

# Chapter 3

## Approach to Assessing Biological Condition

*The NAWQA assessment of ecological health in the Nation's streams is based on an analysis of the condition of three biological communities—algae, macroinvertebrates, and fish. The biological condition assessment followed a study design using nationally consistent sampling and analytical methods in streams within 51 river basins across the Nation. Assessment methods accounted for variability in biological communities associated with natural differences among geographic regions. Chemical, hydrological, and other environmental data were integrated with biological condition to examine relations between land use and stream health. This chapter summarizes the primary features of the study design and provides the context for understanding findings about stream health across the Nation.*



U.S. Geological Survey photo by Stephen Moulton.

*The objectives of this assessment were to determine the health of streams in various land-use settings and investigate the factors related to reduced stream health.*

## Targeted Sampling Across the Nation's Diverse Land Uses and Natural Settings

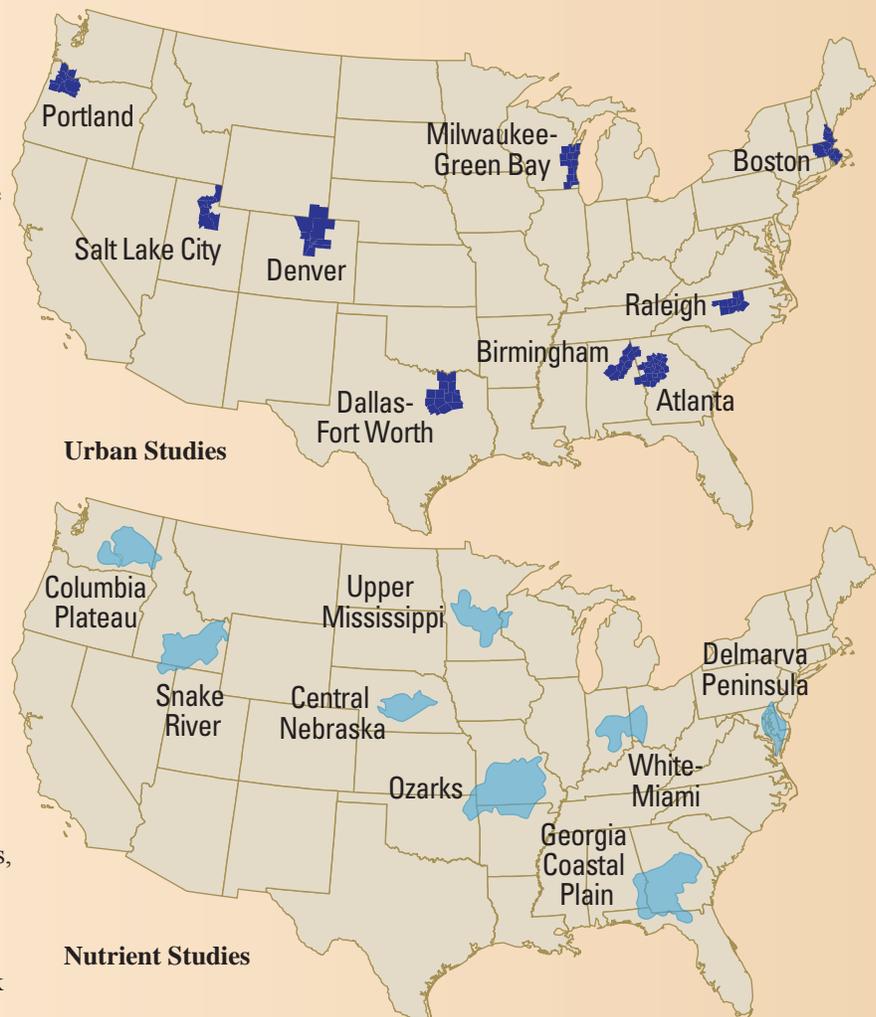
This report is based primarily on results of NAWQA assessments from 1993–2005 that were conducted in 51 major river basins across the United States (referred to as study units). Collectively, the 51 NAWQA study units cover a substantial part of the Nation's land area, accounting for more than 70 percent of total water use and spanning a wide range of hydrologic and environmental settings. Such an approach gives priority to understanding the chemical and physical factors—natural and manmade—affecting stream health in diverse environmental settings.

The primary objectives of this biological assessment were to (1) determine the health of streams—based on assessments of the condition of biological communities—in agricultural, urban, and mixed land-use watersheds and (2) investigate how land and water use influence the chemical and physical factors that reduce biological condition and ultimately stream health. Streams in this report are defined as being wadeable, regardless of named designation (for example, brook, creek, river). In addition to this report, two companion NAWQA studies also assessed stream biological condition (see sidebar, below).

### Focused Biological-Condition Assessments in Urban and Agricultural Settings

NAWQA investigated the effects of urbanization on stream ecosystems in nine metropolitan areas in the conterminous United States (upper map). These studies were done to provide information and understanding to urban planners and those seeking ways to restore stream health in urban areas. A summary of these studies is provided in U.S. Geological Survey Circular 1373 (<http://pubs.usgs.gov/circ/1373/>), and further details are available at <http://water.usgs.gov/nawqa/urban/>.

NAWQA conducted an intensive study of nutrient enrichment—elevated concentrations of nitrogen and phosphorus—in streams in eight agricultural basins in the conterminous United States (lower map). These studies were done to improve understanding of how nutrients influence stream ecosystems, which will provide information for developing nutrient criteria to protect stream health in different geographic regions. Details on these studies and a link to reports is available at <http://wa.water.usgs.gov/neet/>.



