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## **Assessment and conservation of aquatic life in the subsurface of the Pilbara region, Western Australia**

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### **ABSTRACT**

To provide a framework for assessment of mine development projects in the Pilbara, as well to plan conservation of groundwater biodiversity, the Department of Conservation and Land Management (CALM) is undertaking a five-year survey (2002 to 2007) of Pilbara stygofauna. The project aims to map regional patterns in subterranean biodiversity by sampling the range of groundwater environments that can be accessed via bores, wells, caves, springs, spring-brooks and hyporheic habitats. The first year of sampling has revealed many new stygal species, and indicate that stygofauna is abundant and widely distributed across the region, and occurs in several different aquifer types. Seventy-one per cent of 355 bore/well samples yielded stygofauna. Modifications to sampling equipment and sampling protocol may have contributed to higher recovery rates of stygofauna than reported in other survey work. The Pilbara stygofauna comprises at least 150 species belonging to 77 genera and 39 families. The Pilbara is an important region for subterranean biodiversity.

### **KEYWORDS**

Australia, Pilbara, stygofauna, survey, conservation, groundwater

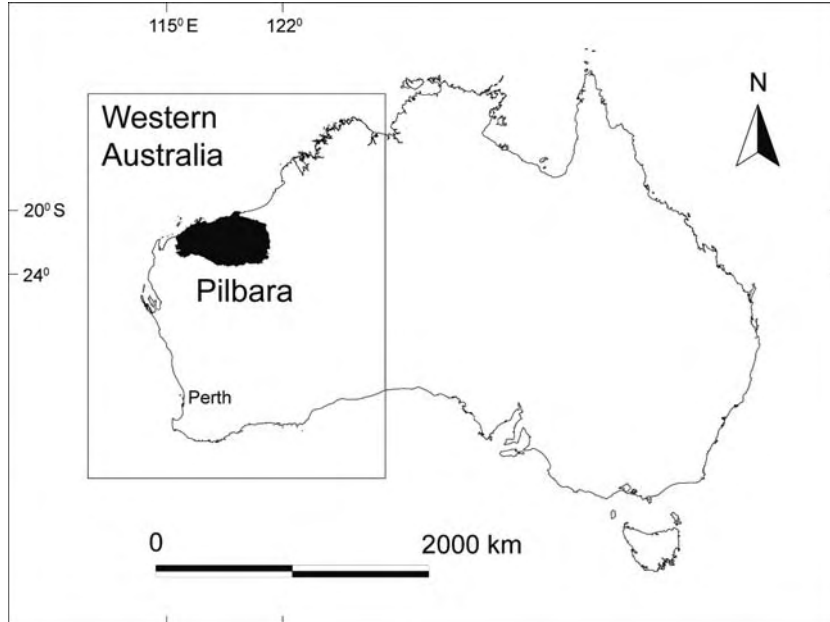
## INTRODUCTION

The Pilbara region (20-24° S and 115-122° E) covers an area of approximately 178,000 km<sup>2</sup> in north-west Western Australia (Figure 1). The Pilbara lies adjacent to Cape Range and Barrow Island, two areas well known for containing rich stygofaunas (Humphreys 2000). Work in the Pilbara during the 1990s, spearheaded by W.F. Humphreys and colleagues, established the presence of a diverse and scientifically interesting stygofauna there as well (Humphreys 2001). To date about 50 stygal species (nearly all Crustacea) have been described or recorded, including: Amphipoda (Barnard and Williams 1995; Bradbury 2000), Spelaeogriphacea (Poore and Humphreys 1998, 2003), Isopoda (Knott and Halse 1999; Wilson 2003), Copepoda (Pesce *et al.* 1996; Laurentiis *et al.* 1999, 2001; Lee and Huys 2002; Karanovic 2004), Ostracoda (Martens and Rossetti 2002; Karanovic 2003; Karanovic and Marmonier 2003), Acarina (Harvey 1998), and Oligochaeta (Pinder 2001).

