Regional Ecological Monitoring and Assessment Program

Ecological Assessment of Streams in the Little Colorado River Watershed, Arizona, 2007

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Ecological Assessment of Streams in the Little Colorado River Watershed, Arizona, 2007

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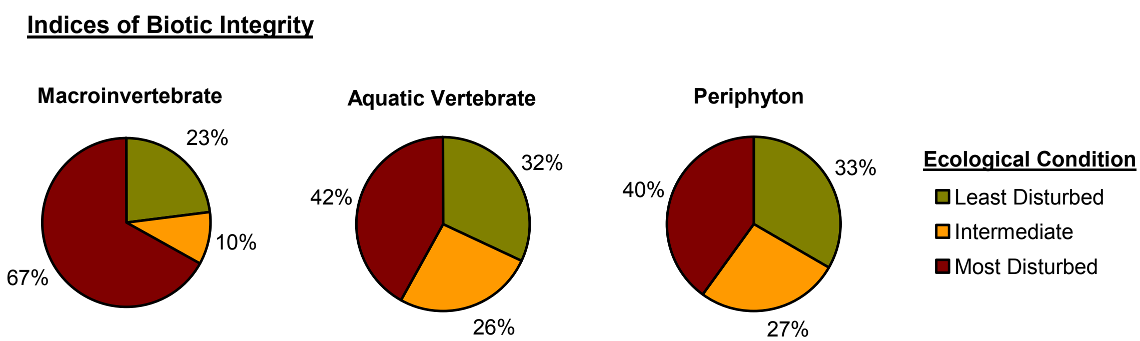
Cover Photo: Chevelon Canyon at telephone ridge above Horse Trap Canyon in the Little Colorado River basin (site ID AZ06631-183).

Executive Summary

As part of the Regional Ecological Monitoring and Assessment Program grant project, the ecological condition of the Little Colorado River (LCR) watershed was assessed based on the biological, chemical, and physical habitat data collected from 30 randomly selected wadeable perennial stream locations within the LCR basin. Indicators of ecological condition and anthropogenic stress were categorized into three condition classes (most disturbed, intermediate, and least disturbed) based on the established standards or thresholds derived from reference condition. A large proportion of the assessed LCR stream length was found to be in most disturbed condition with respect to biotic indicators of ecological condition, such as the indices of biotic integrity for macroinvertebrates, aquatic vertebrates, and periphyton (see figure below). The most pervasive stressors observed in the LCR basin were non-native aquatic vertebrate species (most disturbed in 53% of the stream length), non-native crayfish (present in 43% of the stream length), and habitat integrity (most disturbed in 40% of the stream length). Stressors associated with poor biotic integrity were degraded habitat integrity, crayfish presence, low riparian vegetation cover, and poor streambed stability. Combined with their high prevalence, crayfish and degraded habitat integrity were identified as the most important stressors to be targeted for improvement of the overall ecological condition of the LCR streams.



Percent of Assessed Perennial Stream Length in the LCR Basin

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Introduction

The Environmental Monitoring and Assessment Program (EMAP) was initiated by the U.S. Environmental Protection Agency (EPA) to develop tools necessary to monitor and assess the status and trends of national ecological resources. One of its goals is to evaluate the feasibility of using a probabilistic monitoring design that is consistent spatially and temporally such that local and state ecological condition estimates can be aggregated to regional and national levels. When EMAP is implemented over time, these estimates can be used to detect and quantify changes in condition through time. An EMAP assessment estimates the ecological condition of aquatic resources based on direct measures of key biotic assemblages such as benthic macroinvertebrates, aquatic vertebrates, and periphyton. An EMAP assessment also helps to identify anthropogenic stressors associated with the disturbance of these aquatic resources (U.S. EPA 2002).

In 2000-2004, the Arizona Game and Fish Department (AGFD) and the U.S. Geological Survey (USGS) Arizona Water Science Center cooperated with EPA to participate in the Western EMAP (WEMAP) study (Stoddard and others 2005b). Based on WEMAP sampling in Arizona, AGFD and USGS completed the first state-wide ecological assessment of streams and rivers (Robinson and others 2006; referred to as the 'Arizona assessment' for the remainder of this report). However, there were shortcomings in the 'Arizona assessment', which are addressed through this Regional EMAP (REMAP) grant funded project. The major shortcoming of the previous study was the lack of an accurate perennial stream map from which to select random sites. In the present study ADEQ utilized an improved perennial stream map, produced by a modeling study conducted by the USGS in the Phase I portion of this REMAP grant. In Phase II of the REMAP grant ADEQ obtained a random site list from USEPA, and implemented a probabilistic monitoring design in one basin to try out and evaluate this monitoring design for incorporation into our surface water ambient monitoring program. This report is the product of the basin-wide study. Also in Phase II, we conducted a comparison study of ADEQ and EMAP macroinvertebrate and habitat data collection methods which are presented in a separate report (Spindler and Paretti 2009).

The present study constitutes one of the two objectives of the REMAP grant; to assess the ecological condition of wadeable perennial streams within the Little Colorado River (LCR) watershed using EMAP protocols in order to evaluate how ADEQ might adopt EMAP methods into the surface water monitoring program. To conduct this basin-wide probabilistic survey of the Little Colorado River basin, ADEQ partnered with AGFD and USGS to collect biological, chemical, and physical-habitat data from 30 randomly-selected wadeable, perennial stream sites in the LCR watershed in 2007 (Figure 1 and Appendix 1). This report is an EMAP assessment of the LCR streams based on the EMAP framework outlined in Stoddard and others (2005b) and Robinson and others (2006).

Study Area

The LCR watershed is located in northeastern Arizona (Figure 1). The watershed drains a total of 79,880 square kilometers, almost the entire northeast quarter of the state and a small portion of northwestern New Mexico. Approximately 50% of the watershed area is on Native American Indian Reservations and is out of the state's jurisdiction. This study focuses on the non-tribal area within the Arizona state border as shown in Figure 1.

The LCR watershed includes several large mountain ranges with some of the highest peaks in Arizona (Figure 2). The highest is Humphreys Peak at 3,850 meters on San Francisco Mountain just north of Flagstaff. Much of the watershed's southern edge is defined by the 480-kilometer long Mogollon Rim, a steep escarpment, with an average elevation of 2,100 meters. The Mogollon Rim transitions into the White Mountains near the New Mexico border, where Mount Baldy and Escudilla Mountain are two prominent peaks with 3,500 and 3,000 meters elevations, respectively. The lowest elevation in the basin is 820 meters at the mouth of the LCR.

Figure 1. Little Colorado River watershed and study area.

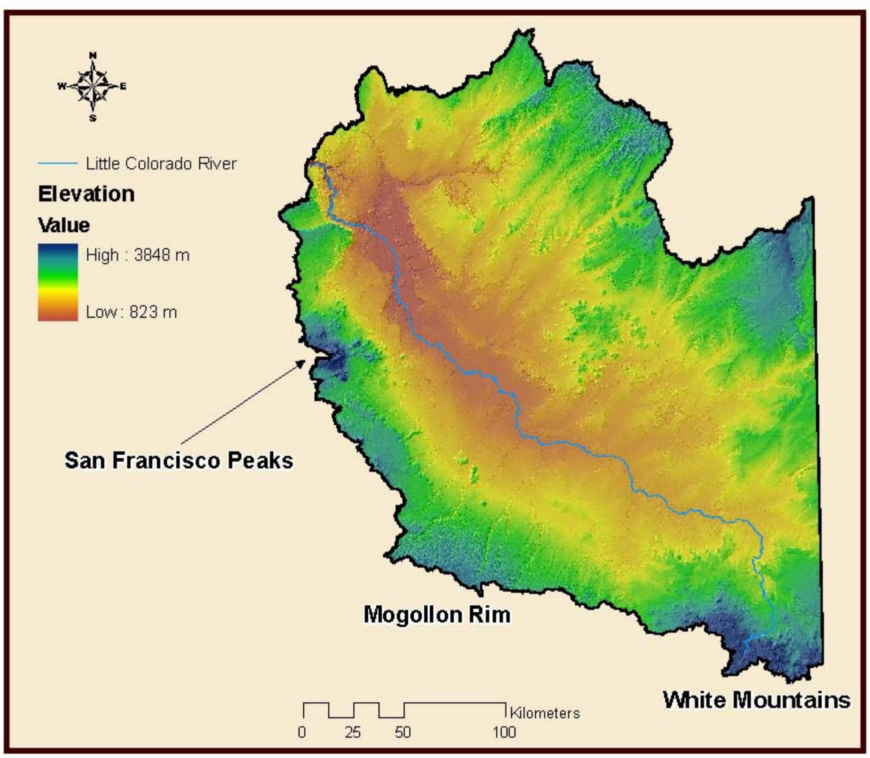

Figure 2. Little Colorado River watershed topography

The LCR headwaters originate in the White Mountains and form the main stem of the LCR in Greer. From Greer the LCR flows generally north to Lyman Lake and continues, mostly intermittently, northwest to the Colorado River in Grand Canyon (Figure 1). Flow alterations caused by impoundments and diversions are common throughout the watershed, causing a number of stream reaches to flow only intermittently or ephemerally. For example, 4 out of 6 sites along Silver Creek were determined to be "non-target" or intermittent due to irrigation diversion in the Snowflake-Taylor area.

Perennial flows are found in the higher elevations due to winter snow, monsoon storms, and springs. The largest tributary, Silver Creek, is fed by the largest spring in the basin (Silver Creek Spring) southeast of Snowflake-Taylor with a discharge of 3,648 gpm (measured in 1990, ADWR 2006). Main sources of perennial flows at the 30 sites sampled for this assessment were snowmelt at 37% and springs at 27% (Figure 3). Ten percent of the sites were located downstream of reservoirs and had regulated flows.

The LCR and its tributaries flow through a variety of landforms such as mountain meadows, coarse colluvial deposits, bedrock canyons, and alluvial deposits. Rosgen and Silvey (1996) devised a stream classification system, in which the Level 1 stream classification involves characterizations of channel morphology, valley types, and landforms. Figure 4 shows Level 1 stream types observed in the LCR basin, and their general descriptions are given in Table 1. Most dominant stream types among the 30 sites were B streams (50%) and C streams (20%).

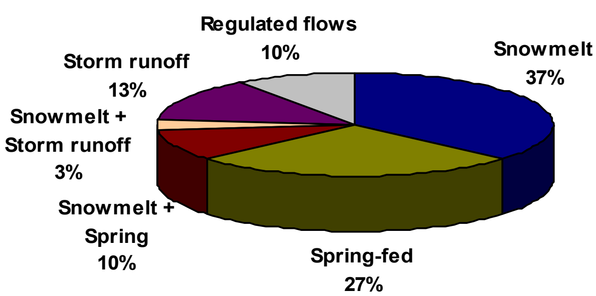

Figure 3. Main water sources contributing to perennial flows at random sampling sites (n=30).

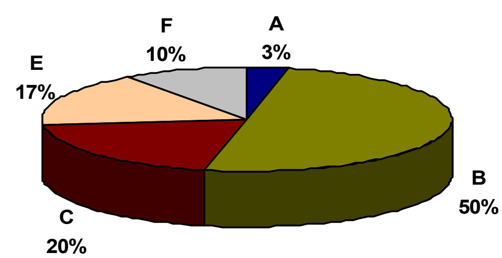

Figure 4. Rosgen Level 1 Stream Types in the LCR watershed (n=30).

Table 1. Rosgen level 1 stream type descriptions.

Stream Type	General Description
A	Steep, entrenched, and cascading step/pool channel.
B	Riffle-dominated channel on moderate gradient in narrow valley.
C	Meandering riffle/pool channel with point bars and well-defined floodplains.
E	Highly sinuous riffle/pool channel in broad valley/meadows.
F	Entrenched and meandering riffle/pool channel on low gradient.

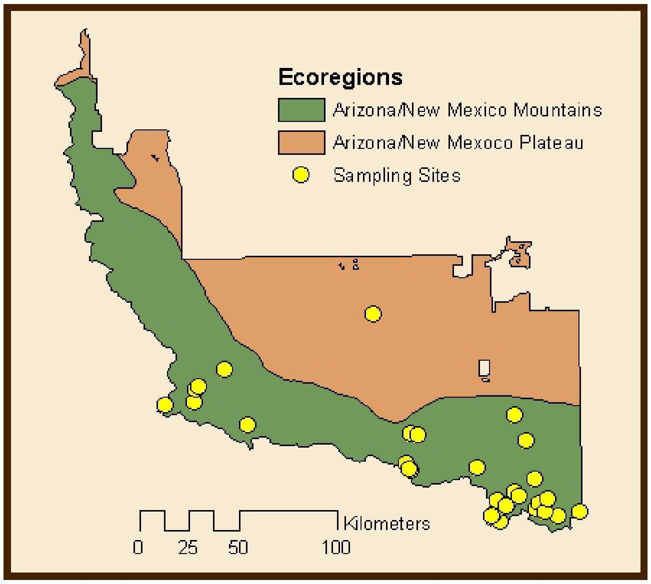

Figure 5. Ecoregions in study area.

