Northern Territory guidelines for targeted surveys of threatened and significant plant species

NORTHERN TERRITORY GOVERNMENT

Department of Environment, Parks and Water Security

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Acronyms	Full form
AOO	Area of Occupancy
CAM	Common Assessment Method
DENR	Department of Environment and Natural Resources (now DEPWS)
DEPWS	Department of Environment, Parks and Water Security (formerly DENR), Northern Territory Government
DNA	Deoxyribonucleic acid
EOO	Extent of Occurrence
EP Act	Environment Protection Act 2019 (NT)
EPA	Environment Protection Authority
EPBC Act	Environment Protection and Biodiversity Conservation Act 1999
GDA	Geocentric Datum of Australia
GIS	Geographic Information System
GPS	Global Positioning System
IUCN	International Union for the Conservation of Nature
MNES	Matters of National Environmental Significance
NT	Northern Territory
NVIS	National Vegetation Information System
NT VSD	Northern Territory Vegetation Site Database
S&P	Standards and Petitions
TPWC Act	Territory Parks and Wildlife Conservation Act 1976

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1. Introduction

The Northern Territory (NT) landscape provides habitat for a broad diversity of native flora, fauna and other organisms, many of which have intrinsic ecological, cultural, social and/or economic value. The Territory has enacted environmental legislation to secure the value of these species for the benefit of present and future generations, by ensuring that economic development follows ecologically sustainable principles.

The NT Environment Protection Authority (EPA) has developed guidelines to assist in determining the potential impacts of a proposed action on terrestrial biodiversity (NT EPA, 2013). While the EPA's guidelines allow a preliminary desktop assessment of impacts, targeted on-ground surveys and assessments are usually required to determine whether an action is likely to have a significant impact on these species[1].

This document provides a best practice protocol for threatened flora survey and data collection. It forms part of a series of guidelines for sampling and describing flora and vegetation specifically in the NT. Other useful documents (Figure 1) are referenced in the document. The Flora and Vegetation Guidelines Series covers the following topics:

- Targeted surveys of threatened and significant plant species;
- Field methodology for vegetation mapping and flora survey (Lewis *et al*. in prep.); and
- Guidelines for the collection of plant specimens (Jobson *et al*. in prep.), and Policy for accessioning specimens to the NT Herbarium collection (DEPWS in prep.).

A subset of more specific survey guidelines for some species and plant groups is also planned.

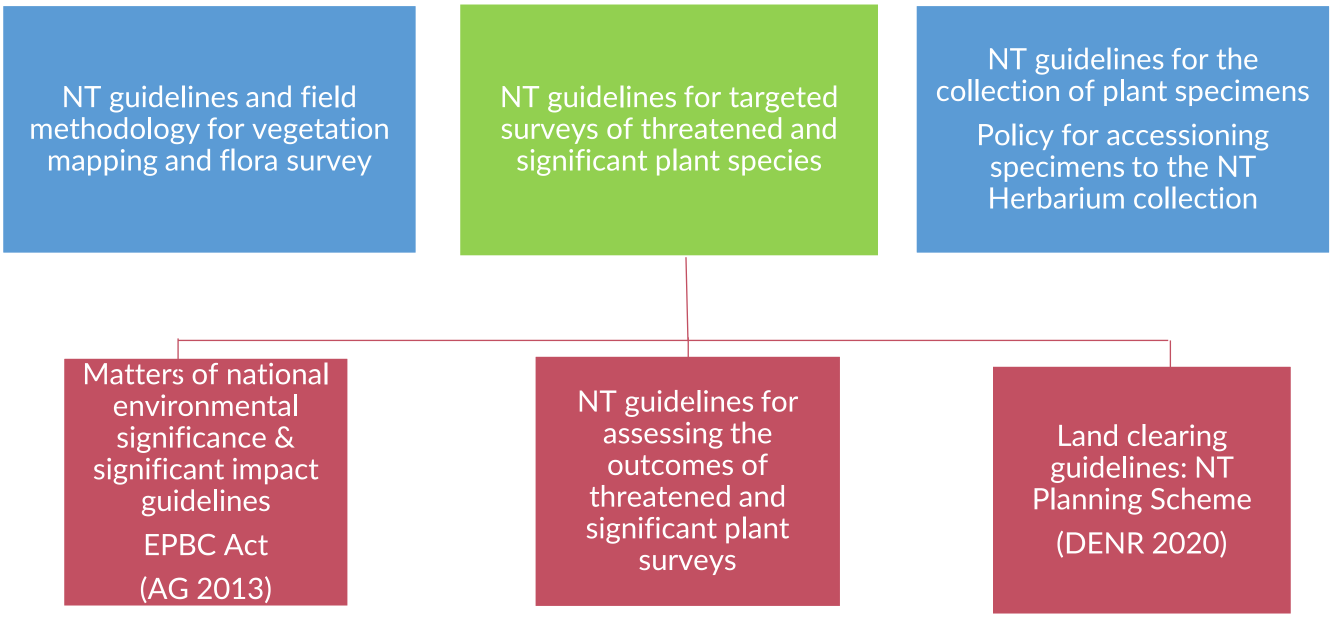

Figure 1: The Flora and Vegetation Guidelines series and documents related to the Threatened Plants Guidelines.

[1] For the purposes of this document, the word 'species' is used in preference to 'taxon' or 'taxa'. It is used as an umbrella term, and it is implied that 'subspecies' and other infra-specific entities are included under this term.

1.1. Purpose

The purpose of this document is to provide conservation managers, environmental consultants, development proponents and regulators in the NT with a set of guiding principles and methods for surveying threatened and significant flora. These guidelines have been prepared with the aim of standardising survey effort and techniques and increasing confidence in survey results. Specifically, these guidelines are designed to:

1. Facilitate targeted surveys to provide reliable information on the presence or absence of threatened or significant plant species at a site[2]; and
2. Ensure that data collection provides accurate estimates of the number and distribution of individuals, demographics or the area of habitat.

Threatened species surveys may need to be supplemented by general vegetation and/or flora surveys and the collection of Herbarium voucher specimens, for the purposes of:

- Identifying the presence and extent of potential suitable habitat for threatened flora and fauna;
- Identifying the presence of Data Deficient, Near Threatened or Significant species that with additional survey effort may qualify for a provisional Threatened Category, especially where the area is poorly known floristically;
- Developing baseline assessment data for management purposes;
- Informing revegetation guidelines and revegetation planning (where applicable);
- Assessments to appraise land clearing applications, or similar proposed actions with potential to impact on threatened and significant species or communities;
- Validating any sightings of threatened or significant plants;
- Identifying habitat suitable for translocation of a threatened or significant species; and
- Identifying threatening processes to habitat critical to a threatened or significant species

1.2. Legislative context

Land managers and development proponents are required to take into consideration a range of NT and Commonwealth legislation, including those relating to potential impacts for threatened and significant species.

1.2.1. Environment Protection Act 2019 (NT)

The *NT Environment Protection Act 2019* (hereafter the EP Act) stipulates that proposed development actions in the NT are to be directed through an environmental impact assessment process, with a requirement to provide varying levels of information depending on the potential for significant environmental impacts. Under the EP Act, the potential impact on certain environmental factors must be considered. Included in these biological factors are the protection of NT's threatened and significant flora and fauna.

