Physiological, Population, and Genetic Responses of an Aquatic Insect (*Isonychia bicolor*) to Chronic Mercury Pollution

A Dissertation submitted to the graduate faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

by

Craig David Snyder

APPROVED

Albert C. Hendricks, Chairman

Ernest F. Benfield

George M. Simmons

Bruce Wallace

Bruce Turner

J. R. Cowles, Head

December 1992
Blacksburg, Virginia

Physiological, Population, and Genetic Responses of an Aquatic Insect (Isonychia bicolor) to Chronic Mercury Pollution

by

Craig D. Snyder

Chair: Dr. Ablert C. Hendricks

(ABSTRACT)

Responses to sublethal concentrations of mercury were determined and compared for several populations of the mayfly, Isonychia bicolor from sites on two mercury polluted rivers in Virginia (USA). Results from laboratory respiration experiments indicated that small nymphs were more sensitive to mercury than larger nymphs, and that sensitivity increased with temperature. In addition, mayfly nymphs from polluted sites on the less contaminated South River were found to be more resistant to sublethal doses of inorganic mercury than nymphs from a reference site as indicated by smaller changes in respiration following exposure. Results of preexposure treatments suggested a genetic basis for the observed tolerance. No evidence of tolerance was observed in nymphs from the more contaminated Holston River.

Field surveys revealed no significant differences in age structure, growth, or survival between populations at reference and polluted sites on the South River. However,

growth and survival of nymphs from the polluted site on the Holston River were significantly slower than at the reference site during the summer. In fact, shortly after the summer generation hatched, nymphal density at contaminated sites declined to zero. This apparent local extinction may have been due to increased toxicity of mercury at warmer temperatures.

Results of reciprocal transplant experiments conducted at sites on the South River suggested that population differences in maintenance costs observed in short-term laboratory experiments, may have been reflected by population differences in fecundity in long-term field experiments. When nymphs from the reference site on South River were transplanted and allowed to develop at a contaminated site, lower fecundities were observed than for the native population. No population differences in fecundities were observed on the Holston River.

The relationship between allozyme genotype to survival of I. bicolor nymphs to acute mercury exposure was also tested. The probability of survival and individual times to death (TTD) were found to be significantly different among genotypes at the Glucose Phosphate Isomerase (GPI) locus. This was true at both summer and winter temperatures and for populations from two separate, unpolluted streams. However, genotypes identified as sensitive and tolerant in these experiments showed no consistent relationship with environmental mercury levels in polluted rivers. Therefore,

the use of allozyme variants as a biomarker to assess evolutionary change in populations due to mercury pollution may be impractical in freshwater systems.

ACKNOWLEDGEMENTS

Special thanks to Dr. Albert Hendricks for his guidance and support during my years at VPI. His accessibility and genuine interest in the work of his students has fostered a great respect from me. Our shared belief in a strong work ethic and his sense of fairness and humor made Dr. Hendricks a pleasure to work for. I also wish to thank the other members of my advisory committee; Drs., Benfield, Simmons, Wallace, and Turner. Their contributions were immeasurable and I only hope this document does them credit. Other members of the VPI faculty deserving special thanks are Dr. Jackson Webster for general advise on research and ecological issues, and, Dr. Robert Newman for advise on statistics and experimental design. For accepting me as a student, a collegue, and a friend, I thank Lawrence Willis and his wife Lucinda. Without question, my life and this research project were enhanced by my relationship with them.

It is with great pride that I acknowledge my parents, Richard and Frances; my brother, Thomas; and my sister, Renee. This achievement is due, in large part, to the values they instilled in me and their love and support throughout the years. And finally, for the sacrifices she has endured, her patience, and unconditional love, I thank my wife, Sharon. Without her, this achievement would have little meaning, and therefore, it is to her that I dedicate this work.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

INTRODUCTION

Pollution control and abatement programs have resulted in significant reductions in aquatic pollution problems. Most of the success has resulted from increasingly strict discharge limits designed to reduce acute toxicity of industrial and municipal effluents (Armstrong and Scott 1979). However, the fauna of polluted waters are more commonly exposed to relatively low concentrations of poisons for long periods rather than to levels of pollution which cause rapid mortality (Abel 1989). This is becoming increasingly true as pollution laws become more strict, pollution sources more diffuse, and pollutants more persistent in the environment (Mitra 1986, Mance 1987). Hence, aquatic organisms will continue to face chronic exposure to low levels of contaminants and information regarding the long term effects of such exposure is needed.

Among the most far-reaching effects are genetic changes in natural populations that result in increased resistance to the toxicant. Evolution of such tolerance is the logical consequence of two factors. First, extensive variation in the response of individuals to pollutants exists within populations (Macek and Sanders 1970, Sprague 1970) and to whatever extent this variation is genetically based, natural selection should increase the frequency of those alleles and allelic combinations conferring resistance. Secondly, the chronic, but low-level nature of most modern pollutants

suggests that while most members of a population may survive insult, sublethal effects should allow individuals with resistant genotypes a competitive advantage and favor their increase in the population (Rahel 1981).

In a recent review, Klerks and Weiss (1987) summarized what is known of metal tolerance in aquatic organisms. Only one study was cited dealing with aquatic insects (Wentsel et al. 1978). Because of the sparsity of information, and the fact that metal contamination continues to be a pervasive problem in aquatic systems, information on whether aquatic insects can adapt to these toxicants is needed.

The overall objective of this research was to determine if, and to what extent natural populations of the aquatic insect, *Isonychia bicolor*, have adapted to mercury stress in two contaminated rivers in Virginia. The project was approached pluralistically, using data from laboratory energetics experiments, field growth experiments, bioassays and genetic surveys of populations at reference and contaminated sites. Furthermore, an effort was made to link response variables at different levels of biological organization (i.e., genetic, individual, and population). Such integrative approaches should provide a clearer understanding of mechanisms and therefore add to the predictive power of toxicological studies.

Recently, some investigators have suggested an energetics

approach to the study of stress tolerance. Two competing hypotheses have been formulated which attempt to integrate physiological responses with population level processes to explain stress tolerance. Koehn and Bayne (1989) suggest that from an energetics point of view, any environmental change represents a stress when the balance between assimilation and metabolism is disturbed (i.e. net increase in maintenance costs) with a consequent reduction in "scope for growth" (i.e., production of reproductive and somatic material). This hypothesis leads to the prediction that individuals or genotypes better adapted to a particular stress will have lower metabolic rates and consequently higher "scope for growth" when exposed to that stress.

However, Sibly and Calow (1989) define two types of stress and argue that adaptive responses depend on the type incurred. They hypothesize that in resource-poor environments (i.e., growth stress) less energy should be invested in defense and more in growth. Conversely, the effect of a "mortality stress" (eg. pollution or predation) may be alleviated by larger investments in defense (eg. neutralizing and excreting toxic compounds, production of degradation enzymes, or evolution of immune systems) since this will reduce the probability of death between birth and breeding. This hypothesis leads to the alternative prediction that populations exposed to chronic pollution will evolve life

cycles that optimize survival and developmental time at the expense of individual growth. These two competing hypotheses were tested by comparing respiratory responses, growth rates, and fecundities of individuals from populations with differing histories of exposure to mercury.

Another recent approach to the study of stress tolerance centers on the use of genetic markers to measure the extent to which natural populations have changed (i.e. adapted) in response to a stress. Methods involve isolating polymorphic enzyme loci with variation in relative fitness among genotypes when exposed to a stress, and subsequently measuring the frequency of these genotypes in populations with varying histories of exposure to the stress. This approach has been used successfully to measure evolutionary changes in marine invertebrates in response to heavy metal pollution (see Nevo et al. 1983 for review). In the last three years, the use of allozyme markers has been extended to freshwater systems (Gillespie and Guttman 1989, Newman et al. 1989, Chagnon and Guttman 1989, Benton and Guttman 1990). However, only one study has focused on freshwater invertebrates (Benton and Guttman 1990) and the efficacy of this approach to freshwater organisms has not been fully evaluated. In this study the potential use of allozyme variants to measure the genetic response of natural populations of *I. bicolor* to sublethal mercury exposure was evaluated.

LITERATURE REVIEW

Mercury Toxicity

Mercury is classified as a type IIB metal along with zinc and cadmium based on its location on the periodic chart. These metals are highly electropositive and therefore have an affinity for amino, imino, and sulfhydryl groups. Metals in this group are also water soluble and thus tend to be biologically available.

At the molecular level, toxicity of type IIB metals is thought to be related to their profound capacity to chelate with sulfhydryl containing ligands (Luckey et al. 1975). Both mercury and cadmium have been found to compete with essential trace metals such as zinc and magnesium for thiol-rich binding sites in some metalloproteins and enzymes (Eichhorn 1975). By irreversibly binding to active sites they are thought to disrupt normal metabolism and behave as "toxic metals".

At the organismal level, the mode of toxicity is not well known in invertebrates. However, in fish, mercury is thought to initially affect membrane permeability and subsequently organ function and osmoregulation (Stickle et al. 1982).

In terms of relative toxicity to aquatic organisms, mercury is generally ranked as the most toxic of heavy metals (Ketchum 1975). It is well known that methylmercury is more toxic than the ionic form (Ketchum 1975, Benes and Havlik

1979) and although other forms of mercury such as HgS, Hg(II), and Hg(0) are generally more prevalent in aquatic environments, the preponderance of mercury (>90%) in the tissues of fish is in the methylated form (Huckabee et al. 1979). However, the relative amount of mercury that is methylated in the tissues of benthic invertebrates is more variable. Reports range from as low as 5% (Jernelov and Lann 1971) to as high as 76% (Huckabee and Hildebrand 1974).

Concentrations of mercury required to induce toxic effects in aquatic organisms are highly variable among taxa. Short-term toxicity tests have indicated that in general aquatic insects may be more tolerant of metal pollution than fish (Warnick and Bell 1969). However, the ecological significance of these tests is unclear because of highly unnatural conditions (eg. toxicant levels orders of magnitude higher than found in even severely polluted habitats)(Clubb et al. 1975). In tests using lower doses over longer periods of time, insects have been found to be equally sensitive to metals and in some cases more so (Spehar et al. 1978). Such results underline the importance of testing for toxic effects over a broad range of environmental conditions and life cycle stages (Elder and Gaufin 1974).

Aquatic insects also exhibit considerable variation in sensitivity. Evidence suggests that there is increasing tolerance to heavy metal pollution in the sequence from

mayflies, to caddisflies, to midges (Savage and Rabe 1973, Solbe 1977, Winner et al. 1980, Armitage 1980). Illies (1978) found that the probability that some species of a particular group will be tolerant is related to the number of species in that group. For example, the family Chironomidae (midges) is a species rich group while the order Ephemeroptera (mayflies) is relatively species poor.

Physiological Responses to Metals

A number of physiological and whole-organism processes have been found to be sensitive to metal toxicity (Bayne et al. 1979). Processes shown to be sensitive to metal exposure in benthic invertebrates include feeding rate (Capuzzo and Sasner 1977), respiration (Calabrese et al. 1977), and protein use (Widdows 1985). Reproductive processes have also been shown to be sensitive to metals because of direct effects on sexual maturation and gamete formation (Myint and Tyler 1982, Eyster and Morse 1984). To a lesser extent, growth rates of aquatic organisms have been found to be sensitive to metal exposure (eg. Hatakeyama and Yasuno 1980).

Increases in respiration rates in response to sublethal levels of chemical pollutants have been reported in the crab, *Cancer irrorattus*, to cadmium and copper (Johns and Miller 1982); in the shrimp, *Mysidopsis bahia*, to the pesticide endrin (McKenney 1982); in rainbow trout, *Salmo gardnerii*, to

cadmium (Slooff 1977); and in the mysid shrimp, *Neomysis americana*, to naphthalene (Smith and Hargreaves 1985). However, decreases in respiration in response to chemical pollution have also been reported (eg. Johnson 1977). In addition, Calabrese et al. (1977) reported both enhancement and depression of respiration rates in marine bivalves in response to sublethal exposure to heavy metals and that the direction of the response was dependent on dose.

Reduction in growth rates of benthic invertebrates have also been observed in response to chemical pollution. For example, Macek et al. (1976) found that the biocides Atrazine and Lindane retarded growth and delayed pupation and emergence of *Chironomus tentans*. Wentsel et al. (1977) using naturally contaminated sediments containing zinc and cadmium also found retarded growth in *Chironomus tentans*. Copper was found to significantly reduce growth rates in the parthenogenetic midge, *Paratanytarsus parthenogeneticus* (Hatakeyama and Yasuno 1981).

Metal Tolerance

There have been numerous reports of metal tolerance in both the flora and fauna of aquatic ecosystems. Tolerant or resistant populations of bacteria, algae, fungi, oligochaetes, mollusks, crustaceans, insects, and fish have all been isolated from contaminated habitats (see Klerks and Weiss

1987, for review). The number of examples in which metal tolerant populations have been observed suggests that responses to metals at the population level may be widespread. Klerks and Weiss (1987) even suggest most populations occupying contaminated habitats may exhibit some degree of tolerance. However, they also point out that many species that otherwise might be expected to occur could be absent from such habitats because they have lesser abilities to adapt or acclimate. Based on the extensive number of reports of metal tolerance, Luoma (1977) suggested that evidence of tolerance could be used as a biomonitoring tool in evaluating metal-contaminated environments. This is because elevated tolerance to a metal in one population, compared to other populations of the same species constitutes a metal-specific response.

Intraspecific variability in sensitivity among individuals of a population is commonly genetically based, or adaptive, in aquatic invertebrate populations (Levington 1980). In this case, increased tolerance develops as a result of natural selection for the most tolerant individuals in the population.

A genetic basis for metal tolerance has been reported in both macroinvertebrates and fish. For example, Bryan and Hummersone (1971) found individuals of the polychaete, *Nereis diversicolor*, from two copper-polluted sites to be more resistant to copper than individuals from clean sites.

Animals from clean sites were subsequently preexposed to copper. No increase in tolerance was observed suggesting that increased tolerance observed in individuals from contaminated sites was genetically based. Klerks and Levington (1987) found a similar pattern in populations of the oligochaete, *Limnodrilus hoffmeisteri*, from a site with high levels of cadmium and nickel. Offspring born and raised for two generations in clean sediment were still highly resistant, strongly suggesting a genetic basis for the observed metal tolerance.

Brown (1976) studied tolerance to copper and lead in the isopod, *Asellus meridianus*, from two rivers polluted with metals from mine drainage. Animals from a lead polluted site were found to be more resistant to copper as well as lead. However, evidence for a genetic basis for the differences in tolerance was only significant for lead.

Only a few cases of adaptation to metals have been confirmed in fish. Smith (1983) found populations of the long-nosed dace (*Rhinichthys cataractae*), from zinc contaminated sites to be significantly more resistant than populations from reference sites. Results of preexposure experiments suggested that much of difference in tolerance between populations was adaptive.

Two studies have reported metal tolerance in aquatic insects (Wentsel et al. 1978, Krantzberg and Stokes 1989).

However, neither study performed experiments to distinguish between acclimation and adaptation.

There is also evidence, particularly in fish, that variability among individuals to metals is the result of acclimation. Acclimation is defined as reversible changes in the morphology or physiology of an organism in response to some environmental change (Ricklefs 1973). In the case of metals, such compensatory changes are brought about by preexposure to low levels of metals which lead to increased resistance. The specific mechanisms of metal-acclimation may be complex involving several metabolic systems. Perhaps the most important mechanism is the stimulation of metallothionein, small sulfur-rich metal-binding proteins)(eg. Lauren and McDonald 1987). Another mechanism proposed to explain sequestering of heavy metals involves uptake into lysosomes or other storage granules (Carpene and George 1981).

Evidence of physiological compensation (i.e. acclimation) has primarily resulted from preexposure experiments. Increases in metal tolerance of a previously sensitive population as a result of previous exposure is evidence of acclimation. Likewise, because compensatory responses are reversible, preexposing individuals from metal-tolerant populations to clean conditions should result in a decrease in tolerance.

Metal tolerance has been related to physiological

acclimation in numerous species of fish. For example, tolerance to cadmium in the white sucker, *Catostomus commersoni*, (Duncan and Klaverkamp 1983); the Atlantic Salmon, *Salmo salar*, (Peterson et al. 1983); and the bluegill, *Lepomis macrochirus*, (Bishop and McIntosh 1981) were all increased by preexposure to sublethal concentrations of cadmium. In addition, Dixon and Sprague (1981) found mortality rates of the rainbow trout, *Salmo gardnerii*, exposed to arsenic were reduced following preexposure.

To date, there have been no reports of acclimation in aquatic insects. However, this may largely be due to the fact that few studies have conducted experiments to distinguish between acclimation and adaptation. In fact, this is true of most reports of metal tolerance in aquatic organisms in general (Klerks and Weiss 1987). Of course, the ability to acclimate may itself be under genetic control and/or constraints. Although it is generally assumed that the ability to acclimate is species specific, few studies have attempted to describe intraspecific variation in acclimation. Despite this, the above definitions will be used to distinguish acclimation from adaptation, and therefore, consistency in terminology with earlier literature will be maintained.

Allozyme Variation: Theory and Applications

With the advent of electrophoresis came the discovery that the amount of genetic variation in natural populations was far greater than previously thought (Lewontin and Hubby 1966). This presented a dilemma to modern evolutionary biologists because if genetic variation is adaptively significant, this discovery is in direct conflict with the genetic load arguments of Muller (1950).

Genetic load is defined as the difference between the average individual fitness and the fitness of the best possible genotype (Muller 1950). Given the large number of gene loci present in individuals, it is clear that individuals can be heterozygous at only a small fraction of loci or the fitness of the individual approaches zero. This is the case even if fitness differences among allozyme variants are very small. Therefore, based on the recent findings that genetic variation is extensive in natural populations, it is clear that classical evolutionary ideas no longer explain experimental observations and that more advanced theories are necessary.

Two theories have emerged, both of which explain the data and are still contested today. The neutralist theory (Kimura 1968, Nei 1975) accepts the genetic load arguments arguing that most intraspecific variability at the molecular level is caused by random drift of mutant genes that are selectively

equivalent in functional efficiency. Conversely, the selectionist theory (Dobzhansky 1970, Lewontin 1974) disregards the genetic load principal arguing that it is erroneous to assume that the load associated with each locus is independent of other loci and/or environmental conditions. Therefore, the load associated with each locus is not multiplicative.

Despite this debate, there is considerable evidence that at least some proportion of variation observed at the allozyme level is adaptively significant. Much of the evidence rests in the preponderance of genotype-environment interactions reported. A significant body of literature demonstrates associations between the genetic structure of populations and some aspect of the environment. For example, allozyme variation has been correlated with osmotic (Hilbish and Koehn 1985, Burton and Feldman 1983), thermal (Mitton and Koehn 1975, Watt 1983), and moisture (Parsons 1980, Nevo and Yang 1982) gradients.

However, positive results of such surveys provide only inferential evidence that variation at the allozyme level is adaptively significant. Such correlations have been criticized because they lack any evidence of causality and therefore such correlations may be spurious (eg. Schnell and Selander 1981, Nei 1975). Unequivocal demonstration of the adaptive significance of an enzyme polymorphism must also show

evidence of physiological differences among allozyme variants, and that these differences contribute to fitness (Levins and Lewontin 1980).

Some efforts have been made to eliminate nonselective causes such as genetic drift or gene flow as explanations for genotype-environment interactions by establishing direct evidence of molecular differences among allozymes (eg. Gibson 1970, Koehn et al. 1971, Thorig et al. 1975, De Jong and Scharloo 1976, Johnson 1976, Hickey 1977). These studies provided both biochemical evidence for fitness differences among allozymes as well as strong correlations between environmental variables and allozyme variation.

In later studies, Nevo and colleagues at the University of Haifa in Israel used a similar approach to examine the effects of chemical pollutants on allelic isozyme variation. In laboratory experiments, they found differential survival of genotypes at the PGM locus in the shrimp, *Palaemon elegans*, to acute mercury exposure (Nevo et al. 1981); at the GPI locus in two marine gastropods, *Monodonta turbinata* and *M. turbiformis*, to both acute zinc and copper exposure (Lavie and Nevo 1982) as well as nonionic detergent and crude oil-surfactant mixtures (Lavie et al. 1984); at eight enzyme loci in the gastropod, *Cerithium scabridum*, to both cadmium and mercury (Lavie and Nevo 1986); and at the GPI locus in two other marine gastropods, *Littorina nertoides* and *L. punctata*, to

mercury (Lavie and Nevo 1987). In all of these studies, both sensitive and tolerant genotypes were identified.

In subsequent field surveys along the Mediterranean coast, the frequencies of tolerant genotypes to mercury were highest at mercury polluted sites in the shrimp, *Palaemon elegans*, and the gastropod, *Monodonta turbinata*, (Nevo et al. 1984). The same pattern was observed in the marine gastropods, *Littorina punctata* and *L. neritoides*, along a mercury pollution gradient in the Mediterranean Sea (Nevo et al. 1987). These surveys validated the results of previous laboratory experiments and clearly showed the genetic structure of marine organisms to be sensitive to various chemical pollutants. Based on these results, some have suggested that such methods can potentially be used as a promising biological monitoring system in marine ecosystems (Battaglia et al. 1980, Beardmore et al. 1980, Berry 1980, Nevo et al. 1983).

This approach has only recently been extended to freshwater systems. Chagnon and Guttman (1989) found two enzyme loci (GPI and IDH) to be sensitive to copper and cadmium in a population of the mosquitofish, *Gambusia affinis*. Newman et al. (1989) found the GPI locus to be sensitive to mercury but not arsenate in the same species. Benton and Guttman (1990) found fitness differences among allozyme variants at the GPI locus to acute copper exposure in the

aquatic insect, *Stenonema femoratum*. In a field study, Gillespie and Guttman (1989) found allele and genotype frequencies for allozymes of the PGM locus differed significantly between populations of the central stoneroller, *Campostoma anomalum*, collected from sites above and below impact from industrial activities.

To date the efficacy of this approach has not been sufficiently tested in freshwater systems. The studies cited consisted of experimental evidence of fitness differences among allozyme variants to chemical pollutants, or of comparisons of allele and genotype frequencies in populations from variably polluted environments, but not both.

MATERIALS AND METHODS

DESCRIPTION OF STUDY SITES

Field surveys and experiments were conducted at sites on two mercury polluted rivers in Virginia (USA). This design allowed replication of contaminated streams and provided the opportunity to compare responses of populations between streams with differing levels of mercury.

The South River is a fourth order tributary of the South Fork of the Shenandoah River in northern Virginia (Fig. 1). The river drains an area of 373 km^2 and its basin is composed primarily of limestone and shale. Some of the area along the river is forested but most is used extensively for agriculture and livestock. The river is characterized by a series of riffles and pools and moderate flow. Substrate in riffles is composed primarily of various sized cobbles on sand.

Between 1929 and 1950, mercuric sulphate was used by the Dupont Plant in Waynesboro, Virginia, as a catalyst in the production of acetate fiber. During that period an undetermined amount of mercury entered the South River. Plant officials discovered the leak in 1977 and subsequent surveys by the Virginia State Water Control Board revealed excessive levels of mercury in sediments and fish tissues (i.e. above EPA action levels). A ban on the consumption of fish caught

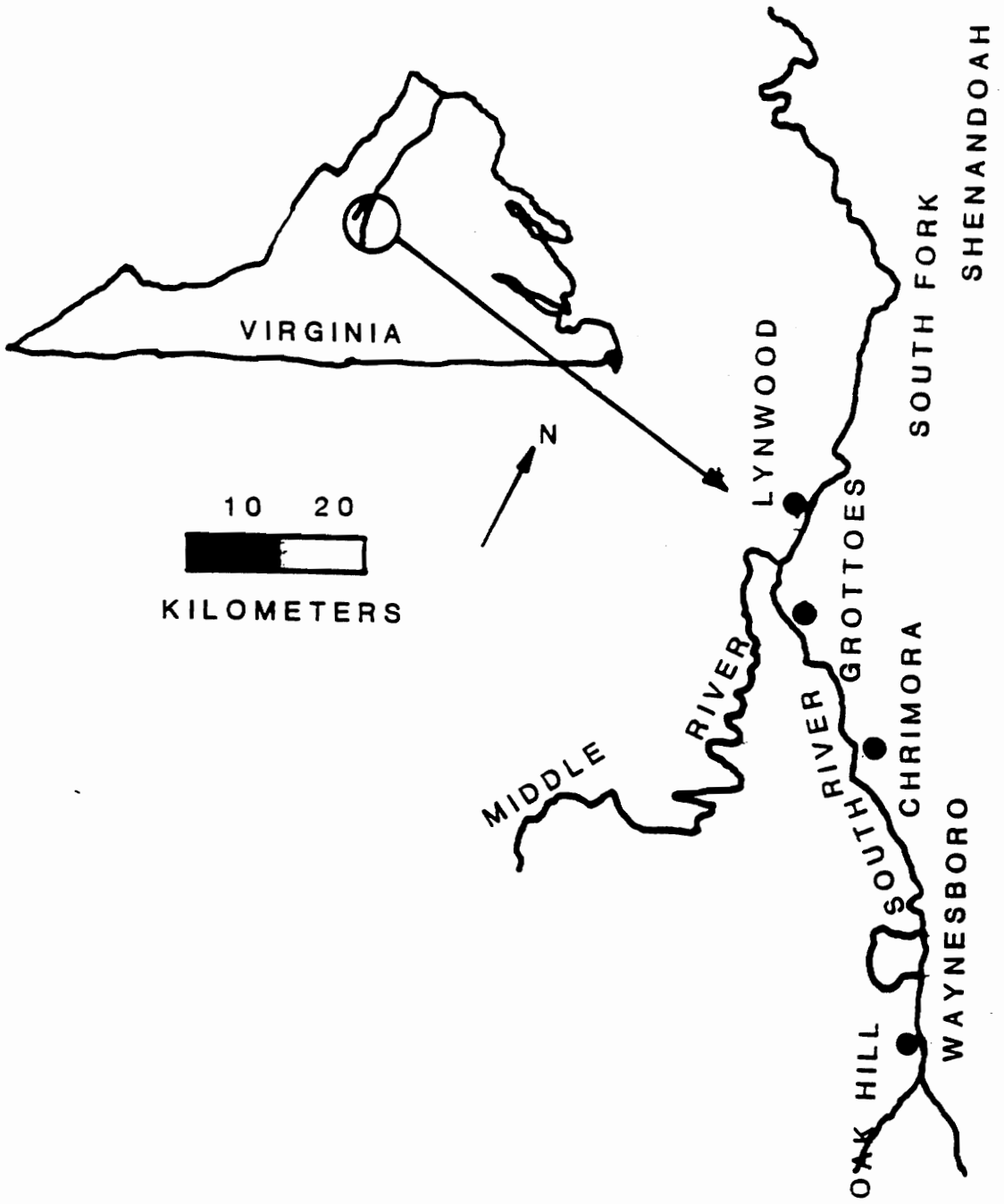

Fig. 1. Map of study sites on the South River.

anywhere on the South River was put in place by Virginia Department of Health. However, the ban has since been lifted and replaced with a health advisory.

The North Fork of the Holston River is a fifth order stream in southwestern Virginia (Fig. 2). It flows southwest into eastern Tennessee where it joins the South Fork of the Holston to form the Holston River proper. Like the South River, the Holston is characterized by the riffle-pool sequence typical of mountain streams in the eastern United States. The river has a relatively steep gradient and therefore high average flows (Bailey 1974). Substrate of riffle areas is a mixture of limestone bedrock, large boulders, and cobble overlaying sand.

The source of mercury contamination was a chloralkali plant located on the North Fork Holston near Saltville, Virginia, approximately 83 miles from the confluence with the South Fork Holston River. In the 1950's the plant began using a mercury cathode in the electrolytic process to produce chlorine and sodium hydroxide from salt (Bailey 1974). The plant continued this process until its closing in 1972 which was due to the high cost of complying with existing water quality standards. The plant site is now classified as a "superfund site" by the U.S. E.P.A. and the Virginia Department of Health has issued a ban on the consumption of fish between the plant site and the Virginia-Tennessee line.