Omernik (1987) divided the United States into 104 Level III ecoregions. Both the WEMAP assessment (Stoddard and others 2005b) and the 'Arizona assessment' (Robinson and others 2006) reported results within broader ecoregions aggregated from Omernik's ecoregions. Two of the Omernik Level III ecoregions occur in the study area: Arizona/New Mexico Mountains and Arizona/New Mexico Plateau (Figure 5). The Mountains region, which lies along the southern border of the watershed, accounts for about 50% of the total study area. The region is characterized by mountainous terrain with pinion-juniper and oak woodlands at low to mid-elevations and ponderosa pine forests at high elevations. The Plateau ecoregion, the other 50% of the study area, is characterized by desert vegetation at low elevations, grass and shrublands at mid-elevations, and pinion-juniper woodlands at high elevations. Most perennial stream sites identified in our study occurred in the Mountains region, and only one probability site (LCR at Holbrook) was located in the Plateau region (Figure 5). Because of our limited sample size, the results in this assessment were only reported on a basin-wide scale. All sampling sites were located above 1,524 meters (5,000 feet) and were categorized as "cold water" streams for the purpose of assessment using Arizona water quality standards (ADEQ 2009).

Precipitation in the LCR basin generally increases with altitude and varies widely season to season. Precipitation is usually highest during summer months of July and August and peaks again during winter months with the driest period in April through June. This study took place during the spring index period of May and June of 2007 when baseflow conditions prevail in cold water streams as per ADEQ sampling requirements (ADEQ 2008). Spring of 2007 was especially dry throughout Arizona with temperatures well above average. For the 3-month period from April to June in 2007, precipitation in the LCR basin was well below normal, ranking below the 25th percentile among the monthly means over the period of 1971-2007 for the LCR basin (Office of the Arizona State Climatologist 2007). Similarly, stream flows measured at select USGS gages in the LCR basin show that flows during the spring months of 2007 were considerably lower than the 30-year average monthly flows measured at the same stations (Figure 6, USGS 2008). The snowpack records from Mt. Baldy indicate consistently dry and warm conditions for the LCR basin since 1999 (ADWR 2006).

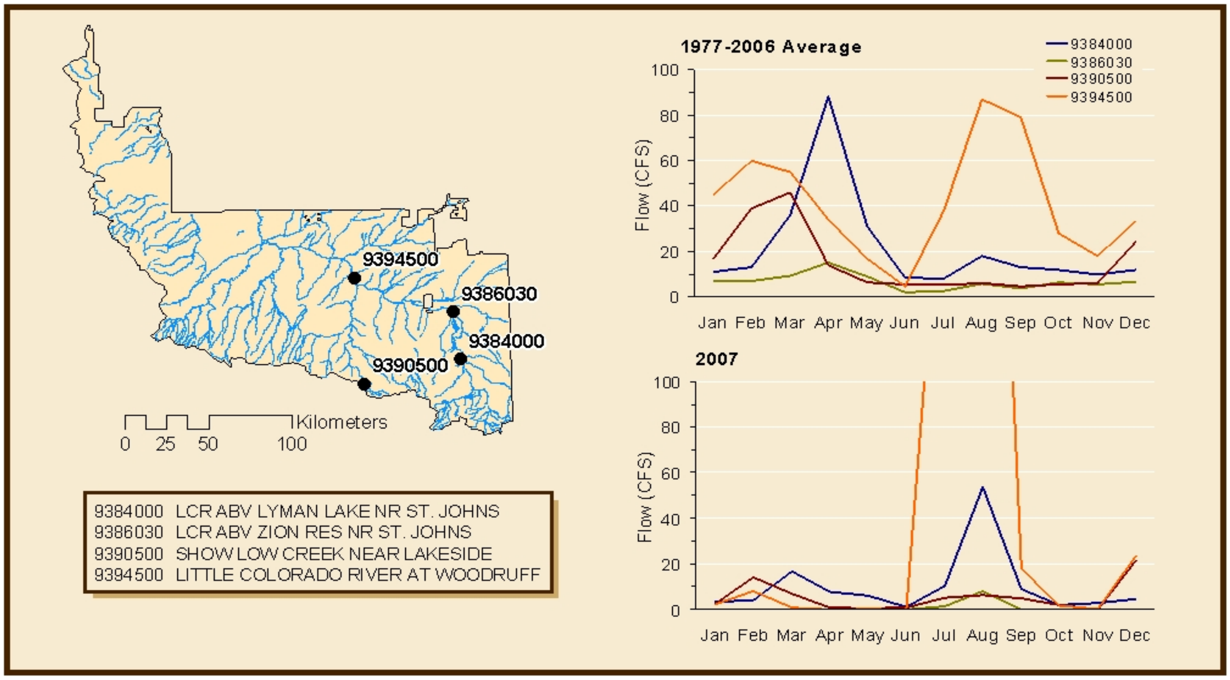

Figure 6. Select USGS gage locations in study area (left) and their monthly mean stream flows for the period of 1977-2006 as compared to 2007 (right). Stations were selected based on the completeness of data. Note that monthly mean flows for the station 9394500 (LCR at Woodruff) were 214 cfs in July and 395 cfs in August, 2007, which are off the chart and not shown on the graph.

Methods

Sites were selected using the EMAP probabilistic survey design to insure the results would be representative of all wadeable perennial streams in the LCR basin. The probabilistic design implemented by EMAP allows (1) the results from a small number of samples to be extrapolated to the entire target population and (2) confidence intervals to be calculated for any estimates (U.S. EPA 2002). The sampling frame used to select the sites was based on the ADEQ Perennial Streams GIS cover that was updated from the original version of an AGFD map from 1993. The sites were evaluated in the order listed in the sample list until at least 30 sites that met the EMAP criteria (flowing, wadeable, and accessible) were identified. Each site was assigned an equal survey design weight (in kilometers) as the sampling frame was not stratified (i.e., the weights were obtained by dividing the total stream length in the sampling frame by the number of evaluated sites). Detailed descriptions on the survey design are given in Stoddard and others (2005a).

The monitoring and assessment were conducted using EMAP protocols (Peck and others 2006) except for water chemistry data, which were collected using ADEQ methods. The EMAP team sampled a stream reach length that was 40 times the average stream channel width (minimum 150 meters). Each reach was divided into 10 sub-reaches of equal length (11 transects) for the necessary habitat observations and measurements and biological sample collections.

Sample Collection Methods

Benthic Macroinvertebrates

Benthic macroinvertebrates were collected using a D-frame kick net (30-centimeter [cm] wide, 500-micron mesh size). Samples were collected at a randomly selected location along each of the 11 transects, combined into a single composite sample, and preserved with alcohol. The sampling effort at each sampling location consisted of placing the D-framed net on the stream bed in the path of flowing water, kicking or scrubbing a 30 x 30 cm^2 area of substrate vigorously for 30 seconds, and adding the contents of the net to the bucket to form a composite sample. For pool habitat, macroinvertebrates were netted by actively sweeping the net through a 30 x 30 cm^2 area for 30 seconds. Samples were mailed to EcoAnalysts, Inc. laboratory in Moscow, Idaho for taxonomic identification and enumeration.

Aquatic Vertebrates and Crayfish

Aquatic vertebrates (fish and amphibians) were collected using a Smith-Root Inc. Model LR-24 backpack electrofisher, making a single pass through the entire reach; crayfish were also collected and documented because they were vulnerable to electroshocking. The fishing effort (7 to 73 total shocking minutes) was allocated equally among all the sub-reaches. Vertebrates captured were processed at the end of each sub-reach: species were identified, counted, and checked for the presence of any external anomalies, and returned to the stream. Voucher specimens were not collected, but threatened or endangered species were photographed.

Periphyton

Periphyton samples were collected from each of the 11 transects adjacent to the location of the benthic macroinvertebrates sample. These 11 samples were combined and preserved with Lugol's iodine. Epilithic samples were collected from transects containing coarse substrate (cobble and gravel). The substrate was placed in a plastic dish and a 12-cm^2 area of the rock surface was delineated with a template. Using a scalpel the periphyton were scraped from the sampling area of each rock and the algal material was rinsed into a dishpan. Following the scraping, the area was brushed thoroughly and rinsed again. Fine sediment was collected if coarse substrate was absent or the transect was located along a pool. For the fine-sediment habitat, a 12-cm^2 area was delineated, and the top 1 cm of sediment was vacuumed into a syringe and added to the composite sample (Peck and others 2006). Samples were mailed to EcoAnalysts, Inc. laboratory in Moscow, Idaho for taxonomic identification and enumeration.

Mercury in Fish Tissue

Fish tissue muscle filets were used for mercury analysis. Two classes of fish tissue samples were collected. The small-fish tissue sample was comprised of small fish species, less than 100 millimeters in total length, with a combined weight of 400 grams (≈100 fish). The large-fish tissue sample consisted of three whole individuals of different species each greater than 120 millimeters in total length. The species selected for sampling were prioritized from most piscivorous to least piscivorous (e.g., bass, trout, catfish, sunfish, minnows, and suckers). This species hierarchy was used because piscivores have more potential for the bioaccumulation of contaminants than do herbivores or detritivores. Specimens were kept on ice while in the field and then frozen until they could be analyzed. The Arizona Department of Health Services (ADHS) state laboratory analyzed the specimens following EPA method 7473 (mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry; February, 2007).

Water Chemistry

Water samples were collected in 1 L plastic bottles by the ADEQ team and submitted to the ADHS state laboratory for total nitrogen, total phosphorus, suspended sediment concentration (SSC), and general inorganic chemistry analyses. Specific conductivity, dissolved oxygen (DO), and pH were measured in-situ with a Hydrolab® multiprobe meter. Turbidity was also measured in the field using a Hach® Environmental turbidity meter.

Physical Habitat

Habitat measurements at each site included (1) a thalweg profile, (2) woody debris tally, (3) substrate and channel dimensions, (4) riparian vegetation characterization and (5) fish cover estimates.

- A thalweg profile comprised of measuring the maximum channel depth, classifying channel units such as pools, and checking for the presence of backwaters, side channels and deposits of fine sediment. The measurements were taken at 10 - 15 equally-spaced intervals between each of 11 transects (100 - 150 measurements along entire reach).
- Large woody debris was counted in each sub-reach and classified into groups based on length, width, and location within or above the bankfull channel.
- Substrate size and embeddedness were visually estimated at five points along equally spaced transects. Channel incision, wetted width, and bankfull channel dimensions were also measured at each transect. The stream slope and compass bearing were obtained between successive transects.
- Riparian vegetation canopy cover density was measured with a densiometer at each bank and in the center of the stream, and riparian vegetation was classified by type and density structure. The proximity of anthropogenic disturbances were noted.
- Fish cover provided by instream habitat features, such as undercut banks, overhanging vegetation, large wood, boulders, and tree roots, was visually estimated at each transect.

Assessment Methods

Indicators of Ecological Conditions

The diversity and abundance of biotic communities in rivers and streams can be used as indicators of the ecological condition of those water bodies (Stoddard and others 2005b). For the LCR stream assessment, the biotic integrity of macroinvertebrate, aquatic vertebrate, and periphyton communities was assessed using indices of biological integrity (IBI). An IBI is the averaged or summed score of several individual measures or metrics that reflect important components of assemblage structure or function, including taxonomic richness, composition, and sensitivity to pollution (Stoddard and others 2005a). The index score ranges from 0 to 100 with the higher values indicating a balanced community with species composition, diversity, and functional organization comparable to that of least-disturbed habitat in a region.

Table 2 lists the IBIs and briefly describes the metrics used for each. The macroinvertebrate IBI was developed by ADEQ using a statewide network of historical reference site data between 1992 and 2003 (ADEQ 2008). The ADEQ cold water IBI used 30 reference sites located above 1,524 meters (5,000 feet). The aquatic vertebrate IBI was primarily developed by EPA during the WEMAP assessment (Stoddard and others 2005a and 2005b, Whittier and others 2007b). Metrics used in the aquatic vertebrate IBI for the LCR basin were the same as given for the Mountains climatic region in Whittier and others (2007b), except that the ‘native sensitive long-lived species’ metric in the life history metric category was not included because there were no aquatic vertebrates in the LCR basin samples in that category (i.e., all values for the metric were zero). More details on determinations of macroinvertebrate and aquatic vertebrate IBIs are found in Appendix 2. New in this assessment is the periphyton IBI developed by the USGS for ADEQ for the LCR basin streams above 1,524 meters (5,000 feet). A brief description of the development of the periphyton IBI is provided in Appendix 3. Indicator data are presented in Appendix 4.

