
25 Biological Surveys

About thirty years ago there was much talk that geologists ought only to observe and not theorize, and I well remember someone saying that at this rate a man might as well go into a gravel-pit and count the pebbles and describe the colours. How odd it is that anyone should see that all observations must be for or against some view if it is to be of any service.

Charles Darwin

Biological surveys of effects include a variety of techniques for enumerating and characterizing biological populations and communities so as to relate them to exposure to some agent. In the simplest case, the measure of effect for the biological survey is an estimate of the assessment endpoint. In such cases, the effects analysis consists of summarizing the data in such a way as to define the relationship of effects to exposure. Examples include plotting the species richness of the soil microinvertebrate assemblage against an exposure axis such as kilometers from a source, soil compaction, or concentrations of a particular chemical. The US Environmental Protection Agency (US EPA) has recommended the use of biological surveys for ecological risk assessment of contaminated sites when feasible and appropriate (Office of Emergency and Remedial Response 1994b; Sprenger and Charters 1997) and in assessments of water quality (EPA 1991b).

A frequent problem in the use of biological surveys is that the entities and properties measured bear an undefined relationship to the assessment endpoints. They are often referred to as indicators or surrogates without defining what they indicate or for what they are surrogates. If the measures of effect do not directly estimate the assessment endpoint, the relationship between them must be clearly characterized by risk assessors. For example, if data are available for stream macroinvertebrates and the assessment endpoint is some property of the fish community, the relationship between them must be characterized in terms of the trophic dependence of fish on invertebrates, the relative sensitivity of fish and invertebrates, the similarity of their exposure, and other relevant properties. Clearly, this difficulty should be avoided in the problem formulation by selecting measures of effects that correspond as nearly as possible to the assessment endpoint (Chapter 18).

The following points should be considered when deciding whether biological surveys are appropriate for analysis of effects in an assessment.

Scale: Highly mobile organisms and the populations and communities that include them are seldom appropriate for biological surveys of a site. For example, a survey of breeding birds was conducted on the East Fork Poplar Creek flood plain in Oak Ridge, Tennessee, but it contributed nothing to the results of the ecological risk assessment. Territorial birds are highly mobile and are nearly always space limited, so all sites that contain physically suitable habitat are quickly occupied whatever the longevity or reproductive success of the resident birds may be. However, if the goal of an assessment is to estimate risks to a regional population or risks from an agent that acts at regional scales, mobility is not a constraint.

Interpretation: In order to interpret the variation observed in results of biological surveys, the properties measured must be stable and consistent across similar sites, in the absence of contamination or disturbance, relative to the magnitude of effects that is considered significant. For example, population densities of microtine rodents are notoriously variable across time and space, varying by orders of magnitude in the absence of any anthropogenic effects. In contrast, properties of stream fish communities are relatively stable and are commonly used to detect anthropogenic effects by comparing exposed communities to reference.

Difficulty: Clearly, biological surveys are inappropriate if they are costly and time consuming, are likely to fail due to the difficulty of proper execution, or if the necessary conditions for success are unlikely to occur. For example, determining the reproductive success of kingfisher populations has proved to be quite difficult, but the reproductive success of birds that nest colonially and in the open is relatively simple (Henshel et al. 1995; Halbrook et al. 1999a). Similarly, the fish communities of wadeable streams can be easily quantified with great accuracy, but the abundances of fish populations and communities of large bodies of water cannot be quantified with sufficient accuracy or precision for many assessments.

Appropriateness: Techniques employed must be suitable for the species or community, season, and habitat of interest and should produce results that meet the objectives of the risk assessment.

Technical expertise: In some cases, the expertise or experience needed to perform a particular survey is not available. In such cases, the need for technical expertise can be reduced by a simple change in the survey techniques or endpoint. For example, technicians who can identify benthic invertebrates to species are in short supply, but identification of families may be sufficient and individuals with very little training can sort invertebrates into higher taxa without knowing their names.