1.2.2. Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act)

The *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) provides protection for nine identified Matters of National Environmental Significance, or MNES (AG 2013a). The EPBC Act also covers actions on, or impacting on, Commonwealth land or actions by Commonwealth agencies (AG 2013b). The species and ecological communities listed as nationally threatened under the Act are considered protected

[2] Defined herein as an area proposed for development or an area identified for conservation planning and assessment purposes.

matters (MNES). Any proposed development or activity with potential for significant impact on a protected matter will require referral under the EPBC Act.

1.2.3. Territory Parks and Wildlife Conservation Act 1976 (TPWC Act)

The *Territory Parks and Wildlife Conservation Act 1976* (TPWC Act) states that all plants and animals native to the NT must be accorded a conservation status. Those species determined to have a threatened status (see below) within the borders of the NT must be managed in a manner that either increases their population and distribution or maintains them at a sustainable level.

All species native to the NT are assessed and accorded a conservation status classification, using the same IUCN Red List criteria (IUCN 2012; IUCN Standards and Petitions Committee 2019) and following the Common Assessment Methodology (DAWE 2020) used by the majority of jurisdictions within Australia. Species classified as Extinct in the Wild, Critically Endangered, Endangered or Vulnerable within the Northern Territory are considered threatened under the TPWC Act.

1.2.4. Other Significant Species

Development proponents in the NT are also required to consider significant species for some purposes (e.g. DENR 2020). These include:

- Regionally important occurrences of Near Threatened[3] or Data Deficient[4]species listed under the TPWC Act;
- Range Restricted[5] species within the NT; or
- Fauna listed as migratory under the EPBC Act.

1.3. Background

Species can become threatened for a variety of reasons, often more than one. They may have undergone a reduction in numbers (or are likely to in the future), or have limited distribution, a relatively small population, a limited number of subpopulations[6] or unviable subpopulations due to a known or inferred threat (IUCN Standards and Petitions Committee 2019). Threatened species may be subject to threats, or they may be naturally rare within the landscape with plausible threats. Populations may be either sparse and at low density within their preferred habitat or similarly they may be highly clumped in their distribution and concentrated within small geographic bounds or patches. This can make detection of these species inherently more difficult than for widely occurring species or those with higher density distributions.

Importantly, some plant species are cryptic and can be difficult to locate and identify, requiring specialist attention. Cryptic species may have one or more of the following characteristics:

- Difficult to detect or identify in the field;
- Poorly known habitat parameters and distribution patterns that are hard to predict; or
- Strongly seasonal or episodic vegetative growth and extremely hard to detect outside of these growth phases.

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[3] Species with a status approaching Vulnerable in the NT, but not meeting the criteria for Vulnerable (see IUCN 2012; IUCN Standards and Petitions Committee 2019)

[4]Species for which insufficient information exists to make a reliable assessment

[5]Species for which a proposed development or action is likely to have a significant impact on the species' area of occupancy and/or extent of occurrence (see IUCN Standards and Petitions Committee 2019)

[6]For definitions of *Population* and *Subpopulation* refer to IUCN Standards and Petitions Committee (2019)

For these latter, the timing of survey is crucial in determining their presence at a site.

In the Top End, species' detectability may be predictable and closely linked to season, while in the NT's arid region, some species are present in the seed bank for years, emerging episodically with rare and unpredictable high-rainfall events. The timing of rainfall in the drier south (winter versus summer) will also determine which species are growing at a site, and whether they can be detected during any one survey period.

Cryptic species with a restricted geographic distribution are amongst the most challenging, in terms of their detection and management. For example, the seasonal variability of the monsoonal tropics and the specific challenges of access can severely limit the window of opportunity for surveying cryptic species found in the Top End. Additionally, surveys in Central Australia for some target species may only be worthwhile in high rainfall years. These factors should be considered in planning development proposals.

2. Survey guidelines

2.1. Survey planning

A field survey plan should be prepared with consideration of the biology and life history of the species and its known habitat characteristics. The survey approach should incorporate two elements:

1. A survey methodology that maximises the likelihood of detecting the target species, taking into consideration seasonal and temporal constraints; and
2. A field survey design that aims to search and/or sub-sample sufficient potential habitat[7], at an appropriate intensity to provide confidence in the likelihood of detection.

The following steps (detailed in Sections 2.2. and 2.3.) should be undertaken in developing a survey plan to identify and target field survey timing and effort:

- Determine the target species;
- Determine current level of survey effort for target species in the area of interest;
- Identify the diagnostic characteristics of the target species and the optimal timing for field survey;
- Determine the potential habitat for target species, including potential habitat modelling available for some species (only the areas of potential habitat require survey);
- Determine the survey effort required for each target plant species; and
- Group species according to the potential habitat, survey timing and survey method.

It should be noted that if an intensive flora survey has been conducted on a site within recent years, and there has been no major disturbance during this period (e.g. fire, flood, drought, disease or weed invasion), then the results of this recent survey may be sufficient to ascertain the presence or absence of the target species at the site without undertaking additional detection surveys. The prerequisites are that the previous survey:

a) Met all the requirements of this guideline (e.g. suitably qualified surveyor(s); specifically targeted the species in question; used the correct survey technique); and

b) Provided an appropriate assessment of the entire area. If applicable, the results of this assessment could be used to design mitigation measures relevant to the site and species as they pertain to the proposed development and other identified threats.

NT scientific permit conditions (see 2.1.2 below) require flora observations to be made available through the NT Flora Atlas. When planning a survey, the NT Flora Atlas and other information sources (if available) should be consulted to determine if appropriate and contemporary surveys have been conducted within the area of interest.

2.1.1. Choosing suitable surveyors

The terrestrial biodiversity component of an environmental assessment requires targeted plant surveys to be designed and implemented by individuals with demonstrable botanical skills and expertise in identifying and surveying for the target species. With inexperienced surveyors, the risk of misidentification or non-detection of some plant groups is likely to be much greater. Experience is also needed to reliably identify potential habitat, plan an appropriate survey, measure variables and calculate parameters.

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[7] Defined herein as all habitat in which a species is likely to occur, based on current scientific knowledge of the ecology of a species. For further discussion of potential habitat, see Section 2.2.3.

The surveyor's botanical skills can be demonstrated by relevant qualifications and a history of experience in survey methods and plant identification in the NT, and/or a history of threatened plant survey projects conducted in the relevant region, preferably in recent years. Simply meeting these requirements does not automatically ensure that the surveyor has the required skills to survey for some species. A surveyor lacking familiarity with the target species will also need to research and consult herbarium specimens, and ideally, will locate and observe known records in the field, to become familiar with the species in question and reliably identify them.

2.1.2. Permits

Under the TPWC Act, anybody wishing to conduct targeted surveys of native flora or fauna, is required to apply for a Permit to undertake scientific research on wildlife. For surveys of species listed as threatened under the Act, the permit requires approval from the Minister for the Environment or their delegate. Furthermore, for the collection of specimens for identification, or of samples for a DNA study, a Permit to take or interfere with wildlife is also required. Information regarding wildlife permits can be obtained from the Northern Territory Parks and Wildlife Commission. Both permit application forms can be found at: https://nt.gov.au/environment/animals/wildlife-permits/permits-take-interfere-with-wildlife.

Proponents should also be aware that there may be further obligations regarding nationally threatened species and threatened ecological communities listed under the EPBC Act, above and beyond the requirements outlined in these guidelines. Further information on relevant Commonwealth assessments and approvals can be found at http://www.environment.gov.au/epbc/environment-assessment-and-approvals.

2.2. Survey design

2.2.1. Determine the target species

Desk top analysis can be used to identify the threatened or significant species known, or with potential, to occur within or near the site in question. Species considered to have a moderate to high likelihood of occurrence within the site should be given highest priority for targeted survey.

