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Acronyms	 CWA – Clean Water Act EPA – Environmental Protection Agency HUC – Hydrologic Unit Code IBI – Index of Biotic Integrity MDNR – Minnesota Department of Natural Resources MPCA – Minnesota Pollution Control Agency MSHA – Minnesota Stream Habitat Assessment SI – Stressor Identification Process TALU – Tied Aquatic Life Use TMDL – Total Maximum Daily Load
Introduction	Each natural river or stream exhibits unique water quality characteristics which are influenced by the physical processes and characteristics of its watershed. If left undisturbed, stream systems typically reach a state of equilibrium. However, as land use activities within a watershed increase and as changes in the landscape occur, stream systems can eventually become destabilized, resulting in physical and chemical changes that affect biological communities (MDNR, 2005). Changes in temperature, dissolved oxygen, habitat, nutrient levels, alkalinity, turbidity, toxic pollutants, and application of road salt can all wreak havoc on biological communities in streams.

Rivers are inherently complex systems to study and understand. The challenge for environmental professionals is to assess and understand human impacts on these vitally important systems and the aquatic life they support.

What is Biological monitoring is the practice of measuring and evaluating biological systems to determine the effects of human activities. Biological monitoring Biological tracks the health of biological systems by measuring the ability of a waterbody Monitoring? to support and maintain a balanced and adaptive community of aquatic organisms. In the past, chemical criteria and related monitoring have been the traditional mechanisms employed by regulatory agencies responsible for assessing and protecting aquatic life. However, human actions impact a far wider range of water resources attributes than water chemistry alone can measure. The degradation of Minnesota's surface waters can be attributed to a multitude of sources including: • chemical pollutants from municipal and industrial point sources • agricultural runoff of pesticides, nutrients and sediment • hydrologic alteration from stream channelization • dams • artificial drainage • habitat alteration from all types of development • introduction of exotic species Biological communities are subjected to the cumulative effects of all activities and are continually integrating environmental conditions over time. The health of aquatic organisms is a reflection of the condition of their aquatic environment. The value of biological monitoring is that it is often able to detect water quality impairments that other methods miss or underestimate. It provides an effective tool for assessing water resource quality regardless of whether the impact is chemical, physical or biological in nature. What is **Biological integrity** is commonly defined as "the ability of an aquatic environment to support and maintain a balanced, integrated, and adaptive **Biological** community of organisms such that it has the kinds, number and diversity of Integrity? aquatic species found in natural habitats within the region". The biological integrity of a waterbody is determined by comparing the health of its component biological communities to a regional reference condition representative of minimal or no human disturbance. On a practical level, there

are few truly pristine waterbodies in Minnesota which would be comparable to pre-settlement conditions. Most of the state's waterbodies have been impacted to some degree by human activities. If pristine conditions exist, they would be confined to undeveloped areas of some northern regions of Minnesota. As a result, MPCA must compare study sites within similar bio-geographical regions to minimize natural variability.

Multiple factors affect biological integrity. Determining biological integrity requires an understanding of the complex mix of physical features and functional attributes that affect biological communities. For most of these parameters there are no federal and state standards against which to gauge compliance.

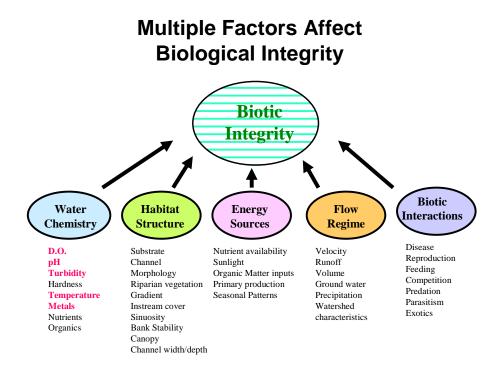


Figure 1: Factors affecting biological integrity

Measuring Biological Condition

The Minnesota Pollution Control Agency (MPCA) and the Minnesota Department of Natural Resources (MDNR) examine fish, macroinvertebrates or plant communities to assess the overall health of a waterbody. When measuring the health of streams, the health of invertebrate and fish communities are assessed. Wetland health is determined by examining invertebrates and plant communities. To date, there are no biological indicators used to assess lakes, however the MDNR is currently working to develop fish and plant indicators. Measuring biological integrity in conjunction with other monitoring efforts makes a holistic watershed assessment possible.

