APPROACHES TO SAMPLING MACROFUNGI

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INVENTORYING VERSUS MONITORING

At present most inventory work is conducted to increase our knowledge of fungal diversity and to learn about habitat preferences and geographic distributions of different taxa. A recent development in that respect is the inventory of target taxa for conservation purposes legally mandated as part of the federally approved management plan for old growth forests in the Pacific Northwest (Forest Ecosystem Management Assessment Team 1993; O'Dell et al. 1996; Molina et al. 2001). Although no nonlichenized fungus is protected yet by that plan, 225 species of old-growth-associated macrofungi in the Pacific Northwest are legally required to be surveyed and managed (USDA Forest Service and USDI Bureau of Land Management 2000; O'Dell et al. 2003). Reasons for undertaking inventories include prioritization of

potential sites for protection and assessment of the impacts of different land management practices on biodiversity (e.g., O'Dell et al. 1992a; Luoma et al. 1996b, 1996c, 1996d). Monitoring is undertaken to assess effects of commercial harvest on edible fungal sporocarp production and on population size (Pilz and Molina 1996), to determine long-term trends in populations of target species, and to determine effects of pollutants on different fungi (Gulden et al. 1992). Baseline data on the diversity and distributions of the taxa to be monitored (i.e., inventories) of the region under study are normally necessary before monitoring can begin.

Several differences exist between sampling for biodiversity, as discussed throughout this chapter, and monitoring. Monitoring programs are designed for determining population trends (rather than overall richness), extend for longer periods, generally focus on fewer taxa (often indicator species), and include many more sites. Because monitoring efforts need to be carried out over long periods, archiving of data and careful documentation of sites and methods are priorities. One should consider the documentation required to facilitate the repetition of a study at a site 20 years in the future and plan accordingly.

The use of fungal "indicator species" to monitor environmental change or assess ecosystem health should be considered carefully. Pearson (1994) proposed several criteria for the selection of indicator taxa, concluding that they should: (1) be taxonomically stable and well known; (2) have a well-known natural history; (3) have readily surveyed and manipulated populations; (4) be sensitive to some disturbances; (5) be a component of a widely distributed higher taxon; (6) include species exhibiting habitat specificity; (7) include species with economic value; and (8) have distributions or patterns of species richness that are correlated with those of other, unrelated groups.

Compared with vertebrates and plants, macrofungi are poorly known taxonomically, especially at the species level (although some taxa are well known); they are difficult to survey and manipulate, especially in natural ecosystems; and their natural histories, beyond a cursory level, are poorly known. However, microfungi can be sensitive to human-generated disturbances, such as air pollution (Gulden et al. 1992; Fellner 1993; Carreiro et al. 2000) and logging (O'Dell et al. 1992a), as well as to natural disturbances such as hurricanes, volcanic eruptions, and landslides (Lodge and Cantrell 1995b; Lodge 1996; Miller and Lodge 1997; Heilmann-Clausen 2001). They have globally distributed genera as well as locally endemic species; include economically valuable taxa (Molina et al. 1993); and influence the distribution of other organisms such as fungivorous insects (Worthen and McGuire 1990), rodents (North 1993), and plants (e.g., Bidartondo et al. 2000; Blackwell 2000; Kretzer et al. 2000). Thus, macrofungi appear to be of intermediate value (five or eight attributes) as indicator organisms.

Drawbacks to monitoring macrofungi include limited taxonomic expertise, annual fluctuations in sporocarp occurrence and abundance, and lack of well-tested protocols for sampling. The first problem can be overcome through careful selection of taxa and species documentation with voucher specimens, which are essential for addressing taxonomic problems and authenticating the taxa being monitored. Annual variation in occurrence of species remains troublesome. The fact that a particular species may not fruit on a site for a number of consecutive years but then be abundant in other years detracts from the utility of macrofungi for monitoring efforts. Species once present at a site that fail to fruit at that site in subsequent years may be present and physiologically active but not fruiting, present as dormant propagules, or extirpated. The causes of such variation are poorly understood, but efforts to improve detection of, for example, ectomycorrhizal species, have met with limited success. Several studies comparing fungi that fruit versus those colonizing roots (as detected by polymerase chain reaction–amplified DNA) have reported a low correlation between ectomycorrhizal fungal diversity and species composition estimates based on sporocarp data and analysis of mycorrhizal roots (e.g., Gardes and Bruns 1996; Peter et al. 2001). Therefore, each phase of monitoring macrofungal species–that is, detection of the species and assessment of its status–requires many years of observations. Macrofungi are most useful for monitoring in areas for which long-term records of their occurrences are available (Arnolds 1991; Ohenoja 1993) or in which sites being compared follow a known environmental or disturbance gradient (Gulden et al. 1992; O'Dell et al. 1992a; North 1993).

