

# Identifying Sources of Stress to Native Aquatic Fauna Using a Watershed Ecological Risk Assessment Framework

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The free-flowing Clinch and Powell River Basin, located in southwestern Virginia, United States, historically had one of the richest assemblages of native fish and freshwater mussels in the world. Nearly half of the species once residing here are now extinct, threatened, or endangered. The United States Environmental Protection Agency's framework for conducting an ecological risk assessment was used to structure a watershed-scale analysis of human land use, in-stream habitat quality, and their relationship to native fish and mussel populations in order to develop future management strategies and prioritize areas in need of enhanced protection. Our analyses indicate that agricultural and urban land uses as well as proximity to mining activities and transportation corridors are inversely related to fish index of biotic integrity (IBI) and mussel species diversity. Forward stepwise multiple regression analyses indicated that coal mining had the most impact on fish IBI followed by percent cropland and urban area in the riparian corridor ( $R^2 = 0.55$ ,  $p = 0.02$ ); however, these analyses suggest that other site-specific factors are important. Habitat quality measures accounted for as much as approximately half of the variability in fish IBI values if the analysis was limited to sites within a relatively narrow elevation range. These results, in addition to other data collected in this watershed, suggest that nonhabitat-related stressors (e.g., accidental chemical spills) also have significant effects on biota in this basin. The number of co-occurring human land uses was inversely related to fish IBI ( $r = -0.49$ ,  $p < 0.01$ ). Sites with  $\geq 2$  co-occurring land uses had  $>90\%$  probability of having  $<2$  mussel species present. Our findings predict that many mussel concentration sites are vulnerable to future extirpation. In addition, our results suggest that protection and enhancement of naturally vegetated riparian corridors, better controls of mine effluents and urban runoff, and increased safeguards against accidental chemical spills, as well as reintroduction or augmentation of threatened and endangered species, may help sustain native fish and mussel populations in this watershed.

## Introduction

The Clinch and Powell River Basin in southwestern Virginia and northeastern Tennessee historically contained one of

the most diverse fish and mussel assemblages in North America (1-4), yet most of these populations have declined dramatically or been eliminated during the past century (5-8). Still, the Clinch and Powell River Basin currently supports more threatened and endangered aquatic species than almost any other basin in North America (9). Despite implementation of recovery plans for most Federally protected species, recent fish and mussel surveys indicate continuing decline of numerous rare species in this part of the basin (10, 11) and in North America (9). Resource managers in the Clinch and Powell River Basin recognized that a comprehensive examination of the available data, for the basin as a whole, was needed to evaluate the relative effects of different human activities on native mussels and fish.

Ecological risk assessment is a process to collect, organize, analyze, and present scientific information to improve the use of science in decision-making. According to EPA guidelines (12), the assessment framework is a structured format including problem formulation and planning, analysis of exposure and effects, risk characterization, and communication of results to the risk managers. Because of the valued and threatened ecological resources and the already existing partnerships working to protect those resources, the Clinch and Powell Basin was selected by the U.S. EPA for application of an ecological risk assessment at a watershed scale. Applying ecological risk assessment within a watershed approach provides resource managers and the public with a logical and systematic method to incorporate scientific information into decision-making (12, 13). However, these risk assessments face many challenges, and their applicability to watershed-scale management is limited (14, 15). Inferring cause-effect relationships between stressors and ecological resources is especially complex in watersheds in which multiple stressors and interactions among stressors are likely to be present. Watershed-scale assessments have been conducted for single stressors (e.g., ref 16), and multiple chemical stressors (e.g., ref 17). In addition, there have been a number of studies that examined relationships between land uses and particular habitat or biological end points within a watershed (18-22). However, most of these studies focused on specific land uses or types of fauna and did not attempt to examine multiple uses and fauna on a watershed scale.

This paper describes an analytical approach to watershed ecological risk assessment that has helped provide resource managers with information to address the impacts of anthropogenic physical and chemical stressors on the valued ecological resources in the Clinch and Powell River Basin. Risk assessment is a cyclical process in which uncertainties are typically large at first and then reduced with further data collection and/or analyses in later stages (12, 15). The study presented here is an initial risk assessment based on existing information.

## Methods: Planning and Problem Formulation

**Description of the Watershed.** The Clinch and Powell River Basin covers 9,971 km<sup>2</sup> and generally ranges between 300 and 750 m in elevation. The Powell River is a tributary of the Clinch River, and the confluence of the two is now submerged at Norris Lake in northeast Tennessee (Figure 1). The majority of the watershed is composed of forest (69%) and agricultural (28%) land; however, the relative proportion of land uses vary among subwatersheds in the basin, particularly with respect to coal mining and pasture lands (Table 1). Major agricultural products in the basin include beef and tobacco. Because of topographic constraints, the majority of the

