To the Graduate Council:

I am submitting herewith a dissertation written by Nicholas Joseph Cheper entitled "The Effect of Temperature on Lirceus brachyurus (Crustacea: Isopoda)." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Zoology.

Dewey L. Bunting
Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

4

Thesis
79b
.C336
cop.2

THE EFFECT OF TEMPERATURE ON LIRCEUS BRACHYURUS (CRUSTACEA:ISOPODA)

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Nicholas Joseph Cheper

June 1979

ACKNOWLEDGMENTS

The author would like to express his sincere appreciation to Dr. Dewey L. Bunting II, Department of Zoology, University of Tennessee, for all his help, patience and guidance throughout this study, and for serving as the chairman of the doctoral committee. I also wish to thank Drs. David A. Etnier, Melbourne C. Whiteside, and James L. Liles, Department of Zoology, and Dr. Clifford C. Amundsen, Department of Botany, for their advice and criticism on this project and for serving on my doctoral committee. Special appreciation is expressed to Dr. R. Bagby, Department of Zoology, for suggestions made during the study, and to Dr. M. A. Handel, Department of Zoology, for her review of the manuscript.

The companionship and assistance of numerous colleagues and fellow graduate students, without whom the author would have never finished this endeavor, is gratefully acknowledged. In particular, special appreciation is expressed to G. W. Wolfe, J. M. Cox, and C. V. Manning for their assistance in collecting animals; to L. B. Goss, J. Conney, R. Faulk, and F. Andrews Burkhart for their help in laboratory procedures; and to S. de Kozlowski, G. Kauffman, and R. Wainberg for assisting in the preparation of this document.

Finally, I sincerely thank my parents, Nicholas and Frances Cheper, for without their love, understanding, and encouragement, this study would have never been completed.

ABSTRACT

The thermal tolerance of the aquatic isopod Lirceus brachyurus (Harger) was determined, and the effect of temperature on photoperiodicity and respiration was investigated. Survival, as measured by the time to 50 percent mortality (LT50), increased with increasing acclimation temperature. A thermal trapezium was constructed which defined the range of temperatures (0 to 23°C) tolerated by the isopod for two weeks. Upper lethal temperatures were lower than recorded for other isopod species, but did correspond to temperatures recorded in the study creek. The animals tolerated cyclic temperature fluctuations (ΔT) of $\pm$ 10°C (between 5 and 30°C) for two weeks. There was no significant difference in tolerance of an elevated temperature among animals exposed to three photoperiod regimes. However, animals acclimated to a long-day photoperiod survived a little longer due to increased metabolic activity used for evaporative cooling.

Metabolic rates, which were measured by a differential respirometer, were highly modified by the movement of animals within the experimental chambers. Mean oxygen consumption per hour decreased with increased time spent in the respirometer, regardless of temperature. Oxygen consumption was similar for animals exposed to low (5°C) and high (25 and

30°C) temperatures, and to intermediate (10, 15, and 20°C) temperatures, regardless of acclimation. This result implies that L. brachyurus may regulate its metabolism without changes in energy expenditures, and that increased metabolism occurs at both elevated and reduced temperatures.

The ecological significance of these results with respect to the biology and habitat of Lirceus brachyurus is discussed.

TABLE OF CONTENTS

CHAPTER		PAGE
I.	INTRODUCTION	1
	Experimental Organism	3
II.	LITERATURE REVIEW	4
	Isopod Biology and Ecology	4
	Temperature Tolerance	7
	Fluctuating Temperature	11
	Photoperiod	12
	Respiration	13
III.	MATERIALS AND METHODS	19
	The Study Area	19
	General Culture	20
	Acclimation at 25°C	22
	Temperature Tolerance	25
	Fluctuating Temperature	30
	Photoperiod	31
	Respiration	32
IV.	RESULTS	35
	Temperature Tolerance	35
	Fluctuating Temperature	37
	Photoperiod	41
	Respiration	44
V.	DISCUSSION	48
	Acclimation and Testing at 25°C	48
	Temperature Tolerance	51
	Fluctuating Temperature	57
	Photoperiod	59
	Respiration	61
VI.	SUMMARY AND CONCLUSIONS	69
LITERATURE CITED		72
APPENDIX		81
VITA		97

LIST OF TABLES

TABLE		PAGE
I.	Chemical Analysis of Water in Study Stream . . .	23
II.	Chemical Analysis of One Week Acclimated Water	24
III.	LT50, Standard Errors, and 95 Percent Confidence Limits for Lirceus brachyurus—Thermal Tolerance Experiments	38
IV.	LT50, Standard Errors, and 95 Percent Confidence Limits for Lirceus brachyurus—Photoperiod Experiments	42
V.	Results of Analysis of Variance Applied to the Three Photoperiod Regimes for Lirceus brachyurus Acclimated to 5, 10, 15, 20, and 25°C	43
A-1.	Results of Significant T-Tests Applied to Lengths and Weights of Dead Versus Alive Animals Used in Thermal Tolerance Experiments	82
A-2.	Results of Modified Duncan's Multiple Range Test Applied to Mean Length and Weight of Animals Collected July, 1977	83
A-3.	Results of Modified Duncan's Multiple Range Test Applied to Mean Length and Weight of Animals Collected September, 1977	84
A-4.	Results of Modified Duncan's Multiple Range Test Applied to Mean Length and Weight of Animals Collected November, 1977	85
A-5.	Results of Modified Duncan's Multiple Range Test Applied to Mean Length and Weight of Animals Collected February and July, 1978 . . .	86
A-6.	Results of Modified Duncan's Multiple Range Test Applied to Significant Mean Lengths of Animals Used in Photoperiod Experiments	87

TABLE PAGE

A-7. Results of Modified Duncan's Multiple Range Test Applied to Significant Mean Weights of Animals Used in Photoperiod Experiments 88

A-8. Mean and Standard Error of Oxygen Consumption for Each Acclimation-Test Temperature Combination 89

A-9. Results of Modified Duncan's Multiple Range Test Applied to Mean Oxygen Consumption of Animals Used in Respiration Experiments 90

A-10. Mean and Standard Error of Animals in Respiration Experiments Acclimated at Varying Temperatures and Tested at 5°C 91

A-11. Mean and Standard Error of Animals in Respiration Experiments Acclimated at Varying Temperatures and Tested at 10°C 92

A-12. Mean and Standard Error of Animals in Respiration Experiments Acclimated at Varying Temperatures and Tested at 15°C 93

A-13. Mean and Standard Error of Animals in Respiration Experiments Acclimated to Varying Temperatures and Tested at 20°C 94

A-14. Mean and Standard Error of Animals in Respiration Experiments Acclimated to Varying Temperatures and Tested at 25°C 95

A-15. Mean and Standard Error of Animals in Respiration Experiments Acclimated to Varying Temperatures and Tested at 30°C 96

LIST OF FIGURES

FIGURE		PAGE
1.	The Effect of Heat Resistance with Acclimation	39
2.	Tolerance Polygon for Lirceus brachyurus	40
3.	The Effect of Environmental Temperature Upon Oxygen Consumption in Lirceus brachyurus	45
4.	The Effect of Time Spent in the Respirometer Upon Oxygen Consumption in Lirceus brachyurus . .	47

CHAPTER I

INTRODUCTION

There have been many studies on environmental parameters that affect aquatic animals. Temperature in particular has been examined by numerous investigators interested in adaptations of freshwater organisms. They have found a general correspondence between upper and lower lethal temperatures and the normal environmental temperature range to which freshwater animals are exposed. However, in some cases, the upper (and lower) limits of thermal tolerance exceed that which the animals normally encounter.

