Identification and Quantification of Reference Conditions Associated with Lotic Ecosystems of the Mid-Continent Region of the US:
A summary of approaches and factors applicable to Heartland Network facilities

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Introduction

One approach to effectively manage and restore ecosystems is to identify sites that experience relatively minimal levels of disturbance and therefore represent healthy or acceptable conditions (Bailey et al., 2004; Dodds and Oakes, 2004). These "reference" sites are sampled for a range of measurements, metrics, or indices and the resulting ecological conditions are used as benchmarks in the development of bioindicators and biocriteria (Barbour et al., 1995; Hughes et al., 1995; Barbour et al., 1999; Reynoldson et al., 1997; Bailey et al., 2004; Dodds and Oakes, 2004). Using this approach, the ecological condition of an individual test site can be compared to a set of defined reference conditions, and deviations from these conditions can be used to assess ecosystem health.

Specifically, a reference site is a "locality on a waterbody which is minimally impaired and is representative of the expected ecological integrity of other localities on the same waterbody or nearby waterbodies." (USEPA, 1996). Reference conditions therefore, are sets "of selected measurements or conditions of minimally impaired waterbodies characteristic of a waterbody type in a region." (USEPA, 1996). Furthermore, several definitions or interpretations of reference condition have been identified to account for the fact that all ecosystems experience some level of human disturbance, and truly pristine sites are virtually nonexistent. These definitions include 1) Minimally Disturbed Conditions (MDC's) which represent the "physical, chemical and biological conditions of a site, reach, segment, or water body in the absence of significant, or with minimal, human disturbance. Historical information or models may be used to help describe the minimally disturbed condition. Minimally disturbed conditions change little over time mostly due to natural processes and, therefore, provide a "target" or upper bound of water quality potential"; and 2) Least Disturbed Conditions (LDC's) which represent the "physical, chemical and biological conditions of a site, reach, segment, or water body that has the least amount of human disturbance in comparison to others within the water body, class, region, or basin. Least disturbed conditions change over time as land use and management practices change and, therefore, are not a "target" or upper bound of water quality (and habitat and hydrological?) potential" (http://www.epa.gov/bioindicators/html/chapter5_acronyms_definitions.html). Most current studies use LDC's based on a lack of available historical data and the unrealistic attainability of MDC's in most regions. However, these two definitions can be used to help define the Best Achievable Conditions (BAC's), which are conditions that are equivalent to LDC's where the best possible management practices are in use. The MDC's and LDC's set the upper and lower limits of the BAC's. Using the population distribution of measures of biological condition associated with a reference population might provide some insights regarding the potential relationship between the MDC and LDC for a particular region (Figure 1). The lack of historic information about the biota associated with undisturbed or nearly undisturbed ecosystems prevents us from directly estimating MDC's and comparing them to LDC's and we can only indirectly estimate what these MDC values may have been.

Over the past decade aquatic ecologists and environmental scientists have invested considerable effort in better formalizing the processes used in the identification and quantification of reference sites and conditions. While the concepts and use of reference conditions in biological assessment studies have been relatively straight

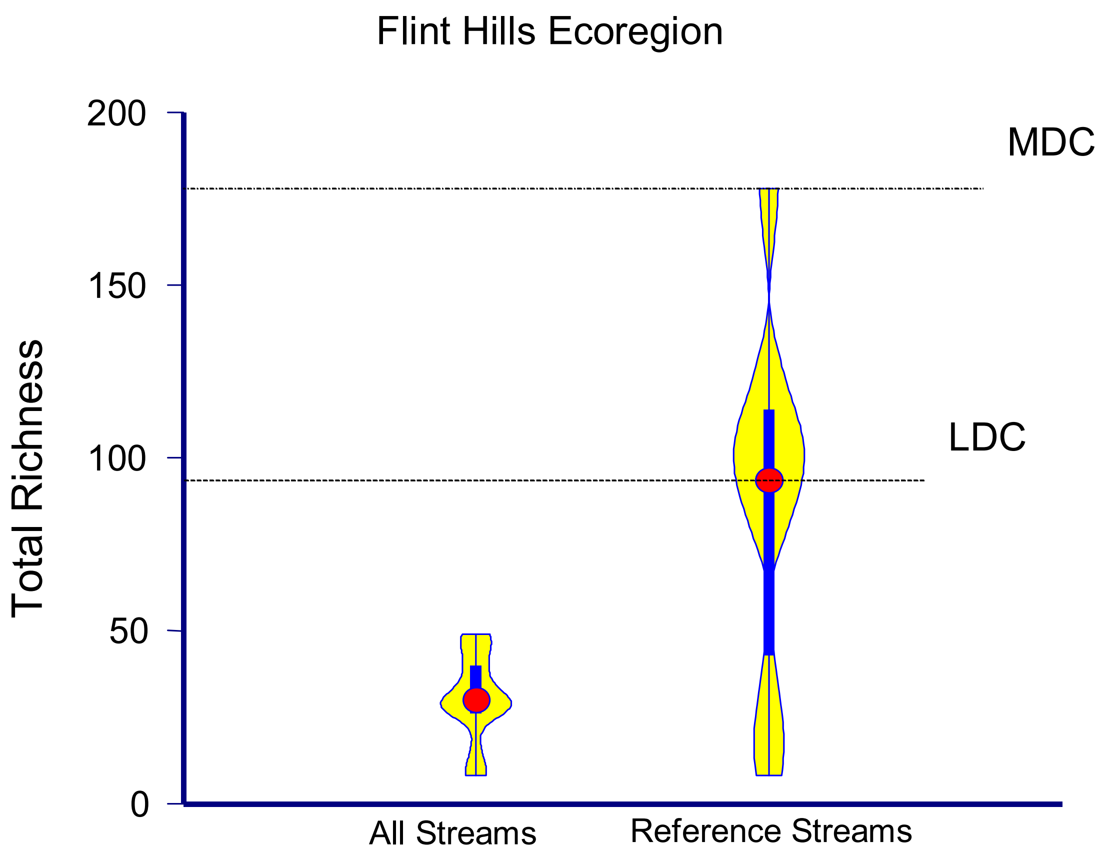

Figure 1. Violin plot of macroinvertebrate total richness for wadeable streams of the Flint Hills Region of the Central Plains showing the hypothesized LDC (median value for reference) and MDC (maximum valve) based on currently recognized reference streams.

forward, the actual determination of reference conditions and sites has proved more difficult. Approaches in determining potential reference conditions and what these conditions represent along a human disturbance gradient vary as a result of the history, duration, spatial extent, natural landscape features, and cumulative nature of human use and management of the regional landscape. The purpose of the current report is to summarize some of the specific approaches and methods used in the determination of reference conditions for aquatic resource assessment and management. We also present a case study of the development of reference site selection procedures and criteria for USEPA Region 7.