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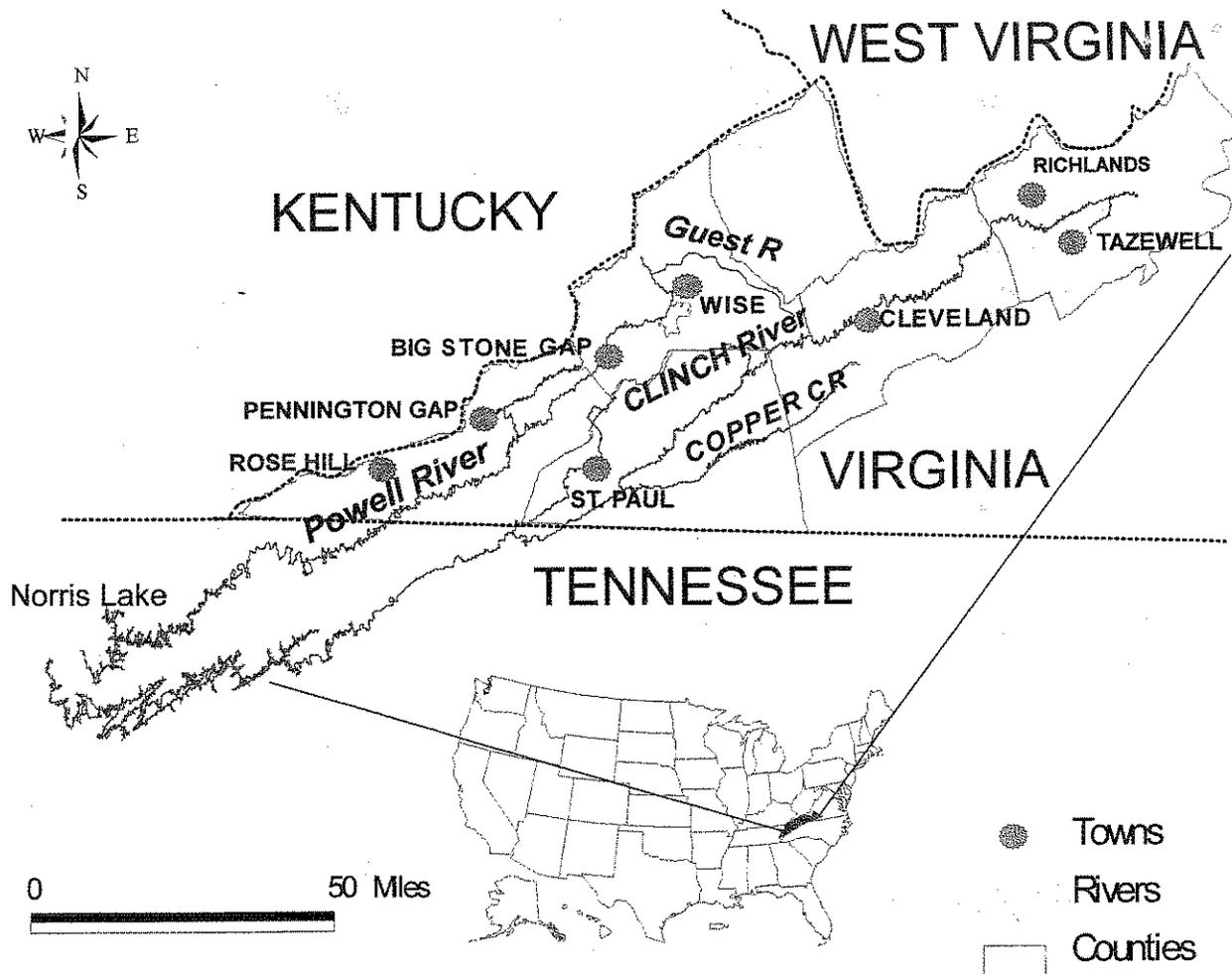


FIGURE 1. Map of the Clinch/Powell River Basin analyzed in this risk assessment.

TABLE 1. Comparison of Land Cover for Four Major Subwatersheds Examined in the Clinch/Powell River Basin Risk Assessment

	Upper Clinch River	Upper Powell River	Guest River	Copper Creek
forest (%)	53.7	89.6	84.1	57.7
cropland (%)	0.6	3.1	<0.1	1.3
pasture (%)	44.5	2.4	10.4	40.9
urban (%)	1.1	4.2	2.6	<0.1
no. of mines <sup>a</sup>	8	21	26	0

<sup>a</sup> Active mines and coal preparation plants.

agricultural activity is limited to flood plains where livestock and row crops are most productive. Nearly 75 000 grazing livestock depend on the rivers or their tributaries for water. Most of the pasture land exceeds Soil Conservation Service soil loss tolerance criteria due to the shallow soils and steep topography (23). Coal mining is generally limited to the upper Powell and Guest River subwatersheds (Figure 1).

**Planning the Risk Assessment.** The U. S. Fish and Wildlife Service, Tennessee Valley Authority, The Nature Conservancy, Virginia Department of Game and Inland Fisheries, Virginia Cave Board, U.S. Environmental Protection Agency, and U.S. Geological Survey participated in this ecological risk assessment. To accomplish the planning aspect of this assessment, resource managers participated in a series of planning meetings and public workshops in which a watershed

management goal and objectives for the risk assessment were developed. The participating organizations agreed to focus on the unimpounded basin above Norris Lake, TN (see Figure 1), for the risk assessment since this is the most productive part of the basin for native species in the Cumberlandian region (1-3, 5, 7).

It was also agreed to use only previously collected data due to resource limitations. Biological and habitat quality data were obtained primarily from Tennessee Valley Authority (TVA) monitoring data, particularly the Clinch Powell River Action Team Surveys (CPRATS; N = 155 sites) and the Cumberlandian Mollusc Conservation Program (CMCP; N = 60 sites). An extensive literature search was also employed to obtain additional relevant data in this watershed.

