STANDARD OPERATING PROCEDURE
BIOLOGICAL MONITORING

STREAM FISH COMMUNITY ASSESSMENT

&

FISH TISSUE

NORTH CAROLINA
DEPARTMENT OF ENVIRONMENT
and NATURAL RESOURCES
Division of Water Quality
Water Quality Section
Environmental Sciences Branch
Biological Assessment Unit

March 14, 2001

NCDENR

Standard Operating Procedures
Stream Fish Community Assessment and Fish Tissue

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NORTH CAROLINA DEPARTMENT OF ENVIRONMENT
AND NATURAL RESOURCES
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This report has been approved for release

(original signed by Jimmie R. Overton)
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Date: March 14, 2001

INTRODUCTION

It is the purpose of this manual to provide details on standard operating procedures of the Biological Assessment Unit of the Division of Water Quality (Division) for the collection and analysis of fish community and fish tissue data. Consistency in data collection and analysis is the cornerstone for evaluating biological integrity. The procedures provided in this manual are a synthesis of widely used methods and methods developed from the experience of personnel within the unit. These methods have been shown to provide repeatable and useful data for water quality evaluation.

This manual will be reviewed regularly and revised as necessary. The prior approved version of this manual was dated January 1997 and contained both fish and benthic macroinvertebrate procedures. In 2001, the decision was made to produce two separate manuals because revision needs for the two programs are different. All current employees and new employees within the unit will be provided with this manual to serve as a guideline of the unit's activities, methods, and procedures. Revisions of this manual will be provided to each employee and it will be the responsibility of the employee to keep his or her manual current.

The standard operating procedures (SOP) and quality control procedures (QC) in this manual will be the basis for all fish community and fish tissue monitoring in the waters of North Carolina, and the subsequent data provided in memoranda and reports prepared by the Biological Assessment Unit. Deviations from these procedures for unusual sampling situations shall be documented in the appropriate report or memorandum.

SAFETY PROGRAM

The Biological Assessment Unit is required to sample throughout North Carolina at times and places where medical facilities may not be readily available. It is imperative that all employees are instructed in and follow safety precautions when using sampling equipment and hazardous materials. The Environmental Sciences Branch has a Safety Committee which is responsible for maintenance and development of current safety procedures. The Committee also maintains the safety standard operating procedures document which all personnel should be familiar. In addition, all personnel involved in electrofishing activities should be trained in First Aid and CPR and should be familiar with standard electrofishing safety procedures.

Sampling conditions are the primary safety factor to be considered for field work. If any field conditions, such as high flows or thunderstorms, raise the question of whether a sample can be safely collected, then decisions should always be made with the safety of personnel of prime concern. This same concern for safety of staff must be of primary importance when scheduling the amount of time to be spent in the field. Long days combined with strenuous effort increase the probability of accidents occurring. "**Safety first**" must always be the rule.

Employees should promptly report on-the-job accidents to their supervisor. If an accident occurs during field operations, the first responsibility of the team leader is to get first aid treatment for the injured employee; their second responsibility is to promptly notify their supervisor. The Safety Committee maintains a written record of accidents.

STUDY PLANS

All investigations conducted by the Biological Assessment Unit will follow a written study plan including but not limited to the:

- **Introduction** - Will identify the nature and history of the area being investigated and the person or agency requesting the study.

- **Objectives** - The purpose of the investigation and expected accomplishments.

- **Sampling Location Selection** - Locating sampling points is of extreme importance in the initiation of fish community and fish tissue monitoring. The variables in watersheds are many and should be considered in as much detail as possible before sites are selected to monitor any body of water. Land use (*i.e*., urban, rural, forested, agricultural, and industrial) should be considered when locating sample sites, because man-made activities significantly affect the amount of sedimentation, nutrients, and organic or inorganic compounds entering a given segment of a river, lake, or stream. The location of permitted dischargers should be reviewed, using the database provided by the Division's NPDES Unit. Discussion of the proposed study with regional office personnel can also provide additional information useful for determining sampling locations. Pre-study planning of this nature will enhance data interpretation once collections and analyses begin.

- **Methods** - Sampling techniques should be listed with reference to those described in this manual. Any deviation from these standard methods must be noted and described.

- **Analytical Requirements** - All water chemistry and quality parameters to be collected, and analyses that will be required, should be noted.

- **Logistics** - Shall include estimates of manpower requirements, equipment needed, time requirements, methods of sample transport to laboratories, *etc*. The study plan must be submitted and approved by the employee's supervisor prior to conducting the investigation.

A study is complete when a written memorandum is sent to and approved by the appropriate level of management (typically the Environmental Sciences Branch head) within the Division. Each memorandum should contain these sections: an **Introduction or Background**, **Sampling Sites**, **Methods**, **Results and Discussion,** and **Summary or Recommendations**. Any figures, maps, and photographs needed to allow a reader to easily locate the sampling sites should also be included. When the report or memorandum is approved, a Biological Assessment Unit file number is assigned. Finally, the report or memorandum is filed in a Projects File that is organized by basin and subbasin.

STREAM FISH COMMUNITY ASSESSMENT

The North Carolina Division of Water Quality has been monitoring the biological integrity of stream fish communities since the early 1990s. The biological monitoring tool that is used is referred to as the North Carolina Index of Biological Integrity (NCIBI). The NCIBI is a modification of the Index of Biotic Integrity (IBI) initially proposed by Karr (1981) and Karr, *et al*. (1986). The IBI method was developed for assessing a stream's biological integrity by examining the structure and health of its fish community. The scores derived from this index are a measure of the ecological health of the waterbody and may not directly correlate to water quality. For example, a stream with excellent water quality, but with poor or fair fish habitat, may not be rated excellent with this index. However, a stream which rated excellent on the NCIBI should be expected to have excellent water quality.

The North Carolina Index of Biological Integrity incorporates information about species richness and composition, trophic composition, fish abundance, and fish condition. The NCIBI summarizes the effects of all classes of factors influencing aquatic faunal communities such as water quality, energy source, habitat quality, flow regime, and biotic interactions. While any change in a fish community can be caused by many factors, certain aspects of the community are generally more responsive to specific influences. Species composition measurements reflect habitat quality effects. Information on trophic composition reflects the effect of biotic interactions and energy supply. Fish abundance and condition information indicates additional water quality effects. It should be noted, however, that these responses may overlap. For example, a change in fish abundance may be due to decreased energy supply or a decline in habitat quality, not necessarily a change in water quality.

Application of the NCIBI

The NCIBI is continually being refined for greater applicability to wadeable streams in North Carolina. Currently, the NCIBI is applicable **only** to **streams** that are **wadeable** from one shoreline across to the other and for a distance of 600 feet. The NICIBI is only applicable to wadeable streams in the Western and Northern Mountains (French Broad, Hiwassee, Little Tennessee, New, and Watauga River basins), the Inner Piedmont, Foothills, and Eastern Mountains (Broad, Catawba, Savannah, and Yadkin (exclusive of the Sandhills) River basins); and the Outer Piedmont (Cape Fear, Neuse, Roanoke, and Tar River basins).

The delineations of the mountains, piedmont, and sandhills in these river basins are based upon a North Carolina State University Co-operative Extension Service map (*North Carolina Watersheds* by J. Fels published in 1997) (Figure 1). More specifically, the Outer Piedmont includes:

- Cape Fear River Basin -- except for the streams draining the Sandhills in Moore, Lee, and Harnett counties, the entire basin upstream of Lillington, NC;
- Neuse River Basin -- the entire basin above Smithfield and Wilson, NC, except for the south and southwest portions of Johnston County and the eastern two-thirds of Wilson County;
- Roanoke River Basin -- the entire basin in North Carolina upstream of Roanoke Rapids, NC and a small area between Roanoke Rapids and Halifax, NC; and
- Tar River Basin -- the entire basin above Rocky Mount, NC, except for the lower southeastern one-half of Halifax County and the extreme eastern portion of Nash County.

The Index is undergoing revisions for the Upper Coastal Plain (Chowan, Neuse, Pasquotank, Roanoke, Tar, and White Oak River basins), the Lower Coastal Plain (Cape Fear and Lumber River basins), and the Sandhills (Cape Fear, Lumber, and Yadkin River basins).

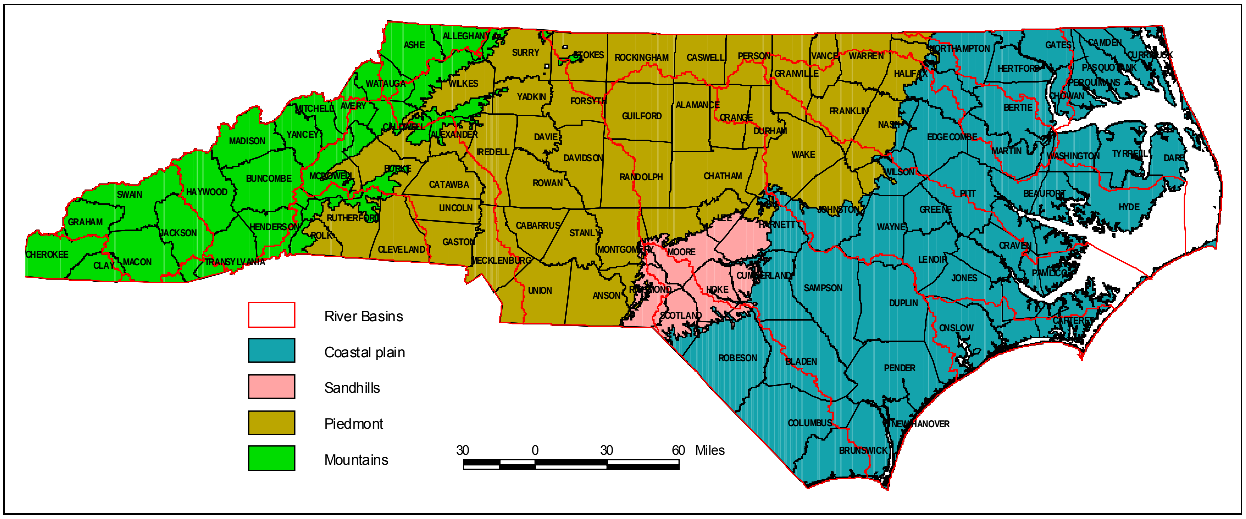


Figure 1. **Physiographic regions and river basins in North Carolina.**

NCIBI Qualifiers

The North Carolina Index of Biological Integrity is only applicable if the methods of collection and data analysis are strictly followed. The Index has not been tested using other collection techniques. Nonwadeable streams and larger rivers that must be sampled with a boat are not currently evaluated with the NCIBI. Neither are high elevation, cold water trout streams. Finally, young-of-year fish are excluded from all NCIBI calculations.