The Flora NT website (http://eflora.nt.gov.au), NR Maps (http://nrmaps.nt.gov.au/nrmaps.html) and the Australasian Virtual Herbarium (AVH) (http://avh.chah.org.au/) can be used to identify species whose occurrence and/or potential distribution may overlap the development footprint. The Department of Environment, Parks and Water Security (DEPWS) has also developed species distribution models (also known as environmental or ecological niche models) for specific threatened plant species in the greater Darwin region (See Appendix 1).

The list of target species can be further refined by considering the presence or absence of key habitat requirements for the species identified, such as:

- Associated native plant community types and species;
- Topographic, soil or geological preferences;
- Microhabitats (e.g. preference for rocky outcrops, ground soaks or tree canopies); and
- Response to disturbance, such as fire, and the level and frequency of disturbance (e.g. slashing, canopy removal).

Species may be excluded from further consideration at a site if:

- The species is considered Data Deficient under the TPWC Act, but only for taxonomic reasons, and not due to uncertainty about their conservation status;
- An assessment of habitat components on a site finds that specific habitat requirements for a species are not present;

- The habitat is found to be substantially degraded[8] or modified structurally and floristically, by weeds or pest animals, so that the species is unlikely to occupy the site; or
- Records that appear to indicate the presence of a potential target species within or near the site are found to be unreliable (e.g. the locality description associated with a record indicates it is not at the site in question).

The justification, including any relevant sources of information, for removing a candidate species from the list must be documented in the report or development application.

2.2.2. Identify the optimal timing for the field survey

The field survey should be conducted at a time when detection and field identification of the target species is optimal. Surveyors should use key biological or regionally specific information, including extant known localities where the species has been recorded in recent times, previous survey or monitoring reports and other published literature (see below) to further refine survey times and thus optimise the detectability of the species. For example, survey times may need to be adjusted to account for:

- The species being visible (e.g. geophytic, annual or ephemeral plants may only emerge after sufficient rainfall; also detectability may decline as the surrounding ground stratum vegetation becomes more dense);
- Flowering or fruiting times (where flowers or fruits are necessary for positive species identification);
- Adequate leaf tissue being available (when genetic sampling and analysis are required for identification);
- Natural or anthropogenic disturbance, or climatic events that trigger germination, re-sprouting or emergence (e.g. recent fires, floods, drought or heavy rainfall); and
- Year to year variation in seasonal conditions which affect emergence, flowering and fruiting.

Surveyors should consult available resources (see Appendix 1) on the life history and biology of target species to best identify a suitable window for survey timing. Where information is scarce, contact the Flora and Fauna Division, DEPWS (Biodiversity.DEPWS@nt.gov.au) for further advice. If it is necessary to time the survey for the beginning or end of this window, the surveyor should consider the following:

- Will the species be detectable and identifiable? Has it emerged or flowered yet, or will it still be flowering or fruiting based on the current seasonal conditions? and
- Does the site represent seasonally intact potential habitat for the species (e.g. at the proposed time of survey has it been substantially affected by fire, flood etc.)?

Justification should be provided for conducting surveys outside of the recommended time frames or when seasonal conditions are poor.

In some situations, surveying at the optimal time for a target species may not be possible or feasible. Examples include cases where project time frames are unavoidably constrained; when a species may be only present for a short period after fire or flooding; or where suboptimal conditions, such as prolonged drought or an extreme, random event has substantially affected the site. In such situations, where potential habitat for a target species is present but a survey cannot be conducted, the species should be assumed to be present at the site and the likelihood of important occurrences of the target species at the site should be considered in relation to the broader distribution and population status of the species. Further guidance

[8] Some species prefer disturbed habitats, or can persist or survive removal of the overstorey stratum (e.g. *Atalaya brevialata*, *Cleome insolata* and some *Typhonium* spp.). In such cases, habitat degradation would not necessarily justify removal of the species from the candidate list.

on the assessment of (potential) threatened species occurrences can be found in Bickerton *et al.* (in prep) and should be considered as part of the broader environmental impact assessment process.

For many species, including some common Eucalypts, the use of vegetative morphological characters for identification is difficult and unreliable. Furthermore, for some groups such as *Typhonium* species, the window of availability for morphological identification of appropriate flowering material can be particularly narrow and highly unpredictable, with plant detectability during this growth phase also being extremely low. For such species, molecular methods are the recommended approach to identify plants at the species or subspecies level. In the absence of such confirmation, a precautionary approach should be applied to the identification and/or taxonomic status of plants within the area of interest. Refer to Appendix 2 for a discussion of the use of molecular methods.

The timing of surveys will usually depend on which method of species confirmation is being utilised. In many cases, species can be reliably identified on diagnostic morphological characteristics and a range of taxonomic keys (see Appendix 1) and Herbarium reference collections can aid this process.

2.2.3. Potential habitat for target species

Targeted searches for species that potentially occupy a site must focus on suitable habitat. The surveyor and / or proponent should provide evidence that the areas chosen to survey satisfy the known habitat requirements of the species. For plants, relevant parameters include vegetation type, surface lithology, elevation, rainfall, soil type and landform pattern and element. Where there is uncertainty, survey effort might be partitioned across a relatively wide range of habitat types.

There is a range of resources and tools available (see Appendix 1) to assist in the identification of potential habitat. The scale and detail of the information varies, depending on the current knowledge of the species life history and ecology, and includes:

- Primary data such as species occurrence records and remotely sensed data (e.g. optical, radar or geophysics imagery);
- Baseline land resource information relating to topography, geology, soil, land systems, land units or vegetation mapping;
- Derived land information (e.g. vegetation attribute (e.g. Foliage Projective Cover), land use and land cover mapping); and
- Species distribution or habitat models (see below) consolidating many of the above data sources into single products identifying the likelihood of individual species occurrences within their known or projected Extent of Occurrence (EOO)[9] (see Appendix 1).

Box 1 (below) provides examples of some of the resources available, and the range in detail of information they provide on associated plant communities and specific niches of some NT threatened and significant plant species. It highlights that some resources do not provide detailed information for species occurring in small areas of rare or unusual habitat, and underscores the need to thoroughly research all available resources when determining potential habitat. Specifically, Threatened Plant Fact Sheets (Appendix 1) are the most likely to contain detailed and accurate habitat information for many species.

In some cases, potential habitat can be modelled effectively using presence and/or absence records of the species, along with a range of spatially explicit covariates that adequately describe the habitat attributes of the species. DEPWS has developed models for some species to assist in assessing the potential likelihood of occurrence of target threatened species (Appendix 1).

Where species distribution or habitat modelling indicates highest likelihood potential habitat, this should form the highest priority for the identification of survey boundaries. However, given the limitations of

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[9] Defined as the "area contained within the shortest continuous imaginary boundary which can be drawn to encompass all the known, inferred or projected sites of present occurrence of a taxon" (or species). (IUCN 2019)

scale associated with mapping products, this information should be further refined on the ground at the property scale to account for fine scale variation in habitat quality or any recent changes in land cover. This may result in amendments to the area identified for priority survey effort based on on-ground assessment.

Similarly, where 'moderate' or 'low' likelihood potential habitat exists within an area of interest, an on-ground assessment of the habitat's suitability for the target species is recommended. Comparison of this habitat to known highest likelihood habitat and regional distribution of the species should be undertaken to guide the requirement for targeted survey. For example, the presence of lower-likelihood potential habitat classes in isolation may negate the need for targeted survey. Further advice on the interpretation of modelled potential habitat information can be obtained by contacting the Flora and Fauna Division, (Biodiversity.DEPWS@nt.gov.au) DEPWS.