Previous thermal regimes can affect upper and lower lethal temperatures. Bullock (1955) and other authors indicate that both the rate of temperature change and length of time spent at a new temperature affect the ability of poikilotherms to compensate for changes in metabolism and activity. In addition, temperature has been shown to affect growth, metabolism, and behavior as well as survival. Consequently, an understanding of temperature and its effects upon biological processes is crucial in evaluating environmental impacts upon aquatic organisms.

One of the major uses of water is associated with the production of electricity by both nuclear and steam-generated power plants (Speakman and Krenkel, 1972). Heated water from

power plant condensers is returned to lakes and streams. This discharge creates temperature fluctuations which can drastically alter the thermal regime of the bodies of water into which it is released. Therefore, it is extremely important to know how temperature and temperature changes will affect (or not affect) aquatic life. Temperature changes can result in alterations of aquatic ecosystems, which may or may not be beneficial to aquatic life. These changes include the elimination of desired organisms (i.e. fish), an increase in the abundance of eurythermal species (Naylor, 1955), and the elimination of potential food resources (either plant or animal). In short, temperature changes can affect the entire biota of a lake or stream.

Freshwater isopods have received little attention with respect to temperature tolerance. Isopods are malacostracan crustraceans which are members of the superorder Peracardia. These animals feed on organic matter and are an important component in the detritivore food chain. They are also an important food source for fish (Ellis and Gowing, 1957) and aquatic amphibians and reptiles. In addition, Hynes (1960) includes isopods as members of the fauna of polluted waters.

The purpose of this study was to investigate the temperature tolerance of a group of animals which are an important component of the freshwater environment. Furthermore, the influence of temperature on photoperiodism and

respiration was examined. The results of these investigations provide data on the temperature tolerance of a freshwater macro-invertebrate, thus extending laboratory studies of lethal levels in invertebrates. Also, changes in biological processes as influenced by temperature are described. Methodologies were employed such that reproducibility could be easily achieved. Consequently, it is hoped that the results of this laboratory study, coupled with field investigations, will lead to a better understanding of the influence of temperature on the dynamics of aquatic ecosystems.

Experimental Organism

The animal used in this study was the isopod *Lirceus brachyurus* (Harger), which is a member of the suborder Asellota, family Asellidae. *Lirceus brachyurus* is distributed in springs and small streams in the Ohio and Tennessee valleys (Hubricht and Mackin, 1949, Beatty, 1974). The genus *Lirceus* is restricted to eastern North America (Birstein, 1964), and its distribution is sympatric with that of the genus *Asellus*, the other predominant asellid in North America. General reviews on North American isopods are found in Hubricht and Mackin (1949), Pennak (1978), Ward and Whipple (1959), and Williams (1970, 1972). An extensive study on freshwater isopods is found in Birstein (1964).

CHAPTER II

LITERATURE REVIEW

Isopod Biology and Ecology

A very limited number of studies have been conducted on freshwater isopods of the genus Lirceus. Hubricht and Mackin (1949) indicate that Lirceus probably consisted of a single entity, which has evolved into a number of distinct species under the influence of geographical isolation. The morphology and life history of Lirceus fontinalis (as Mancasellus macrourus) was studied by Markus (1930). He found that gravid females were present at all times of the year but were most abundant between March and April. Based upon his field samples, he concluded that the lifespan of L. fontinalis was approximately one year. Most animals reproduced and hatched young in the late spring and early summer and then died. The offspring grew during autumn and winter, and reached sexual maturity the following spring. Sexes appeared to be present in equal number with the males being larger in size than the females. Most of the data on the ecology of Lirceus is given in Styron (1968, 1969) and Styron and Burbanck (1967). These authors demonstrated that two populations of Lirceus fontinalis separated by 25 kilometers are evolving into ecotypes due to different environmental conditions. One population inhabits intermittent creeks and drainage pools

over a granite outcrop where high temperature and drought limit survival of the species. The other population inhabits a spring-fed stream where biotic factors and human activities control its survival. Temperatures of 25°C were not uncommon for isopods in the first habitat while temperatures rarely approached 25°C for isopods in the stream. However, isopods in both populations showed similar reactions to low temperature, namely, that survival was limited by the formation of ice crystals in the stream. Both populations were photonegative and positively rheotaxic (oriented upstream towards flowing water). Numerous other analyses, including numerical taxonomy, lead these authors to suggest that isopods living in these different habitats are evolving different behavioral and physiological responses to environmental factors.

The ecology and distribution of Asellus in North America has been reviewed by Williams (1970). A life history study of Asellus intermedius was conducted by Ellis (1961). He demonstrated that brood size tended to increase proportionately to the cube of the length of the animal in this species. In addition, the length of incubation was inversely related to temperature; the young being released sooner at higher temperature. This phenomenon is generally characteristic of aquatic animals. He also found that growth rates generally increased with temperature but were reduced with an increase

in age. Furthermore, Ellis showed that most of the life history data for these animals were similar to those of Lirceus and Asellus aquaticus (a European species). These similarities included length of the breeding season, abundance of gravid females, sex ratios, number of young produced, and life span.

Other life history studies on North American Asellus have been conducted by Hatchett (1947) on A. communis, Ellis (1971) on A. tomalensis, and Martien and Bender (1977) on A. obtusus. Fleming (1972, 1973) has reviewed the evolution of eastern North American Asellus. Studies on ecological aspects of European Asellus species are summarized by Steel (1961), Økland (1978), and Birstein (1964). The latter author's study also includes references to the works of other Russian authors. Other studies have been conducted by Moon (1957), Hynes and Williams (1965), and Williams (1962a,b, 1963).

Hynes (1960) indicated that Asellus aquaticus is characteristic of a "polluted river" fauna along with oligochaetes and chironomids. Wolff (1973) also noted that A. aquaticus was tolerant of polluted areas of the Rhine River. Ellis (1961) also noted in his study of A. intermedius that the distribution of this isopod was positively correlated with the concentration of domestic sewage dumped into his study stream. However, this tolerance of polluted waters is

not found in Lirceus. Styron (1967, 1968) noted that a marked decrease in the population density of Lirceus fontinalis occurred due to human activities. These activities included silt build-up from nearby construction sites and an herbicide application to a nearby tennis court, which reduced the density of the Lirceus population by about 85 percent.

Temperature Tolerance

A tremendous body of literature exists on the effects of temperature on freshwater organisms. General reviews on biological and physiological effects of temperature are found in Prosser and Brown (1961), and Precht et al. (1973). Speakman and Krenkel (1972), and Prosser (1973) reviewed the concept of a temperature tolerance trapezium. This is a diagram which indicates the high and low lethal temperatures at which an animal can survive or reproduce after acclimation to different temperatures. They also allow for predictions to be made on the probability of animal's survival at a given temperature, given the past history of that animal (its acclimation temperature). The value of tolerance polygons is that they are fairly characteristic of a given species.

Most of the studies on temperature effects on aquatic animals have focused on upper temperature limits. These studies include Bursell (1974), and Sherberger et al. (1977) on aquatic insects, McMahon (1975, 1976), and Al-Habbib et al. (1977) on freshwater snails, Bunting (1974), Goss and Bunting

(1976), and Goss (1978) on zooplankton, W. Smith (1970) on mysids, and Krog (1954), and W. Smith (1972, 1973) on freshwater amphipods.

A few studies have concentrated on the effects of cold temperatures on aquatic animals. General reviews are found in A. Smith (1970) and Theede (1973). Specific reviews include Becker et al. (1977) on crayfish and Breganzi (1972) on amphipods.