The NAWQA approach targeted specific land-use settings among the diverse natural settings across the Nation. Assessed streams were primarily located in areas of agricultural and urban development because of (1) the possible physical and chemical effects of these land-use activities on biological condition (chapter 2) and (2) to meet the needs of local stakeholders. The agricultural areas are diverse in climate, geography, and crop types, including corn and soybeans in the Midwest; wheat and other grains in the Great Plains; rangeland in the Southwest; and grains, fruits, nuts, vegetables, and specialty crops in California and the Pacific Northwest. The urban areas also represent diverse environmental settings, including New England coastal basins, the southern Appalachians, the mid-Atlantic Piedmont, northern and southern Midwest plains, arid western basins, and the Pacific Northwest. Other assessments were made in Alaska and Hawai‘i. Most assessments in urban areas focused on residential land with low-to-medium population densities (300 to 5,600 people per square mile) (Hitt, 1994). Some commercial or industrial areas also were included, but point sources and extensive industrial and urban areas generally were not assessed (Gilliom and others, 2006).

### Features of NAWQA’s Biological-Condition Assessment

This biological-condition assessment provides a national perspective on understanding water-quality issues in relation to land use and water-resources management. Listed below are several characteristics and limitations of the NAWQA approach that are important to consider when interpreting the findings presented in this report.

- Assessments include measures of three biological communities (algae, macroinvertebrates, and fish), which is not common among monitoring programs in the United States. A survey of 65 State and other monitoring programs in 2001 showed that macroinvertebrates are the most widely used community (86 percent of programs), followed by fish communities (63 percent) and algal communities (31 percent). In addition, 69 percent of programs use two or more communities in biological assessments, whereas 25 percent use all three communities (U.S. Environmental Protection Agency, 2002).
- Assessments include both geographically extensive and time-intensive sampling. Many sites are visited once and are generally distributed throughout a large geographic area or region. In addition, many repeated measurements of chemistry and biological communities are made at a smaller set of selected sites, because they are indicative of specific land-use features, such as urban development. Time-intensive sampling at a few fixed sites provides much needed understanding of the temporal dynamics and long-term trends of important chemical and physical factors, whereas geographically large study areas provide a broader regional context of water-quality conditions.
- Daily streamflow measurements are included in most fixed-site monitoring. Long-term streamflow monitoring provides crucial understanding of the hydrological context (that is, wet, dry, or average seasonal rainfall) of study sites and the streamflow conditions crucial to stream health.
- Specific land-use settings in a wide range of hydrologic and environmental settings are targeted across the Nation. This approach gives priority to understanding crucial factors influencing water quality and biological condition in these land-use settings but does not provide a representative sample of all stream segments within a given region of the Nation (see next page).

**Land-use classification**

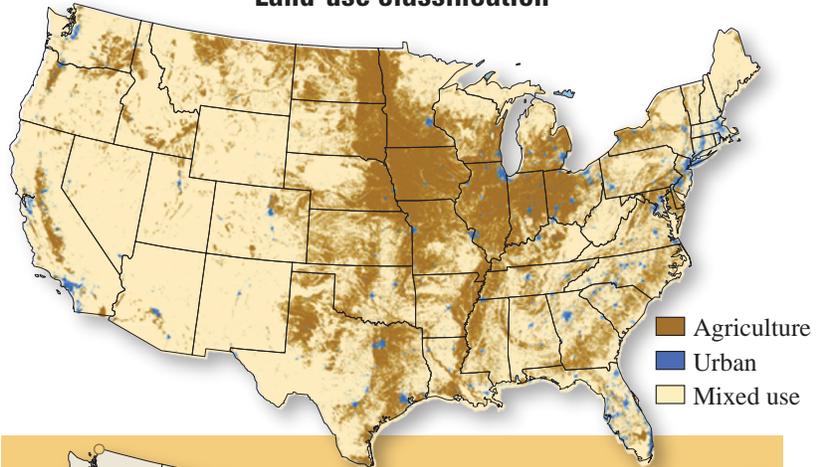
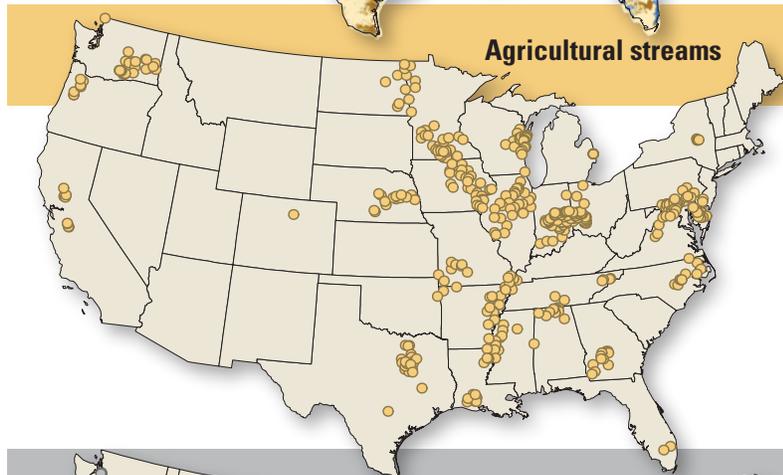


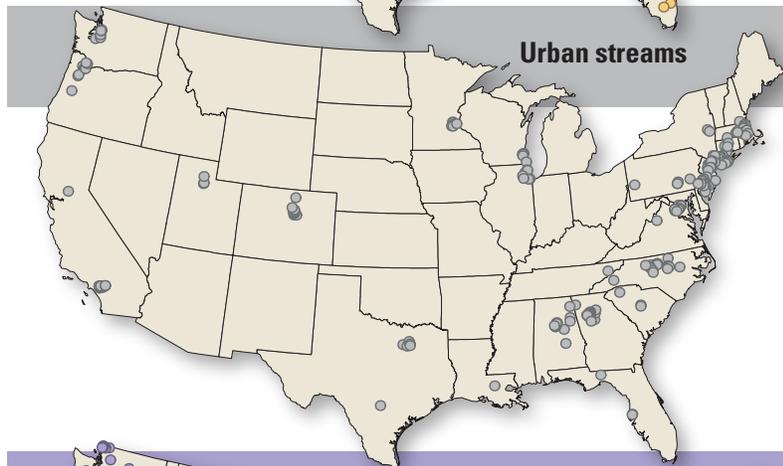
Table showing criteria used by NAWQA to classify streams assessed for biological condition by the dominant land use in their watersheds (modified from Gilliom and others, 2006; Dubrovsky and others, 2010).