If distribution or habitat models are not available for a species, the surveyor should identify and map potential habitat from the range of primary data sources available (Appendix 1). This should be based upon expert interpretation of the published information regarding the species' life history and biology. Where information is limited, contact the Flora and Fauna Division (Biodiversity.DEPWS@nt.gov.au), DEPWS for further advice.

To meet the assessment requirements, the surveyor needs to conduct fieldwork not only to determine the general biodiversity values at the site; further surveys are also needed to confirm the site's characteristics, because:

- Mapping and digital data may be at a broad scale, and may not accurately indicate all plant community types present or all soil and topographic details (See Appendix 3);
- The history of the site and its disturbance events (e.g. fire or flood) is not always reliably evaluated from imagery;
- The small habitat features that provide niches for some species may not be easily identified in a desktop assessment; and
- The reliability of potential species distribution or habitat models varies between species and areas, depending on the data available for modelling and the methods used.

The resulting map of potential habitat at a site will form the basis for the field survey design for the target species. Further information on flora and vegetation survey and mapping at various intensities can be found in Brocklehurst *et al*. (2007) and Lewis *et al*. (in prep.).

Box 1: These two examples highlight the importance of researching a wide range of appropriate resources when determining potential habitat and the specific niche of target plant species. The following tables show the variation in habitat information available from three different sources, for two native NT plant species.

Table 1: *Adiantum capillus-venerus* (growth form: fern) - Vulnerable (TPWC Act)

Source	Habitat Description
NR Maps[10]	**Victoria River Subpopulation:** *Corymbia dichromophloia*, *Eucalyptus miniata* +/- *C. ferruginea* low open woodland \ *Grevillea angulata*, *Acacia dunnii*, *Grevillea refracta* tall sparse shrubland \ *Triodia bitextura*, *Eriachne ciliata* +/- *Triodia stenostachya* mid open hummock and tussock grassland. **West MacDonnell Ranges Subpopulation:** *Acacia macdonnellensis*, *Eucalyptus pachycarpa*, *Acacia aneura* tall sparse shrubland \ *Triodia brizoides*, *Triodia* spp., *Eriachne mucronata* open hummock and tussock grassland.
Flora NT Factsheet	Seepage areas on cliff faces and along water courses in sheltered rocky gorges
Threatened Species Factsheet	On limestone, sandstone or quartzite rock, or on alkaline soils. In crevices in deep sheltered gorges where the root zone is fed by permanent streams or seepage.

The National Vegetation Information System (NVIS) layer of NR Maps details the dominant vegetation of the broad landscape where *Adiantum capillus-venerus* has been recorded near Victoria River and Hugh Gorge (West MacDonnell Ranges). However, the layer is mapped at too broad a scale to capture the fern's microhabitat, e.g seepage areas in sheltered gorges with species such as *Syzygium angophoroides*, *Xanthostemon paradoxus*, *Carallia brachiata* and vine thicket species at the Victoria River site. Details of the vegetation association at the West MacDonnell Ranges location are unknown.

In contrast, the two factsheets provide no vegetation description, but good detail on the specific niche within which the species is found. Furthermore, in the West MacDonnell Ranges, where the fern is also found, the broad landscape is comprised of a wholly different vegetation community to that of Victoria River.

Table 2: *Atalaya brevialata* (growth form: shrub < 1 m) – Critically Endangered (EPBC Act), Data Deficient (TPWC Act)

Source	Habitat Description
NR Maps	*Eucalyptus tetrodonta*, *E. miniata*, *Corymbia bleeseri* mid woodland \ *Livistona humilis*, *Planchonia careya*, *Brachychiton paradoxus* tall sparse shrubland \ *Sorghum intrans*, *Heteropogon triticeus*, *S. plumosum* mid tussock grassland. Well drained rises and low hills, gently sloping plateaux/plains with sandy and lateritic red earth soils.
Flora NT Factsheet	Woodland and open woodland with *Eucalyptus tectifica* and *Corymbia foelscheana*, also *Xanthostemon paradoxus*, *Terminalia grandiflora* and *Acacia hemignosta* with an open stratum of perennial grasses.
Threatened Species Factsheet	Often found in a narrow band upslope of sandy, poorly drained flats with *Grevillea pteridifolia* and *Dapsilathus spathaceus*. Also known upslope of rear-mangrove communities. Occurs in open vegetation on deep coarse sandy soils on foot slopes, but is absent from fine sandy soils or rocky soils with a dense grass or shrub stratum.

For *Atalaya brevialata*, the vegetation description available from NR Maps is accurate at a broad regional scale but at the site level gives a misleading indication of potential habitat for the species. This indicates that the NVIS layer is too broad to show small bands of suitable *A. brevialata* habitat. The Flora NT Factsheet provides only broad detail on the upper and mid strata. In contrast, the Threatened Species Factsheet provides extensive vegetation detail (not shown here), as well as specific niche characteristics, with useful soil and vegetation density information.

[10] Using the layer: Vegetation>National Veg Information System>NVIS2007 – 'V1 Community desc'

2.2.4. Group species within potential habitat

In many cases, individual species will require separate surveys to adequately meet the requirements of this guideline, due to differences in seasonal detectability, habitat or survey intensity requirements. However, it is not uncommon for two or more threatened or significant species to co-occur; especially for those restricted to specific niches, e.g. clay soils, rocky outcrops or swamps. In such circumstances, surveys for multiple species can be designed and conducted concurrently in areas of common potential habitat. Brennan *et al*. (2015) provides an example of a survey for multiple plant species.

To avoid compromising detectability, multi-species searches should be restricted to species within the same stratum (e.g. ground- or mid-stratum). However, multi-species searches do not always equate to an efficient use of time. Instead it is advisable to allocate sufficient time for search strategies that reduce over- or under-estimates of a population.

2.2.5. Demonstrate detectability – reference sites

It is recommended that the surveyor research and locate existing populations (reference sites) of the target species from published records, e.g. Flora NT or AVH records or reports (see Appendix 1), and visit one or more of these sites before undertaking the intended survey. The surveyor can then determine the detectability of the species at the proposed survey time, as well as learn to reliably identify the species in the field before conducting the survey.

The proponent should provide details of the reference site locations and confirmation of the identity of the presumed target species with the survey report or development application, including:

- Photographs (with GPS[11] coordinates, using either WGS84, GDA94 or GDA2020 as the datum) of the detected plants; and
- If required for identification purposes, herbarium specimens (and/or molecular sequence data) to confirm the identity of the detected plants.

2.3. Field survey methods

Many threatened plant species have patchy distributions, small populations, low densities or habitat characteristics that pose difficulties for effective survey. Therefore, the survey effort and method must be sufficiently rigorous to ensure confidence in the results. Additionally, since plants are immobile, the probability of detection for most species depends on the total search effort in the appropriate season rather than the number of site visits (Moore *et al*. 2011).

There are three methods commonly used to survey plant species, with variations of these approaches used for specific species or habitats (Cropper 1993, Keith 2000, McCaffrey *et al*. 2014):

- Parallel field traverses or transects (Cropper 1993), for relatively smaller areas, usually ≤ 100 hectares (ha) or high intensity land uses (e.g. residential development, mineral extraction or mining);
- Targeted meander-traverses, either for medium-sized survey areas, usually 100 – 500 ha, or for linear habitats and narrow ecotones; and
- Targeted quadrat-based methods, for large (> 500 ha) and/or predominantly inaccessible areas (e.g. where broad-scale land clearing is proposed). A quadrat-based method (Lewis *et al.* in prep) is also used in preliminary surveys to characterise habitat where little or no habitat data exist although this method is unlikely to produce sufficient information for threatened species detection surveys or population assessments.