INVENTORYING MACROFUNGI

As with most fungi, it is difficult to quantify populations of macrofungi. Individuals rarely can be distinguished in the field because an individual mycelium may produce from one to many sporocarps. The sizes, numbers, and dry weights of sporocarps vary greatly among taxa, and sporocarps represent an unknown fraction of total biomass, which also includes the vegetative mycelium and sclerotia.

Mycologists traditionally have sampled sites by walking through the area collecting conspicuous specimens of select taxa. That opportunistic approach, however, does not allow for rigorous comparisons of different sites, which requires that sampling intensity be standardized at each site. Collection of all fungi within a series of plots or transects ensures that all taxa fruiting at the time are

scrutinized and reduces the likelihood that cryptic species will be overlooked. Often with such an approach many specimens are identifiable initially only to genus. If the same plots or transects are sampled repeatedly for several years, however, most taxa eventually will be identified.

Currently, the best measure of species abundance is plot frequency (the number of subplots in which a taxon occurs) because it reflects the minimum area occupied by that species in the study site. Because the sporocarps of a species distributed over several sampling units (subplots) may have originated from one large or several small mycelia (cf. Jacobson et al. 1993; Dahlberg and Stenlid 1994), however, plot frequency provides only a rough estimate of abundance and importance (Schmit et al. 1999). Consequently, size, shape, and spacing of sampling units (plots, transects) are important aspects of sampling design.

Types of Sampling Units

Studies should be designed to encompass the spatial and temporal scales appropriate to the taxa of interest. A fundamental distinction must be made between fungi that occur on substrata that form readily observable, discrete, natural sampling units and those that occur on more continuous or concealed substrata that require arbitrary plot boundaries. Natural sampling units are appropriate for species fruiting on leaves, logs, or cones, whereas arbitrary sampling units are required for decomposers of litter or humus and for mycorrhizal fungi.

Studies in which terrestrial macrofungi are surveyed usually use arbitrary sampling units, or plots. Plots range in size from 1 m^2 to 1000 m^2 and can be square, rectangular, or circular. The same plots often are scrutinized for several years. When studies involve removal of most sporocarps (e.g., to determine sporocarp productivity), however, some investigators move plots on each sampling occasion to avoid effects of disturbance (Luoma 1991; O'Dell et al. 1999). An important drawback of changing plot location, deriving from the fact that macrofungi have patchy distributions, is that annual variation in the occurrence of fruiting species becomes confounded with variation in plot location, which can greatly influence the results. Moving the plot location results in a single sampling event over the area, and the data collected are not comparable to data from permanent plots sampled over time. Permanent plots provide a good estimate of diversity for a defined area, as well as information on annual variation in fruiting phenology. They also are easy to relocate and measure. Although plots may underestimate the diversity in a larger area beyond the plot borders, such problems are associated with any sampling method. The effects of long-term removal of sporocarps and perturbation of a site by repeated sampling are not well known, but preliminary studies have provided little evidence of negative effects (Egli et al. 1990; Norvell and Roger 1998). Moving plots may be appropriate, however, when they are raked for truffles (see Chapter 10) in addition to mushroom sampling.

Sampling Vegetative Mycelia

Most of our discussion has focused on the sampling of sporocarps because these are the most readily identifiable structures of macrofungi. Other structures that can be sampled include sclerotia, mycorrhizae, mycelia, mycelial strands, cords, and rhizomorphs. Those vegetative structures can be useful in determining the proportion of taxa that are present but not fruiting. Molecular analyses of ectomycorrhizae have shown that substantial numbers of species present on host roots at some sites rarely fruit (Gardes and Bruns 1996). Standard methods for sampling ectomycorrhizae are given in Johnson and associates (2000).

Although we rely largely on field sampling of sporocarps, encouraging fruiting of some ascomycetes and small agarics by placing substrata in humid chambers may increase detection of litter inhabiting taxa. See Appendix I.