Reference Site Selection

A number of factors must be considered when selecting reference sites. References sites should be selected to characterize natural ecological conditions associated with a particular geographical region (Stoddard, 2005). For example, they should have representative substrate, riparian vegetation, biota, channel morphology, and flow regimes. One approach has been to use ecoregions, which account for natural variability due to landscape features such as geology, soils, vegetation, and climate, to delineate natural variability (Hughes et al., 1986; Larsen et al., 1998; Dodds and Welch, 2000; Hohm et al., 2002; Stoddard, 2005). Therefore, all sites within an ecoregion should experience comparable natural conditions and contain similar "ecological potential" (Stoddard, 2005). Furthermore, reference sites should not be so unique or anomalous that they misrepresent an ecoregion, such as refugia habitat or spring fed streams unless the ecoregion is characterized by such habitats. In some instances unique habitats are all that remain of candidate reference sites (based on selection criteria listed below), in which case they should be used, but with knowledge and caution as to the implications of any comparisons.

Several methods have been developed to identify reference conditions. These include best professional judgment (BPJ), simple or formulaic ranking, percentile determination, multivariate or multimetric analyses, and covariance/regression analyses. These methods vary in whether reference conditions are identified before a study using existing data, or after a study using generated data. Furthermore, methods vary in the inclusion or exclusion of selection variables. The pros and cons associated with these methods have been summarized elsewhere (Bailey *et* al., 2004; Dodds and Oaks, 2004).

The selected examples summarized here are representative of this larger body of literature on reference site selection that continues to grow, as does our understanding of the structure and function of aquatic ecosystems. Detailed information on the methods, core factors used, and study outcomes for each of the 16 studies are presented in Appendix 1.

Review of Reference Selection methods

The majority of methods used some form of steam classification to partition natural variability within the sample sites. Classification allows managers to group sites into relatively homogenous classes with respect to their physical, chemical, and biological attributes (Barbour *et al*., 1995). All of the studies reviewed used a region, or ecoregion classification framework (Table 1). However, a number of additional classification schemes were used in combination with region/ecoregion including stream order, stream flow, and altitude (see Table 1 for a complete listing of classification schemes).

Our review identified a number of different methods that were used in the identification of reference sites (Table 2). The most commonly used methods were screening factors (81% of the studies) and BPJ (69% of the studies; Table 2). Additional reference site selection methods included the use of historical data, field reconnaissance, screening factors, areal photos and topographic maps, paleological data, experimental data, stepwise, sequential methods, and specific criteria. Of these, historical data (19% of the studies), field reconnaissance (19% of the studies), and sequential methods were most commonly used (25% of the studies; Table 2).

Classification Method	Number of Studies
Region/Ecoregion	16 (100%)
Stream Order	2 (13%)
Watershed Size	1 (6.3%)
Geology	1 (6.3%)
Discharge	1 (6.3%)
Altitude	1 (6.3%)
Presence of Trout	1 (63%)
Vegetation Type	1 (63%)

Table 1. Summary of the classification schemes used in the selected studies. Values represent the number of studies (and percentage of total studies) that used a specific classification scheme. See Appendix 1 for detailed overview of studies.

The majority of studies used several combined several methods when selecting reference sites. For example, Burton and Gerritsen (2003) identified reference sites based on biological and non-biological screening. Regional biologists first selected candidate reference sites based on BPJ. Sites were also selected through a screening process that used a set of non-biological criteria that including: water quality characteristics (e.g. dissolved oxygen and conductivity), in stream characteristics, channel characteristics, and bank and riparian characteristics (Appendix 1). Following this screening, biologists were then able to reject non-biological selected sites based on their knowledge of disturbance within the region (Burton and Gerritsen, 2003). Furthermore, Mrazik (1999) combined BPJ, screening factors, field reconnaissance, and fieldwork to identify reference conditions.

Reference Site Selection Method	Number of Studies
Screening Factors	13 (81%)
Best Profession Judgment (BPJ)	11 (69%)
Field Reconnaissance	3 (19%)
Historical Data	3 (19%)
Maps and Aerial Photos	1 (6.3%)
Paleoecological Studies	1 (6.3%)
Experimental Data	1 (6.3%)

Specific Criteria	1 (63%)
Stepwise/Sequential	4 (25%)

Table 2. Summary of the reference site selection methods used in the selected studies. Values represent the number of studies (and percentage of total studies) that used a specific reference selection method. See Appendix 1 for detailed overview of studies.

The number of factors used to screen reference sites varied between studies. However, there were several commonly used factors including land use/land cover information (55% of the studies), point source pollutants (55% of the studies), riparian habitat (38% of the studies), and instream habitat (38% of the studies; see Table 3 for complete listing). Our review indicates that very few methods use biological metrics to

Screening Factor	**Number of Studies**
Land Use	9 (56%)
Point Source Pollution	9 (56%)
Riparian Habitat	6 (38%)
In Stream Habitat	6 (38%)
Non-Point Pollution	4 (25%)
Altered Flow	4 (25%)
Water Quality	3 (19%)
Biotic Assemblages	3 (19%)
Road Density	3 (19%)
Dams/Impoundments	2 (13%)
Population Density	2 (13%)
Morphological Alterations	2 (13%)
Sediment Deposition	2 (13%)
Recreational Use	1 (6.5%)
Alien Species	1 (6.5%)
Fisheries	1 (6.5%)
Floodplains/Wetlands	1 (6.5%)
Amount of Woody Debris	1 (6.5%)
Color/Odor Problems	1 (6.5%)

Table 3. Summary of the screening factors used in the selection of reference site. Values represent the number of studies (and percentage of total studies) that used a factor. See Appendix 1 for detailed overview of studies.

identify reference sites. Several studies suggested that biological metrics should not be used as stand-alone criteria, but instead as a check on the validity of the site being considered for reference status (Ohio EPA, 1987; Huggins et al., 2001). Furthermore, Bailey et al. (2004) recommend that reference criteria are established without regard to macroinvertebrate community structure. In contrast, however, Yoder (2001) specifically identified reference sites in a "biological context" – sites were selected based on known biotic community data.

