *E. reducta* and *E. subcoerulea*, the physically larger species with dimorphic claws, appear to favour sandy soils, *E. walpolea* and *E. similis* prefer peaty soils (with considerable variation particularly for *E. similis*) and *E. pseudoreducta* is exclusively found in clay soils.
6. *Engaewa* habitats can be divided in to three broad categories; narrow valleys, broad valleys and flat plains. Although most species will occupy more than one type the general trend is for *E. reducta*, *E. pseudoreducta* and *E. walpolea* to be found in narrow valleys, *E. subcoerulea* to be most common on flat plains and *E. similis* to occupy either broad or narrow valleys.
7. The major threatening factors that appear likely to have impacted *Engaewa* populations in the past and continue to pose a significant threat are directly

related to habitat loss and involve alterations to wetlands (drainage, damming, extraction), removal of vegetation and cattle grazing.

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INTRODUCTION.

The distribution of the crayfish genus *Engaewa*, in terms of extent, is reasonably well set having been described originally by Riek (1967; 1969) but in far greater detail by Horwitz and Adams (2000). The known distribution of the genus (as shown by the map of sites presented by Horwitz and Adams (2000)) follows the south-west coast of Western Australia from Dunsborough in the north-west to Bow Bridge in the south-east (although this study will show that a slight extension to the south-east as far as Kent River is required). Therefore the major purpose of this study was to reduce the grain of distribution descriptions to the point where a list of specific sites was created. This objective was *partially* achieved. The emphasis on partially is due to the nature of studying distributions.

Whilst every attempt was made to ensure the site list presented in this report was complete it is only possible to confirm the presence of a species, not its absence, particularly cryptic species such as *Engaewa*. Throughout this study it has become clear that variations in habitat and season affect the likelihood of detecting *Engaewa* species and the variable nature of access throughout various regions within this study site also has an effect on detecting their presence. As a result of these unavoidable aspects of studying distributions the list of sites produced in this report should be considered only a current hypothesis of the distribution of the genus and the variation within it, and sites where crayfish were not found should be by no means ignored in future survey work.

The second part of this report focuses on the habitat characteristics of sites where *Engaewa* specimens were collected. Although as previously stated no sites should be dismissed outright in future survey work the habitat data presented in this report could help guide researchers to sites where the probability of finding these crayfish may be higher. The habitat data may also help predict which species is likely to be present when it is believed that the crayfish inhabit a particular site.

DISTRIBUTION.

Sampling methods

As may be expected from an animal which spends virtually its entire life below ground the first challenge when attempting to collect *Engaewa* specimens is finding them. In order to direct collection efforts potential habitat was identified using a combination of maps and satellite imagery, looking for small creek/swamp systems that possessed a significant amount of vegetation. Although this concept created a list of potential habitat it was only once viewed that a creek/swamp system could be assessed with any degree of certainty. Many areas that appeared to be suitable from maps and imagery were clearly not so once viewed and vice versa, hence the only reliable way to assess an area was to visit it. As such virtually every creek/drainage line/swamp or seepage with some degree of native vegetation remaining within the search area was examined. At this point it is again important to point out that the non-detection of *Engaewa* at a particular site should by no means be viewed as a definitive statement of its absence.

Potential habitat was assessed for the presence/absence of these crayfish by looking firstly for characteristic chimneys of soil, which can signal the entrance to a burrow system. These chimneys form from the accumulation of small spherical pellets of soil, which can be up to 1-2 cm in diameter though generally are much smaller, which are the soil that has been expelled as the tunnel systems have been excavated. The chimney can range from less than

half a dozen small pellets surrounding a small hole to a conical shaped chimney up to 35 cm high and formed from tens or even hundreds of individual pellets. The soil forming the chimney may be distinctly pelleted or it may appear as a simple pile of soil, due to the effect of weathering. Where obvious chimneys were lacking closer attention was paid to any patches of different coloured soil or even simple holes in the ground which, due to species specific differences (as shall be discussed later) or weather conditions, may also signal the entrance to a burrow.

Engaewa burrows generally can be distinguished from those of *Cherax* as the chimneys are usually far more substantial and the pellets of soil are much smaller. *Cherax* species typically dig short, straight tunnels and, as such, have small chimneys with much larger pellets due to their larger body size. *Cherax* chimneys also often form a miniature caldera, whereas *Engaewa* chimneys almost always appear conical. The diameter of the tunnel extending vertically from the chimney is also characteristic as *Engaewa* burrows are much smaller in diameter (approximately a 'pinky' to index finger in width) when compared to a *Cherax* burrow (often in the range of middle finger to thumb in width and, at times, larger). Once excavation of a burrow commences it generally becomes quickly apparent whether it belongs to a *Cherax* or *Engaewa* crayfish as *Engaewa* burrows will be far more elaborate, often changing direction and/or branching, whereas *Cherax* burrows are relatively straight and simple.

Collection of crayfish was most often achieved by digging them out of the burrow system, however, on rare occasions it was possible to collect them by spotlighting in shallow puddles or channels at night (predominantly for *E. walpolea*, though occasionally for *E. similis*). Collecting crayfish with the standard digging method involved digging a hole centred on a chimney or group of chimneys and then following any tunnels uncovered. A combination of shovelling and exploring tunnels by hand was used as the excavation of the burrow system proceeded in order to uncover the crayfish. Often the crayfish were below the water level in the burrow so it was necessary to bail water out of the hole as the excavation continued. Using this method crayfish were located either in a tunnel and pulled out by hand, in the water in the hole and bailed out or shoveled out with the soil. A variety of different sized holes were dug ranging from 0.2 x 0.2 to 4 x 2 m, although most holes were less than 0.5 x 1.0 m and whilst the deepest holes were 1.5-2 m deep they were rarely dug deeper than 0.5 m. The success rate of this method varied greatly with a maximum of four crayfish from any single hole dug, however, due to the size and complexity of tunnel systems (it was virtually impossible to reach the end of a tunnel) often no crayfish were found as it may have been possible for crayfish to escape, potentially aided by reaching the water table where they may be able to more rapidly descend into a deeper chamber? The majority of crayfish were discovered in the layer of soil corresponding to the interface of the ground water and upper soil layers, or below the water, with very few found closer to the surface.

Maps

A complete list of sites from which *Engaewa* specimens were collected during this study is provided in Appendix A. The most important outcomes of the surveying undertaken is the identification of a number of new *E. reducta*, *E. pseudoreducta* and *E. walpolea* populations (which using IUCN criteria can be considered as endangered, critically endangered and vulnerable respectively) as well as a number of new populations which, from an initial morphological appraisal, appear to require taxonomic clarification. The species boundaries have been narrowed somewhat, most obviously between *E. similis* and *E. pseudoreducta*, however it appears that the boundary between *E. reducta* and *E. pseudoreducta*/*E. similis* may have been broadened by habitat loss resulting in population level extinctions and the broad boundary between *E. similis* and *E. subcoerulea* may be caused by a natural barrier. The habitat component of this study may also be able to explain the separation between the overlapping but non-sympatric distributions of *E. subcoerulea* and *E. walpolea* and the separation between the geographically close populations of *E. pseudoreducta* and *E. similis*. These should still be considered hypotheses until further study is undertaken.

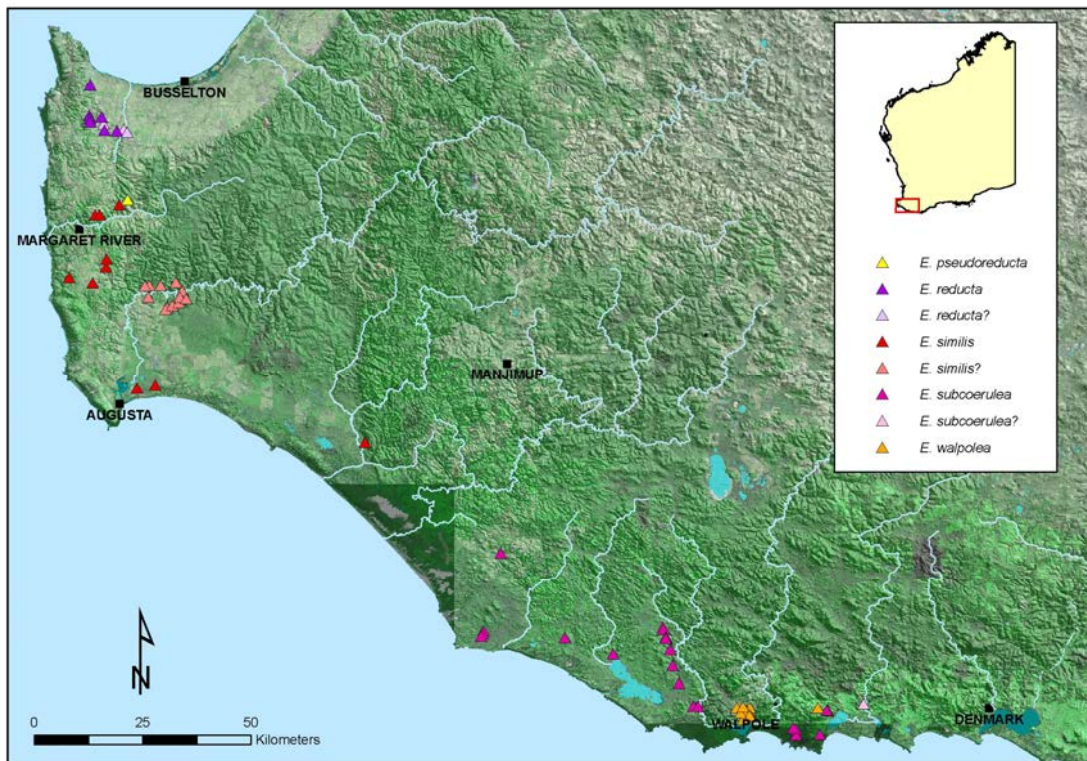


Figure 1: Distribution of populations of the five *Engaewa* species (*E. pseudoreducta*, *E. reducta*, *E. similis*, *E. subcoerulea* and *E. walpolea*) sampled in this study. The populations identified in this report as requiring taxonomic clarification are indicated by the ? after the current species name in the key. The single site for *E. subcoerulea?* was a single individual from near Kent River and it is currently unclear whether it is definitely *E. subcoerulea*, however, it is most likely from the genus *Engaewa* and represents an extension of the range of this genus.

