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BIOLOGICAL CRITERIA

Technical Guidance for Streams and Small Rivers

Revised Edition

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CHAPTER 4: (continued)

A critical decision in the design of biocriteria programs is how to select appropriate indicators of biotic condition.

Biosurveys of Targeted Assemblages

A critical decision in the design of biocriteria programs is how to select appropriate indicators of biotic condition. Biosurvey of the targeted assemblages is the most widely employed approach to biocriteria development. This approach, which has been used by Ohio, Illinois, North Carolina, Maine, Arkansas, New York, and Vermont, focuses on a selected component of the biological community; it samples one or several specific aquatic community segments to measure biological condition. Monitoring the specific characteristics of these assemblages helps assess the effects of a variety of environmental conditions (Ohio Environ. Prot. Agency, 1987).

A number of different organisms associated with lotic systems (i.e., streams and rivers) lend themselves to bioassessment procedures. Commonly measured assemblages include, but are not restricted to, macrophytes, algae, macroinvertebrates, and fish. The targeted assemblage approach to bioassessment can also focus on a single assemblage (e.g., periphyton) or several assemblages (e.g., periphyton, macroinvertebrates, and fish). The attributes measured may be functional parameters, such as photosynthesis or respiration, or other attributes, such as individual health. Examples of widely used methods and techniques for targeted assemblages are found in Karr (1981), Karr et al. (1986), Ohio Environ. Prot. Agency (1987), Plafkin et al. (1989), Standard Methods (1989), U.S. Environ. Prot. Agency (1990), and Weber (1973). The primary advantages of this approach are its flexibility, practicality, cost-effectiveness, and relative scientific rigor.

The importance of the periphyton assemblage within most stream ecosystems makes it a prime candidate for consideration as a bioassessment–biosurvey target.

Attributes of Selected Assemblages

■ **Periphyton.** The periphyton assemblage is composed of benthic algae, bacteria, their secretions, associated detritus, and various species of microinvertebrates (Lamberti and Moore, 1984). Periphyton are an important energy base in many lotic situations (Dudley et al. 1986; Minshall, 1978; Steinman and Parker, 1990) and serve as the primary nutrient source for many stream organisms (Lamberti and Moore, 1984). The capacity of benthic assemblages to colonize and increase in biomass is influenced by variability in stream channel geomorphology, flow rates, herbivore grazing pressure, light intensity, seasonality, and random processes (Coleman and Dahm, 1990; Grimm and Fisher, 1989; Hamilton and Duthie, 1984; Korte and Blinn, 1983; Lamberti et al. 1987; Patrick, 1949; Poff et al. 1990; Steinman and McIntire, 1986, 1987; Steinman et al. 1987; and Stevenson, 1990).

The importance of the periphyton assemblage within most stream ecosystems makes it a prime candidate for consideration as a bioassessment-biosurvey target. More specific advantages are outlined by Plafkin et al. (1989):

- The rapid algal reproduction rates and short life cycles of periphyton make them valuable indicators of short-term impacts.
- Physical and chemical factors have direct effects on the structure and functions of periphyton and on their production.
- Periphyton sampling methods are straightforward, and the samples are easily quantified and standardized.

- Methods have also been standardized for recording functional and nontaxonomic characteristics of periphyton communities, such as biomass and chlorophyll measurements.

- Algal components of periphyton are sensitive to some pollutants to which other organisms may be relatively tolerant.

■ **Macrophytes.** The macrophyte assemblage consists of large aquatic plants that may be rooted, unrooted, vascular, or algiforms. Both emergent and submergent macrophytes provide numerous benefits to streams and small rivers thus helping them to support healthy, dynamic, biological communities (Campbell and Clark, 1983; Hurley, 1990; and Miller et al. 1989). Some understanding of the distributional characteristics and environmental conditions affecting macrophytes (Hynes, 1970) enhance their use in bioassessment strategies. Hynes (1970) and Westlake (1975) discuss differences in lotic macrophyte assemblages based on habitat factors such as water hardness, pH, gradient, and propensity for siltation.

Some investigators have emphasized the influence of macrophytes on habitat structure (Carpenter and Lodge, 1986; Gregg and Rose, 1982, 1985; McDermid and Naiman, 1983; Miller et al. 1989; Pandit, 1984); others have studied water chemistry, nutrient cycling, and macroinvertebrate colonization (McDermid and Naiman, 1983; Miller et al. 1989). Pandit (1984), Seddon (1972), and Westlake (1975) pointed to the use of macrophytes as an indicator assemblage in lotic situations.

Aquatic macrophytes are an important food source for birds and mammals. Fassett (1957) lists 36 species of waterfowl, nine marshbirds, four shorebirds, and nine upland game birds that feed on these plants. He also lists beaver, deer, moose, muskrat, and porcupines as aquatic macrophyte herbivores. The use of macrophytes in bioassessment programs has numerous advantages:

- Macrophyte taxonomy to the generic level is relatively straightforward.

- Because the establishment of macrophyte populations in a specific habitat depends partly on local environmental conditions, they are potentially very useful as site-specific indicators.

- Because their specific microhabitat structure does not limit germination, macrophytes are potentially found in high population densities.

- The growth patterns of individual macrophytes are directly influenced by herbivore activity.

- The longevity, distribution, and rate of their population growth may directly reflect prevailing conditions.

Benthic macroinvertebrate assemblages are important indicators of localized environmental conditions.

■ **Macroinvertebrates.** Macroinvertebrates are the visibly distinguishable crustaceans, molluscs, insects, and other fairly large aquatic invertebrates. Benthic macroinvertebrate assemblages are important indicators of localized environmental conditions because they inhabit the degraded or contaminated resources and can be exposed to degradation directly throughout their life history. Their characteristics can be regarded as a reflection of the integration of short-term environmental variability (Plafkin et al. 1989). At sensitive life stages, they respond quickly to stress; how-

Fish assemblages are well suited to help define environmental conditions because fish inhabit the receiving waters continuously, and with lifespans up to 10 years, they can easily represent the integrated historical effects of chemical, physical, and biological habitat factors.

ever, the overall assemblage responds more slowly. Other advantages of using macroinvertebrates include the following:

- Sampling methods are well developed and require minimal personnel and inexpensive gear.
- Macroinvertebrates play a major role in the nutritional ecology of commercial and sport fisheries.
- Most streams support sufficient abundance levels for assessment.
- Molluscs, many species of crustacea, and some insects are largely immobile. As residential organisms, they are particularly valuable indicators of site conditions over time.
- Many states have already performed background benthic surveys, have personnel trained in benthic biology, and can often get assistance in sampling from lay groups.

