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A Quantitative Health Assessment Index for Rapid Evaluation of Fish Condition in the Field

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Abstract.—The health assessment index (HAI) is an extension and refinement of a previously published field necropsy system. The HAI is a quantitative index that allows statistical comparisons of fish health among data sets. Index variables are assigned numerical values based on the degree of severity or damage incurred by an organ or tissue from environmental stressors. This approach has been used to evaluate the general health status of fish populations in a wide range of reservoir types in the Tennessee River basin (North Carolina, Tennessee, Alabama, Kentucky), in Hartwell Reservoir (Georgia, South Carolina) that is contaminated by polychlorinated biphenyls, and in the Pigeon River (Tennessee, North Carolina) that receives effluents from a bleached kraft mill. The ability of the HAI to accurately characterize the health of fish in these systems was evaluated by comparing this index to other types of fish health measures (contaminant, bioindicator, and reproductive analysis) made at the same time as the HAI. In all cases, the HAI demonstrated the same pattern of fish health status between sites as did each of the other more sophisticated health assessment methods. The HAI has proven to be a simple and inexpensive means of rapidly assessing general fish health in field situations.

The biotic integrity of an ecological system is often reflected by the health of organisms that reside in that system. In aquatic ecosystems, fish, and particularly those species near the top of the food chain, are generally regarded as representative indicators of overall system health. Because of their position in the food chain, fish integrate the effects of many biotic and abiotic variables acting in the system and reflect secondary impacts of chronic stress mediated through the food chain (Larkin 1978; Adams and McLean 1985).

Fish in their natural environments are typically subjected to numerous stressors including unfavorable or fluctuating temperatures, high water velocities and sediment loads, low dissolved oxygen concentrations, limited food availability, and other types of episodic variables. In addition, anthropogenic stressors such as contaminant loading can add to the insults that fish may already experience in many systems. All these factors, individually or together, can impose considerable stress on physiological systems of fish and impair their health (Wedemeyer et al. 1984). When an organism is challenged by environmental stressors, energy is required to deal with that stress, diverting physiologically useful energy away from the critical functions of growth and reproduction (Barton and Schreck 1987; Wedemeyer et al. 1990). Depending on its severity, stress can load or limit physiological systems, reduce growth, impair reproduction, predispose fish to disease, and reduce the capacity of fish to tolerate additional stressors (Adams 1990b; Barton and Iwama 1991).

A variety of approaches has been used to evaluate the effects of stress on the health of fish populations. These approaches have been applied to many types of aquatic ecosystems that experience a variety of environmental stressors. Some of the more commonly used approaches for assessing fish health are age and growth analysis and the condition factor (Le Cren 1951; Bagenal and Tesch 1978; Busacker et al. 1990), various condition or organosomatic indices (Goede and Barton 1990), and numerous measures of biochemical, physiological, and pathological condition (Neff 1985; Adams 1990a; Niimi 1990). Each of these types of health measure has its own set of advantages and limitations, depending on the objectives of the particular study, but most of them cannot be rapidly and inexpensively applied to field studies. In many cases, samples have to be processed and analyzed in a laboratory, which requires varying degrees of time and expense. For example, determination of condition indices may involve only simple length and weight measures, whereas biochemical, physiological, and pathological analyses often require specialized training, instrumentation, and possibly large time commitments.

As a rapid and inexpensive alternative to these more sophisticated approaches for evaluating fish health and condition, Goede and Barton (1990) developed a field necropsy method that provides a health profile of fish based on the percentages of anomalies observed in the tissues and organs of individuals sampled from a population. The major purpose of the necropsy method is to detect gross changes in the health of fish poulations early enough for corrective or remedial actions to be taken. The primary value of this approach is that it provides a means of establishing a data base for detecting trends in the health of a fish population over time. If, by applying this method, a change or trend is indicated in the health of members of a population, then more detailed and specialized procedures can be applied to the problem. The concept of this strategy is analogous to that used by the medical profession. A physician may use a battery of generalized tests on a patient to help diagnose an illness. If the results of a general health screening help to focus on the nature of the problem, then more specific measures can be used for further diagnosis.