A Regional Approach in Defining Reference Conditions

In December of 2000 the USEPA Region 7 Biocriteria Workgroup begin to identify and define factors and approaches that that might be used to identify and quantify potential reference site and the conditions primarily for wadeable streams. All of the state agency members of the Workgroup had already begin to define, identify and use reference sites with in there individual states but it was agreed that a broader, more uniformly define approach and set of assessment factors would be desirable in establishing reference conditions and sites that were representative of ecological units (e.g. ecoregions, physiographic regions) across the region (e.g. Iowa, Nebraska, Kansas and Missouri).

The Workgroup first determined what the regional reference sites and conditions should represent and how they might be used within USEPA Region 7. It was agreed that the reference sites (or streams and watersheds) should accurately characterize the range of variability present in healthy natural systems. Reference conditions should provide an objective definition of what stream ecosystems should be and thus could be used as a "barometer, ruler, benchmark or reference against which can be measures the extent of disturbance of other streams." These reference sites or streams might serve as a regulatory benchmark to identify "biological integrity" with respect to the Clean Water

Act (CWA). It was suggested that the reference streams could be used to identify appropriate pollution-specific conditions for purposes of criteria development (e.g. nutrient criteria). Additionally, stream reference conditions might be used to identify streams of exceptional quality or worthy of additional protection.

The Workgroup focused on the identification of major factors that are important in describing and defining reference sites and streams. Workgroup members had somewhat differing needs and objectives for the use of reference sites/conditions, as well as differing levels of information regarding stream and watershed quality in their region. For these reasons and to accommodate broader ecological conditions occurring across the USEPA region, it was agreed upon that a "core group" of factors would be identified that could be used across geopolitical and agency boundaries for designation of reference site conditions. The starting point for the development of this "core group" of factors was a list of 36 candidate factors generated by the Biocriteria Workgroup on April 27, 2000 (Table 40). This list of factors and conditions were proposed by individual Workgroup members based on the initial workgroup's general definition of "reference" as referring to streams (and watersheds) that represent natural or near natural lotic systems with minimal anthropogenic impacts.

Characteristics and factors that may define and quantify reference sites/conditions	
WWTPs (waster water treatment plants)	Dewatering/ water diversion
CAFOs (confined animal feeding operations)	Threatened or endangered species
Channelization	Species in "need of conservation"
Impoundments	Crossover sites (streams occurring in multiple ecoregions)
Fish kills	Stream gradient

Habitat (instream)	Springs
Habitat (riparian)	Level 3 and 4 Ecoregion maps
Fish surveys	Aquatic life classification
Land use/ land cover (broadscale condition)	Natural salinity
Land use/ land cover (site specific)	Migration Barriers/ Influences
Urban and suburban development	Natural anomalies (rare or unique sites)
RCRA (Resource Conservation and Recovery Act sites)	Geological anomalies (atypical stream segments)
Mining	Habitat anomalies (e.g. car bodies)
Road density	Flow regime anomalies
Physical and chemical parameters	Artificial waterbodies
Biological metrics	Wadeable streams (90-95% of area)
Identified critical habitat	Oil field coverages
Faunal assemblages (e.g. fish, macroinvertebrates)	Center pivot irrigation

Table 4. Factors and conditions recognized by the Biocriteria Workgroup as potential factors that need to be assessed in identifying and quantifying candidate reference streams (watersheds) or stream segments.

Many of these factors were later thought to be redundant or inappropriate, or were redefined and joined with other factors to create a new factor representing a more comprehensive condition or characteristic. Eventually this original list was reduced to the “core group” listing that is composed of 11 factors that could be used to evaluate reference sites/conditions (Table 5). These core factors were defined and delimitated with the understanding the availability of data and application of these factors into an assessment model would vary among uses due to their level of resources and experience Appendix 2 provides a detailed outline of each core factor and potential methods to define and quantify (or quality) the factor and its conditions.

Factors	Primary or secondary evaluator
Wastewater treatment plants and other point sources	Primary
Animal feeding/grazing operations	Primary
Instream habitat	Primary
Riparian habitat	Primary
Land use and land cover – broad scale	Primary
Land use and land cover – site-specific	Primary
Physical and chemical parameters	Primary
Altered hydrologic regime	Primary
Biological metrics	Secondary/ confirmatory
Biotic assemblages	Secondary/ confirmatory
Representativeness	Primary

Table 5. Summary of the factors used in identifying and defining reference sites and conditions within USEPA Region 7 (Biocriteria Workgroup, 2000).

This regional approach appears to have considerable merit and part or all of these factors have been used by various states and other entities within USEPA Region 7. In its current form it is most applicable to wadeable streams or small rivers with drainages that do not transcend widely differing ecoregions or regions with contrasting land uses. The application of this approach and consideration of these core factors within somewhat unique areas or regions (e.g. geological anomalies, managed parks or preserves, large rivers) might also prove of value. However, recognizing and quantifing reference conditions associated with large rivers may prove to be difficult as these lotic ecosystems typically suffer from cumulative effects that offer extend thoughout the main channel system as the drain become large and complex. In other cases, some systems because they currently represent (or are thought to represent) near pristine ecosystems, may have to serve as their own reference. When site specific conditions are used to represent the ecosystem's reference condition, it is still important that these systems be compared to appropriate reference populations to help initially define natural variation and to place these systems along a known ecosystem health and human disturbance gradient.

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Appendix 1. Detailed summaries of reference sites selection methodologies from 16 pertinent studies.