■ **Fish.** Fish assemblages are well suited to help define environmental conditions — either natural or impaired. Fish are long-lived and inhabit the receiving waters continuously. With lifespans up to 10 years, they can easily represent the integrated historical effects of chemical, physical, and biological habitat factors (Ohio Environ. Prot. Agency, 1987). Power (1990) found that fish exert significant influence on the food chain in lotic systems. More specific advantages of using the fish assemblage for bioassessment (Karr et al. 1986; Plafkin et al. 1989) include the following:

- Fish are usually present in lotic systems except for some headwaters.
- Their populations generally include species that feed at a variety of trophic levels.
- Species composition and dominants are relatively stable in most areas.
- The migration patterns and wide-ranging foraging behavior of some fish allow investigators to accumulate effects from relatively large-scale habitats.
- In comparison to other potential bioassessment groups, fish are relatively easy to identify.
- Autecological studies for many freshwater species are extensive, so their life histories are relatively well known.
- Public, and therefore, legislative appreciation for fish is apparent in the fishable goal of the Clean Water Act, the Endangered Species Act (50 percent of "endangered" vertebrate species are fish), and in more specific commercial and sport fisheries legislation.
- Historical survey data are probably best documented for fish.
- Investigators can often get assistance from lay groups.

■ **Wildlife.** Mammals, birds, reptiles, and amphibians can also provide valuable information for bioassessment decisions. Croonquist and Brooks (1991), applying the concept of response guilds, found that bird species with high habitat specificity decrease with increasing habitat alteration.

This approach has considerable potential for development of an avian index of biotic integrity. Birds have been shown to reflect the condition of riparian systems.

Because amphibians live part of their life cycle in an aqueous or damp environment, they are a link between the aquatic and terrestrial environments. They are also sensitive to littoral zone and riparian disturbances and to changes in their food resources (macroinvertebrates and periphyton). The latter may affect their fitness or force them to emigrate from the home range to another foraging zone. Other advantages of including a biosurvey of mammals, birds, and amphibians in biomonitoring programs are the following:

- Their longer life spans make them well suited for evaluation of cumulative effects.
- The relatively large body size of birds and their behaviors (e.g., singing) allow visual and auditory observation to supply most of the necessary information.
- Birds are sensitive to riparian alteration.
- Wildlife taxonomy is well understood.
- Many biomarkers — physical and chemical alterations in the species in response to contamination — appear in these organisms, and an increased likelihood for sublethal effects in non-emigrating individuals.
- Trapping techniques for small mammals are relatively straightforward, and their tracks and droppings also provide easily attainable survey data.
- The public is usually able to assist in conducting wildlife assessments.

Synthesis

Many bioassessment programs focus on a single assemblage for reasons of regulatory focus or mandate, available expertise, resource limitations, or public awareness and interest. However, state agencies are encouraged to incorporate more than one assemblage (e.g., fish and benthic macroinvertebrates) into their assessment programs. Biological programs that use two or three assemblages and include different trophic levels within each group (e.g., primary, secondary, and tertiary consumers) will provide a more rigorous and ecologically meaningful evaluation of a system's biological integrity (U.S. Environ. Prot. Agency, 1990) and a greater range of temporal responsiveness.

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Impairments that are difficult to detect because of the temporal or spatial habits or the pollution tolerances of one group may be revealed through impairments in different species or assemblages (Ohio Environ. Prot. Agency, 1987). Mount et al. (1984) found that benthic and fish assemblages responded differently to the same inputs in the Ottawa River in Ohio. Benthic diversity and abundance responded negatively to organic loading from a sewage treatment plant and exhibited no observable response to chemical input from industrial effluent. Fish exhibited no response to the organic inputs and a negative response to metals. In a more recent assessment, the Ohio EPA found that distinct response signatures (Yoder, 1991) in both fish and macroinvertebrate assemblages indicated an adverse effect from the sewage treatment plant. Selection of aquatic community components that show different sensitivities and responses to the same disturbance will help identify the nature of a problem (U.S. Environ. Prot. Agency, 1990).

Selecting a single assemblage for assessment may provide inadequate resolution for certain impacts that are highly seasonal in occurrence. Organisms having short life cycles may not reflect direct exposure to highly variable impacts at critical times or when complex cumulative impacts are present. Depending on the collection period, those organisms may provide a false sense of ecosystem health if other assemblages of longer-lived populations are under stress. In cases in which periodic pulses of contaminants may occur, long-lived populations may be slow to exhibit response, whereas short-lived organisms may be severely affected.

The occurrence of multiple stressors and seasonal variation in the intensity of stressors require that more than one assemblage be incorporated into biocriteria programs whenever practical. Not all assemblages discussed here are in constant contact with the aquatic habitat component. Those that are — the macroinvertebrates, macrophytes, fish, and periphyton — will exhibit direct, and potentially more rapid, responses to water resource degradation. The assemblage comprising mammals, birds, and amphibians indicates the quality of the riparian corridor and can reflect local land use impacts on the water resource.

Aquatic organisms respond to stress in a variety of ways ranging from alterations in community composition and structure to increases or decreases in the biomass of a single or multiple species, or mortality. Fish and drifting macroinvertebrates also exhibit avoidance behavior by seeking refugia from short- and long-term disturbances.

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Careful selection of taxonomic groups can provide a balanced assessment that is sufficiently broad to describe the composition and condition of an aquatic ecosystem, yet practical enough for use on a routine basis (Karr et al. 1986; Lenat, 1988; Plafkin et al. 1989). When selecting community components to include in a biological assessment, primary emphasis should be given to including species or taxa that (1) serve as effective indicators of high biological integrity, that is, those likely to live in unimpaired waters, (2) represent a range of pollution tolerances, (3) provide predictable, repeatable results from consistent sampling, (4) can be readily identified by trained state personnel (U.S. Environ. Prot. Agency, 1990), (5) show a consistent response to pollution stress, and (6) closely represent local, indigenous biota.

