Vocational Aqualabs

TRAINING

Vocational Aqualabs – Vocational Generic Skills For Researchers

Experimental Design
Unit 4 – Fundamentals of field design

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Field Sampling Design

Let us now turn our attention to the field sampling of a variety of different field sites (Figure 1). Before embarking on the collection of samples, there are a number of things which should be considered. These include some of the following:

- What are the sampling objectives of the trip?
- Where do you intend to sample? Which sites?
- What type of samples do you intend to take?
- What facilities are available for conducting the sampling?
  e.g. What manpower is needed and / or available to assist in the sampling?
  e.g. What sampling equipment is available or required to do the sampling?
  e.g. Is the sampling limited by a budget? What finances are needed / are available?
- What analytical equipment do we need to work the samples up? What laboratory facilities (for example) are available?

Figure 1: Field sampling. There are a number of practical and feasibility factors that must be considered before undertaking sampling in the field (original).

If we, for example, imagine that the reason we wanted to sample a particular lake was to investigate a parasite problem, then we might want to establish what are **objectives** are i.e. why are sampling this particular site and how can I achieve it effectively. With this in mind, our objectives might:

- Conducting a field survey to look at the layout of the site
- Identify the parasite problem from moribund fish
- Screening a number of fish for infectious agents
- Establishing what the parasite prevalence is (of course we must remember that this will also change in time and space)
- Sampling so that we can target different stages of the parasite life-cycle (where appropriate, so with this mind it is good to establish the parasite life-cycle)
- Sampling enough stages / parasites to allow for a comprehensive taxonomic study or to allow us to determine the host specificity of the parasite
- Sampling different fish, either from different locations in the lake if sampling a natural water body or sampling fish of different ages and different sizes from different tanks, cages or ponds if conducting an investigation on a farm site. The reason we may do this is to establish the pathology of the parasite on the host.
- Our sampling objectives should also allow us to think further ahead and how we might treat for the parasite or how we might manage it.
- Finally, we should be aware that many parasites are seasonal and so our sampling strategies should allow for this. Parasites may occur in lower numbers in certain seasons and therefore we have to increase our sample sizes etc etc.

Now we have thought about our sampling objectives, what other **sampling considerations** should we think about? What about:

- **Timing?**
    - Event-driven. Are we sampling in response to a particular event such as the death of fish – if this is the case, then we may have a narrow window in which to conduct our sampling, otherwise we miss finding out what was responsible for causing the mortalities.
    - Environmental seasonality. If we think about parasites again, some only occur in certain seasons – the hosts may be there all year round but the parasites may have a discrete season of occurrence. Our sampling may wish to monitor their numbers to make sure that they are not building up to levels that may threaten the health and welfare of fish stocks.
    - Host seasonality. Alternatively, the appearance of the host may be season, which is certainly true of some marine species which come into coastal waters to feed, breed or spawn. Other species may migrate as part of their life-cycle.
    - Repeated sampling interval for the optimal resolution. Thinking carefully about what you are wanting to sample will allow you to design a sampling schedule and how frequently you need to go sampling.

- **Sample selection**. How many samples do you need to take? Of what size? From where?
- **Capture method**. The method you use has to take account of the samples you want and you have to make sure that the sampling method does not affect your sample. A good example of this is when you are sampling for parasites. Imagine a fish covered in small skin flukes. If you use a gill net, either the fish will struggle frantically when caught in the net and rub many of the parasites off or if the fish dies in the gill net, many skin flukes quickly leave the dead fish to try and find a new host. If you intend to put down a gill net down overnight with the view of sampling skin parasites, therefore, you will probably find that when you come to remove and sample the fish next morning that they have no parasites.
- **Sample number**. How many are needed to give you a statistically robust result?
- **Sensitivity of detection methods**. How appropriate are the methods / techniques you are using to measure the thing you are after?

Sample selection

There are two ways of sampling test specimens such as fish within your experimental design. But as with previous considerations, which is used will have implications for the results, statistical power and outcomes of the experiment you are carrying out.

Targeted samples: choice of particular animals for e.g. parasite collection, parasite identification (e.g. molluscs which are commonly the intermediate host for many parasite species).

