Troglofauna in the vadose zone: comparison of scraping and trapping results and sampling adequacy

S.A. Halse¹, G.B. Pearson¹

¹ Bennelongia Environmental Consultants, 5 Bishop Street, Jolimont WA 6014, Australia

Corresponding author: S.A. Halse (stuart.halse@bennelongia.com.au)

Abstract

Most sampling of troglofauna occurs in caves but troglofauna species are widespread across the vadose zone in Western Australia in iron ore deposits and calcrites. Other than in karstic calcrite, the subterranean spaces in the Western Australian vadose zone are small and often of similar size to the troglofauna inhabiting them. Here we describe how troglofauna can be sampled in the vadose zone using a technique called scraping, in which a haul net is dropped down a hole drilled for geological exploration. We analysed the results of 10,895 sampling events in which both the scraping and trapping techniques were used. In the Pilbara region of Western Australia, where most of the fieldwork occurred, scraping collected approximately three-quarters more troglofaunal animals than trapping and more than twice as many troglofauna species per sample. Most orders of troglofauna were collected in greater numbers by scraping than trapping. However, the yields from both troglofauna sampling techniques are low and, even when the results of both techniques are combined to constitute a single unit of sample effort, the currently prescribed effort for environmental impact assessment will document only about half the species present at a site. It is suggested that a larger number of samples should be collected.

Keywords

Trolobite, subterranean fauna, Australia, sampling method, environmental impact assessment
Introduction

The study of troglofauna still occurs predominantly in caves across most of the world (e.g. Schneider and Culver 2004, Culver et al. 2006, Skubala et al. 2013). However, in Western Australia there has been focus on the occurrence of animals in the smaller spaces distributed, often at considerable depths, in vadose zones across much of the landscape in arid areas (Guzik et al. 2010). The habitat in the upper parts of these non-karstic vadose zones, where subsurface colluvium and weathered conglomerates are present, may be considered to comprise Juberthie et al.’s (1981) milieu souterrain superficiel (MSS). At depth, the vadose zone comprises various types of bedrock in which spaces are mostly the result of fracturing and weathering (Fig. 1).

Sampling of troglofauna in the vadose zone is challenging, especially in deeper rocky areas. Most of the sampling undertaken in Western Australia is for the purpose of assessing the potential impacts of mining on the conservation of troglofauna (EPA 2007, 2013) and occurs in drill holes installed for geological exploration (Fig. 2B). The geologies most frequently sampled for troglofauna are iron ore formations, granitoids and mafic rocks hosting gold deposits, and calcrites associated with potential water supplies or containing minerals such as uranium. Drill holes in iron formations may extend more than 100 m below the ground surface (e.g. Biota 2006, Bennelongia 2010).

Until recently, troglofauna were collected from drill holes using traps baited with leaf litter (EPA 2007). Capture rates from these traps in the Pilbara region (Fig. 3) tended to be very low, with yields of 0.25 troglofaunal animals per trap regarded as satisfactory sampling (Subterranean Ecology 2007). Given that the Pilbara is rich in troglofauna and may support more than 45 species in a few square kilometres (Bennelongia 2010), there has been concern that modest trapping efforts are unlikely to document most of the troglofauna species present in an area.

The primary objective of this paper is to describe, and to document the capture efficiency of a new troglofauna sampling technique called scraping. The implications of low troglofauna capture rates for the completeness of environmental impact assessments are also examined. As a second set of objectives, we briefly describe the troglofauna we have collected in the Pilbara, give some ecological information to improve knowledge of a region with high subterranean fauna conservation values, and highlight the potential richness of non-karstic vadose zones as troglofauna habitat.

