

Underwater Sampling Techniques

Leon E. Hallacher

University of Hawaii at Hilo

Modified in March 2004 for QUEST

Introduction

Coral reefs are one of the world's most precious natural resources. In an effort to simply better understand coral reefs, biologists worldwide continue to study aspects of their biology and ecology. In addition, basic biological monitoring is often necessary to detect changes in reef systems that might be warning signals of reef degradation. It is the intent of this chapter to introduce surveying techniques commonly utilized in the study of coral reef ecology.

A vast array of methods have been developed and used by biologists to survey marine plants and animals in both soft-bottom and hard-bottom communities. It is beyond the scope of this chapter to cover the myriad of sampling methods available to marine ecologists working in the nearshore environment. Instead focus will be on sampling methods taught and practiced during QUEST which are low to moderate cost techniques for estimating the density of coral reef biota. For a more complete review of sampling methodology, design, and analysis consult Andrew and Mapstone (1987), Cochran (1963), Coyer and Witman (1990), Dewees (1982), Elliot (1977), Green (1979), Greene and Alevison (1989), Holme and McIntyre (1971), Hurlbert (1984), Krebs (1989), Pringle (1984), Reed (1980), Russell *et al.* (1978), Sampford (1962), Southwood (1978), Thresher and Gunn (1986), and Underwood (1981).

Ecological Surveying - General Comments and Considerations

If researchers about to embark on a biological survey of a coral reef had unlimited resources, their study might ideally consist of a search of the entire reef system, with as many animal and plant residents as possible being recorded. Obviously, a survey of this detail would be neither practical or possible. It would be far too expensive and time consuming.

The solution, one that is the foundation underlying ecological estimates of abundance in general, is for researchers to sample subsets of the community. These subsets are commonly in the form of transects or quadrats which are searched for their occupants. The fundamental assumption being made is that the species composition of these subsets reflects or represents the species composition of the community as a whole. If only one or a few species are being targeted for study, then the assumption is that the density of target organisms in the subsets represents their density in the greater community.

A replicate series of samples (i.e. subsets of the community), when properly delineated and searched, can provide an acceptably accurate estimate of the composition and abundance of organisms inhabiting a coral reef. In addition, by subjecting samples to certain types of mathematical analyses, it is possible to determine how particular organisms are dispersed in space, i.e. whether they are randomly distributed, aggregated, or uniformly spaced over the reef. That, in turn, will tell the investigator something about the natural history of the organism in question. For example, aggregated individuals frequently cluster around some needed resource or are attracted to each other for a social function like mating, while uniformly spaced individuals are often responding agonistically toward each other as a result of competition for some limiting resource like food or mating sites. For a detailed review of techniques for estimating the abundance and distribution of animals and/or plants in ecological communities see Krebs (1989 pages 11-169).

Many things need to be considered by the ecological investigator. Perhaps the most fundamental consideration in regard to estimating abundance in animal or plant populations is deciding between measuring *absolute density* or *relative density* (Krebs 1989). Relative density is the density of one population relative to that of another population. For example, on a hypothetical reef in Hawaii, a researcher might ascertain that saddle wrasses (*Thalassoma duperrey*) are more abundant than brown surgeonfishes (*Acanthurus nigrofuscus*) which are in turn more abundant than blue-spot groupers (*Cephalopholis argus*) without ever estimating *how many* individuals of each species there are on the reef flat. Absolute density is simply the number of organisms occupying a unit of area or volume. For example, for each 100 m² of our hypothetical Hawaiian reef flat there might be an average of 18 saddle wrasses (0.18 per m²), five brown surgeonfishes, and one blue-spot grouper (0.05 and 0.01 per m² respectively). Relative density is easier and cheaper to determine than absolute density. However, absolute density is required for most ecological investigations including: 1) studies attempting to correlate density to any vital population statistic like reproductive rate, mortality, growth rates, etc.; 2) implementation of fisheries models; or 3) studies estimating energy flow or nutrient cycles in the community (Krebs 1989).

The researcher is also faced with a decision on whether to utilize *fixed* (permanent) or *random* transects/quadrats. A fixed or permanent transect is located in a specific area, and replicate searches are repeatedly taken from that same location. By contrast, random transects are selected "randomly" (often by random number tables) and the same location is probably never searched twice. Random transects are most appropriately used to estimate species composition and abundance within a community. Each transect will be slightly different in regard to substrate relief and biota present, but when many transects are pooled for analysis, they provide an estimate of the community as a whole. Fixed transects can be used to monitor change in the community at one location. Any differences in biota that are observed from one search

to the next are indicative of temporal change because the transect location is held constant. Fixed transects are often employed where environmental perturbation is suspected or anticipated. If any changes observed on the fixed transect(s) are to be attributed to the perturbation in question, the researcher must remember to delineate and search a control transect(s) in a similar environment where the perturbation is not expected to occur (Green 1979).

Another factor that the researcher must take into consideration is the time at which transects are searched. All too often, a sampling regime is structured around the biological clock of the researcher (i.e. during daylight hours) rather than the activity pattern of the target organism(s). For example, if the goal of an investigation is to identify organisms present on a reef and estimate their relative abundances, then transects need to be searched more or less around the clock since many species are primarily or exclusively active at night (Hobson 1972, Helfman 1983, Ebeling and Hixon 1991). Furthermore, some species will be most active at a particular time of night (or day) so it is important to search transects at different times of day or night. Finally, long-term temporal variations in the presence or abundance of target organisms may need to be considered. A transect regime correlated to lunar phase or seasonal cycle might be appropriate in many situations. In the case of seasons, the researcher must be cognizant to the fact that hydrographic seasonality may not coincide with terrestrial seasons, but rather with periods of upwelling, heavy coastal rainfall, or some other hydrographic dynamic.

Another fundamental question that needs to be addressed is, "*How much sampling is enough?*" This is an important question, one that ultimately forces the researcher to make a cost-benefit decision. The "benefit" of increasing sample size, i.e. increasing the number of replicate transects searched, is an increase in the accuracy of species composition and abundance estimates. In general, the more replicates searched, the more accurate the estimate of community composition. Unfortunately, each transect searched incurs some level of "cost" in the form of monetary expenditure and surveyor time. Clearly, a limit needs to be assigned to the number of transects that are searched, the final goal being a sampling regime which provides an acceptable level of accuracy without being excessive in regard to the number of transects searched.

One simple yet reliable method of determining when a community has been adequately sampled is the plotting of a species accumulation curve. Transect number is plotted on the x-axis, while the cumulative number of species observed after the n^{th} transect is plotted on the y-axis (Fig. 1). Early in the transect series, many of the species observed on each transect are seen for the first time, so the cumulative number of species increases rapidly as mirrored by the steep slope of the plot. As the sampling regime proceeds, fewer and fewer previously unrecorded species are discovered on transects, and the plot begins to level out. When the plot reaches an asymptote, no previously unrecorded species are being discovered on transects, and the community,

by convention, is considered to have been adequately sampled.

Species/Area Curve

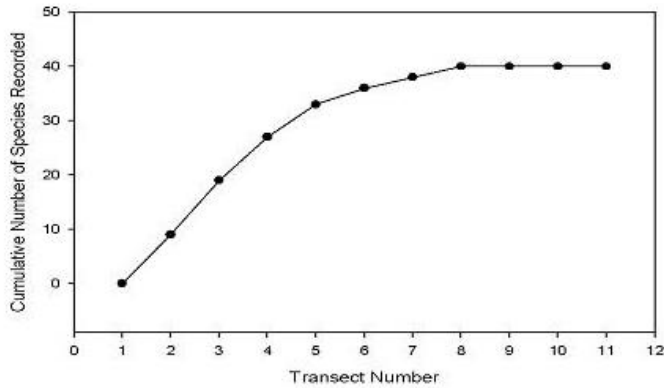


Figure 1. Species area curves depict the relationship between sample number (x-axis) and the cumulative number of species observed (y-axis).

Yet another important factor to be considered in the development of a sound experimental design is the application of statistical analyses to the transect data. It is vital that results be subjected to statistical analysis because without it, most kinds of ecological data cannot be interpreted with any confidence. A paper (or presentation), which reports results of an ecological study that have not been subjected to statistical analysis or that have been analyzed improperly, will not withstand the peer review characteristic of modern ecology.

While statistical design and analysis are treated in detail in a separate chapter, a few basic considerations should be mentioned at this time. First, while weather conditions sometimes make this difficult, the power of statistical comparison of two or more treatments (i.e. Reef A vs a control reef; Reef A during upwelling vs Reef A during nonupwelling; etc.) is enhanced by equal sample size. Effort should be expended to insuring that sample sizes are equal. Second, if treatments are to be compared with any confidence, it is vital that surveying procedures be as identical as possible so that the number of uncontrolled variables are reduced. For example, if the reef slope off Puako, Hawaii was to be compared to that off Honaunau, Hawaii in regard to the composition and abundance of fishes, then all of the following sampling procedures should be observed if possible: 1) the same transecting method should be utilized and the transects should be searched at the same swimming speed; 2) transects should be searched by the same personnel; 3) the same depth should be searched at each location; 4) the transects should be searched at the same time of day, under similar weather and water visibility conditions; and 5) transects at each location should be searched over as short a period of time as possible and the two locations should be sampled as temporally close together as possible to reduced any longer term seasonal effects. Obviously, this sort of "identical" sampling may not always be possible, but an

effort should be made to try to keep unwanted variables to a minimum.

What to Measure - The Need for a Hypothesis

What to measure is a problem fundamental to scientific investigation, and unfortunately, the history of science is filled with examples of measurements that turned out to be irrelevant (Krebs 1989). Even more unfortunate is the fact that many ecologists seem not to have learned from the mistakes of others. All too frequently, especially in field investigations, a wide array of parameters are measured without careful thought being given to whether those data are really needed (i.e. are they applicable to the hypothesis in question?) (Gallagher, personal communication).