- Not replicable (observers / time / space)
- Not useful for prevalence / abundance estimation

Single or multi-stage random samples: all individuals in the population have an equal probability of being selected for e.g. screening.

- Replicable
- Useful for prevalence / abundance estimation

Targeted samples

"Which fish to sample?" - This is perhaps the most important consideration. If you are, for example, conducting a routine health assessment of a stock of fish then your method of sampling must be random. Bias in your sampling could result in a misleading interpretation and caution is required.

Try to avoid sampling of only:

- Fish drawn to food
- Heavily infected fish (known parasite)

- Fish near the surface
  If you sample only those fish near the surface you may obtain a biased sample. If the fish think that they are about to be fed, then the healthiest fish may swim and reach the surface first. If you were to sample only these fish then you may overlook slower, unhealthier fish, which are still members of the experimental population. Conversely, some parasites cause damage that alters the fish's behaviour. Heavy infections of the eye fluke *Diplostomum*, for example, can cause blindness in the fish causing it to swim near the water surface where it is more prone to predation / capture.

- Sampling of sick fish
  The sampling of sick fish only may lead the investigator to believe that the prevalence and intensity of a disease or infection is higher than the actual level present. Immuno-depressed fish may also be prone to further infection by further species, thus masking the true causative agent.

- Sampling dead fish
  By only sampling dead fish you will also get a false picture of the condition of the fish at the site. On death of the fish, the condition and chemistry of body tissues rapidly deteriorates and most ectoparasites (Protozoa, Monogenea and Crustacea) detach or fall off of their fish host and leave to search for an alternative host. However, if fish mortalities are due to large metazoan endoparasites, then the sampling of only dead fish may again lead the investigator to believe that the level of a certain disease or parasite is higher than the actual level.

- Sampling big fish
  Sampling only the largest fish in a system may give a false picture. Large fish may either be the healthiest fish, responding quickly and appearing at the surface first at feeding time, or big fish may be the most heavily parasitised individuals. Bacteria, Protozoa or large intestinal helminths may cause distension of the intestine giving the fish a larger appearance. Certain parasites may sterilize the host causing them to put on more body weight (e.g. parasite infections in some molluscs causes gigantism).

Random samples

Random sampling and randomisation of treatments for experiments are investigated in the section of the programme on "Basic Experimental Design". However, random samples are also a powerful tool in selecting samples as part of a design for a field study, though there are several issues which should be considered:

- True random samples from the field are extremely hard to obtain, particularly from wild populations.
- On farms, harvesting / grading / restocking provide opportunities for good random sampling. Random number tables may be used to select the fish to be sampled with the order of capture / handling determining the number of each fish.

- Random samples from wild populations are often compromised by the capture method, sampling location etc.

Capture method

As discussed briefly above, the method of capture used for sampling can have a significant impact on the parameter you are trying to investigate. Look at the data presented below in Figure 2, taken from the research of Nagasawa (1985).

