To the Graduate Council:

I am submitting herewith a thesis written by Kenneth Ladell Shepard entitled "Use of Standard Metabolic Rate as an Indicator of Environmental Stress in Redbreast Sunfish, Lepomis auritus." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology.

S. Marshall Adams, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Vice Provost
and Dean of the Graduate School

USE OF STANDARD METABOLIC RATE AS AN INDICATOR OF ENVIRONMENTAL STRESS IN REDBREAST SUNFISH, LEPOMIS AURITUS

A Thesis

Presented for the

Master of Science

Degree

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Kenneth Ladell Shepard

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ABSTRACT

Fish exposed to sub-lethal levels of toxicant-induced stress exhibit physiological and biochemical changes that may result in significant population and community level consequences. Standard metabolic rate integrates the total system response of the organism and thus may provide a good indicator of how well the fish is functioning in its environment. In this study, standard metabolic rate was determined for redbreast sunfish (Lepomis auritus) from two streams: 1) a test stream subject to inputs of various heavy metals, organics, and other toxicants, and 2) a reference stream of similar size and hydrology without industrial or municipal inputs. Fish were tested in their home stream water, in dechlorinated tap water, in test stream water containing suspended contaminated sediment, and in water from a section of the test stream composed primarily of industrial effluent and supporting no resident fish population. No significant difference was noted among any of these test groups with the exception of the group tested in the industrial effluent. This group had significantly reduced standard metabolic rates, probably due to gill damage resulting from exposure to chlorine in the water. These results suggest that standard metabolic rate is not a adequate indicator of the effects of chronic exposure to sublethal stressors.

TABLE OF CONTENTS

SECTION

I. INTRODUCTION. 1
Stress and Metabolism 4
Aspects of Standard Metabolism. 9
Variables that Affect Standard Metabolism. 10
Scope of the Study. 13

II. MATERIALS AND METHODS 17
Description of the Study Area 17
The Respirometer. 19
Test Procedure for Metabolism Determination. 22
Temperature/Weight-Metabolism Relationship 24
Electrofishing Effects. 26
Stream Tests. 27
Simulated Storm Events. 31
Tests Using Water from EFPC Above New Hope Pond. 31
Statistical Procedures. 34

III. RESULTS 36
Temperature/Weight-Metabolism Relationship 36
Electrofishing Effects. 37
Stream Tests. 37
Simulated Storm Events. 40
Tests Using Water from EFPC Above New Hope Pond. 40

IV. DISCUSSION. 47

V. SUMMARY AND CONCLUSIONS 54

LIST OF REFERENCES 56

VITA 68

LIST OF TABLES

TABLE

I. Contaminants in New Hope Pond Sediment. . . 32

II. Contaminants in EFPC Above New Hope Pond. . 33

III. Results of Home Stream Water Tests. 41

IV. Results of De-chlorinated Water Tests . . . 42

V. Results of Simulated Storm Event Test . . . 44

VI. Results of Upper EFPC Water Tests 46

LIST OF FIGURES

FIGURE		
I.	Partitioning of Energy in the Fish.	8
II.	Map of the Study Area Showing the Test Streams, East Fork Poplar Creek (EFPC) and Brushy Fork (BF).	18
III.	Schematic of the Apparatus Used to Measure Standard Metabolism in the Redbreast Sunfish	20
IV.	Typical Diel Oxygen Consumption for Redbreast Sunfish (Lepomis auritus)	25
V.	Experimental Protocol for Testing the Effects of East Fork Poplar Creek and Brushy Fork Water on the Standard Metabolism of Redbreast Sunfish	30
VI.	Relationship Between Oxygen Consumption and Temperature for Sunfish	38
VII.	Relationship Between Body Weight and Oxygen Consumption for Sunfish	39
VIII.	Relationship Between Body Weight and Oxygen Consumption for Redbreast Sunfish (Lepomis auritus).	43

I. INTRODUCTION

Significant population and community level effects can result from altered growth and reproduction in fish subject to sub-lethal stress induced by chronic exposure to water-borne toxicants (Sastry and Miller 1981). Chronic exposure to toxicants has been shown to cause tumor formation, DNA damage, immunosuppression, reduced respiration efficiency, and other biochemical and physiological changes which result in reduced growth and reproduction (Wedemeyer and McLeay 1981). These effects rarely occur singly and are often a result of exposure to multiple stressors which renders extrapolation from single-exposure/single-response laboratory experiments to the field difficult. Physiological and biochemical effects do, however, have a metabolic cost which may be reflected in holistic measurements of organism response such as the standard metabolic rate (Umminger 1977, Mehre and Meyer 1985).

The standard metabolic rate is a measurement of the rate of energy expenditure by an organism. Metabolism in this context is catabolism, the breakdown of complex substances into simpler substances consuming oxygen and releasing energy (Fry 1971). Since the rate of energy metabolism integrates more aspects of animal performance than any other single physiological parameter (Bartholomew 1982),

is functioning in its environment. Direct measurement of heat production (direct calorimetry) in fish has been attempted, but with little success because of technical difficulties, primarily the great heat capacity of water compared with the small amount of heat produced by a fish (Smith 1976, in Heath 1987). Since under most conditions (and under the conditions of this study) fish are obligate aerobes, the universal method of measuring the energy expenditure of a fish is the measurement of oxygen consumption (indirect calorimetry) (Heath 1987). Metabolic rates, as measured by oxygen consumption, are classified into three types (Fry 1971).

1) Standard metabolism is the rate of oxygen consumption of a fish in a state of rest, isolated from external disturbances. At a given temperature, it is a measure of the rate of energy use of the animal which reflects the costs of physiological maintenance and corresponds to the basal metabolic rate used in human energetics.

2) Routine metabolic rate is similar to the standard metabolic rate but includes random locomotor activity and is an approximation of the energy usage of the fish in its normal daily activity, including foraging for food, mating behavior, predator avoidance, etc. For sedentary fish, this rate may be close to the standard rate while more active species have a routine rate much higher than the standard rate.

3) Active metabolic rate is the rate obtained when the fish is forced to swim at maximum speed for a period of time and approximates the maximum metabolic capabilities of the organism.

Routine and active metabolism are, in some respects, "optional" to the organism in that they may be behaviorally adjusted in response to environmental conditions to conserve energy. For example, to optimize energy usage, a fish could spend less time (and energy) foraging in times of low food abundance and more time (and energy) in times when food is readily available. Standard rate, on the other hand, is the minimum maintenance level and any reduction must, by definition, incur a corresponding reduction in some essential physiological activity. Reductions in standard metabolic rate can be a result of exposure to stressors, such as toxicants or low dissolved oxygen levels (Beamish 1964, Hughes 1976, Kloth and Wohlschlag 1972), and, unless alleviated, the stress is usually lethal. The standard rate also may increase when an animal is stressed by handling, experiences rapid changes in temperature, or is exposed to toxicants or other biotic and abiotic perturbations. A permanent, chronic increase in the standard rate would reflect an increase in metabolic costs which could result in decreased growth and reproduction while a transient increase may be a result of physiological compensation to

maintain homeostasis. In the latter case, significant population level effects may be manifested depending on the timing, intensity, and concentration of the exposure. In this study I explored the use of standard metabolic rate of redbreast sunfish as an integrative evaluation of chronic stress effects caused by long-term exposure to toxicants.

Stress and Metabolism

The metabolic changes of an organism can be due to a single stressor or it can be an integrated response to the effects of multiple stressors in the environment. The "stressor" is the environmental alteration itself, and "stress" is the organism response i.e., the sum of all physiological responses that occur in an attempt to establish or maintain homeostasis (Wedemeyer and McLeay 1981). The literature is replete with examples of aquatic organism response to single toxicant exposure under constant environmental conditions (e.g. O'Hara 1971b, Malte 1986, Waiwood and Beamish 1978, and those reviewed in Heath 1987). In nature, however, such constant conditions and exposures occur rarely, if ever, and measurement of organism stress should take into account the effects of multiple stressors. Integrating the total effect from a variety of component stressors is important in understanding the consequences of this stress on

fitness of the individual and the population. Exposure to chemicals or other toxic agents may be acute (short-term) or chronic (long-term). In acute exposure, organisms are exposed to a toxicant in single or multiple contacts that occur within hours to days. The effects produced usually occur rapidly, but may be delayed and occur sometime after the exposure. During chronic exposure, organisms are exposed to either a continuous or periodic exposure to low levels of toxicants over a period of weeks to years. Chronic exposure may produce immediate effects as well as effects that develop over a longer period. Chronic exposure typically involves the entire reproductive life cycle of the organism while acute exposure involves a short period of time relative to the life cycle.

Effects may also be classified as acute or chronic. Acute effects occur quickly as a result of short-term exposure to a stressor. In fish, responses occurring within a few hours, days, or weeks are considered acute and generally are severe. Acutely toxic concentrations of chemicals are those that kill 50% or more of the exposed population within the test time, usually 96 h to 14 d (Rand and Petrochelli 1985). Chronic effects may be the result of a single exposure, but generally are a consequence of long-term continuous or repeated exposure. These chronic effects may be lethal (failure of the population to reproduce), or sublethal (behavioral,

physiological, biochemical, or histological changes). Some of these sublethal effects may lead indirectly to deat h. For example, increased metabolic demands for maintenance may result in less energy available for reproduction or foraging activity. Whether an acute or chronic response to a toxicant results in an increase or decrease in standard metabolic rate depends on the mode of action of the particular toxicant.