Temperature tolerance in Asellus aquaticus has been investigated by Williams (1962b). He found that the upper limit of temperature tolerated by these animals without significant increase in mortality was 28°C, with upper lethal temperatures (temperatures that killed half of the population in five days) between 30 and 32°C. Birstein (1964) also records an upper temperature limit of 28°C for A. aquaticus, and indicates that these animals cease moving and feeding at 4°C. Eltringham (1967) examined the relationship between temperature tolerance and development in Limnoria lignorum, a marine wood-boring isopod. He observed that the lowest temperature allowing development was 7-8°C while 23-25°C was the highest temperature at which complete development occurred.

Edney (1964) has looked at temperature tolerance in the terrestrial isopods Porcellio laevis and Armadillidium vulgare. He found that upper lethal temperatures increased

with acclimation temperature for both species after a 30-minute exposure to elevated temperatures. Furthermore, these upper lethal temperature limits decreased with increased time of exposure to the test temperature (30 minutes versus 12 hours). Similar results were observed for the low lethal temperature limits. Edney concluded that acclimation to high temperature raises both the upper and lower lethal temperature limits while acclimation to lower temperature lowers both of these limits. He also demonstrated that lethal temperature limits were not affected by either size of the animal or by molting animals. Finally, he demonstrated that there was a positive correlation between local environmental temperature of wild populations and their upper lethal temperature limits. Sprague (1963) examined the thermal tolerance of four freshwater crustaceans, including Asellus intermedius, to lethal high temperatures. Animals were acclimated for six to thirty days depending upon the collection and test temperatures. He demonstrated that for the acclimation temperatures examined (10, 20, 25, and 30°C), the upper lethal temperature tolerated by half of the population ranged from 30 to 33°C. Sprague also showed that the highest temperature these isopods could resist for twenty-four hours was 34.6°C. In addition, he observed that resistance times decreased as the size of the animal increased, indicating that similar sized animals should be

used in this type of study. He found neither sexual differences nor differences between gravid and nongravid females with respect to resistance to upper temperatures in A. intermedius. Furthermore, innate seasonal differences in resistance were absent since acclimated June and December populations showed similar resistance to lethal temperatures. Finally, the author indicated that temperature resistance was much greater than one would expect based on the water temperature where the animals were collected. He concluded that this may serve as a "safety factor" between the temperature for normal activity and onset of death due to high temperature. This has been observed by numerous other authors.

Very few studies have dealt with temperature tolerance in the genus Lirceus. Styron (1967, 1968) examined temperature as part of his factorial experiments looking at gamma radiation, high temperature, and drought stress in two populations of Lirceus fontinalis. He observed that larger sized animals showed a decrease in resistance to high temperatures. In addition, he found that temperature tolerance was reduced in a creek population which experienced a narrow water temperature range. The other population, which lived in weather pools and an intermittent creek in a granite outcrop, had a much wider water temperature range and was also more tolerant of temperature stress.

Fluctuating Temperature

The thermal tolerance of animals that have been acclimated to fluctuating temperatures have been examined by many authors. Rockstein and Miguel (1973) and Bursell (1974) have reviewed the effect of fluctuating temperatures upon development in insects. They observed that in some cases a fluctuating temperature regime caused retardation of development, in other cases, development was enchanced, while in still other cases no effect was observed. Edney (1964) showed that Porcellio laevis and Armidillidium vulgare acclimated for 14 days to a daily cycle of 12 hours at 30°C and 12 hours at 10°C have a similar thermal tolerance to animals acclimated to a constant 30°C.

Other studies have looked at various durations of exposure to an elevated temperature, and defined tolerance based upon 50 percent mortality after a specified period of exposure (W. Smith, 1973). However, the effect of a diurnal cycle of exposure temperatures upon survival after acclimation at one given temperature has not been investigated. This type of exposure might be considered typical of periodic entrainment in power plant effluents. Some studies have been conducted on the effect of one time short term exposure to heated waters. Ginn et al. (1974, 1976) indicated that temperature changes (ΔT) of 8.3°C above 25.5°C resulted in little or no mortality in Gammarus species exposed to the ΔT

for 60 minutes after 10 days, but a ΔT of 11.1°C above 26.5°C resulted in significant mortality of animals after exposures of 30 and 60 minutes.

Photoperiod

The relationship between photoperiod and temperature in aquatic invertebrates has just begun to receive attention in the literature. Beck (1968) in a review of photoperiodism indicates that sudden shifts in temperature can result in a change in the initiation of some activity. However, temperature does not appear to affect the length of endogenous rhythm periods.

Photoperiodism has been examined somewhat extensively in insects, especially with respect to diapause and molting. Lees (1968) states that the relationship between photoperiod and temperature is quite variable within insects groups, but that temperature-photoperiod interactions show a seasonal effect. Other works dealing with temperature-photoperiod interactions are Stephens (1955) and Armitage et al. (1973) on crayfish, and Kruschwitz (1971) and De March (1973) on Hyalella azteca. In general, molting rates were higher in these two groups under long-day photoperiod-high-temperature regimes. Short-day photoperiods resulted in ovarian growth in females and accumulation of nutrients in both sexes, with temperature influencing the rate of all changes. Thus photoperiod generally affects growth rates.

A few papers have dealt with the effects of photoperiodicity in isopods. Enright (1976) investigated the effect of a circadian rhythm in Excirolona chilteni, an intertidal isopod. Enright demonstrated that this rhythm served to reinforce the tide-cycle and day-night cycle in these animals. Most studies on light have dealt with the effects of light on terrestrial isopods (Allee, 1926; Cloudsley-Thompson, 1952; Warburg 1964, 1968). Generally, these studies have been used to indicate the relationship between photo-kinesis and nocturnal habits of these animals. Few studies on light have been conducted on freshwater asellids. Pardi and Papi (1961) reviewed the response of crustaceans to light, and indicated that these animals were generally negatively phototropic. In addition, they noted that Asellus responds to light stimulation by orientating upstream (positive rheotaxis). Finally, Sther (1931) investigated the influence of light upon Asellus militaris (and other aquatic arthropods). He found that for dark-acclimated animals exposed to sudded illumination, hiding time decreased as light intensity increased.

Respiration

Numerous articles have been written on the influence of temperature on metabolic rates (respiration) of aquatic invertebrates. Wolvekamp and Waterman (1961) reviewed respiration and its relationship to temperature in crustaceans.

Vernberg and Vernberg (1970) reviewed physiological aspects of thermal regulation and its effect on metabolic rates. Newell (1973) indicated that for an ectotherm of a given weight, oxygen consumption is temperature dependent, and warm acclimation results in a shift (change in translation and rotation) in a metabolic rate-temperature curve. However, oxygen consumption decreases with time in a respirometer to a "standard" rate which is independent of thermal acclimation (Newell et al., 1974).

A variety of instruments have been used to measure respiratory rates. In recent years, the Gilson differential respirometer has been used as the main respiration instrument, replacing the Warburg apparatus, which for many years was used almost exclusively for oxygen consumption studies. A comparison of the various instruments used by ecologists interested in respiratory rates is given in Lawton and Richards (1970).

Many studies have been conducted on the effects of temperature on respiration in aquatic organisms. They include Kiester and Buck (1974) and Mill (1974) for insects; Berg and Ockelmann (1959) and Calow (1975) for snails; Comita (1965), Kring and O'Brien (1976), and Goss (1978) for copepods and cladocerans; Knight et al. (1974) for fairy shrimp; McFarland and Pickens (1965) and Laird and Haefener (1976) for decapods; and Culver and Poulson (1971) and Wright and Wright (1976)

for amphipods. The results, while varying from group to group, do illustrate similarities in the following respects. Respiration tends to increase with temperature except at extreme temperatures. Sexes tend not to differ in rates of oxygen consumption. There may or may not be a seasonal effect in respiratory rates, depending upon the group and the temperature range examined. Finally, oxygen uptake and body weight tend to be related to each other, usually to some power of body weight.