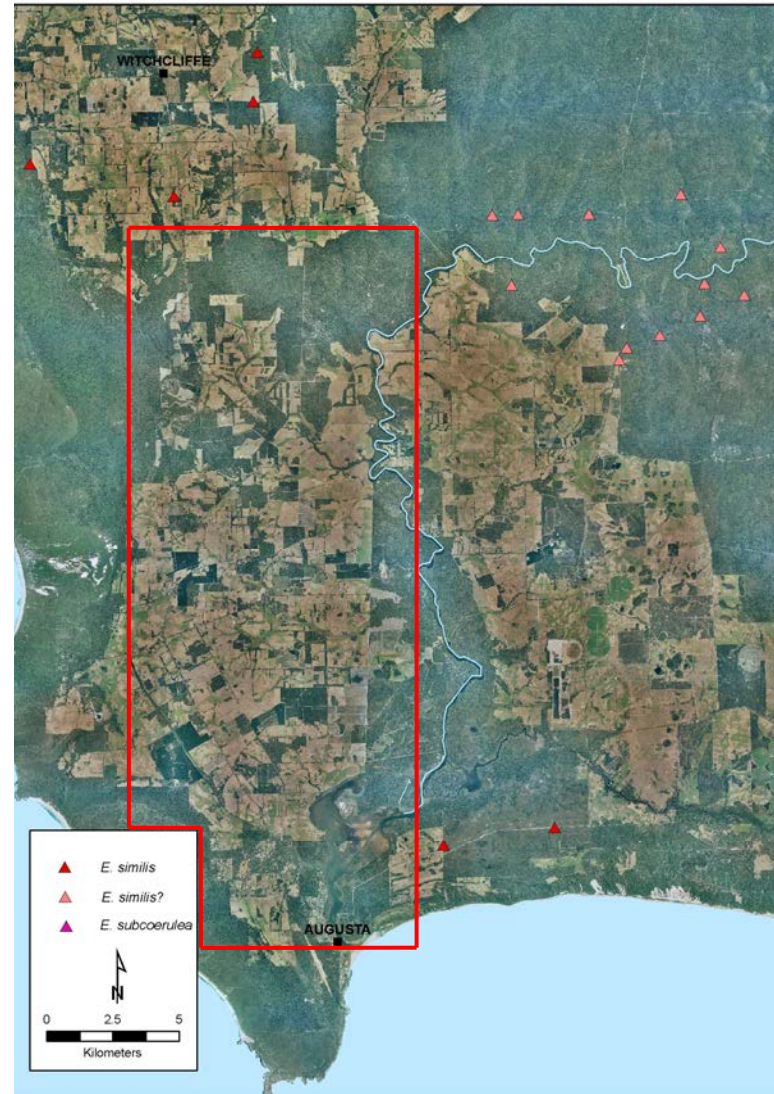
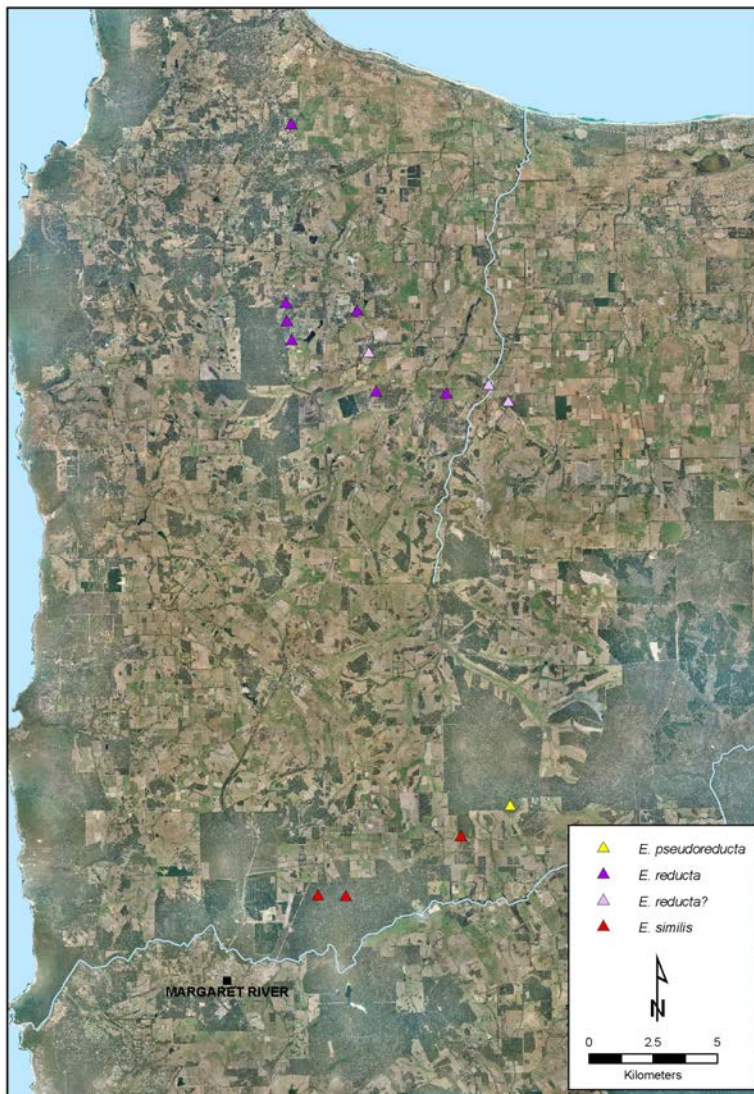


Figure 2 (left): Distribution of *E. pseudoreducta*, *E. reducta* and the northern most *E. similis* populations, highlighting the boundaries between these species. The populations labelled as *E. reducta?* appear to possess some morphological characters not typical of this species.

Figure 3 (right): Distribution of selected *E. similis* populations showing the location of the Blackwood sites which appear to require taxonomic clarification due to an unusual combination of morphological character states. Although not discussed in this report, historical records do exist within the region highlighted by the red rectangle.

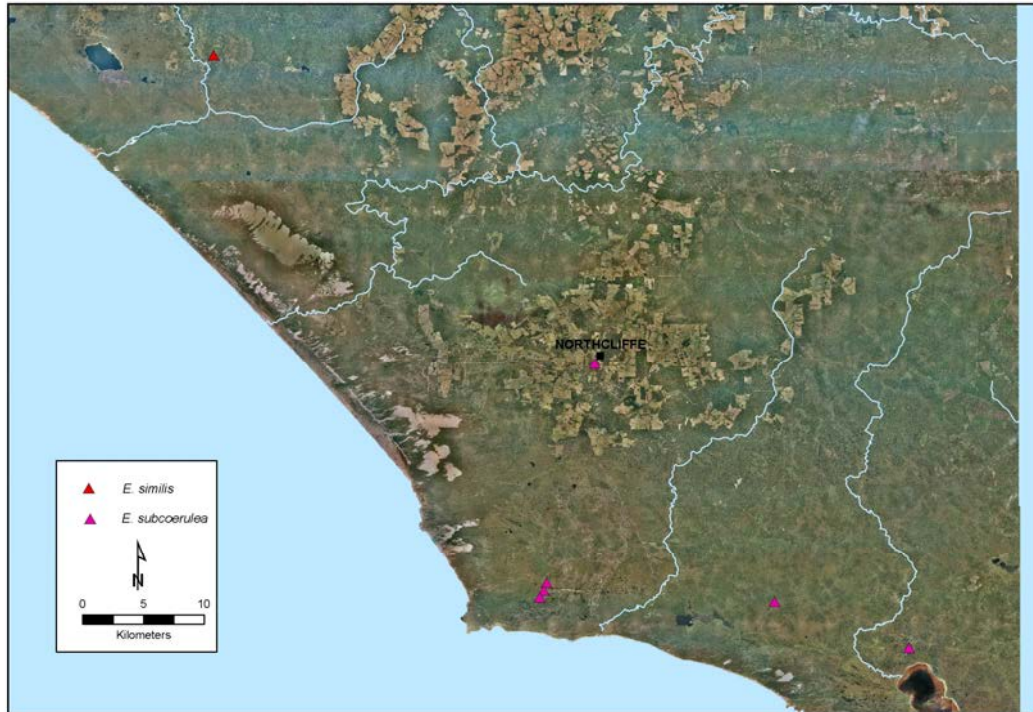


Figure 4: Distribution of the southern most *E. similis* population and the northern most *E. subcoerulea* populations uncovered during this study, highlighting the boundary between these two species.

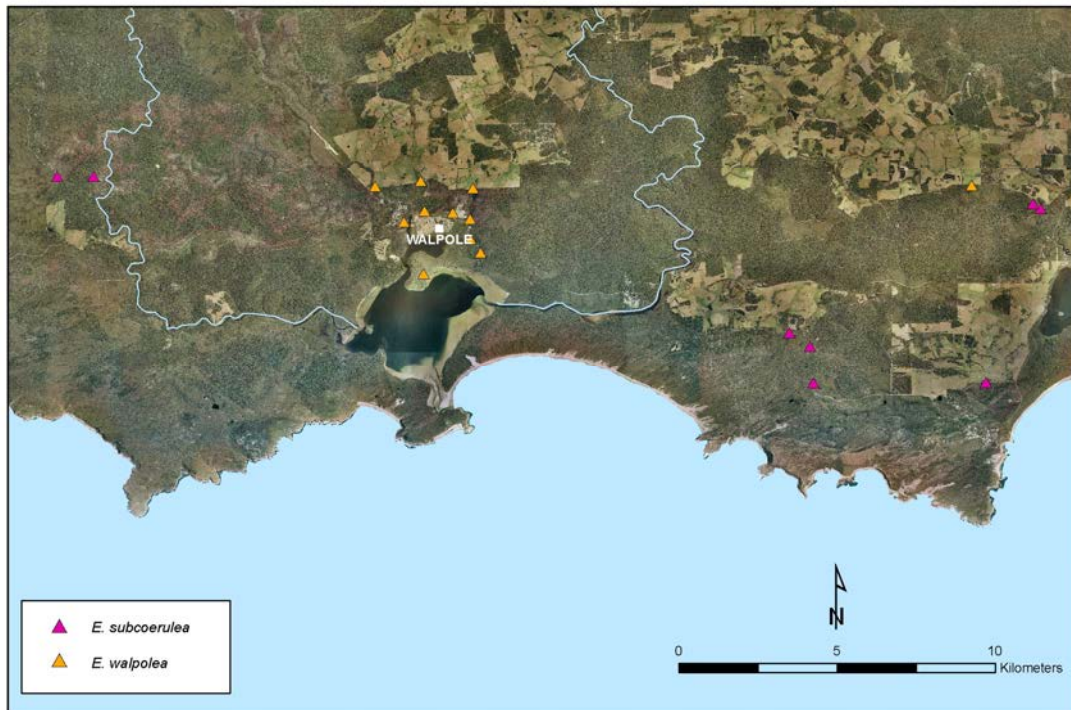


Figure 5: Distribution of *E. walpolea* and selected *E. subcoerulea* populations, highlighting the overlapping distribution of these closely occurring species.