MPCA is Minnesota Rules Chapter 7050 requires that the biological health of all surface waters be assessed and compared to the regional reference condition (those that Required to represent the most natural condition for that surface waterbody type within a Assess the geographic region). The expectation is that healthy, diverse and successfully **Biological** reproducing populations of aquatic organisms (including fish and invertebrates) Condition of all are maintained for most Minnesota waters (the exception is Class 7 waters). Waters Water Quality Minnesota water quality standards consist of : Standards 1. Beneficial uses that describe the specific, intended use of a waterbody (i.e. for recreation, aquatic life, drinking water, etc.) 2. Numeric and narrative criteria which are designed to protect beneficial uses. In theory, if the numeric standard is not exceeded, the beneficial use will be protected. 3. Nondegradation requirements that provide extra protection for high quality waters The majority of state waters are Class 2 waters. Class 2 waters are protected so that they can sustain healthy populations of aquatic life and support a variety of recreational activities such as swimming and fishing. The MPCA is developing a more refined approach to assigning beneficial uses, known as tiered aquatic life uses (TALU). TALU would more precisely define the beneficial use classes based on the biological potential of specific water bodies. **Tiered Aquatic** TALU will allow the MPCA to set goals for the waterbody that are neither under-protective of existing high quality resources nor over-protective of Life uses waterbodies that have been extensively and irretrievably altered. For example, rivers and streams might be classified in the following manner: 1. Exceptional warmwater stream: (Best in each stream class; exceeds Clean Water Act interim goal of meeting beneficial uses; meets or nearly achieves pristine conditions) 2. Warmwater stream: (Meets beneficial uses; the default classification) 3. Modified warmwater stream: (Irretrievably altered habitat; does not meet beneficial uses) 4. Limited warmwater stream: (Very little biological potential) MPCA will examine existing biological expectations for modified streams as well as the characteristics of potentially exceptional waters. MPCA staff will then define habitat criteria and biological thresholds for designation of modified uses and propose new biological thresholds for all remaining uses as part of its proposed changes to Minnesota Rules Chapter 7050. After public comment, and approval, all monitored sites would be placed into their new beneficial use classifications.

Before proposing new thresholds for biological monitoring, a rigorous assessment process would be initiated including:

	 Conducting site visits using intensive watershed monitoring approach (i.e., concentrated surveys within 11 digit hydrologic unit code (HUC), phased approach) Applying standard bioassessment protocol (i.e., fish, invertebrates, habitat, water chemistry) Gathering temperature data using data loggers at potentially coldwater sites Determining biological health (as measured by <i>Index of Biotic Integrity</i> score), habitat and water temperature (for coldwater streams) used to designate use class Gathering historical data used to support determination of the existing use. Convening interagency team to review assessment data and proposed use classifications
Numeric and Narrative Criteria	 Presently, the MPCA does not have numeric criteria to assess biological communities. The MPCA relies on narrative standards. Narrative standards are used to protect surface waters from: impairment of the biological community nutrient enrichment (particularly for lakes) contamination of fish and other aquatic life
	Narrative standards are not quantitative. As such, MPCA must use a weight of evidence approach when analyzing data. Using weight of evidence, staff looks for associations between available data that indicate a consistent pattern of impairment. Professional judgment plays a significant role when applying narrative water quality standards to surface waters.
	MPCA has developed non-numeric (qualitative) criteria that allow staff to determine whether narrative standards are being met. MPCA uses the <i>Index of Biotic Integrity</i> (IBI) as a numerical translator to interpret the narrative biological standard.
	MPCA is in the process of developing numeric criteria (quantitative) for biological communities across Minnesota. These are expected to be completed in 2012. Wetland numeric criteria will likely be approved by 2015.
Index of Biotic Integrity	The <i>Index of Biotic Integrity</i> is the main tool MPCA uses in developing biocriteria. The IBI is a scientifically validated tool which examines the health of various attributes of a biological community. A typical IBI examines 8-12 attributes of a biological community to determine its health.

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To assess the health of streams and rivers, MPCA examines fish and invertebrate populations, as well as the quality of the habitat. To determine wetland health, MPCA studies plant and invertebrate communities.

The IBI score for a specific waterbody is compared to the biological conditions of reference waterbodies within the region. The reference waterbody represents the most natural or best attainable condition for the geographic area in which it is located.