The major limitation of the current necropsy or health profile method is that it does not provide quantitative results that are amenable to statistical comparison of data among sites, species, or years. This method also does not currently account for severity or degree of damage in some of the variables within the necropsy system. Our objectives in this study, therefore, were to modify and refine the necropsy-based approach to (1) provide a quantitative index so that statistical comparisons can be made between data sets, (2) include variables in the health index that reflect the degree of damage incurred as a result of environmental stressors, and (3) provide examples of the use of this index in different types of aquatic systems to demonstrate and validate its applicability.

Approach

The necropsy method of Goede and Barton (1990) currently consists of 16 variables that can be grouped into the following categories: (1) three blood parameters (hematocrit, leukocrit, and plasma protein); (2) length, weight, and condition factor; (3) percentage of fish with normal and abnormal eyes, gills, pseudobranchs, spleens, kidneys, and livers; and (4) index values of damage to skin, fins, thymus, hindgut inflammation, fat deposits, and bile color. Analysis and evaluation of the fish necropsy data involve a summary of the means for variables in group 4, a summary of the percentage of normals in group 3, means and variances of blood constituents in group 1, and length and weight data in group 2. Even though the necropsy method provides a health status profile of a fish population, there is no quantitative basis for comparing statistically the entire index with all its variables to another population sample either in time or space. In addition, past experience with the original necropsy method has shown that some variables within the index, such as conditions of the skin, gills, and fins, demonstrate varying levels of damage that should be taken into account when the health of a fish population is evaluated. The health assessment index (HAI), which we present here, is intended to minimize these limitations of the necropsy method by rendering it quantitative for statistical analysis and comparisons among data sets.

Variable Quantification

For the HAI to have a statistical basis, all variables within the index must be assigned a numerical value. To assign a numerical value of condition to each variable within the HAI, all variables are first given a field code designation according to the original necropsy classification criteria of Goede and Barton (1990). A numerical substitution is then made for each assigned code (Table 1). All codes that represent a normal condition are replaced by a zero, and all codes that represent an abnormal condition or anomaly assume a value of 30. For example, the normal liver color for centrarchids such as black basses *Micropterus* spp. and sunfish *Lepomis* spp. is dark or light red, which is therefore assigned a value of zero (Table 1). Other liver conditions that are considered to be abnormal are assigned a value of 30 (Table 1). A maximum value of 30 was chosen to provide a suitable range for value ranking. The criteria by which variables in the HAI are assigned various conditions or rankings are admittedly somewhat subjective. The nature of this variable ranking system is consistent, however, with the principal purpose and objective of the HAI approach, which is to provide a rapid and simple field methodology for characterizing the general health of members of a fish population.

All variables except bile color and the mesenteric fat level were used in the calculation of the HAI. Bile color could not be assigned a numerical value based on normality because bile can take on differing colors depending on feeding regimes of the fish (time since last meal, meal quantity and quality, etc.). Mesenteric fat deposits in the fish, or the lipid index, can vary widely depending not only on food availability and feeding regimes, but on other interacting factors such as fish size, sex, time of year, and stress level. Because of these interacting variables, the lipid index could not be assigned normal or abnormal condition values. For hematocrit and plasma protein, the normal ranges should be established for each species or groups of similar species for major geographical areas of the country (e.g., southeast, northwest).

Variable Ranking

To account for differences in severity of damage or level of effect, some variables of the HAI are assigned values of 10, 20, or 30, depending on the extent of the abnormality or observed damage (Table 1). For example, if fins with light active erosion are noted, a value of 10 is assigned. A fin condition characterized by moderate active erosion with some hemorrhaging rates a value of 20, and severe erosion with hemorrhaging receives a ranking of 30. Other variables of the HAI that receive assigned values based on the severity of damage or condition are parasite loads, thymus condition, hindgut inflammation, skin condition, and hematocrit and plasma protein levels (Table 1).

Calculation of the HAI

To calculate an HAI for each fish within a sample, numerical values for all variables are summed. The HAI for a sample population is then calculated by summing all individual fish HAI values and dividing by the total number of fish examined for that sample. A standard deviation for each sample is calculated as

$$SD = \frac{\sum_{i=1}^{N} (V_i - X)^2}{N - 1};$$

N = number of fish per site;
X = average index for each site;
V_i = index value for fish i.

