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# Exploring the relationship between sampling efficiency and short-range endemism for groundwater fauna in the Pilbara region, Western Australia

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## SUMMARY

1. Identifying the existence of short or narrow range endemic species is an important issue when planning for conservation of groundwater fauna in the face of threats to groundwater quantity and quality.
2. Fourteen bores were sampled six times over 3 or 4 years to assess the reliability of net-hauling sampling in broad-scale survey to collect the groundwater fauna present at a site and to identify short-range endemic (SRE) species.
3. Species accumulation curves suggested that one sample from a bore collected 23% and 46% of species occurring in low and high abundance, respectively, and two samples collected 38% and 65% of such species. False-negative rates provided a slightly higher estimate of the collection probability of species with low abundances.
4. The frequent failure to collect species present at a site means that some apparent short-range endemism was probably an artefact of low sampling effort. Nevertheless, as is typical for subterranean fauna, a high proportion of the known species in the Pilbara region appeared to be SREs. About 55% had probable ranges <10 000 km$^2$, the criterion proposed by Harvey (2002) for short-range endemism.
5. Consideration of species occurrence patterns, natural barriers and the scale of most disturbances suggest that 1000 km$^2$ is a more satisfactory threshold for short-range endemism than 10 000 km$^2$ but, as the threshold is reduced, more intensive sampling is required to determine whether a species qualifies as an SRE.
6. Extrapolation of the results of regional sampling suggested the Pilbara contains about 500–550 species of groundwater fauna, with the density of species being relatively uniform across the region. Attempts to use a T-S curve approach (*sensu* Ugland & Gray, 2004) highlighted the lack of information about within-population dispersal of these species and the area of an aquifer that is effectively sampled by a bore.

*Keywords*: false negative, narrow range endemism, species accumulation, stygofauna, survey design

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## Introduction

The task of conserving biodiversity in the face of increasing threat from human activities is a challenge to biologists worldwide (Danielopol *et al*., 2003; Mace *et al*., 2005; Dudgeon *et al*., 2006). One of the central tenets of conservation is that all species should be prevented from extinction and there is much legislation to support this aspiration at international,

 

national and local scales (Caughley & Gunn, 1996; Krishnamurthy, 2003). However, legislation requires the existence of a species to be documented, and its conservation status assessed, before protection occurs (e.g. IUCN Red List of Threatened Species). The conventional approach to such assessment involves sampling a number of locations to produce a presence–absence matrix of species occurrence across spatial units. Inferences are then drawn about species distributions, abundances and habitat preferences, with consequential decisions about conservation status (e.g. Mace, 1995; Paran *et al*., 2005; Dole-Olivier *et al*., 2009). The obvious importance of reliable survey data in reaching appropriate conservation decisions has led to considerable interest in quantifying the errors associated with species detection.

One approach to calculating detection errors uses species accumulation curves to measure the relationship between sampling effort and species detection (see Colwell & Coddington, 1994; Colwell, Mao & Chang, 2004). More recently, focus has shifted to explicit calculation of the probability of failing to collect a species when in fact it is present (MacKenzie *et al*., 2002; Tyre *et al*., 2003). Such false-negative (FN) records lead to underestimates of species' ranges and overestimates of extinction probabilities.

Several studies have documented errors associated with sampling interstitial and groundwater species (e.g. Rouch & Danielopol, 1997; Mauclaire, Marmonier & Gibert, 1998; Pipan & Culver, 2005). They show that sampling error prevents easy translation of the results of most surveys into conservation planning (Castellarini *et al*., 2007a,b) but provide little information about the factors affecting species detectability, nor whether there is any relationship between detectability and conservation status.

Locally restricted species tend to have high conservation status because they are more vulnerable to extinction, following habitat destruction or environmental change, than are widespread species (Ponder & Colgan, 2002). The more extreme examples of locally restricted species are referred to as narrow-range or short-range endemics (SREs). Harvey (2002) defined SREs as those species with distributions covering <10 000 $km^2$. Subterranean faunas usually contain higher proportions of SREs than nearby surface communities (Gibert & Deharveng, 2002) so that issues associated with conservation of SREs are particularly important below ground.

Identifying SREs is difficult and many highly visible plant species suspected to comprise small localised populations remain classified in formal conservation lists as 'data deficient', rather than being assigned to a category of distribution, because survey effort is considered inadequate (Coates & Atkins, 2001). Paradoxically, despite cryptic occurrence and much less being known about invertebrate biology and distributions, SRE status is often readily inferred for invertebrate species known only from a single site. The chance of error is high, however, when such assessments are based on only one, or few, surveys of the region. Many of the species recorded at a single site may be widespread but occupying poorly sampled habitats, be at the limit of a broader distribution contiguous with the areas surveyed, or occur only sporadically (see Halse *et al*., 2000; Pinder *et al*., 2004).

The Pilbara region in north-western Australia contains the richest known groundwater fauna in Australia, with up to 54 species at individual bores and a total of about 350 species recorded (Eberhard, Halse & Humphreys, 2005a; S. A. Halse *et al*., unpubl. data). Although the fauna is still being documented, it is apparent that the Pilbara contains globally significant numbers of groundwater species (see Culver & Sket, 2000; Gibert & Deharveng, 2002). The Pilbara also contains the largest concentration of mining in Australia, with much of it occurring in open pits that extend below the watertable and require de-watering (Johnson & Wright, 2001). Thus, there is potential for substantial conflict between mining and the conservation requirements of groundwater fauna (Boulton, Humphreys & Eberhard, 2003; Humphreys, Watts & Bradbury, 2005).

Early sparse, somewhat clustered sampling of groundwater fauna in the Pilbara identified many SREs (e.g. Bradbury, 2000) and strongly suggested that a systematic, broad-scale survey was needed to provide a framework for mining, groundwater use and fauna conservation. Thus, a 4-year survey of the Pilbara began in 2002 with the aims of (i) mapping regional patterns of diversity of the groundwater fauna; (ii) identifying species of conservation significance (mostly SREs) and (iii) relating diversity of groundwater fauna to environmental parameters such as geology and water chemistry (Eberhard *et al*., 2005b).

This paper reports the results of intensive sampling, undertaken at selected sites within the Pilbara survey,

to explore the validity of biodiversity patterns obtained from lower intensity sampling in the regional survey as a whole. Specific objectives were (i) to determine the probability of species present at a site being retrieved in a single sampling event; (ii) to determine whether that probability was affected by species abundance and (iii) to examine whether low sampling intensity may have contributed to the high proportion of site singletons (and inferred SREs) in Pilbara groundwater.

## Methods

The semi-arid Pilbara region is a geologically complex and ancient landscape, consisting of five major catchments and covering an area of approximately 178 000 km$^2$ (Fig. 1). Groundwater is predominantly fresh (total dissolved solids <3000 mg L$^{-1}$), occurring in unconsolidated alluvium, chemically deposited sediments with high secondary porosity (calcrete and pisolitic limonite) and fractured rocks. Groundwater fauna occurs in all these deeper groundwater environments, as well as in shallow ground water in springs and the hyporheos (Halse, Scanlon & Cocking, 2002; Eberhard *et al*., 2005a).

The Pilbara contains >3700 bores and wells. A total of 424 were sampled twice during the Pilbara survey, once after the wet season (April–July) and once towards the end of the dry season (August–October) (Eberhard *et al*., 2005b). Bores and wells were sampled by dropping a weighted phreatobiological net to the bottom of the water column, agitating the net to disturb bottom sediment, and then retrieving the net. Nets of varying diameter were used, according to size of the bore or well. A McCartney vial was fitted to each net, with the base of the vial ground off and replaced with 50-μm mesh screen to improve water flow through nets as they were hauled up.

