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JOINT NATURE CONSERVATION COMMITTEE

Marine Monitoring Handbook
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UK Marine SACs Project

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Procedural Guideline No. 3-7 in situ quantitative survey of subtidal epibiota using quadrat sampling techniques

Eleanor Murray, English Nature[1]

Background

Quadrats provide a quantifiable technique for measuring changes in diversity and abundance of conspicuous species. They provide quantitative data that can be analysed statistically, which helps us understand changes in communities in a monitoring context.

Quadrats facilitate accurate abundance measurements of numbers of species, thus reducing the errors incurred by inter-worker variability and achieving more consistent results, in both a spatial and temporal context.

Quadrats are traditionally used for monitoring the distribution of plant species. They are generally large in area, made of string, and laid out using pegs. Such a quadrat is impractical to use for subtidal quantitative sampling, where frame quadrats of $1m^2$ or smaller are used.

Purpose

Quadrats are generally used for the quantitative assessment of biodiversity for a particular feature occurring within a site. The objective generally relates to the quality of a particular feature or biotope, where species richness may be an important or valued attribute of that feature.

Quantitative counts using quadrats provide a structured way to estimate abundance of species to estimate their population size, and/or to assess species richness and diversity of a biotope. The quadrat provides a simple, repeatable method, which is also suitable for a whole series of statistical tests; this makes it ideal for use in a long-term monitoring strategy. Quadrats are very versatile in terms of shape and size, and can be easily tailored to provide the best application for a whole range of different community types.

Quantitative counts in quadrats can also be used to determine biotopes, but it is generally easier and less labour intensive to use semi-quantitative methods to assign biotopes to particular areas.

It is important to recognise when communities and habitats are not appropriate for monitoring using quantitative quadrat methods. Ephemeral communities may change annually and could not be reliably monitored at the species level on a long-term basis. Similarly, mobile substrata are subject to considerable seasonal disturbance and would be inappropriate to monitor using quantitative methods.

Advantages

Quantitative sampling by quadrat is advantageous as it:

- is generally non destructive;
- can be applied to a wide range of habitats, is easily repeated, and thus provides consistency to sampling;
- can provide very accurate and precise estimates of abundance;
- does not require any specialist equipment;
- provides a robust dataset for statistical analysis.

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Disadvantages

The disadvantages include:

- one quadrat size will generally not encompass all of the species being monitored;
- it is time-consuming compared to semi-quantitative or qualitative methods;
- it only samples very discrete areas within a larger feature.

Logistics

A pilot survey of the area should be undertaken to identify representative examples of species or biotope, depending on the monitoring objective. For the assessment of species richness within a biotope, areas representative of a biotope encompassing most of the characterising species should be chosen. Where key species are being recorded, a transect or individual quadrat locations encompassing the majority of those species in reasonable numbers should be established.

It is essential that the following steps be undertaken:

(1) Define the area in which the quantitative sampling will take place. Moore (2000) recommended an area of uniform habitat, e.g. with consistent characteristics of substratum, inclination, water movement and depth.

(2) Determine community composition of the chosen area by undertaking a broad-scale baseline survey, using MNCR methods[2] or 'by eye' percentage cover estimates.

(3) Decide which species will be monitored within the quadrat and create a 'pro-forma' to aid quadrat counting.

(4) Determine what is the most appropriate quadrat size to use, depending on the size of species and community you wish to monitor.

Equipment

The appropriate transport, navigation and safety equipment is required for undertaking all types of subtidal survey work. The appropriate diving equipment and underwater recording equipment, such as writing boards, underwater communication equipment and cameras (if required) are also necessary to undertake subtidal surveys.

The following additional equipment would also be necessary for quantitative recording work.

Species 'pro-forma'

A list of species should be compiled from the pilot study, including the commonly occurring and characterising species of the community. Moore (2000) recommended that unreliably recorded species not be included, such as cryptic species, very small species, very infrequently encountered species, or ephemeral species that are not characteristic of the chosen community. Mobile species such as crustacea and fish should not be recorded due to their transient nature. There are dangers in adopting this approach, as you are already limiting the assessment of species richness and community information (de Kluijver 1993), and particularly sensitive species groups may be missed: e.g. amphipod species are known to be sensitive to dispersed oil (SEEEC 1998).

Pro-formas should also have space for adding species that perhaps were not recorded during the pilot exercise. They should also provide identification notes for the species which are more difficult to identify in order to aid consistency of recording.

