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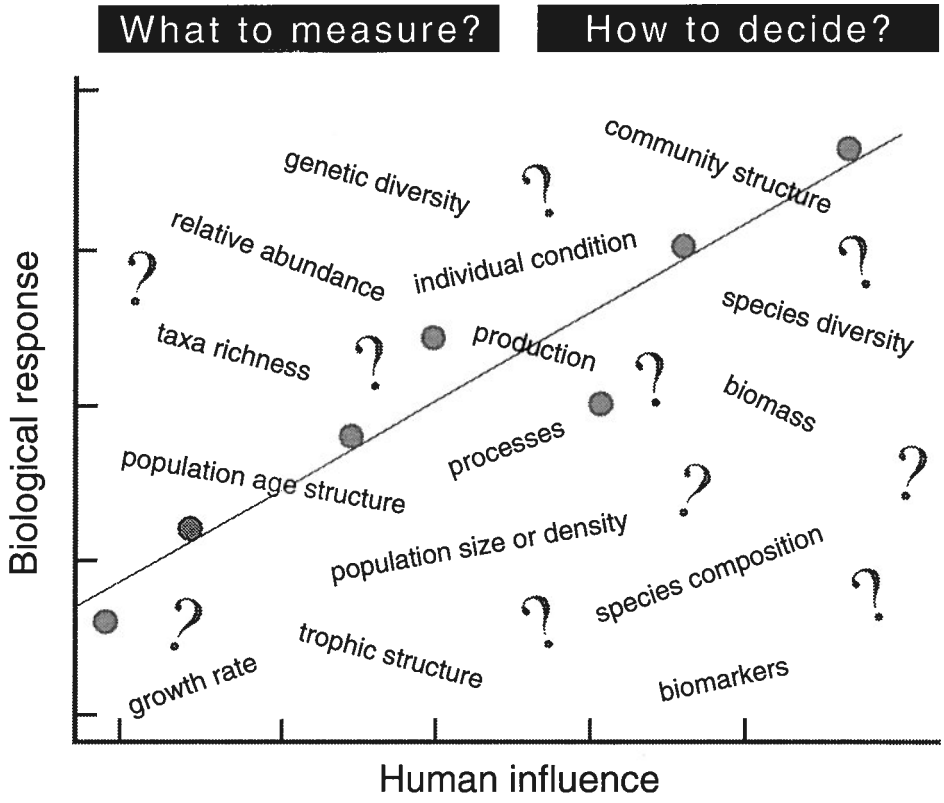
Biological Monitoring and Assessment: Using Multimetric Indexes Effectively

James R. Karr and Ellen W. Chu



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Biological Monitoring and Assessment: Using Multimetric Indexes Effectively

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INTRODUCTION

Can we afford clean water? Can we afford rivers and lakes and streams and oceans, which continue to make life possible on this planet? Can we afford life itself? . . . These questions answer themselves.

—Senator Edmund Muskie (1972)

The story of a continent is reflected in the biology of its rivers. And what a biologist sees in North America's rivers is a history of damaged landscapes and undervalued fresh waters. As a century of dramatic cultural and ecological change in the United States draws to a close, outdated legal doctrines and weak implementation of good laws dominate water resource policy throughout the nation. Will they continue to do so in the twenty-first century?

The most direct and effective measure of the integrity of a water body is the status of its living systems

Water resources are not simply water; their value to a society comes from more than the quality and quantity of liquid water. Humans depend on living waters for many essential goods and services, from drink and food to cleansing of our wastes to aesthetic and recreational renewal. One explicit, visionary statement in the 1972 Water Pollution Control Act Amendments (PL 92-500, now called the Clean Water Act) acknowledged the overarching importance of whole water resources: "The objective of this Act is to restore and maintain the chemical, physical, and biological integrity of the Nation's waters" [Clean Water Act (CWA) § 101(a)].

Although some progress has been made under this law in controlling point-source pollution, especially organic effluent, other harmful and pervasive forms of degradation—nonpoint pollution, altered hydrological regimes, habitat destruction, and invasions by alien species—continue to degrade aquatic ecosystems. In short, despite the clarity of the legal mandate, the condition of America's waters says unequivocally that we have failed to achieve the Clean Water Act's objectives. How can we reverse this trend?

The most direct and effective measure of the integrity of a water body is the status of its living systems. Life depends on water. Do we expect waters that cannot support healthy biological communities to provide us with the goods and services

we need? Choosing and monitoring biological endpoints is thus fundamental for assessing water resource quality and for charting a course for federal and state programs to protect society's most basic interests.

Biological monitoring tracks the health of biological systems in much the same way that investors track the health of the US economy. Biological monitoring aims to detect change in living systems—specifically, change caused by humans. To detect the effects of human activities on biological systems, biological monitoring must study human disturbance apart from disturbances that occur naturally—a crucial distinction that biological monitoring programs have too often lost sight of. Tracking, evaluating, and communicating the condition of biological systems, and the consequences of human activities for those systems, lie at the heart of biological monitoring.

To put it another way, biological monitoring identifies ecological risks that are as important to human health and well-being as the more obvious threats of toxic pollution or vector-borne disease. Indeed, EPA's Scientific Advisory Board (SAB 1990) stipulated, "Attach as much importance to reducing ecological risk as is attached to reducing human health risk." Halting the deterioration of the nation's waters cannot be done if we continue to behave as if our actions had no ecological risks (Karr 1995a).

Assessing ecological risks accurately depends on effective biological monitoring. Included by EPA in its framework for ecological risk assessment (USEPA 1992b, 1994a,b, 1996d), biological monitoring aims to identify problems by assessing biological condition (what EPA calls "characterization of ecological effects") and to define the nature and magnitude of any problem. The results of these analyses must then be communicated to citizens and decision makers, who will determine what to do. Like human-health risk assessors, ecological risk assessors need reliable, conceptually sound tools for each of these steps.

Assessing ecological risks accurately depends on effective biological monitoring

During a century of evolution, through changing human impacts on water and its associated resources, biological monitoring programs have taken a variety of approaches (Davis and Simon 1995; Karr 1998). The approach in this report—development of multimetric indexes of biological condition—began in 1981 with the index of biological integrity, or IBI (Karr 1981). Now well documented as effective for assessing ecological condition in a variety of management settings, with many taxa, and in diverse geographic regions, multimetric biological indexes are a logical next step in biological monitoring's evolution. Why? Principally because these indexes evaluate ecological condition in terms of a system's ability to support unimpaired living systems—in terms of the biota's ability to sustain itself—ultimately the most relevant endpoint for sustaining human society.

In much the way economic indexes such as the Dow Jones industrial average and the index of leading economic indicators combine many financial measures to assess the state of the national economy, the index of biological integrity integrates measurements of many biological attributes (metrics) to assess the condition of a place. Metrics are chosen on the basis of whether they reflect specific and predictable responses of organisms to human activities. Ideal metrics should be relatively

easy to measure and interpret. They should increase or decrease as human influence increases. They should be sensitive to a range of biological stresses, not narrowly indicative of commodity production or threatened or endangered status. Most important, biological attributes chosen as metrics must be able to discriminate human-caused changes from the background "noise" of natural variability. Human impact is the focus of biological monitoring.

Numerous studies have documented the responses of biological attributes to human disturbance. Across diverse taxa and regions, similar biological attributes (e.g., taxa richness and the relative abundance of tolerant organisms) work consistently and reliably as indicators of resource condition. Across regions and agencies, consensus is emerging about the appropriate level of sampling needed to assess the condition of biological systems accurately.

Successful multimetric efforts combine biological insight with appropriate sampling design and statistical analyses. Knowledge of regional biology and natural history—not solely a search for statistical relationships and significance—should drive both sampling design and analytical protocol. Rigorously done, multimetric biological monitoring and assessment offer a systematic approach that measures many dimensions of complex ecological systems—dimensions that have too long been ignored.

Of course challenges remain. Biologists must extend what they have learned about monitoring in fresh water to other environments and other taxonomic groups. On the other hand, they must avoid gathering more data than are necessary for better management decisions. Like any scientific method, biological monitoring generates many new and interesting questions, methods, and refinements. But scientists and managers need to realize that they already know enough about how biological systems respond to human influence—enough to make decisions that will stop the decline of water resources. Managers and policymakers must *use* what they already know.

Most important, however, biologists must communicate ecological condition more effectively outside biological circles. In a society that does not value the integrity of aquatic or other natural systems, no amount of scientific nagging will improve resource policy. Biologists and all who understand both the value and the declining health of natural life-supporting systems must share their knowledge widely. In the end, only an informed public can put adequate pressure on decision makers to change business as usual. The precision and clarity of information gathered through multimetric biological monitoring and assessment can help this process.

This report discusses the state of US running waters and the value of multimetric biological indexes in assessing and communicating their condition. The extent to which better decisions are made—decisions that maintain or restore aquatic systems as opposed to the status quo—will be a measure of these indexes' success.

The report is built around numbered statements, each representing a step in the logical development of multimetric biological indexes or a bone of contention in the assessment literature. The table of contents offers a document map, from trends in aquatic resource condition (Section I), to changing scientific and societal views of water resources Section II), to how and why multimetric indexes work (Section III), through the most common pitfalls associated with use of multimetric indexes (Section IV). In Section V, we quote others' objections to multimetric indexes and try to show that those assertions are at best misleading and often false. Section VI is a call to arms.

Who will find this document useful? Several audiences, we hope: an agency scientist trying to decide whether and how to use fish or invertebrates in monitoring work; a researcher designing a study to detect human effects; and a state agency responding to EPA's mandate to develop biocriteria. This is a handbook for those working to protect the nation's waters; we hope it will become dogeared and dirty.

SECTION I

Aquatic Resources Are Still Declining

This first section sets forth the condition of aquatic ecosystems, to inform those unfamiliar with them of the damage that has already occurred and to arm those already concerned with specific details on the extent of degradation.

PREMISE 1

WATER RESOURCES ARE LOSING THEIR BIOLOGICAL COMPONENTS

Despite strong legal mandates and massive expenditures, signs of continuing degradation in biological systems are pervasive—in individual rivers (Karr et al. 1985b), US states (Moyle and Williams 1990; Jenkins and Burkhead 1994), North America (Williams et al. 1989; Frissell 1993; Wilcove and Bean 1994), and around the globe (Hughes and Noss 1992; Moyle and Leidy 1992; Williams and Neves 1992; Allan and Flecker 1993; Zakaria-Ismail 1994; McAllister et al. 1997). Aquatic systems have been impaired, and they continue to deteriorate as a result of human society's actions (Table 1).

Devastation is obvious, even to the untrained eye. River channels have been destroyed by dams; straightening and dredging; and water withdrawal for irrigation, industrial, and domestic uses. Degradation of living systems inevitably follows. Biological diversity in aquatic habitats is threatened; aquatic biotas have become homogenized through local extinction, the introduction of alien species, and declining genetic diversity (Moyle and Williams 1990; Whittier et al., 1997a). Who remembers that a freshwater fishery existed in the Illinois River in the early 1900s that was second only to the Columbia's? Now that fishery is gone, and the one in the Columbia is nearly gone. Since the turn of the twentieth century, commercial fish harvests in US rivers have fallen by more than 95%.

As recently as a century ago, a commercial freshwater fishery second only to the one in the Columbia River flourished in the Illinois River; now it is gone

Even where commercial and sport catches of fish and shellfish are permitted, one can no longer assume that those harvests are safe to eat (USEPA 1996a). In 1996, fish consumption advisories were imposed on 5% of the river kilometers in the US (*www.epa.gov/OST/fishadvice/index.html*). The number of fish advisories is rising. The 2193 advisories reported for US water bodies in 1996 represent an increase of 26% over 1995 and a 72% increase over 1993. For millennia, humans have depended on the harvest from terrestrial (including agricultural), marine, and freshwater systems for food. But the supply of freshwater foods has collapsed. How would society respond if agricultural productivity declined by more than 80% or if eating "farm-fresh" products threatened our health? Why then do we continue to ignore such changes in "wild-caught" aquatic resources?

Current programs are not protecting rivers or their biological resources because the Clean Water Act has been implemented as if crystal-clear distilled water running down concrete conduits were the act's ultimate goal (Karr 1995b). For example, at least $473 billion was spent to build, operate, and administer water-pollution control facilities between 1970 and 1989 (Water Quality 2000 1991). Still, the

decline continues while money is wasted on inadequate or inappropriate treatment facilities (Karr et al. 1985a; see Box 1, page 12).

In many respects, society has been lulled into believing that our individual and collective interests in water resources have been protected by national, state, and local laws and regulations. We have had faith in the outdated "prior appropriation doctrine" of American frontier water law, the implementation of the Clean Water Act, or "wild and scenic river" designation when, in fact, our habits as a society and the way we have implemented our laws have progressively compromised our fresh waters.

TABLE 1. Examples from United States rivers of degradation in aquatic biota (from Karr 1995a).

Proportionately more aquatic organisms are classed as rare to extinct (34% of fish, 75% of unionid mussels, and 65% of crayfish) than terrestrial organisms (from 11% to 14% of birds, mammals, and reptiles; Master 1990).
Twenty percent of native fishes of the western United States are extinct or endangered (Miller et al. 1989; Williams and Miller 1990).
Thirty-two percent of fish native to the Colorado River are extinct, endangered, or threatened (Carlson and Muth 1989).
In the Pacific Northwest, 214 native, naturally spawning Pacific salmon and steelhead stocks face "a high or moderate risk of extinction, or are of special concern" (Nehlsen et al. 1991).
Since 1910, naturally spawning salmon runs in the Columbia River have declined by more than 95% (Ebel et al. 1989).
During the twentieth century, the commercial fish harvests of major US rivers have declined by more than 80% (Missouri and Delaware Rivers), more than 95% (Columbia River), and 100% (Illinois River) (Karr et al. 1985b; Ebel et al. 1989; Hesse et al. 1989; Patrick 1992).
Since 1933, 20% of molluscs in the Tennessee River system have been lost (Williams et al. 1993); 46% of the remaining molluscs are endangered or seriously depleted throughout their range.
In 1910, more than 2600 commercial mussel fishers operated on the Illinois River; virtually none remain today.
Since 1850, many fish species have declined or disappeared from rivers in the United States (Maumee River, Ohio: 45% [Karr et al. 1985b]; Illinois River, Illinois: 67% [Karr et al. 1985b]; California rivers: 67% [Moyle and Williams 1990]). This decline, combined with the introduction of alien species, has homogenized the aquatic biota of many regions (an average of 28% of the fish species in major drainages of Virginia are introduced; Jenkins and Burkhead 1994).
Thirty-eight states reported fish consumption closures, restrictions, or advisories in 1985; 47 states did in 1991. The 2193 advisories reported for US water bodies in 1996 represent a 26% increase over 1995 and a 72% increase over 1993 (USEPA 1996a). Contaminated fish pose health threats to wildlife and people (Colborn et al. 1990, 1996), including intergenerational consequences such as impaired cognitive functioning in infants born to women who consume contaminated fish (Jacobson et al. 1990; Jacobson and Jacobson 1996).
Riparian corridors have been decimated (Swift 1984).
Native minnows have declined while alien minnows have spread throughout northeastern US lakes (Whittier et al. 1997a).

PREMISE 2

"Clean water" is not enough

Society relies on freshwater systems for drinking water, food, commerce, and recreation as well as waste removal, decomposition, and aesthetics. Yet in the Pacific Northwest alone, recent declines in salmon runs and closures of sport and commercial fisheries have led to economic losses of nearly $1 billion and 60,000 jobs per year (Pacific Rivers Council 1995). Retaining the biological elements of freshwater systems (populations, species, genes), as well as the processes (mutation, selection, fish migration, biogeochemical cycles) sustaining these elements, is crucial to retaining the goods and services fresh waters provide (Table 2).

Waters and fish travel over vast distances in space and time. The integrity of water resources thus depends on processes spanning many spatial and temporal scales: from cellular mechanisms producing local and regional adaptations to a massive transfer of energy and materials as fish migrate between the open ocean and mountain streams. Protecting the elements and processes society values therefore demands a broad, all-encompassing view—one not yet encouraged by conventional management strategies and terminology.

Pollution is anything that alters the physical, chemical, biological, or radiological integrity of water

In particular, the word *pollution* must take on broader connotations. In conventional usage and agency jargon, *pollution* refers to chemical contamination. A more appropriate, yet little-used, definition that more accurately represents what is at stake as water resources decline is the definition given by the 1987 reauthorization of the Clean Water Act: *pollution* is any "manmade or man-induced alteration of the physical, chemical, biological, or radiological integrity of water." Under this definition, humans degrade or "pollute" by many actions, from irrigation withdrawals to overharvesting, not merely by releasing chemical contaminants.

TABLE 2. Elements, processes, and potential indicators of biological condition for six levels of organization within three biological categories. Indicators from multiple levels are needed to assess the condition of a site comprehensively. (Modified from Angermeier and Karr 1994.)

Biological category	Elements (levels)	Processes	Indicators
Taxonomic	Species	Range expansion or contraction	Range size
		Extinction	Number of populations
		Evolution	Population size
			Isolating mechanisms
Genetic	Gene	Mutation	Number of alleles
		Recombination	Degree of linkage
		Selection	Inbreeding or outbreeding depression
Ecological	Individual	Health	Disease
			Deformities
			Individual size and condition index
			Growth rates
	Population	Changes in abundance	Age or size structure
		Colonization or extinction	Dispersal behavior
		Evolution	Presence of particular taxa (e.g., intolerants)
		Migration	Gene flow
	Assemblage	Competitive exclusion	Number of species
		Predation or parasitism	Dominance
		Energy flow	Number of trophic links
		Nutrient cycling	Spiraling length
	Landscape	Disturbance	Fragmentation
		Succession	Percentage of disturbed land
		Soil formation	Number of communities
		Metapopulation dynamics	Sources and sinks
			Number and character of metapopulations

PREMISE 3

Biological monitoring is essential to protect biological resources

Despite their faith in and reliance on technology, humans are part of the biological world. Human life depends on biological systems for food, air, water, climate control, waste assimilation, and many other essential goods and services (Costanza et al. 1997; Daily 1997; Pimentel et al. 1997). Biological endpoints are therefore fundamental. Furthermore, the status of living systems provides the most direct and most effective measure of the "integrity of water," the resource on which all life depends.

Degradation of water resources begins in upland areas of a watershed, or catchment, as human activity alters plant cover. These changes, combined with alteration of stream corridors, in turn modify the quality of water flowing in the stream channel as well as the structure and dynamics of those channels and their adjacent riparian environments. Biological evaluations focus on living systems, not on narrow chemical criteria, as integrators of such riverine change. In contrast, exclusive reliance on chemical criteria assumes that water resource declines have been caused only by chemical contamination. Yet physical habitat loss and fragmentation, invasion by alien species, excessive water withdrawals, and overharvest by sport and commercial fishers do as much if not more harm than chemicals in many waters.

The status of living systems provides the most direct and most effective measure of the "integrity of water," the resource on which all life depends

Even measured according to chemical criteria, water resources throughout the United States are significantly degraded (USEPA 1992a, 1995; see Table 1, page 7). In 1990 the states reported that 998 water bodies had fish advisories in effect, and 50 water bodies had fishing bans imposed. More than one-third of river miles assessed by chemical criteria did not fully support the "designated uses" defined under the Clean Water Act. More than half of assessed lakes, 98% of assessed Great Lakes shore miles, and 44% of assessed estuary area did not fully support designated uses (USEPA 1992a).

By September 1994, the number of fish consumption advisories had grown to 1531 (USEPA 1995). Seven states (Maine, Massachusetts, Michigan, Missouri, New Jersey, New York, and Florida) issued advisories against eating fish from state waters in 1994. Fish consumption advisories increased again in 1995, by 12%; the advisories covered 46 chemical pollutants (including mercury, PCBs, chlordane, dioxin, and DDT) and multiple fish species. Forty-seven states had advisories, representing 15% of the nation's total lake acres and 4% of total river miles. All the Great Lakes were under advisories. For the first time, EPA reported that 10 million Americans were at risk of exposure to microbial contaminants such as *Cryptosporidium* because

their drinking water was not adequately filtered (USEPA 1996c). For the same year, the Washington State Department of Ecology reported that "80 percent of the hundreds of river and stream segments and half of the lakes tested by the state don't measure up to water quality standards" (*Seattle Times* 1996). Outbreaks of *Pfiesteria piscicida*, the "cell from hell," have killed millions of fish and were also implicated in human illnesses from Maryland to North Carolina in 1997 (Hager and Reibstein 1997).

Alarming as they are, these assessments still underestimate the magnitude of real damage to our waters because they generally do not incorporate biological criteria or indicators. When compared with strictly chemical assessments, those using biological criteria typically double the proportion of stream miles that violate state or federal water quality standards or designated uses (Yoder 1991b; Yoder and Rankin 1995a). The reasons for this result are simple. Although humans degrade aquatic systems in numerous ways, chemical measures focus on only one way. Some states rely on chemical surrogates to infer whether a water body supports the "designated use" of aquatic life; others measure biological condition directly (Davis et al. 1996). Only 25% of 392,353 evaluated river miles were judged impaired according to chemical standards intended to assess aquatic life. But when biological condition was assessed directly, 50% of the 64,790 miles evaluated in the US showed impairment.

Perhaps more important, these numbers suggest that we know more about the condition of water resources than we actually do. Sadly, despite massive expenditures and numerous efforts to report water resource trends, "Congress and the current administration are short on information about the true state of the nation's water quality and the factors affecting it" (Knopman and Smith 1993). Because assessments emphasize chemical contamination rather than biological endpoints, state and federal administrators are not well equipped to communicate to the public either the status of or trends in resource condition. Further, because few miles of rivers are actually assessed, and because those that are assessed are not sampled appropriately (e.g., using probability-based surveys; Larsen 1995; Olsen et al., in press), percentages of impaired river miles are extremely rough at best.

In short, despite explicit mandates to collect data to evaluate the condition of the nation's water resources, and the existence of a program intended to provide an inventory under section 305(b) of the Clean Water Act, no program has yet been designed or carried out to accomplish that goal (Karr 1991; Knopman and Smith 1993).

The strength of these observations is clearly an important force driving recent state actions; 42 states now use multimetric assessments of biological condition, and 6 states are developing them. Only 3 states were using multimetric biological approaches in 1989 (Davis et al. 1996), and none had them in 1981 when the first multimetric IBI paper was published. Indeed, hardly any effective biological monitoring programs were in place before 1981. Most states still have a long way to go toward collecting and using biological data to improve the management of their waters.

Because they focus on living organisms—whose very existence represents the integration of conditions around them—biological evaluations can diagnose chemical, physical, and biological impacts as well as their cumulative effects. They can serve many kinds of environmental and regulatory programs when coupled with single-chemical toxicity testing in the laboratory. Furthermore, they are cost effective. Chemical evaluations, in contrast, often underestimate overall degradation, and overreliance on chemical criteria can misdirect cleanup efforts, wasting both money and natural resources (Box 1). Because they focus on what is at risk—biological systems—biological monitoring and assessment are less likely to underprotect aquatic systems or waste resources.

Biological evaluations and criteria can redirect management programs toward restoring and maintaining "the chemical, physical, and biological integrity of the nation's waters." Assessments of species richness, species composition, relative abundances of species or groups of species, and feeding relationships among resident organisms are the most direct measure of whether a water body meets the Clean Water Act's biological standards for aquatic life (Karr 1993). To protect water resources, many states should track the biological condition of water bodies the way society tracks local and national economies, personal health, and the chemical quality of drinking water.

BOX 1. Narrow use of chemical criteria can damage water resources and waste money.

Chlorine is added to effluent from secondary sewage treatment because it kills microorganisms that cause human disease. But the effects of this chlorine continue after effluent is released into streams or other water bodies (Colborn and Clement 1992; Jacobson and Jacobson 1996). In three Illinois streams receiving water from a secondary treatment plant, an IBI based on fish declined significantly as residual chlorine concentration increased (Karr et al. 1985a; Figure 1); the biological effects of chlorine appeared in fish assemblages downstream of the effluent inflow (Figure 2). With chlorination (treatment phase I), IBIs were much lower downstream than upstream. In contrast, when chlorine was removed from secondary effluent (phase II), downstream and upstream IBIs did not differ significantly. In other words, chlorine added to wastewater effluent continues to kill organisms after the chlorinated water is released. Furthermore, biological condition did not improve when expensive tertiary denitrification was added (phase III), even though this treatment brought the plant into compliance with chemical water quality standards for nitrates.

This example illustrates three important points. First, biological integrity may be damaged by too narrow a focus on chemical criteria. Second, such a narrow focus can waste money. Third, many current management approaches and policies are, in essence, untested hypotheses. Managers do not always make the effort to look for broader effects or to test beyond their initial criteria.

Had managers looked for biological effects or reconsidered the levels of chlorine in the effluent instead of assuming that their chlorine criteria worked, the biota of these Illinois streams might have suffered less.

FIGURE 1. In three streams in east-central Illinois, the fish indexes of biological integrity (IBIs) declined significantly in response to wastewater inflow from secondary treatment with chlorination. Fish IBIs declined as residual chlorine concentration increased (from Karr et al. 1985a).

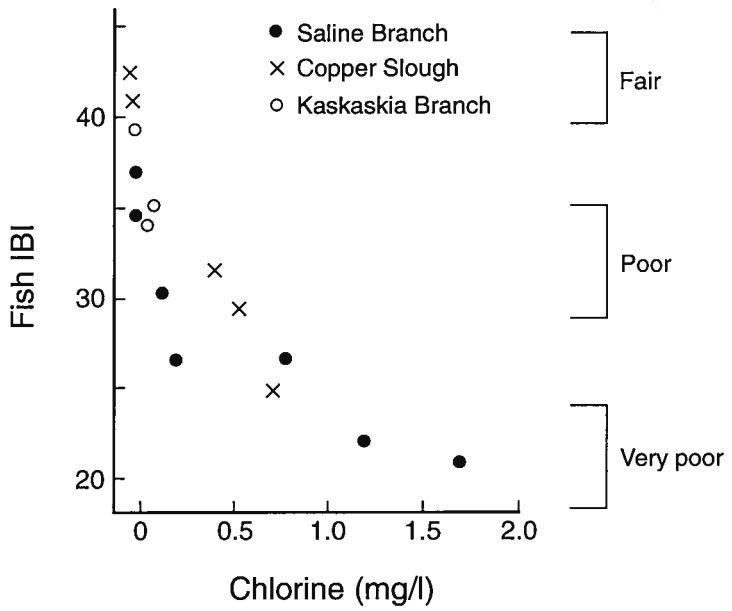
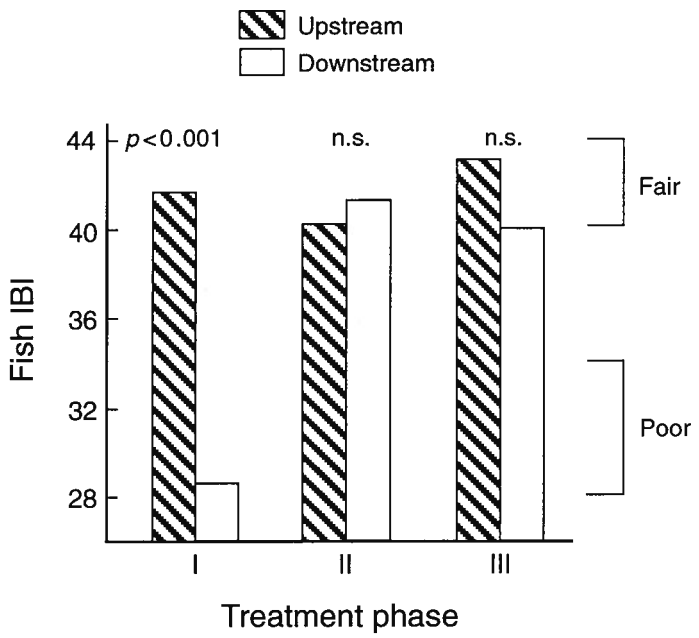


FIGURE 2. Fish IBIs for stations upstream and downstream of wastewater treatment effluent in Copper Slough, east-central Illinois. Phase I: standard secondary treatment; phase II: secondary treatment without chlorination; phase III: secondary treatment without chlorination but with tertiary denitrification. With chlorination (phase I), IBIs were much lower downstream than upstream of effluent inflow. Upstream and downstream sites did not differ statistically after removal of chlorine from secondary effluent (phase II). The addition of expensive tertiary denitrification (phase III) did not increase IBIs (from Karr et al. 1985a).



SECTION II

Changing Waters and Changing Views Led to Biological Monitoring

Biological monitoring is evolving as societal and scientific thinking changes. Growth in knowledge about aquatic systems—and humans' effects on them—has provided a substantial body of theory as well as empirical evidence about how to measure their condition. Multimetric biological indexes synthesize and integrate that expanding knowledge. The goals of biomonitoring include improving risk assessment and risk management.

PREMISE 4

CHANGING WATERS AND A CHANGING SOCIETY CALL FOR BETTER ASSESSMENT

At the end of the nineteenth century, discharge of raw sewage was a major cause of water resource degradation in the United States. Concern about the effects of excessive organic effluent on the potability of water, the spread of disease, problems with navigation, and the status of fish populations led Congress to pass the 1899 Rivers and Harbors Act, also called the Refuse Act. The act's goal was to clean up human wastes and oil pollution in navigable waterways. Protection of the nation's waters thus came under the jurisdiction of the US Army Corps of Engineers.

During the World War years and afterward, legal, regulatory, and management programs concentrated on controlling organic effluent and a growing array of toxic chemicals; declining populations of sport and commercial fishes and shellfish were also targeted. Technology to clean water and to make more fish became the watchword. Point sources of pollution were dealt with by wastewater treatment using "best available" or "best practical" technologies (Ward and Loftis 1989). Although the dust bowl of the 1930s prompted an early effort to protect water resources from nonpoint pollution due to soil erosion, soil and water conservation continued to take a back seat to augmenting agricultural production (Thompson 1995). From the mid-1800s, hatcheries were built and operated because, like agriculture, they promised control over production and, thus, unlimited numbers of fish through technology. Technological arrogance fostered a proliferation of hatcheries (Meffe 1992), masking the degradation of river environments that was happening at the same time; yet some of that very degradation was caused by the hatcheries themselves (White et al. 1995; Bottom 1997). It was not until the 1970s—encouraged by growing public environmental awareness and passage of the 1972 Water Pollution Control Act Amendments (PL 92-500)—that management strategies began to recognize waters as a whole and the need to protect "the integrity of water" (Ballentine and Guarraia 1977).

Chemical criteria based on dose-response curves for single toxicants cannot account for interactions of multiple chemicals or for other human impacts

The past 30 years have brought important gains in the science of water resources. Societal values, too, have been changing as human-imposed stresses have become more complex and pervasive. In addition to sewage and toxic chemicals, the nation's freshwater environments have suffered from physical destruction, increasing water withdrawals, the spread of alien species, and overharvest by sport and commercial fishers. The names and language of water laws—Refuse Act, Soil and Water Conservation Act, Water Pollution Control Act, Clean Water Act—reflect

both society's changing values and attempts to cope with widening problems. Field monitoring and assessment programs have been evolving as well (Karr 1998).

Early water quality specialists developed *biotic indexes* sensitive to organic effluent and sedimentation (Kolkwitz and Marsson 1908); this focus continues in modern biotic indexes (Chutter 1972; Hilsenhoff 1982; Armitage et al. 1983; Lenat 1988, 1993). The most common approach involves ranking taxa (typically genus or species) on a scale from 1 (pollution intolerant) to 10 (pollution tolerant). For each sample site, an average pollution tolerance level (the biotic index value) is expressed as an abundance-weighted mean to facilitate comparisons among sites. Some classifications use only three levels; others (Armitage et al. 1983) classify to family, calculate an average score per taxon, and reverse the scale (1 is pollution tolerant, and 10 is pollution intolerant).

As toxic chemicals became more widespread, water managers recognized the limitations of early biotic indexes and began to screen for the biological effects of synthetic as well as "natural" chemicals. Biologists experimentally exposed fish or invertebrates—typically fathead minnow *(Pimephales promelas)* or *Daphnia* spp.—to contaminants and documented the responses, creating dose-response curves for individual chemical toxicants. For a given body size, they observed, very low doses of a contaminant might lead to little or no response (e.g., few or no deaths among a group of individuals). As dose increased, response increased. The goal was to establish quantitative chemical criteria to use in water quality standards. These criteria were presumed to protect human health or populations of desirable aquatic species by keeping toxic compounds below harmful concentrations—the dilution solution to pollution.

But just as biotic indexes measure primarily the effects of organic pollution, chemical criteria based on toxicology apply only to chemical contamination and a small number of contaminants. Toxicological studies, the foundation for chemical criteria, typically examine the tolerances of only a few species, usually the most tolerant taxa, leading to underestimates of the effect of a contaminant in the field. Chemical criteria based on dose-response curves for single toxicants cannot account for synergistic or other interactions of multiple chemicals in the environment. And criteria for one species (e.g., fathead minnow) do not ensure protection for others not tested. Moreover, an exclusive focus on toxicology ignores other human impacts on aquatic biota, such as altered physical habitat or flow.

Much early work to detect the influence of human actions on biological systems emphasized abundance (or population size or density) of indicator taxa or guilds, often species with commodity value or thought to be keystone species. But population size is notoriously variable even under natural conditions, especially in comparison with physical or chemical water quality criteria. Data from long-term studies of marine invertebrates, for example (Osenberg et al. 1994), show that temporal variability for population attributes (e.g., densities of organisms) is about three times as high as for individual attributes (e.g., individual size or body condition), and nearly four times as high as chemical-physical attributes (e.g., water

temperature, sediment quality, water-column characteristics). Such high variances make analyses of population size problematic for general monitoring studies.

Efforts to overcome that problem have led to increasingly sophisticated sampling designs. Early field assessment protocols commonly used "control-impact" (CI) or "before-after" (BA) sampling designs. In CI designs, abundance is measured at unaffected control sites and at sites affected by an impact; in BA designs, abundance is measured before and then again after the event of interest. Despite the strength of these designs, the high variance of population size makes it difficult to distinguish between changes caused by the event and variation that would occur naturally in time or space.

Population size changes in complex ways in response to changes in multiple natural factors such as food abundance, disease, predators, rainfall, temperature, and demographic lags. Increasingly complex designs (e.g., BACI) were developed (Green 1979) to separate the effect of human activity from other sources of variability in space or time. But BACI confounds interactions between time and location; knowing the magnitude of the interaction and whether the effects are additive is critical to interpreting biological patterns—for example, understanding whether different streams respond in different ways to the same human activity. Still other statistical approaches were proposed to deal with such challenges: "before-after-control-impact paired series" (BACIPS; Stewart-Oaten et al. 1986) and "beyond BACI" (Underwood 1991, 1994). [See Schmitt and Osenberg (1996) for an excellent review of these sampling designs and their use.]

Use of these designs for biological monitoring raises a number of difficulties. First, even though assigning samples to treatment and control groups may account for local spatial variability in doses of contaminants, contaminant dispersal from a point source may be better detected by a more sensitive "gradient design" (Ellis and Schneider 1997)—that is, one that ensures sampling from sites across a range of contaminant levels. When many human activities interact, influencing biological systems in complex ways across landscapes, sampling across sites subject to various degrees of influence will often be more appropriate for discerning and diagnosing the complex biological consequences of that influence (see also Premise 29, page 107).

A second, and the primary, difficulty posed by these designs is the initial decision to focus narrowly on something as variable in nature as population size. In studies to determine environmental impacts, the interaction between variability and the size of the potential impact (effect size) must also be taken into account because that interaction affects statistical power (Osenberg et al. 1994). High variation in population size, even in natural environments, interacts in complex ways with changes in abundances stimulated by human actions. Thus it can be very difficult to detect and interpret the effects of human actions even with these advanced designs. The minimum level of sampling effort may often exceed the planning, sampling, and analytical capability of many monitoring situations. By shifting the focus to better-behaved indicators, such as those used in a proper multimetric

index (changes in taxa richness, loss of sensitive taxa, or changes in trophic organization), it is possible to use these designs, often in their less complex versions.

When ecological research embraced species diversity as a central theme in the 1960s, diversity indexes (e.g., Shannon, Morisita, Simpson) came into vogue for evaluating biological communities (Pielou 1975; Magurran 1988). Not long afterward, however, Hurlbert (1971) raised concerns about the statistical properties of these indexes; others later questioned their biological properties (Wolda 1981; Fausch et al. 1990). Diversity indexes are influenced by both number of taxa and their relative abundances; some are more sensitive to rare taxa, others to abundant taxa. Different diversity indexes may therefore produce a different rank order for the same series of sites, making it impossible to compare the sites' biological condition. Diversity indexes are often inconsistent because they respond erratically to changes in assemblages; thus they can lead to ambiguous interpretations (Wolda 1981; Boyle et al. 1990).

