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 conditions 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. We used boosted regression trees to account for natural sources of biological variation (e.g. stream size, gradient) and to then model the influence of reach and watershed-level disturbances on stream fish and aquatic invertebrate community characteristics of wadeable streams in Missouri. Sensitive biotic metrics (e.g. EPT richness, lithophilic fish species richness) increased with increased stream width/depth ratio, coarse gravel substrates, and dissolved oxygen, and decreased as total chlorophyll increased. At the landscape-level, these metrics tended to increase with higher percentages of forest in the watershed, and decreased with increased densities of headwater impoundments, road crossings, and pasture lands. After using the results of our watershed-level models to predict individual metric values to unsampled reaches across the state, we summed predicted values to generate an estimate of overall biological integrity at each site, retaining streams scoring in the top 95th percentile of each assessment unit to serve as candidate least-disturbed reference reaches. These candidates will allow the refinement of existing biological indices, and development of a companion physical habitat index for Missouri streams.