Technical Issues

The methods and procedures used in bioassessment programs should be based on the study objectives and associated technical issues, including the selection of the proper sampling period, sites, and sampling regime; and the determination of the appropriate habitats to be sampled.

Selection of the Proper Sampling Periods

The ideal sampling procedure is to survey the biological community with each change of season, then select the appropriate sampling periods that accommodate seasonal variation. Such indexing makes the best use of the biological data. It ensures that the sources of ecological disturbance will be monitored and trends documented, and that additional information will be available in the event of spills or other unanticipated events.

In this way, the response of the community to episodic events (e.g., chemical spills) can be assessed throughout the year. Seasonal impacts, which may be highly variable, can be more effectively characterized through more frequent sampling. Impacts from certain stresses may occur or be "worst-case" at specific times of the year, and it may be important to provide adequate documentation of the biological condition during these times. EPA's Science Advisory Board (SAB) suggests that sampling should — at a minimum — include the major components of the fall-winter and spring-summer (or wet season-dry season) community structure. The Florida Department of Environmental Protection has instituted a program that encompasses sampling during two index periods that correspond to this approach.

If some fish and invertebrate life cycles (e.g., spawning, growth, migration, and emergence) cause marked seasonal changes in stream assemblages, then each sampling season will require a separate reference database, metrics, and biocriteria. When such multiple index periods are used, the operational costs, at least initially, may be considerably higher than if surveys were conducted only once a year. Therefore, states must weigh their needs and the long-term value of this information against these costs. Seasonality must always be considered, and where possible, year-round data should be developed even if it has to be phased in slowly over time and as budgets allow.

The alternative, a single index period, will be deficient; it will not document spills or other single episode or transitory events including stresses that take place in other seasons. It should be selected only if seasonality is not a factor in the program objectives. Still, the major or initial applications of state biocriteria are likely to be assessment and management planning related to chronic habitat alteration and point and nonpoint sources. Such chronic stress impacts are more efficiently assessed with a single index period approach. Resident fish and benthic invertebrate assemblages integrate stress effects over the course of a year, and their seasonal cycles of abundance and taxa composition are fairly predictable within the limits of interannual variability. Single season indexing also represents a cost savings compared to seasonal or more frequent sampling.

Given these considerations, state managers must choose the approach most appropriate to their needs and budgets. They must avoid the temptation to spread multiseason sampling so thin that neither seasonal measurements nor indexing are properly achieved. It is better to do a single index period well than to do two poorly. Presuming, therefore, that most states will initially design their biological criteria programs around single season surveys, the following discussion emphasizes index period designs.

The optimal biological sampling period will be consistent with recruitment cycles of the organisms from reproduction to emergence and migra-

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tion, such that the maximum amount of information can be derived from the data. Optimal conditions for biological sampling can be defined as that period of time during which the target assemblages have stabilized after larval recruitment and subsequent mortality and the use of their niche space is at its fullest. Where necessary, a compromise between biologically optimal conditions and water and flow conditions appropriate for the sampling gear must be made. Therefore, selection of the sampling period should be based on efforts to

- minimize between-year variability resulting from natural events,
- maximize gear efficiency, and
- maximize target assemblage accessibility.

Field collections scheduled to correspond to the optimal biological sampling period provide the most accurate assessment of community response to adverse conditions over an annual cycle. Sampling during these periods may not be logistically feasible, however, as a result of adverse weather conditions, staff availability, scheduling constraints, or other factors. The nature of the suspected stressor is an especially important consideration. An agency may be required to perform biological sampling during periods of greatest environmental stress, such as low flow and high temperature periods for point source discharges or high flow and runoff periods for nonpoint source discharges.

Although an estimate of aquatic community structure during optimal biological conditions should reflect the effect of, or recovery from, environmental stress periods (Ohio Environ. Prot. Agency, 1987), assessment of worst-case conditions may be needed under certain permitting regulations or as a follow-up to sampling during biologically optimal periods in which impairment was detected.

Ecological conditions and, thus, optimal sampling periods, vary seasonally as a result of regional climate patterns and the life cycles of the biota. Seven major climatological regions are represented within the contiguous United States (Fig. 4-3). The primary influence of seasonal changes in temperature and rainfall on stream biota is on biological processes (e.g., production, growth, reproduction, distribution, and locomotion). The level of biodiversity may also change seasonally. Even within an ecological region, some scaling of the optimal collection period may be necessary, depending on the elevation of the site, the habitat type, and other broad environmental variables.

Temperature and rainfall are the principal weather factors influencing the selection of sampling protocols and timing. Sampling will be impossible in frozen streams or during extreme high flows. Even subtle changes in temperature and flow may preclude certain kinds of sampling by affecting the equipment or the distribution of target assemblages.

The purpose of the biological sampling program (trend monitoring, special studies) also influences the sampling protocol. Special studies may be conducted at any time depending on need; but trend monitoring studies will focus on annual sampling events with varying sampling frequencies. The most appropriate season for such collections is determined by considering all technical and nontechnical factors. Technical factors include the selected assemblage, recruitment cycles, and severity of degra-

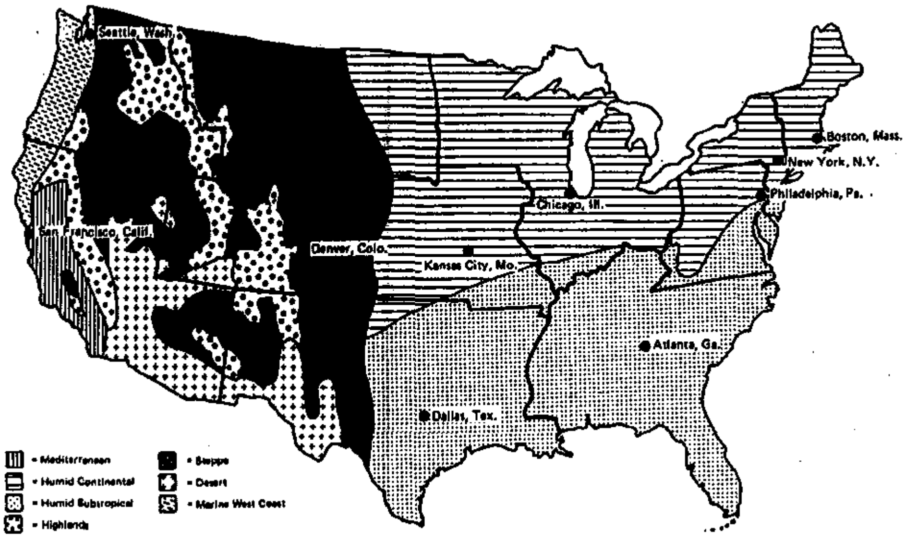

Figure 4-3.—Classification of U.S. climatological regions.

dation or contamination; nontechnical factors include such matters as logistics and personnel. From a practical standpoint, many states may select a sampling period that includes the summer and early fall months.

