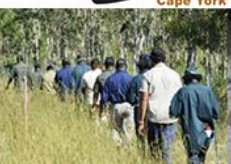
A Ranger's Handbook

Environmental DNA - Soil and Water Biodiversity

Managing Feral Pigs for Biodiversity Conservation in Cape York

Balkanu

Cape York Development Corporation P/L



This series of handbooks helps you choose suitable methods for the control of feral pigs and the monitoring of their impacts on biodiversity in your region. The techniques it describes have been used on Cape York Peninsula, Australia, but the ideas can be applied in similar environments in other regions.

To choose what will work best in your area, it is important to understand the techniques that are available and their limitations. These handbooks provide a brief overview of the available options.

There are multiple techniques for both control and monitoring. Often the best approach for successful control is a combination of techniques (as opposed to just one). Knowing what impacts you want to monitor will drive your decision for a monitoring technique.

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Handbooks in this series:

eDNA - Soil and Water Biodiversity

Background

This method for measuring biodiversity and the health of wetlands over time was designed so rangers can take the samples in the field themselves and rely only on scientists for analysis. Water and soil samples are taken from sites (ideally paired exclusion sites) and sent to a laboratory, where the DNA of micro-organisms can be extracted. By analysing the DNA compositions and comparing these between a fenced and unfenced site (pigs are excluded vs pig have access) over time, it is possible to assess the health of a wetland. Using this method, if control is effective, the micro-organism communities should be similar within the fenced pairs and the unfenced pairs.

This approach allows rangers to get a robust sample of one element of the fauna without having to have the high level fauna skills required for other common methods. Another potential benefit of this approach is that micro-organisms may respond more rapidly to changes in the wetlands than larger species like fish and birds, providing a better indicator of changes happening in shorter time frames. Although this method is promising for future use, it is still experimental and requires more detailed research before it will be a useful replacement for more traditional methods of monitoring.

Purpose

This method examines how soil and water fauna species composition changes following exclusion of feral animals (installation of a fence). This method is very good at determining the similarity of wetlands before any management occurs. This is critical for measuring the impact of control on biodiversity because the natural differences between wetlands can be a bigger driver of species change than the impact of feral animals. Similar water holes need to be selected when the aim is to measure the impact of control on biodiversity. This type of biodiversity survey works well with fenced, paired lagoons, however, it can also be applied generally to any wetland or terrestrial site.

Prerequisites

- Soil and water sampling equipment
- A freezer for freezing samples
- Training in soil collection and managing samples
- Ability to access dry ice and courier samples
- Access to a research institution to undertake the analysis.

Dry ice is a solid form of carbon dioxide and a great cooling agent. It can be purchased through BOC, however, it can be difficult to organise as dry ice is classed as a dangerous good. Dry ice also leaves behind no liquid as it 'melts' making it ideal for couriering.

Limitations

These surveys require attention to detail to ensure the samples are correctly collected and that there is limited contamination of the samples.

- Taking samples needs to be done with extreme care and according to a specific sampling method
- Samples need to be kept frozen once collected, which can be difficult in remote areas
- Sending samples for analysis requires dry ice to keep the samples frozen
- The eDNA results from samples can be difficult to interpret and may require more research to link outputs with wetland function.
- Collecting and storing samples in remote areas can be very difficult. If samples are not collected in the right way the data will not be useful for analysing patterns.

Site Selection

- As with fauna surveys, site selection is one of the most important parts of this survey and requires the same sampling strategy at each site
- Determining the different wetland typologies (water holes with similar size, depth, vegetation, landscape position etc.)
- For each typology select replicates that most closely represent the similar characteristics
- Conduct surveys at the selected sites and use analysis of species composition and abundance to determine how similar the sites are
- Exclude feral pigs from half of the sites as a means of assessing the impact of removing this threatening process.

Method

Once sampling sites have been identified the field sampling method is relatively straight forward. Sampling is done using a standardised method that allows researchers to assess the variability in soil and water fauna across the site. Figure 1 shows the sampling strategy for collecting lagoon soil and water samples.

Equipment Required

Sampling requires the following equipment:

- Steel sampling tubes (40 cm long with 5 cm opening) - thick bore casing with a bevelled edge for penetrating the soil and marked internally and externally at 10 cm increments
- A metal cap that fits over the top of the sampling tube for the mallet to hit
- A mallet for pounding in the sampling tube
- Screwdriver to remove the sampling tube from the soil and to scrape out the soil
- Plastic bags for collecting and mixing the soil samples (one for the upper 10 cm of the soil profile and one for the lower 30 cm of the soil profile) (Figure 2)
- A PVC water sampling pipe with a holder to insert a 50 ml falcon tube into (Figures 3 & 4)

- 50 ml falcon tubes (Figure 4) for storing the soil and water samples
- 12V car freezer for initial freezing of the samples and later transport
- Permanent marker for writing the site and sample information on the falcon tubes.