Citation: (Mrazik 1999)
Agency: Oregon DEQ
Geographic Area: Oregon, USA
Method: "six-step method"
Purpose: to identify 20 reference quality streams in Oregon to use as a standard for evaluation of other streams
Type of approach: based on actual sites, desk and field approaches, multiple factors used and sequentially applied
Core factors and how scored:

1. Define area on map
    a. GIS coverage's, divide state into four large regions/basins, encompassing 168 fifth-field watersheds
    b. These are reporting units – NOT related to ecoregion
2. Survey local professionals for candidate sites
    a. Regional biologists, land managers, geologists, etc.
    b. List compiled of 115 candidates – recorded stream location and approximate size plus other info
3. Determine human disturbance in watersheds
    a. Road density (miles/acre) used as "human disturbance" indicator
    b. Selected top 25% of "least roaded" watersheds from each region, this targeted 31 sites
    c. (future factors to be considered include historical and current land use patterns, road/stream crossings)
4. Establish classes for reference sites
    a. Factors selected to establish classes: regional location, elevation, stream size. Boundaries of the size categories "S,M,L" were particular to each region – based on rainfall & drainage area
    b. Result = 24 classes
    c. Already had 35 "reference sites" identified from previous years – added these to database – to determine which of the 24 classes needed to be populated – identified needs – this allowed them to narrow down to 20 sites most needed
5. Conduct field recon
    a. Verified status of 20 streams selected: walked 200 meters, observed riparian vegetation, recorded other factors – made qualitative judgment of whether it fit reference quality
    b. 80% of sites (17/20) were deemed good; three replacements were chosen for the rejected sites.
6. Evaluate biological health
    a. Field work including: water chemistry, stream habitat, macroinvertebrates, and fish. Also observations on land use and quantified vegetation info.

   b. Preliminary results suggest that all streams are high quality in most dimensions.

Findings: The mapping (including measuring road density) was time consuming but valuable. Dividing into regions allowed work to be done in parallel. In the future they plan to describe conditions based on finer resolution.
Other details:
Definition: "Reference sites should represent attainable stream conditions for a particular region. The high quality condition of reference sites should be represented through their biological, chemical and physical stream properties. Ideally… upstream watershed… free from human modifications… this definition has limitations… so we considered sites that were minimally affected by humans.

Citation: (Wallin, Wiederhom et al. 2003)
Agency: European Union's "REFCOND" working group
Geographic Area: Europe
Purpose: non-legally-binding technical guidance for unified scientific/regulatory coordination
Method: (no name)
Type of approach: stepwise/sequential (see report figure 4)
Core factors and how scored:

1. Establish infrastructure including databases
2. Differentiate water body types
   a. Water bodies are classified according to predefined factors including geographic position, altitude, geology, and size (and depth, for lakes), as well as ecoregional or other factors.
   b. Use pressure criteria (such as land use, recreational use, point and nonpoint pollution, morphological alteration, dewatering & flow regulation, alien species, fisheries, riparian vegetation, etc) and ecological criteria as screening tools
   c. Establish values for 'good' (in all dimensions)
3. Do preliminary analysis of existing waterbodies
   a. If potential RC sites not available, use historical data, expert judgment etc. to set conditions
   b. If potential RC sites available, establish spatial network of them and proceed through stepwise testing. If they pass all then they may meet 'reference' quality:
      1. Do estimated values for biological quality elements meet reference conditions?
         a. Composition & abundance of aquatic flora (including phytoplankton), benthic invertebrate and fish fauna
      2. Do physical-chemical conditions meet high status?
         a. Thermal, oxygenation, salinity, ph, nutrients, specific pollutants (pollutants seem to be the only factors for which specific numeric values have been set)

        3. Do hydromorphological conditions meet high status?
            a. Qty and dynamics of water flow, connection to groundwater, river continuity, depth & width variation, structure & substrate of riverbed, structure of riparian zone
    c. Use predictive models to reinforce either
4. Establish RC for each quality element
5. Test & validate to set class boundaries, do this process iteratively (steps 3-5)
6. After member states have completed systems and defined ecological class boundaries (e.g. between "reference" and "good," all their systems will be compared and "intercalibrated"

Findings: The result is a set of biological, physical-chemical and hydrologic VALUES (not necessarily a set of sites) that serve as a standard against which other sites are tested.

Other details:

Definition: "Reference conditions do not equate necessarily to totally undisturbed, pristine conditions. They include very minor disturbance which means that human pressure is allowed as long as there are no or only minor ecological effects. RC equal high ecological status… physical-chemical, hydromorphological and biological… elements. RC can be a state in the present or in the past… require that specific synthetic pollutants have concentrations close to zero… [and] specific non-synthetic pollutants have concentrations remaining within the range normally associated with undisturbed conditions (background values)."

Citation: (Burton and Gerritsen 2003)

Agency: Tetra Tech Report – prepared for USEPA Office of Science & Technology, Office of Water, and for USEPA Region 3 Environmental Services Division, and for Virginia Department of Environmental Quality

Geographic Area: Virginia, USA

Purpose: develop a stream condition index for freshwater streams

Method: [no name]

Type of approach: combined evidence

Core factors and how scored:

1. Database set up of all samples from 1st to 4th order streams in region (based on 1:100,000 scale maps)
    a. Several classifications considered, based on stream order, ecoregion, and one based on a combination of conductivity & gradient
2. Independent Biological BPJ screening: DEQ regional biologists submitted initial set of candidate sites based on experience (47 sites)
3. Independent non-biological screening:
    a. Samples screened using set of criteria so that values were within particular numeric ranges:
        i. Dissolved oxygen
        ii. PH
        iii. Conductivity

iv. Epifaunal substrate score
v. Channel alteration score
vi. Sediment deposition score
vii. Bank disruptive pressure score
viii. Riparian vegetation zone width score
ix. Total habitat score

b. Sites chosen by "evaluating how consistently" its samples met these criteria (note: 35 final sites had from 1 to 10 samples each)

4. Candidate sites combined and reviewed (agreement on 20 sites, plus other 42 = 62 sites total)
    a. Biologists rejected some numeric sites based on knowledge of pollution, anthropogenic disturbance, etc.
5. Further review based on watershed land cover data
6. Final set of reference sites selected

Other details: Note that sites chosen by different methods are combined for final analysis and that there does not appear to be any final field verification. Note that there is no use of numeric biological scores but this may be to avoid circularity, since the purpose of this report was to develop a benthic macroinvertebrate index.