Perhaps one reason for this "shotgun approach" to data collection is that a hypothesis was not formulated by the investigator *prior* to the start of field work. They may have in mind the accumulation of "baseline" data or the detection of "change", yet they may not have formalized their thoughts in the form of a hypothesis. That is a mistake. ***Every scientific investigation needs at least one hypothesis!*** Before an investigator goes into the field they should not only have formulated and written down the hypothesis to be tested, but they should have spent a healthy amount of time thinking about data parameters that are relevant to the test of that hypothesis. To do otherwise is to take a chance, albeit slim, that none of the data will be useful (i.e. that they will have completely wasted their time). Most likely, a substantial amount of time and money will be expended not only recording valuable data, but also acquiring useless data. The chances of this happening will be reduced substantially if a hypothesis is formulated before any field work is attempted.

Ten Principles for Sampling

Green (1979), in a book on sampling and statistics for environmental biologists, formulated ten principles for sampling. Those ten principles, paraphrased from Green, are as follows:

1. Be able to state your hypothesis concisely to others as your results will only be as coherent as your initial conception of the problem.
2. Take replicate samples within each combination of time, location, etc., since differences among variables can only be demonstrated by comparison to differences within those variables.
3. Take an equal number of **random** replicate samples for each treatment being compared.
4. To test if a condition has an effect, samples should be collected where the condition

is present ("experimental treatment") and where it is absent ("control").

5. Carry out preliminary sampling to evaluate sampling design and statistical analysis options.
6. Your sampling methods should be equally effective over the range of sampling conditions to be encountered if among-area comparisons are not to be biased.
7. If the area to be sampled has large-scale environmental pattern (i.e. lagoon vs reef flat vs reef slope), divide the area into relatively homogeneous subareas and allocate samples to each in proportion to the relative size of the subarea.
8. Your sampling method and/or sample unit should be appropriate to the size, density, and spatial distribution of the organisms being sampled.
9. Error variation in most field data is *not* homogeneous, normally distributed, or independent of the mean and where appropriate should be transformed for parametric statistical analysis or should be analyzed by nonparametric statistical procedures.
10. After choosing the best statistical method for hypothesis testing, accept the results since unexpected or undesired results are *not* a valid reason to reject the initial method for the purpose of hunting for a "better" one.

Although adherence to these principles may seem like a lot of work, they may actually save the researcher time (especially principle #5) and are likely to improve the final product substantially if followed.

Surveying Fishes

With the possible exception of the corals themselves, fishes are perhaps the most conspicuous residents of coral reefs. No ecological survey of a coral reef could be considered adequate without the resident fishes being characterized. Unfortunately, because of their mobility and behavioral plasticity, fishes are much more difficult to survey accurately compared to sessile biota like invertebrates and plants. Nonetheless, over the years techniques have been developed for estimating the species composition and abundance of fishes. In the most basic sense, fish populations can be estimated by one of two categories of sampling techniques: 1) destructive sampling, and 2) nondestructive sampling (Sale 1980a).

Destructive techniques entail the removal of individuals from the community, so that in the process of estimating the fish population in an area, the researcher alters the

community composition, at least over the short term. Some common destructive techniques used to catch fishes include hook & line, nets, traps, spearing, electroshock, chemical toxins like rotenone, and explosives. All of these methods generally involve killing of the fishes caught, although in mark and recapture estimates of population size, individuals are captured, tagged and released although the eventual goal is to capture them again at a later date.

Most destructive techniques are biased toward the capture of particular species or size frequencies, with some species/size classes avoiding capture completely. For example, carnivores are easier to catch by hook & line than herbivores, although the species caught may depend in part upon the bait used and hook size. The minimum size of fishes caught by nets is dependent upon mesh size, and the species caught will depend in part upon where the net is placed (i.e. on the bottom vs subsurface, lagoon vs reef flat vs reef slope). Explosives and poisons are notoriously nonselective and very effective at capturing the fishes in a particular area of reef [respectively estimated by Sale (1980a) at 98% and 100% effective], but their use entails environmental destruction which is generally unacceptable within the scientific community, not to mention illegal in most situations. Researchers utilizing destructive techniques like nets, hooks, and spears frequently employ more than one of these methods, and will vary hook and net eye sizes. In this way, a more accurate estimate of the overall resident fish population is obtained.

Nondestructive methods do not entail capturing of fishes, so individuals are not removed from the community. In effect, the process of estimating the fish populations by nondestructive techniques does not involve substantive community perturbation. Nondestructive surveying techniques all are based on *in situ* visual surveying of fishes: the researcher enters the community, visually counts fishes present, and then leaves the community. While *in situ* the researcher's presence may result in modification of the normal behavior of some fishes (i.e. some species will be attracted to divers while others will hide), but these behavioral shifts are only temporary. In addition to the obvious conservation benefits associated with nondestructive sampling, these techniques harbor additional advantages. They are relatively inexpensive and efficient in regard to data obtained per underwater hour of observation. Perhaps more important, they are relatively unbiased in regard to species recorded, in comparison to techniques like hook & line and nets.

Thresher and Gunn (1986), in a review of visual surveying techniques, divide all visual surveying techniques into three general categories: spot mapping, transects, and point counts. Spot mapping involves intensive mapping of territories and home ranges of target species at randomly chosen points within the area of interest. While this method is widely used by ornithologists, it has not been used to estimate density of reef-associated fishes. However, territory mapping is commonly done in association with behavioral studies on reef fishes. Thresher and Gunn recognize two general types of

transect survey methods: fixed width strip transects and variable width line transects. In strip transects, the most widely used method for counting fishes, the observer proceeds down a transect line counting all subjects visible within a specified distance from the line. In variable width line transects, used almost exclusively in terrestrial systems, the observer proceeds down a transect line and records the perpendicular distance between the line and each individual sighted. Those data are then used to calculate density of the various species seen along the transect line. The third survey category, point counts, involves counting individuals at randomly or systematically determined locations (points) within an area of interest. Point counts can involve counting individuals in an area or traversing some specified position for a specified period of time (interval counts), or can involve counting all individuals within a specified area independent of time required (instantaneous area count). For further details, consult Thresher and Gunn (1986).

The balance of this section will focus on the fixed-width strip transect procedure because it is so widely employed in the surveying of reef fishes in both tropical and temperate habitats. However, some variations of the strip transect and point count methods will be discussed, though in less detail.

Fixed Width Strip Transect

The most widely used method of visual surveying of fishes is fixed width strip transect, commonly referred to in the literature by a number of terms including strip transect, line transect, belt transect, or "Brock" method. In an early paper, Brock (1954) described the effectiveness of the strip transect in evaluating reef fish populations at selected localities around the islands of Hawaii and Oahu. As a result of this pioneering paper, the strip transect method is also referred to as the "Brock" method by many ecologists around the Pacific Basin. The strip transect method is used to estimate absolute densities, as opposed to relative densities, because it not only provides information on species present, but also on their actual abundance per unit of survey area.

The basic methodology is simple. A transect line is deployed on the bottom, most often parallel to shore along a specific depth contour. After the line is fully deployed, the divers wait for a minute or two for fishes disturbed by the deployment process to resume normal activity patterns. The divers then proceed down the transect line, one on each side, recording all fishes seen in the water column and on the bottom on their side of the transect line (Fig. 2). Fishes seen are recorded, most often on underwater writing slates or data sheets, although full face masks with voice recording devices have been employed so that the diver verbally records fishes seen (Coyer and Witman 1990). Although most often the transect line is deployed and searched near the bottom, in some habitats it may sometimes be appropriate to deploy and search additional lines in midwater and/or near the surface (for example, in *Macrocystis* kelp

forests).

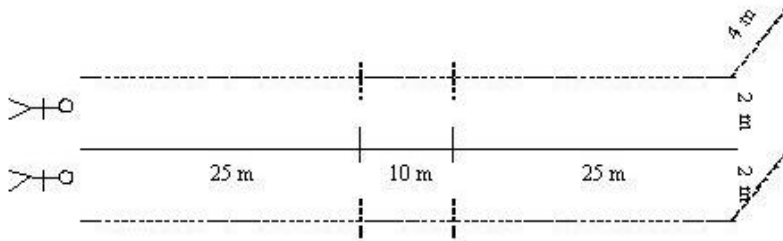


Figure 2. Divers searching two 25 m strip transects, separated by a 10 m buffer zone.

During the search of the transect, divers should remain side by side. They should proceed at a constant slow swimming speed, or they may stop at regular intervals to record fishes within a predetermined distance in front of them, say five meters, before proceeding down the line to the next stop. Divers should decide *a priori* whether or not to record fishes that swim onto their side of the transect from the other side of the line. Whatever methods are decided upon, replicate transects should be searched in the same manner.

At QUEST, each diver searches a lane approximately two meters wide on each side of a transect line extending 25 meters in length. If greater precision in defining the transect is necessary, two lines can be deployed parallel to and on each side of the transect line at a distance of two meters, effectively making a rectangular quadrat 25 meters by 4 meters. It is prudent to remember, however, that deployment of additional lines, while improving precision, also adds significantly to the time it takes to complete a transect search.

The transect size that is finally decided upon will depend upon the community being sampled, although the length should allow transects to be searched completely within the boundaries of a particular habitat type (i.e. lagoon, reef flat, reef slope). While transect size proposed by Brock (1954) was 450 m long by 12 m wide, most workers have opted for far shorter transect lengths, usually between 25 and 100 m in length (see Sale 1980a, Sale and Sharp 1983).