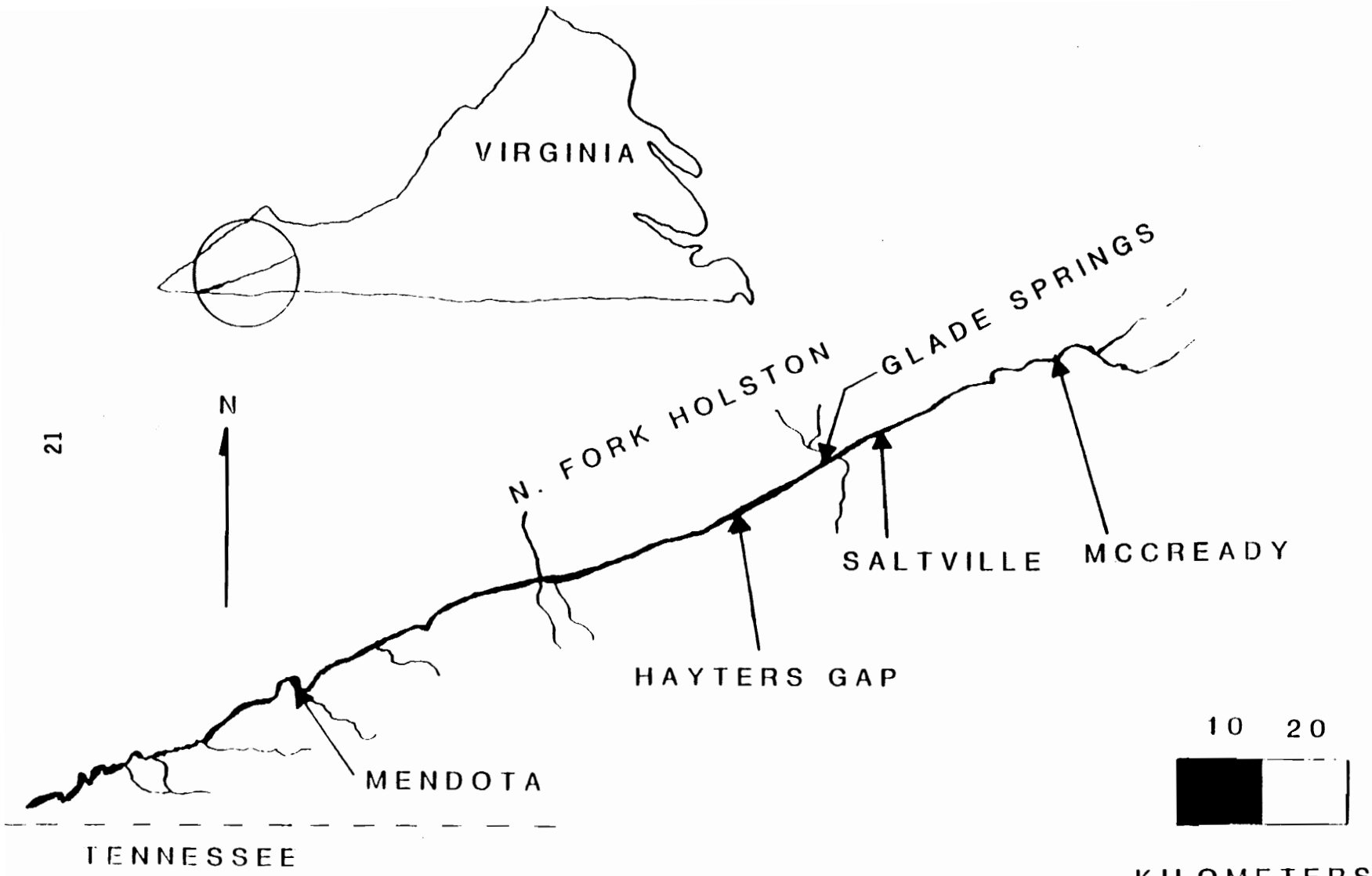

Fig. 2. Map of Study sites on the Holston River.

Most of the work in this study was conducted at a reference and a polluted site on each river. On the South River, a riffle area approximately 1.5 miles upstream of Waynesboro, near Oak Hill was used as the reference site. A site near Grottoes, located approximately 13 miles downstream of Waynesboro was used as the contaminated site (Fig. 1). On the Holston River, a reference site at McCready (located about 5 miles upstream of Saltville) and a contaminated site at Hayters Gap (located about 12 miles downstream of Saltville) were used (Fig. 2).

With the exception of mercury levels, both reference and contaminated sites on both rivers are fairly similar in terms of water quality (Table 1). The only significant differences are in nutrients. The contaminated site on the South River (i.e. Grottoes) has significantly higher levels of nitrates and phosphates than the other three sites. This is due to discharges from the municipal sewage treatment plant in Waynesboro. In addition to differences in nutrients, between-site differences in summer stream temperatures were also evident. The contaminated sites on both streams were considerably warmer than their corresponding reference sites during the summer (Fig. 3). This pattern has been consistently observed on the South River for the last four years (Snyder and Hendricks 1991).

Previous surveys of both rivers indicate considerably

Table 1. Comparison of water chemistry variables at reference and contaminated sites on the South and Holston rivers. Values reported are means (+/- 1 SE).

		SOUTH RIVER[1]		HOLSTON RIVER[2]	
		Reference	Contaminated	Reference	Contaminated
Variable	Units	(Oak Hill)	(Grottoes)	(McCready)	(Hayters)
pH		7.4 (0.2)	8.0 (0.2)	7.3 (0.1)	7.1 (0.1)
Alkalinity	$mgCaCO_3/L$	95 (25)	102 (18)	82.0 (22)	74.0 (29)
Hardness	$mgCaCO^3/L$	152 (84)	182 (100)	110 (70)	100 (44)
Conductivity	$\mu Mhos/cm^2$	198 (99)	285 (133)	168 (104)	329 (117)
Total Nitrates	mg/L	0.70 (0.22)	3.55 (1.50)	1.19 (0.45)	1.25 (0.37)
Total Phosphates	mg/L	0.07 (0.04)	0.27 (0.12)	0.035 (0.08)	0.035 (0.05)

[1] Means of measurements taken approximately monthly between 1985 and 1989. Taken in part from Hendricks et al. 1989.

[2] Means of measurements taken seasonally (N=4) between September 1990 and July 1991.

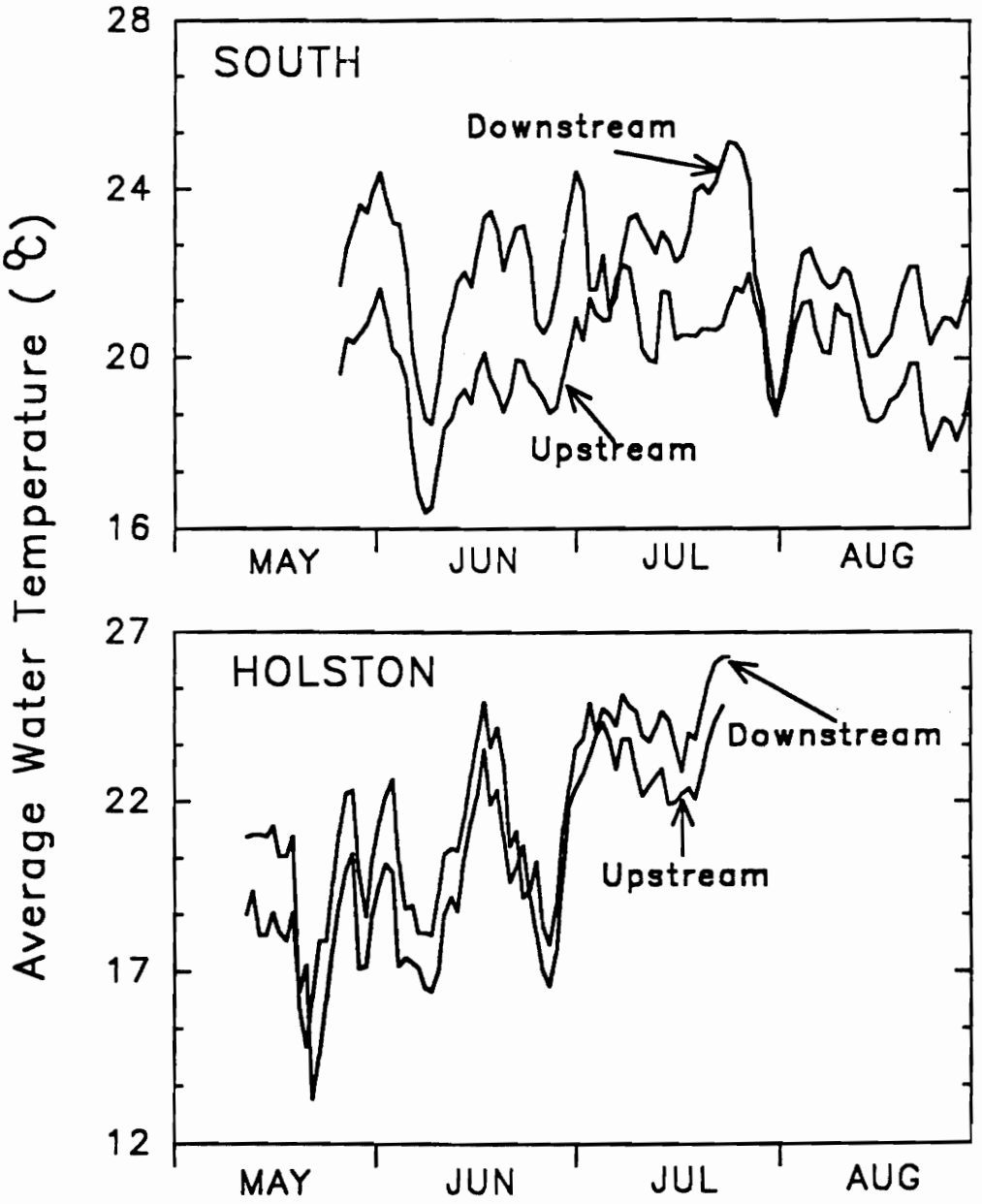

Fig. 3. Comparisons of average stream temperatures between upstream and downstream sites on both rivers. See table 1 for names and descriptions of sites.

higher mercury levels in fish tissues at contaminated sites on the Holston River (Table 2). This was true for all trophic levels. Differences in mercury levels in sediments were less obvious (Table 2). However, mercury concentrations in sediments on the South River are concentrated in the deeper zones (Lawler et al. 1989). Conversely, on the Holston, highest levels are found in the top one inch of sediment (Bailey 1974). This suggests that mercury is less available to organisms in the South River.

DESCRIPTION OF STUDY ORGANISM

Isonychia bicolor is an aquatic insect of the order Ephemeroptera and the family Oligoneuriidae. It is a common member of the aquatic insect assemblage in swift-moving streams of all sizes throughout the eastern half of North America (Edmunds et al. 1958). Nymphs are strong swimmers, spending much of their time in riffle areas filtering seston from the water with long hairs located on their forelegs (Unzicker and Carlson 1982). The life cycle is dominated by the immature stage; adults generally have a life span of about one day during which time mating and egg laying occur (Edmunds et al. 1958).

Insects are commonly used in biomonitoring activities because 1) they represent a wide range of sensitivities to pollution, 2) they are an important link in the food web in

Table 2. Comparison of mercury levels reported in sediments and fish from sites in the South[1] and Holston[2] drainages. Sediment values were obtained from a composite of the first three inches of sediment at each site. Values for fish represent the range in average mercury concentrations observed among species within each trophic level and therefore do not include estimates of total variance. (*) refers to sites surveyed in this study.

				Mercury Concentration (µg/g DW)			
					FISH		
River	Site	Location	RM	Sediments	Predators	Foragers	Scavenger
SR	Oak Hill*	38°2'N,78°55'W	-1	<0.05	0.02-0.08	0.07-0.08	0.07-0.13
HR	McCready*	36°54'N,81°44'W	-5	0.02-0.05	0.38-0.71	0.08	0.12-0.17
SR	Dooms	38°6'N,78°51'W	5	0.95-5.08	0.44-0.52	0.16-0.19	0.14-0.18
HR	Glade Spring	36°52'N,81°51'W	5	0.36-5.23	-----	1.02-1.10	1.03
SR	Grottoes*	38°15'N,78°50'W	13	1.11-8.04	0.66-1.67	0.46-0.57	0.51-0.74
HR	Hayters Gap*	36°48'N,81°56'W	12	2.89-5.24	-----	1.54	-----
SR	Lynwood*	38°19'N,78°46'W	25	-----	0.58	0.25	0.26-0.31
HR	Holston	36°46'N,82°3'W	24	-----	0.97	1.16	1.08-1.16
SR	Elkton	38°24'N,78°38'W	45	-----	-----	0.22	0.26
HR	Mendota	36°42'N,82°18'W	47	3.92-5.65	0.77-1.64	0.96-1.31	0.91-1.35
SR	Shenandoah	38°29'N,78°38'W	58	-----	0.28-0.94	0.23	0.29
HR	Hilton Gap	36°38'N,82°27'W	61	1.05-4.53	1.29-1.91	0.25-0.86	0.65-2.47
SR	Luray	38°42'N,78°29'W	77	-----	0.31	0.17	0.16
HR	Weber City	36°36'N,82°33'W	75	2.51-6.15	1.17-1.20	1.11	1.06

1 Sediment values from Lawler et al. (1989) and fish values from data collected in the summer of 1983 (Hendricks unpublished).

2 Both sediment and fish data from Bailey (1974).

streams, and 3) many species are relatively immobile and thus generally unable to retreat from impacted sites (Cairns and Dickson 1971). Furthermore, the potential for adaptive responses are great in insects because they have relatively short life cycles, high average densities, and high reproductive potentials.

Isonychia bicolor was specifically chosen for this study because 1) it is widely distributed throughout the eastern United States, 2) relatively abundant on both the South and Holston Rivers, 3) has been found to be highly sensitive to heavy metals (Clements 1991), 4) the biology of this species is reasonably well-known on the South River (Hendricks et al. 1989), and 5) techniques for electrophoretic work have recently been worked out for several related species of mayflies (Funk et al. 1988).

RESPIRATION

General Procedures

Oxygen consumption was measured with a Gilson differential respirometer. In all experiments, nymphs were collected from the stream, returned to the lab, and placed in crystallizing dishes containing 1 liter of aerated, filtered (0.45μm) stream water. Because oxygen consumption is dependant on feeding state (Lampert 1984), bowls containing nymphs were placed into an environmental chamber for between 60 and 72 hr

to facilitate clearance of guts and allow nymphs sufficient time to acclimate to test temperatures. Test temperatures were no more than 2°C different from stream temperatures from which nymphs were collected.

Following the acclimation period, nymphs were separated by size and placed into respiration flasks containing 6 ml filtered stream water (0.45 μm). At least two flasks were left empty (i.e. 6 ml water only) to act as controls. Light has been shown to affect respiration rates in invertebrates presumably by affecting activity. In most cases, respiration rates have been found to be lower and less variable under low light intensity (Konstantinov 1971, Buikema 1972). Therefore, the respirometer was covered with black plastic sheeting during experiments. No additional efforts were made to monitor or control activity levels. Oxygen consumption was recorded every 1-4 hr depending on the number of animals per vessel (N=1-13), body size, and temperature. In general, oxygen consumption was considerably higher the first 1-3 measurement intervals due to handling stress. Thus, these initial measurements were discarded and baseline consumption was calculated for each vessel as the average of those measurements taken after O_2 consumption stabilized. Baseline consumption is defined here as the rate of oxygen consumption of starved nymphs in clean water.

After establishing baseline oxygen consumption, mercury (mercuric nitrate, standard reference solution-Fisher Scientific) was added to each test vessel (concentrations varied with experiments). At least two vessels were left untreated as controls during each experiment. Measurements were recorded and averaged in the same way as for baseline consumption.

At the end of each experiment, nymphs were removed from flasks and checked to make sure they were alive. Data from flasks incurring mortality were discarded. Nymphs were then dried at 60°C for 24 hr, desiccated at room temperature for 24 hr and weighed individually on a Kahn 28 electrobalance to the nearest microgram. Mean weight and standard deviation were calculated for each flask. Size-specific respiration rates were then determined for both treatments (i.e. baseline and Hg treated) for each flask and expressed as μl O_2/mg/hr. Percent change in respiration rate was calculated for each vessel by the equation:

$$RR = \frac{(R_t - R_b) * 100}{R_b}$$

where, RR is the Respiratory Response (i.e. % change in respiration rate following treatment), R_t is the respiration rate of nymphs exposed to mercury, and R_b is the baseline respiration rate (starved nymphs in clean water).

Dose-Specific Respiratory Responses

Two experiments were conducted to characterize the dose-specific responses of *Isonychia bicolor* nymphs to mercury. Nymphs for the first experiment were collected from Mill Creek (37°15'N,80°20'W), a first order tributary of the North Fork of the Roanoke River in southwest Virginia. The test was run at 15°C. After establishing baseline respiration rates (R_b) for each test flask, a known amount of mercury was added to each flask to bring the total mercury concentration in each flask up to one of six treatments: 0, 10, 25, 50, 100, or 1000 μg/L. At least three replicates were set up for each mercury concentration. Respiration rates of nymphs in treated flasks (R_t) were then calculated.

Nymphs for the second experiment were collected from Sinking Creek (37°27'N,80°34'W), a third order tributary of the New River. Both streams represent unpolluted streams near the Virginia Tech campus with large natural populations of *I. bicolor*. This experiment was conducted under the same conditions as the first, except that the test temperature was 20°C and an additional test concentration of 400 μg/L mercury was used.

Paired T-tests (Sokal and Rohlf 1981) were used to determine whether respiration rates changed significantly after exposure to mercury (i.e. H_o: $R_t-R_b=0$), for each dose.

Regression analysis was used to determine whether there was a significant relationship between weight and R_b, R_t, or RR (H_o:B_1=0), for each dose.

Population-Specific Responses

Seven experiments were conducted to determine the extent to which natural populations of *I. bicolor* vary in their respiratory response to mercury. In these experiments nymphs were collected from 2-4 sites on the South and Holston Rivers which differed in the degree of contamination. One of three experimental regimes was chosen for each experiment: 100µg/L at 15°C, 50µg/L at 20°C, or 400µg/L at 20°C (Table 3). These initial conditions were chosen to maximize respiratory responses and were based on the results of the previous set (i.e. dose-specific responses) of experiments. Population differences in basal respiration rates (R_b), respiration rates when exposed to mercury (R_t), and respiratory responses (RR), were tested using Analysis of Covariance with population as the class variable and weight as the covariate. The relationship between size-specific respiration rate and body size in aquatic animals is usually expressed as a power function, $y=ax^b$ (Lampert 1984). Thus, respiration rates and body weight were log transformed prior to analyses. Respiratory response was linearly related to body weight and therefore no transfor-

Table 3. Description of respiration experiments designed to test for population differences in response to mercury.

	River	Population[1]	Temp (°C)	[Hg] (μg/L)	Animals/ Vessel	Duration of Exp. (hr)	Measurement Interval (hr)
Apr 1991	South	OH,GR	15	100	2-13	15	2
May 1991	Holston	MC,HG	15	100	2-10	14	2
Jun 1991	South	OH,GR	20	50	2-12	9	1
Aug 1991	South	OH,GR	20	400	2-13	6	1
Aug 1991	South	OH,CH,GR,LN	20	50	2-10	7	1

[1]Population abbreviations: OH=Oak Hill, CH=Chrimora, GR=Grottoes, LN=Lynwood, MC=McCready, HG=Hayters Gap. See Figures 1 and 2, and Tables 1 and 2 for description of sites.

mations were made when testing for population differences in respiratory responses. I report type I mean squares (SAS 1985), which estimate the variance attributed to each effect (i.e. population and weight) before removing other sources of variance.

FIELD OBSERVATIONS AND EXPERIMENTS

Survey Data

Approximately 50 nymphs were collected each month with a D-frame kick net from two sites on the South (i.e. Oak Hill and Grottoes) and Holston (i.e. McCready and Hayters Gap) Rivers (Figs 1 and 2). These *qualitative* samples were collected between May 1990 and August 1991 on the South river, and between September 1990 and September 1991 on the Holston river. Nymphs were separated in the field into size classes based on body length (range=3.5-17mm). Developmental patterns were determined for each site by assessing changes in size-frequency distributions through the year.

In addition, *quantitative* sampling was done during the summer of 1991 at the same two sites on both rivers. Six Hess samples (0.1 m^2, net mesh= 230 μm) were collected at each site every two weeks. All nymphs from each sample were counted and measured using a Vidas Image Analysis System with Videoplan software. Both body length and area measurements were taken

for each nymph. Average density (#/m^2) and mean individual size (cm^2) were calculated for each sample date. Relative growth rate was calculated between each sample interval for all four sites using the equation: $GR = \ln(S_f/S_i)/T \times 100$, where GR is the relative growth rate expressed as percent change in average size per day, S_f is the average final size expressed in cm^2, S_i is the average initial size, and T is the number of days between sample dates.

Temperature was recorded every hour at both sites on the South river throughout the summer of 1991 using Ryan Tempmentor submersible thermographs. Temperature was continuously recorded on the Holston river during the same time period using Ryan Model J submersible thermographs.

Field experiments

Reciprocal transplant experiments were conducted during the summer of 1991 on both rivers. Enclosures were constructed from 15 inch sections of 5 inch plastic sewer pipe. Fiberglass screening was attached to both ends of the pipe with radiator hose clamps. Enclosures were oriented in the stream so that screens were perpendicular to stream flow thus allowing water and seston to pass through the enclosures. Two 18 inch sections of 3/8 inch rebar were driven into the stream bottom and each enclosure was attached to the pair of rebar

using small radiator hose clamps.

Two experiments (early-summer/late-summer) were conducted on the South river. For both experiments, twelve enclosures containing 10 nymphs each were placed at one site upstream (i.e. Oak Hill) and one downstream (i.e. Grottoes) of Waynesboro, Virginia. Six enclosures at each site contained nymphs from the upstream population, and the other six contained nymphs from the downstream population. Ten large rocks were also placed into each enclosure to provide substrate for nymphs. Small nymphs (< 9 mm in length) were collected with a D-frame kick net. The ten nymphs for each enclosure were placed into a petri plate and the plate put on top of a piece of graph paper. Each group of nymphs were then photographed and placed into enclosures.

For the *early-summer* experiment nymphs were allowed to develop for about 4 weeks. Screens were cleaned each week to prevent clogging. At the end of this time, nymphs were removed from enclosures, the number alive in each enclosure was recorded, and each group of nymphs were again photographed as described above.

Surviving nymphs were transported back to the lab along with site stream water. Survivors from each enclosure were placed in separate bowls with 1 liter of stream water and placed into an environmental chamber kept at a constant 22°C.

Flow was maintained with magnetic stirrers and fiberglass screens were placed over the stir bar to prevent injury to nymphs. Periodic replacement of unfiltered stream water kept food supplies stable, and flow created by stirrers made it available to filter-feeding nymphs. Stream water was replaced approximately every 48 hr. Nymphs were kept until emergence to adults or death. Emerging adults were immediately placed in labelled 1.5 ml Eppendorf tubes and frozen. Subsequently, eggs were removed from gravid females and filtered onto 0.45 Millipore filter paper. The filters were cleared with emersion oil and mounted onto slides with glycerine. All adults were then dried at 60°C for 24 hr, desiccated for an additional 24 hr, and weighed individually to the nearest μg on a Kahn 28 electrobalance. Eggs were later counted under a dissecting scope.

Photographs taken of nymphs were used to determine the average individual size of nymphs in each enclosure at the beginning and end of the experiment. Individual area of each nymph was determined from the photographs using the Vidas Image Analysis System described above. The graph paper in the photographs were used to calibrate each measurement. These data were used to calculate the average growth rate of nymphs within each enclosure during the experiment. Ultimately, population differences in growth will be interpreted from an

energetics point of view. In this context, it is change in weight that is important because it directly relates to the relative amount of energy consumed that is used for growth.

The *late-summer* experiment conducted on the South river had essentially the same experimental design except that nymphs were removed from the enclosures each week, counted and photographed. The number of individuals completing their life cycle (i.e. emerged and drowned) was also recorded each week. Relative growth rates for each enclosure were then calculated as the slope of the line generated by regressing the natural log of size on day.

The field experiment conducted on the Holston river in the *early-summer* of 1991 had the same experimental design as the *early-summer* experiment on the South river, except that an additional site was used. The additional site, referred to as the Middle site, was located near Glade Springs, about 5 river miles downstream of Saltville (Fig. 2). Initial surveys indicated that *I. bicolor* nymphs were generally absent here, and therefore mercury levels at this site are likely acutely toxic. At this site, six enclosures contained nymphs from the upstream site (i.e. McCready) and six contained nymphs from the downstream site (i.e. Hayters Gap). No enclosures contained nymphs indigenous to this site.

For all three experiments, differences in growth rate, mortality rate, and fecundity between upstream and downstream

nymphs at both sites were compared statistically using the Two-sample t-test (Sokal and Rohlf 1981). For growth and mortality rates each enclosure was a replicate for each population (df=10 for each test). For fecundity each individual of each population was a replicate (df varied with number of survivors of each population). Because fecundity has been shown to be related to female size in some mayflies (Kondrotieff and Voshell 1980, Sweeney and Vannote 1981), analysis of covariance (Sokal and Rohlf 1981) was also used to compare fecundities between upstream and downstream nymphs at each site. In these analyses, population (upstream nymphs vs downstream nymphs) was the class variable and female weight was the covariate.

ALLOZYME DATA

Preliminary Work

Preliminary electrophoretic surveys were conducted between October 1989 and June 1990. Nymphs for the survey were collected from four sites representing three streams: two sites on the South River (Oak Hill and Grottoes) and single sites on Sinking Creek and Mill Creek. A total of 375 individuals were collected over the nine month period with a D-frame kick net. Nymphs were frozen on dry ice in the field, returned to the lab and stored at -80°C.

Allozymes were separated by horizontal starch gel electrophoresis using methods like those described by Selander et. al. (1971) with modifications outlined in Sweeney et. al. (1987). A total of 29 enzyme systems were screened (Table 4). Of these, only 10 were consistently scorable yielding data on 12 presumptive loci (Table 5). Most of the others could not be reliably scored in nymphs because of insufficient activity on gels (i.e. too faint to read). Three loci were found to be polymorphic: GPI, MPI, and PGM (Table 5). However, at the MPI locus allozyme variants were rare (<2%). Therefore, the remaining work focused on GPI and PGM. Banding patterns were interpreted by classifying the fastest migrating allozyme as "F" and the slowest as "S". In the case of GPI where three alleles were scored, an "M" was ascribed to the medium migrating allele. For example, the slowest migrating allele at the GPI locus was designated GPI^{S} and the slow-medium heterozygote GPI^{S},GPI^{M}.

Laboratory Experiments

Differential survivorship of genotypes was determined by acute bioassays coupled with electrophoretic analyses. Large (1.5L) crystallizing dishes were used as test containers for acute, static bioassays. Flow was maintained in each dish using magnetic stirrers. A fiberglass screen was placed over

Table 4. List of Enzyme systems screened in *Isonychia bicolor* nymphs during the initial electrophoretic surveys.

Enzyme Code	Enzyme	Enzyme Number
ACO	Aconitase	4.2.1.3
ACP	Acid phosphatase	3.1.3.2
ADH	Alcohol dehydrogenase	1.1.1.1
AK	Adenylate kinase	2.7.4.3
AKP	Alkaline phosphatase	3.1.3.1
ALD	Aldolase	4.1.2.13
AO	Aldehyde oxidase	1.2.3.1
DIP	Dipeptidase (Leu-Ala)	3.4.13.11
FUM	Fumarase	4.2.1.2
G3PD	Glyceraldehyde-3-phosphate-dehydrogenase	1.2.1.12
G6PD	Glucose-6-phosphate-dehydrogenase	1.1.1.49
GDA	Guanine deaminase	3.5.4.3
GDH	Glucose dehydrogenase	1.1.1.47
GPI	Glucose-phosphate isomerase	5.3.1.9
HK	Hexokinase	2.7.1.1
IDH	Isocitrate dehydrogenase	1.1.1.42
LAP	Leucine aminopeptidase	3.4.11.1
LDH	Lactate dehydrogenase	1.1.1.27
MDH	Malate dehydrogenase	1.1.1.37
ME	Malic Enzyme	1.1.1.41
MPI	Mannose-phosphate isomerase	5.3.1.8
NP	Purinenucleoside phosphorylase	2.4.2.1
PGM	Phosphoglucomutase	2.7.5.1
PRO	Proline dipeptidase (Pro-pro)	3.4.13.9
SDH	Sorbitol dehydrogenase	1.1.1.14
SOD	Superoxide dismutase	1.15.1.1
TRE	Trehalase	(Unknown)
TRI	Tripeptide aminopeptidase (Leu-gly-gly)	(Unknown)
XDH	Xanthene dehydrogenase	1.2.3.2

Table 5. Number of loci, number of alleles, and buffer system used for those enzyme systems found to have sufficient activity and resolution to score reliably in *Isonychia bicolor* nymphs.

Enzyme Code[a]	No. Loci	No. Alleles	Buffer System
AO	2	1,1	P-7[b]
ACP	1	1	TC 6.4[c]
AKP	1	1	TBE 8.6[c]
G3PD	1	1	LiOH 8.3[c]
GPI	1	3	LiOH 8.3
ME	1	1	P-7
MPI	1	2	P-7
PGM	1	2	P-7
TRE	2	1,1	P-7
XDH	1	1	TBE 8.6

[a] See Harris and Hopkinson (1976) and Pasteur et. al. (1988) for recipes.

[b] See Sweeney et. al. (1987) for recipe.

[c] See Pasteur et. al. (1988) for recipe.

the stir bar to prevent injury to nymphs. Mercuric nitrate was used as the test toxicant and actual stream water, collected from reference sites was used as the dilution water. All containers were well aerated and held at constant temperature in an environmental chamber. Two separate bioassays were conducted using a modified version of methods described by Lavie and Nevo (1982).

In the first test, nymphs (N=80) and stream water were collected from Sinking Creek on 21 July, 1990. Nymphs were randomly placed into test containers (10 individuals/container) with 1 liter of filtered stream water and allowed to acclimate for 24 hr prior to the introduction of mercury. At this point, mercuric nitrate was added to all but one dish (i.e. control) to obtain a nominal concentration of 0.2 mg mercury/L in each dish. Acclimation and testing was done at 22°C.