The investigator must carefully define the objectives of a monitoring program before these design issues can be resolved. Will specific questions be answered by sampling during periods of optimal biological condition or during periods of maximum impact? (These two periods may coincide.) Seasonal considerations are important because community taxonomic structure and the functional composition of some assemblages undergo natural changes in each season and annual cycle.

Natural cycles may also be influenced by chemical or physical alterations. From the traditional perspective of evaluating pollution impacts, summertime low flow conditions are often chosen to assess effects from point source discharges. Low flow conditions capture the effects of minimal effluent dilution in combination with the natural stressors of low water velocity and high temperature. Minimal effluent dilution occurs in summer because the lower quantity of water decreases the ability of the receiving waters to reduce the concentration levels of discharged compounds.

The effects of nonpoint source pollution on the aquatic community are evaluated during the recovery period following high flow because these effects are largely driven by runoff in the watershed. Nonpoint source loadings are estimated using samples collected during periods of high flow. Their actual effects, however, should be based on sampling outside the flow extremes. The effect of regulated and minimum flows are a particular problem during the winter season in the western United States. Regulated flows are a function of anthropogenic activity, usually associated with dams and reservoirs. Sampling activities should be avoided during high and low extremes.

Special studies conducted by state agencies in response to specific regulatory requirements or catastrophic events (e.g., oil spills) may not occur in an optimal season. In these situations, the data should be inter-

Special studies may be conducted at any time depending on need; but trend monitoring studies will focus on annual sampling events with varying sampling frequencies.

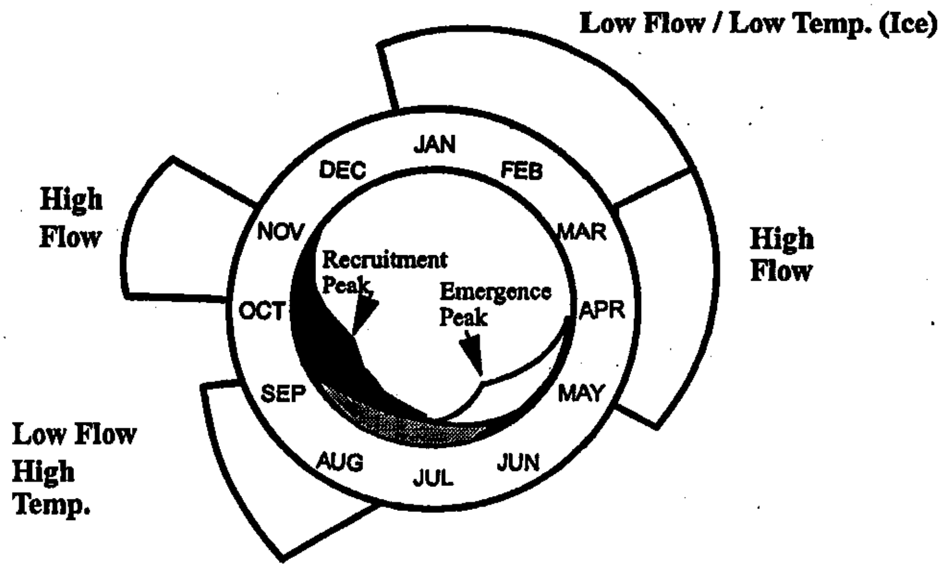

Figure 4-4.—Biological and hydrological factors for sampling period selection in the Northeast (macroinvertebrates). The gray area is the overlap between emergence and recuitment.

In this example (Fig.4-4), sampling in July and early August satisfies most of the criteria for collecting a representative sample at a time of significant chemical contaminant stress. It should be noted that chronic nonpoint source impacts such as sedimentation will be reflected in the quality of the benthic community after flow has returned to near normal following high flow conditions.

In the context of a single population, seasonality may be a significant factor. The early instars are small and difficult to identify, and the young nymphs have a generalized feeding strategy of collecting and scavenging. Only in later instars does feeding specialization occur and the quality of the food source become reflected in the condition of the population. In the case of *Stenonema,* the middle and late instars specialize as scrapers. Scrapers are often considered a pollution sensitive functional feeding group because their food source — diatom algae — responds to the early effects of pollution within the stream.

Periphyton

Periphyton assemblages are associations of algae, bacteria, and fungi that colonize the substrates in a stream. For purposes of bioassessment, most periphyton evaluations focus on diatom algae. The periphyton assemblage exhibits different seasonal abundance patterns than fish or benthos. The key difference is that periphyton assemblages are sufficiently abundant to be collected year-round from streams in temperate zones. Their biologically optimal sampling period may be based on relatively stable conditions but must also account for the comparison of diatom assemblages within similar stages of seasonal succession.

The limiting factors for diatoms are light, temperature, nutrients, water velocity, grazing, and interactions among algae via metabolites. Obviously, the abiotic factors go through an annual cycle of change and, like benthos, the assemblage composition shifts as the changing conditions fa-

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vor new species. This process of seasonal succession creates significant seasonal differences in periphyton assemblages that must be considered in developing a study design. Besides changes in periphyton species composition, additional seasonal issues must be controlled to compare collections among sites and annual trends. Two major considerations are (1) the differences in biomass related to light and temperature regimes and (2) the comparisons of periphyton assemblages that have been subjected to heavy rains and scour with those that have matured under more stable hydrological conditions. Differences in light and temperature regimes may reflect human influences, for example, alterations of the stream channel and removal of riparian vegetation.

Fish

Like periphyton and benthic invertebrates, the fish fauna at a site is likely to vary seasonally. In the Northwest, for example, annual spawning migrations of anadromous salmonids set in motion a seasonal cycle of major importance to the biota. Seasonal migrations of fish are less striking but common in other areas as well. Most frequently, fish movements involve upstream movements in search of spawning areas to serve as nesting and nursery areas for young fish. Upstream areas often provide richer food supplies and lower predation rates than downstream areas.

