# Technical Guidance

## Sampling methods for Subterranean fauna

The content of this Guidance has not yet been updated to reflect the EPA's framework for environmental considerations in environmental impact assessment

**Environmental Protection Authority**

December 2016

# FOREWORD

The Environmental Protection Authority (EPA) is an independent statutory authority and is the key provider of independent environmental advice to Government.

The EPA's objectives are to protect the environment and to prevent, control and abate pollution. The EPA aims to achieve some of this through the development of environmental protection Guidance Statements for the environmental impact assessment (EIA) of proposals.

This document is one in a series being issued by the EPA to assist proponents, consultants and the public generally to gain additional information about the EPA's thinking in relation to aspects of the EIA process. The series provides the basis for EPA's evaluation of, and advice on, development proposals subject to EIA. The Guidance Statements are one part of assisting proponents in achieving an environmentally acceptable proposal. Consistent with the notion of continuous environmental improvement and adaptive environmental management, the EPA expects proponents to take all reasonable and practicable measures to protect the environment and to view the requirements of this Guidance as representing the **minimum** necessary process required to achieve an appropriate level of environmental protection.

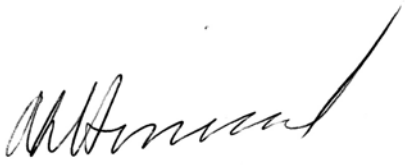
Formal environmental impact assessment (EIA) under the *Environmental Protection Act 1986* is likely to be required if a proposal may cause significant change to a habitat containing subterranean fauna (either stygofauna or troglofauna). Information presented on subterranean fauna can be evaluated only if it has been collected with adequate sampling effort and appropriate methodologies.

This document outlines the EPA's position in relation to what are acceptable sampling efforts and methodologies for subterranean fauna. A framework is provided for determining whether an area is likely to have significant subterranean faunal values.

While guidance is provided specifically in relation to the Western Australian *Environmental Protection Act, 1986,* proponents are reminded to ascertain any responsibilities they may have in regard to this issue under the Commonwealth *Environment Protection and Biodiversity Conservation Act, 1999.*

This Guidance Statement has the status "**Draft**" which means that it has been endorsed by the EPA for release for stakeholder and public review use and comment for 12 months.

I am pleased to release this document and encourage you to comment on it. Information on where to send your comments is provided on the following page.



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**ENVIRONMENTAL PROTECTION AUTHORITY**
**GUIDANCE FOR THE ASSESSMENT OF ENVIRONMENTAL FACTORS**

**DRAFT GUIDANCE STATEMENT No. 54A**

# SAMPLING METHODS AND SURVEY CONSIDERATIONS FOR SUBTERRANEAN FAUNA IN WESTERN AUSTRALIA

**(Technical Appendix to Guidance Statement No. 54)**

## How to comment on this document

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# Guidance Statement No. 54A

# Guidance Statement for Sampling Methods And Survey Considerations For Subterranean Fauna In Western Australia

**Key Words: stygofauna, troglofauna, subterranean, groundwater, caves, sampling**

## 1 PURPOSE

**1.1** Guidance Statements generally are developed by the EPA to provide advice to proponents, and the public generally, about the minimum requirements for environmental management which the EPA would expect to be met when the Authority considers a proposal during the assessment process. The generic process is set out in Appendix 1.

This Guidance Statement is termed "Draft", and should be viewed as a general guide to EIA. While the content of the guidance has not yet been signed off by the EPA at this stage, it should be regarded as the latest thinking in the mind of the EPA if it is asked to consider the issue for assessment. Users would be well advised to be mindful of the guidance at this early stage.

**1.2** This Guidance Statement specifically addresses survey design and sampling methods for subterranean fauna. The Guidance provides information which the EPA will consider when assessing proposals where subterranean fauna is a relevant environmental factor in an assessment. It takes into account:

(a) protection of the environment as defined by the *Environmental Protection Act 1986 (WA*) with a focus on survey design and sampling;

(b) the factor of subterranean fauna.

**1.3** This is a Guidance Statement and proponents are encouraged to consider their proposals in the light of the guidance given. A proponent wishing to deviate from the minimum level of performance set out in this Guidance Statement would be expected to put a well-researched and clear justification to the EPA arguing the need for that deviation.

# 2 THE ISSUE

The EPA seeks to ensure there is adequate protection for important habitats for subterranean fauna and that no subterranean species is threatened with extinction (EPA, 2003). The latter objective reflects a requirement of the *Wildlife Conservation Act 1950*.

Formal environmental impact assessment (EIA) under the *Environmental Protection Act 1986* is likely to be required if a proposal may cause significant change to a habitat containing subterranean fauna (either stygofauna or troglofauna). The EPA's position on the assessment of subterranean fauna is set out in Guidance Statement No. 54 (EPA, 2003). Information presented on subterranean fauna can be evaluated only if it has been collected with adequate sampling effort and appropriate methodologies. Sound information will also contribute to overall knowledge of the subterranean fauna of Western Australia.

# 3 THE GUIDANCE

## 3.1 Introduction

This document outlines the EPA's position in relation to what are acceptable sampling efforts and methodologies for subterranean fauna. It serves as a technical appendix to Guidance Statement 54 (EPA, 2003). A framework is provided for determining whether an area is likely to have significant subterranean faunal values. Stygofaunal sampling is covered in more detail than troglofaunal sampling because the latter is less well understood. Further research into sampling methods for troglofauna is necessary.

The document also describes reporting requirements. Results of subterranean fauna surveys should be available for public review in the EIA review documentation. It is therefore important that proponents make allowance, when planning project timelines, for the substantial period of time required to undertake and report on subterranean fauna surveys.

## 3.2 Subterranean fauna and short range endemism

Subterranean fauna are an important issue in EIA because a high proportion of subterranean species have geographically restricted ranges. It is also becoming apparent that subterranean habitats contain far more species than previously recognized and, in fact, contain a significant proportion of global biodiversity (Gibert & Deharveng, 2002).

While many of the species occupying shallow groundwater are widespread (Halse et al., 2002), and the same is probably true of many terrestrial species in shallow subterranean habitats, species that occupy deeper subterranean habitats and never come to the surface tend to have localized distributions and to be short range endemics. Harvey (2002)

defined short range endemism as having a range < 10,000 km$^2$, while Eberhard et al. (2007) suggested < 1000 km$^2$ constitutes a more appropriate range criterion. Ranges are difficult to determine precisely, however, and the characteristic that makes short range endemics vulnerable to extinction is being confined to highly restricted habitats or individual geological features. Examples of such vulnerable fauna are troglofauna in Channel Iron Deposit in mesas of the Pilbara (EPA, 2007) and stygofaunal beetles and amphipods in calcretes of the Yilgarn (Leys et al., 2003; Cooper et al., 2007).

### 3.2.1 Listed species

There are currently a small number of stygofaunal and troglofaunal species listed for protection under the *Wildlife Conservation Act 1950* and the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*. Approval from the Ministers responsible for the relevant Acts is required to take such species or destroy their habitat.

## 3.3 Desktop review and pilot study

While stygofauna and troglofauna appear to occur throughout Western Australia, there is considerable variation in species density at both regional and local scales. This can be viewed as a matrix of probabilities that provide the basis for determining whether subterranean fauna need formal consideration in an EIA (Table 3.1). At present, the matrix is incomplete and intended only as a starting point for discussions between regulators and proponents. The information on which it is based is summarized below.