Respiration has been studied fairly extensively in isopods. Jones (1974) and Vlasblom et al. (1977) have looked at respiration in the genus Idotea, a marine isopod. Frier (1976) examined oxygen consumption in Sphaeroma, another marine genus. Newell et al. (1976), using multiple regression, found that respiration of Ligia oceanica was mainly affected by exposure temperature, followed in importance by dry weight, starvation period, feeding period, and acclimation temperature. Acclimation, however, did increase the temperature at which the maximum metabolic rate occurred. Levels of metabolism in starved animals were affected by acclimation temperature, body size, and duration of starvation. These parameters did not affect metabolic rates in fed animals.

Newell et al. (1974) investigated factors that affect oxygen consumption in the terrestrial isopod, Porcellio scaber. Animals were acclimated in the dark at temperatures of 5, 15,

20, and 30°C for at least three weeks. Organisms were then placed in a Gilson respirometer and readings taken at test temperatures of 5 to 35°C. They observed that the animals showed a high level of oxygen consumption just after transfer due to handling and an increase in light intensity. However, as the time spent in the respirometer increased, the rate of oxygen consumed decreased until a "standard" rate of oxygen consumption was reached. This usually occurred after at least three hours in the respirometer. This standard rate was characterized by no change in oxygen consumed with time. The authors also observed that no significant differences could be detected in respiration of animals at the various acclimation temperatures at any exposure temperature but respiration increased with exposure temperature. Thus, the authors indicate that their data can be used to compare the metabolic responses of organisms from different environments to temperature itself.

Most of the information on respiration in Asellus is derived from Edwards and Learner (1960) and Wolvekamp and Waterman (1960). Edwards and Learner, using a Warburg apparatus, found that A. aquaticus had an oxygen consumption proportional to .7 times its wet body weight. They also observed that oxygen consumption increased with temperature over the range tested (10 to 30°C). No differences were detected between males and non-oviferous females, nor between

starved and fed animals. Finally, Edwards and Learner indicated that oxygen consumption decreased by 15 to 20 percent when dissolved oxygen levels were low (about 1.5 mg/l). Wolvekamp and Waterman indicated that isopods from swift flowing streams have a higher metabolic rate than animals from slow flowing streams or ponds. They indicated that isopods can "gulp" air through their oral and anal openings as well as respire through their pleopods. They also recorded oxygen consumption rates for A. aquaticus similar to those reported by Edwards and Learner.

Very little work has been done on the oxygen consumption of North American asellids. Most studies have concentrated on tolerance of isopods to low oxygen levels. Sprague (1963) looked at effects of temperature on tolerance of Asellus intermedius to water of reduced oxygen content. Dissolved oxygen values in his study ranged from 0 to 2.7 mg/l. Animals acclimated at 10, 20, and 30°C tolerated .5 mg/l dissolved oxygen for more than a week when tested at 10° and 20°C. Animals acclimated at 10°C and tested at 30°C tolerated an oxygen concentration of 1.6 mg/l for one week. The author concluded that this species' tolerance of low oxygen levels agreed with its ability to survive in polluted and deoxygenated waters. Hynes (1960) also reported Asellus as a member of the "pollution" fauna that tolerates low levels of oxygen.

Styron (1967, 1968) tested tolerance to anaerobic conditions in lotic and lentic populations of Lirceus

fontinalis. He sealed animals of a constant size (5 millimeters) in a chamber and recorded the time of their death after the oxygen consumption reached 0.00 ml/liter. He found that LD50 values were 16.26 hours for the creek population, and 23.32 hours for the pool populations. He indicated that these results were correlated with the habitat of the two populations.

CHAPTER III

MATERIALS AND METHODS

The Study Area

Isopods were collected from a small stream in Anderson County, Tennessee. This creek originates as a spring fed seep, and eventually flows into Buffalo Creek, a tributary of the Clinch River. The actual collection site was .7 miles north of state route 61 in Andersonville, Tennessee. In the area where animals were collected, water depth averaged approximately .5 meter and average stream width was approximately 2 meters under normal (nonflooded) conditions. Substrates consisted of sand and gravel with a slow to moderate current. The creek contained a fair amount of organic matter, since it was used as a source of water for a small herd of cattle. In addition, falling leaves from trees along the banks served as another allochthonous source of organic matter.

Water cress (Nasturtium sp.) was very abundant along the banks of the creek, and this was where most of the isopods were found. Lirceus brachyurus was the most abundant animal in the stream. Other animals found included nematodes, amphipods (Gammarus minus), crayfish (Cambarus spp.), insect larvae (chironomids, coleopterans, ephemeropterans, gerrids, odonates, plecopterans, trichopterans, and tipulids), snails

(Goniobasis and Physa spp.), fish (Semotilus atromaculatus and Cottus carolinae), and salamanders (Desmognathus sp.).

General Culture

Isopods were collected from August, 1977 through July, 1978, by means of a hand-held strainer and placed in glass jars along with some vegetation to be transported to the laboratory. Extra vegetation and creek water were also collected at the same time. The extra vegetation was placed in glass jars along with some water while the creek water was transported to the lab in 20ℓ Naglene plastic, screw cap bottles. Upon return to the laboratory, the animals and extra vegetation were placed in an incubator approximating creek temperatures for twenty-four hours. The incubators that were used throughout the study were GSA Precision Scientific Freas Model 818 incubators. They were set at 5°C intervals from 0 to 35°C. The photoperiod (except for photoperiod experiments) was set at 14 hours of light and 10 hours of dark. Light intensity inside the incubators was approximately 100-foot candles (Goss, 1978).

The day following a collection, isopods were sorted from other debris in a white enamel pan. Approximately 25 to 50 animals were placed either in small one-gallon aquaria or large circular (1.5ℓ volume) culture dishes; the number of animals placed in each container depending upon the size of the dish or the number to be acclimated at a given temperature.

In general, 35-40 animals were placed in the aquaria while 20-25 were placed in the circular dishes. Sufficient water and vegetation were added to the dishes, and they were then placed in incubators at the temperature from which they were removed. Some animals were then switched to incubators 5°C higher or lower depending upon the final acclimation temperature. Acclimation temperatures used in the study were 5, 10, 15, 20, and 25°C. The rate of change from initial storage temperature to final acclimation temperature was 5°C per day. Once animals reached the final acclimation temperature, they were held at this temperature for two weeks before any tests were conducted. All containers were aerated using Hush air pumps. Animals were presumed to be feeding upon vegetative matter and debris in the containers, however an additional source of food was provided. This consisted of a .2 percent solution of Cerophyl, a vegetable and vitamin enriched supplement. Cerophyl was used to insure that animals were not food limited during the period of acclimation. Each culture dish of animals received one Kahn serum pipette (approximately 3.5ml) of Cerophyl per day. A fresh solution was prepared each week.

The extra vegetation that was collected was stored in the 5°C incubator. This vegetation was added to the culture dishes as needed, usually twice a week. Fresh vegetation was collected each week during the period of acclimation.

Water collected at the stream was aerated for twenty-four hours after it was returned to the laboratory. Then, the aerated water was filtered through a number 28 plankton net, and allowed to equilibrate in glass gallon jars to temperature in the incubators for 8 to 12 hours. It was then placed in the culture dishes along with the animals. Extra water was stored in the incubators and added to the culture dishes when needed, usually twice a week. Fresh water was collected once a week. Water chemistry was performed in the laboratory on the stream water at routine intervals. In addition, part of the water discarded after one week of acclimation was retained and analyzed to see whether changes in chemical parameters occurred during acclimation. Tables I and II present water quality parameters measured. All tests were performed following procedures outlined in the Hach Chemical Company Manual for use with a portable Hach kit, while pH was measured by an Accument Model 220 pH meter.