NCIBI Analysis

The assessment of biological integrity using the NCIBI is provided by the cumulative assessment of 12 parameters or metrics (Table 1-3). The values provided by the metrics are converted into scores on a 1, 3, or 5 scale. A score of 5 represents conditions which would be expected for undisturbed or minimally disturbed reference streams in the specific river basin or ecoregion, while a score of 1 indicates that the conditions deviate greatly from those expected in undisturbed or minimally disturbed reference streams of the region. All metrics for each of the three regions were calibrated using regional reference sites.

These 12 metrics are grouped into five categories with each metric designed to contribute unique information to the overall assessment:

1. Species richness and composition (Metric Nos. 1and 3 - 5)
2. Indicator species (Metric Nos. 6 and 7)
3. Trophic function (Metric Nos. 8 - 10)
4. Abundance and condition (Metric Nos. 2 and 11)
5. Reproductive function (Metric No. 12)

Species Composition, Indicator Species, and Trophic Function Assignments
Eight of the metrics involve species composition, pollution tolerance, and trophic composition. Table 4 lists, phylogenetically, the pollution tolerance ratings and trophic guild assignments of the freshwater fish found throughout North Carolina. Many of the species (for example, paddlefish, bigmouth buffalo, and white perch) will not be encountered in streams that are sampled adhering to these procedures. Estuarine species, extirpated species, and species found in nearby drainages of bordering states (but not in North Carolina) are not included. Revisions and updates to this table will be published periodically.

SPECIES RICHNESS AND COMPOSITION (METRIC NOS. 1 AND 3 - 5)
Distributional data for these four metrics were obtained from Menhinick (1991), Lee, *et al*. (1980), Biological Assessment Unit studies, North Carolina State Museum of Natural Sciences, Tennessee Valley Authority, and many other sources.

Metric No. 1. Number of Species
The total number of species supported by a stream of a given size in a given region decreases with environmental degradation. In addition, some streams with larger watersheds or drainage areas can be expected to support more species than streams with smaller watersheds. In other instances, the number of species and the watershed size are not correlated. This metric is rated according to the river basin from which the sample was taken and, in the case of the Inner Piedmont, Foothills, and Eastern Mountains region, the drainage area size at the sampling point. Drainage area size is calculated from USGS 7.5 minute series topographic maps, if not otherwise known (ambient database, USGS publications, or a USGS masterfile printout which gives drainage areas for many streams at given road crossings). This metric is a count of all the species in the sample.

Metric No. 3. Number of Species of Darters
Darters are sensitive to environmental degradation particularly as a result of their specific reproductive and habitat requirements (Page 1983, Kuehne and Barbour 1983). Darter habitats are degraded as a result of channelization, siltation, and reduced oxygen levels. The collection of fewer than the expected number of species of darters can indicate that some degree of habitat degradation is occurring. This metric is a count of all the species of *Etheostoma* and *Percina* in the sample (Table 4).

As with Metric No. 1, the total number of species of darters supported by a stream of a given size in a given region decreases with environmental degradation. In addition, some streams with larger watersheds or drainage areas can be expected to support more species than streams with smaller watersheds. In other instances, the number of species and the watershed size are not correlated. This metric is rated according to the river basin from which the sample was taken and, in the case of the Inner Piedmont, Foothills, and Eastern Mountains region, the drainage area size at the sampling point.

Metric No. 4. Number of Species of Rockbass, Smallmouth Bass, and Trout (Western and Northern Mountains)
Rock bass, smallmouth bass, and the three species of trout are particularly responsive to habitat degradation such as the filling in of pools with sediment and the loss of instream cover. This metric is a count of these five species in the sample. Stocked trout (characterized by pale colors and worn or deformed fins) are not counted.

Metric No. 4 Number of Species of Sunfish, Bass, and Trout (Inner Piedmont, Foothills, and Eastern Mountains)
Sunfish, black bass, and trout species are particularly responsive to habitat degradation such as the filling in of pools with sediment and the loss of instream cover. This metric includes *Lepomis* (all species), *Centrarchus macropterus*, *Ambloplites rupestris*, *Micropterus* (all species), and all three species of trout (Table 4). Stocked trout (characterized by pale colors and worn or deformed fins) are not counted.

Metric No. 4 Number of Species of Sunfish (Outer Piedmont)
Sunfish species are particularly responsive to habitat degradation such as the filling in of pools with sediment and the loss of instream cover. This metric includes *Lepomis* (all species), *Enneacanthus* (all species), *Centrarchus macropterus*, *Acantharchus pomotis*, and *Ambloplites cavifrons* (Table 4).

Metric No. 5 Number of Species of Cyprinids(Western and Northern Mountains)
Many species of minnows are intolerant of habitat and chemical degradation and, because some of the species may have life spans up to six years, provide a multiyear integrated perspective. They also reflect the condition of the benthic community which may be harmed by sedimentation or by sediment contamination. In the Western and Northern Mountains, the Number of Species of Cyprinds (Minnows) is used as a substitute metric for the number of species of suckers. This metric is a count of all the species within the family Cyprinidae in the sample (Table 4).

Metric No. 5. Number of Species of Suckers (Inner Piedmont, Foothills, and Eastern Mountains and Outer Piedmont)

Many species of suckers are intolerant of habitat and chemical degradation and, because they are long lived, provide a multiyear integrated perspective. They also reflect the condition of the benthic community which may be harmed by sedimentation or by sediment contamination. This metric is a count of all the species within the family Catostomidae in the sample (Table 4).

INDICATOR SPECIES (METRIC NOS. 6 AND 7)

The tolerance ratings for these two metrics were derived from Karr, *et al*. (1986), Saylor and Scott (1987), from polling various university, federal, and state fisheries management personnel using the Delphi Technique (Zuboy 1981), Etnier and Starnes (1993), Jenkins and Burkhead (1993), Rohde, *et al*. (1994), and from Biological Assessment Unit data.

Metric No. 6 Number of Intolerant Species

Intolerant species are those which are most affected by environmental perturbations and therefore should disappear, at least as viable populations, by the time a stream is rated as "Fair". Intolerant species also includes some species that have a very restricted zoogeographic distribution or are considered rare, endangered, or threatened. Of the approximately 212 species of freshwater fish found in North Carolina, 52 species are considered intolerant. This metric is a count of all intolerant species in the sample (Tables 4 and 5).

Metric No. 7 Percentage of Tolerant Individuals

Tolerant species are those which are often present in a stream in low or moderate numbers but as the stream degrades, they can become dominant. Of the approximately 211 species of freshwater fish found in North Carolina, 22 species are considered tolerant. This metric is a percentage metric. The number of individuals of the tolerant species (Tables 4 and 5) is summed and divided by the total number of fish collected to obtain the percentage of tolerant fish in the sample.

TROPHIC FUNCTION (METRIC NOS. 8 - 10)

These three trophic composition metrics are used to measure the divergence from expected production and consumption patterns in the fish community that can result from environmental degradation. The main cause for a shift in the trophic composition of the fish community, generally a greater proportion of omnivores and lesser proportion of insectivores than what is expected, is nutrient enrichment. However, in some instances, the percentage of insectivores, especially redbreast sunfish *Lepomis auritus*, may increase dramatically due to environmental degradation and nutrient enrichment. And where the herbivorous central stoneroller *Campostoma anomalum* is found, canopy removal, riparian alteration, and nutrient enrichment may lead to its dramatic increase.

The trophic guild data for these three metrics were derived from the literature (Lee, *et al*. (1980), Karr, *et al*. (1986), Plafkin *et al*. (1989), Etnier and Starnes (1993), Jenkins and Burkhead (1993), Rohde, *et al*. (1994)), and from Biological Assessment Unit data.

Metric No. 8 Percentage of Omnivorous + Herbivorous Individuals

This metric is a percentage metric. The number of individuals of omnivores and herbivores (Table 4) is summed and divided by the total number of fish collected.

Metric No. 9 Percentage of Insectivores

The number of individuals of insectivores (Table 4) is summed and divided by the total number of fish collected.

Metric No. 10 Percentage of Piscivores

The number of individuals of piscivores (Table 4) is summed and divided by the total number of fish collected.

This metric was not used in the Western and Northern Mountains region because the metric failed to discriminate between the impaired and the reference sites and was not significantly correlated with the total NCIBI score. No substitute or alternative metrics were found suitable.

ABUNDANCE AND CONDITION (METRIC NOS. 2 AND 11)

Metric No. 2 Number of Fish

The total number of fish supported by a stream of a given size in a given region decreases with environmental degradation. However, in some instances, nutrient enrichment or environmental degradation may actually increase the number of fish supported by the stream. This metric is a count of all the fish in the sample.

Metric No. 11 The Percentage of Diseased Fish

This metric occurs infrequently, and in most instances, is absent entirely. The metric does occur below point sources and in areas where toxic chemicals are concentrated (e.g., Sanders, *et al.* 1999). This metric is: "*an excellent measure of the aesthetic value of game and nongame fish*" (Barbour, *et al.* 1999).

DELT (Disease, fin Erosion, Lesions, and Tumors) may not be observed in streams the size of which are typically sampled because the worst (urban and industrial) streams are not often sampled and neither are the larger streams and rivers where NPDES dischargers are typically sited and which have a greater DELT rate than the smaller streams. Generally, North Carolina fish are healthy.

To rate this metric, the number of fish in the sample which have sores, lesions, skeletal anomalies (as evident externally), or diseased, damaged, or rotten fins is summed and divided by total number of fish collected to obtain the percentage of diseased fish. Fin or other external damage as a result of spawning should not be counted. Fish are considered to be in spawning condition when tubercles or breeding colors are evident.

This metric was not used in the Western and Northern Mountains region because the metric failed to discriminate between the impaired and the reference sites and was not significantly correlated with the total NCIBI score. No substitute or alternative metrics were found suitable.