Land-use classification	Watershed land-cover criteria
Agricultural	50 to 100 percent agricultural land and 0 to 5 percent urban land
Urban	25 to 100 percent urban land and 0 to 24 percent agricultural land
Mixed use	All other combinations of urban and agricultural land

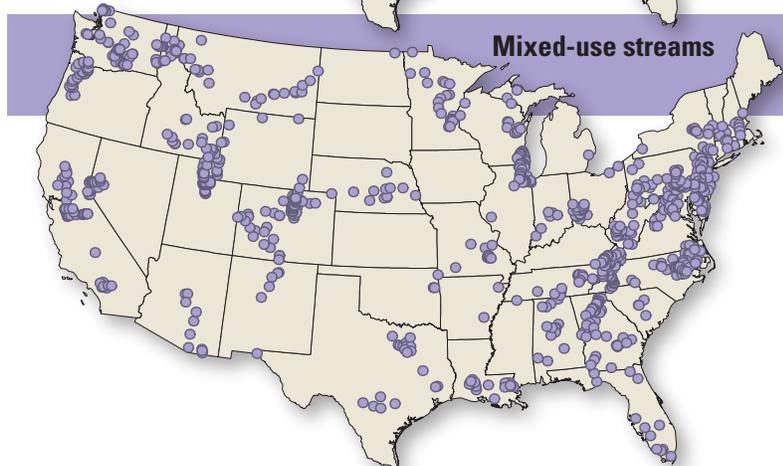
**Agricultural streams**



**Urban streams**



**Mixed-use streams**



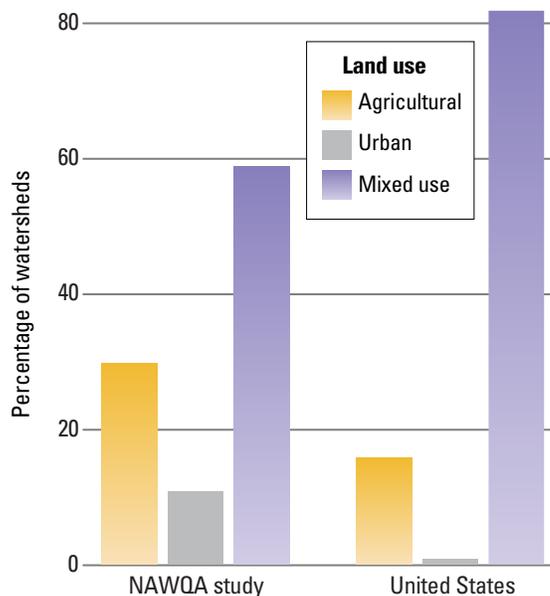
These maps of the conterminous United States show land-use classifications and stream-sampling sites classified as agricultural, urban, or mixed use by NAWQA. Stream sampling sites (dots) were distributed across the Nation's diverse environmental settings to assess biological condition within specific types of land uses.

## NAWQA's Biological Assessment in a National Context

Consistent with the design of NAWQA's investigations, which targeted specific land-use settings, the biological condition findings in this report are presented by land-use category. Each stream assessed by NAWQA was classified into one of three land-use categories—agricultural, urban, or mixed use (see opposite page)—on the basis of the predominant land cover in its watershed (Gilliom and others, 2006). Most streams that were classified as agriculture or urban also commonly have small amounts of other land uses in their watersheds. Streams classified as “mixed use” represent a mix of two or more land uses and do not meet the criteria for individual agricultural or urban settings. Mixed-use streams range in their intensity of development, including some (about 5 percent) that are influenced by large amounts of agricultural and urban land (draining greater than 50 percent of agricultural land and 25 percent of urban land) and some with little agricultural or urban development.

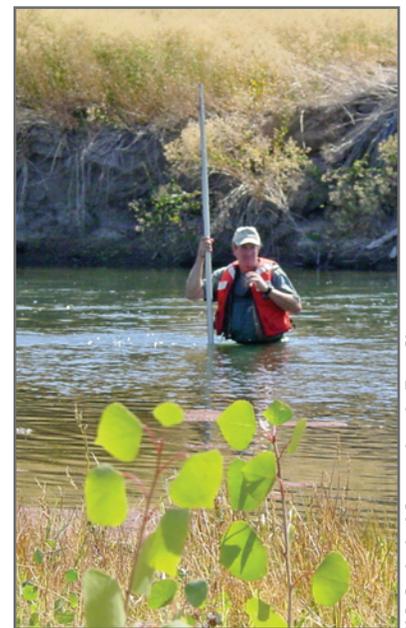
By design, NAWQA's assessment over represented urban and agricultural streams and under represented those within all other land uses, relative to their occurrence throughout the conterminous United States. For example, urban streams represent about 1 percent of all streams in the conterminous United States but represent nearly 10 percent of the sites sampled by NAWQA. Agricultural streams represent less than 20 percent of all streams in the conterminous United States but represent about 30 percent of the sites sampled by NAWQA.

As shown by this bar graph, NAWQA's targeted sampling design for assessing the biological condition of the Nation's streams emphasized streams that drain agricultural and urban watersheds. Targeted watersheds are shown relative to the actual percentage of such watersheds in the United States. (Modified from Gilliom and others, 2006.)