Citation: (Huggins 2001)
Agency: USEPA Region 7 Biocriteria Workgroup
Geographic Area: USEPA Region 7 (Iowa, Kansas, Missouri, Nebraska)
Purpose: Goal was to develop a rule-based procedure for identifying and quantifying reference conditions/sites. Purpose is for diverse agencies in region to define shared core factors for defining habitat quality – a "minimum" set of standards upon which they can agree
Method: (no name)
Type of approach: list of factors, apparently primarily as an guide and aid to "best professional judgment" process
Core factors and how scored: Eleven important core factors identified by consensus (listed below). For each factor, there is a qualitative or semi-quantitative list of considerations given that further define how sites should be screened (not listed here).

a. Wastewater treatment plants and other point sources
b. Animal feeding/grazing operations
c. Instream habitat
d. Riparian habitat
e. Land use and land cover –broad scale
f. Land use and land cover –site-specific
g. Physical and chemical parameters
h. Altered hydrologic regime
i. Biological metrics
j. Biotic assemblages
k. Representativeness

Other details: The group notes that biological metrics are not to be used as a stand-alone criterion, but rather as a check on the validity of the site being considered for reference status.
Definition: "For a given ecoregion in the Central Plains, 'reference conditions' should represent a population of sites that: (1) Accurately characterizes the range of variability present in healthy natural stream systems (2) Provides an objective definition of the best attainable aquatic conditions (3) Provides a barometer, ruler, benchmark, or reference against which we can measure the condition of other waterways (4) Provides a measurement tool to identify "biological integrity" with respect to the Clean Water Act"

Citation: (Bailey, Norris et al. 2004)
Agency: (none)
Geographic Area: (global)
Purpose: outline the steps in the reference condition approach
Method: empirical modeling of conditions based on sites
Type of approach: conceptual model based on real database
Core factors and how scored:

1. Define the spatial and temporal size of the population/study
2. Determine how many reference sites are to be selected
3. Select reference sites (does not detail a method, but endorses methods of (Davies 1994))
4. Describe variation in reference sites & model "reference condition"
5. Use reference condition to describe sites

Other details: "Although some 'ground truthing' of candidate reference sites is carried out… it is important that criteria for defining potential reference sites are established in advance of the actual sampling, and without regard to the invertebrate community found at a site. Put simply, the structure of the community itself is not used to identify the site as reference or otherwise." Also, "this approach acknowledges that there is variability in biota and associated environmental characteristics among sites that are in Reference condition."
Definition: The reference condition is based on a set of data derived from reference sites. The reference condition is used as a standard for comparison. Deviation from Reference Condition is a measure of the effect of stressors on the ecosystem.

Citation: (Davies 1994)
Agency: [Australian] National River Processes and Management Program Monitoring - River Health Initiative (of the Australian Government – Department of the Environment and Heritage)

Geographic Area: Australia
Purpose: reference site selection is part of an overall standard system for assessing river health in the nation
Method: part of the methodology developed for AUSRIVAS, the Australian River Assessment Scheme
Type of approach: iterative, model-based
Core factors and how scored:
Identify region of concern.
1. Classify [rivers] – for example by stream order, discharge, altitude, climatic region, geology
2. Select at least 20 reference sites. Sites selected based on the absence of impacts:
    a. Major impoundments, extractions or diversions
    b. Vegetation clearance
    c. Catchment's urbanization
    d. Presence of roads or corridors
    e. Channel and bottom modification
    f. Bank and shoreline degradation
    g. Floodplain and wetland drainage
    h. Forestry
    i. Mining or extractive industry
    j. Pollution sources
    k. Intensive agriculture
    l. Grazing
3. Collect physical, chemical and macroinvertebrate data (identify to family level) from selected reference sites – at least twice per year
4. Analyze data using discriminate analysis and cluster analysis
5. Develop and refine model
6. Use model to predict
7. TREAT THIS AS AN ITERATIVE PROCESS – ADJUST MODEL AND DEFINITIONS

Citation: (Yoder 2001)
Agency: Ohio EPA / Midwest Biodiversity Institute
Geographic Area: Ohio
Purpose: establish a bioassessment standard for Ohio
Method:
Type of approach:
Core factors and how scored: Reference sites are selected:
1. To represent best attainable background conditions for a homogenous area.
2. To represent distribution in accordance with principal stratifying factors such as regions,
watershed size, physical attributes, and other factors that drive biological variance.
3. In a biological context

4. Based on the ‘‘cultural setting’’ independent of basic sampling data, especially chemical/physical data.
5. To avoid sites with obvious impacts such as point sources, intensive urbanization, direct habitat degradation, gross nonpoint source impacts, and other influences (spills, kills).

Findings: Note that this method uses the "inverse" from many others (i.e., site selection based on known biotic community quality, presumably as determined by best professional judgment).
Other details: Reference data is used to calibrate indices (IBI, ICI); numerical biocriteria established in line with stratification factors.
Citation: (Lattin and Ringold 2003)
Agency: Dynamac, Inc. and USEPA
Geographic Area: Pilot study was for Utah
Purpose: coarse screening for reference sites
Type of approach: stepwise
Core factors and how scored:

1. Coarse-screen (GIS) and rank by stressors; choose best of each stratum
    a. Use map database like RF3 or NHD
    b. Use "nested networks" to organize data. This is related to stream order. For example a 2nd-order network contains a 2nd-order stream and its 1st-order tributaries. Two second-order networks combine to form a 3rd-order network.
    c. Characterize each network by dominant ecoregion (assign to one ecoregion)
    d. Quantify % network disturbance
        i. Focus is on disturbances in order to avoid the "circularity" problem.
        ii. Data are from a variety of sources and include: LU/LC, transportation, point sources, estimates of livestock density, census data, dams/impoundments, and other state/regional data sources
        iii. GIS buffers created around human activities/stressors – buffer widths crudely represent zone of potential impact
        iv. Within each ecoregion/stream order class (or other desired stratum) select those with low "Coarse Screen Disturbance" scores
        v. In highly disturbed landscapes, an alternative to % network disturbance is a measure of riparian continuity calculated using a sliding window.
        vi. Choose about 10 networks for each reference site you eventually want.
2. Fine-screen (using online topography maps and orthphotos, air photos, BPJ, reconnaissance)
    a. Apply finer resolution on stressors – type & severity, scored on a numeric scale

    b. A sample list of 15 standard stressors includes: Urban/Residential Development, Commercial/Industrial, Roads, OHV trails, Pack trails, Railroads / Power lines, Agriculture, Grazing, Confined animal feeding operations, Logging, Irrigation withdrawals, Water diversions, Impoundments, Rock / gravel extraction, Mineral/oil extraction.
    c. Atypical stressors might include things like abandoned ammunition plant…
    d. Make sure you have 3-4 candidates left (out of 10) for each stratum; if not, resample.
    e. Rank by standard scoring
3. Best professional judgment
    a. Local contacts are given maps, instructions, scoring sheets
    b. Best BPJ sites (perennial and 'least disturbed') identified
4. Field verification
    a. Aerial and ground reconnaissance to confirm flow and status
    b. These sites can now be sampled

