Protocol for the installation and monitoring of fish in RAPELD modules and grids.

Why monitor fish?

Globally, aquatic ecosystems are the most affected by human activities and fish are sensitive indicators of the effects of logging. (Dias et al. 2010).

From the video: It is very convenient to study fish when you want to study the impact of human activities on the aquatic environment. Fish have a lot of popular appeal because of their role in the human food chain and also as aquarium fish trade. Beside this, their diversity enables researchers to study life cycles, different habitats and the different properties of those habitats. Aquatic habitats can also reflect what is happening in the wider context of the catchment area. Hence it is possible to analyse the aquatic environment and measure the impact of terrestrial activities.

In contrast to aquatic invertebrates, fish are relatively easy to identify down to genus level.

How is the RAPELD system of modules constructed?

The basic infrastructure for access or localization of plots consists of straight-line trails in a grid or rectangular formation. In some places, the trails are virtual and plots are located with a GPS.

The calibration of remote sensing techniques requires precise geographic locations obtained using a differential GPS, and in some cases precise estimates of altitude. However, many questions can be answered in grids installed using sighting poles and standard GPS equipment. Grids installed using simple techniques can be mapped later using more precise methods if necessary.

A variety of different RAPELD plots can be installed. To monitor and sample fish, riparian plots are used.

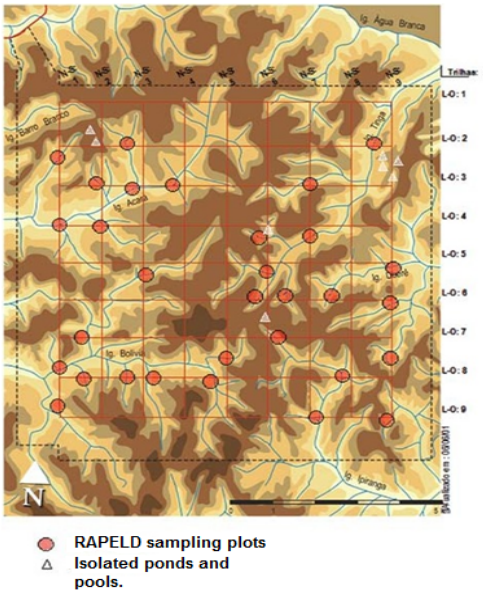

Illustration 1: Map showing the location of aquatic survey plots in Reserva Ducke.

Protocol for the installation and monitoring of fish in RAPELD modules and grids.

The trails are marked every 50m with pickets labelled with the name and distance along the trail.

Photograph of a trail with a 500m marker.

Photograph showing an aluminium marker at 3000m

Procedure

1) **Installing aquatic sampling plots.**

Equipment List:

i. At least 2 people

ii. 8-10, 100cm x 20mm (1/2") PVC pipe sections.

iii. 50m measuring tape

iv. Coloured plastic tape

v. Plastic, wood or aluminium folding ruler

vi. Waterproof field notebook or printed spreadsheets (protected against the rain) to record environmental data.

vii. Waterproof marker pen

viii. Good quality GPS that can receive signal under the canopy.

ix. Map of the grid or module showing the relevant area.

x. Concave densitometer for measuring the amount of light under the canopy. Or, a digital camera with a 28mm lens which can take pictures for later analysis by computer.

xi. Flowmeter (digital or analogue). Or, stopwatch and something which can be floated downstream to calculate the flowrate.

Every plot consists of a section 50m in length measured along the river bed. This measurement must be made along the bank going upstream and following the bends and curves of the water course.

If the movement corridor of another permanent plot crosses the water course, the survey should be carried out upstream in the direction of the source so that the survey is not disturbed by researchers crossing the survey zone thereby altering the water conditions.

If it is not possible to repeat the measurements on exactly the same plot, re-sampling should be carried out in the same conservation unit. It is important to NOT avoid sampling just because of seasonal variation (heavy rainfall or drought) because this will bias the observed results.