## Chemical and Physical Measurement and Assessment

USGS scientists with NAWQA made a wide variety of water-chemistry and physical measurements at sites where biological communities were sampled. Chemical sampling of water included analyses of nutrients (1,504 sites), major ions (1,309 sites), dissolved pesticides (593 sites), and contaminants associated with streambed sediments (414 sites). All chemical samples were analyzed at the USGS National Water-Quality Laboratory, Denver, Colorado, and all field and laboratory protocols are available at <http://water.usgs.gov/nawqa/bib/>. Measures of physical habitat were also made at 920 sites where biological condition was assessed. Habitat measurements included the characterization of channel morphology, substrate types, riparian canopy, and water depth and velocity. NAWQA protocols for characterizing stream physical habitats are available at <http://water.usgs.gov/nawqa/bib/>.



U.S. Geological Survey photo by Terry Short.

Photograph of a U.S. Geological Survey scientist making physical measurements of a stream.



U.S. Geological Survey photo.

U.S. Geological Survey gage measuring stream flow.

Sampling sites were considered to have elevated salinity if measured electrical conductivity of the stream water exceeded regional background levels established in a recent national assessment (Van Sickle and Paulsen, 2008). The occurrence of biological alteration was compared between streams with and without excess salinity at 1,808 sites where conductivity and biological communities had been sampled at the same times.

Sampling sites were classified into one of three broad categories of nutrient status using existing criteria (Dodds and others, 1998). This simple classification scheme was used to compare biological condition to nutrient status at 1,504 stream sites across the Nation where nutrients and biological communities had been sampled at the same times.

Streamflow modification was assessed at 2,888 sites with USGS gaging stations by comparing observed magnitudes of annual (1980–2007) high and low flows to those expected in the absence of manmade disturbances in the watershed. Expected flows were estimated for each assessed site with statistical models developed from a set of 1,059 hydrologic reference sites (Falcone and others, 2010; Carlisle and others, 2010). Daily streamflows were monitored for at least 5 years before making assessments of algal, macroinvertebrate, and fish condition at 283, 274, and 237 sites, respectively.

Water temperature modification was assessed at 2,149 stream sites where continuous monitoring had been conducted for at least one summer during 1999–2009. The observed summertime mean water temperature at each site was compared to an expected natural temperature, which was estimated from statistical models (Hill and others, 2013) similar to those used for assessing streamflow modification.



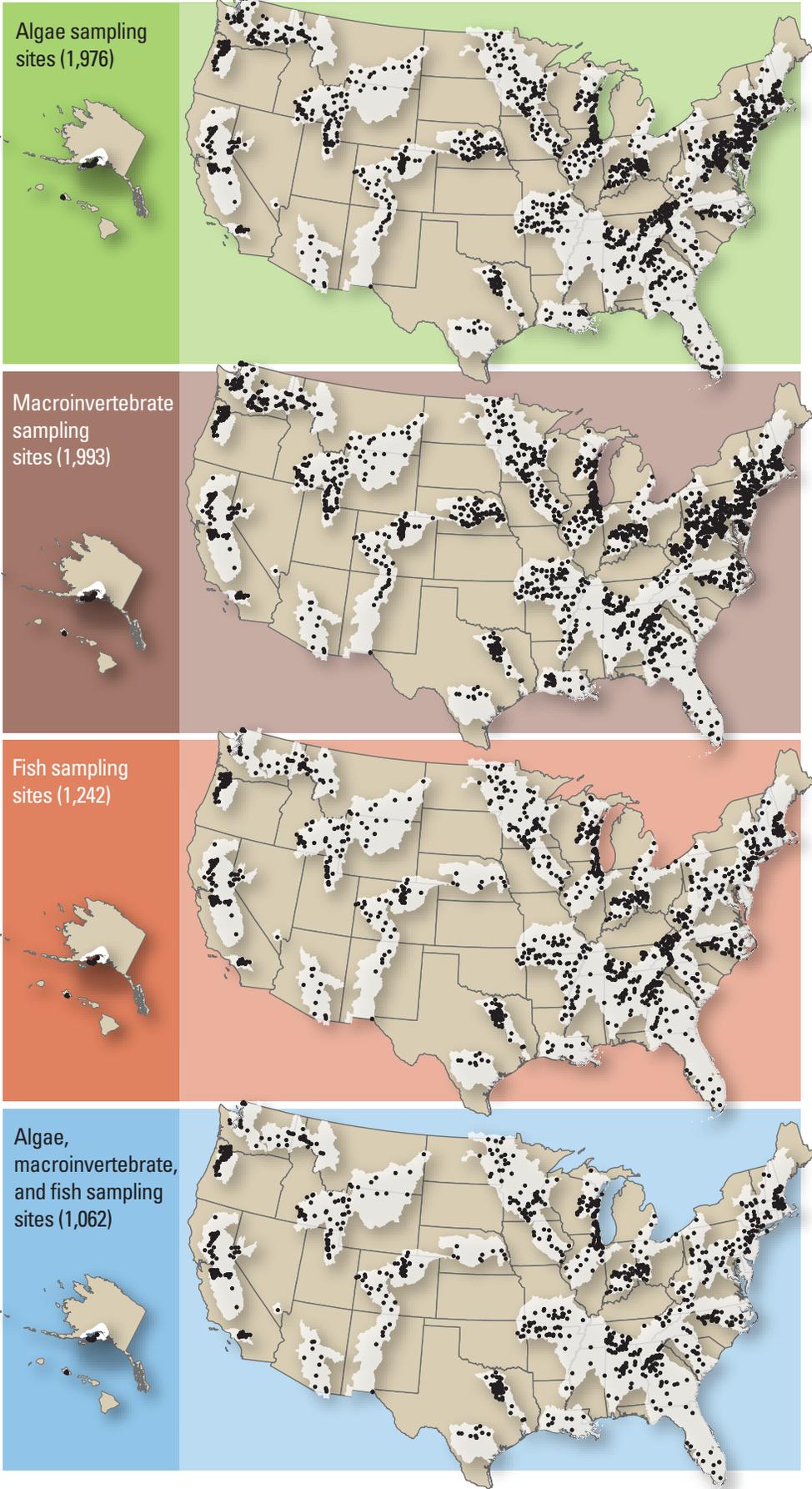
U.S. Geological Survey photos by Martin Gurtz, Douglas Harned, and Rodney Knight.