Definition: 'Least Disturbed Condition' – found in conjunction with the best available physical, chemical, and biological habitat given today's state of the landscape

Citation: (Hughes 1995)
Geographic Area: any
Purpose: general discussion of defining reference conditions
Type of approach: iterative, multifactor
Core factors and how scored:
This document identifies six approaches, to be used in conjunction with one another:
1. <u>Regional reference sites</u>
2. Historical data
3. Paleoecological data (e.g. lake sediment cores)
4. Experimental laboratory data
    a. Note: "water quality data are especially unsuited for establishing reference conditions for systems disturbed by other stressors, such as structural, hydrological, and biological alterations. Instead, such data are most useful for screening out sites selected by other means or for improving model predictions."
5. Quantitative models (from field and historical data)
6. Best professional judgment

It then outlines how to select <u>regional reference sites</u>: "There are eight steps for selecting regional reference sites. Although they are listed in order of recommended occurrence, the process is not linear, but iterative." The steps are:
1. Define areas of interest on maps
    a. Whenever possible, base the areas on natural rather than political boundaries

2. Define water bodies of interest
    a. Best done hierarchically
    b. Include relevant factors such as temperature, size, river basins, extremes in gradient or velocity, bed materials, connectivity…
3. Delineate candidate reference watersheds
    a. Use maps, data, remote sensing and consult with local experts to identify sites: to reject disturbed areas while retaining minimally disturbed areas
    b. Disturbance can be related to factors such as ownership, land use/land cover, proximity to transportation/utility corridors, landfills, feedlots, fish hatcheries, etc.
4. Conduct field reconnaissance
    a. Recommends use of small airplane observation at about 1500 m
    b. Recommends using a qualitative habitat evaluation form
    c. Look for presence of: complex, extensive, old riparian vegetation, complex shoreline and channel, wildlife and benthos.
    d. Look for absence of: roads, chemical stressors, channel/flow manipulation, sedimentation/turbidity, odors/scums, pipes/drains, human/livestock activity.
5. Subjectively evaluate quality of candidate reference sites
    a. "In some extensively disturbed regions it takes considerable effort to locate minimally disturbed sites…It is not a trivial undertaking."
    b. "If candidate sites in [an ecoregion] are unsuitably disturbed, reference sites in a similar region should also be included."
    c. "Occasionally, minimally disturbed sites are less disturbed because they are markedly different than the majority for natural reasons… such anomalous sites should not serve as reference sites."
    d. Decision making process includes tradeoffs. Reasons for making decisions should be documented.
6. Determine the number of ecoregional reference sites needed
    a. A function of regional variability and size, desired level of detectable change, resources, and study objectives
    b. Three is a commonly used minimum for any waterbody class/size in a homogeneous region.
    c. Statisticians recommend a minimum of twenty sites, total.
7. Quantitatively evaluate biological health of reference sites
    a. Survey a minimum of two biotic assemblages and evaluate rigorously
8. Refinements of the general approach for large rivers, lakes and wetlands
    a. Because there are fewer large waterbodies, it's harder to find reference quality sites.

Other details: "Six approaches to determine reference conditions are discussed… these approaches must be politically acceptable and scientifically defensible. This means that they cannot be based solely on current conditions where those conditions reflect fundamentally altered landscapes."

Citation: (Ohio Environmental Protection Agency 1987)
Agency: Ohio EPA
Geographic Area: Ohio
Purpose: Select reference sites for Ohio streams
Core factors and how scored:

1. SMALL STREAMS:
    a. Five Omernik ecoregions were identified
    b. Within each ecoregion, identified watersheds with drainage areas of 10-300 square miles and 300-6000 square miles.
    c. Preliminary cut made by examination of maps. Eliminated watersheds with evidence of substantial human disturbance Factors identified on maps include:
        i. Population density
        ii. Current and past land use
        iii. Point source discharges.
        iv. A "watershed disturbance ranking" was compiled [not clear whether this is combination of above factors, or something different]
    d. Next cut selection was made after aerial and land reconnaissance. Factors considered included:
        i. Amount of stream channel modification
        ii. Condition of riparian vegetation
        iii. Water volume
        iv. Channel morphology
        v. Substrate character and condition
        vi. Obvious color/odor problems.
        vii. Amount of woody debris
        viii. General 'representativeness' of site within ecoregion
    e. Remaining sites were sampled for macroinvertebrates, fish, and chemical/physical water quality, with detailed descriptions of instream habitat
2. LARGE STREAMS/RIVERS (drainage area >300 sq mi)
    a. Basically same procedure as for smaller
    b. Unavoidably, some sites were located (as far as possible) downstream from urban centers and point sources
3. Searched the existing database to look for additional candidates [not specific about how this was done]
4. In the end, about 200 reference sites were selected.

Findings: based on approach of (Hughes, Larsen et al. 1986) and (Whittier, Larsen et al. 1987)

Definition: "'Least-impacted' watersheds were selected. These are not 'pristine' or 'undisturbed' watersheds (none really exist in Ohio), but they do represent the best watershed conditions within an ecoregion given the background activities prevalent in our society… the character of these streams should reflect the reasonably attainable biological conditions and water quality within a particular ecoregion."