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[11] Global Positioning System

These three methods are detailed in Sections 2.3.3. – 2.3.5.

When and where such methods are to be adopted will largely need to be assessed by the proponent on a case by case basis and balanced against factors such as:

- The requirement that the survey effort must be rigorous and sufficient to ensure confidence in the results;
- Cost/logistic constraints – the cost, access or workplace health and safety constraints of employing high intensity methods over a large site may not be practical;
- Target detectability – trees/shrubs/cycads etc. are inherently more detectable and require less survey effort per unit area than geophytic herbs or annual species; and
- Species knowledge base – are detection and occupancy probabilities known for the target species, or can they be inferred from similar species? This may allow alternative, less intensive methods to be employed with some degree of certainty in determining the presence/absence of the target species at a site.

Alternatively, survey guidelines are available for some specific plant families or genera, such as the Commonwealth's survey guidelines for threatened orchids (COA 2013) or the *Northern Territory threatened plant survey guidelines, Supplement 1:* Typhonium *field surveys* (Bickerton *et al.* 2020). Such guidelines are specific to a family or genus, and address aspects of life history and biology. Where they exist, and are endorsed by other jurisdictions or organisations, it may be appropriate to use them in preference to the general approaches outlined here.

2.3.1. Two-step survey approach

The two-step survey approach is suitable for use with any of the above-mentioned survey methods, and is recommended particularly for larger areas (> 100 ha), i.e. either the Targeted meander or the Quadrat-based method. With this approach, the first step determines presence/absence only and then the second step focuses on the distribution and abundance of target species found to be present. It allows for a reduced survey intensity; however, the detection probability is likely to be much lower, especially for cryptic species.

Box 2 summarises one example of a presence/absence survey. For more information on the use of the two-step method for cryptic species across larger areas, refer to Bickerton *et al*. (2020, Box 1 and Appendix).

Box 2: Using a two-step approach for a quadrat-based survey

A survey is required for a sedge that grows to 1 m in height in open sedgeland (See Table 3 below), and detection probability is low (c. 0.2 – 0.5). The minimum survey intensity required to determine the presence or absence of the species should be:

1. Select a portion of 1 ha grid cells to sample approximately 10 - 40% of the total area of potential habitat at a site (depending upon effective transect width);
2. Traverse a minimum of 500 – 1000 m in total of potential habitat per hectare (equivalent to 10 – 20 m survey transect spacing) across 6 independent survey samples (i.e. not statistically correlated); and
3. Utilise multiple independent observers working concurrently.
4. If the target species is detected, a second survey will be required to determine abundance, density and population age structure etc. for the purpose of informing assessments.

It is important to remember that the first stage of this two-step approach does not provide accurate information on the distribution, patch size, density and/or abundance of the species across the site. It should be used to inform the design of a second, independent, more targeted systematic survey using, for example, more closely spaced parallel traverses within areas where the species was detected. This will be crucial for both environmental impact assessments and for conservation management planning (see Bickerton *et al*. in prep.).

2.3.2. Width, length and area of field traverses

Table 3 provides the maximum distance recommended between presence/absence survey traverses for different growth forms. These recommendations account for the distances over which growth forms can be observed, or are able to disperse to, and for different habitats (including different types of cover). For abundance or density surveys, especially for small, highly cryptic herbs and forbs more closely spaced transects (e.g. 1 – 5 m) are required, as they are often obscured by other vegetation and are very difficult to observe from a distance.

It is recommended that the surveyor undertake appropriate research and provide justification of effective transect widths and survey effort as part of the process of designing and reporting on a field survey. Consideration should also be given to the ease with which juveniles of the target species can be identified, when determining transect width.

The total length of field traverses will depend on the area and shape of the potential habitat to be surveyed. Table 4 provides a general estimate of potential lengths of field traverses based on the separation widths identified in Table 3 and a set of general habitat areas if a parallel field traverse method is employed. Estimates given are on the assumption that complete coverage of an area (i.e. 100% of potential habitat) is required.

In many circumstances, full coverage will not be achievable, especially for areas of 50 ha or more, nor will it always be required to determine presence/absence of the target species. Any decision to vary the transect width, separation distances and total length traversed will rely on the proponent’s ability to demonstrate high confidence of detectability of the target species as justification.

It should also be noted that effective transect width and thus area sampled on any one traverse is likely to vary across a season. For example, the detectability of small forbs varies significantly with the changing density of tall tussock grasses over the course of a growing season. For such growth forms, effective transect width may vary by 1 – 5 m over the course of a survey season as understorey density increases. Wherever practicable, survey timing should attempt to achieve high detectability and reduce the effect of understorey density.

Regardless of the method and specific parameters used to survey for a target species in the field, the adequacy of the survey effort should be clearly demonstrable. It is recommended that all survey effort is logged using handheld GPS devices set to record field traverses at the minimum practicable distance interval (typically 10 m). These track logs should be made available to DEPWS (along with any relevant species data recorded) as part of the reporting requirements of a survey.

Table 3: Indicative maximum distances between traverses for presence/absence surveys of different growth forms in the NT. Further taxon specific information regarding survey intensity may also be found in supplements accompanying these guidelines (e.g. *Northern Territory threatened plant survey guidelines, Supplement 1:* Typhonium *field surveys* (Bickerton *et al.* 2020)).

Growth form[12]	Target species (examples)	Maximum separation width of traverses (metres)	
		Open/sparse vegetation[13]	Closed[14] vegetation
Tree; tree mallee; shrub > 6 m	*Acacia latzii*	40	20
Cycad; shrub 1 - 6 m; fern or grass > 1 m	*Cycas armstrongii, Ptychosperma macarthurii*	20	10
Shrub, chenopod shrub or samphire shrub < 1 m	*Acacia praetermissa, Helicteres macrothrix*	15	10
Fern, grass, sedge, rush or vine < 1 m; forb	*Cleome insolata, Eleocharis papillosa, Schoenus centralis, Stylidium ensatum, Typhonium* spp., *Utricularia* spp.	10#	5#

The distances recommended here indicate ideal circumstances with maximum observability. Actual separation distances may need to be much less than these figures.

Table 4: Estimated potential lengths of field traverses.

Separation width of traverse (see Table 3)	Area of potential habitat (ha)						
	1	**2**	**10**	**25**	**50**	**75**	**100**
	Field Traverse Length (km)						
5	2	4	20	50	100	150	200
10	1	2	10	25	50	75	100
15	0.75	1.5	7.5	18.75	37.5	56.25	75
20	0.5	1	5	12.5	25	37.5	50
40	0.25	0.5	2.5	6.25	12.5	18.75	25

[12] As defined by NVIS Technical Working Group (2017): Table 6

[13] Defined as 50-80% crown cover for forests, and < 50% crown or foliage cover for all other structural formations (NVIS Technical Working Group 2017: Table 7)

[14] Defined as 70-100% foliage cover or > 80% crown cover (NVIS Technical Working Group 2017: Table 7)

2.3.3. Parallel field traverses (small areas/intense development)

Parallel field traverses of potential habitat are usually the preferred survey method for target plant species, as they systematically cover the entire area of potential habitat and the detectability of the species is relatively high. This method can be applied to a diverse range of species, habitats and sites, and has the added advantage of allowing a single survey to collect presence/absence, distribution and abundance data, whereas other methods require a two-stage process to firstly assess presence and then distribution and abundance.