HABITAT CHARACTERISTICS.

Vegetation & Landform

The floral species present at twenty-two sites was recorded covering the entire range of the genus (a full list of species recorded is provided in Appendix B). At the time of sampling a small number of species could not be identified as there were no diagnostic characters available (e.g. flower or fruit). The unidentifiable species were primarily reed or rush species as well as a few herbaceous species. The height and abundance of each species was estimated as well as the density of each strata at each of the sites surveyed. It is evident that the vegetative communities associated with the presence of *Engaewa* species are dominated by approximately twelve plant species (Figure 6) from ten genera (Figure 7), although some unidentified reed species may belong in this category. These dominant species, in terms of occurrence, also generally formed the major structural components due to both abundance and density (and therefore biomass).

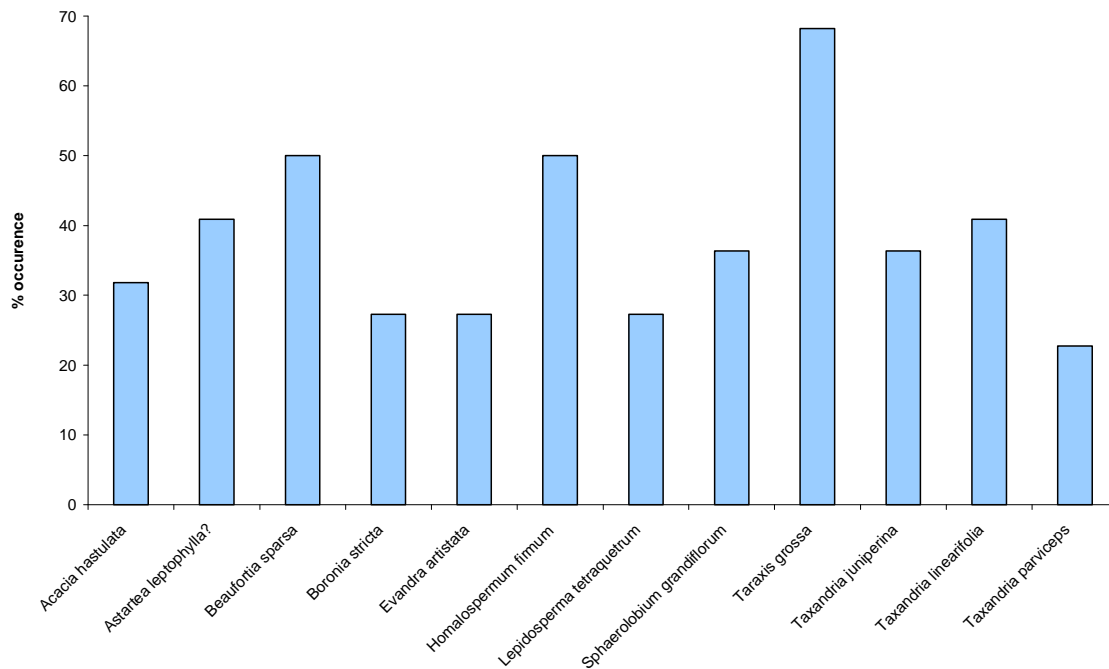


Figure 6: The percentage occurrence of major floral species at twenty-two *Engaewa* sites throughout south-west Western Australia.

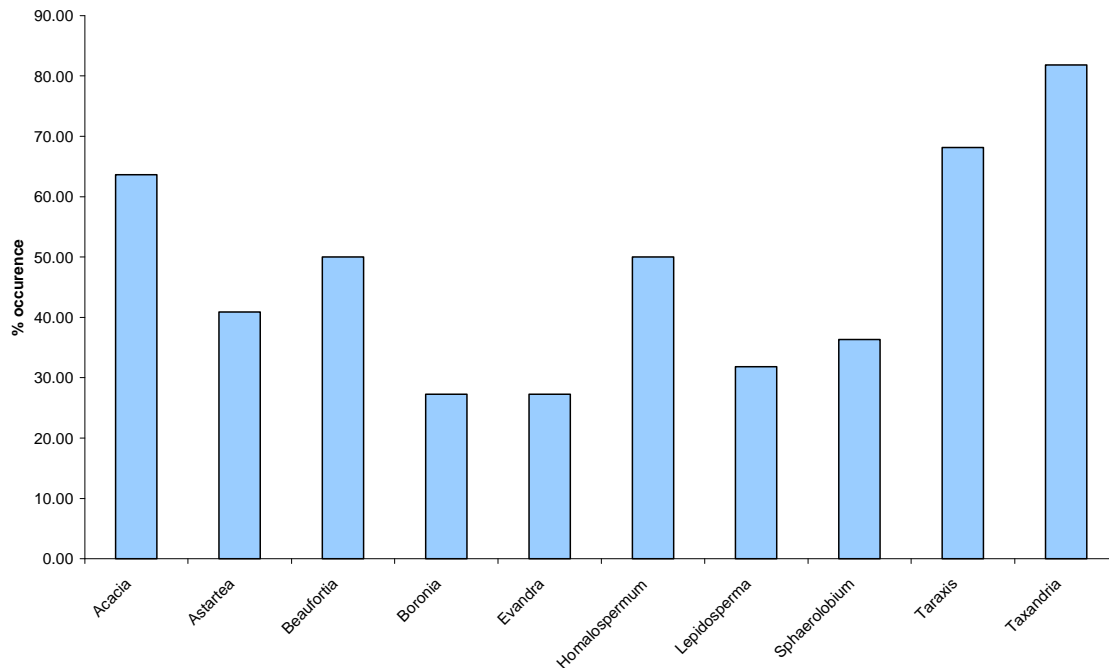


Figure 7: The percentage occurrence of major floral genera at twenty-two *Engaewa* sites throughout south-west Western Australia.

At this stage there is no clearly evident trend relating specific plant species to *Engaewa* species, however, this will be further investigated in the on-going research. The plant species that dominate specific sites appears to be related to a combination of geographic location and landform. The landform of *Engaewa* habitats can generally be divided into three categories; narrow valleys, broad valleys and flat plains (Figure 7 a-c).

Narrow valleys can be categorised as valleys rarely exceeding a maximum width of 30 meters with vegetation in narrow valleys being taller and denser than in the other two categories. The vegetation generally reaches approximately 3-5 meters high with a dense understory resulting in areas of 100% coverage by the vegetation, although in some cases a further overhead canopy at tree height results in a more open understory but still provides a high coverage value. Burrows in narrow valleys tend to cluster around areas where the channel becomes broad and diffuse resulting in high burrow densities in these areas but there may also be stretches devoid of burrows where the channel is more defined. This trend requires such narrow valleys to be explored laterally as well as transversely, although where roads have been built crossing such valleys the impeded water flow that generally occurs may result in favourable conditions near the road. Chimneys in narrow valleys are often small and relatively poorly formed due to the very wet nature of these environments and may only be evident during the winter months.

Broad valleys can be categorised as valleys exceeding a minimum width of 30 meters and may be hundreds of metres wide. The tallest shrub species in broad valleys generally reaches approximately 2-3 meters high and with a variety of reedy or creeping plants growing up to 2 metres high producing a more or less continuous vegetation form these valleys do not have significant stratification. Burrows in these systems are often scattered throughout the entire valley although they may cluster around and along drainage lines occurring in both the lateral and transverse direction. Chimneys in these broad valleys are often large-very large consisting of well formed pellets and may be largely permanent structures.

Flat plains can be categorised as areas devoid of any obvious slope ranging from tens to hundreds and perhaps thousands of meters wide. They generally have fairly stunted vegetation rarely exceeding 1-1.5 meters in height with coverage values varying considerably from areas with exposed soil to less commonly areas with a low/ground layer of vegetation providing almost complete coverage. Burrows in these systems generally follow drainage lines or are clumped around depressions which form seasonal waterbodies, although they can be scattered throughout the entire area. Chimneys on flat plains vary considerably in size but are often large-very large and are often poorly formed, probably due to the sandy nature of the soils in these areas producing pellets that crumble easily. These chimneys are sometimes only recognisable as conical piles of sand.



a



b



c

Figure 7 a-c: The three different landforms associated with *Engaewa* species showing the characteristic vegetation of each.
 (a) Narrow valley (Blackwood Road)
 (b) Broad valley (Blackwood Road)
 (c) Flat Plain (Windy Harbour Road).

Soils & Burrowing

Engaewa species appear capable of burrowing in virtually any soil type, as evidenced by burrows found in hard white clay, gravely sand and peaty loam within a 25 metre region of one site (Figure 8 a-c). This sort of adaptation is often found where burrows are dug in to the gravel along road side edges, which can be of completely different nature to the soil found in the adjoining natural habitat. Despite being able to adapt to different soil types, the different species show an affinity for specific soil types, as shown in Table 1. More detailed descriptions of specific soil profiles was produced when the piezometers were installed to monitor groundwater (Appendix C).

Table 1: Description of the soil type associated with each of the *Engaewa* species.

Species	Soil Type
<i>E. reducta</i>	Generally grey sand with minimal organic content, though occasionally in sands containing organic matter beneath a peaty surface layer.
<i>E. pseudoreducta</i>	Exclusively in heavy grey/yellow clays
<i>E. similis</i>	Generally in dark peaty loam soils though occasionally in lighter coloured sands or deeper light clay.
<i>E. subcoerulea</i>	Generally in grey sand with or without organic matter but also rarely with dark peaty loam soils overlaying deeper sands. Also common in gravely road edges.
<i>E. walpolea</i>	Generally in dark peaty loam soils though some with a higher proportion of sand.

