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Diversity and distribution of groundwater fauna in a calcrete aquifer: does sampling method influence the story?

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Abstract. There has been an increase in the number of studies examining the spatial and temporal patterns in species richness, community structure and population dynamics of groundwater organisms. These studies have raised the issue of uncertainty about the comparability of different sampling methods, and questions of whether sampling bias may exist. Recently, a diverse subterranean fauna was discovered in calcrete (carbonate) aquifers of the Yilgarn Region of central Western Australia. Little is known about the community structure and population dynamics in these aquifers. One important issue is whether current sampling methods adequately sample the species richness and abundance of the fauna to allow for comparative studies. Here we investigate the effectiveness of three sampling methods: haul net sampling, pumping with a 12-V impeller pump, and a discrete interval sampler. The methods were trialled over 16 months with >250 samples taken from 55 uncased bore holes. No significant taxonomic bias was detected across the sampling methods. However, sampling using a haul net was found to be the most efficient method for capturing the available taxa per unit time when sampling bores are less than 10 m deep, with pumping being the least efficient. These results are discussed in relation to the problems of studying stygofauna in Western Australian calcrete aquifers, and of groundwater faunas more generally.

Additional keywords: discrete interval sampling, haul net, pump sampling, stygofauna.

Introduction

As more than 97% of the world's freshwater lies underground (Gibert *et al*. 1994), groundwater is an important natural resource that is facing increasing threat from human activities, including mining, agricultural, industrial, and domestic consumption (Danielopol *et al*. 2003; Eberhard *et al*. 2004). Groundwater is not an inert resource, but sustains a rich community of microorganisms and invertebrates that are poorly understood (Marmonier *et al*. 1993; Boulton *et al*. 2003*a*). In Australia, it has only recently been discovered that groundwater in many parts of the arid zone harbours diverse invertebrate communities, many of which contain unique (Humphreys 2001, 2006), short-range endemic species (Harvey 2002). This finding has important consequences for the management of groundwater in the Australian arid zone and there is a need to develop robust methods to assess the conservation significance of these ecosystems and to monitor any human impacts.

In 1998, a series of subterranean communities was discovered in isolated calcrete aquifers (~10 m thick carbonate deposits) in the arid Yilgarn region of central Western Australia (Humphreys 2001), which are the focus of this study. The fauna found within these calcretes comprise obligate subterranean aquatic taxa (collectively known as stygofauna) including water beetles (Dytiscidae; Watts and Humphreys 2006; and references therein) and Crustacea such as Bathynellacea (Cho 2005; Cho *et al*. 2006*a*, 2006*b*), Oniscidea (Taiti and Humphreys 2001), Amphipoda (J. Bradbury, pers. comm.), Copepoda (Karanovic 2004) and Ostracoda (Karanovic and Marmonier 2002). Intensive fieldwork in recent years within the central and northern Yilgarn region has shown that most stygobitic species are restricted to individual calcrete bodies, and do not occur in the surrounding matrix, which usually comprises fine unconsolidated sediments (Boulton *et al*. 2003*b*; W. Humphreys, pers. comm.). Ongoing molecular studies also revealed that each calcrete represents a closed 'island' habitat, with no apparent gene flow between them for millions of years (Cooper *et al*. 2002, 2007; 2008; Leys *et al*. 2003; Guzik *et al*. 2008). The invertebrate communities are similar between calcretes, with most calcretes containing between 1–3 species of beetles, either chiltoniid (previously referred to as hyalid; Cooper *et al*. 2007) or crangonyctoid amphipods, and copepods.

Although most research into groundwater organisms has focussed on describing species (Edler and Dodds 1996), studies that compare sampling methods and design for assessing groundwater communities are becoming more common and nec-

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essary (e.g. Dumas and Fontanini 2001; Hahn 2002, 2005; 2006; Scarsbrook and Halliday 2002; Schmidt *et al*. 2004). However, these are mostly associated with alluvial habitats, and in locations where the geology, climate and vegetation are very different to that of the arid zone calcrete aquifers. Further, there has been little attention paid to standardisation of methods, both for biodiversity assessment and for ecological research (e.g. examining population changes in space and time) (Juberthie 2000; Hancock and Steward 2004; Hahn 2006). As much of the work on the Yilgarn calcretes has been once-off sampling of potentially sparsely populated aquifers, it is important to have some idea of the likelihood of not catching all taxa present, particularly if some are relatively rare (Dumas and Fontanini 2001). Although a large number of species have now been recorded from Yilgarn calcretes, a lack of replicated sampling has severely limited understanding of their basic biology and ecology.

The methods available for groundwater sampling all have inherent strengths, weaknesses, and different required levels of time and effort. A combination of methods is proposed as most effective for obtaining information, such as numbers of species present or their relative abundance (Hancock and Steward 2004). Methods useful for once-off diversity surveys may not necessarily be suitable for long-term monitoring of populations or community structure (Schmidt *et al*. 2004). Ideally, for ecological studies, sampling methods should allow for a stratified approach, sampling organisms from specific depths within the aquifer (Hahn 2006). Such an approach is likely to be more amenable to determining the role of physical factors (e.g. salinity, pH, temperature) in the ecology and life history of the organisms present within an aquifer.

Three criteria have been proposed for assessing the effectiveness of a sampling method for groundwater invertebrates (Bretschko and Klemens 1986; Scarsbrook and Halliday 2002). These are: (1) that specific species are not attracted or repulsed by the sampling process;, (2) that the method works in different sediment types;, and (3) that samples are taken in a well defined location, with respect to space and time. Although it is possible to apply these criteria to sampling more accessible groundwater communities, such as in the hyporheic zone, it is far more difficult to assess the first two criteria for sampling calcrete aquifers because of their inherent inaccessibility.