In some situations, the researcher will need to record more than just the number of individuals of each species seen. If the position of each individual is recorded relative to the bottom, it is possible to evaluate habitat specialization among species present (for example, whether a particular species which lives on the reef slope is benthonic, demersal, midwater, or neustonic in regard to habitat utilization). If size estimates are obtained on individuals of a particular species, those data can be used to estimate the biomass of that species in the area being sampled. Lengths can then be transformed into weights by the equation, $W = A(L^3)$, where W is the calculated weight, L is the

estimated length, and A is a species constant based on known weights and lengths for the species involved (Brock 1954, Russell *et al.* 1978).

Obviously, accurate estimates of vertical position or size are necessary if these data are going to be of any value (for example, the accuracy of biomass calculations is directly correlated to the accuracy of length estimates made in the field). Unfortunately, accurate estimation of vertical position of individuals on transects or their size can be extremely difficult. Because objects viewed through a face mask appear closer (larger) than they really are, distance can easily be underestimated and size overestimated. For example, a fish which appears to be about three feet above a diver is in fact approximately four feet above them (true distance = apparent distance x 1.33). In effect, objects are magnified by a factor of 1.33, so a fish which appears to be 4" in length is in fact just 3" long (true length = apparent length x 0.75). Some attempt must be made to minimize error in the estimation of distance or size. Calibrated lines suspended vertically above the transect by floats can be used for estimating the distance of fishes above the bottom, but the process of deploying additional lines is logistically cumbersome. An alternative is to simply correct distance estimates by multiplying them by a factor of 1.33. To reduce error in size estimation, diver training programs have been employed which utilized preserved fish of known size, pipe of various lengths, and even calibrated face masks (see Bell *et al.* 1985, Coyer and Witman 1990, and Swenson *et al.* 1988 for details).

As stated earlier, the principal advantage of the visual transect for counting fishes is that it is the least destructive method available for surveying fishes. Additionally, the visual transect gives a more accurate estimate of fishes present than most destructive sampling methods. However, the visual transect is not bias-free, and several sources of error are discussed below.

Perhaps the most common source of error in visual surveying is the underestimation of individuals present because of the observer's failure to see every fish (Sale 1980a, Brock 1982, Sale and Douglas 1981, Sale and Sharp 1983, Ebeling and Hixon 1991). In addition, cryptic or rare species will often be missed during visual transects (Brock 1982, Sale and Douglas 1981, Sale and Sharp 1983). Brock (1982) did a rotenone follow-up of three replicate visual surveys of a 1500 m² patch reef in Kaneohe Bay, Hawaii. Brock's results indicated that diurnally exposed species were usually detected by visual survey (91% were detected), but that highly abundant species were often underestimated (by more than 50%) by the visual method. In addition, many cryptic or rare species were missed completely (53 species were recorded on the visual survey, while 81 were obtained during the subsequent rotenone collection). Sale and Douglas (1981), working on the Great Barrier Reef (GBR), visually surveyed all fishes residing on a replicate series of small patch reefs (0.75 - 2.4m in diameter). Using this visual method they were able to record 82% of species present and 75% of individuals.

An additional source of error, in regard to the underestimation of individuals on visual transects, has been reported for schooling fishes which are consistently underestimated by observers (DeMartini and Roberts 1982). Visual estimates of schooled or aggregated species on transects may record less than half of the individuals actually there (Davis and Anderson 1989). In summary, the visual surveying method is most often biased toward the underestimation of both individuals and species present including: large, mobile, solitary species like jacks and sharks; mobile schooling fishes like halfbeaks or opelu; nocturnal species like squirrelfish or cardinalfish; and especially fishes which are diurnally cryptic or rare.

Also, though less of a problem, certain species may be *overestimated* by the visual method. For example, at slow observer swimming speeds, individuals of mobile species like wrasses may be counted more than once and hence their abundance overestimated. Lincoln Smith (1988) evaluated the effect of observer speed on visual transects searched on reefs near Sydney, Australia and discovered that it has a pronounced effect on the efficiency of the strip transect. At slow speeds, mobile species were overestimated because they moved around on the transect and were often counted multiple times. At fast speeds, cryptic or small fishes were severely overlooked, although subsequent use of rotenone confirmed that highly cryptic species were overlooked at all speeds. When replicate visual transects are to be searched, observer speed should be standardized to minimize variance from this source of error. An alternate approach is to split fish surveys into two types of transect searches; one at slow observer speed to search for cryptic or rare species and the other at regular speed to search for other fish species (Lincoln Smith 1989).

Transect size can also influence accuracy of density estimates and is hence another potential source of bias. Sale and Sharp (1983), again working on the GBR, visually surveyed transects of variable width (1 to 3m wide). They found that density estimates were negatively associated with width (i.e. a higher percentage of individuals were missed on wider transects) and proposed a technique for correcting this source of bias on visual transects. McCormick and Choat (1987) also evaluated the influence of transect size on accuracy and cost-efficiency of visual transects. Working in the temperate waters of New Zealand, they surveyed mapped populations of a common morwong species (Cheilodactylidae, *Cheilodactylus spectabilis*), using visual strip-transects of five different sizes (15x5, 20x5, 25x10, 37.5x10, 50x10 m). They found the 20x5 m transect size to be the most consistently accurate estimator of true density of their target species. When the time needed to search the various transects was factored in, they found the 20x5 and 37.5x10 m transect sizes to be essentially equal in cost-efficiency. While the 20x5 m size had slightly higher statistical precision, the 37.5x10 m transect was more time-effective because fewer transect deployments were required to search an equivalent area of habitat (for example, 16 deployments for the larger transect vs 30 for the smaller one to search 3000 m² of reef). Clearly,

researchers planning to survey fishes visually should be aware that transect size can influence both the accuracy of their density estimates and the efficiency of their search.

Another factor that can affect the accuracy and efficiency of visual survey methods are water conditions like visibility, surge, and water temperature. In situations where visibility drops below 3-4 m, which are rare in the tropics but common in temperate waters, it is difficult for the observer to see and identify fishes (Ebeling *et al.* 1980, Larson and DeMartini 1984, personal observation). In addition, when water visibility is poor, fishes may be more easily startled by a diver's presence so that they hide more readily and are therefore difficult to observe (personal observation). Surge, which can be substantial in waters less than 100' deep during even mild storm events, causes many fishes to seek shelter in cracks and crevices making them much more difficult to record during visual surveying (Quast 1968a,b,c; Gotshall 1987; personal observation). In addition, during heavy surge the observer may find it difficult to concentrate on data recording because at least part of their attention must be devoted to avoiding contact with the bottom as surge whips them back and forth. Diver hypothermia, whether caused by relatively short exposure to very cold water or by improper thermal protection on long tropical dives, can cause divers to suffer loss of body movement, concentration, or even mental acuity, which makes their data highly suspect in regard to accuracy (Bowen 1968, Ebeling and Hixon 1991, personal observation). Because of reduced visibility, water temperature, and storm surge, visual surveying is very difficult to do on many temperate reefs during a substantial portion of the year (Ebeling and Hixon 1991). Conversely, coral reefs can be visually surveyed almost year around, except during tropical storms (Ebeling and Hixon 1991).

In spite of biases and other potential short-comings, the strip transect method is still one of the simplest, most effective, and ecologically "benign" ways of estimating composition and abundance of fishes on coral and temperate reefs. Also, because of its nondestructive nature, the visual method may be the only politically viable method available to the researcher, even if they have the time and money to utilize destructive sampling techniques. Because of its major advantages and minor liabilities, visual surveying, whether by strip transect or some modification of that method, is almost certainly the most common technique utilized to evaluate reef fish communities worldwide.

Variations on the Strip Transect

A number of variations on the strip transect exist, most of which provide an estimate of absolute density of fishes. Perhaps the simplest modification is the recording of strip transects on cinemagraphic film or video tape (Alevizon and Brooks 1975, Ebeling *et al.* 1980, Larson and DeMartini 1984, Coyer and Witman 1990). The observer swims the transect with a movie camera or video camera in an underwater housing, recording fishes on the transect. Video cameras can also be deployed on

tripods for continuous recording of fishes in one area without potential diver interference with normal fish behavior (Coyer and Witman 1990). Cine/video can also be combined with deep submersible or remotely operated vehicle (ROV) technology to search transects far below the depth range of divers on scuba (Stein *et al.* 1992). Of course, deep submersibles can also be used as a platform for real-time transect searches by scientific personnel aboard the submersible (Colin 1974, Stein *et al.* 1992).

The obvious advantage of cine/video technology is that a permanent record of each transect is obtained, and it can be reviewed later in the laboratory. This methodology also has potential problems including expense, equipment failure, difficulty in estimating size of fish from the film/video format, and difficulty in identifying mobile and cryptic species because of film/video acuity which is, even by modern standards, less than that of the human eye.

Coyer and Witman (1990) briefly describe a circular plot method for visually surveying fishes. While this method could arguably be viewed as a variant of the interval point count method which is discussed in the next subsection, it is treated here because the size of the survey areas searched by this method can be large, approaching that of conventional strip transects. In the circular plot method, a transect line is deployed in the habitat to be surveyed. At randomly located points along the transect line, the surveyor records all fish within a 360° arc during a defined period of time. The time needed to adequately sample fishes within the circle will depend upon the radius of the arc. The radius can be a measured distance or can be arbitrarily set as the limit of water visibility. If water visibility is used, it should be recorded from the transect line so that sample area can be determined later. In clear tropical waters, visibility limits would not be a practical method for defining the arc's radius, because the sample area would be too large to survey accurately. Since each plot can be treated as a separate replicate, the plots along the transect line should not overlap if they are to be considered independent samples for statistical purposes. This method is generally faster than transects but the results may be more variable (Coyer and Witman 1990).