REPRODUCTIVE FUNCTION (METRIC NO. 12)

Metric No. 12 Percentage of Species with Multiple Age Groups

This metric was developed by the Division in 1989 as an indicator of the suitability of the habitat for reproduction. Other researchers have used proportion of individuals as hybrids, proportion of individuals as introduced species, simple lithophils (species of fish that spawn where the egg can develop in the interstices of sand, gravel, and cobble substrates without parental care), and number of simple lithophils (Barbour, *et al*. 1999). This metric is strongly influenced by rare species (species represented by 1 or 2 fish) that are not reproducing in the stream. A community may be diverse but if a large proportion of the species are represented by only 1 or 2 fish per species, these rarer species may depress the metric value.

For each species, the total length distribution data are used to determine the presence of different age groups and, thus, the degree of reproductive success. This metric is calculated by first counting the total number of species present in the sample. Then, the total lengths of all the fish of each species are examined to determine whether or not all the fish of that species are of one or multiple age groups. Finally, the percentage of species with multiple age groups is determined by dividing the number of species with multiple age groups by the total number of species collected in the sample. Although some species are rare and some species have fewer age groups than others, at least three individuals per species must have been collected to determine the presence of multiple age groups within the population. In some instances, professional judgment may also be used to determine the reproductive success of a particular species.

Publications such as Carlander (1969 and 1977), Kuehne and Barbour (1983), Page (1983), Manooch (1984), Etnier and Starnes (1993), Jenkins and Burkhead (1993), and Rohde *et al*. (1994) may also be consulted to determine length-age class relationships.

Table 1. **Scoring criteria for the NCIBI for wadeable streams in the Western and Northern Mountains of the French Broad (including the Pigeon River), Hiwassee, Little Tennessee, New, and Watauga River basins with watersheds ranging between 3.1 and 161 mi^2.**

No.	Metric		Score
1	**No. of species**		
	≥ 16 species		5
	12-15 species		3
	< 12 species		1
2	**No. of fish**		
	320-1,000 fish		5
	205-319 fish		3
	< 205 fish		1
	> 1,000 fish		3
3	**No. of species of darters**		
	French Broad & Little Tennessee River Basins	New River, Pigeon River, Watauga[1], & Hiwassee River Basins	
	≥ 4 species	≥ 3 species	5
	2 or 3 species	1 or 2 species	3
	0 or 1 species	0 species	1
4	**No. of species of rock bass, smallmouth bass, and trout**		
	≥ 2 species		5
	1 species		3
	0 species		1
5	**No. of species of cyprinids**		
	All basins, except Pigeon River Basin	Pigeon River Basin	
	≥ 8 species	≥ 6 species	5
	6 or 7 species	4 or 5 species	3
	≤ 5 species	≤ 3	1
6	**No. of intolerant species**		
	All basins, except New River Basin	New River Basin	
	≥ 3 species	≥ 5 species	5
	2 species	3 or 4 species	3
	0 or 1 species	0, 1, or 2 species	1
7	**Percentage of tolerant individuals**		
	≤ 2%		5
	2-10%		3
	> 10%		1
8	**Percentage of omnivorous + herbivorous individuals**		
	10-36%		5
	37-50%		3
	> 50%		1
	< 10%		1
9	**Percentage of insectivorous individuals**		
	55-85%		5
	40-54%		3
	< 40%		1
	> 85%		1
12	**Percentage of species with multiple age groups**		
	≥ 65% of all species have multiple age groups		5
	45-64% all species have multiple age groups		3
	< 45% all species have multiple age groups		1

[1]Tentative for the Watauga River basin; also includes *Cottus bairdi* (mottled sculpin) and *Noturus insignis* (margined madtom). The Watauga River basin is the only basin in North Carolina where these three benthic, insectivorous groups (darters, mottled sculpin, and margined madtom) are sympatric.

Table 2. Scoring criteria for the NCIBI for wadeable streams in the Inner Piedmont, Foothills, and Eastern Mountains of the Broad, Catawba, Savannah, and Yadkin River basins with watershed drainage areas ranging between 2.8 and 245 mi^2.

No.	Metric		Score
1	**No. of species**		
	where Y is the number of species in the sample and X is the stream's drainage area in mi^2:		
	$Y \geq 9.5*Log_{10}X+1.6$		5
	$4.8*Log_{10}X+0.8 \leq Y < 9.5*Log_{10}X+1.6$		3
	$Y < 4.8*Log_{10}X+0.8$		1
2	**No. of fish**		
	Mountains	Piedmont	
	≥ 300 fish	≥ 150 fish	5
	200-299 fish	100-149 fish	3
	< 200 fish	< 100 fish	1
3	**No. of species of darters**		
	where Y is the number of species of darters in the sample and X is the stream's drainage area in mi^2.		
	$Y \geq 1.6*Log_{10}X$		5
	$0.8*Log_{10}X \leq Y < 1.6*Log_{10}X$		3
	$Y < 0.8*Log_{10}X$		1
	If the drainage area is > 70 mi^2, then ≥ 3 species = 5, 2 species = 3, and 0 or 1 species = 1		
4	**No. of species of sunfish, bass, and trout**		
	≥ 3 species		5
	2 species		3
	0 or 1 species		1
5	**No. of species of suckers**		
	≥ 2 species		5
	1 species		3
	0 species		1
6	**No. of intolerant species**		
	Mountains	Piedmont	
	≥ 3 species	≥ 1 species	5
	1or 2 species	(no middle criteria or score)	3
	0 species	0 species	1
7	**Percentage of tolerant individuals**		
	Mountains	Piedmont	
	≤ 12%	≤ 25%	5
	13-25%	26-35%	3
	> 25%	> 35%	1
8	**Percentage of omnivorous + herbivorous individuals**		
	10-35%		5
	36-50%		3
	> 50%		1
	< 10%		1
9	**Percentage of insectivorous individuals**		
	60-90%		5
	45-59%		3
	< 45%		1
	> 90%		1
10	**Percentage of piscivorous individuals**		
	≥ 1.0%		5
	0.25-1.0%		3
	< 0.24%		1
11	**Percentage of diseased fish (DELT = diseased, fin erosion, lesions, and tumors)**		
	< 0.75%		5
	0.76-1.25%		3
	> 1.25%		1
12	**Percentage of species with multiple age groups**		
	Mountains	Piedmont	
	≥ 65% of all species have multiple age groups	≥ 55% of all species have multiple age groups	5
	45-64% all species have multiple age groups	35-54% all species have multiple age groups	3
	< 45% all species have multiple age groups	< 35% all species have multiple age groups	1

Table 3. Scoring criteria for the NCIBI for wadeable streams in the Outer Piedmont of the Cape Fear, Neuse, Roanoke, and Tar River basins ranging between 3.1 and 328 mi^2.

No.	Metric		Score
1	**No. of species**		
	≥ 16 species		5
	10-15 species		3
	< 10 species		1
2	**No. of fish**		
	≥ 225 fish		5
	150-224 fish		3
	< 150 fish		1
3	**No. of species of darters**		
	Cape Fear	Neuse, Roanoke, and Tar	
	≥ 2 species	≥ 3 species	5
	1 species	1 or 2 species	3
	0 species	0 species	1
4	**No. of species of sunfish**		
	≥ 4 species		5
	3 species		3
	0, 1, or 2 species		1
5	**No. of species of suckers**		
	Cape Fear	Neuse, Roanoke, and Tar	
	≥ 2 species	≥ 3 species	5
	1 species	1 or 2 species	3
	0 species	0 species	1
6	**No. of intolerant species**		
	Cape Fear	Neuse, Roanoke, and Tar	
	≥ 1 species	≥ 3 species	5
	no middle score	1 or 2 species	3
	0 species	0 species	1
7	**Percentage of tolerant individuals**		
	≤ 35%		5
	36-50%		3
	> 50%		1
8	**Percentage of omnivorous and herbivorous individuals**		
	10-35%		5
	36-50%		3
	> 50%		1
	< 10%		1
9	**Percentage of insectivorous individuals**		
	65-90%		5
	45-64%		3
	< 45%		1
	> 90%		1
10	**Percentage of piscivorous individuals**		
	≥ 1.4-15%		5
	0.4-1.3%		3
	< 0.4%		1
	> 15%		1
11	**Percentage of diseased fish (DELT = diseased, fin erosion, lesions, and tumors)**		
	≤ 1.75%		5
	1.76-2.75%		3
	> 2.75%		1
12	**Percentage of species with multiple age groups**		
	≥ 50% of all species have multiple age groups		5
	35-49% all species have multiple age groups		3
	< 35% all species have multiple age groups		1

Table 4. North Carolina freshwater fishes tolerance ratings, adult trophic guild assignments, and young-of-year (YOY) cut-off lengths (total length in millimeters).