Methods

Scraping

The scraping method of collecting troglofauna was developed by modifying a haul net used to sample stygofauna in wells. The principle is simple: a cone-shaped net is dropped down a drill hole and dragged back up against the wall of the hole. Troglofauna crawling on the wall are ‘scraped’ into the net and collected.
Different diameter nets are used for scraping according to the size of holes being sampled, with the ideal net diameter being about 60% of the diameter of the drill hole. The net itself consists of a metal ring, a cone-shaped net of 150 micron mesh and a
The leading edge of the net, which is wrapped over the metal ring, can be reinforced with Kevlar to reduce wear as the net is retrieved. A cylindrical brass weight is attached to the narrow base of the net using cable ties and the 120 mm polycarbonate sample collecting vial is screwed into the brass weight, which has an internal thread. A protective metal base can be fitted around the base of the vial as shown in Fig. 2A.

When collecting data on the troglofauna yield of scraping, the net was lowered to the base of the drill hole and retrieved four times, with the net being dragged against a different sector of the hole during each retrieval. A short metal cylinder was fitted into the collar at the top of the holes while sampling to reduce friction and wear on the nylon cord (see Fig. 2B). In the sampling analysed in this paper, scraping occurred immediately before setting a troglofauna trap.

After each retrieval of the net, the contents of the polycarbonate vial, including sand and stones from the sides of the drill hole, were emptied into a sample jar.
the jar were preserved in 100% ethanol at 4°C after completion of four hauls. In the laboratory, samples were elutriated to separate animals from heavier sediment and screened into size fractions using Endecotts sieves (250, 90 and 53 µm) to remove debris and improve searching efficiency. Samples were then sorted under a dissecting microscope.

**Trapping**

The troglofauna traps used were developed from the design of Biota (2006) and consisted of a short length of PVC tube of 50 mm internal diameter. Holes were drilled in the upper part of the tube to allow access of fauna (Fig. 2A). Prior to setting the trap, it was half-filled with wetted leaf litter that had previously been sterilised by microwaving. The trap was left in place eight weeks before being retrieved, with trap contents being emptied into a plastic bag and freighted to the laboratory. In every fourth drill hole, two traps were set (about one third of the distance between surface and bottom of the hole and a few metres above the bottom); in the remaining holes a single deep trap was set a few metres above the bottom of the hole.

In the laboratory, the contents of each plastic bag were placed in a Tullgren funnel under 25 watt incandescent lights for 72 h. Most troglofauna moved down through the funnel and dropped into the vial of ethanol below. However, leaf litter was quickly checked under a microscope for any remaining animals and more thorough searching conducted if animals were present.

**Drill holes**

The geological exploration holes sampled for troglofauna were drilled with a reverse circulation process, whereby rock is broken up by a pneumatic hammer and the rock chips are sent to the surface by air pressure. After drilling, holes were fitted with a short, capped PVC collar extending approximately 1.5 m below ground surface. The purpose of the collar was to prevent collapse of the hole near the surface where substrates are most unstable. The remainder of the hole was open to the surrounding rock matrix. Most holes were 150 mm in diameter and drilled between six months and several years prior to sampling. Approximately 30% of the holes sampled were <30 m deep and 70% were <50 m deep but 2% of holes were >300 m deep. All drill holes sampled in the Pilbara were vertical but about a quarter of the holes sampled in the Yilgarn were inclined 30° from vertical.

**Identifications**

With the exceptions of nematodes, oligochaetes, mites and collembolans, all invertebrate animals exhibiting some troglomorphies were considered to be potential troglofauna and
were identified to species or morphospecies level. While a few species could be identified using available keys, most of the time the characters employed in keys for surface taxa were used to construct morphospecies taxonomy. In addition to the use of keys, expert taxonomists were consulted and many specimens were examined genetically to help determine species boundaries (see Acknowledgments).

Nematodes, oligochaetes, mites and collembolans were excluded from study partly because existing information provided insufficient basis to separate Pilbara and Yilgarn species of troglofauna from surface relatives but also because it was not a regulatory requirement to identify these groups (EPA 2007).