Containers were checked every four hours and dead nymphs removed and frozen at -80°C for later electrophoresis. When approximately 50% of the total number of nymphs died, the experiment was terminated and survivors were frozen separately. All nymphs were later electrophoresed as described above and the genotype of each individual was determined at the GPI and PGM loci.

If the probability of surviving acute mercury exposure is

independent of genotype, then the frequency of each allele and genotype should be equally represented in the surviving and dead fractions. For example, if allele X has a frequency of 0.1 in the starting population, then approximately 10 percent of the alleles in both the surviving and dead fractions should be X. This null hypothesis was tested for each allele and genotype at both the PGM and GPI loci using the G-test of independence (Sokal and Rohlf 1981).

In the second test, nymphs (N=140) and stream water were collected from Mill Creek on 13 February 1991. Bioassay procedures were essentially the same as those described above except for three major factors. First, the duration of the test was considerably longer (nearly 1 week) to ensure higher mortality. Second, because the duration of the test was longer, water was not filtered so food was available to nymphs. This was to ensure that mortality was not related to starvation. *I. bicolor* is a filter-feeder and current supplied by the magnetic stirrer kept an adequate supply of seston in the water column available to nymphs. Finally, the test solution was renewed every 48 hr by replacing about 75% of the water with new water with the same mercury concentration (0.2 mg/L). This ensured that most metabolic wastes were eliminated and the mercury concentration remained stable.

Containers were checked every three hours until the end of the experiment. Dead nymphs were removed, placed into 1.5

ml Eppendorf tubes, and frozen for later electrophoresis. In addition, the body length was measured and the time at death was recorded for each individual. Time-to-death (TTD) was scored as the number of three hr intervals since the original addition of mercury. For example, a nymph that died 5.5 hr after mercury was added would receive a score of two for TTD. Nymphs were then electrophoresed as described above and the genotype of each nymph was determined for the GPI locus.

For this experiment, the effects of genotype and body size on TTD were tested using the SAS LIFEREG procedure (SAS 1985), which fits parametric models to failure-time and right-censored (i.e. survivorship) data. The effect of each independent variable (body length and GPI genotype in this analysis) is tested individually. Also, LIFEREG employs a log-likelihood ratio method which is robust for small sample sizes. Dixon and Newman (1991) give a thorough treatment of Time-to-Death models and their use in toxicity testing.

Field Surveys

Approximately 50 nymphs were collected each month from reference (Oak Hill on the South River, McCready on the Holston River) and contaminated (Grottoes on the South River and Hayters Gap on the Holston River) sites on both rivers. Nymphs were placed in 1.5 ml Eppendorf tubes and frozen on dry ice in the field. These are the same individuals used in

determining growth and developmental patterns of natural populations (see FIELD WORK above). Nymphs were later electrophoresed and the genotype at the GPI and PGM loci was determined for each nymph. Chi-square test was used to test genotype frequency of each population for conformity to Hardy-Weinberg distribution. The G-test of independence (Sokal and Rohlf 1981) was used to test for spatial and temporal differences in allele and genotype frequencies.

RESULTS

RESPIRATION EXPERIMENTS

Effects of Size and Temperature

Basal respiration rates (R_b) were highly variable and were dependent on both body size and temperature. As with most aquatic organisms, the relationship between body size and respiration was best explained by a power function with the equation: $R = aW^b$ (or in logarithmic form: log R = log a + b log W), where R = size-specific respiration rate (μl O_2/mg/hr), W = body weight (mg DW), and a and b are the regression coefficients (y intercept and slope respectively) (Fig. 4). Small individuals (1 mg) respired between 40 and 71% faster than large individuals (5 mg) depending on temperature. Only individuals from uncontaminated sites were used in this analysis to control for any confounding effect of mercury on R_b. No significant differences in the relationship between respiration and body weight were observed among the four populations used in this analysis at either temperature (ANOVA test on slopes, $F>1.12$, $p>0.30$ for each experiment).

Temperature also had a profound effect on R_b. Size-specific respiration rates were between 50 and 63% faster at 20°C than at 15°C depending on body size. Regression equations of log R by log W (Fig. 4) were used to predict respiration

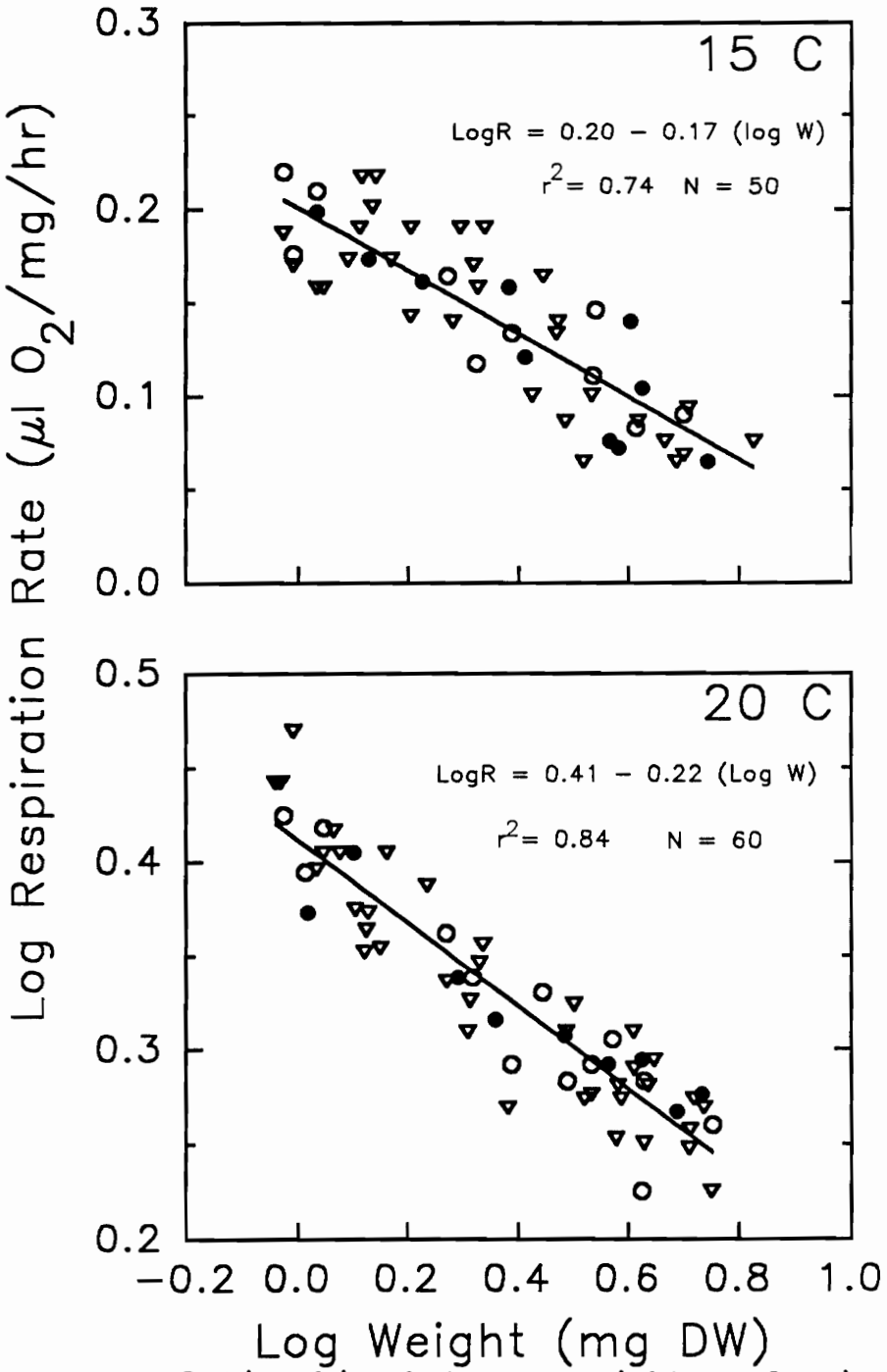

Fig. 4. Relationship between weight and size-specific respiration rates in I. bicolor nymphs at two experimental temperatures. Different symbols represent data from different unpolluted sites to illustrate repeatability of techniques.

rates over a wide range of body sizes at both temperatures. Subsequently, these predicted rates were used to calculate Q_{10} values using the equation, $Q_{10} = (R_2/R_1)^{10/t2-t1}$ where, R_1 and R_2 are the predicted respiration rates and T_1 and T_2 the corresponding temperatures (Lampert 1984). Q_{10} values ranged between 2.18 and 2.65 and were considerably higher for smaller individuals (Fig. 5). Therefore, not only were size-specific respiration rates higher in smaller individuals, but smaller nymphs were also more sensitive to changes in temperature. That is, for each incremental change in temperature, the corresponding change in metabolic rate was greater in smaller individuals.

Dose-specific respiratory responses

Significant changes in respiration (i.e. respiratory response) occurred following treatment with mercury. Both the magnitude and direction of the responses were dose dependent at both temperatures. At lower concentrations (25, 50, 100 µg/L at 15°C; and 25, 50 µg/L at 20°C) respiration rates were enhanced. Conversely, at higher concentrations respiration rates were depressed (Fig. 6). In addition, respiratory response (RR) was greater in small nymphs (<2 mg) at all concentrations except 10 µg/L at both temperatures, and greater at 20°C than at 15°C for all test concentrations except 10 and 100 µg/L. At 100 µg/L a net increase in respiration

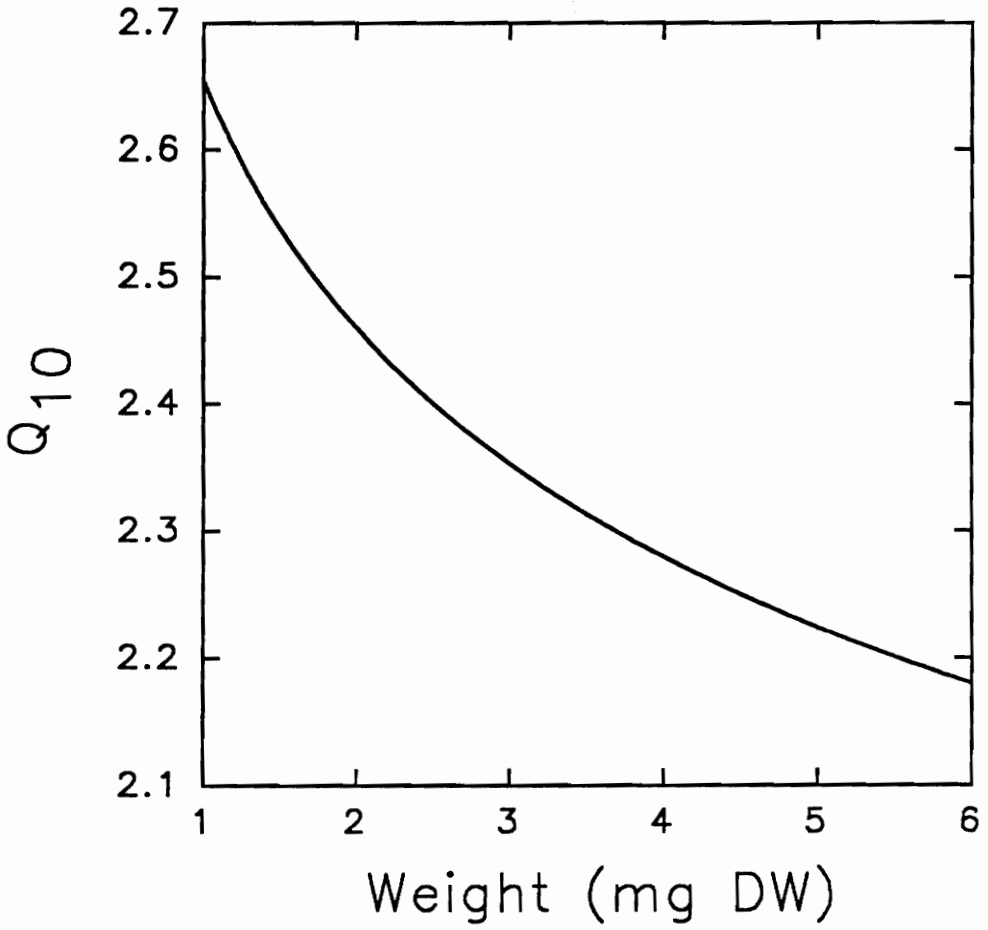

Fig. 5. Relationship between weight and Q10 for I. bicolor nymphs from unpolluted sites. See text for discussion of methods.

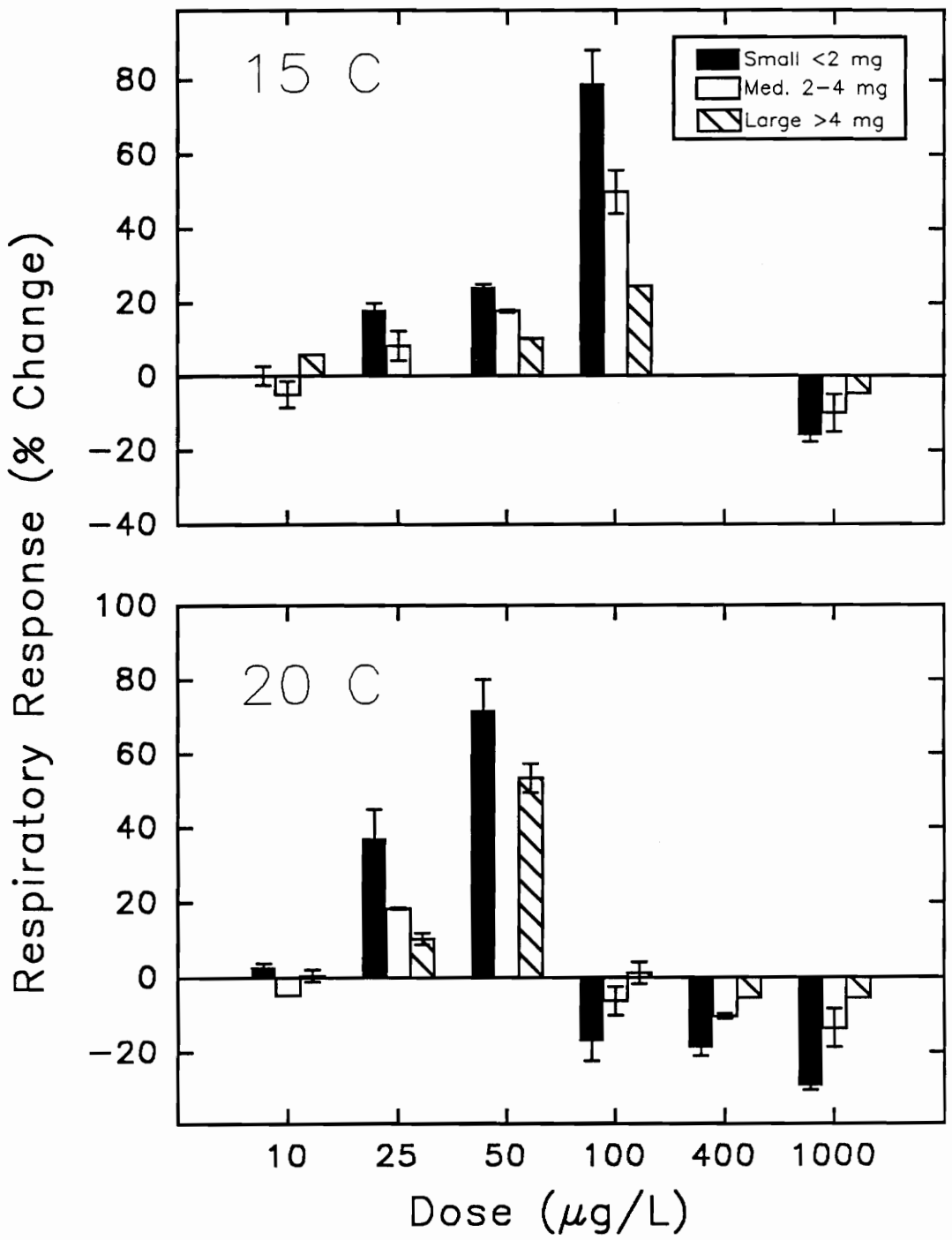

Fig. 6. Relationship between mercury concentration and percent change in respiration of I. bicolor nymphs at two experimental temperatures.

was observed at 15°C but a net decrease (except in large nymphs) was observed at 20°C (Fig. 6).

All changes in respiration following the addition of mercury were statistically significant ($p<0.05$) for all doses except 10 µg/L at both temperatures and 100 µg/L at 20°C (Table 6). It is likely that 10 µg/L is not sufficient to elicit a significant response over short time periods (<15 hr). However, responses at 20°C were significant at 50 and 400 µg/L but not at 100 µg/L. The transition at 20°C between positive and negative changes in respiration following treatment occurred between 50 and 100 µg/L (Fig. 6). Thus, lack of a significant response at 100 µg/L was probably due to some individuals within each test vessel exhibiting increases and some decreases in metabolism and not to a lack of response. The relationship between respiratory response (RR) and weight was also statistically significant for all doses except 10 µg/L (Table 6).

Population-specific responses

The first two experiments were conducted in the spring of 1991 using nymphs from a reference and a contaminated site on both the South and Holston rivers (Table 3). No significant population differences were observed in either R_b or R_t on

Table 6. Results of statistical analyses for dose-dependant responses of *Isonychia bicolor* nymphs to mercury at two experimental temperatures (top = 15°C; bottom = 20°C).

	Paired T-Test H_o: $R_t-R_b=0$			Regression Analyses H_o: Slope (RR X wt)=0		
Dose	**DF**	**T**	**P-value**	**DF**	**T**	**P-value**
10	5	0.03	p>0.70	4	-0.73	p>0.50
25	5	5.39	p<0.01	4	-3.85	p<0.02
50	5	5.68	p<0.01	4	-6.91	p<0.01
100	5	4.91	p<0.01	4	-5.55	p<0.01
1000	5	-4.17	p<0.02	4	3.23	p<0.05

Dose	**DF**	**T**	**P-value**	**DF**	**T**	**P-value**
10	5	0.86	p>0.50	4	-1.23	p>0.20
25	5	3.21	p<0.05	4	-3.36	p<0.03
50	5	6.90	p<0.001	4	-2.42	p>0.10
100	5	-1.88	p>0.10	4	5.18	p<0.01
400	6	-4.72	p<0.01	5	3.48	p<0.02
1000	6	-4.26	p<0.01	5	3.81	p<0.02

either river (Tables 7,8). In addition, there was no significant population difference in RR on the Holston river (Table 8). However, RR of nymphs from the contaminated site on the South river (i.e. Grottoes) were substantially lower than those of nymphs from the reference site (i.e. Oak Hill) ($p=0.063$). And although the interaction term (i.e. Weight X Population) was significant ($p=0.034$), this appeared to be mainly due to lack of population differences in RR of larger individuals (Fig. 7). Smaller nymphs (<2 mg) at the reference site consistently had a higher RR.

These data indicate there was no difference in the way individuals from the two sites on the Holston river responded to mercury. However, the data on the South river was less conclusive. Therefore, a third experiment was conducted on the South river in the early summer using nymphs from the same two sites (Table 3). However, for this experiment, nymphs from the reference site (i.e. Oak Hill) were acclimated for 8 days in water from the contaminated site (i.e. Grottoes) and nymphs from the contaminated sites were acclimated in reference site water. The purpose was to determine if preexposure to mercury would enhance tolerance in individuals from the reference site. Likewise, would preexposure of individuals from the contaminated site to clean water result in a decrease in tolerance.

Table 7. Results of Analysis of Covariance for experiment conducted April 1991 to determine effects of population and weight on respiratory responses of *I. bicolor* nymphs to mercury. Treatment 100 ug/l at 15°C using nymphs from a reference and polluted site on South River. Top = Effects on R_b, Middle = Effects on R_t, Bottom = Effects on RR.

Source	DF	Type I MS	F Value	Pr>F
Log Weight	1	0.03455	121.09	0.0001
Population	1	0.00024	0.85	0.37
Log Weight X Population	1	0.0000007	0.00	0.96

Source	DF	Type I MS	F Value	Pr>F
Log Weight	1	0.2618	186.33	0.0001
Population	1	0.0013	0.91	0.35
Log Weight X Population	1	0.0019	1.32	0.27

Source	DF	Type I MS	F Value	Pr>F
Weight	1	12436.35	104.47	0.0001
Population	1	467.14	3.92	0.063
Weight X Population	1	627.47	5.27	0.034

Table 8. Results of Analysis of Covariance for experiment conducted May 1991 to determine effects of population and weight on respiratory responses of I. bicolor nymphs to mercury. Treatment 100 ug/l at 15°C using nymphs from a reference and polluted site on Holston River. Top = Effects on R_b, Middle = Effects on R_t, Bottom = Effects on RR.

Source	DF	Type I MS	F Value	Pr>F
Log Weight	1	0.03054	32.30	0.0001
Population	1	0.00021	0.22	0.642
Log Weight X Population	1	0.000028	0.03	0.864

Source	DF	Type I MS	F Value	Pr>F
Log Weight	1	0.2460	141.57	0.0001
Population	1	0.0007	0.43	0.522
Log Weight X Population	1	0.0056	3.23	0.093

Source	DF	Type I MS	F Value	Pr>F
Weight	1	11205.32	115.39	0.0001
Population	1	68.15	0.70	0.415
Weight X Population	1	490.30	5.05	0.040

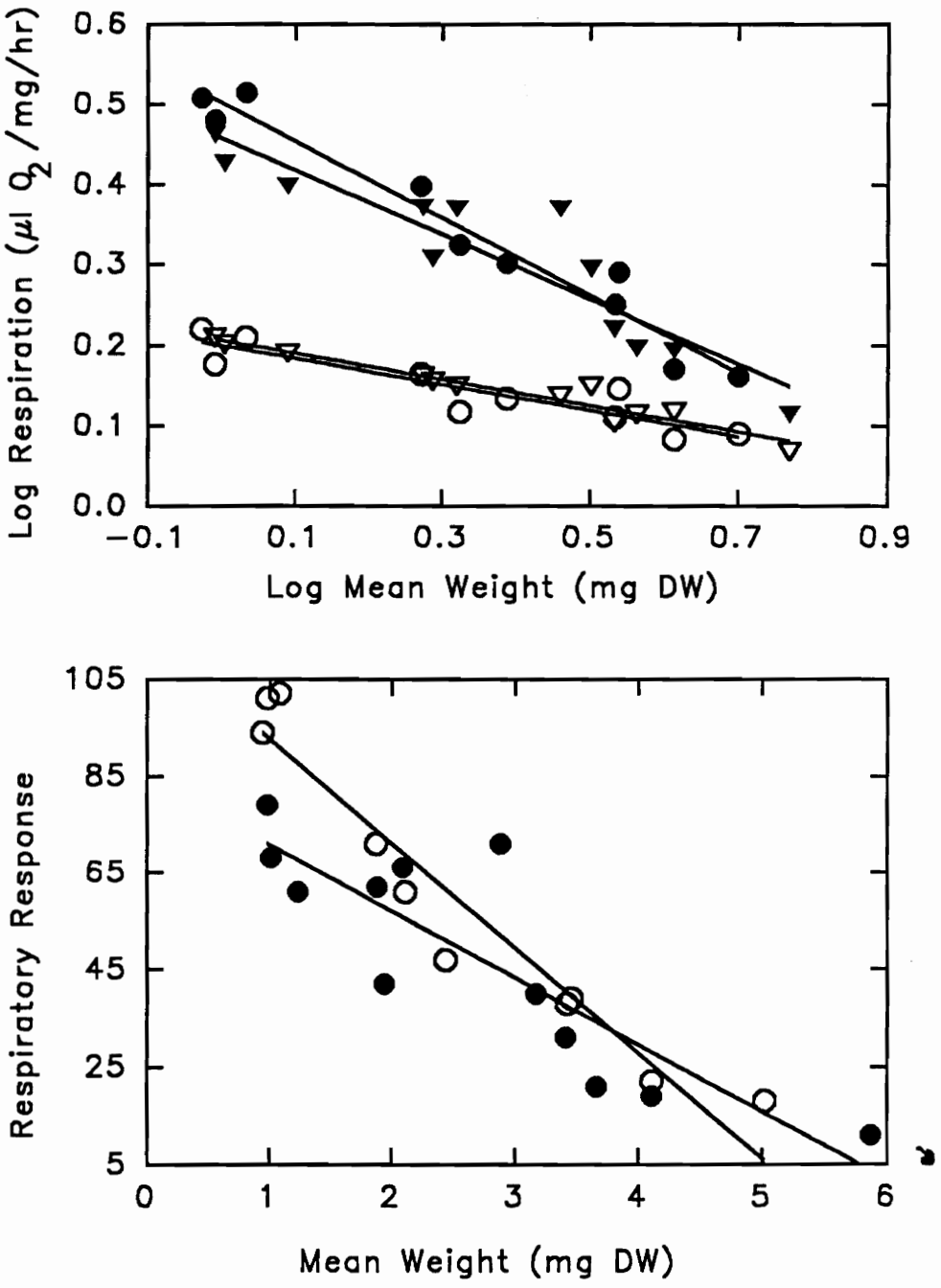

Fig. 7. Top - Size specific respiration rates of I. bicolor nymphs from a reference (circles) and a contaminated (triangles) site on the South River. Open symbols refer to rates in clean water and filled symbols refer to rates in 100µg/L Hg. Bottom - Respiratory responses (i.e. relative change in respiration following exposure). Closed circle refers to nymphs from the contaminated site.

Again, no significant population differences in R_b were observed. However, significant population differences in R_t and RR were observed (Table 9). Individuals from the reference site consistently respired more when exposed to mercury and had consistently higher RR (Fig. 8). These data indicate population differences in RR to mercury were due to differences in metabolism when exposed to mercury (R_t) and not to population differences in basal metabolism (R_b). The interaction between weight and population was again considerable for RR ($p=0.068$, Table 9) making interpretation of the analysis difficult. However, it is again clear that this was due to small population differences in RR among larger individuals. Small and medium-sized nymphs (<4 mg) from the reference site consistently exhibited larger increases in respiration after exposure (Fig. 8). In addition, preexposure resulted in no increase in tolerance in nymphs from the reference site and no decrease in tolerance in nymphs from the contaminated site. Thus, small individuals from the contaminated sites on the South River were more resistant to mercury despite preexposure treatments.

A forth experiment was conducted using nymphs from the same two sites on the South river in the late summer of 1991 (Table 3). However, for this experiment an acute concentration of mercury was used (i.e. a concentration that

Table 9. Results of Analysis of Covariance for experiment conducted June 1991. Treatment = 50 ug/l at 20°C using nymphs from a reference and polluted site on the South River. Top = Effects on R_b, Middle = Effects on R_t, Bottom = Effects on RR.