Measures of diversity were nevertheless advocated for water management (Wilhm and Dorris 1968). Florida established water quality standards based on a diversity index, although the state is are now moving away from them in favor of multimetric evaluations (Barbour et al. 1996a). The index of well-being (IwB), a sum of diversity indexes based on number of individuals and biomass (Gammon 1976; Gammon et al. 1981), has not been widely used, except by the Ohio Environmental Protection Agency (Ohio EPA) (Yoder and Rankin 1995a). Few scientists and managers recommend these diversity indexes today, largely because approaches are available that are both biologically more comprehensive and statistically more reliable. Unfortunately, however, diversity indexes have left a negative semantic legacy that surfaces whenever the word *index* appears (e.g., Suter 1993).

Recognizing the need for approaches better suited to considering the many attributes of biological condition simultaneously, many water resource managers have turned to two approaches with very different strengths: *multivariate statistical analysis* and *multimetric indexes*. Combinations of the two are especially useful (e.g., Hughes et al., in press). Multivariate analysis was developed to facilitate detection of pattern, not impact assessment. Multimetric indexes were designed specifically to document which components of biological systems provide strong signals about the impact of humans and to use those signals to define biological condition and diagnose the factors likely to have caused degradation when it is detected.

Multivariate statistics "treat multivariate data as a whole, summarizing them and revealing their structure" (Gauch 1982: 1). Many researchers advocate multivariate analyses of field assessment data because these approaches are assumed to be the most objective. (Premise 32, page 112, discusses some drawbacks and misuses of multivariate analyses.) Indeed, multivariate statistics are useful when an exploratory survey is called for (Karr and James 1975; Larsen et al. 1986; Whittier et al. 1988); they can help uncover patterns when only a little is known about the underlying natural history of a place or biota (Gerritsen 1995). But because scientists know a great deal about streams and landscapes, invertebrates and fish, and the effects of humans on those places and organisms, we advocate actively and explicitly apply-

ing that knowledge in choosing which biological attributes to monitor and which analytical tools to use—the approach taken in developing multimetric indexes.

Multimetric indexes build on the strengths of earlier monitoring approaches, and they rely on empirical knowledge of how a wide spectrum of biological attributes respond to varying degrees of human influence. Multimetric indexes avoid flawed or ambiguous indicators, such as diversity indexes or population size, and they are wider in scope (Davis 1995; Simon and Lyons 1995).

The biological attributes ultimately incorporated into a multimetric index (called metrics) are chosen because they reflect specific and predictable responses of organisms to changes in landscape condition; they are sensitive to a range of factors (physical, chemical, and biological) that stress biological systems; and they are relatively easy to measure and interpret. Multimetric indexes are generally dominated by metrics of taxa richness (number of taxa) because structural changes in aquatic systems, such as shifts among taxa, generally occur at lower levels of stress than do changes in ecosystem processes (Karr et al. 1986; Schindler 1987, 1990; Ford 1989; Howarth 1991; Karr 1991). The best multimetric indexes explicitly embrace several attributes of the sampled assemblage, including taxa richness, indicator taxa or guilds (e.g., tolerant and intolerant groups), health of individual organisms, and assessment of processes (e.g., as reflected by trophic structure or reproductive biology).

A multimetric index comprising such metrics integrates information from ecosystem, community, population, and individual levels (see Premise 12, page 47; Karr 1991; Barbour et al. 1995; Gerritsen 1995), and it can be expressed in numbers and words. Most important, such a multimetric index clearly discriminates biological "signal"—including the effects of human activities—from the "noise" of natural variability.

Standard samples of invertebrates from one of the best streams in rural King County, Washington, for example, contained 27 taxa of invertebrates; similar samples from an urban stream in Seattle contained only 7 taxa. The rural stream had 18 taxa of mayflies, stoneflies, and caddisflies; the urban stream had no stoneflies or caddisflies and only 1 mayfly taxon. The rural stream had 3 long-lived taxa and 4 intolerant taxa, but the urban stream had none. The rural stream had 17 taxa of "clinger" insects; the rural none. No predatory taxa were present in the urban creek, but 12% of individuals from the rural creek were predators. When these and other metrics were combined in an index based on invertebrates, the resulting benthic index of biological integrity (B-IBI) provided a numeric description of the condition, or health, of the streams. The B-IBI for the rural stream in King County was 44 (from a maximum index of 50); that for the urban stream, 10 (from a minimum index of 10).

Biological monitoring detects biological changes caused by humans

The aim of any resource evaluation program is to distinguish relevant biological signal from noise caused by natural spatial and temporal variation (Osenberg et al. 1994). In ambient biological monitoring of water resources, signals of biological condition are measured and used to predict impacts of human activity on aquatic systems. But not all attributes of these systems, or all analytical methods, provide signals that reveal patterns relevant for managing water resources. In choosing biological indicators, one should focus on attributes that are sensitive to the underlying condition of interest (e.g., human influence) but insensitive to extraneous conditions (Patil 1991; Murtaugh 1996). Periodically over the past century, water managers and researchers have failed to choose from the many variables, disturbances, endpoints, and processes those attributes that give the clearest signals of human impact. The nation's waters declined as a result.

This confusion is not difficult to explain. Like all scientists, biologists in the field are always eager to explore new places, catalogue new habitats and their inhabitants, and apply new principles in the name of "baseline research." Most scientists want to know more, rarely questioning the desirability of more research or basic research. But confusing the perspectives and goals of basic and applied ecological research has been a major reason that biological monitoring programs have seldom halted resource degradation. Compounding this problem, water managers have long sought surrogate measures of human impact or resource condition. The search for surrogates was often too narrow, and much that humans do to degrade resources was overlooked.

The goal of biological monitoring is to measure and evaluate the consequences of human actions on biological systems

Basic-research ecologists try to understand natural variation over space and time within communities of organisms, along with the evolutionary and thermodynamic principles that mediate this variation. For the most part, they work in natural systems subject to relatively little influence from human activities. They ask questions such as, Why does the number of species vary from place to place on the surface of the Earth? What regulates the size of animal and plant populations? How do global biogeochemical cycles regulate ecosystem structure and function?

Like taxonomists trying to distinguish, identify, and name species, basic-research ecologists try to distinguish unique habitat types, communities, or ecosystems, and to classify them. They have long interpreted differences among environments in terms of changing species composition or abundances and energy flow or nutrient

cycling; they focus on differences attributable to natural biogeographic and evolutionary processes. They identify indicator species—for example, to diagnose a particular type of natural community, biome, or environment [e.g., sand or gravel heathlands, alluvial grasslands, or tall- or short-grass prairie; see Dufrêne and Legendre (1997) for a recent example].

Applied ecologists, too, seek to recognize natural variation but also to study how natural systems respond to human activities—in particular, how humans can manipulate natural systems to achieve certain ends. For the past several decades, most applied ecologists have focused on the "engineering" side of their discipline. They have concentrated on producing higher crop yields; increasing the water supply or purifying contaminated water; and enhancing fish productivity by building hatcheries and removing woody debris from streams or, later, putting it back in. They raised waterfowl harvests by building wetlands or engineering mitigation for wetland losses. Many applied ecologists back the intentional introduction of alien taxa, as in fish-stocking programs or "natural" pest control programs, often with substantial negative effects (Simberloff et al. 1997). Even conservation biologists have narrowly aimed to protect endangered species—another rare commodity—instead of seeking to protect life-support systems more broadly. Today, despite public awareness and legislation prompted by visibly degraded biological systems, applied ecology generally still pursues its commodity goals.

Thus for many years, public environmental policy has been driven primarily by application of narrow physical and chemical principles. When biological targets entered the policy arena, they were narrow (cleaner water, hardier corn, more ducks). This problem persists despite clear mandates such as the Clean Water Act's call for protecting biological integrity, despite the rhetoric of "ecosystem management" that has surfaced in the past decade. Part of the problem lies squarely with ecologists trained to use narrow commodities as their indicators; the solution will come from applying ecology to find better, broader indicators of biological condition.

A broader applied ecology should, for example, seek to discover the consequences of activities such as grazing, logging, and urbanization on particular places. Applied ecologists should ask, What do we measure to understand responses to human activities? What methods and measurements best isolate the signal produced by human impact from noise? How do we interpret the results? What are the likely consequences of changes we see? How do we tell citizens, policymakers, and political leaders what is happening and how to fix it?

The first step toward effective biological monitoring and assessment, then, is to realize that the goal is to measure and evaluate the consequences of human actions on biological systems. The relevant measurement endpoint for biological monitoring is biological condition; detecting change in that endpoint, comparing the change with a minimally disturbed baseline condition, identifying the causes of the change, and communicating these findings to policymakers and citizens are the tasks of biological monitoring programs (Figure 3). Keeping this framework in mind can help keep biological monitoring programs on track.

FIGURE 3. Relationships among kinds of variables to be measured, understood, and evaluated through biological monitoring. Biological condition is the endpoint of primary concern.

Both basic-research ecologists and applied ecologists concern themselves with the top tier of Figure 3, the baseline condition minimally disturbed by human actions. Biogeochemical processes give rise to a geophysical setting and a biota defined as possessing biological integrity (Frey 1977; Karr and Dudley 1981; Angermeier and Karr 1994). Natural geophysical settings and biotas unaltered by humans in historical times constitute the main focus for basic-research ecologists, but understanding and documenting these processes and components also provide the foundation for biological monitoring studies.

In essence, understanding baseline, or reference, conditions in different places is analogous to veterinarians' learning what indicates health in different kinds of animals. "Healthy" for a lizard is not the same as "healthy" for a dog. Likewise, the expected *quantitative* values for indicators of ecological health in small midwestern North American streams are not the same as for Pacific Northwest streams or for large South American rivers, even though many of the same biological attributes may work as indicators in those disparate situations (e.g., taxa richness, relative abundance of predators). Knowing geophysical setting and undisturbed biological condition—in other words, knowing what produces and constitutes biological integrity—must underpin any biological monitoring effort.

Through time, geophysical setting and biological integrity are altered by natural events, so that over evolutionary time, biogeochemical processes may change the conditions defining regional integrity. But the rapid growth of human populations and their technologies during just the past 200 years has been a new, radically different force for change. Regional biological systems are no longer what they were 300 years ago, and the change threatens the very supply of goods and services humans depend on (Hannah et al. 1994; Costanza et al. 1997; Daily 1997; Pimentel et al. 1997). As a result, the historical dichotomy between basic ecology and applied ecology must give rise to a seamless "new ecology." Whereas basic ecology has tried to understand the natural world and applied ecology has largely concentrated on extracting human commodities from that natural world, a new ecology must protect local, regional, and global life-support systems.

This more integrative ecology shares its emphasis on human activities with the commodity branches of applied ecology. But whereas commodity ecology sought to increase human influence and to use that influence to maximize harvests of wild and cultivated species, a better applied ecology seeks to understand the biological *consequences* of human activity and to minimize the harmful ones. Biological monitoring measures the condition of biological systems in the broadest sense and thus lies at the heart of this new ecology. The sampling and analytical tools used in monitoring must focus on detecting and understanding human-caused change.

Conceptual frameworks, protocols, and procedures designed for basic research on near-pristine systems are not necessarily those that will identify change caused by human activity. Among 20 randomly selected sites sampled for benthic insects in a cold-water stream, for example, some of the variation in the samples will have natural causes (e.g., among microhabitats within a stream reach or among reaches of streams of different sizes). Sampling itself—the use of a method, the choice of a

method, or the efficiency of different field teams—also produces variation (see Premise 19, page 80). But the most important variation comes from differences in human activity among segments of a watershed. Understanding that variation and communicating its consequences to all members of the human community is perhaps the greatest challenge of modern ecology.

In sum, biological monitoring studies must measure present biological condition and compare that condition with what would be expected in the absence of humans. Biological monitoring documents any divergences from expected baseline conditions and associates divergences with knowledge of human activities in the area; the goal is to find out why conditions have moved away from integrity. In biological monitoring, then, managers need to evaluate five kinds of information all together: (1) present and (2) expected biology, (3) present and (4) expected geophysical setting, and (5) the activities of humans likely to alter both the biology and the geophysical setting. Managers, policymakers, and society at large can use this information to decide if measured alterations in biological condition are acceptable and set policies accordingly. In other words, by identifying the biological and ecological consequences of human actions, biological monitoring provides an essential foundation for assessing ecological risks.

PREMISE 6

ECOLOGICAL RISK ASSESSMENT AND RISK MANAGEMENT DEPEND ON BIOLOGICAL MONITORING

Over the past decade or so, risk assessment has focused on human health effects, usually the effects of single toxic substances from single sources. As practiced since a 1983 report of the National Research Council (NRC 1983; see also NRC 1994, 1996; Risk Commission 1997), human health risk assessment asks five questions (van Belle et al. 1996), each with its own technical jargon:

- Is there a problem? (hazard identification)
- What is the nature of the problem? (dose-response assessment)
- How many people and what environmental areas are affected? (exposure assessment)
- How can we summarize and explain the problem? (risk characterization)
- What can we do about it? (risk management)

Responding to growing interest in ecological risk assessment specifically, EPA in 1992 issued its own *Framework for Ecological Risk Assessment* (see also USEPA 1994a,b), which was superseded in September 1996 by the *Proposed Guidelines for Ecological Risk Assessment* (USEPA 1996d). In these documents, EPA modifies the human health assessment terminology and process to evaluate "the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors" (USEPA 1996d). The agency's framework asks questions very similar to those asked in human health risk assessment:

Tracking biological endpoints, rather than pollution-control dollars, will improve our ability to reduce ecological risks

- Is there a problem? (problem formulation)
- What is the nature of the problem? (characterization of exposure and characterization of ecological effects)
- How can we summarize and explain the problem? (risk characterization)
- What can we do about it? (risk management)

Unfortunately, most risk assessments still take a single-source-single-effect approach, ignoring the multiplicity of stressors to which individual humans, as well as ecological systems, are subjected. In the most recent attempt to shift government thinking in this area, a Presidential/Congressional Commission on Risk issued its *Framework for Environmental Health Risk Management* (Risk Commission 1997), which simultaneously enlarges the context for "risk" to include ecological as well as public health risks and emphasizes the importance of involving the public throughout the risk assessment and management processes.

The commission's report recommends six risk management steps. It explicitly broadens the definition of risk management to include ecological risks. It urges testing of "real-world mixtures" of pollutants, such as urban smog or pesticides left on vegetables. The report recommends looking at whole watersheds and "airsheds," and it makes specific recommendations to Congress and to regulatory agencies including EPA. It also builds public involvement into all six steps, especially in defining a problem and putting it into public health context. The report advises risk managers and citizens to: (1) define the problem and put it in context; (2) analyze the risks associated with the problem in context; (3) examine options for addressing the risks; (4) make decisions about which options to implement; (5) act to implement the decisions; and (6) evaluate the action's results. A primary challenge is to translate these goals into assessment and protection of ecological health.

All these attempts to reinvent risk management allow, even encourage, managers to broaden the questions, context, and tools they apply to the nation's environmental challenges. And although all seem to agree that risk assessment and risk management must be iterative—that conclusions must be revisited and the process repeated so that decisions may be adjusted on the basis of new information—debate still rages over which risks to assess and the "right" way to assess and manage them.

Still, we argue that, whatever the framework for assessing ecological risks, each step must be informed by data from biological monitoring. For accurate, relevant ecological risk assessment, the measurement endpoints (what is measured) and the assessment endpoints (the ecological goods and services society seeks to protect) must be explicitly biological. Biological monitoring provides better information about actual environmental quality than chemical and physical measures alone (Keeler and McLemore 1996) because biological attributes are one step closer to the factors that constitute environmental quality. Microeconomic models based on chemical levels as surrogates of environmental quality may be useful for approximating the costs of pollution control, for example, but they are limited in their ability to explain the ecological, explicitly biological, damage caused by that pollution (Keeler and McLemore 1996). Economic models incorporating biological measures, on the other hand, can potentially contribute more accurately to a whole-system approach to resource management.

To see the benefits of biological monitoring, consider the waste implicit in decisions to invest increasing amounts of money in wastewater treatment in North America while paying little attention to whether water resource condition was improving or to the influence of other limiting factors. The nonlinear nature of ecological systems makes conventional wastewater treatment very inefficient (Statzner et al. 1997). Eventually, environmental improvement per dollar spent declines because other factors begin to limit overall environmental quality. But judicious use of biological monitoring can track living components of environmental quality directly, thereby improving management efficiency. Tracking environmental quality through biological monitoring can guide investment strategies toward those that would yield the greatest benefit per dollar spent. In short, the use of biological endpoints, rather than pollution control dollars or numbers of

permits issued, will improve decision making, achieve greater environmental improvements for each increment of expenditure, and improve our ability to reduce ecological risks.

Ecological risk assessment will miss its mark if it simply folds ecological terminology into a new pollution control or human health–focused process. To protect biological resources, we must measure, monitor, and interpret biological signals. For if we do not understand how biological systems respond—and the consequences of those responses for human well-being—we cannot understand what is at risk or make wise choices.

SECTION III

Multimetric Indexes Convey Biological Information

Five activities are central to making multimetric biological indexes effective:

1. Classifying environments to define homogeneous sets within or across ecoregions (e.g., streams, lakes, or wetlands; large or small streams; warm-water or cold-water lakes; high- or low-gradient streams).
2. Selecting measurable attributes that provide reliable and relevant signals about the biological effects of human activities.
3. Developing sampling protocols and designs that ensure that those biological attributes are measured accurately and precisely.
4. Devising analytical procedures to extract and understand relevant patterns in those data.
5. Communicating the results to citizens and policymakers so that all concerned communities can contribute to environmental policy.

PREMISE 7

Understanding biological responses requires measuring across degrees of human influence

Our ability to protect biological resources depends on our ability to identify and predict the effects of human actions on biological systems, especially our ability to distinguish between natural and human-induced variability in biological condition. Thus, even though measures taken at places with little or no human influence (e.g., only from "reference" sites) may tell us something about natural variability from place to place and through time in undisturbed sites, they cannot tell us anything about which biological attributes merit watching for signs of human-caused degradation. To find these signs, sampling and analysis should focus on multiple sites within similar environments across the range from minimal to severe human disturbance.

One could choose sampling sites that represent different intensities of only one human activity, such as logging, grazing, or chemical pollution. It would then be possible to evaluate biological responses to a changing "dose" of a single human influence. Though rare, such a study opportunity could help identify the biological response signature characteristic of that activity (Karr et al. 1986; Yoder and Rankin 1995b). Knowledge of such biological response signatures would give researchers a diagnostic tool for watersheds influenced by unknown or multiple human activities. In reality, however, it is virtually impossible to find regions influenced by only a single human activity.

Sampling from sites with different intensities and types of human activity is essential to detect and understand biological responses to human influence

In most circumstances, diverse human activities interact (e.g., during urbanization) to affect conditions in watersheds, water bodies, or stream reaches. In such cases, sites can be grouped and placed on a gradient according to activities and their effects: industrial effluent is more toxic than domestic effluent, for example, and both pose more-serious threats than low dams, weirs, or levees (Figure 4). Removal of natural riparian corridors damages streams; conversion to a partially herbaceous riparian area is less damaging than conversion to riprap. Streams grouped this way show striking and systematic differences in biological condition across the gradient (Figure 5).

In other circumstances, a single variable can capture and integrate multiple sources of influence: the percentage of impervious area in a watershed summarizes the multiple effects of paving, building, and other consequences of urbanization, as in a recent study of Puget Sound lowland streams (Figure 6). This measure provides a simple surrogate of human influence that works well across a gradient of impervious area from near 0% to 60%. Unfortunately, it is less useful in understanding the

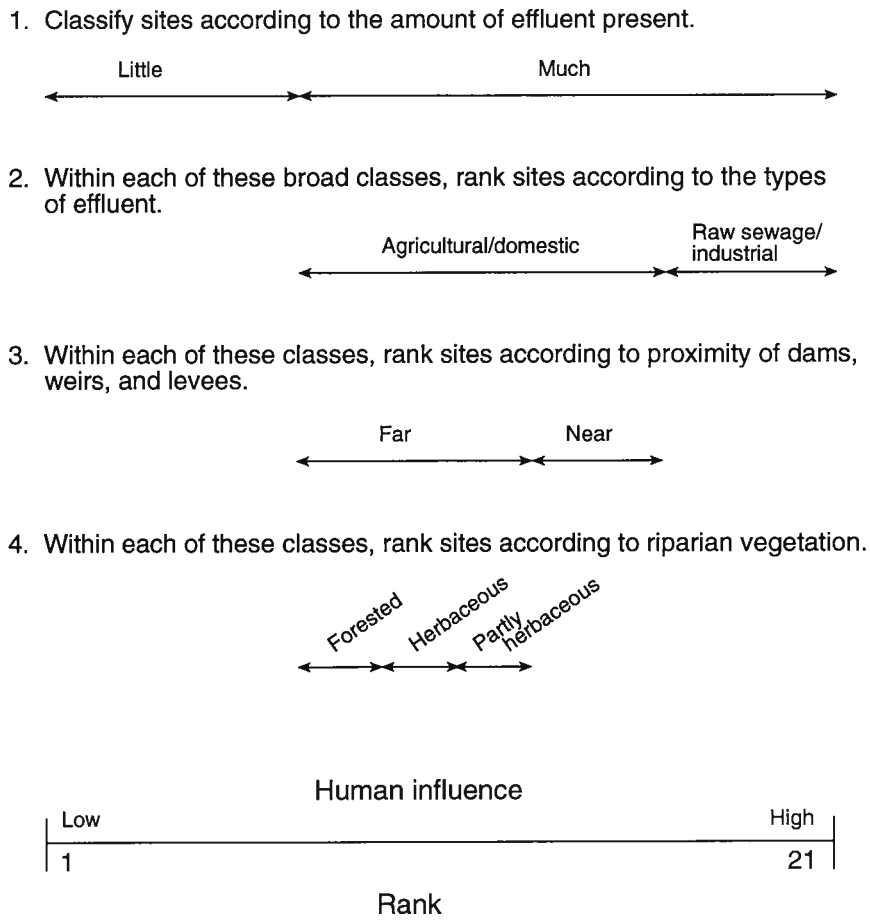


FIGURE 4. A priori classification system for ranking Japanese streams according to intensity of human influence (Rossano 1995). Sites were assigned to one of 21 possible categories based on amount and type of effluent, proximity of dams and other structural alterations, and type of riparian vegetation. Even without quantitative measures from each site, this approach allowed sites to be ranked across a range of human influence.

often large variation in biological condition at some percentages of imperviousness (e.g., 3% to 8%; see Figure 6, page 33). Finding the differences in human activity that can explain these biological differences requires information from the watersheds that is more detailed.

Alternatively, sites may be grouped into qualitative disturbance categories. In a study of recreational influence on stream biology in the northern Rocky Mountains (Figure 7), Patterson (1996) classed sites into four categories associated with different levels of human activity: (1) little or no human influence in the watershed; (2) light recreational use (hiking, backpacking); (3) heavy recreational use (major trailheads, camping areas); and (4) urbanization, grazing, agriculture, or wastewater discharge. Patterson demonstrated that light recreational activity did not substantially reduce B-IBIs in comparison with undisturbed watersheds, whereas heavy recreational use did significantly alter the benthic invertebrates but not as much as more-intensive uses such as urbanization or agriculture.

A similar approach was used in a study of biological response to chemical pollution on three continents: South America, Africa, and southeastern Asia (Thorne and Williams 1997). The authors classified sites according to a pollution gradient based on the integration of six measures of chemical pollution. Biological condition, as indicated by metrics such as total taxa richness (families) and mayfly,

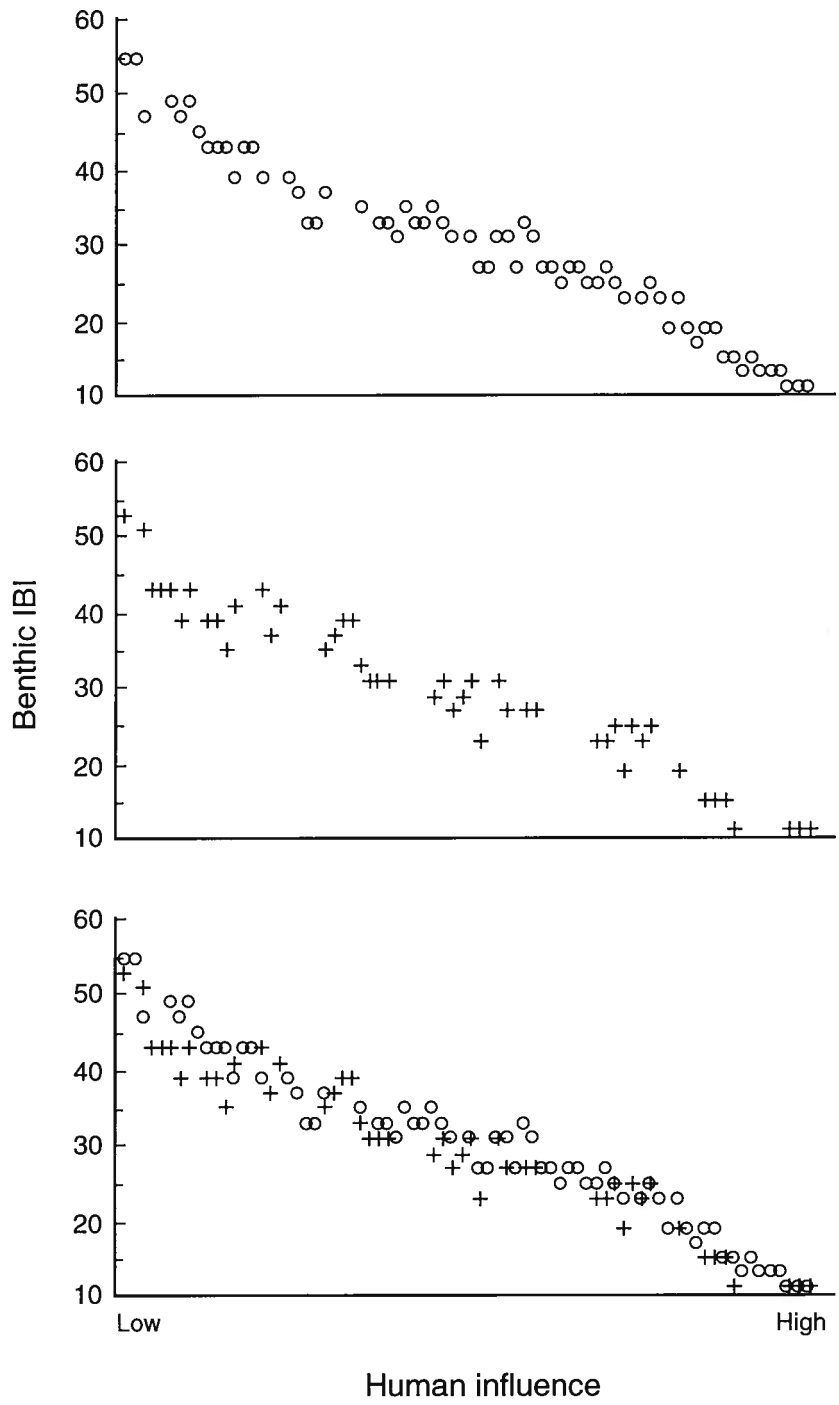


FIGURE 5. Benthic indexes of biological integrity (B-IBIs) for 115 Japanese streams (from Rossano 1995). The top panel shows B-IBIs calculated from half of the 115-stream data set (circles), which was used to initially select and test metrics for use in the B-IBI. The middle panel shows B-IBI values calculated from the second half of the data set (pluses); the metrics and scoring criteria used for these data were the metrics and criteria developed from the first half. In the bottom panel, all 115 B-IBIs are plotted together; the indexes from both sets correspond closely, ranking the streams comparably according to intensity of land use from low to high. The range of human influence against which the B-IBIs are plotted comes from the classification scheme shown in Figure 4.

stonefly, and caddisfly richness, clearly went down as pollution went up. The biological responses in the three tropical regions were similiar; they parallel patterns documented in temperate regions even though the faunas are all very different.

Data collected over a number of years at the same site(s) can also reveal biological responses as human activities change during that period. Regardless of how one

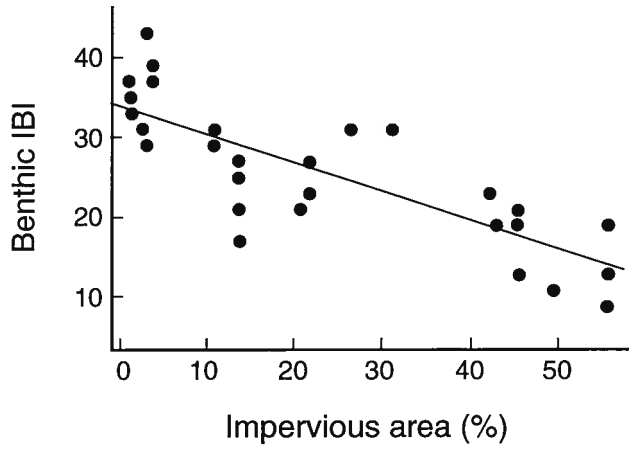


FIGURE 6. Benthic index of biological integrity (B-IBI) plotted against the percentage of impervious area for urban, suburban, and rural stream sites in the Puget Sound lowlands, Washington (from Kleindl 1995). Though B-IBI clearly decreases with increasing impervious area, this plot offers no insight into B-IBI differences among sites with similar percentages of impervious area, especially at low percentages (3% to 17%).

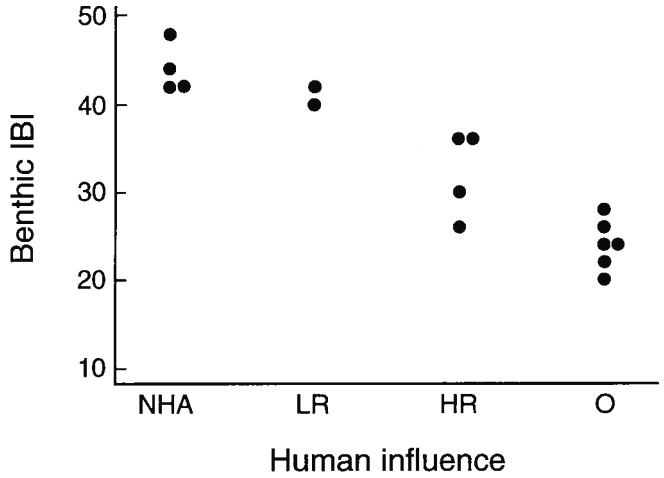


FIGURE 7. Benthic indexes of biological integrity (B-IBIs) for stream sites in Grand Teton National Park, Wyoming (from Patterson 1996). Before B-IBIs were determined, these sites had been placed into four categories of human influence: little or no human activity (NHA), light recreational use (LR), heavy recreation use (HR), and other (O). B-IBIs revealed no significant difference between sites with little or no human activity and those having low recreational use. But B-IBIs were significantly lower for sites used heavily for recreation and lower still for sites subjected to other uses—specifically, urbanization, grazing, agriculture, and wastewater discharge.

represents a range of human influence among study sites, sampling from sites with different intensities and types of human activity is essential to detect and understand biological responses to human influence. Thus the goal is to compare like environments with like environments—to isolate and understand patterns caused by human activities at sites within those like environments.

Too many existing studies confound patterns of human influence with natural variation over time at undisturbed sites or across different environment types. In other situations, researchers combine measures of human activity, the physical and chemical manifestations of those activities, and their biological consequences in a heterogeneous analysis with ambiguous results. Those analyses may even include measures of physical environment such as stream gradient. When this range of factors (different human influences on different environment types) is lumped in a

single analysis, it becomes almost impossible to understand causes or consequences of natural versus human events.

Consider the following analogy. Three experiments are designed: one to understand variation in natural biological systems as a function of stream size; another to distinguish the effects of pesticide runoff on streams of first, fourth, and sixth order; and a third to define the effects of pesticides on plants and insects. Analyzing samples from the first series of stream sites would tell one about biological responses to changing stream size; samples from the second series, about changing human influence as a function of stream size; and samples from the third would distinguish responses of different taxa. It would be silly to mix the data from the three studies in a single statistical analysis, without regard to which study the individual samples came from. Yet by using analytical procedures that mix the effects of natural and human-induced variation (in a single correlation matrix, for example), researchers are essentially doing just that: they are ignoring the context of the different components of their data, making it difficult to distinguish the biological signs relevant to resource management or protection. They then confound the sources of the variation they see, even if their initial sampling setup would have permitted discrimination among those sources. Univariate and multivariate analyses all too often suffer from this flaw.

Sampling only from "reference" sites creates a similar problem because it does not provide a way to document which biological attributes vary with human influence (see Premise 30, page 108). Careful thought about which variables best summarize human influence and the relationships among those variables should be the foundation of monitoring protocols. Creating opportunities to discover biological patterns in relation to human activity must be foremost.

ONLY A FEW BIOLOGICAL ATTRIBUTES PROVIDE RELIABLE SIGNALS ABOUT BIOLOGICAL CONDITION

The success of biological monitoring programs and their use to define and enforce biological criteria is tied to identifying biological attributes that provide reliable signals about resource condition (Table 3). Choosing from the profusion of biological attributes (Figure 8) that could be measured is a winnowing process, in which each attribute is essentially a hypothesis to be tested for its merit as a metric. One accepts or rejects the hypothesis by asking, Does this attribute vary systematically through a range of human influence? When metrics are selected and organized systematically, an effective multimetric index can emerge from the chaos displayed in Figure 8.

Knowledge of natural history and familiarity with ecological principles and theory guide the definition of attributes and the prediction of their behavior under varying human influences. But successful biological monitoring depends most on demonstrating that an attribute has a reliable empirical relationship—a consistent quantitative change—across a range, or gradient, of human influence. Unfortunately, this crucial step is often omitted in many local, regional, and national efforts to develop multimetric indexes (e.g., RBP I, II, III; Plafkin et al. 1989).

Successful biological monitoring depends on demonstrating that an attribute changes consistently and quantitatively across a gradient of human influence

The study of populations has dominated much ecological research for decades (see section II), so researchers still assume that population size (expressed as abundance or density) provides reliable signal about water resource condition. But because species abundances vary so much as a result of natural environmental variation, even in pristine areas, population size is rarely a reliable indicator of human influence (see Premise 13, page 51, and Premise 24, page 95). Large numbers of samples (>25) were required, for example, to detect small (<20%) differences in number of fish per 100 m^2 of stream surface area in small South Carolina streams (Paller 1995b). Other attributes—such as taxa richness (number of unique taxa in a sample, including rare ones) and percentages of individuals belonging to tolerant taxa—have, in contrast, been found to vary consistently and systematically with human influence. Such attributes, when graphed, give rise to analogues of the toxicological dose-response curve—which we call ecological dose-response curves—where the y-axis represents the measured attribute and the x-axis measures of human influence (Figure 9).

Ecological dose-response curves differ in one critical aspect from toxicological dose-response curves. Toxicological dose-response curves usually measure biological response in relation to dose of a single compound. Ecological dose-

TABLE 3. Terms used in defining biological condition.

Term	Definition
Attribute	Measurable component of a biological system
Metric	Attribute empirically shown to change in value along a gradient of human influence
Multimetric index	A number that integrates several biological metrics to indicate a site's condition
Biological monitoring	Sampling the biota of a place (e.g., a stream, a woodlot, or a wetland)
Biological assessment	Using samples of living organisms to evaluate the condition or health of places
Biological criteria	Under the Clean Water Act, numerical values or verbal (narrative) standards that define a desired biological condition for a water body; legally enforceable

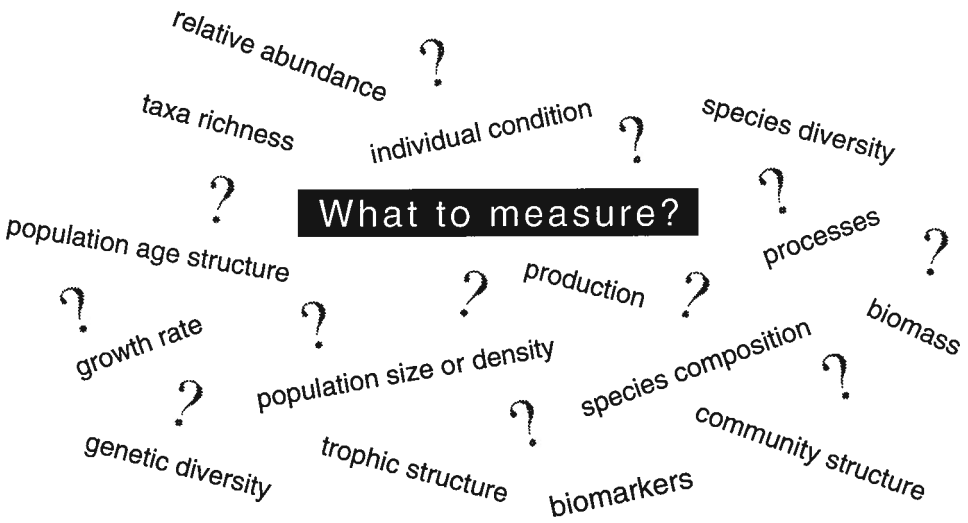
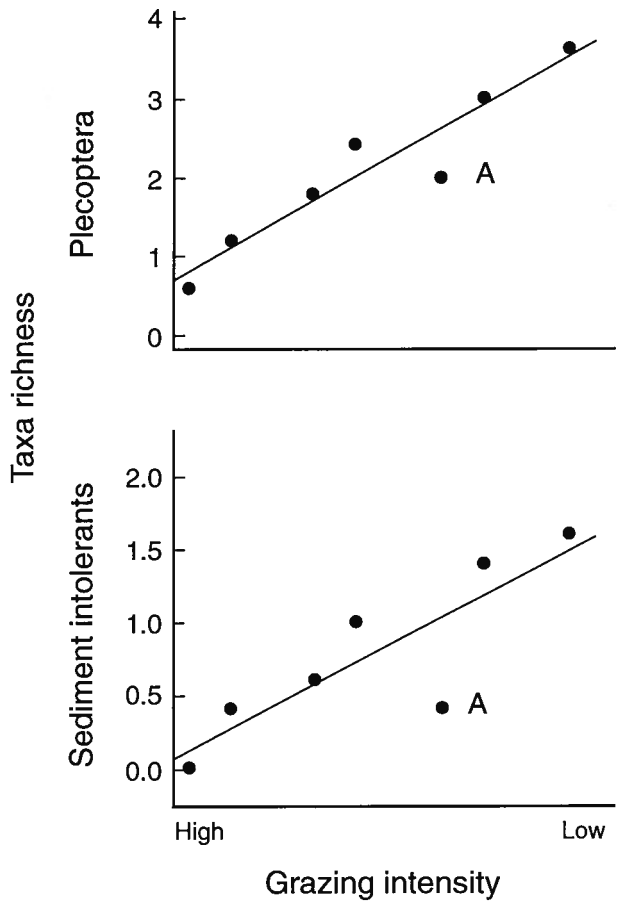


FIGURE 8. Almost any biological attribute can be measured, but only certain attributes provide reliable signals of biological condition and therefore merit integration into a multimetric index.