Table 3.1. Probability that a site contains a rich subterranean fauna is largely determined by the region in which the site occurs and local geology. ▭ very high, ▭ high, ▭ low.

| Region | Geology | Stygofauna | Troglofauna |
|---|---|---|---|
| Kimberley | *Karst, limestone, sandstone, alluvium, islands* | ▭ | ▭ |
| Pilbara[1] | *Most geologies* | ▭ | ▭ |
| | *Barrow Island* | ▭ | ▭ |
| Inland deserts | *Calcrete, alluvium* | ▭ | |
| Gascoyne/Murchison | *Calcrete, alluvium, banded ironstone* | ▭ | ▭ |
| | *Cape Range* | ▭ | ▭ |
| Yilgarn/Goldfields | *Calcrete, alluvium, banded ironstone* | ▭ | ▭ |
| South-West | *Most geologies* | | |
| | *Karst* | ▭ | ▭ |
| Nullabor | *Karst* | ▭ | ▭ |

[1] Probability of a rich troglofauna assemblage is very high in parts of the Pilbara, e.g. Robe Valley.

The Pilbara, and to a lesser extent, the Yilgarn/Goldfields stand out as global hotspots for subterranean biodiversity, especially stygofauna. For example, it is estimated that the Pilbara contains 500-550 species of stygofauna (Eberhard et al., 2005a, 2007), which far exceeds the 300 stygofaunal species known from all cave systems in the United States (Culver et al., 2000). Similarly, the Yilgarn and Goldfields contain more species of subterranean beetle than known from anywhere else in the world (Watts & Humphreys, 2006). It should be assumed that all sites in the Pilbara and Yilgarn/Goldfields will support significant stygofauna and troglofauna assemblages, unless there is strong evidence that subterranean habitats lack pore spaces, have a geology that renders conditions completely anoxic, or contain groundwater of salinity > 60,000 mg $L^{-1}$. Note, however, that fresh water lying above a hypersaline lens may support stygofauna.

The Kimberley is poorly surveyed but sampling to date has shown that alluvium and karstic limestone, dolomite and sandstone systems, as well as offshore islands in the region, support stygofauna (e.g. EPA, 2005). The communities recorded are not as rich as in the Pilbara but there has been less sampling effort. It is likely significant troglofauna communities occur in the Kimberley.

Cape Range, at the northern boundary of the Gascoyne/Murchison region supports rich subterranean communities, including anchialine communities on the freshwater/ocean water interface (Knott, 1993; Humphreys, 1993, 1999). Some other parts of the Gascoyne/Murchison have also been shown to support stygofauna, although assemblages have not been as rich as in the Pilbara or Yilgarn/Goldfields. Calcrete deposits, other karstic areas and banded ironstone formations should be regarded as likely to support both stygofauna and troglofauna.

Relatively little is known about subterranean fauna from the Nullabor cave systems, despite their global significance and the large amount of caving activity undertaken there, but a significant number of troglofauna species have been described (spiders, beetles and cockroaches), as well as some stygofauna (e.g. Gray, 1992; Bradbury & Eberhard, 2000). All karstic systems in the region should be regarded as prospective for subterranean fauna.

Stygofauna and troglofauna have been recorded from few locations in the South-West, other than caves, although the existence of interstitial faunas has been documented (Schmidt et al., 2007) and isolated studies have demonstrated the occurrence of subterranean communities, albeit not particularly rich, in a variety of settings. The occurrence of significant subterranean faunas in the South-West is likely to be associated with discrete geological features, particularly limestone formations.

## 3.4 Scope of desktop assessment

The probabilities in Table 3.1, local geological setting, and other emerging information should be used by proponents to indicate whether subterranean fauna surveys are likely to be required as part of an EIA. In areas of low prospectivity, it may be possible to demonstrate through desktop assessment that a project area is unlikely to have any significant values for subterranean fauna. Such desktop studies should address (with documented evidence):

- Characteristics of the subterranean fauna of the region (based on existing sampling results);
- Geological, hydrogeological and other information suggesting local habitat is unsuitable for subterranean fauna; and
- Ways in which the local subterranean fauna population is likely to differ from the regional characteristics.

Note that desktop assessment of risk (followed by survey, if appropriate) is likely to be required for expansion of projects approved prior to the requirement for subterranean fauna assessment and for projects where changes to approved design or implementation is being sought under Section 45C of the *Environmental Protection Act 1986*.

## 3.5 Benefit of pilot study

In some cases, proponents may believe there is little likelihood of subterranean fauna occurring in a project area but desktop review does not provide convincing evidence to support this position. A pilot study may be an effective method of determining whether subterranean fauna occur. Much less sampling is required to characterize the type of community present than to document all species. If the area supports significant subterranean fauna, the results of the pilot study can be used to focus the more comprehensive survey that will be required to document all species and assess their conservation status (refer to Section 3.12 for more information on pilot studies).

## 3.6 Planning for subterranean fauna sampling

It is essential that proponents leave ample time for subterranean fauna investigation when planning timelines for project development and environmental approval, particularly if projects occur in areas that are ranked as “very high” or “high” in Table 3.1. In addition to time spent on desktop assessment, long periods of investigation may be required to determine which species occur in a project area and their wider distributions.

Table 3.2 summarizes key characteristics of the sampling requirements for subterranean fauna and provides some generic timeframes that should be taken into account when scoping development proposals and planning timelines. It usually becomes obvious only

at the analysis stage whether any species are restricted to the impact zone of a project. The likely requirement for further subterranean fauna investigations, if there are restricted species, may cause considerable delay in project assessment unless subterranean studies commenced during the early stages of project development.

Table 3.2. Timeframes associated with subterranean fauna studies

| | Colonization of bores/traps | Sampling rounds | Identification, analysis and reporting |
|---|---|---|---|
| Stygofauna | Ideally allow 6 mo. for bore to colonize | Ideally sample in 2 different seasons (see section 3.11). 2nd round of sampling should be > 3 mo. after 1st to maximize chances of collecting most fauna. | Takes weeks to many months per round depending on number of samples and availability of expertise (see section 3.9). It is possible that analysis will show that additional sampling or taxonomic study is needed |
| Troglofauna | May trap immediately. Traps remain in place ≥ 6 weeks | Ideally 2 sampling rounds in different seasons (see section 3.11) | Similar timelines to stygofauna but the likelihood additional sampling will be required is high and adequate survey may take several years |

## 3.7 Sampling methods for subterranean fauna

The aims of subterranean fauna sampling in an EIA are to document the species present and to assess their distribution and conservation status. How well this is done will depend on the appropriateness of sampling methods, effort and survey design. Sampling methods for stygofauna and troglofauna are discussed below.

Note that a Licence to take fauna for scientific purposes is required under Regulation 17 of the *Wildlife Conservation Act 1950*. Regulation 17 licences are available from the Department of Environment and Conservation.

### 3.7.1 Stygofauna

Most published subterranean faunal research has a non-quantitative, biological focus with researchers either describing new species or documenting the biology of the more accessible species. It is only in the last decade that the efficiency of sampling methods has received much attention.

Stygofauna occurring in caves can be sampled using similar techniques to those employed for surface water invertebrates (including diving) and are not considered in

detail in this document. Similarly, epigean species occurring under or beside streams in shallow groundwater can be sampled using Bou-Rouch pumps or by digging pits beside a stream, bailing out the incoming groundwater and filtering it through a net (often referred to as the Karaman-Chappuis method) (see http://groundwater-ecology.univ-lyon1.fr/nouveau/methodes-souterraines.htm).

The focus of this document is sampling stygofauna in deeper groundwater. Access to this groundwater is usually by bores and wells (for brevity, these will henceforth be referred to as bores). Projects that have significantly improved our understanding of bore sampling are the PASCALIS project in Europe (see http://www.pascalis-project.com/), the Pilbara Biological Survey in WA (Eberhard et al., 2007), and studies in Queensland and New South Wales funded by the Queensland Department of Natural Resources and Water, Ecowise Environmental, Australian Research Council and University of New England (Hancock, 2007; Hancock & Boulton, 2007).