Two assessment end points were selected as measurable ecological characteristics based on their relevance to management objectives, susceptibility to stressors, and ecological relevance (12): (i) reproduction and recruitment of threatened, endangered, or rare native freshwater mussels and (ii) reproduction and recruitment of native, threatened, endangered, or rare fish species. Stakeholders assumed that protection of these rarer species would help maintain integrity of the watershed as a whole, including nonthreatened or endangered aquatic species.

A preliminary analysis of data collected from the Copper Creek subwatershed (see Figure 1) was performed to identify an appropriate spatial scale with which to relate uses and biological measures and to define biological measures of effect that would be appropriate for analysis of the basin as a whole (24). Since mussel development and dispersal is

dependent on the presence of appropriate fish hosts to carry and nurture the parasitic mussel glochidia (larvae), it seemed plausible that fish index of biotic integrity or IBI could serve as a reasonable predictor of mussel diversity. The IBI score used by TVA is a composite of the 12 different measures or metrics developed by Karr et al. (25) that address characteristics of fish communities at the individual, population, and ecosystem levels (see Table A-1 in Supporting Information; 25, 26). IBI metrics and TVA's scoring criteria were not specifically tailored to this watershed at the time data were collected for this study. Therefore, there may be uncertainties in our analyses associated with the accuracy of IBI measures (26, 27).

IBI scores are derived by comparison to data from reference sites located within the same ecoregion or physiographic province (27). Reference sites represent a condition that is minimally influenced by human actions and serve as a control or baseline. The individual scores are added, and the composite scores are then grouped into integrity categories (poor, fair, good, or excellent) based upon comparison with the reference site (27). Sites with IBI scores that are significantly lower than those observed at minimally disturbed reference sites generally correspond to an impaired or stressed fish community. Such sites are rated by the TVA as either poor or fair condition, depending on how low the IBI score. Sites that have IBI scores within the range of reference site scores are considered minimally impaired, although it is recognized that reference sites themselves may be subject to uncontrolled stressors (e.g., atmospheric deposition). Consistent with the TVA's interpretive criteria, we categorized the former situation as "impaired" and the latter as "unimpaired" for purposes of certain analyses. IBI data used in this study are associated with a high degree of confidence as they were collected by TVA fisheries biologists, who have extensive experience sampling and identifying fish in this watershed. Although it would have been useful to examine the 12 separate IBI measures individually (see Table A-1 in Supporting Information and ref. 28), only the composite IBI scores were accessible for analyses. Analysis of only the composite IBI scores in our study may have masked the risk analysis relationships with land uses in some cases (15).

The preliminary study in Copper Creek demonstrated that fish IBI scores correlated well with mussel species richness at various sampling sites within this subwatershed (Wilcoxon Matched Pairs Test,  $p < 0.05$ ; Figure A-1 in Supporting Information). Thus, we used IBI scores to supplement the relatively few mussel data available. We recognized that there was some uncertainty extrapolating this relationship to the entire watershed. However, anecdotal and published information supplied by resource agencies supported a relationship between fish assemblage integrity and mussel species richness (6, 8, 29).

Our preliminary study also revealed that a riparian corridor 200 m wide (100 m on each side of the stream) and 2000 m upstream from a given sampling site exhibited the most significant relationships between land uses and fish IBI scores (mean Pearson correlation coefficient = 0.35,  $p < 0.05$ ; 24). In this initial watershed risk assessment, we used these dimensions to define the riparian corridor for each biological sampling site in the basin as a whole. We recognized that the riparian buffer size determined for Copper Creek may not necessarily be applicable to all parts of the watershed and was a source of uncertainty in our analyses. However, subsequent analyses of mussel data collected in the upper Clinch River (10) generally supported the riparian corridor results obtained for Copper Creek (24).

Land cover data were derived from classified Landstat Thematic Mapper imagery. All terrain data (i.e., elevation and slope) were obtained from a mosaic of 30-m resolution U.S. Geological survey digital elevation models (DEM). The

**TABLE 2. Summary of Stepwise Multiple Regression Analyses on Instream Habitat Score (IHS) As a Function of Human Land Uses<sup>a</sup>**

independent variable	coeff	SE of coeff	t value	p level
intercept	136.68	61.17	2.32	0.031
urban	-0.58	0.15	-3.82	0.001
cropland	0.26	0.15	1.79	0.089
mining	-0.29	0.16	-1.74	0.095
pasture	0.25	0.16	1.55	0.137

<sup>a</sup> Measured in riparian corridors 200 m × 2 km upstream of each sampling point ( $N = 24$ ). Habitat data are from Tennessee Valley Authority's Clinch Powell River Action Team Survey.

U.S. EPA's River Reach 3 File (RF3) provided stream network data. Several habitat quality measures were also available from TVA sampling, which we used to characterize habitat-related stressor exposure (see Table A-2 in Supporting Information). Data were also available for the location of coal mines (both active and inactive) and preparation plants, major transportation corridors, and urban centers (including wastewater facilities) in the basin (14). All data were entered into a geographical information system (GIS, Arc/INFO, v. 7.04, and Arcview, v. 3.0, ESRI, Redlands, CA; see Table A-3 in Supporting Information) and partitioned in various ways using ACCESS (Microsoft) to develop databases amenable to statistical analysis (Statistica, v. 5.0, Statsoft, Tulsa, OK).

