North American freshwater resources have grown increasingly imperiled as a result of human-induced landscape alterations. Consequently, resource managers need the ability to predict areas of high and low biological integrity to inform management decisions and meet conservation needs. Previous efforts to quantify anthropogenic disturbances to aquatic systems have resulted in indices lacking the ability to identify specific stressor impacts, describe the ways stressors alter the physical and chemical condition of receiving waters, and predict biological integrity. Thus, the need exists for a flexible approach to characterizing stream impairment and identifying candidate least-disturbed stream reaches to serve as benchmarks for high quality physical habitat and biological integrity. After accounting for natural sources of biological variation (e.g. watershed drainage area, reach gradient, spring density), we modeled the influence of channel morphology, substrate, cover, and water quality, and watershed-level flow modification and fragmentation, urbanization, agriculture, and point-source pollution on stream fish and macroinvertebrate community characteristics of Wadeable streams of Missouri. Reach-level environmental variables generally explained greater amounts of biotic variation than did watershed-level variables, with metric values indicative of high biological integrity (e.g. Ephemeroptera, Plecoptera, Trichoptera richness, number of native lithophilic fish species) increasing with increased stream width/depth ratio, coarse gravel substrate, and dissolved oxygen, and decreasing as total chlorophyll increased. At the landscape level, these biotic metrics increased with higher percentages of forest in the watershed, and decreased with increased densities of headwater impoundments, road crossings, and pasture lands. In general, invertebrate metrics showed higher sensitivity to row-cropping and water quality impairment than did fish, particularly in the Ozark Highlands aquatic subregion. We used the results of our watershed-level models to predict biotic metric values to over 28,000 wadeable stream reaches across the state and rescaled and summed individual metric scores to generate an overall estimate of biological integrity at each site. We identified streams scoring in the top 95th percentile to serve as candidate least-disturbed reference reaches predicted to exhibit relatively high quality habitat and biotic conditions. Our method represents a novel approach to characterizing and forecasting stream impairment, and represents a critical step in refining existing biological indices, developing a companion physical habitat index, and ultimately conserving the diversity and integrity of Missouri’s flowing waters.