#### 3.7.1.1 Validity of sampling bores

Since the species occurring in the aquifer, rather than those in an artificial bore, are the focus of assessment, bore sampling is based on the assumption that the species in bores are representative of all those in the surrounding aquifer. The scientific literature offers contradictory opinions about the validity of this assumption, with some authors hypothesizing that a bore is an enriched and unnatural habitat and, therefore, will contain a strongly biased subset of the aquifer assemblage. However, tests based on comparison of the first water pumped from within the bore cavity and that sucked in from the aquifer as pumping continues strongly suggested that bores contain all species found in the aquifer, albeit sometimes in different proportions (Hahn & Matzke, 2005). The results of these tests provide the basis for the EPA accepting that satisfactory assessments of risk to species in the aquifer can be obtained from sampling bores.

The most common methods of sampling stygofauna in bores are haul nets and pumping but traps are also used. The equipment and techniques of each method are described below.

#### 3.7.1.2 Haul nets

A haul net is a weighted ‘plankton’ net (Fig. 3.1), which is lowered to the bottom of the bore, bounced up and down to agitate sediments at the base of the bore, and then slowly retrieved, filtering stygofauna out of the water column on the upward haul. Net hauling requires relatively little equipment, can be done quickly and works equally well for all depths of bore. However, it can only be used in vertical bores and is a relatively inefficient method of sampling: several hauls must be made to obtain a sample of the stygofauna present at the time of sampling. It is recommended that the net is lowered and retrieved six times, with the operator being aware that, in most cases, the majority of animals will be near, or in, the sediments at the base of the bore, so that the yield will increase if the sediments are vigorously agitated.

Many stygofauna are <0.5 mm in length and are elongate in bodyform. A small mesh size (about 50 µm) is required for reliable collection of the smaller species of stygofauna. However, small mesh sizes tend to become clogged with sediment and also create a pressure wave in front of the net as it is retrieved, which may cause animals to be pushed clear of the net. Use of a larger meshed net (about 150 µm) on some hauls is likely to improve capture rates of larger animals. Thus, it is recommended that three hauls are made with a 150 µm net, then three with a 50 µm net.

The contents of the net should be emptied after each haul because any animals present are likely to swim free as the net is dropped back down the bore. Emptying the contents into a sample jar is easier if the bottom of the net consists of a removable vial that can be unscrewed and tipped straight into the sample jar. Cutting off the base of this vial and replacing with 50 µm mesh improves flow through the net (Fig. 3.1). Animals that may be adhering to the mesh of the net should be washed into the vial before it is removed from the net.

Fig. 3.1. Stygofauna nets of different diameters, showing the machined brass weight fitted to the bottom of the net and McCartney vial, with mesh base, that fits into the brass weight

The most common internal diameters of cased bores in WA are 50, 100 and 150 mm. Ideally, net diameter should be about two-thirds of bore diameter so that the net drops easily to the bottom of the bore and sediment and animals can lift up past it as the sediments are agitated. However, constraints in manufacture may lead to the net designed for 50 mm bores occupying most of the bore. Suitable net designs are shown in Fig. 3.1, Hancock (2007) and http://groundwater-ecology.univ-lyon1.fr/nouveau/methodes-souterraines.htm).

### 3.7.1.3 Pumping

Collecting animals by pumping water out of a bore and through a net often recovers more species than net hauling but requires a significant amount of equipment, is more time-consuming, is unsuitable for deep bores, and can damage many of the animals collected. Whether the method can be used on inclined bores is dependent on slope and condition of the bore (most inclined bores are uncased). Considerable prior preparation may be required to develop a suitable method of pushing the pump to the bottom of an uncased bore.

Three types of pumps may be used: an inertia (e.g. Watera), pneumatic piston or an impeller-driven pump (e.g. Grundfos). Ideally, the pump intake should be lowered until 1-2 m above the bottom of the bore and water pumped to the surface, where it is passed through a 50 µm mesh net. If the intake cannot be positioned near the bottom of a cased bore, it must be adjacent to, or below, the slotted section of the casing rather than above it. The sample retained in the net, which will consist of animals and silt, should be transferred to a sample jar. Inertial pumps can be used in bores with diameters as small as 50 mm, while pneumatic piston and impeller-driven pumps require bores with diameters > 80 mm.

Inertia and pneumatic piston pumps cause less damage to larger stygofauna (e.g. amphipods, isopods) than an impeller-driven pump. Little damage should occur to smaller animals in any of the pumps. The pump used should be able to deliver water to the surface at a rate > 10 L $min^{-1}$ from a watertable 40 m below ground to ensure that animals are drawn in from the surrounding aquifer. The rate of delivery can be calculated by filling a 20 L bucket.

The standard procedure when purging bores prior to sampling water quality involves removing three times the bore volume to ensure water in the bore has been replaced by aquifer water. It is recommended that the greater of either three times the bore volume or 300 L be pumped when sampling for stygofauna. Sampling in eastern Australia has shown 300 L yields at least as many stygofauna as net-hauling, and often captures more (Hancock and Boulton, 2007). The volume of water in a bore can be calculated using the formula

Volume in litres = $\pi \times r^2 \times l$

where $l$ = difference between static water level and depth of the bore expressed in metres, and $r$ = half the internal diameter of the bore expressed in millimetres.

### 3.7.1.4 Stygofauna traps

Traps are not often used to catch stygofauna, partly because they require setting one day and pulling up a few days later. Designs are based on a suitable weighted, baited container or substrate being placed within a plankton net and lowered into the bore. Any animals washed out of the container or substrate as the trap is retrieved are caught by the net. Tuna or prawn is commonly used as bait and pieces of mop head or other tufted

material may be used as substrate. The substrate may be wired to the net rather than weighted.

The principal drawbacks with traps are expense (both in terms of the number of nets required and the field-time needed to set and then re-visit them) and taxonomic bias. They preferentially capture large animals such as isopods and amphipods and tend to miss taxa that occur in bore sediments (Hancock, 2007; see also http://groundwater-ecology.univ-lyon1.fr/nouveau/methodes-souterraines.htm.)

#### 3.7.1.5 Preserving samples

Whatever sampling method is adopted, samples should be preserved in the field and returned to a laboratory for sorting under a dissecting microscope. The best all-round preservative is 70 % ethanol but 100 % analytical grade ethanol should be used if DNA is likely to be required from animals. Ensure that animals for DNA studies are placed in 100 % ethanol while still alive and that there is full contact with the preservative. A weak solution of buffered formalin (ca. 5 %) gives crisper fixation of crustaceans for morphological studies but it must be replaced after a couple of weeks with 70 % alcohol. Formalin is difficult to transport, needs to be handled with great care, and prevents later extraction of DNA.

#### 3.7.1.6 Sorting samples

Accumulating live samples each day and sorting them in the evening under a microscope is sub-optimal. The number of species recovered from a sample is related to the number of animals seen and it is unlikely that all species will be recovered unless the whole sample is examined carefully. This rarely occurs in field sorting, which tends to be rushed and often occurs with poor lighting and difficult working conditions.

While preserved invertebrates may be sorted using a range of techniques in the laboratory, sorting is easier if a well defined search image is developed. Elutriating a sample to get rid of as much sediment as possible and sieving the sample into size fractions assists this process. A common strategy is to use three sieves (250, 90 and 53 µm mesh size) to separate the sample into > 250, 250-90, and 9-53 µm categories. Even after elutriation and sieving, sediment will be present and the volume of sample added to a sorting tray should be small enough that sediment is not more than one particle thick across the bottom of the tray.

Animals to be used in DNA work should be sorted in 100 % ethanol rather than water. Some people place a small amount of stain (e.g. Rose Bengal) in each sample at the time of preservation or soon after to assist in detecting smaller animals during sorting.