Each net-haul sampling event consisted of dropping and retrieving nets six times: the first three hauls were made with a 150-μm mesh net principally to catch macrofauna and the second three hauls were made with a 50-μm mesh net to capture microfauna. After sampling, nets were washed in a decontaminant (5%

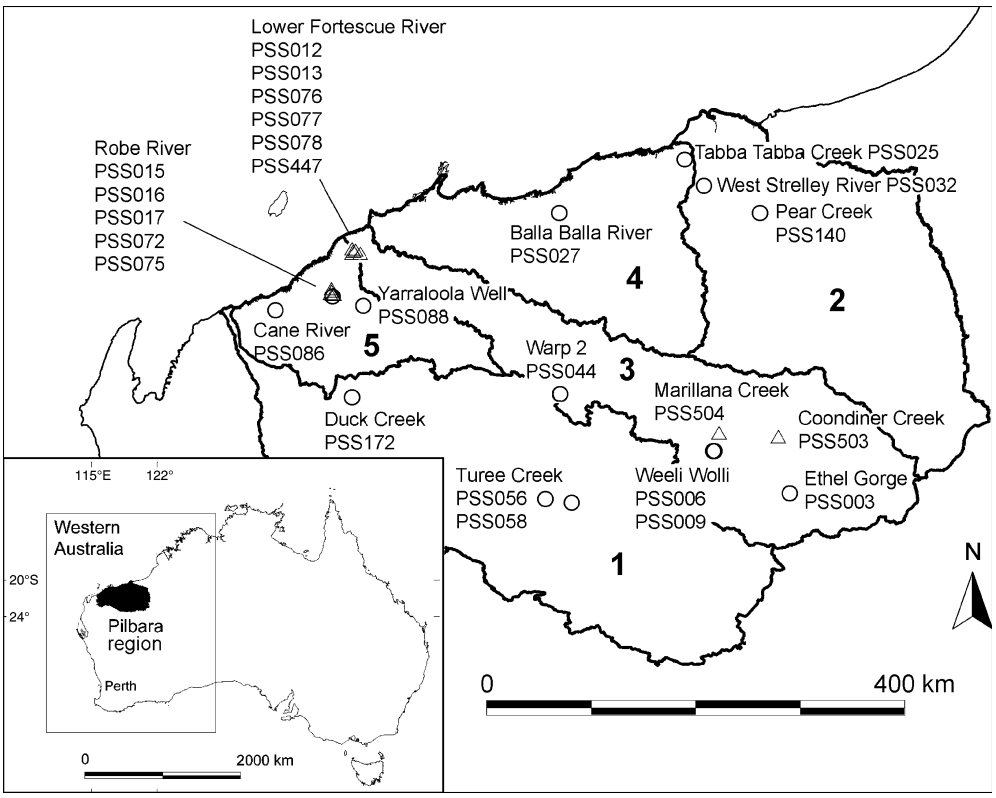


**Fig. 1** Pilbara region showing the five major catchments and locations of 14 net-sampling (circles) and 13 combination bores (triangles). (1) Ashburton River Basin, (2) De Grey River Basin, (3) Fortescue River Basin, (4) Port Hedland Coast Basin, (5) Onslow Coast Basin.

solution of Decon 90; Bacto Laboratories, Sydney Australia) and rinsed with distilled water to reduce the possibility of faunal contamination between sampling sites.

### *Net sampling*

The data set on which most of the presented analyses are based came from 14 bores that were sampled by net hauling on six occasions (three wet season and three dry season collections) over a 3- or 4-year period (2002–05) (Table 1). The bores, which were chosen after initial sampling had occurred at many sites across the Pilbara, represented a range of hydrogeological conditions and associated groundwater fauna. They covered all five major catchments and both coastal and inland settings (Fig. 1).

### *Combination sampling*

The effectiveness of net-haul sampling was further investigated at 13 bores by combination sampling, which consisted of a net-haul sampling event followed immediately by pumping three times the bore volume of groundwater through a 50 μm net. As soon as the bore refilled, another set of net hauls was taken. When calculating sampling effort, combination sampling was considered to consist of three sampling events (one pump sample and two net hauls).

Eleven of the combination sampling bores were located in alluvial aquifers near the coast; the other two were inland (Fig. 1 and Table 1). Combination sampling was undertaken twice at an interval of 2 years at six of the bores and once at the remaining seven bores. All the bores were sampled with nets only on at least two other occasions as part of the standard Pilbara survey protocol.

### *Sample processing and identification*

Each time the net was pulled to the surface, contents of the McCartney vial were transferred to a 120 mL polycarbonate container. On completion of the six net hauls, water was drained off and the sample was preserved in 100% analytical grade ethanol. Samples of animals collected in pump water were similarly preserved.

Prior to sorting specimens under a dissecting microscope, samples were separated into three size fractions in the laboratory by sieving through 250, 90 and 53 μm metal Endecott sieves (Endecott Ltd, London, U.K.). All animals were identified to the lowest taxonomical rank possible using published and informal keys, and the numbers of individuals of each taxon were recorded. Identification frequently required dissection and examination under a compound microscope. All ostracods were identified by I. Karanovic or J. Reeves and T. Karanovic identified copepods collected in 2002 and 2003.

### *Analyses*

Species abundance was categorised in two ways for the purpose of calculating species accumulation rates. First, for each species the mean number of individuals retrieved from all samples in which the species occurred was calculated and species in the lowest 50 percentiles of abundance were designated 'rare' and others 'abundant'. In a second analysis, each species was assigned to an abundance category for each bore, based on the species' average abundance only in the samples from that bore in which it occurred (rare ≤3 animals and abundant >3 animals). A species was sometimes classified as rare at one bore and abundant at another.

Accumulation curves at each bore for rare species, abundant species and all species were generated using Colwell's (2005) ESTIMATES software (version 7.5.1). The total number of species at each bore was estimated using the Chao2 estimator (or ICE if the coefficient of variation for incidence was >0.5 and the ICE estimate was higher) because of the patchy nature of species recovery through time (Colwell & Coddington, 1994; Foggo *et al.*, 2003). Results from all bores were combined to examine the general pattern of accumulation of rare and abundant species, although it should be emphasised that rates in individual bores were heterogeneous because of variation in geology and differences in the biology and behaviour of the particular species present.

False-negative rates for abundant species, rare species and all species (based on abundance in all net-sampled bores) were calculated for each bore from the six surveys as

$$\mathrm{FN} = 1 - n_o/6S \quad (1)$$

where $n_o$ is sum of occurrences of all species at the bore and $S$ is number of species recorded. If all species

recorded at a bore were collected during most sampling events, $n_o$ will approach 6*S* and the FN rate will be low. An important assumption is that all species present at the bore were collected sometime during the sequence of sampling events. As with estimates of species accumulation rates, the FN rates of all intensively sampled bores were averaged to estimate overall FN rates for abundant, rare and all species. Using FN rates, the proportion of species at a bore collected by several samples was calculated as

$$f = 1 - \mathrm{FN}^k \quad (2)$$

where $k$ is number of samples taken.

Species accumulation rates during the first three sampling events (i.e. two net hauls and one pump sample) at combination bores, which took place over a few hours, were compared with rates for three seasonally separated sampling events at repeat bores to assess the extent of species turnover between seasons. If turnover occurred, a higher proportion of species would be expected in the first sample from combination than repeat bores, assuming any increased efficiency of pump sampling was less than the extent of seasonal turnover. In a further test of whether groundwater communities exhibited seasonal change, variations in species richness and abundance at net-sampled bores were examined using repeated-measures ANOVA. Abundance data were logarithmically transformed [log $(x + 1)$] to ensure approximate normality and homoscedasticity of residuals; species richness data did not require transformation.