Effects seen at the cellular, tissue, or organ level are indicators of changes at these particular levels of biological organization, but integrated organism responses could have population and community implications as well. Sub-organism level effects assume greater ecological significance when viewed in the context of their actual effect on populations and communities. Physiological and behavioral responses that directly affect fecundity (through growth, longevity, reproductive success) can be predictors of long-term population and community response to stressors in the environment. These impacts at higher levels of biological organization are more ecologically meaningful than those indicators representative of lower levels that indirectly influence populations (i.e. organs, tissues, and cells). Therefore these long-term responses generally provide environmental assessment end points, such as population and community level effects that can be easily evaluated by human values (Oviatt et al. 1984).

Responses to toxicant-induced stress can include, for example: 1) reduced food assimilation efficiency, 2) reduced oxygen uptake efficiency caused by gill damage 3) blood chemistry changes, 4) DNA damage leading to tumor formation, 5) immune system dysfunction, 6) increased mucous production, 7) detoxification enzyme induction, and a host of others (Mehre and Mayer 1985, Sastry and Miller 1981, Hughes 1976, Heath 1987, Zeeman and Brindley 1981, Parke 1981). Biochemical effects due to pollutants impose a demand on the organism for accelerated protein turnover which is positively related to oxygen consumption (Bayne 1987). With an increase in protein synthesis, there is a concomitant increase in energy allotted to maintenance. The whole-organism manifestation of these biochemical effects may entail such responses as reduced appetite, reduced prey capture efficiency, change in preferred temperature, increase in activity, and lowered reproductive success. Since the physiological processes that underlie these effects require energy, the measurement of the rate of energy expenditure by the fish can be an integrative indicator of the organism response to its environment (Mehrle and Mayer 1985).
Figure I illustrates the functional relationship between standard metabolism and energy flow through the organism.

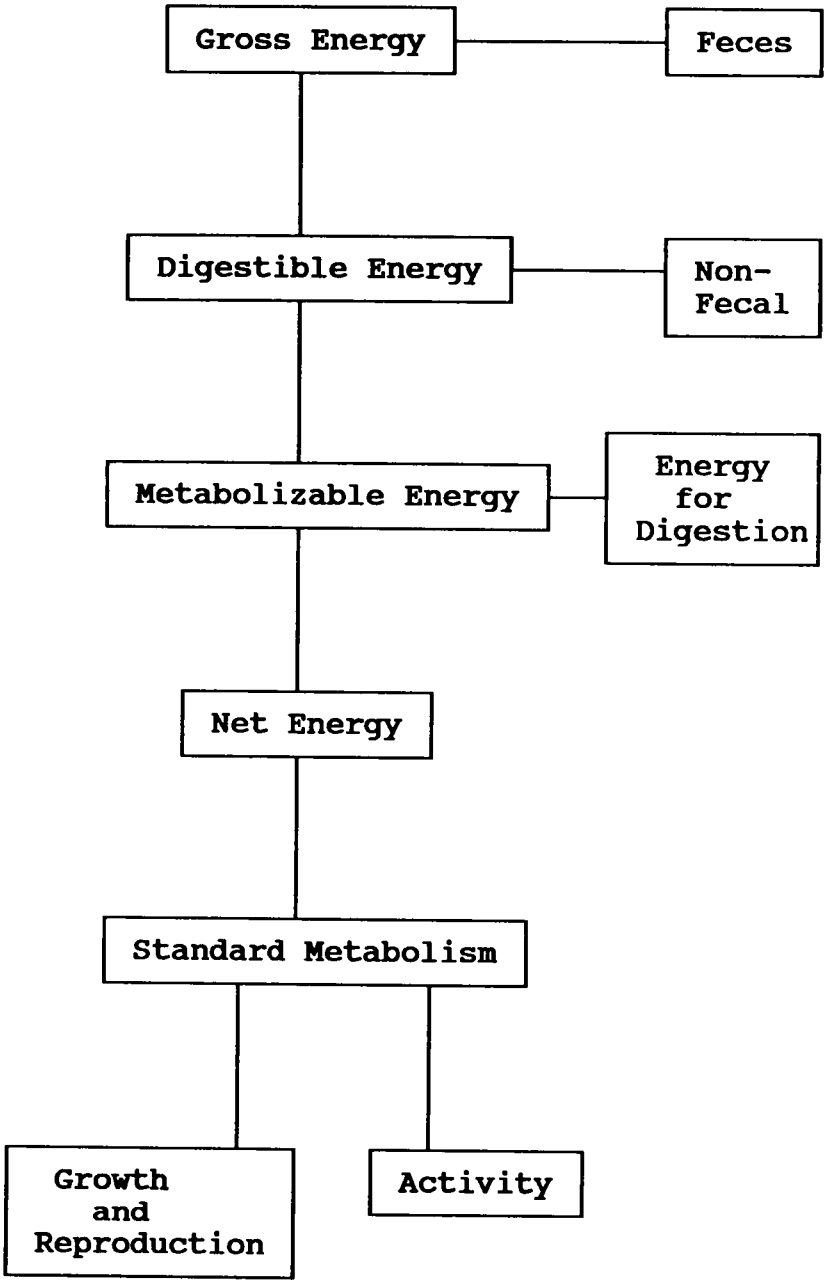


Figure I. Partitioning of energy in the fish.
(After Heath 1987).

Aspects of Standard Metabolism

Standard metabolism is the cost of homeostatic regulation in the animal; the minimum energy necessary for preservation of essential physiological processes. This rate of energy expenditure, comparable to basal metabolism in mammals, was first defined by Krogh (1916, in Beamish and Mookherjii 1964) as the "nearest attainable approximation to the basal metabolism which would obtain when all organs were absolutely at rest." This is a good theoretical definition, but in practice, determining when all organs are "at rest" is difficult, if not impossible. Subsequent researchers have defined standard metabolism as: "oxygen consumption measured at minimum motor activity" (Dolinin 1973); "lowest value of oxygen consumption obtained by an adequate technique" (Brett 1964); and "the minimum value measured over a 24 hour period" (Huppop 1986). Most of the literature values for standard metabolism are calculated from active rates extrapolated to zero activity (e.g. Brett 1964). These rates are suspect for two reasons: the risks involved in extrapolating outside the data points (Snedecor and Cochran 1967), and the possibility that a swimming fish may be physiologically very different from a resting (Cech, in press, Priede and Holliday 1980). Such extrapolation from active rates may be unavoidable for some innately restless fishes (e.g. Salmonidae). This

study, however, examined the relatively sedentary redbreast sunfish (Lepomis auritus) which facilitated the direct determination of standard metabolic rate. Another consideration is the energy utilized for digestion. Digestion and assimilation can increase the inactive metabolic rate by 50 to 500% (Beamish 1964, Brett and Groves 1979). Therefore, food must be withheld from the fish for at least 24 h prior to testing. This post-absorptive state insures that the costs of digestion are not included in the rate measured. To facilitate comparison of metabolic rates among fish of different sizes the measured rate is often expressed as the specific standard rate, which is the oxygen consumption in mg $O_2 \cdot l^{-1} \cdot h^{-1}$ divided by unit mass in grams or kilograms. For this study, the standard metabolic rate will be defined as the minimum rate of oxygen consumption measured over a 24 h period for a fish in the post-absorptive state, isolated from outside disturbances, at zero locomotor activity, and after the rate has reached equilibrium.

Variables that Affect Standard Metabolism

The rate of standard metabolism is a function of three classes of variables (Winberg 1956). First, water quality parameters, such as dissolved oxygen level, temperature, or the presence of toxicants, can affect fish directly,

causing short-term changes in metabolic rate. Aquatic systems can experience rapid temporal changes in water quality with a corresponding rapid metabolic response in fish. The second class of variables that can influence standard metabolism is the specific characteristics of the fish. Metabolic rate can vary with species, sex, age, and weight, but usually sex and age have little effect compared with species and weight. In general, smaller fish have a higher specific standard metabolic rate than larger fish of the same species. Interspecific differences are due to life history differences ranging from sedentary species that are adapted to low dissolved oxygen (e.g. carp, Cyprinus carpio) to active species with high dissolved oxygen requirements (e.g. rainbow trout, Salmo gairdneri). This "base rate" is a product of the phylogeny of the fish and changes only on an time scale of many generations (Priede 1985, Castleberry and Cech 1986, Huppop 1986). The third category of variables, the physiological condition of the fish, operates on a time scale intermediate between the first two. It is a function of the environment to which the fish is exposed during its life cycle. Food quality and availability, temperature regime, and exposure to toxicants that stress physiological systems outside the range of recovery can all induce stress response mechanisms that can in turn affect metabolic rate. These are long-term chronic

exposures that result in permanent physiological, behavioral, biochemical, or histological changes in the organism.

For the purposes of determining the metabolic effects of exposure to chronic low-level stress in the form of water-borne toxicants, the respective contribution to standard metabolic rate of all three of these classes of variables must be addressed. The standard metabolic rate of healthy fish of a variety of weights and at a range of temperatures must be determined to establish the "base line" species-specific rate. If a fish exhibits a change in metabolic rate from base line when tested in water from its natural environment, and then exhibits a return to base line rate when tested in clean water after a short recovery period, the change is probably an acute response, a short-term metabolic compensation to maintain homeostasis (Duthie and Houlihan 1982, McKim, et al. 1987a,b). A chronic response would be indicated if the metabolic rate difference remains unchanged even with improved water quality (Waiwood and Beamish 1978, Rand and Petrochelli 1985). In all cases, the fish must be acclimated to the test temperature, and tested under conditions of high ambient dissolved oxygen levels to avoid confounding the effects of pollutants with the secondary effects of low dissolved oxygen.

