Urbanization affects many chemical and physical characteristics of stream ecosystems, yet relatively little is known about the specific effects of urbanization on stream microhabitats or fish assemblages. In this study we measured fish diversity and abundance, stream velocity, and turbidity in three microhabitats (pools, riffles, runs) at nine stream reaches (designated as primarily rural, residential, or commercial) in the Enoree River basin of northwestern South Carolina. In addition, we measured geomorphologic characteristics (percent impervious surface cover, incision, entrenchment and width/depth) at each site. Because commercial sites had the greatest impervious land cover, we hypothesized that species richness and diversity would be lowest at commercial sites, and that fish assemblages would be more homogeneous among the microhabitats at the commercial sites. Additionally, we hypothesized that stream velocity, turbidity, and geomorphic characteristics would reflect the greater instability of commercial sites. Commercial sites had the lowest species richness and diversity. Richness and diversity did not differ significantly among microhabitats within each land cover and otherwise reflected differences seen at the reach scale. Abundance was highest in commercial sites, but assemblages were dominated by pioneer species. Turbidity was greatest at rural sites and within runs among microhabitats. Index of Biotic Integrity scores were significantly negatively correlated with percent impervious surface cover. Within commercial sites, entrenchment and total fish abundance were significantly negatively correlated, and at rural sites there was a significant negative correlation between width/depth and diversity. Our study demonstrates that urbanization negatively impacts fish communities both at the stream reach and microhabitat scales.