Data analysis
The data analysed in this paper came from a large number of sampling events when both a scrape and a trap sample were collected from the drill hole at the same time. Given that the trap was set immediately after scraping, it was regarded as a contemporaneous sample, even though it was retrieved eight weeks later. Sampling occurred in 65 different areas within the Pilbara (90% of effort) and the eastern Yilgarn regions of Western Australia (Fig. 3), with most drill holes being sampled twice in different seasons. The areas varied in size from about 2–400 km² but were mostly <10 km². When two traps were set in one drill hole, trapping results were combined prior to making a comparison with the equivalent scrape sample.

Two types of analysis occurred using the entire dataset. First, the total numbers of species and animals collected in trap and scrape samples were compared. Second, the numbers of animals in traps and scrapes were compared for various orders represented by ≥6 specimens. The differences between numbers of animals in traps and scrapes were expressed as bias factors by dividing the number of specimens caught in the higher yielding sampling technique by the number caught in the lower yielding one and converting this ratio to its base-10 logarithm. When the higher yielding technique was trapping, the logarithm was assigned a negative value. Chi-squared tests were used to examine the significance of differences in numbers of animals collected by the two techniques.

In a third analysis, based on data from three areas in the Pilbara, species accumulation curves for scraping, trapping and combination sampling (i.e. collection of both trap and scrape samples) were calculated for each area using EstimateS software (Colwell et al. 2012). The three areas contained iron ore deposits and were near the towns of Nullagine (Area 1, c. 40 km²), Newman (Area 2, 2.5 km²) and Pannawonica (Area 3, 4.5 km²) (Fig. 3). The proportions of troglofauna species collected by each sampling technique were calculated by comparing the yield of the technique against the ICE metric estimate of the total number of species present based on combination sampling.

In addition to the above analyses of sampling efficiency, we used convex hulls to calculate the ranges of all species represented in the entire dataset by records from ≥3 drill holes. We also examined the depth below ground surface at which species were
trapped, using records from single traps or the deeper trap if two traps were set in a drill hole. Chi-squared goodness of fit tests were used to test for variations in occurrence of invertebrate orders with depth.

**Results**

A total of 9882 individual specimens considered to be troglofauna, representing 658 species, were collected in the 10,895 troglofauna sampling events. Of these, 9252 individuals of 566 species were collected from the Pilbara and 630 individuals of 92 species were collected from the Yilgarn (six species were considered to occur in both areas). Diplurans, isopods, beetles, pseudoscorpions and schizomids are the more speciose troglofauna groups in the Pilbara and Yilgarn (Fig. 4).

Scraping collected 76% more troglofaunal specimens than trapping (5115 vs. 2907) and more than twice as many species per sample (0.25 vs. 0.11, Table 1) in the 9815 sampling events in the Pilbara. Differences between the two techniques were less pronounced in the Yilgarn, with the scraping component of 1080 sampling events collecting 29%
fewer specimens than trapping but 10% more species. The lower success of scraping in the Yilgarn was probably partly the result of many Yilgarn drill holes being inclined and logistically difficult to sample by scraping (although retrieving traps was also difficult).

Bias among orders

Altogether, 20 orders of troglofauna represented by ≥6 individuals were collected from the Pilbara (excluding nematodes, oligochaetes, mites and collembolans). Thirteen orders yielded substantially more specimens in scrapes (1.2–100 times more) than traps (Fig. 5, Table 2). These orders included symphylans, pauropods and palpigrads, which were almost exclusively collected in scrape samples. Coleoptera were collected in equal numbers in scrapes and traps, while millipedes, isopods and dipterans were more abundant in traps. However, among the four millipede orders, the higher abundances in traps were significant only for Polydesmida and Spirobolida.

Dipterans collected in the Pilbara nearly all belonged to the family Sciaridae, which were collected as larvae or recently hatched adults. Nearly all were caught in traps. Eggs were also found in traps, which appeared to constitute favourable breeding habitat for sciarids, with eggs hatching and producing larvae and even adults while the traps were in place.

The results of sampling in the Yilgarn were similar to those in the Pilbara, with 10 orders represented by ≥6 animals and six of these collected mostly in scrapes (Fig. 5). Coleoptera, the centipede order Scolopendromorpha and millipede order Polyxenida were collected in approximately equal numbers in scrapes and traps, while isopods were more abundant in traps.