Scope of the Study

A National Pollutant Discharge Elimination System (NPDES) permit was issued to the Y-12 Munitions Production Plant in Oak Ridge, Tennessee on May 24, 1985 and specified that a Biological Monitoring and Abatement Program (BMAP) be implemented for East Fork Poplar Creek to demonstrate that the effluent limitations established for the Y-12 Plant protect and maintain the classified uses of this stream (Loar et al. 1985). These uses are primarily recreation (fishing and swimming) and growth and propagation of fish and aquatic life. My study, which is a component of the BMAP project, involved standard metabolic rate determinations for fish collected from the receiving stream for Y-12 Plant discharges, East Fork Poplar Creek (EFPC), and for fish from the reference (control) stream, Brushy Fork. Upper EFPC consists primarily of industrial effluent from the Y-12 Plant and flows directly into a retention basin, New Hope Pond. From the pond, the stream flows 23 km through the city of Oak Ridge and ultimately to its confluence with Poplar Creek, a tributary of the Clinch River. An exhaustive analysis of EFPC water, sediment, and aquatic biota (TVA 1985b, 1985c) found seventeen toxicants at concentrations exceeding background levels, established guidelines and/or analytical detection limits.

Of these toxicants, mercury and PCB concentrations in fish exceeded Food and Drug Administration action level for human consumption. Analysis of sediment samples from EFPC indicated approximately 16 million cubic feet of mercury-contaminated sediment with concentrations exceeding 5.0 $mg \cdot kg^{-1}$; the sediment of the entire EFPC contains an estimated 170,000 pounds of mercury (Tennessee Valley Authority 1975b). During storm flow, mercury is mobilized from the sediment and the total mercury concentrations frequently exceed drinking water standards and water quality criteria for aquatic life. Most of this mercury is bound to particulate matter rather than being in solution. Dissolved mercury, the form most readily taken up by aquatic life, remains at a relatively constant concentration during increased flow (Tennessee Valley Authority 1985a). Primarily because of the elevated mercury levels, the Tennessee Department of Health and Environment prohibited swimming and fishing in East Fork Poplar Creek in November 1982.

In addition to PCBs, other organic compounds found in flesh from fish at concentrations at or above the detection limit include flouranthene, bis(2-ethylhexyl) phthalate, pyrene, phenanthrene, benzo-a-pyrene, chrysene, antracene, and benzo-a-anthracene 1,2 benzanthracene. Metals, other than mercury, found at elevated concentrations include arsenic, cadmium, chromium, lead,

nickel, silver, and zirconium. Chlorine is also frequently detected in upper EFPC above Hew Hope Pond at levels acutely and chronically toxic to Ceriodaphnia and fathead minnows (Loar 1987).

As part of the BMAP for EFPC, a study was undertaken of the stream biota at various levels of biological organization ranging from the biochemical/physiological level to populations and communities. One component of this investigation involved measuring a suite of biochemical, physiological, organ and condition indices from redbreast sunfish taken from the stream. A canonical discriminant analysis procedure was performed on the data to evaluate the integrated multivariate response of the fish in relation to the level of stress encountered. The results indicated (1) a significantly different response between fish collected near the contaminant source and those from the reference stream, and (2) a gradient of pollutant stress responses in EFPC from just below the Y-12 Plant downstream to near the mouth of the stream (Adams, in press).

The primary objective of the present study was to determine if differences in integrated stress response in redbreast sunfish are reflected in a difference in standard metabolism. Another goal was to determine the metabolic response of reference (control) fish during storm events when toxicants could be elevated for short

periods. A third objective was to describe the metabolic response of redbreast sunfish to industrial effluents from a section of the creek that supports fish and has a depauperate benthic community. A final objective involved accurate determination of the weight, temperature, metabolism response of redbreast sunfish for incorporation into a bioenergetics model being developed for this species.

II. MATERIALS AND METHODS

Description of Study Area

East Fork Poplar Creek

The headwaters of East Fork Poplar Creek (EFPC) originate on the north slope of Chestnut Ridge west of the Y-12 Plant in Oak Ridge, Tennessee (Figure II). The majority of the stream flow (9-12 cfs) consists of discharges from the plant which flow into New Hope Pond (NHP), a 2.2 hectare impoundment constructed in 1963 for neutralization, pH equalization, sediment retention, and spill control (Pritz and Sanders 1982). The stream above NHP (upper EFPC) has been channelized and receives the discharge from more than 200 individual sources, accounting for as much as 90% of the stream flow (Butz 1984, in Loar et al. 1985). Below NHP, the stream flows 22.9 km before entering Poplar Creek, a tributary of the Clinch River. East Fork Poplar Creek drains an area of approximately 77.2 km^2 along the northern boundary of the Department of Energy Oak Ridge Reservation.

Brushy Fork

The reference (control) stream for these tests was Brushy Fork, a third order stream which flows between Clinton and Oliver Springs, Tennessee (Figure II). The watershed, discharge volume, other hydrologic characteristics, and availability of fish habitat are

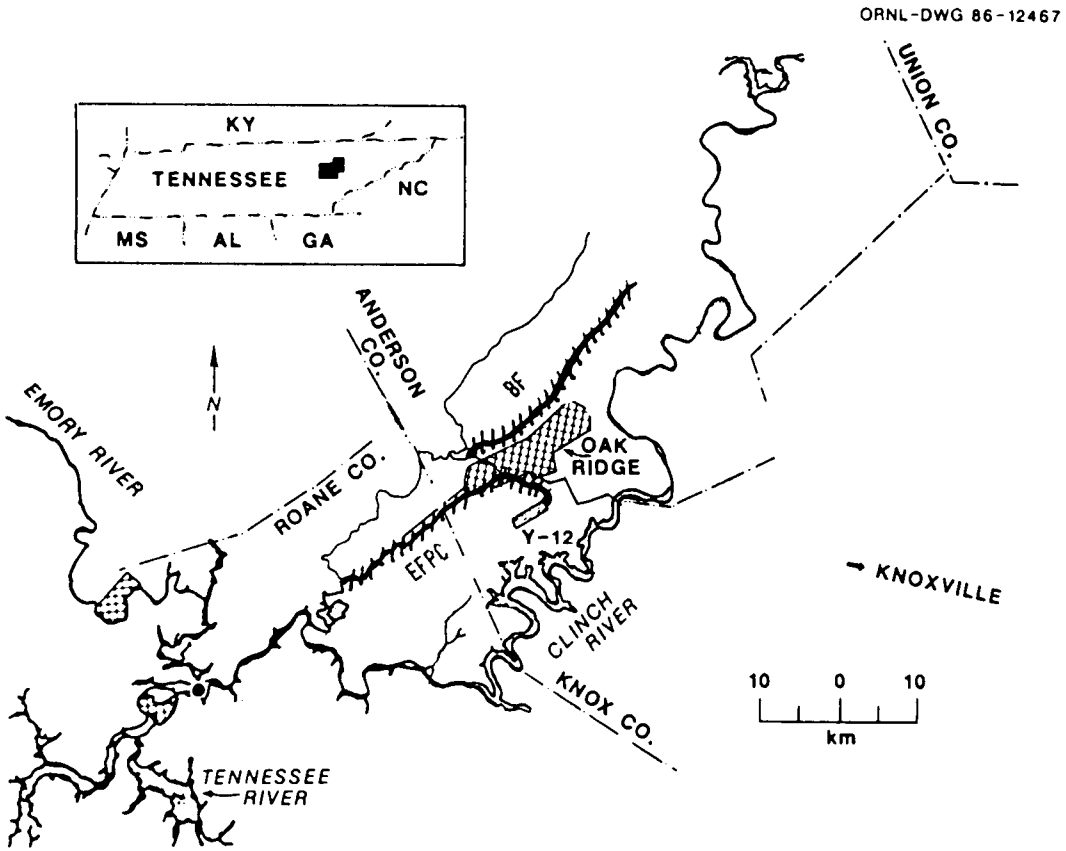


Figure II. Map of the study area showing the test streams, East Fork Poplar Creek (EFPC) and Brushy Fork (BF).

similar to EFPC. Brushy Fork, however, does not receive inputs of industrial or municipal discharges.

The Respirometer

To determine oxygen consumption, a continuous-flow respirometer was constructed and consisted of the following components: 1) two respiration chambers, 2) a reservoir tank, 3) two precision metering pumps for water circulation, 4) in-line dissolved oxygen (DO) probes to monitor the oxygen level of water entering and leaving the chambers, 5) in-tank thermocouples for temperature monitoring, 6) in-tank electrodes, amplifier, and strip chart recorder to monitor ventilatory activity and gross movement, 7) an analog/digital interface to read the DO and temperature sensors, and 8) a microcomputer to control the interfacing device and to log the data to disk (Figure III).

The respiration chambers (12 L) are sized to allow some movement of the fish and to avoid confinement stress. They are vertical cylinders (17 cm x 29 cm diam) constructed of opaque PVC except for a plexiglass port in the top for insertion and removal of the fish. The water enters the bottom of the chamber through a distribution manifold to insure adequate mixing and exits through a line in the top.