The coefficient of variation (CV) is calculated as

$$CV = 100 \cdot SD/X.$$

The HAI for the sample population is composed of multiple HAI observations calculated for each fish within that sample. Therefore, statistical comparisons can be made among sample sites, sample times for the same site, and even species. Since the HAI value for a given site or system is a mean based on the number of sampled fish in the population, the central limit theorem is a justification for using parametric procedures for statistical comparisons among data sets. The HAI for each sample site is a mean based on a relatively large sample size (e.g., $N = 30$); therefore, the distribution of individual HAI values for a site tends to be normally distributed according to the central limit theorem (Snedecor and Cochran 1980). In addition to these parametric procedures, nonparametric rank statistics could also be used for testing differences among data sets.

In addition, the CV of each sample can be calculated and used to indicate the level or degree of stress experienced by a fish population. For fish experiencing stress resulting from poor water quality, for example, we might except the CV to be lower for that sample than for a reference population because all individuals within the stressed population should be equally exposed to water quality stressors. In contrast, when fish are exposed to bacterial infection, the vulnerability of individuals may differ according to the competence of their immune systems and other inherent biochemical and physiological factors. In this situation, variability in a stress response should be higher among members of the stressed than of the reference population. Caution must be exercised in the interpretation of the CV because other factors such as fish size distribution, sex, and species may influence the inherent variability of stress responses in fish.

Table 2 provides an example of how an HAI is calculated for a sample fish population. An index is first determined for each fish based on the summation of the anomaly values for that fish. The individual index values are then used to calculate a population sample mean for the HAI. Letter and number codes for each variable in Table 2 follow those in Table 1. The parenthetical numbers in Table 2 are the values (10, 20, or 30) assigned to the observed anomaly that are summed to calculate an individual fish index value.

In the example given in Table 2, pathologies were most often observed in the liver and spleen;

TABLE 1.—Description of variables used in the health assessment index (HAI). Values are assigned to each of these variables according to the type and severity of the observed anomoly (modified from Goede and Barton 1990).

Variable	Variable condition	Original field designation	Substituted value for the HAI
Thymus	No hemorrhage	0	0
	Mild hemorrhage	1	10
	Moderate hemorrhage	2	20
	Severe hemorrhage	3	30
Fins	No active erosion	0	0
	Light active erosion	1	10
	Moderate active erosion with some hemorrhaging	2	20
	Severe active erosion with hemorrhaging	3	30
Spleen	Normal; black, very dark red, or red	B	0
	Normal; granular, rough appearance of spleen	G	0
	Nodular; containing fistulas or nodules of varying sizes	D	30
	Enlarged; noticeably enlarged	E	30
	Other; gross aberrations not fitting above categories	OT	30
Hindgut	Normal; no inflammation or reddening	0	0
	Slight inflammation or reddening	1	10
	Moderate inflammation or reddening	2	20
	Severe inflammation or reddening	3	30
Kidney	Normal; firm dark red color, lying relatively flat along the length of the vertebral column	N	0
	Swollen; enlarged or swollen wholly or in part	S	30
	Mottled; gray discoloration	M	30
	Granular; granular appearance and texture	G	30
	Urolithiasis or nephrocalcinosis; white or cream-colored mineral material in kidney tubules	U	30
	Other; any aberrations not fitting previous categories	OT	30
Skin	Normal; no aberrations	0	0
	Mild skin aberrations	1	10
	Moderate skin aberrations	2	20
	Severe skin aberrations	3	30
Liver	Normal; solid red or light red color	A	0
	"Fatty" liver; "coffee with cream" color	C	30
	Nodules in the liver; cysts or nodules	D	30
	Focal discoloration; distinct localized color changes	E	30
	General discoloration; color change in whole liver	F	30
	Other; deviation in liver not fitting other categories	OT	30
Eyes	No aberrations; good "clear" eye	N	0
	Generally, an opaque eye (one or both)	B	30
	Swollen, protruding eye (one or both)	E	30
	Hemorrhaging or bleeding in the eye (one or both)	H	30
	Missing one or both eyes	M	30
	Other; any manifestation not fitting the above	OT	30
Gills	Normal; no apparent aberrations	N	0
	Frayed; erosion of tips of gill lamellae resulting in "ragged" gills	F	30
	Clubbed; swelling of the tips of the gill lamellae	C	30
	Marginate; gills with light, discolored margin along tips of the lamellae	M	30
	Pale; very light in color	P	30
	Other; any observation not fitting above	OT	30
Pseudobranchs	Normal; flat, containing no aberrations	N	0
	Swollen; convex in aspect	S	30
	Lithic; mineral deposits, white, somewhat amorphous spots	L	30
	Swollen and lithic	S&L	30
	Inflamed; redness, hemorrhage, or other	I	30
	Other; any condition not covered above	OT	30
Parasites	No observed parasites	0	0
	Few observed parasites	1	10

TABLE 1.—Continued.