Transects

A transect can be used to help place the quadrats. The transect can be located either randomly or fixed in space; the quadrats can be located randomly the length of the transect, or at fixed positions along its length. Belt transects can be used for the quantitative counts of larger, more widely dispersed species (see Munro 1998; Howson *et al.*). 2000. A transect should be constructed with reasonably thick rope to avoid excessive tangling or knotting and should be weighted or negatively buoyant to prevent it moving in any water current.

2 See Procedural Guideline No. 3-3.

Fixing materials

If fixed stations are to be used, suitable fixing materials such as ring bolts are needed. The method of fixing to the rock depends on the geology and accessibility of the site.[3]

Quadrats

The design of quadrat will vary depending on the species or biotope to be surveyed. Quadrats are of a known area and may be round or rectangular, but are generally square, as these are easiest to construct, easily subdivided into grid-squares and are most amenable to percentage cover estimates (Kingsford and Battershill 1998). Quadrats can be made of any corrosion-resistant material, but should be neutrally or slightly negatively buoyant. When working in areas of kelp where it is difficult to place a full quadrat on seabed, a quadrat with an 'open' end may be used, where the open end can be 'closed' by a line to make a full quadrat; alternatively a two-sided quadrat may be used, with the position of the other two sides judged by eye.

The size of quadrat will vary depending on the survey objective, and the following conditions:

(1) The size range and distribution of organisms to be surveyed. This must take into account the fact that the larger, widely spaced organisms may need to be sampled by a different sized quadrat/transect approach. If there is a high species richness, then smaller quadrats should be considered in order to cut down the time of recording within a single quadrat.
(2) The heterogeneity of the community in terms of species patchiness or variability of substrata. The quadrat should aim to cover a representative range of species/substrata in order to obtain a representative sample of the community.
(3) The diving conditions: e.g. currents and limited visibility make recording by quadrat difficult, so it may be easier to take smaller quadrats to ease the diver's movement through the water. Depth is also a limiting factor in terms if survey time, and the appropriate size quadrat and counting method should be used in order to enable sufficient replicate samples to be taken in a single dive.

The table below provides guidance on the appropriate size of quadrat for the particular community sampled.

Quadrat size	*Community to sample*
$1m^2$	Areas with widely spaced, larger species and colonies, e.g. seafans
$0.25m^2$	Areas with cover of foliose and filamentous algae, e.g. kelp forests
$0.1m^2$	Areas of densely packed small, e.g. circalittoral faunal turfs

Personnel

Divers should posses the required diving qualifications to undertake underwater survey work (for the specified requirements, see Holt 1998).

Experienced marine surveyors, who possess the appropriate identification skills, should undertake this work. The workers should be familiar with the community and the species present: it is recommended that they are shown the checklist in advance of the survey so they can familiarise themselves with the species to be recorded. Pre-survey training in estimating percentage cover in quadrats would also be advantageous to ensure consistent records.

Method

Divers should be fully briefed on how to deploy the quadrat before commencing the survey work. Instructions include the positioning of the quadrat to the relevant marker or transect line, and the rules of what species to count must be established.

In areas where the topography does not vary too greatly, belt transects may be used instead of placing quadrats along a single transect line. These are generally a fixed width and marked into intervals; counts/cover estimates are made in the marked area within the transect.

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3 See Procedural Guideline No. 6-2 for site fixing methods.

Sampling strategy: how many samples to take?

To achieve an efficient and cost-effective monitoring programme, a minimal sampling strategy must be designed in order to gain the correct amount of information with the least effort. This will vary depending on the biotope/species being surveyed.

To determine the number of samples to be taken, a baseline survey has to be undertaken to preferably over-sample the area to gain enough records to undertake analysis. The number of quadrats to reliably monitor change can be assessed using power analysis. Power analysis is a statistical technique which enables estimates to be made of the number of samples required to detect a given level of change (Snedecker and Cochran 1980).[4]

Cumulative species curves can also be used to assess when a population has been sufficiently sampled by a number of quadrats. The cumulative number of species is recorded with each increase in quadrat number until a point is reached when all of the common species have been identified and a further increase in quadrat number will not lead to any further significant increase in species number. Gamble (1984) gave a rough guide to the minimum number of samples as 'that which, if doubled, would yield only a 10% increase in information'.

Kingsford and Battershill (1998) and Moore (2000) recommended that 10 quadrats sampled within a discrete area would give adequate precision to detect notable changes in the whole community. Howson et al. (2000) concluded that between 8 and 12 quadrats would be adequate to detect a change in community of between 15 and 20%.

Sampling strategy: fixed or random quadrats?

In areas where it is difficult to establish fixed locations, random sampling by quadrat may be the most appropriate technique to assess species richness/presence in a monitoring context. Random quadrats have also been used where destructive sampling techniques have been undertaken, e.g. population and condition of seagrass beds (Fowler and Pilley 1992).