At least four techniques have been used for sampling stygofauna from wells and boreholes: (1) trapping by means of artificial substrate or baited traps; (2) collecting by bailing (including use of an interval sampler); (3) pumping bore water; or (4) using haul nets (= phreatic nets). These and other methods for collecting stygofauna have been examined previously in other localities and groundwater habitat types in Australia (Hancock and Steward 2004; Eberhard *et al*. in press). However, there are major differences in the matrix and depths where stygofauna are found; for example, between alluvial habitats and calcretes in the Yilgarn region. Given the intensity of research focussed on the Western Australian calcretes, and the Yilgarn in particular (e.g. Cooper *et al*. 2002, 2007; 2008; Leys *et al*. 2003; Guzik *et al*. 2008), evaluation of the performance of specific sampling methods for this habitat are required if more detailed information is to be forthcoming of the unique communities they harbour. Therefore, the aims of this study were to investigate the performance of three methods for sampling stygofauna, with a specific focus on exploratory and ecological studies within calcrete aquifers. The three methods, haul net sampling, pumping with a 12-V impeller pump, and a discrete interval sampler, were tested individually and in combination, and the results used to assess the efficiency and taxonomic bias of each method.

Methods

Site description

The study site was part of an extensive groundwater calcrete on the Sturt Meadows pastoral station (28°41′S 120°58′E), ~50 km from Leonora in Western Australia. The terrain is open Acacia woodland, interspersed with low shrubs. Calcrete is exposed at the surface in some areas, whereas in others it is up to 2 m below the surface. Several small abandoned calcrete quarries are found throughout the area. The calcrete formation is typical of the Yilgarn area, in that it was formed within a paleodrainage channel upstream of a salt lake (Morgan 1993), in this instance, Lake Raeside (see fig. 1 in Cooper *et al*. 2008).

The Sturt Meadows calcrete has an area of ~43 km^2 and the study site comprises two contiguous grids of mineral exploration bores orientated north–south (Fig. 1). The northern grid is 0.9 × 1.4 km with bore spacing at 100 m in each direction; the southern grid is 1.2 × 0.9 km with bore spacing 100 m east–west and 200 m north–south (Fig. 1). The bores were reverse-circulation percussion-drilled (~100 mm in diameter) in 2001 to an average depth of 10.3 m to sample the quality of the calcrete and then sealed with a concrete plug. These plugs were removed in October 2004 and replaced with a 100 mm PVC sleeve, 1.5 m long, cemented in place to stabilise the top of the bore, and the bore was capped. Thus, the bores are uncased for most of their length, including for the entire water column. The resulting siltation and collapse of the bore walls has affected differentially access to individual bores, such that the amount of watertable accessible varies from 1.9–4.0 m below the surface, with a water depth between 0.4–8 m. The mean permeability was 1.9–4.6 × 10^{-4} m s^{-1} (Anaconda 2001), which is similar to that of sand and suggests an average porosity of ~25%.

Sampling methods

Three methods were employed to sample the water column in the bores: drawing a haul net through the water column, filtering water pumped from the bore, and collecting bore water with a discrete interval sampler. A fourth method, using artificial substrate traps, has recently been trialled at Sturt Meadows, but was unsuitable for the sampling design used in the current study (see below), due to the need for a significant lag time before colonisation of the traps.

The haul net consisted of a weighted fine mesh net (250 μm), with a 65 mm diameter wire ring around the top to ensure the mouth remained open during retrieval, and a socket for the attachment of a collection tube at the opposite end (Fig. 2). As the bores were unlined, the water column contained suspended silt, and a large mesh size was chosen to limit clogging of the net while still capturing all the macroinvertebrate species. The net was lowered to the bottom of the bore and then retrieved slowly through the water column, concentrating the fauna into the collection tube. After each haul, the collection tube was replaced and each tube was sorted individually.

The discrete interval sampler, a 2.2-L Kemmerer SS Water Sampler (Wildco Supply Co., Buffalo, NY, USA), consisted of a stainless steel cylinder (75 mm diameter, 500 mm long) attached to a retrieval rope, which is open at the top and bottom and has a spring-loaded clamp (Fig. 3). When the appropriate depth is reached, the release of a messenger weight triggers the sampler to shut at both ends. On recovery of the sampler, water is drained by means of a tap and then filtered through a fine mesh net (250 μm) to collect any invertebrates. Sampling was repeated every 0.5 m of water depth throughout the water column and each stratum sampled was sorted individually.

Pumping utilised a 12-V impeller pump capable of sustaining pumping rates of 5 L min^{-1}, and had an outlet diameter of 10 mm to extract water from just below the water surface. This region of the water column was targeted to avoid impellor damage by sucking up sediment from the bottom of the bore. Also, pilot studies (described below) found the highest species richness within the top 500 mm of water, with all known macroinvertebrate species obtained after ~14 pump samples, whereas only four species were collected at the bottom (Fig. 4). The extracted water was filtered through a fine mesh net (250 μm), and the fauna concentrated into a collection tube.

After each sampling event, the haul nets and the discrete interval sampler were rinsed using filtered rainwater, and the pump and associated tubing was rinsed by pumping 10 L of filtered rainwater, to ensure no organisms were erroneously counted in subsequent samples or transferred between bores. Samples were sorted in a field laboratory using a dissection microscope, using 10–20× magnifications. In samples with a high silt load or with large numbers of small invertebrates, easily detected organisms were first removed; the sample was then treated with ethanol and stained with Bengal Red, and sorted a second time.

Sampling design

A pilot study using haul net sampling was conducted over two 1-week blocks, six months apart, to identify boreholes containing stygofauna and to ensure sampling in one period would not adversely impact on the number of animals collected in subsequent trips. Repeat sampling was conducted across 20 holes; the holes were resampled one week apart, and a one-way Analysis of Variance showed no significant difference in the number of taxa or number of individuals collected between the two times (data not shown). For the main study, sampling was conducted in five intensive 1-week blocks over a period of 18 months, with a minimum of two months between sampling times. Five combinations of three methods were trialled as follows: (1) five repeated haul samples; (2) 10 repeated haul samples; (3) five repeated haul samples, followed by one 25-L pump sample; (4) two 25-L pump samples; and (5) one complete water column profile using the discrete interval sampler progressively sampling towards the bottom of the bore.