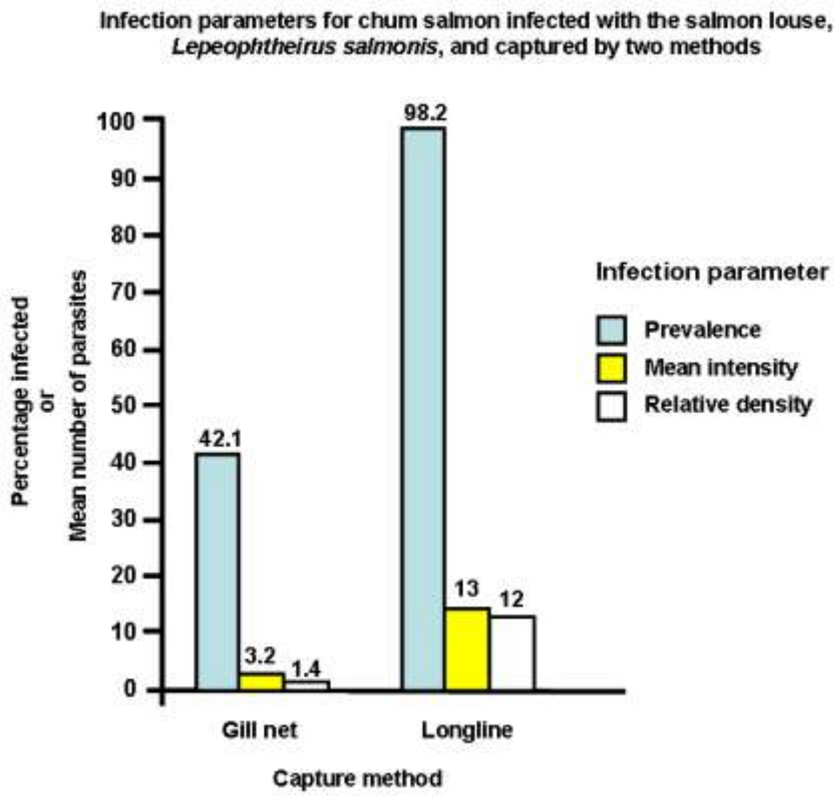

Figure 2: A comparison of two capture methods and the number of salmon louse, Lepeophtheirus salmonis, recovered by each. Data taken from Nagasawa (1985).

These data show how the sampling/capture method can affect the apparent "reading" of disease infection, potentially leading to erroneous conclusions on disease transmission.

Field sampling and surveys

Not all experiments are manipulation of conditions within a lab or in the field to test the hypothesis. Sometimes we wish to test theories or hypotheses in the real world using actual effects and changes.

For example, we may want to investigate the effect of aquaculture discharges on the local and wider environments. To do this we could use tests within the field such as transplanted sentinel organisms or set up simulations of this discharge in the lab. Such designs are dealt with previously. However, we can also look at the true effect on the environment using surveys and look at changes in the physical, chemical or biological parameters within that environment.

However, field surveys must also be well designed to ensure that:

- The results are meaningful and testable using statistical methods
- Ensure there is no (or at least a minimisation of) biased within the results
- Work within the available resources or budget of the study, i.e. available sampling and analytical effort.

Example:

Say we wish to survey a marine cage fish farm to see if there is any environmental impact of its discharge of nutrient rich wastes, and where those impacts were. We would be testing a hypothesis something like “Nutrient discharges from cage fish farms cause significant affects on the physical, chemical and biological nature of the surrounding environment”. How would we design this experiment? We must ask ourselves two initial questions:

- What form is this waste?
- What samples do we need to take and from where?

We need to first question to help answer the second. Of course it is the second question which is the “experimental design”.

The answer to the first question is that the waste is in two forms; soluble nutrients (ammonium/ammonia and urine) which are discharged to and remain in the water column and solid wastes (uneaten feed and faecal material) which tend to settle to the seabed. Thus we must investigate the hypothesis by sampling in two environmental partitions - the water column and the seabed.

So the answer to the first part of the second question is, we need to take water samples and sediment samples for analysis of their physical chemical and biological nature. These will then be analysed for physical (e.g. temperature, suspended solids, particle size of sediments etc), chemical (pH, concn of C, N and P based nutrients, dissolved oxygen, BOD/COD, redox potential of sediments etc) and biological (presence/absence of indicator species, species richness, species diversity etc).

The second question though has a spatial component - where should we sample? This is an important part of the experimental design as we need to be able to sample where any affect is taking place, but also must ensure no biased. There are three main methods of sampling design under such field conditions:

- Randomised design
- Even grid design
- Targeted design

These should not be considered as alternatives in terms of statistical power but as alternatives based on expediency of the field work. In terms of experimental power the three designs above are in order of strength. Were randomised design is the strongest (see Expt Design I) and targeted design the weakest. Figure 3 to 5 shows the three design approaches to this problem of location of samples.