Because of geographic variation in flows and temperatures, no general pattern occurs across all regions. A seasonal timetable representative of physical conditions and fish assemblage activities in the New England region is illustrated in Figure 4-5. Unless the sampling objective includes the study of unusual flow conditions and concurrent biotic responses, field sampling protocols should avoid extreme flow conditions (low or high) that may represent unusual stress, assemblage instability, or result in danger to field crews.

Sampling in several regions of the country has demonstrated that optimal fish sampling periods can be defined with relative ease. Generally, sampling periods should follow the spring spawning migrations that coin-

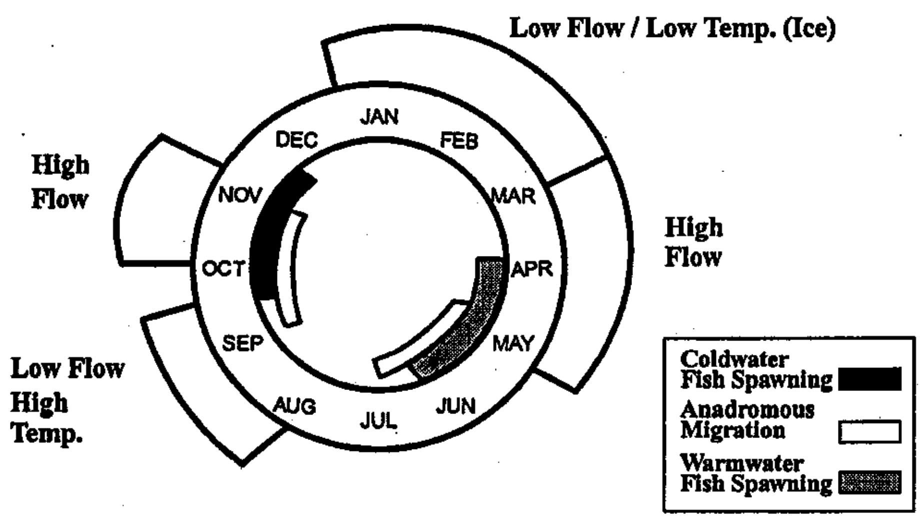

Figure 4-5.—Biological and hydrological factors for sampling period selection in the Northeast (fish).

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preted through concurrent reference data or through a seasonal adjustment to established reference data. If base biocriteria are established for a reference database for a single season, then data collected from the test sites during this season are directly comparable.

Two options are available for collections at test sites during seasons other than that used for base criteria. First, selected reference stations can be sampled concurrently with the test sites to provide baseline comparisons for data interpretation. Criteria established during the optimal season represent a range of values that can be extrapolated to other seasons. In this manner, a percentage of the reference may be acceptable as an alternate criterion.

The selection of an appropriate sampling season depends on the seasonal attributes of the aquatic community, but the administrative issues of sampling efficiency, safety, regulatory requirements, and appropriate metrics for data analysis are equally significant.

The second option may be to develop adjustments for an annual cycle. This can be done through seasonal collections of the reference database to document natural seasonal variation. Alternatively, a knowledge of seasonal appearance and disappearance of particular forms can be used to develop adjustments.

This discussion has focused on the seasonal attributes of the aquatic community. The administrative issues of sampling efficiency, safety, regulatory requirements, and appropriate metrics for data analysis are equally significant and must also be considered in light of the sampling objectives. The following paragraphs consider the sampling protocol in relation to the seasonal attributes of benthic, periphyton, and fish assemblages.

Benthos

Maximum information for a benthic community is obtained when most of its populations are within a size range (later instars) that can be retained during standard sieving and sorting and be identified with the most confidence. Reproductive periods and different life stages of aquatic insects are related to the abundance of particular food supplies (Cummins and Klug, 1979). Peak emergence and reproduction typically occur in the spring and fall, although onset and duration vary somewhat across the United States. During peak recruitment of the young, approximately 80 percent are too small to be captured in sufficient numbers to characterize the community accurately, and the food source requirements for early instars may be different from those for later instars. Therefore, the biologically optimal sampling season occurs following the period of initial recruitment and high mortality of young, and when the food resource has stabilized to support a balanced indigenous community.

The comparative time frames for sampling the benthic community are illustrated in Figure 4-4. The seasonal timetable shows annual high and low flow periods, emergence peaks for aquatic insect communities, and biologically optimal sampling periods (BOSP) for a stream in the New England region. High and low flow correspond to periods of high and low rainfall and associated runoff. Emergence is triggered by average daily temperature and photoperiod and usually occurs at peak intervals in spring and fall. The biologically optimal sampling period falls between the peaks in late winter and late summer and occurs after the population has been exposed to two-thirds of the aquatic phase of the organism's life cycle measured in degree days (that is, in units calculated as the product of time and temperature over a specified interval).

cide with periods of high flow. Most states in eastern North America select the summer period for sampling (June through August) to coincide with periods of low to moderate stream flow and avoid the variable flow conditions of early spring and autumn (Karr et al. 1986). Fish assemblages during summer are relatively stable and contain the full range of resident species, including all major components of age-structured populations. Angermeier and Karr (1986) have outlined sampling rationale, including the merit of excluding young-of-the-year (YOY) from spring and late summer samples. This exclusion reduces variability and the problem of identifying and sampling very small fry. Excluding YOY from most analyses improves reliability and does not weaken the interpretation of the system's condition.

The scenario presented in Figure 4-5 identifies high and low flow periods in early spring and late summer for streams in the northeastern United States. The number of species is likely to peak in the spring with the spawning migration; the number of individuals will peak in the early autumn with the addition of YOY. The biologically optimal sampling period (BOSP) corresponds to seasonal effects within the fish assemblage and to the flow dynamics that influence sampling efficiency. Because the physical condition of the streams affects the efficiency of fish sampling gear, it also affects the nature or quality of the resulting data. For example, the effectiveness of passive equipment (e.g., trap nets) can be substantially reduced during periods of high or low flow, and the efficiency of active equipment (e.g., electrofishing gear) is reduced by turbidity, water temperature, and conductivity.

Decisions about which habitats to sample are critical to the success of a biocriteria program.