Davis and Anderson (1989) experimented with a new technique which combined a variation of the Schnabel mark-and-recapture method with the strip transect method. They compared the results of this technique to population estimates from visual transects, color video, and timed counts at baited stations. Working in the kelp forests of southern California's Channel Islands, they captured four "target" species of fish underwater with a diver-controlled electrofishing apparatus, tagged them, and then released them. Over the next two weeks, 16 equally spaced lines were searched (22.5° apart, up to 800 m long) radiating from the point of release of the captured fishes. The tag status (tagged, untagged, or unknown) of all target fishes sighted within 10m of each radial transect line and within 3m of the bottom was recorded. Modified Schnabel population estimates were then calculated using Ricker's (1975) equation:

$$N = \sum_{x=1}^{n \text{ dates}} (C_t M_t) (R+1)^{x-1}$$

N = Population estimate
 C_t = Total fish seen on day t
 M_t = Total marked fish at large at beginning of day t
 R = Total re-sightings during the experiment

Davis and Anderson found that all of the three alternate methods utilized (visual transects, color video, and timed counts at baited stations) consistently and significantly underestimated densities of the target species relative to density estimates derived from the *in situ* mark-and-resight method. Of the three alternate methods, they concluded that visual transects were the most accurate, but advised caution in interpreting results of visual transects (at least in kelp forest environments) because of their apparent pronounced underestimation of fish densities. They also concluded that the *in situ* mark-and-resight technique was probably prohibitively expensive for regular use (13-15 times more diver time is needed to produce a mark-resighting estimate of population density compared to a visual transect based estimate).

Point Counts

In addition to transects, point counts represent another category of visual surveying technique which have been used to estimate composition and abundance of fishes (Thresher and Gunn 1986). This technique involves counting individuals at randomly or systematically selected locations within a study area. Point counts can be made during a set period of time (interval counts) or can involve the counting of all individuals at a designated point without a time limit being imposed (instantaneous area count).

Interval counts have been utilized to survey fishes, but not widely. This method assumes that each individual traversing the survey location is counted only once, although in reality this assumption is likely to be erroneous to some degree (Thresher and Gunn 1986). In addition, interval counts provide only a relative index of abundance since they are based on rates of movement through a limited survey area (Thresher and Gunn 1986).

Gotshall (1987) utilized an interval count method at baited stations to estimate relative abundances of fishes inhabiting inshore temperate waters. At a series of randomly selected stations on the bottom, divers deployed a bait-filled canvas bag attached to the middle of two lengths of 2m long chain. When deployed, the chains formed a cross, the arms of which represented the radii of a circle two meters in

diameter. In the center of the imaginary circle was the baited canvas bag. For a period of ten minutes, divers counted all fishes that entered the circle within 1m of the bottom. While the baited station method is biased toward counting of species attracted by the bait, it nonetheless has some advantages over conventional strip transects. Each circle replicate takes about one third the time needed to search a 50m transect so more replicates can be searched. During periods of surge and low visibility (common in temperate waters), divers are able to concentrate on counting fishes and are not distracted by difficulties in swimming a transect. Also, baited stations attract some cryptic species that would normally be missed by divers searching a strip transect.

Interval counts taken without the influence of bait eliminate the bias of species-specific attraction. Slobodkin and Fishelson (1974), working in the Red Sea, studied the influence of the cleaner wrasse, *Labroides dimidiatus*, on the distribution of other fishes on the reefs off Eilat. They deployed a 10m transect line along the reef slope. They then stopped at the end of the 10m line, moved out perpendicularly from the reef face about 5m, and counted all fishes coming within 1m of either side of the transect's end during a 20-minute survey period. This procedure was repeated 60 times so that 600m of reef face was systematically surveyed. Slobodkin and Fishelson were able to convincingly demonstrate that cleaning wrasse territories acted as a focus for the aggregation of other reef fishes. Cleaners were observed at 13 of the 60 point-count replicates, and species richness and the number of individuals observed was highest at those 13 stations.

Instantaneous area counts have been more widely employed to survey fishes than interval counts. In this method, all individuals within a specified area are painstakingly recorded, with the survey area usually being small and well-defined (Sale 1980a). This method has been used to survey fishes on small patch reefs in the Caribbean and Indo-Pacific, on artificial reefs, on limited areas of naturally demarcated habitat on coral reefs, and on quadrats established on otherwise undifferentiated benthic substratum (Sale 1980a).

In instantaneous area counts, mean density can be estimated by dividing the grand total of individuals seen by the cumulative size of the survey area. Potential biases in this method are similar to those of strip transects including errors in area estimation, observer effects on fish distribution, and problems of subject detection (Thresher and Gunn 1986). The typically small survey areas that are searched by this method may help to reduce the effect of these inherent biases. In cases where the search areas have been larger than about 5m in diameter, attempts to count all individuals present are usually frustrated (Sale 1980a).

Estimates of Relative Density

Most of the methods discussed so far provide an estimate of the *absolute* density of fishes (i.e. number of individuals per unit area). As stated earlier, estimates of absolute density are time intensive and may provide a level of precision that is not needed. For example, a researcher may simply need to know what species of fishes reside on a particular reef, or they may need to have some idea about which species are the most common on the reef without needing to know their density. In those situations, methods providing estimates of *relative* density are more appropriately utilized because they require less time.

The simplest and fastest of *in situ* surveying methods is the presence/absence timed swim survey (Reed 1980), a method providing no information on relative or absolute abundance. Prior to entering the water, the surveyor sets a specified length of time for the visual survey. The surveyor then swims freely within the habitat of interest recording species seen. No other data are taken. Search time should not be excessively long (i.e. it should not result in surveyor fatigue or decompression problems). Replicate timed swims should be searched until the habitat has been adequately sampled (for example, the point at which a species accumulation curve levels off). This method provides an estimate of species composition and has the advantage of being fast and therefore inexpensive.

There are a number of relatively fast *in situ* methods which can be used to estimate the relative abundance of fishes on the reef. All of these methods are based on the assumption that the probability of encountering a species increases with its abundance (Jones and Thompson 1978, Sale 1980a, Coyer and Witman 1990). In essence, the more common the species, the more likely the researcher is to encounter it. Stated another way, the more common the species, the *sooner* the researcher is likely to encounter it after entering the water or starting a transect. All relative abundance estimating methods are based on this inverse relationship between abundance and the average time to first encounter. The methods described below are the "Reed" method, the rapid visual transect method (RVT), and the species accumulation survey method (SAC).

Reed (1980) described a relative abundance estimation method which employs a large number of divers who simultaneously survey a target area. In this method, 5-10 surveyors enter water simultaneously. Each observer swims freely within the target habitat for a specified period of time (determined prior to the survey). Each diver records species seen in order of sighting (ex. Sp. B=#1, Sp. J=#2, Sp. A=#3, etc.). Once recorded, a species is ignored on subsequent sightings. Surveyor scores are then summed for each species (they can be averaged). The species with the lowest score is considered to be the most abundant species in the target area while the species with the highest score is regarded as the rarest species.

Jones and Thompson (1978) developed the rapid visual transect or "RVT" method for *in situ* estimating of the relative abundance of fishes. The observer swims freely within the target habitat for a specified period of time (ex. 50 mins). The survey period is divided into intervals (ex. 10 mins). Each species sighted is recorded only once and assigned to a time interval in which it was first seen. Species are assigned scores based on the interval in which they were seen:

<u>Time</u>	<u>Interval</u>	<u>Score</u>
00:00 - 09:59	1	5
10:00 - 19:59	2	4
20:00 - 29:59	3	3
30:00 - 39:59	4	2
40:00 - 49:59	5	1

This procedure is replicated until the community has been adequately sampled (i.e. species accumulation curve, etc.). The species with the highest average score is the most abundant, while the species with the lowest average score is regarded as the rarest species.

Compared to the strip transect method, RVT overemphasizes the importance of widespread though rarer species while under-estimating patchy but abundant fishes (DeMartini and Roberts 1982). The effect of this bias depends upon the habitat being sampled. For example, on coral reefs where fishes would best be characterized as having a large number of relatively rare species, the RVT method is likely to overestimate the relative abundance of many species (Ebeling and Hixon 1991). Conversely, temperate kelp forest fishes are often dominated by a few abundant species that tend to aggregate, and the RVT method underestimates their relative abundance (Ebeling and Hixon 1991). Because of the inherent differences in water conditions and the way fish behave between temperate and tropical reefs, sampling accuracy makes it difficult to compare results of visual surveys taken in each environment, even if the same method is utilized (Ebeling and Hixon 1991). As a final note, Sanderson and Solonsky (1986) compared RVT to strip transect technique (STT) and found RVT to be less precise (i.e. subject to higher among replicate variance).

Kaufman and Ebersole (1984) developed the species accumulation survey method of estimating relative abundance of fishes. The surveyor swims freely within the target habitat carrying a stopwatch. They stop the watch when the first species is seen, record the time, and then restart the watch. They stop the watch when second species is seen, record the time, and then restart the watch. This process continues until the rate of new species sightings drops to some specified level (for example, a rate equal to or less than 2 new species in a 10 min interval). At the end of each survey, every species' time of first encounter is entered into a data base. Surveys are replicated until

the community has been adequately sampled. The species with shortest average sighting time is regarded as the most abundant species while the species with the longest average sighting time is the rarest. An added advantage of this method is that different areas can be compared for species richness via their species accumulation curves (Fig. 3).