Convenience versus Plot Sampling

Methods of sampling must be chosen to fit the goals of a particular study. If the goal is to get a preliminary estimate of richness at a site, then some sort of survey, or convenience sampling, may be most efficient. In that type of sampling, experienced individuals visit a location and document the taxa that they find. The approach is a traditional one among taxonomists and can be an efficient way to obtain a snapshot of the diversity present. It also may be useful for rapid assessment of potential sites for reserves. For example, convenience sampling was used to survey the macrofungi of Barlow Pass, Washington (Ammirati et al. 1994). Volunteers from the local amateur mycological society made weekly visits to a site for 2 years. During each visit, they walked through the site collecting samples of each taxon encountered. Although more of the taxa present, at least of the large and showy species, may have been detected with this approach than would have been detected with plot sampling, that hypothesis remains untested.

Relying solely on convenience sampling, however, precludes comparisons of diversity and species composition of macrofungi between sites, or at a single site over time, because convenience sampling does not provide for the necessary standardization of sampling effort. Addition-

ally, collector bias during convenience sampling may cause some habitats or taxa to be overlooked. Sampling in plots provides quantitative data because sampling effort is standardized, either through the sampling of an explicitly defined area or by sampling for an explicitly defined amount of time. Removal and examination of all sporocarps in plots also ensures that species difficult to distinguish in the field are collected, and the imposed scrutiny of a defined area results in the detection of more inconspicuous taxa.

Dispersed Subsamples

Ecologists have been sampling macrofungi for several decades but have made little effort to optimize or standardize sampling approaches (Cooke 1955; Arnolds 1992). For example, in many studies, large rectangular plots, divided into subplots, are observed over several years. Permanent plots have the advantage of being monitored easily over time. Because of the clumped distribution of sporocarps of many fruiting macrofungi, however, subplots adjacent to one another are more likely to contain the same species than those at a distance (Murakami 1987). Therefore, the use ofcontiguous subplots may underestimate species richness (O'Dell et al. 1999). That spatial autocorrelation can be addressed by dispersing plots in various ways (Luoma et al. 1991; Lodge and Cantrell 1995a; O'Dell et al. 1999; Mueller and Mata 2000), which are discussed under "Recommended Protocols for Sampling Macrofungi," at the end of this chapter.

More spatially explicit studies for various groups of macrofungi are needed. For example, saprobes may exhibit distributions different from those of ectomycorrhizal species. G. Walker and J. Ammirati (unpublished data) found that species-area curves for saprobic fungi leveled off sooner than those for ectomycorrhizal fungi in four Sitka spruce *(Picea sitchensis)* stands on the Olympic Peninsula in Washington State. Schmit and colleagues (1999), however, did not observe a difference in the shape of species-effort curves among the different ecological guilds of macrofungi sampled in a northwest Indiana oak-dominated forest over a 3-year period.

Adaptive Sampling

Studies of macrofungi typically use sampling designs of fixed area. In other words, investigators decide prior to sampling how many plots of what size to examine at each site. A recent innovation with possible value to mycologists is adaptive sampling, which refers to any case in which sampling effort is modified in response to observations made in the course of sampling. Usually some threshold value for the number of samples of the target organisms is set. When the number in a plot exceeds that value, another area is sampled in some standard predetermined way. Adaptive sampling is useful for increasing the number of interesting observations (e.g., macrofungal sporocarps), particularly for organisms that are rare and have patchy distributions. Thompson (1992) has provided statistical methods for dealing with bias resulting from unequal sampling effort that must be accounted for when comparing values from different sites sampled using adaptive sampling methods (Figs. 8.35A and B).

Determining Adequate Sampling

Deciding how much area to sample; the size, number, and distribution of plots; and the frequency of sampling is not a trivial undertaking and, with regard to fungi, deserves much more attention than it has received. When possible, investigators carrying out biodiversity studies should apply methods that allow for spatially and temporally explicit analyses (e.g., see Colwell and Coddington 1994; Lodge and Cantrell 1995a; Schmit et al. 1999).

One rule regarding the amount of area to sample that sometimes is applied to diversity studies states that sampling is adequate when every taxon occurs in at least two sampling units. Our experience indicates that this criterion is difficult, or probably impossible, to meet when sampling macrofungi. Ecologists have developed species-effort curves for other organisms to address this issue, and such curves occasionally have been applied to fungi (Arnolds 1992). For a given landscape, stand, or habitat, the number of species observed tends to increase steeply with increasing sample area and then level off as fewer new taxa are encountered.