## 3.8 Troglofauna

Most troglofauna sampling in caves is by active searching for animals, which are often relatively large. Pit traps and other techniques employed for ground-dwelling terrestrial invertebrates can also be used to increase sampling effort (e.g. Schneider & Culver, 2004). The focus of this document, however, is sampling the troglofauna occupying much smaller subterranean spaces, to which there is no direct access. Bores provide the best method of reaching these habitats and animals are collected by lowering traps into bores and leaving them several weeks to be colonized by animals.

### 3.8.1 Troglofauna traps

There has been little research into effective methods of trapping troglofauna using bores. In Western Australia (WA) it is common to lower PVC pipe, usually closed at either end with aviary mesh and containing leaf litter (Fig. 3.2), into the bore to align with fissures and voids in the surrounding habitat (e.g. Biota, 2005). Bore logs can be used to identify an appropriate bore height at which to place the trap, although use of down-hole video has the potential to improve placement.

Trap designs and protocols are likely to develop further as more troglofauna investigation occurs but adherence to three principles should assist current trapping. Firstly, there must be a connection between the trap and surrounding habitat. Small traps suspended in a large diameter bore may not be accessible to troglofauna and, if bores are cased, traps must be set opposite slots or dropped to the bottom of the bore, where troglofauna can access the trap through the open base of the bore. Secondly, there is a considerable amount of observational data suggesting recoveries are greater in humid conditions, such as after a cyclone when substrates above the watertable contain extra moisture (Humphreys, 1991; Biota, 2006b). Maintaining a moist environment around the trap is likely to increase trapping success. Making sure the bore is capped and sealing around the cap with plastic will help maintain humidity. Thirdly, carbon and nitrogen are in short supply in the subterranean environment and sources of these are likely to attract animals. The leaf litter used in most traps in WA acts as bait. It is common to sterilize this litter but that inhibits breakdown and reduces attractiveness to troglofauna. While it is important to ensure that any litter used does not contain live invertebrates, trapping recoveries may be increased by inoculating the litter with some widespread bacteria that will assist decomposition. The addition of further bait, such as a small quantity of dog biscuit or cheese, may also increase capture rates.

It is suggested that troglofauna traps should remain in place for 6-8 weeks, based on observations that capture rates appear to increase with length of the trapping period. However, the relationships between trapping time, number of animals caught and their taxonomic composition have not been quantified and warrant further investigation. At present, a sampling protocol is probably best justified by plotting species accumulation curves rather than by relating it to existing standards.

Fig. 3.2. Troglofauna trap being lowered into bore. Traps are made of PVC and ends are often closed with aviary mesh to provide access, rather than having holes drilled along the pipe

#### 3.8.1.1 Preserving samples

Troglofauna samples are best returned live to the laboratory. Traps can be sealed in zip lock bags for transport. Once sorted, animals should be preserved in 70 % ethanol unless they are likely to be needed for DNA analyses when 100 % ethanol should be used.

#### 3.8.1.2 Sorting samples

Troglofauna and other soil invertebrates display strong negative phototaxis, which can be used to facilitate sorting by transferring the contents of the troglofauna trap (leaf litter, bait and animals) to a Berlese Funnel, containing 70 or 100 % ethanol, for 24 hrs. Animals should move towards the bottom of the sample and fall into the ethanol. The heat from the lamp will dry samples (another cause of downward animal movement) but, while drying times will vary, litter in the bottom of the sample should retain some moisture for at least 12 hours. Litter should be checked under a dissecting microscope after removal from the Berlese Funnel to ensure that all species have moved out of the litter.

Most of the invertebrates caught in uncased bores will be species utilizing the upper layers of the soil profile because uncased bores act as giant pit traps for these species. Biota (2006a) found only 4 % of specimens were troglofauna and, to minimize wasted identification time, an efficient process needs to be developed to distinguish troglofauna from other species as early as possible in the sorting and identification chain.

## 3.9 Species identification

Despite some scientific debate about the validity of different species concepts, species are the most easily defined taxonomic unit (other than the individual) and form the operational unit at which the *Wildlife Conservation Act 1950* seeks to maintain biological diversity. An important part of that *Act* in relation to EIA is that it is illegal to undertake activities which may reasonably be considered likely to lead to the extinction of any species.

Assessing risks of species extinctions requires identifications at species, or morpho-species, level. The EPA is mindful that conservation objectives will be best served if subterranean fauna identifications are competently undertaken and based on the latest available taxonomic information. While there is no accredited list of experts for identification of subterranean fauna, proponents should endeavour to employ appropriately qualified and experienced biologists. Nevertheless, at times the imperfect state of invertebrate taxonomy makes it difficult for even the best taxonomists to achieve species, or even morpho-species, identifications for many groups of subterranean fauna. The problem exists for most troglofauna but there are some groups of stygofauna for which there is almost no taxonomic framework at all. In practice, species conservation objectives cannot be readily applied until a framework for at least morpho-species identifications exists. Assessment by the EPA will recognize this constraint (see Table 3.3).

### 3.9.1 Time requirements for sorting and identification

The time required to sort and identify the specimens in a sample is extremely variable and is affected by the amount of experience the operator has, the ease with which taxonomic characters can be discerned, and how well developed the taxonomic frameworks are for the particular animals being identified. As an approximate guide to the amount of time required, stygofaunal samples with a moderate amount of sediment that yield no specimens should be searched for a minimum of 2 h. Most of the time taken to extract troglofauna from samples is spent setting up Berlese funnels but the litter from a subset of samples should be checked under a microscope for at least 1 h per sample.

Both stygofauna and troglofauna samples containing animals are likely to take, on average, at least a day to sort and identify to species. However, processing times are likely to be substantially greater when 'new' animals are discovered. Documenting their characteristics so that these animals will be recognized when encountered again may take a day. In many cases, similar animals will occur elsewhere and preliminary taxonomy will be required to determine whether they belong to the same, or different, species and this may require another day. Any genetic studies to support the validity of the decisions about species relationships, or formal morphological descriptions to enable publication of a new species name, may require weeks of work.

Proponents are advised to allow a minimum of 3-4 months for species identification and verification after each round of sampling.

### 3.9.2 Expectation of morphological identifications

All specimens collected for EIA purposes, including juveniles, should be assigned a morphological identification, although it is recognized that in many cases only genus or family identifications will be possible for juveniles.

Adult subterranean species are often small and nearly always fragile but species identification is expected wherever possible. Identification should be feasible for most groups. Workers must recognize that even members of groups whose surface representatives are readily identifiable as whole animals under low magnification are likely to require dissection and examination under a compound microscope. In some animal groups, only one sex will possess the species-specific characters that are used in taxonomy and, in these cases, the expectation for identification of the other sex is the same as for juveniles.

#### 3.9.2.1 Stygofauna

A very large amount of stygofaunal taxonomy has been undertaken on Western Australian species over the past decade, such that species in the groups listed in Table 6.1

are expected to be identified at least to morpho-species level. It should be noted that two species of stygobitic vertebrate, a fish and eel, are known from WA.

Table 3.3 Stygofaunal invertebrate groups able to be identified to species or morphospecies with existing taxonomic resources*.

| Phylum or Class | Orders known to have stygofaunal members in WA |
|---|---|
| Crustacea | Remipedia,Copepoda, Ostracoda, Spelaeogriphacea, Thermosbaenacea, Syncarida, Amphipoda, Isopoda, Decapoda |
| Arachnida | Trombidiformes (only Hydracarina studied) |
| Insecta | Coleoptera |
| Oligochaeta | Tubificida |
| Polychaeta | Phyllodocida (family Nereididae) |
| Aphanoneura | Aelosomatida |
| Gastropoda | Neotaeniglossa (family Hydrobiidae), Basommatophora (family Planorbidae) |

*Groups not expected to be identified to species include Nematoda, Turbellaria, Rotifera and mites other than Hydracarina.