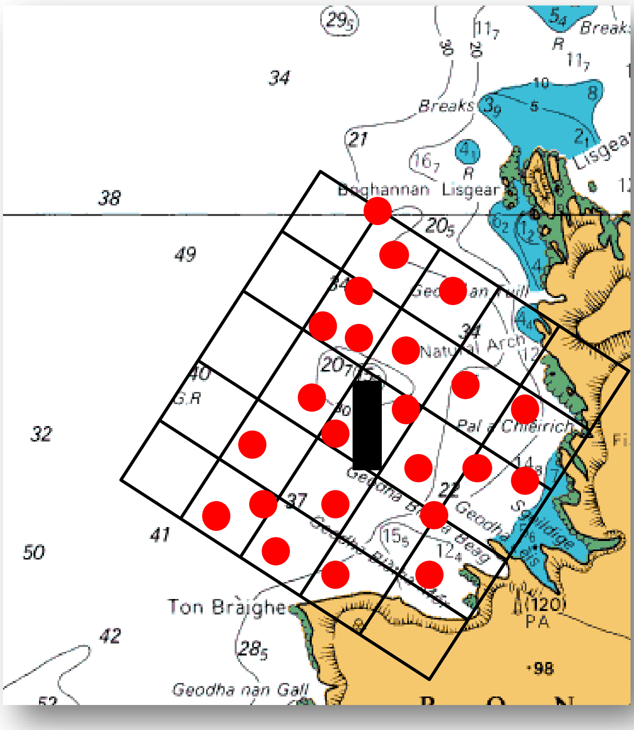

Figure 3: Randomised field sampling design to sample water or sediments in the locality of a fish farm

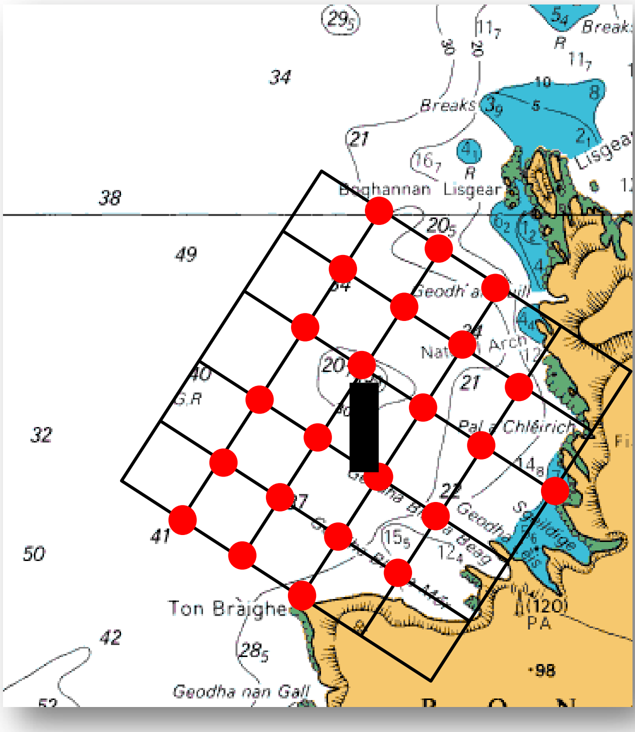

Figure 4: Grid pattern field sampling design to sample water or sediments in the locality of a fish farm

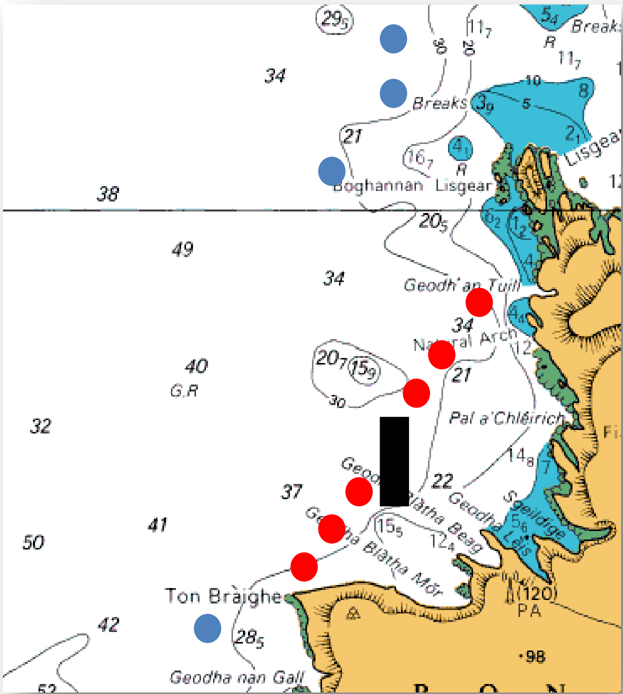

Figure 5: Targeted (transect) field sampling design to sample water or sediments in the locality of a fish farm