FIGURE 9. Average taxa richnesses of Plecoptera and sediment-intolerant taxa plotted against grazing intensity for seven stream sites in the John Day Basin, Oregon, in 1988. Site A had fewer taxa than expected because although cattle were excluded, intense grazing upstream had affected the site's biota.



response curves measure a biological response to the cumulative ecological exposure, or "dose," of all events and human activities within a watershed, expressed in terms such as percentage of area logged, grazing intensity, or percentage of impervious area in a watershed. The number of unique native fish taxa in a midwestern stream sampled today, for example, reflects the cumulative effects of human influence up to the present.

PREMISE 9

Simple graphs reveal biological responses to human influences

"Often the most effective way to describe, explore, and summarize a set of numbers (even a very large set) is to look at pictures of those numbers. . . . [O]f all methods for analyzing and communicating statistical information, well-designed data graphics are usually the simplest and at the same time the most powerful" (Tufte 1983: 9; see also Tufte 1990, 1997). Tufte's message is nowhere more important than in the display, interpretation, and communication of biological monitoring data.

Graphs reveal the biological responses important for evaluating metrics more clearly than do strictly statistical tools because they exploit "the value of graphs in forcing the unexpected" (Mosteller and Tukey 1977) on whoever looks at them, including researchers, who must then confront and explain the pattern in those graphs. For samples where the relationship between human influence and biological response is strong, statistics and graphs agree (Figure 10). In other cases, meaningful biological patterns can be lost by excessive dependence on the outcome of menu-driven statistical tests. Statistical correlation can miss an important relationship if the x-variable (e.g., percentage of area logged) is measured with low precision or if additional factors beyond those plotted on the x-axis influence metric values but are not included in the statistical analysis.

Graphs force us to confront the unexpected

In Figure 11, for example, we plot two different aspects of biological condition against one measure of human influence, such as the percentage of upstream watershed that has been logged. Sites are assigned a plus or minus based on that measure and other aspects of human influence that are visible and documented but not plotted on the same graph. In forested watersheds, these other aspects might include whether roads were near or far from the stream channel, time since logging, or traits unique to particular watersheds. In some cases such interacting factors may have degraded biological condition (roads near the stream channel would exacerbate logging's effects), or they may have allowed good conditions to persist (roads on distant ridges have less effect on streams). The distribution of pluses and minuses in Figure 11 illustrates the fallacy of assuming that a biological metric says nothing about condition because it does not correlate strongly with a single surrogate of that condition, as researchers perennially assume when a biological measure does not correlate with some measure of chemical pollution. Rather, we should conclude that the surrogate is not capturing significant components of human influence and look more closely for the biological explanations behind the data.

FIGURE 10. Example of two hypothetical metrics plotted against a gradient of human influence. Here statistical correlation and graphical analysis agree: metric A is a good indicator, and metric B is not. (Compare Figure 11.)

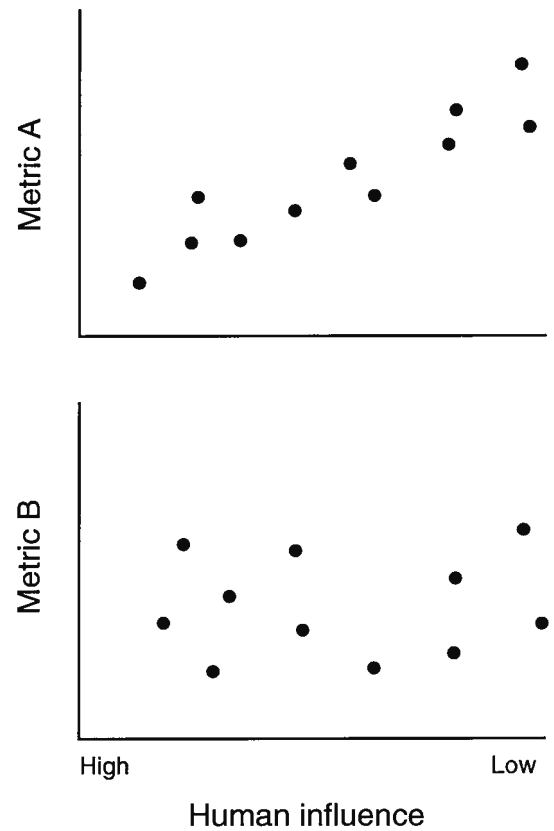
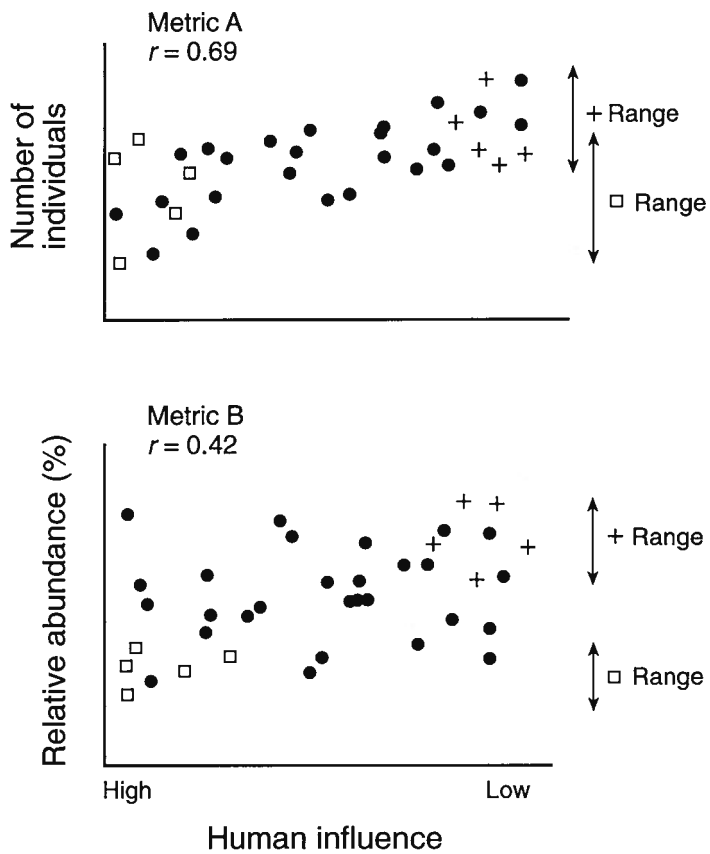


FIGURE 11. Hypothetical relationships between human influence and candidate biological metrics (from Fore et al. 1996). Metric A is more strongly correlated with resource condition (or r^2 is higher if using regression) than Metric B, initially suggesting that it is a better metric. But comparing the metrics' ability to distinguish between minimally disturbed sites (denoted by plus signs) and severely degraded sites (open boxes; ranges noted by arrows) shows that Metric B is actually a more effective measure of biological condition despite its smaller statistical correlation. (Compare Figure 10.)



Not all aspects of human influence can be easily captured in a single graph or statistical test. When a number of variables influence condition, a single plot against one dimension of human influence will not tell the whole story (Figure 12); neither will a single statistical test. Graphs force one to search for insights that rote application of statistical tests cannot discover.

Weak statistical correlation can also miss important biological patterns when the distribution of the data (e.g., Figure 13) does not lend itself to tests based on standard correlation techniques that detect only linear relationships. Yet nonlinear patterns are common in field data (Figure 14). Consider the plots in Figure 15, for example. The points fall into a wedge-shaped distribution, whose scatter shows little or no statistical significance but can be interpreted biologically. The upper bound of each plot is the hypotenuse of a right triangle (the maximum species richness line) that defines the number of species expected in minimally disturbed streams as a function of stream size (Fausch et al. 1984). The plots illustrate what Thomson et al. (1996) term a "factor ceiling distribution"; in this case, the ceiling, maximum species richness, is defined by the evolution of the regional biota. Generally, at sites where the number of fish species falls below the ceiling, some human activity in the adjacent or upstream watershed has reduced the number of species present; or sampling might have been inadequate, "dragging" species richness below the line.

Graphs highlight idiosyncrasies in data distributions that, when examined closely, may provide insight into the causes of a particular biological pattern. At one extreme, outlying points on a graph may offer key insights about the complex influence of human activities in watersheds; one can, for example, explore what unique situations at those sites cause them to appear as outliers.

Even the spread of data can offer insights, as illustrated by the large range in B-IBIs at sites with 20% to 30% impervious area shown in Figure 16. Sites with high mayfly taxa richness (B and C) lie in reaches of two streams with relatively intact riparian corridors and wetlands. The site with low mayfly taxa richness (A) is located in a stream that receives fine material from an old coal mine. Sites A, B, and C had unique characteristics that were best understood by examining their specific contexts, not by applying a regression or correlation analysis. Finding these patterns then led to subsequent studies in the same and in other places to determine if those patterns were more general.

Graphs also illustrate variation in behavior among taxa in response to a specific disturbance (Figure 17). For example, numbers of taxa for three orders of insects (stoneflies, mayflies, and caddisflies) declined downstream of the outflow from a streamside sludge pond in the Tennessee Valley, but the magnitude of change varied among the taxa (see also Premise 13, page 51). The same graph also reveals the direction and magnitude of change along a longitudinal transect down the stream.

Graphs may sometimes allow researchers to avoid naive application of elaborate multivariate techniques (Beals 1973). Principal components analysis, the most often used ordination technique (James and McCullough 1990), defines statistically orthogonal factors, which, biologically, may or may not be independent; interpret-

FIGURE 12. Taxa richness of Trichoptera plotted against the percentage of watershed area that was logged for 32 stream sites in southwestern Oregon. Metric correlation (Spearman's *rho*) was not significant because, alone, the percentage of area logged was an inaccurate measure of human influence; other factors, such as type of logging, presence of roads, and other human influences, were not included. When these other human influences were considered to identify minimally disturbed sites (denoted by plus signs) and severely degraded sites (open boxes), the response of Trichoptera taxa richness visibly distinguished between different degrees of human disturbance.

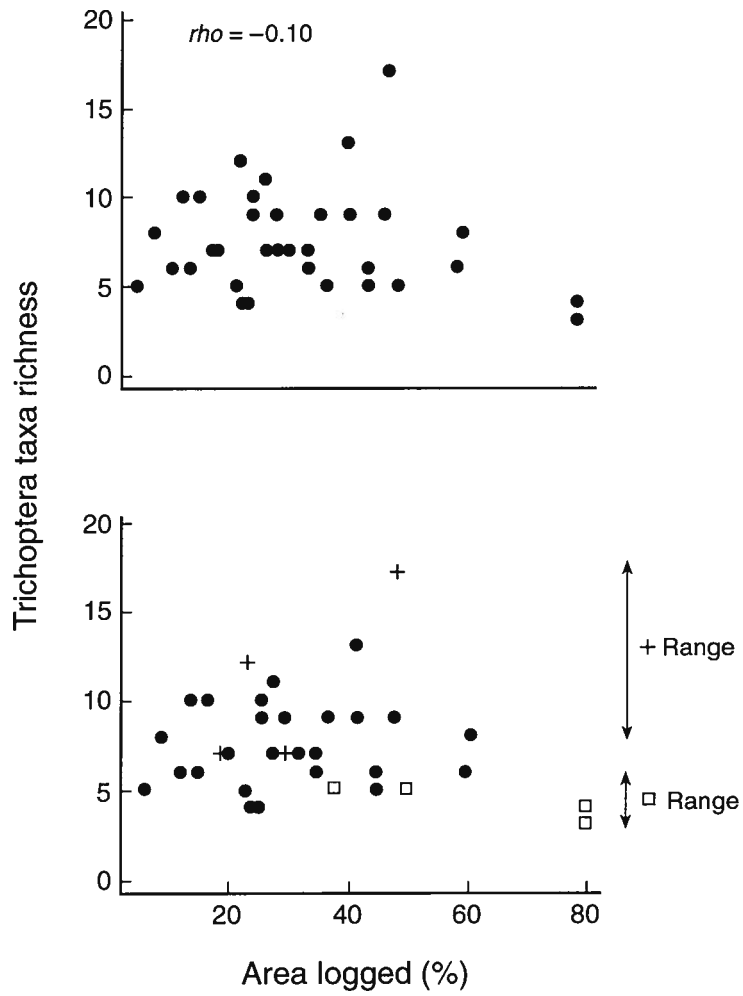


FIGURE 13. Hypothetical relationship between human influence and a Metric A. Statistical correlation (Spearman's *rho*) is not significant, yet the graphic pattern strongly suggests a biological response. At low levels of human influence, Metric A is not a reliable indicator of biological condition, but where human disturbance is high, the metric does respond.

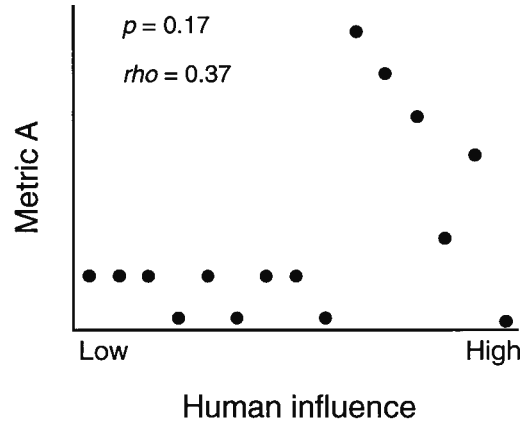


FIGURE 14. Relative abundance (percentage of total) of individuals belonging to tolerant taxa in samples of benthic invertebrates from 65 Japanese streams ranked according to intensity of human influence (see Figures 4, page 31 and Figure 5, page 32). (Data provided by E. M. Rossano.)

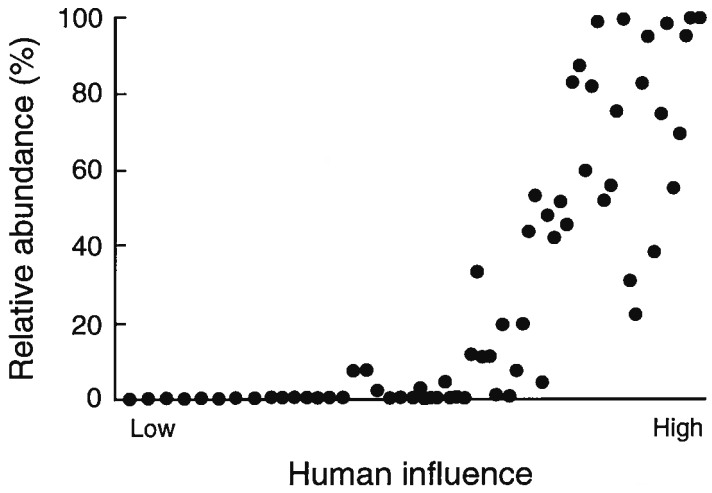
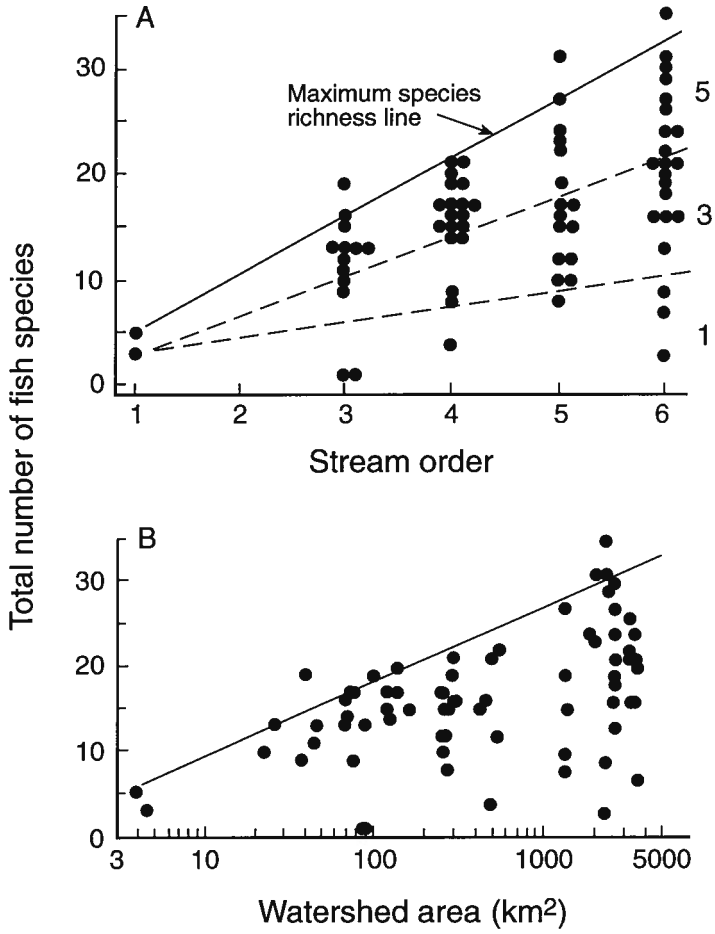


FIGURE 15. Number of fish species in relation to stream size (top) and watershed area (bottom); each point represents a site. The maximum species richness line through the highest points on each graph defines the number of species expected in minimally disturbed streams or watersheds. Points below that line represent sites where human activity has reduced the number of species present. (From Fausch et al. 1984.)



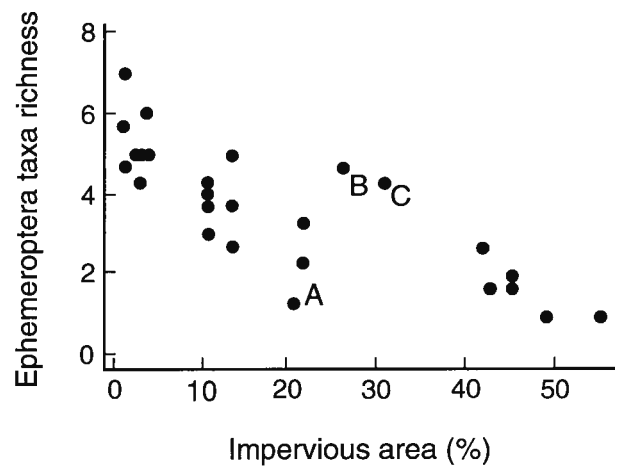


FIGURE 16. Average taxa richness of Ephemeroptera plotted against percentage of impervious surface area surrounding Puget Sound lowland streams (from Kleindl 1995). Site A, Coal Creek, had fewer Ephemeroptera than expected. This site has an active mine in its headwaters, and Ephemeroptera are known to be sensitive to mine waste. Sites B and C had relatively intact riparian areas (wetlands).

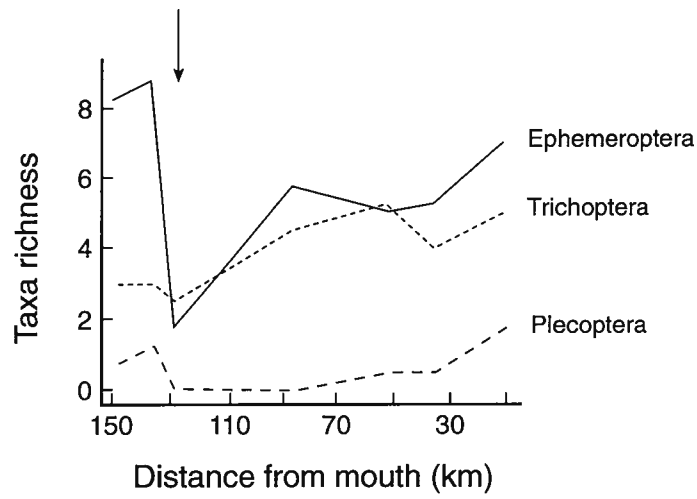


FIGURE 17. Taxa richness of mayflies, stoneflies, and caddisflies for sites along the North Fork Holston River in the Tennessee Valley in 1976 (from Kerans and Karr 1994). Arrow indicates the position of the streamside sludge pond. Taxa richnesses for all three orders decline at the sludge pond and slowly recover for sites downstream.

ing the results can therefore be complicated (Goodall 1954). Graphs can be a superior approach to methods that focus on maximum variance extracted because, when used correctly, they emphasize ecological rather than mathematical associations, a more appropriate criterion for organizing and understanding complex information (Beals 1973).

Complex ecological situations require unusual analytical means. Graphs can often be ecologists' most useful tools, permitting the exploration of ecological data "before, after, and beyond the application of 'standard analyses' " (Augspurger 1996). Rather than choose an inappropriately linear statistical model before plotting their data, ecologists should exploit the power of graphs for "reasoning about quantitative information" (Tufte 1983), and then choose and apply appropriate statistics. It is myopic to be a slave of standard statistical rules and procedures or to avoid statistics altogether.

PREMISE 10

Similar biological attributes are reliable indicators in diverse circumstances

A striking conclusion from 15 years' research in selecting metrics is that the same major biological attributes serve as reliable indicators in diverse circumstances. This result has its advantages and disadvantages. On the advantage side, every small project (e.g., at the county or community level) need not test and define its own locally applicable metrics. Scientists and resource managers can implement local biological monitoring and assessment programs based on results from other studies. When local studies cite earlier work, readers can know that the methods have been tested elsewhere; the accumulating body of tests refines, or refutes, the generality of patterns others have defined.

On the disadvantage side, some applications of multimetric indexes uncritically borrow theoretical or empirical metrics from other studies. This borrowing becomes problematic when the theory is wrong or does not apply in the study circumstance, or when metrics are applied to systems or regions other than those for which they were tested. For example, human impacts may increase taxa richness in cold-water streams (Hughes and Gammon 1987; Lyons et al. 1996) as cool- and warm-water species enter areas where water temperatures have been raised by activities such as logging of riparian vegetation. In contrast, in eastern warm-water streams, human influence commonly decreases species richness except for aliens (Karr et al. 1986). Thus, one cannot make identical assumptions about metrics of fish taxa richness in the two contexts. Similarly, a benthic invertebrate metric for soft-bodied organisms (e.g., oligochaetes, tipulid flies, and other grublike forms) often indicates degraded conditions in North America, but in Japan, the better metric consists of legless organisms, a grouping that includes the soft-bodied organisms but also shelled snails and mussels. In North America, mussels and snails are more often indicators of high-quality environments, but in Japan, most are alien or otherwise indicative of degraded conditions.

The bottom line is that metrics should be based on sound ecology and adapted only with great care beyond the regions and habitats for which they were developed. Exploring biological patterns to discover the best biological signals (that is, metrics) should mix graphs, conventional statistics, and thoughtful consideration of regional natural history.

TRACKING COMPLEX SYSTEMS REQUIRES A MEASURE INTEGRATING MULTIPLE FACTORS

Scientists, citizens, and policymakers faced with making decisions about complex systems—economies, a family member's health, an ecological system—need multiple levels of information. Consider some of the indexes used to track the health of the national economy: the index of leading economic indicators, the producer price index, the consumer price index, the cost-of-living index, or the Dow Jones industrial average. All these indexes integrate multiple economic factors.

The index of leading economic indicators (Mitchell and Burns 1938) tracks the US economy in terms of 12 measures: length of work week; unemployment claims; new manufacturing orders; vendor performance; net business formation; equipment orders; building permits; change in inventories, sensitive materials, and borrowing; stock prices; and money supply. These measures are combined to form the overall index, which takes as its reference point a standardized year (e.g., 1967); the value of the current year's index is expressed in terms of its value in the reference year. Composite economic indexes like these have survived six decades of discussion and criticism and remain widely used by economists, policymakers, and the media to interpret economic trends (Auerbach 1982).

We use multimetric indexes to monitor the economy; we should use them to monitor water resources

Similarly, physicians and veterinarians rely on multiple measures and multiple tests to assess the health of individual patients. On a single visit to the doctor, a patient might be "sampled" for urine chemistry, blood-cell counts, blood chemistry, body temperature, throat culture, weight, or chest X-rays. Clearly, these measurements are not independent of one another, for they come from a single individual whose health is affected by many interacting factors. Further, you would not expect your doctor to rely on only one specialized blood test to diagnose your overall health; rather, you assume that multiple measures will give a more accurate diagnosis. Patterns emerging from these multiple measurements would enable the doctor to recognize the signature of a particular ailment and suggest more targeted measurements if she suspected a certain disease. Only then could she prescribe treatment.

Multimetric biological indexes calculated from ambient biological monitoring data provide a similar integrative approach for "diagnosing" the condition of complex ecological systems. The same logical sequence applies in compiling multimetric economic, health, or biological indexes. First, identify reliable and meaningful response variables through testing; then measure and evaluate the system against expectations; finally, interpret the measured values in terms of an overall assessment of system condition. The resulting index (for economic or biological

resources) or diagnosis (for patients) allows people without specialized expertise to understand overall condition and to make informed decisions that will then affect the health of those economies, resources, or patients.

Most multimetric biological indexes for use in aquatic systems comprise 8 to 12 metrics,[1] each selected because it reflects an aspect of the condition of a biological system. These metrics are not independent because they are calculated from a single collection of organisms, just as multiple personal health tests are done on a single individual. But even if metrics are statistically correlated, they are not necessarily biologically redundant. Rather, just as a fever plus a high white-blood-cell count reinforces a diagnosis of bacterial infection, multiple metrics all contribute to a diagnosis of ecological degradation (ecological disease).

The two most common IBIs for streams have been developed, tested, and applied using fish (Karr 1981; Miller et al. 1988; Lyons 1992a; Fore et al. 1994; Lyons et al. 1995, 1996; Simon in press) and benthic invertebrates (Kerans and Karr 1994; Kleindl 1995; Rossano 1995, 1996; Fore et al. 1996; Patterson 1996). Both incorporate known attributes from multiple levels of biological organization and different temporal and spatial scales. Typically, patterns emerge that are the signatures of biological responses to particular human activities (Karr et al. 1986; Yoder 1991b; Yoder and Rankin 1995b).

Based on the success and widespread use of these two indexes, similar indexes are now being developed by a number of state agencies to use with invertebrates and vascular plants in wetlands (Karr 1997); with algae and diatoms in streams (Bahls 1993; Kentucky DEP 1993; Florida DEP 1996; Barbour et al., in press); and with plants, invertebrates, and vertebrates in terrestrial environments (CRESP 1996; Chu 1997; Bradford et al., in press; see also Premise 21, page 84). Extending IBI to new taxa, environment types, and geographic areas is like learning to practice medicine in humans, pets, livestock, and so on: the expectation of what constitutes "health" depends on the animal, but the same fundamental diagnostic strategy applies in all cases.

[1] For species-poor environments such as cold-water streams, the total number of metrics is likely to be smaller (e.g., Lyons et al. 1996).

MULTIMETRIC BIOLOGICAL INDEXES INCORPORATE LEVELS FROM INDIVIDUALS TO LANDSCAPES

The success of multimetric approaches such as IBI in assessing biological condition depends on choosing and integrating metrics that reflect diverse responses of biological systems to human actions. Ideally, a multimetric index would cover all such responses, but the costs of developing such an index would be much too high. A suite of chosen metrics is necessarily a compromise between "too narrow" and "too broad"; it is also a compromise of choices among conveniently measured biological surrogates of important biological phenomena. Present IBI and B-IBI metrics represent our choices in these compromises, but we expect metrics to evolve and expand over the next decade. Still, a fundamental tenet of IBI is that the user makes a conscious effort to choose metrics that cover the range of biological signals available from disturbed systems.

IBI is not a community analysis in either of the common uses of the word *community*. IBI does not examine all taxa but is generally based on one or two assemblages (phylogenetically related groups of organisms; Fauth et al. 1996), such as fish or benthic invertebrates. Neither does a multimetric IBI focus on the community level in the standard textbook hierarchy of biology (individual, population, assemblage, community, ecosystem, and landscape). Rather, the choice of measures in a multimetric index reflects an attempt to represent as many of those levels as possible, preferably directly but at least indirectly. The resulting indexes are likely to produce the strongest multimetric view of biological condition (Table 4). The best multimetric indexes are more than a community-level assessment because they combine measures of condition in individuals, populations, communities, ecosystems, and landscapes.

Users should deliberately choose metrics to encompass the range of signals from disturbed biological systems

Individual level. Individual health manifests itself in many ways both internally and externally, with physiological or morphological signs and in metabolic or genetic biomarkers reflecting organismal stress. We have not yet seen reliable metabolic or genetic biomarkers that can be applied broadly in the field, although in certain situations (see Summers et al. 1997 for a promising example), biomarkers may work as secondary tools for diagnosing biological condition; we hope for progress in this area in the next decade. To date, however, IBI metrics of individual health consist of easily detected external abnormalities; their frequency in an assemblage indicates stress on individuals.

In fish, for example, visible signs of stress include skeletal deformities; skin lesions; tumors; fin erosion; and certain diseases that are associated with impaired

TABLE 4. Types of metrics, suggested number of metrics of each type, and represented levels in the biological hierarchy. Well-constructed multimetric indexes contain the suggested number of metrics from each type and therefore reflect multiple dimensions of biological systems.

Metric type	Number	Individual	Population	Community	Ecosystem	Landscape
Taxa richness	3–5	√	√	√	√	
Tolerance, intolerance	2–3		√	√		
Trophic structure	2–4			√	√	√
Individual health	1–2	√				
Other ecological attributes	2–3	√	√	√	√	√

environments, especially large amounts of toxic substances. Early studies of fish in the seven-county area around Chicago indicated high incidence of external abnormalities (Karr 1981), for example—a pattern also apparent in Ohio (Yoder and Rankin 1995a). Among benthic invertebrates, head-capsule deformities in chironomids (midges) are strong indicators of toxics (Hamilton and Saether 1971; Cushman 1984; Warwick et al. 1987; Warwick and Tisdale 1988). Anomalies in fish are often used as IBI metrics, but chironomid head-capsule deformities are rarely incorporated into the benthic IBI because so much laboratory work is required to stain, mount on slides, and count the individual insects.

In other studies, tadpoles collected in a coal ash deposition basin had fewer labial teeth than tadpoles from reference areas (Rowe et al. 1996). They also had deformed labial papillae, which would limit the types of food they could eat and limit their growth. Fish in Gulf of Mexico estuaries showed higher numbers and frequencies of several pathologies at heavily disturbed sites than at minimally disturbed sites (Summers et al. 1997). Finally, periphytic diatoms of the genus *Fragilaria* in a metal-contaminated Rocky Mountain river in Colorado had deformed cells (McFarland et al. 1997). The percentage of deformed cells ranged from 0.2% ± 0.2 to 12% ± 2.0 from low to high levels of heavy metal (Cd, Cu, Fe, Zn) contamination.

Population level. Several metrics in both the fish and benthic IBIs indicate, if not the details of population demography, the relative condition of component groups. For example, the lack of intolerant taxa among fish or invertebrates or of clingers (taxa that cling to rocks) among the invertebrates is a strong signal that populations of these organisms are doing poorly. The absence of darters, sunfish, and suckers among the fishes and of mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera) among the invertebrates, suggests that viable populations of many species within these taxa cannot maintain themselves.

Usually, a population must be viable at a site before one can consistently detect a species' presence.

Assemblage level. Changes in the chemical, physical, and biological environment resulting from human activities alter assemblages. These changes may appear as changes in species composition or species richness (conventional measures of community structure); in trophic structure, such as decreases in top carnivores or increases in omnivores; or in shifts from specialists to generalists in food or reproductive habits (reflecting shifts in food-web organization, including energy flow and nutrient cycling). Multimetric indexes incorporate this information by including metrics such as the percentage of predators, omnivores, or other feeding groups and also species richness and the relative abundance of alien fishes (in streams) or of vascular plants (in wetlands and terrestrial environments).

Considerable theoretical discussion has centered on "functional feeding groups" of North American benthic invertebrates (Cummins 1974; Cummins et al. 1989; Cummins et al. 1995). In particular, according to the river continuum hypothesis (Vannote et al. 1980), the relative abundance of these groups is predicted to change along the length of a river or stream. For example, in comparison with headwaters, which are presumed to receive mostly allochthonous organic matter, downstream reaches might have more filter-feeders or net-spinning caddisflies taking advantage of high in-stream production. But the river continuum hypothesis does not seem to apply consistently across North American streams (Vannote et al. 1980; Winterbourn et al. 1981; Minshall et al. 1983). Metrics based on functional feeding groups among benthic invertebrates (with the possible exception of relative predator abundance) likewise respond differently in different streams.

This inconsistent response differs from what might be a more general pattern of trophic metric behavior in fishes; perhaps the trophic structure of fish assemblages in North America is more consistent than for benthic invertebrates. Alternatively, perhaps more is known about the natural history of fishes, permitting better delineation of feeding groups. Or our knowledge of invertebrates may be less precise, or invertebrates may be more opportunistic. The generality of trophic group response to disturbance deserves more careful analysis, but, meanwhile, we urge caution. Despite a widely accepted theory, metrics pertaining to functional feeding groups among benthic invertebrates may or may not be good indicators; their dose-response relationships to human influence must be carefully tested and established for multiple data sets and circumstances before they should be used in a multimetric index.

Landscape level. Regardless of level in the biological hierarchy (individuals, species, ecosystem), the persistence of living things depends on heterogeneities in space and time. Spatial heterogeneities are visible in littoral zonation, in vegetation bands associated with water depth in marshes, or in association with soil moisture and slope gradients on drier land. Stream fish spend their lifetime in many microhabitats; they are exposed to different flows and other shifts in time as days and seasons change. Eggs laid in main-channel gravels become fry hiding in side channels and along the banks; fry grow into juveniles large enough to avoid the

predators that would otherwise eat them; juveniles may then move into deep pools where those predators are and where food supplies also differ.

Finding food, avoiding predators, seeking spawning habitat—any activity in an organism's life cycle—are subject to and dependent on such heterogeneities in space and time. For some species, the scale of movements may extend only a few centimeters; for others, the scale can be hundreds or thousands of miles. The loss of spatial or temporal components of these heterogeneities can change the distribution or abundance of a species or cause it to disappear altogether. The presence or absence of anadromous or other migratory fishes (e.g., salmon, bull trout) is thus a landscape-level indicator. Dams, alien predators, and altered water flows and temperatures interfere with their movements through a landscape, decimating these species.

Incorporating several multimetric indexes (fish IBI, benthic IBI, algal IBI) into a biological monitoring program is a good way to reflect the condition of assemblages that respond to human disturbances at different scales. Different taxa in the same or different assemblage reflect the presence of a broad range of heterogeneities. If top predator taxa needing large home ranges or long-lived taxa requiring years to mature are present, for example, one can infer that the spatial and temporal components they require are also present. Excessive in-stream production or many herbivorous fishes or invertebrates are characteristic of heavily grazed landscapes, where riparian corridors may be damaged and excessive nutrients from livestock wastes are entering the stream.

Development of IBI to date has involved a conscious effort to span the range of biological context. But much remains to be done. Better measures of individual health are needed, as are measures better defining demographics. Strengthening the connections between measures of food web and trophic structure and more-direct measures of nutrient cycling and energy flow would also improve multimetric assessment. Finally, landscape metrics that emphasize overall biological condition (number of native community types or cumulative taxa richness across a watershed) are also needed. Ideally, metrics of landscape condition should be more than a sum of site-specific assessments.

Great care must be taken to measure biological condition, not stressor intensity. We believe that biological surrogates of biological condition are essential; chemical and physical surrogates of biological condition are not adequate.

Developed and applied properly, the multimetric IBI incorporates and depends on known components of biology—components specific to localities and taxa—across the organizational hierarchy and from disparate spatial and temporal scales. The result is a synthesis of biological signals revealing the effects of human activities at different levels, in different places, on different scales, and in response to a range of human activities.