Table 2. Aquatic indices of biological integrity and metric components.

Index of Biological Integrity	Metric Category
Macroinvertebrate IBI (value range 0-100)	• Taxonomic Richness – Number of total taxa, number of true fly larvae • Taxonomic Composition – Percent abundance of stoneflies • Feeding Groups – Percent abundance and number of taxa in the scraper functional feeding group • Pollution Tolerance – number of intolerant taxa, abundance-weighted average tolerance of assemblage (Hilsenhoff Biotic Index)
Aquatic Vertebrate IBI (value range 0-100)	• Habitat Use – Proportion of individuals that are sensitive and rheophilic (sensitive to pollution and prefers to live in fast-flowing water) • Pollution Tolerance – Assemblage tolerance index (Whittier and others 2007a) • Feeding Groups – Proportion of individuals that are sensitive and invertivores-piscivores (sensitive to pollution and eat insects and fish) • Reproductive Strategy – Proportion of all species that are lithophilic (spawn on rocks) • Taxonomic Composition – Proportion of vertebrate abundance in family Salmonidae (trouts) • Non-native Species – Proportion of individuals that are non-native
Periphyton IBI (value range 0-100)	• Composition – Percent abundance of Achnanthes minutissima (Disturbance Index), percent abundance of the motile genera Navicula, Nitzschia, Cylindrotheca, and Surirella (Siltation Index) • Organic Pollution Tolerance – Lang-Bertalot modified by Bohls, Van Dam Saprobity (Van Dam and others 1994) • Habit – Percent abundance of individuals that are not motile

Aquatic Indicators of Stress

The EMAP assessment involves not only the assessment of the ecological condition indicators such as IBIs, but also the assessment of some human-caused stressors that have negative effects on aquatic ecosystems. These stressors can be chemical, physical, or biological. Table 3 lists stressors examined in this study, which includes the same set of stressors examined in the WEMAP study plus additional chemical stressors regulated in Arizona. Indicator data are presented in Appendix 4.

The EMAP protocol includes several measures of habitat, and in WEMAP assessment (Stoddard and others 2005b) identified four habitat metrics that significantly contributed to the condition of aquatic invertebrate and fish assemblages. These four habitat metrics are streambed stability, habitat complexity, riparian vegetation cover complexity, and riparian disturbance. The underlying habitat features that these metrics are attempting to capture perform robustly in describing the effects of stressors on stream biota along local and regional scales (Kauffman and others 1999).

Table 3. Indicators of anthropogenic stress examined in the study. *Stressors added by ADEQ

Stressor Type	Aquatic Stressor	Description
Chemical	Total phosphorus	Common ingredient in fertilizers, contributes to excessive algae growth
	Total nitrogen	Sum of nitrate, nitrite, and total Kjeldahl nitrogen. Found in fertilizers, human and animal waste, and atmospheric deposition, contributes to excessive algae growth.
	Specific conductivity	A measure of dissolved minerals or salinity in water. High salinity common in irrigated areas.
	Dissolved oxygen (DO)*	A measure of oxygen in water, regulated by the state for the aquatic and wildlife designated use.
	pH*	A measure of acidity in water, regulated by the state for the aquatic and wildlife designated use.
	Turbidity*	A measure of water clarity or ability to pass light in water.
	Suspended sediment concentration (SSC)*	A measure of the amount of sediment suspended in water, regulated by the state for the aquatic and wildlife designated use.
	Mercury in fish tissue	A measure of the amount of mercury in fish muscle tissue. Bioaccumulation in fish due to mercury contaminated water from mining, coal combustion, waste incineration, herbicides, fungicides, and pulp, paper, and textile effluents.
Physical habitat	Streambed stability	Measured as Log_{10}[relative bed stability (RBS)], a ratio of median particle size to critical particle size determined from measures of bankfull depth, large woody volume, residual pool depth, and slope. Highly negative values indicated excessive sediment, and large positive values indicate armoring.
	Habitat complexity	A measure that sums the amount of in-stream habitat consisting of undercut banks, boulders, large wood, tree roots, and overhanging vegetation. Values close to zero indicate low habitat complexity.
	Riparian vegetation cover complexity	A measure of riparian vegetation complexity that sums the amount of woody cover provided by canopy, understory, and ground cover layers. Values close to zero indicate low complexity of riparian vegetation cover.
	Riparian disturbance	A measure of the presence and proximity of 11 types of human disturbances (e.g., roads, landfills, pipes, buildings, mining, channel revetment, cattle, and agriculture) along the stream reach. Values close to the maximum indicate high levels of riparian disturbance.
	Index of habitat integrity (IHI)*	Summed score (0-100) of habitat metrics including riparian disturbance, canopy cover, percent sand and fine substrate, percent fast water, and percent glide.
Biological	Non-native vertebrate species	Percent of individuals that are non-native. Non-native fish and amphibians can prey on, compete with, and exclude natives.
	Non-native crayfish	Presence or absence of non-native crayfish species.
	Asian clam	Presence or absence of non-native Asian clam.

- **Streambed stability** provides information about the relationship between sediment supply and transport. Human activities can alter the balance between sediment inputs and sediment that is transported by stream flow. The filling of interstitial spaces between coarser substrate and the subsequent habitat loss have negative effects on aquatic invertebrates and fish. The EPA measures streambed stability as a logarithm ratio, comparing the particle size of observed sediments (or median diameter of particle) to the "critical" or mobile bed particle diameter (Kaufmann and others 1999). The critical particle is a function of shear stress which incorporates measures of bankfull depth, large woody debris, residual pool dimensions, and slope. This is used to estimate the size of sediment each stream can move or scour during its flood stage (Kaufmann and others 1999). Lower values of the index indicate that a site is less stable and dominated by finer substrates and higher values identify a stream that is highly armored. Moderate index values (near zero) indicate that stream sediment loads are balanced, i.e., as much sediment is transported out of the system as it is entering.
- **Habitat complexity** or instream habitat-diversity plays a prominent role in the structuring of macroinvertebrate and fish communities. Instream habitat features such as large wood, boulders, undercut banks, and tree roots provide refugia for a variety of organisms. Habitat homogenization, such as channelization, can have negative effects on stream biota (Peck and others 2006). EMAP measures habitat complexity by summing several visual estimates of instream habitat consisting of undercut banks, boulders, large pieces of wood, bush, and cover from overhanging vegetation.
- **Riparian vegetation cover complexity** integrates landscape effects and local function of stream ecosystems. Activities within a watershed will eventually impact downstream ecosystems where riparian vegetation can act as a buffer from anthropogenic activities by providing bank stabilization and protection of floodplain soils. Riparian vegetation also provides shade for temperature control and nutrients through allochthonous inputs. EMAP uses an average of visual estimates to gauge the complexity of riparian woody vegetation cover adjacent to the stream reach.
- **Riparian disturbance** or human activities within the watershed can act as direct or indirect stressors on stream networks. To gauge these impacts EMAP visually estimates eleven specific forms of human disturbances (e.g., roads, landfills, pipes, buildings, mining, channel revetment, cattle, and agriculture) along the stream reach. Human activities were weighted depending on their proximity to the stream. The index generally varies from 0 (no observed disturbance) to 6 (disturbance is measured instream and on the banks throughout the reach).

In addition, a preliminary multi-metric index of habitat integrity (IHI) was developed by USGS for Arizona streams above 1,524 meters (Appendix 5) and is presented as a stressor in this study. Although the IHI can be used as an indicator of the ecological condition, habitat measures are not as sensitive as fish and macroinvertebrates since the degree that habitat responds to human activities is much different temporally and spatially. Also there is a greater degree of sampling error and natural variability associated with habitat sampling which makes fish and macroinvertebrate more desirable for environmental monitoring. There is no general framework for calculating or analyzing multiple habitat metrics. The lack of consensus about what features should be measured and what methodology should be used results in more inconsistencies in the analysis stage. Therefore, the IHI should be used as a stressor indicator on biota rather than an ecological indicator of disturbance until more studies focus on developing biologically relevant and consistent habitat data collection methods.

Condition Classes

IBI scores and stressor values were reported in terms of three condition classes (most-disturbed, intermediate, and least disturbed) based on the established water-quality standards or threshold values derived from reference condition (Appendix 6). Exceptions included (1) non-native crayfish and Asian clams that were classified based on the presence/absence, (2) total nitrogen and phosphorus that were not classified due to absence of the state-wide standards, and (3) specific conductivity and non-native vertebrates for which WEMAP thresholds were applied (Appendix 6).

Figure 7 shows a flow chart of processes involved in the determination of condition classes based on thresholds developed from reference condition. Potential reference sites included: (1) hand-picked and probability sites above 1,524 meters (5,000 feet) that were sampled during the 2000-2004 WEMAP, and (2) all probability sites that were sampled during 2007. All potential reference sites were evaluated and filtered based on procedures outlined by Stoddard and others (2005a) and Whittier and others (2006 and 2007c) and summarized in Appendix 7. Different sets of reference sites were selected for biological and physical indicators without referring to the results of the specific indicator being assessed to avoid circularity. For example, chemical, physical habitat, and land use variables were used to screen potential reference sites for biological indicators, and chemical and land use variables were used to select reference sites for habitat stressors (Appendix 7). The end results were a set of 17 reference sites (5 from this study and 12 from the WEMAP assessment) for aquatic vertebrates and a total of 16 reference sites (6 from this study and 10 from the WEMAP assessment) for physical habitat stressors. The WEMAP data was not available for periphyton, so periphyton reference sites included only 6 sites from this study. See Appendix 1 for reference site designations on the probability sites sampled in this study.

The range of conditions found in these reference sites describes a distribution of values, and extremes in the distribution are used as thresholds to distinguish sites in relatively good condition from those that are clearly not. For the most part, the EPA used the 5^{th} and 25^{th} percentiles of the reference distribution as thresholds (Stoddard and others 2005a). Caution should be taken, however, when establishing thresholds with small sample sizes. If the distributions are skewed, then the percentiles used to set the threshold may be less useful and another approach should be explored for setting the thresholds. For example, it was deemed inappropriate to use percentile thresholds for periphyton since the distribution of reference sites was so limited. Thus periphyton thresholds for determining reference and impaired conditions were set at the mean and the mean minus one standard deviation, respectively.

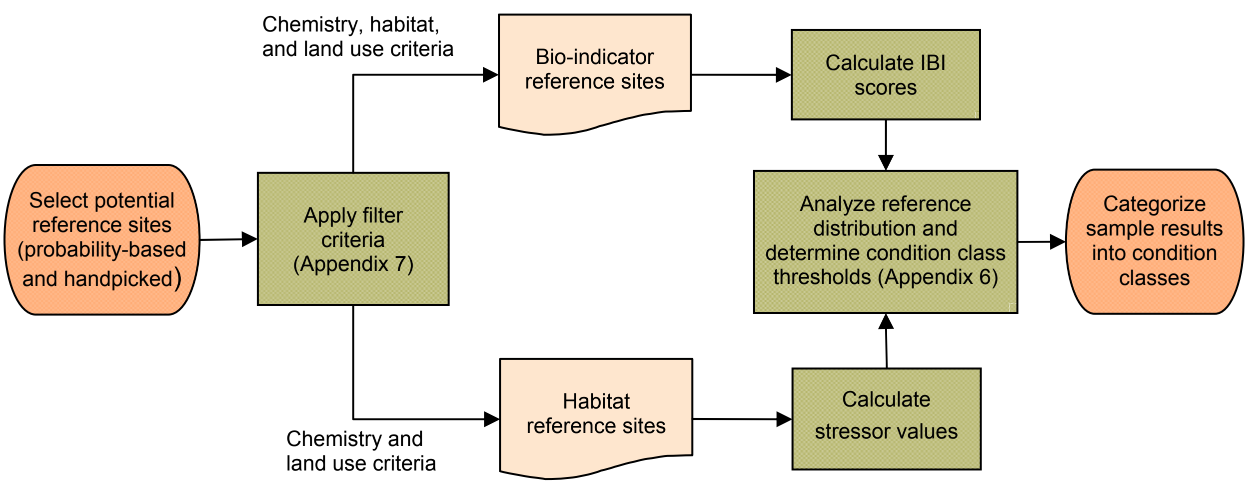

Figure 7. Flow chart for the determination of condition classes using reference sites.