The sampling methods were trialled using a rotating block design. Eleven blocks, consisting of five bores per block, were sampled on each sampling occasion. Bores were selected from those from which invertebrates had previously been captured, and in which the water was deeper than one metre. At the outset of the study, one bore in each block was randomly assigned the treatment 'five repeated haul samples' and was so sampled each trip. The other four treatments were each randomly allocated to the remaining bores in each block and then rotated across the remaining bores on subsequent trips, using a Latin square approach to minimise the impact of any sampling interference of the previous treatments. The rotating block design was selected to minimise environmental artefacts, such as variation due to differences in bore hole size and physio-chemical properties, given that pilot studies had shown considerable within- and between- bore variation that could potentially mask any differences among methods. During the final sampling period, the time taken to perform each method for each of the 11 blocks was recorded.

Taxon identification

Stygofauna collected in the Sturt Meadows calcrete included three species of dytiscid beetle (Watts and Humphreys 2006), Amphipoda: Chiltoniidae, Oligochaeta and Copepoda: Harpacticoida and Cyclopoida. As part of an independent study, taxa sampled from the Yilgarn calcretes were sequenced for the cytochrome *c* oxidase I (CO1) gene, to provide diagnostic molecular 'barcodes' and to test for the presence of cryptic species. These analyses verified the presence in samples of three dytiscid beetle species, a single species in each of the two copepod orders and multiple species of chiltoniid amphipods and oligochaete worms (T. Bradford, pers. comm.). A lack of clear

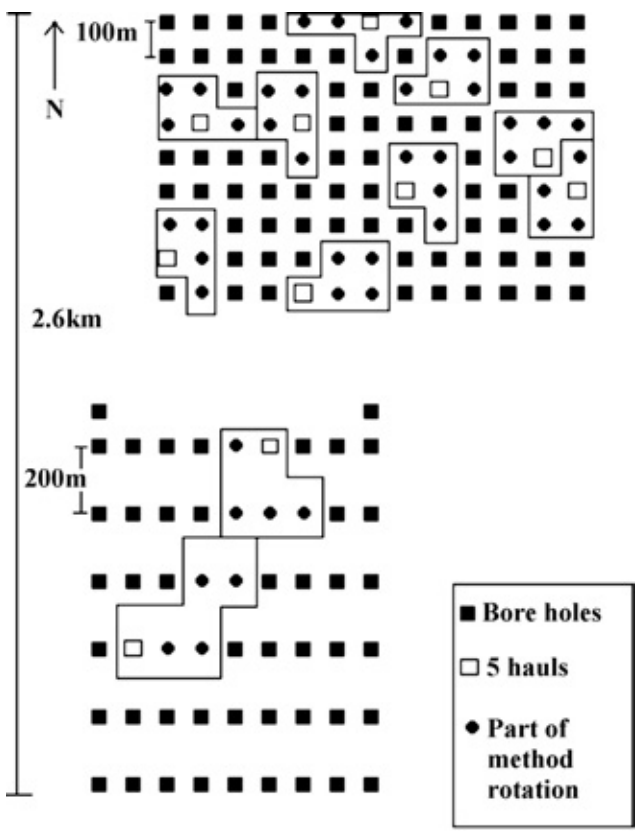

Fig. 1. Layout of the bore field at Sturt Meadows showing the groups of five bores used to test the sampling methods. Solid squares represent individual boreholes; solid circles represent the bores used in this study; hollow squares denote bores used for five repeat hauls.

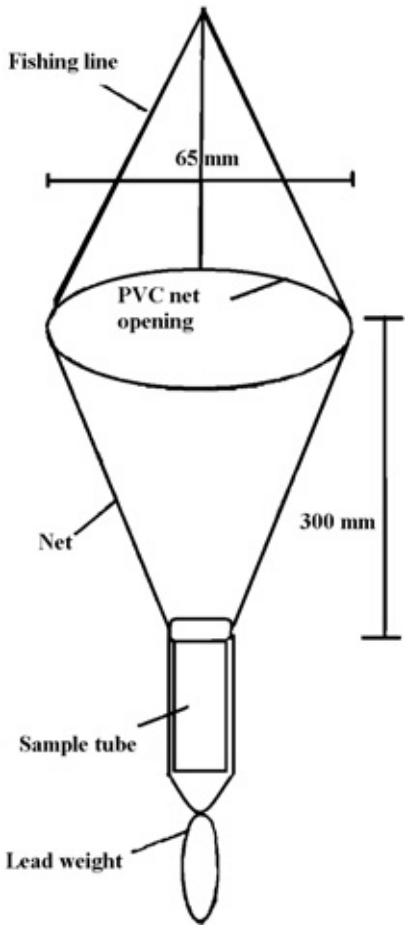

Fig. 2. Line drawing showing the main components of a haul net. The opening consists of a rigid ring made of PVC piping or cable to ensure the mouth remains open. The conical net, comprised of synthetic mesh (mesh size of 250 μm) concentrates the sample into a plastic collection vial, which is screwed onto the threaded receiver. A lead weight is connected to the bottom of the net.

diagnostic morphological characters for the latter two groups made it unfeasible to identify them to species level and they were grouped at a higher taxonomic level for the analyses presented in the current study. Species in both groups appeared to be similar in overall size and shape, and would likely show similar mobility and resistance to flow within the borehole. These attributes suggest that grouping of species at a higher taxonomic level should not overly influence the comparison of sampling methods. Dytiscid beetles were identified to species, but larvae were pooled as similar size classes (approximating instars) could not be easily identified. These were treated as a separate group because the physical and behavioural differences between adults and larvae may impact the effectiveness by which they are captured.

Data analysis

Data from each sample were grouped within treatment type. Terrestrial taxa captured in haul nets, such as Collembola and troglobitic arachnids (e.g. Barranco and Harvey 2008), were not included in further analyses, as the primary aim of this study was to investigate the efficiency of capturing the stygofauna.

Taxon accumulation curves were generated using Colwell's (2005) EstimateS software (ver. 8.0.0), utilising data from the 10 haul method to estimate the number of hauls required to capture all the species within the aquifer. Separate accumulation curves were generated for common taxa (highest 50 percentile of abundance) and rare taxa (lowest 50 percentile of abundance) taxa. Taxon accumulation curves were also generated for each of the sampling methods trialled to ascertain how many times repeat sampling is required to capture all the known taxonomic groups within a bore, with 95% confidence intervals calculated and plotted.