Consequences of the survey: Biological survey may cause unacceptable injury to the sampled population or ecosystem. The destructive sampling of rare species is an obvious example.

Data relevance: Data not generated by the assessment program should be used if pertinent and of adequate quality. However, care must be taken to appropriately analyze and interpret them. For example, fish survey data have been collected by the Tennessee Valley Authority for the purpose of comparing the quality of their reservoirs. These data were used to determine that the Oak Ridge Reservation had not altered the fish community of Watts Bar Reservoir relative to other reservoirs in the system, but they could not be used to infer risks at the scale of embayments, which was the scale of remedial actions (Suter et al. 1999).

25.1 AQUATIC BIOLOGICAL SURVEYS

Aquatic biota surveyed for waste site and water quality assessments may include periphyton, plankton, fish, and benthic macroinvertebrates (Office of Emergency and Remedial Response 1994b; Gibson et al. 1996; EPA 1996a, 1997a, 1998b; Barbour et al. 1999). The choice of assemblage and sampling method depends on the endpoints and habitat characteristics. Care should be taken to ensure that the survey locations capture the variation in exposure while recognizing the scale of the system relative to the habitat requirements and mobility of the surveyed organisms.

Habitat quality information is critical to the ability to discriminate between contaminant effects and natural variability. They must be accounted for in the survey design and should be quantified to the extent possible for all sites. The relevant habitat factors depend on the types of organisms being surveyed. For example, photosynthetically active radiation is important for algal and periphyton surveys, cover type and stream structure are important for fish surveys, and water chemistry (e.g., pH, hardness, and conductivity) is important for all assemblages.

The common use of biological surveys in aquatic systems, particularly streams, presents both advantages and disadvantages for risk assessors. The primary advantages are that methods are well established, and the expertise to perform surveys is commonly available. In addition, in states such as Ohio where bioassessment programs are underway, community types are already classified, reference conditions are established, and criteria for injury are defined (Yoder and Rankin 1995b, 1998). The primary disadvantage is that the metrics and indices chosen for monitoring or regulatory programs may not be appropriate for a contaminated site risk assessment and remediation. In particular, the biotic indices that are commonly used are designed to discriminate sites with common sorts or disturbances, particularly organic enrichment (Karr and Chu 1997). They are relatively insensitive to toxic effects (Dickson et al. 1992; Hartwell et al. 1995). Biological surveys for risk assessments should focus on measures of effect that are sensitive to the agents being assessed and are sufficiently valued to support a remedial or regulatory action.

25.1.1 PERIPHYTON

Algae and other aquatic plants are much less often included in biological surveys than fish or invertebrates. Except for estuarine species, aquatic macrophytes are more likely to be considered noxious weeds than valued endpoint entities. However, periphyton have long been used as indicators of stream quality because they are ubiquitous, constitute the base of most lotic food chains, are in direct contact with water, are sessile, are sensitive to a wide range of stressors, respond quickly to changes in water quality, are more stable than phytoplankton, and are easily sampled (Rosen 1995). Periphyton have the practical advantages of being easily associated with a site and being easily collected by scraping from natural or artificial substrates. However, it is often difficult to demonstrate to decision makers that a change in algal community properties is adverse, particularly when pollution and disturbances commonly increase algal production due to increased light or nutrient levels.

Periphyton samples can be collected from natural substrates, or from artificial substrates (e.g., frosted glass slides), which are placed in the water for a set period of time (e.g., 2 to 4 weeks) and then removed for analysis. The principal advantages of artificial substrates are ease of use, repeatability of measurements, reduced variability of taxonomic composition, and relative abundance. However, they are selective for particular species and the results may not be representative of the entire periphyton community. Both approaches have their supporters and detractors in the regulatory community (Rosen 1995), and the choice of natural or artificial substrates should be made in cooperation with the relevant agencies.