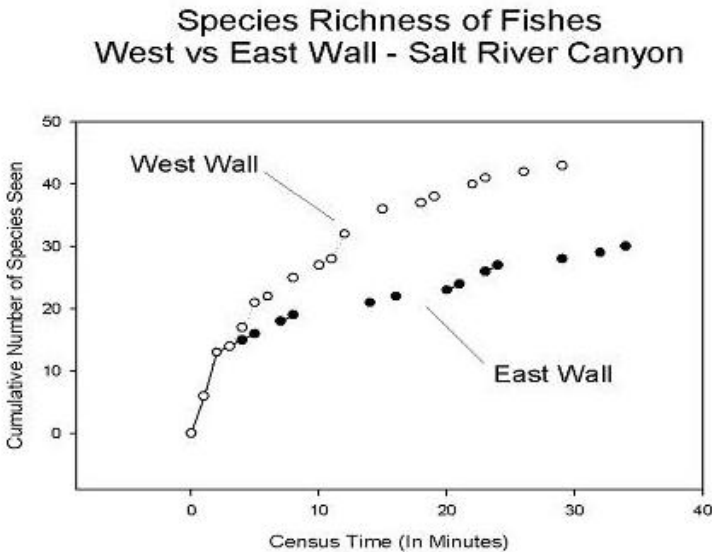


Figure 3. Species richness comparisons of two Caribbean reef areas made by species-time accumulation curves (modified from Kaufman and Ebersole 1984).

Surveying of Coral Reef Invertebrates and Algae

In contrast to the fishes, which are exceedingly mobile animals, much of the life on coral reefs is either permanently attached to the bottom or moves very slowly. These taxa include such things as macrophytes, the corals themselves, molluscs, annelids, crustaceans, and echinoderms. The limited mobility of most of the species in these taxons makes estimating their abundance somewhat easier than for fishes.

There are a host of sampling procedures that can be used to estimate the abundance of algae and invertebrates in marine systems. Two basic categories of sampling technique that are frequently used are 1) line transects and 2) area sampling via quadrats. In line transects, organisms or substrate lying under a transect line are commonly recorded. This is a quick method for determining the relative abundance of epifauna like corals and algae. In a common variant of the line transect, the distance between individual macroinvertebrates like urchins and randomly selected points along a transect line are employed to calculate the density of the target species. In quadrat sampling, a known area is searched for organisms or nonliving substrate. The procedure can involve surveying everything contained within the quadrat's borders, or

just the enumeration of a target species like a polychaete or an echinoderm. Basically, depending upon the technique employed, line transects or quadrats can be utilized to survey attached epifauna like corals and algae, or macroinvertebrates like echinoderms, snails, polychaetes, and crustaceans. Line transect and quadrat sampling procedures utilized during QUEST are reviewed in greater detail in subsequent subsections. Before proceeding, however, several considerations concerning quadrat selection need to be evaluated.

When selecting a quadrat to be used in a study, an important question that needs to be answered is, "What size and shape is best?" Unfortunately, the answer is not simple. Two factors frequently considered in regard to quadrat selection are: 1) statistical precision and 2) searching cost (Krebs 1989). Searching cost is proportional to the time it takes to search a quadrat; the more time spent searching the greater the cost. In general, the larger the quadrat, the less time it takes to search *per unit area* of quadrat (Pringle 1984). In other words, to sample 100 m² of bottom with a 1/4 m² quadrat might take over twice as long compared to sampling with a 1 m² quadrat and almost five times as long compared to a 4 m² quadrat (based on actual data from Pringle 1984). If cost alone was the deciding factor determining quadrat size, then larger quadrats would almost certainly be chosen.

However, if statistical precision were more important (as is often the case in ecological investigations), an investigator would want to select the quadrat that produces the minimum standard error of the mean (SE) (Krebs 1989). According to Krebs, statistical precision can be increased by minimizing edge and habitat heterogeneity effects. Edge effect occurs when an animal or plant falls under the border of the quadrat. Is that organism inside or outside the quadrat? Surveyor indecision leads to increased variance among replicate quadrats. Edge effect can be reduced by decreasing edge length relative to quadrat area. In other words, a circle-shaped "quadrat" would have less edge effect than a square. A square, in turn, would have less edge effect than a rectangle.

If edge effect alone determined statistical precision, a circle would be the best shape quadrat to select. Unfortunately, habitat heterogeneity effect also needs to be considered. Sample areas are rarely uniform in regard to the distribution of contained biota; organisms are usually distributed somewhat patchily (Krebs 1989). Replicate long and thin quadrats almost always have lower variance than circles or squares because they cross more patches (Krebs 1989). In other words, taking only habitat heterogeneity into consideration, rectangular quadrats would produce the greatest statistical precision.

Clearly, quadrat selection involves the interaction of at least three variables; searching cost, edge effect, and habitat heterogeneity effect. So what shape of quadrat is the best to use? There is apparently no *a priori* method to answer this question. The

researcher can consult the literature to see the size and shape of quadrats used by earlier workers, but often those workers have not explained why a particular quadrat shape or size was used (Pringle 1984). Pielou (1974) recommends that before conducting a sampling program, a separate investigation on sampling design be done to ascertain the most efficient quadrat size. One commonly utilized way to ascertain the best quadrat design is to test a number of sizes and select the quadrat that is the most efficient (i.e. produces the lowest product of searching cost x statistical precision).

Pringle (1984) for example, prior to beginning a sampling program to estimate biomass of Irish moss (*Chondrus crispus*) an important commercial algae, conducted a study to determine the best quadrat size. Pringle began by reviewing quadrat shapes and sizes used in 21 previous studies of biomass or density in habitats similar to that occupied by Irish moss. The results of this survey indicated that the most commonly utilized quadrat shape was a square (66.7%), followed by circles (19%) and rectangles (14.3%). The most commonly used quadrat size was <0.25 m² (42.8%), followed by 1.0 m² (28.6%), 0.25 m² (23.8%), and >1.0 m² (4.8%). The results of this survey were as follows:

	Area (m ²)				Total
	<0.25	0.25	1.0	>1.0	
Squares	3	4	6	1	14
Circles	3	1	0	0	4
Rectangles	3	0	0	0	3
Total	9	5	6	1	21

In regard to these data Pringle remarked, "The workers did not give reasons why particular sample unit shapes or sizes were chosen."

Pringle then tested various sized quadrats (0.25, 1.0, 1.56, 2.25, 2.99, and 4.0 m²) to ascertain their efficiency in sampling 20 m² of benthos for Irish Moss biomass. He found a general inverse relationship between quadrat size and search time per unit area. Using the two smallest quadrats (0.25 and 1.0), the total time required to search 20 m² was 696 and 364 minutes respectively. For the four larger sizes, the range of times needed to search 20 m² was 183-326 minutes. In Pringle's investigation, the quadrat that sampled the 20 m² in the least amount of time was 2.25 m² (183 minutes required). The percent savings in time using this quadrat, compared to the other quadrat sizes, ranged from 12.9% (vs 4.0 m²) to 74.8% (vs 0.25 m²). Based on time alone, the 2.25 m² quadrat would be selected. However, when Pringle considered sampling efficiency (statistical precision x searching time) the final selection was quite different. The 0.25 m² quadrat size was by far the most efficient size because of a much lower SE relative to the other quadrat sizes. Pringle concluded, "Based on these results, I suggest the use of a 0.25 m² sampling unit or smaller when sampling benthic organisms that are the size and distribution of macrophytes sampled here."

In conclusion, selection of the best quadrat size and shape for benthonic sampling is not a simple process and depends upon which selection criterion is most important to the investigator (search time, statistical precision, or efficiency). In any event, a preliminary study to empirically determine the best quadrat size may save the worker considerable time and expense in the long run. For more information on determining optimal quadrat size and shape see Krebs (1989, pp. 64-72) or Pringle (1984).

Surveying Attached Epifauna

A large portion of the biomass on coral reefs consists of organisms that are permanently attached to the substratum, like corals and algae. To survey attached epifauna two methods are employed during QUEST: point quadrat search and percent cover estimation. Both methods require the use of quadrats. Quadrats used during QUEST to survey epifauna are square, one half meter on a side, making them 0.25 m² in area of coverage. While there are many types of quadrat construction (see Reed 1980 or Coyer and Witman 1990), QUEST quadrats are made of PVC pipe that has been weighted with lead and drilled to permit water to fill the piping upon submergence.

Both point quadrat search or percent cover estimation require the deployment of a transect line which has meter graduations on it. Once the transect line is deployed, the divers proceed down the line to a search location designated by the first number of a random number series. The quadrat is then placed on the substrate and searched using the desired method (at QUEST, both methods are used during each quadrat placement). Quadrat placement can be to either side of the line or in the middle of the line as long as the placement location is consistently utilized at subsequent search locations on the transect line. Once the quadrat has been searched, the observer proceeds down the line to the next random number position and the search procedure is repeated.

Quadrat Intersect Method

The point quadrat or quadrat intersect method utilizes a quadrat that is partitioned by a series of lines which intersect at regular intervals. Quadrats at QUEST are strung with a total of six lines arranged in two parallel sets of three, oriented at right angles to each other (Fig. 4). This design creates nine points of intersection. After the quadrat has been placed on the bottom, organisms or substrate type located under each intersection are recorded. QUEST quadrats are actually "double-strung", so that one complete set of lines lies about 1" above an identical set. The observer must line up the upper and lower points of intersection before recording what lies under them. This eliminates parallax problems based on the observer's position (i.e. what appears to lie under a single set of intersecting points may differ depending upon the observer's

position relative to the quadrat).

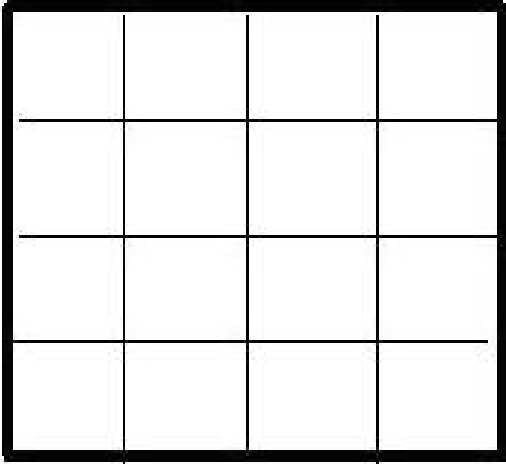


Figure 4. A quadrat strung with parallel lines to produce nine intersecting points.