Many of the animals collected in scrapes, especially hemipterans, were found in root mats broken off by the net and scraping may be a particularly efficient way of sampling this microhabitat.

Species accumulation curves

The greater efficiency of scraping, compared to trapping, as a means of documenting the troglofauna of an area was confirmed when species accumulation curves were plotted for three areas in the Pilbara. Scraping yielded 33–57% more species than trapping (Fig. 6). However, both scraping and trapping collected low numbers of animals, so
New sampling technique for troglofauna

Figure 5. Bias in capture of different orders of troglofauna in the Pilbara using scraping and trapping.

Table 2. Differences between orders in numbers of animals collected by scraping and trapping. $P$ values for $\chi^2$ goodness of fit testing assuming equal numbers of animals in traps and scrapes. NS, non-significant.

<table>
<thead>
<tr>
<th>Order</th>
<th>Trap</th>
<th>Scrape</th>
<th>$P$</th>
<th>Trap</th>
<th>Scrape</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudoscorpiones</td>
<td>9</td>
<td>158</td>
<td>0.001</td>
<td>0</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Palpigradi</td>
<td>4</td>
<td>114</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizomida</td>
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<td>309</td>
<td>0.05</td>
<td>1</td>
<td>9</td>
<td>0.05</td>
</tr>
<tr>
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<td>11</td>
<td>183</td>
<td>0.001</td>
<td>1</td>
<td>9</td>
<td>0.05</td>
</tr>
<tr>
<td>Opiliones</td>
<td>2</td>
<td>15</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geophilomorpha</td>
<td>6</td>
<td>18</td>
<td>0.05</td>
<td>5</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>Scolopendromorpha</td>
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<td>36</td>
<td>0.05</td>
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<td></td>
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<td></td>
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<tr>
<td>Polyxenida</td>
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<td>325</td>
<td>NS</td>
<td>4</td>
<td>5</td>
<td>NS</td>
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<tr>
<td>Spirobolida</td>
<td>4</td>
<td>2</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spirostreptida</td>
<td>36</td>
<td>6</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pauropoda</td>
<td>3</td>
<td>124</td>
<td>0.001</td>
<td></td>
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<tr>
<td>Symphylia</td>
<td>2</td>
<td>204</td>
<td>0.001</td>
<td>6</td>
<td>18</td>
<td>0.05</td>
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<tr>
<td>Isopoda</td>
<td>312</td>
<td>234</td>
<td>0.01</td>
<td>263</td>
<td>109</td>
<td>0.001</td>
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<tr>
<td>Diplura</td>
<td>47</td>
<td>162</td>
<td>0.001</td>
<td>0</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Thysanura</td>
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<td>392</td>
<td>0.001</td>
<td>2</td>
<td>23</td>
<td>0.001</td>
</tr>
<tr>
<td>Blattodea</td>
<td>465</td>
<td>1117</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemiptera</td>
<td>20</td>
<td>1370</td>
<td>0.001</td>
<td>2</td>
<td>21</td>
<td>0.001</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>288</td>
<td>288</td>
<td>NS</td>
<td>5</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>Diptera</td>
<td>889</td>
<td>36</td>
<td>0.001</td>
<td></td>
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</tr>
</tbody>
</table>
that combining the results of trapping and scraping for each drill hole yielded 25–38% more species than were recorded by scraping alone.

While combination sampling (i.e. collecting both scrape and trap samples) is more efficient than either scraping or trapping alone, a very large sampling effort may still be required with combination sampling to collect most (e.g. 80%) of the troglofauna species present in an area. The 127 combination samples from Area 1 and 91 combination samples from Area 2 collected only 69% and 66%, respectively, of the estimated troglofauna species in these areas. Area 1 yielded 66 animals and 5 singletons (species represented by a single individual), whereas Area 2 yielded 145 animals and 3 singletons. Sampling appeared to collect additional species faster at Area 3 but the fauna there was also richer, so that only 58% of species were collected by 44 combination samples (Fig. 6). The 59 animals collected included 5 singletons. No species was collected from more than one area, despite a small number of species being wide-ranging in the Pilbara (see below).
New sampling technique for troglofauna