### 3.9.2.2 Troglofauna

Relatively few troglofaunal species are known from WA compared with stygofauna. This reflects less fieldwork and taxonomic effort. Although the relative richness of the two groups in WA is unclear, it should be noted that globally subterranean habitats contain similar numbers of species of troglofauna and stygofauna.

The variety of terrestrial invertebrate groups to which troglofauna might belong is large (Table 3.4). It is likely that not all groups with troglofaunal members have been recognized in WA and, therefore, workers confronted with an unusual specimen should consult keys to higher level classifications of invertebrates before trying to fit a specimen into a group currently recognized as having troglofaunal representatives in WA. It should be possible to identify adults of all groups currently recognized as troglofaunal to morpho-species (although the genus will often be unknown). Juvenile identifications are likely to be to higher taxonomic level.

Table 3.4 Invertebrate orders known to have troglofaunal representatives in WA. Adults of all groups are expected to be identified to morpho-species level.

| Class | Orders known to have troglofaunal members in WA |
|---|---|
| Crustacea | Isopoda |
| Arachnida | Schizomida, Pseudoscorpionida, Opiliones, Aranea, Palpigradida, Scorpionida |
| Chilopoda | Scolopendrida |
| Diplopoda | Polydesmida |
| Hexapoda | Diplura, Collembola |
| Insecta | Thysanura, Blattodea, Coleoptera, Orthoptera, Hemiptera |

### 3.9.3 Genetic identifications

While species represent the most clear-cut biological grouping used in science other than the individual animal and are the unit at which the *Wildlife Conservation Act 1950* operates, there is considerable continuing debate about how species can be defined (Claridge et al. 1997; Wheeler & Meier, 2000). No particular definition of species has been adopted for the purposes of Guidance Statement No. 54 or this appendix but the Biological Species Concept as described by Paterson (1992) is considered to provide a useful framework for species conservation. In essence, the concept defines a species as a group of organisms that share the same mate recognition system and which, if occurring together, will interbreed.

During the last decade the method used in most genetic studies of populations and species has changed from an examination of the structure of proteins produced by an animal (allozymes) to direct examination of genetic sequences (DNA and RNA studies). One benefit of using DNA sequences is that mutational processes are well understood at the DNA level, enabling phylogenetic relationships (i.e. evolutionary histories) to be inferred more confidently from DNA than allozymes. Another is that DNA techniques are very versatile because mutation rates and the amount of variation in the sequence of base pairs (Adenine, Thymine, Guanine, Cytosine) differs along the genome. Conservative genes may exhibit little or no variation even within an order, while plastic genes may show at least 30 % variation between individuals of the same species. Conservative genes provide more reliable information about relationships at high taxonomic levels, while highly plastic genes are more appropriate for forensic work and examining paternity.

Valuable uses of DNA in subterranean fauna work include matching juveniles with adults of the same species, so that a species distribution can be fully described even though most animals collected are juvenile and not identifiable by morphological means. Another is to determine how many species are represented by the animals in a particular area. This is usually done to differentiate morphologically similar species, often in conjunction with a study of morphological variation to identify characters that separate the species. Sequence variation provides a very useful, though not infallible, guide to

species membership. A third use of DNA has been to examine the evolutionary history of species and date speciation events. Genes that are frequently used to examine species membership are the mitochondrial genes cytochrome oxidase subunit 1 (CO1), 12s small subunit RNA (12S) and 16s large subunit RNA (16S). The more slowly evolving nuclear ribosomal gene 18S usually provides information about higher taxonomic relationships.

There is no absolute amount of difference in gene sequence between two animals that indicates they belong to different species but the principle underlying the use of sequence variation to identify species is the well documented fact that differences accumulate between reproductively isolated populations as a result of mutations. Across the animal kingdom, Hebert et al. (2003) found an average of 11.3% difference in the CO1 gene sequence between closely related species although the range of differences was considerable(4-32 % for 95 % of species pairs). Recently separated species, and species in groups with slow mutation rates (the rate of mutation varies between animal groups and is also affected by environmental conditions), show lower levels of difference. Interpreting sequence differences is most straightforward when animal populations are in geographic contact and can potentially meet to exchange genes. In that case an absence of gene flow strongly suggests the populations belong to different species. When populations are separated by long-term geological barriers, as usually occurs with subterranean animals, genetic divergence is expected independent of whether speciation has occurred and the meaning of sequence differences may be more difficult to interpret.

Biota (2006b) used DNA results to distinguish several species of Schizomida, in different mesas of the Robe Valley, on the basis that sequence differences between populations were > 12 %. Genetic divergence between existing morphologically described species from Cape Range suggested 12 % was the appropriate benchmark for determining species boundaries among mesa animals. All the Robe Valley species identified on the basis of sequence divergence were subsequently shown to have sufficient morphological differentiation to be regarded as separate species on morphological criteria (except in a couple of cases where no adults were collected and morphology could not be evaluated). Similar kinds of results were obtained for stygofaunal beetles in the Yilgarn (Leys et al., 2003). In contrast, geographically isolated amphipods in the Pilbara and Yilgarn may display sequence diverge of 10-35 % between populations that show no morphological differences (Finston et al., 2007; Cooper et al., 2007). While species status may be inferred on genetic grounds alone, obtaining other concordant evidence of speciation is to be preferred because breeding experiments have usually failed to support species status for genetically identified taxa lacking morphological differences (Finston et al., 2007 ).

Whatever reliance is being placed on genetics, all specimens should be identified morphologically before genetic analysis. In most cases, morphological identifications can be more easily integrated with existing taxonomic descriptions and the results of other surveys. Another important reason for requiring a recorded morphological identification is to ensure there is some record of the specimen for EIA purposes in the event genetic work is unsuccessful or does not provide adequate information for conservation assessment.

### 3.9.4 Vouchering and sharing of information

Representative samples of all species collected during EIA should be deposited with the Western Australian Museum to build up collections for future taxonomic and zoogeographic work that will enable the State's subterranean fauna to be better documented and provide an improved framework for assessment. Proponents should liaise with the Museum about what information should accompany each sample.

There is also considerable information in most EIA documents on the distribution and habitat preferences of subterranean fauna that can contribute to knowledge of the State's fauna and all documents should be put in the public domain. This can be achieved by lodging the documents in libraries of State Government agencies and placing them on proponent's websites.

## 3.10 Physico-chemical data

Information on geology and depth at which bores are slotted should be presented in the EIA to relate the habitats being sampled to those within the project area as a whole. Hydrogeology reports may be a source of information on regional water quality to provide context for data collected from individual bores and in the project area as a whole.

The Australian and New Zealand standards for water quality and groundwater sampling should be consulted prior to designing a sampling program (AS/NZS 5667.1:1998 and AS/NZS 5667.11:1998). Salinity (e.g. electrical conductivity) and pH should be measured on site with a hand held meter at all bores where stygofauna are sampled to provide information on habitat tolerances of species. The information may explain the absence of species from project areas considered likely to contain stygofauna. Dissolved oxygen provides useful information but is difficult to measure well. Depth to the water table and the bottom of the bore should be measured because they are often significant factors in determining stygofaunal species richness and abundance of animals (Datry et al., 2005).

Salinity and pH may be measured *in situ* by dropping the probe of the meter down the bore or they may be taken from a water sample collected with a bailer or pump. Measurements should come from water 1-2 m below the surface. Many aquifers are stratified, with lenses of fresh water above more saline water, and the salinity measurement may be misleading for such aquifers, especially if pumped samples are used. Stratification can be documented by profiling the water column of the bore at intervals of about 1 m using a salinity meter. Such information is biologically useful though not required to be done routinely. Profiling needs to be done carefully and occur prior to sampling, which will disturb any stratification that exists. Dissolved oxygen can be measured in pumped water or by lowering a probe beneath the water table and should be recorded where possible. Depth to the water table should be measured with a depth

tape prior to sampling each bore and depth to the bottom of the bore can be measured while sampling.