After measuring out the 50m section, leave the tape in place and mark the points 0m, 16m, 34m and 50m with tubes or coloured tape attached to trees, so that they be easily seen. All the environmental measurements will be taken at these points for each day of the survey.

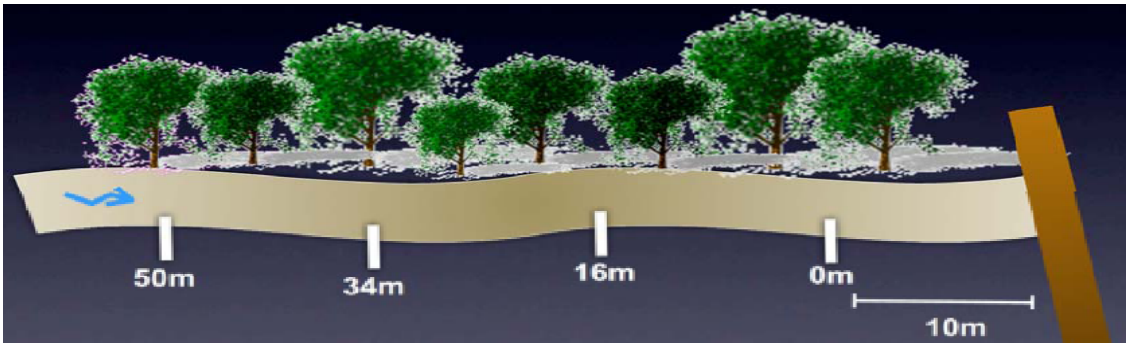

Illustration 2: Showing where along the plot environmental measurement are taken.

Environmental parameters.

Before sampling and collecting fish, measure the physical and limnological parameters of the water course.

For each of the 4 marked points of the segment measure;

- **The width** (L)(from water margin to margin at the water surface).
- **Depth** (Z) (divide the width by 10 and take 9 equally spaced (l) depth measurements)

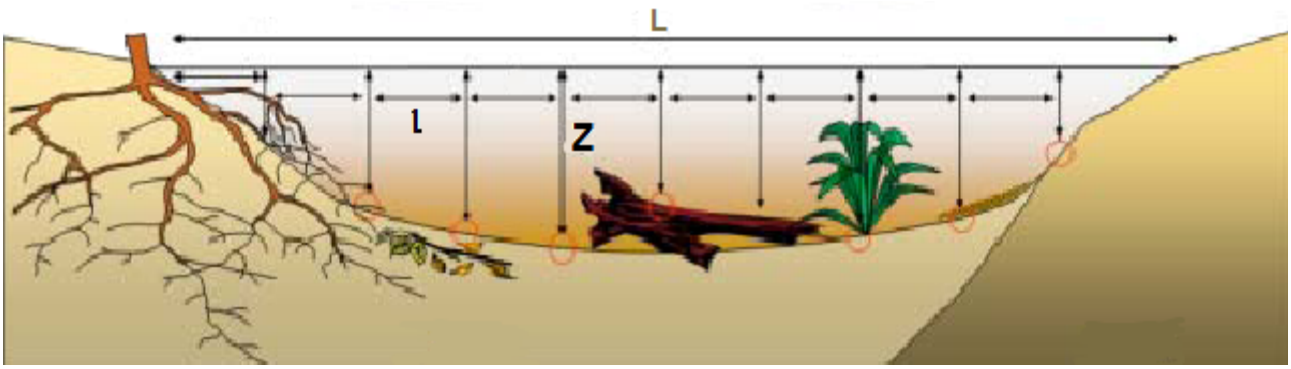

Illustration 3: Showing the location of depth and substrate measurements.

- **Type of substrate**

  In this example, from left to right, 1, 2 - roots, 3 - coarse litter, 4- sand, 5, 6 - branch, 7 - macrophyte, 8 - fine litter, 9 - clay.

- **Flowrate** (m/s). A flowmeter should be located a little below the water surface in the middle of the water. Alternatively measure the time taken for a floating object to travel uninterrupted for a distance of at least 1m. Record the average of 3 measurements taken at each of the 4 points in the 50m segment.