Photographs of biological samples being collected by U.S. Geological Survey scientists. Left, collecting algae from the surface of a rock; middle, sampling macroinvertebrates in a stream riffle; right, using electrofishing to stun fish for collection and examination.

*As a measure of stream health, NAWQA sampled and assessed the condition of three unique biological communities—algae, macroinvertebrates, and fish.*

## Biological Sampling

Nationally consistent field sampling methods developed by NAWQA for algae, macroinvertebrates, and fish made it possible to compare results across a wide variety of stream types and geographic locations. NAWQA's assessments of algae, macroinvertebrates, and fish were derived from samples collected at 1,976, 1,993, and 1,242 stream sites, respectively, using published methods (<http://water.usgs.gov/nawqa/bib/>). Samples of all three communities were collected at 1,062 stream sites. Biological sampling was generally conducted during predetermined time periods, typically during low stream flows. The analysis of these samples was accomplished using consistent methods with continual quality assurance and data management (see Assessment Tools sidebars). Macroinvertebrate and algal samples were processed at the USGS National Water Quality Laboratory (Denver, Colorado) and Academy of Natural Sciences of Drexel University (<http://ansp.org>), respectively, using published methods (NAWQA field and laboratory protocols can be accessed at <http://water.usgs.gov/nawqa/bib/>). All biological data collected by NAWQA are publicly available (see Assessment Tools sidebars).



Maps showing sites in the United States (dots) where biological samples were collected during NAWQA studies. Algal, macroinvertebrate, and fish communities were sampled in streams within NAWQA study units (tan shading) across the Nation. All three communities were sampled at a subset of these sites (bottom panel). Alaska and Hawai'i not shown to scale.

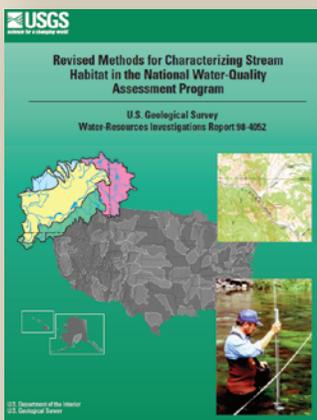
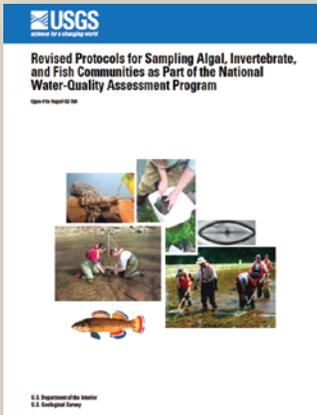


# Assessment Tools

## Field Sampling and Taxonomic Quality Assurance

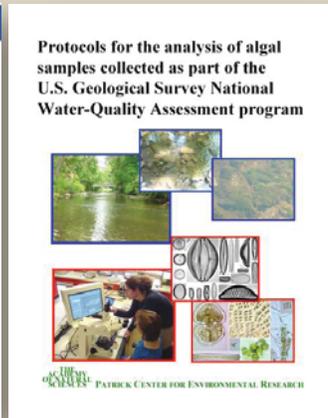
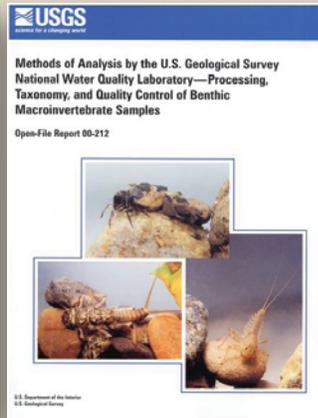
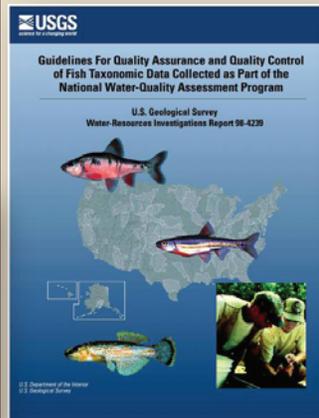
### Field Sampling

Nationally consistent field sampling methods developed for algae, macroinvertebrates, and fish, and their habitats made it possible for U.S. Geological Survey (USGS) scientists to compare results across a wide variety of stream types and geographic locations.



### Taxonomic Quality Assurance and Sample Processing

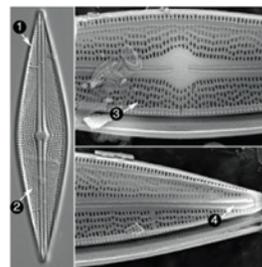
Data sharing depends on strict quality assurance of taxonomic identification, consistency, and resolution. Standard procedures for quality assurance and control are published for algal, macroinvertebrate, and fish community samples. Representative individuals of each taxon (taxonomic unit) collected are maintained in "voucher" collections that allow comparisons with other contemporary and future sampling programs and will potentially be useful for evaluating changes in species and genetic composition (see, for example, Walsh and Meador, 1998).



Links to these and other resources are available at <http://water.usgs.gov/nawqa/bib/>.



**Diatom identification guide & ecological resource**  
for water resource managers, ecologists, taxonomists, analysts, systematists, students, and the public.



*Brachysira serians*

**Getting Started**  
[ABOUT THIS WEBSITE](#)  
[WHAT ARE DIATOMS?](#)  
[IDENTIFY YOUR TAXA](#)

**Special News**  
25 March, 2011  
Information for new contributors  
  
01 March, 2011  
2011 North American Diatom Symposium,  
14-18 September

### Tool for Taxonomic Consistency

USGS and the U.S. Environmental Protection Agency cooperated to develop a single comprehensive source of taxonomic and ecological information for diatoms of the United States. The guide can be accessed at <http://westerndiatoms.colorado.edu/>.



