Transactions of the American Fisheries Society 109:532–536, 1980

Stress and Body Condition in a Population of Largemouth Bass: Implications for Red-Sore Disease

GERALD W. ESCH

Department of Biology, Wake Forest University
Winston-Salem, North Carolina 27109

TERRY C. HAZEN

Department of Biology, Faculty of Natural Sciences
University of Puerto Rico, Rio Piedras, Puerto Rico 00931

Abstract

The body conditions, $K = 10^5(\text{weight, g}) \div (\text{standard length})^3$, and various hematological characters were examined for largemouth bass (*Micropterus salmoides*) taken from Par Pond, a reservoir heated by effluent from a nuclear production reactor at the Savannah River Plant near Aiken, South Carolina. Largemouth bass with K less than 2.0 had significantly lower ($P < 0.05$) hematocrits, hemoglobin concentrations, total red blood cell counts, total white blood cell counts, and lymphocyte fractions, and significantly higher granulocyte fractions and cortisol concentrations, than those with K greater than 2.0; monocyte, thrombocyte, and reticulocyte fractions were not different between the two K-factor groupings. When data were pooled, all blood variables except the reticulocyte fraction were significantly correlated with K. Hematocrit, the lymphocyte fraction, and cortisol concentration account for 20.5% of the variation in K. These data support a previous hypothesis that elevated water temperature promotes stress. Stress within the Par Pond largemouth bass population may play an impotant role in the epizootiology of red-sore disease caused by the gram-negative bacterium, *Aeromonas hydrophila*.

In this report, we provide data on the hematological changes which accompany a reduction in body condition of largemouth bass (*Micropterus salmoides*) and characterize the relationship between body condition and concentrations of cortisol and circulating white blood cells. These data are relevant to the epizootiology of red-sore disease in largemouth bass from Par Pond, a 1,012-hectare cooling reservoir for a nuclear production reactor on the Savannah River Plant near Aiken, South Carolina.

A 10-year study has provided substantive evidence that elevated temperatures promoted significant reductions in body condition of the Par Pond largemouth bass population. Gibbons et al. (1978) suggested that a number of factors were important in producing body condition changes, and Quinn et al. (1978) concluded the large majority of largemouth bass in the heated portions of the impoundment are permanent residents. It is also known that largemouth bass in heated parts of Par Pond have elevated body temperatures (Bennett 1971) and that elevated body temperature promotes increased metabolism (Hazen, et al. 1978). Moreover, Gibbons et al. (1978) noted that stored fat reserves are mobilized for use during times when food intake is not sufficient to meet the energy demands of vertebrate animals and that, in Par Pond, various prey species, primary *Lepomis* spp., are dramatically reduced in numbers during the warmer, summer months (Bennett and Gibbons 1972). Thus, as a consequence of increased metabolism, reduction in food intake, and the depletion of stored body fat, largemouth bass lose body weight and body condition is reduced.

Many largemouth bass in Par Pond suffer from red-sore disease, caused by the gram-negative bacterium, *Aeromonas hydrophila* (Esch et al. 1976). Based on data from over 5,000 largemouth bass taken during 1974–1978, Esch and Hazen (1978) proposed that stress, induced by elevated temperatures, played a significant role in increasing susceptibility of largemouth bass to red-sore. Moreover, they observed a significant ($P < 0.05$) positive correlation between reduced body condition and the probability of largemouth bass acquiring red-sore disease.

Stress, in the classical sense (Selye 1950), necessarily implies the production and release of

TABLE 1.—*Blood characteristics of Par Pond largemouth bass with body conditions (K)[a] less than or greater than 2.00.*

Blood constituent	$K < 2.00$		$K > 2.00$			
	N	Mean ± SE	N	Mean ± SE	F	P
Hematocrit (% cell volume)	143	34.8 ± 0.7	322	42.0 ± 0.03	112.42	<0.0001
Hemoglobin (g/dl)	143	7.6 ± 0.2	322	9.2 ± 0.01	54.23	<0.0001
Cortisol (μg/dl)	121	14.9 ± 0.8	276	12.4 ± 0.4	8.53	<0.01
Total red blood cells (10^6 cells/ml)	143	5.6 ± 0.3	322	6.8 ± 0.2	21.27	<0.0001
Total white blood cells (10^4 cells/ml)	143	22.0 ± 1.1	322	28.7 ± 0.9	19.02	<0.0001
Granulocytes (%)	84	7.1 ± 3.1	219	4.6 ± 0.9	13.26	<0.0001
Lymphocytes (%)	84	52.8 ± 43.2	219	57.5 ± 30.0	8.11	<0.0001
Monocytes (%)	84	3.3 ± 1.0	219	2.6 ± 0.5	2.71	>0.50
Thrombocytes (%)	84	39.1 ± 28.1	219	37.8 ± 16.5	0.55	>0.50
Reticulocytes (%)	84	1.9 ± 0.4	219	2.5 ± 0.3	4.83	>0.25

