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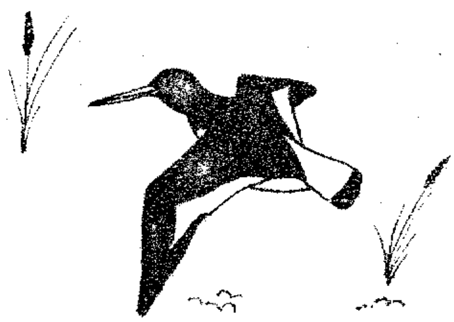

A GUIDE TO THE SAMPLING OF INTERTIDAL FLAT MACRO-INVERTEBRATE FAUNAS

by

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Persons working in intertidal areas must be aware of the inherant dangers involved. The Coastal Ecology Research Station cannot be held responsible for damage to persons or property incurred while following sampling procedures recommended in this paper.

A GUIDE TO THE SAMPLING OF INTERTIDAL FLAT MACRO-INVERTEBRATE FAUNAS

By SELWYN McGRORTY (C.E.R.S.)

ABSTRACT

A detailed procedural guide is given to a particular systematic sampling scheme used at the Coastal Ecology Research Station to investigate aspects of the invertebrate faunas of extensive intertidal flats. Random sampling procedures and some theoretical aspects of sampling are considered briefly, but the paper is intended to indicate the practical problems of working in 'soft-sediment' intertidal areas and the reader is urged to seek the advice of a statistician when deciding upon the most appropriate sampling scheme to fulfil his specific aims.

CONTENTS

INTRODUCTION

It is the primary purpose of this paper to present an account of a particular systematic sampling scheme, developed by members of the Coastal Ecology Research Station (e.g. Anderson 1972) to investigate aspects of the invertebrate faunas of extensive intertidal areas for which little or no previous information is available, but a brief account is also given of random sampling procedures. The paper has been written primarily as a guide for the Nature Conservancy regional staff, bird watching societies and similar organisations, who from time to time seek our advice on invertebrate sampling.

The theory of sampling is duscussed in a number of standard statistical reference works e.g. Cochran (1953) and Stuart (1968) and the reader is directed to these if a detailed account is required of any specific theoretical problem. The aim of any survey is to describe in qualitative or quantitative terms chosen parameters of a population or group of populations present within a defined area from a series of samples taken from the population(s). Biologists have discussed at length the most appropriate ways of sampling the faunas and floras of different habitats to answer specific questions, but there still lingers with some the idea that if the correct sampling scheme is in some way very difficult to execute, then the statistician or analyst will still be able to find a way of getting the best out of data collected in a more convenient way. It cannot be over-emphasised that the statistician should be consulted at the planning stage not merely when difficulties are experienced with analysis.

The variety of sampling procedures found in the literature fall into a limited number of categories. One group of sampling procedures, often recommended as rapid survey methods, can be called 'subjective sampling'.

Samples are taken from areas which the worker considers typical, representative and/or different from adjacent areas. There are many objections to this type of sampling. The results must be strongly influenced by the observers interpretation of the area being sampled, transition zones are unlikely to be recognised and sampled, some species are almost certain to be under-sampled or even missed completely dependant on the observers experience and the fauna or flora will appear to be more discontinuous than in reality. Nevertheless, where the object of sampling is to record maximum diversity of habitat type and species content within a minimum of time, subjective sampling based on observable differences is acceptable for primary survey and can be useful for planning subsequent objective sampling more effectively.

There are three basic techniques of objective sampling, each appropriate to particular situations.

a) Systematic sampling - the position of each sampling unit (e.g. core, quadrat) is fixed with regard to all other sample units.

b) Simple random sampling - samples are placed at random within the entire area occupied by the population, so that each sample unit is located independently of all other sampling units.

c) Stratified random sampling - the study area is divided into a number of smaller areas or strata and at least 2 randomly selected sample units are located in each strata.