The quadrat intersect method provides an estimate of the abundance of epibenthic organisms like corals and algae. The principal advantage of this method, and the reason that it is commonly utilized during underwater sampling, is that each quadrat can be searched quickly. This means that many replicate quadrats can be searched during a dive. The method's principal disadvantage is that uncommon and rare species rarely fall under intersecting points and are therefore not recorded.

Percent Cover Estimation

The percent cover estimation method requires the observer to estimate the percentage of quadrat area filled by each taxon or substrate type. Although this can be done for an entire quadrat, accuracy is enhanced if the quadrat is partitioned into smaller subunits, with percent cover being independently estimated for each subunit. In general, the smaller the subunit, the easier it is to estimate percent cover of included organisms. However, the more subunits there are requiring separate percent cover estimations, the longer it takes to search the entire quadrat. At QUEST, the 0.25 m² quadrat is partitioned into 16 equal subunits by the two sets of intersecting lines (Fig. 4). However, students are asked to estimate percent cover from four of these units combined (i.e. as if the 0.25 m² quadrat were divided into four equal subunits) (Fig. 5).

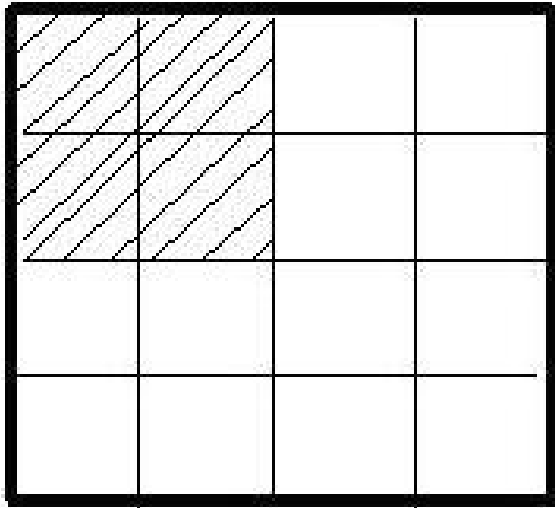


Figure 5. During percent cover estimation, a 0.25 m² quadrat may be searched by quartiles (cross-hatched area) of approximately 0.063 m² each.

The principal advantage of the percent cover estimation method is that it provides a census of the actual area of bottom covered by each taxon in the quadrat. Also, rare or uncommon species are less frequently overlooked in comparison to the point-intersect method. Its primary disadvantage is that it is a time-intensive method that severely limits the number of replicate quadrats that can be searched on a dive. Also, since percent cover is being estimated, observer error or bias is more likely in comparison to the point-intersect method.

Photoquadrat

Another method taught at QUEST for surveying epibenthic flora and fauna is the photoquadrat, which is a modification of the point-intersect method. Using a submersible camera mounted on a rigid framework the observer takes photographs of organisms contained within the quadrat formed by the base of the mounting framework (Fig. 6). An alternative to mounting the camera on a rigid framework is to attach a rod, of set length, to the camera so that it extends in front of the camera. The observer holds the camera at right angle to the bottom and when the tip of the rod touches the bottom, takes a photograph. This insures that all frames will be taken from the same distance above the bottom (i.e. that all will cover an equal area of the bottom). The second method allows for the additional attachment of two lasers, a known distance apart, set parallel to each other, and aimed forward. This provides a size reference in each frame, because the laser beams will show in the photograph and are a known distance apart from each other.

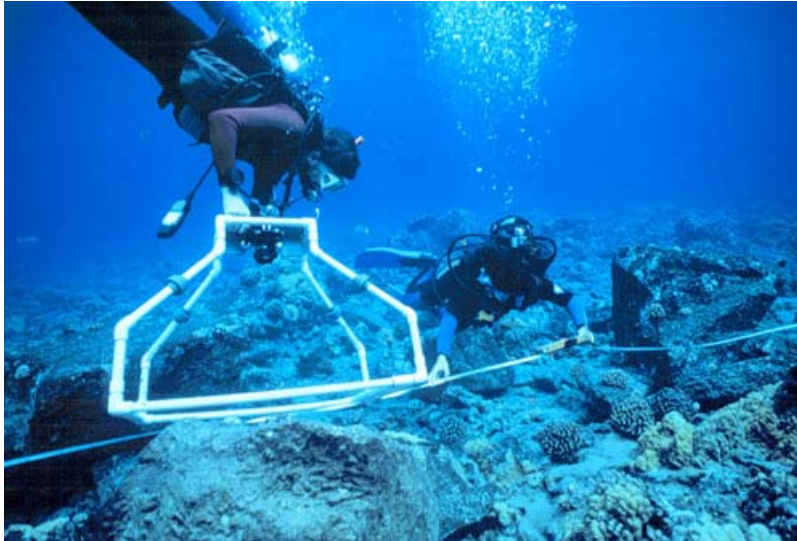


Figure 6. Divers using a camera mounted on a photoquadrat stand to photograph randomly selected locations along a transect line.

Cameras used can be film or digital. Before digital photography became common, color transparency film was used. Images were projected onto a white surface with random points marked on it. Organisms or substrate under each of the points was recorded, producing essentially a variation of the point intersect method. An alternative to wall projection of images was digitizing of slides with a scanner and analysis of the digital images with software designed for that task. Increasingly, digital cameras are being used because they produce high-resolution digital images directly for software analysis (like PhotoGrid or PointCount). Digital images should be, at a minimum, 4 megapixels in resolution.

Photoquadrat sampling procedures are similar to those previously described for quadrats. Typically a transect line is deployed and quadrat search positions on the line are designated by random numbers. With the advent of digital photography and increased frame capacity compared to film cameras, some investigators prefer to shoot every meter of a transect line. These photographs can then all be analyzed, or can be randomly selected for analysis.

It is important that a careful record be maintained for the start of each transect, so that photos can later be matched to the transect line from which they came. Typically, a number or letter identifying the transect line is written on a diver's slate and photographed prior to quadrat photos being taken on that line. The position of each quadrat on the line can be ascertained by matching the sequence of random number positions photographed to the numbering sequence of the photographs.

There are at least two potential advantages of the photoquadrat over conventional point quadrats and percent cover estimation. First, because the

photographic process is relatively fast, it is possible to photograph many more quadrats on a dive than could be searched by divers during the dive. Those photographs can then be analyzed in the laboratory. Second, the photoquadrat method provides a permanent photographic record of each quadrat.

There are, however, potential liabilities to the photoquadrat method. Equipment failure or film development problems can result in no photographs or photographs of such poor quality that they cannot be analyzed with any degree of confidence. This is becoming less and less of a problem as digital photography evolves. Even with excellent photographic quality, however, it is sometimes difficult to identify organisms if they are in regions of shadow. Finally, use of photographic imagery still doesn't solve the problem of overlooking small or rare organisms.

Video Quadrat

In addition to underwater still photography, video can be used to support underwater surveying techniques including point quadrat, percent cover estimation, and line transects. Divers use a video camera inside an underwater housing. In the past video format used was 8 mm or Hi-8. Now, modern digital video formats like Mini DV are utilized and frames can be analyzed by software like PhotoGrid or PointCount. The procedures previously described for quadrat search or line transects are followed, except that a videotape record is made. The video is then analyzed in the laboratory. As with photoquadrats, each transect line should be referenced prior to being video taped.

The advantages and potential disadvantages outlined for photoquadrats also apply to video. In addition, as mentioned previously in the subsection on fish surveying, even the highest quality video format does not provide sufficient resolution for identification, with confidence, of some organisms.

Line Transect

While not employed at QUEST, a line transect can be used to estimate abundance of epibenthic organisms like coral and algae. A transect line is deployed on the bottom and pulled tight. The length of line overlying various kinds of organisms or nonliving substrate is then measured, either by graduations on the transect line or by hand-held meter stick (Fig. 7). The minimum unit of measurement utilized depends in part upon the length of the transect, although Reed (1980) recommends that measurements be made to the nearest centimeter. Some workers have used chain instead of transect line, counting the number of chain links overlying each kind of substrate instead of measuring distance of coverage (Porter 1972). Chain should probably be avoided, because unless it is handled with great care it can damage

delicate organisms and can abrade or snag on corals causing mechanical damage.

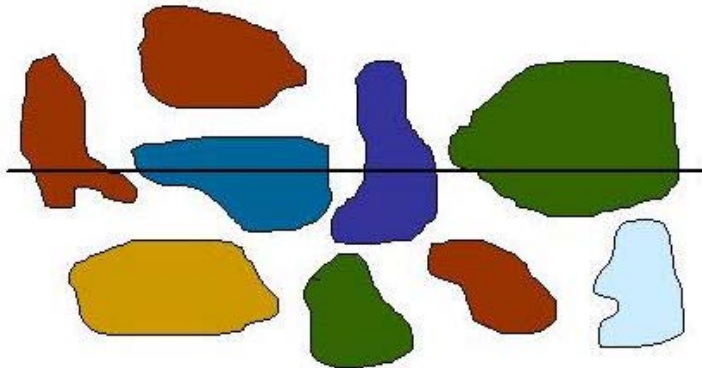


Figure 7. A transect line deployed across different substrate types (modified from Reed 1980).

The advantage of the line transect over other methods is that it is a rapid technique for estimating substrate cover. Its disadvantage is that small or uncommon patches tend to be excluded from the data (Reed 1980). A second observer may wish to record species in the vicinity which were not recorded on the transect.