Relative Extent and Relative Risk

As in the WEMAP report, each stressor was assessed for its extent and its likelihood of posing a relative risk to aquatic biota when it is present in the environment. In a relative extent analysis, each stressor is ranked based on the proportion of the assessed stream length in most-disturbed condition, identifying the most common stressors in the LCR basin. Relative risk analysis determines how much more likely a stream is to have poor biotic integrity if the stressor condition in the same stream is most-disturbed than if it is least-disturbed.

Relative risk (RR) is defined as the ratio of two conditional probabilities (Stoddard and others 2005b):

$$RR = \frac{P(\text{most-disturbed biological condition} \mid \text{most-disturbed stressor condition})}{P(\text{most-disturbed biological condition} \mid \text{least-disturbed stressor condition})}$$

where the numerator is the probability of finding most-disturbed biological condition in streams having most-disturbed stressor condition and the denominator is the probability of finding most-disturbed biological condition in streams having least-disturbed stressor condition. Relative risk of one indicates no association between the stressor and the biological indicator. A relative risk value greater than one indicates greater probability of finding the biological indicator in most-disturbed condition given that the stressor is in most-disturbed condition.

Relative risk (RR) is calculated from the estimated lengths of stream that have various combinations of biological and stressor conditions (Stoddard and others 2005b), as illustrated below in a contingency table for the macroinvertebrate IBI versus the crayfish stressor:

Estimated stream length (km) sampled in the LCR basin		Crayfish condition class	
		Most-disturbed	Least-disturbed
Macroinvertebrate IBI condition class	Most-disturbed	116	63
	Least-disturbed	0	54

From this table, RR of crayfish to macroinvertebrates is estimated to be

$$RR = \frac{116/(116+0)}{63/(63+54)} = \frac{1.0}{0.54} = 1.9$$

In other words, it is 1.9 times more likely to find a most-disturbed macroinvertebrate condition in streams where crayfish are present (most-disturbed) than in streams without crayfish (least-disturbed).

Results

Site Evaluations

The total perennial stream length within the LCR basin was approximately 2121 kilometers (km). This was the total stream length identified in the ADEQ Perennial Streams GIS cover for the LCR basin, which was also the sampling frame basis for the list of probability sites. A total of 237 sites were evaluated through GIS and field reconnaissance. Each site represented 8.95 km of stream length in this assessment.

Each site was classified into a target (i.e., flowing and wadeable) or non-target status (i.e., either non-perennial or non-wadeable reaches, streams on Indian land, or wrong waterbody types such as ditches, washes, wetlands, and lake shores). A large proportion (83%) of the total frame length (2121 km) was determined to be non-target (Figure 8). The remaining 367 km or 17% of the total frame length was target, of which 99 km was inaccessible due to physical barriers or lack of access permissions.

The results of this assessment are representative of the entire target population, that is, all the wadeable perennial stream length found in the LCR basin (367 km). However, the unsampled portion of the stream resource cannot be assessed for condition, and no inferences should be made by applying the results of this assessment to the unsampled portion of the stream population (Stoddard and others 2005b). The assessed length in this report refers to the stream length represented by the sites that were actually sampled, which is 268 km (73% of the target stream length) represented by the 30 sites. For aquatic vertebrates, the assessed length is 170 km because it excludes the sites that did not have any fish or had permit restrictions.

The ADEQ Perennial Stream map was modified in 2007 to improve on the accuracy of finding target sites; however, the result indicated only a 17% chance in finding target sites for the LCR basin. This was largely due to map errors in water body types, which accounted for nearly 30% of the total frame length. In addition, the map included stream reaches on Indian Reservations that were considered to be non-target for this assessment. With map errors corrected and streams on Indian Reservations excluded, the accuracy of identifying the target sites would have been 41%. It is also likely that prolonged drought conditions had affected this accuracy; 20% of the total frame length was found dry.

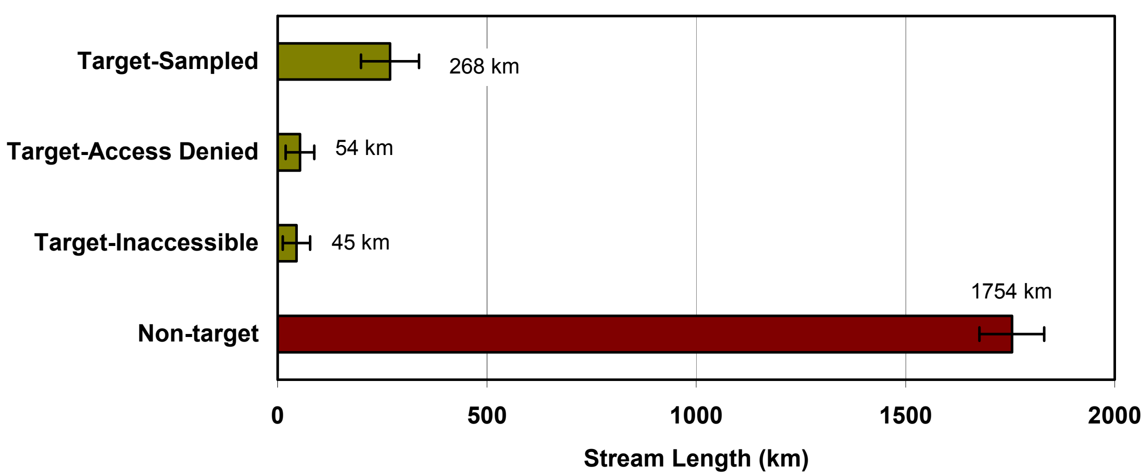

Figure 8. Stream length estimates with 95% confidence intervals in the LCR basin. Non-target category includes non-perennial or non-wadeable reaches, streams on Indian land, and wrong waterbody types labeled as streams.

Ecological Condition

The assessments of ecological condition of streams in the LCR basin based on the three IBIs (macroinvertebrate, aquatic vertebrate, and periphyton) are shown in Figure 9. It should be noted that 11 of the 30 sites were not assessed for aquatic vertebrate biotic integrity because they either were sampled but no aquatic vertebrates were captured (9 sites) or were not sampled because they were Apache Trout recovery streams (2 sites). Therefore, the assessment for aquatic vertebrates was based on only 19 sites, representing 170 km of stream length. All 30 sites, representing 268 km of stream length, were assessed for macroinvertebrates and periphyton. The assessed stream length may simply be referred to as the 'stream length' in the following sections.

- **Macroinvertebrate IBI**: Approximately 67% of the assessed stream length was determined to be in most disturbed condition, and 23% was in least disturbed condition with respect to macroinvertebrate integrity in the LCR basin (Figure 9). Roughly 10% of the stream length was intermediate or inconclusive meaning IBI scores were between the 10th and 25th percentile of reference condition and verification samples are needed to make further assessment (ADEQ 2008). Three metrics that responded more to stress (i.e., displayed the largest differences between the least-disturbed and the most-disturbed categories) were the number of intolerant taxa, number of total taxa, and percent abundance of stoneflies.

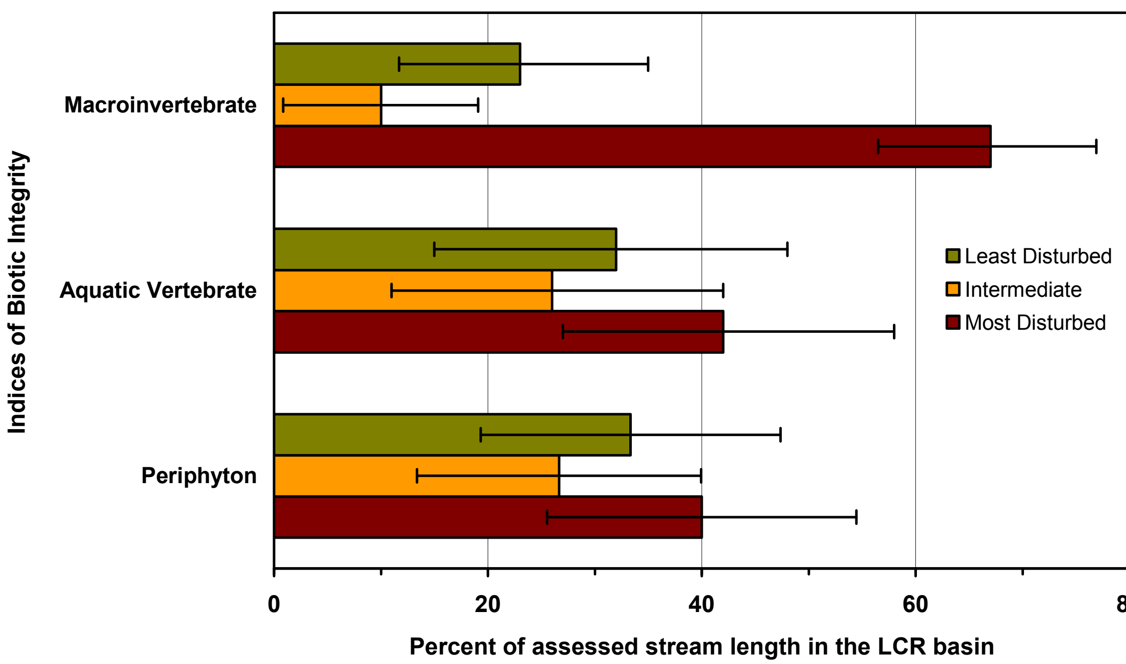

Figure 9. Summary of results for ecological condition indicators for the LCR basin during 2007. Bars (with 95% confidence intervals), show the percentage of perennial stream length within each condition class. Assessed stream lengths were 268 km for macroinvertebrates and periphyton and 170 km for aquatic vertebrates (98 km could not be assessed due to small stream size/absence of fish and denied permits).

- **Aquatic Vertebrate IBI**: Forty-two percent of the stream length in the LCR basin was assessed to be in most-disturbed condition, 26% in intermediate condition, and 32% in least disturbed condition with respect to aquatic vertebrate integrity (Figure 9). The most sensitive metrics to stress were the relative abundance of lithophilic species, assemblage tolerance index, and relative abundance of family Salmonidae.
- **Periphyton IBI**: Basin-wide, 40% of the stream length was considered to be in the most-disturbed condition, 27% in intermediate condition, and 33% in least disturbed condition with respect to periphyton integrity (Figure 9). The metrics that responded most to stress were the percent abundance of Achnanthes minutissima and percent abundance of immotile individuals.

Stressor Condition

The assessment results for indicators of chemical, physical, and biological stress are shown in Figure 10. References for the condition class thresholds are presented in Appendix 6.

Chemical Stressors

- **Nutrients**: Total nitrogen and phosphorus were not assessed in terms of condition classes due to a lack of reasonable statewide criteria. However, ADEQ has large historic datasets of total nitrogen and phosphorus concentrations in streams throughout Arizona. Box plot analyses were performed using nitrogen and phosphorus data between 1994 and 2007 from the LCR basin to determine if the box plot distributions would discriminate a-priori reference from a-priori impaired sites. These sites were classified based on the ADEQ reference/impaired site criteria, and macroinvertebrate assemblages have been shown to display statistical differences between the a-priori classes (ADEQ 2007). The box plot distributions of nutrient data showed large overlaps of the interquartile ranges between the reference and impaired classes, suggesting that nutrients might not be important stressors in the LCR basin. Concentrations of total phosphorus in the LCR basin ranged from 0.01 mg/L to 0.19 mg/L with an average of 0.06 mg/L. Concentrations of total nitrogen in the LCR basin ranged from 0.04 mg/L to 1.31 mg/L with an average of 0.20 mg/L.
- **General Chemistry**: The following additional chemical parameters were assessed because of existing statewide standards for protecting aquatic life use: specific conductivity, dissolved oxygen (DO), pH, turbidity, and suspended sediment concentration (SSC). For conductivity, roughly 90% of the assessed stream length in the LCR basin was in least disturbed condition, and 10% was in most disturbed condition. For DO, nearly 87% of the assessed stream length was in least disturbed condition while 13% was in most disturbed condition. Most stream length (97%) was in least disturbed condition for pH. For turbidity, approximately 57% of stream length was in least disturbed condition, and 33% was in most disturbed condition. The entire assessed stream length was in least disturbed condition for SSC (Figure 10).
- **Mercury in Fish Tissue**: Of the 19 sites where fish were found, at only 14 sites were fish-tissue samples collected and analyzed for mercury. Eleven samples had mercury concentrations above the method reporting limit of 0.05 mg/kg. All 14 sites, or 47% of the stream length in the Little Colorado River Basin were estimated to be in least-disturbed condition with respect to mercury in fish tissue (Figure 10); 53% was not assessed because sites were not sampled (2 sites), did not contain fish (9 sites), or tissue samples were not collected because too few and small fish were captured (5 sites).