Periphyton communities vary widely in response to microhabitats even within the scale of individual sampling units, especially on rocks or other natural substrates. One can reduce variability by compositing multiple samples collected from a single type of habitat within a stream reach. For the assessment of water quality, collecting only from riffles and runs in streams is generally sufficient and periphyton communities in these habitats (particularly in those with current velocities of 10 to 20 cm/s) are less variable than in pools and edge habitat (Rosen 1995). Also, sampling the periphyton of soft substrates is more difficult and time consuming than sampling hard substrates.

The measures of effect may be structural or functional. Structural measures include measures of taxonomic composition and measures of standing crop (biomass). Common measures of taxonomic composition are species richness and relative abundance. Enough periphyton cells should be counted to ensure that uncommon species are included (Rosen 1995). Taxonomic identification should be at least to genus for soft algae and to species for diatoms (Rosen 1995). Because diatoms are common, abundant, and relatively easily identified by their siliceous frustules, they are often counted to the exclusion of soft algae. Standing crop is

measured as *chlorophyll a*, ash-free dry weight, cell counts, and cell volume. Each method has limitations, but all are generally acceptable for comparisons between exposed and reference sites. Indices of structural characteristics include diversity indices and indices of similarity between sites. Although useful in conjunction with other structural measurements, such indices should not be used as the only measure of periphyton structure.

The functional measure used for periphyton is primary productivity. The most common and widely accepted methods for estimation of primary productivity are based on the production of oxygen (O_2 method) or the uptake of radioactive carbon (^{14}C method) (Rosen 1995). Choose the method that best fits the budgetary, logistical, and quality requirements of the assessment. The O_2 method is inexpensive, relatively simple to perform, and readily used in the field. The advent of microelectrode technology has simplified and improved the measurement of oxygen production. The ^{14}C method is more expensive, more complicated to perform, and much less amenable to field use than is the O_2 method. However, it is a direct measure of primary productivity and is more sensitive than the O_2 method (Rosen 1995).

Physicochemical parameters to be measured and controlled in selecting reference sites include substrate composition, current velocity, temperature, photosynthetically active radiation, dissolved oxygen, conductivity, alkalinity, hardness, and nutrients.

25.1.2 PLANKTON

Plankton are the algae (phytoplankton) and small invertebrates (zooplankton) suspended in the water column with little or no ability to resist currents. Plankton are traditionally used as indicators of water quality in lakes and saltwater ecosystems. They are ubiquitous, are in direct contact with the water, are sensitive to a variety of stressors, respond quickly to changes in water quality, and have a direct impact on water quality. However, phytoplankton species composition and abundance are highly variable over periods of a few days, so they are seldom useful as measures of effects except for long-term changes in nutrient loading.

Plankton may be collected from discrete depths or be integrated over a range of depths or horizontal distances, depending on the expected distribution of the stressor(s). Methods include nets, pumps, and bottles. Their selection depends on the target organisms, target depths, and desired sample quality. Measurements include species richness, relative abundance, and community indices (e.g., diversity and similarity). Phytoplankton are often used as the sole representative of the plankton community. They are sufficiently diverse to permit the evaluation of a variety of stressors. It is especially important to collect physical data and water samples for analyses (temperature, photosynthetically active radiation, dissolved oxygen, conductivity, alkalinity, hardness, contaminants, and nutrients) in conjunction with plankton samples; otherwise, it is difficult to associate exposure with effects in large open bodies of water.

25.1.3 FISH

Biological surveys commonly include fish, because the value of fish is generally acknowledged and fish respond to a variety of aqueous contaminants. In addition, fish have practical advantages; their environmental requirements are well known, they integrate effects at lower trophic levels, and identification is relatively simple. Collection methods include electrofishing, nets, and traps. Method selection depends on the habitat characteristics and study design. Relevant habitat characteristics include cover type, stream structure, flow rate, pH, hardness, alkalinity, conductivity, and temperature.