Surveying Solitary Macroinvertebrates

Most corals and algae are permanently attached epifauna that live colonially or aggregated, and frequently cover relatively large areas of bottom. However, many coral reef invertebrates exist as solitary individuals, either permanently attached to the substrate or capable of moving slowly over the bottom. These kinds of invertebrates are usually referred to in the scientific literature as "macroinvertebrates" and commonly include individuals from the following taxons: echinoderms like sea urchins, sea stars, and sea cucumbers; molluscs like marine snails, nudibranchs, and octopuses; polychaete worms like Christmas tree worms or fire worms; and a host of crustaceans like mantis shrimps, crabs, and shrimps. Among the most commonly utilized methods for surveying macroinvertebrates are strip transect, quadrat search, line transect, and nearest neighbor.

Strip Transect

The strip transect is deployed as previously described for visual surveying of fishes. Divers then proceed down the transect line, counting macroinvertebrates within a predetermined distance from each side of the transect line. In this method, specific invertebrate species or groups are usually targeted for enumeration; for example, collector urchins or maybe even all echinoderms. Density of the target organism is then calculated via the equation:

$$D = \frac{n}{(2lw)}$$

D = density

n = number of individuals seen

l = transect length

w = width of the search area to *one side of the transect line*.

Because of the size of the strip transect search area, it is not possible for divers to accurately count all individuals of all the common macroinvertebrate species. Also, certain invertebrate species may be too numerous on the transect to be counted during a single scuba dive. For example, on portions of the Puako reef at night, the boring urchins *Echinometra mathaei* and *oblonga* are frequently too abundant to be accurately counted by the strip transect method.

The principal advantage of the strip transect method for counting invertebrates is that it can be quick, and that it requires no additional sampling equipment beyond the transect line and a writing slate. A major disadvantage is that rare species will be overlooked. Also, as just mentioned, very common species can overwhelm divers trying to count them.

Quadrat Search

Another method of sampling mobile invertebrates is the quadrat search. Quadrats are placed at randomly selected locations along a transect line and are then searched for macroinvertebrates. At QUEST, quadrats used for this procedure are squares, one meter on each side, providing a search area of 1 m². To facilitate shoreline entry and exit with this relatively large quadrat, a collapsible quadrat was developed by John Coney. While entering and exiting the water, the quadrat is carried in a compact collapsed form. At the transect line location, it can be quickly assembled and disassembled.

The quadrat search method has two major advantages over the strip transect. First, the search area is much smaller and more precisely defined which reduces sampling error in comparison to the strip transect. Invertebrates can be more accurately counted and rare species are not as readily overlooked. Furthermore, numerous replicate quadrats can be used to calculate how sample organisms are distributed in space, specifically whether they are randomly distributed, aggregated, or uniformly spaced. The principal, and relatively minor, disadvantage of this method is the need to carry additional sampling equipment in the form of a quadrat.

Line Transect

The line transect is another method by which the density of macroinvertebrates can be estimated. After the transect line is deployed, the observer proceeds down the line and counts all individuals of the target species that are seen from the line. Unlike a strip transect in which all individuals seen within a fixed distance of the transect line are enumerated, on a line transect search each individual is recorded *separately* along with information on the perpendicular distance (y) between that individual and the transect line (Fig. 8). The perpendicular distance (y) can also be calculated from the sighting angle (α) and the distance between the observer and subject (r) via the equation:

$$y = r \sin \alpha$$

With these data, density of target species can be calculated via a number of different mathematical methods. For a discussion of the line transect computational methods see Krebs (1989, pp 113-121).

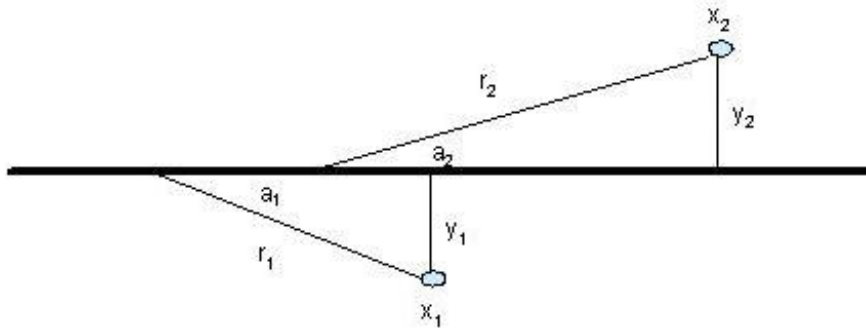


Figure 8. An observer moving along a transect line sights target organisms x_1 and x_2 from the line. From sighting distance (r_1 and r_2) and angle (α_1 and α_2), perpendicular distances from the transect line to the organism (y_1 and y_2) can be calculated. The perpendicular distance may also be measured directly (modified from Krebs 1989).

On line transects, like strip transects, some individuals are undetected within the transect and therefore an under-counting bias occurs (Thresher and Gunn 1986, Krebs 1989). However, this method has an advantage over the strip transect in that subject detectability ("detection function") is incorporated into calculations of target species density so that the under-counting bias is reduced (see Krebs 1989, pp 113-121; or Thresher and Gunn 1986, pp 96-97). This method's principal disadvantage is that it is more time consuming than the strip transect or quadrat search methods.

Nearest Neighbor Measurements

For invertebrates that are widely spaced over the reef, like some urchin species, any one of several Nearest-Neighbor Distance methods can be used to estimate their density on the reef. In addition, some nearest-neighbor procedures allow the observer to determine if the individuals measured are randomly distributed, aggregated, or uniformly spaced. Nearest-neighbor methods most frequently utilize two kinds of distance measurements: 1) from a random point to the nearest organism, and 2) from that organism to its nearest neighbor. There are a number of different nearest-neighbor methods that can be employed, each with its own underlying biases and advantages (see Krebs 1989, pp 126-148 for a more detailed discussion). At QUEST, the relatively simple nearest-neighbor method described by Reed (1980) is used to estimate the density of selected macroinvertebrates.

A transect line is deployed and the diver proceeds down the line to the first of a series of locations selected by randomly generated numbers. The observer then measures the distance from the transect line location to the nearest target organism (at QUEST the target species is usually the collector urchin *Tripneustes gratilla*). The observer then measures the distance from that individual to its nearest neighbor. Once that measurement is taken, the observer proceeds down the transect line to the next random location and the procedure is repeated. Reed (1980) recommends that a minimum of 25 pairs of measurements be recorded.

These measurement data are then used to calculate an estimated density for the species in question. Each distance measurement is considered to be the radius of an invisible circle, the center of which is occupied by the target individual (Fig. 9). The area of that imaginary circle is calculated by the equation:

$$A = \pi r^2$$

A = area

π = 3.1416

r = radius (distance measurement)

Areas are computed for the circles surrounding all individuals measured. Mean circle area is then computed from all the individual circles and represents the estimated density of the target organism on the reef.

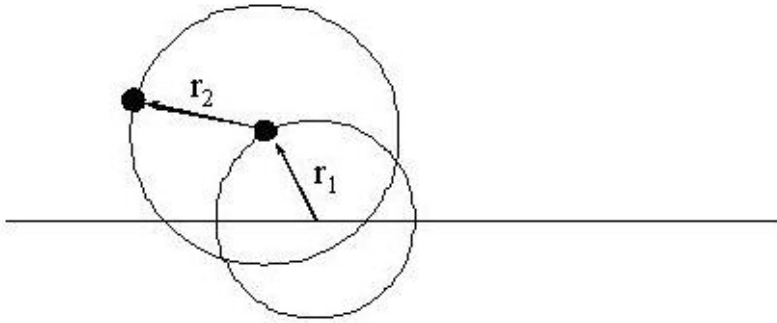


Figure 9. One pair of nearest neighbor measurements taken from a random point along a transect line. Distance from the line to the nearest individual (r_1) and from that individual to its nearest neighbor (r_2) represent radii of imaginary circles (modified from Reed 1980).

Tow Boarding

Occasionally researchers are faced with the task of surveying large expanses of coral reef in relatively short periods of time. For example, they may be asked to do a coral reef biological survey around Pacific islands that have not been previously studied. Or, they may need to quickly locate a series of study sites along an extended coral reef coastline like the Big Island's Kohala Coast. In these types of situations, the tow board surveying technique may be useful.

This technique involves an observer holding onto a hydrodynamically designed water-sled that is towed slowly behind a small boat. The observer, by a series of body twists and rolls, can direct the sled from side to side or make it descend and ascend. Using hand signals, the observer communicates with the boat's pilot and spotter. The pilot's primary function is to operate the boat, keep it on course, and watch for objects in front of the boat. The spotter watches the person being towed to alert the pilot should they lose their grip on the tow board or have some other unforeseen problem. The spotter also relays hand-signal information from the observer to the pilot. Tow board survey can be done with mask and snorkel or scuba if sustained submergence is required.

Using the tow board as a sampling vehicle, a variety of surveying techniques can be employed. Usually, the tow board survey is used to delineate areas of interest for subsequent detailed surveys, or to make a qualitative reconnaissance in which species are recorded as being present or absent. However, if a photographic record of the bottom is systematically made along a tow board transect of known length, it is possible to treat each photograph as a replicate quadrat, and hence to utilize them for quantitative analysis of benthic organisms. Furthermore, since the tow board is in essence "flying" over the bottom, sampling techniques applicable to aerial surveys of

terrestrial wildlife populations and marine mammals may be appropriately modified for tow board surveying (see Krebs 1989, pp 93-113 for a discussion of aerial surveying methods).

The advantage of this technique is obvious. Just a few divers can survey vast expanses of reef in a short period of time. However, this technique has many disadvantages. First, it is difficult to accurately identify and record organisms as they move rapidly past the observer's field of view. Even large and common species may go unrecorded. Small, cryptic, and rare species are likely to be missed. Often, when tow boarding is employed, just a few target or indicator species are recorded. Disadvantages also include cost of boat and sled, and the inherent dangers involved in towing a diver behind a boat, particularly if the diver is maneuvering the sled close to the bottom. All persons involved, boat driver, spotter, and observer must remain attentive at all times.