Fish IBI Many organisms have been proposed as indicators of environmental quality. Ideally, a biological monitoring program will integrate multiple data sets in order to better assess environmental quality. Fish are on of the most widely used organisms for measuring water resource quality. They are typically present even in the smallest stream and are easily sampled and identified with the proper equipment and training. The Clean Water Act mandates "fishable" waters and the public widely recognizes fish for their economic and aesthetic value.

Fish are a diverse group of organisms and have a wide range of life history requirements. Some fish are more sensitive to changes in water temperature, substrate composition, stream flow, or various water chemistry parameters, while others are more tolerant of environmental change. Fish occupy positions throughout the aquatic food web and characterize a range of trophic levels (planktivores, herbivores, omnivores, insectivores, and piscivores). The structural and functional variety of fish communities make them excellent indicators of water quality and provide an integrated view of waterbody condition.

The MPCA has been using fish community data to assess water quality for the last decade. IBIs have been developed for streams in the Minnesota, Red, St. Croix, and Upper Mississippi River Basins. IBIs are currently being developed for the entire state of Minnesota; this includes a re-evaluation of all data used in previous IBIs.

Macro-Invertebrate IBI Aquatic invertebrate biomonitoring has long been a tool of choice in assessing and monitoring the impact of anthropogenic stress in aquatic systems. Aquatic invertebrates include insects, crayfish and other crustaceans, snails, clams, aquatic worms, and leeches.

> Aquatic macroinvertebrates (or benthic macroinvertebrates) are found in all types of surface waters, including large rivers, small streams, lakes and wetlands. They are most commonly found living on submerged substrates such as aquatic vegetation, woody debris, or rocks and clobbles, but are also found living freely in the water column.

	Benthic macroinvertebrates are a very diverse group of organisms. They display a wide range of sizes, habitat requirements, life histories, and sensitivities to water quality impairment. Some are sensitive to changes in substrate composition; others are sensitive to fluctuations in dissolved oxygen. Some require cold water temperatures, while others can tolerate a wide range of temperatures. This wide range of living requirements makes benthic macroinvertebrates excellent indicators of anthropogenic stress on aquatic systems.
	Minnesota has used benthic macroinvertebrates as indicators of human disturbance of aquatic resource integrity for the past 25 years. From 1976 – 1979 the MPCA collected macroinvertebrate community data from 21 stream stations throughout the state to assess water quality.
	From 1990 – 1992, macroinvertebrates were collected at 45 stream stations through the Minnesota River Basin. An IBI developed by Ohio EPA for the Eastern Cornbelt Plain was used to assess biological integrity.
	In the past six years, IBIs have been developed for streams in the St. Croix and Upper Mississippi River Basins as well as depressional wetlands in the Central Hardwood Forest and Prairie Ecoregions. Macroinvertebrate IBIs are currently being developed for all streams in Minnesota; this includes a reevaluation of existing IBIs. Macroinvertebrate IBIs are also being developed for wetlands in the Northern Lakes and Forests ecoregion.
Plant Community IBI	The Plant Community IBI is used in conjunction with macroinvertebrate IBI scores and water chemistry data to determine biological integrity within depressional wetlands. Depressional wetlands are defined as wetlands that occur within a hallow depression in the landscape that are not directly associated with streams or lakes, have a semi-permanent to permanent flooding regime, and have predominantly emergent marsh to shallow open water vegetation types (typically seen in wetland types 3, 4, and 5).
	A plant community IBI is used in conjunction with a macroinvertebrate IBI and water chemistry data to determine the biological integrity of depressional wetlands. Depressional wetlands are defined as wetlands that occur within a shallow depression in the landscape that are not directly associated with streams or lakes, have a semi-permanent to permanent flooding regime, and have predominantly emergent marsh to shallow open water vegetation types (typically seen in wetland types 3, 4, and 5).