The distribution of random quadrats is subject to bias by the worker. It is essential that the placing of a quadrat is not influenced by a diver trying to include a particular species, and it is important to stipulate this prior to sampling. In order to achieve even coverage of an area it may be appropriate to divide the area into compartments, and take random samples within each compartment, or use a randomly placed transect and take random samples along its length. Howson *et al.* (2000) used a 'ladder' transect to aid with randomising quadrats on sublittoral reef communities (see Figure 1). For a full explanation of different methods of randomisation, see Kingsford and Battershill (1998).

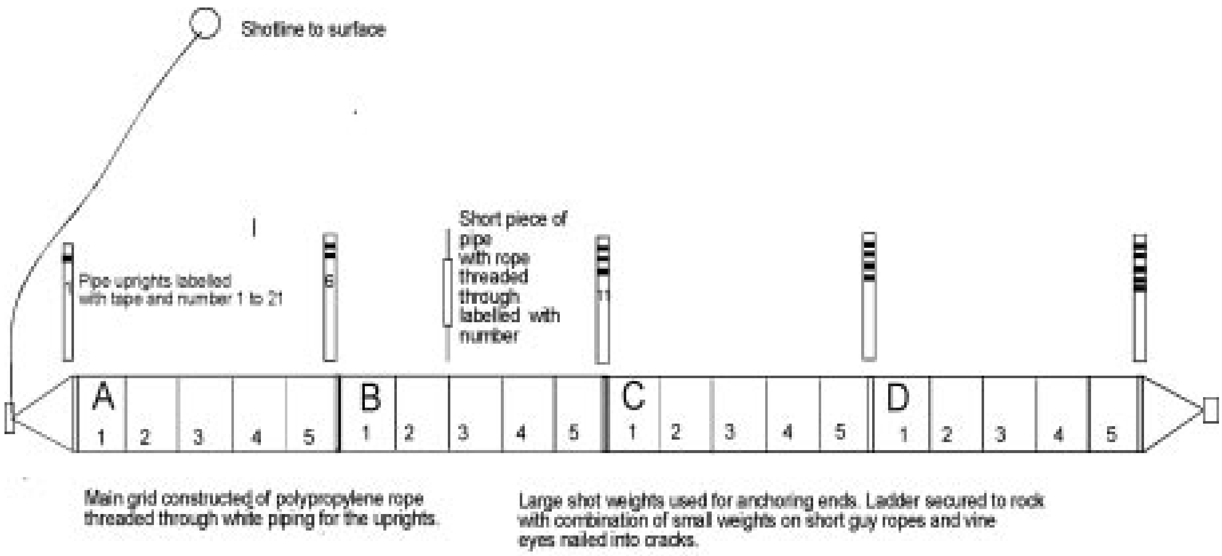


Figure 1 Construction of ladder transect. Each square on ladder measures 1m x 1m (drawing not to scale). To aid orientation by both the divers, the ladder was divided into four blocks, A–D, of five squares, 1–5. Each quarter of a square was a potential position for a quadrat. This enabled random positions to be selected for the quadrat sampling.

4 A comprehensive review of software for power analysis is available at: **http://sustain.forestry.ubc.ca/cacb/power/review/review.html**

Sites for single quadrats may be established and marked for relocation. A permanent marker for the site should be established (possibly a ring bolt in the rock face) and instructions or photographs for relocation constructed. Relocation time will vary with the quality of this information, and the familiarity of workers with the site. One or a number of the corners of the quadrat should be marked for exact repositioning. Moore (2000) used a permanently fixed transect to locate a number of permanent quadrats.

Counting in quadrats

The quantity of a species within a quadrat can be assessed either by numbers of individuals, percentage cover or frequency of occurrence. Usually, for most recording schemes, there will be either a mixture of counts or % cover, depending on the species being assessed. The rules for deciding which is the most appropriate technique are given below:

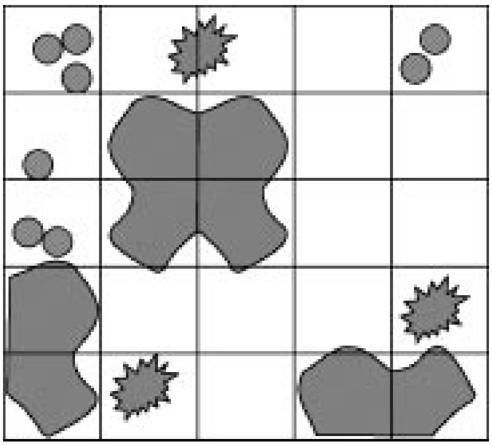
Counts	*% Cover*
Mobile fauna	Flora and fauna forming crusts, mats or turfs
Sessile animals in low abundance, e.g. cup corals	Other ground-covering sessile fauna in high abundance, e.g. barnacles
Sessile erect animals, e.g. hydroids	Canopy cover of foliose algae
Tall algae, e.g. kelp	

There are exceptions to these rules, which will have to be judged on a species-by-species basis, e.g. small patches of hydroids may be easier to assess using % cover, and uniformly sized sponge colonies may be easier to count.