Source	DF	Type I MS	F Value	Pr>F
Log Weight	1	0.08158	219.32	0.0001
Population	1	0.0000082	0.02	0.88
Log Weight X Population	1	0.0000004	0.00	0.97

Source	DF	Type I MS	F Value	Pr>F
Log Weight	1	0.18655	309.50	0.0001
Population	1	0.00449	7.45	0.012
Log Weight X Population	1	0.00099	1.65	0.212

Source	DF	Type I MS	F Value	Pr>F
Weight	1	2395.55	39.96	0.0001
Population	1	554.18	9.24	0.0058
Weight X Population	1	220.16	3.67	0.0678

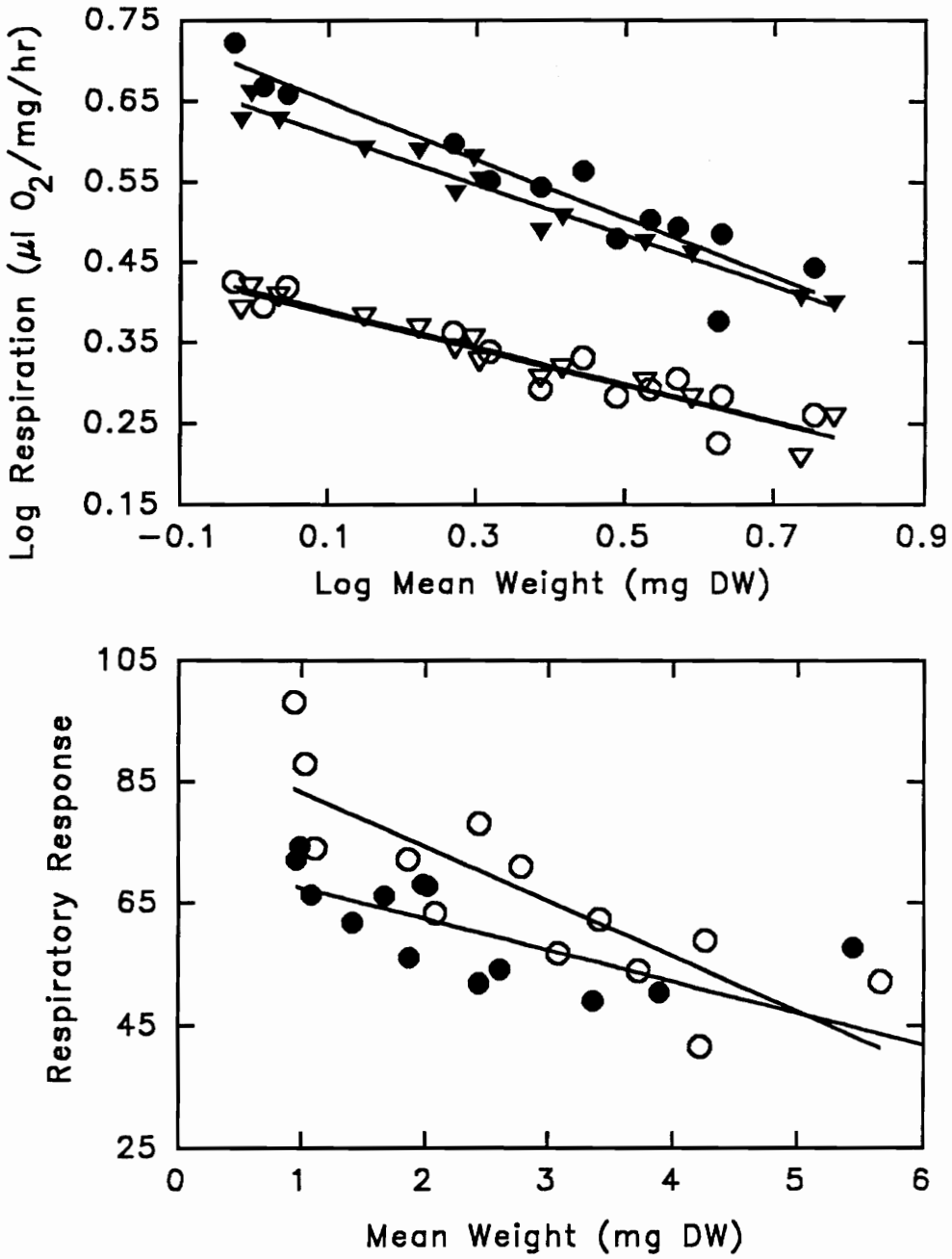

Fig. 8. Top - Size specific respiration rates of I. bicolor nymphs from a reference (circles) and a contaminated (triangles) site on the South River. Open symbols refer to rates in clean water and filled symbols refer to rates in 50µg/L Hg. Bottom - Respiratory responses (i.e. relative change in respiration following exposure). Closed circle refers to nymphs from the contaminated site.

resulted in a decrease in respiration relative to basal rates). The purpose was to determine if population differences in RR observed in the previous experiments using chronic concentrations, were also observed under more acute exposures.

No significant population differences in R_b, R_t, or RR were observed (Table 10). Although the difference in RR was not significantly different between the two populations ($p=0.061$), small nymphs (<3 mg) from the reference site exhibited on average a larger decrease in respiration following exposure (Fig. 9). Therefore, it is not clear whether nymphs from contaminated sites are more resistant to acute levels of mercury.

Experiment 5 was also conducted in the late summer of 1991 (Table 3). Nymphs used in the experiment were collected from three sites on the South river and one site on the South Fork of the Shenandoah river, each representing different levels of contamination (Table 2 and Fig. 1). The purpose was to determine if there was a relationship between response to mercury and extent of exposure. No significant population differences in R_b or R_t were observed. However, differences in RR among populations were highly significant ($p<0.0001$, Table 11). RR ranged from a 56.48 percent increase in size-specific respiration following exposure at Grottoes to 67.20 at Oak

Table 10. Results of Analysis of Covariance for experiment conducted August 1991. Treatment = 400 ug/l at 20°C using nymphs from a reference and polluted site on the South River. Top = Effects on R_b, Middle = Effects on R_t, Bottom = Effects on RR.

Source	DF	Type I MS	F Value	Pr>F
Log Weight	1	0.14549	43.12	0.0001
Population	1	0.00054	0.16	0.693
Log Weight X Population	1	0.00876	2.60	0.126

Source	DF	Type I MS	F Value	Pr>F
Log Weight	1	0.08808	16.99	0.0008
Population	1	0.00911	1.76	0.204
Log Weight X Population	1	0.00058	0.11	0.742

Source	DF	Type I MS	F Value	Pr>F
Weight	1	448.20	9.32	0.0076
Population	1	104.55	2.18	0.161
Weight X Population	1	113.22	2.36	0.142

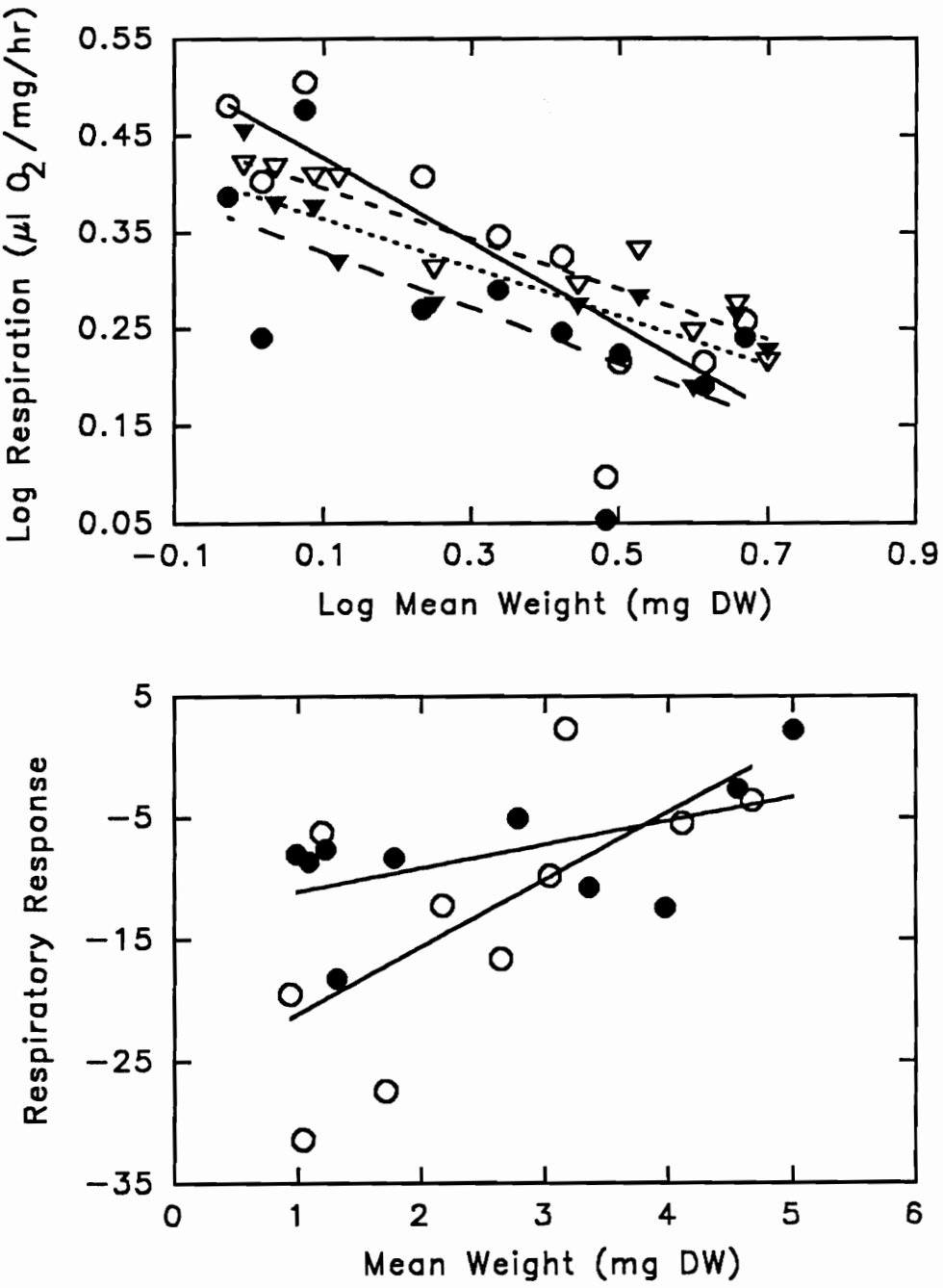

Fig. 9. Top - Size specific respiration rates of I. bicolor nymphs from a reference (circles) and a contaminated (triangles) site on the South River. Open symbols refer to rates in clean water and filled symbols refer to rates in 400µg/L Hg. Bottom - Respiratory responses (i.e. relative change in respiration following exposure). Closed circle refers to nymphs from the contaminated site.

Table 11. Results of Analysis of Covariance for experiment conducted August 1991. Treatment = 50 ug/l at 20°C using nymphs from one reference site and 3 polluted sites on the South River. Top = Effects on Rb, Middle = Effects on Rt, Bottom = Effects on RR.

Source	DF	Type I MS	F Value	Pr>F
Log Weight	1	0.11058	63.70	0.0001
Population	3	0.00069	0.40	0.757
Log Weight X Population	3	0.00214	1.23	0.317

Source	DF	Type I MS	F Value	Pr>F
Log Weight	1	0.24078	150.83	0.0001
Population	3	0.00285	1.79	0.173
Log Weight X Population	3	0.00211	1.32	0.288

Source	DF	Type I MS	F Value	Pr>F
Weight	1	3415.46	206.17	0.0001
Population	3	219.90	13.27	0.0001
Weight X Population	3	24.00	1.45	0.250

Table 12. Least-square mean respiratory response (i.e. mean response at the grand mean weight) and standard error for four populations from the South River (top) and a table of p-values showing the results of paired t-tests for population differences in LS mean responses (bottom).

Population	[Hg] (μg/g)	LS Mean Response (% Change)	S.E.
Oak Hill	<0.01	67.20	1.36
Chrimora	2.23	57.64	1.40
Grottoes	1.27	56.48	1.37
Lynwood	0.12	61.98	1.48

	Oak Hill	Chrimora	Grottoes	Lynwood
Oak Hill	----	0.0001	0.0001	0.015
Chrimora	0.0001	----	0.556	0.042
Grottoes	0.0001	0.556	----	0.011
Lynwood	0.015	0.042	0.011	----

Hill (Table 12). This represents about a 19% difference among the 4 populations. RR was negatively correlated with whole-body mercury levels measured in nymphs. Responses were highest in nymphs collected from the reference site (Oak Hill), lowest in nymphs from Chrimora and Grottoes where mercury levels were highest, and increased again in nymphs collected from Lynwood, where mercury levels were intermediate (Table 12). Mean RR of nymphs collected from all three contaminated sites were significantly lower than those collected from Oak Hill (Table 12). In addition, RR of nymphs from Chrimora and Grottoes were significantly lower than nymphs from Lynwood. However, there was no significant difference in RR between nymphs from Chrimora and Grottoes even though mercury levels in the body tissues of nymphs were nearly twice as high at Chrimora (Table 12).

FIELD SURVEY

Growth and Development

I. bicolor was bivoltine at both sites on the South river. Both the upstream (Oak Hill) and downstream (Grottoes) populations consisted of a winter generation between September and May and a summer generation between late May and August (Fig. 10). Most of the growth by the winter generation occurred in fall and spring. Little growth occurred between November and late April. Individuals at the upstream site

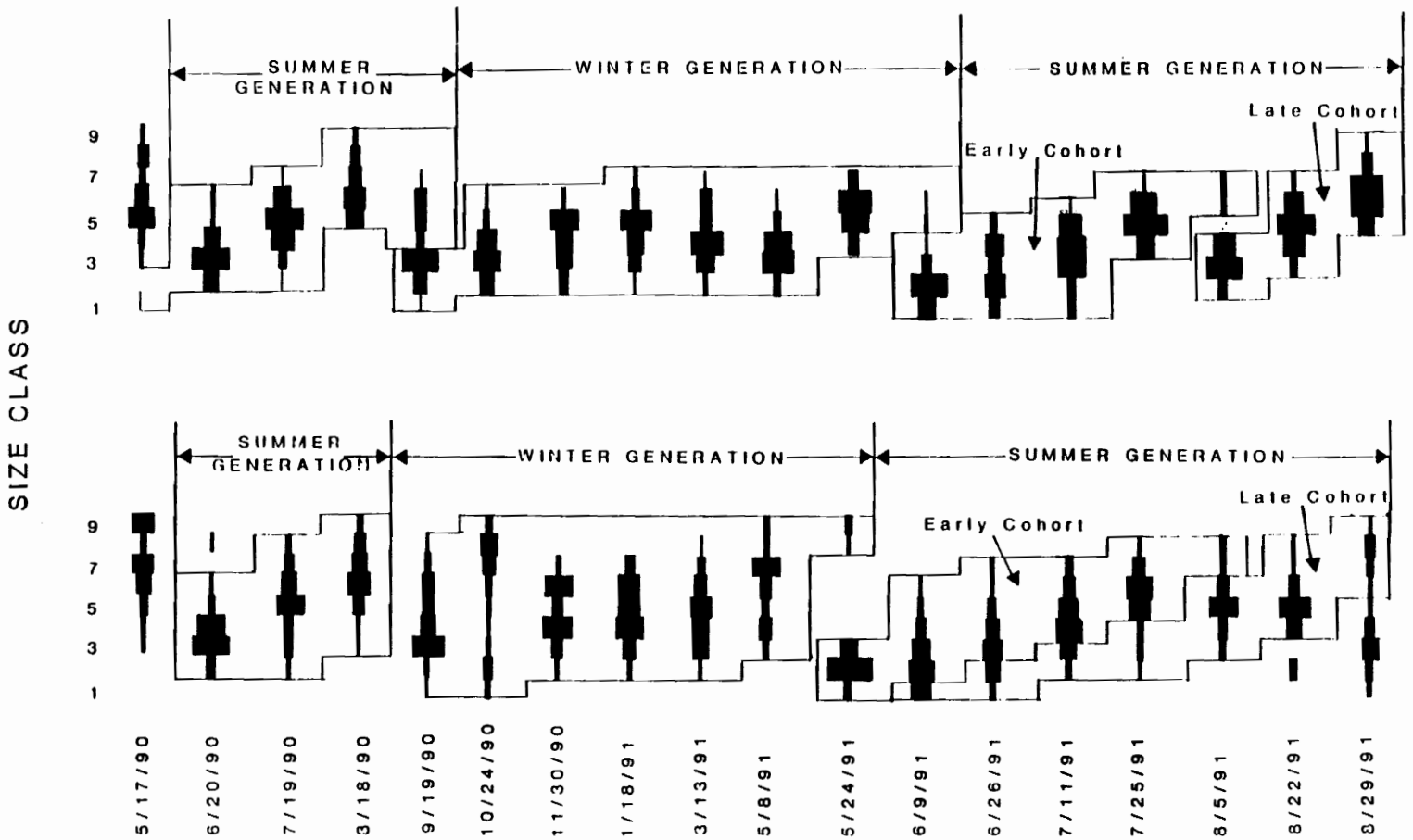

Fig. 10. Size-frequency histograms for populations of I. bicolor from upstream (Oak Hill) and downstream (Grottoes) sites on the South River. Size classes 1-9 are based on body length measurements (see text). The width of each box refers to the percentage of total nymphs collected in each size class.

overwintered primarily in size classes 3-5. Most downstream nymphs overwintered slightly larger (4-6) because more growth occurred during the fall.

Small individuals were found earlier in the spring at the downstream site (Fig. 10). However, at both sites, hatching of the summer generation was asynchronous resulting in a relatively large percentage of both populations consisting of small nymphs throughout much of the summer. In fact, at the downstream site, hatching began sufficiently early, and growth was sufficiently fast, that a small portion of the population may have completed two generations in the summer. At any rate, at least two recognizable cohorts (i.e. groups of individuals within a generation with common phenologies) developed during the summer at both sites. However, I was able to discern the two summer cohorts only for the summer of 1991 when sampling was more frequent (Fig. 10).

Average individual size fluctuated considerably at both sites during the summer of 1991 (Fig. 11, top). Decreases in size during the summer generation were associated with recruitment of small individuals due to asynchronous hatching throughout the summer. Cohorts were split out as shown in figure 10 and relative growth rates were calculated separately for each cohort. Growth rates of each cohort on the South river ranged between 2.09 and 2.81 %/day (Fig. 11,bottom; Table 13). Little between-site differences in growth rate

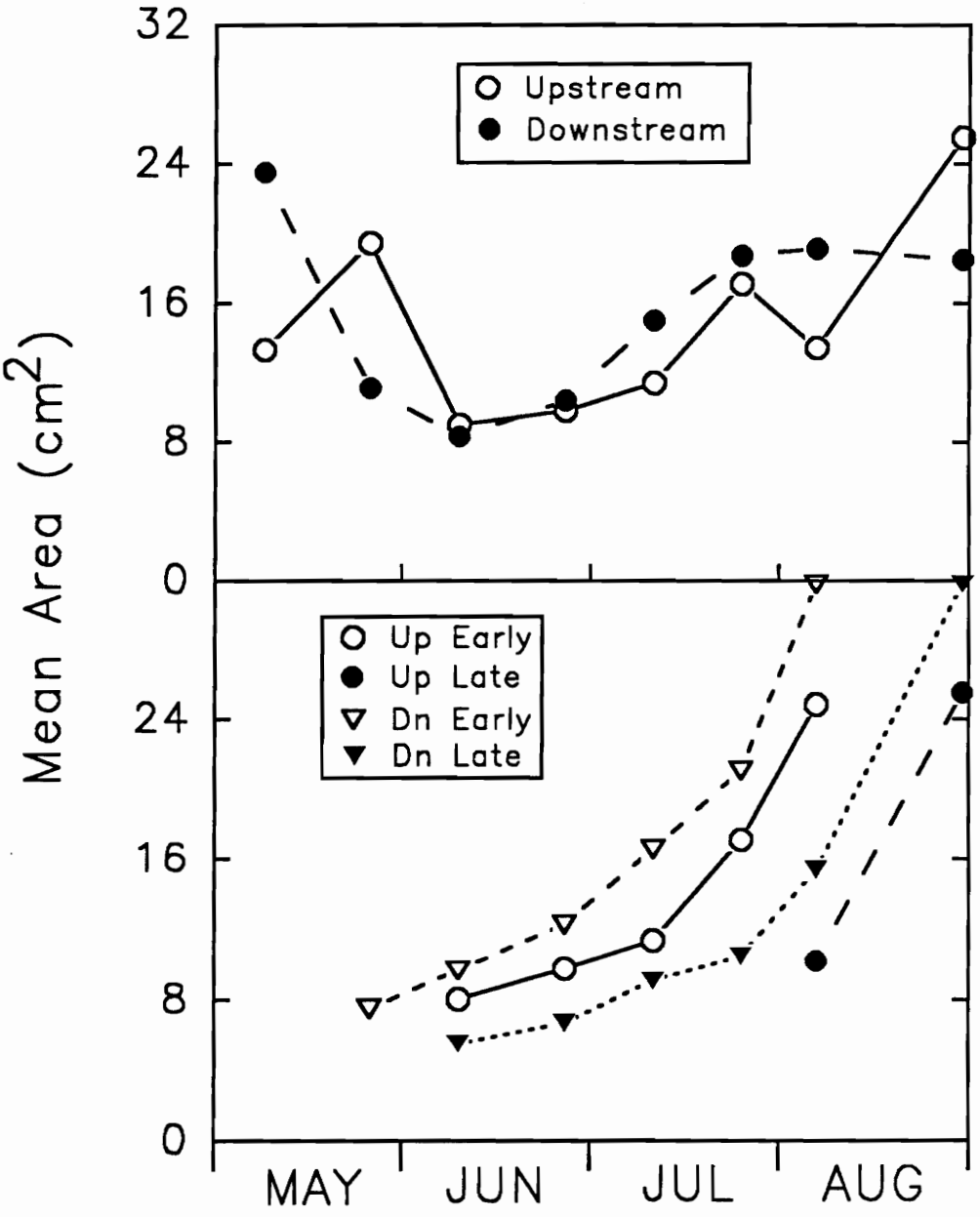

Fig. 11. Top - Mean individual size of I. bicolor nymphs at the upstream (Oak Hill) and downstream (Grottoes) sites on the South River during the summer of 1991. Bottom - Growth patterns for the same time period after cohorts were seperated (see text for details).

Table 13. Growth rates of *Isonychia bicolor* nymphs based on field collections taken from sites upstream and downstream of the source of mercury pollution on the South (top) and Holston (bottom) rivers. Samples were collected during the summer of 1991 and growth rates were calculated between each time interval for each cohort (early/late) using the equation: GR = Ln (S_f/S_i)/day X 100, where GR is the relative growth rate expressed as percent change in mean size, S_f is the final size (cm^2) for the time interval, and S_i is the initial size for the time interval.

	UPSTREAM		DOWNSTREAM	
Sampling Interval	**Early**	**Late**	**Early**	**Late**
5/24 - 6/9			1.82*	
6/9 - 2/26	1.21*		1.43*	1.23
6/26 - 7/11	1.14		2.12	2.18
7/11 - 7/25	2.91		1.68	2.47*
7/25 - 8/6	3.12	1.72*	3.39	3.21*
8/6 - 8/29		3.90*		
OVERALL	**2.10**	**2.81**	**2.09**	**2.27**

	UPSTREAM		DOWNSTREAM	
Sampling Interval	**Early**	**Late**	**Early**	**Late**
5/17 - 5/27	1.48		-0.13	
5/27 - 6/14	1.91		1.27*	
6/14 - 6/28	4.00	1.92*		
7/3 - 7/19		2.58*		
7/19 - 7/28		3.12		
OVERALL	**2.46**	**2.54**	**1.27**	

* Growth rates of nymphs during time intervals when enclosure experiments were underway.

were observed for the early-summer cohorts. However, nymphs from the upstream site grew 19% faster than downstream nymphs in late summer. This was likely due to differences in phenology. Late-summer nymphs at the upstream site were only present in August when stream temperatures were very high. Conversely, late-summer nymphs at the downstream site were present between June and August and thus growth was averaged over a period with a wide range in water temperature (Fig. 11).

Growth and development patterns at the upstream site (McCready) on the Holston were similar to those observed on the South river. The life cycle consisted of a long winter generation and a short summer generation with two recognizable cohorts (Fig. 12). The downstream population (Hayters Gap) exhibited a similar pattern through the winter although qualitative collections took considerably more time suggesting lower densities. However, no nymphs were found at the downstream site in April which represents the end of the winter generation. Small individuals were collected in the spring but nymphs were again conspicuously absent from the downstream site throughout the summer (Fig. 12). Growth rates of the summer cohorts at the upstream site on the Holston river were comparable to those observed on the South River (Fig. 13,bottom; Table 13). Growth rate of the early summer cohort at the downstream site was much slower than at the

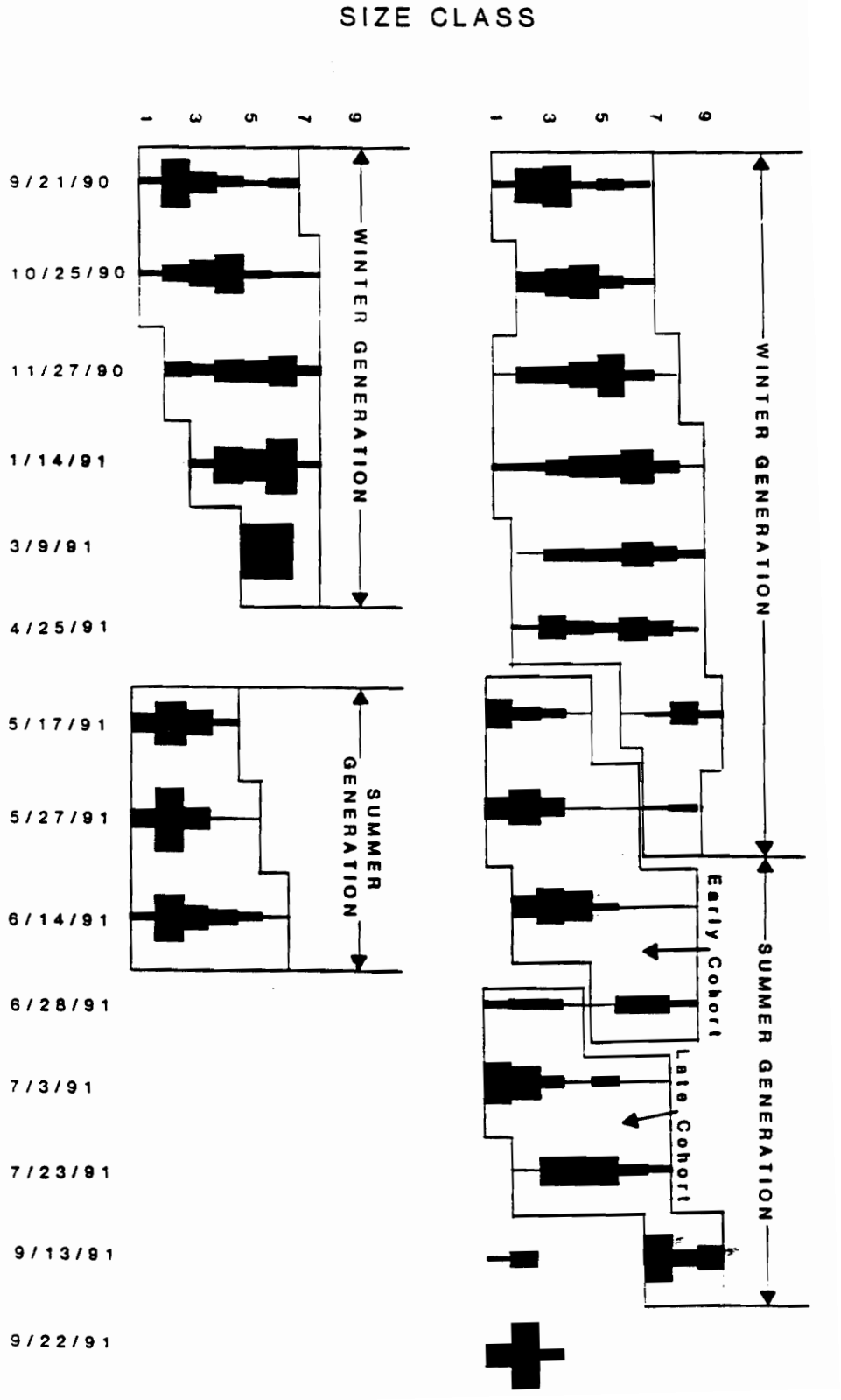

Fig. 12. Size-frequency histograms for populations of I. bicolor from upstream (McCready) and downstream (Hayters Gap) sites on the Holston River. Size classes 1-9 are based on body length measurements (see text). The width of each box refers to the percentage of total nymphs collected in each size class.

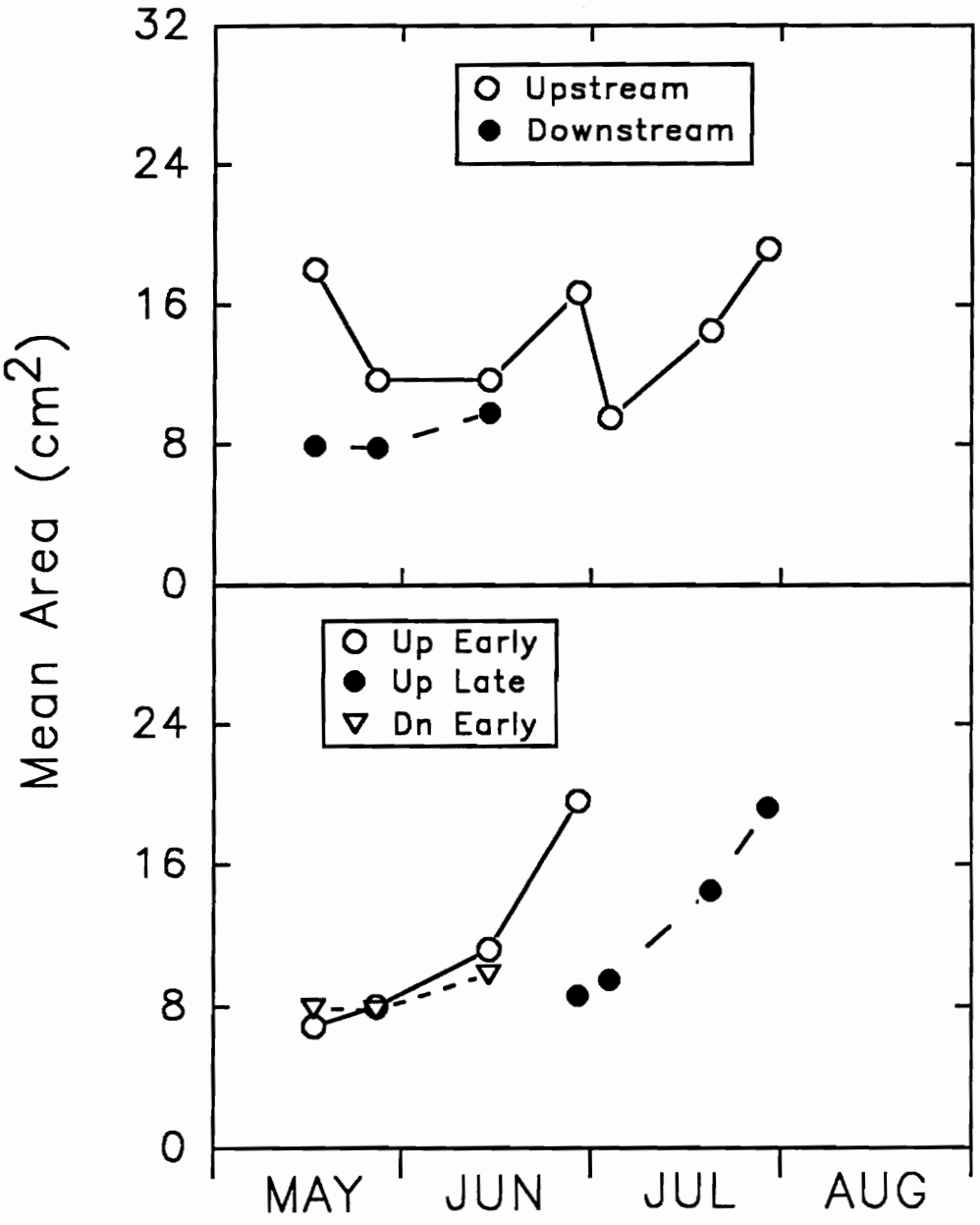

Fig. 13. Top - Mean individual size of I. bicolor nymphs at the upstream (McCready) and downstream (Hayters Gap) sites on the Holston River during the summer of 1991. Bottom - Growth patterns for the same time period after cohorts were seperated (see text for details).

upstream site. As noted above, no nymphs were present at the downstream site in the late summer.