All glassware used in culturing was washed by hand in tap water with Sparkleen detergent, soaked in an acid wash solution, washed twice in a dishwasher, and rinsed in distilled water.

Acclimation at 25°C

Estimates of natural mortality were determined for isopods at each acclimation temperature during the two week acclimation period. Mortality at 5, 10, 15, and 20°C ranged

TABLE I

CHEMICAL ANALYSIS OF WATER IN STUDY STREAM

Chemical	08/02/77	11/17/77	12/15/79	02/22/78	03/26/78	07/17/78
Ammonia	0[a]	.02	.01	.01	.01	.03
Nitrate	1.5	2.5	2.0	8.0	9.0	8.0
Nitrite	.01	.01	.05	.04	.04	.01
Chlorine	0	.01	.01	0	0	0
Iron	.05	.03	.02	.01	0	0
Silica	32	33	33	32	33	33
Sulfate	13	27	5	5	5	8
Turbidity	5[b]	0	10	10	8	10
Total Alkalinity	185	150	160	160	150	170
Calcium Hardness	140	100	120	140	110	110
Total Hardness	190	160	150	170	200	180
Dissolved Oxygen	3.0	3.4	3.8	4.2	4.0	4.0
pH	7.0	7.1	7.1	6.9	7.0	6.8

[a]All measurements except Turbidity and pH in mg/l.

[b]Formazin Turbidity Units.

TABLE II

CHEMICAL ANALYSIS OF ONE WEEK ACCLIMATED WATER

Chemical	Date	Acclimation Temperatures 5°C	10°C	15°C	20°C	25°C
Ammonia	08/09/77	.70[a]	---	1.05	---	3.2
	10/22/77	.50	---	.80	1.40	3.3
	11/25/77	.50	.40	1.10	.85	3.0
	03/01/78	.40	.20	1.30	1.00	3.1
	07/24/78	----	.40	1.20	1.10	3.0
Nitrate	08/09/77	1.0	---	2.0	---	3.4
	10/22/77	2.0	---	2.5	4.0	3.0
	11/25/77	8.0	7.0	10.0	10.0	10.3
	03/01/78	8.0	8.0	10.0	8.0	10.2
	07/24/78	----	2.0	5.0	7.0	8.0
Nitrite	08/09/77	.04	---	.04	---	.25
	10/22/77	.07	---	.09	.20	.30
	11/25/77	.07	.05	.18	.12	.30
	03/01/78	.06	.05	.14	.12	.30
	07/24/78	----	.01	.02	.01	.20
Total Alkalinity	08/09/77	190	---	200	---	210
	10/22/77	170	---	170	180	180
	11/25/77	160	150	190	190	200
	03/01/78	200	210	200	200	220
	07/24/78	----	210	210	220	250
Calcium Hardness	08/09/77	110	---	110	---	100
	10/22/77	120	---	110	110	100
	11/25/77	100	90	130	120	110
	03/01/78	120	130	130	140	150
	07/24/78	----	110	120	120	150
Total Hardness	08/09/77	180	---	190	---	180
	10/22/77	180	---	190	190	190
	11/25/77	140	110	160	170	180
	03/01/78	190	180	190	170	200
	07/24/78	----	190	200	200	220
Sulfate	08/09/77	10	---	12	---	20
	10/22/77	6	---	9	9	20
	11/25/77	6	1	13	11	18
	03/01/78	5	5	13	8	19
	07/24/78	----	6	12	13	20
Turbidity	08/09/77	5[b]	---	5	---	20
	10/22/77	10	---	10	5	30
	11/25/77	4	11	18	20	30
	03/01/78	5	10	20	30	80
	07/24/78	----	10	15	12	60
pH	08/09/77	6.9	---	7.0	---	7.0
	10/22/77	6.8	---	6.8	6.8	7.0
	11/25/77	6.7	6.7	6.8	6.8	7.0
	03/01/78	6.7	6.8	6.8	6.9	7.1
	07/24/78	----	6.8	6.8	6.9	7.0

[a]All measurements except Turbidity and pH are in mg/l.

[b]Formazin Turbidity Units.

from 0 to 25 percent with an average of 10 percent. However, animals acclimated at 25°C showed mortalities of 70 to 100 percent. It was postulated that changes in water quality due to decaying vegetation (see Table II) or light intensity were responsible for this mortality. Sprague (1963) also observed mortality in Asellus intermedius acclimated at 25°C. Consequently, the following procedures were employed to reduce mortality at this acclimation temperature. First of all, water and vegetation were changed more often; usually the water was changed every day and the vegetation every other day. Also, strips of black opaque polyethylene were cut to the dimensions of the acclimation containers to reduce light intensity. Mortality at 25°C was reduced to 50-60 percent using these procedures. While this was an improvement over the mortality of animals before these steps were taken, it was still much higher than the natural mortality observed at the other acclimation temperatures.

Temperature Tolerance

At the end of the acclimation period, animals were isolated for tolerance tests. They were removed from the acclimation containers, placed in a white pan, and separated from the associated vegetation. Individual animals were placed in 8-dram shell vials filled with approximately 30 ml of filtered water acclimated at the same temperature as the animals. All transfers were made with a large bore pipette

which did not injure the animals. Groups of 24 vials with animals were placed in rubber coated test tube racks covered on the sides and bottom by black opaque polyethylene to reduce light intensity. Racks were left at the acclimation temperature overnight for animals to adjust to the vials. Animals were not fed after they were placed in the vials. Thermal tolerance was tested for each acclimation temperature except 10°C at 0, 5, 10, 15, 20, 25, 30, and 35°C. Animals acclimated at 10°C were not tested at 5, 15, 20, and 25°C after initiation, since the results of 5°C and 15°C acclimated animals were similar at these test temperatures. All experiments were conducted in the Freas incubators with a photoperiod of 14 hours light, and 10 hours dark.

At the beginning of each experiment, animals were transferred to the test temperature in two ways. The first method, designated as the immediate exposure method, involved transferring animals in vials at the acclimation temperature to vials (with water) that had been acclimated for 24 hours at the test temperature. The transfer was accomplished by means of a pipette and was accomplished as quickly as possible. The second method of transfer, designated as the gradual transfer method, simply involved placing the acclimated vials (with animals) at the test temperature. Thus, the first method examined an immediate exposure to the test temperature as compared to the second method which

provided a gradual rate of change to the test temperature. This rate of change to the test temperature varied with both acclimation (initial) and test (final) temperatures.

Preliminary experiments were conducted to examine the value(s) of rate of change. Vials were filled with 30 ml of filtered water acclimated at 5, 10, 15, 20, and 25°C. Tests were conducted at 0, 5, 10, 15, 20, 25, and 30°C. For each test, a pair of vials were placed at the test temperature and temperature readings were recorded each minute using a thermistor attached to an electronic thermometer until the test temperature was reached. The rates of temperature change were found to be exponential instead of linear and ranged from 1°C every 2 minutes to 1°C every 9 minutes. In all cases, temperature change was accomplished in one hour or less. These rates, which tended to average about 1°C per 5 minutes, are defined as the gradual rate of change employed in the tolerance tests.

For each tolerance test, 24 animals were used, 12 of which were immediately exposed to the test temperature and 12 of which were gradually exposed to the test temperature. Only visually healthy animals were used in each experiment. The animals were checked for mortality at 1/2, 1, 2, 4 and 8 hours after the beginning of each experiment. Subsequent checks were made every 8 hours either for two weeks or until all the animals had died, whichever occurred first.