Biological Stressors

- **Non-native Vertebrate Species**: Presence of non-native aquatic vertebrates was assessed in 28 of the 30 sites; two sites (7%) were not sampled. Non-native aquatic vertebrate species were widespread and common in Little Colorado River Basin streams, with 53% of the stream length having more than 10% of individuals that were non-native (most-disturbed condition). Non-natives were absent (least-disturbed condition) in 33% of the stream length (Figure 10). Note that aquatic

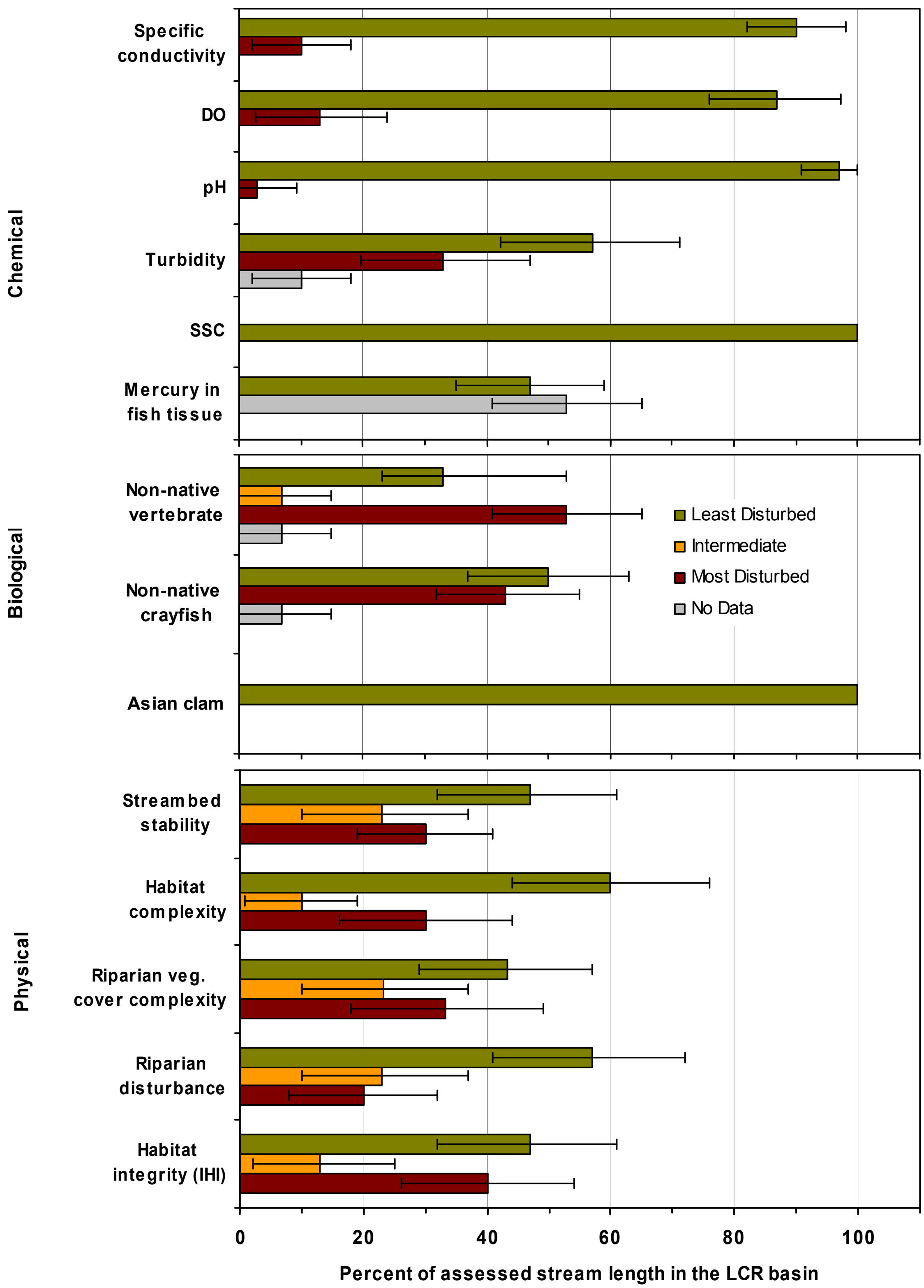

Figure 10. Summary of results for chemical, biological, and physical indicators of stress for the LCR basin during 2007. Bars (with 95% confidence intervals), show the percentage of perennial stream length within each condition class. Proportions are based on the 268-km assessed length.

vertebrates were absent from nine of the sites (30% of stream length) within the least disturbed category.

- **Non-native Crayfish**: Crayfish are not native to Arizona (Hobbs 1989), so the presence of crayfish indicates that a site is disturbed. Presence of crayfish was evaluated in 28 of the 30 sites; two sites (7%) were not sampled. Crayfish were absent from 50% (15 sites) but present in 43% (12 sites) of stream length in the LCR basin (Figure 10).
- **Asian Clam:** The Asian clam (*Corbicula fluminea*) was absent from all sites; the entire assessed stream length was in least-disturbed condition with respect to Asian clams.

Physical Habitat Stressors

- **Streambed Stability**: Thirty percent of the stream length in the LCR basin was categorized as most-disturbed with regard to sedimentation. Most of streams that were in the most-disturbed condition exhibited problems with finer substrates rather than armoring. Almost half of the stream-length in the LCR was in the least-disturbed condition (47%). Sites contributing to the most-disturbed percentage are characterized as having lower stream gradient, finer substrates, and less riparian habitat.
- **Habitat complexity** was degraded in 30% of the assessed stream length, but most (60%) of the stream kilometers were considered least-disturbed. This indicates that most streams had a diverse set of features providing cover and potential refugia for fish and macroinvertebrates. Rocks and aquatic macrophytes were the dominant forms of cover in the LCR basin.
- **Riparian Vegetation Cover Complexity**: Basin-wide, approximately 43% of riparian vegetation structure has been simplified or modified along stream banks (i.e., most-disturbed condition). The most-disturbed percentage is roughly double the estimate of riparian disturbance (see below) suggesting that the effects of local anthropogenic activities are not entirely accounting for the condition of the riparian vegetation cover and that watershed effects may be important. Approximately one-third of the stream length was in the least-disturbed condition with regard to riparian vegetation cover.
- **Riparian Disturbance**: Overall, anthropogenic impacts were limited in the areas adjacent to streams in the LCR basin. Riparian disturbance exceeded the cold-water thresholds in approximately 20% of the stream length while 57% of the stream length was in the least-disturbed condition. The most prevalent anthropogenic stressors were related to grazing and presence of roads.
- **Index of Habitat Integrity**: Approximately 40% of the stream length in the LCR was considered to be in the most-disturbed condition using the habitat integrity index developed for the REMAP sites. Forty seven percent was in the least-disturbed and the remaining stream length was considered intermediate.

Ranking of Stressors

In order to evaluate the importance and magnitude of stressors affecting the biota, stressors were ranked by extent and by relative risk. The EPA addressed stressor relevance in two ways in the Western EMAP Assessment (Stoddard and others 2005b). First, how common is the stressor or what is the extent of the stressor with regard to actual stream kilometers affected by the stressor? Second, what is the severity of each stressor on the biotic integrity? Ideally these two factors should be combined to address relative importance; however, no such methodology exists. Instead extent and risk are used separately with the goal of ranking stressors relative to their importance to stream biota.

Relative Extent

Figure 11 presents the stressors ranked according to the proportion of stream length in the LCR basin that is in the most-disturbed condition. The most extensive stressors were a combination of chemical, biological, and physical stressors. Similar to the previous 'Arizona assessment' (Robinson and others 2006), non-native aquatic vertebrate species was the most prominent stressor occurring in 53% of the perennial-stream length (Figure 11). Other extensive stressors occurring in the LCR basin were the presence of crayfish and degraded habitat integrity (43%, and 40%, respectively). Intermediate stressors were all habitat-related and included relative bed stability (30%), poor habitat complexity (10%), riparian disturbance (20%), and high turbidity (33%). It should be noted that the habitat index is somewhat redundant since it incorporates similar habitat metrics, but the multi-metric accounts for more of the variability in the data than any single habitat metric. The least common stressors were chemistry-related and included low dissolved oxygen (13%), high salinity (10%), and unsuitable pH levels (3%).

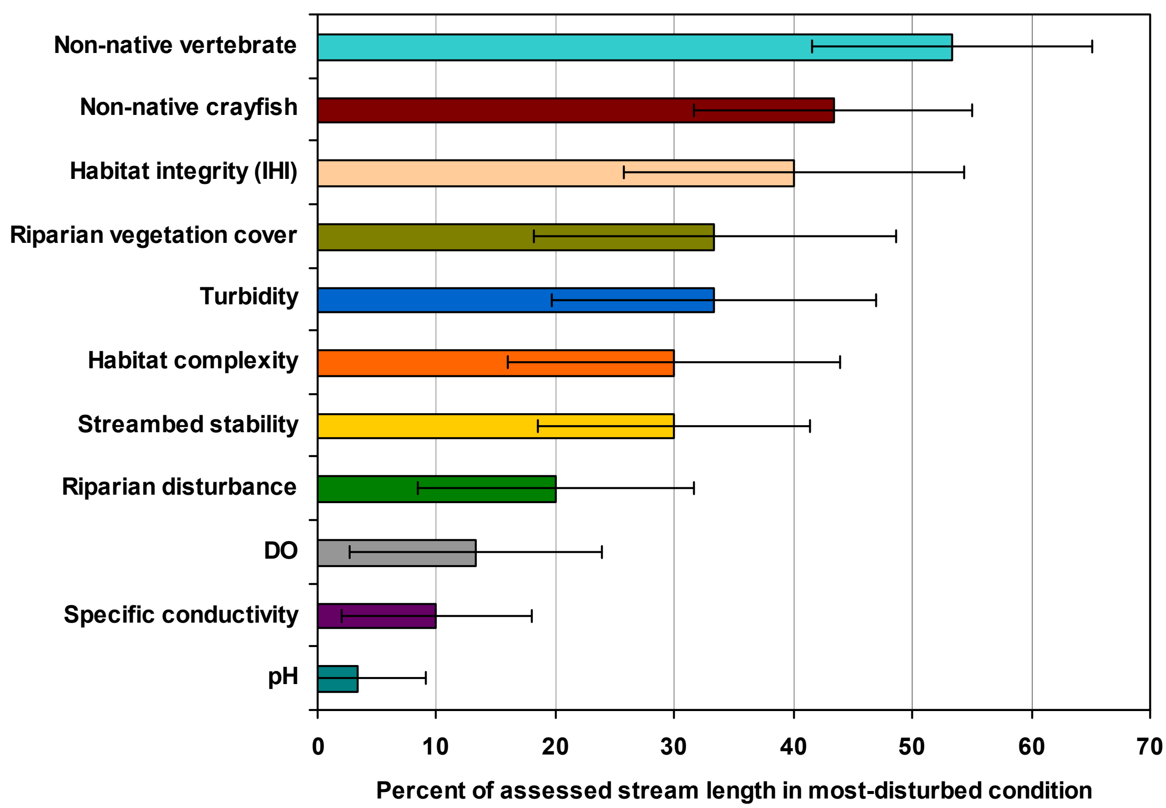

Figure 11. Relative extent of stressors (proportion of stream length with stressor in most disturbed condition) for the LCR basin.

Relative Risk

To address the severity of a stressor the EPA uses the concept of relative risk which is a statistic that measures the proportional increase in the likelihood of finding a biological indicator in the most-disturbed condition while having a stressor in the most-disturbed condition within the same stream (Stoddard and others 2005b). A relative risk value of one or less indicates that there is no association between the stressor and the biological indicator. Confidence intervals are used to identify statistically significant risk-ratios (i.e., any ratio with the lower confidence limit that falls below 1.0 is not considered significant). The goal is to provide managers with quantitative approximations of the severity of each stressor and the potential effect on stream biota.