Citation: (US EPA 2002)
Agency: Indiana Department of Environmental Management
Geographic Area: Indiana
Purpose: select reference sites for streams in Indiana?
Method: not entirely clear
Type of approach: <u>"IDEM uses a non-typical process for developing reference condition: reference condition is represented by a percentage of the total population of the sites sampled."</u>
Core factors and how scored:

1. Reference Site/Condition Development
    a. "IDEM uses a non-typical process for developing reference condition: reference condition is represented by a percentage of the total population of the sites sampled. Reference condition is defined by a historical cross-section of sample sites representing the full gradient of ecological conditions as they existed during statewide or ecoregion specific investigation."
2. Number of reference sites: unknown
3. Reference site determinations:
    a. Based on best professional judgment, on a regional/aggregate basis
    b. Not using site specific or paired watershed approaches
4. Reference site criteria:
    a. "Deviation from central tendencies on multimetric indices and the qualitative habitat evaluation index (QHEI) is also taken into consideration when evaluating impairment. Field chemistry is measured and probabilistic sites are sampled for broad chemical analysis."
5. Characterization of reference sites within a regional context
    a. Based on: historical conditions**, least disturbed sites, gradient response – NOT solely through BPJ.
    b. IBI is calculated on drainage area for headwater streams, wadeable rivers, large rivers, and great rivers
6. Stream stratification within regional reference conditions
    a. Based on ecoregions or aggregates, multivariate groupings, and 8-digit HUCs
    b. Note based on elevation, stream type, or jurisdiction
7. Additional information:
    a. Reference sites linked to ALU *(in a statistical sense)*
    b. Some reference sites represent acceptable human-induced conditions *(it is understood that all sites have a human-induced condition)*
    c. Reference sites/conditions are NOT referenced in water quality standards

Definition: <u>"IDEM uses a non-typical process for developing reference condition: reference condition is represented by a percentage of the total population of the sites sampled…Reference condition is defined by a historical cross-section of sample sites representing the full gradient of ecological conditions as they existed during statewide or ecoregion specific investigation."</u>

Citation: (Kansas Department of Health and Environment 1995)
Agency: Kansas Department of Health and Environment
Geographic Area: Kansas
Purpose: Reference sites in the context of overall macroinvertebrate monitoring network
Type of approach: [Best professional judgment]
Core factors and how scored:
Not explained in any detail – presumably "best professional judgment." One of the questions in the list of criteria for including a site in the monitoring network is whether it represents an "unusually pristine location, suitable for use as a long-term ecoregional reference location." Six reference sites ("relatively unpolluted locations" that represent the state's major river basins) are each *sampled quarterly* to provide a comparison for the 50-100 other sites that are regularly monitored.
Other details: and "Reference sites will likely increase in number and play an increasingly important role in surface water quality assessments performed by the department."
Definition: "Reference sites serve to identify the variation in community structure and species abundance associated with relatively unperturbed streams in a given land use setting, geological or geographical area, or 'ecoregion'"

Citation: (Missouri Department of Natural Resources 2004)
Agency: MDNR
Geographic Area: Missouri
Purpose: protect aquatic life
Type of approach: stepwise, based on (Hughes, Larsen et al. 1986) and (Rabeni, Sarver et al. 1997)
Core factors and how scored:
Very explicitly identified as a six-step process, with an alternative. Quoted verbatim, below:

1. Minimization of Human Impacts and/or Disturbance.
    a. Select water bodies that have reduced levels of human activity as suggested by population density, point source pollution, channel/basin modification, land use or otherwise abnormal diffuse sources of water contamination.
2. Comparative Water Body Size
    a. Select water bodies that have similar flow patterns, watershed areas, and average annual discharge or residence times. If possible, discharge differences between the impacted and the reference site should be less than an order or magnitude.
3. Water Body Channel or Basin Similarity
    a. Locate and investigate inflows, outflows, sinks and major receiving waters. Identify drainage patterns, channel/basin morphology and

stream/basin gradients. Select reference water body most representative within the region.

4. Locate Refuges
   a. Locate areas where human impacts are minimized due to federal, state or county mandates. Examples include state or federal parks, monuments, grasslands, forests or wilderness areas. Select candidate reference water bodies within refugia unless the landscape is atypical of the region.

5. Investigate Historical Patterns
   a. Determine migration barriers, historical connections between streams and lakes, and known zoogeographic patterns of distribution. Select water bodies that have the potential to have similar biologic richness.

6. Select Reference Water Body. Select from remaining candidate reference streams those that have the least degraded or disturbed watersheds.

ALTERNATIVE If it is judged by the department that a reference water body does not exist within a similar ecoregion, the "trisection," method will be applied to the water body of concern to determine reference conditions. The trisection method involves partitioning the statistical distribution of data values into three equal sections after the worst 5th percentile has been discarded from the data set. Then the highest or lowest one third (whichever represents the less polluted condition) of the remaining distribution is selected as the reference condition.

Findings: a very explicitly outlined methodology based on the 1997 regional report

Definition: "Reference Stream Reach (approved by EPA, 2000) 10 CSR 20-7.031(1)(R): Stream reaches determined by the Missouri Department of Natural Resources to be the best available representatives of ecoregion waters in a natural condition with respect to habitat, water quality, biological integrity and diversity, watershed land use, and riparian conditions."

Citation: (US EPA 2002)

Agency: Nebraska Department of Environmental Quality

Geographic Area: Nebraska

Purpose: Assessment of aquatic life support

Core factors and how scored:

1. Number of reference sites: 38
2. Reference site determinations:
   d. Based on best professional judgment, on a regional/aggregate basis, or site specific
   e. Not using paired watershed approaches
8. Reference site criteria:
   a. "No waste water treatment plants, other point sources, or concentrated animal feeding operations (CAFOs); good instream habitat, riparian habitat, land use and cover, physical and chemical parameters, biological metrics, and faunal assemblages; no altered hydrologic regimes; representativeness. At a minimum, sites need to be in the top 10 to 20 percent of all sites sampled in the ecoregion, with little disturbance and no spills or discharges within sites area."

9. Characterization of reference sites within a regional context
    a. Least disturbed sites are selected; also should be regionally representative and reasonably attainable.
10. Stream stratification within regional reference conditions
    a. Based on stream types
    b. Based on ecoregions or their aggregates,; there are three ecoregions and six strata with roughly five reference sites in each.
    c. Not based on elevation, jurisdiction, or multivariate groupings.
11. Additional information:
    a. Reference sites linked to ALU
    b. Some reference sites represent acceptable human-induced conditions

Findings: This report is two years old; information may have changed.
Other details: "Nebraska agrees with the reference site concept but needs to determine if appropriate reference sites exist in Nebraska. NDEQ is currently evaluating macroinvertebrate and fish data to locate both excellent and severely impaired sites in order to determine the appropriate habitat conditions that correspond to both extremes. Reference site criteria have not yet been finalized."