Which method is used is often based on expediency in terms of maximising the returns for the amount of effort/cost given. This often means a transect approach is the most useful for this purpose. Depending on the area size and the outcome of the survey, locations and extent of transects can be defined randomly. Though for conditions such as our example, a point source effect, it is more usual to place (or target) the transect to maximise output.

In this case we would place the transect to allow us to sample to see how much the effect is and where it is (how far away from the farm). For marine cage farms this is highly dependent on the physical conditions of the location, i.e. where will be water currents move the waste to. We can answer this question using models of dispersion of the waste, an example of which is given in Figure 6. Such models allow us to target transects in the direction of most waste dispersion and also across this axis of dispersion. An example of a survey design which results is given in Figure 7. In addition to these sampling stations there would also be a number of reference stations at some distance from the fish cages beyond the influence of the fish cage waste, for comparison with normal background conditions.

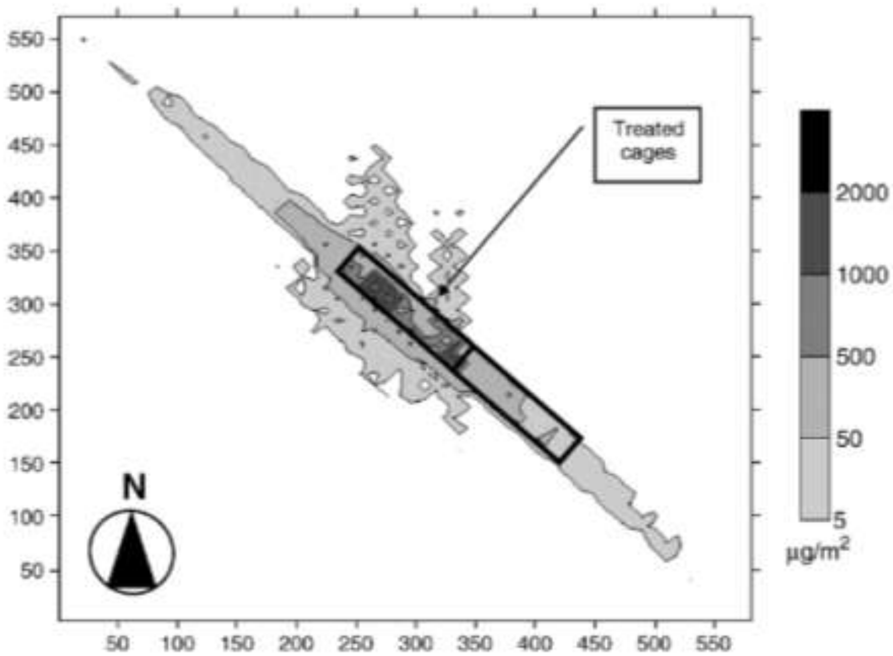

Figure 6: Spatial model of the dispersion of wastes to the seabed from a marine cage fish farm (from Telfer et al, 2006). The cages are given in white. The axis scales are in m.