The Pilbara has a sparse human population but is economically important because of its mineral wealth. In 2000, Western Australia produced 158 million tonnes of iron ore representing 15 % of world production with forecasts of expanded future markets and new mine developments (Department of Mineral and Petroleum Resources 2001). Groundwater is a critical issue in the Pilbara because mining below the watertable in large open-cut pits requires dewatering of surrounding aquifers. Other major impacts on groundwater resources include abstraction for water supplies to towns, industry, and agriculture (Water and Rivers Commission 1996). In recent years, there has been some conflict between groundwater abstraction or mine dewatering operations, and the protection of groundwater-dependent ecosystems, including stygofauna (Water and Rivers Commission 1996; Johnson and Wright 2001; Playford 2001).

In the most extreme cases, mine development proposals have been halted or delayed because it was considered they posed a threat to the conservation of stygofauna (Playford 2001; Finston *et al.* 2004). In Western Australia, all native fauna species (though not necessarily individual animals) are protected under the Wildlife Conservation Act 1950 and environmental impact assessment of development proposals requires that proponents demonstrate that no species are placed at risk of extinction (Playford 2001; Department of Conservation and Land Management 2004). This requires accurate recognition of species and knowledge of their distributions.

To provide a framework for assessment of development projects in the Pilbara, as well to plan conservation of groundwater biodiversity, the Department of Conservation and Land Management (CALM) is undertaking a five-year survey (2002 to 2007) of Pilbara stygofauna. The project aims to map regional patterns in subterranean biodiversity by sampling the range of groundwater environments that can be accessed via bores, wells, caves, springs, spring-brooks, and hyporheic habitats. This short paper describes the approach and methods used in this survey, and presents some preliminary results from the first year of sampling.

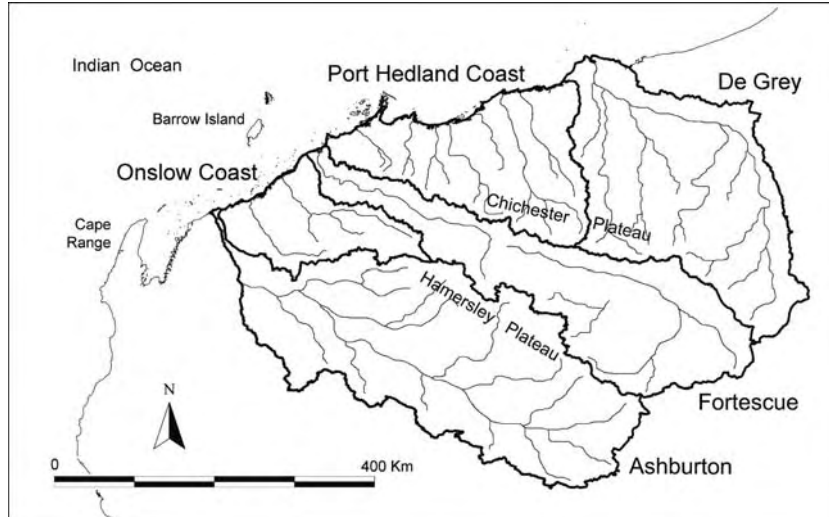


**Figure 1.** Map of Australia showing the Pilbara region in Western Australia.

### **Climate and physiography**

The Pilbara experiences extreme climatic conditions characterised by high daytime temperatures in summer (average maximum 36-39° C), low winter minima (average minimum 6-12° C), and high evaporation (average annual potential evaporation 3200-4000 mm). Annual average rainfall is very low (200-350 mm) but with high inter-annual variation associated with irregular tropical cyclones (Gentili 1972; Bureau of Meteorology 1977).