Sampling can typically begin in May or June in most areas and proceed into September unless unusually low flow periods occur during late summer drought. The probability that low flow periods will occur in late summer increases in watersheds that have been severely modified by urbanization or agricultural land use, in which case low flow sampling should be avoided.

Selection of Habitat for Aquatic Assemblage Evaluations

Stream environments contain a number of macro-and microhabitat types, including pools, riffles, and raceways, or surface and hyporheic zones. The latter refers to regions of saturated sediment beneath or beside the stream (Lincoln et al. 1982). Larger rivers have even more complex habitat configurations. Because no single sampling protocol can provide accurate samples of the resident biota in all habitats, decisions about habitats are critical to the success of a biocriteria program. These decisions are usually made in concert with the decision about the assemblages to be sampled, the sampling methods to be used, and the seasonal pattern of sampling.

Selection of habitats for sampling may be influenced by institutional requirements, such as sampling and analysis protocols that are part of an existing monitoring program, or the need to develop data that are consistent with a historical database; however, historical approaches should not be retained without careful evaluation of their ability to provide the data necessary to make informed resource decisions in future years.

Periphyton, invertebrates, and fish species in a stream vary in their distribution among major habitats. Depending on the data quality objectives established for the specific project or program, one or more assemblages may be targeted for inclusion in biosurvey activities. Attributes of several potential assemblages and their several advantages were described earlier in this chapter.

A major consideration in the development of bioassessment procedures is whether sampling all habitats is necessary to evaluate biological integrity or whether selected habitats can provide sufficient information.

A major consideration in the development of bioassessment procedures is whether sampling all habitats is necessary to evaluate biological integrity or whether selected habitats can provide sufficient information. The selection of single habitat over multiple habitat, or vice versa, influences study design and may influence selection of the biotic assemblage to be sampled. Some taxa include individuals whose mobility or natural spatial distribution requires multiple habitat sampling.

Generally, fish sampling reduces the need to make more detailed habitat decisions because most fish in small to medium rivers can be sampled using seines or electrofishing methods that efficiently sample all major surface water habitats except hyporheic zones and bank burrows. By sampling the full diversity of stream habitats for fish, the importance of fish movements among microhabitats for resting and foraging is reduced. Efficient sampling of all local habitats limits the problem of correcting evaluations of taxa in case the intensity of sampling varies among the range of available habitats.

Habitats to be sampled for periphyton require different analytical approaches. For example, periphyton assemblages may develop more easily on rigid or hard substrates. Though periphyton can grow on the leaves and stems of macrophytes, more prolific growths are generally seen on the hard surfaces of large substrate particles (e.g., cobble or small boulders). Steinman and McIntire (1986) found that substrate type is one of several characteristics that affect the taxonomic structure of lotic periphyton assemblages. Other factors are the dispersal and colonization rates of taxa in the species pool, competitive interactions, herbivory, chemical composition of the environment, and the character of ecological disturbances. Because it is difficult to remove or collect periphyton from natural substrates (Austin et al. 1981), hard surfaces (either natural or artificial) are usually the focus of sampling efforts. Most strategies for sampling periphyton assemblages are single habitat though other variables introduce additional complexity.

Benthic macroinvertebrates inhabit various habitats in lotic situations, for example, riffles, pools, snags, or macrophyte beds. Complete characterization of the assemblage requires a multihabitat and multisampling protocol such as that advocated by Lenat (1988). The benthic macroinvertebrate protocols for rapid bioassessment advocated by Plafkin et al. (1989) were developed for sampling the most productive and dominant benthic habitat in wadable streams. Consequently, riffles and cobble substrate were the primary focus of the rapid bioassessment protocols because that habitat is predominant across the country.

This approach works for small streams and streams that are dominated by riffles; however, it requires additional evaluation and technical development for use in other habitats. Plafkin et al. (1989) argue that the habitat where riffles predominate, will often be the most productive and stable habitat for the benthic community. The production of the habitat is related to provision of refugia, food resources, and necessary community interactions. It may be necessary to document the extent and character of the habitat because streams differ in these qualities, which differences may be related to natural and anthropogenic causes. In some streams, riffles are not a dominant feature, and the emphasis on them may be misleading.

Since the issuance of the Rapid Assessment Protocols (RBPs) in 1989, rapid assessment techniques have evolved to focus on sampling of more than one habitat type, usually in the proportion of their representation at the sites of interest. These techniques have been primarily designed for low gradient streams (Mid-Atlantic Coastal Streams Workgroup, 1993; Florida Dep. Environ. Prot. 1994) and encompass the sampling of four or five habitat categories.

The sampling of a single habitat type (e.g., riffles or runs) is intended to limit the variability inherent in sampling natural substrates and to enhance the evaluation of attributes in an assemblage that will vary substantially in various habitats. Double, composited square meter kick net samples (2 m^2) are used in RBPs to collect large representative samples from riffle or run areas. Other gear can also be used to collect such composite samples.

Multihabitat sampling allows the evaluation of a broad range of effects on the benthic assemblage. However, it may also introduce variability into comparisons of the benthic assemblage among sites. Multihabitat investigations of water resource integrity are potentially confounded by (1) the absence of a particular habitat at a station, and (2) the potential differences in the quality and quantity of a habitat. As more habitats are sampled, the more difficult it is to control for comparable habitat among sites; and the absence of a habitat type at one or more stations exacerbates the problem. However, some states, such as North Carolina, have been successful in using a multihabitat sampling approach and advocate this technique as being more appropriate than simply sampling the riffle or run (Lenat, 1988).

A case study in association with the North Carolina Department of Environmental Management addressed the issue of sampling strategy and indicated that the riffle assemblage and the multihabitat assemblage responded similarly to differences among stations (Plafkin et al. 1989). For example, under stress, taxa richness was reduced by the same proportion in both the riffle and the multihabitat assemblage samples at a given station. These responses suggest that either the riffle assemblage or the multihabitat assemblage can be used to assess biotic integrity in streams in which riffles are prevalent.

The choice of sampling habitats also entails a choice of sampling methods.