Notwithstanding economic or logistic constraints, parallel field traverses are recommended for smaller sites, ≤ 100 ha, or those where a proposed development would result in a significantly intense utilisation of the land, such as small extractive tenements or residential-style developments. One exception is if a target species occupies linear habitats or narrow ecotones, e.g. < 20 m wide (e.g. *Stylidium ensatum*, *Clauseana excavata*), where meander surveys (Section 2.3.4.) may be more appropriate.

The parallel field-traverse technique involves searching along a grid of parallel traverses a set distance apart (Table 3, Section 2.3.2.), across areas of potential habitat for each target species. Traverses should be conducted in the following manner:

- Use a GPS track log to record the field traverses, configured to record at a minimum distance interval (e.g. 10 m), to illustrate the intensity at which the traverses are conducted. These logs become the basis for demonstrating survey adequacy;
- Move at a slow walking pace, making a visual sweep either side of the traverse, and using the different angles of light to spot the floral, fruit and leaf characteristics and habit of the target plant;
- Use the GPS to record the location of each occurrence of target species along the traverse;
- Record the number of mature individuals at each occurrence, either a count or an estimate; and if appropriate, record or estimate the area of occupancy (AOO) of the species[15] (See Section 2.4. for more details);
- Record any environmental factors that may impede detection, e.g. wind, rain, cloud, poor light, steep slopes, dense vegetation (Moore *et al*. 2011);
- Plot the potential habitat, field traverses and occurrences on a site map for the survey report or development application, including the datum and projection used. The map should be a geographic information system (GIS) topographic map or preferably geo-referenced aerial imagery (or similar).

2.3.4. Targeted meander traverses (medium sized areas or linear habitats)

The targeted meander traverse is a commonly employed survey method with a less structured approach, and can be a more pragmatic option where the area of potential habitat to be surveyed is in the range of 100 – 500 ha. It is generally more suited and applicable to growth forms that are easier to detect, and in arid communities with more sparse vegetation, where larger transect widths can be used.

This method is also suitable for species that occupy linear habitats or narrow ecotones, where the potential habitat is easily identified in the field but too narrow to effectively apply a grid method. Some cryptic, seasonally evident species, such as *Stylidium ensatum*, are suitable target species for this type of survey.

The targeted meander traverse method usually relies on the availability of potential habitat mapping at a suitable scale for interpretation in the field, and the surveyor's ability to identify suitable habitat for the

[15] There are two definitions of AOO, and the purpose of the survey will determine which definition to use. IUCN Standards and Petitions Committee (2019) defines AOO as the area of suitable habitat currently occupied by the species, whereas the CAM (DAWE 2020) estimates AOO using a series of 2 km x 2 km grids over each recorded point. The former is appropriate for most surveys, whereas the latter is to be used when assessing the conservation status of a species.

species in the field. With these prerequisites, survey effort can be more efficiently directed towards the highest likelihood potential habitat areas.

The surveyor must ensure that the meander traverses cover the full extent of the target species' potential habitat within a survey site, within logistic constraints, to achieve adequate coverage and high levels of confidence in the survey results.

By extension of the general recommendations relating to the parallel traverse method, DEPWS suggests that the targeted meander approach will be most effective when suitably identified potential habitat is surveyed using:

- Multiple independent observers; and
- Non-systematic, non-overlapping traverses at a maximum effective transect width based on species detectability and understorey density (Table 3, Section 2.3.2.), and aiming to maximise spatial coverage of the highest likelihood potential habitat and the encounter rate of the target plant species.

The traverse should continue until:

a) The entire potential habitat area is covered; or

b) At least 10% of the potential habitat is sampled from across the entire survey site, based on the effective transect width used and the total transect length covered.

This decision will be governed firstly by the level of certainty required to inform a future significant impact assessment for an occurrence within the area of interest, and secondly by the size of the area being considered, the cost effectiveness of undertaking field survey and the potential risk.

Areas of potential habitat should be traversed following no set pattern but in a roughly zig-zag manner within a belt of known effective width, systematically searched in a similar fashion to the parallel field traverse method.

The targeted meander technique can allow for greater spatial coverage than plot-based methods and is less time consuming than parallel traverses. Thus, it can be an attractive option when either the size of the potential habitat area is too large for the use of parallel field traverses, and/or the effective transect width (Table 3, Section 2.3.2.) is sufficiently wide to allow increased observability of the target species across the surveyed area.

It should be noted that the results of the targeted meander traverses alone cannot be used to quantify mature individual abundance or species distribution on a site, and it will be necessary to employ additional survey effort to assess these parameters (where necessary), as discussed in Section 2.4.

DEPWS recommends that the targeted meander approach is not used for most survey areas ≤ 100 ha given the reduced certainty associated with the survey results and the relative cost effectiveness of the parallel traverse approach. An exception to this is the case of linear distribution of habitat as mentioned above. Similarly, there may be medium sized sites (100 - 500 ha) where the detectability of the target species is low and transect-based approaches may not be economically or logistically feasible. In these cases, quadrat-based methods (below) may be considered appropriate.

2.3.5. Quadrat-based sampling (large areas)

Quadrat-based methods are the most effective means of determining presence/absence of a species over large areas, and are recommended for surveys of ≥ 500 ha. Generally, the development of such an approach will need to consider:

- Vegetation mapping over the study area at a scale fine enough to accurately identify potential habitat of any target species (see Appendix 3);

- Further to this, stratification (Section 2.3.5.1.) of the study area to ensure the sampling effort provides a greater likelihood of detecting the target species. This is required where the available vegetation mapping is broad (Appendix 3), and does not identify the specific niche of a target species;
- Any existing field methods that have previously been employed for the target species (e.g. Boxes 4 and 5, Section 2.4.);
- Target species observability (refer to Bickerton *et al*. 2020, Box 1 and Appendix) and estimated survey (p*) and site (p) detection probabilities (based on published literature or field-based expert opinion);
- Estimated probability of occupancy (ψ) of the target species (Bickerton *et al*. 2020, Appendix), based on published literature or field-based expert opinion;
- The acceptable level of precision required to support the conclusions of the study (i.e. the acceptable level of error in determining the likelihood of presence or absence of a species);
- Appropriate site and sample scales for the target species; and
- Spatial distribution of surveys within a study area (See Gallant *et al*. 2008 Table 3.1).

2.3.5.1. Site stratification

Stratification, or stratified sampling, as alluded to above, accounts for the different habitat types that are identified at a fine scale within a survey area[16]. Different habitat types may be sampled at different intensities (or not at all) depending on their perceived suitability for the target species. Box 3 provides an example.

Box 3: Stratification

In a survey for threatened species on Bathurst Island (Brennan *et al*. 2015), *Typhonium mirabile* was selected as one of the target species, due to previous records within the survey area.

Ecological data and expert knowledge (Kerrigan *et al.* 2007a, 2007b; Liddle & Elliott 2008) indicated that *T. mirabile* occurs in association with Eucalypt open forest, and records suggested the species is usually found at > 60 m altitude. Potential habitat of 10,000 ha of *Eucalyptus* forest and woodland was then identified and stratified at two levels according to elevation (> 40 m vs. < 40 m).