For each biological sampling point, proximity to the nearest urban centers, major roadways, or coal mine activities upstream were calculated and categorized as either <1, 1-2, or >2 km based on the preliminary riparian corridor analyses mentioned above. Biological and habitat data were subjected to one-way analysis of variance (ANOVA,  $p < 0.05$ ) using these three proximity categories as class variables. In addition, for each site, percent urban or agricultural area was also computed within the riparian corridor as defined above and related to habitat and biological measures using forward stepwise multiple regression analyses ( $p < 0.05$ ). Type I error was controlled by limiting analyses to no more than one factor (independent variable) per 5 sites and by including only those variables that increased the overall  $R^2$  by at least 10%. Variables with  $p$  values  $> 0.05$  were considered in multiple regression analyses only if their  $F$  value was sufficiently high to be entered into the model and if the resulting  $R^2$  value was at least 10% greater. While this study focused on effects of human land uses, several other stressors, such as water quality contaminants from nonpoint sources, were recognized as potentially important in this risk assessment but could not be statistically analyzed because of a lack of monitoring data.

## Results and Discussion

**Effects of Land Use on Habitat Quality.** About 57% of the variability in the habitat quality index score was explained by land uses within the riparian corridor if the analysis was limited to a relatively homogeneous topographic range (350–400 m in elevation) ( $R^2 = 0.57$ ,  $F = 6.72$ ,  $p = 0.01$ ,  $N = 24$ ; Table 2). Urban and mining uses were negatively related and pasture and cropland were positively related to habitat quality index score in this small data set (Table 2). The positive relationships were counter to previous visual observations by resource personnel in the watershed (1, 8, 11) and may be an artifact of limited sample size in this analysis. Conversely, given that this data set consists of relatively low elevation streams with wide, flat flood plains, and consequently lower erosion rates, pasture and cropland may have relatively less effect on overall instream habitat than urban areas or mining, both of which are associated with greater runoff and/or discharge potential.

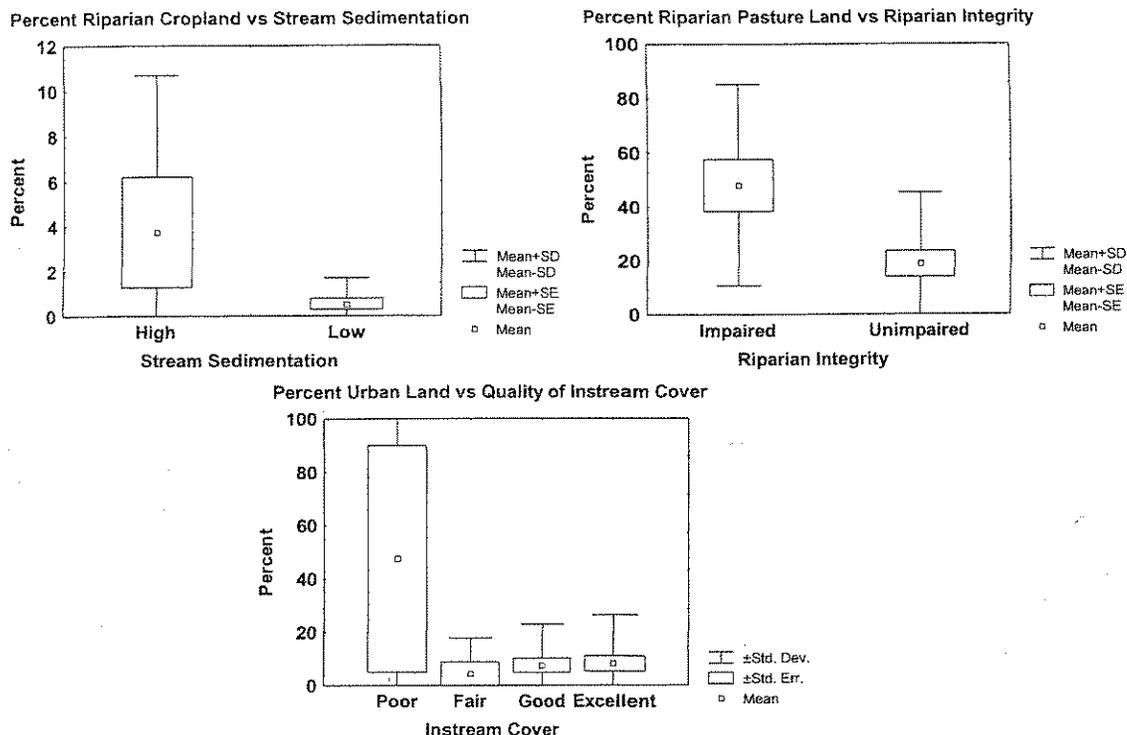


FIGURE 2. Significant relationship between land uses and instream physical habitat quality measures.

The results presented in Table 2 were not confirmed for the watershed as a whole; if all sites were considered in the analysis, regardless of topography ( $N = 108$ ), only 26% of the variability in habitat index score was explained by land use. The remaining variability may be due to upland land uses that were not included in the riparian corridor analyses, other topographic features that may affect habitat quality (e.g., drainage slope), and/or uncertainties due to either the visual habitat assessment technique or the aggregate habitat index measure used by TVA.