In Figure 12, relative risk (RR) values are presented for the biological and stressor data for streams in the LCR basin. The stressors presenting the greatest relative risk ratios are similar for vertebrates and macroinvertebrates. For example, LCR streams that have poor habitat integrity (IHI) are roughly 2 and 2.9 times more likely to have poor aquatic macroinvertebrate and vertebrate integrity, respectively. Crayfish and low complexity riparian vegetation cover present a significant risk to macroinvertebrates (RR = 1.9

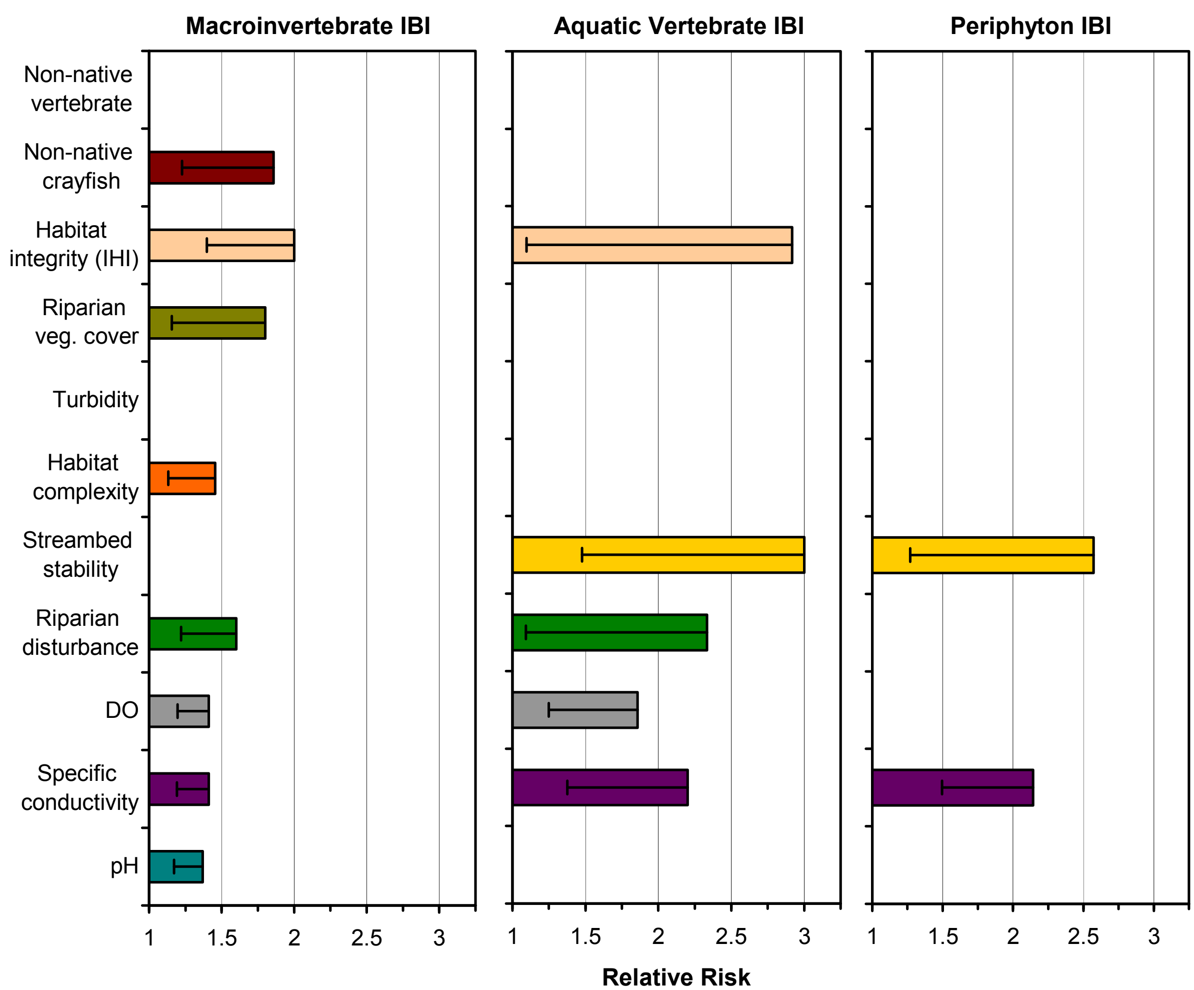

Figure 12. Relative risk of stressors to integrity of macroinvertebrates, aquatic vertebrates, and periphyton. Relative risk ratios of less than 1 are considered insignificant and are not shown. 95% lower confidence limits are shown to indicate significance of ratios (confidence intervals that encompass 1.0 are not considered significant).

and 1.8, respectively). Crayfish were found to present a risk to aquatic vertebrates (2.25) but as a result of high variability it was not included and should be noted as observational. Crayfish were identified as a significant risk to aquatic macroinvertebrates and vertebrates in the 'Arizona assessment' report (Robinson and others 2006). Streambed stability had the greatest risk to vertebrate integrity, and vertebrate integrity was three times more likely to be impaired if fines were in excess at a site. Riparian disturbance also showed a significant risk but this risk ratio may be redundant since it is also incorporated in the habitat integrity index. Specific conductivity (salinity) and low dissolved oxygen concentrations were the only other significant risks to aquatic vertebrate integrity (RR = 2.2 and 1.9, respectively). Salinity also ranked high as a relative risk to fish in the WEMAP report (Stoddard and others 2005b). Salinity, dissolved oxygen, and pH presented a significant relative risk to the macroinvertebrate integrity, but overall the presence of crayfish and habitat degradation (riparian disturbance, habitat integrity, habitat complexity) presented the most risk to macroinvertebrate integrity. Significant risks to periphyton integrity were identified as specific conductivity and streambed stability. Periphyton integrity was two times more likely to be in the most-disturbed condition when sites had elevated salinity or poor streambed stability.

The most useful analysis for managers is to combine relative extent and relative risk which address the stressors that are the most common and whose effects are potentially the most severe. The most extensive stressors in the LCR basin are non-native vertebrates, degraded habitat (vegetation and integrity) and presence of crayfish. Crayfish presence and habitat degradation occur in 40% or more of the basin and present the highest relative risk to macroinvertebrate integrity. Habitat integrity is also a significant risk for aquatic vertebrates (RR = 2.9). Poor streambed stability occurs in 30% of the streams and poses a significant risk to fish and periphyton. Salinity and dissolved oxygen present an elevated relative risk to aquatic invertebrates and periphyton, but the relative extent of these stressors is minor (10% and 13%, respectively). The relative risk posed by non-native vertebrates on aquatic vertebrates could not be calculated because there were no sites that were categorized as most-disturbed for the aquatic vertebrate IBI and least-disturbed for the non-native aquatic vertebrate stressor. More data are needed to make this risk assessment. Overall the risk analyses suggest that habitat and crayfish are the most obvious targets of remediation efforts for managers.

Conclusions

A high percentage of stream length in the Little Colorado River (LCR) basin was assessed to be in most-disturbed condition with regard to all three biotic indicators of ecological condition. For macroinvertebrates and aquatic vertebrates, this general assessment is in agreement with the previous 'Arizona assessment' (Robinson and others 2006). However, in this study, a smaller proportion of the total LCR stream length was found to be in most disturbed condition for aquatic vertebrates (42±15%), as compared to 69% in the mountains region in the 'Arizona assessment'. It may be that aquatic vertebrate assemblages are in worse condition in mountain streams outside of the LCR basin than within the basin. The difference might also be related to the fact that condition category thresholds used in this study were lower and therefore easier to meet than those used in the 'Arizona assessment' (Robinson and others 2006). All of the reference sites in this study were within Arizona, whereas in the 'Arizona assessment' reference sites were located west-wide and were the same as those used in the WEMAP assessment (Stoddard and others 2005b).

The scores of the aquatic vertebrate IBI were significantly correlated with the macroinvertebrate IBI scores (aquatic vertebrate IBI = 0.927*macroinvertebrate IBI – 7.459; $r = 0.77$, $p < 0.001$), indicating that ecological condition of the aquatic vertebrate community can be represented by the macroinvertebrate community. The stressors presenting the greatest relative risk ratios were also similar for the two indicators. For example, LCR streams that have poor habitat integrity, presence of crayfish, high salinity, and low dissolved oxygen (DO) were more likely to have poor macroinvertebrate and aquatic vertebrate integrity. One significant difference between the two indicators was the relative risk of streambed stability, which was found to pose a risk to aquatic vertebrates but not to macroinvertebrates. Low streambed stability can result from excess fine sediment due to erosion, which would inevitably pose stress to biota including macroinvertebrates by filling in habitat spaces between stream substrates. The macroinvertebrates in LCR streams, however, were more responsive to different habitat measures such as the low complexity of riparian vegetation cover and instream habitat features.

Non-native crayfish appear to pose one of the greatest risks to macroinvertebrate assemblages in the LCR basin; a similar result was found within the mountains climatic region in the 'Arizona assessment' (Robinson and others 2006) and in a multivariate analysis of environmental stressor variables and macroinvertebrate IBI scores of the LCR samples (Spindler and Paretti 2009). Arizona is the only state in the conterminous United States where all crayfish are not native (Hobbs 1989). The conclusion of this report that crayfish are a major stressor to macroinvertebrate communities and ecosystems is also supported by a variety of literature (Lodge and others 2000, Nyström and others 2001, Stenroth and Nyström 2003). Non-native crayfish would be a good target for management action to improve the ecological condition of LCR basin streams. Another important target would be the improvement of habitat integrity. The Index of Habitat Integrity (IHI) was developed to better assess the overall condition of physical habitat that was associated with the health of aquatic biota. The IHI was below the optimum condition for 40% of LCR streams and found to present significant risk to both macroinvertebrates and aquatic vertebrates. The IHI appears to reflect the biological condition of the LCR streams more accurately than individual habitat metrics.

Although both specific conductivity (salinity) and DO are some of the least extensive stressors, their relative risk results are not that surprising, as most freshwater fish do not tolerate low dissolved oxygen concentrations (Matthews 1998), or high salinities (Myers 1949). Salinity was found to be a stressor with significant risk to the aquatic vertebrate assemblage in the 'Arizona assessment' (Robinson and others 2006), and in the WEMAP assessment (Stoddard and others 2005b). Dissolved oxygen concentration was not analyzed as a stressor in the Arizona or WEMAP assessments, so it is unknown if the same pattern detected in this study would be evident at a broader scale. These two stressors are likely to increase in extent and severity in the future because of increased land and water use concomitant with increasing human population. Therefore, these two stressors might also be good targets for management action to improve the overall ecological condition of LCR streams.

Similar to the ‘Arizona assessment’ (Robinson and others 2006) non-native aquatic vertebrates were the most extensive stressor. However, we could not calculate the relative risk because of a division by zero; there were no sites categorized as most-disturbed for the aquatic vertebrate IBI and least-disturbed for the nonnative aquatic vertebrate stressor. Therefore, we cannot draw any conclusions about the risk that non-native vertebrates pose to the aquatic vertebrate assemblage. A larger sample size would have likely resulted in a calculable relative risk value. However, there is a large body of evidence that non-native fishes negatively affect native fishes (Miller 1961, Moyle 1986, Minckley and Deacon 1991). Therefore it seems likely that non-native aquatic vertebrates are a significant risk to the fish assemblage and should be a management target. Another measure of non-native vertebrates, such as proportion of fish-eating non-native species, might have been a better indicator of non-native vertebrate stress. It may also be that the aquatic vertebrate IBI is not really a good indicator of ecological condition of the aquatic vertebrate assemblage in Arizona. A stressor should by definition have a negative effect. If a stressor is not negatively related to a measure of aquatic vertebrate assemblage condition, then it begs the question as to whether it is a stressor at all, or if it is, whether the aquatic vertebrate IBI is really a good measure or the ecological condition of the aquatic vertebrate assemblage.

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Appendix 1: Random Sampling Sites in the Little Colorado River Basin

Table A1-1. Sample locations in the LCR basin. Reference sites for fish, periphyton, and habitat are indicated by superscripts next to EPA site ID: F = fish, P = periphyton, and H = habitat.