METRICS ARE SELECTED TO YIELD RELEVANT BIOLOGICAL INFORMATION AT REASONABLE COST

The index of biological integrity as first developed for fish (Karr 1981; Karr et al. 1986) incorporated 12 metrics from three biological categories: species richness and composition, trophic composition, and individual condition. Later work with both fish and invertebrates led to somewhat different groups: specifically, species richness, taxonomic composition, individual condition, and biological processes (Karr 1993; Barbour et al. 1996b) or community structure, taxonomic composition, individual condition, and biological processes (Fore et al. 1996). Within each broad category, some metrics are proven for many regions and faunas. Others work in some regions or studies but not in others. Still other potential metrics based on theoretical ecology or toxicology may work but have not been adequately tested,[2] because they are either too difficult to measure or too theoretical to define (Table 5). The categories in Table 5 guide metric selection for new regions, faunas, or habitats, but no metric should become part of a multimetric index before it is thoroughly and systematically tested and its response has been validated across a gradient of human influence.

No metric should become part of a multimetric index before its response has been validated across a gradient of human influence

The choice of how to actually express each metric is as important as selecting the metric itself. One could simply count the number of individuals in a target group and express it as population size, abundance, or density (Figure 18, top); one could determine the proportion, or relative abundance, of the total number of individuals belonging to a target group (number of individuals in the target group divided by the total number of individuals in the sample; Figure 18, middle); or one could count the number of taxa in the entire sample or in particular subgroups (taxa richness; Figure 18, bottom). One could also determine (not shown) the proportion of the biota from specific taxa (e.g., number of mayfly taxa/total number of taxa). Approaches vary in their ability to reveal consistent dose-response relationships, as Figure 18 shows; knowledge of natural history and of which sampling protocols are most efficient should guide one's choice.

Population size—besides being difficult and often costly to determine with sufficient precision (Paller et al. 1995b), especially for rare species—is not a good measure because it is naturally too variable, irrespective of human impacts (Karr

[2] Unfortunately, untested or too-theoretical attributes have been central to EPA's rapid bioassessment protocols (RBP I, II, III), used since 1989. Many measures incorporated into RBP III were never tested adequately, and recent tests (Barbour et al. 1992; Kerans et al. 1992; Kerans and Karr 1994; Barbour et al. 1996a; Fore et al. 1996) indicate that they do not meet rigorous standards for metric acceptance.

TABLE 5. Sample biological attributes, in four broad categories, that might have potential as metrics. Actual monitoring protocols have proven some of these attributes effective; other attributes may work but need more testing; still others are difficult to measure or too theoretical. Ideally, an IBI should include metrics in each of these categories, but untested or inadequately tested attributes should not be incorporated into the final index.

Category	Demonstrated effective	Need more testing	Difficult to measure or too theoretical
Taxa richness	Total taxa richness Richness of major taxa, e.g., mayflies or sunfish	Dominance (relative abundance of most-numerous taxa)	Relative abundance distribution, after Preston (1962)
Tolerance, intolerance	Taxa richness of intolerant organisms Relative abundance of green sunfish Relative abundance of tolerant taxa	Number of rare or endangered taxa	Chironomid species (difficult to identify)
Trophic structure	Trophic organization, e.g., relative abundance of predators or omnivores		Productivity
Individual health	Relative abundance of individual fish with deformities, lesions, or tumors Relative abundance of individual chironomids with head-capsule deformities Growth rates by size or age class	Contaminant levels in tissue (biomarkers)	Metabolic rate
Other ecological attributes		Age structure of target species population	

1991). Our recent work in Puget Sound lowland streams, for example, found no systematic relationship in two successive years between benthic invertebrate abundance and the percentage of impervious area in the upstream watershed, one measure of human influence (Figure 19).

Similarly, ratios of two groups in an assemblage do not respond systematically to human influence, largely because ratios are composed of two factors that can respond, and thus vary, independently of each other, making it impossible to draw firm conclusions about the relationship of those ratios to human influence (see Premise 24, page 95). Further, two large numbers and two small numbers may yield the same ratio, although the biological meaning of small and large numbers may be very different (Kerans and Karr 1994). If both components of the ratio are

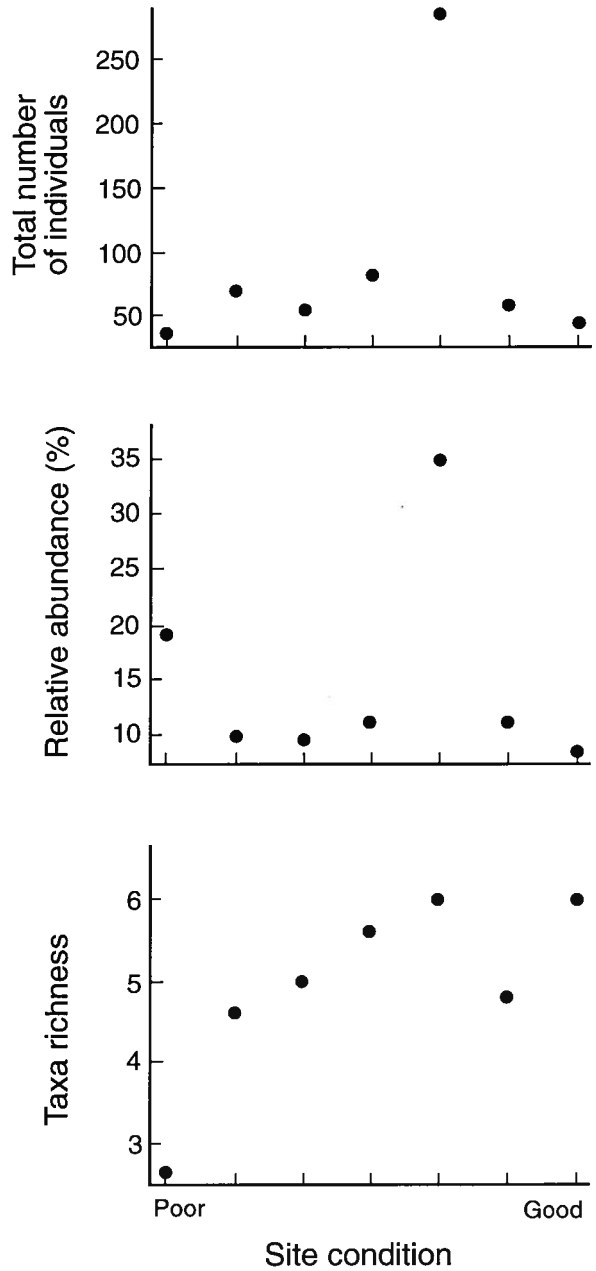


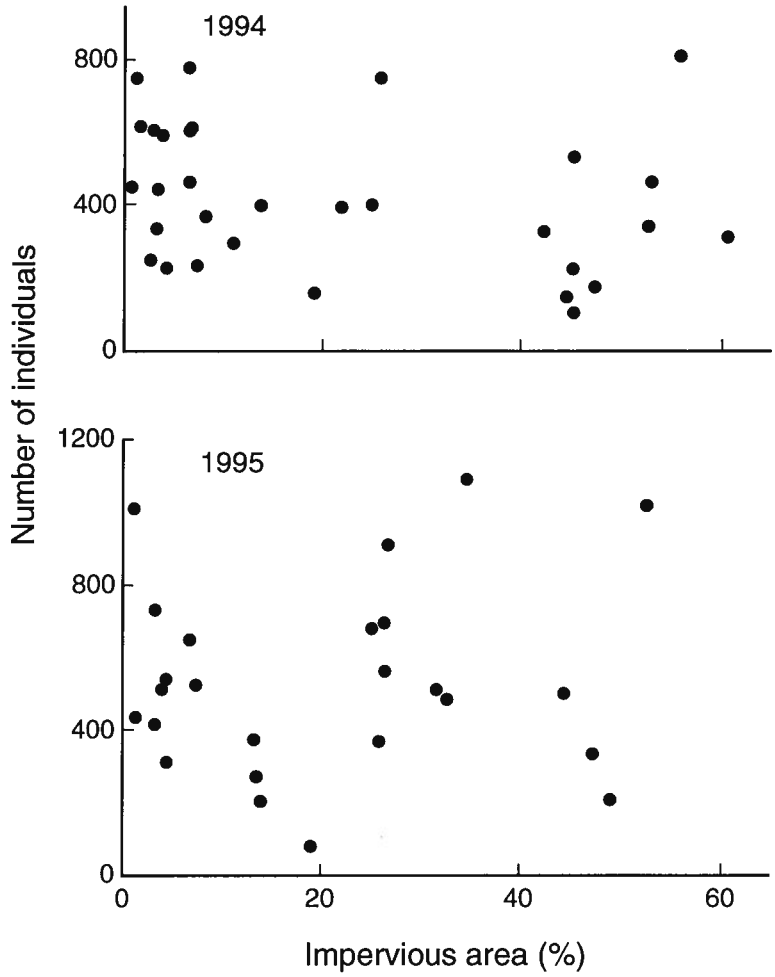
FIGURE 18. Presence of Trichoptera (caddisflies) in a standard sample, expressed as total number of trichopteran individuals (top), relative abundance of trichopteran individuals (middle), and richness of trichopteran taxa (bottom). These three biological attributes are plotted against grazing intensity as an indicator of site condition at seven stream sites in the John Day River basin of southwestern Oregon.

important, they might more appropriately be considered separately. (This reasoning also applies in the case of diversity indexes, which combine richness and relative abundances. We prefer to keep those issues distinct with separate metrics.)

Metrics related to feeding ecology or trophic structure are best expressed as relative abundance—for example, the number of individual predators, omnivores, or scrapers divided by the total number of sampled individuals.[3] The relative abundance of organisms at various levels in a stream's trophic organization reflects the condition of the food web, including energy flow and nutrient dynamics, but relative abundances are much easier to measure than true production or energy

[3] Although this metric looks like a ratio, it is a ratio of a variable over a constant for the sample. In contrast, the ratios of two taxa or two functional feeding groups are ratios of two variables from the sample.

FIGURE 19. Number of invertebrates plotted against impervious area for lowland Puget Sound streams in two successive years.



flow. If we know what to expect from minimally disturbed sites in a region, we can then distinguish the deviations caused by human activities from that expectation. The relative abundance of fish-eating fish in minimally disturbed streams, for example, is likely to be 20% or more; omnivores, 20% or less. In degraded streams, the relative abundance of omnivores is likely to be much higher (> 40%).

Major taxonomic groups are best evaluated in terms of taxa richness[4] because, as human activities damage a stream and its watershed, native taxa tend to disappear. A decline in taxa richness is generally one of the most reliable indicators of degradation for many aquatic groups (Ford 1989; Barbour et al. 1995), including periphyton (Bahls 1993; Pan et al. 1996); phytoplankton (Schelske 1984); zooplankton (Stemberger and Lazorchak 1994); riverine fish (Karr 1981; Miller et al. 1988; Ohio EPA 1988; Rodriguez-Olarte and Taphorn 1994; Rivera and Marrero 1994; Lyons et al. 1995, 1996); lake fish (Minns et al. 1994); estuarine fish (Thompson and Fitzhugh 1986; Deegan et al. 1993; Weaver and Deegan 1996; Deegan et al. 1997; Hartwell et al. 1997); freshwater invertebrates (Ohio EPA 1988; Reynoldson and Metcalfe-Smith 1992; Kerans and Karr 1994; DeShon 1995; Fore et al. 1996;

[4] Taxa richness can be standardized per unit of area (e.g., taxa/0.1 m^2) or per unit count of individuals (e.g., taxa/500 individuals). The proper choice is hotly debated, a topic we cover in more detail in Premise 28, page 101.

Thorne and Williams 1997); and marine invertebrates (Summers and Engle 1993; Engle et al. 1994; Weisberg et al. 1997).

Taxa richness may be calculated for an entire sample or for subgroups, such as fish families or insect orders, that use the stream environment in a particular way. Sunfish, for example, feed in the water column or at the surface of pools, whereas suckers feed in benthic pool environments, and darters or sculpins feed in benthic riffle environments. Each requires the unique structural complexity and cover associated with those particular feeding environments; the interactions of cover, structural complexity, and changing food abundances resulting from human actions may cause declines in all these groups. Because their natural histories differ, these three taxa provide information about the condition of three different habitat types within a stream. Loss of sucker taxa points to a problem, such as sedimentation, within the benthic pool environment. Loss of sunfish suggests loss of physical cover and their invertebrate food in the pelagic and surface zones of pools; indeed, insects decline at the surface when riparian vegetation is lost. Similar information may be gained from the taxa richness of lithophilous spawners or nursery species.

Among benthic invertebrates, we calculate the taxa richnesses of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) because they too reflect different types of degradation. Ephemeroptera taxa are lost when toxic chemicals like those from mine wastes foul a stream (see Figure 17, page 43; Hughes 1985; Kiffney and Clements 1994). Plecoptera taxa disappear as riparian vegetation is lost and sediment clogs the interstitial spaces among cobbles. Plecoptera tend to decline at less intense levels of human influence than Trichoptera or Ephemeroptera. Therefore, combining these three taxa into a single "EPT"[5] metric (as in RBP III and others; Plafkin et al. 1989; Lenat and Penrose 1996) may obscure real differences that could help define both the types and sources of degradation at a site.

The signals provided by intolerant and tolerant taxa mean that the best expression of metrics based on these taxa differs between intolerants and tolerants. The mere presence of very sensitive, or intolerant, taxa (as apparent from taxa richness) is a strong indicator of good biological condition; the relative abundance of these taxa, in contrast, is difficult to estimate accurately without extensive and costly sampling efforts. Presence alone of tolerant taxa, on the other hand, says little about biological condition since tolerant groups inhabit a wide range of places and conditions, but as conditions deteriorate, their relative abundance rises (see Figure 21, page 61). In general, we recommend that only about 10% (no fewer than 5% or more than 15%) of taxa in a region should be classed as intolerant or tolerant. The point of these metrics is to highlight the strong signal coming from presence of the most intolerant or most tolerant taxa. We avoid the average tolerance value as reflected in biotic indexes because the strong signals of tolerants and intolerants are swamped by the remaining 70% to 90% of taxa with intermediate tolerances.

(For a more statistical rationale for choosing taxa richness and relative abundance, see Premise 19, page 80, and Figure 33, page 81.)

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[5] EPT is the sum of the mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera) found in a benthic invertebrate sample.

PREMISE 14

MULTIMETRIC INDEXES ARE BUILT FROM PROVEN METRICS AND A SCORING SYSTEM

Across taxonomic groups, many of the same biological attributes indicate human-induced disturbance (see pages 54–55, Premise 13; Table 6). Over the last 15 years, numerous studies have helped define those most broadly applicable metrics (Karr 1981; Miller et al. 1988; Kerans and Karr 1994; Fore et al. 1996; see Barbour et al. 1996b for summary table of metrics). After testing in a series of independent studies, 10 attributes of stream invertebrates and 10 to 12 attributes of stream fishes consistently emerge as reliable indicators of biological condition at sites influenced by different human activities in different geographic areas[6] (Tables 7 and 8; see also Table 5, page 52).

Consistently reliable metrics include the total number of taxa present in the sample (total taxa richness), the number of particular taxa or ecological groups (e.g., taxa richness of darters or mayflies), the number of intolerant taxa, and the percentage of all sampled individuals (relative abundance) belonging to stress-tolerant taxa (e.g., tubificid worms). Among fishes, a high percentage of individual fish with disease, fin erosion, lesions, or tumors indicates toxic chemicals in a stream. Increased frequency of hybrids seemed a useful metric in early IBI studies (Karr 1981; Karr et al. 1986), although relatively few studies since then have used it successfully. Increased hybridization could indicate a loss of habitat variety and consequent mixing of gametes from different species spawning in a homogenized environment (Hubbs 1961; Greenfield et al. 1973).

Metric values are scored by comparison with the value expected at a minimally disturbed site

The values of metrics such as these provide the best and most complete assessment of a site's condition, but to compare sites and communicate their relative condition to the widest possible audience, metric values at a site are summarized in the form of an aggregate index—the index of biological integrity. Because human actions affect biological resources in multiple ways and at multiple scales, 10 to 12 metrics from four broad categories (see Table 4, page 48, and Table 5, page 52) are selected and then scored using standardized scoring criteria; these metrics are the building blocks of the multimetric index (Karr 1981; Karr et al. 1986; Karr 1991).

Because we now know a great deal about which metrics respond consistently to different levels of human effect, agency biologists with limited budgets do not

[6] The number of metrics in the fish IBI is somewhat smaller in relatively simple systems such as cold-water streams (Lyons et al. 1996). Wetlands may be most appropriately assessed with multiple taxa (e.g., plants, insects, fish , birds) with fewer metrics for each of the taxa- or assemblage-based IBIs.

TABLE 6. Regardless of taxon used or habitat sampled, similar metrics respond predictably (√) to human influence. As human influence increases, taxa richness declines, the relative abundance of generally tolerant organisms increases, and generally sensitive taxa disappear. (Sources: see page 54, Premise 13.)

Taxon	Habitat	Taxa richness	Relative abundance of tolerants	Number of sensitive or intolerant taxa
Fish	River	√	√	√
Fish	Lake	√	√ (generalists)	√
Fish	Estuary	√		√ (nursery specialists)
Benthic invertebrates	River	√	√	√
Periphyton	River	√	√	√

have to test all attributes to begin using a multimetric index; instead, they can take advantage of and build on studies that have been done before. Nevertheless, whenever more than five sites with different human influences can be sampled, we encourage testing of metric responses in particular locales to see whether the patterns observed in other regions can be generalized.

Before one can build a multimetric index, one must convert metric data into a common scoring base. Typically, metrics are quantified with different units and have different absolute numerical values (e.g., numbers of taxa may range from 0 to a few dozen; relative abundances of certain groups may range from 0% to 100%). Also, some metrics increase in response to human disturbance (e.g., percentage of omnivores) while others decrease (e.g., overall taxa richness). To resolve such differences, each metric is assigned a score based on expectations for that metric at minimally disturbed site(s) for that region and stream size. Metrics that approximate what one would expect at minimally disturbed sites are assigned a score of 5; those that deviate somewhat from such sites receive a score of 3; those that deviate strongly are scored 1 (Karr 1981; Karr et al. 1986; Karr 1991). The final index is the sum of all the metrics' scores (Figure 20).

In all cases, the basis for assigning scores is "reference condition," that is, the condition at sites able to support and maintain a balanced, integrated, and adaptive biological system having the full range of elements and processes expected for a region; thus IBI explicitly incorporates biogeographic variation into its assessment of biological condition. In some regions, biologists can actually find and sample from sites that have not been influenced, or have been influenced only minimally, by humans; in other regions, where pristine sites are unavailable, biologists may have to infer reference condition based on knowledge of the evolutionary and biogeographic

TABLE 7. Potential metrics for benthic stream invertebrates. Metrics that responded to human-induced disturbance as predicted are indicated by a check (√); those marked with a dash (–) were not tested. Percent sign (%) denotes relative abundance of individuals belonging to the listed taxon or group(s). Metrics marked with an asterisk (*) have been included in a 10-metric multiregional B-IBI (Karr 1998; see also Table 11, page 103). Human influence in Tennessee Valley consisted primarily of mining and agriculture; in southwestern Oregon, logging and road building; in eastern Oregon, grazing; in Puget Sound lowlands, urbanization (measured by percentage of impervious surface); in Japan, multiple human influences; and in Wyoming, recreation.

Metric	Predicted response	Tenn. Valley	SW Ore.	Eastern Ore.	Puget Sound	Japan	NW Wyo.
Taxa richness and composition							
Total number of taxa*	Decrease	√	√		√	√	
Ephemeroptera taxa*	Decrease	√	√		√	√	√
Plecoptera taxa*	Decrease	√	√	√	√		√
Trichoptera taxa*	Decrease	√	√	√	√	√	
Long-lived taxa*	Decrease	–	√		√	–	
Diptera taxa	Decrease						
Chironomidae taxa	Increase			–	–		
Tolerants and intolerants							
Intolerant taxa*	Decrease	√	√	√	√	√	√
Sediment-intolerant taxa	Decrease	√[a]	√	√	–	–	–
% tolerant*	Increase	√	√		√	√	√
% sediment-tolerant	increase	–	√	√	–	–	–
% planaria + amphipods	Increase	–	–	–	√	–	–
% oligochaetes	Increase	√				√	
% chironomids	Increase						
% very tolerant	Increase	–	–	–	–	√	–
% "legless" organisms	Increase	–	–	–	–	√	
Feeding and other habits							
% predators*	Decrease	√		√			√
% scrapers	Variable	√		√			√
% gatherers	Variable			√			
% filterers	Variable	√					
% omnivores	Increase	√					
% shredders	Decrease			√			√
% mud burrowers	Increase	–	–	–	–	√	–
"Clinger" taxa richness*	Decrease	–	–	–	–	√	–
Population attributes							
Abundance	Variable	√					√
Dominance*	Increase	√	√			√	√

[a] Sediment-surface taxa richness

TABLE 8. Metrics used in the original fish index of biological integrity (IBI) for midwestern US streams and equivalents for more general application.

Original fish IBI	General fish IBI[a]
Number of fish species	Number of native fish species
Number of darter species	Number of riffle-benthic insectivores
Number of sunfish species	Number of water column insectivores
Number of sucker species	Number of pool-benthic insectivores
Number of intolerant species	Number of intolerant species
Relative abundance of green sunfish	Relative abundance of individuals of tolerant species
Relative abundance of omnivores	Relative abundance of omnivores
Relative abundance of insectivorous cyprinids	Relative abundance of insectivores (specialized insectivores)
Relative abundance of top carnivores	Relative abundance of top carnivores
Number of individuals	*Not a reliable metric*
Relative abundance of hybrids	*Not often used successfully*
Relative abundance of diseased individuals	Relative abundance of diseased individuals

[a] Metrics chosen vary as a function of stream size, temperature class (warm-, cool-, cold-water), and ecological factors to reflect biogeographic and other patterns, including sensitivity to different human influences.

processes operating in the region (see Premise 30, page 108). In still other cases (Fausch et al. 1984; Hughes 1995; Hughes et al., in press), researchers must depend on historical data, collected when human activity was less, to define reference condition.

Simple, uniform rules for setting scoring criteria—the range of numerical values that qualify a metric for a score of 5, 3, or 1—are therefore difficult to define because they depend in part on the sampling design that generated the data. In a hypothetical watershed where one-third of sampled sites were pristine, one-third moderately disturbed, and one-third highly disturbed, one could simply divide the values for each metric at the thirty-third and sixty-seventh percentiles. But human activities tend to homogenize landscapes and living systems so that a majority of sites in a given watershed are likely to be moderately or even severely degraded, such as in the Japanese study illustrated in Figure 21. In the real world, therefore, it makes sense to err on the conservative side by expanding the middle score (3) or

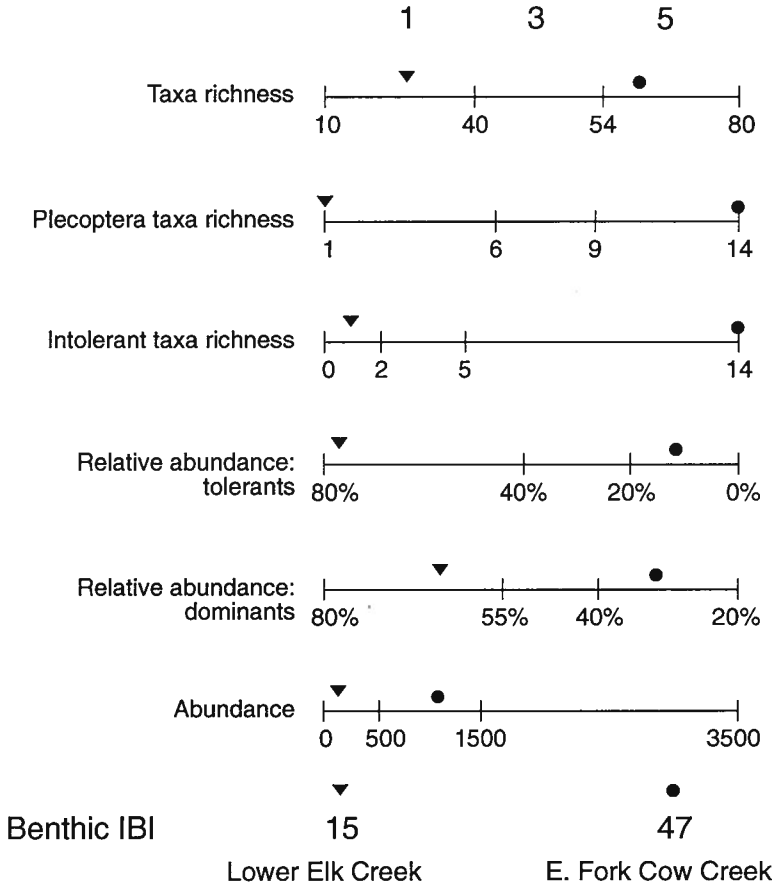


FIGURE 20. Range and numeric values for six invertebrate metrics from a severely disturbed site (lower Elk Creek, ▼) and a less disturbed site (East Fork Cow Creek, ●) in southwestern Oregon. Because the metrics have different quantitative values, they are given scores (5, 3, 1) to put them on the same scale: 5 indicates little or no deviation from expected, or reference, condition; 3 indicates moderate deviation from expected condition; and 1 indicates strong deviation from expected condition. Vertical lines in the figure represent the cutoff points for assigning these metric scores. Total benthic IBI (B-IBI) value for these two sites equals the sum of these metric scores and five others (from Fore et al. 1996).

even the low score (1) to include more sites rather than fewer, thus making it more difficult for a site to attain a high score.

Natural shifts or breaks in the distribution of metric values can guide the setting of scoring criteria; indeed, scoring criteria should be adjusted to fall at these points because the points often reflect a biological response. Where metric values increase or decrease linearly across the gradient of human influence (Figure 21, top), as in total taxa richness, the values are typically trisected into three equal divisions, each representing the criteria for assigning a score of 1, 3, or 5. Other metrics, such as relative abundance of tolerant organisms or particular trophic groups, respond in a more skewed pattern (Figure 21, bottom; Figure 22); for these metrics, natural break points suggest setting scoring criteria in unequal divisions. Setting scoring criteria is an iterative process and should be revisited as regional databases and biological knowledge expand.

FIGURE 21. Plots of two sample metrics showing different ways to set the criteria for assigning metric scores of 1, 3, and 5. For metrics with a monotonic, or linear, distribution (e.g., total taxa richness: top), one divides into equal thirds the range from 0 to the highest value (here 36). For metrics that are not distributed monotonically, one uses natural breaks in the distribution to define score boundaries (shown in the bottom plot by vertical dotted lines). Metric values and classification scheme for human influence come from Rossano (1995) (see also Figure 3, page 23, and Figure 4, page 31).

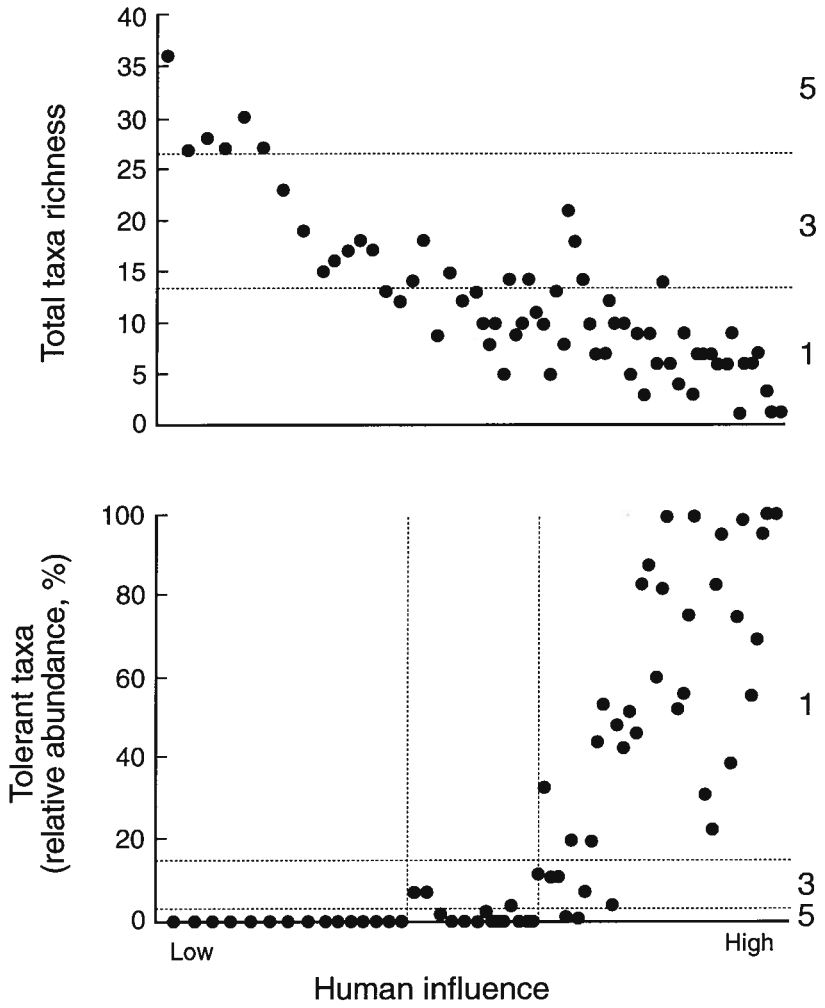
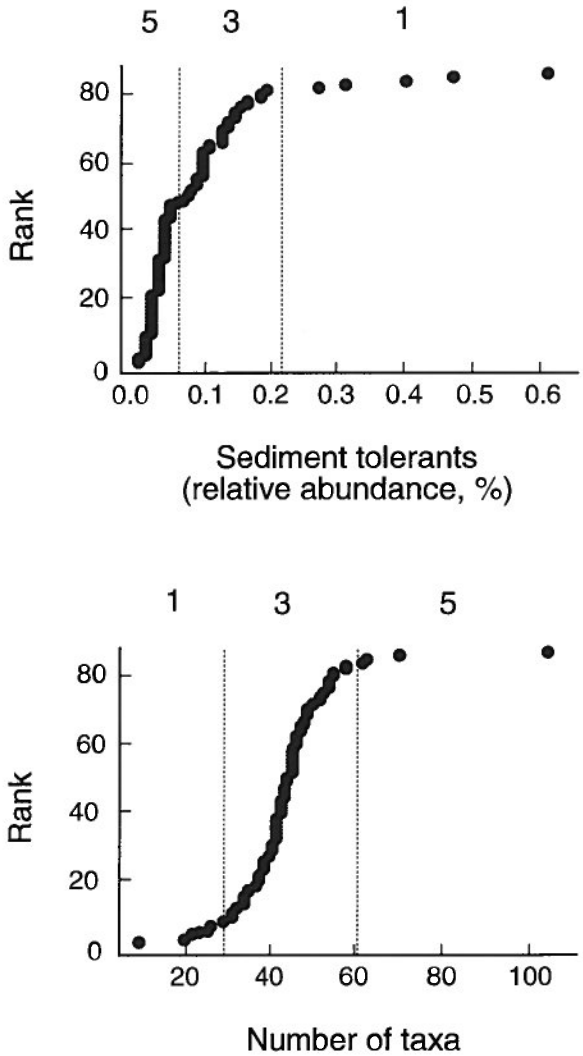


FIGURE 22. Relative abundance (percentage of sediment-tolerant individuals) and taxa richness (number of taxa) plotted against the rank order of that metric value for 86 stream sites sampled in southwestern Oregon. Dotted vertical lines mark the range of values (scoring criteria) for scoring metrics as 5, 3, or 1. Most sites have near 0% sediment-tolerant individuals; only very degraded sites show higher values of this metric. In other words, the distribution pattern for this metric is skewed. Taxa richness, in contrast, is less skewed. Scoring criteria are divided into unequal divisions for skewed metrics, reflecting a biological response in the data (top); the divisions are more equal for unskewed metrics (bottom). In both cases, most sites receive a score of 3, the most conservative interpretation of condition.



THE STATISTICAL PROPERTIES OF MULTIMETRIC INDEXES ARE KNOWN

Multimetric indexes are statistically versatile. We can use familiar statistical tests, such as *t*-tests or analysis of variance (ANOVA), to look for significant differences in index values because IBI satisfies the model's assumptions (Fore et al. 1994). In addition, because IBI is a single integrating number, it serves as a yardstick to rank (compare) sites according to their relative condition. Finally, from statistical power analysis, we know that an IBI formulated and developed as we propose can detect six distinct categories of resource condition (Fore et al. 1994; Doberstein, Karr, and Conquest, in prep.). Because we know the statistical precision of a given IBI, we can use IBIs to discover and define differences among sites caused by changes through time or space.

Using bootstrap[7] analysis of fish data from Ohio, we determined that the distribution of IBI at one stream site is unimodal (Figure 23); integrating metric scores into a multimetric index thus allows us to take advantage of properties of the mean. Integration can be done by summing or averaging the metric scores; the results are equivalent. For the fish IBI, averaging metric scores reduced the variance and increased precision (Fore et al. 1994). The values for multimetric indexes approximate a normal distribution (Fore et al. 1994), probably because averages tend to be distributed normally by the central limit theorem (Cassella and Berger 1990); consequently, multimetric indexes can be tested with familiar statistics such as ANOVA or regression.

Integrating metric scores into a multimetric index allows us to take advantage of properties of the mean

The IBI distribution satisfies the assumptions of ANOVA, even though the strong unimodal peak but no tails (expected given the way scores are calculated) is not strictly normal (see Figure 23). These assumptions are: (1) the error term is unbiased; (2) measurement error is not correlated among sites; (3) variance is homogeneous; and (4) the distribution of the error term is normal (assumed only for hypothesis testing).

Some regulatory situations require statistical evidence that a significant change has occurred in the field. The statistical properties of IBI make it an appropriate choice for these situations. In reality, however, management decisions are rarely based on the outcome of a statistical test or its associated *p*-value. Often, sites

[7] The bootstrap algorithm creates new samples by randomly selecting and replacing elements from the original sample. Random sampling with replacement continues until the bootstrap sample contains the same number of elements as the original sample. Many such samples are generated to approximate the distribution of IBI at a site.

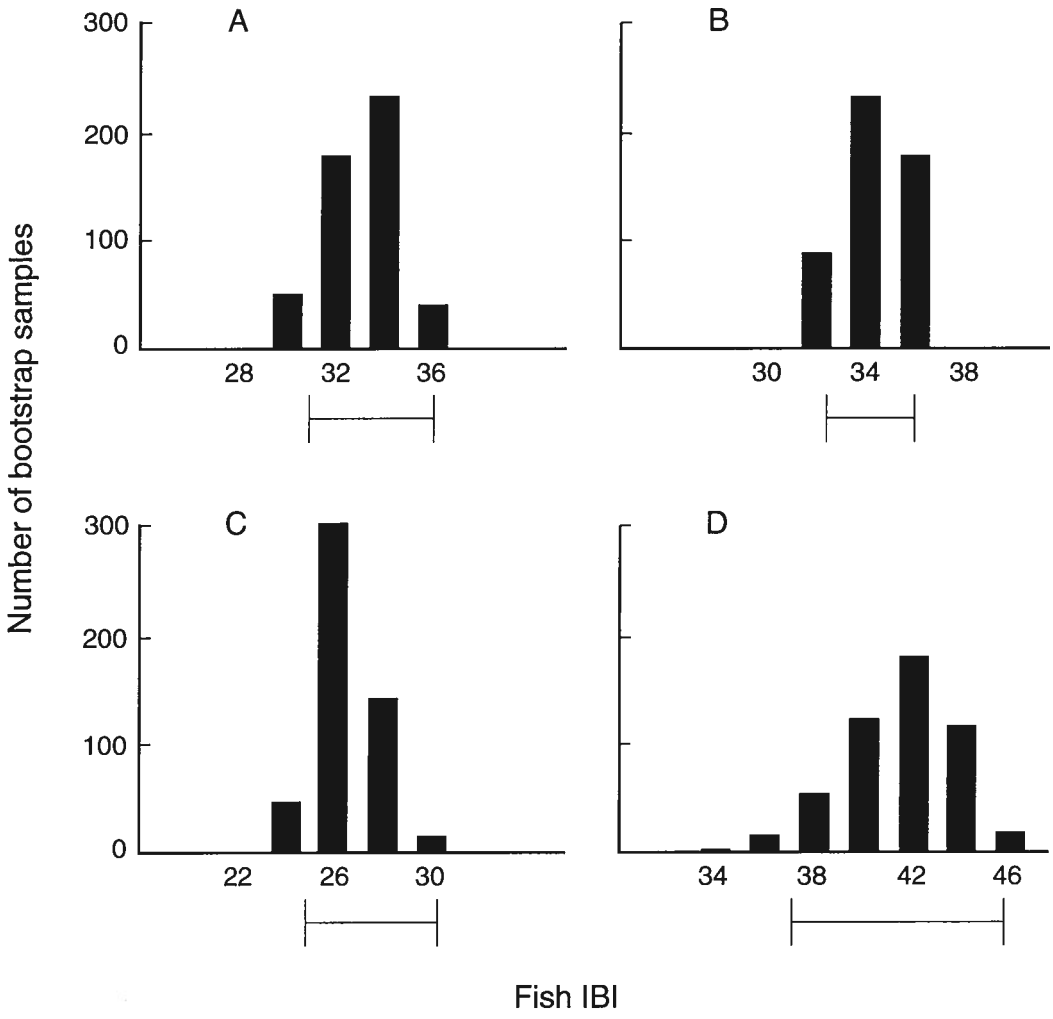


FIGURE 23. Distribution of fish IBI values from bootstrapping analysis for four typical stream sites in Ohio; the unimodal distributions approximate a normal distribution. The line below each *x*-axis marks the 95% confidence interval (< 8). A difference of ± 4 points in IBI values therefore represents a statistically significant change in biological condition (Fore et al. 1994).