Streams are typically sampled using electrofishing or, less commonly, seining methods. High-quality estimates of species presence and abundance can be obtained by blocking the

upstream and downstream approaches with nets and then repeatedly sampling the reach. The resulting attributes are expressed per unit area, rather than per unit effort, which is less precise for this method. Electrofishing, seines, or hoop nets are used for large streams and rivers, whereas electrofishing, gill nets, fyke nets, and subsurface trawls are best used for lakes and marine environments. Boat-mounted electrofishing units are used in portions of large rivers and lakes that are sufficiently shallow such as shorelines and embayments. The inability to restrict fish movement in open bodies of water results in relative measures of fish community metrics (i.e., numbers per unit effort). Stationary nets are highly selective; the results should not be compared to results obtained with other sampling techniques.

Commonly in the United States, fish community properties or indices that combine several properties are used as measures of effect. The properties may include the number of species, the number of trophic groups, the abundance of species or trophic groups, the biomass of species or the community, and size distributions. The indices may include conventional diversity indices or arithmetic combinations of heterogeneous variables, most notably the Index of Biotic Integrity (IBI) and its derivatives (Karr et al. 1986). These indices are preferred by many state agencies because they are used in water quality management programs (Simon and Lyons 1995). They have many disadvantages as effects measures in risk assessment that can be largely mitigated by disaggregating the index to its component metrics (Suter 1993b, 2001). Properties of individual fish populations are less commonly used as endpoints in surveys, but they would be appropriate where game, commercial, rare, or otherwise particularly valued species are present. Appropriate population properties include abundance, size distribution, and production. The only commonly used properties of individual fish are frequencies of gross pathologies and anomalies. These are easily noted while counting and measuring fish from a community survey and are often of concern to the public and risk managers. The EPA recommends species richness and relative abundance as fish survey metrics for contaminated sites (Office of Emergency and Remedial Response 1994b).

Because fish are mobile, attention must be paid to the range of movement relative to the scale of contamination or disturbance. For this reason, fish surveys are used more in streams, where movement is relatively limited, than in lakes or estuaries. Where movement is a problem, it may be desirable to focus on species such as sunfish that are relatively sessile rather than on community properties that may be influenced by highly mobile or schooling species such as shad.

25.1.4 BENTHIC INVERTEBRATES

Benthic macroinvertebrate communities are commonly surveyed for ecological assessments because they are ubiquitous, important components of aquatic food chains, in direct contact with water or sediment, relatively immobile, and sensitive to a wide range of agents.

Benthic invertebrates in streams are frequently collected from cobble substrates in riffles and runs. The techniques are well established and the results can be compared with many other similarly sampled sites (DeShon 1995). These riffle communities are exposed to water-borne contaminants and conditions such as temperature but have relatively little exposure to sediment-associated contaminants. Benthic invertebrates in riffles are exposed primarily by respiration of contaminated water, whereas benthic invertebrates in sediment depositional areas are often immersed in the contaminated sediment and may ingest sediment. Respiration of overlying water may still be an important pathway for sediment-dwelling organisms, especially for those that ventilate their burrows (e.g., *Hexagenia* mayflies), but not to the exclusion of sediment-associated pathways, which include respiration of sediment pore water.

Surveying riffles but not pools can produce misleading results, as revealed by a survey of paired riffle and pool surveys in multiple streams in Tennessee (Kerans et al. 1992). In many

instances the results for both riffles and pools correctly classified the streams regarding human impacts (based on a fish community index). However, when the classifications differed between riffles and pools, the results for pools were nearly always "correct" in the sense of being consistent with the classification based on the fish community index. Hence, benthic invertebrate communities in sediment depositional areas may be surveyed in addition to the riffle communities if any of the contaminants of concern are likely to be particle associated. The exception is streams in which sediment depositional areas constitute a relatively small fraction of the habitat. For example, the benthic invertebrate communities in sediment depositional areas of Upper East Fork Poplar Creek in Oak Ridge, Tennessee, were not surveyed, because such areas constituted less than 5% of the total available habitat (DOE 1995). In this case a preliminary stream survey was conducted to measure the size, distribution, and total surface area of deposited fine sediments. This proved to be a very useful tool for selecting assessment endpoints, habitats, and exposure pathways for a detailed analysis.