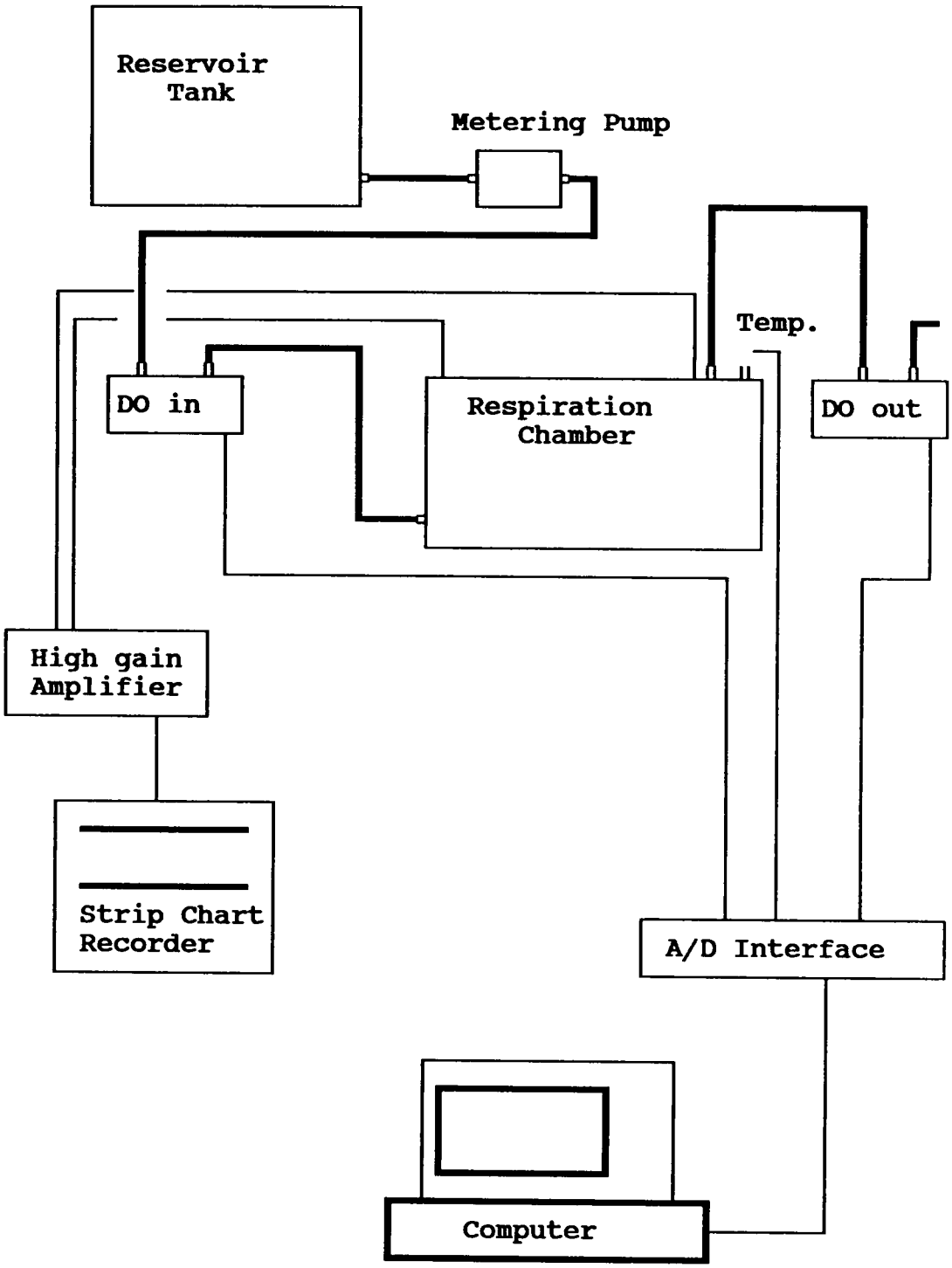


Figure III. Schematic of the Apparatus Used to Measure Standard Metabolism in the Redbreast Sunfish

Water collected in the field was held in a 200-L reservoir tank where temperature was controlled by a thermostatic heating element and aeration was provided to assure constant temperature and dissolved oxygen levels throughout each test. To insure constant flow conditions through the chambers, precision metering pumps (FMI Lab Pump Model RP-D) were used to pump the water from the reservoir through the chambers. The flow rate can be varied over a range of 0-113 $ml \cdot min^{-1}$.

The dissolved oxygen of the water entering and leaving the chambers was monitored by Yellow Springs Instrument (YSI) Industrial Products Division Model 5501 polarographic DO sensors connected to YSI Model 1255 transmitters. These probes were mounted in glass bottles containing a stirring bar and set over a magnetic stirrer so that the stirring circulated the water past the sensor with sufficient velocity to allow maximum oxygen diffusion through the membrane. Thermocouples in each tank monitored the temperature. The signal was processed by a thermocouple to analog converter (Omega TAC-386).

To monitor the ventilatory frequency and the locomotor movements of the fish, electrodes (1.0 mm diam. stainless steel wire) were placed in the tank near the walls on either side. These electrodes were connected to a high-gain amplifier (designed and built by ORNL Instrument and Controls Shop) which amplifies the potential differences

that develop between the electrodes with each respiratory or other movement of the fish (Spoor et al. 1971, Spoor and Drummond 1972). The resulting signal is traced on a chart recorder.

The DO transmitters and thermocouples are connected to an Omega WB-40 analog/digital interface which received the analog voltage output of the sensors and converted it to digital output. This output was sent via serial communications port to a Zenith Z-181 microcomputer where it was logged to disk for subsequent retrieval and analysis. The microcomputer also controlled (through the analog/digital interface) the rate and order in which the temperature and DO sensors were read.

Test Procedure for Metabolism Determination

Standard procedures were developed and followed for all standard metabolism experiments. Before each test, the DO probes were calibrated according to the procedure recommended by the manufacturer. The electrolyte and teflon membranes were changed after every series of tests or after two weeks. To initiate an experiment, two fish were netted and immediately anesthetized in a 50.0 $mg \cdot L^{-1}$ solution of MS-222 (tricane methanesulfonate), then weighed and placed in the respirometers containing water at the acclimation temperature. Food was withheld for 24 h prior to an experiment to insure a post-absorptive

state. The lids of the respirometers were fastened, pumps started, and air purged from the chambers and supply tubes. The flow rate of water through the chambers was set to 3.5-4.5 $L \cdot h^{-1}$, a rate which usually resulted in a 1-2 $mg \cdot O_2 \cdot L^{-1}$ drop in oxygen concentration between the inflows and outflows of the chambers. After purging, the WB-40 interface was set to read DO in, DO out, and temperature for each chamber at one minute intervals. These readings were converted from analog to digital and sent to the microcomputer where they were logged to disk. The standard oxygen consumption was calculated by using the formula:

$$SM = (RO_2) \cdot (V) \qquad (1)$$

where SM = standard metabolic rate ($mg\ O_2 \cdot h^{-1}$), RO_2 = oxygen consumption ($mg\ O_2 \cdot L^{-1}$) and V = water flow rate ($l \cdot h^{-1}$). To facilitate comparisons of rates among fish of different sizes, the specific standard metabolic rate, SSM, is often calculated as:

$$SSM = SM \cdot W^{-1} \qquad (2)$$

where M = weight in kg.

After 24 h, the readings for the period were examined to determine if the metabolic rate, as reflected by oxygen consumption, had stabilized. In virtually all cases, the metabolic rate peaked and subsided to a constant level 8-10 h into the test. Examination of stripchart recordings of movement indicated little locomotor activity

after the first 2-4 h in the chamber. Only ventilatory movement was detected after the initial acclimation period. A graph of the results of a typical test is shown in Figure IV. To account for the lag between the actual response of the fish and the measured response of the system, the measured values were adjusted by using the method suggested by Niimi (1978). If the oxygen consumption rate had reached and maintained equilibrium within 24 h, the fish was removed to the holding tank and the next test begun.

In all cases, the initial test was performed with a fish in one chamber and the second chamber "blank", with no fish, to determine if any biological activity in the water might influence the results. After this initial test, where no activity was found in the blank respirometer, all tests were performed using two fish, one in each chamber.

Temperature/Weight-Metabolism Relationship

Redbreast sunfish were collected by electrofishing from the reference stream, Brushy Fork, and held in four well-aerated, continuous-flow holding tanks (Living Stream, Model LS-700) in the lab. During the recovery period, the water in these tanks was kept at 18°C, the approximate mean temperature of the stream at time of capture. Twelve fish were held in each tank and fed daily with Purina Trout Chow (No. 4). After allowing 30 days to recover