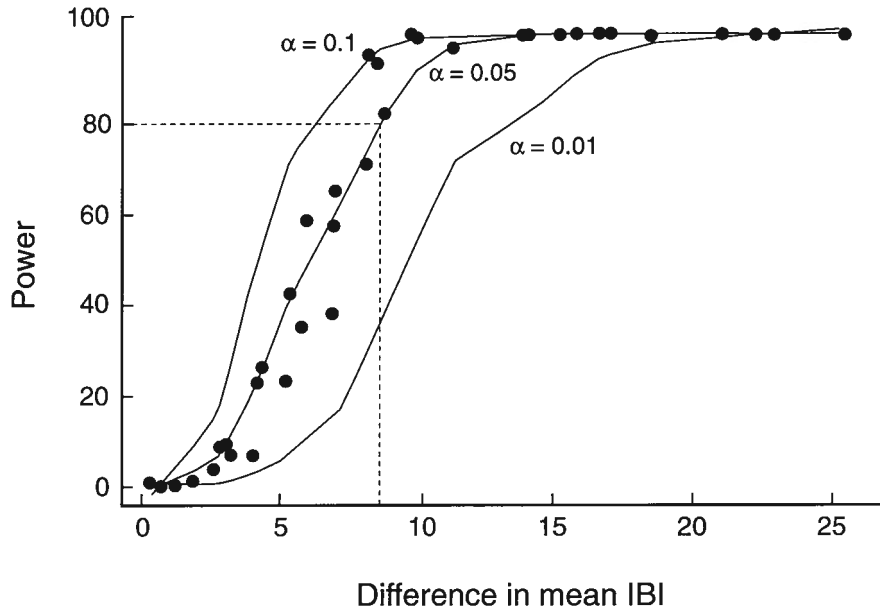


FIGURE 24. Power curves for the fish IBI estimated from nine locations sampled three times by the Ohio EPA (from Fore et al. 1994). Actual points are shown only for $\alpha = 0.05$; other values of α are pictured as smoothed lines. For 80% power (a value accepted by most researchers), IBI can reliably detect a difference of about 8 points at an α–level of 0.05 (projected onto the x-axis, as indicated by dashed lines). Total IBI can range from 12 to 60, a difference of 48; thus IBI can detect six non-overlapping categories of biological condition.

within an area need to be ranked so that funds for restoration can be allocated, or policies to determine human use can be evaluated. Managers and policymakers therefore need to know something about the magnitude of differences across sites and, most important, whether observed differences are biologically meaningful. Without this kind of information, they cannot ascertain the causes of those differences.

A multimetric index provides a yardstick for measuring and communicating the biological condition of sites, but how many tick marks are on the yardstick? In other words, what is the precision of the index? On the basis of a statistical power analysis of fish data from Ohio EPA, IBI can detect six distinct categories of biological condition (Figure 24). Ohio EPA's version of IBI, like the original IBI, ranges from 12 to 60. For this index, 95% of the variability in IBIs generated by the bootstrap procedure fell within ± 4 points of the observed IBI (Fore et al. 1994). These results confirmed previous estimates of confidence intervals based on field observations through time (Angermeier and Karr 1986; Karr et al. 1987).

PREMISE 16

MULTIMETRIC INDEXES REFLECT BIOLOGICAL RESPONSES TO HUMAN ACTIVITIES

Human activities degrade water resources by altering one or more of five principal groups of attributes—water quality, habitat structure, flow regime, energy source, and biological interactions—often through undetected yet potentially devastating effects on water resources (Table 9; Karr 1991, 1995b). Human activities such as logging, agriculture, and urbanization affect water quality by introducing sediment and raising water temperature (Bisson et al. 1992; Megahan et al. 1992; Gregory and Bisson 1997; Williams et al. 1997). Habitat structure changes when large woody debris is removed from a channel, or when sediment fills the spaces among cobbles. When vegetation is removed from a watershed, streams and rivers flood more heavily and more often, or they may dry up entirely. Logging of riparian areas also alters the energy sources in a stream: removing riparian vegetation removes one source of allochthonous organic material, disrupts entry of large woody debris to the channel, and also increases light reaching the stream, which in turn increases water temperature and algal growth and thus the algal material available to fish and invertebrates. Overfishing and introducing alien species, including native fish raised in hatcheries, alter relationships among predators and prey or competitors. As these changes stress the normal assemblage of stream organisms, they degrade the stream.

Because IBI can detect many influences in time and space, it is an ideal tool for judging the effectivenss of management decisions

Because multimetric indexes are sensitive to these five factors, they quantify the biological effects of a broad array of human activities. The effects of logging were generally reflected in benthic IBIs from southwestern Oregon (Figure 25), even though logging was quantified simply as the percentage of total watershed area that was logged (Fore et al. 1996). Secondary influences on B-IBIs in these watersheds included road density and location. In east-central Illinois (Karr et al. 1986), fish IBIs revealed the influences of agriculture: IBIs were lowest at sites where cultivation reached streamside, and stream channels had been dredged and straightened; IBIs were higher downstream, where the riparian area was left either as pasture or forest, and the stream channel was intact (Figure 26). In the Pacific Northwest, urbanization generally produces lower IBIs than logging (Kleindl 1995; Fore et al. 1996).

Multimetric indexes can reflect changes in resident biological assemblages caused by single point sources in one river or stream as well as differences over a wide geographic area. For example, taxa richness of mayflies, stoneflies, and caddisflies (see Figure 17, page 43), as well as overall B-IBI (Figure 27), fell sharply immediately

TABLE 9. Five attributes of water resources altered by the cumulative effects of human activity, with examples of degradation in Pacific Northwest watersheds (from Karr 1995b).

Attribute	Components	Degradation in Pacific Northwest watersheds
Water quality	Temperature, turbidity; dissolved oxygen; acidity; alkalinity; organic and inorganic chemicals; heavy metals; toxic substances	Increased temperature and turbidity Oxygen depletion Chemical contaminants
Habitat structure	Substrate type; water depth and current speed; spatial and temporal complexity of physical habitat	Sedimentation and loss of spawning gravel Obstructions interfering with movement of adult and juvenile salmonids Lack of coarse woody debris Destruction of riparian vegetation and overhanging banks Lack of deep pools Altered abundance and distribution of constrained and unconstrained channel reaches
Flow regime	Water volume; flow timing	Lower low flows and higher high flows limiting survival of salmon and other aquatic organisms at various phases in their life cycles
Food (energy) source	Type, amount, and size of organic particles entering stream; seasonal pattern of energy availability	Altered supply of organic material from riparian corridor Reduced or unavailable nutrients from carcasses of adult salmon and lampreys after spawning
Biotic interactions	Competition; predation; disease; parasitism; mutualism	Increased predation on young by native and alien species Overharvest by sport and commercial fishers Genetic swamping by hatchery fish of low fitness Alien diseases and parasites from aquaculture, including hatcheries

downstream of a streamside sludge pond on the North Fork Holston River in Tennessee (Kerans and Karr 1994). Across six midwestern regions or watersheds with different degrees of land development, fish IBIs differed markedly (Figure 28; Karr et al. 1986). Yet despite their different fish faunas, one can compare the condition of these regions on a single quantitative scale.

FIGURE 25. Benthic IBI values plotted against the percentage of area logged in watersheds in southwestern Oregon in 1990. Percentage of watershed area logged alone is an incomplete measure of human influence because information about type of logging, time since logging, or location and type of roads is not included. Nevertheless, B-IBI clearly distinguishes the best available (+) from the degraded (–) sites.

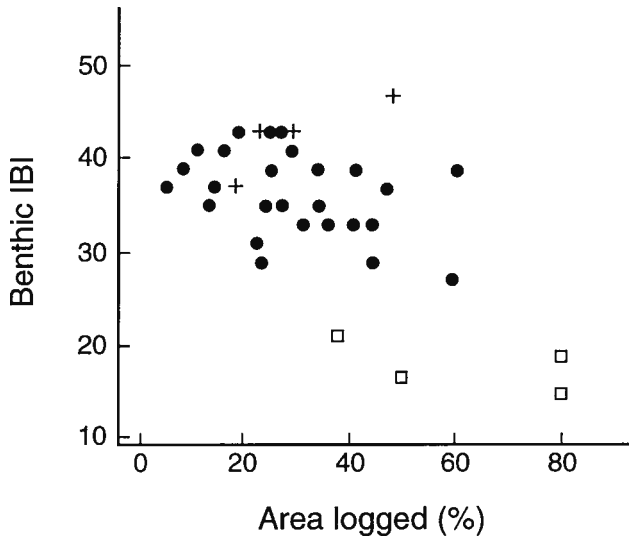


FIGURE 26. Fish IBI values for Jordan Creek, a first- to third-order stream in east-central Illinois (from Karr et al. 1986). Higher values represent changes in the fish assemblage that reflect improved biological conditions from stations 1 through 4.

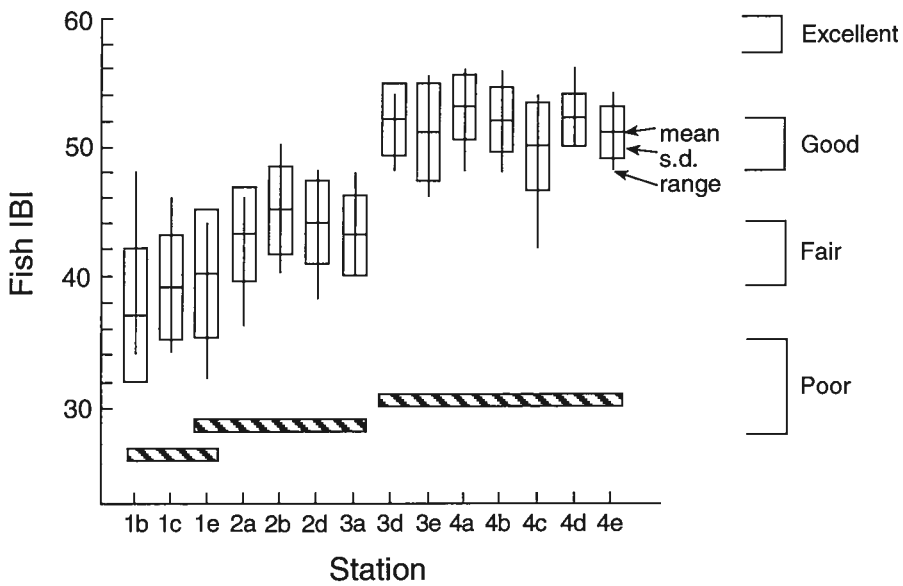
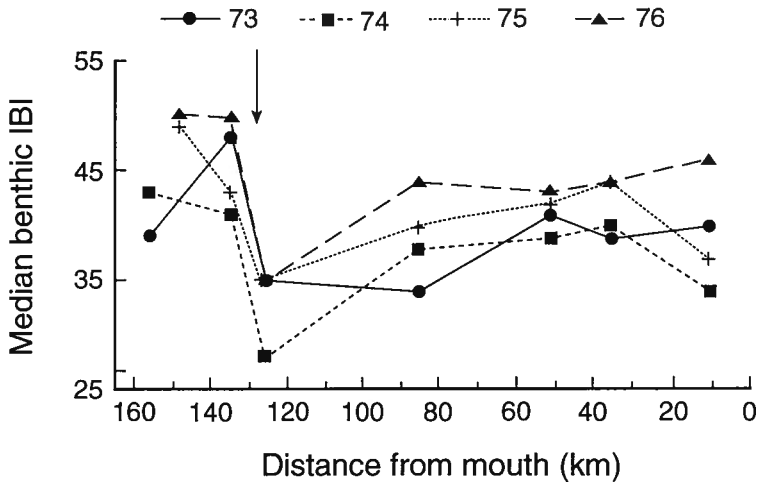


FIGURE 27. Median B-IBI values for the North Fork Holston River in the Tennessee Valley from 1973 to 1976 (from Kerans and Karr 1994). The arrow marks the location of a streamside sludge pond. (Compare Figure 12, page 41.)



Because IBI can detect many influences, both in time and space, it is an ideal tool for evaluating the efficacy of management decisions. Along the Scioto River, Ohio, for example, fish IBI values for data collected in 1979 paralleled degradation resulting from regional habitat deterioration and wastewater effluent. By 1991, improvements in effluent treatment processes had substantially raised IBI (Figure 29); in this case, the benefits of management can be seen as increased IBI. Management actions may also decrease IBI. A local effort to stabilize the channel upstream of a woodlot in Indiana resulted in substantial sediment transport into the woodlot reach of the stream and a sharp decline in IBI (Figure 30). The graphs of IBI values from these places can be quickly interpreted by policymakers and concerned citizens as well as research biologists.

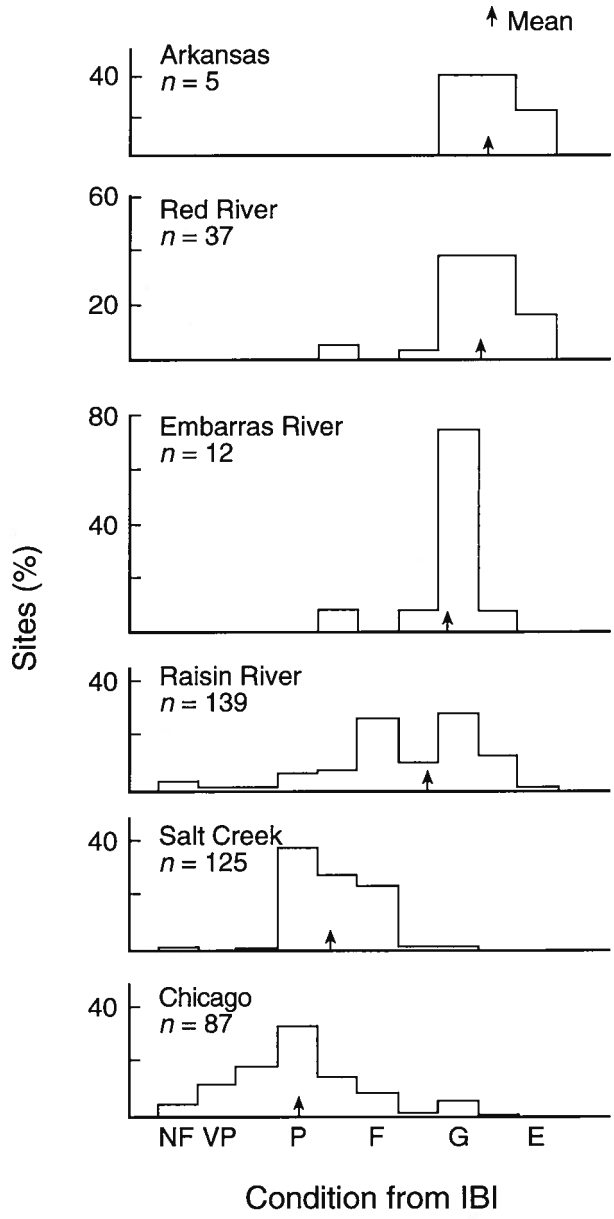
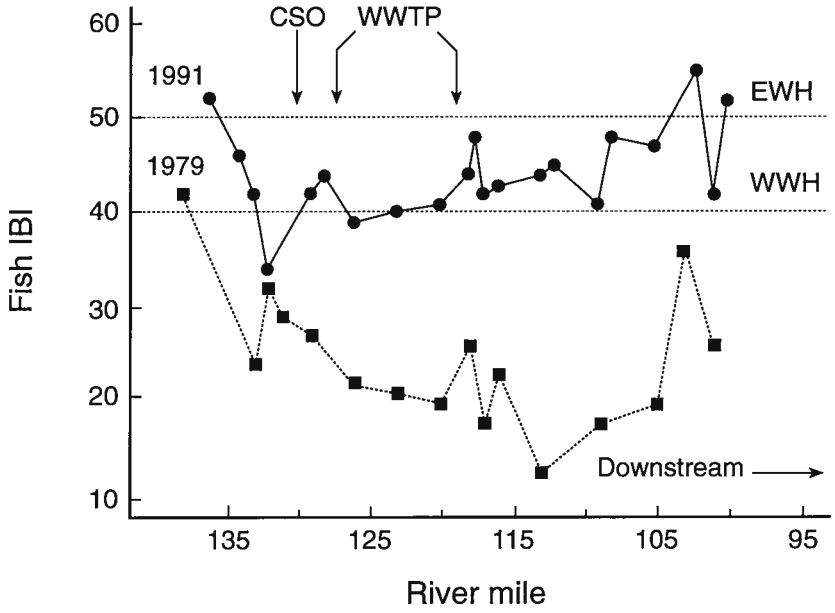


FIGURE 28. Distribution of sites in six midwestern regions or watersheds according to biological condition. The fish IBI was used to distinguish six categories of condition: NF, no fish; VP, very poor; P, poor; F, fair; G, good; and E, excellent. The IBI values varied across the six regions depending on the type and intensity of human land use (from Karr et al. 1986).

FIGURE 29. Fish IBI values along the Scioto River, Ohio (from Karr 1991). The lower IBIs reflect degradation associated with combined-sewer overflow (CSO) and wastewater treatment plants (WWTP). Improvements in effluent treatment, reflected in an overall increase in IBIs from 1979 to 1991, brought most of the sites into compliance for warm-water habitat (WWH); some sites even scored as excellent warm-water habitat (EWH).



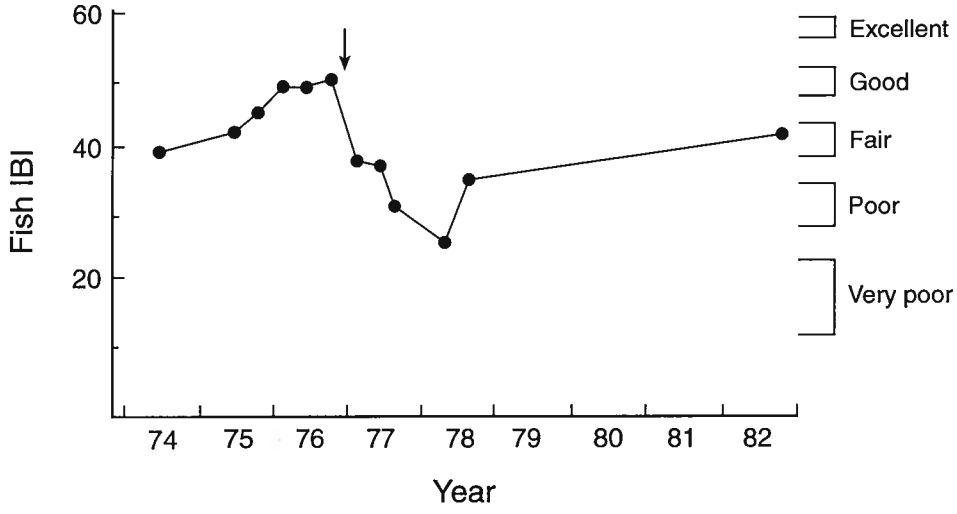


FIGURE 30. Changes in fish IBI values over time in Wertz Drain in Wertz Woods, Allen County, Indiana. During 1974–76, Wertz Drain had relatively high IBI values for a first-order stream in an area of intensive agriculture. The channel was sinuous, pools and riffles were well developed, and there were trees shading the channel. Although this site was not intentionally modified, a poorly executed bank stabilization project upstream during 1976 transported sediment to the site. Consequently, habitat quality deteriorated, as did the resident fish community. IBIs clearly trace the decline and slow improvement in stream condition over time.

How biology and statistics are used is more important than taxon

The taxonomic group most appropriate for assessing environmental condition depends on the region to be assessed; agency resources; special staff expertise; and, most important, how biological knowledge is applied in designing sampling and analysis protocols (Karr 1991). Of the 47 states with bioassessment programs in place, 20 use fish, 44 use benthic invertebrates, and 4 use algae (periphyton or diatoms) (Davis et al. 1996). Twenty-six states use more than one major group, such as fish as well as invertebrates. No one taxon is correct or incorrect in a monitoring program. Like using 10 to 12 IBI metrics, sampling more than one taxon creates some redundancy. But in many circumstances, that redundancy pays off by substantially improving one's ability to diagnose the causes of degradation, causes that may be apparent only if more than one assemblage is sampled (e.g., fish and invertebrates, fish and algae).

In the Pacific Northwest, benthic invertebrates have some advantages over fish as the primary subjects for biological monitoring (Fore et al. 1996). Macroinvertebrate taxa are numerous, ubiquitous, abundant, and relatively easy to sample; their responses to a wide spectrum of human activities are relatively easy to interpret. Moreover, because the life cycles of some benthic invertebrates extend several years, they are excellent integrators of past human influences. But fish also have advantages. Taxa such as sculpins, cyprinids, and suckers are often well represented in numbers of species and individuals in Pacific Northwest streams. Broadly ranging species such as anadromous salmonids offer a tool for monitoring large landscapes and the effects of harvest, hatcheries, and barriers to migration (R. M. Hughes, pers. commun.). Some biologists recommend including more than one vertebrate class (e.g., fish and amphibians) in any IBI based on vertebrates (e.g., Peter Moyle, cited in Miller et al. 1988; Hughes et al., in press).

In many circumstances, the redundancy that comes from sampling more than one assemblage permits better diagnosis of degradation

Convenience, money, time, or place will also affect the choice of taxon to sample. Chosen taxa should be cost effective to collect and identify. Most fish (exceptions include some sculpins, minnows, and darters) can be identified at once in the field. More equipment may be required for fish (e.g., electrofishing gear) than for invertebrates, although both require more-complex equipment in deep-water environments. Permit requirements, too, may be more complicated for sampling fish than benthic invertebrates or algae. Insects and diatoms, on the other hand, are easier to sample in the field but more difficult and time-consuming to identify in the laboratory.

Watershed size and location can affect the consistency of results obtained using different taxa. Fish- and invertebrate-based assessments may disagree, depending on river size or region. In large watersheds (> 500 mi^2), for example, fish and benthic IBIs ranked sites the same only 44% of the time (Yoder and Rankin 1995a). The two kinds of IBIs gave the same results 65% of the time for midsize streams and rivers (50 to 500 mi^2) and 75% of the time for small streams (Yoder and Rankin 1995a). According to R. M. Hughes (pers. commun.), species richnesses of fish and invertebrates rarely agree for Appalachian streams and New England lakes. A high-priority challenge is to determine if these apparent inconsistencies reflect real differences in the sensitivity of the different assemblages or if they result from differences in sampling effectiveness for fish and invertebrates as a function of water body size.

Finally, one has to be careful that taxa chosen for biological monitoring reflect real changes in the local and upstream landscape. The absence of anadromous fishes may not indicate that a site is in poor condition; a natural waterfall may simply be blocking fish passage, or their absence may reflect ocean conditions or overharvest rather than site condition. Migratory birds or fishes inhabiting estuaries or the ocean for part of their life cycles may be affected more by conditions elsewhere than by those in the monitored streams. Indeed, landscape-level factors may well have more effect on local and regional biological integrity than do traditionally monitored alterations in physical or chemical habitat (Richards et al. 1996, 1997; Roth et al., 1996; Allan et al. 1997; Wang et al. 1997; Hughes et al., in press). Species listed as threatened or endangered under the Endangered Species Act reflect landscape conditions well, and including them in an IBI may even improve management of these species by putting them squarely into their larger biological context (Karr 1994).

In short, different taxa have different advantages for different places. As for all aspects of designing a biological monitoring program, researchers need to tease out the patterns of response among taxa from artifacts of defining reference condition or of sampling itself; they need to consider carefully how different taxa might permit a better diagnosis of the causes of degradation in different geographic areas and situations. The most accurate assessments of biological condition may well come from determining biological condition using IBIs based on more than one assemblage.

Sampling protocols are well defined for fishes and invertebrates

The utility of any measure of biological condition in a stream depends on how accurately the original sample represents the fauna present in that stream—that is, how successful it is in avoiding statistical "bias." Indeed, a fundamental assumption of the fish IBI is that the sample on which it is based reflects the taxa richness and relative abundances of the stream's fauna, without bias toward taxa or size (Karr et al. 1986). Implicit in this assumption is that sampling effort is standardized. Any fish sampling protocol must therefore be consistent, comprehensive, and representative of the stream's microhabitats, including pools, riffles, margins, and side channels. Many researchers during the last 15 years have helped to refine the protocols for sampling fish to evaluate or implement an IBI (Ohio EPA 1988; Lyons 1992a,b; Lyons et al. 1995; Lyons et al. 1996). Other protocols for sampling fish and invertebrates have also been described, although their goals and applications vary somewhat from development of an IBI [Klemm et al. 1990, 1993, for USEPA's Environmental Monitoring and Assessment Program (EMAP); Cuffney et al. 1993 and Meador et al. 1993 for US Geological Survey's National Water Quality Assessment (NAWQA)].

One sampling method doesn't fit all, but sampling must be standardized

Early work on the fish IBI identified sampling gear, the range of microhabitats in a stream, and stream size as important factors affecting sampling accuracy (Karr et al. 1986; Ohio EPA 1988). These researchers showed that, with standard procedures, it is feasible to sample virtually all fish from all microhabitats in small- to medium-size streams. Boat-mounted electrofishing gear is the most effective and most efficient in the widest variety of stream types. Early work by Angermeier and Karr (1986) suggested that fully sampling from two entire meanders typically captures the variety of stream microhabitats, yielding enough individual fish to calculate taxa richness and relative abundances for IBI metrics. More recent work in several geographic areas suggests about 40 channel widths as the appropriate length of sampling efforts (Lyons 1992b; Paller 1995a,b; Angermeier and Smoger 1995). In relatively homogeneous systems (e.g., low-gradient streams), longer distances may be needed (Angermeier and Smogor 1995).

Large rivers, lakes, reservoirs, and coastal and estuarine environments contain a diversity of habitats. No single sampling method is appropriate to every one of those habitats, yet using multiple sampling methods is difficult, expensive, and thus impractical. As a result, selective sampling protocols, which measure biological condition based on one or a few local microhabitats, have been developed for

these systems (Thoma 1990; Weaver et al. 1993; Jennings et al. 1995; Deegan et al. 1997; Whittier et al. 1997b; Whittier 1998).

Invertebrates, such as benthic insects, pose different sampling challenges: more species to deal with than among fishes, more microhabitats, more sampling techniques and protocols appropriate for the variety of microhabitats. Therefore, one must either use many different protocols to get a representative invertebrate sample or first test whether sampling from a single microhabitat accurately represents stream condition. In their study of streams in the Tennessee Valley, Kerans et al. (1992) sampled invertebrates from pools (Hess sampler) and riffles (Surber sampler) and evaluated 18 invertebrate attributes as indicators of human influence. They concluded that monitoring designs "that quantitatively sample multiple habitats, are spatially replicated, and use many different attributes for assessment provide a good method for determining biological condition" (Kerans et al. 1992: 388). Although a number of invertebrate attributes behaved similarly for pools and riffles, others (e.g., mayfly taxa richness, caddisfly taxa richness) matched expected stream health rankings better for pools than for riffles. When the researchers combined metrics to create a B-IBI, patterns were stronger for pools than for riffles. Rankings were not always consistent for pool and riffle data (Kerans and Karr 1994), perhaps because these studies were done in relatively large rivers with substantial sedimentation, which might be detected more readily in pool environments (B. L. Kerans, pers. commun.).

Debate still rages over whether single- or multiple-habitat sampling is best with invertebrates. Some contend that a single habitat is adequate; others insist that sampling multiple habitats is essential. Our experience suggests that sampling a single habitat is appropriate and adequate, although our reasons for this conclusion do not always agree with others'. Sampling riffles, for example, is often justified on the grounds that riffles are the most diverse, the most productive, or the dominant habitat (Plafkin et al. 1989; Barbour et al. 1996b; Barbour et al., in press). We are not convinced that these claims are true or even at issue. Still, because we have successfully and cost-effectively used single-habitat samples to discern human effects on small streams (Kerans et al. 1992; Kerans and Karr 1994; Kleindl 1995; Rossano 1995, 1996; Patterson 1996), we recommend a single-habitat sampling protocol that concentrates on riffles.

Because a Surber sampler samples only part of a riffle, a single sample may not be precise enough to judge stream condition. We therefore tested the effects of replicate sampling of invertebrates, using data from the John Day River basin of north-central Oregon (Fore and Karr, unpubl. manuscript). Five replicates were collected, and their contents were identified for each of seven sites (Tait et al. 1994). Using a bootstrap resampling algorithm, Fore and Karr simulated the effects of taking one, three, or five replicates at a site. Fore and Karr changed the number of replicates for each site to test whether metric precision varied as a function of the number of replicates (Figure 31). With only one replicate, a metric could either increase or decrease depending on which of the five replicates was chosen by the bootstrap algorithm. In practice, therefore, the numerical value of a metric calculated using a single Surber sample at a site would depend on where in the riffle that

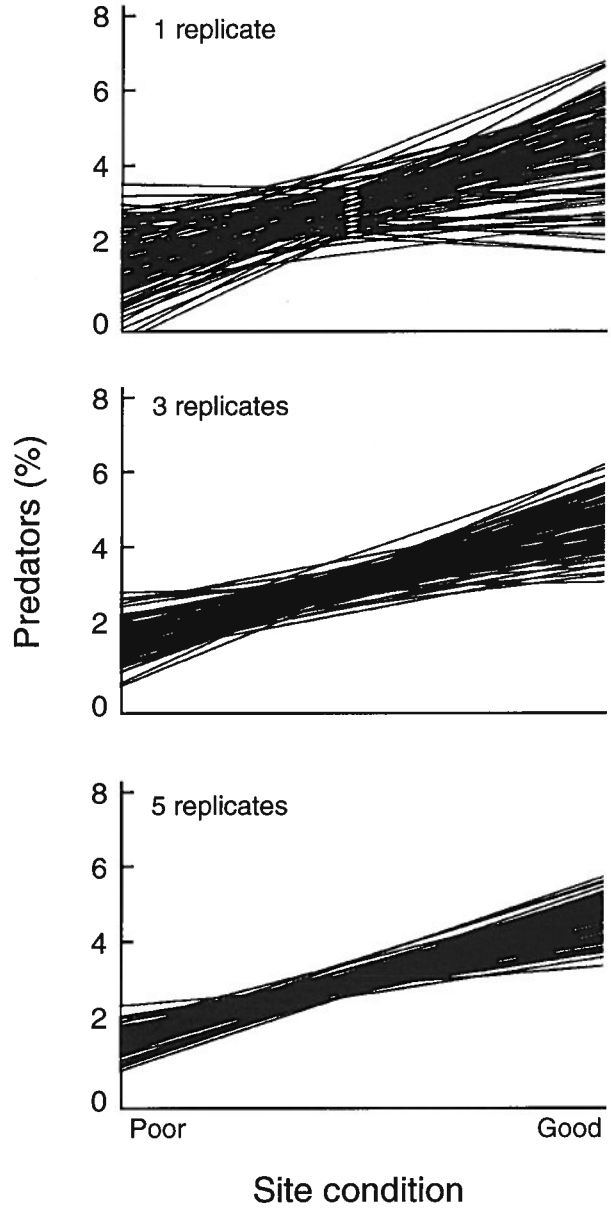


FIGURE 31. Results of bootstrapping analysis (random sampling with replacement) of the relative abundance (percentage) of predators for seven stream sites along a gradient of grazing intensity in the John Day Basin, Oregon. For each site, one, three, or five replicates were randomly selected, and least-fit regression lines (100 in each graph above) were plotted. The lines in the upper graph are based on means for one replicate (out of five possible) per site; in the middle, for three replicates per site; in the bottom graph, for five replicates per site. Precision increases with number of replicates, especially between one and three replicates; in fact, the relationship between site condition and proportion of predators may appear either negative or positive with only one replicate. Note, however, that precision increases relatively little from three to five replicates. The lower two graphs clearly show that the relative abundance of predators increases as resource condition improves.

sample had been taken. When the mean of three replicates is plotted, however, the relationship between metric scores and human influence is more consistent (see Figure 31). Metric precision increases little if five replicates are collected instead of three. Thus we conclude that the increased costs of sample collection and analysis for three replicates over one are justified, but not those for five replicates.

For invertebrates, therefore, we recommend a standard sampling area of approximately 0.1 m^2 (0.3 m-by-0.3 m Surber sampler frame) and three replicate samples for each site. We also recommend collecting from riffles for three reasons: (1) riffles are easier to define and identify by field crews than are pools or margins; (2) riffles are more uniform than other stream microenvironments and thus easier to compare across watersheds; and (3) riffles are shallow, and the current through them is

fast, making sampling with kicknets or Surber samplers easier. We also take all replicates in a single riffle; this strategy characterizes one site more fully than does the alternative of sampling once in each of several riffles, as some protocols propose (e.g., EMAP; R. M. Hughes, pers. commun.).

It is especially important to collect and count a sufficient number of insects to characterize the biota in multiple dimensions. If sampling fails to yield a total of 500 or more organisms (for example, in regions where natural invertebrate densities are low), the number of replicates or the sampled area may need to be increased. We believe that sampling enough organisms is far more important than how sampling is organized (e.g., single or multiple riffles, composite samples, or no composite samples). Subsampling that counts only 100, 200, or even 300 organisms, as recommended by RBP and some other protocols, tends to reduce the utility of many metrics that have become standard in multimetric assessments (Doberstein, Karr, and Conquest, in prep.; see Premise 28, page 101).

It is probably not always necessary to identify insects to species; strong patterns emerge from samples where most insects are identified only to genus (except for chironomids). Identification to genus provides distinct advantages over identification only to family, however—in particular, by strengthening the ability to discriminate among sites of intermediate quality (Figure 32).

Using standard methods for sampling invertebrates (Box 2), we have been able to detect changes in biological condition caused by a whole range of human influences from the Grand Tetons (Patterson 1996) to streams in several areas of Oregon and Washington (Kleindl 1995; Karr, Morley, and Adams, in prep.).

Finally, for both fishes and invertebrates, timing of sampling is important. Karr et al. (1986) recommended periods of low to moderate stream flow for sampling fishes. For benthic invertebrates, recent experience leads us to recommend late summer, before autumn rains begin. We sample stream insects in the Pacific Northwest in September. Water flows are generally stable and safe for field work at that time of year, and invertebrates are abundant. Sampling at this time also minimizes disturbance to the redds, or nests, of anadromous fish. Optimal sampling period will, of course, vary regionally and should be set based on knowledge of the regional biota, precipitation patterns, and other relevant factors.

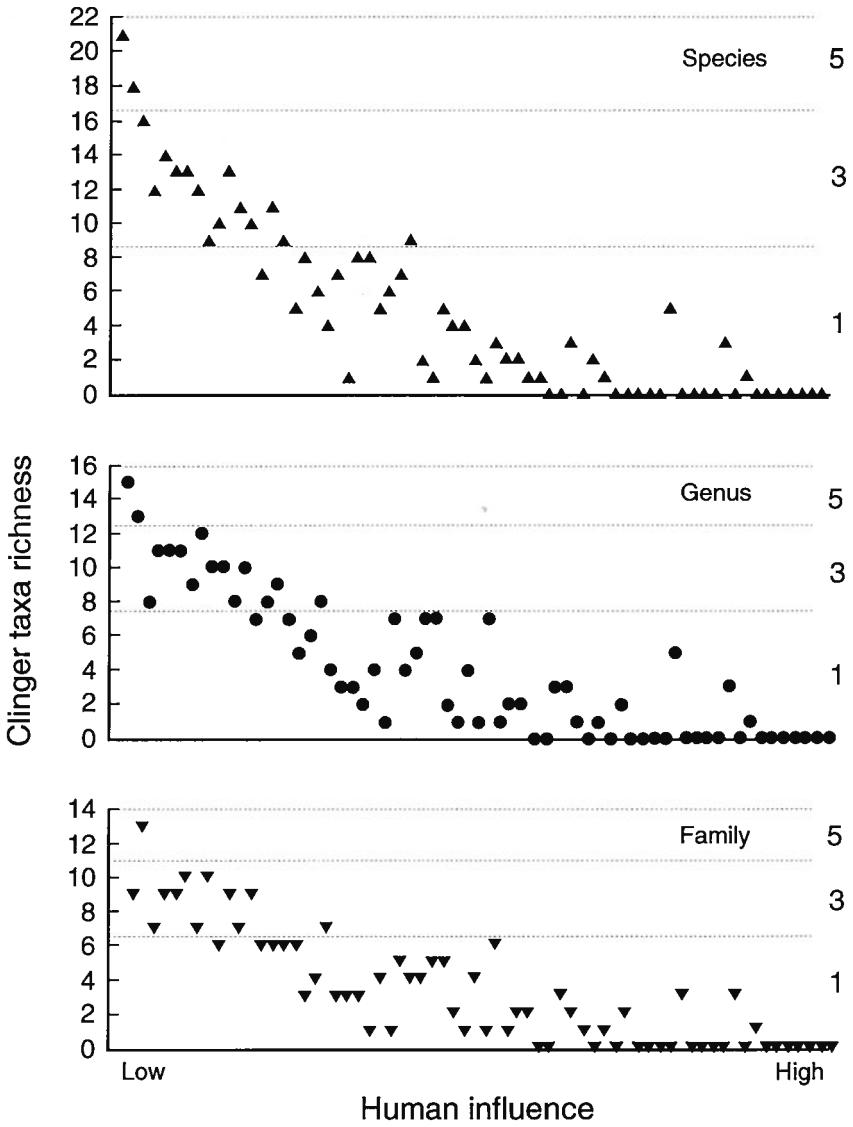


FIGURE 32. Number of clinger taxa present in samples of benthic invertebrates from 65 Japanese streams ranked according to intensity of human influence (see Figure 4, page 31, and Figure 5, page 32). The pattern is consistent across the influence gradient, regardless of the level of taxonomic identification, but the slope becomes smaller from species to genus to family, reducing the metric's usefulness for discriminating among sites at higher taxonomic levels. (Data provided by E. M. Rossano.)