Determining Whether an Impairment Exists	In addition to biological sampling activites, MPCA/MDNR staff conduct quantitative and qualitative assessments of riverine habitat (instream and riparian). This information is used in conjunction with IBI scores to determine whether riverine or wetland systems are impaired.
	If a waterbody is determined to be biologically impaired, it means that it is not supporting the aquatic life beneficial use classification that it was designated. In order to identify a waterbody as biologically impaired, we must have supporting data that showing that fish, invertebrates and/or plant communities are impacted.
	Who conducts these assessments? Currently, MPCA staff conducts most of the monitoring activities that measure biological integrity. In the future, as more waterbodies are listed as biologically impaired, consultants are likely to play an increasingly significant role in biological monitoring for TMDL studies statewide.
	The proper implementation of biological monitoring activities requires significant training due to the need for specialized equipment and rigorous, data intensive field collection. In addition, field technicians must have expertise in fish identification, and a strong knowledge of aquatic ecology. Therefore, it is important to work closely with MPCA biological monitoring staff when developing your biological monitoring plan.
Biological Monitoring Methods	MPCA uses an integrated approach to assessing stream condition, which requires that fish, macroinvertebrate, physical habitat and water chemistry data are collected. To facilitate consistency in data collection, MPCA has developed standard operating procedures. Below, the methods used to collect each kind of data are briefly described.
	 Fish communities are typically assessed between mid-June and mid –September. For wadeable streams, a fish community assessment is conducted in conjunction with a physical habitat assessment. The following equipment is typically used to assess fish community health: Backpack shocker or stream shocker in wadable streams, mini-boom or boom shocker in non-wadeable streams. Nets Fish holding areas (tub or bucket) Scale, measuring board Formalin for preserving specimens that must be identified in lab Labels for specimen jars
	 Staff collect the following kinds of field data: 1. The common name of each species collected 2. The minimum and maximum length for each fish species collected 3. The total weight of each species collected 4. The total number of individuals of each fish species 5. The total number and type of fish anomalies

6. The number of individuals of each fish species



Figure 2: MPCA staff conduct fish community monitoring



Figure 3: Fish are collected using a backpack shocker



Figure 4: MPCA and DNR staff measure and identify fish species

Macroinvertebrate Sampling Streams are the resource most commonly monitored for aquatic macroinvertebrates. MPCA staff members typically collect the majority of aquatic invertebrate samples for TMDL projects. The MPCA uses D-frame aquatic dipnets to collect macroinvertebrates within multiple habitats at each stream station. Samples are subsampled to 300 organisms in the lab, and identified to their genus.

Sieve buckets, sample bottles, and alcohol to preserve specimens are also used when collecting aquatic invertebrate samples.

Macroinvertebrate sampling involves collecting a composite sample from up to five different habitat types. The goal of this method is to get a sample representative of the invertebrate community of a particular sampling reach. This is done by walking the length of the stream and determining which productive habitats dominate the stream reach.

The habitats to be sampled include:

- 1. Rocky bottom (riffle/cobble/boulder)
- 2. Aquatic macrophytes (submerged/emergent/vegetation)
- 3. Snags (snags/rootwads)
- 4. Undercut banks and overhanging vegetation
- 5. Leaf packs



Figure 5: "Scrubbing" the rocks for invertebrates

Sampling consists of dividing 20 different sampling efforts equally among the dominant, productive habitats present in the reach. A sample effort involves placing a D-net on the substrate and disturbing the area directly in from of the net opening equal to the net width (1 sq. foot).

Physical
HabitatHabitat assessments describe the physical characteristics of the stream channel
and condition of the riparian area. Habitat assessments are conducted during the
summer months (mid-June through mid-September), when streams are at or near
base flow. The habitat evaluation is conducted concurrently with fish sampling
to obtain a comprehensive description of the fish habitat conditions within the
reach. Qualitative and quantitative habitat characteristics are recorded at each
sampling location.

When conducting qualitative habitat assessments, the MPCA uses the Minnesota Stream Habitat Assessment (MSHA) which was designed to assess important characteristics influencing Minnesota streams. The MSHA incorporates measures of watershed land use, riparian quality, bank erosion, substrate type and quality, instream cover, and several characteristics of channel morphology. The following kinds of information are collected:

- 1. Surrounding land use
- 2. Riparian zone characteristics (width, degree of erosion, percentage of shade)

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- 3. Insteam characteristics (substrate, channel type, embeddedness, water color, cover types and amount)
- 4. Channel morphology (depth variability, channel stability, velocity type, sinuosity, pool width, channel development, water level)
- 5. Percentage of macrophytes and algae

When conducting quantitative habitat assessments, a specific MPCA protocol is followed. First, a description of the sampling station is completed, including the field number, and the length and location of the major morphological features within it (e.g., number and length of bends, pools, riffles, runs, log jams, islands, beaver dams, etc.). Next, habitat quality within the sampling station is assessed utilizing a transect-point method. Thirteen separate transects are established within each sampling station. Measurements are made at five equally spaced points within each transect. Measurements are taken or visual estimates are made to characterize key components of the physical habitat structure which impact stream ecology within each transect. Key components that are measured include channel morphology, substrate type, fish cover, and riparian condition, stream canopy, and stream vegetation.