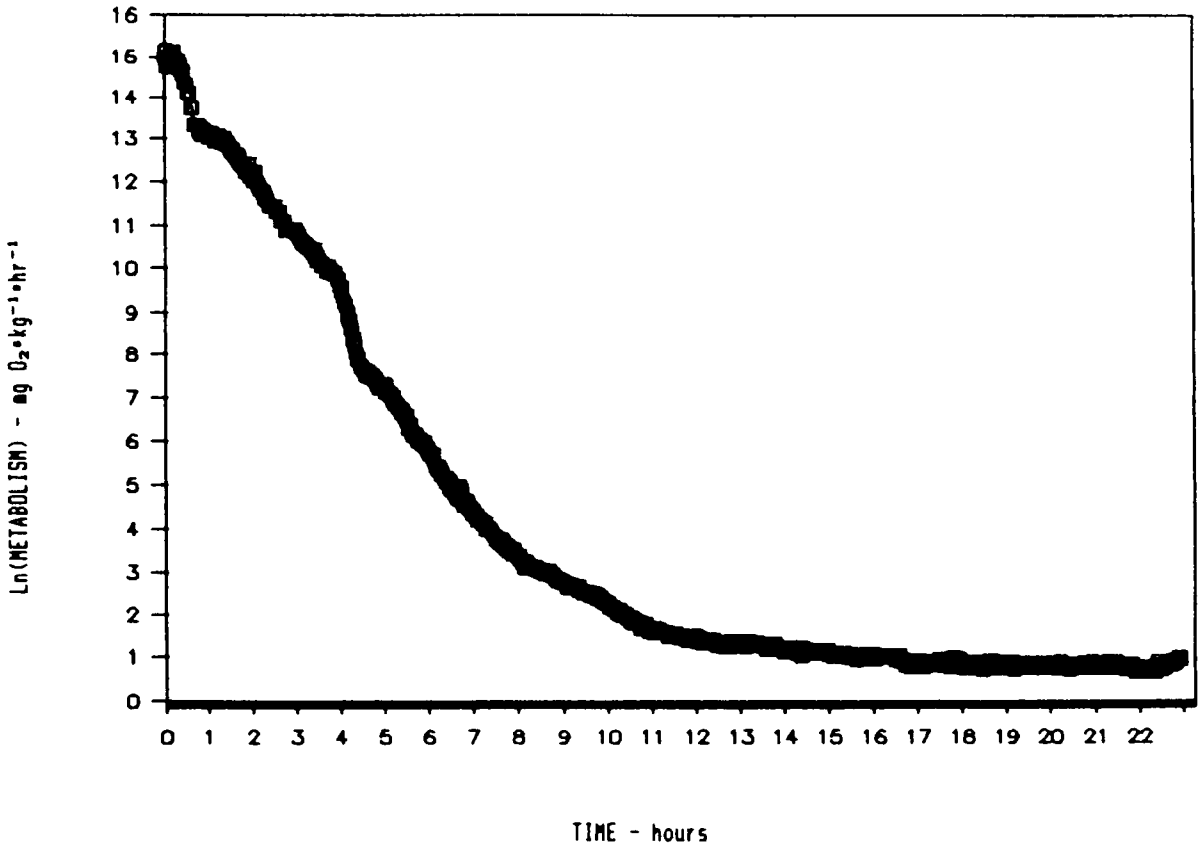


Figure IV. Typical diel oxygen consumption for redbreast sunfish (Lepomis auritus).

from the trauma of capture and handling, groups of fish were acclimated in each of the four tanks. Fish were chosen so that the range of weights (30 g to 185 g) of the entire group was represented in each tank group and that the mean weights of each group were similar. The acclimation temperatures (12°, 18°, 22°, 28° C) were chosen to approximate the normal temperature range the fish were likely to experience in the wild. Temperature changes during acclimation were 1°C per day, and fish were held at the acclimation temperature (±1.0°C) for at least 2 months before testing. Sixteen fish weighing between 34 g and 183 g (95.5 g mean weight) were tested using the previously described procedure. The tests at 12°, 18°, and 28°C were performed with the respiration chambers immersed in temperature controlled baths, a step not necessary for the 22°C test which was near ambient laboratory temperature.

Electrofishing Effects

Although it was demonstrated that occasional (biweekly) electroshocking had no effects on growth (Gatz and Adams, 1987), it was necessary to determine the recovery time of metabolic rate after electrofishing. To accomplish this, six fish (70 g mean weight) which had been held in laboratory tanks for six months were shocked with the same electrofishing unit used in the field (Smith-Root Model

15-A) for a duration and intensity comparable to that used in the field. One fish was immediately removed and tested. Test fish were removed after each succeeding day so that fish representing recoveries ranging from zero to nine days were tested. The activity and metabolic rate were monitored for 24 h and time to stabilization was recorded.

Stream Tests

To investigate intersite differences and to determine the presence of acute or chronic water quality effects on metabolic rate, a series of tests were performed on fish collected from EFPC and from Brushy Fork Creek, the reference stream. Initially, ten redbreast sunfish (77 g mean weight) were taken by electrofishing from the site just below the outfall of New Hope Pond. These fish were placed in cages in the stream in an area of sufficient depth and flow to minimize confinement stress, and allowed to recover 24 h prior to testing.

For each test, water was pumped from the stream into clean carboys, a fish was removed from the in-stream cage and anesthetized in a 50.0 $mg \cdot L^{-1}$ solution of MS-222, and the test water and fish were taken to the laboratory. At the laboratory, the test fish was weighed and placed in the respiration chamber where the metabolic rate was measured as the fish was exposed to EFPC water. At the

time of the tests, EFPC had daily temperature fluctuations between approximately 18°C and 28°C and BF fluctuated between 18°and 25°C; therefore all tests on both EFPC and BF fish were done at 23°C to remove the confounding effect of temperature and to facilitate comparison among sites. Diana (1983) determined that (for largemouth bass) fish undergoing daily temperature fluctuations react similarly metabolically to fish acclimated to constant temperature. That is, if the metabolic rate of a fish is measured at a temperature within its daily temperature regime, metabolism will be indistinguishable from that of a fish held at that constant temperature within those same limits.

The fish used in this study were not fed during their 10 d confinement, but this length of food deprivation should not have influenced standard metabolism since fish starved for periods as long as 25 d exhibited no demonstrable effect on standard metabolic rate (Beamish 1964).

Initial laboratory tests indicated that the metabolic rate stabilized within 8 to 12 h of entering the test chamber, and then remained relatively constant. Recordings of the output of the in-tank electrodes confirmed that locomotor activity was virtually absent after the test fish had been in the chamber for 4-6 h. Oxygen consumption was measured every minute over the duration of

the test and the minimum after stabilization was taken to be the standard metabolic rate. At the conclusion of each test, the fish was placed in a stock tank of de-chlorinated tap water maintained at the acclimation temperature of the fish to allow recovery from any short-term metabolic changes due to toxicants in the test water. This procedure continued daily until ten fish had been tested. These fish were then retested in tap water in the same order as the first series of tests to determine if differences in standard metabolism were due to acute effects of water quality or chronic physiological changes that would not be alleviated by a brief recovery period in clean water. The first fish tested in stream water was also the first fish tested in clean water to insure that each animal had the same recovery time.

At the conclusion of the EFPC tests, the fish were returned to the stream and the entire procedure was repeated with fish (83 g mean weight) from the reference stream. Care was taken to insure that the capture, handling, caging, and time from capture to testing for control fish were the same as for EFPC fish. This procedure insured that any effects of caging, feeding regimes, handling, etc. were comparable between sites. The experimental protocol for EFPC and BF is illustrated in Figure V.

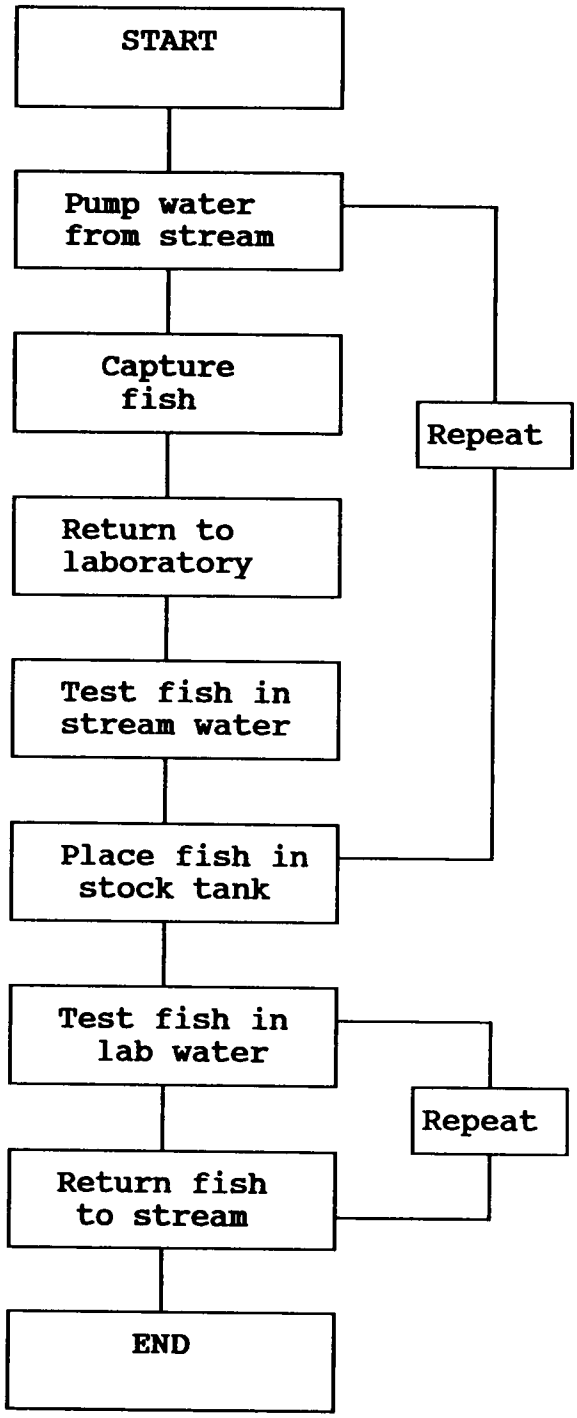


Figure V. Experimental protocol for testing the effects of East Fork Poplar Creek and Brushy Fork water on the Standard Metabolism of Redbreast Sunfish.

Simulated Storm Events

To investigate the effects of storm events which may mobilize toxicants from the sediment, seven fish (77 g mean weight) that had been held in laboratory tanks for eight months were tested in EFPC water in which sediment from New Hope Pond was suspended. The sediment samples were taken from the top 3 cm of the bottom of the pond near the outfall. Table I summarizes concentrations of metals, organics, and phenols measured in a typical sediment sample from EFPC. The total suspended solids (TSS) concentration in the test water was maintained at approximately 500 $mg \cdot l^{-1}$, a level comparable to that expected in the stream after a one-inch rainfall (Tennessee Valley Authority 1985c). All test temperatures were 23°C.

Tests Using Water from EFPC Above New Hope Pond

Water from upper EFPC above New Hope Pond, which consists almost entirely of effluent from the Y-12 Plant, was collected and returned to the laboratory. Table II summarizes a typical water quality analysis of this section of EFPC. Ten redbreast sunfish (79 g mean weight), which had been held in laboratory tanks for eight months, were tested in this water at their acclimation temperature (23°C) using the standard procedures.