Newly formed chimneys appear rapidly throughout the winter months throughout the entire range of the genus (as seen in the example provided in Figure 9 a&b). Whether this indicates a peak in digging activity is unclear as it may simply represent a change in the depth at which soil is being removed and/or deposited. They may dig only at or below the water level or they may be clearing out silt and soil deposits occurring due to water running into the burrow. Chimneys of vastly differing sizes are created during this time, which may be related to juveniles dispersing from their familial burrow when water levels are at a maximum and creating their own burrow system. Either way they can (and clearly do) move significant amounts of soil as apparent from the size of the chimneys that can be formed. Their rapidity in digging new burrows was also evidenced from the appearance of a new chimney in the remnants of an excavated hole only a day or two after it was refilled and also through recording the activity of a collected specimen, which dug a tunnel in excess of 20cm overnight and may have only stopped due to reaching the corner of the container.



Figure 8 a-c: Example of different soil types at a single site, all of which had *Engaewa* burrows.
 (a) a peaty layer with high organic content overlaying a fine sand
 (b) pale heavy clay
 (c) coarse sand with gravel (possibly originating from road construction)



Figure 9 a&b: An example of chimney construction occurring throughout winter at a site in Yelverton with burrows absent in March (a) and present in October (b). The burrows are evident within the red circle.

Although there appears to be a common seasonality relating to chimney construction burrow depth and structure is highly variable throughout the range of the genus, but again some general patterns can be seen. Both *E.*

reducta and *E. subcoerulea* appear to dig hugely expansive burrow systems often reaching many meters along both the horizontal and vertical axes. The sand that these two species are most commonly found in appears to be ideal for digging as it is soft when wet but “sets” hard. Corresponding to these large burrow systems the chimneys of these species tend to be extremely large, although once the sand dries out it does often crumble, losing the typical pelleted appearance and becoming a pile of sand. Interestingly individuals of these two species appear to grow to the largest size within the genus and always have dimorphic claws with the large dimorph being noticeably inflated. These two species can be found in all landform types but *E. subcoerulea* are most common in flat plains and *E. reducta* in narrow valleys.

Very few *E. pseudoreducta* burrows have been excavated and they have only been found in the heavy clay soils of narrow valleys in and adjoining Treeton reserve. The burrows found during this study were identified by small piles of slightly different coloured soil. This soil is likely to have represented washed down chimneys as there had been significant rainfall and the burrows were within a small creek line. As the water table was so high at the time of collecting this species the burrow systems were not fully explored though they appeared to branch laterally at a shallow depth as well as possessing tunnels proceeding deeper. Although largely speculation at this time it may be that *E. pseudoreducta* burrows are relatively small by *Engaewa* standards (following the general pattern of burrow size relating to body size *E. pseudoreducta* are relatively small). The small chimneys seen may be due to any or all of small burrows, little maintenance occurring (due to the clay holding its shape well) and a high degree of weathering in the area.

E. walpolea burrows also appear to be quite small and quite often lack chimneys altogether, being identified only as small holes. *E. walpolea* can be found primarily in narrow valleys but one site is best described as a broad valley and one as a flat plain. They tend to be in areas that are particularly wet by *Engaewa* standards, including in the bottom of depressions or in channels that become completely submerged for significant periods of time. The burrow systems of *E. walpolea* are generally quite shallow and relatively simple compared to the other species and specimens collected have generally been small and lack dimorphic chela, perhaps suggesting that they do not burrow as much as other species. They have also been found occupying (sharing?) burrows with small *Cherax*, perhaps digging short tunnels of the main *Cherax* burrow? Where soil profiles have been examined *E. walpolea* burrows appear to be in shallow soils overlaying a layer of gravel and/or clay, which if largely impervious to water may hold water at a relatively high level (compared to the deeply draining sands elsewhere), allowing the crayfish to remain in contact with the groundwater without needing to burrow deeply (this may provide an ecological explanation for the separation of *E. walpolea* and *E. subcoerulea* around Walpole). *E. walpolea* can be readily found by spotlighting in shallow puddles and channels at night, where they appear to be content to walk around, often in the presence of much larger *Cherax*, a situation rarely

encountered for the other species. When collected in this manner they are much more “flighty” and regularly attempt to “tail flip” (where the tail muscles are rapidly contracted causing the crayfish to move rapidly backward; a classic escape movement by aquatic crayfish), whereas specimens from the other species are docile when collected, even when placed into water. Whether this is related to species specific differences or due to the different collecting method is not clear.

E. similis appears to inhabit the most variable habitat and, as such, adopts different burrowing strategies with a combination of large burrows and small burrows being seen across and even within some sites. They can be found in both narrow and broad valleys and in soils ranging from highly organic peaty loams to sands. The size and complexity of burrows seems to relate to the amount of surface water present with large *subcoerulea/reducta* type burrows in broad valleys with little surface water and smaller shallower burrows in very wet narrow valleys.

Water

In order to compare the water characteristics throughout *Engaewa*’s range nine piezometers were used to sample groundwater as well as collections made of burrow water and surface water when available. The nine piezometer sites were chosen to provide multiple sites for each species where available (at the time of installation there were no other suitable *E. reducta* or *E. pseudoreducta* sites)(Table 2). The depth to which the piezometers were installed varied, however, attempts were made to ensure that they were at least 2 metres deep except at Blackwood where the water table is consistently higher than the other sites. Generally they were dug as deep as was possible using a hand auger (The depth of each one can be determined from the soil profiles shown in Appendix C). Auguring usually stopped due to either the substrate being too hard (e.g. a gravel layer) or too soft (e.g. sand that was collapsing in to the hole). Unfortunately using this method meant that all piezometers were dry for the scheduled summer sampling and the Ebbett Road and Osmington piezometers remained dry in the autumn sampling. An example of a piezometer is shown in Figure 10.

Table 2: Location, co-ordinates and species present at each of the piezometer sites used for sampling groundwater throughout the Warren and South-West DEC regions.

Location	Co-ordinates	Species present
Beach Road	50 487537E 6125636N	<i>subcoerulea</i>
Blackwood Road	50 344956E 6224254N	<i>similis?</i>
Collier Creek	50 476818E 6128911N	<i>walpolea</i>
CNR Ebbett & Highway	50 492859E 6130642N	<i>walpolea</i>
Ebbett Road	50 494829E 6129881N	<i>subcoerulea</i>
Haag	50 326990E 6267364N	<i>reducta</i>
Osmington	50 331065E 6246797N	<i>similis?</i>
Treeton	50 332684E 6247831N	<i>pseudoreducta</i>
Witchcliffe	50 328087E 6234227N	<i>similis</i>



Figure 10: Piezometer used for sampling groundwater.

Where they were available ground, burrow and surface water samples were collected in autumn and winter 2007 and analysed for a suite of chemical parameters (Appendix D). From these initial results it was decided that the water samples collected in spring 2007, which represents the most complete data set, would be analysed for a slightly adjusted suite of chemical parameters (Appendix E). The final list of chemical parameters excluded the metals Cd, Cu, Mn, Ni and Zn as they were largely absent and therefore non-informative.

Conductivity ($\mu\text{S}/\text{cm}$) and pH provide useful overviews of any seasonal variation as they are influenced by a number of water characteristics. Conductivity values varied substantially between sites and did show some seasonal variation (Figure 11). Two patterns were seen whereby conductivity either increased from autumn to winter before falling in spring (e.g. CNR Ebbett & Highway and Treeton) or declined steadily from autumn through winter to spring (e.g. Blackwood Road and Collier Creek). pH generally had an inverse relationship to conductivity where it was highest in autumn then declined in winter before beginning to rise again in spring (Figure 12). As all sites showed similar patterns in all values analysed throughout the different seasons (refer to Appendices C & D) and the increase in surface and burrow waters in spring made it the most complete data set the remaining analyses will focus exclusively on these spring data.

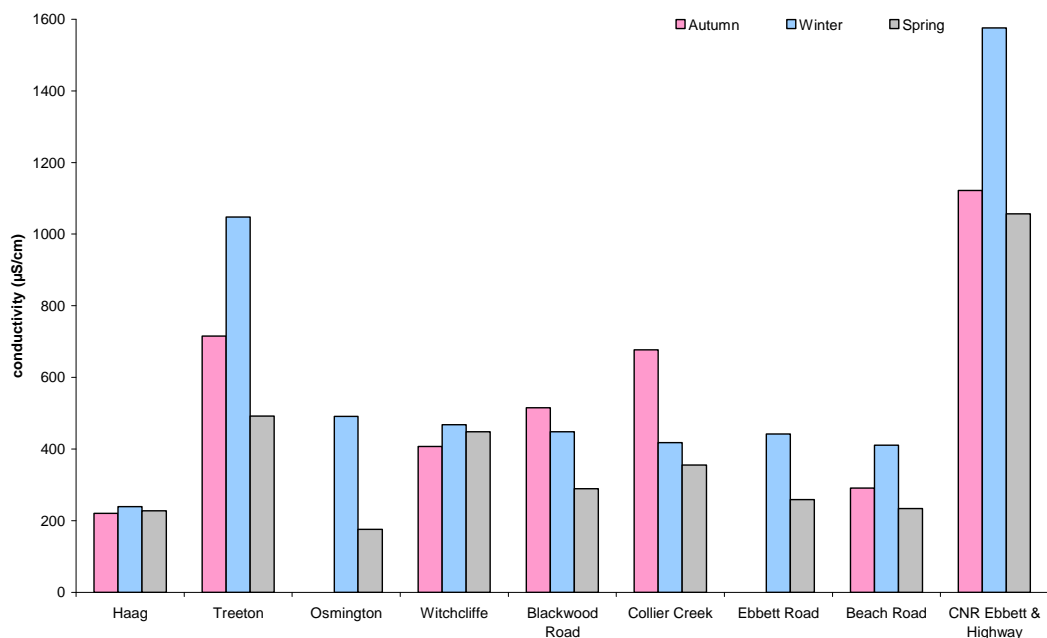


Figure 11: Conductivity ($\mu\text{S}/\text{cm}$) of groundwater samples collected in autumn, winter and spring of 2007 across 9 sites. The missing columns for Ebbett Road and Osmington in autumn are due to the piezometers being dry at these sites.