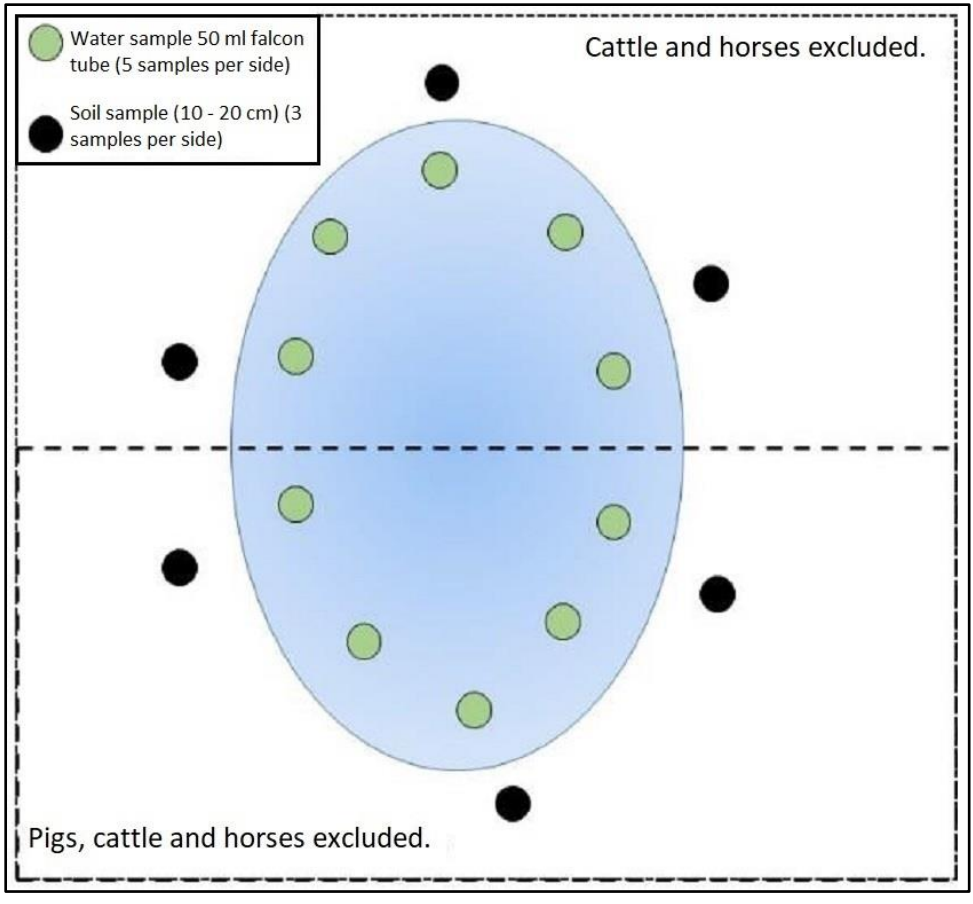

Figure 1: An example of a paired lagoon, excluding pigs one side, and excluding horses/cattle on the other but allowing pigs. Shown are examples of where water and soil samples can be taken.

Soil Sampling

Rangers use the metal tubes, caps and hammers to collect soil samples (left) and an extended handle to collect water samples (right).

Some of the tools needed for soil sampling: snap lock bags, metal tubes, metal caps for the tubes, small and large hammers and screwdrivers.

At each fenced and unfenced site, three soil samples are taken (see Figure 1), totalling six soil samples per paired site. If the site is just a singular unfenced terrestrial or wetland site, three samples will suffice. The metal sampling tubes are pounded into the ground 30 cm deep, leaving 10 cm of the tube protruding so it can be removed. Two holes drilled into the steel at the top of the 40 cm tube are used to remove the sample by inserting a large screwdriver and twisting the tube left and right. A new tube should be used for each sample and cleaned with bleach between sites (or before collecting another sample).

Once the sampling tube is removed the top 10 cm (depth class: 0 – 10 cm) of soil is placed into a new bag and the bottom 10 cm (depth class: 20 – 30 cm) is placed into another bag to be shaken and mixed. The remaining soil (10 – 20 cm) in the tube is discarded. After mixing in the bags, a soil sample is taken from each bag (different depth classes) and is put into a new 50 ml falcon tube up to the 30 ml mark. The sample source (e.g. 10 cm or 30 cm soil depth), date and site name are written on the falcon tube. The six samples are placed into a new site-specific plastic bag and the site locations are written on the bag. At each site a photograph and GPS location are also taken.

Figure 2: Soil from the metal tube is separated into new individual bags.