Sample survey cells were selected within both habitat types, with a 60% bias (i.e. placement of 60% of quadrats) towards the higher altitude areas. The survey results support the assumption that *T. mirabile* prefers a higher elevation, with 73% of all plot-based records and 72% of all incidental records found at > 40 m elevation (Brennan *et al*. 2015). The results highlight the value in surveying across both elevation classes, while justifying the decision to stratify with a 60% bias.

See Bickerton *et al*. (2020, Box 1 and Appendix) for more details.

Bickerton *et al*. (2020) provides an example of the use of a quadrat-based method to survey for a cryptic species, and outlines recommendations for appropriate survey design.

[16] Further guidance on the scale of habitat recognition required to support property level assessments can be found in the *Land Clearing Guidelines: Northern Territory Planning Scheme (version 1.2)* (DENR 2020) but typically requires high to very-high resolution of survey and mapping at a scale greater than 1:20 000.

2.4. Patch abundance, density, area of occupancy and population structure

Once a target species is detected at a site, the abundance and/or AOO of the species should be recorded or estimated as far as is practicable. Estimates of local or subpopulation size will generally be needed to assess the impacts on a species under the significant impact criteria (Bickerton *et al*. in prep.).

Surveys to determine population parameters of interest for significant impact assessments will typically require more intense effort at a scale appropriate to the target species (e.g. Bickerton *et al.* 2020). The information provided in Table 3 regarding growth form detectability should be used to assist this process. These more intensive surveys should be focussed on the extent of potentially occupied habitat identified in the first (detection) phase of the survey program and do not need to be conducted within habitat where no plants were detected.

Various methods exist for determining the focus area(s) for intensive survey and could include more detailed habitat recognition or the use of mathematical approaches (such as minimum convex hulls) bounding the detected plants identified during phase 1 surveys. Further advice regarding the extent, intensity and suitability of a proposed survey for specific species can be obtained from the Flora and Fauna Division (Biodiversity.DEPWS@nt.gov.au), DEPWS.

The accepted standard measure for assessment of populations and densities is the number of mature individuals[17] (IUCN Standards and Petitions Committee 2019). Numerous considerations and nuances arise from the definition of this term, and the definition needs to be understood before undertaking a field survey. Surveyors are advised to refer to the IUCN document for guidance, and proponents will need to document and justify the concept of mature individuals that was chosen. For more detailed information on defining mature individuals refer to Appendix 4.

If the identification of mature individuals is straightforward, then the IUCN standard should be followed for subpopulation estimates or counts. If the parallel traverse method has been employed across the entire area of potential habitat, the surveyor should be able to collect abundance and distribution data sufficient to enable a reliable assessment of mature individual density. However, there will be circumstances when it is not so simple. Here we provide two examples.

In some instances, it is recommended that the surveyor count or estimate all individuals observed, not just mature plants. For example, if the species is an ephemeral, annual or short-lived perennial (i.e. 2 – 3 years), then all individuals should be counted or estimated.

For species with the capacity to reproduce vegetatively (e.g. *Cycas armstrongii*), it may be difficult to determine how many stems within a clump are mature ramets[18] (See Appendix 4). It may be preferable to count the number of clumps observed and estimate the proportion of mature ramets within each clump.

In a situation where the targeted meander method has been used, and a target species has been detected, the surveyor should systematically search the vicinity of each detected target plant for more plants. The parallel traverse method, or an outward spiral of concentric traverses can be used until the reasonable maximum limit of propagule dispersal (if known or inferred) is reached. Additional occurrences of individual plants or clusters should be recorded on GPS, including the number of mature individuals observed. This will allow a more accurate estimate of the local abundance or known AOO to be made. Box 4 provides an example of this method.

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[17] The number of individuals known, estimated or inferred to be capable of reproduction (IUCN Standards and Petitions Committee 2019).

[18] The term 'ramet' is defined in Appendix 4.

Box 4: Using the Spiral method to search the vicinity of a detected individual

Surveyors wish to estimate population abundance for a small shrub (< 1 m) at a site, and have employed the targeted meander method for their search. The preferred habitat of the species is Eucalypt open woodland. Research has determined that seeds are dispersed by ants, and the maximum dispersal distance is thought to be 20 m.

1. After consulting Table 3 (Section 2.3.2.), the surveyors agree that the width of their meander traverses should be 15 m.
2. One of the surveyors comes across a single target plant on their meander transect. They record the location with a GPS, and mark the spot with an easily observed stake.
3. The surveyor then searches in an outward spiral from the individual plant, using a 15 m wide traverse.
4. The surveyor records and marks every target individual they come across in their outward spiral traverse.
5. The surveyor stops searching when the outer layer of the spiral is > 20 m from every individual recorded, as the point of maximum dispersal has been surpassed.

Where the unit of population measure is abundance, and the local AOO appears to be relatively small, the number of mature individuals can be effectively counted in a manner as outlined above. However, larger populations or occupied areas cannot be counted without noticeable error (Cropper 1993, Keith 2000). In such cases it is best to extrapolate the density by sampling over the area of known occupancy[19]. Once the limits of the patch are defined by circumnavigating the perimeter of the patch with a handheld GPS unit, the surveyor should either:

1. Estimate the magnitude of the number of mature individuals within the patch (e.g. 10's, 100's, 1000's etc.); or
2. Subsample from within the patch to provide a patch size estimate (i.e. mean number of mature individuals per unit area multiplied by total patch area). The specific sub-sampling regime will depend upon the growth form of the species.

See Box 5 for an example of this.

Similarly, further information on population age structure may be important in the assessing the significance of survey results in the broader context of the species distribution and population status. In these cases, information on reproductive status, mode of reproduction (e.g. resprouts vs. sexual), individual size and other surrogates for evaluating population age structure may be collected in conjunction with density measures during intensive survey. Further information on how population parameters may be used to aid the interpretation of survey results can be found in Bickerton *et al.* (in prep).

2.5. Survey report

Upon completion of the survey, a report should be compiled that outlines the survey design, the technique implemented and the results. The report should include:

- The qualifications, suitability and level of experience of the surveyors involved;

[19]When measuring or estimating population density, the IUCN Standards and Petitions Committee (2019) definition for AOO is to be used in preference to the CAM (DAWE 2020) definition (see footnote, Section 2.3.3.).

- The permit or authority under which the survey was conducted;
- The list of targeted threatened or significant species, including the derivation (or reference source) of the list and the reasons for deleting species from the list;
- A map of the survey area, showing potential habitat and specific niches of target species (if applicable);
- The timing of the survey, and the reasoning behind it;
- The survey design for each target species or group;
- A map of the field traverses taken (using the tracklog function on a GPS unit), or the location of sampling quadrats;
- The results of the survey, including
    - Species presence and/or absence;
    - Abundance and/or density of the target species present;
    - The age class, whether flowering or fruiting, and physical condition of plants detected;
    - The methods used to calculate or estimate abundance and/or density; and
    - A map showing the location and distribution of target species detected.

All maps provided within the report should be using the best and most recent available imagery of the survey site.