Differences in taxon assemblages among sampling methods were assessed by transforming the invertebrate data to presence/absence of each taxon, to reduce the weighting of the presence of relatively rare taxa. These data were then analysed using non-metric multidimensional scaling (NMS) (Kruskal 1964) with random starting configuration, using Bray–Curtis distance.

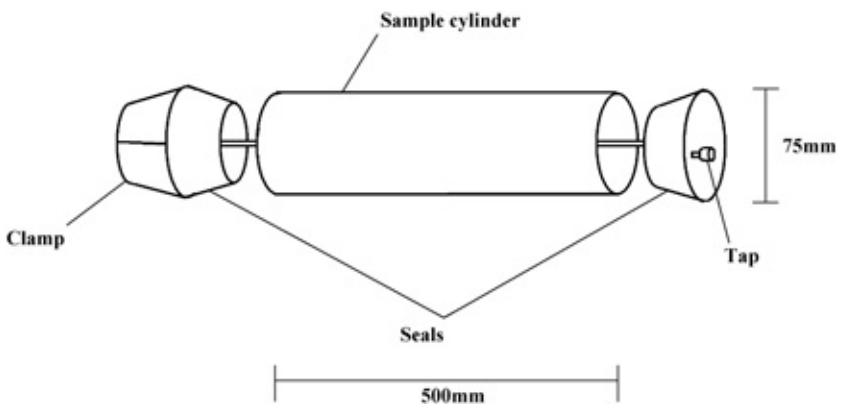

Fig. 3. Components of the discrete interval sampler. The main tube of the sampler is stainless steel, with a stainless steel rod passing through the middle to keep both ends aligned. The end caps are plastic with rubber seals, and a plastic spring loaded clamp is kept opened by a steel trigger weight. A steel tap in the base is utilised for sample recovery.

The output of the ordination was constrained to three dimensions with 200 iterations performed.

Multi-response permutation procedure (MRPP) using Bray–Curtis distance analysis was utilised to test for any significant difference between number of invertebrate species captured by the different sampling techniques. MRPP is a non-parametric test for multivariate differences between two or more *a priori* defined groups (Neitlich and McCune 1997), in this instance, the five sampling treatments. All multivariate analyses and Bray–Curtis measures were undertaken using PC-ORD ver. 5 (MjM Software Design, Gleneden Beach, OR) (McCune and Mefford 1999).

Results

In total, 6254 macroinvertebrates across eight taxonomic groups were collected in the 262 samples taken during the study (Table 1).

Methodological efficiencies

The average time taken to perform each sampling regime is shown in Table 2. Regimes utilising five and 10 haul net samples took less field time (5 and 8 min, respectively), but had higher sorting times (15 and 25 min, respectively), whereas pumping 50 L of water or using the discrete interval sampler took more field time (16 and 36 min, respectively), but had lower sorting times due to there being fewer individuals per sample (5 and 16 min, respectively). The combination of haul sampling and pumping of 25 L took the same time in the field (16 min) as pumping 50 L, and took a similar time to sort (17 min) compared with samples from five hauls (15 min).

Performance of methods

Comparison of the total number of species captured for each method shows that the haul sampling regimes (5 hauls or 10 hauls) were the most efficient overall with regards to number of taxonomic groups and number of individuals captured (Fig. 5, Appendix 1). Five haul samples combined with pumping 25 L was marginally less efficient than haul sampling alone, but more efficient than pumping 50 L or the discrete interval sampler. When the performance of methods is examined in regard to the abundance of specific taxonomic groups, the haul net sampling regimes were most efficient for cyclopoid and harpacticoid copepods, whereas the other three methods performed equally, but less well for these groups. Five haul samples combined with pumping was most efficient for amphipods and adults of the three species of dytiscid beetles (*Nirripirti* Watts & Humphries spp.), followed by 10 hauls and five hauls, whereas pumping alone and the discrete interval sampler performed relatively poorly for these two groups. Although collected in low numbers, the combined haul sampling and pumping performed slightly better than the haul net regimes for dytiscid larvae and oligochaete worms, whereas pumping and the discrete interval sampler collected very few or no individuals for these groups. Importantly, the discrete interval sampler showed that although some species (e.g. *Nirripirti mesosturtensis* Watts & Humphries, 2006) were found throughout the water column, much of the fauna was restricted to the upper 500 mm of the water column (e.g. *Nirripirti macrosturtensis* Watts & Humphries, 2006, A. Allford, pers. observ.).

The taxon accumulation curves for 10 hauls show that, on average, all taxonomic groups were captured within the first six hauls (Fig. 6). The more common taxa (harpacticoid and cyclopoid copepods, amphipods, and *N. mesosturtensis*) were generally captured within the first two hauls, whereas the rare taxa (*Nirripirti microsturtensis* Watts & Humphries, 2006, *N. macrosturtensis*, *Nirripirti* larvae, and oligochaetes) could take up to seven hauls before they were detected. It should be noted that the amphipod taxonomic group included one rare