Kerans et al. (1992) examined patterns of variability and the contribution of pool versus riffle invertebrate samples to the evaluation of biotic integrity and the detection of different kinds of degradation. They evaluated over a dozen attributes of the invertebrate assemblages including numbers of species (total and for a number of taxa) as well as several ecological classifications. At least eight attributes exhibited spatial or temporal trends, or both, depending on whether the habitat was pools or riffles. Attributes that were temporally and spatially unpredictable included some that are most commonly used in stream bioassessment. Kerans et al. conclude that measures of human impact on biotic integrity may be biased if sampling is restricted to only one habitat.

The choice of sampling habitats also entails a choice of sampling methods because conventional sampling methods for invertebrates vary in their efficiency among habitats. Surber and Hess samplers are used for riffles, while grab samplers are used most efficiently in the soft substrate of pool habitats. Several forms of net samplers have been developed for various stream habitats: kick nets or seines (Plafkin et at. 1989; Lenat, 1988), D-frame nets (Montana Dep. Health Environ. Sci., 1990), and slack (rectangular frame) samplers (Cuffney et al. 1993). Passive colonization-dependent samplers (e.g., Hester-Dendy samplers) may also be used for evaluation of invertebrate assemblages (Ohio Environ. Prot. Agency, 1987).

Substrate Choices

In either the single habitat or multihabitat approach, the most prevalent and physically stable habitat that is likely to reflect anthropogenic disturbance in the watershed should be chosen.

In either the single habitat or multihabitat approach, the most prevalent and physically stable habitat that is likely to reflect anthropogenic disturbance in the watershed should be chosen. These habitats will vary regionally because of differences in topography, geology, and climate. The biological community in a particular stream may also change in response to increasing stream size (Vannote et al. 1980). The key to sampling, pertinent to benthic invertebrate surveys, is to select the habitats that support a similar assemblage of benthos within a range of stream sizes. Habitats that have been used for benthos are riffles, snags, downed trees, submerged aquatic vegetation, shorezone vegetation, and sediments, such as sand, silt, or clay (Table 4-2).

The habitat with the most diverse fauna is emphasized by most investigators because it offers the highest probability of sampling the most sensitive taxa. Riffles usually fit this criterion, and when present, are preferred. This habitat type is followed by hard, coarse substrates, snags, aquatic vegetation, and soft substrates. If multiple habitats are selected, similarity in habitat quality and comparable levels of effort among sampling sites must be considered.

Natural and Artificial Substrates

The habitat with the most diverse fauna is preferred — riffles followed by hard, coarse substrates, snags, aquatic vegetation, and soft substrates.

Most benthic surveys employ direct sampling of natural substrates. This method is particularly important if habitat alteration is suspected as the cause of impairment. A major assumption is that every habitat has a biological potential, which is reflected in the resident biotic community. Be-

Table 4-2.—Common benthic habitats.

SNAGS/DOWNED TREES	SHOREZONE VEGETATION
• Productive in blackwater streams (Benke et al. 1984)	• Present in most streams
• Diversity of epifauna	• Measures riparian impacts
• Community dependent on well-prepared substrate	• Dominated by shredders and collectors
	• May be seasonal
SUBMERGED AQUATIC VEGETATION	**SILT/MUD**
• Productive in coastal zones	• Pool communities
• High standing crop	• Dominated by fauna
• Seasonal habitat	• Sediment quality and water quality effects
• Snails usually abundant	• Fauna usually tolerant to low oxygen
SHIFTING SAND	**LEAF LITTER/DEBRIS**
• Prevalent in erosional areas	• Prevalent in forested streams
• Dominated by opportunistic infauna	• Measures riparian impacts
• Sediment quality and water quality effects	• Dominated by shredders
• High dominance by monotypic fauna	• Microbial preparation of substrate

cause interpretation depends on the level of assemblage development within the existing habitat, sampling natural substrates is recommended. If, however, an artificial substrate can be matched to the natural substrate (e.g., using a rock basket sampler in a cobble substrate stream), then such artificial substrates may also be used (Sci. Advis. Board, 1993). Maine uses this rock basket approach. The Ohio EPA biocriteria program (Ohio Environ. Prot. Agency, 1987) has successfully used Hester-Dendy multiplate artificial substrate samplers supplemented by qualitative, natural substrate samples to assess biological integrity using benthic assemblages.

The advantages and disadvantages of artificial substrates (Cairns, 1982) relative to natural substrates are the following:

■ **Advantages of Sampling with Artificial Substrates**

1. Enhances sampling opportunities in locations that are difficult to sample effectively.
2. Permits standardized sampling by eliminating subjectivity in sample collection technique.
3. Minimizes confounding effects of habitat differences by providing a standardized microhabitat.
4. Directs the interpretation to specific water quality questions without interference of habitat variability.
5. Increases the ease of placing samplers in discrete areas to discriminate impacts associated with multiple dischargers.

■ **Disadvantages of Sampling with Artificial Substrates**

1. Requires the investigator to make two trips for each artificial substrate sample (one to set and one to retrieve).
2. Measures colonization potential rather than resident community structure.
3. Allows problems such as sampler disturbance and loss to occur.
4. Complicates interpretation of the effects of habitat structure.

If artificial substrates are selected, the surface area of the materials should be standardized among units. Introduced substrates, in the context of biological monitoring, are artificial substrates that are constructed to match natural bottom materials at the site of the survey. An example of introduced substrates are rock baskets, such as those used by Maine (Davies et al. 1991), in which baskets that contain rocks native to the region of known surface area are partially buried in the bottom sediment. Where possible, the use of introduced substrate is preferable to other types of artificial substrate as recommended by the SAB (1993). Rock baskets or other substrates should be placed in waters of similar depths, velocities, and daily sun and shade regimes.

Standardization of Techniques

Standard operating procedures should be adhered to in all phases of fieldwork, data analysis, and evaluation. Such standards are essential for maintaining consistency and comparability among data sets and for appropriate quality assurance and control (Kent and Payne, 1988; Klemm et al. 1990; Smith et al. 1988). Without standard operating procedures to mimic previous studies, the difficulties encountered in comparing temporal and spatial data or analytic results may be substantial. The inherent variability of the sampling process (Cairns and Pratt, 1986) can be reduced through standardization of sampling gear, gear efficiency, level of effort, subsampling methods, handling and processing procedures, and computer software. Standardization of project activities provides considerable strength in reducing, controlling, and understanding variability.

Standard operating procedures should be adhered to in all phases of fieldwork, data analysis, and evaluation. Such standards are essential for maintaining consistency and comparability among data sets and for appropriate quality assurance and control.