When the aims of the survey are concerned with identifying the species composition of the fauna and/or flora of an area and detecting the pattern of species distributions a systematic sampling scheme is appropriate and the ideal arrangement of sample sites on a relatively undifferentiated area like a tidal flat is a rectangular grid with equidistant spacing in both directions. Any pattern detected will be large in relation to the spacing employed but the scale will be uniform throughout the area and there will be no doubt if an irregularly shaped distribution pattern is detected that it is in some way connected with the irregular pattern of randomly placed sampling units. This is the prime objection to random sampling (simple or stratified) in this context; no meaning can be assigned to the irregularity and inconsistency of scale. This, of course, is precisely the requirement of probablistic statistics; each individual in a population must have the same chance of selection in a particular sample and there must be no meaning assignable to the sampling pattern or selection bias is implied. Selection bias is avoided in a systematic sample (i.e. the collection of sampling units) by placing the grid in a random manner in the area to be sampled. If the distance between sampling points on a grid is not regular, then a conscious decision has been made to investigate pattern on a finer scale in one spatial direction. A band transect consisting of a row of contiguous quadrats may be used in this way to investigate variation along a recognisable gradient - but great difficulties are created if the results of several irregularly spaced transects are pooled; of course, if the transects are evenly spaced then they constitute a grid.

If, however, the aims of the survey are to provide density estimates of one or more species, and the density estimator (e.g. sampling mean) is required with its fiducial limits, then the sample units should be set out at random. Only when this condition is met can all possible bias be eliminated and the sample variance be regarded as an unbiased estimator of the population variance. A systematic sample will also be free from selection bias if the grid is placed at random in the area to be sampled and both random and systematic samples will be good estimators of the sample mean free from estimation bias, since in the long run, after repeated sampling both would give a sampling mean equal to the population mean. However, the two estimators will differ in their respective variances (for a particular sample size). The random sample will give an unbiased estimate of the variance of the sampling mean but this is not usually the case with a systematic sample and it is not usually possible to say, without comparing the two schemes in the field situation, whether the systematic sample will have a smaller variance (positive bias) or a larger variance than a random sample.

Therefore, where an unbiased error estimation is required, a random (simple or stratified) sampling scheme is essential, but where this is not of great importance a systematic scheme may be preferred to estimate population density. Where previous information is available, suggesting an uneven population distribution, a stratified random scheme would usually be preferred since this would allow greater precision of estimation of the population density.

To conclude this introduction; the aims of the survey and the way in which the results are to be analysed determine the sampling procedure.

Sampling Intertidal 'Soft-Sediment' invertebrate faunas

Every natural habitat presents its own set of problems to the biologist - surveyor. Soft-sediments, used here to describe sands (2.0 - 0.02mm median particle diameter), silts (0.02 - 0.002mm. m.p.d.) and clays (<0.002mm m.p.d.) are deposited in areas of progressively greater shelter and produce shores of increasingly shallower gradient, where the distance between the high water mark and low water mark may be several kilometres. These vast distances and the resultant speed of the flood tide, changes in the weather resulting in bad visibility, the often complicated creek patterns and the actual softness of 'muds' and quicksands present very real DANGERS to the unwary.

On rocky shores a large proportion of the fauna can be seen at relatively little trouble to the observer, but on soft-sediment shores the fauna is almost entirely burrowing and few species produce indications of their presence at the surface. Thus in order to determine what, if any, species of macroinvertebrate are present, substrate samples must be dug, sieved and the animals sorted from the resultant mixture of coarse particles and organic debris. Again, on a rocky shore the pattern of species distribution, in many cases, is obvious to the observer and a species may occupy a vertical height of several metres on a rock face, with clear upper and lower boundaries. On a soft-sediment beach, the same vertical height intercept may be represented by several hundred metres or even kilometres on the ground and species pattern can only be determined, except in a few tube or caste producing species, by extensive sampling and analysis of the results.

Systematic sampling

Having decided that a systematic sampling scheme is appropriate, with regard to the aims of the project, the most important problem is that of scale. With so few clues to indicate the presence of species on intertidal flats the surveyor will generally have no prior indication of the scale of any pattern of species distribution upon which to base the grid size. In practice, however, since funds are rarely unlimited, the number of sampling units collected will be determined by manpower and transport costs and availability. As pointed out previously, any pattern revealed can only be at a scale larger than the sampling scale, so that the smaller the grid interval the finer will be the scale of pattern detectable. In practice the dimensions of the sample unit (the greater the volume of sediment sampled the greater the transport and processing costs per unit) must be balanced against the frequency of sampling - but if the aim is a 'total' fauna survey one must guard against collecting samples which are too small in relation to animal density in order that a greater cover can be achieved.