Variable	Variable condition	Original field designation	Substituted value for the HAI
	Moderate parasite infestation	2	20
	Numerous parasites	3	30
Hematocrit[a]	Normal range	30–45%	0
	Above normal range	>45%	10
	Below normal range	19–29%	20
	Below normal range	<18%	30
Leukocrit	Range defined as normal	<4%	0
	Outside the normal range	≥4%	30
Plasma protein[b]	Normal range	30–69 mg/dL	0
	Above normal range	>70 mg/dL[b]	10
	Below normal range	<30 mg/dL	30

[a] Normal ranges for centrarchid species such as largemouth bass and redbreast sunfish.
[b] Values greater than 70 mg/dL are generally inaccurate because of factors that interfere with the protein analysis such as elevated serum lipids.

lesser frequencies of anomalies were recorded for kidney, parasite loads, plasma protein concentrations, and leukocrit. All index variables were anomalous for at least one fish in the sample. Fish 3 and 11 were in very poor health, as indicated by their high index values. For example, fish 3 with an index value of 190 had a fatty liver (30), moderate skin aberrations (20), light active fin erosion (10), a swollen pseudobranch (30), mild hemorrhaging of the thymus (10), moderate inflammation of the hindgut (20), a granular kidney (30), a few internal parasites particularly in the heart and liver (10), and an abnormally high leukocrit (30). The condition of most of the fish in this sample, however, was not this poor, as indicated by the population mean HAI of 97.3.

An HAI for the sample population can be calculated in the field within 5 min after the health assessment procedure is completed. Data for each fish are entered into a portable computer, and a spreadsheet program with a series of macros assigns each observed anomaly a numerical value as in Table 1, tabulates the HAI for each fish, and calculates a mean, SD, and CV for the sample population. Comparisons of the HAIs can then be made among sample sites, between different aquatic systems, or at the same site over time to establish temporal patterns in fish population health. Statistical comparisons can also be made among indices and environmental conditions to determine correlations or relationships between fish health and environmental variables such as temperature, dissolved oxygen, or turbidity. Normal or expected HAI values for a population are established by sampling unaffected areas over time and comparing results to affected sites.

Application

The use of the HAI to assess the health of fish populations in the field is demonstrated in this section by three examples of the index's successful application. This approach is currently being used by the Tennessee Valley Authority (TVA) to evaluate the general health status of fish in a wide range of reservoir types over the entire Tennessee Valley (North Carolina, Tennessee, Alabama, Kentucky). The HAI has also been applied in reservoirs and rivers to assess the effects on fish health of polychlorinated biphenyl (PCB) contamination in Hartwell Reservoir (South Carolina, Georgia) and of pulp and paper effluents in the Pigeon River (North Carolina, Tennessee).

TVA Reservoir Survey

The TVA conducts an annual monitoring survey on 28 reservoirs, including 11 mainstream systems and 17 tributary storage impoundments. The objectives of this "vital signs" survey are to provide basic information on reservoir health or integrity and to obtain screening-level information that is used to evaluate each reservoir with respect to the mandates of the U.S. Clean Water Act. The HAI is used along with other measures of reservoir condition such as physical and chemical characterization of the water and sediment, surveys of benthic macroinvertebrates, and acute toxicity screening to assess the overall health of the reservoir ecosystem. To obtain information

TABLE 2.—An example of the calculation of the health assessment index based on a sample of 15 fish. Letter and number designations for each variable of the HAI follow those given in Table 1; the first entry is the field designation and the number in parentheses is the value assigned to an observed anomaly. Parenthetic numbers are summed to calculate an index value for each fish. Fat and bile designations do not enter the index.