Both trapping and scraping mostly collected animals inhabiting the surface soil and gravel layers rather than troglofauna. Approximately 97.7 and 93.6% of specimens collected by trapping and scraping, respectively, were classified as surface species rather than troglofauna. While some species classified as surface may be living at depth (see Discussion), the high proportion of surface fauna in the traps and scrapes was probably mostly the result of two inter-related processes. First, the drill hole was likely to have acted as a conduit for surface fauna to explore the vadose zone by travelling down the outside of the collar onto the walls of the hole. Second, the drill hole was also likely to have acted as a pit trap for much of surface fauna exploring it.

Table 3. Percentage of various troglofauna groups in trap samples from different depths. Polydesmida, Polyxenida, Spirobolida and Spirostreptida combined as Diplopoda. No traps set at >80 m depth.

<table>
<thead>
<tr>
<th></th>
<th>&lt;10 m</th>
<th>10–19 m</th>
<th>20–39 m</th>
<th>40–80 m</th>
</tr>
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<tbody>
<tr>
<td>Schizomida</td>
<td>21</td>
<td>22</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>Diplopoda</td>
<td>16</td>
<td>13</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Isopoda</td>
<td>22</td>
<td>15</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Diplura</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Blattodea</td>
<td>21</td>
<td>25</td>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Diptera</td>
<td>2</td>
<td>5</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Other</td>
<td>12</td>
<td>11</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>No of species records</td>
<td>110</td>
<td>317</td>
<td>288</td>
<td>119</td>
</tr>
</tbody>
</table>

Terrestrial fauna

Examination of the depths at which species of different groups were collected showed that all groups recorded frequently in traps were found at depths >40 m (Table 3). No groups showed significant variation in occurrence with depth, although isopods showed a possible tendency to occur more frequently in shallow depths, dipterans to be more common at >20 m depth and schizomids to prefer depths of <40 m.

The ranges of species represented by few records are likely to be underestimated by our convex hull calculations. Nevertheless, it appeared that troglofauna species in the Pilbara and Yilgarn predominantly had very small ranges. Of the 230 species recorded in ≥3 drill holes, 77% had calculated ranges of <10 km² and only 4% had ranges >10,000 km². Groups with particularly small calculated ranges included isopods, spiders, schizomids and harvestmen (although the latter was represented by only two species) (Table 4). The ranges estimated here for schizomid species fit well with estimates for schizomids elsewhere in the Pilbara (Harvey et al. 2008).

All groups other than schizomids and harvestmen contained some moderately or very widespread species and, in many cases, these may represent troglophiles. The
widespread species included a ubiquitous polyxenid millipede Lophoproctidae sp. B01 found both in the Pilbara and Yilgarn, the pseudoscorpion *Tyrannochthonius aridus* found on the surface and below ground, the hemipteran Meenoplidae sp. B03 of which adults have remnant eyes (eyeless species had ranges of 461 and 10 km²), and the dipteran Sciaridae sp. B01.

Discussion

**Richness of troglofauna in Pilbara**

While the main purpose of this paper is to highlight the value of scraping as a technique for collecting troglofauna from the vadose zone, the results of the sampling reported here also show that the Pilbara region of Western Australia supports a significant troglofauna community and complements the results of other subterranean surveys showing the Pilbara is rich in stygofauna (Eberhard et al. 2009, Halse et al. 2014). Sampling of 59 mostly small areas in the Pilbara collected 549 species and morphospecies considered to be troglofauna. Other environmental impact assessments in the Pilbara have collected many additional troglofauna species (e.g. Harvey et al. 2008, Baehr et al. 2012, Smith et al. 2012).