## 3.11 Sampling effort and design

The most important ingredient of any survey is the sampling design. EIA, as it relates to determining species conservation status, has two components: listing all species present in the impact zone and documenting their conservation status, with particular emphasis on whether these species also occur outside the impact zone.

Clear definition and delineation of the impact zone must accompany the EIA. The impact zone for stygofauna will be the area of aquifer where significant project-related drawdown occurs, as well as any area where contamination or other disturbance from the proposal is likely. Information about the extent of aquifer drawdown and any changes in chemical parameters should be provided with the aim of quantifying the portion of each habitat unit in the aquifer that will be disturbed. The impact zone for troglofauna will be an area that includes all subterranean habitat likely to be removed or disturbed (through altered humidity, vibration, pollution etc) and an appropriate buffer to protect quality of surrounding habitat (i.e. a transition zone between impacted and undisturbed). Changes to the surface, which may alter the natural input of nutrients on which subterranean fauna may rely, should also be considered.

### 3.11.1 Sampling the impact zone

The number of species collected from any habitat containing subterranean fauna is likely to increase with sampling effort and time. Some species occur in low abundance and will be collected only after a large sampling effort, while others may occasionally move into the habitat from the surrounding area and so may be collected late in the sampling sequence (Eberhard et al., 2007). However, as a general principle, fewer new species will be collected with each additional sample collected and a very large number of samples will be required to collect all species using the site. The requirement for most EIAs is that they employ a reasonable sampling effort that will collect most species and provide sufficient information to demonstrate whether the project is likely to impact on species of conservation concern. It is suggested that proponents should aim to collect 95 % of species using the area to be impacted by development.

One way of determining what is adequate sampling effort within the impact area is to commit to a level of inventory (e.g. collection of 95 % of subterranean species present) and then progressively sample until this is achieved, using species accumulation curves and richness estimators (Colwell and Coddington, 1994). However, this is an open-ended process that ignores the logistics of fieldwork and the tight timelines of many resource development projects. It is usually more efficient to take a number of samples that has been agreed in advance as likely to collect most species.

### 3.11.1.1 Stygofauna

The sampling protocol used in the Pilbara Biological Survey has high efficiency compared with most stygofaunal protocols previously used in Western Australia (Eberhard et al., 2005b) but, even with that protocol, 12 samples are required to collect 95 % of species using a single bore (Eberhard et al., 2007).

In theory, adequate conservation assessment for 95 % of species present in a small development of uniform geology could be achieved from a single, well-placed bore sampled 12 times, or six bores sampled twice. This is rarely adequate in practice, however, as explained below. Geology will probably not be uniform throughout the project area and different species assemblages are likely to occur in the different geological formations (Table 3.5a). More importantly, there is always substantial variation in yield between bores in the same geological formation, probably because of small differences in construction, localized minor geological variation and chance events, such as contamination. Only half to one-third of bores are high-yielding (Table 3.5b).

The uneven yield from bores shows that, in practice, an assessment based on 12 samples from a single bore is highly unlikely to document most species. Bearing in mind that often only one-third of bores are high-yielding and accessing the full range of species in the surrounding aquifer, the minimum sampling requirement for adequate documentation of all species in a small homogeneous area should be 3 bores, each sampled 12 times (36 samples), to provide a reasonable chance of sampling a high yielding bore. However, taking 12 samples from a bore requires two or three years sampling. A more efficient design, especially where several geological formations occur within the project area, is to apply the same effort across a large number of bores. Therefore, in areas where it is likely there are significant stygofaunal values, a total of 40 samples taken from at least 10 bores within the impact zone will be required.

Table 3.5. Pilbara Biological Survey results from Weeli Wolli, Harding Dam and Palm Spring (Millstream) showing results for bores within 0.5, 1.5 and 0.8 km of each other, respectively.
(a) Weeli Wolli. Shallow bores in alluvium, deep bores in Brockman Iron Formation. Sampling effort was unrelated to number of species collected (29 in total), which differed according to formation. The number of species unique to each formation is also shown

| Bore | Slotted (m BDL) | Geology | No. samples | No. species | Unique to formation |
|---|---|---|---|---|---|
| PSS006 | 11-23 | Brockman | 6 | 10 | 19 |
| PSS008 | 22-34 | Brockman | 2 | 17 | |
| PSS007 | 4-10 | alluvium | 2 | 8 | 5 |
| PSS009 | 2-8 | alluvium | 6 | 3 | |

(b) Harding Dam and Palm Spring. All bores at each locality in similar geology and slotted for full length. Each bore sampled twice. Total number of species collected was 19 at each locality

| No. bores | Max. depth range (m) | Geology | Species numbers |
|---|---|---|---|
| *Harding Dam* | | | |
| 6 | 7-22 | alluvium | 3, 9, 3, 2, 12, 4 |
| *Palm Spring* | | | |
| 5 | 6-26 | calcrete | 8, 4, 9, 2, 5 |

Sampling should occur in at least two seasons and bores should encompass the full range of geomorphology present, with the more prospective habitats assigned significant sampling effort (greater than their proportional abundance in most cases). In most cases, the most efficient sampling design will be to sample 20 bores in two seasons, spaced at least three months apart. This sampling design, if properly implemented, will usually be accepted by the EPA as adequate sampling effort, although the results of a species accumulation analysis may be taken into account also.

Proper implementation of any sampling design requires appropriately constructed bores but it is recognized that drilling bores specifically for stygofaunal survey may be impractical. The key features of adequately constructed bores are that animals have access to the borehole from the geology being targeted, the bore is free of drilling muds, hydrocarbons and other contaminants, and sufficient time has elapsed since construction for the bore to be colonized. It is recommended that all bores sampled are at least six months old. Ideally, relatively new bores will have been 'developed' by pumping to remove contaminants and improve water flow into the bore before sampling occurs.

Sometimes the timelines for EIA are such that proponents wish to undertake stygofaunal assessment using bores less than six months old. This may be done provided bores are more than three months old. However, sampling of bores less than six months old must be conducted over two seasons and, if the yield per bore is significantly greater in the second season than it was in the first, a third round of sampling will be required (i.e. first round will be deemed to have been inefficient). Thus, it is important to spend time developing and increasing chances of colonization of new bores.

Vertical, cased bores with appropriate slotting are the easiest bores to sample and should provide information about the depth and habitat (if there are different geological strata) in which stygofaunal species occur. Very narrow slots (e.g. 0.5 mm) are likely to exclude larger species of stygofauna. Uncased vertical bores will often provide larger numbers of animals and more efficiently reveal the presence of stygofauna in an area but they can be difficult to sample because of intruding tree roots or partial bore collapse and are unsuitable for repeated sampling. Another disadvantage is that they provide no information about the depth at which animals occur. Uncased inclined bores, which are commonly used for exploration, are a significant sampling resource that has been little utilized for stygofaunal sampling, although they are subject to the same limitations as uncased vertical bores. These bores may potentially be sampled by pumping but the logistical challenges of doing so are considerable (many pumps work only when vertical) and bore collapse at the water table is common. Local geology and drilling method will determine whether the bores can be used without further collapse.

#### 3.11.1.2 Troglofauna

The general principles outlined for the design of stygofauna sampling programs also apply to troglofauna. Although the amount of sampling necessary to document 95 % of the troglofauna in a geologically homogeneous area is unclear, the limited data available suggest it will be considerably more than is needed for stygofauna and that additional species will continue to be collected from speciose areas after hundreds of samples have been collected (Biota, 2006a,c).