It is generally recommended that a sample of sediment with a surface area of at least 0.1 - 0.25m^2 is collected (Holme and Macintyre 1971), otherwise the (often larger and deeper burrowing) less dense species will be undersampled or even missed entirely. Also, as the sample unit is reduced in size the proportion of specimens in the sample damaged by the corer is likely to increase and this obviously creates difficulties with the identification of many species. Worms are particularly prone to being cut in half (not only in the sampling process but also if due care is not taken in the sorting process) and it is usual to adopt the convention when counting specimens that only heads are regarded as being present.

The dimensions of the sampling unit are, however, less important than is often assumed, in particular when a density estimate of a species is required, it is the number of sampling units (cores) which determines the precision of the estimate, not the volume of sand collected in each sample unit. For practical purposes however the number of animals contained in the sampling unit may be a very important consideration; a minimum number of animals may be required e.g. for size frequency analysis of the population. Normally the sampling unit should not contain more than 2-300 animals, so that counting errors due to observer fatigue are minimised, but it is advisable when choosing the size of the sampling unit to err on the side of generosity basing sample size more on the number of larger species. If smaller species are thereby sampled in very large numbers (e.g. Corophium sp., spionid worms), then sub-samples can be taken and counted.

Similarly the number of cores taken need not be excessive; a data matrix of say 200 samples x 100 species would be very cumbersome to handle and be costly in terms of computer time. Jeffers (Merlewood Research Station Research and Development paper No.17) stated that for the purposes of trend surface and components analysis the 275 invertebrate and chemical samples taken from the whole of Morecambe Bay ($310km^2$) ~~could have been~~ reduced to approximately 120 samples and have given similar results with an acceptable precision.

One must be aware with systematic sampling of the possibility that the sampling frequency might coincide with some natural frequency in the environment. This could give a misleading picture of the distribution of the animals and will make the sampling variance of the sample average relatively large. For example, occasionally drainage creeks run down the beach roughly parallel and equidistant - if the sampling interval coincided with the distance between creeks - then the collection of sample units might show a strong over or under representation of the faunas of creek edges.

PROCEDURE

The following is a detailed account of a systematic sampling scheme used at the Coastal Ecology Research Station. It is realised that the sample size may be too small to give a true picture of the total fauna; some large, deep burrowing species are certainly undersampled, but this is not a serious objection in the context in which it has been used. The intertidal invertebrate surveys which we undertake at the present time are almost always aimed at describing the invertebrate resources present in very large areas of intertidal sand and mud flats and potentially available, as food to wading birds and wildfowl.

This consideration also limits the depth of sampling to 15cm. and determines to a large extent the time of sampling. Sampling in autumn and early spring gives respectively estimates of the potential food available to over-wintering birds and the invertebrate stocks remaining when the birds have departed for the summer breeding areas. Sampling at such times also ensures that only small numbers of juvenile invertebrates are taken, which can give rise to serious identification problems ~~in~~ summer samples.

The species, which are poorly sampled, have rarely, if ever, been observed to be taken by these birds and the smaller samples allow a considerable saving in transport and processing costs.

The technique would be eminently suitable for a preliminary extensive study of a large previously unworked area, to provide basic information regarding species densities, distributions and faunal associations and to provide questions for further study.

1. A preliminary site visit should be made if possible to arrange for access to the sampling site, assess any dangers due to tide, deep creeks, soft mud etc., and arrange transport and accommodation. The sampling procedure can be tested on site and the possibilities of sieving on site (if sufficient standing water is available on the beach and no danger is thereby incurred), at a 'seawater-tap' in a nearby laboratory or fisheries building, or if necessary at a freshwater tap as close to the sampling site as possible. The aim is to reduce to the minimum transport of sediment samples. It is also essential to sieve as soon as possible after sampling to preserve the condition of the animals.