Note. The average volumetric flowrate Q (m3/s) can be calculated using the formula Q = A. Vm where Q = volumetric flow; Vm = average speed of the current; A = average cross-sectional area of the water course.

The average cross-sectional area is calculated as follows: $At = \sum[(Z_n + Z_{n+1}) / 2 .l]$; from n= 1 to 10

Where At = transect area, n= the number of the partial cross-section and l = L (the width)/10.

Canopy openness (%) - calculated as a percentage using a concave densiometer (Robert E. Lemmon Forest Densiometer, C model). The average is determined from 4 cardinal point readings (N, E, S, W) taken at each of the 4 points along the segment.

Water quality measurements

Materials needed:

• Portable electronic equipment to measure dissolved oxygen (DO), pH, conductivity, temperature, and turbidity.

• Plastic bottles (0.5 litres capacity, with a tight seal) for collecting and transporting water samples for laboratory analysis.

Protocol for the installation and monitoring of fish in RAPELD modules and grids.

The minimum required measurements to be taken are for D.O. (mg/l and % saturation), conductivity (µS/cm), water and air temperature (^{o}C), and pH.

Additionally, water samples may be taken for laboratory analysis.

NOTE. Sometimes there may evidence of human activity which may affect the sampling data. It is important to make accurate quantitative and qualitative records of the situation.

There are specific protocols for evaluating environmental impacts, some of which have been adapted to the Amazon region. These allow a reliable and fast evaluation of the overall conditions of the location.

Sampling of fish fauna

Fish can be sampled and collected by a wide variety of methods. If it is easy to return to the survey area, combining passive and active sampling and collection methods can minimise the effects of selective capture on the survey results.

The recommended equipment is as follows:

- fine mesh (1mm) dip nets (40x30cm), with a short or long handle depending on preference
- a small trawl net (about 3.0 x 1.5 m), with a fine mesh (1.0 to 5.0 mm between opposing knots) with floats on top and light sinkers on the bottom;

• At least 3 nets to fence in the sampling zone. 3.0 x 1.5 m with a fine mesh (1.0 to 5.0 mm between opposing knots), with floats on top and light sinkers on the bottom;

• Tent pegs, to fix nets to the substrate.

• Optional / additional collection devices:

- small plastic pots (made from PET bottles),
- collapsible (Fyke) traps
- Pots made from cloth or netting containing baits with exit barriers;
- detectors for electric fish;
- Other devices, depending on the location and questions being asked. E.g. electro-fishing equipment.

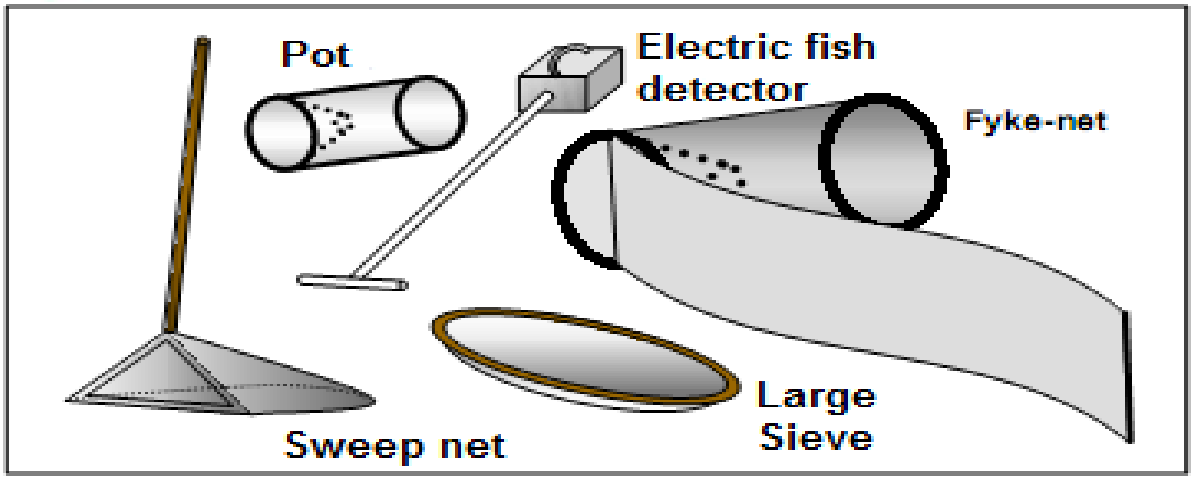

Illustration 4: Showing the variety of equipment used for catching fish.