Validity of the hypothesis that many apparent SREs detected during the Pilbara-wide sampling program were sampling artefacts, because in fact they have much larger ranges than suggested by sampling results, was examined by comparing spatial occurrences of sites with species recorded at only two or three sites (site doubletons and tripletons, respectively) with the spatial distribution of sampling sites. Data from 397 bores and wells sampled twice were used in this analysis; the net-sampled and combination sampling bores were excluded. The distance between records of an SRE species should be closer to the minimum distance between bores, reflecting localised distribution, than overall bore spacing (i.e. the average distance between each bore and every other bore).

The total number of species of groundwater fauna in the Pilbara was estimated using the ICE estimator within ESTIMATES (see above) and the more recently derived T-S curve technique (Ugland, Gray & Ellingsen, 2003; Ugland & Gray, 2004). The maximum number of sites that could be processed in the software available to calculate T-S curves was 240, so regional survey bores where no species was recorded were omitted and additional sites were randomly dropped until only 240 bores or wells and 239 species remained in the data set. Extrapolations with ESTIMATES were made using both this reduced data set and the regional data set of 397 bores and wells. For calculation of T-S curves, sites were stratified according to catchment.

## Results

### *Net sampling*

Ninety-three species belonging to 10 higher taxonomic groups were collected: Crustacea (69 species), Oligochaeta (10), Nematoda (3), Arachnida (3), Rotifera (2), Gastropoda (2), Aphanoneura (1), Polychaeta (1), Hirudinea (1) and Turbellaria (1). Six orders of Crustacea were collected: Ostracoda (32 species), Copepoda (20), Amphipoda (7), Isopoda (5), Syncarida (4) and Thermosbanacea (1). Cumulative species richness at individual bores ranged from 0 to 36 (12 ± 3; mean ± SE). The total number of animals collected per bore ranged from 0 to 1402 (76 ± 24) and the number of animals per species was 33 ± 10. No animal was collected from bore PSS056. The three bores (PSS003, PSS016 and PSS058) with relatively high species richness (>20 species) also had high numbers of animals (350–475) but the site with most animals (PSS032) had only 12 species.

More species were collected in small than large numbers and the abundance distribution was overdispersed (Fig. 2). Twenty-four species were represented by only one animal in the sample(s) in which they occurred and 69 species were represented by ≤5 animals per sample. Forty-seven per cent of all species were found in only one sample (here referred to as sample singletons), 24% were recorded in only two samples (sample doubletons) and only 29% were recorded more than twice (Fig. 3a). Sample singletons were usually represented by fewer animals in a sample than sample doubletons and other more frequently occurring species (Fig. 3b).

Unsurprisingly, the species that occurred in high numbers were mostly collected earlier in the sampling

**Table 1** Bores and wells sampled, aquifer characteristics including bore depth (metres below ground level), standing water level (SWL), number of sampling events (net sampling or combination sampling), geology and selected physicochemical parameters at −1 m SWL (mean of sampling events)

| Hydrographic basin (catchment area in km$^2$) | Aquifer name | Geology | Sampling type | No. events | Bore code | Species richness | Bore depth (m bgl) | SWL (m bgl) | Temperature (°C) | pH range | Salinity (mg L$^{-1}$) | DO (mg L$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 Ashburton River (78 893) | Duck Creek | Colluvium: unconsolidated sand and gravel | Repeat | 6 | PSS172 | 16 | 7 | 6 | 31.4 | 6.5–6.9 | 678 | 2.0 |
| | Turee Creek | Alluvium: fluvial sand, silt and gravel | | 6 | PSS056 | 0 | 49 | 39 | 31.5 | 6.6–7.5 | 494 | 4.2 |
| | | | | 6 | PSS058 | 27 | 10 | 6 | 30.3 | 6.9–7.2 | 660 | 3.3 |
| 2 De Grey River (56 717) | Pear Creek | Alluvium: silt, sand and gravel with calcrete | | 6 | PSS140 | 9 | 54 | 5 | 32.0 | 6.4–7.4 | 100 | 0.2 |
| | West Strelley River | Alluvium: silt, sand and gravel | | 6 | PSS032 | 12 | 51 | 5 | 31.9 | 5.7–7.2 | 504 | 3.1 |
| 3 Fortescue River (49 232) | Ethel Creek | Alluvium: silt, sand and gravel with calcrete | | 6 | PSS003 | 22 | 23 | 3 | 27.9 | 6.6–8.0 | 844 | 1.5 |
| | Weeli Wolli Creek | Alluvium: unconsolidated silt, sand, gravel and cobbles overlying fractured-rock (Brockman iron formation) | | 6 | PSS006 | 7 | 22 | 3 | 28.0 | 6.7–7.2 | 285 | 1.6 |
| | | | | 6 | PSS009 | 2 | 34 | 4 | 26.1 | 6.9–7.3 | 295 | 1.8 |
| | Warp 2 | Alluvium: unconsolidated silt, sand and gravel | | 6 | PSS044 | 5 | 84 | 15 | 28.1 | 6.9–7.4 | 348 | 4.4 |
| 4 Port Hedland Coast (35 172) | Balla Balla River | Calcrete | | 6 | PSS027 | 2 | 46 | 10 | 30.8 | 6.4–7.0 | 465 | 1.2 |
| | Tabba Tabba Creek | Alluvium: silt, sand and gravel | | 6 | PSS025 | 12 | 16 | 6 | 31.9 | 6.4–7.7 | 627 | 1.5 |
| 5 Onslow Coast (15 689) | Cane River | Alluvium: clay, sand, silt and gravel partly calcreted | | 6 | PSS086 | 4 | 30 | 10 | 31.2 | 7.1–8.0 | 257 | 3.8 |
| | Yarraloola Well | Unconsolidated fluviatile deposits | | 6 | PSS088 | 17 | 54 | 16 | 31.0 | 6.0–6.3 | 277 | 0.6 |
| | Robe River | Alluvium, calcrete and limestone | | 6 | PSS016 | 36 | 13 | 6 | 31.1 | 6.7–7.1 | 480 | 4.1 |

 