2. Equipment required will include: one simple tubular corer ($\frac{1}{100}$ m^2 surface area x 15cm. deep) and a spade per sampling team, polythene bags, plastic bottles, labels and pencils, notebook, compass, haversack and/or mud sledge and/or mechanical transport (e.g. tractor).

3. The size of grid (i.e. distance between sampling units) will be determined before sampling begins, probably on the basis of the number of cores that can be collected within the constraints of the time and manpower available. There may be some slight advantages in relating the grid to the Ordnance Survey 1km. grid.

4. The first site and hence the grid position should be located by a random process (thus avoiding selection bias). This can be done by selecting random co-ordinates (from random number tables) within the first grid 'square', or choosing the point on the map with a pin and locating the sampling site by compass bearings on at least two prominent landmarks or by measuring the distance along a fixed compass bearing from a suitable landmark (e.g. the point where a seawall makes a sharp angled bend, the corner of a building etc.). The important point is that the sampling site should not be chosen because there are a lot of animals present or because of some special topographic feature. Having located the first site, four cores are taken from undisturbed sediment within an area of 1-2m^2. The most efficient number of cores taken at each sampling site will depend on the distribution of the fauna. If the variation between cores at a sampling site is small compared with the variation between sampling sites (i.e. grid squares) then it would be more efficient to take fewer cores at each site (say 2) and increase the number of sampling sites (i.e. reduce the scale of the grid). The reverse also applies. Thus in general the greater the species diversity and the greater the (small scale) aggregation of the species present, the greater the number of cores required at each sampling point on the grid. Experience has shown that four cores per sampling point is generally satisfactory.

The cores are either placed in separate polythene bags (15" x 10") or bulked (24" x 18"). The site is allocated a number and this is written on both sides of a card which is placed inside a plastic bottle (100 - 200ml. capacity) which in turn is placed in the polythene bag with the sample. The bag is sealed with a plastic and wire tie. The number of <u>Arenicola marina</u> and/or <u>Laniche conchilega</u> (important food organisms which do burrow below 15cm) are estimated from the number of castes or tubes respectively counted in 5, 1m^2 quadrats adjacent to the sample site. A site description should be made noting e.g. plant growth at the surface, depth of black layer indicating anaerobic conditions in the soil profile etc.

5. To locate the next and subsequent sampling sites one person is appointed 'pacer' and his or her pace is checked against a standard measure ON SITE. The number of paces required per grid interval is calculated and the pacer proceeds along the correct compass bearing for the appropriate distance. Placing canes or piles of sand at intervals can help to keep a straight line if there is no convenient object on shore to line up with the first site. When the pacer arrives at what he considers to be the exact location of site 2, the cores are taken as previously described. The exact location of the site can then be determined by compass bearings on two or more landmarks. This method of site location may appear inaccurate, but has been found to work out well even in difficult conditions. It is important to note that regardless of the accuracy of site location the samples must be taken at the exact spot determined by the 'pacer'; it may be useful to adopt the convention of taking the first core at the tip of the pacer's leading foot on arrival at the site and the remaining cores as close as possible to this on undisturbed ground. In the rare event of the site lying exactly on the boundary of two substrates or e.g. a mussel bed, then the cores could be apportioned between the two, but one should NOT take samples more than 1-2m from the site because there is something different close by, as the samples are then liable to gross selection bias determined by the operators personal preferences.

6. Experience has shown that the safest method of sampling is to follow the ebb tide so that the sample nearest the low-water mark will be taken at approximately low tide. If the distance between high and low water mark is more than 3-4 kilometres and the substrate soft, the lowest sample sites may not have been reached by this time; if ON FOOT the return

journey must be commenced at a safe time in relation to ~~the hazards of the~~ site e.g. by 1 hr. after the predicted time of low water or if there is a strong onshore wind, then the return journey should be started even earlier. Samples are collected on the return journey and transported to suitable sieving sites. Teams of three people are the most efficient and can be organised as follows:- a 'pacer-writer', a 'core-man', a 'polythene bag/odd-job man'. Three samples (i.e. 12 cores) will weigh in the region of 70 lbs. and the team should be able to collect at least 9 samples on one tide, but the possible load per person is reduced as the substrate becomes softer. If the substrate is firm sand and the beach quite narrow, then of course this may be considerably improved upon.