[a] $K = 10^5$(weight, g) ÷ (standard length, mm)^3.

adrenocorticosteroids, some of which have a striking anti-inflammatory action that promotes increased susceptibility to invasion by pathogenic agents (Esch et al. 1976). If stress occurs when body conditions of largemouth bass are reduced, then we should also expect a concomitant rise in concentrations of circulating cortisol and a decline in white blood cells. Other hematological changes may also occur within largemouth bass subjected to chronic exposure to elevated water temperature. Esch and Hazen (1978) did not address the physiological relationships between body condition, stress, and red-sore disease, a gap we partially fill in this paper.

Methods

The biotic and physicochemical characteristics of Par Pond have been described by Parker et al. (1973) and Hazen (1978). Temperatures in heated parts of the reservoir averaged 5 C–10 C above those in other areas.

By a combination of angling and electrofishing, mostly the latter, largemouth bass were collected throughout the reservoir during 48-hour periods midway in each season from summer 1976 through winter 1978. Largemouth bass used in the present study were all over 20 cm total length; the majority were 30–40 cm long. Most fish were captured between 1200 and 1400 hours. Blood was removed by cardiac puncture in heparinized, 10-ml-draw vacutainers. Blood was placed on ice and returned to the laboratory where hematocrit, hemoglobin, total red blood cells, total white blood cells, and differential white blood cell counts were measured by standard methods (Hazen, et al. 1978); cortisol was measured by protein-binding assay (Baum et al. 1974).

Red-sore disease was noted by visual examination for surface lesions (Hazen, et al. 1978; Huizinga et al. 1979). In the majority of cases, the presence of *Aeromonas hydrophila* in surface lesions was confirmed by isolation on R-S medium (Shotts and Rimler 1973; Hazen 1978).

The body condition, $K = 10^5W \div L^3$, where W = body weight (g) and L = standard length (mm), was computed for each fish. In previous studies (Esch and Hazen 1978; Gibbons et al. 1978), relationships between quantities of dissectable body fat, K, and red-sore disease for Par Pond largemouth bass showed significant distinctions between individuals with $K < 2.0$ and those with $K > 2.0$ and these two K-groupings are retained here. However, due to seasonal variability in body conditions, variation in reactor activity, seasonal differences in prevalence of disease, and relatively small seasonal sample sizes, data for all fish were also pooled for statistical analysis.

Data were analyzed by analysis of variance, multiple regression, and multiple correlation by programs developed for a Hewlett-Packard 3000 computer. Statistical packages used included IDA (University of Chicago). All data were heteroscedastic, as determined by skew and kurtosis, and were subjected to $\log(X + 1)$ or arc-sine square-root transformation as appropriate (Zar 1974). All probabilities less than or equal to 0.05 were considered significant.

Results

The mean values for hematocrit, hemoglobin, total red blood cells, and total white blood

TABLE 2.—*Correlation coefficients of body condition* (K)[a] *with blood constituents of 303 largemouth bass from Par Pond. All values except that for reticulocytes are significant* (P < 0.05).

Blood constituent	r
Hematocrit	0.3296
Hemoglobin	0.2054
Total white blood cells	0.1592
Total red blood cells	0.2132
Cortisol	−0.1635
granulocytes	−0.1453
Lymphocytes	0.2583
Monocytes	−0.1274
Thrombocytes	−0.1556
Reticulocytes	0.1129

[a] $K = 10^5$(weight, g) ÷ (standard length, mm)^3.

cells were significantly lower for largemouth bass with K less than 2.00 than for those with K more than 2.00 (Table 1). Importantly, cortisol concentrations were significantly higher among fish in relatively poor body condition. Percentages of monocytes, thrombocytes, and reticulocytes did not significantly vary between the two K-groupings, but granulocytes were significantly higher and lymphocytes significantly lower among fish with K below 2.00 than for those with K above 2.00.

To preclude the possibility that a division of these fish into only two K groups caused an analytical artifact, data were pooled over 303 largemouth bass (all those for which white cell fractions could be determined) and subjected to multiple regression analysis with K as the dependent variable. The correlation matrix (Table 2) confirms our conclusions above. All blood variables except reticulocytes are significantly correlated ($r = 0.4525$) with body condition. With decreasing body condition, hematocrit, hemoglobin, total red and white blood cell counts, and the lymphocyte fraction declined while cortisol concentrations and percentages of granulocytes, monocytes, and thrombocytes rose. The overall r^2 value for blood data from all 303 largemouth bass in the sample indicated that the independent variables explain 25.5% of the variation in K. Hematocrit, lymphocyte percentage, and cortisol concentration accounted for 20.5% of the variation in K (Table 3).