Table I. Contaminants in New Hope Pond sediment. Summary of the major heavy metals and organic compounds in the fine particle surface layer.[a]

Parameter	Max	Min	Mean
Heavy Metals (ppm)			
Mercury	165.0	11.0	40.0
Arsenic	14.0	3.8	6.9
Cadmium	8.2	0.5	1.6
Chromium	58.0	24.0	37.0
Lead	170.0	36.0	80.0
Nickel	74.0	20.0	37.0
Silver	45.0	2.0	8.0
Zirconium	590.0	350.0	448.0
Organics (ppb)			
PCB 1254	2,000	200	680
PCB 1260	4,000	200	850
Fluoranthene	4,600	870	1,850
Benzo-a-pyrene	900	900	900
Chrysene	920	920	920
Anthracene	1,000	1,000	1,000

[a]Tennessee Valley Authority (1985b)

Table II. Contaminants in EFPC above New Hope Pond. Summary of chlorine and ICP metals analysis for water from upper East Fork Poplar Creek above New Hope Pond.[a]

Parameter (mg/l)	Mean	s.d.
Total Residual Chlorine[b]	0.19	0.15
Barium	0.043	0.016
Calcium	46.50	9.110
Magnesium	10.53	2.470
Manganese	0.034	0.026
Molybdenum	0.119	0.038
Sodium	13.33	0.577
Phosphorus	0.455	0.221
Silicon	1.748	0.815
Strontium	0.143	0.025

[a]Means of quarterly samples taken 8/86-7/87, A. J. Stewart, unpublished data.

Statistical Procedures

The relationships of temperature and body weight to standard metabolic rate were examined by multiple linear regression analysis (General Linear Model, SAS Institute, Cary, NC). The stream tests, simulated storm event tests, and upper EFPC water tests were also analyzed by this least squares linear regression procedure, but to account for differences in metabolic rate between the test groups (EFPC vs. BF, sediment exposed vs. not exposed, etc.), a "dummy" or indicator variable multiple regression analysis was used (Younger 1985). For example, the dummy variable (X_2) was assigned the values "1" for EFPC and "0" for BF.

The resulting model is:

$$Ln(M) = a + b \cdot Ln(W) + c \cdot X_2 \qquad (3)$$

where:

M = specific standard metabolic rate in mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$

a = regression constant

W = weight in grams

b = weight constant

X_2 = dummy variable

c = dummy variable constant

and:

X_2 = 0 if BF or,

X_2 = 1 if EFPC

To predict metabolic rate for a fish from BF,

$$\begin{aligned} Ln(M) &= a + b \cdot Ln(W) + c \cdot (0) \\ &= a + b \cdot Ln(W) \end{aligned} \qquad (4)$$

which is essentially a simple regression for BF fish. To predict metabolic rate for EFPC fish ($X_2 = 1$), the regression equation is:

$$\begin{aligned} Ln(M) &= a + b \cdot Ln(W) + c \cdot (1) \\ &= (a + c) + b \cdot Ln(W) \end{aligned} \qquad (5)$$

The coefficient "c" represents the difference in magnitude of the metabolic rates of EFPC fish and BF fish.

This procedure incorporates all features of two separate regressions into one multiple regression, and an F-test on the regression coefficient of the dummy variable ("c") reveals whether there is any significant difference in metabolic rates between the two groups.

III. RESULTS

Temperature/Weight-Metabolism Relationship

Experiments to relate temperature and body size to oxygen consumption were performed on fish acclimated at four temperatures (12°, 18°, 22°, 28°C) and with a mean weight of 95 g. Body weight and temperature were found to significantly affect standard metabolism. The relationship between body weight, temperature, and metabolic rate can be described as:

$$\text{mg } O_2 \cdot kg^{-1} \cdot h^{-1} = a \cdot W^b \cdot e^{c \cdot T} \quad (6)$$

or in linear form:

$$Ln(\text{mg } O_2 \cdot kg^{-1} \cdot h^{-1}) = Ln(a) + b \cdot Ln(W) + c \cdot T \quad (7)$$

where:

M = Specific Standard Metabolic rate in mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$

a = regression constant

W = weight in grams

b = weight constant

T = temperature in °C

c = temperature constant

After exponential transformation to linearize the data, multiple linear regression by the method of least squares

using the General Linear Model yielded the relationship:

$$M = 240.76 \cdot W^{-0.665} \cdot e^{0.0568 \cdot T} \quad (8)$$

or

$$Ln(M) = 5.844 - 0.665 \cdot Ln(W) + 0.0568 \cdot T \quad (9)$$

These values compare favorably with previously published information on oxygen consumption of closely related species (Evans 1984, Brett and Sutherland 1965, Roberts 1964, Wohlschlag and Juliano 1959) (Figures VI and VII).

Electrofishing Effects

Of the six fish that were electroshocked, only the individual tested immediately after electroshocking failed to reach a stable metabolic rate within 24 h. The other five reached equilibrium six to ten hours after being placed in the respiration chamber, a response comparable to recovery from handling stress (Moss and Scott 1961, Smit 1965, Randall and Smith 1967, Hoelton 1974) and comparable to recovery times recorded in the temperature/weight-metabolism tests. These results indicated a 24 h recovery period after shocking was sufficient to allow the standard metabolic rate to stabilize.

Stream Tests

Fish from the reference stream, Brushy Fork Creek, and from the polluted stream, East Fork Poplar Creek, were

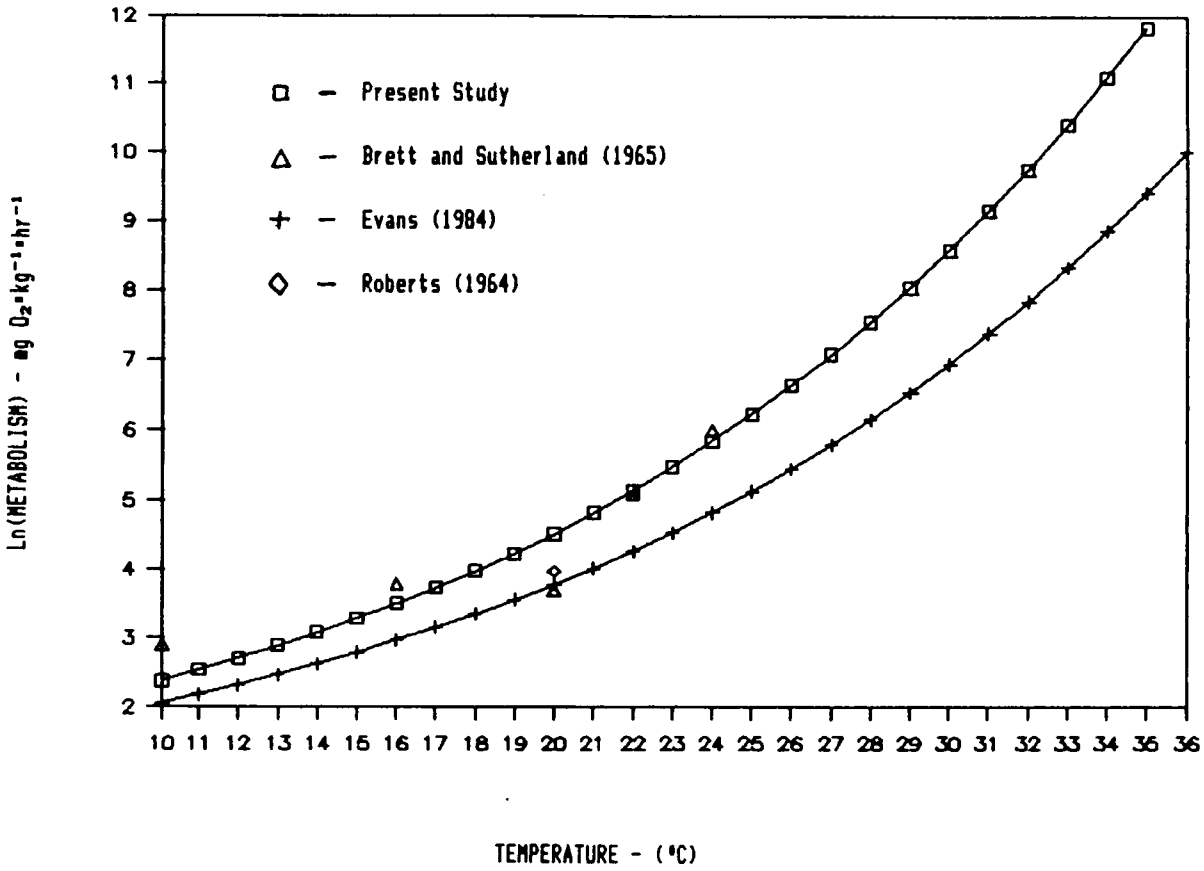


Figure VI. Relationship between oxygen consumption and temperature for sunfish. All data correspond to a 100 g fish.