Survival

Average density on the South river were higher at the downstream site throughout most of the summer of 1991 (Fig. 14). Density increases throughout the spring at both sites were the result of recruitment through asynchronous hatching. Two additional increases were observed for the downstream population in late July and August. These also represent times when the number of births exceed the number of deaths and support the conclusion of more than one cohort developing during the summer, as noted above. The peak in August was likely associated the with the onset of hatching of the subsequent winter generation. Aside from the peak in the spring, there were no additional peaks in average densities at the upstream site despite the presence of an additional cohort. Thus, the rate of recruitment was lower than the death rate.

Average densities were similar at both sites on the Holston river in the spring (Fig. 14). However, densities continued to increase at the upstream site, reaching a peak in late June of 227 individuals/m^2. Conversely, average density at the downstream site fell to zero in the early summer and

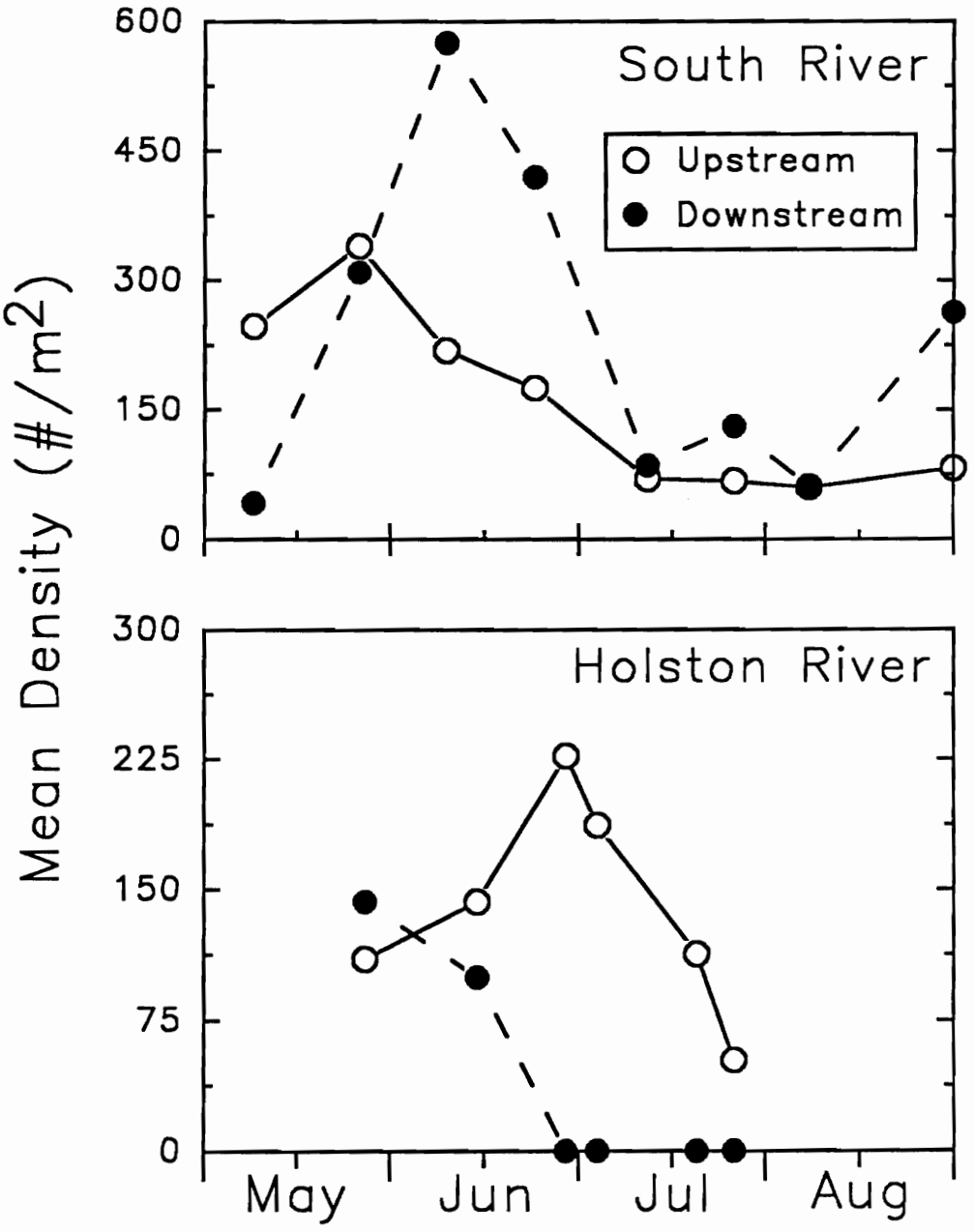

Fig. 14. Mean densities of I. bicolor nymphs from upstream and downstream sites on the South and Holston Rivers during the summer of 1991.

never recovered. During this time, both qualitative (i.e. kick net) and quantitative (i.e. Hess sampler) samples were taken over a variety of habitats including both pools and riffles, deep and shallow areas, and a range of substrate sizes. Also, additional sites (N=6-10) downstream of Saltville were sampled frequently during the summer. Even so, very few *I. bicolor* nymphs were found between the Olin Plant and Mendota (Fig. 2), a distance of 47 river miles. Therefore, small nymphs observed in the spring either died, drifted far downstream, or migrated upstream perhaps into smaller tributaries.

FIELD EXPERIMENTS

Growth Rates

No significant differences were observed in growth rates between upstream and downstream nymphs at either site on the South river during the *early-summer* experiment ($p>0.50$)(Fig. 15, top). Essentially the same pattern was observed at all three sites on the Holston river during the *early-summer* experiment ($p>0.13$)(Fig. 15, bottom). However, I clearly overestimated the time necessary for nymphs to complete their life cycle. In many of the enclosures, especially those with downstream nymphs, a large fraction of individuals had molted to adults and drowned prior to their removal. As a result, I

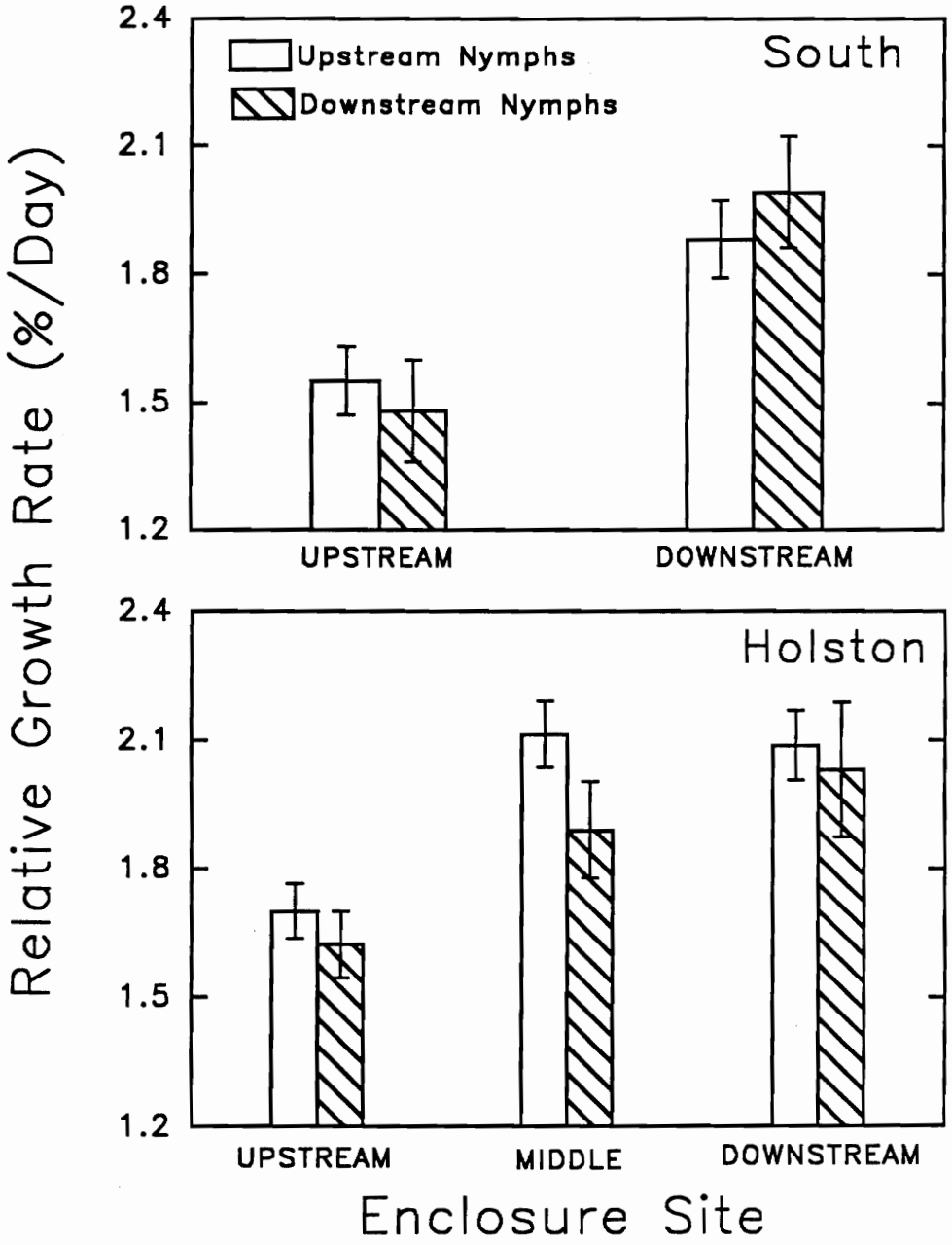

Fig. 15. Comparison of relative growth rates between upstream and downstream nymphs reared at both upstream and downstream sites during the early-summer field experiment Data for both the South and Holston Rivers are presented.

was concerned that I may have underestimated growth of downstream nymphs. Therefore, the experiment was repeated on the South river, but this time, nymphs were removed from enclosures, counted and photographed each week (see Methods). Also, nymphs were returned to the lab as they matured (evidenced by dark wing pads) instead of simultaneously at the end of the experiment. An attempt was made to repeat the experiment on the Holston river also, but as reported earlier sufficient numbers of individuals could not be found anywhere below Saltville, Virginia.

No significant differences were observed in growth rates between upstream and downstream nymphs at the upstream site on the South river during the *late-summer* experiment ($T=0.34$, $p=0.74$). However, downstream nymphs grew significantly faster than upstream nymphs at the downstream site ($T=2.20$, $p=0.05$)(Fig. 16). In order to compare the results of these experiments, it was necessary to ensure that nymphs from both sites were approximately the same size at the beginning. Unfortunately, hatching occurred earlier at the downstream site on both rivers (Figs. 10 and 12), and therefore enclosures containing downstream nymphs were placed in the stream between 9 and 22 days earlier. Thus, upstream and downstream nymphs at all sites except the middle site on the Holston, where both upstream and downstream nymphs were begun at the same time, were subjected to potentially different

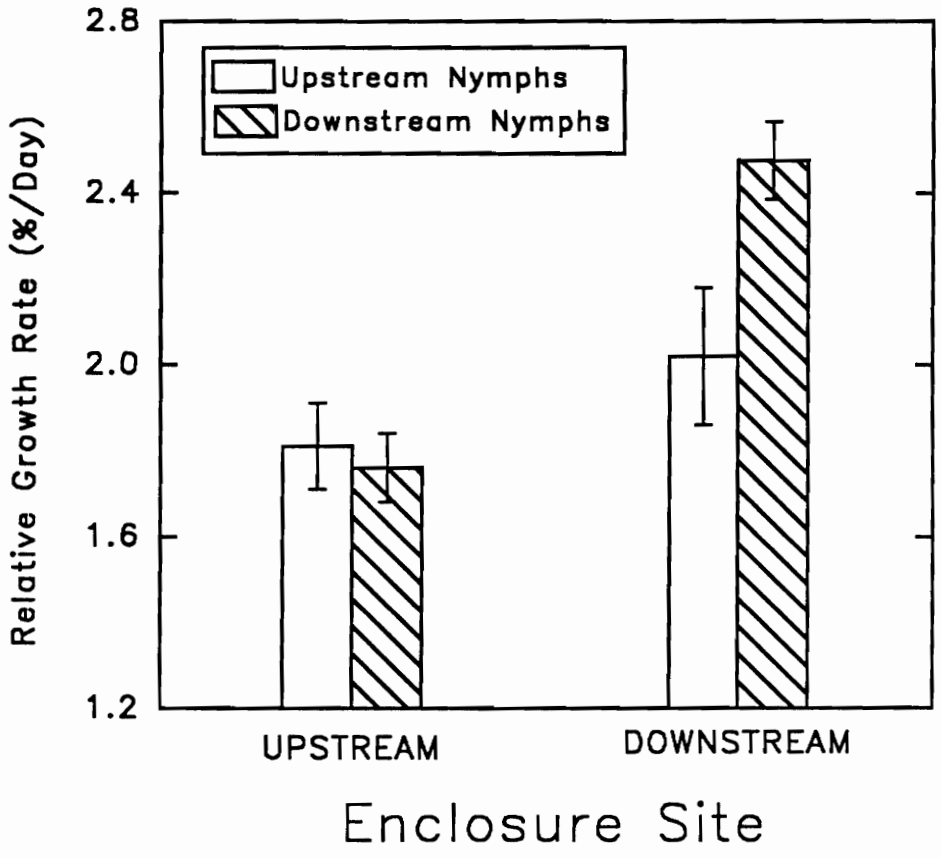

Fig. 16. Comparison of relative growth rates between upstream and downstream nymphs reared at both upstream and downstream sites during the late-summer field experiment conducted on the South River.

temperature regimes during the experiment. Because temperature is known to influence growth (Beck 1983), it was necessary to account for these differences. Therefore, growth rates were recalculated as percent change in weight per "degree-day > 10°C" instead of per "day" (i.e. temperature-corrected growth rates). A threshold temperature of 10°C was chosen because little growth and development occurred during the winter when stream temperatures were below 10°C.

No significant differences in temperature-corrected growth rates were observed between upstream and downstream nymphs at any of the sites on either river ($p>0.29$)(Fig. 17). These data suggest that faster growth of downstream nymphs at the downstream site on the South river in *late-summer* was accounted for by temperature differences and probably not due to higher tolerance of downstream nymphs to a mercury contaminated habitat.

Mortality

No significant differences in mortality rates were observed between upstream and downstream nymphs at either site on the South river during the *early-summer* experiment ($p>0.18$)(Fig. 18). Likewise, no significant differences were observed at the middle and downstream sites on the Holston ($p>0.40$). However, downstream nymphs had a significantly

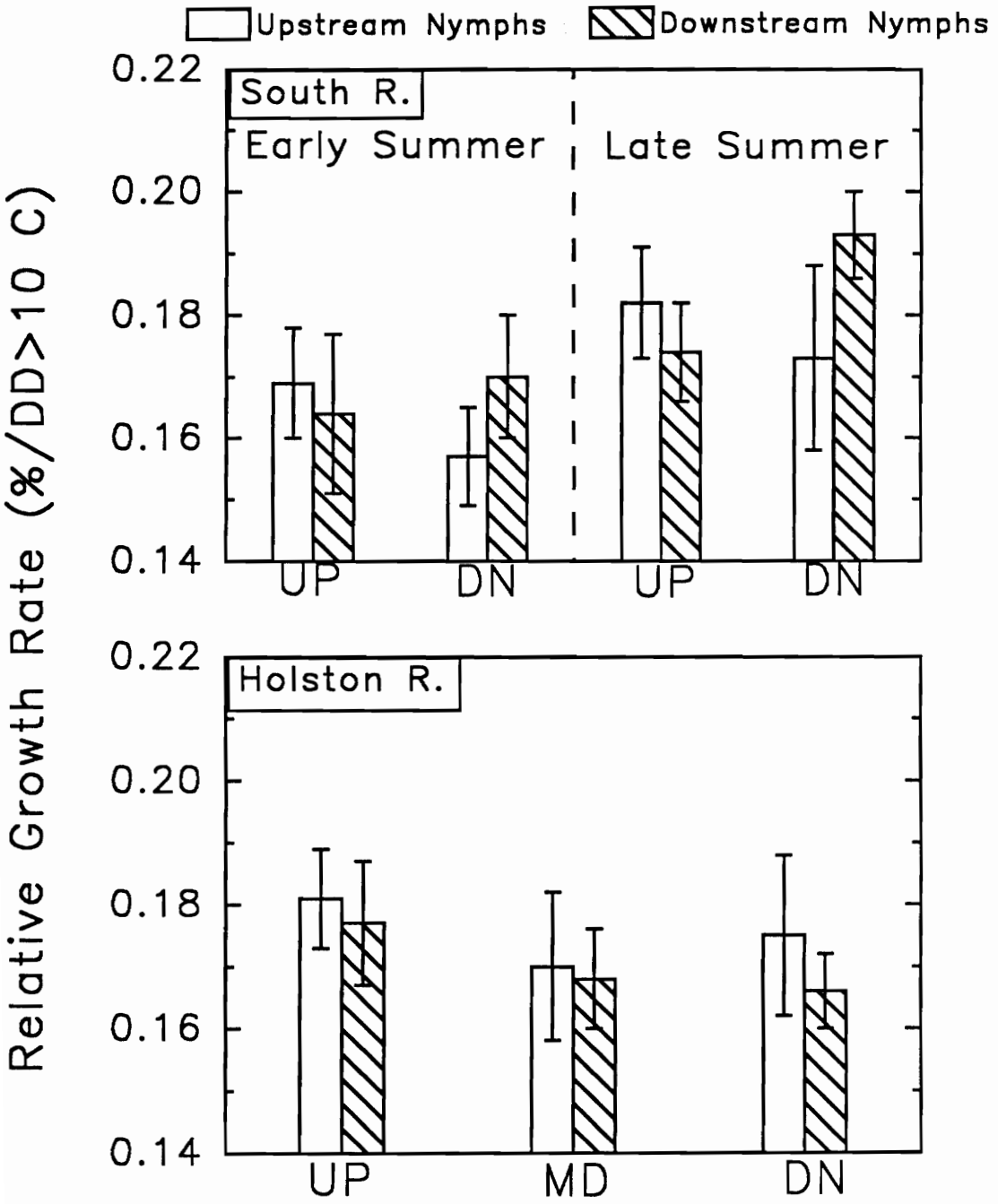

Fig. 17. Comparison of relative growth rates between upstream and downstream nymphs reared at both upstream and downstream sites after correcting for temperature (i.e. % change in size/degree day > 10°C). See text for complete description.

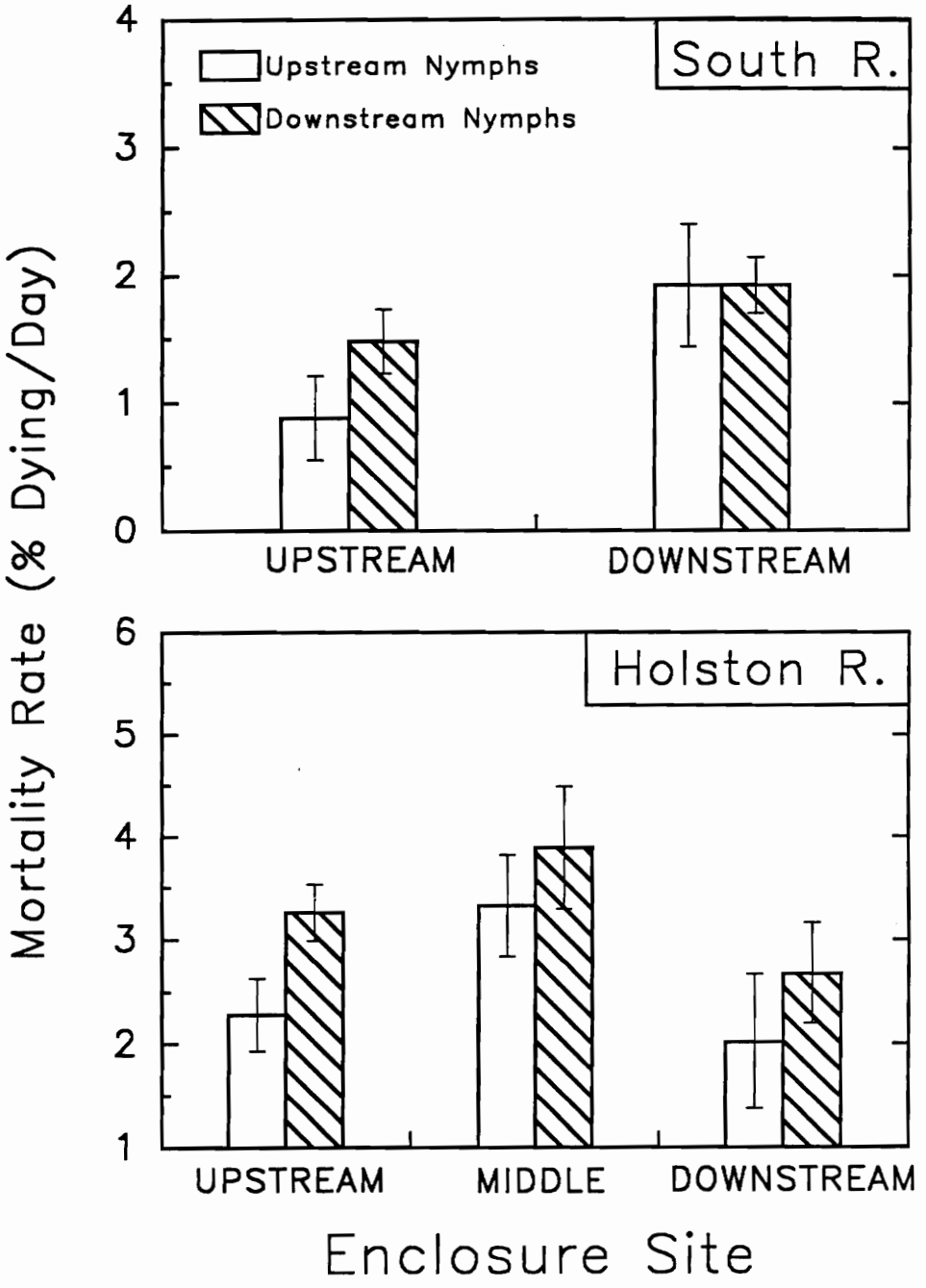

Fig. 18. Comparison of mortality rates between upstream and downstream nymphs incurred during transplant experiments at the upstream and downstream sites during the early summer experiment. Data for both the South and Holston Rivers are presented.

higher mortality rate at the upstream site on the Holston (T=2.22, p=0.05)(Fig. 18).

During the *late-summer* experiment, pattern as well as the rate of mortality could be examined because enclosures were checked weekly. Early in the experiment when nymphs were small, mortality was higher in downstream nymphs at the upstream site, and in upstream nymphs at the downstream site (Fig. 19). However, no significant differences in mortality rate were observed at the end of the experiment (p>0.35). This pattern might suggest that individuals were stressed or killed during transplant. No obvious differences in emergence patterns were observed between upstream and downstream nymphs at either site (Fig. 19).

Fecundity

No significant differences in fecundity were observed between upstream and downstream females when reared at the upstream site for both experiments on the South river (p>0.47)(Fig. 20). Conversely, females from the downstream population were considerably more fecund than females from the upstream population when reared at the downstream site. This pattern was consistent for both experiments (*Early-summer*: T=1.61, p=0.13; *Late-summer*: T=1.99, p=0.07)(Fig. 20).

To determine the extent to which population differences in fecundity on the South river were influenced by female

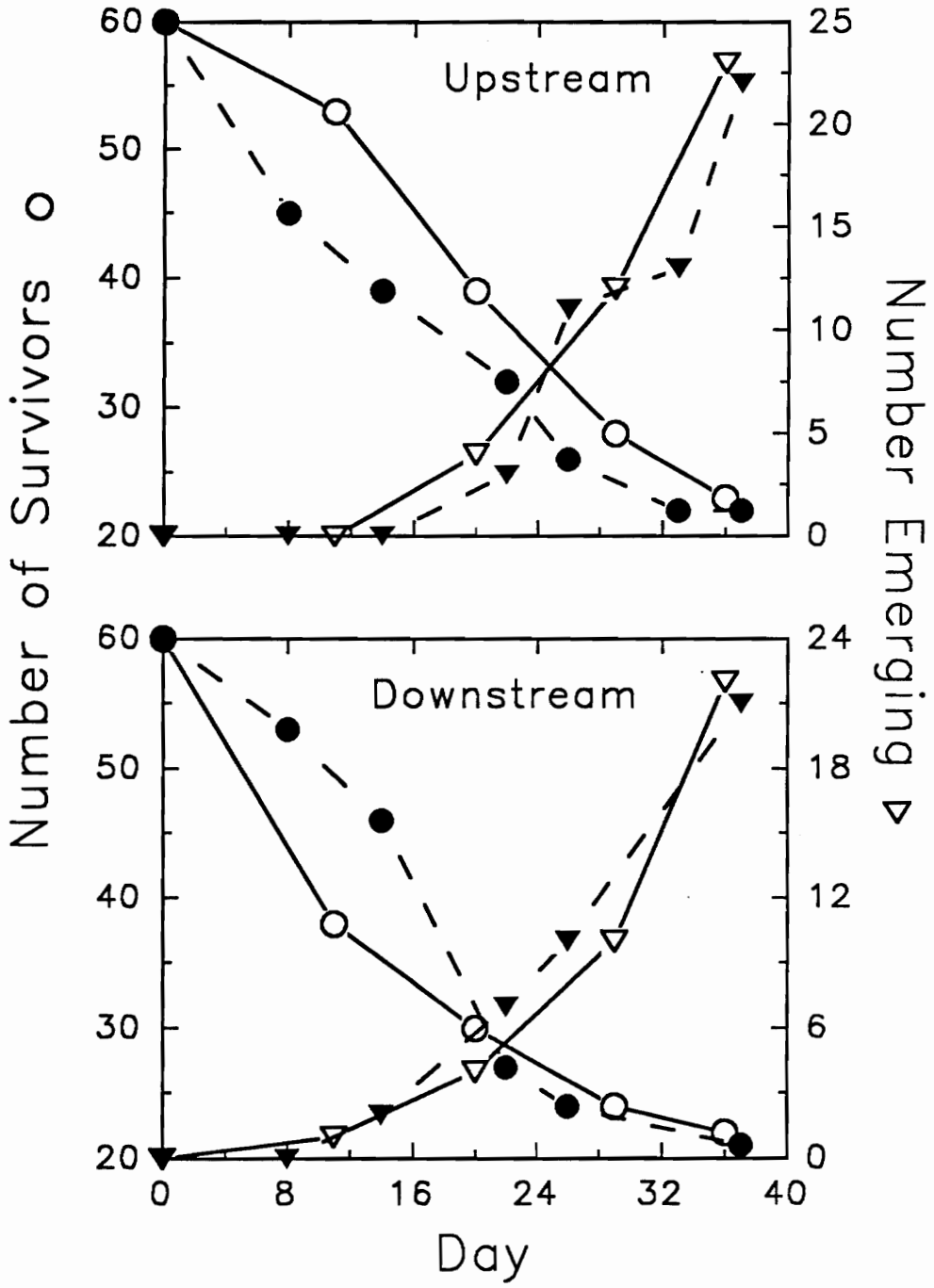

Fig. 19. Comparison of mortality and emergence patterns between upstream and downtream nymphs reared seperately at upstream and downstream sites on the South River during the late-summer experiment. Open symbols refer to upstream nymphs and closed symbols refer to downstream nymphs.