Survey methods vary in rigor from qualitative (e.g., sampling all habitats with a D-frame net) to semiquantitative (e.g., sampling for a specified time or distance with a kicknet) to quantitative (e.g., sampling 0.1 m² with a Surber sampler). Kerans et al. (1992) compared the results of quantitative (Surber and Hess samplers) and qualitative (sampling all habitats with D-Frame net and hand picking) surveys for multiple streams in Tennessee. The quantitative surveys consisted of three to eight replicate samples per site, whereas the qualitative survey consisted of a single composite sample with collection time limited to 2 h. The qualitative surveys failed to detect human impacts that were detected by the quantitative surveys, probably due to the lack of replication. Thus, the assessor should select methods and survey designs that are quantitative and replicated within sites. A preliminary site evaluation may be limited to qualitative and semiquantitative surveys to establish the presence or absence of certain groups of invertebrates and provide qualitative taxa richness and semiquantitative abundance estimates. However, a definitive risk assessment should include quantitative, replicated estimates of community metrics. Definitive assessments should also consider including qualitative surveys of all habitats when the quantitative samples are collected using artificial substrates (DeShon 1995), because artificial substrates are selective and may not be representative of rare taxa or the actual taxa richness at a site.

Data from benthic invertebrate surveys typically consist of counts of individuals of species or higher taxa and, in some cases, biomass. One may simply use the numbers or biomass of individual taxa as the results. Alternatively, from these data, species richness (or taxonomic richness if some taxa are not identified to the species level) or other diversity metrics such as evenness, total numbers, and biomass can be derived. They may be aggregated into multi-metric indices such as Ohio's Invertebrate Community Index (DeShon 1995). The total abundance of Ephemeroptera, Plecoptera, and Trichoptera (EPT taxa) is also a common metric. However, it is based on the sensitivity of these taxa to organic loading and siltation and may not be relevant to site contaminants. For example, the nominally sensitive ephemeropteran *Hexagenia limbata* was so abundant and so contaminated with mercury and polychlorinated biphenyl (PCB) in Poplar Creek that it posed a risk to its predators (Baron et al. 1999). In addition, some waters are unsuitable for EPT taxa, even in the absence of contamination. The EPA recommends biomass, species richness, density, diversity, and relative abundance as benthic invertebrate survey metrics (Office of Emergency and Remedial Response 1994a). Functional measures are seldom used, but may be assumed to be related to these structural measures (Clements 1997).

Kerans and Karr (1994) evaluated 18 attributes of benthic invertebrate communities as indicators of biological condition in streams. The authors conclude that all of the attributes should be used because they appear to be responsive to different human impacts (Kerans and Karr 1994). This is a reasonable approach for contaminated sites, provided the possible

explanations for the status of each attribute are considered (see Kerans and Karr 1994 for examples). However, emphasis should be placed on metrics that are related to the assessment endpoint. Carlisle and Clements (1999) found taxa richness measures to be the most sensitive and statistically powerful metrics for evaluating metal pollution in Rocky Mountain streams. Abundance attributes were generally found to be insensitive to metal pollution or highly variable. The species richness of mayflies, which are generally sensitive to metals, is particularly noteworthy (Clements 1997).

Population or organism properties are seldom considered in benthic invertebrate surveys. In some cases, however, abundance of a particular sensitive and valued species may be an endpoint. One such endpoint was the abundance of the widgeon clam (*Pitar morrhuana*) at Quonset Point, Rhode Island (Eisler 1995).

It is important to determine the texture, organic matter content, depth of overlying water, and any other habitat properties that might influence the benthic invertebrate community at sampled locations. Elevated ammonium concentrations are particularly common and likely to result in toxicity that is unrelated to site contaminants. Even at a highly contaminated site, habitat variables are likely to explain more of the variance in invertebrate community properties than contaminant concentrations (Jones et al. 1999).