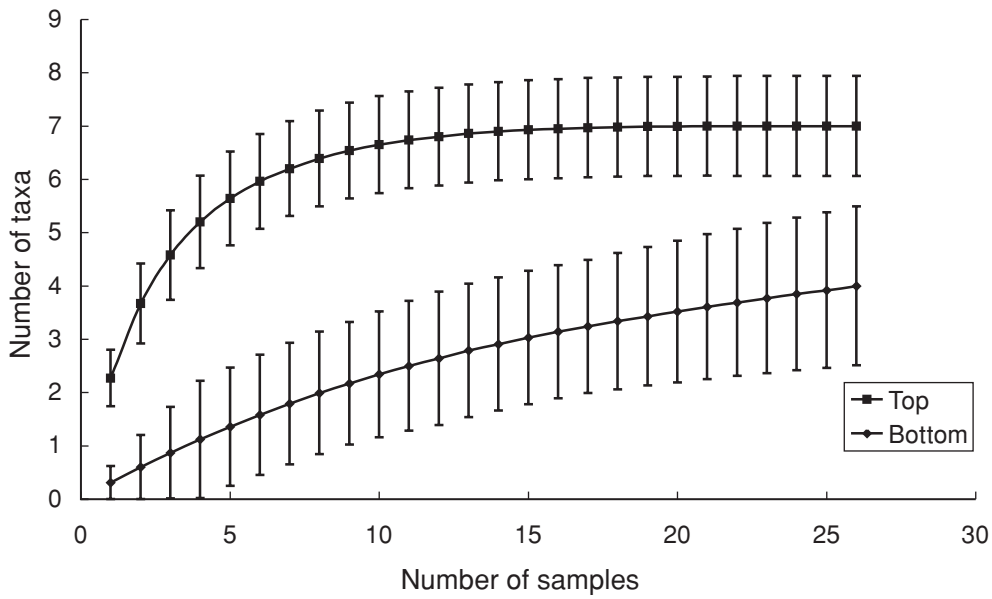

Fig. 4. Taxon accumulation curve, based on pilot study data, for pumping samples collected at the top and bottom of the bore. Error bars represent 95% confidence intervals.

Table 1. Number of groundwater taxa collect across 262 samples from the Sturt Meadows calcrete

Taxonomic group or species	# species[A]	# individuals	# samples
Cyclopoid copepods	1	1813	155
Harpacticoid copepods	1	2512	157
Chiltoniid amphipods	3	690	133
Nirripirti macrosturtensis	1	78	41
Nirripirti mesosturtensis	1	474	114
Nirripirti microsturtensis	1	324	105
Nirripirti larvae	3	192	52
Oligochaetes	~6	171	58
Total	18	6254	

[A]Based on molecular identification using COI (T. Bradford, pers. comm.).

species according to recent genetic analyses (T. Bradford, pers. comm.) and we cannot be certain that two hauls would be sufficient to detect this species.

The three-dimensional NMS ordination (stress = 13.55; variance explained 88%) showed no apparent grouping of points for different sampling methods (Fig. 7), suggesting that they do not have a significant effect on taxon composition in samples. Therefore, any variation in taxa present in a sample is more likely to be due to variation in the fauna among boreholes. This finding is further supported by the results of the MRPP analysis, which yielded a weak chance corrected within-group agreement ($A = 0.001$, $P = 0.568$), showing that within-group (i.e. sampling method) variation was similar to between-group variation. This result suggests that the variation in community structure with respect to the number of taxa captured is due to factors other than the sampling method utilised.

Table 2. The average time taken for each sampling regime

Method	Field time (min)	Sorting time (min)	Total time (min)
5 hauls	5	15	25
10 hauls	8	25	33
5 hauls + pumping 25 L	16	17	33
Pumping 50 L	16	5	21
Discrete interval sampler[A]	36	16	52

[A]Based on the average number of strata sampled: field time 4 min, laboratory time 2 min per strata, 2 min setup and cleanup time per hole.

The taxon accumulation curves for each of the haul net regimes and haul net plus pumping 25 L show that there is no significant difference (degree of 95% confidence intervals overlap) among the three methods (Fig. 8*a*). The accumulation curves for pumping 50 L and the discrete interval sampling also show that there is no significant difference (degree of 95% confidence intervals overlap) in the number of samples required from each method to ensure all taxa are captured (Fig. 8*b*). However, comparison of haul netting plus pumping 25 L with pumping 50 L does show a significant difference, with haul + pumping requiring fewer samples (n~14) to capture all available taxa (Fig. 8*c*).

Discussion

Groundwater ecosystems are particularly difficult habitats to sample effectively (Edler and Dodds 1996) owing to the varying inaccessible nature of these systems and the limitations of available sampling methods (Palmer 1993). As 'perfect' sampling methods are likely not to exist, it is particularly important for researchers to select an appropriate method for the study being

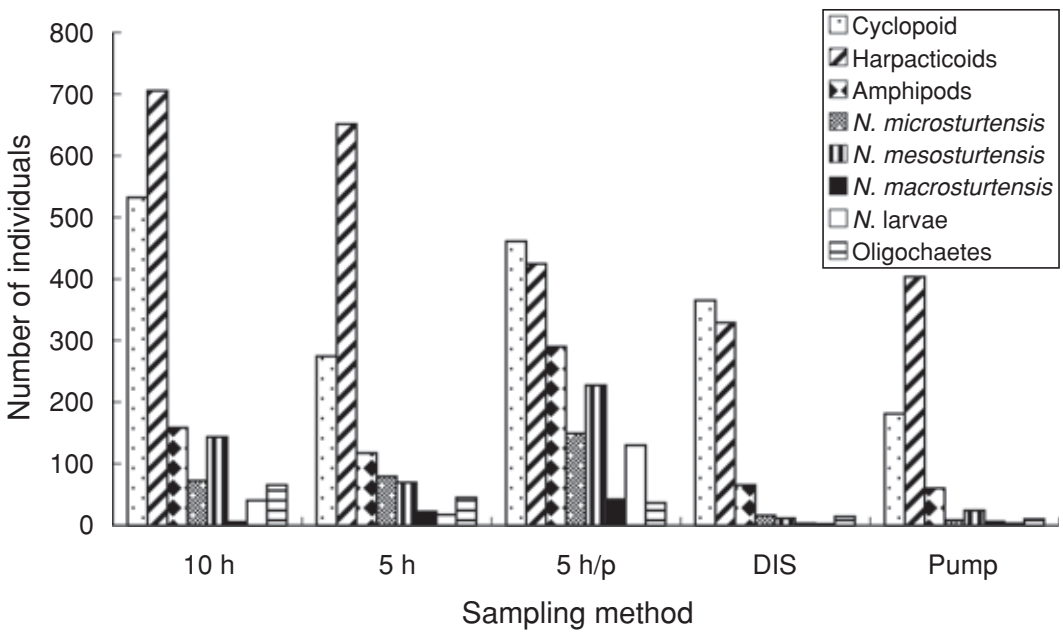

Fig. 5. Histogram showing the total number of each taxonomic group captured by the five sampling methods at the Sturt Meadows calcrete. The numbers were calculated from 55 repeats of each method (11 blocks × 5 field trips from 2005 to 2007). The code for each method is as follows: 5 = 5 hauls; 5+P = 5 hauls + pumping 25 L; P = pumping 50 L; 10 = 10 hauls; DIS = discrete interval sampling.

undertaken. The optimal compromise between accuracy, efficiency and the ability to collect stratified information is dependant on the aims of the study, with once-off biodiversity surveys placing a greater emphasis on efficiently collecting representatives of all taxa in the system, whereas ecological research requires more detailed information, e.g. the distribution of species in space and time, and must be quantifiable to allow comparisons between sites.