**Table 1** (*Continued*)

| Hydrographic basin (catchment area in km$^2$) | Aquifer name | Geology | Sampling type | No. events | Bore code | Species richness | Bore depth (m bgl) | SWL (m bgl) | Temperature (°C) | pH range | Salinity (mg L$^{-1}$) | DO (mg L$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 Onslow Coast | Robe River | Alluvium, calcrete and limestone | Purge | 2 | PSS016 | 50 | 13 | 6 | 31.1 | 6.7–7.1 | 480 | 4.1 |
| | | | | 2 | PSS015 | 25 | 23 | 8 | 31.8 | 6.3–7.2 | 742 | 4.9 |
| | | | | 2 | PSS017 | 12 | 16 | 6 | 31.0 | 6.7–7.2 | 840 | 4.3 |
| | | | | 1 | PSS072 | 8 | 28 | 7 | 31.8 | 7.1–7.4 | 540 | 4.0 |
| | | | | 1 | PSS075 | 10 | 20 | 6 | 30.7 | 6.8–7.1 | 825 | 3.8 |
| 3 Fortescue River | Lower Fortescue River | Alluvium, calcrete, limestone and conglomerate | Purge | 2 | PSS012 | 2 | 49 | 7 | 29.5 | 8.1–8.9 | 174 | 0.4 |
| | | | | 2 | PSS013 | 14 | 25 | 7 | 31.1 | 6.4–6.8 | 446 | 2.2 |
| | | | | 1 | PSS076 | 4 | 70 | 9 | 30.7 | 7.7–8.8 | 278 | 0.4 |
| | | | | 1 | PSS077 | 11 | 20 | 9 | 30.7 | 6.8–7.1 | 535 | 4.1 |
| | | | | 2 | PSS078 | 15 | 25 | 8 | 30.6 | 6. 8–6.9 | 1108 | 5.2 |
| | | | | 1 | PSS447 | 13 | 16 | 9 | 30.7 | 6.7–6.9 | 383 | 4.4 |
| | Coondiner Creek | Alluviumand colluvium: sand and clay | | 1 | PSS503 | 2 | 57 | 11 | 30.3 | 6.7–7.0 | 590 | 3.3 |
| | Marillana Creek | Pisolitic limonite (vuggy porosity) | | 1 | PSS504 | 11 | 62 | – | 29.6 | 6.4–6.5 | 410 | 3.4 |

sequence at a bore than those occurring in low numbers, reflecting a strong relationship between abundance and detectability (Fig. 4). Based on across-bore abundance categories and Chao2 estimates of the true numbers of species at each bore, the first sampling event collected only 23 ± 6% of rare species present at a site, 46 ± 7% of abundant species and 33 ± 5% of all species, while six sampling events collected 79 ± 22%, 92 ± 16% and 82 ± 16, respectively (Fig. 4a). The effect of abundance was even more pronounced when within-bore abundance categories were used (Fig. 4b).

Analysis of FN rates provided a similar picture, although they overestimated the efficiency with which rare species were recovered. The probability of collecting a rare species in a single sample was 36 ± 3%, for abundant species 50 ± 3% and for all species 39 ± 3%. FN rates suggested six samples would probably collect 95% of all species. The discrepancies between FN and species accumulation estimates were mainly the result of FN calculations overestimating the rate of accumulation of rare species.

### *Combination sampling*

Results from combination bores suggested there was no significant seasonal turnover in species composition at a site. The first net-hauling event at these bores collected a smaller proportion (<16%) of all species collected by the net-pump-net samples than did the first of three net-hauling events in different seasons at net-sampled sites (43 ± 8% versus 51 ± 3%). The probable reason for the first event at combination bores yielding a lower, rather than similar, proportion of species to that obtained at net-sampling sites was that pump sampling was more efficient and inflated the total species list at combination bores. Pumping collected on average 6.8 ± 1.5 species compared with 5.4 ± 1.2 from a net-hauling event at the same bores, although the difference was not significant ($P = 0.2$, paired $t$-test, $n = 18$) (but see Hancock & Boulton, 2009).

Inclusion of bore PSS0016 in both net and combination sampling meant that 11 sampling events occurred at this site, which allowed predictions based on species accumulation curves and FN rates to be tested. Cumulative species richness appeared to stabilise after 10 sample events (Fig. 5), which was

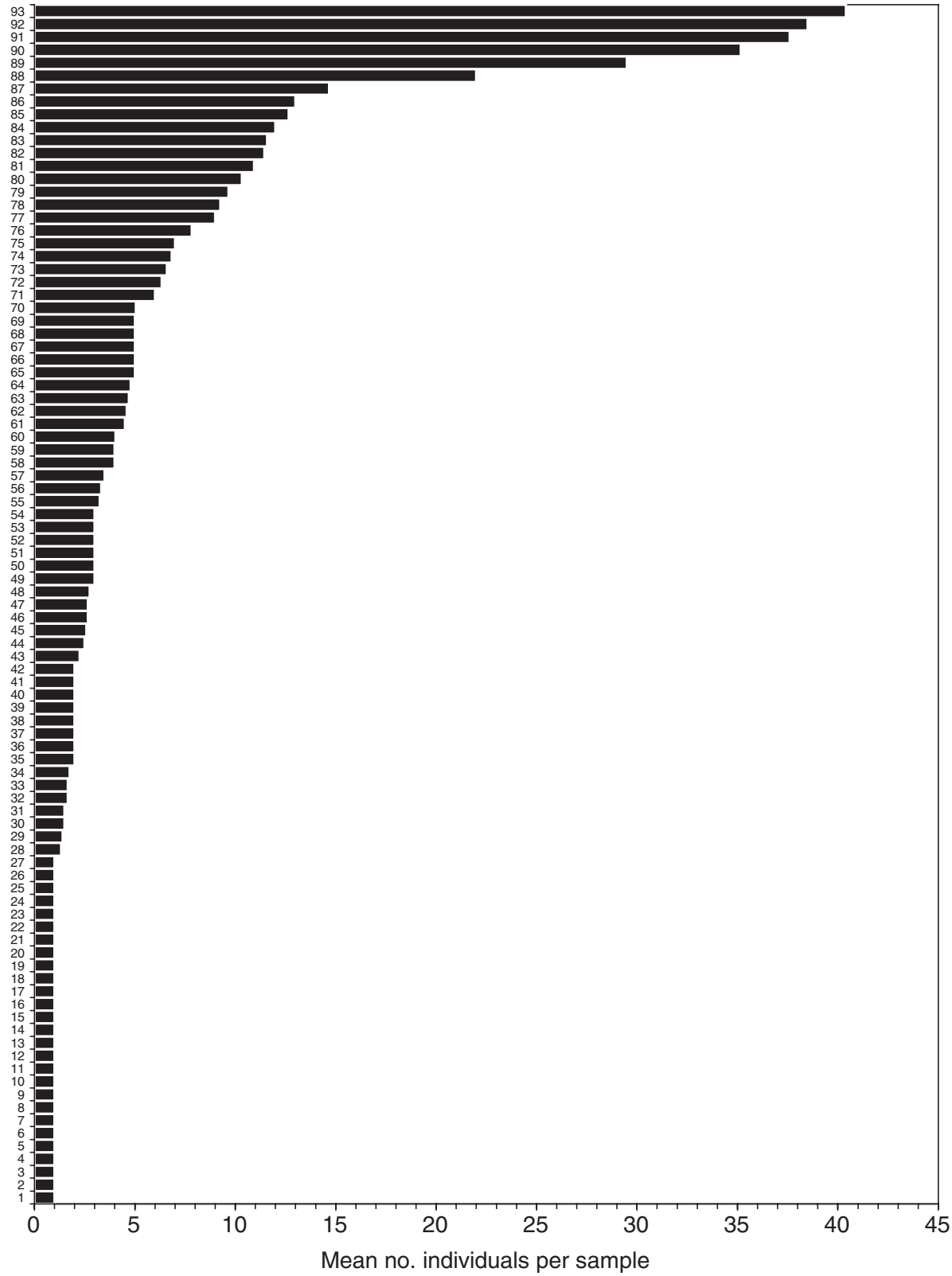


**Fig. 2** Distribution of animal abundances among species from net-sampled bores. Mean abundance was calculated for each species only from samples in which the species was present. See Appendix for species names according to numbers on *y*-axis.