If conditions are favourable, the collection pots and Fyke-nets can be set up in the early afternoon (when the environmental measurements are being done) and left in place until the next morning.

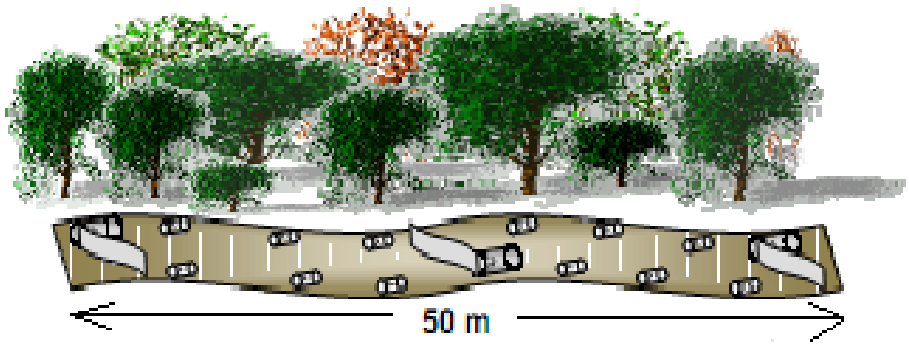

Illustration 5: Showing the positioning of nets and collection devices for passive collection.

After this passive capture phase the stream is blocked up and down stream and active collection using dip nets, trawl nets and sieves is carried out for 1 or 2 hours.

A third net can be used to reduce the collection areas and facilitate the catch. The catch is carried out moving downstream. Several passes are made in order to try to remove all the fish in the sampling stretch. (This rarely happens).

Mounds of leaf litter, root tufts and submerged logs should be turned over in order to collect the species that live there. Excavate sand and other soft substrates to collect benthic species.

When available, electric fish detectors can be used to make finding and catching electric fish (Gymnotiformes) easier.

Protocol for the installation and monitoring of fish in RAPELD modules and grids.

Video still showing part of the installation process.

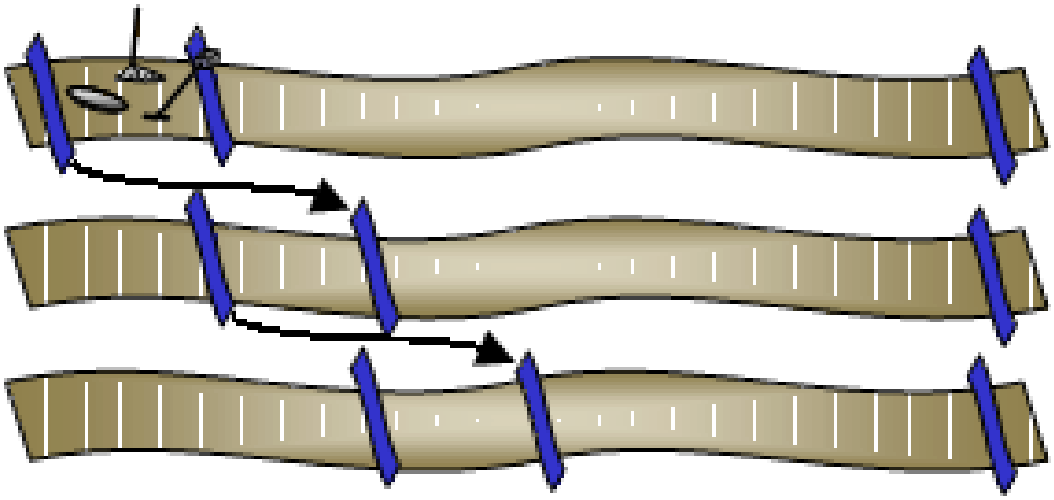

Illustration 6: Showing the net leap-frogging process used to reduce individual collection areas.