BOX 2. How to sample benthic invertebrates.

Equipment

Modified 500-micron Surber sampler with cod end (receptacle)
2.5-gallon bucket (dishpan works well too)
Squirt or spray bottle
Forceps
Marking tape
500-micron soil sieve
Sample jars (8-oz or 4-oz; 4-oz urine specimen bottles are an inexpensive alternative)
Plastic sandwich bags (Ziploc) for loose lids
Pure ethanol; diluted by sample to about 70%
Permanent markers (Sharpies)
Pencils
2 white, deep-sided sorting pans for large debris
Small rake, trowel, or other implement (e.g., piece of rebar or old screwdriver) with marking tape at 10 cm
50-m measuring tape
Flagging
Stopwatch
Camera to photograph site and surrounding environment
Kitchen spatula for transferring material from sieve to sample jar
Pocket knife (always handy)
Spares of selected items above

Selecting a Sample Reach

The choice of a stream reach to sample should be guided by a study's specific aims and by watershed characteristics. But sampling for biological monitoring must never lose sight of the ultimate goal: to detect and measure human influence in watersheds. Factors to consider include stream size, stream gradient, range of microhabitats in the reach, and length of sample reach.

Selecting a Sample Site

The distribution of invertebrates in small streams is patchy, driven by associations among the animals and stream microhabitats (e.g., riffles, pools, and raceways, or erosional and depositional areas). For that reason, our standard protocol calls for collecting three replicate samples as follows:

1. Sample in the "best" natural riffle segment within a study reach, even if doing so does not give an exact match of substrates for all study streams. Sediment types may vary among streams, especially in association with different human activities within watersheds. Ideal sampling substrates consist of rocks 5 to 10 cm in diameter sitting on top of pebbles. Avoid substrates dominated by rocks larger than 50 cm in diameter.
2. Sample within the stream's main flow.
3. Sample at water depths of 10 to 40 cm.
4. Collect three replicate samples in a single riffle; depth, flow, and substrate type should be similar for the three replicates.
5. Begin sampling at the downstream end of the riffle and proceed upstream to collect the three replicates; avoid the transition zone from the riffle to a downstream pool or other habitat.

Sampling the Site

Sampling teams may consist of two to four people. Collecting the macroinvertebrates requires two people; others can assist with equipment, labeling, taking notes, and other tasks. Sample as follows:

1. Place the Surber sampler on the streambed with the opening of the nylon net facing upstream. Brace the brass frame and hold it firmly on the substrate, especially on the side attached to the net to prevent invertebrates from slipping under the net.
2. While one person holds the brass frame under water, the other person should lift any large rocks within the frame and wash into the stream any organisms crawling or loosely attached to the rocks, so the organisms drift into the nylon net. Put the washed rocks into a bucket for further picking on shore.
3. When large rocks have been removed, cleaned, and placed in the bucket, thoroughly stir the remaining substrate with the rake or trowel. Stir to a depth of 10 cm for a short period (about one minute) to loosen organisms in the interstitial spaces and to wash them into the net. If you find more large rocks with organisms on them, wash the organisms into the net and put the rocks into the bucket.
4. Now slowly lift the brass frame off the substrate, tilting the net up and out of the water. Use the action of the water to wash trapped or clinging organisms into the Surber sampler's cod end.
5. Carry the net and the bucket to shore for picking or for transferring to alcohol to sort, count, and identify in the lab. The Surber sampler's removable receptacle makes the transfer relatively simple. Use the squirt bottle to wash down the sides of the net before removing the cod end. Using the magnifying glass and forceps, collect and preserve *every* organism from the Surber sampler as well as from the rocks and water in the bucket. After removing the cod end, wash its contents through the soil sieve, picking out large rocks, detritus, and other debris for hand sorting. Transfer any organic matter remaining on the sieve to sample jars, taking care not to damage invertebrates. A plastic kitchen spatula and squirt bottle work well to dislodge clingers from the sides of the net or the sieve.
6. Put a pencil-on-paper label into each sample jar and label the outside with permanent ink; include the date, sample location (name and number), and replicate number.
7. Rinse the net *thoroughly* after each sample to avoid cross-contamination.

When to Sample

Species composition and population sizes of macroinvertebrates vary substantially through a river's seasonal cycles. Because the goal is to assess the influence of human actions, not natural variation through time, collect samples during a short period. For Pacific Northwest streams, late summer or early autumn is best. This timing gives representative samples of stream invertebrates and simultaneously:

1. Avoids endangering field crews (as in seasons of high water).
2. Standardizes seasonal context.
3. Maximizes efficiency of the sampling method because flows are neither too high nor too low.
4. Avoids periods when flows are likely to be too variable.

In the Pacific Northwest, we sample in September, before the autumn rains begin. Shifting the sample period a bit earlier into August or extending it into October is acceptable. But all samples should be collected within a period of not more than four weeks.

PREMISE 19

THE PRECISION OF SAMPLING PROTOCOLS CAN BE ESTIMATED BY EVALUATING THE COMPONENTS OF VARIANCE

Calculating components of variance is a simple and useful technique for estimating the relative contribution of measurement error and site differences to the overall variance of a metric or index. In general, our goal is to select metrics that have small measurement error relative to the differences we want to measure: changes related to human activities.

For example, we used zooplankton data from northeastern lakes studied under EPA's EMAP to estimate the relative contribution of three sources of variability to the overall variance observed for each of three metrics: taxa richness, relative abundance, and density (Hughes et al. 1993; Stemberger and Lazorchak 1994; Stemberger et al. 1996). In that study, one to three zooplankton samples were collected from each of seven lakes. The data were then subsampled in the laboratory and the organisms taxonomically identified. In our analysis of those data, we identified three sources of variability and, thus, three components of variance: variability caused by differences among lakes (lake effects), variability caused by differences in sample location within the lake (crew error), and variability caused by different subsamples identified in the lab (lab error). These three sources of variance for metric scores can be summarized in an ANOVA model as:

Statistical analysis of metric and index variance is useful for fine-tuning protocols

$$\text{Metric score} = \text{Lake}_i + \text{Crew error}_{j(i)} + \text{Lab error}_{k(ij)}$$

where Lake_i = the effect of the ith lake on metric score; $\text{Crew error}_{j(i)}$ = the variability caused by crew differences, sampling time, or location within the ith lake; and $\text{Lab error}_{k(ij)}$ = the variability that arises from the laboratory subsampling protocol used in the initial study.

In statistical language, this model is a two-level nested ANOVA that is unbalanced because the number of replicates varies at each level. Using the sums of squares from the computer output and a little algebra (Sokal and Rohlf 1981: Chapter 10), one can estimate the variance of each term in the model.

For this analysis, we assumed that lakes differed in human influence and thus biological condition. We were interested in how the lakes differed from one another. We were not interested in evaluating differences within lakes or within subsamples; therefore, these two sources of variability were considered sources of error. A variable is typically labeled an "effect" when one wants to measure or compare values for that variable; if, on the other hand, one does not care whether

crew A collects more animals than crew B ("crew effects"), for example, then one seeks to avoid that source of variability altogether, and so it is labeled "error."

Based on our analysis of the components of variance in the zooplankton samples (Figure 33), we concluded that the sampling protocol was adequate to detect lake differences when taxa richness or relative abundance were calculated. We also discovered that lab variability was relatively small and that using lab time to identify replicate samples is not necessary. In contrast, metrics varied relatively more depending on where crews collected samples within the lake. Consequently, we recommend that future studies like this one should put more effort into sampling from the lakes while reducing the number of lab subsamples.

We arrived at another important conclusion by comparing taxa richness, relative abundance, and density. The error components of variance for density were much larger than the lake component; for density, any signal at the lake level was lost in the noise of variability. In contrast, for taxa richness or relative abundance, most of the variability occurred among lakes rather than among replicate samples and subsamples (see Figure 33). If the goal is to distinguish among lakes, then one should select metrics that minimize variability caused by within-lake and within-lab differences and maximize variability resulting from human influence. Taxa richness and relative abundance are metrics that do so.

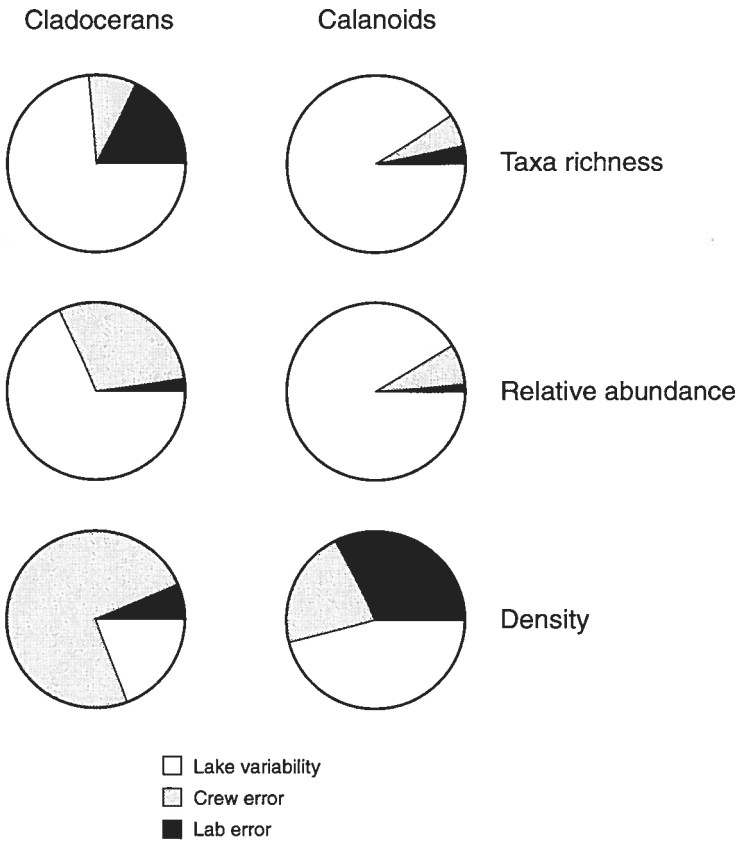


FIGURE 33. Sources of variance for two groups of herbivorous zooplankton (cladocera, such as Daphnia, and calanoid copepods), calculated for northeastern lakes (using data collected by R. S. Stemberger under EPA's Environmental Monitoring and Assessment Program). Taxa richness, relative abundance of individuals, and density were calculated for each group. The lab protocol used to subsample ("lab error") and replicate samples taken from each lake ("crew error") constituted two sources of error; differences from lake to lake ("lake variability") were the effect of interest. Number of lakes, 7; number of crew replicates, 1–3; number of lab replicates, 1–3. Components of variance were estimated with ANOVA.

We analyzed components of variance in two other locations, the Puget Sound lowlands and Grand Teton National Park, Wyoming, to compare the sources of variability with total variance in benthic IBIs for homogeneous sets of streams (Figure 34). Rather than looking at individual metrics, these studies focused on the indexes themselves, *after* individual metrics had been tested and integrated. For samples within riffles in Puget Sound lowland streams, approximately 9% of the total variance in index value arose from differences within streams (Figure 34, top). (For this study, human influence was measured as a continuous variable, the percentage of impervious area; see Figure 6, page 33.)

The Grand Teton study did not measure human influence in each watershed. Instead, all sampled streams were assigned to one of four categories of human influence, and variation was apportioned according to its source: among members of a group or among groups. B-IBI differences among members of the groups contributed 11% to the overall variance in B-IBI. Eighty-nine percent of the variance came from differences among the groups that reflected discrete human influence classes: little or no human activity; light recreational use; heavy recreational use; and urbanization, grazing, agriculture, or wastewater discharge (see Figure 7, page 33). In the Puget Sound and Grand Teton studies, the sources of error were low relative to variability resulting from different types of human land use.

Statistical analysis of metric and index variance is thus useful for tuning sampling protocols; it is important in defining where to put one's efforts and in determining the usefulness of an index to detect human effects. But it cannot replace the more important aspects of testing and analysis that link metric and index values to human influence. The most desirable statistical properties are no substitute for a biologically meaningful response to human disturbance.

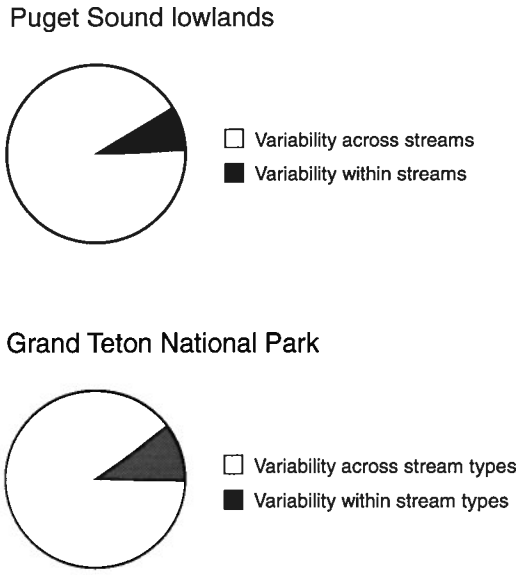


FIGURE 34. Components of variance for the B-IBIs for sites ($n = 30$) in the Puget Sound lowlands and ($n = 16$) Grand Teton National Park, Wyoming. In Puget Sound, variability associated with stream differences was large relative to variability associated with microhabitat (within-riffle) differences. In Wyoming, variability associated with different categories of streams (grouped according to land use) was much higher than variability associated with streams within each group. Components of variance were estimated with ANOVA.

MULTIMETRIC INDEXES ARE BIOLOGICALLY MEANINGFUL

A multimetric IBI for a site is a single numeric value, but one that *includes* the numeric values of individual indicators of biological condition. The actual measured values of the component metrics—each explicitly selected because it represents a specific biological element or process that changes reliably as human influence increases—are not lost when an IBI is calculated. An IBI itself, along with patterns in the component metrics, focuses attention on biologically meaningful signals. Each numeric metric value and the IBI as well can be translated into words for a variety of audiences, including nonscientists, enabling them to understand immediately how the biology at high-scoring sites differs from that at medium- or low-scoring sites.

A site labeled "excellent" on the basis of a fish IBI, for example, is comparable to the best streams without human influence (Karr 1981). A full complement of species expected for the habitat and stream size is present, including the most sensitive or intolerant forms. (Note especially that not all regionally distributed species will be found in any single sampling site; even the best sites contain only a fraction of regional species.) In addition, long-lived taxa are present in the full range of age and size classes; the distribution of individuals and taxa indicates a healthy food web with a balanced trophic structure or organization. In contrast, a fair-quality site has very few sensitive or intolerant forms and a skewed trophic structure (e.g., larger numbers of omnivores and relatively few top predators, especially in older age classes). At a very poor site, few fishes are present, except for introduced or tolerant forms, and more than a few individual fish are likely to show deformities, lesions, and tumors. Similar descriptions can convey the details of biological condition for benthic invertebrate assemblages. In contrast, the ecological context of many chemical criteria, bioassays, and biomarkers is often unclear.

Each metric and IBI value translates into a verbal and visual portrait of biological condition

The combination of numeric and narrative descriptions that come from a multimetric IBI makes communication possible with virtually all academic disciplines, stakeholders, and communities. The opportunity for education is thus part and parcel of a multimetric approach.

PREMISE 21

MULTIMETRIC PROTOCOLS CAN WORK IN ENVIRONMENTS OTHER THAN STREAMS

The principles for developing sampling protocols and analytical procedures for monitoring streams are broadly applicable to other environments. Progress has been made in assessing estuaries (Deegan et al. 1993; Engle et al. 1994; Weaver and Deegan 1996; Deegan et al. 1997), lakes (Stemberger et al. 1996; Pinel-Alloul et al. 1996), wetlands (Adamus 1996; Karr 1997), riparian areas (Brooks and Hughes 1988; Croonquist and Brooks 1991), and reservoirs (Jennings et al. 1995).

Applying multimetric concepts to terrestrial environments has so far been limited. Most of the relevant studies examined individual biological attributes rather than a set of metrics. Species richness, for instance, declined with declining size of forest fragments (Williamson 1981). In midwestern agricultural landscapes, the relative abundance of omnivorous birds increased as the size of forest fragments fell; other feeding groups did not change systematically with fragment size (Figure 35; Karr 1987).

In a mist-net study of tropical forest birds, Karr (1987) detected disturbance-associated shifts in species composition, capture rates, and trophic organization within the undergrowth assemblage. Species richness in standard samples declined by 26%, and capture rates doubled, in a disturbed forest relative to an undisturbed forest; in this case, the disturbance was a recent history of intensive research within the forest. Although the number of species changed little in the major foraging guilds, spiderhunters, which feed on insects and nectar, increased sharply with a change in undergrowth plants in the disturbed area.

The first full-scale terrestrial IBI is now under development at the Hanford Nuclear Reservation

In 1996, Karr et al. (1997) began developing the first full-scale IBI for a terrestrial locale, the Hanford Nuclear Reservation in eastern Washington State. Under the jurisdiction of the US Department of Energy since 1943 for weapons production, the 560-mi^2 reservation was closed to public access and development for more than half a century. As a result, Hanford is a paradox. On the one hand, it poses an enormous toxic-cleanup challenge to the Department of Energy, whose Office of Environmental Management has been at it since 1989; on the other, the reservation and its surroundings comprise some of the state's largest continguous patches of native shrub-steppe vegetation and the last spawning run of chinook salmon in the mainstem Columbia River. The vegetation before European settlement consisted of shrubs (*Artemisia* spp., *Chrysothamnus* spp., and *Purshia tridentata*) and

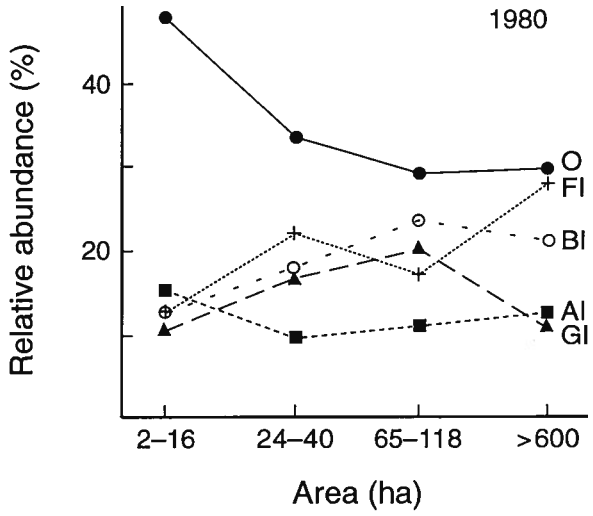


FIGURE 35. Percentage of individuals in several trophic groups among birds of forest islands in east-central Illinois: O, omnivores; FI, foliage insectivores; BI bark insectivores; AI, aerial insectivores; and GI, ground insectivores. The relative abundance of omnivores increases as size of the forest fragment decreases; relative abundances of the other groups do not change as systematically.

perennial bunchgrasses (*Agropyron spicatum, Festuca idahoensis, Stipa* spp., and *Poa* spp.). The number of alien annual plants increased with increasing human activity (Daubenmire 1970; Rickard and Sauer 1982), persisting even long after the activity ceased. The abundance of insect taxa shifted after wildfires (Rogers et al. 1998).

The Hanford area is ideal for testing potential metrics for an IBI because it presents a full array of kinds and degrees of human impact. Initial field work established 13 study sites across this gradient, including agricultural lands and lands altered by heavy equipment, fire, and grazing (Figure 36). A site was also chosen from the neighboring Arid Lands Ecology Reserve (ALE), which has been minimally disturbed. Plants and insects were the two organismal groups chosen for metric testing and IBI development.

After one spring field season, the researchers have now begun establishing which plant and insect attributes will give consistent ecological dose-response curves across the gradient of disturbances at Hanford. Measured plant attributes include species present; number of individuals; and percentage of cover for grasses, forbs, shrubs, and the cryptogamic crust. Insects were collected from pitfall traps, sweep nets, butterfly transects, and individual shrubs; galls on the shrubs were also counted.

Altogether 58 plant species, representing 20 families, have been found from the 13 sites; 72% of these are native and 16% are introduced aliens. The distribution of particular species (e.g., the alien cheatgrass *Bromus tectorum* and native grasses) and the proportion of native vs. alien species varies across the sites. The proportion of alien species per site ranges from 28% to 92%; it is highest at the most disturbed sites. The percentage of alien species and the percentages of native grass and shrub taxa may offer potential plant metrics (Figure 37).

On the basis of insects from 4 of the 13 sites, taxa richness appears to be higher at the minimally disturbed ALE site (49 insect families) than at the old town of

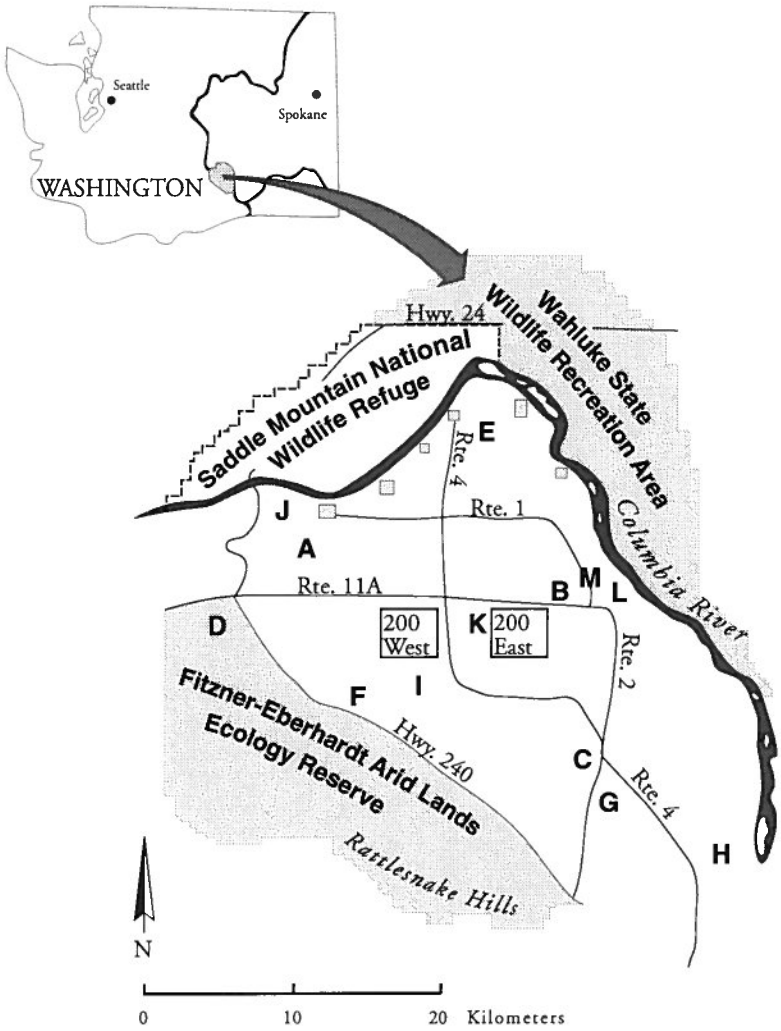
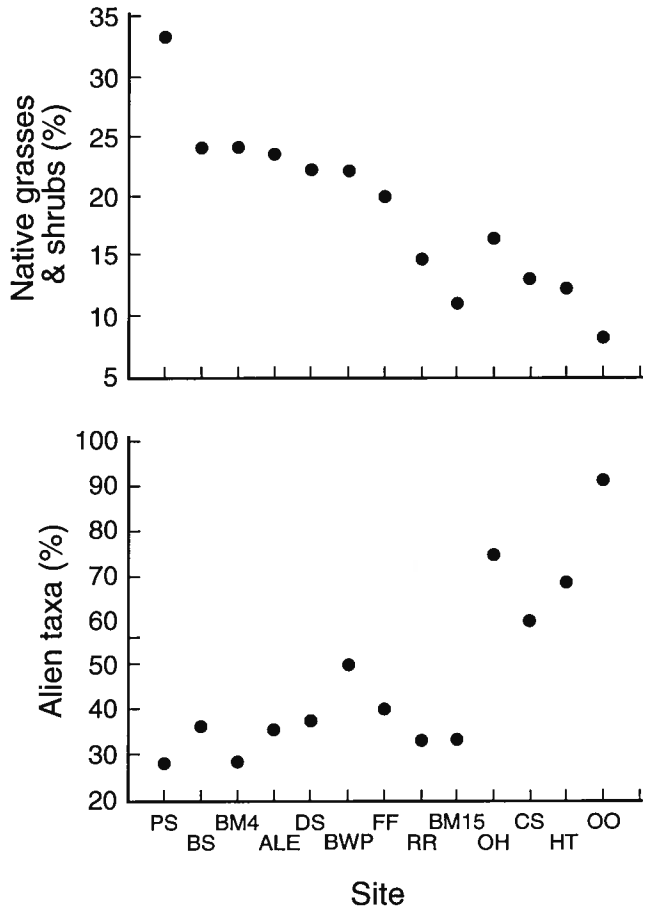


FIGURE 36. The Hanford Nuclear Reservation, including central Hanford, the Arid Lands Ecology Reserve (ALE), Wahluke State Wildlife Recreation Area, and Saddle Mountain National Wildlife Refuge. Letters indicate location of study plots. Sites C, G, and H have been affected by fire; site D by an early history of grazing; sites J and M by agriculture; and sites F, K, and L by physical disturbances. Sites A, B, and D show only minimal disturbance (reference sties). Sites E and I have unknown disturbance histories.

Hanford (29 families), a burn site (23 families), or an abandoned agricultural field (23 families) (Figure 38). Relative abundances also vary across these sites. A common agricultural pest (cutworm, a noctuid moth) made up 89% of the Lepidoptera at an abandoned agricultural site, but no species dominated among the butterflies and moths at the other sites. Beetles, especially one species (*Eusattus muricatus*, family Tenebrionidae), dominate at the burn site but not at the others. Other promising attributes include the number of predators and parasitoids; food web effects that may show up as shifts in species composition from site to site; and the numbers, taxa richness, and taxa composition of bees, wasps, and ants (Hymenoptera). The Hymenoptera are particularly interesting because they occupy a wide range of trophic levels. At the old town site, an area dominated by the alien yellow star thistle *(Centaurea solstitialis),* hymenopterans had the highest relative abundance (38%) of the insects collected there. Perhaps there is a link between hymenopteran pollinators and the introduced weed, an interaction that may offer a useful metric.

FIGURE 37. Preliminary ecological dose-response curves for two potential metrics for plants at 13 Hanford sites: top, relative abundance of native shrubs and grasses (percentage of total), and bottom, relative abundance of alien species.



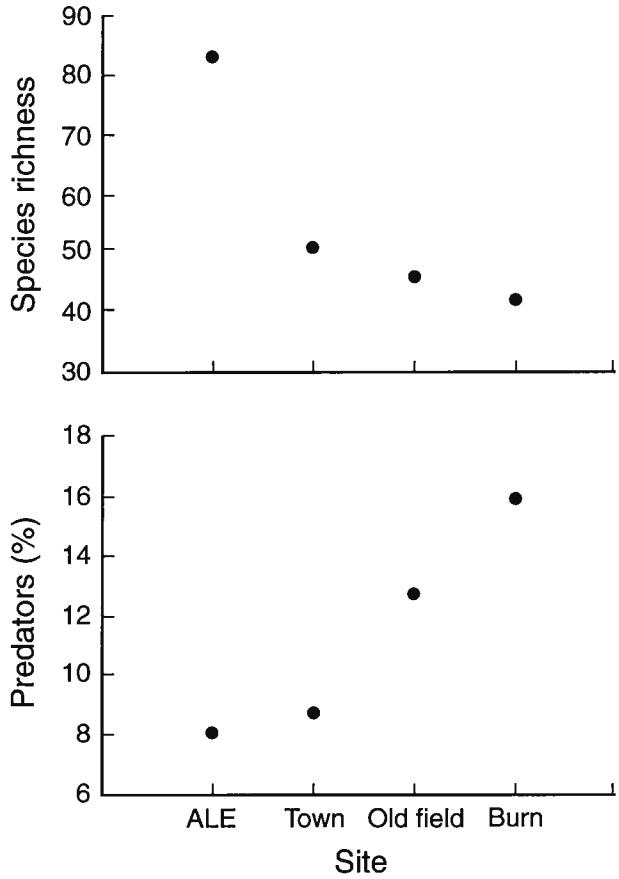


FIGURE 38. Preliminary ecological dose-response curves for two potential metrics for insects at four Hanford sites: top, species richness, and bottom, relative abundance of predators (%).

SECTION IV

For a Robust Multimetric Index, Avoid Common Pitfalls

Although properly constructed multimetric indexes are robust measurement tools, various pitfalls can derail their development and use. The failure of a monitoring protocol to assess environmental condition accurately or to protect running waters—or any other environment—usually stems from flaws in sampling or analysis. Multimetric indexes provide an important tool for measuring the condition of ecological systems. They can be combined with other tools in ways that enhance or hinder their effectiveness, and, like any tool, they can be misused. That multimetric indexes can be, and are, misused does not mean that the multimetric approach itself is useless. Like any scientific procedure, multimetric procedures must be tailored appropriately to a particular situation.

For streams, for example, it is unrealistic to expect a single "off-the-shelf" multimetric index to be appropriate everywhere. Regional variations that adhere to some basic biological, sampling, and statistical principles maintain the strengths of a multimetric assessment while reflecting the reality of regional variation in biological condition (Miller et al. 1988). The goal is not to measure every biological attribute; indeed, doing so is impossible. Rather, the goal is, first, to identify those biological attributes that respond reliably to human activities, are minimally affected by natural variability, and are cost effective to measure; and, second, to combine them into a regionally appropriate index.

PREMISE 22

Properly classifying sites is key

Successful biological monitoring depends on judicious classification of sites. Yet excessive emphasis on classification, or inappropriate classification, can impede development of cost-effective and sensible monitoring programs. Using too few classes fails to recognize important distinctions among places; using too many unnecessarily complicates development of biocriteria. Inappropriate levels of classification also lead to problems. The challenge is to create a system with only as many classes as are needed to represent the range of relevant biological variation in a region and the level appropriate for detecting and defining the biological effects of human activity in that place.

Like a taxonomy of places, classification attempts to distinguish and group distinct environments, communities, or ecosystem types; the proper approach to classification may vary, however, according to specific goals. Biological (community) classification generally lags far behind classification by physical environment or habitat type for aquatic systems (Angermeier and Schlosser 1995). The characteristics that make streams similar or different biologically—and thus make classification important for biological monitoring—are determined first by the geophysical setting (including climate, elevation, and stream size), and second by the natural biogeographic processes operating in a place (see Premise 5, page 21, and Figure 3, page 23). Together they are responsible for local and regional biotas. Coastal rainforest headwaters on the Olympic Peninsula, for example, are likely to be biologically comparable, as would be headwater streams in central Illinois.

Characterizing ecoregions should not get in the way of testing and using metrics diagnostic of human impact

But even though geophysical context is a fundamental determinant of variation in biological systems, classification based on the geomorphologists' view of stream channel types, or on other landforms occupied by biological systems, is not necessarily the proper level for assessing the biological condition of those systems. In the Pacific Northwest, geomorphologists identify some 50 to 60 channel types based on the interplay of physical and chemical processes that shape stream channels (MacDonald et al. 1991). But recognizing these channel types does not necessarily mean that an equal number of biological classes is needed for biological monitoring. The native biota may not be unique to each of those channel types in terms of species composition, taxa richness, or other important aspects of ecological organization; even if some species replacement occurs, metric norms may not change. Fewer biological categories may therefore work just as well.

Many agency programs rely on geographically delineated ecological regions reflecting prevailing geophysical and climatic regimes (Omernik 1995; Omernik and

Bailey 1997). Such ecoregion divisions are valuable, but they are not the be-all and end-all of classification schemes. Indeed, classification at the ecoregion level alone is unlikely to give appropriate weight to every factor important to creating homogeneous sets for comparing the biological condition of streams. Other factors, including topography, geological substrate, and stream size or gradient may be more significant biologically. In addition to ecoregion, a good classification scheme should consider the defining characteristics of local and regional physical and biological systems. It would make little biological sense, for example, to group large, meandering stream reaches with small, fast-flowing streams even if they are in the same lowland ecoregion; the habitats these stream reaches provide, and therefore the biota that live there, are very different. Likewise, the biological attributes signaling the effects of human activities in two high-elevation first-order streams may not differ just because they are in different ecoregions. In short, ecoregions (or equivalent units) are a necessary but not sufficient basis for a stream classification used in biological monitoring.

Furthermore, no matter how much it enhances our knowledge of natural landscape variation, characterizing ecoregions should not get in the way of testing and using metrics diagnostic of human impact. The point of classification is to group places where the biology is similar in the absence of human disturbance and where the responses are similar after human disturbance. In some cases, these groupings may coincide with ecoregion boundaries; in others, they may cross those boundaries. To evaluate sites over time and place, we need groupings that will give reliable metrics and accurate criteria for scoring metrics to represent biological condition (see Premise 14, page 56).

On the east and west sides of the Cascades, and elsewhere in the Northwest, for example, many of the same metrics respond to the effects of grazing, logging, and urbanization, even though climate, vegetation, terrain, and human land use differ (Table 10). The expected values of these metrics differ—taxa richness, for example, is lower east of the Cascades—which may result from "natural" differences or differences stemming from more widespread human influence on a more fragile eastside landscape. Nevertheless, in both westside and eastside ecoregions, the same metrics respond across a range of human influence, and IBIs composed of these metrics reflect and distinguish among the effects at different sites. Elsewhere, such as across eastern deciduous forests and midwestern prairies, maximum species richness also transcends ecoregion boundaries (Figure 39). Expected species richness seems to be higher for forested landscapes than for prairie or grassland landscapes. Other metrics, such as trophic structure, however, are reliable indicators of human influence across ecoregions for some places and taxa (e.g., North American fishes) but not for others (e.g., benthic invertebrates) (see Premise 12, page 47).

Thus, classification based on ecological dogma, on strictly chemical or physical criteria, or even on the logical biogeographical factors used to define ecoregions is not necessarily sufficient for biological monitoring. The good biologist uses the best natural history, biogeographic, and analytical resources available to choose a classification system.

TABLE 10. Similar metrics emerge as reliable indicators of human influence across the Pacific Northwest, regardless of ecoregion. Percent sign (%) denotes relative abundance of individuals belonging to the listed taxon or group. Metrics marked with a check are those that responded across a range of intensity for grazing (eastern Oregon and Wyoming) or logging (western Oregon and Idaho).