Certain relationships between individual habitat measures and land use, however, were evident for the watershed as a whole. Categorical analysis indicated that stream sedimentation was lower where cropland comprised  $\leq 3\%$  of riparian land; instream fish cover was poor if urban use in the riparian corridor was  $\geq 10-20\%$ ; and riparian vegetation integrity was higher in areas in which pasture land in the riparian corridor was  $< 50\%$  (ANOVA,  $N = 108$ ; Figure 2). These relationships suggest that instream habitat should have a higher probability of being satisfactory for aquatic life if agricultural land and urban influences are small within the riparian corridor. Similar results have been reported by Lenat (30), Karr and Chu (26), and Cooper et al. (31). Our results suggest that individual habitat measures may be more informative than an aggregated habitat index in this case. These data also suggest that the mitigating effects of riparian corridor vegetation are sensitive to human influences within the stream flood plain in this mountainous watershed.

Habitat quality scores and important habitat metrics such as embeddedness or sedimentation were not significantly different in relation to distance from coal mines or transportation corridors for the watershed as a whole (ANOVA,  $p > 0.50$ ;  $N = 108$ ), contrary to expectations based on the literature (29, 32). These results may be due, in part, to the fact that other land uses such as cropland or pasture also result in increased sedimentation and therefore may have masked a specific effect of mining on sedimentation.

**Relationships between Habitat Quality and Biological Measures of Effect.** Linear relationships were not observed between habitat quality metrics and IBI, possibly due to the

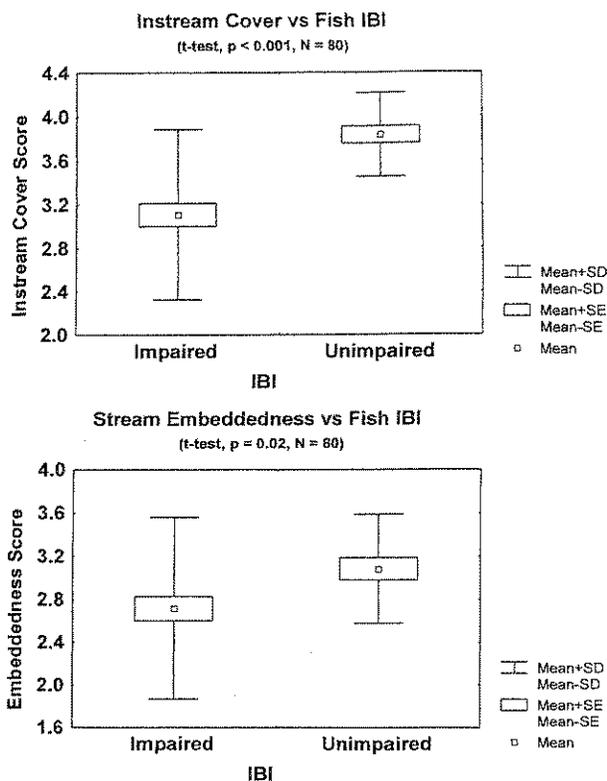
TABLE 3. Summary of Stepwise Multiple Regression Analyses on Fish Index of Biological Integrity (IBI) As a Function of Instream Habitat Measures<sup>a</sup>

independent variable	coeff	SE of coeff	t value	p level
intercept	9.84	13.83	0.17	0.86
embeddedness	-0.05	0.19	-2.50	0.028
riparian forest cover	0.66	0.22	3.04	0.007
epifaunal substrate	0.76	0.21	3.60	0.002
bank integrity	0.54	0.19	2.73	0.014
instream cover	0.34	0.18	1.89	0.07
sediment erosion rate	-0.25	0.21	-1.16	0.25

<sup>a</sup> Reported by Tennessee Valley Authority's Clinch Powell River Action Team Survey ( $N = 24$ ).

fact that habitat metrics are categorical as discussed above. Since IBI is a composite measure, it may also conceal continuous relationships between fish assemblages and habitat quality measures (15). Stepwise multiple regression analysis indicated that approximately 57% of the variability in IBI values was explained by individual habitat metrics if we again limited our analyses to sites within a narrow elevation range ( $R^2 = 0.57$ ,  $F = 4.04$ ,  $p = 0.09$ ,  $N = 24$ ; Table 3). Embeddedness and instream cover were clearly related to IBI if it was categorized as either impaired or unimpaired (i.e., IBI scores  $\leq 40$  vs scores  $> 40$ , respectively; Figure 3). Sites with either substrate embeddedness scores  $\leq 2$  (indicating moderate to severe embeddedness) or instream cover scores  $< 3$  (poor to fair cover) according to TVA criteria had greater than a 90% chance of having impaired fish community integrity. Both habitat variables have also been closely linked to mussel species distribution (2, 33-35). Given that we observed an inverse relationship between pasture land use or urban proximity and embeddedness (Figure 3), it is not surprising that these land use activities have effects on fish assemblage integrity.

**Effects of Land Use on Biota.** Proximity to mining was the most significant land use factor related to fish IBI in this watershed (forward stepwise multiple regression,  $R^2 = 0.55$ ,



**FIGURE 3.** Embedded sediments or instream cover as a function of impaired or unimpaired fish assemblage integrity and IBI. Lower habitat scores indicate poorer conditions for aquatic life.