Fish number or statistic	Health assessment index variable									
	Liver	Eye	Skin	Fin	Pseudobranch	Thymus	Fat	Spleen	Hindgut	Kidney	Bile
1	A (0)	N (0)	0 (0)	0 (0)	S (30)	0 (0)	3	D (30)	0 (0)	N (0)	2
2	F (30)	N (0)	0 (0)	0 (0)	N (0)	0 (0)	2	G (0)	0 (0)	N (0)	2
3	C (30)	N (0)	2 (20)	1 (10)	S (30)	1 (10)	1	G (0)	2 (20)	G (30)	0
4	E (30)	N (0)	0 (0)	0 (0)	N (0)	0 (0)	3	OT (30)	0 (0)	S (30)	3
5	A (0)	B (30)	0 (0)	0 (0)	N (0)	0 (0)	4	D (30)	0 (0)	S (30)	1
6	A (0)	N (0)	0 (0)	0 (0)	N (0)	0 (0)	0	E (30)	0 (0)	S (30)	3
7	C (30)	N (0)	0 (0)	0 (0)	N (0)	0 (0)	2	D (30)	1 (10)	N (0)	0
8	C (30)	N (0)	0 (0)	2 (20)	N (0)	2 (20)	1	E (30)	0 (0)	N (0)	0
9	A (0)	N (0)	2 (20)	0 (0)	N (0)	0 (0)	4	G (0)	0 (0)	N (0)	3
10	F (30)	N (0)	0 (0)	0 (0)	N (0)	0 (0)	2	B (0)	0 (0)	N (0)	3
11	C (30)	B (30)	2 (20)	3 (30)	N (0)	0 (0)	3	E (30)	0 (0)	N (0)	3
12	A (0)	N (0)	0 (0)	0 (0)	S (30)	0 (0)	0	D (30)	0 (0)	N (0)	0
13	A (0)	H (30)	0 (0)	0 (0)	N (0)	0 (0)	1	B (0)	0 (0)	N (0)	2
14	A (0)	N (0)	0 (0)	0 (0)	N (0)	0 (0)	3	E (30)	1 (10)	N (0)	1
15	F (30)	N (0)	0 (0)	0 (0)	N (0)	0 (0)	2	G (0)	0 (0)	S (30)	0
Mean											
SD											

for the HAI, 30 largemouth bass *Micropterus salmoides*, ranging in size from 0.5 to 2.5 kg, were collected by boat electrofishing at three areas in each reservoir, including the upper section, transition area (midlake), and the lower section near the dam. An HAI was then calculated for each fish, for each area of the reservoir, and for the entire reservoir.

The average HAI for all reservoirs in the TVA valleywide survey was 62; Watts Bar (63) represented the average impoundment in the system (Table 3). Watts Bar is a large mainstream system characterized by moderate levels of primary and secondary productivity and high standing crops of forage fish. The range of reservoir HAI values for the 22 impoundments in 1991 was 17 (best) for the relatively pristine Watauga system to 79 (worst) for Chickamauga Reservoir. Watauga Reservoir, the system with the healthiest largemouth bass population, is a mountain headwater impoundment that supports a well-balanced warmwater and coolwater fishery. As reflected by the low HAI score, water quality in this system is very good and there are no major nutrient or contaminant inputs into the system. Conversely, Chickamauga Reservoir, a large mainstream impoundment below Watts Bar on the Tennessee River, had the highest HAI score, reflecting a largemouth bass population that is in relatively poor condition compared to the other TVA impoundments. This reservoir receives contaminants from numerous sources, the most notable being the Hiwassee River into which effluents from a pulp and paper plant are discharged.

Hartwell Reservoir

Hartwell Reservoir is a 24,400-hectare U.S. Army Corps of Engineers impoundment of the Savannah River along the South Carolina–Georgia border. The fish in the upper northeast sector of the reservoir in South Carolina contain high levels of PCBs (Gaymon 1988, 1990); body burdens generally decrease downstream toward the dam. The Tugaloo River arm, primarily in Georgia, is relatively free of contaminants and served as a reference site for this study. At each of three sites in this reservoir, 30 largemouth bass, ranging in size from 0.5 to 2.0 kg, were collected by boat electrofishing and the HAI was determined. Site locations and mean PCB concentrations (wet-weight basis) in the fillets of largemouth bass were (1) 12 Mile Creek (21 μg/g), (2) 18 Mile Creek (2.0 μg/g), and (3) Tugaloo River arm (0.3 μg/g).