Plant Community Assessment

The wetland vegetation biological assessment techniques used by the MPCA require that MPCA staff identify the different kinds of plants growing in a wetland and determine how abundant those plants are. MPCA vegetation sampling relies on the observer to select areas within the desired community that are representative of the overall community composition to place a sampling plot where plant data can be quantified.

Wetland assessment uses the following equipment:

- 1. Field data recording device
- 2. GPS for sample location recording
- 3. Laptop computer
- 4. Camera
- 5. Site files and maps
- 6. Garden stakes and measuring tape for laying out sampling plots
- 7. Plastic bags for speciments
- 8. Plant press for collecting plant specimens

Field crews begin by recording information about the site. The major plant communities in the wetland are then identified by walking around the edge or margin of the entire wetland. Next, sample plot locations are selected to best represent the marsh and aquatic plant communities found in the wetland. Either one large (100 m^2) or four small plots (5m^2) are surveyed, depending on the ecoregion in which the site is located. For each plant type occurring in the survey plot, the percent cover is estimated.

Throughout the remainder of the site visit, photos are taken of the site and water chemistry data is collected. The degree of human stressors such as habitat alteration and hydrologic modification are also noted on the data sheets.

Water Chemistry Monitoring The following tests are typically conducted on water chemistry at the same time that biological assessments are occurring. The following parameters are measured:

- 1. Dissolved oxygen
- 2. pH
- 3. Conductivity
- 4. Temperature
- 5. Nutrients (phosphorus and nitrogen)
- 6. Turbidity
- 7. Total suspended solids
- 8. Ammonia



Figure 6: MPCA staff analyze water samples

A Hach or YSI mulitparameter meter is used to test for dissolved oxygen, conductivity and temperature. Nutrients and total suspended solids concentrations must be determined by a certified laboratory. A flow meter is used to document flow at the time of fish sampling.

Calculating IBI Scores Once biological data is collected, it is analyzed to produce a series of community attribute values that are scored and aggregated into a final index. For example, the number of gravel spawning fish species occurring in streams indicates the availability of clean, gravel substrates for spawning.

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A higher number of these species is an indicator of favorable habitat conditions. The range of gravel spawning species in a particular watershed might range from zero to 13. To get the final metric value, the raw value at any given sight is normalized to a 10-point scale. All of the scores for the metrics in the IBI developed for that particular watershed are then added together to obtain a final IBI score.

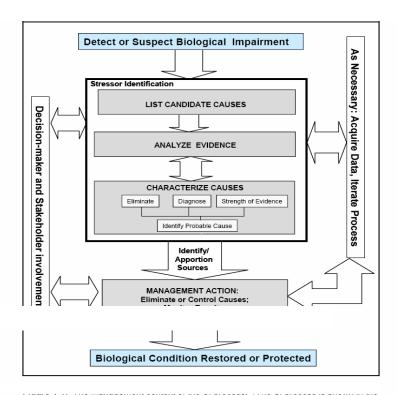
Using the Stressor Identification Process to Identify Causes and Sources of Impairment

With IBI scores in hand, your technical team will now need to determine why the biological impairment exists. What causes or combinations of causes are responsible for the impairment? Linking biological effects with their causes is often complex since there are often multiple stressors which are impacting a waterbody (EPA, 2005).

The EPA has developed a useful process for identifying the probable causes of impairment, called the *Stressor Identification (SI) Process*. The ability to accurately identify causes of impairment and defend the evidence supporting these findings is especially important for controversial TMDL projects. If the TMDL is legally challenged, it is critical to have a high level of confidence in the accuracy of your conclusions (EPA, 2005).

Using the SI process entails three major types of activities:

- 1. a scoping phase in which the technical team critically reviews available information, considering the possible causes of impairment
- 2. a data analysis phase
- 3. a step where evidence is evaluated and the probable cause characterized



The SI process is initiated by the observation of a biological impairment. The first step should be to understand the nature of the impairment: When and under what conditions does the impairment occur? This often means scrutinizing the IBI data very carefully. The next step requires listing all of the potential causes of the impairment. Data is then analyzed to generate evidence for those causes. Note that it is important to use all of the information you have about the waterbody during this step (geological, geomorphologic, etc.). Data must be organized and synthesized in such as way that identification of potential causes can be supported.