Family/ Species	Common Name	Tolerance Rating	Trophic Guild of Adults	YOY (< TL, mm)
Petromyzontidae	**Lampreys**			
Ichthyomyzon bdellium	Ohio lamprey	Intermediate	Parasitic	50
I. castaneus	Chestnut lamprey	Intermediate	Parasitic	
I. greeleyi	Mountain brook lamprey	Intermediate	Non-feeding	40
Lampetra aepyptera	Least brook lamprey	Intermediate	Non-feeding	50
L. appendix	American brook lamprey	Intermediate	Non-feeding	40
Petromyzon marinus	Sea lamprey	Intermediate	Parasitic	100
Acipenseridae	**Sturgeons**			
Acipenser brevirostrum	Shortnose sturgeon	Intermediate	Insectivore	200
A. oxyrinchus	Atlantic sturgeon	Intermediate	Insectivore	200
Polyodontidae	**Paddlefishes**			
Polyodon spathula	Paddlefish	Intermediate	Planktivore	200
Lepisosteidae	**Gars**			
Lepisosteus osseus	Longnose gar	Tolerant	Piscivore	200
Amiidae	**Bowfins**			
Amia calva	Bowfin	Tolerant	Piscivore	200
Anguillidae	**Eels**			
Anguilla rostrata	American eel	Intermediate	Piscivore	100
Clupeidae	**Herrings and shads**			
Alosa aestivalis	Blueback herring	Intermediate	Insectivore	100
A. mediocris	Hickory shad	Intermediate	Insectivore	100
A. pseudoharengus	Alewife	Intermediate	Insectivore	50
A. sapidissima	American shad	Intermediate	Insectivore	100
Dorosoma cepedianum	Gizzard shad	Intermediate	Omnivore	100
D. petenense	Threadfin shad	Intermediate	Omnivore	100
Hiodontidae	**Mooneyes**			
Hiodon tergisus	Mooneye	Intermediate	Insectivore	100
Salmonidae	**Trouts and Chars**			
Oncorhynchus mykiss	Rainbow trout	Intolerant	Insectivore	100
Salmo trutta	Brown trout	Intermediate	Piscivore	100
Salvelinus fontinalis	Brook trout	Intolerant	Insectivore	100
Umbridae	**Mudminows**			
Umbra pygmaea	Eastern mudminnow	Intermediate	Insectivore	50
Esocidae	**Pikes**			
Esox americanus americanus	Redfin pickerel	Intermediate	Piscivore	100
E. masquinongy	Muskellunge	Intermediate	Piscivore	200
E. niger	Chain pickerel	Intermediate	Piscivore	100
Cyprinidae	**Minnows**			
Campostoma anomalum	Stoneroller	Intermediate	Herbivore	60
Carassius auratus	Goldfish	Tolerant	Omnivore	50
Clinostomus funduloides	Rosyside dace	Intermediate	Insectivore	40
Ctenopharyngodon idella	Grass carp	Tolerant	Herbivore	200
Cyprinella analostana	Satinfin shiner	Tolerant	Insectivore	40
C. chloristia	Greenfin shiner	Intermediate	Insectivore	40
C. galactura	Whitetail shiner	Intermediate	Insectivore	50
C. labrosa	Thicklip chub	Intolerant	Insectivore	40
C. lutrensis	Red shiner	Tolerant	Insectivore	30
C. nivea	Whitefin shiner	Intermediate	Insectivore	40
C. pyrrhomelas	Fieryblack shiner	Intolerant	Insectivore	40
C. spiloptera	Spotfin shiner	Intermediate	Insectivore	40
C. zanema	Thinlip chub	Intolerant	Insectivore	40

Table 4. (continued).

Family/ Species	Common Name	Tolerance Rating	Trophic Guild of Adults	YOY (< TL, mm)
Cyprinus carpio	Common carp	Tolerant	Omnivore	150
Erimonax monachus	Spotfin chub	Intolerant	Insectivore	40
Erimystax insignis	Mountain blotched chub	Intermediate	Omnivore	40
Exoglossum laurae	Tonguetied minnow	Intolerant	Insectivore	50
E. maxillingua	Cutlips minnow	Intolerant	Insectivore	50
Hybognathus regius	Silvery minnow	Intermediate	Herbivore	50
Hybopsis amblops	Bigeye chub	Intermediate	Insectivore	50
H. hypsinotus	Highback chub	Intolerant	Insectivore	40
H. rubifrons	Rosyface chub	Intolerant	Insectivore	50
Luxilus albeolus	White shiner	Intermediate	Insectivore	50
L. cerasinus	Crescent shiner	Intermediate	Insectivore	50
L. chrysocephalus	Striped shiner	Intermediate	Omnivore	50
L. coccogenis	Warpaint shiner	Intermediate	Insectivore	50
Lythrurus ardens	Rosefin shiner	Intermediate	Insectivore	50
L. matutinus	Pinewoods shiner	Intolerant	Insectivore	50
Nocomis leptocephalus	Bluehead chub	Intermediate	Omnivore	50
N. micropogon	River chub	Intermediate	Omnivore	50
N. platyrhynchus	Bigmouth chub	Intermediate	Omnivore	50
N. raneyi	Bull chub	Intermediate	Omnivore	50
Notemigonus crysoleucas	Golden shiner	Tolerant	Omnivore	75
Notropis alborus	Whitemouth shiner	Intermediate	Insectivore	40
N. altipinnis	Highfin shiner	Intermediate	Insectivore	40
N. amoenus	Comely shiner	Intermediate	Insectivore	50
N. bifrenatus	Bridle shiner	Intermediate	Omnivore	40
N. chalybaeus	Ironcolor shiner	Intolerant	Insectivore	40
N. chiliticus	Redlip shiner	Intermediate	Insectivore	40
N. chlorocephalus	Greenhead shiner	Intermediate	Insectivore	40
N. sp. cf. *chlorocephalus*	Piedmont shiner	Intermediate	Insectivore	40
N. cummingsae	Dusky shiner	Intermediate	Insectivore	40
N. hudsonius	Spottail shiner	Intermediate	Omnivore	50
N. leuciodes	Tennessee shiner	Intermediate	Insectivore	50
N. lutipinnis	Yellowfin shiner	Intermediate	Insectivore	40
N. maculatus	Taillight shiner	Intolerant	Insectivore	40
N. mekistocholas	Cape fear shiner	Intermediate	Omnivore	40
N. petersoni	Coastal shiner	Intermediate	Insectivore	40
N. photogenis	Silver shiner	Intolerant	Insectivore	50
N. procne	Swallowtail shiner	Intermediate	Insectivore	40
N. rubellus micropteryx	Redface shiner	Intolerant	Insectivore	40
N. rubellus rubellus	Rosyface shiner	Intolerant	Insectivore	40
N. rubricroceus	Saffron shiner	Intermediate	Insectivore	40
N. scabriceps	New River shiner	Intolerant	Insectivore	40
N. scepticus	Sandbar shiner	Intermediate	Insectivore	40
N. spectrunculus	Mirror shiner	Intermediate	Insectivore	50
N. telescopus	Telescope shiner	Intolerant	Insectivore	50
N. volucellus	Mimic shiner	Intolerant	Insectivore	40
Phenacobius crassilabrum	Fatlips minnow	Intermediate	Insectivore	50
P. teretulus	Kanawha minnow	Intolerant	Insectivore	50
Phoxinus oreas	Mountain redbelly dace	Intermediate	Herbivore	40
Pimephales notatus	Bluntnose minnow	Tolerant	Omnivore	30
P. promelas	Fathead minnow	Tolerant	Omnivore	30
Rhinichthys atratulus	Blacknose dace	Intermediate	Insectivore	50
R. cataractae	Longnose dace	Intermediate	Insectivore	50
Semotilus atromaculatus	Creek chub	Tolerant	Insectivore	50
S. lumbee	Sandhills chub	Intolerant	Insectivore	40
Catostomidae	**Suckers**			
Carpiodes carpio	River carpsucker	Intermediate	Omnivore	100
C. cyprinus	Quillback	Intermediate	Omnivore	100
C. velifer complex	Highfin carpsucker	Intermediate	Insectivore	100
Catostomus commersoni	White sucker	Tolerant	Omnivore	100
Erimyzon oblongus	Creek chubsucker	Intermediate	Omnivore	100
E. sucetta	Lake chubsucker	Intermediate	Insectivore	100
Hypentelium nigricans	Northern hogsucker	Intermediate	Insectivore	100
H. roanokense	Roanoke hogsucker	Intermediate	Insectivore	100
Ictiobus bubalus	Smallmouth buffalo	Intermediate	Omnivore	100

Table 4. (continued).

Family/ Species	Common Name	Tolerance Rating	Trophic Guild of Adults	YOY (< TL, mm)
I. cyprinellus	Bigmouth buffalo	Intermediate	Insectivore	100
Minytrema melanops	Spotted sucker	Intermediate	Insectivore	100
Moxostoma collapsum	Notchlip redhorse	Intermediate	Insectivore	100
M. carinatum	River redhorse	Intermediate	Insectivore	100
M. duquesnei	Black redhorse	Intermediate	Insectivore	100
M. erythrurum	Golden redhorse	Intermediate	Insectivore	100
M. macrolepidotum	Shorthead redhorse	Intermediate	Insectivore	100
M. pappillosum	V-lip redhorse	Intermediate	Insectivore	100
M. robustum	Robust redhorse	Intolerant	Insectivore	100
M. sp. cf. *erythrurum*	Carolina redhorse	Intermediate	Insectivore	100
M. sp. cf. *macrolepidotum*	Sicklefin redhorse	Intermediate	Insectivore	100
Scartomyzon ariommum	Bigeye jumprock	Intolerant	Insectivore	100
S. cervinum	Black jumprock	Intermediate	Insectivore	75
S. rupiscartes	Striped jumprock	Intermediate	Insectivore	100
S. sp. cf. *lachneri*	Brassy jumprock	Intermediate	Insectivore	100
Thoburnia hamiltoni	Rustyside sucker	Intolerant	Insectivore	
Ictaluridae	**Catfishes**			
Ameiurus brunneus	Snail bullhead	Intermediate	Insectivore	75
A. catus	White catfish	Tolerant	Omnivore	100
A. melas	Black bullhead	Tolerant	Insectivore	75
A. natalis	Yellow bullhead	Tolerant	Omnivore	75
A. nebulosus	Brown bullhead	Tolerant	Omnivore	75
A. platycephalus	Flat bullhead	Tolerant	Insectivore	75
Ictalurus furcatus	Blue catfish	Intermediate	Piscivore	100
I. punctatus	Channel catfish	Intermediate	Omnivore	100
Noturus eleutherus	Mountain madtom	Intermediate	Insectivore	40
N. flavus	Stonecat	Intermediate	Insectivore	40
N. furiosus	Carolina madtom	Intolerant	Insectivore	40
N. gilberti	Orangefin madtom	Intolerant	Insectivore	40
N. gyrinus	Tadpole madtom	Intermediate	Insectivore	40
N. insignis	Margined madtom	Intermediate	Insectivore	40
N. sp. cf. *leptacanthus*	Broadtail madtom	Intolerant	Insectivore	40
Pylodictis olivaris	Flathead catfish	Intermediate	Piscivore	150
Amblyopsidae	**Cavefishes**			
Chologaster cornuta	Swampfish	Intermediate	Insectivore	25
Aphredoderidae	**Pirate perches**			
Aphredoderus sayanus	Pirate perch	Intermediate	Insectivore	50
Fundulidae	**Topminnows**			
Fundulus diaphanus	Banded killifish	Intermediate	Insectivore	40
F. lineolatus	Lined topminnow	Intermediate	Insectivore	40
F. rathbuni	Speckled killifish	Intermediate	Insectivore	40
F. waccamensis	Waccamaw killifish	Intolerant	Insectivore	40
Poeciliidae	**Livebearers**			
Gambusia affinis	Western mosquitofish	Tolerant	Insectivore	20
G. holbrooki	Eastern mosquitofish	Tolerant	Insectivore	20
Atherinidae	**Silversides**			
Labidesthes sicculus	Brook silverside	Intermediate	Insectivore	50
Menidia beryllina	Inland silverside	Intermediate	Insectivore	50
M. extensa	Waccamaw silverside	Intolerant	Insectivore	50
Moronidae	**Temperate basses**			
Morone americana	White perch	Intermediate	Piscivore	75
M. chrysops	White bass	Intermediate	Piscivore	200
M. saxatilis	Striped bass	Intermediate	Piscivore	175
Centrarchidae	**Sunfishes and Black Basses**			
Acantharchus pomotis	Mud sunfish	Intermediate	Insectivore	50
Ambloplites cavifrons	Roanoke bass	Intermediate	Piscivore	50
A. rupestris	Rock bass	Intolerant	Piscivore	50

Table 4. (continued).