Citation: (Nebraska Department of Environmental Quality 1999)
Agency: NDEQ
Geographic Area: Nebraska
Purpose: reference site selection for 1998 305(b) report
Type of approach: minimum criteria (checklist)
Core factors and how scored:

1. Reference sites identified and stratified through use of landscape and habitat characteristics
    a. Characteristics include: ecoregion, stream flow/discharge, water temperature, substrate type, vegetation type, presence of trout
    b. At finest scale of resolution, identified 85 stream classes.
2. Each site scored using a checklist. Sites for which all of the following were true were considered reference sites:
    a. "Threshold qualification" questions all scored YES (a single "NO" to any of the questions disqualifies the site from consideration). Questions relate to:
        i. Whether site is wholly within ecoregion/subecoregion of interest
        ii. Presence of upstream impoundments
        iii. Presence/type of dischargers upstream
        iv. Presence/history of pollution or contaminants
        v. Presence of high density urban/commercial development
        vi. Channelization
    b. "Riparian/buffer zone conditions" all scored <5
    c. "Potential nonpoint source pollution impact" questions all scored <5
    d. "Instream habitat condition" question scored >7
    e. "Final reference site rating" total scored >16

Citation: (US EPA 2003)
Agency: US EPA Research & Development
Geographic Area: Mid-Atlantic Region (Delaware, etc.)
Purpose: Mid-Atlantic Integrated Assessment
Core factors and how scored:
Two methods

1. "BPJ method" Regional experts hand-selected 60 reference sites based on experience
2. "Objective method" reference sites were selected based on the following six criteria, developed specifically for the Mid-Atlantic region:
    a. Acid-neutralizing capacity of >50 ueq/L
    b. Total P < 20 ug/L
    c. Total N <750 ug/L
    d. Chloride <100 ueq/L
    e. SO4 <400 ueq/L
    f. Mean RBP habitat score >15, based on USEPA's Rapid Bioassessment protocol

Findings: A very important finding: "Reference sites did not always meet criteria for reference conditions." 73% of the handpicked sites (44/60) did not meet the objectively defined criteria. The lesson: "Best professional judgment should always be confirmed with objective criteria"

Appendix 2. Detailed outline of the core factors suggested for uses in assessing potential reference sites located within ecoregions occurring in USEPA Region 7 (Iowa, Kansas, Nebraska and Missouri).

Core factors to be considered when selecting regional reference streams in USEPA Region 7

(Region 7 biocriteria workgroup, April 27, 2000)

1. Wastewater treatment plants and other point sources

- Prefer no point source
- Acceptable if discharge effects are minimal
  - o Minimize number, density, and size of facilities
  - o Site not in close proximity to point source (below effective mixing zone)
  - o Effluent to stream flow ratio low
  - o No impairment of aquatic life beneficial use due to point source discharge
  - o Existing point sources have record of compliance

2. Animal feeding/grazing operations

- Prefer none
- Prefer no cattle access upstream
- Acceptable if influence AND potential of degradation is minimal
  - o Number of facilities low
  - o Number of animal units low
  - o Site not in close proximity to cattle access or feeding operations
  - o Site not in close proximity to land application of livestock waste
  - o No impairment of aquatic life beneficial use due to livestock impacts

3. Instream habitat

Under reference conditions, instream habitat is characterized by the highest quality and diversity of instream habitat relative to stream type, considering:

- No excessive sedimentation or embeddedness
- No riprap
- No unnatural (manufactured) substrates

4. Riparian habitat

Under reference conditions, riparian habitat would provide an effective buffer that maximizes instream habitat potential:

- No row crops
- No removal of riparian vegetation
- Preference to natural riparian conditions
- Width, length of riparian area considered

5. Land use and land cover – broad scale

This consideration involves a two-step evaluation process:

- Step one: Characterize ecoregions or sub-ecoregions using following LU/LC categories:
  - Row crop
  - Timber
  - Grass/herbaceous vegetation
  - Artificial (e.g. buildings, impervious cover)
  - Water
  - Barren (e.g. quarries, mines)
  - Land treatment
- Step two: Summarize the LU/LC percentages by 12-digit HUCs (10-40 thousand acres) to develop summary statistics for the range of each LU/LC category

6. Land use and land cover – site-specific

- For a candidate reference site and its watershed, determine the LU/LC percentages.
- Site-specific LU/LC should not be anomalous compared to the broad-scale LU/LC.
- Percent of land cover that is natural and/or land use is treated (e.g. application of BMPs and appropriate land management) exceeds that of broad-scale ecoregion

7. Physical and chemical parameters

- Prefer sites meet or exceed aquatic life standards over the long term
- Sites should reflect best attainable physical or chemical conditions within ecoregion and flow conditions

8. Altered hydrologic regime

- Minimal channelization effects (no influence is preferred)
- Prefer sites not under influence of dams
- Sites located away from bridges and crossings influences
- Sites located away from outfall structures (e.g. storm sewers, tiles) influences
- No influence from anthropogenic dewatering
- Little or no influence of impervious surfaces or urban runoff

9. Biological metrics

- This is not a stand-alone factor
- Index of metric scores should be among the highest for a defined population in region
- Caveats: This is data-driven to determine if site will be a valid reference site; not a good choice to select your site, but a good check on the validity of the site being considered for reference level

10. Biotic assemblages

- Biotic diversity is consistent with both historical assemblages (where available) and current distributions.
  - Presence of rare/unique communities

    - Limited number of exotics
    - Temporal variations considered
    - Few native species lost
    - Presence of threatened or endangered species
- Take into account stream classification and size
- Migration possibilities should be taken into consideration: dams, reservoirs, drainage divides, etc. can prevent recolonization of reaches

11. Representativeness

- Reference sites should represent the range of biological, physical, and chemical conditions of the ecoregion
- These sites should be minimally disturbed by anthropogenic activities
- A sufficient number of sites should be selected to adequately represent different stream classes (e. g. cold water, saline, large, small) and capture the natural variability within specific classes. A stream classification system should be used when physical, chemical and biological expectations among lotic water types differ enough as to have quantifiable distinct attributes.