Mortality was determined in the following manner. At each check, animals that did not respond to irritation from a probe, or indicated lack of coordinated beating of the pleopods were returned to the temperature at which they had been acclimated for 8 hours. At this time, they were examined under a microscope. Dead animals were recorded as having succumbed at the time when they were removed from the test temperature. Any animal showing some movement was returned to the acclimation temperature for an additional 8 hours. At this time, if any coordinated movement was observed, they were discarded. Otherwise, they were recorded as having died 8 hours after they were removed from the test temperature. In almost all cases, it was readily apparent whether or not an animal was alive or dead. However, some animals did show uncoordinated respiratory movements even after 16 hours at the acclimation temperature. Dead animals were sexed and their lengths measured to the nearest half millimeter. They were then placed in distilled water in small stoppered freeze-dryer flasks, and immediately frozen in a refrigerator freezer.

Water was maintained at a constant level during the course of the experiments by addition of filtered water to the vials. The water in each vial was replaced once a week. Once every day, fecal material and other debris were removed from the vials using a Kahn serum pipette.

At the end of two weeks, any remaining animals were killed in 70 percent isopropyl alcohol, and then placed into

distilled water until the alcohol evaporated. They were then sexed, measured, and placed into freeze-dryer flasks and frozen.

Specimens were freeze dried using a Labconco Model 3 Freeze Dryer. Organisms remained on the freeze dryer for a minimum of 24 hours. Organisms were weighed individually on a Cahn Model 4700 Automatic Electro-balance.

Mean and variance estimates were made on length and weight for all test animals. Size/weight differences in mortality or survivorship were checked using t-tests. In addition, differences in sex, and type of exposure (gradual versus immediate, as described above) were examined using tests for the significance of two proportions (Sokal and Rohlf, 1969). Furthermore, mean lengths and weights were determined for both groups (experimental deaths plus survivors). These means were compared both between and within dates of collection for differences that might influence temperature tolerance. Duncan's Multiple Range Test, modified for unequal sample size (Steele and Torrie, 1960), was employed for these comparisons.

Percent mortality was plotted against time and a straight line was fitted to the data using probit analysis for those experiments where mortality was equal to or greater than 50 percent. Control deaths were adjusted by Abbott's formula (Finney, 1962). From the probit-regression

lines, LT50's were obtained by inverse prediction. The LT50 is defined as the time in hours required to kill 50 percent of the animals. LT50's were then plotted against exposure temperature and a thermal trapezium was constructed following the methods of Prosser (1973).

Fluctuating Temperature

The same methodology was employed for the fluctuating temperature experiments as for the thermal tolerance experiments with a few exceptions as described below. All animals were transferred from the acclimation to the test temperature gradually. Animals were left at the test temperature for the specified time (8 or 16 hours), then returned to the acclimation temperature. This cycle was repeated daily until two weeks had elapsed. The acclimation and test temperatures examined were as follows: 5 to 15°C, 8 hours; 10 to 20°C, 8 hours, 15 to 25°C, 8 and 16 hours; 20 to 30°C, 8 and 16 hours; 15 to 5°C, 8 hours; 20 to 10°C, 8 hours; and 25 to 15°C, 8 hours.

Animals were examined every 8 hours and mortality, sex, and length were measured as described above. The animals were freeze-dried but not weighed and no statistical analysis was performed due to lack of any mortality above 50 percent after correction for control death.

Photoperiod

Animals were acclimated as described above except that three photoperiods were employed. One was 14 hours of light, 10 hours of dark (14L:10D), the same as the tolerance experiments. The other two regimes were 22 hours of light and 2 hours of dark (22L:02D); and 2 hours of light and 22 hours of dark (02L:22D). These regimes were selected to observe if acclimation to a long- or short-day photoperiod altered tolerance of an elevated temperature. Animals were transferred into vials as described for the temperature tolerance experiments. All experiments were conducted at a test temperature of 32.5°C. Acclimation temperatures were 5, 10, 15, 20, and 25°C. These tests were performed in a stainless-steel water bath holding approximately 38ℓ of water. Racks (minus the polyethylene covering) holding the vials were supported in the bath by water filled jars such that the top of the vials were far enough out of the water to prevent mixing.

Heat was provided by a Techne-Tempunit Tu-12 circulating thermoregulator which controlled the set temperature (32.5°C) to ± .5°C. Continuous lighting was employed during these tests and the light intensity was approximately 75 foot-candles. The sample size for these experiments was 30 animals, 15 immediately and 15 gradually exposed to the test temperature.

Checks were made at 1/2, 1, 2, 4, and 8 hours, and every 8 hours until 95 percent mortality was observed. This occurred by 16 hours for all experiments except for tests on 25°C acclimated animals, where 95 percent mortality occurred at 104 hours. Mortality was examined as described above, as was sexing, measuring, freeze-drying and weighing. The data were analyzed by probit analysis and LT50 values were calculated. An analysis of variance was performed to examine the effects of temperature and photoperiod regime upon survival. In addition, standard errors of the LT50 values were calculated and significance tests of LT50's were performed as described by Stanley (1963).

Respiration

Respiration studies were conducted for animals acclimated at 5, 10, 15, 20, and 25°C. Oxygen consumption was determined at test temperatures of 5, 10, 15, 20, 25, and 30°C. The methodology employed was similar to Goss (1978). Respiration was measured using a Gilson Model GRP20 Differential Respirometer.

Animals were acclimated for two to three weeks following the procedures previously described. Two days prior to each experiment, streptomycin sulfate was added to creek water that had been filtered through Whatman #1 filter paper using a Buchner funnel to obtain a 25 mg/l concentration, and this solution was placed at the acclimation temperature.

Streptomycin sulfate was used to prevent bacterial respiration during tests (Goss, 1978). One day prior to testing, animals were removed from the acclimation containers and placed in a liter container with the treated creek water. Polyethylene was placed around the liter container to reduce light intensity. Seven-milliliter respirometer test flasks were used; they held 4 ml of water into which one isopod was placed. The center well of each test flask was filled with cotton saturated with a 10 percent potassium hydoxide solution to absorb carbon dioxide. Reference flasks were filled with 10 mg/l mercuric chloride solution to a point such that the gas volume was equal to that in the test flasks. High vacuum silicone stopcock grease was used on all glassware fittings, and after each test glassware was boiled for at least 2 hours in distilled water with Sparkleen detergent. The glassware was then placed in an acid wash solution for at least 12 hours, thoroughly rinsed in distilled water, and allowed to dry before using again.

All respiration experiments were run for 8 hours, usually between 11:00 a.m. and 7:00 p.m. Readings were taken every hour during the experiments. Organisms were allowed to acclimate to respirometer flasks on the respirometer for 2 hours before readings were made. The respirometer system was open for the first hour of acclimation, and sealed during the second hour of acclimation. After the final reading,

the system was open and the test flasks removed. The animal from each test flask was placed into a freeze-dryer flask one-third filled with distilled water, and frozen. When a sufficient number of flasks were filled, they were freeze-dried and weighed as described above.

Results were analyzed graphically. Oxygen consumption, expressed as microliters O_2 per milligram body weight per hour, was plotted against test temperature; acclimation temperatures combined. Duncan's Multiple Range Test was used to examine differences in acclimation temperatures for each test temperature. In addition, oxygen consumed per hour was plotted against time spent in the respirometer to examine changes in the metabolism of the animals. Again, acclimation temperatures were combined for each test temperature.

CHAPTER IV

RESULTS

Temperature Tolerance

Initial analysis was performed on the animals used in the determination of thermal tolerance to examine if those animals that died during the two week experimental period differed in length or weight than those that survived. Table A-1 in the Appendix records the significant ($\alpha < .05$) t-tests for all acclimation-test temperature combinations. With one exception, the animals that died during these tests were longer and heavier than those that survived. Animals were collected for tests over the period of one year. Analyses of variance were employed to examine if animals collected on the same date, but acclimated at different temperatures, were similar in length and weight. Means from the AOV's were then ranked using Duncan's Multiple Range Test for unequal sample size, and the results of these tests are shown in Tables A-2 through A-5 in the Appendix. No consistant pattern emerged from these results. However, animals acclimated and tested at 25°C were smaller and lighter than animals acclimated and tested at other temperatures. This implies that larger, heavier animals died during acclimation at 25°C. Comparisons of length and weight among collection days could not be tested because of differences

within each collection date. Except for extreme values, the means appeared to be similar for all collection dates. Any differences could be attributed to changes in the dynamics of the isopod population over the course of the sampling period.