The Pilbara region coincides with the emergent part of the Pilbara Craton, which has remained more or less continually above sea level since the Proterozoic (> 545 Ma), bordered by marine environments (including the Tethys Sea) from the Devonian (410-354 Ma) until the fragmentation of Gondwana in the Cretaceous (141-65 Ma) (Cockbain and Hocking 1990). The Pilbara encompasses five major drainage basins, the Ashburton, Fortescue, De Grey, Onslow Coast and Port Hedland Coast Basins respectively (Figure 2). Each basin drains to the Indian Ocean via a series of river systems characterised by highly sporadic flow regimes. The major topographic features of the Pilbara are the Hamersley Plateau, which coincides with the Central Pilbara iron ore region and contains the Hamersley Range that reaches an elevation of 1250 m above sea level (asl), and which is separated by the Fortescue Valley from the Chichester Plateau and Range of more subdued relief (618 m asl). The river drainage systems have deeply dissected the margins of the Hamersley and Chichester Plateaus, but then follow broad low-gradient valleys across extensive lowlands to wide coastal plains.



**Figure 2.** Pilbara region showing the five major hydrographic basins and the physiographic features mentioned in the text.

Groundwater occurs throughout the Pilbara region in Precambrian basement rocks, Phanerozoic sedimentary basins and Cainozoic deposits. Total stored fresh-saline groundwater resources are estimated at 67,140 GL with renewable fresh-marginal resources of 291 GL/year (Allen 1997). The aquifers have been classified into three types by Johnson and Wright (2001): (1) Unconsolidated sedimentary aquifers; (2) Chemically-deposited aquifers; (3) Fractured-rock aquifers. Unconsolidated sedimentary aquifers comprise Cainozoic valley and coastal plain alluvium and colluvium, while the chemically-deposited aquifers consist of calcrete or pisolitic limonite formed within the valley-fill sequences. Fractured rock aquifers are developed in Proterozoic and Archaean sedimentary and volcanic rocks including dolomite, sandstone, shale, chert, banded-iron formation and basalt. The calcretes and dolomites are commonly karstic, and vuggy porosity is developed within the limonites (Johnson and Wright 2001).

## METHODS

### Survey constraints, limitations and approach

The scope of the survey was constrained by several factors, including: (1) Large land area to be covered with relatively few access points for sampling groundwater; (2) Limited existing knowledge of the taxonomy and biology of Pilbara species and their distributions; (3) Taxonomic impediment in that most Western Australian invertebrates are undescribed with few taxonomists working on them and a poor framework for morpho-species identifications; (4) Logistical constraints imposed by field operations in a remote and rugged location with extreme climate. With these constraints in mind, the overall aim was to obtain an overview of biodiversity patterns at a regional scale. Achieving broad spatial coverage in a restricted time frame (4 years for fieldwork) reduced the capacity for repeated sampling at individual sites and for investigation of distribution patterns at smaller spatial, or longer temporal, scales. Parallel studies

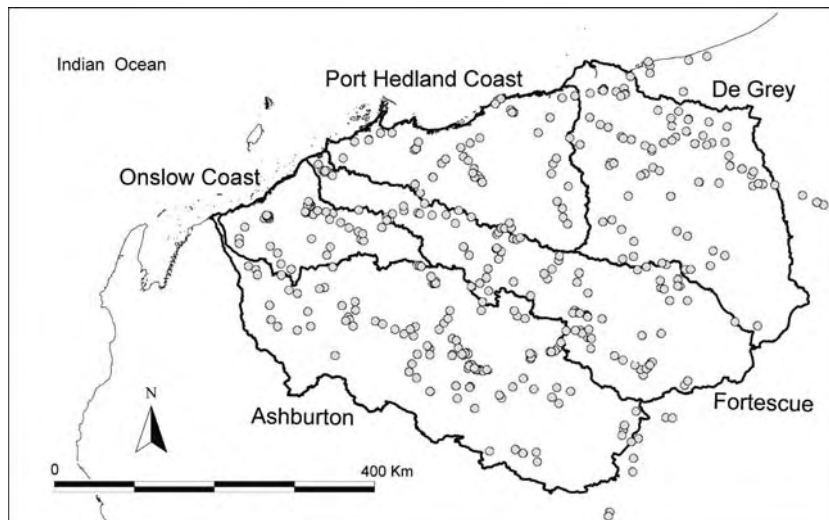
(described below) evaluated issues such as sampling efficiency, and stratification in the water column.