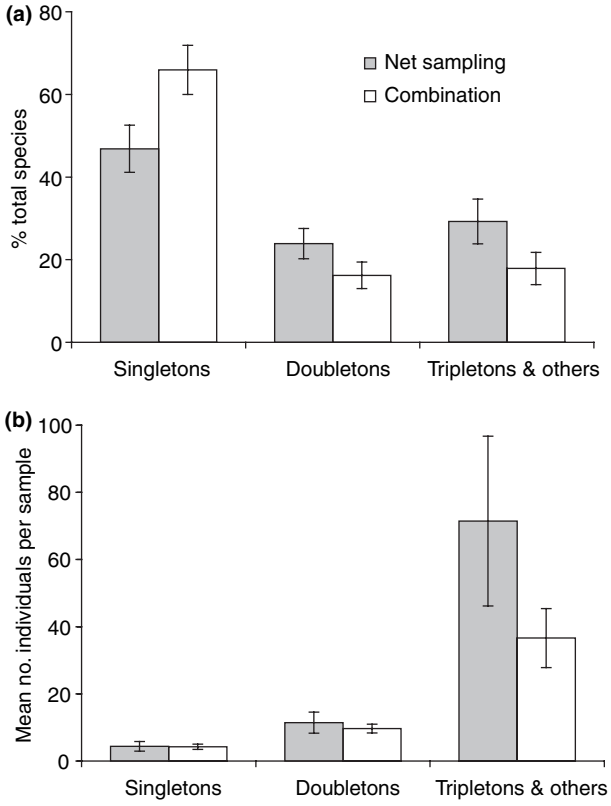


**Fig. 3** Frequency of species occurrence and its relationship with animal abundance. (a) Mean proportion (±SE) of singletons, doubletons and other species at net-sampling and combination bores. (b) Mean animal abundance (±SE) for sample singletons, doubletons and other species at net-sampling and combination bores.

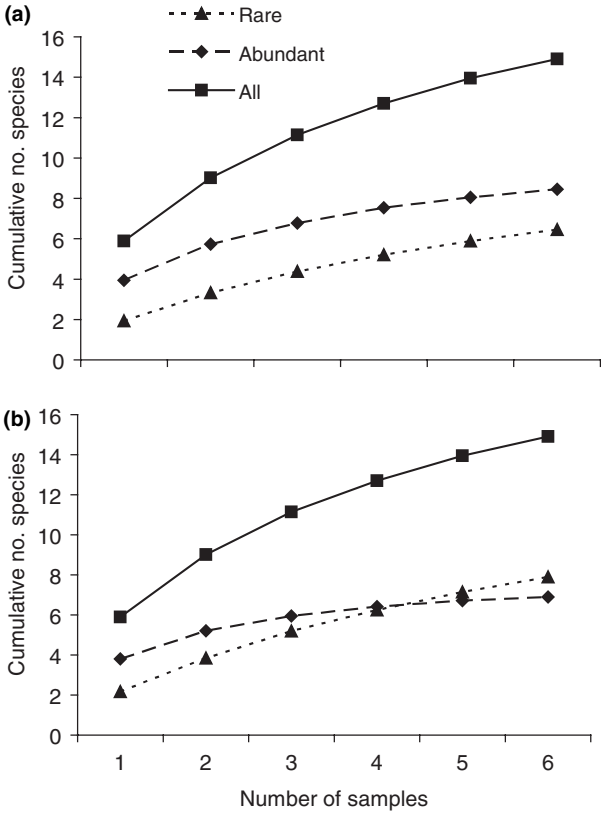


**Fig. 4** Species accumulation curves for rare species, abundant species and all species, based on averaged data from all net-sampled bores. (a) Rare = average species abundance across all bores was in lowest 50 percentiles of abundance. (b) Rare = species abundance within bore being sampled was ≤3 animals per sample in which the species occurs.

in general agreement with predictions of the species accumulation curves (Fig. 4) other than that the actual number of species collected was 15% higher than the prediction generated by Chao2 after six sampling events.

### *Abundance and richness patterns*

Analysis of patterns of total animal abundance at the net-sampling bores showed strong differences between sites but no significant differences between times of year sampled (Table 2). The same lack of response to season was apparent in species richness (results not shown).

### *Distributional patterns in the Pilbara*

Sampling throughout the Pilbara identified about 350 species. The proportions of species represented by sample singletons and doubletons were 37% and 20%, respectively, and the proportions of species recorded at only one, two, three or more bores were 44%, 20%, 10% and 26%.

For 29 of the 50 species represented by site doubletons, both collecting locations were within the same catchment (Table 3). However, even these 29 species had average distances between occurrences that were >50% of the average distance of any bore to any other within the same catchment, which suggests that many of the species had catchment-wide ranges that do not fit with localised distributions expected of SREs. A similar pattern was obtained for site tripletons, although only seven of the 25 species recorded at only three bores appeared to be restricted to single catchments (Table 3).

Despite the relatively weak evidence of localised occurrence among site doubletons and tripletons, 28 of

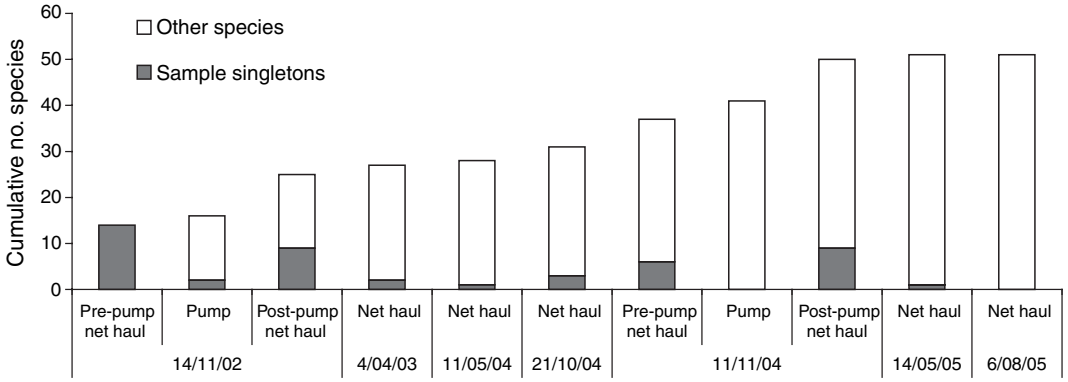


**Fig. 5** Increase in cumulative number of species recorded at bore PSS016, and decrease in number of species recorded only once at the site (referred to in legend as sample singletons), as sampling effort increased.

**Table 2** Comparison of total groundwater fauna abundance across sampling season (as month) using mixed-model ANOVA with sampling month being a fixed and site a random effect. Abundance varied significantly across sites but not months

| Source | d.f. | Type III sum of squares | Mean squares | *F* | *P* |
|---|---|---|---|---|---|
| Site | 13 | 1153 | 88.7 | 15.3 | <0.001 |
| Month | 6 | 68.5 | 11.4 | 1.97 | 0.08 |
| Error | 64 | 371 | 5.8 | | |

the 50 species represented by site doubletons appeared to meet Harvey's (2002) criterion for SREs of range <10 000 km$^2$ if circular ranges were assumed. Eighteen of the site doubletons appeared to have ranges <1000 km$^2$. Of the 25 site tripletons, 11 and three had ranges of <10 000 km$^2$ and <1000 km$^2$ respectively.

*Regional species richness*

Using a reduced matrix of 240 bores and wells that yielded 239 species, the ICE estimator suggested that about 400 species of groundwater fauna occur in the Pilbara (Fig. 6a). This estimate omitted several groups of animals that were excluded from analyses because they are poorly resolved taxonomically in the Pilbara and present identification difficulties. Extrapolating ratio of collected and uncollected taxa (1.67) to the full number of species known from the 240 bores (320) provides a more realistic estimate that about 500–550 species occur in the Pilbara. A similar estimate was obtained using the full regional data set.