Finally, evidence is used to eliminate, to diagnose and to compare the strength of evidence regarding probable causes. New data may need to be gathered to support or refute various causal scenarios. Several iterations of the process may prove necessary in order to determine causes and sources with enough certainty to finalize the TMDL study.

Once the technical team has worked through the process and reached a conclusion, the appropriate management strategies can then be selected (EPA, 2005).

The SI process is dependent upon multiple disciplines. The SI process should be conducted with a team of experts from the fields of biology, geomorphology, water chemistry, and geology. It is important to include local officials and other key stakeholders in the process as well.

The SI process may be applied to any type of waterbody for single or multiple causes of impairment. In some cases, multiple iterations of the SI process may be required to get to the root cause of the problem.

STOP! Complete Worksheet 10-1 Refer to Worksheet 10-1. Your technical team should work together to answer these essential questions as part of the SI process before completing the TMDL study.

> TMDL studies typically also require identification of the sources of pollution that are causing impairment so that pollutant loading can be apportioned among them. This is an especially challenging aspect of the SI process.

> The EPA has developed a detailed guidance for conducting a Stressor Identification process. A copy of the guidance can be found on EPA's Web site, http://www.epa.gov/waterscience/biocriteria/stressors/.

Summary	 Biological (biotic) integrity is one measure of the health of a waterbody and how it is functioning relative to its original state before human impacts were imposed. Biological monitoring allows us to measure the ability of a waterbody to support and maintain a balanced and adaptive community of aquatic organisms. Biological monitoring is important since it can detect water quality impairments that other methods (water chemistry monitoring) can miss or underestimate. Minnesota Water Quality Rules ensure that healthy, diverse, and successfully reproducing populations of aquatic organisms are maintained. MPCA has developed narrative criteria (qualitative) that help to determine whether narrative standards are being met The <i>Index of Biotic Integrity</i> (IBI) is the tool used to determine whether or not a waterbody is impaired. The IBI Score for a specific waterbody must be compared to the biological conditions of reference waterbodies within a river basin and watershed. The MPCA is in the process of developing numeric criteria to protect biological assessment is a complex process, requiring technical expertise. Coordinate with the MPCA and MDNR before beginning any monitoring effort. The <i>Stressor Identification Process</i> can be most successful when the technical team has access to an adequate amount and quality of 	
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	Jeff Jasperson, MPCA TMDL Technical Support	651-297-8483
References	Minnesota Department of Natural Resources, <u>Health</u> <u>Course</u> , 2005.	ny Rivers Water
	US Environmental Protection Agency, <u>Handbook fo</u> <u>Watershed Plans to Restore and Protect Our Waters</u>	
	US Environmental Protection Agency, <u>Stressor Iden</u> <u>Document</u> , 2000, (EPA/822/B-00/025).	tification Guidance

Date:

Worksheet 10-1: Stressor Identification Process

Meet with your technical team to complete the Stressor Identification Process. Answer the following questions as you work through the Stressor Identification Process. Answering these essential questions will help to characterize the causes of impairment for your waterbody.

Causal Considerations	SI Team Response
1. Temporality	
Did the potential cause precede the effect in time?	
A cause can be eliminated if the effect (ex. mortality) precedes the potential cause	
2. Spatial Co-occurrence	
2a. Did the level of the cause increase over the closest upstream location?	
A cause can be eliminated if the effect (ex. mortality) occurs upstream of the candidate cause	

2b. Does the candidate cause	
occur at the reference site?	
A cause can be eliminated if it	
occurs at the reference site as well	
as at impaired sites	
3. Biological Gradient	
Did the effect decrease with a	
decreasing exposure to the	
candidate cause?	
A cause can be eliminated if there is	
an increasing level of impairment	
(e.g. more dead fish) with a	
decrease in exposure to the stressor	

4. Complete Exposure Pathway	
Is the exposure pathway known and is it complete?	
A cause can be eliminated if a necessary link in the known chain of events is missing a step in the causal pathway	
5. Experiment Temporality	
When the candidate cause was removed or reduced, was the effect reduced?	
A cause can be eliminated if the effects continue even after a candidate cause is removed	