**TABLE 4.** Summary of Forward Stepwise Multiple Regression Analyses of Fish IBI Values with Human Land Use Factors<sup>a</sup>

human land use	coeff	SE of coeff	t value	p value
intercept	-373.08	208.53	-0.84	0.41
pasture	0.53	0.15	3.41	0.002
mining	-0.50	0.14	3.48	0.001
cropland	-0.26	0.14	-1.84	0.076
urban	-0.17	0.12	-1.42	0.17
highways	-0.22	0.13	-1.59	0.12

<sup>a</sup> IBI data obtained from TVA's CPRATS data set for sites between 350 and 450 m in elevation in the Clinch/Powell Watershed ( $N = 38$ ).

$F = 9.54$ ,  $p < 0.001$ ,  $N = 34$ ; Table 4). This was evidenced by significantly lower IBI scores at sites within 2 km of a mine (ANOVA,  $p < 0.05$ ; Figure 4). Unimpaired fish communities were also associated with greater pasture area and less urban land within the riparian corridor (Figure 4). The apparently positive effect of pasture land on fish community integrity was consistent with the positive relationship observed between pasture and habitat index score using a limited data set (Table 2). Our interpretation of this result is that mining and urban areas are comparatively far more detrimental sources of stress on fish in this watershed than pasture areas as a whole. This is supported by the fact that percent forested land cover was greater near mining activity than further away (ANOVA,  $F = 5.93$ ,  $p = 0.003$ ,  $N = 152$ ) and negatively correlated with pasture land cover (Spearman Rank Correlation,  $r = -0.80$ ,  $p < 0.05$ ). As a result, higher IBI scores (i.e., better fish community integrity) were associated with less forested cover than sites with lower IBI scores (poorer fish community integrity;  $t$ -test,  $t = 3.01$ ,  $p = 0.003$ ,  $N = 137$ ). Thus, the supposed positive effect of pasture land may, in fact, indicate that mining has a profound negative effect on fish communities in this watershed. However, 45% of the variability in IBI scores was unexplained by multiple regres-

**TABLE 5.** Summary of Forward Stepwise Multiple Regression Analysis of Native Mussel Species Richness As a Function of Riparian Land Use Factors<sup>a</sup>

land use factors	coeff	SE of coeff	t value	p value
intercept	51.00	79.92	0.26	0.74
urban	-0.44	0.16	-3.35	0.007
cropland	-0.13	0.12	-1.27	0.30

<sup>a</sup> Data are from TVA's Cumberlandian Mussel Conservation Program database ( $N = 33$ ).

sion analysis, indicating either that the composite nature of IBI scores conceals relationships between fish assemblages and land uses (15) or that other site-specific factors, such as hydrologic regime or other water quality effects, are significant sources of stress in this system.

The number of native mussel species present was related to several land uses including (in order of significance) percent urban area, proximity to mining, and percent cropland ( $R^2 = 0.26$ ,  $F = 3.01$ ,  $p = 0.03$ ,  $N = 33$ ; Table 5). Sites further away from towns or mining tended to have a greater number of mussel species present. Again, this model explained only a portion of the variability observed. Other factors could include (i) site-specific geomorphic characteristics such as substrate particle size, flow, and current velocity and orientation of bedrock ridges (36); (ii) lack of obligate fish hosts at the necessary spawning times (37, 38); and (iii) proximity to accidental chemical spills.

Accidental spills were not quantitatively included in this assessment due to a lack of appropriate data. However, several toxic spills have been documented in this basin over the past 30 yr (39-41) including a 1999 truck accident that spilled concentrated ammonia into the Upper Clinch River, resulting in a large fish kill and mortality of at least 300 Federally threatened and endangered mussels (42). Mussels have still not recovered from these spills, possibly because of residual sediment contamination (43) that may impair survival of mussel glochidia and juveniles (44, 45). For this reason, we included proximity to major transportation corridors (one major source of accidental spills in addition to industrial sources in urban/developed land) as a major source of stress in this watershed (see Table 4). Results of our analyses suggest that these site-specific chemical spills may be important in explaining variability in fish and mussel abundance and distribution.

**Impact of Cumulative Number of Stressors.** The foregoing analyses examined relationships between either single sources of stress or linear combinations of individual human activities and biological measures of effect. In an attempt to determine effects of co-occurring sources of stress, we calculated a nominal cumulative stressor index for each biological site depending on how many of the four major human land uses previously identified in our analyses (mining activities, urban areas, major transportation corridors, and agricultural area) were within 2 km upstream of a site. Fish IBI was inversely related to the cumulative number of sources present (Figure 5A,  $r = -0.49$ ,  $p < 0.01$ ,  $N = 138$ ). ANOVA indicated that sites with > 1 human activity had significantly lower IBI scores ( $p < 0.01$ ) than sites with none of the four sources present. Sites with three or four sources present had lower IBI scores than sites with one source (ANOVA,  $p < 0.01$ ). Approximately 66% of the sites having two of the four land uses present ( $N = 58$ ) had IBI scores < 35, indicating poor fish community integrity according to the TVA's criteria. In nearly all of these cases (88%), the sources of stress were urban areas and mining.