Applying species-effort techniques to macrofungi has demonstrated the need to sample an area larger than that required for plants. Arnolds (1992) recommended plots of 1000 m^2 in forest or 500 m^2 in grasslands, with a minimum of five plots per community type being sampled. One also can plot cumulative species richness versus the number of years of sampling to estimate adequate duration. J. Ammirati and T. E. O'Dell (unpublished data), however, observed a continuous increase in mycorrhizal species richness over 5 years of sampling. Similarly, Schmit and colleagues (1999) documented an

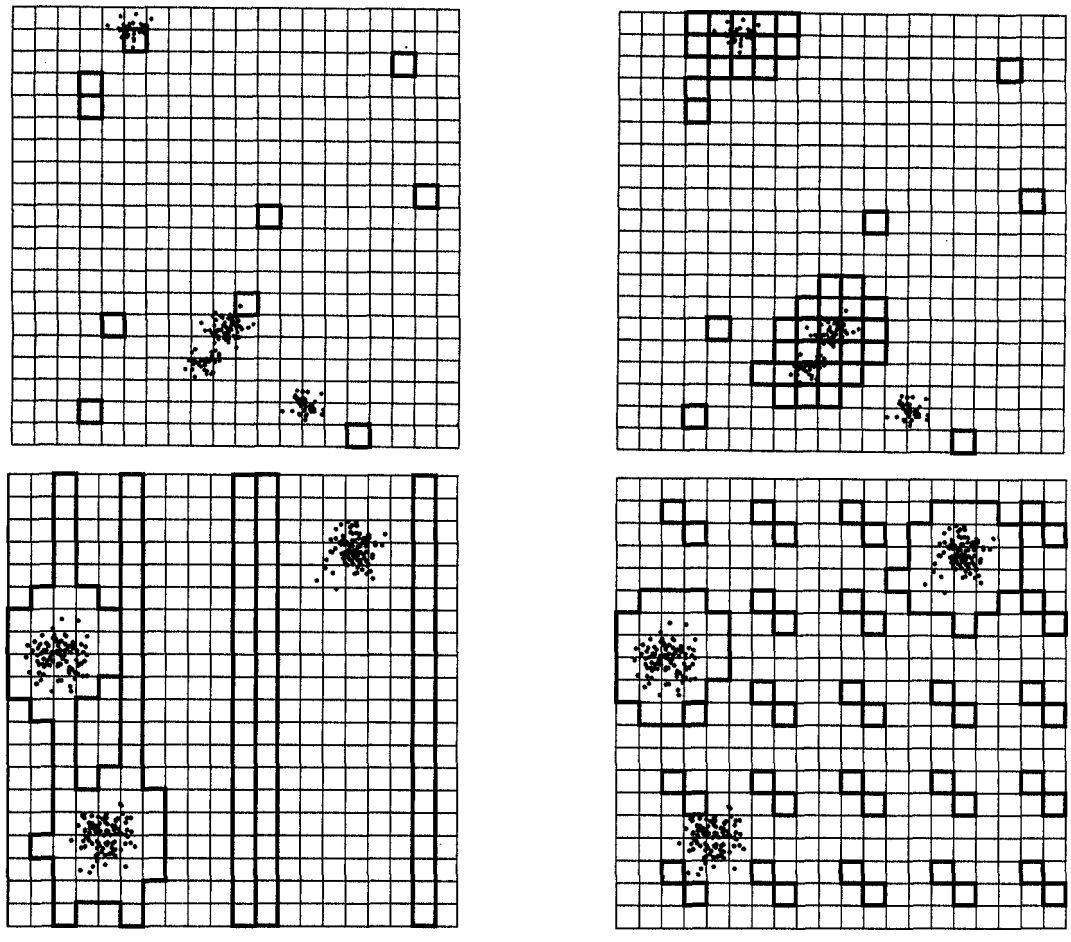

FIGURE 8.35 These figures show how an adaptive sampling is carried out. The upper panels illustrate adaptive cluster sampling to estimate the number of point objects in a study region of 400 units. An initial random sample of 10 units is shown on the left. Adjacent neighboring units are added to the sample whenever one or more of the objects of the population is observed in a selected unit. The resulting sample is shown on the right. (Both upper panels from S.K. Thompson, "Adaptive cluster sampling," 1990, Journal of the American Statistical Association 85: 1050–1059.With permission from the American Statistical Association.) The lower left panel illustrates adaptive cluster sampling with initial random selection of five strip plots. The final sample obtained is indicated with the heavy line. The lower right panel illustrates adaptive cluster sampling with initial random selection of two systematic samples. The final sample obtained is indicated with the heavy line. (Both lower panels from S.K. Thompson, "Adaptive cluster sampling: Designs with primary and secondary units," 1991, Biometrics 47: 1103–1115. With permission from the Biometric Sociery.)

increase in species richness for saprobic and mycorrhizal taxa over a 3-year period, and Straatsma and associates (2001) continued to detect similar numbers of new species during each year of a 21-year study, We are unaware of any recommendation for the minimum duration of an inventory, but based on our experience, 10 years would be desirable.