Surveying Soft Bottom Communities

Soft bottom communities may be found in association with many coral reefs, particularly in lagoon areas or on sand flats at the base of the reef slope. In addition, small pockets of soft sediment can be found interspersed among corals in many other regions of the reef. These soft bottom communities are dominated by infauna, organisms that burrow into the substrate and are hence hidden from view. Infaunal organisms range taxonomically from plants and animals to bacteria. Infaunal plants, bacteria, and fungi are all microscopic, while infaunal animals range in size from protozoans a few microns in length to macrofauna like polychaete worms, and large snails and bivalve molluscs which may be many centimeters long.

Because visual surveying techniques cannot be applied to infauna, sampling methods are utilized which involve collection of organisms hidden in the soft bottom (i.e. destructive sampling methods). While there are many commercially available bottom grabs that collect consistent volumes of sediment (i.e. permit quantitative sampling), home made quantitative sediment grabs can be made out of tin cans of various sizes (Reed 1980). These are pushed into the soft bottom to extract a known volume of sediment. Replicate grabs are made along randomly selected locations on a transect line. In the laboratory, the collected sediment is then passed through a series of sieves of decreasing mesh size to extract various size-classes of organisms that are subsequently identified and enumerated. This technique provides an estimate of the composition and abundance of organisms living in soft bottoms.

References

- Alevizon, W. S. and M. G. Brooks 1975. The comparative structure of two western Atlantic reef-fish assemblages. *Bull. Mar. Sci.* 25:482-490.
- Andrew, N. L. and B. D. Mapstone 1987. Sampling and the description of spatial pattern in marine ecology. *Oceanogr. Mar. Biol.* 25:39-90
- Bell, J. D., G. J. S. Craik, D. A. Pollard, and B. C. Russell 1985. Estimating length-frequency distributions of large reef fish underwater. *Coral Reefs* 4:41-44.
- Bowen, S. H. 1968. Diver performance and effects of cold. *Hum. Factors* 10:445-463.
- Brock, R. E. 1982. A critique of a visual census method for assessing coral reef fish populations. *Bull. Mar. Sci.* 32:269-276.
- Brock, V. E. 1954. A preliminary report on a method of estimating fish populations. *J. Wildl. Management* 18: 297-308.
- Cochran, W. G. 1963. *Sampling techniques, 2nd Ed.* Wiley, New York.
- Colin, P. L. 1974. Observation and collection of deep-sea fishes off the coasts of Jamaica and British Honduras (Belize). *Marine Biology* 24:29-38.
- Coyer, J. and J. Witman 1990. The underwater catalog: A guide to methods in underwater research. Shoals Marine Laboratory, Cornell University, New York. 72 pp.
- Davis, G. E. and T. W. Anderson 1989. Population estimates of four kelp forest fishes and an evaluation of three in situ assessment techniques. *Bull. Mar. Sci.* 44:1138-1151.
- DeMartini, E. E. and D. Roberts 1982. An empirical test of biases in the rapid visual technique for species-time censuses of reef fish assemblages. *Mar. Biol.* 70:129-134.
- Deweese, C. M. 1982. Guidelines for marine ecological surveys: nekton. California Sea Grant College Marine Advisory Program, University of California, Davis. 12 pp.
- Ebeling, A. W., and M. A. Hixon 1991. Tropical and temperate reef fishes: comparison of community structures. Pgs 509-563 in P. F. Sale, ed. *The Ecology of Fishes on Coral Reefs*. Academic Press, San Diego, CA, 92101, 754 pp.
- Ebeling, A. W., R. J. Larson, W. S. Alevizon, and R. N. Bray 1980. Annual variability of reef-fish assemblages in kelp forests off Santa Barbara, California. *Fish. Bull.* 78:361-377.

- Elliott, J. M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. Sci. Publ. No. 25, Freshwater Biological Association, Ferry House, U.K.
- Gotshall, D. W. 1987. The use of baited stations by divers to obtain fish relative abundance data. *Calif. Fish and Game* 73:214-229.
- Green, R. H. 1979. *Sampling design and statistical methods for environmental biologists*. Wiley, New York.
- Greene, L. E. and W. S. Alevizon 1989. Comparative accuracies of visual assessment methods for coral reef fishes. *Bull. Mar. Sci.* 44:899-912.
- Helfman, G. S. 1983. Underwater methods. In "Fisheries Techniques" (L. A. Nielsen and D. L. Johnson, eds.), pp. 349-369. Am. Fish. Soc., Bethesda, Maryland.
- Hobson, E. S. 1972. Activity of Hawaiian reef fishes during the evening and morning transitions between daylight and darkness. *Fish. Bull.* 70:715-740.
- Holme, N. A. and A. D. McIntyre 1971. *Methods for the study of marine benthos*. Blackwell Scientific Publications, London. 334 pp.
- Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* 54:187-211.
- Jones, R. S. and N. J. Thompson 1978. Comparison of Florida reef fish assemblages using a rapid visual technique. *Bull. Mar. Sci.* 28:159-172.
- Kaufman, L. S. and J. P. Ebersole 1984. Microtopography and the organization of two assemblages of coral reef fishes in the West Indies. *J. Exp. Mar. Biol. Ecol.* 78:253-268.
- Larson, R. J., and E. E. DeMartini 1984. Abundance and vertical distribution of fishes in a cobble-bottom kelp forest off San Onofre, California. *Fish. Bull.* 82:37-53.
- Lincoln-Smith, M. P. 1988. Effects of observer swimming speed on sample counts of temperate rocky reef fish assemblages. *Mar. Ecol. Prog. Ser.* 43:223-231.
- Lincoln-Smith, M. P. 1989. Improving multispecies rocky reef fish censuses by counting different groups of species using different procedures. *Env. Biol. Fishes* 26:29-37.
- Krebs, C. J. 1989. *Ecological Methodology*. Harper & Row, New York.

McCormick, M. I. and J. H. Choat 1987. Estimating total abundance of large temperate reef fish using visual strip-transects. *Mar. Biol.* 96:469-478.

Pielou, E. C. 1974. *Population and community ecology*. Gordon & Breach Science Publishers, New York, NY, 424 p.

Porter, J. W. 1972. Patterns of species diversity in Caribbean reef corals. *Ecology* 53:745-748.

Pringle, J. D. 1984. Efficiency estimates for various quadrat sizes used in benthic sampling. *Can. J. Fish. Aquat. Sci.* 41:1485-1489.

Quast, J. D. 1968a. Some physical aspects of the inshore environment, particularly as it affects kelp-bed fishes. [In] W. J. North and C. L. Hubbs (eds.), Utilization of kelp bed resources in southern California. *Calif. Dept. Fish Game Fish Bull* 139:25-34.

Quast, J. D. 1968b. Fish fauna of the rocky inshore zone. [In] W. J. North and C. L. Hubbs (eds.), Utilization of kelp bed resources in southern California. *Calif. Dept. Fish Game Fish Bull* 139:35-55.

Quast, J. D. 1968c. Estimates of the population and standing crop of fishes. [In] W. J. North and C. L. Hubbs (eds.), Utilization of kelp bed resources in southern California. *Calif. Dept. Fish Game Fish Bull* 139:57-79.

Reed, S. A. 1980. Sampling and transecting techniques on tropical reef substrates. [In] Environmental survey techniques for coastal water assessment conference proceedings. Sea Grant Cooperative Report UNIH-SEAGRANT-CR-80-01: 71-89.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. *Bull. Fish. Research Bd. Can. Bull.* 191, 382 pp.

Russell, B. C., F. H. Talbot, G.V.R. Anderson, and B. Goldman 1978. Collection and sampling of reef fishes. pp 329-345 [In] Stoddart, D. R., and R. E. Johannes (eds.). *Coral Reefs: Research Methods*. UNESCO, Paris.

Sale, P. F. 1980a. The ecology of fishes on coral reefs. *Oceanogr. Mar. Biol.* 18:367-421.

Sale, P. F. and W. A. Douglas 1981. Precision and accuracy of visual census techniques for fish assemblages on coral reef patch reefs. *Env. Biol. Fish.* 6:333-339.

Sale, P. F. and B. J. Sharp 1983. Correction for bias in visual transect censuses of coral reef fishes. *Coral Reefs* 2:37-42.

- Sampford, M. R. 1962. *An introduction to sampling theory*. Oliver and Boyd, Edinburgh.
- Sanderson, S. L. and S. C. Solonsky 1986. Comparison of a rapid visual and a strip transect technique for censusing reef fish assemblages. *Bull. Mar. Sci.* 39:119-129.
- Slobodkin, L. B. and L. Fishelson 1974. The effect of the cleaner-fish *Laboides dimidiatus* on the point diversity of fishes on the reef front at Eilat. *Am. Nat.* 108:369-376.
- Stein, D. L., B. N. Tissot, M. A. Hixon, and W. Barss 1992. Fish-habitat associations on a deep reef at the edge of the Oregon continental shelf. *U.S. Fish. Bull.* 90:540-551.
- Southwood, T. R. E. 1978. *Ecological Methods, 2nd Ed.* Methuen, London, ___ pp.
- Swenson, W. A., W. P. Gobin, and T. D. Simonson 1988. Calibrated mask-bar for underwater measurement of fish. *North Amer. J. Fish. Manag.* 8:382-385.
- Thresher, R. E. and J. S. Gunn 1986. Comparative analysis of visual census techniques for highly mobile reef associated piscivores (Carangidae). *Environ. Biol. Fishes* 17:93-116.
- Underwood, A. J. 1981. Techniques of analysis of variance in experimental marine biology and ecology. *Oceanogr. Mar. Biol. Ann. Rev.* 19:513-605.