For smaller quadrats (≤ 50cm x 50cm), it is easy to assess percentage cover by eye (de Kluijver 1993), although the accuracy of visual assessment is increased if the quadrat is subdivided into smaller grid-squares (Dethier *et al.* 1993). These smaller squares represent a percentage of the whole quadrat, and the number of squares filled by a single species can be easily counted which will give a percentage for the whole quadrat, with part records from the smaller squares also contributing to percentage cover. This method increases accuracy and may be appropriate where there is a complicated mosaic of species, such as algal turfs, but is much more time-consuming than unaided visual assessment.

Using the gridded quadrat also allows species to be recorded using frequency of occurrence scores. This is where the occurrence of a species within each of the grid squares is counted, giving a 'score' for each species between zero and the maximum number of grid squares. There are advantages in this approach in that it gives a single, simple measure for all species, and is potentially quicker to assess, although Moore (2000) found that the latter was not the case for small $0.25m^2$ quadrats. Frequencies are used as an indicator of abundance, and should not be directly related to actual counts or abundance of a particular species.

A diagram representing species counted in a gridded quadrat is given in Figure 2 below.



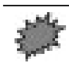
Species	*Count*	*%*	*Freq.*
	8	-	4
	3	-	4
	-	25	8

Figure 2 Representation of a quadrat with corresponding abundance estimates

Data analysis

It is essential that prior to data analysis, the species records are closely scrutinised to eliminate any 'noise' which may affect the analyses. This may involve removing species that are unreliably recorded, e.g. those which are inconspicuous or difficult to identify. Species may be 'grouped' to genus level or higher where there is doubt or some discrepancy between workers as to the identification.

Multivariate and univariate statistics can be used for data analyses. Clustering and ordination methods can look at the variation of replicate samples within a single sampling area, to assess whether sampling had been restricted to a single biotope, and also to assess the variability of the biotope. Packages such as DECORANA and MVSP are very effective at undertaking these analyses. Ordination and clustering methods for community assessment are adequately described in Mills (1994) and Clarke and Warwick (1994).

Multivariate methods can be used to calculate the statistical significance to changes in the whole community (ANOSIM[5]) and highlight the species or suite of species responsible for the changes in community composition (SIMPER[5]). Multivariate techniques are relatively straightforward to interpret as they can present the extent of community change in a single visual graph.

Univariate analyses should be used to assess the significance of any change in the abundance of an individual species, or any changes in the diversity of a biotope. A student's t-test can be used to assess the change in abundance of individual species over time. There are numerous diversity indices that can be used to assess changes in species diversity over time, e.g. the Shannon–Weaver diversity index.

For fully worked through examples of all the statistics mentioned above, refer to Moore (2000) or Howson *et al.* (2000).

Accuracy testing

The assessment of abundance within a quadrat may vary between workers; hence recorders must concentrate on giving an accurate assessment of abundance for each organism. Recording protocols must be prescriptive and carefully adhered to by the survey team. It should be written on the survey pro-formas whether an assessment by actual counts, % cover or frequencies should be undertaken for each species. Pre-survey training out of the water may be useful to familiarise the divers with the recording protocols.

For species counts, knowledge of all species to be counted is essential, especially with the more difficult taxonomic groups where there may be similar species within one quadrat. This should be achieved by using experienced surveyors who have been shown the species checklist in advance so that they can familiarise themselves with the species concerned.

An example of a survey protocol for Plymouth Sound is given in Box 1 (from Moore *et al.* 1999).

5 Both SIMPER and ANOSIM are available as part of the 'Primer' software (see: **http://www1.npm.ac.uk/primer/**).

Box 1 Recording rules established for Plymouth sound monitoring study (from *Moore et al.* 1999)

The primary rule is to ensure that all records are obtained from the same precisely defined habitat. The habitat should be as uniform as possible, i.e. should not include any significant proportion of sub-habitats. This may require the surveyor to exclude or ignore certain sub-habitats (e.g. epiphytes on kelp stipes or the undersides of boulders). The communities present in these sub-habitats may need to be monitored separately; possibly with a different methodology from that used on the main habitat.