Discussion

The use of body condition as an index for estimating a fish's well-being or fitness, or as a means of evaluating the suitability of a given habitat, is a well-established and acceptable practice (Everhart et al. 1975; Lagler 1975). When it is so used, however, caution must be exercised because allometric growth, season, sex, and small sample size may affect K values. All of these variables were carefully considered by Gibbons et al. (1978) in their study of over 10,000 largemouth bass taken during a 10-year period throughout Par Pond. They concluded that chronic exposure to thermal effluent significantly reduced body conditions of largemouth bass.

Our data demonstrate that several blood constituents in largemouth bass are affected by exposure to thermal effluent from a nuclear production reactor. Differences in mean blood values for the two K groupings are, while significant, small; if only a few of the values were different, conclusions regarding the relationships could easily be spurious. However, seven of ten variables were significantly different; moreover, over 20% of the variability in K can be explained by just three constituents: hematocrit, lymphocytes, and cortisol.

It has been shown that as stored energy reserves are depleted, body condition, or fitness, is reduced among the Par Pond largemouth bass population (Gibbons et al. 1978). Esch and Hazen (1978) proposed that as body condition declines, largemouth bass in Par Pond may be stressed and that cortisol levels should rise and leukopenia should develop. Results of the present study certainly support this hypothesis.

The main thrust of this study was to generate data to support an hypothesis relating body condition and stress. We believe this objective has been met. The basis for our interest in this hypothesis is related to a long-term study of red-sore disease in Par Pond and other systems throughout the southeastern United States. Various aspects of the epizootiology of the disease have been investigated (Esch et al. 1976; Esch and Hazen 1978; Hazen et al. 1978; Hazen 1979; Huizinga et al. 1979). Briefly, the disease process in a largemouth bass includes the formation of purulent surface lesions, accompanied by scale erosion. It may ultimately result in hemorrhagic septicemia, culminating in the almost complete destruction of major internal organs, primarily the hind kidney. However, production of a humoral antibody, specific for *A. hydrophila*, appears to afford some

TABLE 3.—*Best-fit regression analysis and analysis of variance for body condition (K)[a], the dependent variable, against hematocrit, lymphocyte percentage, and cortisol concentration for 303 largemouth bass from Par Pond.*

Best-Fit Regression

Variable	B[b]	Standard variance	SE	t	Multiple r	r^2
Hematocrit	0.3590	0.0184	0.0027	6.947		
Lymphocytes	0.2858	0.0083	0.0015	5.501		
Cortisol	−0.1256	−0.0058	0.0024	−2.432		
K	0	1.0708	0.1499	7.142		
Correlation						
Unadjusted					0.4612	0.2127
Adjusted[c]					0.4525	0.2048

Analysis of Variance

Source	Sums of squares	df	Mean squares	F	P
Regression	9.2722	3	3.0908		
Residuals	34.3257	299	0.1148		
Total	43.5980	302	0.1444		
				26.92	<0.00001

[a] $K = 10^5$(weight, g) ÷ (standard length, mm)^3.
[b] B = slope.
[c] Correlation coefficient adjusted to account for the biased estimator of the population parameter (Bliss 1970).

short-term protection against red-sore disease (unpublished observations).

The red-sore disease process at the population level appears to follow one of two courses in most epizootics. The first is associated with sudden and widespread mortality, usually involving several fish species (Shotts et al. 1972; Miller and Chapman 1976; Esch and Hazen 1980). The second is of a chronic nature in which only a few species are affected and mortality is low; this appears to characterize the epizootiology of red-sore disease in Par Pond. For Par Pond, Esch and Hazen (1978) showed that high water temperature, reduced body condition, and high prevalence of the disease are related. They proposed that elevated temperature stimulated metabolic activity among largemouth bass, resulting in decreased levels of body fat and protein, and ultimately, caused body condition to decline. This, they believed, would induce chronic stress; associated increases in cortisol concentrations would cause leukopenia and lead to increased susceptibility to red-sore disease.

Evidence in the present report supports the notion of a relationship between body condition, cortisol, and leukopenia while data of Esch et al. (1976) and Esch and Hazen (1978) indicate a relationship between body condition of largemouth bass and the probability of acquiring red-sore disease. While these hypotheses certainly require additional testing, they offer reasonable explanations for varied disease patterns in such widely scattered and diverse habitats as Lake Apopka in Florida, Albemarle Sound, Lake Norman and Badin Lake in North Carolina, and Par Pond in South Carolina.