Sex ratios of animals used in this study over collection dates ranged from 2:1 to 25:1 male to female. In general there were no significant differences in mortality due to sex. This result includes both mortality of individuals during the experimental period versus survivors in one given test temperature, as well as overall mortality both within and among collection dates. In a few instances, significantly more females than males died. This was caused by the fact that one female death resulted in a much higher percent mortality than the death of one male for any given test.

Animals were exposed to the test temperature in two ways, immediately and gradually. There were no significant differences in mode of exposure to the test temperature over the test range of 5 to 30°C. At the test temperature of 32.5°C, there were also no significant differences except for animals acclimated at 5°C, where animals immediately exposed to the test temperature succumbed more rapidly than those that were gradually exposed. This same result was also observed at the test temperature of 35°C for all acclimation temperatures.

Table III lists the LT50 values for the acclimation-test temperature combinations where mortality was greater than or equal to 50 percent after correction for control death. Again, LT50 stands for the time in hours required for half of the animals to die. The plot of LT50's versus environmental temperature is shown in Figure 1. Figure 2 depicts the tolerance polygon for Lirceus brachyurus. The enclosed region indicates the acclimation-environmental temperature combinations within which the species will survive. The diagonal line represents the set of points where the acclimation and environmental temperatures are the same. Note that this line cuts the polygon at 0°C and approximately 23°C. This indicates that the incipient lower and upper lethal limits of survival for these isopods are, respectively, 0 and 23°C.

Fluctuating Temperature

Animals exposed to a cyclic temperature fluctuation for two weeks exhibited no mortality greater than 50 percent, after correction for controls. These observations occurred regardless of acclimation temperature or length of the period of fluctuation between two temperatures. Changes in temperature (ΔT) were 10°C above or below the acclimation temperature. Periods of exposure to higher or lower temperatures were 8 and 16 hours. Due to the lack of any LT50 values, no statistical tests were performed. However,

TABLE III

LT50, STANDARD ERRORS, AND 95 PERCENT CONFIDENCE LIMITS FOR *LIRCEUS BRACHYURUS*—THERMAL TOLERANCE EXPERIMENTS

Test Temp. (°C)	Acclimation Temp. (°C)	LT50 (hours)	Lower limit(hrs)	Upper limit(hrs)	Standard Error
0	25	109.71	66.78	180.21	.0998
25	25	240.38	221.86	260.45	.0161
30	5	42.83	33.98	54.01	.0452
30	10	101.41	89.24	115.26	.0255
30	15	85.49	72.35	101.01	.0345
30	20	103.19	90.38	117.80	.0268
30	25	110.06	92.22	131.40	.0349
32.5	5	1.84	1.35	2.51	.0486
32.5	10	2.96	1.96	4.47	.0416
32.5	15	5.59	3.94	7.94	.0354
32.5	20	9.46	6.88	13.02	.0322
32.5	25	27.64	23.10	33.07	.0330
35	5	1.46	.89	2.39	.0778
35	10	1.37	.99	1.88	.0500
35	15	1.16	.70	1.28	.0686
35	20	1.85	1.27	2.69	.0515
35	25	6.34	4.77	8.43	.0401