## Assessment Tools

### Nationally Consistent Ecological Data and Tools for Interpretation

#### A National Database of Aquatic Bioassessment Data

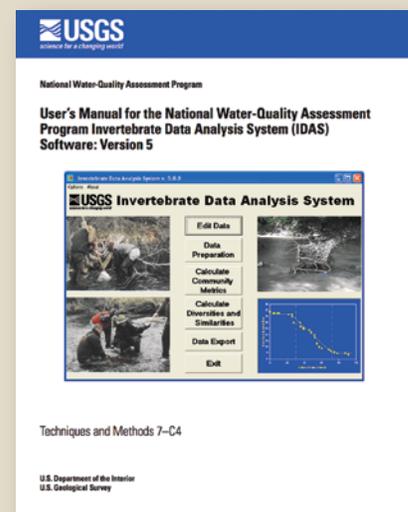
The U.S. Geological Survey (USGS) BioData Retrieval system provides access to aquatic bioassessment data (biological community and physical habitat data) collected by USGS scientists from stream ecosystems across the Nation. USGS scientists collect algal, macroinvertebrate, and fish community data, as well as stream physical habitat data, which is part of the USGS's fundamental mission to describe and understand the Earth. The publicly available BioData Retrieval system disseminates data from more than 15,000 fish, aquatic macroinvertebrate, and algal community samples. Additionally, the system serves data from more than 5,000 physical datasets (samples), such as for reach habitat, that were collected to support the community sample analyses. Scientists, resource managers, teachers, and the public can retrieve data using an online query. BioData can be accessed at <http://aquatic.biodata.usgs.gov>.

#### Nationally Consistent Biological Data Advance Environmental Assessment and Basic Science

Biological data collected by NAWQA have been successfully used by other monitoring organizations and scientists to address questions ranging from local issues to continental-scale phenomena. One of the greatest strengths of the data is the consistency with which they have been collected across a wide geographic area. For example, Passy (2008) used algal data collected across the Nation to describe continental-scale patterns in diatom distributions. Similarly, NAWQA macroinvertebrate data from reference-quality sites across the Nation were used to establish baseline conditions in the U.S. Environmental Protection Agency's Wadeable Streams Assessment (U.S. Environmental Protection Agency, 2006a). Fish-community data collected by NAWQA were used by Mitchell and Knouft (2009a) to examine nationwide patterns of invasive fish species.

#### Data Processing Tools Facilitate Analysis and Interpretation

Tools for analyzing biological data help in making comparisons of results among sites at local, regional, and national scales. For example, the Invertebrate Data Analysis System (IDAS) allows users to resolve taxonomic discrepancies, calculate a wide variety of macroinvertebrate metrics and indices, and export data to other analysis software. Similar software has been developed for analyzing algal community data based on algal attributes compiled by USGS biologists (<http://pubs.usgs.gov/ds/ds329/>).



## Assessing Biological Condition—The Reference Concept

Biological reference sites are needed to establish a baseline or expectation in most biological-condition assessments. Implicit in the concept of reference sites used in this assessment is that such sites are in reality least-disturbed or potentially best attainable given the current degree of human influence on the Nation's landscapes (Stoddard and others, 2006). Few, if any, streams are totally unaffected by human activities, particularly considering historical disturbances (such as timber harvesting) or atmospheric inputs of pollutants from global sources. Also, the level of historical disturbance varies widely across the country. For example, biological reference sites closely approximate pristine conditions in areas that are within protected wilderness, parks, and nature preserves. In contrast, biological reference sites in the Midwest are in watersheds that historically experienced intensive transformations from prairie to farmland but are currently among the least-disturbed watersheds in that region—such as those with protected riparian buffers.

A consequence of variation in the quality of reference sites is that assessments in some areas of the country are based on a lower expectation than those in regions where more natural reference sites exist. Despite this difficulty, biological assessments are still meaningful because they express the degree to which biological communities in a stream differ from those in streams that are least-disturbed in a particular region.

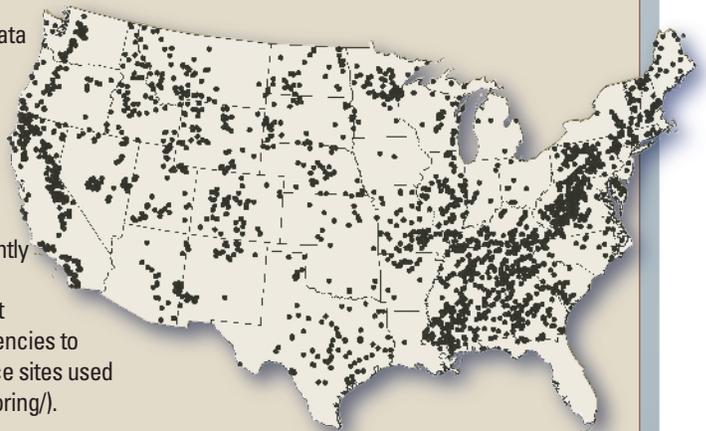
To characterize reference conditions, biological data from USGS, the U.S. Environmental Protection Agency, and select State agencies were combined. Reference sites were identified from this large set of sites by evaluating watershed and riparian land-cover disturbance, applying site-specific measures of habitat and chemical conditions, and using professional scientific judgment (Herlihy and others, 2008). Separate reference sites were identified and used for each biological community. Nationwide, algal and macroinvertebrate communities, as well as fish communities, were assessed using 276, 585, and 1,238 reference sites, respectively. Differences in numbers of reference sites among biological communities are largely due to data availability. Reference site biological data were archived for public use (see sidebar, below).