Culver et al. (2013) recently pointed out the difficulties of comparing species richness between different regions of the world, especially when there is an element of
extrapolation involved in species estimates. While nearly all the troglofauna species collected from the Pilbara are undescribed, most are represented by voucher specimens in the Western Australian Museum and many have been defined by DNA analysis as well as morphological study. Working morphological diagnoses for 130 undescribed species are available on the Western Australian Museum’s website (http://www.museum.wa.gov.au/catalogues/waminals). Thus, it is unlikely that additional taxonomic study will substantially change the number of troglofauna species we have identified.

A much more significant issue is that <1% of the Pilbara has been sampled. While most of the areas sampled are iron formations, which current information suggests support more troglofauna than other geologies of the region (EPA 2007, 2013), even iron formations are very poorly sampled. This, together with the fact that other environmental assessment surveys are known to have collected additional species, suggests the current list of 549 taxa from the Pilbara substantially underestimates the actual number of species in the region, even if a small proportion of the taxa considered to be troglofauna are surface species. It is therefore likely that Guzik et al.’s (2010) estimate that 960 species of troglofauna occur in the western half of Australia, mostly in the Pilbara and Yilgarn, will prove to be too low.

Perhaps the most interesting points to note in relation to the richness of troglofauna in the Pilbara are that firstly it is a fauna of small spaces in the landscape matrix of the vadose zone rather than a fauna of caves and, secondly, it occurs in a very arid setting. Average annual rainfall in the Pilbara is 250–400 mm and average maximum January temperature is 39–41 °C (http://www.bom.gov.au/climate/averages/tables/ca_wa_names.shtml, see Fig. 3 for locations). Annual pan evaporation is approximately 3500 mm (Luke et al. 2003).

**Troglofauna v. soil fauna**

We believe our recognition of species boundaries was mostly sound but we excluded a potentially significant number of species from our troglofauna list by ignoring mites, collembolans and oligochaetes. On the other hand, some of the species we recorded as troglofauna may be soil fauna. The possible inclusion of some soil species reflects the difficulty of assigning animals to ecological categories on the basis of morphological information (Sket 2008). The process was made more difficult by the preponderance of surface species in scrape and trap samples, including some species of specialised soil fauna that lacked eyes and pigmentation. While soil fauna usually have smaller, more compact bodies and shorter appendages than cave troglobites (Juberthie and Decu 1994), it is unclear how well these characters distinguish between soil fauna and troglofauna for species of diplurans, atelurinid thysanurans, pauropods, palpigradids, symphylans and geophilomorph or Cryptops centipedes.

The difficulties of categorising species is illustrated by palpigradids and pauropods. Both groups were collected almost entirely by scraping. Although Western Australian palpigradid species appear to lack the elongated appendages typical of most cave pal-
pigradids, Barranco and Harvey (2008) considered *Eukoenenia guzikae*, which was collected from a well in the Yilgarn, to be troglofauna. While the wide range of the morphologically similar Palpigradi sp. B01 (35,642 km$^2$) suggests it may be a surface species or trogloxene, two other similar-looking species have known ranges of 36 and <1 km$^2$, which suggest they are more likely to be troglobites. Furthermore, some palpigradid specimens brought live into the laboratory desiccated rapidly at room humidity and temperature (unpublished observation), suggesting they are unlikely to be surface species.

After morphological examination, Ulf Scheller (personal communication) concluded that Pilbara pauropods are predominantly, if not entirely, surface fauna. However, two lines of evidence suggest the morphology and ecology of pauropods may not be matched. First, it seems surprising for osmoregulatory reasons that pauropods occur in surface soils of an area as hot and arid as the Pilbara, although they have been collected from arid surface habitats in Israel (Scheller and Broza 1999). Second, while two species have moderately large ranges (ranges of 7148 and 4072 km$^2$), the other four species collected at ≥3 drill holes had ranges more likely to be associated with troglobites (1–47 km$^2$).

There is also evidence from Cape Range, just south of the Pilbara, that species lacking troglomorphies may use subterranean environments as refugia from arid surface conditions. Humphreys (1993) found that many of the beetles considered to be surface species occupy caves without being present in the surrounding surface environments and this may also apply to groups such as pauropods and palpigradids.