Firm guidelines about sampling design and effort for troglofauna cannot be set at present and there is an obvious requirement for research into ways of improving the efficiency of troglofauna sampling. However, as an interim step, it is recommended that at least 60 samples should be collected from areas considered likely to have significant troglofaunal values. Two seasons of sampling are recommended but, if sampling is restricted to one season, it must be the wet season. An efficient design may be to sample 30 bores in the late wet/early dry season and then again late in the dry season. Such a sampling design will usually be accepted by the EPA as an adequate initial investigation of troglofauna. Additional sampling may be required to document the species assemblage at sites that appear rich in troglofauna.

Uncased bores are the most appropriate for troglofauna sampling and those reaching the water table are more likely to contain troglofauna because of their high humidity. Traps may be pushed down uncased inclined bores to depths of 10-15 m. There are few data on the most appropriate depths for setting traps but yields appear relatively constant to depths of at least 40 m (Biota, 2006b) and it may be appropriate to set several traps at different levels in a bore, matching prospective geological strata.

The information available suggests that traps can be set for troglofauna in uncased bores almost immediately after drilling and there is no minimum age requirement of the bores sampled.

### 3.11.2 Sampling beyond the impact zone

There is frequent confusion about the overall number of samples required for EIA. Proponents should note that the principal objective of sampling is to demonstrate to the satisfaction of the EPA and other regulatory authorities that no species is restricted to the impact zone. This is likely to require a significant number of samples to be collected outside the project area

The low efficiency of subterranean fauna sampling creates a significant risk that a widespread species may be recorded only from the project area if that area is sampled much more intensively than surrounding areas. Therefore, it is recommended that at least as many samples are taken from beyond the impact zone as within it and that the habitats sampled beyond the impact zone should be similar to those within the zone.

#### 3.11.2.1 Matching habitats

Many species exhibit strong habitat preferences and are likely to have spatially patchy distributions that match the distribution of their preferred habitat. Sometimes species will occur only in the geological formation proponents intend to develop, rather than throughout the surrounding landscape. Therefore, proponents should endeavour to identify and sample areas of the formation that will not be developed or impacted by development. Assistance from hydrologists and geomorphologists to identify appropriate habitat may enable many apparently restricted species to be collected outside the impact zones in which they occur.

## 3.12 Pilot study design

The design of pilot studies is likely to vary according to situation. The aim will usually be to determine whether a project area has significant subterranean faunal values, which can be achieved with low sampling effort (Culver et al., 2004; Eberhard et al., 2007). It is expected that 6-10 stygofaunal samples or 10-15 troglofaunal samples will be collected in pilot studies. If the pilot study reveals the occurrence of significant subterranean fauna, more intensive investigation is likely to be required (refer to section 3.11).

Occasionally surveys of similar scale to a pilot will be approved for assessment of small projects in areas likely to have significant subterranean faunal values. The justification for such action is that very small impact zones may be unlikely to encompass the full range of any species. This will not always be the case, however (EPA, 2007). If such a study reveals formally listed or scientifically valuable species, fuller survey will be required.

## 3.13 Monitoring

Sometimes it will appear likely that proponents can develop a project and simultaneously maintain the population of a restricted species. The framework for seeking approval to do so is outlined in Guidance Statement No. 54 (EPA, 2003). One of the requirements is that proponents should monitor the abundance of the restricted species so that any population decline will be recognized and management action instigated before project activities threaten species persistence.

Monitoring is likely to be difficult. Data collected during the Pilbara Biological Survey suggest that, unless species are collected in large numbers, detecting declines in species abundance will require many samples. By way of illustration, the data used in Fig. 3.3 suggest that at least 100 samples are required each sampling event for effective monitoring of stygofaunal species for which < 5 animals are collected, on average, per sample. Many restricted stygofauna, and nearly all troglofauna species are collected in lower numbers and will require greater effort. A somewhat different analytical approach in eastern Australia by Hancock & Boulton (2007) reached similar conclusions about the large monitoring effort necessary for stygofauna.

It must be emphasized that Fig. 3.3 is presented only to make the point that large numbers of samples will often be required in monitoring programs. It should not be used to infer the numbers of samples required for a particular program. That will depend on the species involved, the regional situation being monitored and the sampling methods used.

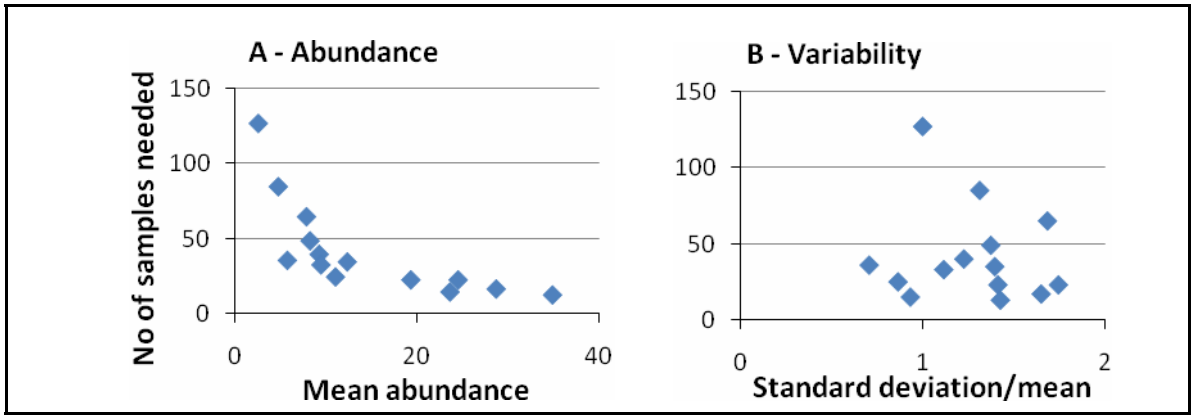


Fig. 3.3. Number of samples required per sampling event to detect 20 % decline in abundance of a restricted species between two sampling events. Necessary sample sizes were calculated using the formula of Snedecor & Cochran (1980, pp. 102-106) and numbers of animals collected per sample for multiple samples of 14 species found during the Pilbara Biological Survey (S. Halse et al., unpublished). Less sampling effort is required for species with high average abundance but the between-sample variation in numbers of animals collected has little impact on the number of samples needed. Species on which this figure is based are: Copepoda, *Abnitrocrella halsei*, *Abnitocrella* sp. 3,

*Diacyclops cockingi*, *Stygonitrocella bispinosa*; Ostracoda, *Areacandona astrepte*, *A. lepte*, *A. scanlonii*; Amphipoda, *Chydaekata* sp., Paramelitidae sp. 4; Isopoda, *Pygolabis eberhardi*, Microcerberidae sp.; Oligochaeta, *Dero nivea*, *D. stomachosa*, *Pristina longiseta***.**

## 3.14 Reporting

An important aspect of EIA is that members of the public have the opportunity to review, and comment upon, information that the EPA uses in its assessment of projects. If subterranean fauna is a significant issue in assessment, a written report giving detailed information about subterranean fauna is required to be released for public review the relevant EIA documentation. This report should provide sufficient background information and context to make it interpretable by the interested reader. Clearly written reports containing all relevant information also assist assessment officers and the EPA in making timely assessments.