Sampling design should be considered an iterative process. Data from early efforts can be used to make decisions about ways to modify later sampling. For example, the occurrence in multiple plots of most taxa observed in a study (an unlikely circumstance) would indicate that sample area could be reduced. Conversely, if most samples included unique taxa, sampling intensity should be increased.

Complementarity (dissimilarity) is an empirical measure of the degree of species uniqueness between different samples. It is expressed as the proportion of total species encountered that is unique to one sample or the other. An empirical test for complementarity is as follows:

$$C_{jk} = U_{jk}/S_{jk}$$

where C_{jk} is the complementarity between samples (or sites) *j* and *k*, U_{jk} is the total number of taxa encountered at only one site, and S_{jk} is the total richness of both sites combined (Colwell and Coddington 1994). If all species are found at both sites, then $C_{jk} = 0$; if no species are found in common, then $C_{jk} = 1$. Sampling for richness is most efficient when complementarity among samples is about 0.5. High complementarity (fewer species in common) indicates that many taxa probably are being overlooked, and a lower value suggests that some samples are redundant. Lodge and Cantrell (1995a; unpublished manuscript) used an analysis of complementarity to determine that sampling 12 1-m^2 plots in each of two blocks was an efficient way to estimate diversity of litter agarics fruiting in a tropical forest on a particular date.

Resources constrain what sampling can be done. Although research indicates the need for long-term studies of macrofungi to assess diversity, it is difficult to obtain more than 3 years of funding at a time. Therefore, most studies incorporate 1–3 years of sampling, although 5–10 years would be more appropriate. Estimates of richness based on modest amounts of sampling may be improved using extrapolation techniques.

EXTRAPOLATION TO RICHNESS

Because large numbers of species inhabit small areas, studies of fungal diversity tend to focus on fairly narrow taxonomic or ecological groups. That is obviously inappropriate when estimates of total species richness are sought. Proposals are being made to assess total biotic diversity. Although that is a worthwhile goal, available resources are obviously insufficient to support all taxa inventories at more than a few sites. More often, total diversity for specific taxa or ecological guilds will have to be estimated from some modest amount of sampling.

Several methods for estimating total diversity from a limited number of samples have been developed by mathematical ecologists (see discussion in Colwell and Coddington 1994). A full discussion of extrapolation procedures is beyond the scope of this chapter, but techniques generally are based on the proportion of rare species, typically those encountered in one or two samples. Simply put, the greater the proportion of rarely encountered taxa, the more a diversity estimate should be revised upward. The jackknife is one such procedure and is carried out according to the formula:

$$S^* = S_{obs} + L[(n - 1)/n]$$

where S^* is estimated total richness, S_{obs} is the total number of species in all samples, L is the number of species observed in only one sample, and n is the number of samples. More precisely, the formula is known as the first-order jackknife because it considers only the rarest taxa; the second-order jackknife includes species observed at two sites, and so on (Colwell and Coddington 1994).

Another measure for estimating richness from samples is Chao's second estimator, "Chao 2." It is one of the simplest measures as well as one of the most reliable when numbers of samples is small. The formula is as follows:

$$S^* = S_{obs} + (L^2/2M)$$

Variables are the same as in the jackknife, and M is the number of species observed in exactly two samples. This algorithm performed particularly well on data with a preponderance of rare taxa (Colwell and Coddington 1994), a situation frequently encountered with fungi. It also highlights a disadvantage of any extrapolation—that is, the potential for artifacts. Note that when the number of taxa in exactly two plots is zero, estimated total richness is infinite.

Schmit and colleagues (1999) examined the utility of these two and other estimators. They found that none of the currently used extrapolation techniques was robust when applied to their data.

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Front cover: The slime mold *Physarum roseum* on a decaying leaf. Slime molds are fungal-like organisms traditionally studied by mycologists. Photo by Ray Simons.

Back cover: A species of *Mycena* found in Yunnan, China. Species of the mushroom genus Mycena are commonly encountered throughout the world. Photo by Gregory M. Mueller.