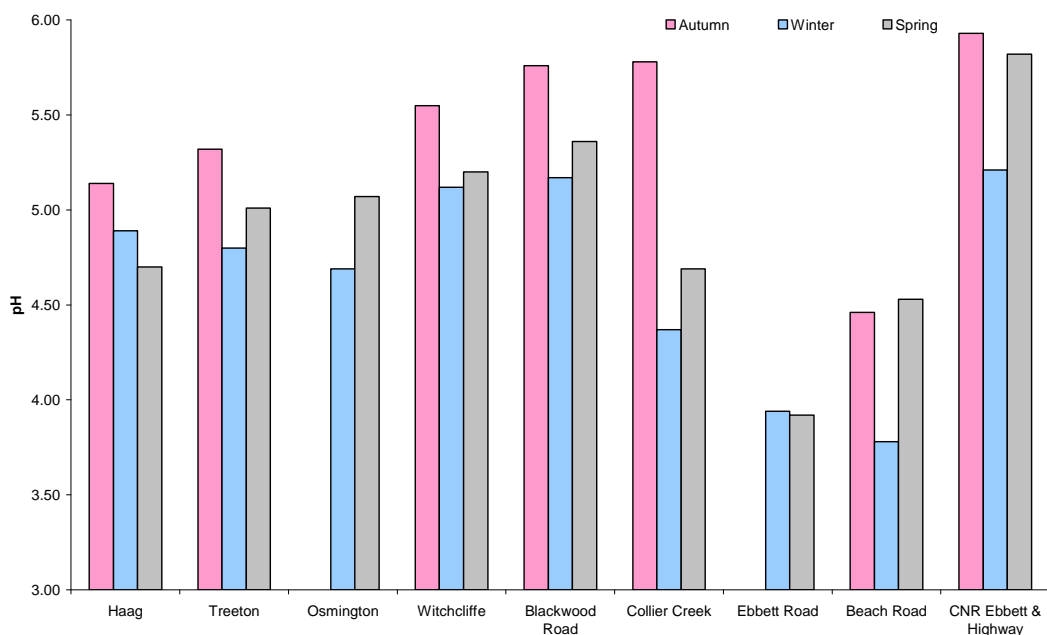


Figure 12: pH of groundwater samples collected in autumn, winter and spring of 2007 across 9 sites. The missing columns for Ebbett Road and Osmington in autumn are due to the piezometers being dry at these sites.

Ammonia and nitrate & nitrite values were higher in the southern *E. walpolea* and *E. subcoerulea* sites (CC-EH) compared to the northern sites (HA-BW)(Figure 13). Nitrate & nitrite values were particularly low in the

ground and burrow waters of Treeton and the adjacent Osmington sites (Figure 13). Burrow water was consistently higher in total phosphate than either surface or groundwater and groundwater was generally higher than surface water at all sites (Figure 14). This pattern was not evident for ortho-phosphate but as with ammonia and nitrate & nitrite it was generally higher in the southern *E. walpolea* and *E. subcoerulea* sites (CC-EH) compared to the northern sites (HA-BW)(Figure 14).

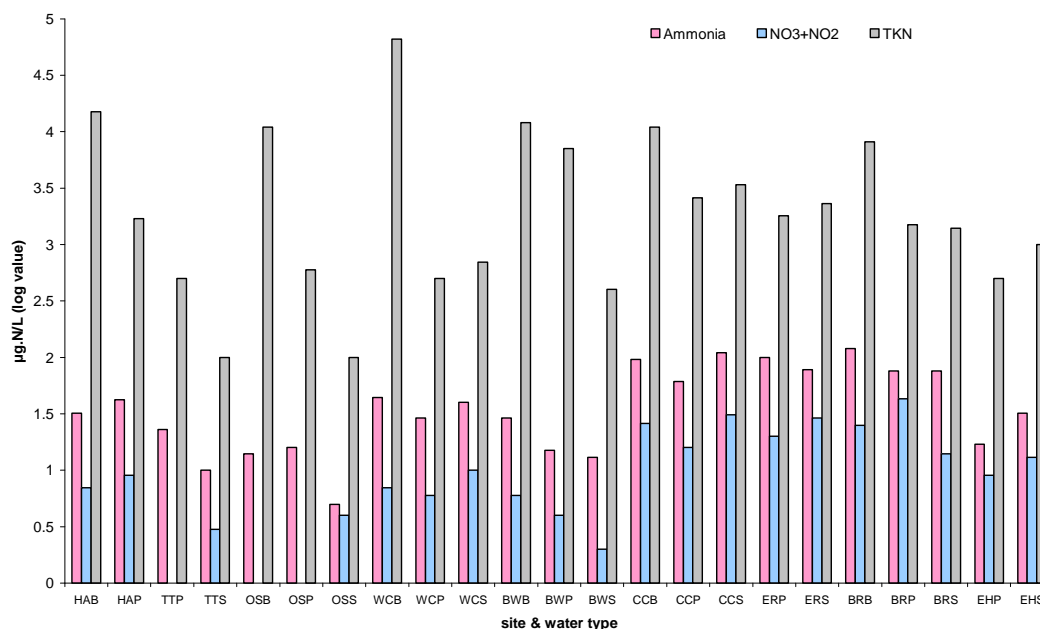


Figure 13: Comparison of the various forms of nitrogen (measured in µg.N/L (log values)) present in water samples collected throughout the range of the crayfish genus *Engaewa*. The sites sampled (and species present) are: HA- Haag (*reducta*), TT- Treeton (*pseudoreducta*), OS- Osmington and WC- Witchcliffe and BW- Blackwood (*similis*), CC- Collier Creek and ER- Ebbett Road (*walpolea*), BR- Beach Road and EH- CNR Ebbett & Highway (*subcoerulea*). The third letter refers to water type: B- burrow, P- groundwater (piezo), S- surface water.

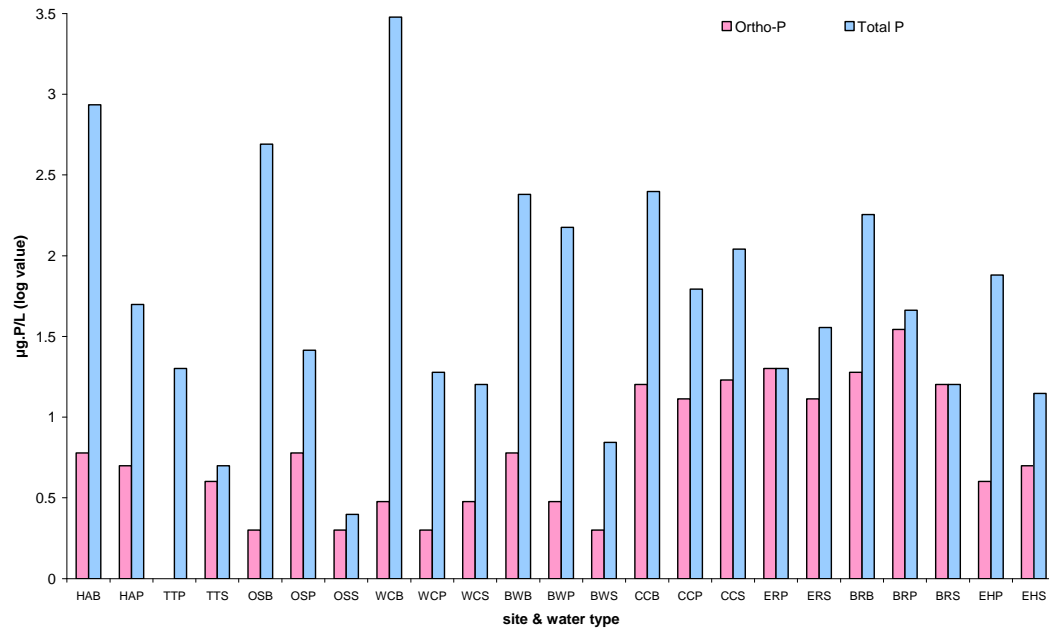


Figure 14: Comparison of the various forms of phosphate (measured in $\mu\text{g.P/L}$ (log values)) present in water samples collected throughout the range of the crayfish genus *Engaewa*. The sites sampled (and species present) are: HA- Haag (*reducta*), TT- Treeton (*pseudoreducta*), OS- Osmington and WC- Witchcliffe and BW- Blackwood (*similis*), CC- Collier Creek and ER- Ebbett Road (*walpolea*), BR- Beach Road and EH- CNR Ebbett & Highway (*subcoerulea*). The third letter refers to water type: B- burrow, P- groundwater (piezo), S- surface water.

A comparison of the ratios of chloride, sodium and magnesium across all sites shows that they are reasonably close, however, the obvious difference is the elevated levels of all three ions in the CNR Ebbett & Highway site compared to all other sites (Figure 15). This was also evident when comparing conductivity values (Figure 11). All sites are $\text{Cl} > \text{Na} > \text{Mg}$ and whilst the values of these are well within the range of what is considered fresh water the ratios (combined with Ca, K & SO_4) highlight the coastal nature of these sites and possibly result from precipitation derived from oceanic evaporation.