Family/ Species	Common Name	Tolerance Rating	Trophic Guild of Adults	YOY (< TL, mm)
Centrarchus macropterus	Flier	Intermediate	Insectivore	50
Enneacanthus chaetodon	Blackbanded sunfish	Intermediate	Insectivore	40
E. gloriosus	Bluespotted sunfish	Intermediate	Insectivore	40
E. obesus	Banded sunfish	Intermediate	Insectivore	40
Lepomis auritus	Redbreast sunfish	Tolerant	Insectivore	50
L. cyanellus	Green sunfish	Tolerant	Insectivore	50
L. gibbosus	Pumpkinseed	Intermediate	Insectivore	50
L. gulosus	Warmouth	Intermediate	Insectivore	50
L. macochirus	Bluegill	Intermediate	Insectivore	50
L. marginatus	Dollar sunfish	Intermediate	Insectivore	50
L. microlophus	Redear sunfish	Intermediate	Insectivore	50
L. punctatus	Spotted sunfish	Intermediate	Insectivore	50
Lepomis sp.	Hybrid sunfish	Tolerant	Insectivore	50
Micropterus coosae	Redeye bass	Intermediate	Piscivore	100
M. dolomieu	Smallmouth bass	Intolerant	Piscivore	100
M. punctulatus	Spotted bass	Intermediate	Piscivore	100
M. salmoides	Largemouth bass	Intermediate	Piscivore	100
Pomoxis annularis	White crappie	Intermediate	Piscivore	75
P. nigromaculatus	Black crappie	Intermediate	Piscivore	75
Elassomatidae	**Pygmy sunfishes**			
Elassoma evergladei	Everglades pygmy sunfish	Intermediate	Insectivore	20
E. zonatum	Banded pygmy sunfish	Intermediate	Insectivore	20
E. boehlkei	Carolina pygmy sunfish	Intolerant	Insectivore	20
Percidae	**Darters and Perches**			
Etheostoma acuticeps	Sharphead darter	Intolerant	Insectivore	
E. blennioides	Greenside darter	Intermediate	Insectivore	40
E. chlorobranchium	Greenfin darter	Intolerant	Insectivore	50
E. collis	Carolina darter	Intermediate	Insectivore	30
E. flabellare	Fantail darter	Intermediate	Insectivore	30
E. fusiforme	Swamp darter	Intermediate	Insectivore	30
E. inscriptum	Turquoise darter	Intolerant	Insectivore	
E. jessiae	Blueside darter	Intolerant	Insectivore	
E. kanawhae	Kanawha darter	Intolerant	Insectivore	40
E. mariae	Pinewoods darter	Intolerant	Insectivore	30
E. nigrum	Johnny darter	Intermediate	Insectivore	30
E. olmstedi	Tessellated darter	Intermediate	Insectivore	40
E. perlongum	Waccamaw darter	Intolerant	Insectivore	30
E. podostemone	Riverweed darter	Intolerant	Insectivore	30
E. rufilineatum	Redline darter	Intermediate	Insectivore	40
E. serrifer	Sawcheek darter	Intolerant	Insectivore	30
E. swannanoa	Swannanoa darter	Intermediate	Insectivore	40
E. thalassinum	Seagreen darter	Intolerant	Insectivore	40
E. vitreum	Glassy darter	Intermediate	Insectivore	30
E. vulneratum	Wounded darter	Intolerant	Insectivore	40
E. zonale	Banded darter	Intermediate	Insectivore	40
Perca flavescens	Yellow perch	Intermediate	Piscivore	80
Percina aurantiaca	Tangerine darter	Intolerant	Insectivore	40
P. burtoni	Blotchside logperch	Intolerant	Insectivore	40
P. caprodes	Logperch	Intermediate	Insectivore	40
P. crassa	Piedmont darter	Intolerant	Insectivore	40
P. evides	Gilt darter	Intolerant	Insectivore	40
P. gymnocephala	Appalachia darter	Intolerant	Insectivore	40
P. oxyrhynchus	Sharpnose darter	Intolerant	Insectivore	40
P. nevisense	Chainback darter	Intolerant	Insectivore	40
P. roanoka	Roanoke darter	Intolerant	Insectivore	30
P. sciera	Dusky darter	Intermediate	Insectivore	40
P. squamata	Olive darter	Intolerant	Insectivore	
Stizostedion canadense	Sauger	Intermediate	Piscivore	
S. vitreum	Walleye	Intermediate	Piscivore	

Table 4. (continued).

Family/ Species	Common Name	Tolerance Rating	Trophic Guild of Adults	YOY (< TL, mm)
Cottidae	**Sculpins**			
Cottus bairdi	Mottled sculpin	Intermediate	Insectivore	50
C. carolinae	Banded sculpin	Intermediate	Insectivore	50
C. caeruleomentum	Blue Ridge sculpin	Intermediate	Insectivore	50
Sciaenidae	**Drums**			
Aplodinotus grunniens	Freshwater drum	Intermediate	Insectivore	

Table 5. Intolerant species of fish found in North Carolina.

Family/ Species	Common Name	Family/ Species	Common Name
Salmonidae	**Trouts and Chars**	**Fundulidae**	**Topminnows**
Oncorhynchus mykiss	Rainbow trout	*Fundulus waccamensis*	Waccamaw killifish
Salvelinus fontinalis	Brook trout		
		Atherinidae	**Silversides**
Cyprinidae	**Minnows**	*Menidia extensa*	Waccamaw silverside
Cyprinella labrosa	Thicklip chub		
C. pyrrhomelas	Fieryblack shiner	**Centrarchidae**	**Sunfishes**
C. zanema	Thinlip chub	*Ambloplites rupestris*	Rock bass
Erimonax monachus	Spotfin chub	*Micropterus dolomieu*	Smallmouth bass
Exoglossum laurae	Tonguetied minnow		
E. maxillingua	Cutlips minnow	**Elassomatidae**	**Pygmy sunfishes**
Hybopsis hypsinotus	Highback chub	*Elassoma boehlkei*	Carolina pygmy sunfish
H. rubifrons	Rosyface chub		
Lythrurus matutinus	Pinewoods shiner	**Percidae**	**Darters and Perches**
Notropis chalybaeus	Ironcolor shiner	*Etheostoma acuticeps*	Sharphead darter
N. maculatus	Taillight shiner	*E. chlorobranchium*	Greenfin darter
N. photogenis	Silver shiner	*E. inscriptum*	Turquoise darter
N. rubellus micropteryx	Redface shiner	*E. jessiae*	Blueside darter
N. rubellus rubellus	Rosyface shiner	*E. kanawhae*	Kanawha darter
N. scabriceps	New River shiner	*E. mariae*	Pinewoods darter
N. telescopus	Telescope shiner	*E. perlongum*	Waccamaw darter
N. volucellus	Mimic shiner	*E. podostemone*	Riverweed darter
Phenacobius teretulus	Kanawha minnow	*E. serrifer*	Sawcheek darter
Semotilus lumbee	Sandhills chub	*E. thalassinum*	Seagreen darter
		E. vulneratum	Wounded darter
Catostomidae	**Suckers**	*Percina aurantiaca*	Tangerine darter
Moxostoma robustum	Robust redhorse	*P. burtoni*	Blotchside logperch
Scartomyzon ariommum	Bigeye jumprock	*P. crassa*	Piedmont darter
Thoburnia hamiltoni	Rustyside sucker	*P. evides*	Gilt darter
		P. gymnocephala	Appalachia darter
Ictaluridae	**Catfishes**	*P. oxyrhynchus*	Sharpnose darter
Noturus furiosus	Carolina madtom	*P. nevisense*	Chainback darter
N. gilberti	Orangefin madtom	*P. roanoka*	Roanoke darter
N. sp. cf. *leptacanthus*	Broadtail madtom	*P. squamata*	Olive darter

Table 6. Tolerant species of fish found in North Carolina.