Spatial variability, rather than temporal variability, is the primary concern for sediment contaminants and sediment characteristics. This is especially true in slow-flowing systems with relatively stable sediments. Samples for sediment analysis should be collected as close to the biological survey sampling points as practically possible. Ideally, subsamples of the sediment included in each benthic survey sample, including replicates, should be analyzed for contaminants and sediment characteristics. This is rarely practical for contaminant analyses, but sediment characterization is relatively simple and inexpensive. Recommended sediment quality characteristics include grain size (percent sand, silt, clay), organic carbon content, ammonia, and pH. Quantitative measurements such as grain size fractions should be preferred over subjective and qualitative designations such as sandy or mucky. This allows the assessor to better compare results within and among studies. It also expands the risk characterization techniques available to the assessor. For example, the benthic invertebrate assessment for the Clinch River included multiple regression analyses of the benthic survey data with both contaminant and habitat characteristics as explanatory variables (Jones et al. 1999).

In addition, water quality may influence benthic communities and can vary significantly through time. Water samples should be taken such that representative exposures can be estimated.

25.2 TERRESTRIAL BIOLOGICAL SURVEYS

Terrestrial biological surveys are much less common than aquatic surveys as input for ecological risk assessments and there are no survey-based soil or air quality criteria like the aquatic biological criteria in the United States. The methods are less well developed and typically must be developed ad hoc or adapted from resource management or research methods.

25.2.1 SOIL BIOLOGICAL SURVEYS

Soil communities are surveyed less often than aquatic communities, even though there are fewer inherent difficulties in obtaining soil samples. Ecological risk assessments rarely use surveys of soil invertebrates, microorganisms, or soil processes. However, examples can be

found in Menzie et al. (1992) and Jenkins et al. (1995). Approaches to surveying soil biota include: (1) collecting samples of soil and extracting taxa in the laboratory; (2) extracting organisms in the field, e.g., with mustard solution; and (3) trapping organisms using pitfall traps. The second method is the least quantitative, as it is likely to extract organisms to variable depths. Although most surveys have focused on invertebrates (Paine et al. 1993; Pizl and Josens 1995), microbial community properties, element transformations, and litter accumulation have also been surveyed (Jackson and Watson 1977; Strojjan 1978; Tyler 1984; Beyer and Storm 1995). The abundance and composition of soil biota are highly dependent on soil characteristics (Nuutinen et al. 1998), so risk assessors must carefully determine that reference locations are appropriate.

25.2.2 WILDLIFE SURVEYS

Many methods are available for the collection of field data for wildlife populations, involving direct observation, trapping, vocalizations, track counts, netting, and attractants (Bookhout 1994; Heyer 1994; Wilson 1996; Suter et al. 2000). These methods may produce data that are useful in ecological risk assessments and may help elucidate the presence, nature, and magnitude of effects. Wildlife surveys may generate presence/absence, abundance, and age structure data as well as food habits information for exposure modeling. By the comparison of these data between the contaminated site and one or more reference sites, effects attributable to contaminant exposure may be differentiated from population fluctuations or habitat alterations that result from other causes. As noted previously, colonial nesting birds lend themselves to surveys for effects of contaminants or other agents (Giesy et al. 1994b; Henshel et al. 1995; Ludwig et al. 1996; Halbrook et al. 1999b; Custer et al. 2003).

Wildlife surveys differ from plant and invertebrate surveys in the extent to which they incorporate necropsy of organisms that are found dead, debilitated, or moribund (US Geological Survey 1999). This is because the techniques are available and because wildlife ecology is more focused on organisms. A good example is the necropsy of waterfowl performed as part of the assessment of lead mining in the Coeur d'Alene basin (Henny 2003). Although necropsy of opportunistically collected organisms is suggestive, a data set that is useful for assessment will usually require collection of other organisms from the site of concern and reference sites to determine the distribution and frequency of pathologies and body burdens.