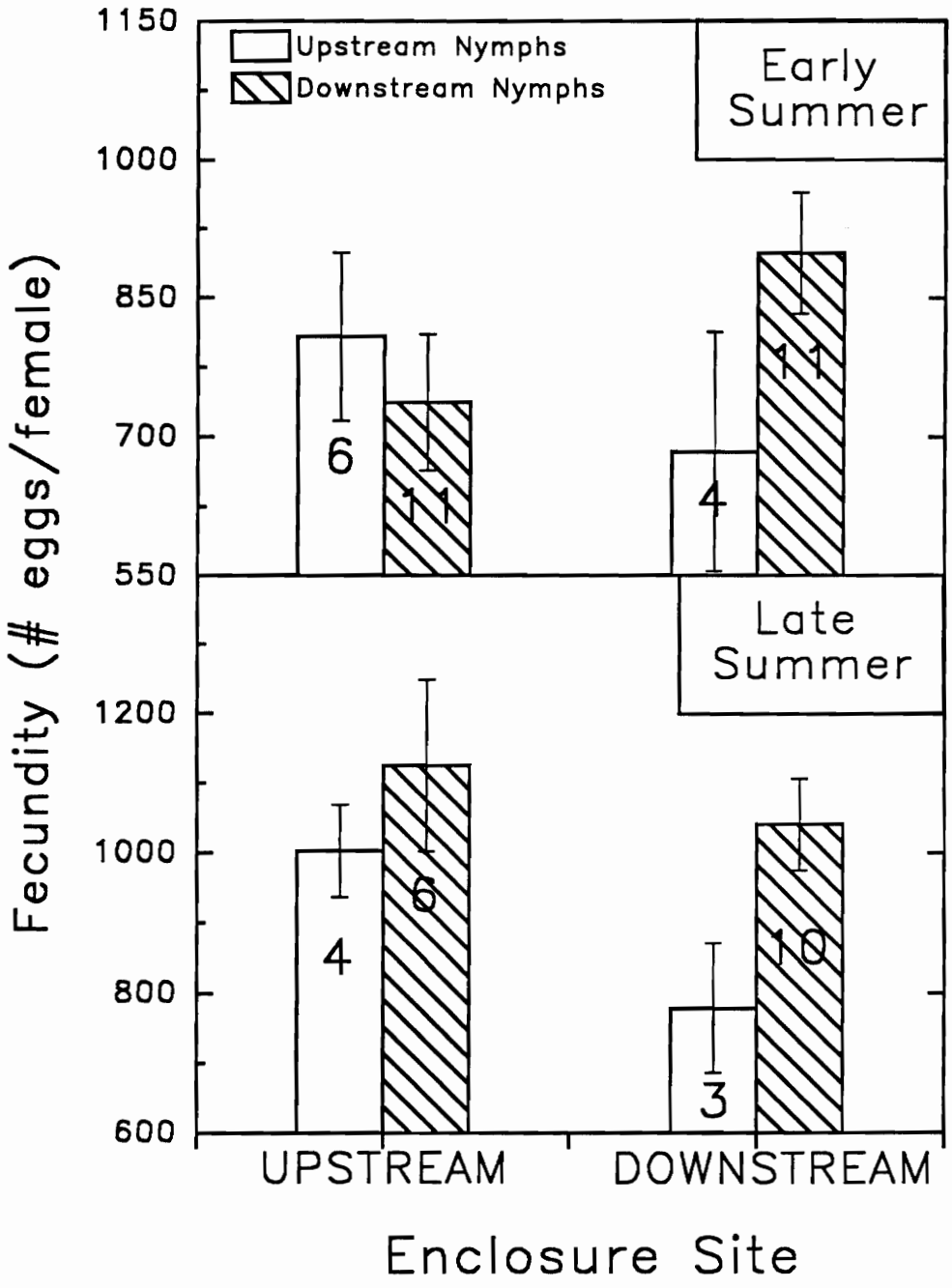

Fig. 20. Comparison of average fecundities of females from upstream and downtream populations when reared seperately at upstream and downstream sites on the South River. Data for both the early and late-summer experiments are presented.

size, the data were reanalyzed using analysis of covariance with population as the class variable and female size as the covariate. For the *early-summer* experiment, no significant population differences in fecundity were observed at either site after taking into account female size (Table 14). However, during the *late-summer* experiment, a considerable portion of the variability in fecundity at the downstream site (58% of the MS Error, p=0.09) was explained by population. Conversely, female size explained only 29% of the variability in fecundity (Table 15). Neither population or size explained a significant amount of variability in fecundity at the upstream site (Table 15).

Mortality was considerably higher on the Holston river than on the South (Fig. 18). As a result, fewer individuals of each population completed their life cycles at any of the sites on the Holston. Therefore, between-population comparisons of fecundity were not feasible. Raw data for individuals that successfully completed their life cycles on the Holston river are summarized in table 16. No population differences in either variable were obvious.

ALLOZYME DATA

Differential Survival Experiments

The first experiment was conducted at 22°C using nymphs

Table 14. Results of Analysis of Covariance designed to test for population differences in fecundity during transplant experiments on the South River in the early-summer of 1991. Source population (i.e. resident/transplant) was the class variable (Pop) and body weight was the covariate (Wt).Top-Results at upstream site; Bottom-Results at the downstream site.

Source	DF	MS Error	F Value	Pr>F
Wt	1	1870523	0.30	0.60
Pop	1	2368613	0.37	0.55
Wt * Trt	1	36741	0.01	0.94

Source	DF	MS error	F Value	Pr>F
Wt	1	278037	6.17	0.03
Pop	1	32100	0.71	0.42
Wt * Pop	1	35354	0.78	0.40

Table 15. Results of Analysis of Covariance designed to test for population differences in fecundity during transplant experiments on the South River in the late-summer of 1991. Source population (i.e. resident/transplant) was the class variable (Pop) and body weight was the covariate (Wt).Top-Results at upstream site; Bottom-Results at the downstream site.

Source	DF	MS Error	F Value	Pr>F
Wt	1	130779	1.90	0.22
Pop	1	1556	0.02	0.89
Wt * Trt	1	2929	0.04	0.84

Source	DF	MS error	F Value	Pr>F
Wt	1	71530	1.81	0.21
Pop	1	143402	3.63	0.09
Wt * Pop	1	30843	0.78	0.40

Table 16. Adult weight and fecundity data for individuals from the Holton river field experiment.

Enclosure Site	Source of Nymphs	Sex	Weight (mg DW)	Number Eggs
Upstream	Upstream	F	5.387	1092
Upstream	Upstream	F	4.418	1106
Upstream	Upstream	F	5.116	855
Upstream	Upstream	M	3.414	
Upstream	Downstream	F	4.777	677
Upstream	Downstream	F	4.941	836
Upstream	Downstream	F	3.886	411
Downstream	Upstream	F	5.221	1038
Downstream	Upstream	F	4.967	876
Downstream	Upstream	F	4.209	799
Downstream	Upstream	M	2.899	
Downstream	Upstream	M	3.112	
Downstream	Downstream	F	4.815	804
Downstream	Downstream	F	5.015	902
Downstream	Downstream	F	3.678	331
Downstream	Downstream	M	2.141	
Middle	Upstream	M	4.030	

from Sinking Creek, a small, unpolluted stream in Virginia. A total of 70 mayflies were treated with mercury. Of these, 42 (60%) died at the end of 76 hr. None of the individuals in the control group died during the experiment.

I was able to score only 41 nymphs at the GPI locus because of bad chemicals or a mistake in staining. Neither PGM or GPI genotype frequencies were found to deviate from Hardy-Weinburg expectations in the Sinking Creek population used in this test (PGM: $Chi^2=0.46$, GPI: $Chi^2=0.83$, $p>0.70$ for each).

Differences in sensitivity to acute mercury exposure were observed among alleles and genotypes at the GPI locus (Table 17). A significantly higher proportion of GPI^S alleles, and a significantly lower proportion of GPI^M alleles were found in the survivor fraction than would be expected by chance. Similarly, a significantly higher proportion of individuals homozygous for the GPI^M allele were found among the dead than would be expected by chance. No such differences were observed at the PGM locus (Table 17).

The second experiment was conducted at 10°C using nymphs from Mill Creek, another unpolluted stream in Virginia. This allowed me to determine if genotype differences observed in the first experiment were consistent across temperatures and populations. In addition, the experimental design and

Table 17. Results of first differential survivorship experiment conducted on 7/21/90 using nymphs collected from Sinking Creek, Virginia. Frequencies of each allele and genotype in survivors relative to frequencies of all others combined was compared to frequencies in the fraction that died using the G test of independence. Results for both the GPI and PGM enzyme loci are reported.

GPI ALLELE (DF=2)

Allele	Survived	Died	N	G_{adj}[1]	Pr>G
S	0.16	0.00	5	9.92	0.007
M	0.78	1.00	75	14.21	0.0008
F	0.06	0.00	2	3.84	0.15
N	32	50	82		

GPI GENOTYPE (DF=3)

Genotype	Survived	Died	N	G_{adj}[1]	Pr>G
SS	0.06	0.00	1	1.92	0.59
SM	0.19	0.00	3	12.96	0.005
MM	0.62	1.00	35	6.02	0.11
MF	0.13	0.00	2	3.93	0.27
N	16	25	41		

PGM ALLELE (DF=1)

Allele	Survived	Died	N	G_{adj}[1]	Pr>G
S	0.93	0.92	129	0.07	0.96
F	0.07	0.08	11		
N	56	84	140		

PGM GENOTYPE (DF=1)

Genotype	Survived	Died	N	G_{adj}[1]	Pr>G
SS	0.86	0.83	59	0.07	0.99
SF	0.14	0.17	11		
N	28	42	70		

[1]Used Williams Correction factor (Williams 1976) for small sample size.

statistical analysis employed in the second experiment (see Methods) were more informative, providing median time-to-death (TTD) estimates for each genotype as well as testing for the potentially confounding effects of body size on TTD. Furthermore, these methods were more robust with small sample sizes. Because survival was related to genotype only at the GPI locus in the first experiment, nymphs in the second experiment were not stained for PGM.

A total of 125 mayflies were treated with mercury and the experiment was allowed to continue until 159 hr at which time only 21 nymphs (17%) were still alive. Two individuals from the control group died early in the experiment. These deaths are believed to be due to stress imposed during transport from the field. Genotype frequencies conformed to Hardy-Weinburg expectations in the Mill Creek population used in this test also.

No relationship between body length and TTD was observed. However, TTD was found to differ significantly among GPI genotypes (Table 18,Top). Median TTD was lowest in the GPI^{S},GPI^{F} heterozygote (Table 18, Middle). However, this genotype was represented by only one individual in the Mill Creek population (i.e. 0.8%) and was never observed in populations on the South or Holston rivers. Among the remaining four genotypes, TTD ranged from 49.24 in the

Table 18. Results of the second differential survival experiment conducted on 2/13/91 using nymphs from Mill Creek, Virginia. The LIFEREG procedure of SAS was used to test for effects of GPI genotype and size on individual time to death (TTD). The results of the lIFEREG procedure are reported at the top, frequencies and median TTD for each genotype in the middle, and a table of p-values computed for each pair-wise genotype comparison is illustrated at the bottom.

RESULTS OF LIFEREG PROCEDURE

Variable	DF	Rank	SE Rank	X^2	Pr>X^2
Intercept	1	1.951	0.736	7.020	0.008
Size	1	0.035	0.051	0.471	0.493
Genotype	4			17.429	0.002

MEDIAN TIME TO DEATH

Genotype	Frequency	N	TTD (hr)
SS	0.06	7	73.61
SM	0.20	24	82.50
MM	0.65	78	49.24
MF	0.08	10	78.56
SF[1]	0.01	1	16.69

PAIRWISE COMPARISONS AMONG GENOTYPES

---	SS	SM	MM	MF	SF[1]
SS	---	0.69	0.13	0.85	0.03
SM	0.69	---	0.001	0.85	0.02
MM	0.13	0.001	---	0.04	0.09
MF	0.85	0.85	0.04	---	0.02
SF[1]	0.03	0.02	0.09	0.02	---

[1] Genotype only found in Mill Creek population

GPI^{M},GPI^{M} homozygote to 82.50 in the GPI^{S},GPI^{M} heterozygote (Table 18, Middle). The common GPI^{M},GPI^{M} homozygote survived a significantly shorter period than the GPI^{S},GPI^{M} and GPI^{M},GPI^{F} heterozygotes (Table 18, Bottom). The other homozygote scored at this locus (GPI^{S},GPI^{S}) was intermediate in terms of TTD. These findings are in agreement with results of the first experiment conducted at summer temperatures using nymphs from another unpolluted stream.

Field Survey - Temporal Patterns

Genotype frequency distributions at the GPI locus did not deviate significantly from Hardy-Weinburg expectations on any of the sample dates. This was true for both upstream and downstream populations on both the South (Table 19) and Holston (Table 20) rivers. The GPI^{M} allele and GPI^{M},GPI^{M} genotype were most prevalent in all four populations and frequencies remained relatively stable throughout the year (Figs. 21,22). No overall temporal differences in allele or genotype frequency distributions were observed at any of the sites (Fishers exact test, Tables 21 ,22). However, both allele and genotype frequencies in September 1990 were significantly different than all other sample dates at the downstream site on the South river (Fig. 21, Table 21). A

Table 19. Gene frequencies of allozyme variants at the GPI locus, number of individuals examined, and chi^2 values obtained when testing for deviations from Hardy-Weinburg equilibrium for I. bicolor populations from upstream (Oak Hill) and downstream (Grottoes) sites on the South river.

	Reference Site (Oak Hill)						Contaminated Site (Grottoes)					
	Allele Frequencies						Allele Frequencies					
Date	S	M	F	N	X^2	p	S	M	F	N	X^2	p
6/20/90	0.067	0.900	0.033	30	6.55	0.26	0.133	0.867	0.00	30	0.71	0.98
7/19/90	0.064	0.923	0.013	39	5.06	0.41	0.043	0.957	0.00	35	0.07	0.99
8/18/90	0.033	0.950	0.017	30	0.08	0.99	0.050	0.950	0.00	30	0.08	0.99
9/19/90	0.100	0.875	0.025	40	0.82	0.98	0.200	0.800	0.00	40	0.35	0.99
1/18/91	0.045	0.932	0.023	22	0.12	0.99	0.056	0.944	0.00	18	0.06	0.99
3/13/91	0.033	0.950	0.017	30	0.08	0.99	0.067	0.933	0.00	30	6.47	0.26
5/24/91	0.090	0.897	0.013	39	0.51	0.99	0.150	0.680	0.00	40	0.02	0.99
10/20/91	0.025	0.950	0.025	40	0.49	0.99	0.075	0.925	0.00	40	0.26	0.99

Table 20. Gene frequencies of allozyme variants at the GPI locus, number of individuals examined, and chi^2 values obtained when testing for deviations from Hardy-Weinburg equilibrium for I. bicolor populations from upstream (McCready) and downstream (Hayters Gap) sites on the Holston river.

	Reference Site (McCready)						Contaminated Site (Hayters)					
	Allele Frequencies						Allele Frequencies					
Date	S	M	F	N	X^2	p	S	M	F	N	X^2	p
8/14/90	0.133	0.833	0.033	30	1.20	0.94	0.204	0.796	0.00	22	0.01	0.99
9/21/90	0.188	0.812	0.000	40	0.52	0.99	0.262	0.688	0.05	40	1.35	0.93
5/17/91	0.167	0.817	0.017	30	1.51	0.91						
7/23/91	0.183	0.800	0.017	30	1.88	0.87						

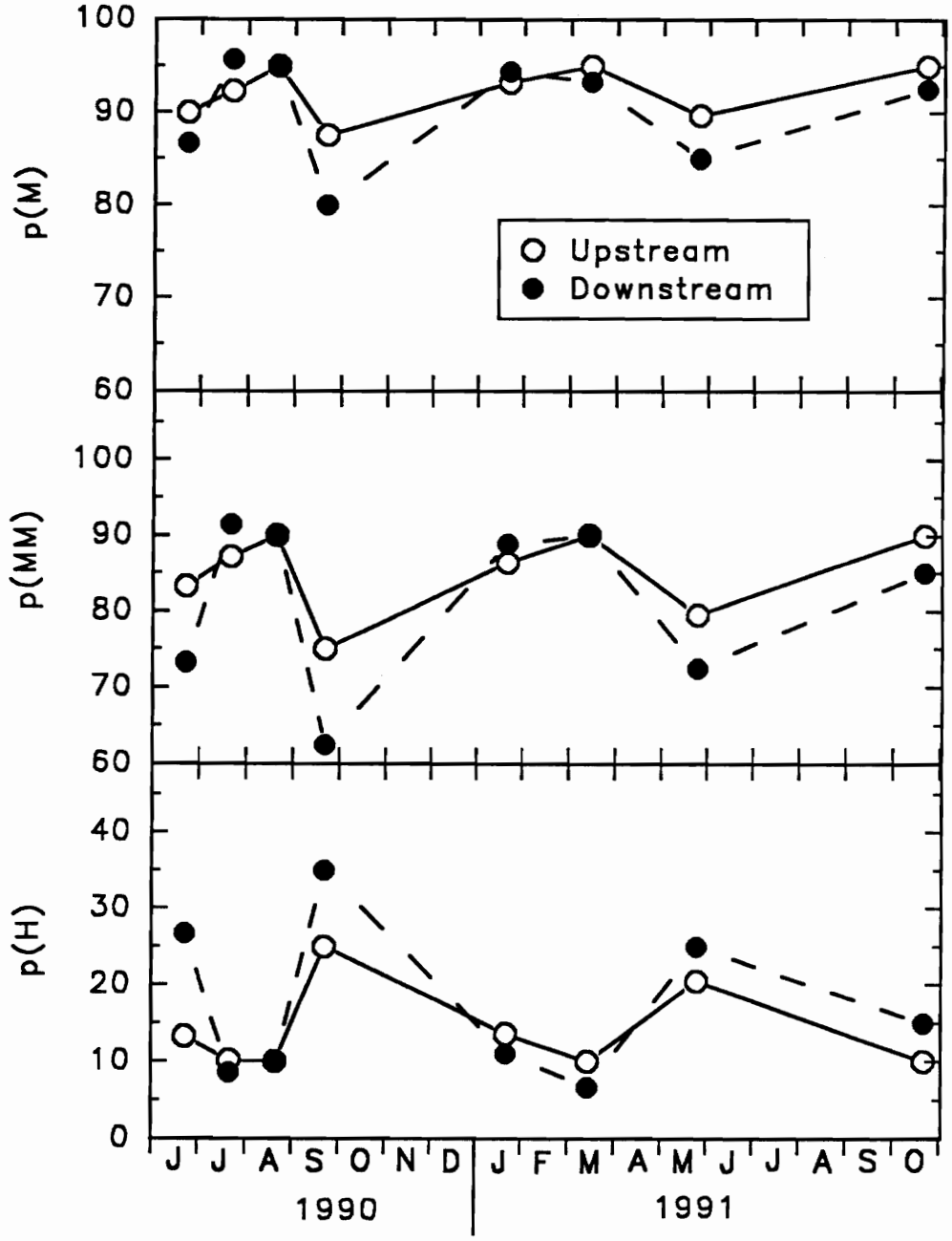

Fig. 21. Comparison of the frequencies of the most common allele (top), the most common genotype (middle) and heterozygotes (bottom) at the GPI locus between natural populations at upstream and downstream sites on the South River.

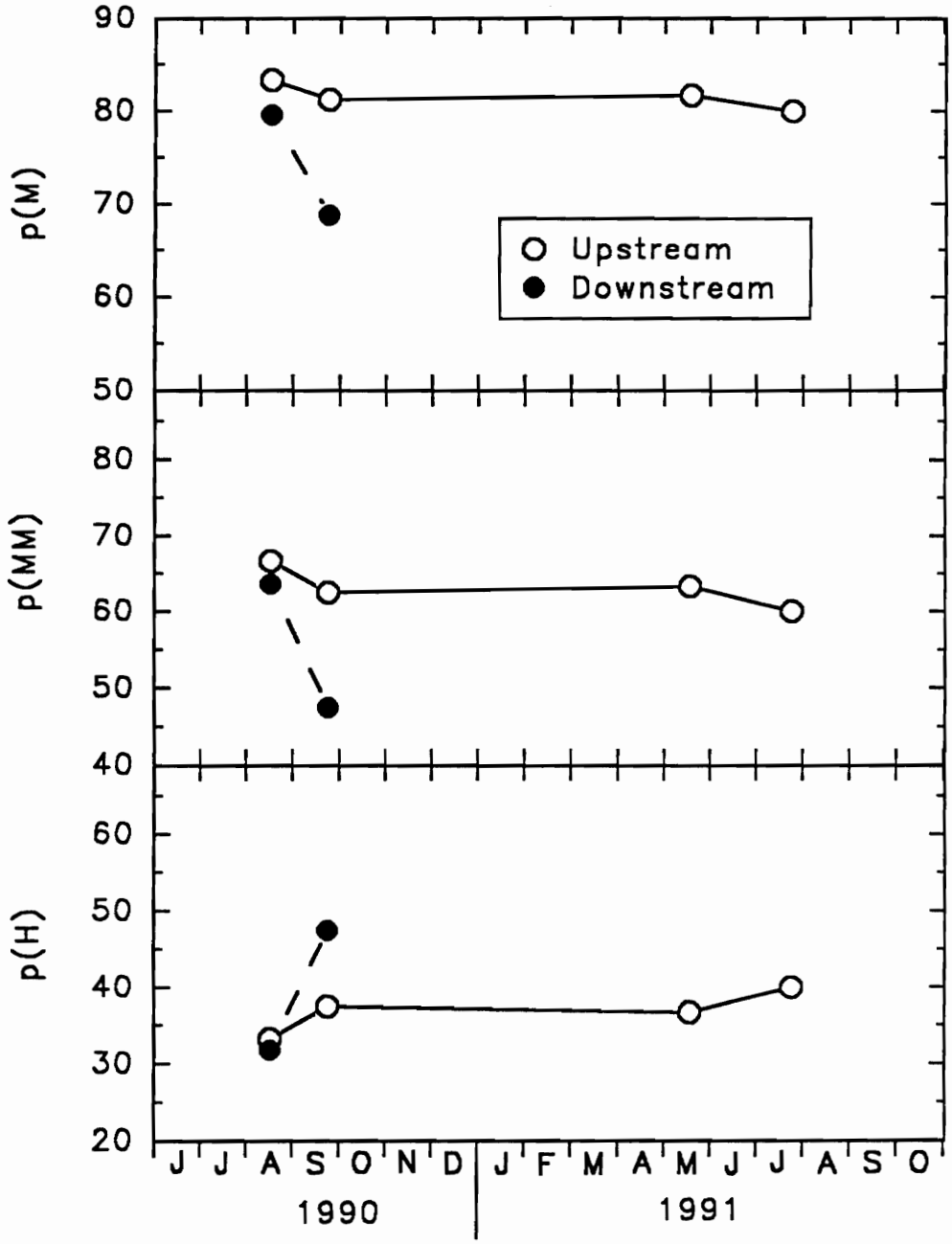

Fig. 22. Comparison of the frequencies of the most common allele (top), the most common genotype (middle) and heterozygotes (bottom) at the GPI locus between natural populations at upstream and downstream sites on the Holston River.

Table 21. Results of the G test of independence to test for temporal differences in allele and genotype frequencies at the GPI locus on the South river. Frequencies observed on each sample date were compared to the frequencies of all other sample dates combined. In addition, Fishers Exact Test was used to test for temporal differences overall (see text). Top - Results at the upstream site (i.e. Oak Hill); Bottom - Results at the downstream site (i.e. Grottoes). Note: Allele frequencies are reported elsewhere.

UPSTREAM SITE

	Allele			Genotype		
Date	**G_{adj}[1]**	**DF**	**Pr>G**	**G_{adj}[1]**	**DF**	**Pr>G**
6/20/90	0.49	2	0.78	3.23	3	0.36
7/19/90	0.31	2	0.86	2.52	3	0.47
8/18/90	1.50	2	0.47	1.59	3	0.66
9/19/90	1.64	2	0.44	3.40	3	0.33
1/18/91	0.39	2	0.82	0.54	3	0.91
3/13/91	1.50	2	0.46	1.59	3	0.66
5/24/91	0.98	2	0.61	2.26	3	0.52
10/20/91	0.20	2	0.87	1.16	3	0.76
	FC=7.17, p=0.97 DF=16			**FC=9.48, p=0.89 DF=16**		

DOWNSTREAM SITE

	Allele			Genotype		
Date	**G_{adj}[1]**	**DF**	**Pr>G**	**G_{adj}[1]**	**DF**	**Pr>G**
6/20/90	0.64	2	0.72	2.12	3	0.55
7/19/90	3.81	2	0.15	3.93	3	0.27
8/18/90	2.41	2	0.30	2.57	3	0.46
9/19/90	8.20	2	0.02	8.66	3	0.03
1/18/91	1.09	2	0.58	1.21	3	0.75
3/13/91	1.06	2	0.59	4.56	3	0.21
5/24/91	2.08	2	0.35	2.08	3	0.56
10/20/91	0.84	2	0.66	1.39	3	0.71
	FC=19.76, p=0.23 DF=16			**FC=17.92, p=0.33 DF=16**		

[1] Used Williams Correction Factor for small sample size.

Table 22. Results of the G test of independence to test for temporal differences in allele and genotype frequencies at the GPI locus on the Holston river. Frequencies observed on each sample date were compared to the frequencies of all other sample dates combined. Top - Results at the upstream site (i.e. McCready); Bottom - Results at the downstream site (i.e. Hayters Gap).

UPSTREAM SITE

	Allele			Genotype		
Date	G_{adj}[1]	DF	Pr>G	G_{adj}[1]	DF	Pr>G
8/14/90	2.03	2	0.36	2.08	3	0.56
9/21/90	3.17	2	0.20	3.17	3	0.37
5/17/91	0.01	2	0.99	0.01	3	0.99
7/23/91	0.12	2	0.94	0.16	3	0.99

DOWNSTREAM SITE

	Allele			Genotype		
Date	G_{adj}[1]	DF	Pr>G	G_{adj}[1]	DF	Pr>G
8/14/90	4.36	2	0.11	2.87	3	0.41
9/21/90	4.36	2	0.11	2.87	3	0.41

[1] Used Williams Correction Factor for small sample size.

similar, although not significant deviation was observed in May 1991. During these same two months, the frequency of heterozygotes at both sites increased proportionately. The pattern was similar, but not significant at the downstream site on the Holston in September 1990 (Fig. 22). However, only one comparison (i.e. Aug. vs Sep) was possible at this site.

Field Survey - Spatial Patterns

Allele frequency distributions at the GPI locus were not significantly different between sites on the South River on any sample date, although the results obtained in September 1990 are marginal (Table 23). Likewise, between-site differences in genotype frequency distributions were not significant except in June 1990 (Table 23). Overall, between-site differences in both allele and genotype frequencies on the South river were not significantly different over the entire sampling period (Fishers combined, Table 23). On the Holston River, allele frequencies were significantly different between sites in September of 1990 (Table 24).

Because the frequency of the GPI^{M} allele was so high, between-site differences in allele and/or genotype frequency distributions were largely determined by between-site differences in the frequency of this allele. As a result,

Table 23. Results of G-test of independence to test for between-site differences in allele and genotype frequency distributions for each sample date on the South river. Between-site differences in frequencies over the entire sample period were evaluated using Fishers Combined test (see text) and results are reported at the bottom.

	Allele			Genotype		
Date	G_{adj}[1]	**DF**	**Pr>G**	G_{adj}[1]	**DF**	**Pr>G**
6/20/90	4.17	2	0.12	8.21	3	0.04
7/19/90	1.64	2	0.44	2.62	3	0.45
8/18/90	1.59	2	0.45	1.59	3	0.66
9/19/90	5.76	2	0.06	6.27	3	0.09
1/18/91	1.24	2	0.54	1.24	3	0.74
3/13/91	2.07	2	0.36	2.77	3	0.43
5/24/91	2.72	2	0.26	3.36	3	0.34
10/20/91	2.80	2	0.25	2.83	3	0.42
	FC=21.85, p=0.15 DF=16			**FC=19.86, p=0.23 DF=16**		

Table 24. Results of G-test of independence to test for between-site differences in allele and genotype frequency distributions for each sample date on the Holston river. Between-site differences in frequencies over the entire sample period were evaluated using Fishers Combined test (see text) and results reported at the bottom.

	Allele			Genotype		
Date	**G_{adj}[1]**	**DF**	**Pr>G**	**G_{adj}[1]**	**DF**	**Pr>G**
8/14/90	3.02	2	0.22	4.05	3	0.26
9/21/90	7.38	2	0.02	6.49	3	0.09
	FC=10.85, p=0.03 DF=4			**FC=7.51, p=0.11 DF=4**		

potential between-site differences in the frequency of the two rarer alleles might be masked. To test for this, allele and genotype frequencies were pooled over all sample dates within each site. Subsequently, between-site comparisons of the frequencies of each allele and genotype relative to the frequency of all other alleles and genotypes combined were made using the 2X2 contingency G-test of independence (Sokal and Rohlf 1981). Pooling of frequency data within each site was justified because no overall temporal differences in allele or genotype frequencies were observed at either site (Tables 21,22).