Family/ Species	Common Name	Family/ Species	Common Name
Lepisosteidae	**Gars**	**Catostomidae**	**Suckers**
Lepisosteus osseus	Longnose gar	*Catostomus commersoni*	White sucker
Amiidae	**Bowfins**	**Ictaluridae**	**Catfishes**
Amia calva	Bowfin	*Ameiurus catus*	White catfish
		A. melas	Black bullhead
Cyprinidae	**Minnows**	*A. natalis*	Yellow bullhead
Carassius auratus	Goldfish	*A. nebulosus*	Brown bullhead
Ctenopharyngodon idella	Grass carp	*A. platycephalus*	Flat bullhead
Cyprinella analostana	Satinfin shiner		
C. lutrensis	Red shiner	**Poeciliidae**	**Livebearers**
Cyprinus carpio	Common carp	*Gambusia affinis*	Western mosquitofish
Notemigonus crysoleucas	Golden shiner	*G. holbrooki*	Eastern mosquitofish
Pimephales notatus	Bluntnose minnow		
P. promelas	Fathead minnow	**Centrarchidae**	**Sunfishes**
Semotilus atromaculatus	Creek chub	*Lepomis auritus*	Redbreast sunfish
		L. cyanellus	Green sunfish
		Lepomis sp.	Hybrid sunfish

INTEGRITY CLASS ASSIGNMENT

The scores for all 10 or 12 metrics are then summed to obtain the overall NCIBI score. Finally, the score (an even number between 12 and 60) is then used to determine the biological integrity class of the stream from which the sample was collected (Table 7).[1]

Table 7. Revised scores and classes for evaluating the fish community of a wadeable stream in select streams using the North Carolina Index of Biological Integrity

River Basin	NCIBI Score	Integrity Class
French Broad, Hiwassee, Little Tennessee, New, and Watauga	58 or 60	Excellent
	48, 50, 52, 54, or 56	Good
	40, 42, 44, or 46	Good-Fair
	34, 36, or 38	Fair
	≤ 32	Poor
Broad, Catawba, Savannah, and Yadkin	54, 56, 58, or -60	Excellent
	48, 50, or 52	Good
	42, 44, or 46	Good-Fair
	36, 38, or 40	Fair
	≤ 34	Poor
Cape Fear, Neuse, Roanoke, and Tar	54, 56, 58, or 60	Excellent
	46, 48, 50, or 52	Good
	40, 42, or 44	Good-Fair
	34, 36, or 38	Fair
	≤ 32	Poor

[1] In the Western and Northern Mountains (French Broad, Hiwassee, Little Tennessee, New, and Watauga River basins), the NCIBI is based upon 10 rather than 12 metrics (Table 1). Using 10 metrics with each metric's criteria scored a 1, 3, or 5 and desiring to keep 60 as the maximum NCIBI Total Score, the total score was multiplied by 1.2 (60/50=1.2). Scores were rounded up or down to the nearest whole even number (e.g., 57.6 rounded up to 58; 50.4 rounded down to 50).

Total Score based upon 10 Metrics before Multiplier	Total Score based upon 10 Metrics after Applying a 1.2 Multiplier	Final Total Score after Rounding (if necessary)
50	60	60
48	57.6	58
46	55.2	56
44	52.8	52
42	50.4	50
40	48	48
38	45.6	46
36	43.2	44
34	40.8	40
32	38.4	38
30	36	36
28	33.6	34
26	31.2	32
24	28.8	28
22	26.4	26
20	24	24
18	21.6	22
16	19.2	20

Using 10 metrics instead of 12 and following the conversions as described, the final Total NCIBI Scores of 54, 42, and 30 are no longer possible. This slight flaw should not affect the usefulness and applicability of the 10 metric NCIBI for the Western and Northern Mountains.

Field Sampling and Laboratory Processing Methods

Fish Collection Licenses and Permits

Collection permits are required to collect fish from North Carolina freshwater ecosystems and must accompany the field staff whenever collections are made. It is the responsibility of the Team Leader to insure that all appropriate permits have been obtained prior to the collection of fish. Permits may be obtained from the North Carolina Wildlife Resources Commission, Division of Boating and Inland Fisheries. An Endangered Species Permit may also be required depending upon the stream being monitored. This license is obtained from the North Carolina Wildlife Resources Commission, Division of Wildlife Management, Nongame and Endangered Species Section.

Site Selection

For wadeable streams, a representative site of approximately 600 ft. is selected. Wadeable streams are those that can be safely waded by the sampling crew while wearing a backpack electrofisher unit and still allow the sampler and netter to reach all areas of the stream with the electrofishing probes and dipnet. The sample site should include all available macro- and microhabitats representative of the stream at the particular road crossing. The site length is measured preferably starting at the access point and proceeding in an upstream direction. If possible, personnel measuring the stream segment should avoid walking in the stream segment to avoid scaring fish out of the sample segment and to minimize habitat disturbance.

Field Variables

A Fish Community Assessment-IBI Data Sheet (Appendix 1) is completed whenever a sample is collected. Data that are collected include: stream name, sample location, county, river basin, subbasin, latitude, longitude, drainage area, sample number, sample date, time, number of shocking units, duration of shocking, and sampling personnel. Physical data that are collected includes specific conductance, dissolved oxygen, temperature, pH, habitat description, average stream width and depth, water clarity, and substrate.

Sample Collection

Essential sampling equipment that should accompany the personnel when sampling are:

County and state maps	Chest waders and rubber gloves
Camera and slide film or digital camera and disks	Measuring boards
Appropriate identification keys and field guides	Data sheets, pens, and pencils
Assorted jars and plastic buckets with lids	Formalin
GPS unit	Tape measure and flagging tape
Dipnets (1/8 in. mesh) and assorted sizes of seines	Identification labels, tags, and rubber bands
Backpack electrofishing units	First aid kit and insect repellent
Electrofishing batteries and chargers	Large fish preservation containers
Probes and replacement rings	Water quality instruments

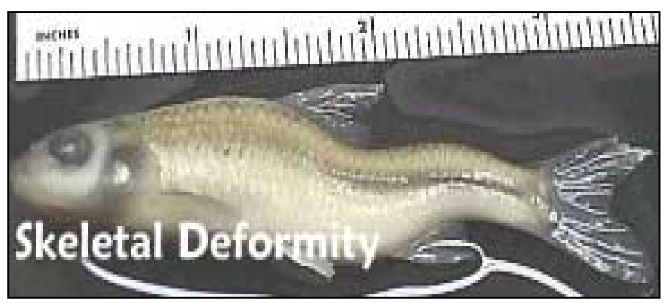
The number of personnel required to efficiently and effectively sample a 600 ft. wadeable section of stream is dictated by the stream's width:

Stream width (m)	No. of electrofishers	No. of netters
≤ 3	1	1
3 to 10	2	2
10 to 15	2 or 3	2 or 3
> 15	3 or 4	3 or 4

Typically, one-half of the sampling crew is outfitted with backpack electrofishing units and the other half with dip nets and buckets. The fish in the delineated stretch of stream are then collected using backpack electrofishing units and persons netting the stunned fish. The entire crew should sample first in an upstream direction and then, after a short break (5-10 minutes to allow the water to clear), proceed back downstream. All micro- and macrohabitats (riffles, pools, runs, snags, undercuts, deadfalls, quiescent leaf-covered substrates, *etc*.) should be thoroughly sampled. Electrofishing downstream into a seine should also occur wherever there are significant riffles. Stunned fish are netted and placed into buckets with water that is frequently changed to minimize stress and mortality.

Details of the backpack electrofisher use and operation are given in the operator's manual and should be read carefully by all staff before using the equipment. Safety concerns require the wearing of chest waders and rubber gloves when the electrofishing unit is in operation.

After collection, all readily identifiable fish are examined for diseases, sores, lesions, fin damage, and skeletal anomalies, measured (total length to the nearest 1 mm), and then released. All data are recorded on the Fish Community Assessment-IBI Data Sheet (Appendix 1). If a species is represented by multiple ages, a "Y" (for yes) is written in the margin of the data sheet across from the species name. If a species is not represented by multiple ages, a "N" (for no) is written. Deformed or diseased fish are also noted on the data sheet by circling the total length measurement of the affected fish. In addition, it is suggested that at least two photographic slides or digital pictures be taken of the site and of any unusually deformed or diseased fish.

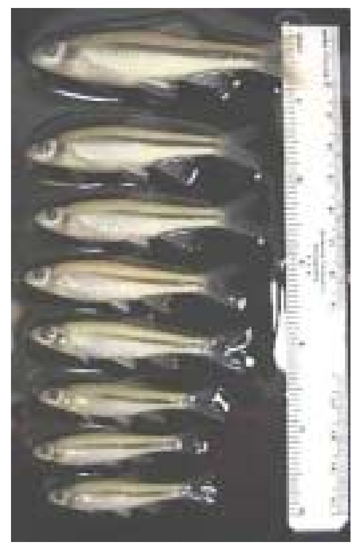


Once the first 50 specimens of a species are measured, the remaining fish of that particular species are just counted and released. All other fish (i.e., those fish that are not readily identifiable) are preserved in 10% formalin and returned to the laboratory for identification, examination, and total length measurement. If large (> 300 mm), unidentifiable fish are retained, the abdominal cavity should be injected with formalin at the time of preservation or as soon as possible before the end of the sampling day. Sample identification (containing waterbody name, road crossing or station, county, date, and collection number) tags are completed and placed inside and attached outside the sample container (plastic bucket or jar).

Laboratory Processing of Fish Samples

After the fish have been properly preserved in formalin (usually 1-2 weeks or until the fish no longer are floating in the preservative), the sample can be processed. The preservative is decanted under a hood (or other means providing appropriate ventilation) and discarded. The sample is rinsed with tap water several times and then allowed to soak in tap water for approximately one hour. The sample is sorted and each fish is identified to the **species** level and its total length measured to the nearest 1 millimeter. All laboratory-derived data are recorded on the Fish Community Assessment-IBI Data Sheet (Appendix 1). Deformed or diseased fish are also noted on the data sheet by circling the total length measurement of the affected fish. If a species is represented by multiple ages, a "Y" (for yes) is written in the margin of the data sheet across from the species name. If a species is not represented by multiple ages, a "N" (for no) is written. Problematic identifications are verified by personnel from the North Carolina State Museum of Natural Science.

Young-of-Year Considerations and Adjustments



Young-of-year (YOY) fish may pose several challenges when applying the IBI metrics to a fish community sample (Angermeier and Karr (1986) and Angermeier and Schlosser (1987). Assessments made during the spring and early summer (April-June) tend to avoid these challenges. However, samples collected later in the summer and fall may contain an abundance of YOY fish. Individuals of a species who spawn in late summer or fall or from a late hatching cohort are not considered YOY when collected the following year (after January 1st) even though such individuals may be noticeably smaller than an earlier hatching cohort.

In some instances, depending upon the mildness of the winter and early spring, YOY fish (for example, redfin pickerel, creek chubsucker, bluegill, and redbreast sunfish), may already be present in samples collected during the spring. Assessments made in mid- to late June require careful attention and sometimes, professional judgement.

Efforts are made to not collect YOY fish, and, if collected, all YOY fish are excluded from all NCIBI calculations. Between July 1 and December 30, when most YOY may be collected, Table 4 should used as a guidance for the determination of YOY cut-off lengths. If a length for a particular species is not listed, best professional judgment or new knowledge of the life history of the species in North Carolina or the Southeast may be used for individuals collected where there may be doubt as to whether or not a fish is a YOY fish.