EPA Site ID	ADEQ Site ID	Stream Name and Location	Sample Date	Latitude (Dec. Deg)	Longitude (Dec. Deg.)	Elev. (ft)	Drainage Area (mi^2)
AZ06631-026 H	LCECL040.69	East Clear Creek at Poverty Flat Along FH147	4/30/2007	34.47695	-111.326	7000	4.2
AZ06631-037	LCHAL008.83	Hall Creek Blw the Wilderness Area Boundary	6/5/2007	33.9725	-109.521	9280	2.3
AZ06631-038	LCMRS043.17	Morrison Creek 0.8 Mile Blw Confluence With Coyote Creek	5/24/2007	33.97013	-109.055	8440	2.9
AZ06631-050	LCMLK001.18	Milk Creek Southwest Corner of Section 34	5/22/2007	33.95183	-109.173	8000	4.3
AZ06631-053	LCHAL010.20	Hall Creek Downstream of Hall Creek Headwaters	6/7/2007	33.95694	-109.536	9580	1.4
AZ06631-061	LCSIL041.04	Silver Creek End of Queen Creek Place	4/24/2007	34.34425	-109.977	6060	105
AZ06631-063 H	LCECL021.13	East Clear Creek Just East of FH095 And FH496 Intersection	5/2/2007	34.55078	-111.161	6500	95
AZ06631-065 F, P	LCSLR001.42	South Fork LCR Above South Fork Campground	5/21/2007	34.0707	-109.41	7620	25
AZ06631-077	LCMIN018.05	Mineral Creek Above Forest Service Road #404	6/29/2007	34.17992	-109.618	8070	6.3
AZ06631-088 P, H	LCCLE063.52	Clear Creek Downstream of Willow Creek Confluence	5/7/2007	34.64472	-110.999	6000	313
AZ06631-093	LCSHL026.50	Show Low Creek Above Morgan Wash	4/17/2007	34.20833	-110.001	6480	69
AZ06631-097	LCLCR342.03	Little Colorado River Above Airport Road	4/12/2007	34.12788	-109.299	6940	133
AZ06631-098	LCRIG004.87	Riggs Creek Above Riggs Reservoir	5/10/2007	33.97598	-109.247	8160	2.5
AZ06631-109	LCHAL004.59	Hall Creek East of Geneva Reservoir	6/4/2007	34.02778	-109.506	9000	6.8
AZ06631-110	LCSHL031.05	Show Low Creek Blw Porter Cr And Billy Cr Confluence	4/16/2007	34.17166	-109.983	6660	63
AZ06631-125	LCLCR360.06	Little Colorado River 1/4 Miles East of the Greer Post Office	6/6/2007	34.00803	-109.454	8330	14
AZ06631-130	LCRUD003.45	Rudd Creek at Sipe Wildlife Area	5/23/2007	34.03335	-109.23	7640	18
AZ06631-133	LCELR007.19	East Fork LCR Above F.S. Rd #113 Crossing	6/13/2007	33.92979	-109.489	9460	2.3
AZ06631-137	LCCOY000.71	Coyote Creek at Richville Valley	4/11/2007	34.30638	-109.346	6060	227

EPA Site ID	ADEQ Site ID	Stream Name and Location	Sample Date	Latitude (Dec. Deg)	Longitude (Dec. Deg.)	Elev. (ft)	Drainage Area (mi^2)
AZ06631-141 [P]	LCBEN002.57	Benton Creek Near Pat Knoll Cabin	5/9/2007	33.98538	-109.291	8600	2.5
AZ06631-145	LCLCR311.31	Little Colorado River South of Salado	4/10/2007	34.42601	-109.402	5840	780
AZ06631-149	LCSIL043.84	Silver Creek Below AGFD Hatchery	6/28/2007	34.33587	-109.939	6103	99
AZ06631-151 [F, P, H]	LCECL018.17	East Clear Creek 3/4 Mile Upstream From Kinder Crossing Trail	5/1/2007	34.56419	-111.147	6460	101
AZ06631-155	LCLCR211.73	Little Colorado River North of Mclaws Bend	4/25/2007	34.89681	-110.181	5070	7945
AZ06631-157	LCELR000.13	East Fork LCR 500 Feet Above West Fork Confluence	6/12/2007	34.00199	-109.457	8410	14
AZ06631-162 [F, P, H]	LCBRB006.74	Barbershop Canyon Creek Blw Merritt Draw Confluence	6/20/2007	34.49442	-111.165	6950	3.2
AZ06631-183 [F, P, H]	LCCHC081.26	Chevelon Canyon At Telephone Ridge Abv Horse Trap Canyon	6/18/2007	34.38736	-110.872	6500	59
AZ06631-186	LCSHL029.75	Show Low Creek Near Lakeside	6/26/2007	34.17944	-109.987	6610	68
AZ06631-210 [F]	LCSLR003.72	South Fork LCR Below Joe Baca Draw	6/21/2007	34.04889	-109.39	8100	17
AZ06631-237	LCRUD007.23	Rudd Creek Above Benton Creek Confluence	6/28/2007	34.01097	-109.281	8100	5.1

Table A1-1. Continued.

Appendix 2: Calculating Metric and Index Scores for Macroinvertebrates and Aquatic Vertebrates

Multi-metric index scores for macroinvertebrates or aquatic vertebrates were calculated by following these general procedures: 1) determine sample metric values, 2) rescale metric values to obtain metric scores, and 3) average or combine metric scores to produce index scores. The ADEQ macroinvertebrate IBI uses scoring thresholds (Table A2-1) to calculate the metric score as the percent of reference. The threshold values were derived from the statewide all-inclusive (reference to impaired sites) data set; the 95th percentile was selected for metrics that decrease with disturbance (positive metrics) and the 5th percentile was selected for metrics that increase with disturbance (negative metrics) to represent reference condition. For a positive metric, for example, the metric score was calculated by (sample metric value / threshold value) x 100. A metric value greater than or equal to the threshold value was given a score of 100. The index score was then determined by taking an average of all metric scores (ADEQ 2008). Aquatic vertebrate IBI scores were calculated according to Whittier (2007b). Metrics were scored on a continuous scale from 0 to 10: ceiling and floor values for each metric were defined as the 5th and 95th percentile values observed in all WEMAP sites (Table A2-1). For positive metrics, values less than the 5th percentile were given a score of 0, those with values greater than the 95th percentile were given scores of 10, and all metric values in between were interpolated linearly. Negative metrics were scored similarly, with the floor (95th percentile) and ceiling (5th percentile) values reversed. Scored metrics were summed, and the summed score was scaled to a range of 0-100 by multiplying each sum by 1.67.

Table A2-1. Macroinvertebrate and Aquatic vertebrate metrics and scoring thresholds.

IBI	Metric	Category	Response to Increasing Impairment	Scoring Threshold or Ceiling/Floor Values
Macroinvertebrate	Total taxa	Richness	Decrease	38
	Diptera taxa (% true flies)	Richness	Decrease	11
	Percent Plecoptera (% stoneflies)	Composition	Decrease	19.1
	Scraper taxa	Trophic	Decrease	11
	Percent scraper	Trophic	Decrease	45.1
	Intolerant taxa	Tolerance	Decrease	6
	Hilsenhoff Biotic Index	Tolerance	Increase	4.23
Aquatic vertebrate	Proportion of individuals that are non-native	Non-native species	Increase	0 / 1
	Proportion of vertebrate abundance in family Salmonidae	Composition	Decrease	1 / 0
	Proportion of individuals that are sensitive and rheophilic	Habitat	Decrease	1 / 0
	Proportion of all species that are lithophilic	Reproductive	Decrease	1 / 0.2
	Assemblage Tolerance Index	Tolerance	Increase	1.16 / 5.32
	Proportion of individuals that are sensitive and invertivores/piscivores	Trophic	Decrease	1 / 0

Appendix 3: Periphyton Metric Selection and Index Development

This section describes the construction of a preliminary multi-metric periphyton index developed for Arizona streams above 1,524 meters (5,000 feet). Diatoms are frequently used as an indicator of water quality and ecological condition (Stevenson and others 2008, Potapova and Charles 2007, Griffith and others 2005, Hill and others 2003). Their ubiquitous presence and sensitivity to contaminants and nutrients lend themselves as ideal ecological indicators for regional assessments. A periphyton index was developed using data only collected in the LCR basin. A suite of 146 metrics was generated that represented 14 major categories (e.g., richness, pollution tolerance, abundance, et cetera). Metric selection followed a similar approach as described in the WEMAP; sites were subjected to several filtering criteria to reduce the number of potential variables to a few. Metric values had to have a range where no more than 75% of the values were the same, otherwise they were eliminated. Redundant metrics were eliminated (one of the pair in the correlation if $r \geq 0.70$), as were metrics unresponsive to stressors such as land use, physical habitat disturbance, and chemical conditions. T-tests were used to compare reference and impaired sites (selected a-priori) for each metric; those with statistically insignificant results ($P > 0.05$) were eliminated. After all filters were applied, the five remaining metrics were included in the index (Table A3-1). The five metrics were within tolerance, composition, and functional-type categories. Metrics were normalized and scored as described in Barbour and others (1999). The ceiling and floor values for each metric were defined as the 5^{th} and 95^{th} percentile (Barbour and others 1999, Stoddard and others 2005a). For positive metrics (e.g., those that are highest in reference sites), values less than the 5th percentile were given a score of 0, those with values greater than the 95^{th} percentile were given scores of 10, and all metric values in between were interpolated linearly. Negative metrics were scored similarly, with the floor (95th percentile) and ceiling (5th percentile) values reversed (Barbour and others 1999, Stoddard and others 2005a). These metrics were then combined into a multi-metric index.

Pearson correlation analysis of the periphyton index and stressors indicates that the index was inversely correlated to riparian disturbance (agricultural related), embeddedness, and percent fines and sand (r = -0.67, -0.66, and -0.66, respectively) and positively associated with larger substrate and bed stability (r = 0.61 and 0.53, respectively). Identifying the most relevant metric is difficult due to multicollinearity between metrics. Specific conductance and sulfate were the chemical stressors with which the index was most negatively associated (r = -0.77 and -0.74, respectively). It should be noted that due to a small sample size, the pool of reference sites was limited (n = 6). Discretion should be used with this index until more reference sites can be included in the analysis and its use should be limited to the LCR basin.

Table A3-1. Periphyton Metrics selected for the LCR basin.

Periphyton Metric	Category	Response to Increasing Impairment	Ceiling Value	Floor Value
Van Dam Saprobity	Tolerance	Increase	2.82	1.96
Pollution Tolerance (Lang-Bertalot modified by Bohls)	Tolerance	Decrease	2.21	2.92
% Achnanthes minutissima (Disturbance Index)	Composition	Decrease	0.83	21.11
% Not Motile	Habit	Decrease	2.02	70.97
% Siltation Index	Composition	Increase	67.72	3.17

Appendix 4: Indicator Data and Ancillary Indicator Information

Table A4-1. Biological integrity and stressor indicator scores for each site sampled. Raw data can be accessed by contacting Patti Spindler, at Arizona Department of Environmental Quality headquarters in Phoenix. For the aquatic vertebrate IBI and Mercury in fish tissue, 'No fish' indicates the site was sampled but no fish were captured, and 'Not sampled' indicates the site was not sampled. For mercury-in-fish tissue, 'Not collected' indicates that insufficient numbers of fish, which were small, were captured so a sample was not collected.

EPA SITE ID	EMAP Vertebrate IBI	ADEQ Macro-Invertebrate IBI	ADEQ Periphyton IBI	Total Phosphorus (mg/L)	Total Nitrogen (mg/L)	Conductivity (Salinity) (µS/cm)	pH	Turbidity (NTU)	Suspended Sediment Conc. (mg/L)	Mercury In Fish (µg/g)	Riparian Disturbance	Riparian Vegetation	Relative Bed Stability	Habitat Complexity	Index of Habitat Integrity	Non-Native Vertebrates (proportion of individuals)	Non-Native Crayfish (count)
AZ06631-026	No fish	18.65	60.63	0.020	0.193	100	8.90	1.51	5.0	No fish	1.886	0.140	-0.010	0.432	46.645	0	0
AZ06631-037	No fish	38.99	63.21	0.050	0.180	38	7.69	20.30	5.0	No fish	0.000	0.020	-1.170	0.925	47.903	0	0
AZ06631-038	No fish	29.82	42.76	0.190	0.150	370	8.45	No data	38.0	No fish	0.939	0.373	-1.776	0.430	45.126	0	0
AZ06631-050	No fish	39.54	65.12	0.050	0.160	150	8.54	No data	28.0	No fish	0.030	0.503	-2.104	0.816	76.207	0	0
AZ06631-053	No fish	50.43	61.73	0.050	0.180	38	7.02	20.30	5.0	No fish	0.030	0.182	-1.243	0.234	75.888	0	0
AZ06631-061	13.34	17.87	72.62	0.070	0.180	150	8.45	11.70	10.5	0.08	0.513	0.131	-0.418	0.527	54.597	0.55	209
AZ06631-063	19.8	24.26	68.24	0.010	0.174	140	8.75	2.57	5.0	0.09	0.962	0.167	-0.783	0.305	58.039	0.14	36
AZ06631-065	43.83	56.98	55.38	0.040	0.060	180	8.79	1.69	5.0	0.13	0.000	0.640	-0.790	1.014	97.769	1	0
AZ06631-077	Not sampled	51.15	80.00	0.100	0.131	100	7.82	6.88	7.5	Not sampled	0.813	0.286	-1.470	0.598	59.013	Not sampled	Not sampled
AZ06631-088	1.04	22.48	72.03	0.010	0.130	230	8.43	1.89	5.0	Not collected	0.000	0.299	0.063	0.634	72.320	0.94	11
AZ06631-093	0	18.35	68.19	0.020	0.350	220	8.96	2.81	5.0	0.13	0.955	0.186	-0.926	0.509	55.964	1	181
AZ06631-097	18.53	29.35	40.57	0.060	0.180	160	7.37	16.10	14.5	0 (<.05)	3.265	0.130	-1.339	0.075	33.697	0.4	32
AZ06631-098	No fish	31.69	75.22	0.080	0.090	240	8.07	9.20	46.0	No fish	2.030	0.116	-3.546	0.164	11.491	0	0
AZ06631-109	No fish	52.15	83.86	0.170	1.310	58	7.51	4.33	5.0	No fish	0.098	0.315	-0.417	0.486	84.915	0	0