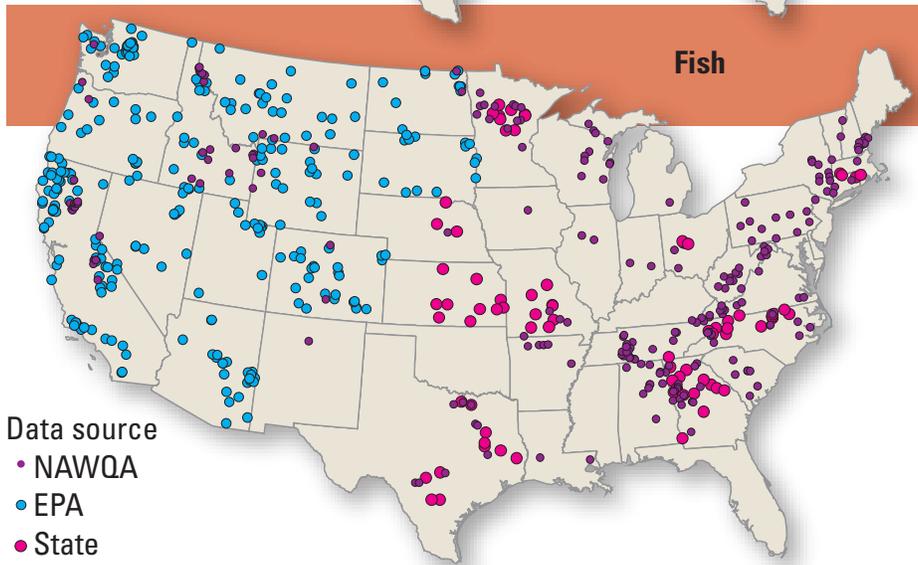
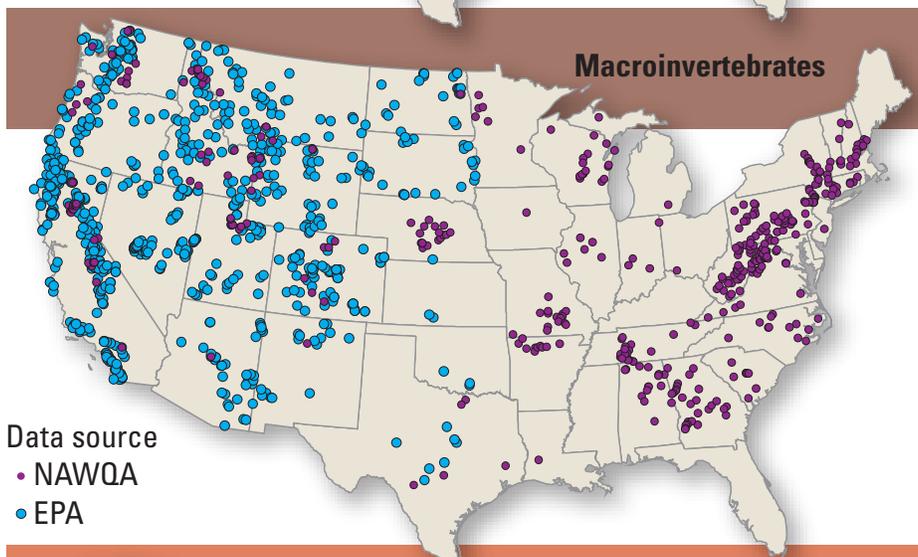
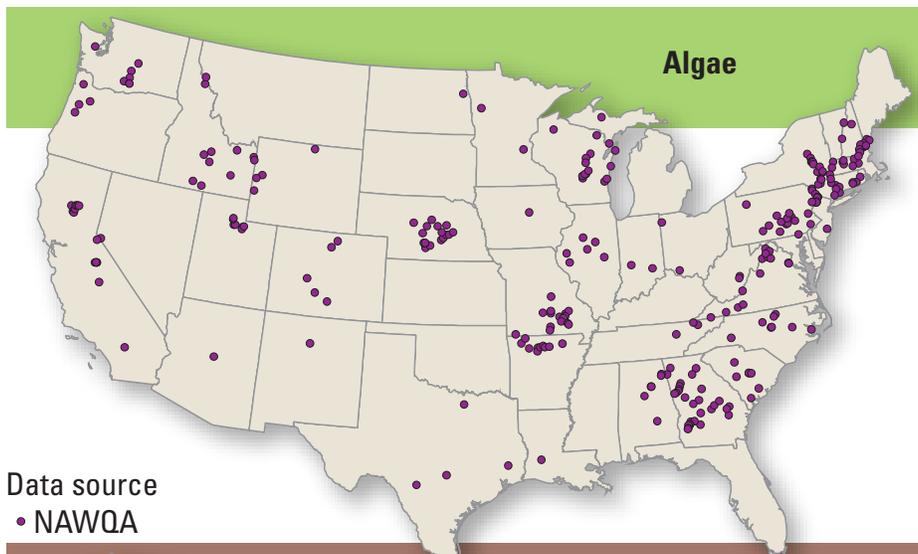


### Assessment Tools

#### National Repository of Reference-Site Data for Rivers and Streams

In cooperation with the Western Center for Monitoring and Assessment of Freshwater Ecosystems, U.S. Geological Survey (USGS) scientists with NAWQA led an effort to compile biological data and map reference-quality sites from Federal assessment projects throughout the Nation (dots on map of the conterminous United States). This effort provides public access to the data collected at these sites, as well as other environmental information, so that future stream bioassessments can benefit from the work of Federal agencies and better characterize reference conditions across the Nation's diverse landscapes. Data for more than 2,000 reference-quality sites originated from sampling efforts of the U.S. Environmental Protection Agency, USGS, Bureau of Land Management, U.S. Forest Service, and university researchers. The types of data currently available include macroinvertebrate taxon counts, habitat features, chemistry data, and geospatial data. Access to these data is available at <http://www.cnr.usu.edu/wmc/htm/data/>. USGS is working with other agencies to further develop this database into a comprehensive network of reference sites used by Federal, State, and local monitoring programs (<http://acwi.gov/monitoring/>).