Despite large deposits of karstified carbonate rocks (Proterozoic dolomites and Cainozoic calcretes), there were very few caves accessible for sampling. There were many springs and spring-brooks, and > 3,700 wells and bores associated with pastoral and town water supply, road and railway construction, and mine dewatering operations (Allen 1997). However, most wells and bores were concentrated either in high-yield valley-fill aquifers used for water supply, or local aquifers where mine dewatering, road and railway construction occurs, rather than being evenly distributed across the landscape.

An important subsidiary aim of the survey was to collaborate with taxonomists to support description of new taxa. Groups in which particular efforts were made are Copepoda, Ostracoda, Isopoda and Amphipoda. The survey has also collaborated with geneticists using DNA techniques to define species boundaries and to reconcile genetic data with morphological characters. The latter have been found to be variable in some Amphipoda (see Bradbury 2000; Finston *et al.* 2004).

### **Sample sites**

Sample sites were selected to provide broad geographic coverage and to encompass the range of geologic, topographic, and physiographic environments across the Pilbara region. Sites were also selected to cover different aquifer types (porous sedimentary, chemically-deposited, fractured-rock, karstic and vuggy aquifers). Approximately 450 (eventually 550) bores and wells were selected (Figure 3). Resource and time constraints prevented comparable sampling coverage of springs, spring-brooks and the hyporheos, so about 40 of these groundwater habitats will be sampled. In conjunction with the stygofauna survey, there is a parallel survey of aquatic fauna in Pilbara surface waterbodies, including springs, brooks and rivers (A.M. Pinder, S.A. Halse and J.M. McRae unpublished data). Where possible, both surveys will sample the same springs, spring-brooks and groundwater-fed river pools to evaluate the fauna in groundwater/surface water ecotones.



**Figure 3.** Pilbara region showing the five major hydrographic basins and location of 450 bores and wells selected for sampling stygofauna. The clustered and linear distribution patterns reflect, respectively, the concentration of bores in water supply borefields within high-yield valley-fill aquifers and alignments of bores sunk for construction of roads and railways. The spatial coverage will be improved by sampling an additional 100 bores/wells.

## Sampling protocol and equipment

### *Bores and wells*

Environmental attributes recorded for each site include latitude, longitude, altitude, bore/well construction details (including where available, the depth and geology at the slotted interval), surface geology, vegetation, land-use, and impacts.

Standing water level (SWL, in metres below ground level) and the maximum depth of each bore was measured to the nearest 0.05 m with a Richter Electronic Depth Gauge or weighted Lufkin tape measure. Temperature, pH, electrical conductivity, dissolved oxygen, turbidity and redox were measured at -1 m SWL using a calibrated Yeo-Kal 611 water quality analyser. Water samples for laboratory analysis (undertaken at Chemistry Centre, Perth) were collected from < 1 m SWL using a sterile bailer (Clearwater PVC disposable 38 x 914 mm), and stored in sterile, rinsed 250 ml plastic bottles. One 250 ml water sample was filtered through a 45 µm membrane and frozen for analysis of nutrients (total soluble N, total soluble P). Highly turbid samples were pre-filtered through a glass-fibre filter using a hand vacuum pump (Millipore Sterifil Aseptic 47 mm OM041). A second 250 ml water sample was refrigerated for laboratory determination of solute concentrations ( $\text{Ca}^{2+}$ ,  $\text{Fe}^{2+}/\text{Fe}^{3+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{HCO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{SiO}_2$ , Si, Sr), alkalinity, hardness, colour, turbidity, electrical conductivity, pH, and total dissolved solids. Laboratory methods followed APHA (1995).