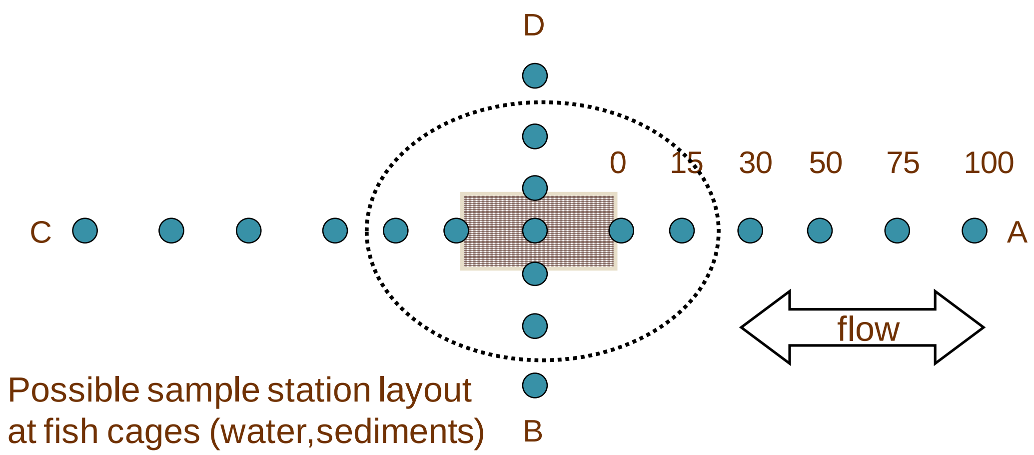

Figure 7: Possible layout of sample stations along transects adjacent to a marine cage fish farm to investigate localised effects of wastes.

Importance of sample size

Sample size in field experiments and surveys is also an important concept. As we have already seen with the number of replicates, normally the larger the sample size the better.

The larger the sample size ….

- the more likely it is to be suitable for the most commonly used statistical methods
- the better your ability to detect low prevalence of a factor of interest
- the greater the precision of estimates
- the smaller the detectable difference between samples

However, as with replication sample size may be limited due to operational considerations such as equipment limitations and cost/available effort. Therefore these are often a compromise.

Many field studies take the form of surveys where samples are taken from different locations in relation to a factor, in order to estimate the effect of that factor. For example the factor may be impacting on the organisms living within the sediments in ponds, for example, or on the seabed. One of the problems of investigating communities, such as sedimentary organisms, is they may be subject to different distribution patterns. As such the sample size must be sufficiently large to sample a representative sample from the distribution.

Samples taken from the environment which are for analysis of physical or chemical parameters are replicates and subject to the same choices and rules in terms of their number as previously discussed. Likewise as a rule of thumb a minimum of 5 replicate samples at each station is recommended, and certainly no fewer than 3.

However, when sampling for biological parameters such as species richness and composition, there are other confounding environmental factors. Benthic (sediment) organisms often exhibit what is known as “clumped”, “patchy” or contagious distribution. This is where there may be an abundance of organisms in one place but little in another, as represented in Figure 3.

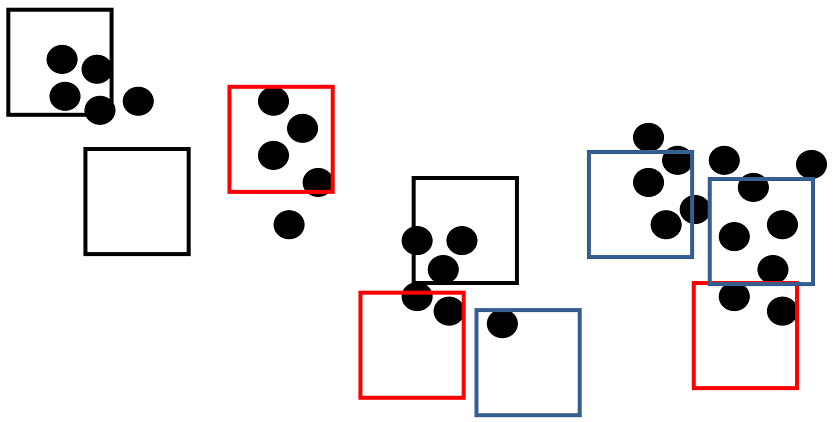

Figure 3: Contagious distribution of benthic organisms and number of samples, Black = 1 to 3 , Red = 4 to 6, Blue = 7 to 9.

Clearly the more samples taken the more animals are sampled (see Figure 3). If only 3 or 6 are take you can see the relative amounts of species captured. However, for such samples not only are they time consuming to take, they are also very labour intensive and expensive to analyse. How do we therefore make sure that we take enough samples to ensure we have an adequate representation of the species within the community? To do this we use a species/area curve. Here we take a preliminary series of samples from an area, or if not possible can use representative sampling from a similar situation. Here we plot a species richness/area curve such as in Figure 4.