Acknowledgments

We acknowledge the technical assistance of James R. Matthews, William F. Crawford, and Anne Mansfield. This research was supported by contract DE-AS09-76EY-00900 between Wake Forest University and the Department of Energy (DOE) and by grant B-112-NC from the North Carolina Water Resources Research Institute. The research was also supported, in part, by contract EY-76-C-09-0819 between the University of Georgia and DOE. We are grateful to J. W. Gibbons and M. H. Smith for their continuing encouragement and support of our work in South Carolina.

References

BAUM, C. K., R. TUDOR, AND J. LANDON. 1974. A simple competitive protein-binding assay for plasma cortisol. Clinical Chemistry Acta 55:147.

BENNETT, D. H. 1971. Preliminary examination of body temperatures of largemouth bass (*Micropterus salmoides*) from an artificially heated reservoir. Archives of Hydrobiology 68:376–381.

BENNETT, D. H., AND J. W. GIBBONS. 1972. Food of largemouth bass (*Micropterus salmoides*) from a

South Carolina reservoir receiving thermal effluent. Transactions of the American Fisheries Society 101:650–654.

Bliss, C. I. 1970. Statistics in biology, volume 2. McGraw-Hill, New York, New York, USA.

Esch, G. W., and T. C. Hazen. 1978. Thermal ecology and stress: a case history for red-sore disease in largemouth bass. Pages 331–363 *in* J. H. Thorpe and J. W. Gibbons, editors. Energy and environmental stress in aquatic systems. CONF-77114, United States Department of Energy Symposium Series, National Technical Information Service, Springfield, Virginia, USA.

Esch, G. W., and T. C. Hazen. 1980. The ecology of *Aeromonas hydrophila* in Albemarle Sound, North Carolina. Special Report 153, North Carolina Water Resources Research Institute, University of North Carolina, Raleigh, North Carolina, USA.

Esch, G. W., T. C. Hazen, R. V. Dimock, and J. W. Gibbons. 1976. Thermal effluent and epizootiology of the ciliate *Epistylis* and the bacterium *Aeromonas hydrophila* in association with centrarchid fish. Transactions of the American Microscopical Society 95:687–693.

Everhart, W. H., A. W. Eipper, and W. D. Youngs. 1975. Principles of fishery science. Cornell University Press, Ithaca, New York, USA.

Gibbons, J. W., D. H. Bennett, G. W. Esch, and T. C. Hazen. 1978. Effects of thermal effluent on body condition of largemouth bass. Nature (London) 274:470–741.

Hazen, T. C. 1978. The ecology of *Aeromonas hydrophila* in a South Carolina reservoir. Doctoral dissertation. Wake Forest University, Winston-Salem, North Carolina, USA.

Hazen, T. C. 1979. Ecology of *Aeromonas hydrophila* in a South Carolina cooling reservoir. Microbial Ecology 5:179–195.

Hazen$_1$, T. C., G. W. Esch, J. W. Gibbons, and A. B. Glassman. 1978. Relationship of season, thermal loading and red-sore disease with various hematological parameters in largemouth bass (*Micropterus salmoides*). Journal of Fish Biology 12:491–498.

Hazen$_2$, T. C., M. L. Raker, G. W. Esch, and C. B. Fliermans. 1978. Ultrastructure of red-sore lesions on largemouth bass: the association of the peritrich *Epistylis* sp. and the bacterium, *Aeromonas hydrophila*. Journal of Protozoology 25:351–355.

Huizinga, H., G. W. Esch, and T. C. Hazen. 1979. Histopathology of red-sore disease in largemouth bass, *Micropterus salmoides*. Journal of Fish Diseases 2:310–321.

Lagler, K. F. 1975. Freshwater fishery biology. William C. Brown, Dubuque, Iowa, USA.

Miller, R. M., and W. R. Chapman. 1976. *Epistylis* and *Aeromonas hydrophila* infections in fishes from North Carolina reservoirs. The Progressive Fish-Culturist 38:165–168.

Quinn, T., G. W. Esch, T. C. Hazen, and J. W. Gibbons. 1978. Long range movement and homing by largemouth bass (*Micropterus salmoides*) in a thermally altered reservoir. Copeia 1978:542–545.

Parker, E. D., M. F. Hirshfield, and J. W. Gibbons. 1973. Ecological comparisons of thermally affected aquatic environments. Journal of the Water Pollution Control Federation 45:726–733.

Selye, H. 1950. Stress and the general adaptation syndrome. British Medical Journal 1:1383–1392.

Shotts, E. B., J. L. Gaines, C. Martin, and A. K. Prestwood. 1972. *Aeromonas*-induced deaths among fish and reptiles in an eutrophic inland lake. Journal of the American Veterinary Medicine Association 161:603–607.

Shotts, E. B., and R. Rimler. 1973. Medium for the isolation of *Aeromonas hydrophila*. Applied Microbiology 26:550–553.

Zar, J. H. 1974. Biostatistical analysis. Prentice-Hall, New York, New York, USA.