Sample Collection

A major influence on the comparability of field ecological projects is the type and intensity of appropriate training and professional experience for all personnel (Barbour and Thornley, 1990). Similar exposure to sampling methods and standard operating procedures can reduce the amount of variation from one sampling event or project to the next. Standardizing the equipment relative to operator efficiency, sampling effort, and the area to be sampled greatly affects data quality. Operator efficiency depends on the operator's experience, dexterity, stamina, and adherence to specified survey requirements. Physical habitat conditions at the time of sampling (e.g., flow levels, current velocity, and temperature) also influence efficiency. Active sampling efforts (e.g., using net samples or electrofishing) may be standardized as a function of person-hours spent at each sampling station and by tracking the physical area or volume sampled. Passive methods (e.g., artificial substrates, trap nets) may be standardized by tracking the person-hours and the exposure time. This choice is often dictated by the earlier selection of the assemblage to be sampled; for some, a relatively small selection of sampling techniques may be available. A certain sampling area or volume may be required to obtain an appropriate sample size from a particular community and to estimate the natural variability of that community at the sampling station.

Once the assemblage, sampling equipment, and method have been chosen, standard operating procedures can be written for field operations, including a clear description of the sampling effort to be applied during each sampling event. All employees should have this documentation, and new employees should be accompanied in the field by experienced staff until they are thoroughly familiar with all procedures (Ohio Environ. Prot. Agency, 1987).

Processing samples in the field requires several critical steps. Sample containers for benthic invertebrates and voucher fish should be marked with appropriate and complete information on internal and external labels. Other identifying information and descriptions of visual observations should be recorded in a field notebook.

Data on birds and mammals, which consist primarily of visual observations and for which accurate field taxonomy is possible, will not require subsequent processing in the laboratory. However, the details of each observation should be carefully recorded so that they may be checked later. Most fish sampling requires sorting, recording, and releasing the fish at the site of capture. Fish sampling crews should have a reference collection available in the field, and specimens should be collected and accurately labeled so that identifications can be confirmed.

Sample containers with preserved specimens should be assigned unique serial or identification numbers. These numbers should be recorded in a logbook along with the appropriate labeling information. All sample containers or specimens should be appropriately packaged for transportation and continued processing in the laboratory.

For assemblages in which extremely large numbers of individuals or associated substrate are obtained in each sample as is often the case with small fish, benthic macroinvertebrates, periphyton, or planktonic organisms, it may be impractical and costly to process an entire sample. In such cases, standardized random subsampling, similar to that recommended by Plafkin et al. (1989), is a valid and cost-effective alternative.

Standardized random subsampling is a valid and cost-effective alternative to processing an entire sample. As a subsampling method is developed, every attempt must be made to reduce bias.

As a subsampling method is developed, every attempt must be made to reduce bias. Therefore, guidelines are needed to standardize the effort and to eliminate investigator subjectivity. Rapid bioassessment protocols, for example, maintain subsampling consistency by defining the mode (a gridded pan), by placing limitations on the mechanics of subsampling and the subsample size, and by assuring that the subsampling technique is consistently random.

Sample Processing

The need for specialized training and expertise is most necessary during the identification of organisms. Unless the project objectives direct otherwise, each specimen should be identified to the most specific taxonomic level possible using current literature. Some techniques may require identification only to the ordinal, familial, or generic level (Ohio Environ. Prot. Agency, 1987; Plafkin et al. 1989), but the most accurate information on tolerances and sensitivities is found at the species level.

Nevertheless, taxonomic resolution should be set at a level achievable by appropriately trained state personnel. State water resource agencies should find it beneficial to establish collaborative working arrangements with local and regional experts who can provide training, technical support, and quality assurance and control. Stream ecology research over the last decade indicates that a specific minimal level of resolution should be set (i.e., the "lowest achievable taxonomic level" is not a helpful criterion) and that additional refinement should be left to individual state groups as their capabilities permit (Sci. Advis. Board, 1993).

The SAB further states that proposed levels of intensity and taxonomic resolution must receive a thorough evaluation by the scientific research community. For example, adult and juvenile fish should usually be identifiable by species (Sci. Advis. Board, 1993). The identification of larval fish may provide useful information; however, it may only be feasible to identify them to the generic or familial levels. Reasonable candidate levels for stream macroinvertebrates are given in Table 4-3.

Once the samples have been analyzed (identified, enumerated, and measured), reference (voucher) material should be placed in the well-estab-

Table 4-3.— Proposed minimal levels of taxonomic resolution for stream macroinvertebrates (taken from Sci. Advis. Board, 1993).

TAXONOMIC LEVEL	GROUPS
Genus	Plecoptera (in part), Ephemeroptera, Odonata, Trichoptera, Megaloptera, Neuroptera, Lepidoptera, Coleoptera (in part, larvae and adults), Hemiptera, Diptera (Tipulidae and Simulidae), Crustacea, Mollusca
Tribe	Chironominae
Subfamily	Chironomidae
Family	Diptera (other than Chironomidae, Tipulidae and Simulidae), Oligochaeta, Plecoptera (in part), Coleoptera (in part)
Order	Other noninsect groups

lished network of federal, state, and university museums for *regionally centralized curation* (Sci. Advis. Board, 1993). This action ensures a second level of quality control for specimen identification. Preferably, collection and identification of voucher specimens will be coordinated with taxonomic experts in regional museums. These repositories, which have always been the centers for systematics, should continue to be used for this function (Sci. Advis. Board, 1993). The SAB recommends that once the information on the samples has been entered into a database and verified, the repository institutions should be encouraged to conduct additional systematic studies on the material. Information from these additional analyses can then be made available to state biocriteria programs.

All identifications should be made using the most up-to-date and appropriate taxonomic keys. Verification should be done in one of two ways: (1) by comparison with a preestablished reference or research specimen collection, or (2) by having specimens confirmed by taxonomic experts familiar with the group in question (Borror et al. 1989). A regional consensus of taxonomic certainty is critical to ensure that the results are comparable both spatially and temporally. The taxonomists should always be contacted by telephone or mail before any specimens are sent to their attention. It is also important to follow their advice on the proper methods for packing and shipping samples. Damaged specimens may be useless and impossible to identify.

Suggested Readings

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