The cumulative numbers of species at terminal points of the catchment plots used to calculate a T-S curve (Fig. 6b) showed a very strong linear relationship with the square root of sample size (rather than

**Table 3** Nearest neighbour and average distance to any other bore within each of the five catchments of the Pilbara compared with distances between occurrences of site doubleton and tripleton species. Weighted averages were used to summarise within-catchment data

| | Bores and wells Average inter-bore distances (km) | | | Doubletons Inter-occurrence distances (km) | | | Tripletons Inter-occurrence distances (km) | | |
|---|---|---|---|---|---|---|---|---|---|
| Catchment | *n* | Nearest | Average | *n* | Average | Max. | *n* | Average | Max. |
| 1 Ashburton | 132 | 7.5 | 151 | 11 | 44 | 146 | 1 | 67 | 67 |
| 2 De Grey River | 112 | 7.0 | 123 | 2 | 22 | 22 | 1 | 47 | 47 |
| 3 Fortescue River | 109 | 6.5 | 174 | 7 | 144 | 363 | 1 | 28 | 28 |
| 4 Port Hedland Coast | 76 | 6.4 | 108 | 4 | 60 | 118 | 2 | 47 | 90 |
| 5 Onslow Coast | 40 | 5.5 | 60 | 2 | 54 | 94 | 2 | 22 | 38 |
| Within catchments | 469 | 6.8 | 135 | 29 | 72 | 263 | 7 | 40 | 57 |
| Across catchments | | | 242 | 21 | 227 | 502 | 18 | 259 | 400 |

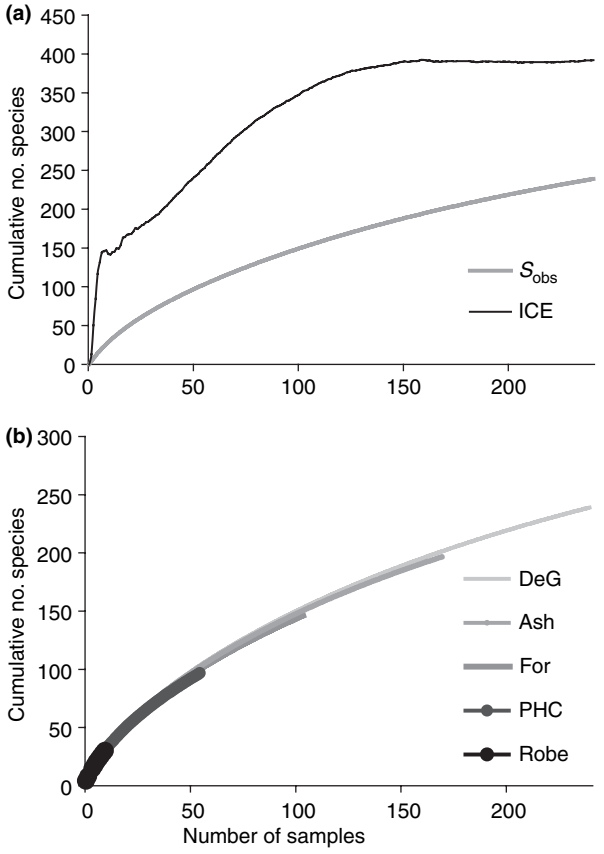


**Fig. 6** Species accumulation patterns in the Pilbara regional survey. (a) Estimated total number of species in the Pilbara according to number of samples collected, using the ICE estimator, and cumulative number of species observed ($S_{obs}$). (b) Species accumulation curves based on data from one, and then two catchments etc (see Ugland *et al*., 2003). Legend indicates the additional catchment in each curve. Note that that the De Grey curve, incorporating all catchments, is the $S_{obs}$ curve from (a).

the logarithm of sample size as found by Ugland *et al*., 2003), yielding the equation

$$S = 17.1\sqrt{a} - 26 \quad (R^2 = 1.00) \tag{3}$$

where $a$ represents area as a multiple of the sampling unit. T-S curve-derived estimates of the regional number of species were very sensitive to estimated size of the sampling unit (i.e. how much of the surrounding aquifer was captured when sampling bores) and are not presented. However, assuming the ICE estimate was of the correct magnitude, T-S curve calculations had the interesting implication that the fauna within a radius of about 5 km of the bore was sampled.

There was little difference between major catchments in patterns of occurrence of the groundwater fauna. The proportion of bores and wells that yielded no species from two sampling events varied between 20% in Port Hedland Coastal and 28% in the Ashburton (see Fig. 1 for locations) and the rate of accumulation of species showed almost no variation among catchments (Fig. 6b).

## Discussion

Results of the intensive sampling in the Pilbara are similar to those of other subterranean fauna studies in that additional species continued to be collected as sampling effort increased (e.g. Culver *et al*., 2004; Hancock & Boulton, 2009). Species accumulation curves showed that, in the Pilbara, one net-haul sampling event collected only 33% of the species present at a bore and six sampling events collected only 82% of species. In eastern Australia, 10 net hauls at a bore (i.e. 1.6 Pilbara sampling events) yielded 31% of the species collected by four combinations of net hauling and pumping (Hancock & Boulton, 2009, recalculated from Table 3). These findings have profound implications for the design of studies that are intended to provide a complete list of the species in an area, as is the case for environmental assessment (EPA, 2003). Currently, it is unusual for bores in Australia to be sampled more than twice in biodiversity surveys or environmental assessment programmes because of time constraints and the cost of fieldwork, particularly in remote regions such as the Pilbara.

### *Species abundance and detectability*

The relationship between species abundance and detectability forms the basis for several frequently used estimators of species richness, such as Chao1 (Foggo *et al*., 2003). Most animal communities contain a few abundant species and many species that occur in low numbers (e.g. Fig. 2) and the abundant species are more likely to be collected in a random sample of a few animals than the rare species. As sampling effort increases, the number of species collected increases according to the general formulation that a species will be collected if its proportion in the community multiplied by the total number of animals in the sample is >1 (Courtemanch, 1996).

We are unaware of previous studies of the relationship between abundance and detectability for subterranean animals, despite the sampling deficiencies and the small numbers of animals collected in studies of subterranean environments (e.g. Eberhard *et al*., 2005b; Hahn & Matzke, 2005; Schneider *et al*., 2005), making them more susceptible to effects on detectability than studies of surface-water fauna. Using only two abundance categories, this study showed that species represented by high numbers of animals were two or three times more likely to be collected in one sampling event than species occurring at low abundance (Fig. 4). In reality, the difference in collection probabilities of the most, and least, abundant species will be much greater and much of what has previously been treated as stochastic variation in species recovery can probably be explained in terms of species abundances. Recognising this will improve our understanding of the structure of groundwater fauna communities and improve interpretation of sampling results.

### *FN rates*

ESTIMATES has often been used to provide estimates of the true number of species at a site, or in a region, from which sampling efficiency can then be inferred. Such estimates are unreliable at low sampling efforts (Foggo *et al*., 2003) and, therefore, we explored use of FN rates as an alternative method of examining sampling efficiency. The FN rate calculated was based on the assumption that all species present at a bore were detected in at least one of the six sampling events. However, this assumption is unlikely to be correct for subterranean fauna, and other organisms occurring in low abundance, as shown by this study and Hancock & Boulton (2009).