We were unable to detect significant differences in average mussel diversity with cumulative human land use sources due to many sites with few mussels present, regardless of

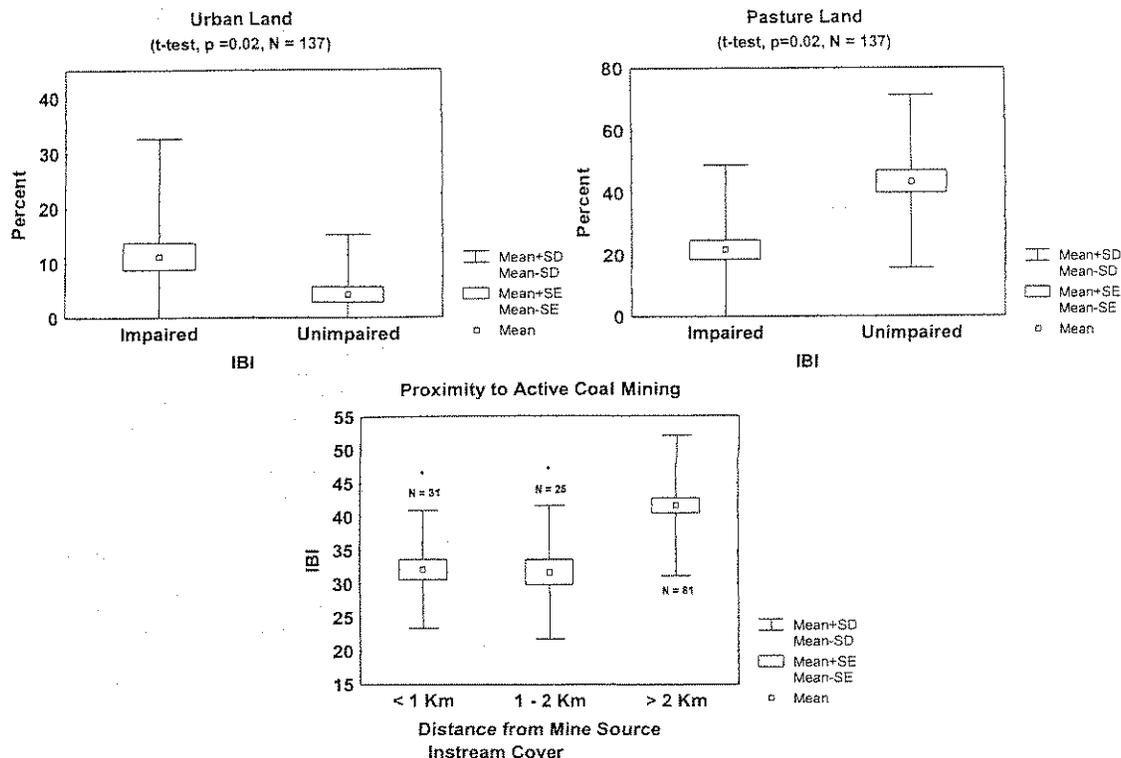


FIGURE 4. Significant relationships observed between land use activities and fish index of biotic integrity (IBI). Impaired fish IBI = IBI scores < 40, and unimpaired fish IBI = IBI scores  $\geq$  40, based on TVA's criteria.

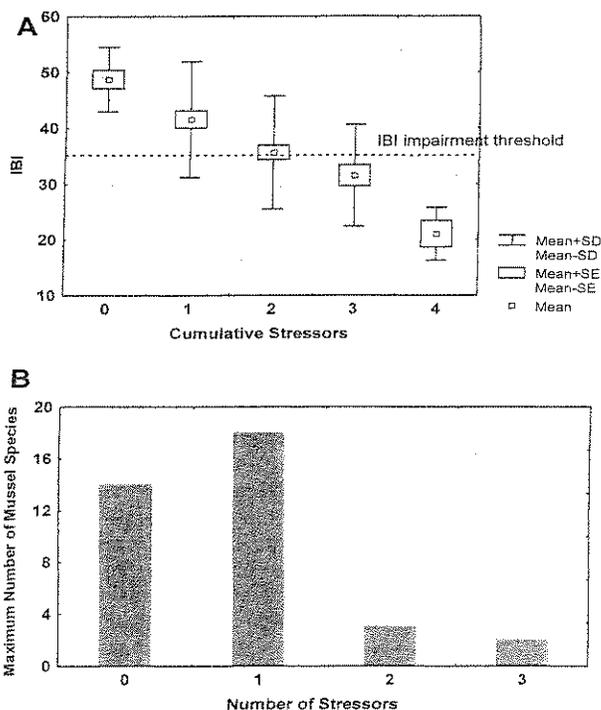


FIGURE 5. Fish IBI (A) and maximum number of mussel species (B) in the Clinch/Powell River Basin as a function of the number of stressors.

surrounding land uses. However, we did observe an inverse relationship between the cumulative number of sources and the maximum number of mussel species present at a site (Figure 5B). Sites having  $\geq 2$  of the four sources had greater than a 90% probability of having fewer than two mussel species present. Sites with one or no sources had a maximum of between 4 and 10 species. Thus, the presence of multiple

human land uses appeared to increase the risk to mussels and fish in this watershed. Similar findings have been reported elsewhere for other fauna and watersheds (9, 46).