Bores and wells were sampled for stygofauna using a plankton net of suitable diameter (47mm, 97mm, 147mm, or 197mm) to match the bore/well. The net, with a weighted McCartney vial attached, was lowered to the base of the bore/well then

agitated up and down ( $\pm 1$  m, 6 times) to disturb the bottom sediment. Six hauls of the entire water column were made, the first three hauls used a 150  $\mu\text{m}$  net to capture macrofauna, the second three hauls used a 50  $\mu\text{m}$  net to capture microfauna such as rotifers. To minimize loss of fauna through bow-wave effects during hauling, the McCartney vial had the bottom removed and replaced with 50  $\mu\text{m}$  mesh. The entire net haul sample was transferred to a 120 ml labeled polycarbonate container and preserved in 100% ethanol. To maximize preservation for possible DNA analysis, the ethanol was replaced after a few hours by decanting the sample through a 50  $\mu\text{m}$  net and refilling the sample bottle with fresh 100 % ethanol. To eliminate the possibility of faunal contamination between sites, the nets were sterilized by washing in a decontaminant (5% solution of Decon 90), then rinsed in distilled water and air-dried.

## **Targeted field studies**

### ***Repeated sampling***

The very large land area to be surveyed meant that each bore/well was sampled on only two occasions, while springs, spring-brooks and the hyporheos were sampled only once. Each bore/well was sampled once in late autumn-early winter (April to July) and once in late winter-early spring (August to October) to capture possible seasonal influences on species occurrence (extremely hot summers preclude field sampling at that time of year). While preliminary data on sampling efficiency suggested two sample events per site was less than optimal for documenting the stygofaunal assemblage of individual bores/wells, whether there are implications for description of regional patterns will be evaluated by repeated sampling of a small subset of the sites. The subset consisted of 14 spatially dispersed sites located in both coastal and inland settings. The sites exhibited a range in species richness (from zero to > 20 taxa recorded during the initial two sampling events). These sites will be sampled twice each year for 2 to 3 years, with > 3 months separating each sample event, and species accumulation curves will be generated.

### ***Sampling efficiency***

The sampling efficiency of the net haul method (cf. Malard *et al.* 1997) is being evaluated in five bores in alluvial aquifers. A standard set of six net hauls is taken and then the bore is purged. Purged water is passed through a 50  $\mu\text{m}$  plankton net over the pump outlet and all animals retained. The volume purged is three times the bore volume, using a centrifugal pump (Grundfos MP-1) with maximum pump rate of 2.4 m<sup>3</sup>/hour. After the bore refills it is re-sampled with six net hauls. Sampling efficiency is calculated by comparing the fauna collected pre-purging with the total fauna collected pre-, during and post-purging.

### ***Water column profiling and down-hole video***

At a few selected sites, physico-chemical profiling of the water column in bores and wells will be undertaken to investigate possible effects from stratification, which may influence the distribution of animals or the interpretation of water chemistry data collected at -1 m SWL. In cased bores where the slotted interval occurs deeper than the SWL, a 'deadwater zone' of uncharacteristic water chemistry may develop (Malard *et al.* 2003). Additionally, stratified anchialine habitats may be present in coastal areas such as the lower Fortescue River where there is a saltwater interface (Commander 1993) and elements of an anchialine fauna occur (Humphreys 2001). Accordingly, the physico-chemistry of the water column will be profiled to maximum



depth 100 m using the Yeo-Kal 611 water quality analyser. A down-hole video camera (Underwater Video Systems UVS-VDS-2001 connected with a JVC GR-DV900AA cam recorder) will be used to relate any physico-chemical stratification to the distribution and abundances of animals within the bore water column.