The mean value of the HAI for the largemouth bass sample population was highest at 12 Mile Creek, lowest at the reference site, and interme-

TABLE 2.—Extended.

Fish number or statistic	Health assessment index variable				Index value
	Parasites	Plasma protein	Leukocrit	Hematocrit	
1	0 (0)	34 (0)	1 (0)	33 (0)	60
2	0 (0)	41 (0)	3 (0)	36 (0)	30
3	1 (10)	45 (0)	4 (30)	40 (0)	190
4	3 (30)	51 (0)	3 (0)	37 (0)	120
5	0 (0)	37 (0)	3 (0)	37 (0)	90
6	2 (20)	28 (30)	2 (0)	42 (0)	110
7	2 (20)	35 (0)	1 (0)	29 (30)	110
8	0 (0)	52 (0)	1 (0)	40 (0)	100
9	1 (10)	26 (30)	4 (30)	37 (0)	90
10	0 (0)	45 (0)	3 (0)	27 (30)	60
11	0 (0)	32 (0)	4 (30)	28 (30)	200
12	0 (0)	33 (0)	3 (0)	41 (0)	60
13	3 (30)	36 (0)	1 (0)	43 (0)	60
14	1 (10)	40 (0)	1 (0)	29 (30)	90
15	0 (0)	29 (30)	1 (0)	44 (0)	90
Mean					97.3
SD					46.5

diate at 18 Mile Creek (Table 3), reflecting the gradient of PCB contamination at these sites. Anomalies in three of the index variables (liver, gill, and kidney) were primarily responsible for influencing the HAI of largemouth bass in Hartwell Reservoir (Table 4). Liver condition, which was based primarily on color, was responsible for 50–57% of all abnormal observations at the 12 Mile and 18 Mile creek sites and 17% of the anomalies at the reference site. Many of the abnormal livers for fish at 12 Mile and 18 Mile creeks had a light or coffee-cream color, indicative of high fat deposition. A fatty liver usually is a pathological state attributable to excessive accumulation of lipids in cellular cytoplasm (Roberts 1978). Fat accumulation can result from an inability to convert lipids in hepatocytes to a phospholipid form suitable for use (Runnells et al. 1965). This pathology has been observed in fish exposed to PCBs and other organic compounds (Lipsky et al. 1978; Klaunig et al. 1979). Spleens and kidneys also presented important anomalies, primarily in largemouth bass from the 12 Mile Creek site. At 12 Mile Creek, kidneys were swollen, which is a general indicator of pathology (Goede and Barton 1990). Spleen abnormalities in fish from both 12 Mile and 18 Mile creeks were primarily the result of enlargement, whereas abnormal spleens from the reference fish were caused by nodules. Because the spleen serves a hemopoietic function, enlargement could indicate bacterial infections or disease. Abnormal levels of plasma protein and hematocrit also contributed to the higher overall HAI at the 12 Mile and 18 Mile creek sites. The low hematocrit values at these two sites may indicate a disease or pathogenic infection (Cardwell and Smith 1971).

TABLE 3.—Health assessment index (HAI) values for largemouth bass from the Tennessee Valley Authority (TVA) survey and sites in Hartwell Reservoir (Georgia–South Carolina), and for redbreast sunfish from the Pigeon River (North Carolina–Tennessee) study.

Aquatic system	HAI	SD	Coefficient of variation
TVA valleywide			
Mean of all reservoirs	62	15.6	25.2
Average reservoir (Watts Bar)	63	8.6	13.7
Healthiest reservoir (Watauga)	17	3.5	20.1
Worst reservoir (Chickamauga)	79	3.2	4.1
Hartwell Reservoir			
Reference site (Tugaloo River)	42	36.0	85.7
Intermediate site (18 Mile Creek)	64	36.9	57.7
Contaminated site (12 Mile Creek)	74	30.8	41.8
Pigeon River			
Pigeon River, km 95	60	35.6	59.7
Pigeon River, km 35	51	35.7	69.5
Little River (reference)	21	23.2	108.8
Little Pigeon River (reference)	35	25.4	72.6