**Risk Characterization.** Several lines of evidence point to the importance of various land use activities and riparian corridor integrity as determinants of native mussel and fish distribution in the Clinch and Powell River Basin with proximity to coal mining operations having the most adverse effects. Site-specific factors that could have also contributed to effects on IBI and mussel species richness are wastewater discharge and other point and nonpoint sources that could release toxic constituents or excessive nutrients downstream (47). Many of these were not explicitly included in our analyses due to a lack of appropriate data.

Similar to results reported in other watersheds (20, 21, 26, 28), we observed that agricultural and urban land use contribute sediment to the stream causing embeddedness, poor cover for fish and invertebrates, and, consequently, impaired fish and mussel assemblages. Riparian areas with more forested land cover and less cropland, urban, or mining activity tended to be associated with less sedimentation, less substrate embeddedness, more instream cover for aquatic fauna, and higher fish and native mussel species richness. Our results suggest that, if agricultural or urban use upstream is great enough within the riparian zone, sedimentation effects and resultant loss of habitat ensue for up to 2 km downstream. Although riparian vegetation can reduce deleterious land use effects on water quality (18, 31, 48), it is not clear that improvement of the riparian corridor in this watershed will necessarily result in recovery of native mussel and fish populations. Little or no recovery of threatened or endangered mussel or fish species has been observed in this basin despite improvement in conventional water quality parameters (e.g., BOD, fecal coliform, suspended solids, nutrients) (49).

Native fish and mussels have a high risk of extirpation due to endemism and habitat fragmentation, resulting in populations that are too inbred, small in size, and more

susceptible to stressors (5, 15, 46). Populations are now more widely separated than they were historically (2-4, 7, 8, 20), which could lead to reduced recruitment success and declining populations, especially in the presence of stressors. Results of this study suggest that native fish and mussel populations are relatively vulnerable to several sources of stress in this watershed and that the risk of extirpation is likely to increase as more sources of potential stress co-occur. GIS-based examination of known mussel beds in the watershed suggests that as many as two-thirds of the sites are vulnerable to at least two sources of stress identified in this risk assessment (24). Therefore, it may be most useful to further protect those populations that appear vulnerable due to proximity to mining, urban and pasture areas, or transportation corridors.

One of the chief means for sustaining threatened, endangered, and other rare mussel species in the Clinch/Powell watershed has been through controlled rearing and stocking programs in new or historically important areas (44). As a result of this risk assessment, resource managers are now considering implementing several resource protection measures including riparian buffer preservation, limited access of livestock to streams, better treatment of mine discharges to streams, and spill prevention along transportation corridors. These measures are probably as important as stocking in terms of sustaining endemic species. If stream habitat as well as water quality can be maintained or improved, present mussel and fish populations might be able to expand into nearby areas, thus increasing the distribution and abundance of these species.

**Uncertainties.** Several analyses in the first stage of this watershed ecological risk assessment indicated that native mussel species richness and fish IBI are strongly influenced by factors not quantified in this study. Riparian corridor land uses, for example, accounted for approximately half of the variability observed in IBI values and even less of the variability observed in mussel species richness. For fish, some of these results could be due to the nature of the IBI value itself, which may mask relationships. Norton et al. (28), for example, showed that individual IBI metrics could be strongly related to land use characteristics and habitat quality measures. Attempts are being made to electronically catalogue the individual IBI metric values in the future as well as the composite IBI score for each site in the GIS database.

The riparian corridor dimensions used in our analyses may be another source of uncertainty. A fixed riparian buffer size (200 m wide  $\times$  2 km upstream), based on analyses in one subwatershed, was applied to all biological sampling sites throughout the watershed, regardless of stream size or drainage area. Larger streams, such as the upper Powell or Upper Clinch Rivers, could conceivably have different relationships between riparian land uses and stream habitat or biological measures. Thus, upland land uses or larger riparian areas may need to be considered in some cases to buffer streams from deleterious land use effects (21, 50). In a second stage of this risk assessment, we are investigating relationships between land uses and mussel species richness or IBI using a variety of riparian and upland buffer dimensions in the context of streamflow or drainage area.

The relatively little variability in biological measures explained by habitat measures suggests that water quality stressors may play a significant role. Unfortunately, we were unable to characterize chemical stressors due to a lack of relevant data. There are only two long-term water quality stations in the entire watershed, both of which are located in the lower part of the watershed. Potential chemicals of concern such as pesticides, coal mining chemicals, and heavy metals were largely unmeasured. Thus, water quality stressors were inferred in this risk assessment based on nearby land use/source activities in association with biological effects

and habitat quality information. However, habitat data were qualitative nominal scores. Our experience suggests that some investigator bias cannot be avoided in using such qualitative assessment protocols. More robust habitat assessment techniques would help reduce this source of uncertainty.

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### Supporting Information Available

Type of data used in the watershed risk assessment including: (1) the metrics or measures comprising the overall fish index of biotic integrity (IBI) used to assess fish community "health" at each location; (2) geographic data (and their spatial resolution) in the geographical information system (GIS) used in watershed analyses; and (3) available biological and instream habitat quality data. Figure showing the observed relationship between fish IBI and native mussel species richness in Copper Creek, a subwatershed of the Clinch and Powell River watershed. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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