7. Sieving should be carried out as soon as possible after sampling, preferably in seawater using a 0.5mm mesh sieve. The use of 1mm sieve considerably reduces the processing time but also loses a large percentage of, for example, the smaller size classes of Hydrobia ulvae which may form part of the diet of small waders such as dunlin. The larger pieces of debris in the sieve can be discarded at this stage but the remaining specimens plus debris mixture is washed into the labelled plastic bottle and preserved in 5% formaline. The sieving process should be as gentle as possible and rather than risk damage to specimens by forcing clay substrates through the sieve with a water jet, the sample should be gently 'puddled' by hand, prior to sieving, in a large volume of water.

8. If sediment samples are required for physico-chemical analysis of soils these can be taken within the same 1-2m^2 area at each sampling site, using a 5cm diameter x 15cm deep perspex or metal corer. Usually between 5 and 10 cores are taken and bulked in a large polythene bag or on a board; the whole thoroughly mixed and an equivalent volume to 1-3 cores retained for analysis. The number of possible analyses are enormous but a limited number have proved to be repeatedly valuable in interpreting species distributions. These include

estimates of the chemical elements Carbon, Nitrogen, Calcium and Phosphorous and the physical parameters median grain size of sediment and site elevation (or exposure time). Jeffers (Merlewood Research Station Research and Development papers, Nos. 14, 15 and 17) showed that for Morecambe Bay, because of the high intercorrelations many physical and chemical factors measured were redundant.

Presentation of results

Although systematic sampling does not comply with the basic requirement of randomness for probablistic statistical techniques, estimates of sample means (e.g. species density), biomass etc., are unbiased estimators of the respective population parameters. A range of diversity and similarity indices are available and can be used to compare sites. Association analysis, correlation analysis and multivariate components analysis, requiring computer facilities, can be used to group sites according to faunal similarities. Trend surface analysis can be used to map the direction of trends of variation in species distributions and to a certain extent can act as predictive models, since values will be assigned to areas which were inaccessible for sampling. Detailed procedural accounts of these analyses can be found in the literature, but the biologist is always advised to seek the aid of a statistician at all stages of his study.

Several of the analyses mentioned above use only the criterion of presence or absence (binary data) in their simpler versions. In much floral survey and some faunal survey where the species are easily observed, the time taken to recognise and record the species present in a quadrat is very much less than the time required to count the number of specimens of each species. In these circumstances it will be possible in a given time to record very many more sets of binary data than fully quantitative data and hence achieve a much more intense cover of the study area. There is the added advantage of a considerable saving of computer capacity when analysing binary data as compared to fully quantitative data. However, it is true in general that more sets of binary data are required in say an association analysis to produce a satisfactory classification of sites than with quantitative data. In general the processing of substrate samples and identification of the species of invertebrates present, is so expensive (in terms of 'man-days') as compared with the time taken to then count the number of specimens of each species, that it is doubtful if the collection of binary data only, could ever be justified in intertidal soft-sediment studies.

Random sampling

If an estimate of population density is required for a particular species with fiducial limits, then a random sampling scheme is necessary. This may simply involve locating a number of sites by randomly chosen co-ordinates, throughout the entire study area or if there is previous evidence (e.g. a preliminary systematic survey) that the species has an uneven distribution pattern or varies in density along a physical or chemical gradient, then a stratified random sampling scheme would give a greater precision of estimation. In either case a preliminary survey would be required to decide the best sample size and the minimum number of samples necessary to give the required level of accuracy of estimation.

Dependent on the size of the species and its density a range of sample unit sizes are taken and a graph of variance (between sample unit replicates) and sample size plotted. From the shape of the curve one must decide at what point the cost and labour involved in processing larger samples cannot be justified in terms of the resultant decrease in variance (i.e. increase in precision).