3. Acknowledgements

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Appendix 1: List of resources

Atlas of Living Australia (ALA): https://www.ala.org.au/

Australian Plant Name Index (APNI): https://biodiversity.org.au/nsl/services/search/names

Australasian Virtual Herbarium (AVH): http://avh.chah.org.au/

EPBC Species Profiles and Threats (SPRAT) Database: http://www.environment.gov.au/cgi-bin/sprat/public/sprat.pl

Flora and Fauna Division, DEPWS: mailto:Biodiversity.DEPWS@nt.gov.au

Flora NT website: http://eflora.nt.gov.au/

- Species distributions
- Specimen voucher data
- Species fact sheets
- Taxonomic keys / treatments

NT Herbarium; Darwin and Alice Springs contact details: http://eflora.nt.gov.au/contactus

NR Maps website: http://nrmaps.nt.gov.au/nrmaps.html

- For Species Distribution and / or Habitat models within NR Maps, access Layers / Maps / Biodiversity / All Biodiversity products, and use the Identify tools. Download the pdf and use Adobe for interactive tools.
- Species-specific spatial data packages are also available from DEPWS on request. Contact Geospatial Services via email at datarequests.DEPWS@nt.gov.au or online at https://denr.nt.gov.au/land-resource-management/info-systems/natural-resource-maps/spatial-data-requests

NT Threatened Plant Factsheets and Recovery Plans: https://nt.gov.au/environment/native-plants/threatened-plants

Other NT and Australian Government agency environmental datasets: https://nt.gov.au/environment/environment-data-maps/environment-data.

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See also Section 4: References

Appendix 2. Molecular methods for cryptic species

In the case of some species, including cryptic, seasonally evident or rainfall dependent species (e.g. geophytes), the use of morphological characters for species identification is difficult and unreliable. For other species, the time window may be particularly narrow, not only for detection but also for the observation of the flowers or fruits required for positive identification, possibly only a few days. For example, the window of availability for morphological identification of many *Typhonium* species can be particularly narrow and highly unpredictable, with plant detectability during this growth phase also low. In such circumstances, molecular methods should be used for identification of species and population differentiation.

DEPWS recommends the use of molecular methods for taxonomic identification when:

1. A reliable method to obtain sequences from tissue samples is readily available;
2. Molecular sequences of a known species are readily available to compare against the sample material;
3. Appropriate expertise is available to analyse and interpret the data; and
4. Morphological identification of vegetative material is not feasible and fertile material is unavailable due to a brief flowering season or logistical barriers in accessing the site at an appropriate time.

This model is currently recommended for *Typhonium* species. A similar approach may be applied to other morphologically or taxonomically difficult genera such as *Cleome* and *Stylidium*, as molecular resources and techniques are developed. Using molecular methods for taxonomic identification is likely to allow greater flexibility in the timing of presence/absence surveys and improved confidence in the delineation of species.

All proposals to use molecular methods for species identification, or for population studies of any species, require prior consultation with the Flora and Fauna Division, DEPWS at Biodiversity.DEPWS@nt.gov.au to determine appropriate permit conditions. Specific information will be provided on the methods used to collect, store and analyse plant tissue samples, and appropriate laboratory facilities. General sample collection recommendations are provided below.

The surveyor will need to:

- Record individual GPS waypoints for each plant sample, and the datum used (e.g. GDA94 / GDA2020).
- Collect small amounts of plant tissue from multiple plants onto silica gel, as below:
  - Sample from the leaf blade.
  - Collect an adequate quantity and quality of plant material to allow DNA extraction and molecular analysis. A preferred tissue sample size is 10 mm x 10 mm or up to 100 mg fresh weight (20 mg dry weight). Ideally the sample should represent < 10% of the total area of living leaf blade for the individual plant and where there are ≥ 10 individual plants evident in the localised area.
  - There will be circumstances where the preferred sample amount is > 10% of the plant's leaf blade area, or where < 10 individual plants are found in the patch or sub-population. For this reason, consultation is required with Flora and Fauna Division of DEPWS prior to sampling, to determine the most appropriate sampling regime and permit conditions for the situation.
  - Collect each sample from a separate plant, and collect and store in separate bags.
  - Collect a minimum of three genetic samples for each occurrence or sub-population. Sampling should encompass any morphological variation observed, the spatial or geographic

extent and any microhabitat variation of the sub-population. In highly variable or extensive sub-populations, more than 3 samples may be appropriate.

  - Avoid and minimise the risk of cross-contamination from other plants when collecting samples. Ideally, sanitise implements (e.g. scissors, tweezers, hole punch) and/or hands with 70% ethanol between individual collections.
  - Place the freshly harvested plant material immediately into a plastic zip-lock bag, along with a 10-fold volume of silica gel, and seal the bag. Ideally, leaf samples should first be placed into an unused, empty, tea bag satchel or small paper envelope before placing into the zip-lock bag, as drying leaf samples can fragment. If practicable, it is preferable to freeze the material in liquid nitrogen and to store it at – 70°C for future use.
  - Label each sample bag using at the minimum a site name and unique sample identifier. Use two labels (one inside, one outside) per sample, to avoid confusion.
  - Transport and store the bags of samples in a rigid airtight container such as a clip-lock plastic box with a silicone O-ring seal.
- Submit all collection data to the DEPWS for entry to the NT VSD database, as per permit conditions.
- Submit specimen vouchers to the NT Herbarium if fertile material is available for collection, or the record is a significant range extension (see Jobson *et al*., in prep.).
- Submit the samples to the appropriate laboratory facility. Surveyors should be aware that processing and response times for commercial or research facilities will vary and this should be considered in survey and project timelines to ensure ongoing certainty regarding assessments.
- Submit all resultant molecular analyses, sequence data and metadata to Flora and Fauna Division for future use, as per permit conditions.

Appendix 3. Mapping at an appropriate scale

Throughout these Guidelines, reference is made to the importance of mapping the survey site at an appropriate scale, i.e. at a scale fine enough to allow identification of potential habitat or specific niches of target species. It is not feasible for DEPWS to adopt a prescriptive approach and define what should be considered fine or broad scale mapping, rather it will depend on the specific features or habitat niches that need to be mapped.

Table 5, by Thackway *et al*. (2008), illustrates the above point. It indicates the minimum resolution possible across a range of mapping scales, and highlights the need for surveyors to consider the size and proportions of any specific niche that is potential habitat for a target species.

Table 5: Size limits of mapped features set by cartographic constraints (from Thackway *et al*. 2008).

	Size on map	Cartographic scale			
		1:25 000	1:50 000	1:100 000	1:250 000
Area of the smallest mapped feature	2 x 2 mm	0.25 ha	1.0 ha	4 ha	25 ha
Minimum width of linear features	1 mm	25 m	50 m	100 m	250 m

For more detail on mapping scale, refer to Thackway *et al*. (2008).

Appendix 4: Defining mature individuals

The number of mature individuals is the number of individuals known, estimated or inferred to be capable of reproduction (IUCN Standards and Petitions Committee 2019). For plant species, this number should be estimated at a point in time when these individuals are available for reproduction. A reproductive individual is regarded as one producing offspring, whether sexually or asexually, which includes clonal or vegetatively reproducing organisms such as *Typhonium* species. This may be complicated by the difficulty in determining a ‘mature individual’.

To quote IUCN Standards and Petitions Committee (2019):

“As a general rule, the ramet, i.e., the smallest entity capable of both independent survival and (sexual or asexual) reproduction should be considered a 'mature individual'. Reproducing units within a clone should be counted as individuals, except where such units are unable to survive alone.”

And:

“In principle, the smallest such entity (ramet) that an organism could be divided into without causing its death or preventing reproduction, should be counted as one mature individual.”

However, what such an entity would be is often not known and in such cases, it may be necessary to adopt a pragmatic approach to defining 'mature individuals’. Thus, it is often more appropriate to count ‘clumps’ of *Typhonium* (and some other species), as an estimate of the number of mature individuals. These clumps may appear clonal in origin, but in fact can consist of one or more mature individuals along with smaller, pre-reproductive plants.