The frequency of the GPI^S allele was found to be significantly higher at the contaminated site on the South river (Table 25). Conversely, the frequency of the GPI^F allele was significantly higher at the reference site. Difference in the frequency of the GPI^M allele was not significantly different between sites. Spatial differences in genotype frequencies were also observed on the South river. The frequencies of the two heterozygotes were significantly different between sites (Table 25). Conversely, no differences in the frequency of the two homozygotes were observed. No spatial differences in allele or genotype frequencies were observed on the Holston river (Table 26).

In October 1991, an additional two sites were sampled to

Table 25. Results of the G-test of independence to determine spatial differences in allele and genotype frequencies at the GPI locus on the South river after data was pooled over sample dates (see text). Between-site differences in the frequency of each allele and genotype were compared relative to frequencies of all others combined. Upstream site = Oak Hill; Downstream site = Grottoes.

Allele	Upstream (OH) Frequency	N	Downstream (GR) Frequency	N	G_{adj}[1]	Pr>G DF=1
S	0.067	36	0.103	54	4.031	0.045
M	0.913	493	0.897	472	0.587	0.444
F	0.020	11	0.000	0	11.013	0.001

Genotype	Upstream (OH) Frequency	N	Downstream (GR) Frequency	N	G_{adj}[1]	Pr>G DF=1
SS	0.008	2	0.011	3	0.884	0.35
SM	0.118	32	0.182	48	4.820	0.03
MM	0.833	225	0.806	212	0.498	0.48
MF	0.041	11	0.000	0	11.106	0.001
HET	0.159	43	0.182	48	0.358	0.55

[1] Used Williams Correction Factor for small sample size

Table 26. Results of the G-test of independence to determine spatial differences in GPI allele and genotype frequencies on the Holston river, after data was pooled over sample dates (see text). Between-site differences in the frequency of each allele and genotype were compared relative to the frequency of all others combined. Upstream site = McCready; Downstream site = Hayters Gap.

Allele	Upstream (MC) Frequency	N	Downstream (HG) Frequency	N	G_{adj}[1]	Pr>G DF=1
S	0.169	44	0.242	30	1.85	0.17
M	0.815	212	0.726	90	0.49	0.48
F	0.015	4	0.032	4	1.49	0.22

Genotype	Upstream (MC) Frequency	N	Downstream (HG) Frequency	N	G_{adj}[1]	Pr>G DF=1
SS	0.000	0	0.048	3	3.25	0.07
SM	0.338	44	0.387	24	0.20	0.65
MM	0.631	82	0.532	33	0.44	0.51
MF	0.031	4	0.032	2	0.002	0.96

determine whether allele and genotype frequencies correlated with environmental mercury levels. Both the GPI^{F} allele and the GPI^{M},GPI^{F} genotype were only found at the reference site (Table 27). In addition, the GPI^{S} allele and the GPI^{S},GPI^{M} genotype increased in frequency in a downstream direction. However, the frequency of both continued to increase even below the confluence with the Middle river (Lynwood) where environmental mercury levels were low (Table 27).

Table 27. Results of allozyme survey conducted at four sites on the South river on 10/20/91. Mercury concentrations in seston, whole-body mercury concentrations in nymphs, and frequencies of each allele and genotype (expressed as percentages) are reported for each site. Number of individuals analyzed was 40 for each site.

SITE	Mercury in seston (µg/g DW)	Mercury in nymphs (µg/g DW)	Allele Frequency p(S)	p(M)	p(F)	Genotype Frequency p(SS)	p(SM)	p(MM)	p(MF)
Oak Hill[1]	<0.01	<0.01	2.5	95.0	2.5	0.0	5.0	90.0	5.0
Chrimora	17.12	2.23	5.0	95.0	0.0	0.0	10.0	90.0	0.0
Grottoes[1]	15.22	1.27	7.5	92.5	0.0	0.0	15.0	85.0	0.0
Lynwood	2.01	0.12	8.8	91.2	0.0	0.0	17.5	82.5	0.0

[1] Sites routinely surveyed on the South river.

DISCUSSION

ENERGETICS

Laboratory Experiments

The first objective of this study was to investigate mercury tolerance in the aquatic insect, *I. bicolor* from an energetics point of view. The initial phase of the work focused on quantifying the natural variability in respiration rates of nymphs collected from unpolluted streams before and after nymphs were exposed to inorganic mercury. Subsequent experiments were designed to test for tolerance by assessing differences in respiratory responses between individuals from reference and polluted sites.

Basal respiration rates (μl O_2/mg/hr) were found to be higher in smaller nymphs and at higher temperatures (Figs. 4 and 5). The inverse relationship between body size and size-specific respiration has been reported in most types of organisms (reviewed in Lampert 1984). In addition, Sweeney (1978) found this relationship in an earlier energetics study of *I. bicolor*. This negative relationship has been explained in allometric terms. Assuming no drastic changes in shape, as individuals increase in size the surface area/volume ratio is reduced and thus so is the per gram output of energy (Callow

1977).

The relationship between temperature and respiration is commonly expressed by the ratio of metabolic rates resulting from a temperature increase of 10°C (i.e. Q_{10} value)(Lampert 1984). Q_{10} values of approximately 2.0 have been reported for a wide range of poikilotherm taxa (Trama 1972). This means that metabolism doubles with every 10°C increase in temperature. However, these values have been shown to vary with body size even within a species (Newell and Roy 1973, Holopainen and Ranta 1977). In this study, Q_{10} values for *I. bicolor* nymphs were between 2.1 and 2.7 and were higher in smaller nymphs (Fig. 5) indicating a greater sensitivity of smaller nymphs to changing temperature.

Sensitivity of *I. bicolor* nymphs to mercury was also related to body size and temperature, as well as dose (Fig. 6). Smaller nymphs were more sensitive to inorganic mercury over a wide range of concentrations as indicated by larger changes in respiration following exposure. Numerous examples of increased sensitivity to metals in early or smaller life cycle stages have been reported in fish (Macek and Sleight 1977, McKim 1977) and in invertebrates (Giudici et al. 1986, Zajac and Whitlatch 1989). Selective toxicity to small or young individuals could be disastrous at the population level if recruitment is predominantly influenced by survival of

young. However, population models show population viability to be less effected by such selective toxicity if fecundity is density-dependent (Meyer et al. 1987, Partridge and Harvey 1988). In this context, higher mortality among nymphs could be offset by higher adult fecundity. This would essentially increase the number of recruits entering the population each generation.

Higher uptake rates of metals due to greater size-specific metabolism in small individuals (see above) has been proposed to explain increased sensitivity of smaller individuals (Williamson 1979). To some degree, this has been corroborated by bioaccumulation studies. A negative correlation between size and metal body burden has been observed for many freshwater invertebrates (eg. Kormondy 1965, Ray et al. 1980, Smock et al. 1983a, Krantzberg 1989, Snyder and Hendricks 1992). However, this relationship has also been explained by surface adsorption which should be relatively more important in smaller individuals because of a higher surface area/volume ratio (eg. Boyden 1977).

Temperature was also shown to affect respiratory responses of nymphs exposed to mercury by effectively shifting the dose-response curve. That is, measurable changes in respiration were observed at lower concentrations in nymphs exposed to higher temperatures (Fig. 6). Although temperature effects on metal sensitivity has not been tested from a

physiological point of view, increasing toxicity of metals at higher temperatures is well known (Cairns et al. 1975, Cairns et al. 1978). Effects of temperature on metal toxicity is presumably due to increased metabolism and consequently higher uptake rates (Cairns et al. 1975).

Respiratory responses of nymphs exposed to mercury were highly dependant on dose. In fact, not only was the magnitude of the response affected by dose, but so was the direction. At lower doses, increased respiration rates were observed relative to controls while at higher concentrations respiration rates were depressed (Fig. 6). Such diphasic response patterns have been reported by others (eg. Calabrese et al. 1977). Increases in metabolism at low doses are thought to be indicative of physiological adjustments while decreases in metabolism at higher doses are the result of pharmacotoxic action associated with the breakdown of homeostatic mechanisms such as, excretory processes (Luckey et al. 1975). Therefore, concentrations which result in an increase in respiration rates may be thought of as sublethal or chronic, and those which result in a depression of respiration rates may by viewed as acute.

In other experiments, population differences in respiratory responses to both sublethal and acute mercury exposures were assessed. On the South River, small individuals collected from a reference site consistently had

larger increases in respiration following sublethal exposures than similar sized individuals from contaminated sites (Tables 7,9,11). Moraitou-Apostolopoulou et al. (1982) found similar results with the shrimp, *Palaemon elegans*, exposed to both cadmium and chromium. In acute bioassays, no differences were observed in survival between animals from polluted and unpolluted sites. However, in subacute tests with cadmium, oxygen consumption was higher in animals from the unpolluted site suggesting physiological responses to sublethal exposures to metals may be a more sensitive indicator of tolerance. As in the present study, population differences in respiration rates were only statistically significant (i.e. $p<0.05$) in small individuals. This represents the only other study using respiratory responses of individuals to sublethal metal exposure to evaluate differences in tolerance.

In a multi-site comparison on the South river, respiratory responses were found to be lowest at the two most contaminated sites, highest at the reference site, and intermediate at a mildly polluted site (Tables 12). Again population differences were only evident in smaller individuals. However, no differences in responses were observed between the two most polluted sites despite large differences in total mercury concentration in nymphal body tissues (Table 12). This finding suggests mercury tolerance may be positively related to environmental mercury levels up

to some limit, above which no further increases in tolerance are possible. No population differences in respiratory responses and therefore no evidence of tolerance was observed on the Holston river (Table 8).

Preexposure of nymphs from the reference site on the South River to low levels of mercury resulted in no increase in tolerance. Similarly, preexposure of nymphs from a contaminated site to clean water resulted in no decrease in tolerance (Table 9). While not conclusive, these results suggest mercury tolerance observed in this species on the South river is genetically based. To prove conclusively that observed tolerance is adaptive, tests need to be conducted on individuals from subsequent generations. However, like most aquatic insects, this species cannot be cultured in the laboratory because of elaborate mating behaviors (i.e. swarming).

A genetic basis for metal tolerance has been shown or suggested in other benthic macroinvertebrates including the polychaete, *Nereis diversicolor*, to copper (Bryan and Hummerstone 1971); the isopod, *Asellus meridianus*, to lead (Brown 1976); and the fiddler crab, *Uca pugnax*, to methylmercury (Callahan and Weis 1983). Although metal tolerance has been noted in other aquatic insects (Wentsel et al. 1978, Krantzberg and Stokes 1989), no effort was made in these studies to distinguish between acclimation and

adaptation.

Respiratory responses to acute exposures showed the same general pattern as chronic exposures. Individuals from contaminated sites exhibited smaller decreases in respiration than nymphs from the reference site. However, population differences were not statistically significant ($p=0.161$, Table 10) and therefore possible inferences about tolerance to acute exposures are speculative.

Field Surveys

Few attempts have been made to relate physiological responses of individuals exposed to metals to population processes. However, comparative surveys of aquatic insect populations from metal polluted and reference sites have provided inferential evidence of population level responses. For example, Sodergren (1976) hypothesized the life cycle of two mayfly species at cobalt polluted sites were adjusted to permit peak development during seasonal peaks in high flows when metal concentrations were lowest. Furthermore, both mathematical (eg. Nisbet et al. 1989) and intuitive (Koehn and Bayne 1984, Sibly and Callow 1984) models have been derived which link effects of stress at the individual and population level. However, to date, these models have not been tested with chemical pollutant stresses.

In this study, population age structure, individual

growth rates, and summer densities were compared between populations at contaminated and reference sites. On the South River, surveys revealed no obvious differences in any of these variables between populations that could be attributed to mercury. Hatching appeared to occur sooner and development slightly faster at the contaminated site (Fig. 10). However, this could be explained by warmer spring and summer temperatures (Fig. 3). Another mayfly species, *Ephoron leukon*, another mayfly species, was also found to grow and develop faster at the contaminated site on this river, in each of three consecutive years (Snyder and Hendricks 1991). In that study, between-site differences in water temperature explained differences in growth and development.

Summer density of *I. bicolor* was actually higher at the contaminated site throughout most of the summer on the South River (Fig. 14). It is possible that this was due to higher nutrient concentrations (Table 1). Hawkins (1986) found densities of ephemerellid mayflies to be highly dependent on nutrients and food availability.

Somewhat different results were obtained from the Holston River survey. Although there were no apparent population differences in age structure or developmental patterns during winter, qualitative sampling at the contaminated site was much more time consuming suggesting lower densities. More importantly, summer densities at the contaminated site fell to

zero soon after hatching and did not recover prior to the end of the summer (Fig. 14). This pattern was consistent at other sites surveyed below Saltville, and probably reflects responses of nymphs to acutely toxic conditions.

In respiration experiments, *I. bicolor* nymphs were found to be more sensitive to mercury at the higher experimental temperature (see above). Therefore, the apparent local extinction of nymphs from the contaminated site during the summer may be explained by the greater toxicity of mercury associated with warm, summer temperatures. However, in reciprocal transplant experiments (discussed below), a large proportion of both transplant and indigenous individuals collected in the spring completed their life cycle at this site. It may be that higher summer temperatures did act to increase toxicity of mercury, but only very small nymphs (i.e. smaller than those used in the transplant experiment) and perhaps eggs were vulnerable. This is supported by earlier findings that respiratory response was inversely related to size (see above).

Another possible explanation relates to the influence of contaminants on competitive and predatory interactions. Toxicants have been shown to reduce the ability of both fish (Goodyear 1972, Kania and O'Hare 1974), and more recently aquatic insect (Clements et al. 1989) prey species to avoid predators. If increased toxicity due to warmer summer

temperatures reduced ability of *I. bicolor* nymphs to avoid predators, than a high rate of mortality might ensue. However, nymphs in enclosures would have been less effected by increased toxicity because they were not exposed to predators.

Field Experiments

Reciprocal transplant experiments were conducted during the summer of 1991 to determine if tolerance observed in laboratory respiration experiments extended to population level processes in the field. Energy assimilated from food must be channeled into one of three areas: growth, reproduction, or maintenance. If maintenance costs increase in response to some environmental stress, decreased production of somatic and/or reproductive material would be predicted (Koehn and Bayne 1989). Therefore, based on population differences in respiratory responses to mercury observed in lab experiments, faster growth and/or higher fecundities would be expected in downstream nymphs when raised at the downstream site. Conversely, higher fitnesses might be expected in upstream nymphs when raised at the upstream site. This is because adaptations to stressful environments are often accompanied by lowered fitness values in unperturbed environments (Parsons 1989).

However, results of field experiments showed no significant population differences in either growth or

mortality rates at either site on the South River that could be attributed to mercury. Higher growth rates of downstream nymphs at the downstream site were accounted for by differences in water temperature (Fig 17). Some evidence of higher mortality was observed in transplanted animals at both sites in the early stages of development (Fig. 19), but this was probably due to mortality incurred during transplant.

In contrast, considerable, although not statistically significant, population differences in fecundity were observed at the contaminated site on the South River. Indigenous females produced higher numbers of eggs than transplants at the contaminated site in both the early and late-summer experiments (Fig. 20). However, differences observed in the early-summer experiment were accounted for by differences in female size (Table 14), while differences observed in the late-summer experiment were independent of female size (Table 15). These conflicting results make inferences regarding the long-term effects of mercury tentative. Even so, the probability of the observed pattern occurring by chance was less than 10 percent in the late-summer experiment (Table 15). Furthermore, based upon the results of laboratory experiments such differences were expected. Therefore, the observed differences in fecundity, albeit not statistically significant, are compelling if not convincing.

GENETIC ANALYSES

Laboratory Fitness Experiments

The second objective of this study was to determine whether single-locus genotypes may influence fitness of *I. bicolor* nymphs exposed to mercury. In previous studies with marine invertebrates, allozyme variation at several loci was correlated with individual responses to acute mercury exposure (Nevo et al. 1981, Nevo et al. 1984, Lavie and Nevo 1986, Lavie and Nevo 1987). Such findings have led some to propose this method as a biomonitoring tool in the study of stressed ecosystems (Battaglia et al. 1980, Beardmore et al. 1980, Berry 1980, Nevo 1983). This study represents an effort to determine the efficacy of this approach in polluted freshwater systems.

Laboratory experiments demonstrated that the probability of survival and mean TTD in *I. bicolor* nymphs exposed to acute concentrations of inorganic mercury were significantly different among genotypes at one of the three enzyme loci examined (Tables 17,18). Among the four genotypes scored at this locus, the lowest probability of survival and shortest mean TTD was associated with the most common homozygous genotype (GPI^{M},GPI^{M}). Conversely, the GPI^{S},GPI^{M} heterozygote was found to have the highest probability of survival and longest mean TTD. These results were consistent at both

summer and winter temperatures and in populations from two unpolluted sites (Tables 17,18).

Allozymes have been found to differ in substrate specificity, temperature optima, and sensitivity to inhibitors (Milstein 1961). Therefore, fitness differences among GPI genotypes to acute mercury exposure observed in this study are not surprising. However, by what mechanism single-locus genotypes may influence fitness is still unclear. Mercury is highly reactive with sulfur and has been shown to compete and sometimes displace coenzymes such as magnesium and zinc for thiol-rich binding sites on metalloproteins and enzymes (Luckey et al. 1974). Pellerin-Massicotte et al. (1989) showed the affinity of the MDH enzyme in tissues of the blue mussel, *Mytillus edulis* decreased with addition of methylmercury and selenium offering experimental evidence for competitive inhibition by mercury. If this is indeed the case, than one would predict those allozyme variants with a relatively large number of the amino acids cystein and methionine near their active sites would be most sensitive to mercury because their side chains contain sulfur. This hypothesis has not been tested.

Field Surveys at GPI locus

Perhaps more important than the biochemical mechanism by which allozyme variants relate to fitness, is the relevance of

the results of these acute, short-term exposures to effects during chronic, low-level exposures which are more common in nature. The results of standard acute bioassays which are used routinely in pollution studies have been criticized (eg. Kimball and Levin 1985) because they are often used to make predictions about population responses to chronic exposures without proper field validation. It does not necessarily follow that relative sensitivities to acute exposure can be extended to chronically polluted situations.

The genetic structure of the natural populations at both reference and contaminated sites surveyed in this study remained relatively stable throughout the year. However, on the South river temporal changes in allele and genotype frequencies at the GPI locus were observed in September of 1990 at the contaminated site, and a similar, albeit not statistically significant pattern was observed in May of 1991 (Fig. 21). On these dates, the frequency of the sensitive GPI^{M},GPI^{M} genotype was lower and the frequency of heterozygotes increased relative to the rest of the year. These sample intervals also correlated with recruitment periods and so the population consisted mainly of small individuals at these times (Fig. 10). Results of respiration experiments showed small nymphs were more sensitive to chronic mercury concentration, and therefore observed changes in

genotype frequencies observed at this time could reflect the outcome of natural selection for mercury resistant individuals at their most vulnerable time. However, caution should be exercised in interpreting these patterns. Allele and genotype frequencies exhibited similar patterns at the reference site on the South river although they were not statistically significant (Fig. 21).

Based on the results of the laboratory experiments, a lower frequency of individuals homozygous for the GPI^M allele would be expected at chronically contaminated sites. However, field surveys conducted on both the South and Holston Rivers found no evidence of such a pattern. The frequency of the sensitive (GPI^M,GPI^M) genotype was not significantly different between reference and contaminated populations on either river (Tables 25,26). However, the genotype identified as most tolerant in the laboratory experiments (GPI^S,GPI^M) was indeed more frequent at the contaminated site on the South river as was the GPI^S allele (Table 25), although there was no evidence for this pattern on the Holston River. In October of 1991, an additional two sites were included in the monthly survey on the South River. While the frequency of the GPI^S,GPI^M genotype was again higher at Grottoes (contaminated) than Oak Hill (reference), the frequency continued to increase even below the confluence of the Middle river where mercury levels

were very low due to dilution (Table 27). While such a clinal pattern does infer some relationship between fitness of this genotype and some environmental variable, there seems to be no evidence that chronic mercury levels are important.

The other heterozygote scored at this locus (GPI^{M},GPI^{F}) was also found to be relatively tolerant to acute mercury exposure. Despite this, the frequency of this genotype was significantly higher at the reference site on the South River (Table 27). These results do not agree with predictions based on laboratory fitness experiments.

In contrast to this study, work with marine invertebrates have shown striking relationships between results of laboratory fitness experiments and genotype frequencies in metal polluted environments (Nevo et al. 1984, Nevo et al. 1987). Possible explanations to account for this discrepancy are numerous. Wider variation in metal concentrations may have been present among field sites in these studies, some of which may have been closer to acute levels used in fitness experiments. The authors do not report metal levels in these studies and instead rely on gross comparisons between highly polluted and reference sites (eg. Nevo et al. 1987). In low, or moderately polluted environments individual fitness may be less associated with the ability to cope with the contaminant. In such cases, fitness may be linked to several, more or less

equally important environmental variables and thus single-locus genotypes would not be predictive.

An alternative explanation involves uptake mechanisms. In this study, laboratory fitness experiments involved dosing of the water. However, mercury levels in both the South and Holston Rivers are primarily associated with sediments and suspended organics (Hendricks et al. 1989, Lawler et al. 1989) and are virtually undetectable in the water. Furthermore, it has been shown that most of the whole-body metal concentrations in aquatic insects inhabiting metal contaminated environments are associated with the gut and gut contents (Smock 1983b, Snyder and Hendricks 1992). This implies that uptake via food is most important. Therefore it is possible that genetic responses observed in fitness experiments were specific to water-contaminated conditions. Although the relative proportion of the total body burden of metals derived from food and water has been intensively studied in fish and to a lesser extent invertebrates (eg. Phillips and Buhler 1978, Alliot and Frenet-Piron 1990), the relationship between relative toxicity and mode of intake has not. However, in mammals, metal toxicity is strongly influenced by mode of uptake (Luckey et al. 1975).

CONCLUDING REMARKS

Most studies designed to detect effects of pollutants on freshwater ecosystems have used changes in community structure as the response variable. However, these community-based metrics have been most successful in measuring impacts in acutely or severely polluted systems (Nriagu 1989). They lack sensitivity in low or moderately polluted environments because they rely on elimination or proliferation of species. Furthermore, changes in structure and function of communities will not be understood or explainable without knowledge of response mechanisms at the individual and population levels (Wiederholm 1984). Thus, some have called for integrative studies designed to link responses at different levels of biological organization (eg. Kimball and Levin 1985). Although such comprehensive studies may be impractical from a regulatory point of view, they are necessary if the science of ecotoxicology is ever to become predictive.

The present study was an effort to integrate responses to sublethal mercury concentrations at the individual, population, and molecular levels. Physiological responses of individuals were found to be useful in predicting vulnerable times in the life history of populations. For example, oxygen consumption rates were most sensitive to sublethal

concentrations of mercury in small nymphs and at higher temperatures. This may explain the inability of populations at contaminated sites on the Holston River to complete their development during the warmer summer months. In addition, there was some evidence that genotype frequencies at contaminated sites on both rivers shifted during recruitment periods when nymphs were small. However, patterns at reference sites were similar although not statistically significant.

Evidence of tolerance observed in populations from the South River was also most pronounced in smaller individuals. A genetic basis for the observed tolerance was suspected. Some have suggested that in order for populations to persist and reproduce in a contaminated site, the tolerant phenotype should be expressed in the most sensitive life history stage (eg, Mulvey and Diamond 1991). Thus, these sensitive stages may be the limiting factor in tolerance evolution and therefore those life history characteristics that directly impinge on the viability of these stages should be most effected. Results of this study support this argument. Nymphs from contaminated sites on the South River not only had lower maintenance costs, but female fecundities also appeared to be higher than for nymphs from the reference site. This would increase the odds that a large number of individuals would persist through the smaller, more vulnerable instars.

Adaptations to stressful environments are often accompanied by trade-offs with other aspects of fitness. In particular, populations adapted to specific stresses have been shown to have reduced fitness in environments without the stress relative to nonadapted populations. There was no evidence of such costs in the present study. However, while fitness differences between adapted and reference populations are often large at contaminated sites, differences at reference sites are more often small and difficult to detect without very large sample sizes (Bradshaw and Hardwick 1989).

The potential use of allozyme variants as a biomonitoring tool to detect adaptive changes in populations due to metal pollution was also evaluated in this study. Although sensitive and tolerant genotypes were identified, their frequencies in natural populations were independent of mercury levels. In addition, there was no correlation between tolerance observed in laboratory respiration experiments and genotype frequencies of these populations. Therefore, this method was not effective in measuring adaptive responses in this species.

In summary, I believe this study supports the contention that the most useful approach to the study of aquatic pollution is to intensively study individual and population response mechanisms of sensitive species. This will help to provide a clearer understanding of long-term effects of low levels of pollutants in aquatic ecosystems. Subtle effects,

such as the evolution of tolerance and its potential costs such as reductions in fitness in clean environments or increased vulnerability to additional natural and/or anthropogenic stress could more easily be assessed using such integrated approaches.

LITERATURE CITED

Abel, P.D. 1989. Water Pollution Biology. Ellis Horwood Limited, West Sussex, England. 231pp.

Alliot, A. and M. Frenet-Piron. 1990. Relationship between metals in sea-water and metal accumulation in shrimps. Marine Pollution Bulletin 21:30-33.

Armitage, P.D. 1980. The effects of mine drainage and organic enrichment on benthos in the River Nent system, Northern Pennines. Hydrobiologia 74:119-128.

Armstrong, F.A.J. and D.P. Scott. 1979. Decrease in mercury content of fishes in Ball Lake, Ontario, since imposition of controls on mercury discharges. Journal of Fisheries Research Board of Canada 36:670-672.

Battaglia, B., Bisol, P.M., and E. Rodino. 1980. Studies on the genetic effect of pollution in the sea. Rapp P-v Cons. Int. Explor. Mer. 179:267-274.

Bailey, D.S. 1974. The occurrence of mercury in the fish and sediment of the North Fork of the Holston River 1970-1972. Basic Data Bulletin No. 41, Virginia State Water Control Board, January 1974.

Bayne, B.L., Moore, M.N., Widdows, J., Livingstone, D.R., and P. Salkeld. 1979. Measurement of the responses of individuals to environmental stress and pollution: Studies with bivalve molluscs. Transactions of the Royal Society of London 286:553-561.

Beardmore, F.A., Barker, C.J., Battaglia, B., and J.F. Payne. 1980. The use of genetical approaches to monitoring biological effects of pollution. Rapp P-v Cons. Int. Explor. Mer. 179:299-305.

Beck, S.D. 1983. Insect thermoperiodism. Annual Review of Entomology 28:91-108.

Benes, P. and B. Havlik. 1979. Speciation of mercury in natural waters. Pages 175-202 *in* J.O. Nriagu (editor). The Biogeochemistry of Mercury in the Environment. Elsevier/North-Holland Biomedical Press, Amsterdam.

Benton, M.J. and S.I. Guttman. 1990. Relationship of

allozyme genotype to survivorship of mayflies (*Stenonema femoratum*) exposed to copper. Journal of the North American Benthological Society 9:271-276.

Berry, R.J. 1980. Genes, pollution and evolution. Rapp P- v Reun Cons. Int. Explor. Mer. 179:253-257.

Bishop, WE. and A.W. McIntosh. 1981. Acute lethality and effects of sublethal cadmium exposure on ventilation frequency and cough rate of bluegill (*Lepomis macrochirus*). Archives of Environmental Contamination and Toxicology 10:519-530.

Boyden, C.R. 1977. Effect of size upon metal content of shellfish. Journal of Marine Biology Association of the U.K. 57:675-714.

Brown, B.E. 1976. Observations on the tolerance of the isopod *Asellus meridianus* to copper and lead. Water Research 10:555-559.

Bryan, G.W. and L.G. Hummerstone. 1971. Adaptation of the polychaete *Nereis diversicolor* to sediments containing high concentrations of heavy metals. I. General observations and adaptation to copper. Journal of Marine Biology U.K. 51:845-863.

Bryan, G.W. 1976. Some aspects of heavy metal tolerance in aquatic organisms. Pages 7-34 *in* A.P.M. Lockwood (editor). Effects of Pollutants on Aquatic Organisms. Cambridge University Press, Cambridge.

Buikema, A.L. 1972. Oxygen consumption of the cladoceran *Daphnia pulex*, as a function of body size, light, and light acclimation. Comparative Biochemical Physiology 42:877-888.

Burton, R.S. and M.W. Feldman. 1983. Physiological effects of an enzyme polymorphism: glutamate-pyruvate transaminase and response to hyperosmotic stress in the copepod, *Tigriopus californicus*. Biochemical Genetics 21:239-251.

Cairns, J.C. and K.L. Dickson. 1971. A simple method for the biological assessment of the effects of waste discharges on aquatic bottom-dwelling organisms. Journal of the Water Pollution Control Federation.

Cairns, J.C., Heath, A.G., and B.C. Parker. 1975. The

effects of temperature upon the toxicity of chemicals to aquatic organisms. Hydrobiologia 47:135-171.

Cairns, J.C., Buikema, A.L., Heath, A.G., and B.C. Parker. 1978. Effects of temperature on aquatic organism sensitivity to selected chemicals. Virginia Water Resources Research Center Bulletin 106. 88pp.

Calabrese, A., Thurberg, F.P., and E. Gould. 1977. Effects of cadmium, mercury, and silver on marine animals. Marine Fisheries Review 39:5-11.