One of the most important factors in any ecological sampling method is the accuracy with which the proportion of taxa collected reflects the true community structure. The inaccessible nature of the Yilgarn calcretes makes this very difficult to test. However, any relative bias due to sampling method should be apparent when contrasted against the results from alternative methods. Assuming a bias exists, it would be expected that in an ordination plot (as in Fig. 7), samples taken using the same methods would group together. Using this criterion, coupled with the MRPP results showing little difference among sampling methods, it is apparent that other factors, such as seasonal variation or environmental factors, are responsible for differences in sample composition observed in this study.

The discrete interval sampler was not found to be a particularly efficient method for sampling stygofauna, but it provided valuable information on the relative location of fauna within the water column, that is virtually impossible to obtain using haul net and pumping methods. This method, therefore, is probably the most effective for ecological studies and, with careful handling, the water collected can also be used for physiochemical analysis. However, the volume of water sampled with this method is reliant on bore depth, making standardisation across heterogeneous calcretes problematic.

Pumping has been used for sampling groundwater fauna in several studies worldwide (Hahn 2002; Scarsbrook and Halliday 2002; Hancock and Steward 2004; Hahn and Matzke 2005). Although it is easy to standardise the volume of water extracted, the area within the water column in which the organisms are sampled is difficult to ascertain given the differences between the type and density of the matrix (e.g. alluvial sediment versus calcrete). Depending on the pump used, species can be extracted from several microhabitats, and this is undoubtedly affected by pumping rates and hydraulic transmissivity of the surrounding substrate. Although it was not apparent in this study, previous studies of hyporheic zones (Fraser and Williams 1997; Boulton *et al*. 2004) have found a capture bias towards small, less tenacious animals, whereas larger sessile or more active animals are more likely to either resist the suction of the pump, or be filtered out by the surrounding sediment matrix. The positioning of the pump inlet also will have an effect on the species represented in a sample, as the chance of capturing an organism clearly decreases as distance from the pump inlet increases. This problem might be overcome by using a more powerful pump than that used in the current study (see Malard *et al*. 1997) or by pumping at several depths of the bore (Datry *et al*. 2005; EPA 2006). The latter approach has the added advantage of detecting changes in community structure associated with depth (Datry *et al*. 2005), or the correlated changes in physico-chemical characteristics, such as the concentration of dissolved oxygen or dissolved organic carbon (Ronen *et al*. 1987). Although specimen damage due to the pump impellors was an *a priori* concern in this study, all species collected were found to pass through the pump with no damage. However, this may not be the case if a more powerful

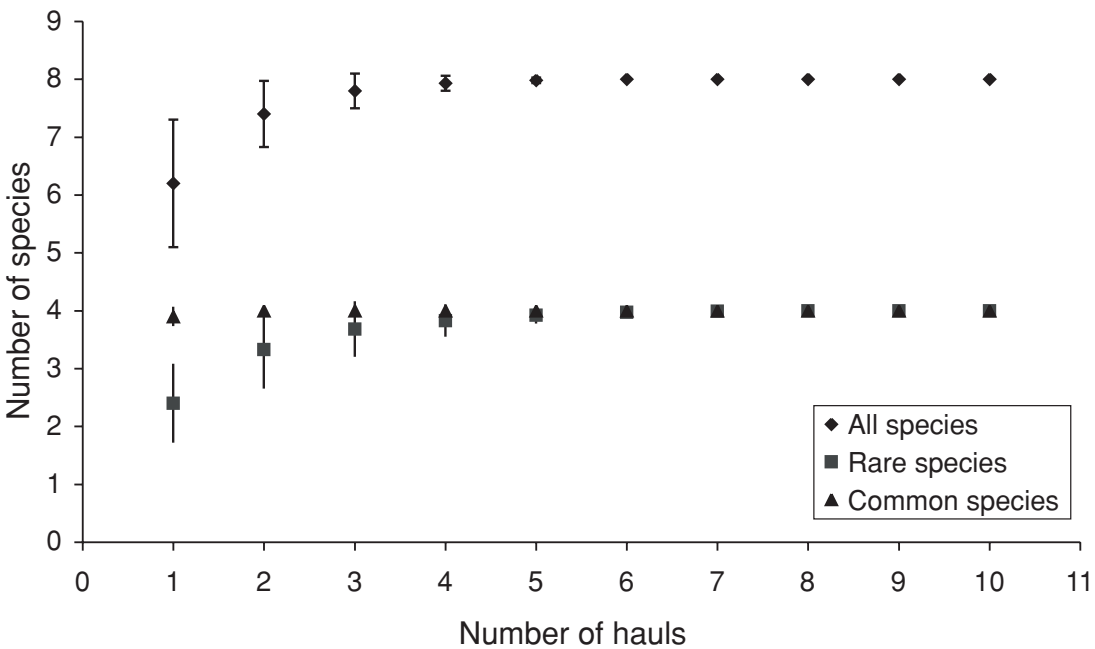

Fig. 6. Taxon accumulation curves based on 10 hauls showing the appearance of new taxa after each cumulative haul with 95% confidence interval displayed. Common taxa = taxa in highest 50 percentile of abundance); rare taxa = taxa in lowest 50 percentile of abundance.

pump (e.g. a pneumatic pump) is used, and where particulate sediment is sucked up.