Water Sampling

At each fenced and unfenced site, five water samples are taken (see Figure 1), totalling 10 water samples per paired site. If the site is just a singular unfenced wetland site, five samples will suffice. Write the sample number, date and site name on a new 50 ml falcon tube. Insert the tube into the holder on the PVC sampling pipe (Figure 4) and remove the lid. Take a sample of water from the wetland (Figure 3), half fill the tube, and without spilling, replace the lid. Repeat this around the wetland until all samples are complete. Water samples should be placed into a freezer and frozen.

Figure 3: Water sampling (above) using a long PVC pipe with a holder for a 50 ml falcon tube (Figure 4 - below) on the end.

Water samples can be used to look at micro-organisms but can also indicate the presence of bigger vertebrate species such as turtles, fish and water birds. James Cook University is currently working on a detailed method for conducting consistent monitoring using eDNA methods.

Sample Management

Each paired site has six soil samples and 10 water samples in total. The soil and water samples are put into the 12V car freezer and kept frozen, including when sent away to a genetics lab for analysis. When samples are ready to be sent, they should be packed into Styrofoam containers (Figure 5) with an appropriate amount of dry ice so that they remain frozen until they arrive at the lab. Seal Styrofoam containers with tape. When sending samples using dry ice it is important to contact your courier to get the sending dangerous goods requirements.

Figure 4: Ensuring all samples are correctly labelled with site, sample and date is critical. This 50 ml falcon tube is ideal for sampling water and recording site information.

Figure 5: An appropriate Styrofoam container for couriering samples. Using dry ice will keep your samples frozen during transport and won't leave behind any residue (melted water), as it evaporates as carbon dioxide gas once it 'melts'.

Outputs

The results from the soil and water sample analysis provide species lists of soil and water fauna using the available database, plus unknown species not in the database. These can be used to see how wetlands are changing over time (Figure 6). The image shows how species composition has changed (in this case very little change is evident) over three years at sites that didn't exclude pigs.

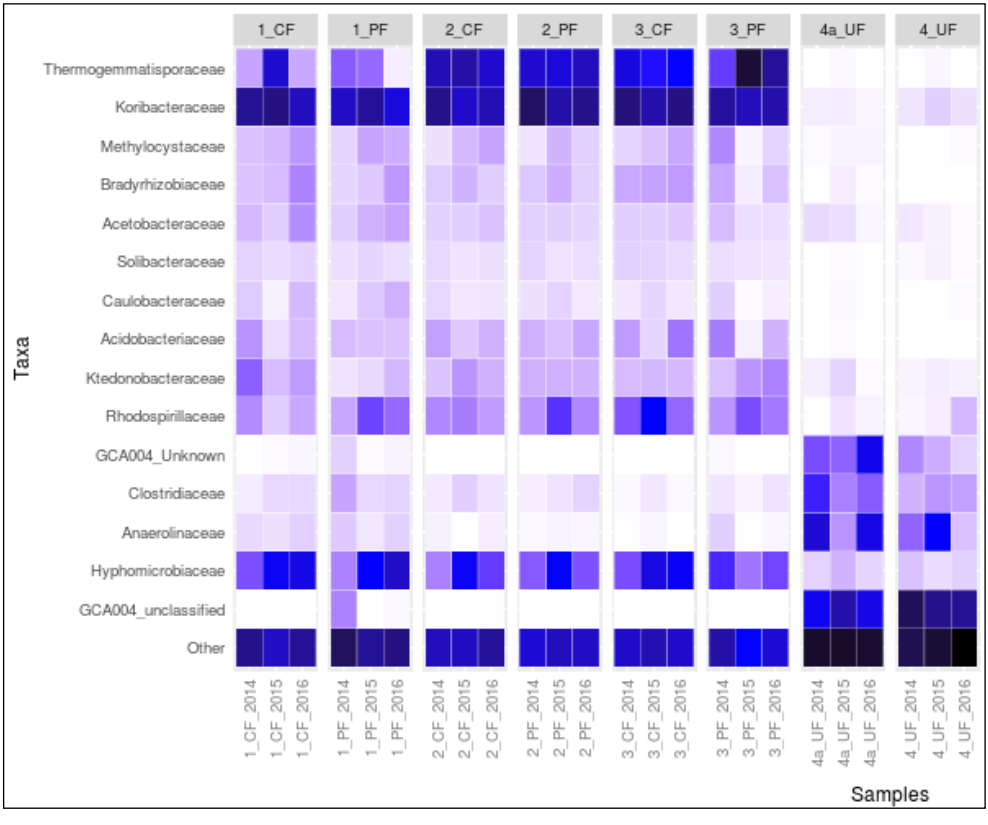

Figure 6: This heatmap shows the most abundant families (with an abundance >1%, with rarer families categorises as 'other'). Each site contains three columns representing three survey years. Sites are paired based on treatment (1 CF & 1 PF are pairs: site 1 cattle fence, site 1 pig fence). Comparisons can be made within sites across years, between pairs or broadly across sites. Colour intensity represents species abundance, the darker the colour the higher the abundance relative to other species.

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