A survey duration should be defined. The length of the survey time will depend on the size of the quadrat/transect and on the biotope type. The time spent should be within 10% of the defined time, but the application of this rule will need to take account of the diving conditions. [Note: it should be possible for the diver to set a watch to beep at the end of the defined time.]

Although not proven by the available data, it is considered likely that the quality of the diving conditions will affect the quality of the recorded data. While some environmental factors cannot be controlled, operating rules should specify the threshold conditions for conducting the survey. These should include: available light and clarity of water, water currents, sea state. At the least, a record of the conditions should be maintained. It may also be appropriate to define the required torch beam characteristics (e.g. bright, medium or broad beam torch with fully charged batteries).

A series of rules should also be developed to define the types and forms of animals and plants that need to be surveyed. The abundance of some taxa is very difficult to record with any reliability because of their growth form, mobility or other characteristic. The presence of these taxa in data that are to be analysed quantitatively could reduce the power of the analysis, by introducing a much greater level of recording variability. It should be possible to reduce this variability by eliminating these species from the analysis. It may also speed up the survey if they are not even recorded. Thus, recording checklists should exclude such species and only include species which can be recorded most reliably.

The following species selection guidelines are considered to be appropriate for most situations where the conservation objectives are based on the composition and species richness of seabed communities of conspicuous species.

Quantitative monitoring should focus on species/taxa that are:

- sessile; i.e. not mobile like fish, crabs and gastropods. This is mainly because the presence of mobile species in full view (i.e. not hidden in crevices) can depend on factors such as time of day and other very short-term environmental fluctuations.
- attached to or living on the hard substratum surface (i.e. not epiphytic, except on encrusting coralline algae). This is partly to do with defining the sub-habitat, but also because it is often very difficult to estimate abundance of epiphytes.
- adult or near adult (i.e. not juveniles, spat, sporelings or eggs). This is because the presence of large numbers of juveniles etc. are usually temporary and can bias multivariate analyses. Furthermore, the juveniles of some species (e.g. *Metridium senile*) are produced in large numbers and often settle in habitats for which they are not suited; thus they may not survive there and cannot be considered true members of the community.
- easily recorded with the chosen units (i.e. either percentage cover or counts). Thus, if the chosen units are percentage cover (which will normally be the most appropriate) this rule will probably exclude many solitary erect species (in particular, many hydroids). This rule would need to be defined in greater detail for the specific biotope.

Note: it is emphasised that the application of these guidelines should not prevent the surveyor recording other species or taxa, either qualitatively or quantitatively. On the contrary, additional information will often be useful for more subjective and qualitative assessment of the data. However, objective analysis of the quantitative data should be restricted to those taxa that can be recorded most reliably and which are true members of the community.

QA/QC

For monitoring purposes, it is essential that sites are relocated accurately to give a continuous accurate dataset. A good map of the site is required, and each quadrat should be given a unique reference, so that time series data for a single station can be easily accessed. In terms of sampling the following rules must be applied:

(1) Re-survey of a site should take place at the same time of year (if appropriate).

(2) The same size and shape of quadrat must be used each time.

(3) The same method of counting species (counts or % cover) should be used each time.

(4) For random samples, the same number of quadrats across a broadly similar area are to be counted each time.

Survey personnel must familiarise themselves with the fauna and flora or the area, and should undertake an inter-worker calibration exercise before starting the monitoring.

Cost and time

A full review of the costs and times involved in subtidal quantitative sampling is given in Moore (2000).

The preparation of a checklist of species and the establishment of recording rules may result in reduced survey time or allow more quadrats to be surveyed in the same number of dives. These benefits must be offset against any additional time for undertaking a pilot study to define appropriate recording rules.

If fixed quadrat locations are used, they reduce the spatial variability element in the data and therefore reduce the number of quadrat records needed to detect any temporal changes. However, this saving must be set against the extra time, and therefore cost, required to establish and maintain the fixed locations. In many locations these costs may be very limited, particularly if it is necessary to mark the site for relocation purposes anyway. However, if there are potential problems with marking the site – e.g. on very mobile mixed substrata or at very popular diving sites – these costs may become excessive.

Health and safety

All field staff must follow approved safety procedures published by their host institution, or that of the contracting agency, whichever are the more stringent.

All diving operations are subject to the procedures described in the Diving at Work Regulations 1997[6] and must follow the Scientific and Archaeological Approved Code of Practice.[7]

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7 Scientific and Archaeological diving projects: The Diving at Work Regulations 1997.Approved Code of Practice and Guidance – L107. HSE Books 1998. ISBN 0 7176 1498 0.

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