In our sampling of the Sturt Meadows calcrete we found that the efficiency of pumping using the 12-V impeller pump was poor compared with the other methods, with relatively low numbers of taxa captured per unit time. This was partly balanced by sorting times being shorter due to the low silt load in most samples. Coupled with higher equipment costs, higher levels of maintenance, and the requirement of a 12-V power source, pumping is a poor option for once-off exploratory surveys where ease of use and high efficiency are important. This method also requires more repeated samples to capture representatives of all taxa present within an aquifer, so that studies using only this method are likely to be inadequate. This conclusion is consistent with that of Dumas and Fontanini (2001), who found that haul net sampling was more efficient, less costly and, most importantly, showed no difference in the taxonomic composition, compared with other methods.

Haul net sampling is a common method of stygofauna sampling (Hancock and Steward 2004), owing to the portability and ease of use associated with this method. However, standardisation is more difficult than with pumping, as the volume of water sampled is a function of the area of the net mouth, the depth of the water column, and the number of repeated samples taken. Sampling effectiveness of haul nets is also influenced by other factors that slow net retrieval, like tree roots or snags in the bore. Bores that are not vertical are more difficult to sample with a haul net, as ensuring the net reaches the bottom is particularly difficult. The filtration capability of the net is also dependant on the amount of particulate matter in the water column as silt can clog the net, which is a particular problem when sampling uncased bores. When coupled with the inability to perform small-scale stratified sampling, and the possibility that it is biased against sediment dwelling animals, haul net sampling alone is less attractive for ecological studies. However, owing to the ease of use, portability and speed of sampling with this method, many samples can be taken in a short period of time, particularly if bores are well constructed and relatively shallow. Coupled with high efficiency in capturing invertebrates, this method lends itself to rapid diversity assessment and for generating material for taxonomic studies (e.g. Watts and Humphreys 2006; Dumas and Fontanini 2001; Hancock and Steward 2004; Reeves *et al*. 2007).

For exploratory subterranean fauna surveys, a related advantage of haul sampling is the higher chance capture of troglobitic organisms during retrieval. Significantly, this approach collected the first troglobitic palpigrade for Australia (Arachnida: Palpigradi), from Sturt Meadows calcrete (Barranco and Harvey

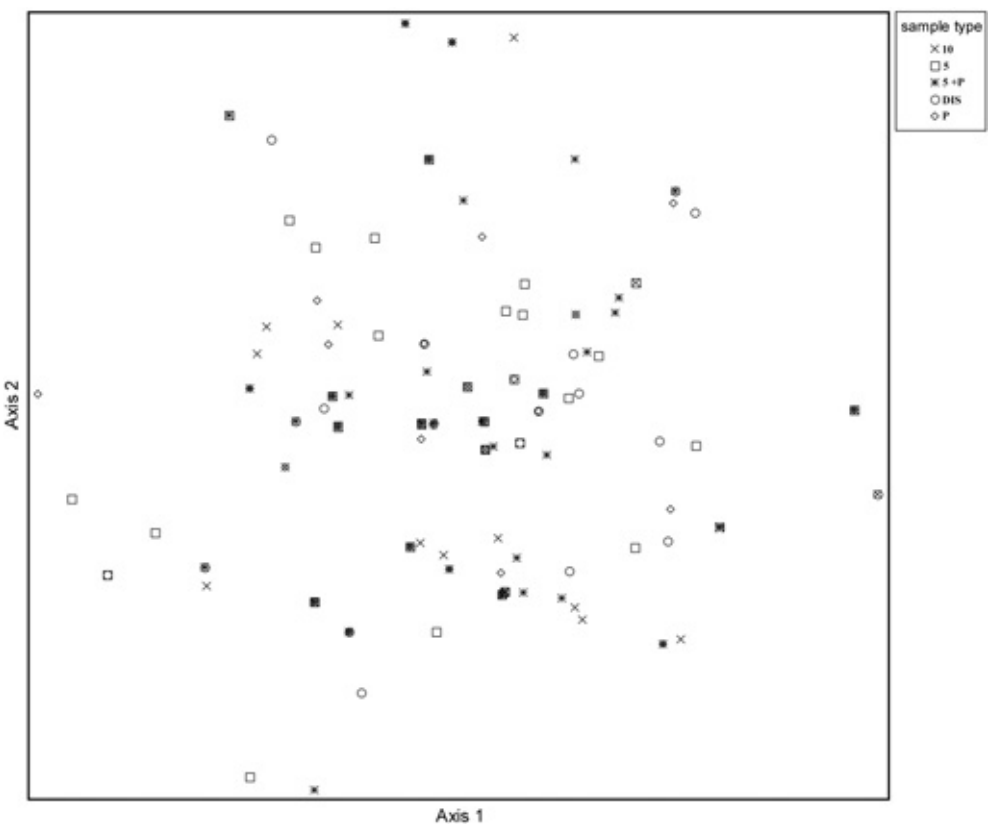

Fig. 7. Non-metric multidimensional scaling ordination plot showing groundwater invertebrate data derived using the five sampling methods. The points represent single sampling events, with symbols to distinguish each treatment (sampling method) used. The distance between points is relative to the dissimilarity among sample composition. Each axis represents an arbitrary dissimilarity measurement. The code for each method is as follows: 5 = five hauls; 10 = 10 hauls; 5+P = five hauls + pumping 25 L; P = pumping 50 L; DIS = discrete interval sampling.