Having chosen a suitable sample unit size the number of samples required to estimate the mean with a particular level of accuracy is approximately given by the formula:

$$N = \left(\frac{ts}{D\bar{X}} \right)^2$$

where $\overline{X}$ = mean, s = standard deviation, D = the required level of accuracy expressed as a decimal (normally 0.1, 10%), t is a quantity dependent on the number of samples and obtained from tables; for more than 10 samples t is approximately 2 at the 5% level. It will be noted that in this equation the standard deviation (s) varies with the square root of the number of samples (N), so that to obtain a small improvement in accuracy a large increase in the number of samples is required.

There are many ways of achieving randomly located samples, but the following has been tried and worked successfully in many habitats. The largest scale map available of the area to be sampled should be chosen and divided into the smallest possible grid (e.g. overlaid with mm graph paper). Each grid line is numbered. Pairs of numbers are taken from random number tables to give co-ordinates which locate the sampling sites on the map by a pinprick through the overlay.

The sites are located on the ground in the same way as grid sites previously described, by pacing the distance along a compass bearing from a recognisable landmark. On arrival at what the pacer considers to be the exact spot, the sample is taken at the tip of his leading foot. The actual site location is then determined by taking compass bearings. It will be obvious that the actual site sampled and the site originally located by the randomly chosen co-ordinates will not be the same, though they should be within a few metres, but this is not important. If the procedure is rigidly followed, the sample site will have been randomly selected and free from operator (selection) bias.

This procedure will be the same whether a simple set of random samples are taken from the entire study area or whether the area is subdivided into smaller areas (strata).

Summary

The AIMS of a survey and the way in which the results are to be analysed

DETERMINE the SAMPLING PROCEDURE. Unbiased estimates of the standard error of the mean of a population (i.e. the fiducial limits of a density estimate) can only be calculated from randomly placed samples. However, where the aim is to detect pattern i.e. species distribution, species associations, the relationship of species with environmental parameters, the classification of sites on a faunal or floral basis etc., then, the ideal sampling arrangement is a rectangular grid over the entire study area.

Procedures are given for carrying out surveys of intertidal soft-sediment invertebrate faunas.

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APPENDICES

APPENDIX I. PRECAUTIONS

1. Working in intertidal areas

a) ALWAYS seek and heed local knowledge when seeking permission for access to the study area.

b) INFORM one or more responsible shore-based person(s), e.g. coastguard or farmer of the plans for the day and the expected time of return. REPORT back on return.

c) NEVER VENTURE ONTO UNKNOWN AREAS ALONE - parties of MORE than 2 are to be preferred at all times.

d) CARRY a compass (each person) and know which direction is landward, each group should also have maps, buoyancy aids and flares at all times.

e) ALWAYS work on the ebb - start the return journey (if on foot) depending on the distance involved and softness of substrate from 1 hr. before to 1 hr. after the predicted time of low-water.

f) AT ALL TIMES keep a 'weather-eye' open - in particular for fog or strong on-shore winds.

2. In the laboratory

a) When working with samples preserved in formalin - if it irritates your nose and eyes, it is doing you harm.

b) ALWAYS wash samples thoroughly in running water before sorting, identifying etc., under a microscope. Add formalin to 2-5% concentration again before storage.

APPENDIX II. PULLOUT SUMMARY OF SAMPLING INSTRUCTIONS

APPENDIX III. SOME USEFUL TITLES

A. General

1. PLYMOUTH MARINE FAUNA - compiled from the records of the laboratory of the Marine Biological Association of the United Kingdom. (Citadel Hill, Plymouth, Devon) - updated periodically. Provides a very useful list of species and information on habitat. Can be used to check nomenclature.

2. Polychaetes

a) R.B.Clark - The fauna of the Clyde sea area: Polychaetes, provides a key to genus and list of species.

b) P.Fauvel - Faune de France - 5, Polychaete errantes.
(in French) - 16, Polychaete sedentaires.

c) J.H.Day - Polychaetes of South Africa - Pt.1 Errantia
(in English) Pt.2 Sedentaria

3. Molluscs

a) N.Tebble - Bivalve Seashells.

b) A.Graham - British Prosobranchs - Linnean Society of London.

4. Crustaceans

a) E.Naylor - British Marine Isopods - Linnean Society of London.

b) Die Tierwelt der Nord-und Ostsee. Vol XIV - Amphipoda.

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