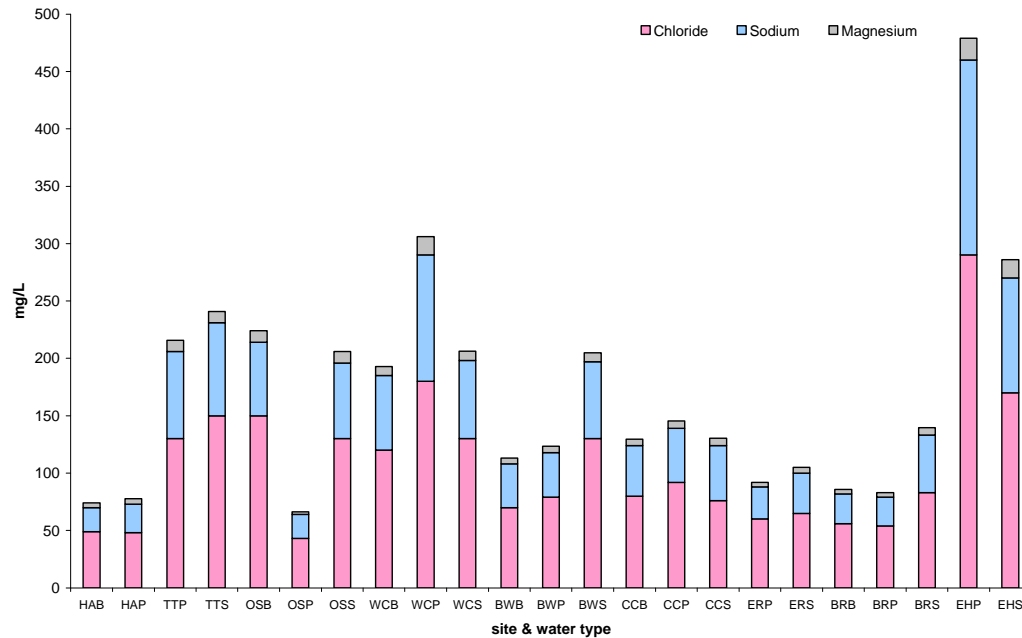


Figure 15: Comparison of the various salts (measured in mg/L) present in water samples collected throughout the range of the crayfish genus *Engaewa*. The sites sampled (and species present) are: HA- Haag (*reducta*), TT- Treeton (*pseudoreducta*), OS- Osmington and WC- Witchcliffe and BW- Blackwood (*similis*), CC- Collier Creek and ER- Ebbett Road (*walpolea*), BR- Beach Road and EH- CNR Ebbett & Highway (*subcoerulea*). The third letter refers to water type: B- burrow, P- groundwater (piezo), S- surface water.

The data from the spring 2007 collection were also analysed via principal component analysis (PCA) using Primer 6 and SPSS 14.0. Total phosphate was log transformed and all data were normalised prior to PCA. Where values were below the reporting limit for the sampling method employed they were estimated to be 50% of the reporting limit. A Draftsman Plot was used to identify highly correlated variables, which were then tested for significance using Pearson's correlation. Where variables were found to have significant correlation one of each pair was removed from the data set before PCA was performed. This resulted in sodium, chloride, magnesium and calcium being removed due to their correlation with conductivity, ammonia being removed due to its correlation with nitrate/nitrite, phosphate and pH, nitrate/nitrite being removed due to its correlation with ortho-phosphate and TKN being removed due to its correlation with total phosphate (Table 3). Variables included in PCA were pH, temperature, conductivity, iron, potassium, sulphur, ammonia, ortho-phosphate and total phosphate. It is important to remember when analyzing the PCAs that any of the excluded variables could be substituted for the corresponding variable that was included in the data set (e.g. saying the data set is separated due to the influence of ortho-phosphate is the same as saying it is due to nitrate/nitrite).

Table 3: Pearson's correlation values for water chemistry pairs which showed significant values ($p < 0.01$).

	Cl	Cond.	Mg	Na	NO3/NO2	Ortho-P	pH	Total-P
Ca	0.833	0.853	0.874	0.834				
Cl		0.985	0.953	0.986				
Cond.			0.977	0.977				
Mg				0.965				
NH4					0.839	0.800	-0.763	
NO3/NO2						0.910		
Total-N								0.987

When analysing the water chemistry data on a site by site basis the first two principal components explained 55% of the variation (Table 4) coming primarily from pH and ortho-phosphate on PC1, iron on PC2 and temperature and conductivity on both PC1 and 2 as shown by their eigenvectors (Table 5). These are represented by the vectors shown in Figure 1 with the length and direction of the blue lines signifying the extent to which each chemical property influences the PCA. It is important to note that sites appear to group more by location than water type (ground, surface, burrow), which can be seen by the general association between colours rather than shapes in Figure 13.

Table 4: Percentage variation and cumulative percentage variation attributable to each principal component of a principal component analysis comparing a suite of water chemistry values (pH, temperature, conductivity, iron, potassium, sulphur, ammonia, ortho-phosphate and total phosphate) amongst sites for all water types (ground, burrow and surface) collected in spring 2007.

PC	% Variation	Cumulative % Variation
1	38.3	38.3
2	17.3	55.7
3	15.0	70.7
4	10.2	80.8
5	8.3	89.1

Table 5: Eigenvectors (correlation coefficients) of the most important water chemistry components for a principal component analysis amongst sites for all water types (ground, burrow and surface) collected in spring 2007.

Variable	PC1	PC2
Iron	0.009	0.822
Ortho-phosphate	0.437	
Temperature	0.388	0.348
pH	-0.464	
Conductivity	-0.427	0.390

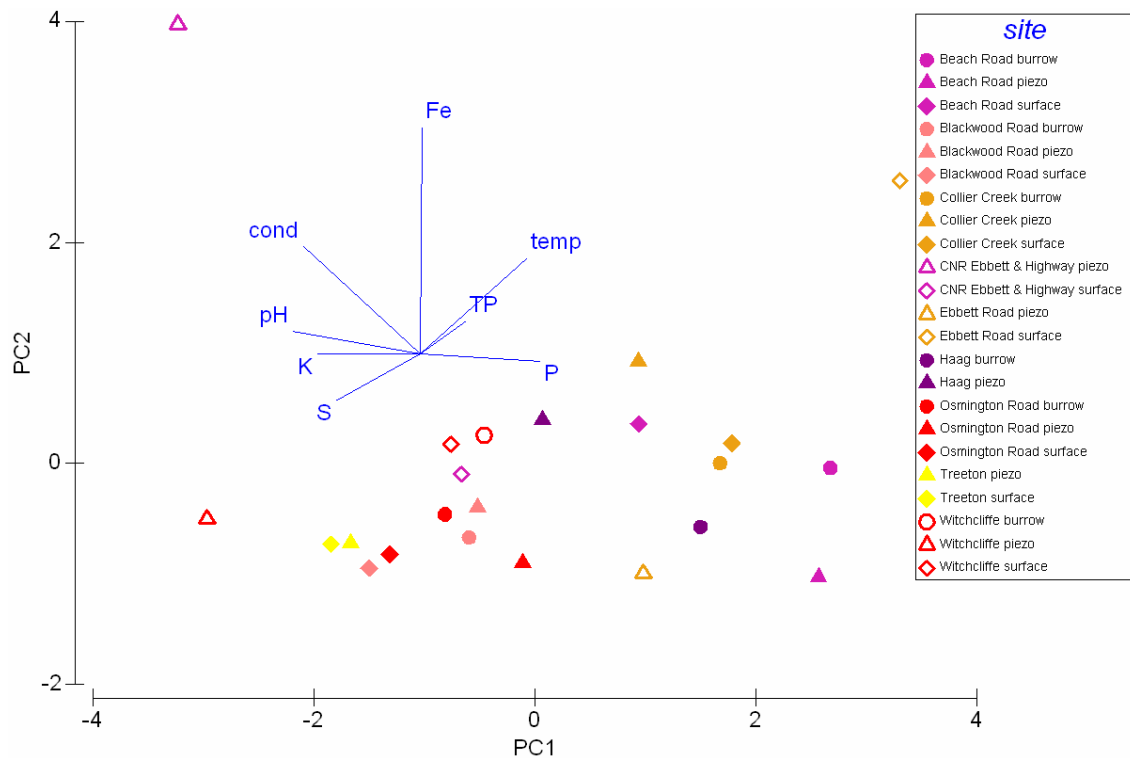


Figure 13: PCA of burrow, surface and groundwater (piezo) identified by the type of water sampled (symbol shape), the species present at each site (symbol colour) and the site from which it was collected (symbol colour filled or outline). Vectors highlighted by the blue lines show the direction and magnitude of influence (cond=conductivity; Fe=iron; K=potassium; P=ortho-phosphate; pH; S=sulphur; temp=temperature; TP=total phosphate).

When analysing the water chemistry data by species (using only the piezometer data as it is a complete data set) the first two principal components explained 72% of the variation (Table 6) coming primarily from iron, sulphate, ortho-phosphate and conductivity as shown by their eigenvectors (Table 7). These are represented by the vectors shown in Figure 14 with the length and direction of the blue lines signifying the extent to which each chemical property influences the PCA. Figure 14 also shows groupings produced by testing the (dis)similarities of sites via Euclidean Distances (represented by the green and blue circles). None of the groups produced by this testing were statistically significant, however, the groupings produced at a distance of 2 and 4 is shown, which are interesting as the similis (Blackwood) site groups with the similis site from Osmington, both of which represent populations that have been flagged as requiring further taxonomic study (this report and Burnham, 2005 respectively). This is despite the similis (Blackwood) site being much closer geographically to the other similis site located at Witchcliffe.

Table 6: Percentage variation and cumulative percentage variation attributable to each principal component of a principal component analysis comparing a suite of water chemistry values (pH, temperature, conductivity, iron, potassium, sulphur, ammonia, ortho-phosphate and total phosphate) amongst sites (distinguished by species present) for only groundwater collected in spring 2007.

PC	% Variation	Cumulative % Variation
1	45.8	45.8
2	26.2	72.0
3	11.6	83.6
4	8.8	92.4
5	4.2	96.6

Table 7: Eigenvectors (correlation coefficients) of the most important water chemistry components for a principal component analysis amongst sites (distinguished by species present) for only groundwater collected in spring 2007.