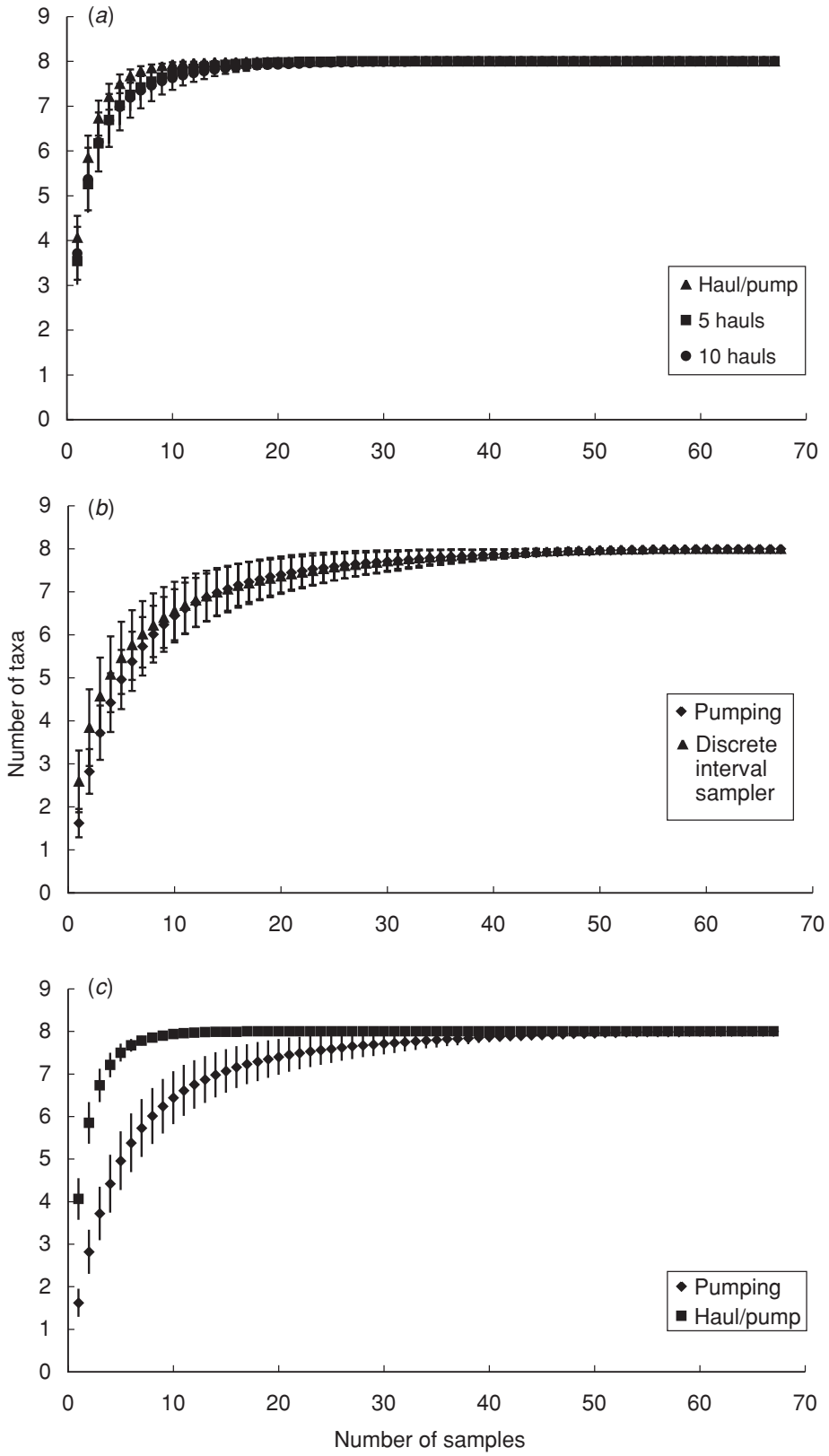

Fig. 8. Taxon accumulation curves with 95% confidence intervals for each method assessed at the Sturt Meadows calcrete: (*a*) haul methods (5 hauls, 10 hauls, and 5 hauls + pumping 25 L); (*b*) pumping and discrete interval sampling; (*c*) pumping 50 L compared with 5 hauls + pumping 25 L.

2008), and numerous other troglobitic taxa. Pumping and discrete interval samplers are not likely to capture troglobites as they are closed to specimen ingress during retrieval. The efficiency, ease of use, low equipment cost and low maintenance requirements also make haul sampling a candidate for long-term monitoring projects, where taxon presence/absence data or changes in abundance are more important than where they exist in the water column. However, the potential to cause physical change to the structure of the uncased bore, by repeated abrasion of the net against the hole walls, leading to increase siltation or bore collapse, needs to be considered before adopting this method.

In the current study, performing 10 hauls per hole for 50 boreholes resulted in capturing all known stygobitic taxa. However, the number of hauls required to obtain a reliable indication of the total faunal composition of a calcrete is less clear. Although there is a decline in the capture rates of more common species in subsequent hauls, rare taxa appear to take up to seven hauls before being detected. This is comparable to the sampling regime proposed by Eberhard *et al*. (in press) who recommend six net hauls to obtain a reasonable indication of the species present in the community. This suggests that, if capturing rare species is paramount to a particular study, the number of hauls should be maximised against time restrictions in the field. Less than five hauls would likely result in some taxa remaining undetected and, as a minimum, 10 hauls should be adopted to increase the chance of detecting rare taxa.

A combination of haul net and pump sampling has been recommended for other groundwater systems (Dumas and Fontanini 2001; Hancock and Steward 2004; Eberhard *et al*. in press). These methods have been found to compliment each other, with haul nets collecting larger, free-swimming organisms, and pumping potentially capturing organisms from the surrounding free water and from within the aquifer matrix. However, for the Yilgarn calcretes, pumping failed to capture any organisms not readily captured by haul nets. This could be due to differences between the pumps used in each study, or differences between sites in relation to both faunal composition and uniformity in substrate type. No significant difference was found in the accumulation of species in this study between standard haul net sampling, and the combination of both pumping and hauling. Therefore, the added workload of pumping after haul sampling appears unnecessary, at least for exploratory surveys of the shallow (<10 m) calcrete aquifers of the Yilgarn Region. The possibility of achieving different results using stronger pumps with higher pump rates or pumping greater volumes could be considered for future studies.

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Note added in proof

Nirripirti Watts & Humphreys has been synonymised with *Paroster* Sharp. See Leys and Watts (2008, this issue).

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Appendix 1. Number of each taxonomic group captured by the sampling methods

Method	Cyclopoid copepods	Harpacticoid copepods	Amphipods	*Nirripirti macrosturtensis*	*Nirripirti mesosturtensis*	*Nirripirti microsturtensis*	*Nirripirti* larvae	Oligochaetes
5 hauls	274	651	117	79	69	22	17	45
10 hauls	532	705	158	72	143	5	40	65
5 hauls + pumping 25 L	461	424	290	149	227	42	130	37
Pumping 50 L	181	403	60	8	24	6	3	10
Discrete interval sampler	365	329	65	16	11	3	2	14