Metric	Predicted response	Eastern Oregon	SW Oregon	Central Idaho	NW Wyoming
Taxa richness and composition					
Total number of taxa	Decrease	√	√		√
Ephemeroptera taxa	Decrease		√	√	√
Plecoptera taxa	Decrease	√	√	√	√
Trichoptera taxa	Decrease	√	√		
Tolerants and intolerants					
Intolerant taxa	Decrease	√	√	√	
Sediment-intolerant taxa	Decrease	√	√		√
% tolerant	Increase		√	√	
% sediment-tolerant	increase	√	√		
Feeding and other habits					
% predators	Decrease	√		√	√
% scrapers	Variable	√		√	√
% gatherers	Variable	√		√	
Population attributes					
Dominance*	Increase		√		

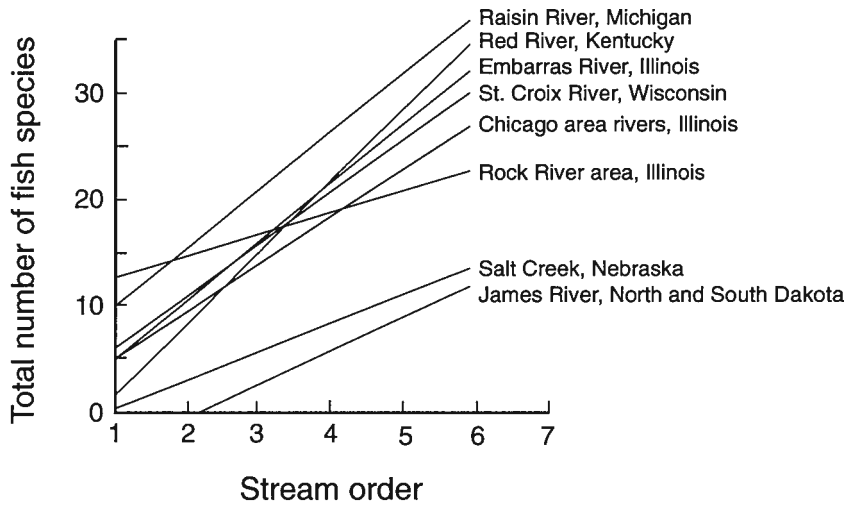


FIGURE 39. Lines of maximum species richness for stream order, based on historical data from midwestern streams. Although the lines differ for the eight watersheds, they fall into two general groups: woodland watersheds in several ecoregions in the eastern Midwest (upper group) and two Great Plains streams in two different ecoregions. (Modified after Fausch et al. 1984.)

Avoid focusing primarily on species

Many water quality specialists begin their analyses of stream data with a matrix of species and abundances. Using species-level community comparisons such as percentage similarity indexes, Pinkham and Pearson's B, the Bray-Curtis index, or multivariate statistics, they then evaluate species overlap among sites and classify the sites based on these evaluations. Unfortunately, the mathematical and ecological properties of these measures (Wolda 1981; Washington 1984; Reynoldson and Metcalfe-Smith 1992) make these procedures problematic. Moreover, regional classifications based on species overlap limit one's view by focusing on species composition rather than higher-level taxonomic and ecological structure.

Consider two undisturbed streams in adjacent Appalachian watersheds (Figure 40). A standard sample from a first-order stream in one watershed contains eight fish species: darters A, B, and C; sunfish D and E; and minnows F, G, and H. The other site contains seven species: darters M, N, and O; sunfish P and Q; and minnows R and S. Comparing the samples using measures of species overlap (0%) would highlight the completely different species composition at the two sites, even though the higher-level taxonomic or ecological overlap (near 100%) is obvious at the family level and in feeding ecology. Both sites support three darters, two sunfish, and either two or three minnows.

Simple species composition is not as good a guide as ecological structure for classifying sites

Consider now what happens after a disturbance at each site: the species composition of both streams shifts as another regional darter, J (a tolerant species), moves in, and two of the original darter species disappear from each stream because they cannot tolerate the changes caused by the disturbance. Similar changes occur in the other taxa (see Figure 40). Now the species overlap index for the two sites is more similar (33%), and both are less similar to their original assemblages (27% and 30%). Assemblages with very different species composition respond in much the same way, becoming more similar in the presence of similar human activity. These responses result from their nearly identical ecological structure, not from similarities in species composition. It is this ecological structure that gives the clearest signals of human disturbance.

In this example, species-level classification suggests that the two areas are very different, even though their higher-level taxonomic and ecological organization are nearly identical. The point is that ecological organization and regional natural history are better guides for site classification than a focus on species composition.

FIGURE 40. Species composition for two hypothetical fish assemblages before and after a human disturbance that changes the biological condition of the sites. The turnover in species is not sufficient reason to conclude that these sites should be classified differently, for their ecologial organization before and after disturbance are the same.

Site 1	Site 2
Before disturbance	
Darter A	Darter M
Darter B	Darter N
Darter C	Darter O
Sunfish D	Sunfish P
Sunfish E	Sunfish P
Minnow F	Minnow R
Minnow G	Minnow S
Minnow H	
After disturbance	
Darter A	Darter M
Darter J	Darter J
Sunfish D	Sunfish D
Sunfish L	Sunfish P
Minnow F	Minnow R
Minnow K	Minnow K

MEASURING THE WRONG THINGS SIDETRACKS BIOLOGICAL MONITORING

A bewildering variety of biological attributes can be measured, but only a few provide useful signals about the impact of human activities on local and regional biological systems. Some attributes vary little or not at all (e.g., the number of scales on the lateral line of a particular fish species); others vary substantially (e.g., weight, which can vary with age and reproductive or environmental conditions). Variation may be natural or human induced, and natural variation may come from temporal (diurnal, seasonal, annual) or spatial sources (stream size, channel type), or both. Biological monitoring must separate human effects from natural variation by discovering, testing, and using those biological attributes that can be measured with precision to provide reliable information about biological condition.

Some attributes are poor candidates for monitoring metrics because of their underlying biology. In particular, abundance, density, and production vary too much to use in multimetric biological indexes (see Figures 18, page 53, and Figure 33, page 81), even when human influence is minimal, and they (especially production) may also be very difficult to measure. Estimated density or species abundance at a site is affected by three sources of variance: sampling efficiency, natural events, and human activities (see Premise 19, page 80).

The belief that a metric should *work is not reason enough to believe that it will*

Population size can vary enormously even when conditions are stable (Botkin 1990; Bisson et al. 1992) because populations respond to natural environmental changes as well as to intrinsic dynamics such as lag times between developmental stages. Identifying correlates of population variance in natural environments is challenging enough, but where human influence is also at work, the complex interaction of human and natural events determining population size makes it almost impossible to separate human effects from sampling and natural variance. Sampling protocols have been developed to overcome this problem (see Premise 4, page 16; Schmitt and Osenberg 1996), but they are often complicated, expensive, and time consuming. Moreover, they may even fail to detect biological signals that may be detected by looking at other components of biological systems or organizing and framing data in other ways. Taxa richness and relative abundance are more effective as indicators of biological responses to human actions (see Premise 6, page 26; Premise 11, page 45; Premise 12, page 47; Premise 17, page 71).

Some attributes, such as ratios (e.g., of the abundances of two trophic groups), are inherently flawed. A ratio consists of measures pertaining to two different groups, one used as the numerator, the other as the denominator. The numerator,

denominator, or both may vary simultaneously and for diverse reasons. For example, very large numbers of scrapers and filterers may yield the same ratio as a pair of very small numbers of each trophic group. Metrics expressed as ratios may intuitively seem useful, but empirical evidence (Barbour et al. 1992) and statistical theory (Sokal and Rohlf 1981) show that when two variables are combined in a ratio, the ratio tends to have higher variance than either variable alone. If two attributes of an assemblage are potentially important, moreover, they should be evaluated independently. With rare exceptions (e.g., relative abundance of individuals in a sample; see Premise 13, page 51 and below), using ratios mixes independent parameters in ways that make it hard to discern their relative influence, much as diversity indexes combine species richness and evenness into a single expression.

Not to be confused with ratios are metrics expressed as proportions (e.g., proportion of darters out of total number of individuals). The relative abundance, or percentage, of a particular group is calculated as the number of individuals in that group divided by the total number of individuals present. That proportion changes only as a function of changing relative abundance of the target taxon. As the number of individuals in a sample becomes very small, such as at seriously impaired or highly oligotrophic systems, however, low numbers may distort these proportions, and assessment procedures may need altering (e.g., Ohio EPA 1988).

Finally, many attributes now in use are based on theoretical arguments that often lack adequate empirical support. Although theory can be a good guide for selecting metrics, the theory must be tested with real-world data before a metric is used. Empirical natural history patterns should always take precedence over ecological theory in choosing which metrics to incorporate into a multimetric index. Theory can suggest metrics, especially when one begins to look at a new geographic region or a new biota. But the belief that a metric *should* work is not enough reason to conclude that it will. Ecology's path as a scientific discipline is littered with the carcasses of "good" theoretical constructs that evidence later showed were flawed. We should not rely on theory to guide decisions about vital goods and services that come from natural systems. Once again, the key test is whether an attribute shows an empirical dose-response relationship across a gradient of human influence.

PREMISE 25

Field work is more valuable than geographic information systems

Although a geographic information system (GIS) can be a powerful tool for mapping satellite and other data, it is not required for a successful monitoring project. The time and money spent on this technique may be better spent doing field work to identify the types and levels of human influence and defining the criteria for selecting and ranking sites.

Local field work leads to understanding and to decisions based on practical local experience observing natural systems, knowledge of the major human activities associated with those systems, and the resulting biological responses. The most successful projects are those that identify major human land uses in a region and study existing information before sampling. GIS can be useful for managing and displaying information, but GIS technology is not a replacement, or even a good surrogate, for biological monitoring.

PREMISE 26

Sampling everything is not the goal

Biological systems are complex and unstable in space and time (Botkin 1990; Pimm 1991; Huston 1994; Hilborn and Mangel 1997), and biologists often feel compelled to study all components of this variation. Complex sampling programs proliferate. But every study need not explore everything. Biologists should avoid the temptation to sample all the unique habitats and phenomena that make biology so interesting. Managers, especially, must concentrate on the central components of a clearly defined research or management agenda—for example, detecting and measuring the influence of human activities on a biological system.

Sites should be selected for sampling that are typical of a region and reasonably homogeneous with respect to important biogeographic features. Special habitat types—such as streams that are spring fed, ephemeral, or very large—may represent important and fascinating gaps in our biological knowledge, but if they represent a small percentage of a region's sites they should be left out of broad surveys (unless, of course, they are the target of a particular monitoring program).

Biologists are trained to focus on the unique because unique environments often yield new insights into how biological systems operate. But for monitoring, it is more important to focus widely on changes caused by humans and to document those effects.

AVOID PROBABILITY-BASED SAMPLING UNTIL METRICS ARE DEFINED

Probability-based sampling selects sites randomly within a region so that an estimate of overall resource condition is statistically reliable (Olsen et al., in press). But the technique is best not applied until after site classification and metric testing are completed—in other words, after dose-response relationships to human activity have been established.

Random sampling may not permit one to develop an integrative IBI to measure human effects: random sampling can even make it difficult to discover patterns caused by human activities. Random sampling of sites does not guarantee that selected sites are homogeneous enough (properly classified) to be included in an analysis. Neither does it guarantee that a full range of ecological states, from heavily degraded to undisturbed, will be studied. In fact, because human influence is so pervasive, most sites within a watershed are likely to be moderately to severely degraded; probability-based sampling is likely to miss the best and worst places if they are rare. Yet the best and worst sites are key for demonstrating biological responses to human influence, for developing and testing new metrics, and for calibrating scoring criteria (5, 3, or 1). By the same token, numerous studies demonstrate that subjective selection of reference sites can also be misleading (Patterson 1996; R. M. Hughes, pers. commun.; also see Premise 30, page 108).

Probability-based sampling allows statistically defensible generalizations to other places—but only after metrics have been verified

Another drawback of probability-based sampling may be the cost of identifying every potential sampling site before a random sample can be selected. Perhaps most important, if an agency commits exclusively to this sampling design before determining the biological responses likely to give the most useful signal about resource condition, considerable money and time can be lost, especially if the sampling design is short-circuited by the problem of getting access to sites because landowners may not grant permission to sample on private lands. Finally, many institutions and agencies may lack the resources for sampling sufficient numbers of sites to apply probability-based surveys.

On the other hand, if we already have robust indicators, probability-based sampling is critical to evaluate the condition of all waters in a region. Whenever probability-based sampling has been combined with strong indicators in recent years, degradation has been found to be more pervasive than originally believed. Probability-based sampling can also help avoid problems with a monitoring strategy that defines sites based on known sources of degradation: a random sample can find sites omitted because their causes of degradation were unknown.

Three early steps are crucial to a robust monitoring protocol: first, classifying of regional biological systems at appropriate levels—neither too detailed nor too superficial (see Premise 22, page 90); second, discovering of biological patterns associated with human actions—the documentation of ecological dose-response curves (see Premise 5, page 21); and third, cross-checking to ensure that the classification system selected is appropriate for the data set (see Premise 22, page 90). Narrowly conceived and implemented probability-based sampling designs too often overlook one (or more) of these three steps, and thus can fail to detect biological patterns associated with human-induced degradation. The failure of some state and federal programs in the past decade can be traced to the failure to define metrics that exhibit dose-response curves before monitoring began.

Nevertheless, when classification and ecological dose-response are appropriately established in concert with probability-based sampling, the result can be especially useful because it allows biologists to make statistically defensible conclusions beyond the sampled sites. For riverine fish, for example, probability-based sampling can help to estimate the condition of rivers over a large region where the fish metrics and a fish IBI have already been tested and validated. For now, probability-based sampling is less useful with other taxonomic groups, such as zooplankton, ants, plants, and to some extent benthic invertebrates, for which tests of metrics—the search for ecological dose-response curves—are incomplete.

Counting 100-individual subsamples yields too few data for multimetric assessment

A number of sampling protocols have been used in multimetric biomonitoring studies. Although there are no absolute standards for sampling design or analytical techniques, certain protocols are more effective than others in avoiding the pitfalls of too few data or poor-quality data.

Since the fish IBI was first developed in 1981, fish-sampling protocols have called for sampling all microhabitats within stream reaches from 100 m to 1 km long, depending on stream size. Fish IBIs have been developed for Ohio (Ohio EPA 1988; Yoder and Rankin 1995a,b), Wisconsin (Lyons 1992a; Lyons et al. 1996), Oregon (Hughes and Gammon 1987; Hughes et al., in press), Canada (Steedman 1988; Minns et al. 1994), Mexico (Lyons et al. 1995), and France (Oberdorff and Hughes 1992). Sampling design has not been controversial, largely because standard sampling methods are effective at sampling most fish in most microhabitats in small to midsize streams.

Why not sample a reasonable area and count the whole sample to begin with?

One study dealing with the effects on fish IBIs of sample size (number of individuals per sample) found that small samples were correlated with high measurement error; that is, the confidence intervals for IBIs increased as sample size decreased (Fore et al. 1994). Among 37 sites in Ohio's Great Miami Basin, 29 had confidence intervals for IBI of 6 or less (Fore et al. 1994; Figure 41). Seven out of eight of the sites with confidence intervals greater than 6 had fewer than 400 individuals per sample. The loss of precision in estimating IBI with samples of 400 or fewer suggests that it is unwise to intentionally use still smaller samples or subsamples.[8]

Sampling protocols are not as broadly accepted for benthic invertebrates as for fish. At least three superficially similar multimetric indexes using benthic invertebrates have been proposed: the invertebrate community index (ICI: Ohio EPA 1988; Yoder and Rankin 1995a,b); the rapid bioassessment protocol III (RBP: Plafkin et al. 1989); and the benthic index of biological integrity (B-IBI: Karr and Kerans 1992; Kerans et al. 1992; Kerans and Karr 1994; Fore et al. 1996; Rossano 1996; Karr 1998). Both ICI and B-IBI were extensively tested before publication or use in research or management; neither the sampling methods nor the metrics were

[8] When small sample sizes are a result of severe degradation, scoring of metrics—especially for relative abundance—can be adjusted to account for this fact (Ohio EPA 1988). Researchers sponsored by EPA's Environmental Monitoring and Assessment Program on Oregon streams and rivers were able to get precise results with samples of as few as 100 to 200 fish (R. M. Hughes, pers. commun.). Perhaps the threshold varies in cold- vs. warm-water streams, an issue that deserves further exploration.

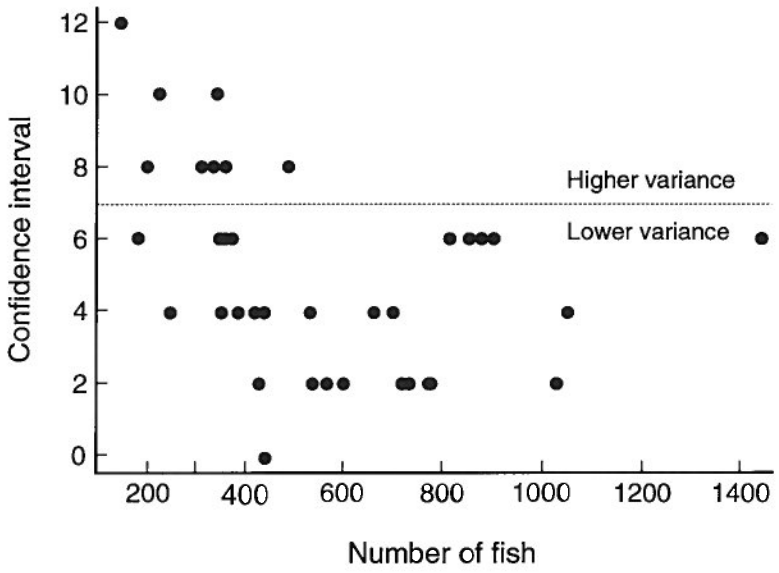


FIGURE 41. Confidence intervals for a fish IBI in relation to the number of individuals in samples collected at 37 sites within the Great Miami Basin, Ohio. (From Fore et al. 1994.)

as carefully evaluated for RBP, although recent tests are helping strengthen the protocol (Barbour et al. 1992; Barbour et al. 1996a; Barbour et al., in press). Tests of B-IBI in several regions (Tennessee, Wyoming, Oregon, Washington, Japan) point to 10 metrics as appropriate for including in a broadly applicable B-IBI (Table 11).

One of the most controversial aspects of these three invertebrate indexes is the number of individual organisms to be counted for an analysis. Both ICI and B-IBI call for counting every individual in each sample. RBP, in contrast, calls for subsampling as few as 100 individuals from each large sample to define a "consistent unit of effort"; the adequacy of this number has been hotly debated (Fore et al. 1994; Barbour and Gerritsen 1996; Courtemanch 1996; Vinson and Hawkins 1996). The need for subsampling with RBP comes out of its initial design: RBP calls for sampling a 2–3 m^2 area "to integrate sampling among a wide range of heterogeneous microhabitats" (Barbour and Gerritsen 1996: 387). A smaller sampling area, such as 0.1 m^2, would reduce the heterogeneity among sampled microhabitats from the outset (Kerans et al. 1992; see Premise 18, page 73).

We have found one effort to justify the adequacy of the 100-individual subsample approach (Barbour and Gerritsen 1996) unconvincing on several grounds, particularly with regard to studies of streams. First, the authors base their conclusions on data from lakes, not streams, and we believe it is not a good idea to extrapolate results across environment types. Second, arthropods were collected in "12 petite Ponar grabs (0.02 m^2)," giving a total sample area of only 0.24 m^2, in comparison with RBP's recommended 2–3 m^2 for streams. Third, only one subsample was generated for each of nine sites; variability was assessed, not with multiple samples from a site, but from multiple sites. Nine sites were grouped according to relative abundance curves, creating a mathematical near-certainty that taxa richness would vary systematically across the groups. A better approach would have been to

TABLE 11. Ten-metric B-IBI based on study in six geographic regions. Metrics were tested in six benthic invertebrate studies done in the Tennessee Valley, southwestern Oregon, eastern Oregon, the Puget Sound region, Japan, and northwestern Wyoming. A + indicates that the metric varied systematically across a gradient of human impact for that data set; – indicates that the metric did not vary systematically; 0 indicates that the metric was not tested for that data set. Sources: Tennessee, Kerans and Karr 1994; southwestern Oregon, Fore et al. 1996; eastern Oregon, Fore et al., unpubl. manuscript; Puget Sound, Kleindl 1995; Japan, Rossano 1995; northwestern Wyoming, Patterson 1996.

Metric	Predicted response	Tenn. Valley	SW Ore.	Eastern Ore.	Puget Sound	Japan	NW Wyo.
Taxa richness and composition							
Total number of taxa	Decrease	+	+	+	+	+	+
Ephemeroptera taxa	Decrease	+	+	–	+	+	+
Plecoptera taxa	Decrease	+	+	+	+	–	+
Trichoptera taxa	Decrease	+	+	+	+	+	+
Long-lived taxa	Decrease	0	+	+	+	0	
Tolerants and intolerants							
Intolerant taxa	Decrease	+	+	+	+	+	+
% tolerant	Increase	+	+	–	+	+	+
Feeding and other habits							
% predators	Decrease	+	–	+	+	–	+
"Clinger" taxa richness	Decrease	0	0	0	+	+	0
Population attributes							
% dominance (three taxa)	Increase	+	+	–	–	–	+

examine sites of different known human influence, to construct multiple random samples from each site, and to examine if the ranking of sites or other inferences about relative condition of the sites (e.g., ability of different metrics to discriminate among sites) was influenced by the subsampling procedure.

The decision to count only 100-individual subsamples (intended to speed laboratory analysis) has serious ramifications for the counts' reliability in multimetric indexes. First, the counting procedure itself becomes a source of error or bias. In RBP, the samples are spread out in a sorting pan with a sampling grid, and grid squares are counted at random until 100 individuals have been counted. The initial process to "randomly distribute" the organisms is one potential source of bias. Bias also arises from differences in the identity, size, mass, density, or distribution of individuals among the squares; these attributes can influence results even if random selection of grid squares is strictly enforced.

In addition, sample size affects estimates of taxa richness and relative abundances, which are central to a robust multimetric index (Courtemanch 1996). Samples must be large enough to accurately reflect the species richness and relative abundances for the resident biota. Yet, argues Courtemanch (1996: 382–383), the 100-individual subsample does not provide an "asymptotic estimate," either of taxa richness (number of taxa per standard number of individuals) or of taxa density (taxa per standard area) in each sampled unit; thus "there is no basis for comparison with either another sample community or with a reference condition."

Courtemanch proposes two remedies for this problem: two-phase processing, in which the entire sample is first searched for large individuals belonging to rare taxa; and serial processing, which involves following the RBP procedure to count individuals in grids up to 100 and then counting more grids until no new taxa are found. The large-individual standard is appealing but, we find, hard to defend on either sampling or biological grounds (see also Walsh 1997). A similar approach is outlined by Vinson and Hawkins (1996).

It may be more efficient to sample a smaller, entirely "countable" area in the first place, rather than spending the time and effort to collect large numbers of organisms that are never counted. The protocol we recommend (see Box 2, pages 78–79) samples smaller areas, focuses on a single microhabitat, collects three replicate samples, keeps samples separate, and counts each sample completely. Such a protocol saves some time in the field and gives more complete results from the laboratory; we thus have greater confidence in both the statistical and biological aspects of the resulting multimetric evaluation. This approach does not, of course, give a complete count of all organisms present in a stream reach or a measure of variability among riffles within the reach. It has, however, provided enough detail to judge relative biological condition among streams—within a region and among regions.

Perhaps the most serious flaw in the 100-individual subsample approach derives from the fact that sample size does not affect all metrics in the same way. Counting only 100 individuals may thus lead to erroneous conclusions or limit a manager's ability to diagnose causes of degradation. In testing the 100-individual standard, for example, Barbour and Gerritsen (1996) found that, for taxa richness, counting 100-individual subsamples and also counting all individuals produced the same rank order for their nine sample sites; they therefore concluded that 100 individuals adequately represented taxa richness across these sites. Yet because these researchers' method is based on analysis of relative abundance curves, not sites ranked according to a known human-influence gradient, the behavior of their taxa richness metric cannot be attributed exclusively to human impact. Further, it is inappropriate to extrapolate from the presumed behavior of one metric to the behavior of all metrics in a multimetric index.

Subsamples of only 100 individuals are less likely than large samples to consistently reveal the presence of intolerant, long-lived, or otherwise rare taxa, regardless of their size; small subsamples are also likely to affect relative abundances of key trophic or other ecological groups (Ohio EPA 1988). Failing to count rare taxa or

rare ecological groups such as intolerant taxa would exclude some of the strongest biological signals about the condition of places. This effect of subsampling is analogous to the exclusion of rare species that is often recommended in multivariate analyses (Reynoldson and Rosenberg 1996; see Premise 32, page 112).

An analysis of random subsamples of stream invertebrates collected in Puget Sound lowland streams (Doberstein, Karr, and Conquest, in prep.) has yielded very different conclusions from those of Barbour and Gerritsen (1996). Using a bootstrap resampling protocol like that described by Fore et al. (1994), Doberstein, Karr, and Conquest generated several hundred subsamples for each of several streams for 100-, 300-, 500-, and 700-individual subsamples and for the entire complement of individuals collected in three 0.1-m^2 samples. (The field sampling procedures were those described in Box 2, pages 78–79.) After determining the variance in parameter estimates (metric values) for the resulting distributions of random samples, Doberstein, Karr, and Conquest then asked how many distinct classes of biological condition could be detected, by each metric and for the integrative B-IBIs.

Using the 10-metric B-IBI shown in Table 11 (page 103), the researchers found they could reliably discern an average of 3.6 classes of biological condition per metric (range, 1.14 to 10.61) when they counted full samples from minimally disturbed streams (Figure 42). This result compares favorably with the 3 classes distinguished by the 5, 3, and 1 scoring protocol. In contrast, metric sensitivity for random (bootstrap) 100-individual subsamples dropped to an average of 1.1 classes (range, 0.31 to 3.16). Counting all sampled individuals and then combining the metrics into a B-IBI permitted detection of 5.8 classes, the same sensitivity found by Fore et al. (1994) for a fish IBI. Counting random 100-individual subsamples from each sample site, in contrast, allowed detection of only 2.1 classes of stream condition (e.g., "good" vs. "bad") (Figure 42). Given the time and energy devoted by state agencies to biological monitoring, this resolution is unsatisfactory.

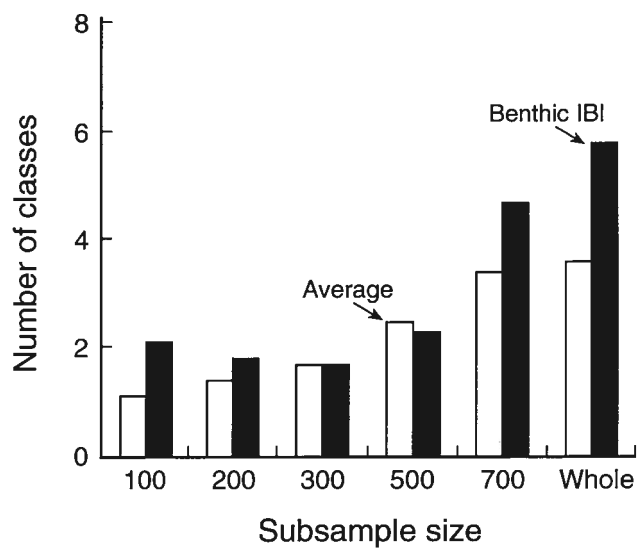


FIGURE 42. Average number of classes detected by metrics in a 10-metric B-IBI (see Table 11) and by the B-IBI itself at different subsample sizes. Data come from a minimally disturbed stream in King County, Washington.

Doberstein, Karr, and Conquest (in prep.) have also found that counting an increasing number of 100-individual subsamples permitted detection of an increasing number of classes. For three minimally disturbed streams, counting three 100-individual subsamples instead of one raised the detectable levels of stream condition from 1.88 to 4.43. Would it not be simpler to count the whole sample to begin with?

In sum, one needs large enough samples and multiple metrics for a truly multimetric picture of biological condition. Multiple metrics together provide a stronger signal than one or two alone and, further, allow diagnosis of the likely causes of degradation.

Avoid thinking in regulatory dichotomies

The framework for environmental regulation necessarily divides actions and places into those that are "in compliance" and those that are not on the basis of legal standards and criteria that are assumed to protect the overall condition of a site and its inhabitants. As a result, agency personnel tend to think in dichotomies and to view sites as "impaired" or "unimpaired," "acceptable" or "unacceptable," and so on (Murtaugh 1996). The trouble is, biological condition is not an either-or affair. The condition of living systems within a region may vary from near pristine to severely degraded. In other words, the biological condition of places falls along a gradient. Therefore, to fully understand, rank, and evaluate those places, researchers should also measure biological condition along a gradient.

Multimetric biological indexes furnish a yardstick for measuring, tracking, evaluating, and communicating actual continuous variability in biological condition. Instead of simply labeling a site "control" or "treatment," "impaired" or "unimpaired," "acceptable" or "unacceptable," a multimetric assessment identifies and preserves finer distinctions among sites, in the index itself and in the values of the component metrics. Multimetric assessment automatically takes account of a site's context, permitting distinctions among urban streams that might all be labeled "impaired" in a dichotomous analysis. Suburban Swamp Creek sites near Seattle, for example, have B-IBIs of 26 to 34, which is clearly better than urban Thornton Creek's range of 10 to 18 but not nearly as good as rural Rock Creek's 44 to 46.

Because biological condition is a continuous variable, it should be measured on a continuous scale

Dichotomous methods for evaluating biological condition lead to a variety of analytical and even regulatory problems. What is or is not an "acceptable" threshold in some biological (or chemical) factor depends on a site's context. Thresholds considered acceptable in an urban stream may be totally unacceptable in a rural or wildland stream. In addition, threshold definitions change over time as science and human values change, people learn more, and measurement techniques become more sophisticated. Through the years, the regulated community as well as regulators and other citizens have become frustrated by what they perceive as arbitrary moving targets in the form of "minimum detectable" thresholds.

In contrast, measuring biological condition with a continuous yardstick such as IBI puts a site along a gradient of condition in comparison with other sites or other times, allowing thresholds to be reset according to context. It also permits a ranking of many sites—which might all be labeled "degraded" in a dichotomous scheme—so that priorities may be set for budget-constrained protection or restoration efforts.

PREMISE 30

Reference condition must be defined properly

The goal of biological assessment is to detect and understand change in biological systems that results from the actions of human society. But change with respect to what? Just as economic analyses define a standard (e.g., 1950 dollars) against which economic activity can be judged, biological assessment must have a standard against which the conditions at one or more sites of interest can be evaluated. This standard, or reference condition, provides the baseline for site evaluation.

In multimetric biological assessment, reference condition equates with biological integrity—defined as the condition at sites able to support and maintain a balanced, integrated, and adaptive biological system having the full range of elements and processes expected for a region. Biological integrity is the product of ecological and evolutionary processes at a site in the relative absence of human influence (Karr 1996); IBI thus explicitly incorporates biogeographic variation. Protecting biological integrity is a primary objective of the Clean Water Act. The value of IBI is that it enables us to detect and measure divergence from biological integrity. When divergence is detected, society has a choice: to accept divergence from integrity at that place and time, or to restore the site.

Programs that measure biological and geophysical conditions in near-pristine environments provide much information about biotas and geophysical contexts in different areas. They inform managers about natural ranges of variability and allow comparisons across watersheds and landscapes among streams of similar elevation, size, or channel type; they provide ecologists with needed information about the interplay of physical processes and biological responses. But reference condition is only half the picture. If the goal of water resource management is to halt degradation of living aquatic systems, then managers must stop focusing exclusively on natural processes and responses, as they have for many years in trying to implement biological criteria. Reference information is not enough.

Furthermore, measuring pristine conditions in one ecoregion or subecoregion after another, year after year, will not slow the degradation of aquatic resources. Sampling pristine environments from every ecoregion or subecoregion does not necessarily add insight about which biological attributes provide reliable signals about resource condition. Putting as much effort into quantifying and evaluating human influence as into collecting biogeographical information is the only way to discern biological signal from the background of natural variability. Sampling sites across a range of human influence provides the means to detect that signal.

The message here is clear. Agency biologists would do well to devote as much effort to understanding how to detect human influence as to collecting biogeographical "reference" information. Until state and federal agencies understand the importance of sampling across a gradient, both time and money will be wasted.

One major challenge is that there are few, if any, places left that have not been influenced by human actions. Thus, defining and selecting reference sites, and measuring conditions at those sites, requires a careful sampling and analysis plan. Common pitfalls include using local sites that are degraded rather than looking over a wider area for minimally disturbed sites; arbitrarily defining reference sites without adequate screening or site evaluation; and classifying sites inaccurately so that degraded sites are put into reference sets, especially when arbitrary statistical rules (e.g., a site is considered "impaired" if it is 25% of reference condition) are used to guide regulatory or other management decisions (e.g., Barbour et al. 1996a). Definition of reference condition in biological assessment may use modern or historical data, or theoretical models (Hughes 1995). Some are better than others.

The Wyoming Department of Environmental Quality, for example, requested nominations for reference streams from water resource personnel in the state. Analysis of biological data from 14 nominated sites (Patterson 1996) indicated that three sites had IBI values substantially below reference condition; sources of degradation could easily be identified even though the sites had been judged as reference sites. Six additional sites also had low scores, suggesting some human-induced degradation. The remaining five Wyoming reference sites were not likely affected to any significant degree by human activity. In this case, even professionals erred in judging sites as unimpaired. Because defining reference condition properly is critical to the success of multimetric indexes, reference sites must actually be minimally influenced by people.

To begin making biological monitoring more effective—that is, to get information in the most cost-effective manner that can begin to protect water resources immediately—biologists need to document and understand dose-response relationships between particular biological attributes and human influence (see Premise 7, page 30). They need to identify metrics that respond to human disturbance and not just to geographical differences among ecoregions. They must shift their focus from exhaustively characterizing ecoregions or defining reference condition to sampling sites that have been subject to different intensities and types of human influence. Finally, they must choose a small set of metrics that provide reliable signals about the effects of human activities in the region. Metrics must be chosen according to their ability to distinguish between different types and intensities of human actions. By integrating those metrics into a multimetric index, we have a scientifically sound and policy-relevant tool to improve management of water resources.

PREMISE 31

STATISTICAL DECISION RULES ARE NO SUBSTITUTE FOR BIOLOGICAL JUDGMENT

The objective of biological monitoring is to detect human-caused deviations from baseline biological integrity (see Premise 5, page 21, and Figure 3, page 23) and to evaluate the biological—not statistical—significance of those deviations and their consequences (Stewart-Oaten et al. 1986, 1992; Stewart-Oaten 1996). In other words, biological change, not *p*-value, is the endpoint of concern. A statistically significant result (small *p*-value) may not equate with a large, important effect, as researchers often assume; similarly, a statistically insignificant effect (large *p*-value) may well be biologically important (Yoccoz 1991; Stewart-Oaten 1996). Without some statement about the probability of detecting an effect of given magnitude, it is almost impossible for anyone to know for certain from, say, a *t*-test whether a biological effect is present. It is too simplistic, and potentially misleading, to assume that lack of statistical significance necessarily means that differences between places do not exist. Only power analysis can define the precision of a finding that two things do not differ.

Statistical significance is not the same as biological importance

Ecologists tend to overuse tests of significance (Yoccoz 1991). It is not enough to detect differences in lieu of determining an impact's magnitude and cause or of understanding its consequences (Stewart-Oaten 1996). It would be wiser to decide first what is biologically relevant and then use hypothesis testing to look for biologically relevant effects, not merely run a general "search for significance." Overreliance on statistical correlation, *t*-tests, or other statistical models can short-circuit the process of looking at data and asking whether they make sense and what they show. Dependence on *p*-values can divert scientists and managers from exploring the biology responsible for the patterns in data, no matter when or by whom they were collected.

To evaluate alternative decisions, scientists and managers should balance hypothesis testing with other statistical tools, such as decision theory (Hilborn 1997); they should explore thoroughly the causes and consequences of differences in biological condition. When a study is based on tested biological metrics, of course hypothesis testing can be appropriate, as when sites upstream and downstream of a point source need to be compared for setting regulations. But when a biologist or statistician reports a significant difference based on a *p*-value, the key next questions are, How different? In what way? What is the effect in biological systems?

By providing a biological yardstick for ranking sites according to their condition, multimetric indexes can answer these questions. Because their statistical properties are known and their statistical power can be calculated (see Premise 15, page 63; Peterman 1990; Fore et al. 1994), they can also be used to compare sites statistically. But a ranking according to biological condition is more appropriate than statistical comparisons for setting site-specific restoration or conservation priorities.

PREMISE 32

MULTIVARIATE STATISTICAL ANALYSES OFTEN OVERLOOK BIOLOGICAL KNOWLEDGE

To many field biologists, "statistics" means "multivariate statistics" because field data are complex and multidimensional. Despite the availability of numerous statistical techniques, monitoring studies have used the same multivariate techniques since the 1960s (Potvin and Travis 1993). These multivariate approaches—including cluster analysis, factor analysis, and widely used ordination techniques such as principal components analysis (PCA; James and McCulloch 1990)—extract the maximum statistical variance in variance-covariance matrices, usually across species or sites (Ludwig and Reynolds 1988). Unfortunately, the contexts in which multivariate methods have been applied have often precluded detecting, understanding, and basing decisions on some of the most important signals from biological systems.

The fault lies not with multivariate statistics themselves, which can provide important insights about the structure of data sets, but rather with how they are used. Multivariate analyses were developed for pattern analysis, not impact assessment. Failure to understand the difference, or to keep it in mind when interpreting biological data, can lead to errors. We believe that misinterpretation is more common with multivariate techniques than with the multimetric approach. Certainly it is easier for people without statistical training to understand the results of a multimetric analysis. Many authors have covered the use of multivariate methods (Wright et al. 1993; Davies et al. 1995; Davies and Tsomides 1997; Walsh 1997), so we focus on some of the problems associated with their misuse in biological monitoring.