It is suggested that reports on subterranean fauna should include, as appropriate, the following information:

- Background (background to the project, its location and proponent, activities that will be undertaken and expected changes to subterranean habitats, why subterranean fauna investigation is necessary, e.g. known presence of subterranean fauna in region, prospective geology);
- Scope and objectives of subterranean fauna investigations (whether level of investigation was desktop study, pilot study, or extensive survey with identification of many species; whether objective was to show no subterranean fauna were likely to occur or to document the species present and their ranges in relation to the impact zone; survey outside the impact zone for subterranean fauna found inside the impact zone);
- Subterranean fauna habitat (description of available habitat and the extent of predicted impacts to this habitat, supported by hydrogeological evidence);
- Survey design and sampling methods (justification for number of bores sampled within the impact zone and the locations of these bores in relation to geology and likely project impacts; number of sampling rounds and their timing; description of sampling techniques, and documentation of sampling effort outside the impact zone. Note that a legible map of the extent of the impact zone and locations of all bores must be provided, together with a table indicating whether bores are inside or outside the impact zone);
- Survey and identification team (with information about taxonomists consulted and the extent of peer review of identifications of difficult or poorly known groups);

- Limitations of the study (anything about the way in which the study was conducted, or the information available, that may affect the validity of results and the appropriateness of the conclusions drawn from them);
- Results and discussion of species collected (results of each round of sampling; description of the conservation and scientific significance of the species collected, including a clear statement about the range of each species in relation to the impact zone; interpretation of the results in a regional context with comments on the significance of the subterranean fauna community rather than species);
- Discussion of risk (evaluation of risk of proposal to long-term survival of the subterranean fauna species and community. Risks to subterranean fauna are largely determined by the extent of habitat disturbance associated with the proposal and the wider distribution of this habitat(s) in which the animals occur. Proponents should address issues such as the extent to which the subterranean fauna habitat will be impacted by the proposal, habitat connectivity, habitat occurrence in a local and regional context, and whether other habitats in the region may support the subterranean fauna species at risk from the proposal. Note that the threat to species or a community apparently restricted to the impact zone may be regarded as acceptable if it can be clearly demonstrated that only a small portion of their likely habitat will be impacted); and
- Conclusions and recommendations (clear summary of the expected change to subterranean fauna habitat, the species collected that have restricted distributions or other high conservation or scientific value, and the risks to these species associated with development; commitments to any additional investigations. Note that if restricted species of high conservation significance occur within a project area and proponents intend to manage impacts on these species during development so as to maintain the species *in situ*, a comprehensive management plan with appropriate commitments will be required).

# 4 APPLICATION

## 4.1 Area

This Guidance Statement applies to the survey designs and sampling methods for all projects subject to assessment by the EPA throughout the state of Western Australia where subterranean fauna is a factor.

## 4.2 Duration and Review

(To be inserted when the final Guidance is released)

# 5 RESPONSIBILITIES

## 5.1 Environmental Protection Authority Responsibilities

The EPA will apply this Guidance Statement during the assessment of proposals under Part IV of the *Environmental Protection Act 1986* where subterranean fauna is a factor.

## 5.2 Department of Environment and Conservation Responsibilities

DEC will assist the EPA in applying this Guidance Statement in environmental impact assessment and in conducting its functions under Part V of the *Environmental Protection Act 1986*.

## 5.3 Proponent Responsibilities

Where proponents demonstrate to the EPA that the requirements of this Guidance Statement are incorporated into proposals, the assessment of such proposals is likely to be assisted.

# 6 DEFINITIONS AND/OR ABBREVIATIONS

| | |
|---|---|
| Anchialine | Land-locked body of water connected to the ocean via an underground conduit. |
| Cave | Subterranean space, sufficiently large to admit a person |
| Epigean | Of the surface of the earth |
| Stygofauna | Aquatic groundwater animals |
| Troglogauna | Air-breathing subterranean animals in caves or voids |
| Void | Subterranean space, of any size smaller than a cave. |

# 7 LIMITATIONS

This Guidance Statement has been prepared by the Environmental Protection Authority to assist proponents and the public. While it represents the contemporary views of the Environmental Protection Authority, each proposal which comes before the Environmental Protection Authority for environmental impact assessment will be judged on its merits. Proponents wishing to deviate from the Guidance provided in this document should provide a robust justification for the proposed departure.

# 8 REFERENCES

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| **Index** | **Draft Guidance** **August 2007**<br>Final Guidance |
| **Status** | Signed-off by the EPA at this stage for stakeholder and public use and review |
| **Citation** | This document cannot be cited at this time but may be used by the EPA for the purposes of environmental impact assessment (EIA) with respect to this factor. |
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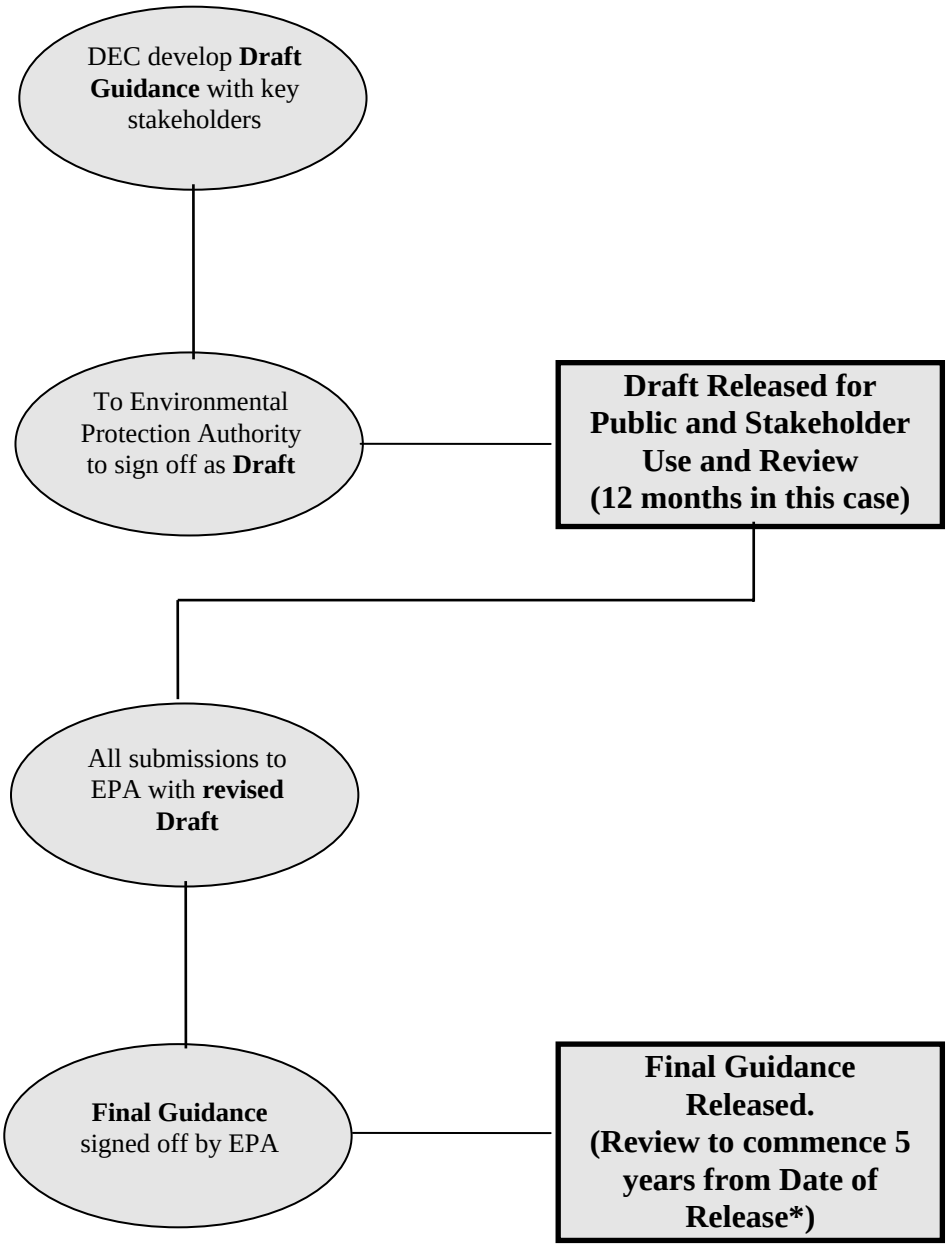
# Appendix 1: Generic Flow Diagram for the Guidance Statement Process




* Guidance may be reviewed earlier if circumstances require it.