A more accurate FN for all species can be calculated by inserting the observed FN rate (0.392), derived from eqn 1, into a formula provided by Tyre *et al*. (2003) to generate collection probabilities that include failure to collect a species

$$L(y|\hat{p},\hat{q}) = p\binom{m}{y}\hat{q}^{y}(1-\hat{q})^{m-y} \qquad y > 0 \tag{4}$$

where $1 - q$ is the FN rate; $p$ is the probability that the species utilised the site throughout sampling (assumed = 1); $m$ is the number of sampling events (6) and $y$ is the number of times the species was observed. For the all-species data set, the likelihood of a species not being recorded in six samples was 0.05, the revised FN rate was approximately 0.65, and revised probability of a species being collected in a single sample was 0.35. This is similar to the estimate based on species accumulation curves (0.33). However, a discrepancy remained between estimates of the detection probability of rare species based on the revised FN rate and Chao2 (0.30 versus 0.23).

### *Regional heterogeneity*

Species richness often exhibits heterogenous patterns across regions (Ugland *et al*., 2003; Culver *et al*., 2004) and taking heterogeneity into account when estimating regional species richness is likely to improve accuracy. Unfortunately, the T-S curve method proposed by Ugland *et al*. (2003) proved to be very sensitive to assumptions about the area of aquifer that was captured by sampling a bore. There is no experimental information about the distance over which groundwater fauna will move into bores but T-S curve calculations suggested ingress occurs from the surrounding 50–80 $km^2$ of aquifer. This is a much larger area than inferred in other studies (e.g. Malard *et al*., 1997; Hahn & Matzke, 2005). While independent confirmation is needed that the mobility suggested by T-S curves is not a mathematical artefact, such mobility has considerable ramifications for management of groundwater fauna. In particular, re-colonisation of de-watered sites from surrounding areas may occur quickly if animals can move several kilometres.

### *Colonisation of bores*

The extent to which the composition of groundwater fauna in bores accurately reflects faunal composition in surrounding ground water is an important, unresolved issue. Results to date are inconsistent, with some studies suggesting bores provide biased samples and others implying a representative selection of aquifer species is obtained (see Hahn & Matzke, 2005). Bias may be caused by the differential attraction of various species to bores because of their feeding and habitat preferences, or by the physical exclusion of larger species from bores.

Construction, slotting and screening methods for bores vary considerably, and are unrecorded for most

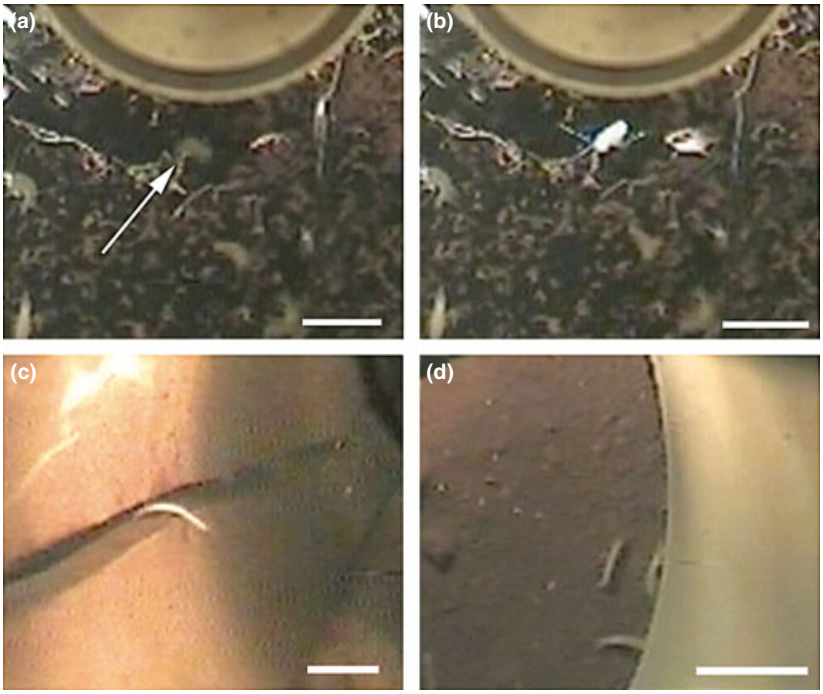


**Fig. 7** Groundwater species entering bores in the Pilbara from down-hole video footage. (a & b) Amphipod (arrowed in a) entering through small void in base of bore PSS190 at 35 m depth in the Fortescue Basin. (c) *Pygolabis* isopod entering through slot of bore PSS172 at 7 m depth in the Ashburton Basin. (d) *Pygolabis* isopods entering under casing of bore PSS179 at 41 m depth in the Ashburton Basin. Scale bar = 1 cm.

bores in the Pilbara, but bore attributes appear unlikely to have had much effect on the number of species collected from bores where net sampling occurred. Down-hole video recordings showed isopods of the genus *Pygolabis*, the largest groundwater invertebrates in the Pilbara and measuring over 1 cm (Keable & Wilson, 2006), moving freely in and out of bores through slotting (Fig. 7c). Amphipods were also observed swimming through slotting. Amphipods and *Pygolabis* species were both observed entering bores through small fissures at the base (Fig. 7a,b) and below the casing (Fig. 7d) of bores. Furthermore, *Pygolabis* or amphipods of the genera *Nedsia*, *Pilbarus* or *Chydaekata* were recorded from a high proportion of bores. The above evidence suggests there were few impediments to colonisation by larger species and that any bias in species composition within bores, relative to the surrounding aquifer, was more likely because of factors associated with the enrichment of bores (Hahn & Matzke, 2005).

### *Singletons and SREs*

One of the defining characteristics of subterranean fauna globally is the high level of regional and short-range endemism. In Europe, subterranean fauna usually comprises >50% SREs, with some regions having >90% (Gibert & Deharveng, 2002), and high proportions of SREs have been reported from groundwater of the Australian arid zone (Cooper *et al.*, 2002; Harvey, 2002). Survey effort in the Pilbara is insufficient to make definitive statements about the proportion of groundwater fauna that are SREs but, nonetheless, it seems likely that very few species collected at more than three sites in the regional survey are SREs (range <10 000 km$^2$). Fewer than 30% of species represented by site tripletons and <50% of site doubletons are likely to be SREs.

Assessing the proportion of species recorded as site singletons that are likely to be SREs is difficult. About 85% of them were also sample singletons, which was similar to the 88% expected if they had an average collection probability of 0.23 (used in calculations below). Average density of bores and wells in the Pilbara survey was about 23/10 000 km$^2$ and species with a collection probability of 0.23 should have been collected from more than one bore if their ranges were ≥10 000 km$^2$ (probability of being collected two or three times was >0.99 and 0.94, respectively). Thus, even allowing for bore distribution being somewhat irregular within a catchment, most of the species recorded as site singletons must have ranges <10 000 km$^2$ and qualify as SREs according to Harvey's (2002) criterion, unless they are restricted to groundwater habitats that

we rarely sampled. If our assumptions about collection probability and distribution of singletons in relation with bore spacings are correct, then about 55% of known Pilbara groundwater species are SREs. A true picture of endemism in the Pilbara, however, must also take into account the species we failed to collect. Despite some small gaps in our sampling coverage, most of these species would have been missed because their ranges were ≤10 000 $km^2$, which implies that about 70% of all Pilbara groundwater species are SREs.