Variable	PC1	PC2
Iron	-0.384	0.576
Sulphur	-0.294	-0.752
Ortho-phosphate	0.446	
Conductivity	-0.590	

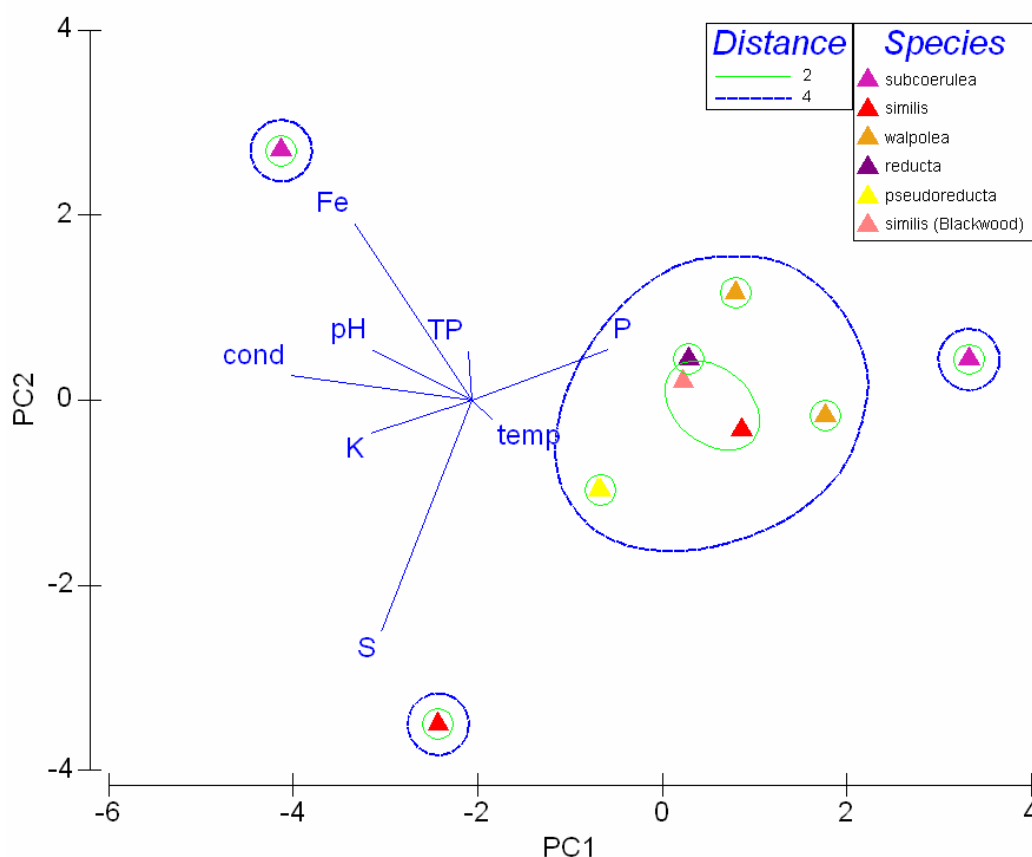


Figure 14: PCA of groundwater identified by the species present at each site. Vectors highlighted by the blue lines show the direction and magnitude of influence (cond=conductivity; Fe=iron; K=potassium; P=ortho-phosphate; pH; S=sulphur; temp=temperature; TP=total phosphate). The green and blue circles represent groupings identified at Euclidean distances of 2 and 4 respectively, though neither are statistically significant.

CONSERVATION.

An important aspect of conservation is formal recognition of variation (whether above or below the species level) so that specific populations can be afforded protection under law. This is one of the major outcomes that will ultimately result from the on-going systematic studies of this genus. Another equally important aspect of conservation is understanding what is required for species persistence, otherwise there is no point providing “protection under law”. To successfully ensure the on-going survival of a species (perhaps more correctly thought of as a certain number of populations) it is necessary to identify what the specific requirements of a species is and, conversely, what factors are likely to provide a threat to their survival. This report (and the continuing research) is largely focused on the first two aspects mentioned, namely recognition and distribution of diversity and recording and analysing the interaction between biology and environment. The paucity of publications relating to this crayfish genus highlight how little is currently known about it and this situation is mirrored when we attempt to analyse the threats that it may face. Despite this we can make some reasonable assumptions.

Horwitz and Adams (2000) listed a number of factors that may be detrimental to the survival of *Engaewa* populations, which were; drainage of wetlands (for peat or sand mining or for grazing), damming of water courses (for viticulture or agriculture), clearing of native vegetation, afforestation practices and cattle grazing. From this list and what has been observed during collecting for this project it appears that the major threat to *Engaewa* species is habitat loss, as much of their presumed natural range has been affected by alterations to water levels (whether from drainage, dam construction or water extraction) and/or clearing of native wetlands and creeks. The maps in this report are evidence to the extreme levels of habitat fragmentation and reduction that has occurred, particularly in the northern part of *Engaewa*'s range. Where habitat was found that appeared suitable for *Engaewa* and was in a relatively undisturbed condition the crayfish were virtually always found to be present. Although they appeared quite able to adapt to assumedly sub-optimal habitat, such as road edges, they were never found in farm paddocks, even when their burrows may have been plentiful in an adjoining property (to the point where they could be found next to the fence post), or even in still vegetated areas to which cattle had been allowed access.

The effect of fire on *Engaewa* appears to be minimal as they can be readily (and often more easily) found in recently burnt areas as their chimneys are clearly apparent. Although fire *per se* does not appear to be a problem the timing of any prescribed burns may be important. The collection of crayfish above ground has shown that at various times of the year they do exit their burrows, possibly for mature crayfish to seek mates and for juveniles to disperse from the familial burrow? It may be that they will be vulnerable to the effects of fire at this time, either directly from the fire, or perhaps more likely from alteration to the surface water chemistry or from increased

predation resulting from the absence of dense vegetation and/or leaf litter which would assist their movements above ground.

To fully understand any impact that fire may have in this regard we need further biological and behavioural data, however, it has been shown that freshwater crayfish can be generally divided in to either summer or winter breeders and may only breed every second year (Hamr & Richardson, 1994). Currently it is unclear when *Engaewa* species breed but Horwitz and Adams (2000) record the collection of berried females in May (*E. similis*) and January (*E. walpolea*) and extremely small juveniles in May and August (*E. similis*) and August (*E. pseudoreducta*). During collecting for this study berried females of *E. walpolea* were found during September (including a specimen that was found by spotlighting and was walking in the bottom of a puddle). For the time being it is probably best to assume that they would rarely leave their burrow when surface water is not present and that they would most likely follow the water table as it rises and falls. This would suggest that prescribed burns should occur ideally at the driest time suitable, and perhaps toward the end of spring opposed to autumn so that the vegetation will be somewhat re-grown when the crayfish are leaving their burrows.

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Appendix A: Sites from which *Engaewa* specimens were collected in this study.

<i>Engaewa reducta</i>		<i>Engaewa subcoerulea</i>	
allan's PP	50332189 6264381	ebbett road 1	50494829 6129881
arnold's PP	50324476 6274546	ebbett road 2	50494584 6130023
blythe road 1	50324254 6267560	beach road	50487534 6125523
blythe road 2	50324294 6266870	beach road	50487640 6124354
blythe road 3	50324484 6266124	ficifolia road	50493098 6124370
credero's PP	50327505 6265642	station road	50486890 6125946
ensor's PP		wheatley coast rd	50419319 6166301
forest rise PP		windy harbour rd	50415385 6148175
haag	50327037 6267255	windy harbour rd	50415139 6147553
payne rd	50332937 6263734	windy harbour rd	50414823 6146963
		chesapeake 1	50434138 6146630
		chesapeake east	50445238 6142816
<i>Engaewa pseudoreducta</i>			
treeton 2	50333028 6247985	805	50460559 6136132
		813	50457384 6146521
<i>Engaewa similis</i>			
adelaide creek	50345724 6226893	kent river	50503138 6131444
blackwood road (1)	50342183 6223054	N to S A	50456743 6149008
blackwood road (1a)	50341893 6222604	N to S B	50456971 6148579
blackwood road (2)	50343427 6223522	N to S C	50457423 6146777
blackwood road (3)	50344956 6224254	N to S D	50457382 6146496
blackwood road (3a)	50345115 6225487	N to S E	50458492 6143827
blackwood road (4)	50346630 6225055	N to S F	50459041 6140293
bramley 1	50356607 6244428	N to S G	50463759 6130886
bramley 2	50325523 6244446	two road	50464919 6130885
calgardup brook	50319588 6230031		
crinia creek	50340750 6228140	<i>Engaewa walpolea</i>	
GA1A	50328039 6232363	butler creek (tourist info)	50475360 6129799
hine road	50338058 6228137	cemetery creek (crossing)	50476796 6129550
leath road	50337080 6228086	cemetery creek (tree fern)	50476900 6130513
miles road	50337824 6225433	chugg street	50476255 6129732
nyandimurra school	50325035 6228807	collier creek	50477144 6128484
osmington	50331104 6246798	collier creek tributary	50476818 6128911
scott river 1	50335248 6204311	ebbett road 3	50492642 6130607
scott river 2	50339452 6205009	the knoll	50475334 6127818
scott road	50387974 6191735	water corp	50473800 6130570
spearwood creek	50344228 6228877	boronia ridge	50474707 6129456
witchcliffe	50328188 6234221	little river track	50475250 6130740

Sites with PP suffix are on private property

GENUS	SPECIES	Allan's	Blythe Road	Boronia Ridge	Bramley	Chesa peake	Chugg Street	Collier Creek	Collier Ck trib	Ebbett & Hwy	Haag	Inlet River	Kent River
Acacia	browniana					1						1	
Acacia	divergens												
Acacia	hastulata			1				1		1			1
Acacia	myrtifolia						1	1					
Acacia	pentadenia												
Acacia	pulchella				1								
Adenanthos	obovatus											1	
Agonis	flexuosa						1						
Anarthria	prolifera											1	
Anarthria	scabra												
Anigozanthus	flavidus						1						
Astartea	leptophylla?		1		1					1	1		
Beaufortia	sparsa			1		1	1	1	1		1	1	
Boronia	stricta			1		1		1			1		
Callistemon	glaucus												1
Comesperma	virgatum					1							
Dasypogon	bromeliifolius											1	
Drosera	macrantha								1				
Evandra	artistata			1		1						1	
Hakea	ceratophylla											1	1
Hakea	linearis												
Homalospermum	firmum	1		1	1					1	1		1
Johnsonia	lupulina											1	
Juncus	pallidus				1								
Lepidosperma	gladiatum												
Lepidosperma	tetraquetrum		1		1								
Leucopogon	australis	1		1									
Melanostachya	ustulata												
Pimelea	lanata									1			
Pteridium	esculentum	1	1					1					
Sphaerolobium	grandiflorum			1	1		1			1			1
Sphenotoma	gracile					1							
Sphenotoma	squarrosum										1		
Taraxis	grossa	1	1	1	1	1		1	1	1	1		1
Taxandria	juniperina	1		1			1	1			1		
Taxandria	linearifolia		1		1	1		1		1			1
Taxandria	parviceps						1	1					
Reedia				1									
unknown		1	1	4	2	6	5	3	5	3	2	8	4
TOTAL		6	6	14	10	14	12	12	8	10	9	16	11