25.2.3 TERRESTRIAL PLANT SURVEYS

Because vegetation provides the habitat for all inhabitants of terrestrial communities, it is important to survey and map vegetation on contaminated or disturbed sites, even before the problem formulation. In addition, if plant populations or communities are assessment endpoints, biological surveys may be an appropriate line of evidence for estimating risks. Because plants are immobile, they are clearly associated with a localized environment, and are easily sampled. However, few ecological risk assessments have been based on plant survey data. Guidance has been provided by the EPA (Environmental Response Team 1994b, 1996). The Agency recommends density, coverage, and frequency metrics as measures of effects for plant populations and communities. Examples are provided by Galbraith et al. (1995) and LeJeune et al. (1996), who took transect measurements of percent cover of tree, shrub, forb, and grass species to aid in the estimation of risks to the plant community in the Clark Fork River floodplain and Anaconda site in Montana. Similarly, surveys of vascular plants, mosses, and lichens showed severe effects in zinc-contaminated areas of the Lehigh Gap, Pennsylvania (Beyer and Storm 1995).

Because plants are valued and ecologically important for their primary production, measures of plant growth or production may be particularly useful for sites with contaminated

to surveying soil biota laboratory; (2) extracting organisms using pitfall traps to extract organisms to (Paine et al. 1993; Pizani et al. 1978; Tyler 1984; Beyer 1994). The accuracy of soil sampling is highly dependent on soil characteristics that determine that reference

populations, involving attractants (Bookhout et al. 1994) to produce data that are representative of the presence, nature, and abundance, and age of the population. By the comparison of sites, effects attributable to fluctuations or habitat changes are identified. Nesting birds lend additional information (Henshel et al. 1994b; Henshel et al. 1993).

extent to which they are moribund (US Geological Survey 1994). Wildlife ecology is often performed as part of an assessment. Although necropsy of animals for assessment will be useful for assessment and reference sites to compare.

in communities, it is important to identify sites, even before the assessment endpoints, to assess the risk. Because plants are easily sampled, they are good indicators. Guidance has been provided by The Agency recommending that sensitive populations and species be identified (Paine et al. 1996), who have identified the 11 and Anaconda sites and severe effects in 1995).

production, measured with contaminated

soils or shallow, contaminated ground water. Tree coring is recommended by the EPA as a means to measure effects of contaminants on tree growth (Environmental Response Team 1994c). The width of annual growth rings may indicate the effects of contaminants, but because of the confounding effects of drought, frost, and other environmental factors, the interpretation should be performed by an experienced dendrochronologist. When vegetation is herbaceous, the EPA recommends that growth be determined by repeated clipping and weighing of the aboveground plant parts (Environmental Response Team 1994a).

It should be emphasized that few risk assessment schedules permit the repeated sampling of vegetation over long periods of time. The usefulness of a vegetation survey depends on whether observed effects can be related to measures at reference sites or reference (precontamination) points in time. Although one tree ring sample provides a time series (and each tree is its own control), the discernment of effects from herbaceous plant clippings generally requires multiple temporal samples. Thus, detrimental effects on production of the forest understory, old fields, or grasslands are not usually evident from a single vegetation survey.

If unhealthy plants or unvegetated areas are observed, the following question should be asked to determine the usefulness of the survey: can factors other than contaminants explain the brown foliage or other adverse response? These factors could include seasonal patterns, nutrient deficiency, insect herbivory, salt from winter applications to roads, acid rain, ozone, drought, grazing pressure, fire, or changes in hydrological patterns associated with the development of adjacent land. For example, when adverse impacts on forest trees were observed within the Bear Creek Watershed on the Oak Ridge Reservation, it was unclear whether dead trees were the result of contamination or altered hydrology associated with logging a neighboring area. Occasionally, specific toxic symptoms may be associated with particular contaminants. For example, "crinkle leaf" of cotton is associated with manganese toxicity, and an accumulation of purple pigment in soybean leaves can signal cadmium toxicity (Foy et al. 1978). However, these symptoms do not necessarily apply to other species, and most symptoms of toxicity such as stunted growth and chlorosis are common to many toxicants and nutrient deficiencies (Skelly et al. 1990).