The proportion of SREs is, however, dependent on the threshold criterion used. Harvey's (2002) decision to use a range of 10 000 $km^2$ to define SREs was arbitrary and we suggest it is rather large. It fails to distinguish groundwater species with sub-regional distributions that are secure from range-related natural and anthropogenic threats from those species with sufficiently localised distributions that most of their population may be at risk from an activity such as de-watering or from a pollution event. No project involving below water table mining and groundwater abstraction in the Pilbara has caused significant groundwater drawdown beyond a 10 $km^2$ radius of pumping (Johnson & Wright, 2001), which equates to an impact area of about 350 $km^2$, and in most cases the impact area has been <100 $km^2$. Therefore, we suggest that a more appropriate criterion for Pilbara groundwater SREs is a range of <1000 $km^2$. Mine de-watering and groundwater extraction are unlikely to threaten species with distributions at the upper end of this range but a threshold of 1000 $km^2$ represents a precautionary approach and is of a scale that matches natural barriers. Many Pilbara groundwater species appear to be restricted to sections of hydrographic basins, or tributaries within them, and to have ranges of the order of 1000 $km^2$ (see Finston *et al*., 2006; Reeves, De Deckker & Halse, 2007).

In conclusion, high regional richness of groundwater fauna is usually attributed to age of the landscape, habitat fragmentation, SRE and existence of suitable habitat (e.g. Christman & Culver, 2001; Humphreys, 2001; Gibert & Deharveng, 2002). The Pilbara is an old landscape with geological heterogeneity (McPhail & Stone, 2004) and >40% of known Australian groundwater species have been recorded from this region (Humphreys *et al*., 2005). Although there is much still to be learned about the groundwater fauna of the Pilbara, the recently completed regional survey is likely to have identified its major characteristics and sub-regional hotspots. Results from the net-sampled bores suggest that, as with Slovenian caves (Culver *et al*., 2004), two rounds of sampling is usually sufficient to identify Pilbara sites that are rich in groundwater species. Continued sampling is, however, required to document the full richness of the sites. This was demonstrated at bore PSS016 where 11 sampling events yielded three times more species than collected in two events (Fig. 5).

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**Appendix 1** Taxonomic list of the 93 taxa collected during repeat sampling of 14 bores in the Pilbara (see Fig. 2). Many of the identifications are only to morphospecies level. Species numbers relate to those in Fig. 2

| | Species numbers |
|---|---|
| Turbellaria | |
| Turbellaria sp. | 70 |
| Nematoda | |
| Nematoda sp. 3 | 24 |
| Nematoda sp. 4 | 30 |
| Nematoda sp. 11 | 23 |
| Rotifera | |
| *Dissotrocha* sp. | 72 |
| Gastropoda | |
| Planorbidae sp. | 42 |
| Hydrobiidae sp. | 22 |

**Appendix 1** (*Continued*)

| | Species numbers |
|---|---|
| Oligochaeta | |
| Hirudinea sp. | 68 |
| *Aeolosoma* sp. 3 | 59 |
| Phreodrilus sp. WA32 | 26 |
| Phreodrilidae sp. DVC | 45 |
| Phreodrilidae sp. SVC | 64 |
| *Ainudrilus* sp. WA27 | 54 |
| Tubificidae sp. 1 | 83 |
| Tubificidae sp. 2 | 29 |
| Tubificidae sp. WA28 | 27 |
| *Dero nivea* Aiyer | 19 |
| *Pristina* sp. WA3 | 69 |
| Enchytraeidae sp. 2 | 25 |
| Enchytraeidae sp. 1 | 86 |
| Polychaeta | |
| Nereidae sp. | 31 |
| Arachnida | |
| *Guineaxonopsis* sp. S1 | 12 |
| *Arrenurus* n. sp. 2 | 52 |
| *Peza* sp. | 41 |
| Oribatida sp. 1 | 53 |
| Ostracoda | |
| *Gomphodella hirsuta* Karanovic | 82 |
| *Limnocythere stationis* Vavra | 16 |
| *Limnocythere* sp. 1 | 15 |
| *Candonopsis pilbarae* Karanovic | 88 |
| *Deminutiocandona aporia* Karanovic | 48 |
| *Deminutiocandona* cf. *atope* Karanovic | 66 |
| *Deminutiocandona aenigma* Karanovic | 44 |
| *Deminutiocandona stomachosa* Karanovic | 62 |
| *Humphreyscandona woutersi* Karanovic & Marmonier | 80 |
| *Humphreyscandona* sp. 2 | 75 |
| *Humphreyscandona ventosa* Karanovic | 67 |
| *Notacandona boultoni* Karanovic & Marmonier | 17 |
| *Origocandona posteriorecta* Karanovic | 18 |
| *Pilbaracandona colonia* Karanovic & Marmonier | 32 |
| *Pilbaracandona eberhardi* Karanovic & Marmonier | 33 |
| *Pilbaracandona kosmos* Karanovic | 85 |
| *Pilbaracandona temporaria* Karanovic | 39 |
| *Pilbaracandona rosa* Karanovic | 43 |
| *Areacandona mulgae* Karanovic (11) | |
| *Areacandona lepte* Karanovic | 61 |
| *Areacandona cylindrata* Karanovic | 58 |
| *Areacandona triangulum* Karanovic | 55 |
| *Areacandona iuno* Karanovic | 91 |
| *Areacandona* cf. sp. 1 | 37 |
| *Areacandona* sp. 7 | 38 |
| *Areacandona astrepte* Karanovic | 50 |
| *Areacandona atomus* Karanovic | 10 |
| *Leicacandona carinata* Karanovic | 51 |
| *Leicacandona jimi* Karanovic | 14 |
| *Kencandona verrucosa* Karanovic | 13 |
| Candonidae n. gen. | 77 |

**Appendix 1** (*Continued*)

| | Species numbers |
|---|---|
| Syncarida | |
| *Bathynella* sp. | 21 |
| *Notobathynella* sp. | 47 |
| *Chilibathynella* sp. | 40 |
| *Atopobathynella* sp. A | 20 |
| Thermosbaenacidae | |
| *Halosbaena tulki* Poore & Humphreys | 79 |
| Copepoda | |
| *Mesocyclops brooksi* De Laurentiis *et al*. | 5 |
| *Inermipes* sp. 2 | 4 |
| *Diacyclops einslei* Karanovic | 3 |
| *Diacyclops humphreysi* s. str. X *unispinosus* Karanovic | 78 |
| *Diacyclops humphreysi humphreysi* Karanovic | 81 |
| *Diacyclops cocking* Karanovic | 76 |
| *Diacyclops scanloni* Karanovic | 90 |
| *Diacyclops sobeprolatus* Karanovic | 60 |
| *Halicyclops (Rochacyclops) rochi* Karanovic | 87 |
| *Orbuscyclops westaustraliensis* Karanovic | 6 |
| *Elaphoidella humphreysi* Karanovic | 84 |
| *Schizopera roberiveri* Karanovic | 46 |
| *Abnitocrella* sp. 3 | 56 |
| *Archinitocrella newmanensis* Karanovic | 92 |
| *Parapseudoleptomesochra tureei* Karanovic | 65 |
| *Stygonitocrella bispinosa* Karanovic | 36 |
| *Stygonitocrella trispinosa* Karanovic | 63 |
| *Stygonitocrella unispinosa* Karanovic | 74 |
| *Parastenocaris jane* Karanovic | 35 |
| *Pseudectinosoma galassiae* Karanovic | 7 |
| Amphipoda | |
| *Chydaekata* sp. | 73 |
| Paramelitidae n. gen. | 2 |
| Paramelitidae sp. 9 | 28 |
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| *Nedsia* sp. | 93 |
| Melitidae sp. 1 | 49 |
| Bogidiellidae sp. | 1 |
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| *Pygolabis eberhardi* Keable & Wilson | 71 |
| *Pygolabis humphreysi* Wilson | 34 |
| *Pygolabis paraburdoo* Keable & Wilson | 9 |
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