		Mam-moth	N'cliffe	Payne Road	Station Road	The Knoll	Treeton	Windy Harbour	Witch cliffe	Wye Road	Yel-verton	TOTAL	%
GENUS	SPECIES												
Acacia	browniana											2	9.09
Acacia	divergens									1		1	4.55
Acacia	hastulata		1		1					1		7	31.82
Acacia	myrtifolia							1		1		4	18.18
Acacia	pentadenia				1							1	4.55
Acacia	pulchella						1	1	1			4	18.18
Adenanthos	obovatus			1								2	9.09
Agonis	flexuosa					1						2	9.09
Anarthria	prolifera							1				2	9.09
Anarthria	scabra							1				1	4.55
Anigozanthus	flavidus											1	4.55
Astartea	leptophylla?	1			1		1		1		1	9	40.91
Beaufortia	sparsa			1	1			1		1		11	50.00
Boronia	stricta				1					1		6	27.27
Callistemon	glaucus											1	4.55
Comesperma	virgatum							1				2	9.09
Dasypogon	bromeliifolius							1		1		3	13.64
Drosera	macrantha							1		1		3	13.64
Evandra	artistata				1			1		1		6	27.27
Hakea	ceratophylla											2	9.09
Hakea	linearis				1							1	4.55
Homalospermum	firmum		1		1	1				1	1	11	50.00
Johnsonia	lupulina							1				2	9.09
Juncus	pallidus	1										2	9.09
Lepidosperma	gladiatum	1				1						2	9.09
Lepidosperma	tetraquetrum	1	1				1		1			6	27.27
Leucopogon	australis											2	9.09
Melanostachya	ustulata			1								1	4.55
Pimelea	lanata									1		2	9.09
Pteridium	esculentum											3	13.64
Sphaerolobium	grandiflorum				1			1		1		8	36.36
Sphenotoma	gracile				1							2	9.09
Sphenotoma	squarrosus									1		2	9.09
Taraxis	grossa		1		1	1				1	1	15	68.18
Taxandria	juniperina				1					1	1	8	36.36
Taxandria	linearifolia	1					1		1			9	40.91
Taxandria	parviceps					1		1		1		5	22.73
Reedia												1	4.55
unknown		3	3	5	3	2	1	8	3	2	2		
TOTAL		8	7	8	15	7	5	20	7	17	6		

185			
190			
195			
200			
205			
210			
215			
220			
225			
230			
235			
240			
245		heavy sticky grey sandy clay, pale mottling	
250			
255			
260			
265			
270			
275			
280			
285			
290			
295			
300			white grey medium- heavy clay
305			
310			
315			
320			
325			
330			

METHOD		5030	5050	4700	2700	ICP001	ICP001	ICP001	ICP001	ICP001	ICP001	ICP001	ICP001	ICP001	ICP001	ICP001	ICP001
SAMPLE	Date	Cl	SO4	TOTAL-P	TOTAL-N	K	Na	Total Ext	Total Ext	Total Ext	Total Ext	Total Ext	Total Ext	Total Ext	Total Ext	Total Ext	Total Ext
Reporting		mg/L	mg/L	µg.P/L	µg.N/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Limit		<1	<1	<5	<50	<0.25	<0.25	<0.005	<0.003	<0.005	<0.05	<0.025	<0.001	<0.02	<0.1	<0.25	<0.025
File		7110101	7110101	7102401		7101602	7101602	7101003	7101003	7101003	7101003	7101003	7101003	7101003	7101003	7101003	7101003
HAPA	4/7/07	58	28	770	12000	7.4	41	10	<0.003	0.006	12	8.7	0.06	<0.02	0.3	10	1.4
TTPA	4/7/07	210	14	130	1100	6.2	110	8.6	<0.003	<0.005	2.9	14	0.032	<0.02	<0.1	5.5	0.35
WCPA	4/7/07	97	34	640	1900	11	69	12	<0.003	0.018	2.2	8	0.089	<0.02	0.3	14	0.82
BWPA	4/7/07	120	38	660	23000	10	74	19	<0.003	0.007	1	14	0.061	<0.02	0.2	15	0.59
CCPA	4/7/07	200	12	2500	33000	3.9	110	12	<0.003	<0.005	7	12	0.029	<0.02	0.3	6.9	1
CCSA	4/7/07	130	33	1400	34000	3	70	4.8	<0.003	<0.005	0.99	8.4	0.016	<0.02	<0.1	4.5	0.56
BRPA	4/7/07	84	32	2000	100000	7.6	54	40	<0.003	0.014	2.9	13	0.052	<0.02	0.3	21	2
EHPA	4/7/07	320	2	3200	3000	6.3	180	14	<0.003	<0.005	8.6	20	0.1	<0.02	0.3	1.4	0.25
HAPJ	7/7/07	58	40	56	1700	2.5	37	9	<0.003	<0.005	2.4	6.2	0.027	<0.02	<0.1	4.8	0.4
OSPJ	7/7/07	120	34	51	510	3.1	82	9.7	<0.003	<0.005	0.07	8.6	0.013	<0.02	<0.1	13	0.54
TTPJ	7/7/07	290	15	14	200	4.8	150	3.9	<0.003	<0.005	0.16	19	0.006	<0.02	<0.1	6.2	0.52
WCPJ	7/7/07	120	31	24	170	5.2	74	5.2	<0.003	<0.005	0.15	7.2	0.055	<0.02	<0.1	12	0.89
BWPJ	7/7/07	110	29	320	12000	5.8	70	7.7	0.003	<0.005	0.24	9	0.026	<0.02	<0.1	8	0.66
CCPJ	7/7/07	100	70	28	2000	4.8	64	6.1	<0.003	<0.005	0.94	8.8	0.01	<0.02	<0.1	10	0.5
CCSJ	7/7/07	77	34	17	1000	2.8	47	6.8	<0.003	<0.005	1.5	6.9	0.021	<0.02	<0.1	7	0.52
BRPJ	7/7/07	68	35	16	1000	1.2	52	3.1	<0.003	<0.005	0.09	8.6	0.002	<0.02	<0.1	6.9	0.57
BRSJ	7/7/07	110	64	10	1300	3.6	78	4.6	<0.003	<0.005	1.5	10	0.002	<0.02	<0.1	9	0.76
EHPJ	7/7/07	370	94	74	440	4.8	220	14	<0.003	<0.005	1.4	35	0.069	<0.02	<0.1	37	0.19
ERPJ	7/7/07	94	140	250	8100	1.6	65	6.8	<0.003	<0.005	0.71	9.7	0.01	<0.02	<0.1	14	0.83
BLANK A	4/7/07					<0.25	2.3	1.9	<0.003	<0.005	<0.05	0.26	0.004	<0.02	<0.1	<0.25	<0.025
BLANK J	7/7/07					0.5	13	0.46	<0.003	<0.005	<0.05	0.07	<0.001	<0.02	<0.1	<0.25	0.03

METHOD SAMPLE CODE	Sampling Date	ICP001 Ca mg/L	ICP001 Fe mg/L	ICP001 K mg/L	ICP001 Mg mg/L	ICP001 Na mg/L	ICP001 S mg/L	5030 Cl mg/L	2000 AMMONIA µg.N/L	4100 ORTHO-P µg.P/L	2100 NO3+NO2 µg.N/L	4700 TOTAL-P µg.P/L	2700-2100 TKN calculated µg.N/L
Reporting Limit		<0.005	<0.01	<0.25	<0.025	<0.25	<0.25	<1	<3	<2	<2	<5	<200
File		7112701	7112701	7112701	7112701	7112701	7112701	7112601		7112201		7112802	
BLKO	5/10/07	1.3	0.06	0.7	0.18	7.4	<0.25	13	10	<2	55	<5	<200
BRBO	5/10/07	1.8	0.98	1.5	3.6	26	1.1	56	120	19	25	180	8100
BRPO	5/10/07	1	0.15	1.1	4	25	2.3	54	76	35	43	46	1500
BRSO	5/10/07	3.6	1.4	3.6	6.5	50	3.2	83	76	16	14	16	1400
BWBO	12/10/07	3.4	0.39	3.7	5.2	38	1	70	29	6	6	240	12000
BWPO	12/10/07	3.2	0.34	3.6	5.4	39	1.5	79	15	3	4	150	7100
BWSO	12/10/07	2.8	0.23	2.6	7.8	67	2.5	130	13	2	2	7	400
CCBO	5/10/07	4.6	0.58	2.5	5.5	44	1.7	80	96	16	26	250	11000
CCPO	5/10/07	3.3	2.1	2.8	6.4	47	0.6	92	61	13	16	62	2600
CCSO	5/10/07	3.5	1	2.2	6.5	48	1.1	76	110	17	31	110	3400
EHPO	5/10/07	11	5.2	4.8	19	170	1.8	290	17	4	9	76	500
EHSO	5/10/07	10	0.7	1.6	16	100	7.5	170	32	5	13	14	1000
ERPO	5/10/07	2.6	0.25	4.8	4	28	1.1	60	100	20	20	20	1800
ERSO	5/10/07	3	3.7	0.7	5	35	1.6	65	78	13	29	36	2300
HABO	12/10/07	1.6	0.69	2	3.9	21	3.1	49	32	6	7	860	15000
HAPO	12/10/07	2.4	2.6	2.4	4.8	25	4.7	48	42	5	9	50	1700
OSBO	12/10/07	5.8	0.21	2.7	10	64	5.1	150	14	2	1	490	11000
OSPO	12/10/07	2.1	0.16	3.7	2.4	21	2.4	43	16	6	1	26	600
OSSO	12/10/07	6.2	0.26	2.7	10	66	5.3	130	5	2	4	3	100
TTPO	12/10/07	2.3	0.53	3.1	9.7	76	7	130	23	1	1	20	500
TTSO	12/10/07	3	0.25	3.3	9.8	81	4.5	150	10	4	3	5	100
WCBO	12/10/07	3.8	1.1	2.5	8	65	3	120	44	3	7	3000	66000
WCPO	12/10/07	5.8	0.25	4.6	16	110	18	180	29	2	6	19	500
WCSO	12/10/07	4	1.1	2.6	8.2	68	3.1	130	40	3	10	16	700