Basic soil data should be obtained during the vegetation survey. These characteristics include major plant nutrients, pH, organic matter content, particle-size distribution, bulk density, and salinity, where relevant. One or more of these factors might explain differences in plant parameters at different locations.

25.3 PHYSIOLOGICAL, HISTOLOGICAL, AND MORPHOLOGICAL EFFECTS

Monitored effects on biochemical, physiological, or cellular properties of an organism that are indicative of toxic effects are commonly referred to as biomarkers. Their use has been inhibited by the fact that few of them are clearly related to the overt effects that constitute assessment endpoints in most ecological risk assessments. Although it has been proposed that remedial goals be based on elimination of any detectable biomarker response (Depledge and Fossi 1994), regulators do not normally take action on the basis of enzyme induction, even for humans.

Biomarkers of effects may play a supporting role in ecological risk assessments. In particular, biomarkers that are characteristic of a particular chemical, class of chemicals, or mode of action can support the inference that apparent effects are caused by particular contaminants (Chapter 4). For example, aminolevulinic acid dehydrogenase (ALAD) activity in the blood of waterfowl was used to diagnose lead toxicosis (Henny 2003). Even damage that is not particularly diagnostic can be useful if it can be even qualitatively related to population-level responses. For example, histological damage to the gonads of largemouth bass in Poplar Creek embayment in Oak Ridge, Tennessee, supported the inference that the

low abundance and species richness of fish was due to toxic effects rather than habitat properties. When biomarkers are used to support inferences concerning causation, it is important to associate their levels or frequencies with contaminant concentrations.

Gross pathologies such as tumors, lesions, and skeletal deformities have played a more important role in ecological risk assessments than biochemical biomarkers. They are a common source of public concern, particularly where they occur in sport or commercial fish. Frequencies of gross pathologies are easily determined when fish are collected for chemical analysis or for biological surveys. Pathologies that are characteristic of chemicals or chemical classes can also contribute to attributing causation to both the pathologies themselves and any population or community effects.

25.4 UNCERTAINTIES IN BIOLOGICAL SURVEYS

Biological surveys potentially provide direct estimates of effects at sites receiving various levels of exposure. The primary uncertainties to be considered in such cases are sampling variance and biases in the survey results as estimators of the assessment endpoint. Sampling variance is estimated by conventional statistics. Biases must, in general, be estimated by expert judgment.

More difficult uncertainties arise when biological survey results are used to estimate effects at sites other than those surveyed. Such estimates may require interpolation or extrapolation. An example of interpolation would be the use of fish surveys at certain locations in a stream to estimate effects at locations lying between sampled locations. This might be done by algebraic interpolation, by spatial statistics, or by process modeling. An example of extrapolation would be the use of survey data for one contaminated stream to estimate effects on another stream with the same contaminant. At minimum, the uncertainty in such extrapolations would be equal to the variance among fish communities at uncontaminated sites (i.e., upstream reference communities in the case of interpolation or regional reference communities in the case of extrapolation). Additional uncertainty results from variance in the effective contaminant exposure due to differences in chemical form, patterns of temporal variance, etc.

25.5 SUMMARY

Biological surveys are used to determine whether a site is biologically impaired or to estimate exposure-response relationships for a site assessment or for a watershed or region. They are inherently realistic, but because exposures and conditions are uncontrolled, apparently causal associations are often misleading (Chapter 4). In addition, because of inherent variability of populations and communities, imprecision of most methods, typically small numbers of samples, and general lack of time series, effects must typically be large before they can be confidently detected. Hence, it is important to avoid accepting negative results without determining whether the methods used could detect levels of effects that are important to decision makers or stakeholders. Therefore, it is important to use biological surveys along with laboratory test results and modeling so that its realism can be weighed against the greater clarity and sensitivity of other lines of evidence (Chapter 32).