Components of the QA/QC Plan

One or two experienced fisheries biologists will be responsible for overseeing the collection of all wadeable stream fish community samples. Personnel from the Biological Assessment Unit will provide primary sampling assistance. Other experienced field biologists may be used as needed.

Prior to sampling, a fish species list will be compiled of all the species known or suspected to occur within the basin or stream under study. Such a list is compiled from species distribution maps (Menhinick 1991 and amended with Biological Assessment Unit data and data from other regional fisheries researchers). The list will also show which species may be afforded protection at the federal or state level and which would require field identification and immediate release.

As discussed in the Sample Collection section, as many readily and easily identifiable fish are processed stream-side as possible. A fish whose specific identity is unknown, questionable, or disputed between the fisheries biologists is properly preserved for later laboratory identification.

Examples of a species or a specimen(s) that should be preserved are ones that:

- can not be readily and easily identified in the field;
- are not represented in the Reference Collection (a list of species in the Reference Collection is kept with the Reference Collection in the Fish Laboratory and should be consulted prior to sampling) ;
- are of known taxonomic value (e.g., a poorly understood or undescribed species (such as the Carolina redhorse) or rarely collected size classes of a species);
- represent a new distributional record; or
- may be a hybrid.

Additional suggested guidelines for when to preserve specimens may be found in Walsh and Meador (1998).

Random samples, identified in the laboratory, are re-processed for accurate and correct determinations of identity and presence or absence of multiple age classes. Because of the relatively limited icthyofauna within any specific river basin, the likelihood of misidentifications is not as great as is the case for other taxonomic groups (e.g., benthic invertebrates or phytoplankton). Consequently, each fisheries biologist is required to roll two dice after every 12 samples have been completed. The sample corresponding with the die number is re-identified and processed by another fisheries biologist for verification. Any misidentifications or inaccuracies in multiple age class determinations are resolved between the two biologists. The data sheet from which the sample was chosen for verification is signed and dated by both biologists attesting to the accuracy and completeness of the sample.

A Reference Collection shall be maintained. Except for federally- and state-recognized rare, endangered, or threatened species (Table 8), the Reference Collection should include at least one specimen of every freshwater species found in the state. Species afforded the extra state or federal protection and which were collected accidentally (incidental take) shall be deposited in the North Carolina State Museum of Natural Sciences (NCSMNS). The Reference Collection shall be maintained and utilized for laboratory identifications of problematic species. Comparisons of such specimens or species may also be made to specimens in the NCSMNS. A list of species in the Reference Collection is kept with the Reference Collection in the Fish Laboratory and should updated as needed.

Table 8. Alphabetical listing of the state and federally protected endangered and threatened species (from LeGrand and Hall 1999).

Species	Common Name	State Status	Federal Status
Acipenser brevirostrum	Shortnose Sturgeon	Endangered	Endangered
Aplodinotus grunniens	Freshwater drum	Threatened	
Cottus carolinae	Banded Sculpin	Threatened	
Elassoma boehlkei	Carolina Pygmy Sunfish	Threatened	
Erimonax monachus	Spotfin Chub	Threatened	Threatened
Etheostoma acuticeps	Sharphead Darter	Threatened	
Etheostoma perlongum	Waccamaw Darter	Threatened	
Exoglossum maxillingua	Cutlips Minnow	Endangered	
Hybopsis rubifrons	Rosyface Chub	Threatened	
Lampetra appendix	American Brook Lamprey	Threatened	
Luxilus chrysocephalus	Striped Shiner	Threatened	
Menidia extensa	Waccamaw Silverside	Threatened	
Notropis mekistocholas	Cape Fear Shiner	Endangered	Endangered
Noturus flavus	Stonecat	Endangered	
Noturus gilberti	Orangefin Madtom	Endangered	
Percina burtoni	Blotchside Logperch	Endangered	
Percina caprodes	Logperch	Threatened	
Percina sciera	Dusky Darter	Endangered	
Polyodon spathula	Paddlefish	Endangered	
Thoburnia hamiltoni	Rustyside Sucker	Endangered	

All specimens returned to the laboratory for identification which do not become part of the Reference Collection or of the Teaching Collection (a collection maintained to educate school groups, tours, or citizens at public fair and forums) will be donated to the NCSMNS. The State Ichthyologist (and staff) will serve as the qualified, independent fish taxonomic specialist(s). All specimens are verified for correctness of species identification prior to being incorporated into the NCSMNS Collection. Any misidentifications or other discrepancies by the Division fisheries biologists will be communicated back by the NCSMNS staff.

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Appendix 1.

FISH COMMUNITY ASSESSMENT-IBI DATA SHEET

STREAM	SAMPLE NO.
SAMPLE LOCATION	SAMPLE DATE
COUNTY	TIME
RIVER BASIN	NO. OF SHOCKING UNITS
SUBBASIN	DURATION (sec.)
LATITUDE	SAMPLING PERSONNEL
LONGITUDE	
DRAINAGE AREA (mi.^{2})	

PHYSICAL DATA

SP. CONDUCTANCE (µS/cm)	AVG. STREAM WIDTH (m)
DISSOLVED OXYGEN (mg/L)	AVG. STREAM DEPTH (m)
TEMPERATURE (^{0}C)	WATER CLARITY (clear, cloudy, turbid, blackwater)
pH	SUBSTRATE TYPE (s)
HABITAT DESCRIPTION	

SPECIES COLLECTED

Species	Total No.	Length	Length	Length	Length	Length	Length	Length	Length	Length	Length

Appendix 1 (continued)

PAGE _____ OF _____

FISH COMMUNITY ASSESSMENT-IBI DATA SHEET

STREAM	SAMPLE NO.
SAMPLE LOCATION	SAMPLE DATE

SPECIES COLLECTED

Species	Total No.	Length	Length	Length	Length	Length	Length	Length	Length	Length	Length

FISH TISSUE

Because fish spend their entire lives in the aquatic environment, they incorporate chemicals from this environment into their body tissues. Contamination of aquatic resources have been documented for heavy metals, pesticides, and other complex organic compounds. Once these contaminants reach surface waters, they may be available for bioaccumulation, either directly or through aquatic food webs, and may accumulate in fish and shellfish tissues. Results from fish tissue monitoring can serve as an important indicator of further contamination of sediments and surface water.

This procedure is used by the Division to collect and process fish tissue samples to be analyzed for chemical contaminants. These procedures are based on established guidelines described in USEPA (1995). The procedure does not include procedures used by the Division's Analytical Chemistry Laboratory.

Study Design

A detailed sampling plan should be developed by the primary researcher and approved by the Biological Assessment Unit supervisor prior to initiating any studies. At minimum, a study should involve a two tiered approach:

- Screening, or Tier I studies, should identify sites where commonly consumed fish species are contaminated with target analytes and may pose a risk to human health.
- Intensive, or Tier II, studies should characterize the magnitude and geographical extent of contamination in harvestable fish at sites identified in Tier I studies. Tier II studies should also be designed to verify results of Tier I screening studies.

Further information on study objectives and sampling design may be found in USEPA (1989 and 1995).

Sample Collection

In most cases the Division will employ electrofishing as the primary means of fish collection. Collections on lakes and non-wadeable streams are usually accomplished using a boat mounted electrofisher powered by a 2.5 watt or a 7.5 watt generator. Collections on wadeable streams are accomplished using back pack electrofishing techniques (refer to the Fish Community Assessment Section for details on this method).

During fish tissue sampling, a measured distance is not sampled, rather sampling is conducted until the required number of fish are collected. All personnel involved should be familiar with standard electrofishing operational and safety procedures (Reynolds 1996).

In certain cases electrofishing may not be effective especially when targeting Ictalurids (catfishes) and other benthic species. In these cases, trot lines, traps, or gillnets may be used (Hubert 1996).

Certain studies may require that fish be collected by other agencies or that fish be purchased from commercial fishermen. Division personnel should provide quality control measures necessary to ensure that samples are collected and handled properly with minimal contamination and that sampling sites are verified.

Sample Shipment and Handling

Fish collected for analyses must be shipped to the processing laboratory in such a manner as to prevent decomposition or contamination. Fish should be removed from live wells, holding tanks, or buckets, rinsed with ambient water to remove foreign matter, and placed on a contaminant free surface for sorting. Skins on fish selected for analysis should be examined for breaks or lacerations from sampling gear - a possible source of contamination. Fish samples should be sorted by species before packaging for shipment.

Fish selected for metals analysis are placed by species in polyethylene bags. After removing as much air as possible, the bags are sealed and tagged with the date, time, station name, species, and collector(s).

Fish selected only for organics analyses, including dioxins, are wrapped whole in clean aluminum foil with the dull side of the foil against the skin of the animal. Large spines on any fish should be sheared to minimize puncturing of the foil. Wrapped fish are sorted by species and placed in tagged polyethylene bags as described for metals samples.

Packaged fish are placed immediately on wet ice and chilled to 4^{o}C for transport back to the laboratory. Samples shipped on wet ice should reach the processing laboratory within 24 hours of collection to allow sufficient time for processing. Samples to be filleted should be processed no later than 48 hours after collection.

If samples cannot be processed within this time frame then they should be frozen as whole fish, delivered to the laboratory as soon as possible, and stored at -20 ^{o}C until processing can be performed. **Freezing samples should be avoided whenever possible due to the possibility of rupturing internal organs and contaminating fillet tissue.** If fish are frozen they should not be allowed to thaw during transport. Prior to processing, frozen fish samples should only be partially thawed before filleting (ice crystals should still be visible in the fillet tissue).

Laboratory Processing
Equipment used in processing samples for metals analysis should be made of stainless steel, glass, or plastic. Chromium and nickel contamination can occur from the use of stainless steel. Therefore, if these metals are of concern, other materials should be used during sample processing. Equipment used in processing samples for organics analysis should be made of stainless steel, glass, or anodized aluminum.

Prior to preparing samples, all surfaces in the processing laboratory are washed with a detergent and rinsed with a metal free water (treated by reverse osmosis). Utensils and equipment are cleaned with a detergent and hot tap water, rinsed with tap water, rinsed in a 20% HNO_3solution for metals analysis or rinsed in pesticide grade isopropanol or acetone for organics analysis, rinsed with metal free water, and allowed to air dry completely.