EPA SITE ID	EMAP Vertebrate IBI	ADEQ Macro-Invertebrate IBI	ADEQ Periphyton IBI	Total Phosphorus (mg/L)	Total Nitrogen (mg/L)	Conductivity (Salinity) (µS/cm)	pH	Turbidity (NTU)	Suspended Sediment Conc. (mg/L)	Mercury In Fish (µg/g)	Riparian Disturbance	Riparian Vegetation	Relative Bed Stability	Habitat Complexity	Index of Habitat Integrity	Non-Native Vertebrates (proportion of individuals)	Non-Native Crayfish (count)
AZ06631-110	0	19.47	75.57	0.010	0.320	300	8.42	11.90	8.5	0 (<.05)	2.106	0.095	-0.579	0.216	41.547	1	39
AZ06631-125	73.34	73.62	66.53	0.050	0.384	53	8.34	15.10	5.0	0.06	0.803	0.389	-0.862	0.118	65.813	0.51	0
AZ06631-130	21.96	24.19	22.09	0.120	0.270	590	8.43	8.99	10.5	Not collected	0.000	0.148	-2.581	0.827	26.442	0	800
AZ06631-133	Not sampled	62.75	67.80	0.050	0.200	45	7.88	2.71	5.0	Not sampled	0.182	0.066	-1.690	0.193	55.780	Not sampled	Not sampled
AZ06631-137	4.86	34.00	0.52	0.020	0.280	1400	8.86	No data	51.0	Not collected	2.258	0.109	-1.763	0.095	31.183	0.88	0
AZ06631-141	No fish	67.92	71.39	0.040	0.035	150	7.97	2.75	5.0	No fish	0.333	0.308	-0.558	0.514	95.418	0	0
AZ06631-145	0	23.79	0.71	0.020	0.230	2500	8.72	18.10	17.5	Not collected	3.242	0.213	-2.906	0.177	25.638	1	5
AZ06631-149	31.22	14.47	73.27	0.070	0.143	150	9.17	13.00	8.5	0 (<.05)	0.188	0.052	-0.632	0.271	49.912	0.01	554
AZ06631-151	29.58	14.16	74.10	0.010	0.183	260	8.09	1.76	5.0	0.08	0.068	0.206	0.534	0.300	73.852	0.14	77
AZ06631-155	0	18.84	2.58	0.010	0.180	4100	8.34	6.67	23.5	Not collected	1.652	0.197	-1.987	0.000	18.200	1	0
AZ06631-157	54.3	51.02	52.62	0.040	0.260	110	8.18	11.80	11.0	0.1	1.610	0.295	-0.485	0.418	72.275	1	0
AZ06631-162	37.41	45.00	97.82	0.020	0.250	92	7.89	2.00	5.0	0.09	0.115	0.390	-0.266	0.375	69.894	0.04	82
AZ06631-183	25.57	21.74	80.07	0.030	0.236	270	7.68	1.11	5.0	0.23	0.167	0.309	-0.424	0.584	76.148	0.2	140
AZ06631-186	0	34.39	59.78	0.040	0.410	300	8.75	15.10	19.0	0.14	0.682	0.414	0.254	1.227	75.014	1	125
AZ06631-210	52.72	70.21	53.57	0.050	0.110	180	8.33	2.23	5.0	0.06	0.000	0.403	-0.700	0.802	86.168	0.98	0
AZ06631-237	No fish	62.18	46.44	0.150	0.209	230	8.22	5.53	9.5	No fish	0.303	0.502	-1.728	0.661	66.604	0	0

Table A4-1 Continued.

Appendix 5: Habitat Metric Selection and Index Development

This section describes the construction of a preliminary multi-metric habitat index developed for Arizona streams above 1,524 meters (5,000 feet). Similar to IBI development used in the WEMAP report, the range of each variable was checked before testing the redundancy and responsiveness of habitat metrics. Values that were similar for 75% or more for a metric were removed since they do not adequately describe a gradient of conditions. Habitat variables were then divided into the 4 groups; geomorphology, instream habitat cover, riparian vegetation, and riparian disturbance. Within each group redundant variables were identified and removed using correlation and principal component analysis. Variables were removed if correlation coefficients were greater than 0.80. Metrics with highest loadings on the first two PC axes were retained. These groups of variables were then combined and a similar reduction process was repeated for the combined group of variables. Next the responsiveness of each metric was checked with scatter plots of each metric versus land use and chemical stressors. Visual assessments and F-tests were conducted to determine if metrics were discriminating between known reference and impaired sites. After all filters were applied, five metrics remained (Table A5-1). Metrics were normalized to a scale from 1 to10 before combining the metrics into a multi-metric index. The ceiling and floor values for each metric were defined as the 5th and 95th percentile (Barbour and others 1999, Stoddard and others 2005a). For positive metrics (e.g., those that are highest in reference sites), values less than the 5th percentile were given a score of 0, those with values greater than the 95th percentile were given scores of 10, and all metric values in between were interpolated linearly. Negative metrics were scored similarly, with the floor (95th percentile) and ceiling (5th percentile) values reversed (Barbour and others 1999, Stoddard and others 2005a).

A second dataset or validation dataset was created consisting of 25 WEMAP sites sampled during 2000 to 2004. Sites near or above 1,524 meters were included in the validation dataset. The EPA already determined the biological condition of these sites for the WEMAP report. These sites were used in the IBI to test how well the calculated condition match the apriori condition set by the EPA. Although these sites were not assessed based on the habitat condition, the assumption was made that a site in the least-disturbed biological condition would also have a similar habitat condition. Presumably this relationship is

Table A5-1. Physical habitat metrics selected for the LCR basin.

Habitat Metric	Category	Description	Response to Increasing Impairment	Ceiling Value	Floor Value
Riparian disturbance	Human disturbance	Riparian disturbance based on proximity of anthropogenic sources	Increase	2.48	0.00
Canopy cover	Riparian cover	Mean Bank Canopy Density (%)	Decrease	8.50	96.63
Percent sand and fine substrate	Substrate quality	Substrate Sand & Fines <2 mm (%)	Increase	95.24	3.05
Percent fast water	Geomorphic/instream habitat quality	Fast Water Habitat (% of reach)	Decrease	0.00	83.61
Percent glide	Geomorphic/instream habitat quality	Glide (% of reach)	Increase	98.13	9.33

close and the conditions can be used as a proxy for habitat condition. Discriminant function analysis was used to test how well the set of habitat variables used in the index grouped according to its predetermined EPA condition. The five selected metrics were used in this test. The habitat integrity index performed successfully using the test variables. WEMAP condition was correctly classified 76% of the time. All six misclassifications were between intermediate and reference sites and not misclassifications between least- and most-disturbed sites.

The habitat integrity index also significantly discriminated between impaired and reference when plotting the index versus stream-biota IBIs for the LCR basin (macroinvertebrates: $F_{2, 29} = 6.19$, $P = 0.006$; fish: $F_{2, 16} = 3.79$, $P = 0.045$; periphyton: $F_{2, 27} = 4.35$, $P = 0.023$). The habitat integrity index explains more than a quarter of the variation in the steam biota IBIs suggesting that the habitat index is a good indicator of the stream biota condition. This index appears to be more robust than using a single metric to explain biological condition.

Appendix 6: Condition Class Thresholds

	Most-Disturbed		Least-Disturbed	
	Threshold	Percentile	Threshold	Percentile
Aquatic Vertebrate IBI	<17	5th	≥31	25th
Macroinvertebrate IBI	≤45	a	≥52	a
Periphyton IBI	<61	Mean - SD	≥72	Mean
Specific Conductivity	>1000 µS/cm	b	≤1000 µS/cm	b
pH	<6.5 or >9.0	a	≥6.5 and ≤9.0	a
Dissolved Oxygen	<7.0 mg/L	a	≥7.0 mg/L	a
Turbidity	>10 NTU	c	≤10 NTU	c
Suspended Sediment Conc. (SSC)	>80 mg/L	a	≤80 mg/L	a
Fish Tissue Mercury	>0.3 µg/g (wet wt)	d	≤0.3 µg/g	d
Riparian Disturbance	>1.19	95th	≤0.14	75th
Habitat Complexity	<0.26	5th	≥0.36	25th
Streambed Stability	<-1.7 or >0.55	5th	≥-1.19 and ≤-0.23	25th
Riparian Vegetation Coverage	<0.16	5th	≥0.28	25th
Habitat Integrity (IHI)	<55	5th	≥0.63	25th
Non-native Vertebrates	>10% of Individuals	b	Absent	b
Non-native crayfish	Present	e	Absent	e
Asian clam	Present	f	Absent	f

a *Arizona Administrative Code Water Quality Standards for surface waters (ADEQ 2009)*

b *Thresholds adopted from the EMAP west assessment (Stoddard and others 2005a)*

c *This is an earlier Arizona water quality standard, which was replaced by the suspended sediment concentration (SSC) standard in 2002.*

d *The state of Idaho uses this concentration to protect human adults, bald eagles and wildlife (Idaho Department of Administration, IDAPA 58 Title 01 Chapter 02—Water Quality Standards).*

e *Presence of crayfish was considered the most-disturbed condition because crayfish are not native to Arizona (Hobbs 1989). Absence of crayfish was considered to be the least-disturbed condition.*

f *The Asian clam (Corbicula fluminea) is not native to Arizona, so the presence of Asian clams was considered the most-disturbed condition.*

Appendix 7: Reference Site Criteria

For aquatic vertebrates and periphyton assemblages as well as for physical habitat variables, potential reference sites included: (1) hand-picked and probability sites above 1,524 meters (5,000 feet) that were sampled during the 2000-2004 WEMAP, and (2) all probability sites we sampled during 2007. All potential reference sites were evaluated and filtered based on procedures outlined by Stoddard and others (2005a) and Whittier and others (2006 and 2007c). Criteria are summarized in Table A7-1 for aquatic vertebrates and periphyton and Table A7-2 for habitat references. For macroinvertebrates, empirically based Arizona IBI cold water thresholds were used.

Table A7-1. Three sets of criteria used to select candidate reference sites for aquatic vertebrates and periphyton. For the Herlihy and Stoddard methods (Stoddard and others 2005a) all criteria must be met to classify a site as reference. For the Whittier method (Whittier and others 2006) three natural gradients (stream width, elevation, and slope) were plotted against the twelve anthropogenic stressors indicated by the "X", and least-disturbed sites were identified along those gradients as the encompassing 15% of the sites.

	Herlihy	Stoddard	Whittier (Elevation, Slope, Stream size)
Total phosphorus (µg/L)	<50	<50	X
Total nitrogen (µg/L)	<750	<750	X
Chloride (µeq/L)	<300	<300	X
pH (laboratory)	<9	<9	
Turbidity			X
Riparian disturbance	<0.5	<0.5	X
Canopy density	>50%	>50%	
Percent sands or finer substrates	<15%		X
Streambed stability		>-2.0	
Natural fish cover			X
Complex riparian vegetation			X
Road density (m/hectare)			X
Human population density			X
% Urban			X
% Agriculture			X

Table A7-2. Chemical and land use criteria used to select physical habitat references. It is likely that chemical stressors are not directly impacting habitat like certain land uses might. However, chemical stressors should serve as an independent indicator-proxy for the condition of a stream. The water chemistry thresholds were decreased (made stricter) in order to properly discriminate between reference and impaired sites. The other thresholds used to determine reference condition were the same as the thresholds established in the WEMAP report (Stoddard and others 2005a).

Salinity (specific conductivity) (µS/cm)	<500
Total phosphorus (µg/L)	<40
Total nitrogen (µg/L)	<125
Road density (road length/square kilometer)	<15%
Total disturbance (% agricultural + %urban land use)	<5%
Population density (population/square kilometer)	<5%
Grazing metric (unitless metric that integrates land-use covers)*	<5000

**The summation of weighted scores calculated for GIS layers (land ownership, land cover, topographic position index, slope, and proximity to water). It should be noted that the grazing metric was not considered for six of the LCR sites.*