Pigeon River

The Pigeon River is a high-gradient stream that originates in the mountains of western North Carolina, receives effluents from a bleached kraft mill at river kilometer 105, and then flows northwest before joining the French Broad River near Knoxville, Tennessee. Along the entire length of the Pigeon River below the mill, the water has a dark tea color, due to staining by dissolved organics, and contains chlorinated phenols and resin acids characteristic of bleached kraft mill effluents (Leach and Thakore 1973). At sites 10 and 70 km downstream from the mill outfall, 35 adult redbreast sunfish *Lepomis auritus* were sampled by boat electrofishing. From two reference streams (Little River and Little Pigeon River, which are similar in size, flow, bottom substrate, and gradient to the Pigeon River), equal numbers of redbreast sunfish were also collected. Fish collected from all three streams were examined, and an HAI was calculated for the sample population at each site.

TABLE 4.—Percentage of fish with tissue and organ anomalies in a sample collected from sites in Hartwell Reservoir (Georgia–South Carolina) and the Pigeon River (North Carolina–Tennessee).

Site	Percentage of fish with anomalies in					
	Liver	Gill	Kidney	Spleen	Eyes	Fins
Hartwell Reservoir						
Reference site	17	7	20	15	0	0
18 Mile Creek	50	33	27	13	0	0
12 Mile Creek	57	17	43	30	0	7
Pigeon River						
Pigeon River, km 95	47	53	20	20	13	0
Pigeon River, km 35	50	50	30	6	3	6
Little River (reference)	17	0	23	3	0	0
Little Pigeon River (reference)	27	20	17	7	0	0

Fish collected 10 km downstream from the paper mill (river km 95) had the highest HAI (poorest health), and the mean index decreased slightly for redbreast sunfish sampled 70 km downstream of the mill (river km 35) (Table 3). The mean HAI values for both reference sites, however, were considerably lower than those from the two Pigeon River sites. The mean HAI for redbreast sunfish from the Little River was less than 50% of the value of the two Pigeon River sites, and the index from the Little Pigeon River was bout 35% lower than at the contaminated sites (Table 3).

Of the 14 variables included in the calculation of the HAI, 6 were responsible for most of the abnormalities observed in fish from the Pigeon River and reference sites (Table 4). Changes in the liver, gill, and kidney were the main anomalies observed; lesser effects were recorded for the spleen, eyes, and fins. As with the anomalies observed in the fish exposed to PCBs in Hartwell Reservoir, many of the livers in the fish collected from the Pigeon River were characterized by high fat levels and focal discoloration. Focal discoloration can be due to various factors including focal necrosis caused by bacterial infections. Gill damage is highly characteristic of fish exposed to pulp mill effluents (Lehtinen et al. 1984; Couillard et al. 1988). Effects on gills include hyperplasia, clubbing of lamellae, and lamellar fusion that not only impairs gas exchange but also affects osmoregulatory and excretory functions in fish. Anomalies in the kidney were observed in 20–30% of the fish examined from the Pigeon River (Table 4). Relatively high proportions (17–23%) of fish from the reference sites also had abnormal kidneys, but the anomalies there were attributed primarily to swelling, probably caused by parasitic infestation. In addition to swelling and parasitic infestation, abnormal kidneys in fish from the Pigeon River could have resulted from tubule damage and necrosis caused by the pulp mill effluents (Santos et al. 1990).

In both the Pigeon River and Hartwell Reservoir studies, the CVs were higher for fish from the reference sites than for those from the contaminated sites (Table 3). One possible interpretation of this finding is that fish living in degraded environments are all exposed equally to constant stressors such as poor water quality and, therefore, the variability in physiological condition in fish from a contaminant-exposed population may tend to be less than for fish in unstressed environments.