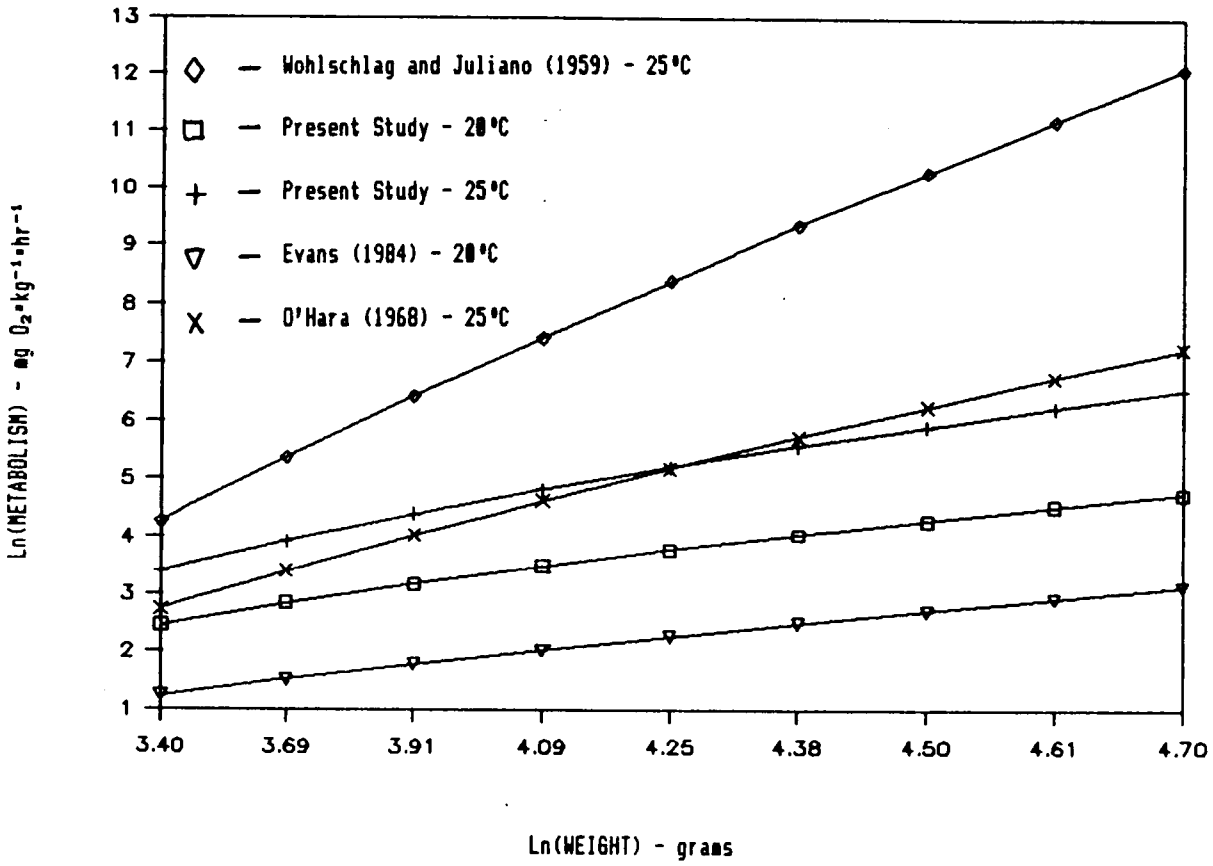


Figure VII. Relationship between body weight and oxygen consumption for sunfish.

tested in water from their home stream and in de-chlorinated tap water. The results of these tests are shown in Tables III and IV and Figure VIII.

Analysis of the results indicates no significant difference between the standard metabolic rate of EFPC and BF fish tested in either their home stream water or dechlorinated tap water.

Simulated Storm Events

The results of this series of tests are shown in Table V and Figure VIII. Under conditions of this study, the metabolic rate of these fish was statistically indistinguishable from that determined for the BF control group. The dummy variable, representing sediment exposure or no exposure, did not add significantly to the regression. It should be noted, however, that although the sediment was maintained in suspension in the reservoir tank, some settling was inevitable in the respiration chambers and the actual exposure concentration may have been somewhat less than in the stream during an actual storm event.

Tests Using Water from EFPC Above New Hope Pond

The ten fish tested in water from upper EFPC above New Hope Pond containing the effluent from the Y-12 Plant had significantly lower metabolic rates than any other group

Table III. Results of home stream water tests. Summary of the "dummy" variable multiple regression analysis of the independent variables weight and test group (EFPC or BF) and the dependent variable metabolic rate for EFPC and BF redbreast sunfish tested in their home stream water.

ANOVA Table

Source of Variation	df	Sum of Squares	Mean Square	F Value
Model	2	0.80426	0.40213	16.27*
Error	13	0.32140	0.02472	
Total	15	1.12566		

Regression Model

Source	df	Regression coefficient	Partial F
LnW	1	-0.55258	31.75*
Stream	1	0.07735	0.78[a]

*Significant at $P \leq 0.001$.

[a]-No significant difference (at $P \leq 0.05$) between EFPC fish and BF fish.

Table IV. Results of de-chlorinated water tests. Summary of the "dummy" variable multiple regression analysis of the independent variables weight and test group (EFPC or BF) and the dependent variable metabolic rate for EFPC and BF redbreast sunfish tested in de-chlorinated tap water.

ANOVA Table

Source of Variation	df	Sum of Squares	Mean Square	F Value
Model	2	1.64193	0.82097	80.95*
Error	12	0.12170	0.01014	
Total	14	1.76363		

Regression Model

Source	df	Regression coefficient	Partial F
LnW	1	-0.73912	61.66*
Stream	1	0.02816	0.24[a]

*Significant at $P \leq 0.001$.

[a]-No significant difference (at $P \leq 0.05$) between EFPC and BF fish.

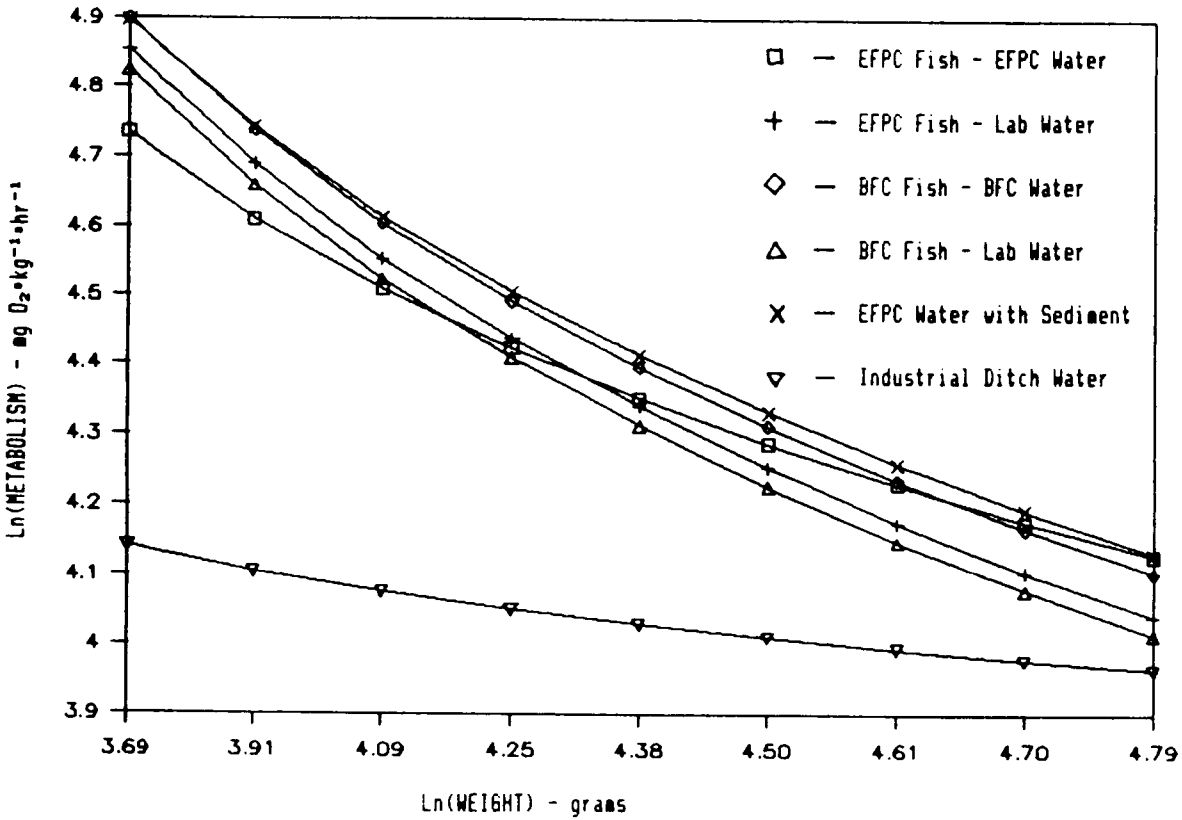


Figure VIII. Relationship between body weight and oxygen consumption for redbreast sunfish (Lepomis auritus).

Table V. Results of simulated storm event tests. Summary of the "dummy" variable multiple regression analysis of the independent variables weight and test group (sediment exposed and not sediment exposed) and the dependent variable metabolic rate for control redbreast sunfish tested in EFPC stream water containing contaminated sediment.

ANOVA Table

Source of Variation	df	Sum of Squares	Mean Square	F Value
Model	2	2.65475	1.32737	78.17*
Error	35	0.59433	0.01698	
Total	37	3.24908		

Regression Model

Source	df	Regression coefficient	Partial F
LnW	1	-0.63511	155.66*
Sediment	1	0.04658	0.67[a]

*Significant at $P \leq 0.001$.

[a]-No significant difference (at $P \leq 0.05$) due to sediment exposure.

tested (Figure VIII, Table VI). The dummy variable (exposure to upper EFPC water or no exposure) added significantly to the regression. When removed from the chambers, these fish were covered with a heavy mucous layer that was obviously much greater than fish from any other test group.

Table VI. Results of Upper EFPC water tests. Summary of the "dummy" variable multiple regression analysis of the independent variables weight and test group (upper EFPC water exposed and not exposed) and the dependent variable metabolic rate for redbreast sunfish from a reference stream (Brushy Fork) tested in water from upper EFPC above New Hope Pond.

ANOVA Table

Source of Variation	df	Sum of Squares	Mean Square	F Value
Model	2	3.67377	1.83689	63.27*
Error	38	1.10331	0.02903	
Total	40	4.77708		

Regression Model

Source	df	Regression coefficient	Partial F
LnW	1	-0.53312	88.28*
EFPC Water	1	0.38642	38.25*[a]

*Significant at $P \leq 0.001$.