Multivariate analyses were developed for finding patterns, not assessing impacts

First, some ordination techniques, including PCA, assume that the data follow a multivariate normal distribution (Tabachnik and Fidell 1989), which is in fact a rare pattern in data from biological monitoring. These methods assume smooth continuous relationships, either linear or simple polynomial, but relationships among environmental variables are often nonlinear. In multivariate analysis, the numerous zeros and frequent high abundances typical of biomonitoring data are outliers with a potentially strong influence on the statistical solution (Gauch 1982; Tabachnick and Fidell 1989), so the data are often transformed to "fix" departures from normality, usually without success (Ter Braak 1986). Second, data are often edited (e.g., rare taxa are deleted), which may result in omitting important biological information (Walsh 1997).

Third, depending on which variables an analysis includes, multivariate techniques may fail to discriminate among important sources of variation, such as natural and human-induced variation or variation caused by sampling, subsampling, and error. Most multivariate data matrices contain a mix of sites, some with little influence from humans, others subject to different degrees of human influence. The matrices often mix data from different seasons or from, for example, different stream sizes or lake types. Although variables may be similarly confounded in multimetric analyses, it is usually easier to recognize and avoid this pitfall because multimetric analyses do not rely on computers to "discover" the relevant pattern.

Finally, multivariate approaches assume that statistically describing maximum variation will identify the most meaningful signal about biological condition. But because multivariate methods reduce the dimensionality of the original data by extracting or "loading" the maximum amount of variation on successive axes, they lose biological information at each step. This problem is compounded if the initial choice of biological variables was made without considering whether the variables responded across degrees of human influence.

The most common applications of multivariate statistics rely on lists of taxa and their abundances to detect differences among sampled sites or times (Reynoldson and Metcalfe-Smith 1992; Norris and Georges 1993; Norris 1995; Pan et al. 1996; Reynoldson and Zarull 1993). PCA, for instance, uses mathematical algorithms to extract variance from a matrix of species abundances, one of the most variable aspects of biology, rather than examining how the animals feed, reproduce, use their habitat, or respond to human activities. When species-abundance matrices are the focus, important ecological attributes never even make it into the analysis. The combined loss of signal, because major important components of biology are ignored and because the statistical procedure cannot apportion variation to definable causes, limits the ability of the most common multivariate applications to discern complex patterns and to help investigators understand them.

In one telling example of the pitfalls of multivariate analyses of species abundances,[9] two investigators advocated excluding rare species, saying that they simply add "noise to the community structure signal and . . . little information to the data analysis. . . . We recommend excluding all taxa that contribute less than 1% of the total number or occur at less that 10% of the sites" (Reynoldson and Rosenberg 1996: 5; see also Marchant 1989; Norris 1995). Yet the presence of rare taxa indicates ecological conditions capable of supporting such often sensitive taxa, thereby offering special clues about a site's environmental quality (Karr 1991; Courtemanch 1996; Fore et al. 1996).

Furthermore, comparing the results of PCA using real data with PCA using matrices of random numbers shows that the percentage of variation described may be similar for both, especially for the second and subsequent principal components; that loadings of original variables on principal axes are often as high for random

[9] From the Ninth Annual Technical Information Workshop on study design and data analysis in benthic macroinvertebrate assessments (North American Benthological Society meeting, June 1996).

numbers as for real data; and that matrix size is an important determinant of the amount of variation extracted (Karr and Martin 1981). Multivariate techniques were unable to discern known deterministic relationships in one study (Armstrong 1967), and in another, they manufactured relationships in data sets containing no such relationships (Rexstad et al. 1988).

PCA reflects the underlying linear correlation (or covariance) among all the variables in the matrix. If no, or small, correlations exist, then PCA can manufacture relationships. The problem can be avoided with a careful examination of the correlation matrix before applying PCA. Without careful choice of variables conveying reliable signals about biological condition or, as Gotelli and Graves (1996) argue, without a comparison of the data against a null model showing pattern(s) that would occur in the absence of any effect, multivariate statistics can misguide resource assessment efforts. General uses of PCA seldom give results that go beyond common sense (Karr and Martin 1981; Fore et al. 1996; Stewart-Oaten 1996). Gotelli and Graves (1996: 137) go so far as to suggest that "multivariate analysis has been greatly abused by ecologists. . . . [D]rawing polygons (or amoebas) around groups of species [or points], and interpreting the results often amounts to ecological palmistry. Ad hoc 'explanations' often are based on the original untransformed variables, so that the multivariate transformation offers no more insight than the original variables did."

The key danger of overreliance on multivariate analyses is that management decisions may be based on statistical properties of data—on the structure of a covariance matrix—rather than on biological knowledge and understanding. In fact, when multivariate analyses examine the same biological attributes used in multimetric indexes, they yield essentially identical results (Hughes et al., in press). The key message, then, is to use procedures to account for biological impacts, not just to describe pattern. Avoid analytical "shortcuts" that are not easily understood or that must be done idiosyncratically for every data set. There is simply no substitute, either in multivariate statistics or in multimetric indexes, for careful application of biological and ecological knowledge, regardless of analytical tool. Careful design of sampling, thoughtful analysis of data, and careful description of biological condition can eliminate the need for general approaches that merely extract variation.

Assessing habitat cannot replace assessing the biota

In its broadest sense, *habitat* means the place where an organism lives, including all its physical, chemical, and biological dimensions; an oak-hickory forest or a cold-water stream is a habitat. Habitat also refers more narrowly to the physical structure of an environment. In streams, *habitat structure* generally means the physical structure of the channel and near-channel environment. Stream biologists see habitat structure as a critical component of environmental condition; they view habitat assessment, which involves measuring physical habitat structure, as a way to compare present structure with some idealized habitat.

Increasingly, scientists and managers have come to equate the presence of such idealized habitat with the presence of an organism; measuring habitat can even take the place of looking for the living inhabitants. But the presence of a given habitat structure does not guarantee the presence of desired biological inhabitants, any more than chemically clean water guarantees a biologically healthy stream.

Stream habitat features include channel width and stability, water depth, streambed particle size, current velocity, and flow volume (Gorman and Karr 1978; Rankin 1995). These factors interact to define the mix of pools and riffles, pattern of meanders, or braiding characterizing a stream channel. Width of the riparian area and floodplain, riparian canopy cover, bank condition, and woody debris are also important components of habitat structure.

Don't assume that if you build "habitat," the inhabitants will come

Habitat assessments focus on such physical features to determine the suitability of a physical environment for an aquatic biota. In a habitat assessment, managers may measure the physical habitat directly, as in the habitat evaluation procedures developed by the US Fish and Wildlife Service (USFWS), or they may infer habitat condition from mathematical models, such as USFWS's in-stream flow incremental method. Unfortunately, some have used these models to justify spending millions of dollars on "in-stream structure" without assessing biological responses or even the persistence of those structures in dynamic channels.

But habitat structure, like water quality, is only one of the five factors affected by human activities in a watershed (see Table 9, page 67). Severe physical damage to a stream channel is easy to see and document, but subtle degradation invisible to human observers may be biologically just as destructive. When resource agencies measure habitat variables in lieu of testing the response of biological systems to

human disturbance, they effectively assume that disturbance affects only physical habitat and that only visible damage harms the biota.

Yet measuring habitat structure may not reflect past sediment torrents or debris flows from upstream or from a road built along the channel. Habitat assessments do not reliably account for how floods or droughts are exacerbated by changes in the extent of impervious area in a watershed or the effects of water withdrawals. Hyporheic connections, too, are difficult to measure and poorly understood, yet the hyporheic zone is a critical refuge for organisms during floods or drought. When groundwater flow patterns are altered by water withdrawal, these connections are broken; the consequences can be judged only by measuring the condition of the biota. Although simple biotic measures may not detect specific changes in the hyporheic zone, a biological change can lead to further investigations to identify the cause.

Measuring physical habitat cannot determine the effects on resident organisms of introduced and alien species, chemical contaminants, changes in temperature, or dissolved oxygen. Measuring habitat structure in a stream where an invisible or unmeasurable form of water pollution is impairing the biota, for example, could lead one to conclude that the biota is healthy when it is not. Measures of stream habitat convey an incomplete picture of a stream's biological condition. Sampling water quality or habitat structure can aid in interpreting data on biological condition; it cannot and should not be used to *define* biological condition.

Fishery managers once neglected the physical structure of stream environments or considered it unimportant. But simply reversing that view is equally misguided. Habitat assessment alone does not capture all the ways that humans influence water resources. Using habitat surrogates to draw inferences about biological condition does not account for interactions between predators and prey, timing of peak or low flows, competition, alien species, or harvesting.

Worse, to talk of protecting "fish habitat" (or, more extreme, "fish*ery* habitat") implies that we know what fish need; it implies that we can "fix" biological condition by fixing the habitat—by adding woody debris, building spawning channels, or bulldozing to create pools. Yet anadromous fish populations continue to decline in the Pacific Northwest despite expensive projects to restore stream channels and construct "spawning channels." A stream is more than a collection of habitat types. Physical habitat criteria are necessary, but entirely insufficient, to ensure commodity production of wild salmon, let alone biological integrity.

SECTION V

Many Criticisms of Multimetric Indexes Are Myths

The multimetric approach has come under fire from toxicologists, ecologists, and water managers on several grounds (Calow 1992; Suter 1993; Wicklum and Davies 1995). Yet numerous successful applications of multimetric biological monitoring and assessment (Yoder 1991a; Davis and Simon 1995; Lyons et al. 1995, 1996; Davis et al. 1996), explicit responses to the critics (Karr 1993; Simon and Lyons 1995; Hughes et al., in press), and the work on which this report is based suggest that biological criteria and multimetric indexes constitute robust tools for monitoring rivers and streams, especially when compared with the virtual lack of biological monitoring in the past. We explore some of the criticisms here.

MYTH 1

"Biology is too variable to monitor"

The success of biological monitoring rests on our ability to select good indicators, indicators that are sensitive to the underlying conditions of interest (i.e., human influence) but insensitive to extraneous factors (Patil 1991). The belief that biology is too variable to monitor comes not from a lack of good indicators but from past failures to find the right indicators.

Because studies of naturally variable attributes such as population size, density, and abundance have dominated ecology for the better part of a century, resource managers as well as ecologists tend to regard biological assessments as less consistent than chemical assessments. But not all biological attributes vary as much as population size, density, and abundance; indeed, attributes such as taxa richness yield clear, consistent patterns in response to human actions. The issue, then, is not "biology vs. consistency" but, rather, which attributes of biology make sense to monitor: Which attributes respond predictably to gradients of human influence? Measuring biological attributes that do respond consistently gives important insights about the condition of water bodies.

The sources of variability in data—whether chemical, physical, or biological—must be controlled in field sampling protocols and laboratory procedures. Standardized lab procedures helped reduce the variability of chemical data but did not eliminate it. In the past decade, major advances have been made to standardize field biological sampling—in particular, to identify those biological attributes whose signal-to-noise ratio is high and that respond predictably to human impact.

Patterns in biological variability also offer some unexpected insights into human impact. Several studies have observed a correlation between mean and variance in IBI (see Premise 14, page 56): as IBI decreases, its variance increases (Karr et al. 1987; Steedman 1988; Rankin and Yoder 1990; Yoder 1991b). This association could reflect real changes in the resident biota at degraded sites, it could be a statistical artifact, or it may not be a general phenomenon. Hugueny et al. (1996), for example, reported lower variation in IBI at a disturbed site than at an upstream site. In the Willamette River, Oregon, standard deviations of IBI were highest at intermediate values (Hughes et al., in press). Using the bootstrap algorithm, Fore et al. (1994) demonstrated that the increased variance of IBI values at degraded sites did reflect biological changes in the resident assemblage; this conclusion supports the observation that biological systems subjected to high human disturbance are less resilient to environmental change. A thoughtful exploration of the specific circumstances in each of these cases might clarify these relationships.

Of course, natural variability cannot be separated entirely from human-induced variability, for human disturbance often exacerbates the effects of natural events (Schlosser 1990); floods or low flows are often more extreme in damaged watersheds, for example (Poff et al. 1997). The higher variability of IBI values observed at degraded sites (Karr et al. 1987; Steedman 1988; Fore et al. 1994; Yoder and Rankin 1995b) does point to effects on the sites' biological systems that mirror physical signs of degradation and suggests that highly variable IBIs may be an early-warning sign of excessive human impact.

MYTH 2

"Biological assessment is circular"

Some have complained that IBI development is circular because biologists look at a site, decide whether it is degraded or pristine, and then develop metrics and an index that show the sites to be degraded or pristine as first observed. This view is flawed on two levels. On a concrete level, comparison of site condition with a regionally defined reference condition and assemblage—not one's own first observations—is built into metric testing and index development.

On a second, more abstract level, index development may appear circular because of the interplay of observation and experimentation that lies at the heart of science. Assessing water resources rarely allows replicated experiments; only one Puget Sound is available, for example, and controlled experiments at that scale are unlikely. Yet the links between certain human activities in watersheds and the biological health of the rivers running through those watersheds are clearly visible. As knowledge accumulates from repeated observation of real-world patterns, our confidence in the generality of those patterns increases.

Circularity can be avoided through repeated rigorous documentation of biological responses to a wide range of human actions (development of ecological dose-response curves) in a wide range of geographic areas. Ecological dose-response curves depict patterns that are both qualitative and quantitative, as well as consistent across a broad range of circumstances. For river fishes, for example, the same metrics (see Table 8, page 59) respond to human influence in studies in many habitats, under many human impacts, and for many regional assemblages (Miller et al. 1988; Lyons 1992a; Lyons et al. 1995, 1996; Oberdorff and Hughes 1992; Hughes et al., in press). The same holds true for invertebrates (see Table 6, page 57; Table 7, page 58; and Table 11, page 103). Indeed, many of the same attributes are consistent indicators for a variety of faunas (see Table 5, page 52, and Table 11, page 103).

In her study of 115 streams in west-central Japan, Rossano (1995, 1996) convincingly demonstrated that IBI development is not circular; her work also verified dose-response patterns previously described for North America. Rossano first classified all 115 streams according to the type and magnitude of human activity within their watersheds (see Figure 4, page 31). After selecting a few streams that appeared the best and the worst, she randomly chose half the streams and plotted the quantitative values for biological attributes expected to change in those streams across her gradient of human influence (see Figure 5, top, page 32). She found distinct dose-response curves for some of the plotted metrics, including total taxa

richness, number of intolerant taxa, number of clinger taxa, and relative abundance of tolerants (see Figure 14, page 42); these attributes also respond to human impact in North America. Rossano then scored these metrics (see Premise 14, page 56), summed the scores to yield a B-IBI for each site, and plotted the B-IBI values against human influence (see Figure 5, top, page 32). Finally, she applied the same metrics and scoring criteria from the first half of the data set to the other half of the 115 streams; B-IBIs from both sets of streams followed nearly identical patterns (see Figure 5, bottom, page 32; Rossano 1995, 1996).

Such systematic documentation and testing of metrics in many places and with many human influences reinforces the validity of those metrics and the resulting IBIs as accurate yardsticks of human impact.

MYTH 3

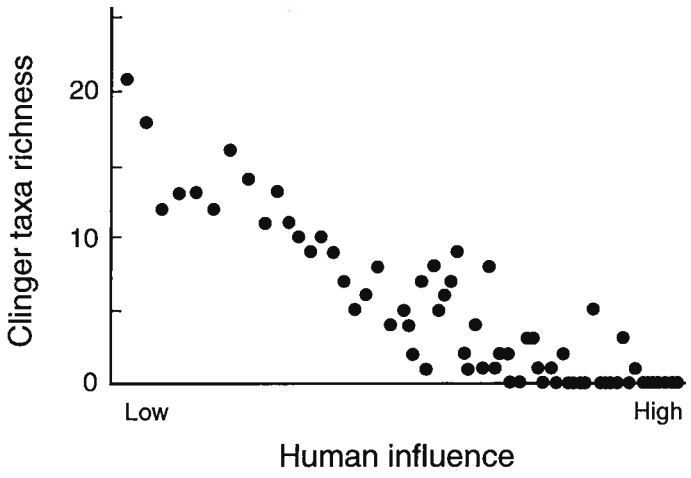
"We can't prove that humans degrade living systems without knowing the mechanism"

This comment implies that we must understand the means by which something happens, not just *that* it happens, before we can act. We hear this comment from two rather different groups. The first is basic natural scientists, who focus on process and cause and effect and subscribe to the mantra of $\alpha = 0.05$ and the null hypothesis of no effect (Shrader-Frechette 1996). Rarely have these scientists been faced with day-to-day environmental decision making. The second group embraces this view as a stalling tactic for overusing ecological systems, sidestepping their own responsibility while blaming "science" for knowing too little.

But where would medicine be now if doctors had to understand how diseases worked before treating them or how drugs worked before using them? For centuries, people have prevented or cured diseases and alleviated symptoms with drugs, such as aspirin, even though they did not know the physiological mechanism by which the drugs acted. Modern medicine recognizes and combats viral and bacterial diseases without fully understanding how each virus or bacterium does its damage. Humans routinely act on the basis of what they see without knowing every mechanism behind it.

Of course, we want to know how observed changes come about in biological systems altered by humans. But those mechanistic explanations are not essential for using biological monitoring to indicate degradation and find likely causes. The number of clinger taxa declines very reliably along gradients of human influence (Figure 43), regardless of what we do or do not know about the specific mechanisms responsible. Perhaps fine sediments fill the spaces among cobbles, destroying the clinger's physical habitat. Perhaps clingers are more exposed to predators as they move out of the sediment-laden spaces. Perhaps upwelling from hyporheic zones no longer supplies cool oxygenated water. Perhaps the diverse foods of many clinger species are no longer available. Perhaps all these factors are operating. Perhaps some other mechanism is responsible. But although the mechanism is not documented, the empirical pattern is clear. We would be foolish not to use it to detect degradation and to take actions to protect water resources.

FIGURE 43. Number of clinger taxa plotted against a human influence gradient for Japanese streams. (From Rossano 1995.)



MYTH 4

"Indexes combine and thus lose information"

Because a multimetric index like IBI is a single numeric value, critics have assumed that the information associated with the metrics is somehow lost in calculating the index itself (USEPA 1985; Suter 1993). Not at all.

Multimetric indexes condense, integrate, and summarize—not lose—information. They comprise the summed response signatures of individual metrics, which individually point to likely causes of degradation at different sites (Karr et al. 1986; Yoder 1991b; Yoder and Rankin 1995b). Although a single number, the index, is used to rank the condition of sites within a region, details about each site—expressed in the values of the component metrics—remain (Simon and Lyons 1995). It is straightforward to translate these numeric values into words describing the precise nature of each component in a multimetric evaluation. These descriptions, together with their numeric values, are available for making site-specific assessments, such as pinpointing sources of degradation (Yoder and Rankin 1995a) or identifying which attributes of a biotic assemblage are affected by human activities (see Figure 17, page 43).

At a site in urban Thornton Creek in Seattle, for example, total taxa richness is 25% of a reference stream minimally affected by human activity, Rock Creek in rural King County. Thornton Creek has only one mayfly taxon and no caddisflies or stoneflies, compared with five, six, and seven taxa of mayflies, caddisflies, and stoneflies, respectively, in Rock Creek. Individuals belonging to tolerant taxa make up more than 50% of the individuals in Thornton Creek samples and only 26% in Rock Creek samples. Thornton Creek has no long-lived or intolerant taxa, while Rock Creek supports four intolerant and two long-lived taxa. Rock Creek has a benthic IBI of 44 (maximum 50), whereas Thornton Creek's IBI is only 10 (minimum 10). Narrative descriptions of the sites as well as the numeric values for each metric and the B-IBI tell us a great deal about these two streams.

Those who advocate multivariate statistical analyses for biological monitoring insist that multimetric indexes lose information selectively. In their view, multivariate statistics extract biological patterns from the whole data set. Yet many multivariate analyses exclude rare taxa (see Premise 32, page 112) or examine only species lists and abundances, an approach that overlooks organisms' natural history and ecology or the known responses of specific taxa to human actions. Multivariate statistical algorithms are based on the structure of variance-covariance matrices, not on specific knowledge of how organisms develop, find food, reproduce, and interact with one another and their physical and chemical surroundings.

Although management decisions can be, and have been, based on multivariate statistical analyses of biological data (Reynoldson and Zarull 1993; Wright et al. 1993; Davies et al. 1995), the decision process is hardly transparent to anyone who does not understand the mathematical algorithms or the models' underlying assumptions. In our view, multivariate statistics' inherent complexity distracts biologists from making clear, testable statements to one another and to nonscientists about how the biota of a place responds to human influence.

MYTH 5

"Multimetric indexes aren't effective because their statistical properties are uncertain"

Although there may have been a basis for this statement in years past, recent work on the statistical properties of biological data and of the multimetric index suggests that, as for any other procedure, careful program design—from sampling and field work to data analysis—can yield data and conclusions that are both biologically useful and statistically robust. More important, perhaps, recent work also shows that the problems associated with biological data of all kinds can be reduced by systematic planning, data collection, and analytical procedures. Conversely, when sampling design and data quality are not rigorously controlled, no procedure or approach can have known statistical properties.

In particular, bootstrap analysis of real data has demonstrated that the fish IBI approximates a normally distributed random variable (Fore et al. 1994; see Premise 15, page 63). In this study, the statistical precision of the fish IBI agrees with data collected over periods of two to eight years for both fish and invertebrates (Angermeier and Karr 1986; Karr et al. 1987). For example, 13 lowland Puget Sound streams were sampled at the same sites in successive years (1994–95) to evaluate between-year variation in the streams when human activities had not changed. B-IBI for these streams changed by no more than 4 during that two-year study; two sites increased by 2, four decreased by 2, three decreased by four; and 4 were unchanged. All changed by 10% or less of the range of B-IBI, an exceptional stability for most biological analyses. Similar concordance among years was detected in studies in Oregon (R. M. Hughes, pers. commun.).

Statistical properties of multimetric indexes are known (see Premise 15, page 63), as are the sources of variation (see Premise 19, page 80). When one knows the sources of variation, one can construct studies to limit their influence. Too often biologists seek to incorporate all sources of variation rather than design a study to focus on the kinds of variation relevant to program goals.

Biological monitoring has come a long way since the early 1980s in identifying the biological attributes to measure and in integrating these measures statistically in ways precise enough to describe the status and trends of biological systems. The declines in living aquatic systems tell us that we cannot afford *not* to use the tools we have or to stop seeking still better ones.

"A NONTRIVIAL EFFORT IS REQUIRED TO CALIBRATE THE INDEX REGIONALLY"

This criticism hinges on the assumption that developing and using a multimetric biological index costs lots of time and money. True, the required effort is nontrivial, but how trivial is it to count permits issued, accumulate fines, collect samples, or produce meaningless "305(b) reports" that are not representative of regional or national conditions? How much money do agencies spend on these activities?

In fact, the cost of biological monitoring is often less than that of more conventional approaches (Yoder 1989; Table 12). Most important, the long-term cost of *not* doing effective biological monitoring is highest of all—the continued degradation and ultimate loss of the most valued components of life in our waters. "The specter of millions of dollars being misspent on environmental controls, without strong evidence of the efficacy of the treatment, indicates that money spent on high-quality monitoring programs is money well spent" (Rankin 1995).

Over the past three years, Karr and several graduate students have developed and implemented region-specific biological standards in small streams and shown that biological responses to human actions can be documented and generally understood from studies lasting months, not years. Two master's students at the University of Washington each sampled about 30 sites in one year and one season (four weeks of field work). Each study yielded enough data to define and calibrate a B-IBI for the Puget Sound lowlands (Kleindl 1995) or Grand Teton National Park (Patterson 1996). Kleindl and Patterson also required approximately three months of laboratory time for counting and identifying three replicate benthic invertebrate samples for each study site. Thus, geographic calibration can be accomplished within the time frame and budget of a master's project. Surely each region's water resources are worth that level of commitment.

TABLE 12. Comparative costs (in US dollars) of collecting, processing, and analyzing samples to evaluate the quality of a water resource. (Data from Ohio EPA provided by C. O. Yoder.)

	Per sample[a]	Per evaluation[b]
Chemical and physical water quality		
4 samples per site	1436	8616
6 samples per site	2154	12,924
Bioassay		
Screening (acute 48-hour exposure)	1191	3573
Definitive (LC_{50}[c] and EC_{50}[d], 48- and 96- hour)	1848	5544
Seven-day (acute and chronic effects, 7-day exposure, single sample)	3052	9156
Seven-day (as above but with composite sample collected daily)	6106	18,318
Macroinvertebrate community	824	4120
Fish community	740	3700
Fish and macroinvertebrates combined	1564	7820

[a] Cost to sample one location or one effluent; standard evaluation protocols specify multiple samples per location.
[b] Cost to evaluate the impact of an entity; this example assumes sampling five stream sites and one effluent discharge.
[c] Dose of toxicant that is lethal to 50% of the organisms in the test conditions at a specified time.
[d] Concentration at which specified effect (e.g., hemorrhaging, pupil dilation, swimming cessation) is observed in 50% of tested organisms.

MYTH 7

"The sensitivity of multimetric indexes is unknown"

This statement implies that multimetric indexes cannot discern and separate patterns of biological consequence from the noise of variation (natural, sampling, crew, seasonal, and so on). But the many examples we cite from scientists and managers show that a modest effort by a few people can systematically document biological patterns that are useful in research, management, and regulatory contexts. The key is to define ecological dose-response curves for a range of geographic areas and diverse human influences (logging, agriculture, recreation, and urbanization). We must connect human actions to biological change.

SECTION VI

The Future Is Now

Twenty-five years after passage of the Clean Water Act, we can be thankful that our rivers no longer catch fire. But the science of biological monitoring is still way ahead of the regulatory and policy framework used to manage water resources. The problem lies not in the letter or spirit of our laws but in a pervasive reluctance to shift from a narrow pollution-control mentality to a broader regard for the biological condition of our waters.

Humans tend to fiddle while Rome burns—not deliberately but because we react ineptly to complex situations. Faced with problems that exceed our grasp, we pile small error upon small error to arrive at spectacularly wrong conclusions (Dörner 1996). We did this when we built Egypt's Aswan Dam, disrupting a cycle of flooding and Nile Valley fertilization that had sustained farmers for millennia; we did it in the series of events leading up to the 1986 explosion of Reactor 4 at Chernobyl. Are we doomed to do it while our rivers, lakes, wetlands, and oceans get deeper into trouble?

PREMISE 34

WE CAN AND MUST TRANSLATE BIOLOGICAL CONDITION INTO REGULATORY STANDARDS

When the 1972 amendments to the Water Pollution Control Act were being debated in Congress, then–EPA Administrator William Ruckelshaus testified in the House of Representatives against the House bill. Referring to its general objective to "restore and maintain . . . chemical, physical, and biological integrity," Ruckelshaus stated, "We do not support the new purpose or 'general objective' that would be provided. The pursuit of natural integrity for its own sake without regard to the various beneficial uses of water is unnecessary" (Committee on Public Works 1973). Later, after President Nixon had vetoed the amendments, the Senate Committee on Environment and Public Works underwent 33 days of hearings, 171 witnesses, 470 statements, 6400 pages of testimony, and 45 subcommittee and full-committee markup sessions—and concluded that "chronic adverse biological impact may be a greater problem than the acute results of discharge of raw sewage or large toxic spills" (Muskie 1992). The 1972 Water Pollution Control Act amendments finally passed, over the presidential veto, setting the restoration and maintenance of the biological integrity of water as the first of three broad goals.

We have the knowledge and the know-how to use biological criteria; let's stop arguing and use them

For Ruckelshaus at the time, apparently, water "use" by humans was the whole story, and consumptive uses of water were legitimate while nonconsumptive uses, such as keeping fish and wildlife alive, recreation, or aesthetics, were not sufficiently "beneficial." Like so many water resource managers before and since, the EPA administrator saw water as a fluid, a commodity to be bought and sold, not as a complex biological system that provides diverse goods and services to society. For him and his agency, clean water was enough.

Clean water still seems to be enough for many in agency circles. Water resource managers schooled in the language and dogma of chemical pollution have been slow to adopt a broader view of resource degradation. Decision makers stay safely with existing rules and standards, most often interpreting them more narrowly than even the letter of the law suggests they should be interpreted. The federal and state agencies responsible for writing regulations, tracking water resource condition, and creating water-protecting incentives are reluctant to embrace biological integrity as a primary goal.

At present, water quality standards—the formalized rules regulators use to protect water resources—contain three components: designated uses, criteria, and the principle of antidegradation. (The antidegradation goal entered the regulatory

agenda in the 1980s under the broad reasoning that water resource decisions should allow no further degradation. In theory, the antidegradation philosophy was supposed to end past acceptance of "dilution is the solution to pollution.") Under these rules, each state must define designated uses, or goals, for all water bodies within its boundaries. Criteria—generally numeric and chemical but sometimes narrative and biological (e.g., that conditions be "fishable and swimmable" or adequate to "protect aquatic life")—are then established on the assumption that preventing violations of the criteria will protect the designated uses.

Chemical water quality measures, permits issued, and fines levied are still the primary currencies in most state water quality programs for protecting designated uses. The lion's share of water resource funding still goes to controlling point-source pollution, despite widespread knowledge that nonpoint pollution and nonchemical factors damage more miles of streams and acres of lakes than do point sources (see Table 9, page 67)—and this despite advances in biological monitoring that have laid a strong foundation for setting *numeric biological* criteria. It is past time to include biological monitoring, and the scientific assessment of resource condition it produces, into decision making. Biological criteria, and the regulations to implement them, would be better able to address society's present values and more appropriate for targeting expenditures to protect the quality of life in our waters and our communities.

As we have tried to show in this report, when supported by classification to minimize the heterogeneity of samples, an appropriate number of metrics proven to vary along a gradient of human influence, and standardized scoring procedures, multimetric biological monitoring and assessment can give decision makers clear signals about the condition of water resources—knowledge that is the essential first step toward wise targeting of expenditures to protect or restore those resources. So why have only two states incorporated biological monitoring and numeric biological criteria into water quality standards? Why have only 15 more begun to develop such criteria (Davis et al. 1996)—despite calls to do so in the law, the scientific literature (Karr and Dudley 1981; Davis and Simon 1995), and the government's own documents (USEPA 1988, 1990, 1996b)?

One may regard the glass as half full or half empty. Virtually no state had biological criteria in 1981 when the first multimetric fish IBI appeared (Karr 1981). And although adoption of numeric biological criteria has been slow (Davis et al. 1996), the last decade has brought progress: 29 more states now have narrative biological water quality standards, and 11 are developing them. Ohio, for example, has used the fish IBI and ICI, an invertebrate derivative of the fish IBI, to define two levels of biocriteria, excellent warm-water habitat and warm-water habitat, expressed as numeric standards. The criterion for excellent warm-water habitat was initially set at IBI = 50 for most of Ohio, to protect the state's highest-quality waters from additional degradation. Warm-water habitat (IBI > 40) applies to moderately degraded areas; this criterion is intended to prevent further degradation and provides an attainable benchmark for restoration of streams in watersheds that humans have heavily influenced.

Thus it is hardly farfetched to imagine use of biological criteria in all states. We have broad national objectives, reasonable criteria, and multimetric indexes that are biologically sound and statistically robust. Isn't it time for researchers and policymakers to stop arguing about whether we know enough to act definitively? Of course we don't know everything; of course water bodies, like forests, are more complicated than we *can* know. But we know a great deal. Perhaps we would make more progress in protecting our waters if researchers all agreed not to ask for further funding until regulatory agencies used the knowledge already piled up in their archives. Can we look forward to a lull in our research programs?

CITIZEN GROUPS ARE CHANGING THEIR THINKING FASTER THAN BUREAUCRACIES ARE

Polls and a fast-rising number of grassroots watershed activities clearly show that the American people are aware of and concerned about the nation's rivers, lakes, wetlands, and oceans. Citizens are more informed scientifically than they were a couple of generations ago, and they are increasingly alarmed by what they see being lost from our waterways. People across the country identify water pollution as the most important environmental issue (e.g., in the Pacific Northwest; Harris and Associates 1995). US coastal county and city managers have ranked safe, clean drinking water as number one among critical national issues (NOAA press release, May 1997, *http://www.noaa.gov/public-affairs*); indeed, 58% of these managers ranked clean water as equal to or more important than health care. In a survey conducted for American Rivers, 94% of respondents identified contamination of drinking water by sewage and industrial waste as a primary concern.

Such concerns have sparked thousands of citizen initiatives to monitor water quality and river health. The 1996–97 *River and Watershed Conservation Directory* (River Network 1996) lists some 3000 organizations and agencies in the United States whose missions directly address river or watershed protection. Mainstream organizations from the Izaak Walton League to Trout Unlimited have also expanded their view of rivers and river health. Local chapters of both these groups have begun to emphasize broader understanding of the causes and treatment of river degradation. New national organizations are developing as well. These include Project GREEN, Adopt-a-Stream Foundation, River Network, and River Watch Network (Karr et al. 1998).

We need not be trapped by our old ways of thinking; rather, we can learn from them

River monitoring done through the schools has become one of the fastest growing elements of volunteer monitoring (USEPA 1994c). Colorado Waterwatch, for example, is a partnership of the State Division of Wildlife and teachers and students at more than 250 schools; students monitor some 500 stations throughout the state of Colorado. In Seattle, Washington, the Thornton Creek Alliance ties together the teachers and students in 28 elementary through high schools in a network, centered on rivers, with local business and political leaders. Rivers provide the theme for interdisciplinary education, and everyone gains a better understanding of local landscapes and a stronger sense of community.

At the same time, individual scientists and historically conservative scientific groups such as the American Fisheries Society, the Ecological Society of America,

and the North American Benthological Society have expanded their efforts to reach governments and citizen groups. The Ecological Society, for example, has started a new series of publications, *Issues in Ecology,* targeted to the press, policymakers, and the public. The Benthological Society is establishing liaisons with major North American conservation organizations, developing a database of professionals willing to share their expertise widely, and selling slides and slide sets for use in educational programs.

A curious, and telling, element in many citizen initiatives is that they are funded in part by local, state, and federal governments. King County, Washington, supports numerous citizen alliances seeking to learn more about their watersheds. A statewide Governor's Watershed Enhancement Board in Oregon makes substantial amounts of money available for local watershed initiatives. EPA has also funded numerous local groups to monitor and restore the condition of rivers. Why, we ask, are these agencies not doing more to broaden perspectives in their own ranks? Why are they not strengthening their own programs to track biological condition, as required under section 301(b) of the Clean Water Act?

If, as Dörner (1996) argues, failure has its own logic, that logic is seldom more obvious than in the workings of our bureaucracies. Humans long ago developed the tendency to deal with problems on an ad hoc basis. We defined and solved problems one at a time; we didn't need to see a situation embedded in the context of other situations; we thought in straight, cause-and-effect lines about one dimension at a time. Contemporary decision makers still (Dörner 1996: 18)

- Act without first analyzing the situation.
- Fail to anticipate side effects and long-term repercussions.
- Assume that the absence of immediately obvious negative effects means that correct measures have been taken.
- Let over-involvement in "projects" blind them to emerging needs and changes in the situation.
- Are prone to cynical reactions.

The inappropriateness of these reactions for solving modern problems is only made worse by the difficulty of separating good information from bad when we are overloaded with information; our reluctance to accept new knowledge even when we see that it's good; and defense of the status quo by bureaucracies and other vested economic, scientific, and social interests. This kind of approach worked fine in simpler, slower times; it doesn't work now in this complex, increasingly high-speed world. We need to respond quickly, and correctly, to our present environmental problems, but bureaucracies seem incapable of fast responses.

Still, there are no magic solutions for overcoming our plodding ways of dealing with complex problems. But it helps to know how we think—that we sometimes think badly, that we often become stuck in old ways when new ways would be far better. It helps to realize that facing up to the next century's challenges does not necessarily require us to tap into some hitherto fallow 90% of our brain potential; rather, it requires the development of our common sense, our flexibility, our ability

to anticipate consequences (Dörner 1996). Albert Einstein put it this way: "You cannot solve a problem by applying the same conceptual framework that created the problem." Environmental educator David Orr (1994) says simply, "Think at right angles."

PREMISE 36

Can we afford healthy waters? We can afford nothing less

Until all states see protecting biological condition as a central responsibility of water resource management, until they see biological monitoring as essential to track attainment of that goal and biological criteria as enforceable standards mandated by the Clean Water Act, life in the nation's waters will continue to decline.

We are all responsible, and we all need to do better. We must take a broader view of the problems we face if we hope to devise effective solutions; we must also explicitly recognize the nature of modern organizational systems and hold them accountable (Bella 1997). Citizens need to increase their understanding of science and continue to put pressure on governments to act. Scientists need to strengthen their biological monitoring approaches, talk with neighbors and relatives, write outside of technical publications, and dare to speak up in the realm of day-to-day decision making. Managers need to reexamine "the way it's always been done" and do what works to keep waters alive. Agency administrators need to allocate funding inside their own agencies to programs that actually protect water resources. They should refocus their own professional energies on activities they are funding citizen watershed groups to do.

"Can we afford rivers and lakes and streams and oceans, which continue to make life possible on this planet?" We must answer Edmund Muskie's question with a resounding yes.

SECTION VII

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