Evaluation of the HAI

The ability of the HAI to characterize the health profile of a fish population can be evaluated by comparing this index to other measures of fish health taken at the same time as the HAI. In Hartwell Reservoir, Adams and Greeley (1991) used three other biomonitoring approaches, in addition to the HAI, for assessing the condition of the largemouth bass population: (1) levels of PCBs in the flesh and gonads; (2) biological indicators including detoxification enzymes, histopathology, blood chemistries, and bioenergetic indices; and (3) reproductive competence. All four methods provided similar conclusions relative to the health status of largemouth bass at the three sampling sites in Hartwell Reservoir (Table 5). The health profile of the largemouth bass population in Hartwell Reservoir, regardless of the method of analysis, follows an inverse gradient with PCB levels in fish, being best at the reference site and worst in the 12 Mile Creek section of the reservoir. One area in which the HAI differs from the three other methods, however, is in the ability of the index to distinguish the condition of fish at 12 Mile Creek from the health of fish at 18 Mile Creek. The HAI

TABLE 5.—Comparison of four biomonitoring techniques used in the Hartwell Reservoir study and their relative ability to distinguish the health status of largemouth bass at each sampling site.

Biomonitoring technique	Relative health status of largemouth bass			Comments
	Best	Intermediate	Worst	
Contaminant analysis	Reference	18 Mile Creek	12 Mile Creek	PCBs highest at 12 Mile Creek in fillets and ovaries
Bioindicator analysis	Reference	18 Mile Creek	12 Mile Creek	Based primarily on integrated response analysis with biochemical, physiological, histopathological, and bioenergetic measures
Reproductive analysis	Reference	18 Mile Creek	12 Mile Creek	Based primarily on abundance of vitellogenic oocytes, ovary size, and estrogen receptor data
Health assessment index	Reference	18 Mile Creek	12 Mile Creek	12 Mile and 18 Mile creek HAI values similar; HAI only indicates departures from normal; not diagnostic

indicates that the health status of largemouth bass from these two sites was similar (e.g., HAI values of 74 and 64 for 12 Mile and 18 Mile creeks, respectively), whereas the other methods suggest lower levels of effects at the 18 Mile Creek site. This apparent discrepancy implies that the HAI is either a much more sensitive early warning index of health effects than the other techniques or that it could be overestimating the condition status of fish, particularly at 18 Mile Creek.

In the Pigeon River, several health assessment methods were also compared with the HAI to evaluate the ability of this index to characterize population health. This study included indicators of (1) biochemical and physiological changes, (2) reproductive abnormalities, (3) population effects, and (4) community-level disturbances. The HAI demonstrated the same patterns of fish health status among sites as did each of the other assessment methods (Adams et al. 1992). For example, the site with the highest HAI value (poorest health) nearest the pulp mill outfall was correlated with the highest levels of detoxification enzyme induction (an indicator of contaminant exposure), the largest number of reproduction-related anomalies, an abnormal size and age structure distribution of the population, and the lowest index of biotic integrity (IBI; a measure of fish community health) (Adams et al. 1992).

Results from these two case histories demonstrate that the HAI can be a reliable method for assessing the general health status of a fish population. Furthermore, the HAI has the added advantage over many other approaches of being relatively simple, rapid, and cost-effective. The simplicity of this approach, however, may also be one of its primary limitations because of its weakness as a diagnostic tool. The HAI is not designed to be diagnostic in character, but to provide a first-level assessment of the health profile of a fish population. If the HAI identifies a general health problem in a population, then more specific assessment approaches could follow, such as the application of biological indicators (Adams et al. 1989; Adams 1990a). Another potential application of the HAI is its inclusion as an additional metric in the IBI. Currently, the IBI consists of about 12 metrics that are collectively used to obtain a single index score on the ecological status of a fish community. The HAI could be included as an additional metric in the IBI that would serve as an indicator of the health of individual fish in that population or community.

The particular approach taken to determine the health status of a fish population depends on the objectives and needs of a particular user group (e.g., fish culture, environmental quality, or fishery management) and the resources available (technical expertise, funding, etc.). For issues dealing with fish culture and fishery management, the necropsy method of Goede and Barton (1990) is useful in helping to diagnose the nature of a specific problem. For example, a fish culturist may want to determine the success of various types of feeding programs or evaluate the effects of disease on condition. The objectives of the fishery manager may be to evaluate the results of a stocking program or the bioenergetic status of fish. In issues dealing with water quality, the HAI could be applied to assessing the general health of fish populations. It should be emphasized that the HAI and the necropsy method are designed to complement each other in the evaluation of fish health. The degree to which they should be used separately or together depends primarily on the needs of a particular user group. In this regard, future refinements in the HAI and necropsy method will involve developing additional components of these two approaches that will address more specific concerns related to environmental quality, fish culture, or fishery management.

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