The total length of each fish is determined to the nearest millimeter and the wet weight of each fish is determined to the nearest gram. Fish are weighed on foil lined trays and the foil is changed between each species. All data are recorded on the laboratory data sheet (Appendix 1).

Scaling is performed on cleaned stainless steel or plastic surfaces covered in heavy duty aluminum foil. Separate cutting boards and utensils are used for scaling and skinning to prevent cross contamination of tissues. Fish are scaled prior to filleting using an automatic rotary scaler and rinsed with water filtered *via* reverse osmosis (R.O.) to remove slime and foreign matter. The scaling surfaces are also rinsed between fish to prevent contamination. Scaleless fish (catfish) are skinned prior to filleting.

Filleting is performed on plastic or stainless steel surfaces covered with heavy duty aluminum foil. Aluminum foil is rinsed with R.O. water between fish from the same station and changed completely between stations. Filleting is performed using cleaned bare hands or talc free disposable gloves. Hands or gloves should be rinsed between samples to prevent cross contamination. Fillets are ressected using high grade stainless steel knives cleaned according to the above directions. Knives are rinsed with R.O. water between fish from the same station and recleaned or changed between stations.

Fillets should be ressected according to the general procedure (Figure 1). Fillets should be removed from the lateral area of the fish behind the head and pectoral fin and should include the belly flap. **Care should be taken not to cut into the gut cavity as it may contaminate the fillet tissue.**

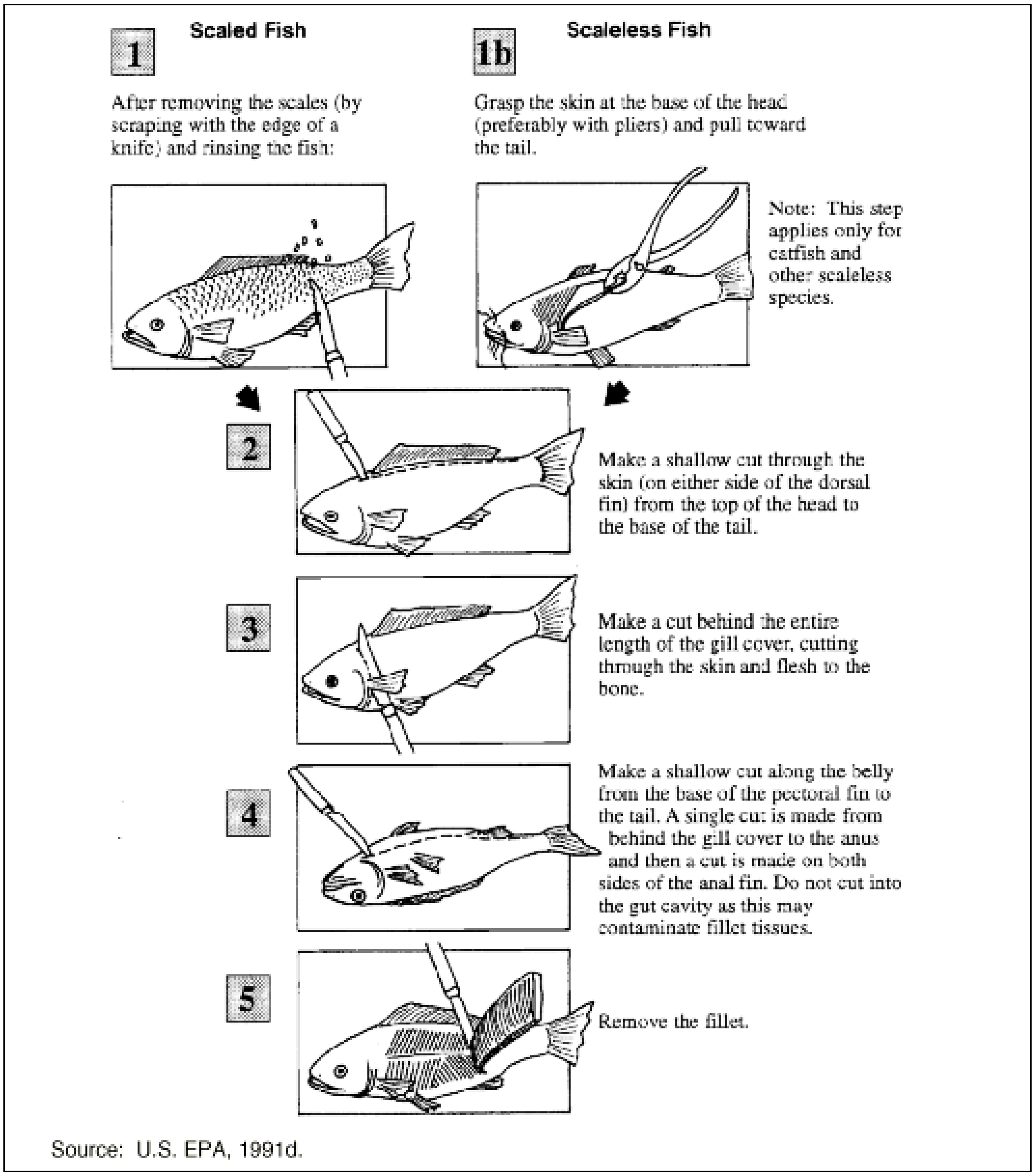

Figure 1. Procedure for filleting fish.

Fillets are ground and homogenized prior to analysis to ensure equal distribution of contaminants throughout the sample. Fillets are ground using a glass and stainless steel high speed blender or Hobart® Model 8145 commercial grinder. Samples are ground until they appear homogenous. Samples processed in the Hobart® Model 8145 commercial grinder are removed from the grinder and further mixed by hand. Hand mixing is accomplished by dividing the sample into quarters, mixing opposite quarters, and then mixing the remaining halves. Composite samples are prepared from at least 4 but no more than 10 individuals of the same species and should be of the same general size class. **Individuals of different species are never mixed to form composite samples.** The final individual or composite samples should be composed of at least 100 g of tissue to ensure an adequate amount of material for analysis. Metals samples are placed in foil cups with foil lined lids, and labeled. Organics samples are wrapped in aluminum foil, dull side against the tissue, then wrapped in plastic to prevent desiccation.

All samples are then sent either directly to the Division's Analytical Chemistry Laboratory (or other agency's laboratory), or frozen immediately and stored at -20 ^{o}C for later analysis.

Quality Assurance/Quality Control

To assess total variability, duplicate samples will be prepared from at least 10% of the fish samples. Duplicates are prepared using tissue from the same fillet or composite homogenate. Duplicates are assigned a "dummy" sample identification which is recorded in the processing laboratory log. The analytical laboratory does not receive this information.

During intensive or Tier 2 studies, portions of at least 10% of the prepared homogenates will be frozen at -20 ^{o}C and archived at the Division's Water Quality Laboratory for a period of at least 6 months after completion of the study. This is done in case of analytical problems or the need for future references.

To assess interlaboratory variability, the Division will attempt to split sample homogenates with other laboratories for analysis at least twice per year. Numbers of splits will depend on time and resource constraints of participating laboratories. Results from splits are tallied and plotted using descriptive statistics. Laboratory variability is considered acceptable if it is within two standard deviations of the mean for all measurements.

Data Analysis and Reporting

Data reported from the analytical laboratory below the method quantitation limit (MQL) are assigned a value of one-half the MQL. Data reported at or above the MQL are used as reported. The following statistics are calculated for each sampled species at each site:

- Range of target analyte concentrations;
- Arithmetic mean of target analytes;
- Standard deviation of mean target analyte concentrations; and
- Number of samples

Comparisons are performed using the Student t-Test (parametric) or the Sign Test (nonparametric).

In evaluating fish tissue analysis results, several different types of criteria are used. Human health concerns related to fish consumption are screened by comparing results with federal Food and Drug Administration (FDA) action levels (USFDA 1980), Environmental Protection Agency (US EPA) recommended screening values, and criteria adopted by the North Carolina State Health Director (Table 1). Individual parameter results which appear to be of potential human health concern are evaluated by the North Carolina Division of Occupational and Environmental Epidemiology by request from the Water Quality Section. All data are routinely provided to the State Health Director for evaluation.

The FDA levels were developed to protect humans from the chronic effects of toxic substances consumed in foodstuffs and thus employ a "safe level" approach to fish tissue consumption. Presently, the FDA has only developed metals criteria for mercury.

The US EPA has recommended screening values for target analytes which are formulated from a risk assessment procedure (USEPA 1995). These are the concentrations of analytes in edible fish tissue that are of potential public health concern. The Division compares fish tissue results with US EPA screening values to evaluate the need for further intensive site specific monitoring.

The North Carolina State Health Director has adopted a selenium limit of 5 µg/g for issuing an advisory. Although the USEPA has suggested a screening value of 0.7 ppt (pg/g) for dioxins, the State of North Carolina currently uses a value of 3.0 ppt in issuing an advisory.

Table 1. Fish tissue criteria. All wet weight concentrations are reported in parts per million (ppm, µg/g), except for dioxin which is in parts per trillion (ppt, pg/g).

Contaminant	FDA Action Levels	US EPA Screening Values	NC Health Director
Metals			
Cadmium		10.0	
Mercury	1.0	0.6	1.0
Selenium		50.0	5.0
Organics			
Aldrin	0.3		
Chlorpyrifos		30	
Total chlordane		0.08	
Cis-chlordane	0.3		
Trans-chlordane	0.3		
Total DDT[1]		0.3	
o,p DDD	5.0		
p, p DDD	5.0		
o,p DDE	5.0		
p,p DDE	5.0		
o,p DDT	5.0		
p,p DDT	5.0		
Dieldrin		0.007	
Dioxins (total)		0.7	3.0
Endosulfan (I and II)		60.0	
Endrin	0.3	3.0	
Heptachlorepoxide		0.01	
Hexachlorobenzene		0.07	
Lindane		0.08	
Mirex		2.0	
Total PCBs		0.01	
PCB-1254	2.0		
Toxaphene		0.1	

[1] Total DDT includes the sum of all its isomers and metabolites (i.e. p,p DDT, o,p DDT, DDE, and DDD).
[2] Total chlordane includes the sum of cis-and trans- isomers as well as nonachlor and oxychlordane.

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Appendix 1. Fish tissue raw data sheet.

Station	Date

Species	Total length (mm)	Weight (g)	DWQ Sample Number