**Comparison of sampling methods**

The non-karstic vadose zone appears to have been relatively little sampled for troglofauna anywhere other than Australia, despite its potential to harbour very significant biodiversity. Consequently, the yields of trapping and scraping cannot readily be compared to sampling methods used elsewhere. However, the traps used in this study were baited with wet leaf litter. Other studies have shown that the addition of water and leaf litter to an arid zone cave attracted troglobitic species, provided a site for their reproduction (Humphreys 1991) and improved the yield of pitfall traps (Weinstein and Slaney 1995). More elaborate versions of the traps used here, incorporating propylene glycol as a preservative, have been used successfully by Lopez and Oromi (2010) and others to sample troglofauna in true MSS. Thus, it is considered reasonable to treat the trapping undertaken here as representative of existing best practice when evaluating scraping results.

In the rocky iron ore formations of the Pilbara, scraping yielded approximately three-quarters more troglofaunal animals and twice as many species per sample as recorded by trapping (Table 1). Nearly all groups of troglofauna were collected more efficiently by scraping, so that use of that technique provided a more complete picture of the troglofauna community than if trapping alone was used (Figs 5, 6). Fewer samples were collected in the rocky iron ore and gold bearing formations sampled in the Yigarn
and capture rates were lower than in the Pilbara. These lower capture rates were probably the result of logistical issues associated with sampling inclined holes and the fact that fewer troglofauna occur in Yilgarn habitats other than calcrete (see Bennelongia 2009, Guzik 2010). The pattern of bias among different orders in the Yilgarn was similar to the Pilbara (Fig. 6) but scraping did not collect greater numbers of animals and species per sample (Table 1). This probably reflected the greater proportion of isopods and lower proportion of arachnids and hemipterans in the Yilgarn samples and the catch biases associated with these groups (Fig. 5).

**Implications for assessment**

The low yields of trapping and scraping have implications for the adequacy of environmental impact assessments in regions where troglofauna occur. In such regions, the identification and protection of areas that are particularly rich in troglofauna should be a conservation priority. Using trapping alone, only about a third of the troglofauna species present in Areas 1 and 2 would be collected by the 60 samples recommended for impact assessment by EPA (2007). Use of both scraping and trapping would result in 60 combination samples collecting about half the species present (Fig. 6).

The relatively small proportion of species collected by troglofauna sampling using currently recommended levels of effort is probably the result of two factors. First, the number of species in a sample and the degree to which the sample collects all species from an area is dependent on the number of animals collected (Fisher et al. 1943). Individual troglofauna samples collect very few animals and, therefore, large sample efforts are needed to document a high proportion of the fauna. Second, it has been shown for cave troglofauna that, depending on environmental conditions, often not all species in an area are accessible to sampling at any one time (Krejca and Weckerly 2007); in the case of the non-karstic vadose zone there may be times when species will not enter drill holes. Sampling on multiple occasions will increase the likelihood that species are present during sampling. The current assessment guidelines recommend sampling occurs in two seasons (EPA 2007).

**Conclusions**

Three broad conclusions are drawn from this study.

First, the non-karstic vadose zone of the Pilbara is rich in troglofauna. This is likely to be the case in many other parts of the world and more surveys should be conducted to identify the general importance of the non-karstic habitats globally. Holes drilled for geological exploration associated with mine development provide easy access to the deeper vadose zone.

Second, scraping appears to be a useful technique for sampling troglofauna in the vadose zone. Under at least some circumstances, it yields more fauna and provides results
faster than trapping because there is no colonisation period required. However, because of the relatively low yields of all sampling methods for troglofauna trialled to date, we suggest scraping and trapping should usually be used in combination to maximise yields.

Third, the low yields obtained from troglofauna sampling highlight the importance of identifying, prior to sampling, the proportion of the fauna that needs to be collected to adequately characterise a troglofauna community for the purposes of environmental impact assessment. Analyses should be conducted during assessment to evaluate whether this target has been met.

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References


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