Here 10 sediment samples of the same area have been taken and the cumulative percentage of total species richness (estimated by extrapolation) against sample number or area. Here we can see that the 10 samples account for about 90% of the total. Now we are in a position to decide on the number of samples needed in such conditions to account for a required percentage of the total species number. It is usual to sample to account for 70% of the total number of species. Here we can see this equates to 6 samples. Now we know we should sample 6 times to achieve the sample size in terms of species number we require within this and similar conditions.

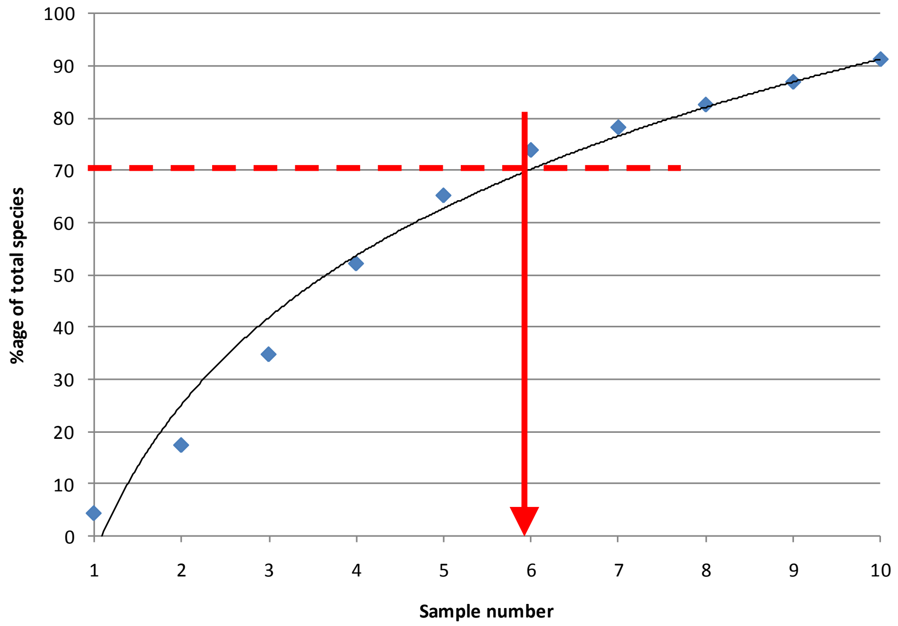

Figure 4: Species/area curve for preliminary faunal samples from sediment. The x-axis can be plotted as number of samples or cumulative sample area.

Sampling methods

Actual sampling at these locations can be done using a variety of methods to collect sediment or water samples for further analysis, or even video or photographs for visual assessment. A variety of methods are shown in the pictures below. More information on sampling methodology can be found in Davies et al (2001)[1]



[1] Davies, J., et al (2001) Marine Monitoring Handbook, March 2001. UK Marine SACs Project. JNCC. Pp 405. ISBN 1 86107 5243 – **given as pdf in support materials**

Time series sampling

This example can also be used to look at change over time as well as over distance. Each sampling methods - random, grid and transect - could be used on a number of occasions and results for each compared. There are several factors that should be considered if this is to be the case:

- The same sampling methods and number of replicates at each sampling location should be used on each sampling occasion
- Apart from random sampling, as need as possible the same sample station locations should be used on each occasion to allow comparison.
- Pre-impact data should be collected from the same places (except for random designs) using the same methods, including at the reference stations for transect approaches.

The final consideration brings into focus the concept of baseline data. This is data collected before impact or development (in this case initial fish production). Baseline data in conjunction with reference stations are important when investigating temporal impacts in the natural environment as they allow us to take into account the natural changes within that environment. The real world environments are not static and are constantly changing, naturally. A good survey design will take this into account and allow correction or adjustment for this.

Baseline data gives the investigator information on what the environment is like, and spatial changes occur over the sampling area before being impacted. The reference stations can allow a spatial comparison at a single time point, but also in conjunction with the baseline data can also provide the investigator with information on what is happening within the environment over the time of the survey. It is therefore important to take suitable baseline data and reference data during any spatial or temporal monitoring study.

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Many thanks for your attention today

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