### **Springs, spring-brooks and hyporheos**

Preliminary sampling of interstitial habitats in springs, spring-brooks and the hyporheos of semi-permanent river pools was undertaken using a Bou Rouch pump. Three subsamples, between 2 and 10 m apart, were collected at each site to account for small-scale heterogeneity. Unless shallow bedrock underlying the sediments prevented it, sampled depth was generally 0.5-1 m below the ground surface. A diaphragm bilge pump (Munster Simms Eng. Ltd.) with 25mm inlet/outlet was used to pump a 10 L volume of sample as fast as possible into a 10 L bucket with 1L graduations marked. Pump discharge rate (L/sec) was recorded and the physico-chemical parameters (temperature, pH, electrical conductivity, dissolved oxygen) of the sample in the bucket were measured with a WTW Multi 340i meter, and 2 x 250 ml water samples were collected for laboratory analysis of nutrients and ionic concentrations. The bucket sample was then elutriated to separate water and animals from sediment, and filtered through a plankton net (50 µm mesh). After elutriation, the volume of sediment remaining in the bucket was estimated to nearest 0.1 L. Between sites, the Bou Rouch equipment was thoroughly rinsed in filtered water, and the net was decontaminated. Samples were preserved in 100 % ethanol. Future sampling will utilise a Grillot pump as recommended in Malard (2003).

Except after heavy rainfall, Pilbara rivers consist of isolated pools with either no, or very limited, flow, which precludes detection of upwelling and downwelling zones by pressure differential using the T-bar method (see Malard *et al.* 2003). Instead, potential zones of groundwater upwelling were inferred from geomorphic structures such as bedrock outcrops which frequently form barrages to hyporheic flow in the alluvium of river channels (eg. Davidson 1975).

Environmental attributes recorded at each Bou Rouch sampling site were the same as for bores/wells. The following additional details were also recorded: channel width, alluvium thickness, estimated discharge (L/sec), flow regime (perennial, semi-permanent, intermittent), spring type (limnocrene, rheocrene, helocrene); type of hyporheic zone (1, 2, or 3 *sensu* Malard (2003).

### **Identification and taxonomy**

Prior to sorting, samples were first separated into three size fractions by sieving through 250, 90, and 53 µm Endicott sieves. Sorting occurred under Leica MZ3, 6, 8 or 12 dissecting microscopes with 0.6x or 10x eyepiece and 10x or 16x objective. Each taxon was identified to the lowest taxonomic rank possible using published keys and descriptions, and the numbers of each taxon were recorded. Identification of microfauna used a Zeiss Axioskop 2 compound microscope with 10x eyepiece and 5x, 10x, 20x, 40x and 100x objective. Identifications were confirmed by specialist taxonomists as necessary. Many taxa were new and undescribed so examples of each new (morpho-) species collected were retained in a voucher collection and used for checking identifications and designating new species. In collaboration with molecular geneticists (at the South Australian Museum and the University of Western Australia, see for example Finston *et al.* this volume), DNA techniques were used to

assist in defining species boundaries, particularly in groups such as Amphipoda that appear to have few reliable morphological characters for distinguishing species. Publication of descriptions of new species collected during the survey will be supported.

### **Database, analysis, and reporting**

All survey data are stored in a relational database using MS Access. The database structure comprises several linked tables that encompass taxa, site, and environmental attribute data. Site x species and site x environmental attribute matrices will be compiled for analysis of regional-scale biodiversity patterns using multivariate analysis software such as PATN (Belbin 1993). Analysis will seek to determine inter alia, groups of species with similar patterns of occurrence, groups of sites with similar stygofauna and the environmental attributes that influence biodiversity patterns. A parallel study will examine groundwater chemistry and its influence on the distribution of ostracods (J. Reeves and P. De Deckker, Australian National University, unpublished data). The survey results are intended to be published in a series of papers in refereed scientific journals.