Preservation and destination of the collected samples

Materials List

• 37% formaldehyde (100% formalin) - 1 litre yields 10 litres of 10% formalin solution. (1 part formaldehyde to 9 parts water);

• Ethanol 96 GL (for eventual preservation of fish or tissue samples, for subsequent genetic analysis);

• “Eugenol” (clove oil) (to euthanize the fish with a lethal dose of anaesthetic before preservation in formalin);

• Paper Labels 90g (approximate size: 10 x 7 cm) for sample identification labels;

Protocol for the installation and monitoring of fish in RAPELD modules and grids.

- Waterproof ink pen or ink, fine point (0.3 to 0.5), or black pencil and sharpener
- Strong plastic bags of various sizes (most commonly used: 35 x 20 cm); some larger plastic bags for shipping large fish.
- Rubber bands to close the bags containing samples;
- Disposable plastic syringes 10 and 20 ml;
- Disposable hypodermic needles, medium and large;
- A variety of large strong plastic containers with sealable lids and strong handles. (5 to 20 litres)

In accordance with generally accepted procedures for avoiding unnecessary suffering, the specimens must be euthanized with a lethal dose of anaesthetic, before being immersed in formalin for preservation. Eugenol has been found to be a low cost efficient product and has a low environmental impact when compared to other products.

Two drops of Eugenol per litre of water is generally sufficient for most of the small fish present in streams. A solution of Eugenol (an oil) in a small amount of alcohol facilitates its dilution in water and accelerates the process.

After euthanasia, the specimens should be preserved in 10% formalin. Small fish (up to 5 or 7cm) may be simply immersed in formalin; larger fish need to be injected with the solution which does not penetrate efficiently by diffusion into animals with larger muscle masses.

The preserved specimens must be packed in sturdy plastic bags containing sufficient formalin to completely cover the fish (fish volume should be half the formalin volume).

A sturdy paper label displaying information about the specimen (place, date, time, plot, collection method, type preservative solution, collectors' names, and other useful data for the study) must be attached to the bag with the specimen.

The bags containing the samples from different sites must be transported in large rigid plastic boxes and/or drums with enough space so that they are not pinched or damaged in any way.

In the laboratory, after a minimum of three days of immersion in 10% formalin, the samples can be quickly washed in running water and transferred to appropriate glassware with 70% alcohol.

The taxonomic identification should be made using the specific classification keys and / or with the aid of experienced taxonomists/researchers. In Brazil, the samples (type specimens) shall be deposited in a Reference Collection in an IBAM accredited public institution.

Protocol for the installation and monitoring of fish in RAPELD modules and grids.

NOTE.

In environmentally sensitive areas, protected areas, or when conducting studies requiring repeated sampling, taking fish for preservation may not be appropriate, either due the characteristics or conservation status of the area or the possibility that this will interfere with subsequent studies.

It is recommended that the collected fish are kept alive in suitable containers until the end of the sampling period. After they have been identified (or photographed) and counted they are then released back into the stream.

This is possible only if the local fish fauna is well known, and can be accomplished with the use of photographic field guides.

The photographic record of live fish can be done in the field, after the daily collections:

- A small glass aquarium (25 cm x 15 cm x 10 cm);
- A piece of glass slightly narrower than the tank serves to reduce space and restrict movement of the fish
- A good quality digital camera (of least 5 MP and a macro lens).

A camera capable of photographing fast moving objects (1 / 125s) and with a flash extension makes it easier to photograph the fish without getting the reflection of the flash off the glass.

Make sure that you upload your data to a public database. This gives added value to your collection efforts by making the data available to a wide audience.