[a]-Significant difference (at $P \leq 0.001$) due to upper EFPC water.

IV. DISCUSSION

When exposed to stressors, such as water-borne toxicants, organisms respond with a series of physiological modifications that have been designated the General Adaptation Syndrome (GAS) (Seyle 1973). The three phases of the GAS are: 1) an alarm reaction which occurs soon (within seconds to minutes) after exposure to the stressor, 2) an adaptation period in which physiological processes are modified in an attempt to maintain homeostasis and, if the stress is too great in length or severity, 3) exhaustion occurs, adaptation is lost, and death is likely as physiological homeostasis fails.

Since the first phase is transient, and the third is usually fatal, the second response is the one that may have long-term ecological consequences. This response may lead to adaptation followed by establishment of a new stabilized condition which, if the environmental change continues, may be followed by acclimation and return to the original level.

The significant reduction in metabolic rate in fish tested in water from EFPC above New Hope Pond is probably at the expense of essential physiological processes and thus cannot be maintained indefinitely. This reduction in rate implies a Phase Three GAS response that will result in death if the exposure is continued. Exposure to copper

and mercury (Hughes 1976), zinc sulfate (Skidmore 1970), and chlorine (Bass et al. 1977) have resulted in depressed metabolic rates in fish. In all these cases, the reduction appears to be an indirect effect of gill damage or excess mucous production. The effect of these toxicants is to interfere with the oxygen transfer at the water-gill interface. Because the gill is the major resistance to gas transfer, any interference at this point can have severe repercussions, notably reduced arterial oxygen levels leading to a shift to anaerobic metabolism. This arterial hypoxia leads to accumulation of blood lactic acid, decline in blood pH, and eventual acute tissue damage and death (Bass and Heath 1977). These reactions are similar to those following reductions in environmental oxygen (Hughes 1981).

The water in upper EFPC above New Hope Pond contains a variety of toxicants, but of those known to depress metabolic rate, only chlorine was present at elevated concentrations (Table II). The mercury is primarily found complexed to organic matter and not readily taken up by the fish (Lock 1975). Chlorine in this reach of EFPC occurs as free chlorine and monochloramine, both of which can induce excessive epidermal mucous formation and deleterious histopathological changes in the gills (Bass and Heath 1977, Bass et al. 1977). The increase in epidermal mucous production and the lowered standard

oxygen consumption rate noted in my study are typical responses to elevated chlorine levels (Brungs 1973). Total residual chlorine concentrations in this section of the stream typically average > 0.20 $mg \cdot L^{-1}$ with peak levels of > 0.65 $mg \cdot L^{-1}$ (A. J. Stewart, unpublished data). These values are considerably greater than Environmental Protection Agency criteria which suggest a maximum total residual chlorine concentration of 0.01 $mg \cdot L^{-1}$ for protection of aquatic life. Bluegill (Lepomis macrochirus) exposed to concentrations of chlorine similar to levels found in this section of the stream have exhibited an increase in the number of mucous cells and deleterious histopathological changes in the gills leading to a severe reduction in respiratory gas transport (Bass and Heath 1976, Bass et al. 1977). Exposure to these levels (0.20 $mg \cdot L^{-1}$) did not cause death in seven days exposure, but the resulting gill damage and reduced respiration efficiency would inevitably be lethal with continued exposure. Exposure to slightly higher concentrations of total chlorine (0.40 $mg \cdot L^{-1}$) can result in death from suffocation, a result of accumulation of mucous in the gills and acute tissue damage from hypoxia (Bass et al. 1977). This evidence points to blockage of oxygen uptake in the gills caused by chlorine damage as the primary cause of the reduction in metabolic rates seen in this study. Continued exposure to this level of

chlorine would lead to death, a conclusion consistent with the observation that this section of the stream supports no resident fish population (J. M. Loar, unpublished data).

This study found no statistically significant difference in standard metabolic rate of fish between the reference site and EFPC site below the outfall of New Hope Pond, indicating the fish (1) are not stressed (or this possibility cannot be rejected), (2) have complete physiological compensation to chronic stress, or (3) do not exhibit metabolic changes as a result of chronic sub-lethal stress.

There is good reason to believe, however, these fish are indeed stressed. Water quality analysis (Tennessee Valley Authority 1985c, A. J. Stewart unpublished data) gives evidence that the fish are periodically exposed to toxicants (e.g. mercury and cadmium) at levels which have been shown to result in significant elevations in metabolic rate (Calabrese et al. 1975, O'Hara 1971b, Sastry and Miller 1981). In addition, Adams et al. (in press) have found significant differences in toxicant induced stress response variables between fish collected in EFPC immediately below New Hope Pond and those collected at other sites farther downstream. Complete compensation infers physiological changes that allow the fish to maintain homeostasis and normal functioning in a

polluted environment with no metabolic penalty. The temporal heterogeneity of both the concentration and composition of the stressors in this stream would make such compensation highly unlikely. It appears probable that chronic sub-lethal stress has little or no significant effect on standard metabolic rate on fish in EFPC, and any metabolic costs incurred are a result of transient acute exposure to toxicants rather than long-term chronic exposure.

The presence of pulses of toxicants such as chlorine in the stream would lend credence to the likelihood that the fish in East Fork Poplar Creek are subject to transient acute stress at sublethal levels rather than chronic sublethal stress. The section of stream above New Hope Pond is subject to frequent periods of high chlorine concentrations that are toxic to Ceriodaphnia and fathead minnows. (A. J. Stewart, unpublished data). In most instances, the retention of the effluent in New Hope Pond probably reduces the concentration of the highly reactive chlorine to sub-acute levels. Consequently, fish downstream from New Hope Pond probably experience periodic pulses of chlorine at acute levels only when the concentration entering the pond is unusually high, and the residence time insufficient to dissipate the chlorine. This section of EFPC supports a population of sunfish which are apparently reproducing, but are significantly

different both biochemically and physiologically from fish in the reference stream, and from fish at other sites farther downstream. For these fish, the energetic costs of exposure are transient rather than chronic, but these costs could still significantly affect the total energy budget during reproductive activities and other times of high energy demands. If pulses of toxicants occur during critical periods such as spawning, the ecological consequences could be particularly severe. The transient chlorine exposures may stress physiological systems beyond the level of recovery and cause the biochemical and condition differences noted between the redbreast sunfish immediately below New Hope Pond and those from other sites downstream (Adams et al. in press). Exposure to toxic chloramines, formed when free chlorine reacts with ammonia compounds, may also contribute to the stress regime these fish encounter.

The energetic costs of transient exposure to toxicants will be difficult to assess without a continuous in situ monitoring system. In addition to metabolic costs, transient exposure to toxicants can have other deleterious effects such as reduction in feeding that last far longer than the time of exposure (Heath 1987). A monitoring program that relies only on periodic sampling may detect the effects of toxicant stress (reduced growth, impaired reproduction, etc.) without identifying and quantifying

the transient exposures that are the underlying cause. An in situ system which continuously monitors oxygen consumption and respiration rate is useful in relating temporal changes in the animal to those in its environment.

Fish in the section of EFPC immediately downstream from New Hope Pond probably are subjected to occasional episodes of toxicant induced stress which cause increased energetic demands due to the Phase One (alarm reaction) and Phase Two (physiological adaptation) General Adaptation Syndrome reactions. In addition these fish are probably periodically subjected to acutely toxic levels of pollutants which elicit a Phase Three response. The transient nature of the acutely toxic exposure prevents mortality from being the usual result except in cases when this stressor is combined with others such as overcrowding, unusual temperatures, or bacterial infection. These combinations of stressors may result in fish kills such as those recently noted in this section of EFPC (Loar et al. 1987).

V. SUMMARY AND CONCLUSIONS

The principal findings of this study are:

1) The relationship between weight of redbreast sunfish, temperature and specific standard metabolism is given by the equation:

$$M = 240.76 \cdot W^{-0.665} \cdot e^{0.0568 \cdot T}$$

This relationship agrees well with values determined for other species in this genus and will be used in developing a bioenergetics model for this species.

2) Effects of electrofishing on standard metabolic rate last for no more than 24 h. After this period, metabolic rate reaches equilibrium in 8 - 12 h, a time comparable to fish that have not been electroshocked.

3) Under the conditions of the test, no significant difference in standard metabolic rate was found between fish from a polluted stream (East Fork Poplar Creek) and an unpolluted stream (Brushy Fork). These results imply that standard metabolic rate is not an adequate indicator of the effects of chronic exposure to sub-lethal stressors for fish in East Fork Poplar Creek.

4) Fish tested in stream water which consists mainly of industrial effluent and supports no resident fish population have reduced standard metabolic rates. This reduction appears to be an indirect result of gill damage

or excessive mucous production which interferes with oxygen exchange at the water-gill interface. This impairment of oxygen uptake may lead to hypoxia, changes in major physiological processes, tissue damage and, if of sufficient duration, death. The deleterious effects noted are probably due to high levels of total residual chlorine in the industrial effluent.

5) Retention of the industrial effluent in New Hope Pond probably allows the highly reactive chlorine to dissipate so that the fish below New Hope Pond are not subject to acute concentrations of chlorine. Periodic sub-lethal exposure to other contaminants, however, may be responsible for differences in biochemical variables and condition indices between fish at this site and those of other sites along EFPC and BF.

6) The transient nature of the toxicant concentration dynamics in EFPC suggests that an in situ continuous monitoring system could be a valuable biomonitoring tool in evaluating the significance of these episodic events on the integrated stress response of fish populations.

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