## **PRELIMINARY RESULTS**

### **General sampling statistics**

In the first year of sampling (November 2002 to November 2003), 355 samples were collected from 253 bores, of which 97 bores were sampled at least twice including five bores that were purged (three samples per event). Fauna was detected in 252 of 355 samples (71 %), with a mean of 3.8 taxa per sample (excluding samples with zero taxa). Across all samples, the mean number of animals per sample was 23.2 (range 0 – 250). For the 97 sites sampled twice, a mean of 5.4 taxa per site was recorded. On average (104 comparisons), two sampling events had 24 % species in common, while each sample comprised 38 % common species.

### **Systematic diversity**

Initial sampling has revealed the presence of many new species of Crustacea and Acarina, in addition to undescribed Mollusca, Oligochaeta, Polychaeta, Tricladida, and Nematoda. Thus far, the Pilbara stygofauna comprises, minimally, > 150 species belonging to 77 genera and 39 families. The classes/orders and families recorded to date include: Acarina (Arrenuridae, Halacaridae, Limnesiidae, Mideopsidae, Pezidae, Oribatida), Amphipoda (Bogidiellidae, Melitidae, Neoniphargidae, Paramelitidae), Aphonaneura, Bathynellacea (Bathynellidae, Parabathynellidae), Copepoda (Ameiridae, Canthocamptidae, Cyclopidae, Diosaccidae, Ectinosomatidae, Parastenocaridae), Decapoda (Atyidae), Gastropoda (Hydrobiidae), Isopoda (Amphisopidae, Cirolanidae, Microcerberidae, Phylloscidae, Tainisopidae), Oligochaeta (Enchytraeidae, Naididae, Phreodrilidae, Tubificidae), Polychaeta (Neiridae), Ostracoda (Candonidae, Darwinulidae, Limnocytheridae), Rotifera (Philodinidae), Speleogriphacea (Speleogriphidae), Thermosbaenacea (Halsobaenidae), Tricladida (family indeterminate), Nematoda (family indeterminate).

## DISCUSSION AND CONCLUSION

The Pilbara is an important region for subterranean biodiversity. It contains a comparatively rich systematic diversity of stygofauna (cf. Botosaneanu 1986). The current survey has uncovered many new stygal species. Preliminary results from the first year of sampling indicate that stygofauna is abundant and widely distributed across the region, and occurs in several different aquifer types. The 71 % recovery rate of stygofauna from 355 samples was higher than anticipated given the generally lower rates previously reported in other survey work, both in the Pilbara and elsewhere in Western Australia. Recovery rates from other surveys in the Pilbara averaged 38 % (range 0 to 69 %, n = 355 samples from 311 bores/wells in 14 aquifers and 15 surveys; data extracted from Eberhard 1998; Eberhard and Humphreys 1999; Biota Environmental Services 2002, 2003, 2004; Knott and Goater 2004). Lower recovery rates were also reported from porous and limestone aquifers in south-west Western Australia, a region where stygofauna appear to be less diverse. One survey (150 bores) in the Perth Basin yielded stygofauna in 2 % of samples (reported in Knott and Goater 2004), while another survey (33 bores) yielded 15 % (Eberhard 2003). The higher recovery rates in the CALM Pilbara survey may be the result of: (1) Use of smaller net mesh sizes (50 and 150  $\mu\text{m}$ ) to capture microfauna, whereas other sampling has used 200, 250 or 350  $\mu\text{m}$  mesh; (2) Modifications to net hauling equipment and sampling protocol (x 6 net hauls with vigorous agitation of bottom sediments) to maximize capture of animals from sediment in the bottom of bores, and to minimize fauna loss through bow-wave effects during hauling. Some other surveys employed only 2 or 3 net hauls, and less vigorous agitation of bottom sediments; (3) Sorting in the laboratory under relatively high magnification, rather than field sorting with lower magnification microscopes.

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