Callahan, P. and J.S. Weis. 1983. Methylmercury effects on regeneration and ecdysis in fiddler crabs (*Uca pugilator, U. pugnax*) after short-term and chronic preexposure. Archives of Environmental Contamination and Toxicology 12:707-714.

Calow, P. 1977. Ecology, evolution, and energetics: A study in metabolic adaptation. Advances in Ecological Research 10:1-62.

Capuzzo, J.M. and J.J. Sasner, Jr. 1977. The effect of chromium on filtration rates and metabolic activity of *Mytisus edulis* and *Mya arenaria*. Pages 225-237 *in* F. J Vernberg, A. Calabrese, F. P. Thurberg, and W. Vernberg (editors). Physiological Responses of Marine Biota to Pollutants. Academic Press, San Diego.

Carpene, E. and S. George. 1981. Absorption of cadmium by gills of *Mytilus edulis*. Molecular Physiology 1:23-34.

Chagnon, N.L. and S.I. Guttman. 1989. Differential survivorship of allozyme genotypes in Mosquitofish populations exposed to copper or cadmium. Environmental Toxicology and Chemistry 8:319-326.

Clements, W.H., Cherry, D.S., and J.C. Cairns, Jr. 1989. The influence of copper exposure on predator-prey interactions in aquatic insect communities. Freshwater Biology 21:483-488.

Clements, W.H. 1991. Community responses of stream organisms to heavy metals: A review of observational and experimental approaches. Pages 363-386 *in* M.C. Newman and A.W. McIntosh (editors). Metal Ecotoxicology: Concepts and Applications. Lewis Publishers, Chelsea, Michigan.

Clubb, R.W., Gaufin, A.R., and J.L. Lords. 1975. Acute cadmium toxicity studies upon nine species of aquatic insects. Environmental Research 9:332-341.

Dejong, G. and W. Scharloo. 1976. Environmental determination of the adaptive significance or neutrality of amylase variants in *Drosophila melanogaster*. Genetics 84:77-94.

Dixon, P.M. and M.C. Newman. 1991. Analyzing toxicity data using statistical models for time-to-death: An introduction. Pages 207-241 *in* M.C. Newman and A.W. McIntosh (editors). Metal Ecotoxicology: Concepts and Applications. Lewis Publishers, Chelsea, Michigan.

Dixon, D.G. and J.B. Sprague. 1981. Acclimation-induced changes in toxicity of arsenic and cyanide to rainbow trout, *Salmo gardneri*. Journal of Fish Biology 18:579-589.

Dobzhansky, T. 1970. Genetics of the Evolutionary Process. Columbia University Press, New York.

Duncan, D.A. and J.F. Klaverkamp. 1983. Tolerance and resistance to cadmium in white suckers (*Catastomus commersoni*) previously exposed to cadmium, mercury, zinc, or selenium. Canadian Journal of Fisheries and Aquatic Sciences 40:128-138.

Edmunds, G.F., Jr., Berner, L., and J.R. Traver. 1958. North American mayflies of the family Oligoneuriidae. Annals of the Entomological Society of America 51:375-382.

Eichhorn, G.L. 1975. Active sites of biological macromolecules and their interaction with heavy metals. Pages 123-142 *in* A. D. McIntyre and C.F. Mills (editors). Ecological Toxicology Research. Plenum Press, New York.

Elder, J.A. and A.R. Gaufin. 1974. The toxicity of three mercurials to *Pteronarcys californica* Newport, and some possible physiological effects which influence the toxicities. Environmental Research 7:169-175.

Eyster, L.S. and M.P. Morse. 1984. Development of the surf clam (*Spisula solidissima*) following exposure of gametes, embryos, and larvae to silver. Archives of Environmental Contamination and Toxicology 13:641-649.

Funk, D.H., Sweeney, B.W., and R.L. Vannote. 1988. Electrophoretic study of eastern North American *Eurylophella* (Ephemeroptera:Ephemerellidae) with the discovery of morphologically cryptic species. Annals of the Entomological Society of America 81:174-186.

Gibson, J. 1970. Enzyme flexibility in *Drosophila melanogaster*. Nature 227:959-960.

Gillespie, R.B. and S.I. Guttman. 1989. Effects of contaminants on the frequencies of allozymes in populations of the Central Stoneroller. Environmental Toxicology and Chemistry 8:309-318.

Giudici, M. de N., Migliore, L., and S.M. Guarino. 1986. Effects of cadmium on the life cycle of *Asellus aquaticus* and *Proasellus coxalis* (Crustacia, Isopoda). Environmental Technology Letters 7:45-54.

Goodyear, C.P. 1972. A simple technique for detecting effects of toxicants or other stresses on a predator-prey interaction. Transactions of the American Fisheries Society 101:367-370.

Hawkins, C.P. 1986. Variation in individual growth rates and population densities of ephemerellid mayflies. Ecology 67:1384-1395.

Hatakeyama, S. and M. Yasuno. 1981. A method for assessing chronic effects of toxic substances on the midge, *Paratanytarsus parthenogeneticus*: Effects of copper. Archives of Environmental Contamination and Toxicology 10:705-713.

Hendricks, A.C., Snyder, C.D., and L.D. Willis. 1989. An ecological study of the South river to ascertain the extent of long-term responses of invertebrates to mercury. Report submitted to the E.I. Dupont De Nemours Co.

Hickey, D.A. 1977. Selection for amylase allozymes in *Drosophila melanogaster*. Evolution 31:800-804.

Hilbish, T.J. and R.K. Koehn. 1985. Dominance in physiological phenotypes and fitness at an enzyme locus. Science 229:52-54.

Holopainen, I.J. and E. Ranta. 1977. Respiration of *pisidium amnicum* (Bivalvia) measured by infrared gas

analysis. Oikos 28:196-200.

Huckabee, J.W. and S.G. Hildebrand. 1974. Background concentrations of mercury and methylmercury in unpolluted freshwater environments. Proceedings of the First International Conference on Mercury.

Huckabee, J.W., Elwood, J.W., and S.G. Hildebrand. 1979. Accumulation of mercury in freshwater biota. Pages 277-302 *in* J.O. Nriagu (editor). The Biogeochemistry of Mercury in the Environment. Elsevier/North-Holland Biomedical Press, Amsterdam.

Illies, J. (editor). Limnofauna Europaea. A checklist of animals inhabiting European inland waters, with accounts of their distribution and ecology (except Protozoa). 2nd edition Gustav Fischer Verlag, Stuttgart, West Germany. 532pp.

Jernolov, A. and H. Lann. 1971. Mercury accumulation in food chains. Oikos 22:403-406.

Johns, D.M. and D.C. Miller. 1982. The use of bioenergetics to investigate the mechanisms of pollution toxicity in Crustacean larvae. Pages 261-288 *in* W.B. Vernberg, A. Calabrese, F.P. Thurnberg, and F.J. Vernberg (editors). Physiological Mechanisms of Marine Pollution Toxicity. Academic Press, New York.

Johnson, G.B. 1976. Genetic polymorphism and enzyme function. Pages 41-57 *in* F.J. Ayala (editor). Molecular Evolution. Sinauer and Associates, Inc. Sutherland, Mass.

Johnson, F.G. 1977. Sublethal biological effects of petroleum hydrocarbon exposures: Bacteria, algae, and invertebrates. Pages 271-318 *in* D.C. Malins (editor). Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms, Volume II: Biological Effects. Academic Press, New York.

Kania, H.J. and J. O'Hare. 1974. Behavioral alterations in a simple predator-prey system due to a sublethal exposure to mercury. Transactions of the American Fisheries Society 103:134-136.

Ketchum, B.H. 1975. Problems in aquatic ecosystems, with special reference to heavy metal pollution of the marine environment. Pages 76-81 *in* A. D. McIntyre

and C.F. Mills (editors). Ecological Toxicology Research. Plenum Press, New York.

Kimball, K.D. and S.A. Levin. 1985. Limitations of laboratory bioassays: the need for ecosystem level testing. Bioscience 35:165-171.

Kimura, M. 1968. Evolutionary rate at the molecular level. Nature 217:624-626.

Klerks, P.L. and J.S. Levinton. 1987. Effects of heavy metals in a polluted aquatic ecosystem. Pages 29-53 *in* S.A. Levin (editor). Ecotoxicology. Springer-Verlag Press, Berlin.

Klerks, P.L. and J.S. Weis. 1987. Genetic adaptation to heavy metals in aquatic organisms: A review. Environmental Pollution 45:173-205.

Koehn, R.K., Perez, J.E. and R.B. Merrit. 1971. Esterase enzyme function and genetic structure of populations of freshwater fish. American Naturalist 105:51-69.

Koehn, R.K. and B.L. Bayne. 1989. Towards a physiological and genetical understanding of the energetics of stress response. Pages 157-171 *in* P. Callow and R.J. Berry (editors). Evolution, Ecology, and Environmental Stress. Academic Press, New York.

Kondratieff, B.C. and J.R. Voshell, Jr. 1980. Life history and ecology of Stenonema modestum (Banks) (Ephemeroptera:Heptageniidae) in Virginia, USA. Aquatic Insects 2:177-189.

Konstantinov, A.S. 1971. Ecological factors affecting respiration in chironomid larvae. Limnologica 8:127-134.

Kormondy, E.J. 1965. Uptake and loss of zinc-65 in the dragonfly *Plathemis lydia*. Limnology and Oceanography 10:427-433.

Krantzberg, G. 1989. Metal accumulation by chironomid larvae: the effects of age and body weight on metal body burdens. Hydrobiologia 188/189:497-506.

Krantzberg, G. and P.M. Stokes. 1989. Metal regulation, tolerance, and body burdens in the larvae of the genus *Chironomus*. Canadian Journal of Fisheries and Aquatic

Sciences 46:389-398.

Lampert, W. 1984. The measurement of respiration. Pages 413-467 *in* J.A. Downing and F.H. Rigler (editors). Manual on Methods for the Assessment of Secondary Production in Freshwaters. IBP Handbook #17, Second edition. Blackwell Scientific Publishers. Oxford.

Lauren, D.J. and D.G. McDonald. 1987. Acclimation to copper by rainbow trout, *Salmo gardnerii*: Physiology. Canadian Journal of Fisheries and Aquatic Sciences.

Lavie, B. and E. Nevo. 1982. Heavy metal selection of PGI allozymes in marine gastropods. Marine Biology 71:17-22.

Lavie, B., Nevo, E., and U. Zoller. 1984. Differential viability of Phosphoglucose Isomerase allozyme genotypes of marine snails in nonionic detergent and crude-oil surfactant mixtures. Environmental Research 35:270-276.

Lavie, B. and E. Nevo. 1986. Interactive effects of cadmium and mercury pollution on allozyme polymorphisms in the marine gastropod *Cerithium scabridum*. Marine Pollution Bulletin 17:21-23.

Lavie, B. and E. Nevo. 1987. Differential fitness of allelic isozymes in the marine gastropods *Littorina punctata* and *Littorina neritoides* exposed to the environmental stress of cadmium and mercury pollution. Environmental Management 11:345-349.

Lawler, Matusky, and Skelly Engineers. 1989. Reassessment of mercury in the South river and South Fork of the Shenandoah river. Report submitted to the E.I. Dupont de Nemours Co.

Levinton, J.S. 1980. Genetic divergence in estuaries. Pages 509-520 *in* V.S. Kennedy (editor). Estuarine Perspectives. Academic Press, San Diego.

Levins, R. and R. Lewontin. 1980. Dialectics and reductionism in ecology. Synthese 43:47-78.

Lewontin, R.C., and J.L. Hubby. 1966. A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila*

pseudoobscura. Genetics 54:595-609.

Lewontin, R.C. 1974. The Genetic Basis of Evolutionary Change. Columbia University Press, New York.

Luckey, T.D. Venugopal, B., and D. Hutcheson. 1974. Heavy Metal Toxicity, Safety, and Hormology. Academic Press, New York. pp.120.

Luoma, S.N. 1977. Detection of trace contaminant effects in aquatic ecosystems. Journal of the Fisheries Research Board Canada 34:436-439.

Macek, K.J. and H.O. Sanders. 1970. Biological variation in the susceptibility of fish and aquatic invertebrates to DDT. Transactions of the American Fisheries Society 99:89-90.

Macek, K.J., Buxton, K.S., Sauter, S., Gnilka, S., and J.W. Dean. 1976. Chronic toxicity of Atrazine to selected aquatic invertebrates and fishes. U.S.E.P.A. EPA-600/3-76-047.

Macek, K.J. and B.H. Sleight. 1977. Utility of toxicity tests with embryos and fry of fish in evaluating hazards associated with chronic toxicity of chemicals to fishes. *In* Aquatic Toxicology and Hazard Evaluation. Proceedings of the First International Symposium on Aquatic Toxicology. Philadelphia, American Society for Testing and Materials, S.T.P. No. 634.

Mance, G. 1987. Pollution Threat of Heavy Metals in Aquatic Environments. Elsevier Applied Science Publishers, Essex, England. 372pp.

McKenney, C.L. 1982. Interrelationships between energy metabolism, growth dynamics, and reproduction during the life cycle of *Mysidopsis bahia* as influenced by sublethal Endrin exposure. Pages 447-476 *in* W.B. Vernberg, A. Calabrese, F.P. Thurnberg, F.J. Vernberg (editors). Physiological Mechanisms of Marine Pollutant Toxicity. Academic Press, New York.

McKim, J.M. 1977. Evaluation of tests with early life stages of fish for predicting long-term toxicity. Journal of Fisheries Research Board of Canada 34:1148-1154.

Meyer, J.S., Ingersoll, C.G., and L.L. McDonald. 1987.

Sensitivity of population growth rates estimated from cladoceran chronic toxicity tests. Environmental Toxicology and Chemistry 6:115-126.

Milstein, C. 1961. On the mechanisms of activation of phosphoglucomutase by metal ions. Biochemical Journal 79:574-584.

Mitra, S. 1986. Mercury in the Ecosystem. Trans Tech Publications, Aedermannsdorf, Switzerland. 327pp.

Mitton, J.B. and R.K. Koehn. 1975. Genetic organization and adaptive response of allozymes to ecological variables in *Fundulus heteroclitus*. Genetics 79:97-111.

Moraitou-Apostolopoulou, M., Verriopoulos, G., and I. Rogdakis. 1982. Evaluation of the stress exerted by a polluted environment to a marine organism by comparative toxicity tests. Bulletin of Environmental Contamination and Toxicology 28:416-423.

Muller, H.J. 1950. Our load of mutations. American Journal of Human Genetics 2:111-176.

Mulvey, M. and S.A. Diamond. 1991. Genetic factors and tolerance acquisition in populations exposed to metals and metalloids. Pages 301-316 *in* M.C. Newman and A.W. McIntosh (editors). Metal Ecotoxicology: Concepts and Applications. Lewis Publishers, Chelsea, Michigan.

Myint, U.M. and P.A. Tyler. 1982. Effects of temperature, nutritive and metal stressors on the reproductive biology of *Mytisus edulis*. Marine Biology 67:209-223.

Nei, M. 1975. Molecular Population Genetics and Evolution. Amsterdam/North Holland Publications.

Nevo, E., Perl, A., and D. Wool. 1981. Mercury selection 6 allozyme genotypes in shrimps. Experientia 37:1152-1154.

Nevo, E. and S.Y. Yang. 1982. Genetic diversity and ecological relationships of marsh frog populations in Israel. Theoretical and Applied Genetics 63:317-330.

Nevo, E., Lavie, B., and R. Ben-Shlomo. 1983. Selection of allelic isozyme polymorphisms in marine organisms: Pattern, theory, and application. Pages 69-92 *in* M. Siciliano (editor). Isozymes: Current Topics in

Biological and Medical Research, Volume 10:Genetics and Evolution.

Nevo, E., Ben-Shlomo, R., and B. Lavie. 1984. Mercury selection of allozymes in marine Organisms: Prediction and verification in nature. Proceedings of the National Academy of Sciences 81:1258-1259.

Nevo, E., Lavie, B., and R. Noy. 1987. Mercury selection of allozymes in marine gastropods: Prediction and verification revisited. Environmental Monitoring and Assessment 9:233-238.

Newell, R.C. and A. Roy. 1973. A statistical model relating the oxygen consumption of a mollusk (*Littorina littorea*) to activity, body size and environmental conditions. Physiological Zoology 46:253-275.

Newman, M.C., Diamond, S.A., Mulvey, M. and P. Dixon. 1989. Allozyme genotype and time to death of mosquitofish, *Gambusia affinis* during acute toxicant exposure: a comparison of arsenate and inorganic mercury. Aquatic Toxicology 15:141-156.

Nisbet, R.M., Gurney, W.S.C., Murdoch, W.W., and E. McCauley. 1989. Structured population models: a tool for linking effects at individual and population level. Pages 79-99 *in* P. Calow and R.J. Berry (editors). Evolution, Ecology, and Environmental Stress. Academic Press, New York.

Nriagu, J.O. Preface. Pages 1-3 *in* A. Boudou and F. Ribeyre (editors). Aquatic Ecotoxicology: Fundamental Concepts and Methodologies. CRC press, Boca Raton, FL.

Parsons, P.A. 1980. Isofemale strains and evolutionary strategies in natural populations. Evolutionary Biology 13:175-217.

Partridge, L. and P.H. Harvey. 1988. The ecological context of life history evolution. Science 241:1449-1455.

Pellerin-Massicotte, J., Pelletier, E., and M. Paquet. 1989. Cellular and biochemical indicators assessing the quality of a marine environment. Hydrobiologia 188/189:587-594.

Peterson, R.H., Metcalfe, J.L., and S. Ray. 1983. Effects of cadmium on yolk utilization, growth, and survival of

Atlantic Salmon alevins and newly feeding fry. Archives of Environmental Contamination and Toxicology.

Phillips, G.R. and D.R. Buhler. 1978. The relative contributions of methylmercury from food and water to rainbow trout (*Salmo Gairdneri*) in a controlled laboratory environment. Transactions of the American Fisheries Society 107:853-861.

Rahel, F.J. 1981. Selection for zinc tolerance in fish: Results from laboratory and wild populations. Transactions of the American Fisheries Society 110:19-28.

Ray, S., McLeese, D., and D. Pezzack. 1980. Accumulation of cadmium by *Nereis virens*. Archives of Environmental Contamination and Toxicology 9:1-8.

Ricklefs, R.E. 1973. Ecology. Chiron Press. Newton, Mass.

SAS Institute. 1985 SAS User's Guide: Statistics, Version 5 Ed. SAS Institute Inc. Cary, NC.

Savage, N.L. and F.W. Rabe. 1973. The effects of mine and domestic wastes on macroinvertebrate community structure in the Coeur d'Alene River. Northwest Science 47:159-168.

Schnell, G.D. and R.K. Selander. 1981. Environmental and morphological correlates of genetic variation in mammals. Pages 60-99 *in* M.H. Smith and J. Joule (editors). Mammalian Population Genetics. The University of Georgia Press, Athens, Georgia.

Selander, R.K., Smith, M.H., Yang, S.Y., Johnson, W.E., and J.B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus* I. Variation in the old-field mouse (*Peromyscus polionotus*). University of Texas Studies in Genetics 6:49-90.

Sibly, R.M. and P. Calow. 1989. A life-cycle theory of responses to stress. Pages 101-116 *in* P. Callow and R. J. Berry (editors). Evolution, Ecology, and Environmental Stress. Academic Press, New York.

Slooff, C. 1977. Biological monitoring based on fish respiration for continuous water quality control. Pages 501-506 *in* O. Hutzinger, L. H. Van Lelyveld, and B. C. J. Zoeteman (editors). Aquatic Pollutants.

Pergamon Press, New York.

Smith, R.L. and B.R. Hargreaves. 1985. Respiratory rate in the mysid, *Neomysis americana*: Effects of Naphthalene, temperature, and other factors. Pages 477-503 *in* F. J. Vernberg, F. P. Thurberg, A. Calabrese, and W. Vernberg (editors). Marine Pollution and Physiology: Recent Address. University of South Carolina Press, Columbia, S.C.

Smock, L.A. 1983a. Relationship between metal concentrations and organism size in aquatic insects. Freshwater Biology 13:313-321.

Smock, L.A. 1983b. The influence of feeding habits on whole-body metal concentrations in aquatic insects. Freshwater Biology 13:301-311.

Snyder, C.D. and A.C. Hendricks. 1992. Effects of seasonally changing feeding habits on whole-body mercury concentrations in *Hydropsyche morosa* (Trichoptera:Hydropsychidae). Hydrobiologia (in Review).

Snyder, C.D., Willis, L.D., and A.C. Hendricks. 1991. Spatial and temporal variation in the growth and production of *Ephoron leukon* (Ephemeroptera). Journal of the North American Benthological Society 10:57-67.

Solbe, J.F. de L.G. 1977. Water quality, fish and invertebrates in a zinc-polluted stream. Pages 97-105 *in* J.S. Alabaster (editor). Biological Monitoring of Inland Fisheries. Applied Science Publishers Ltd., London.

Sodergren, S. 1976. Ecological effects of heavy metal discharge in a salmon river. Environmental Pollution 3:25-36.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry. W.H. Freeman Company, North America. 859pp.

Spehar, R.L., Anderson, R.L., and J.T. Fiandt. 1978. Toxicity and bioaccumulation of cadmium and lead in aquatic invertebrates. Environmental Pollution 15:195-208.

Sprague, J.B. 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Research 4:3-32.

Stickle, W.B., Sabourin, T.D., and S.D. Rice. 1982. Sensitivity and osmoregulation of Coho Salmon, *Onchorynchus kisutch*, exposed to Toluene at different salinities. Pages 331-348 *in* W. B. Vernberg, A. Calabrese, F. P. Thurberg, and F. J. Vernberg (editors). Physiological mechanisms of Marine Pollution Toxicity. Academic Press, New York.

Sweeney, B.W. 1978. Bioenergetic and developmental response of a mayfly to thermal variation. Limnology and Oceanography 23:461-477.

Sweeney, B.W. and R.L. Vannote. 1981. *Ephemerella* mayflies of White Clay Creek: bioenergetic and ecological relationships among six coexisting species. Ecology 62:1353-1369.

Sweeney, B.W., Funk, D.H., and R.L. Vannote. 1987. Genetic variation in stream mayfly (Insecta:Ephemeroptera) populations of eastern North America. Annals of the Entomological Society of America 80:600-612.

Thorig, G.E.W., Schoone, A.A. and W. Scharloo. 1975. Variation between electrophoretically identifiable alleles at the alcohol dehydrogenase locus in *Drosophila melanogaster*. Biochemical Genetics 13:721-731.

Trama, F.B. 1972. Transformation of energy by an aquatic herbivore (*Stenonema pulchellum*) Ephemeroptera. Pol. Archives of Hydrobiologia 19:113-121.

Unzicker, J.D. and P.H. Carlson. 1982. Ephemeroptera. Pages 3.1-3.97 *in* A.R. Brigham, W.V. Brigham, and A. Gnilka (editors). Aquatic Insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterprises.

Warnick, S.L. and H.L. Bell. 1969. Acute toxicity of some heavy metals to different species of aquatic insects. Journal of the Water Pollution Control Federation 41:280.

Watt, W.B. 1983. Adaptation at specific loci. II. Demographic and biochemical elements in the maintenance of the *Colias* PGI polymorphism. Genetics 103:691-724.

Wentsel, R.A., McIntosh, A., and G. Atchinson. 1977. Sublethal effects of heavy metal contaminated sediment on the midge larvae (*Chironomus tentans*).

Hydrobiologia 56:153-156.

Wentsel, R., McIntosh, A. and G. Atkinson. 1978. Evidence of resistance to metals in larvae of the midge *Chironomus tentans* in a metal contaminated lake. Bulletin of Environmental Contamination and Toxicology 20:451-455.

Williamson, P. 1979. Comparison of metal levels in invertebrate detritivores and their natural diets: Concentration factors reassessed. Oecologia 44:75-79.

Widdows, J. 1985. Physiological responses to pollution. Marine Pollution Bulletin 16:129-133.

Winner, R.W., Boesel, M.W. and M.P. Farrell. 1980. Insect community structure as an index of heavy-metal pollution in lotic ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 37:647-655.

Zajac, R.N. and R.B. Whitlatch. 1989. Natural and disturbance-induced demographic variation in an infaunal polychaete, *Nephtys incisa*. Marine Ecology Progress Series 57:89-102.

CURRICULUM VITAE

Craig D. Snyder

August 25, 1992

Address

Department of Biology
Virginia Polytechnic Institute and
State University
Blacksburg, Virginia 24061
Telephone (703) 231-8931

2480 Ramble Road
Blacksburg, Virginia
24060
Telephone (703) 552-5357

Education

PhD., Biology, In progress, VPI&SU, Blacksburg, Virginia.
Dissertation title: Physiological, population, and genetic responses of an aquatic insect (*Isonychia bicolor*) to chronic mercury pollution.
Major Professor: Dr. Albert C. Hendricks.

M.S., Biology, May 1988, VPI&SU, Blacksburg, Virginia.
Thesis title: Effects of seasonally changing feeding habits on mercury uptake and secondary production of a filter-feeding caddisfly.
Major Professor: Dr. Albert C. Hendricks.

B.S., Biology, 1981 Berry College, Mount Berry, Georgia.

Grants and Awards

Acedemic scholarships, Virginia Tech (1987, 1988, 1990, 1991, and 1992).

Grant-In-Aid of Research, Graduate Assembly, Virginia Tech (1990).
Title: Adaptive responses of an aquatic insect to chronic mercury pollution.

Publications

Hendricks, A.C. and C.D. Snyder. Population and community responses of a filter-feeding assemblege to a catastrophic flood. (in preparation).

Snyder, C. D. and A. C. Hendricks. Effects of seasonally changing feeding habits on whole-body mercury concentrations in *Hydropsyche morosa* (Trichoptera: Hydropsychidae). Hydrobiologia (In Review).

Snyder, C.D., Willis, L.D., and A.C. Hendricks. 1991. Spatial and temporal variation in the life history and production of *Ephoron leukon* (Ephemeroptera: Polymitarcidae). Journal of the North American Benthological Society.

Hendricks, A.C., Snyder, C.D., and L.D. Willis. 1991. Production of a hydropsychid caddisfly in a fourth order mountain stream contaminated with mercury. Society of International Limnology.

Abstracts / Presented Papers

Snyder, C.D. and A.C. Hendricks. Physiological responses on *Isonychia bicolor* (Ephemeroptera:Oligoneuridae) to mercury. To be presented at the Fortieth Annual Meeting of the North American Benthological Society, Louisville, Kentucky, May 1992.

Snyder, C.D., Hendricks, A.C., and L.D. Willis. Effect of a catastrophic flood on densities of three species of filter-feeding caddisflies. Presented at the Thirty-eighth Meeting of the North American Benthological Society, Blacksburg, Virginia, May, 1990.

Hendricks, A.C., Snyder, C.D., and L.D. willis. Production of a hydropsychid caddisfly in a fourth order mountain stream contaminated with mercury. Presented at the Twenty-Fourth Annual Meeting of the Society of International Limnology, Munich, West Germany; August, 1989.

Snyder, C.D., and A.C. Hendricks. Relationship between seasonally changing feeding habits and secondary production. Presented at the Thirty-Seventh Annual Meeting of the North American Benthological Society, Guelph, Ontario, Canada, May, 1989.

Snyder, C.D., Willis, L.D. and A.C. Hendricks. The Impact of a Seasonal Shift in Feeding Habits on the Accumulation of Mercury in a Filter-feeding Caddisfly. Presented at the Thirty-Sixth Annual Meeting of the North American Benthological Society, Tusculoosa, Alabama, June, 1988.

Snyder, C.D., Willis L.D., and A.C. Hendricks. Life History and Production of *Ephoron leukon* (Williamson) (Ephemeroptera: Polymitarcidae) from the South River, Virginia. Presented at the Thirty-Fifth Annual Meeting of the North American Benthological Society, Orono, Maine, June, 1987.

Willis, L.D. and C.D. Snyder. Effects of a Sudden Increase in Discharge on the Drift of Aquatic Insects and Larval Fish. Presented at the Thirty-Fifth Annual Meeting of the North American Benthological Society, Orono, Maine, June 1987.

Snyder, C.D., Nicoletto, P.F., and A.C. Hendricks. Sexually Dimorphic Accumulation of Mercury in Four Species of Centrarchid Fishes: A Cost of Reproduction? Presented at the Seventh Annual Meeting of the Society of Environmental Chemistry and Toxicology, Alexandria, VA., November, 1986.

Snyder, C.D., Willis, L.D. and A.C. Hendricks. Life History and Production of *Isonychia bicolor* (Walker) (Ephemeroptera: Siphlonuridae) from the South River, Virginia. Presented at the Thirty-Fourth Annual Meeting of the North American Benthological Society, Lawrence, Kansas, May 1986.

Willis, L.D., Snyder, C.D., and A.C. Hendricks. Life History and Production of Two Species of *Hydropsyche* (Hydropsychidae) from the South River, Virginia. Presented at the Thirty-Fourth Annual Meeting of the North American Benthological Society, Lawrence, Kansas, May 1986.

Professional Societies

North American Benthological Society Ecological Society of America

Society of Environmental Toxicology and Chemistry

References and Complete Dossier Available upon Request

Craig Snyder