U.S. Geological Survey photo by Daren Carlisle.

*Reference sites range from those in near pristine watersheds in protected wilderness areas (photo above) to those in watersheds with substantial landscape alteration that have protected riparian buffers (photo below).*



USDA Natural Resources Conservation Service photo by Lynn Betts.

The baseline by which algal, macroinvertebrate, and fish communities were assessed for biological condition by NAWQA were derived from reference sites sampled by the U.S. Geological Survey, the U.S. Environmental Protection Agency (EPA), and select State agencies (see Measures of Biological Condition sidebar). These maps of the conterminous United States show the locations of these sites (dots).

*A nationally consistent way to measure biological condition is to standardize by each site's biological potential.*

## Assessing Biological Condition—General Approach

Biological condition is used as an indicator of stream health and is defined as the degree to which biological communities differ from their expected natural potential. A major challenge in national assessments is the ability to make comparisons of biological condition in streams across diverse geographic settings. This requires standardized measures of biological condition that adjust for natural factors, such as stream size and climate, that control the types of species present in a given stream (chapter 2). In some geographic areas, including Alaska, Hawai'i, and Florida, such measures of biological condition could not be used because of small numbers of sampled reference sites. Results from these areas are included in this report, although not presented in a national context.

Biological condition at each stream site was assessed by comparing observed community characteristics (such as number of taxa) to those expected if the site was minimally disturbed by human influences. The observed characteristic (O) is obtained from a sample of the biological community at a site, whereas the expected characteristic (E) is predicted with a model developed using data from a collection of reference sites. Because deviation of O from E is expressed as a ratio, the measure is standardized by each site's biological potential and is therefore a comparable measure of biological condition across the Nation, despite large differences in naturally occurring biological communities. In addition, because natural variation in environmental settings is accounted for in estimates of E, departures of O from E are likely the result of human influences. Importantly, O does not always equal E at reference sites because of natural environmental variability (such as storm events), differences in the level of human-caused modification among reference sites, and inevitable error in models used to estimate E.

For clarity of presentation, O:E ratios were modified in two ways. For some analyses, O:E ratios were rescaled to a simple percentage, so that the measure of biological condition ranged from 0 (no similarity to natural potential) to 100 (identical to natural potential). For other analyses, the biological community at each site was classified as "altered" if its O:E score was lower than that of 90 percent of the reference sites within its region and was classified as "unaltered" if not. Importantly, this simple classification of biological condition is based on statistical properties unique to the data in this study and therefore not related to criteria used by States and other monitoring jurisdictions to assess beneficial-use attainment (that is, whether the designated use of a water body can be attained).

Because of differences in the natural distributions of algal, macroinvertebrate, and fish communities, the characteristics used to define O and E also differed. For example, the number of taxa was used as a measure for invertebrate communities, relative abundance of different taxonomic groups was used for algal communities, and a combination of both was used for fish communities (see sidebar at right). In addition, different procedures were used to determine expected conditions for the three different taxonomic groups.

## Measures of Biological Condition Were Tailored to Each Community and Region

Biological condition is assessed by comparing observed (O) community attributes, such as number of native species, to those expected (E) if the community was minimally disturbed by human influences. The observed attribute (O) is derived from a sample collected at a stream site, whereas the expected (E) condition is modeled from data collected at reference sites with similar natural environmental characteristics, such as climate and stream size. The community attributes measured for O and E and the procedures for estimating E differ for the communities assessed, as described below.

For macroinvertebrate communities, the expected characteristic (E) was a site-specific list of taxa derived from statistical models that predict the probabilities of observing each taxon at a site, given its environmental setting (for example, stream size, climate, geographic location). The statistical models were developed for each of three regions of the conterminous United States—the area west of the Continental Divide (Carlisle and Hawkins, 2008), the south-central plains (Yuan and others, 2008), and the remaining part of the conterminous United States, including the Eastern United States and central and northern plains (Carlisle and Meador, 2007). The observed characteristic (O) for macroinvertebrate communities at a stream site was the list of taxa actually observed in the sample collected at that site and that were among those taxa expected to occur there (that is, on the “E” list of taxa for that site). Because O is constrained by the list of taxa in E, the O:E index is not simply a measure of taxa richness but is sensitive to the replacement of taxa that often occurs in disturbed environments. For example, if a pollution-sensitive taxon is replaced by a pollution-tolerant one, total taxa richness does not change. However, the O:E index would indicate a loss of one taxon.

Fish communities were divided into three regions. For fish communities in the Eastern and Central United States, O:E was developed and is interpreted identically to that for macroinvertebrates (Meador and Carlisle, 2009). Fish communities in the Western United States were not assessed with statistical models because natural communities contain very few species. Instead, fish communities were assessed using an index of biological integrity (IBI) developed from 210 reference sites, where the IBI represents measures of community composition other than species richness (for example, proportion of exotic species; Whittier and others, 2007). Thus, in the Western United States, E for each site was estimated as the average IBI value for all reference sites within the region, whereas O was the observed value of the IBI calculated from the sample collected at that site (Meador and others, 2008).

The relatively small number of sampled reference sites for algae precluded the use of statistical models for assessing algal communities. Instead, an IBI was developed in a way similar to that for western fish communities. The diatom IBI represents measures of the relative abundance of diatom taxa collected at a site. Thus, for algae throughout the United States, E for each site was estimated as the average IBI value for all reference sites within its region, whereas O was the observed value of the IBI calculated from the sample collected at that site. Separate IBIs were developed for each of five generalized regions spanning the conterminous United States (Potapova and Carlisle, 2011).