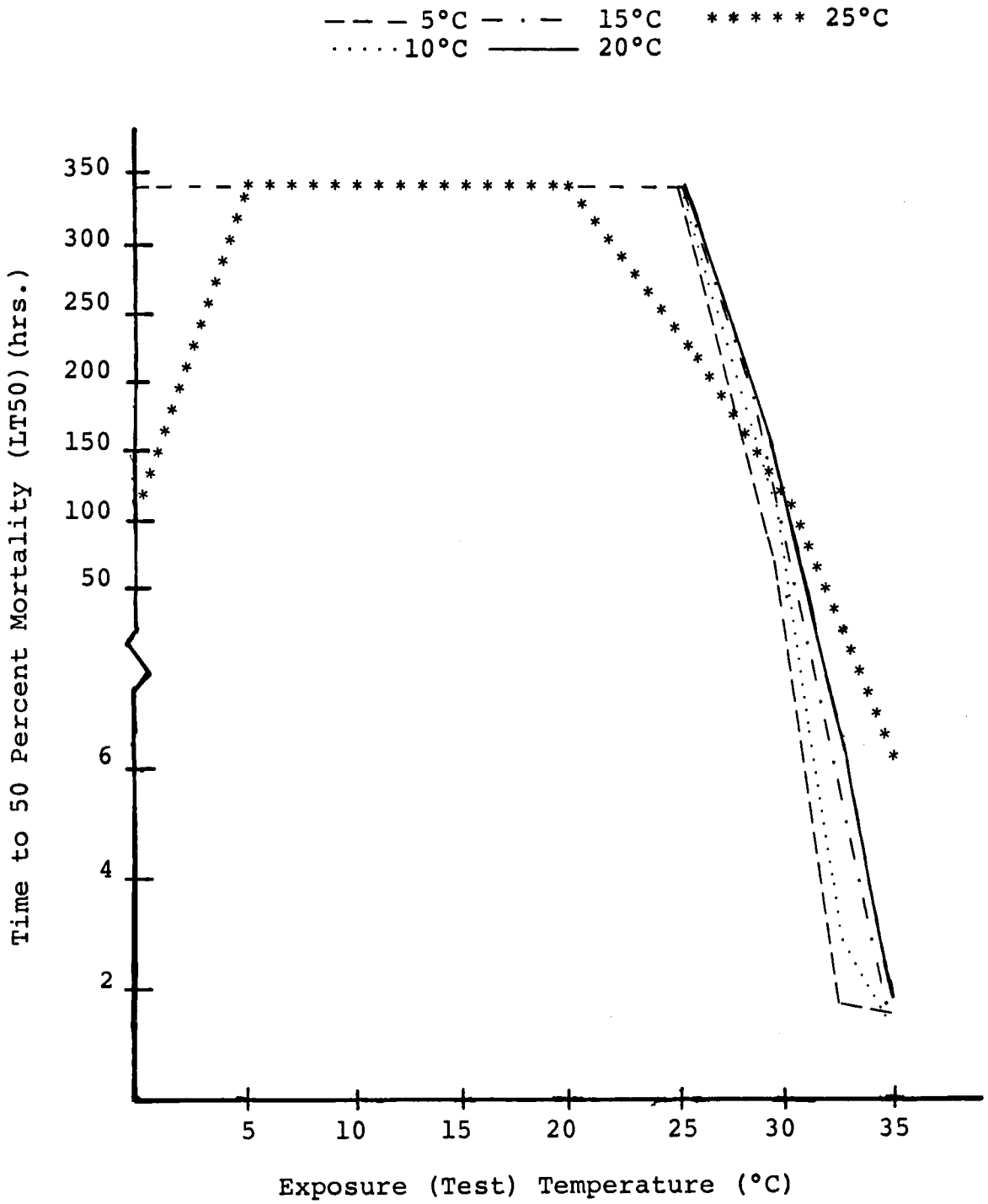


Figure 1

The Effect of Heat Resistance With Acclimation

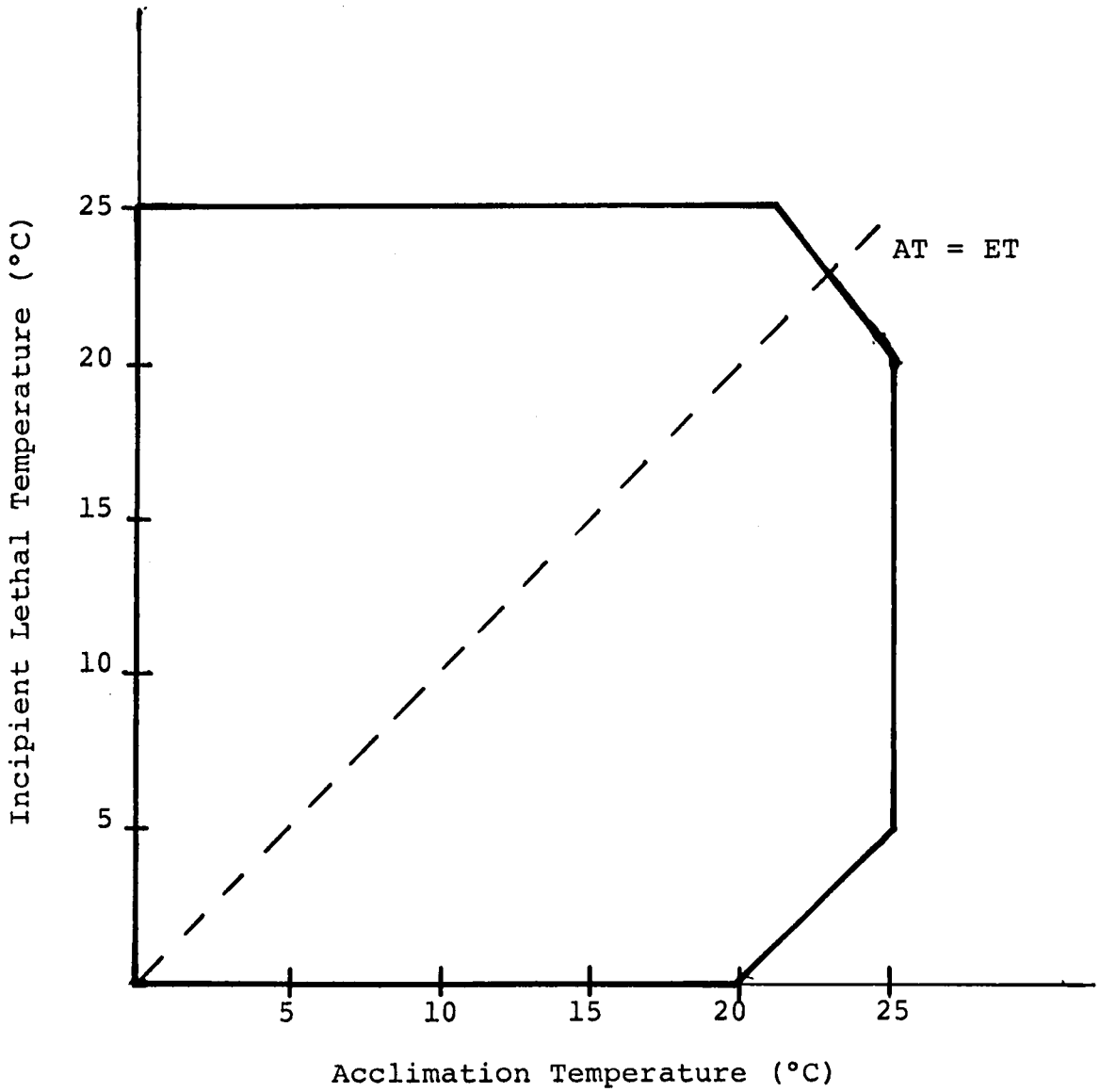


Figure 2

Tolerance Polygon for Lirceus brachyurus

casual inspection of the data revealed that animals acclimated at 25°C were smaller than animals acclimated at the other temperatures. There was no difference in mean length between animals that died during the experiments and those that survived the two week experimental period. There were no differences in mortality due to sex.

Photoperiod

A summary of significant mean lengths and weights of animals used in the photoperiod experiments is given in Tables A-6 and A-7 in the Appendix. Duncan's Multiple Range Test with unequal sample size was used to analyze the data. At three acclimation temperatures (5, 15, and 20°C), animals exposed to the normal photoperiod (14L:10D) had significantly higher mean lengths and weights than animals tested at the other two photoperiod regimes. This difference is probably due to the earlier date of collection of these animals. The low mean of the 22L:02D animals acclimated at 20°C is probably due to an increased number of females in that sample. There were no statistically significant differences in mortality between males and females in any of the experiments.

The LT50 values obtained are found in Table IV. An analysis of variance was performed on these values to examine the effects of photoperiod and temperature upon survival. This analysis is shown in Table V.

TABLE IV

LT50, STANDARD ERRORS, AND 95 PERCENT CONFIDENCE LIMITS FOR *LIRCEUS BRACHYURUS*—PHOTOPERIOD EXPERIMENTS

Acclimation Temp. (°C)	Photoperiods (Light:Dark)	LT50 (hours)	Lower limit(hrs)	Upper limit(hrs)	Standard Error
5	14L:10D	1.84	1.35	2.51	.0486
5	22L:02D	2.16	1.70	2.74	.0327
5	02L:22D	1.53	1.20	1.94	.0328
10	14L:10D	2.96	1.96	4.47	.0416
10	22L:02D	3.68	2.84	4.77	.0405
10	02L:22D	3.64	2.70	4.90	.0405
15	14L:10D	5.59	3.94	7.94	.0354
15	22L:02D	6.40	5.01	8.18	.0247
15	02L:22D	5.89	4.44	7.81	.0285
20	14L:10D	9.46	6.88	13.02	.0322
20	22L:02D	11.90	9.46	14.98	.0360
20	02L:22D	11.56	8.99	15.09	.0261
25	14L:10D	27.64	23.10	33.07	.0330
25	22L:02D	25.68	21.59	30.55	.0319
25	02L:22D	20.84	16.94	25.63	.0390

TABLE V

RESULTS OF ANALYSIS OF VARIANCE APPLIED TO THE THREE PHOTOPERIOD REGIMES FOR *LIRCEUS BRACHYURUS* ACCLIMATED TO 5, 10, 15, 20, and 25°C

Source	Sum of Squares	Degrees Freedom	Mean Square	F-Ratio
Acclimation Temperature	1025.3824	4	256.3456	82.9748***
Photoperiod Regime	4.1413	2	2.0707	<1^{NS}
Temperature X Photoperiod	24.7155	8	3.0894	
Total	1054.2392	14		

***Significant at $\alpha < .001$.

^{NS}Not significant.

There were no significant differences in LT50 values for the three photoperiod regimes employed, but there were significant differences in survival among acclimation temperatures; as the acclimation temperature increased, survival times also increased (Table IV). Tests were also performed at each acclimation temperature for differences among photoperiod regimes taken two at a time, following the method of Stanley (1963). Only at the extreme temperatures of 5 and 25°C, were two LT50 values significantly different. Although the LT50's per acclimation temperature were generally not significant, there appears to be an order in survival at the different photoperiod regimes. Animals acclimated to the control or 14L:10D regime had the shortest survival time, followed by the 02L:22D and the 22L:02D photoperiod regimes.

Respiration

Table A-8 in the Appendix lists the mean oxygen consumption for each acclimation-test temperature combination. Acclimation temperatures were tested by Duncan's Multiple Range Test with unequal sample size to see if they could be combined for each test temperature. This is shown in Table A-9 in the Appendix. Except at 10°C, oxygen consumption was significantly different over the range of acclimation temperatures employed for each test temperature. Figure 3 shows the plot of test temperature versus oxygen consumption, with acclimation temperatures combined. The vertical lines

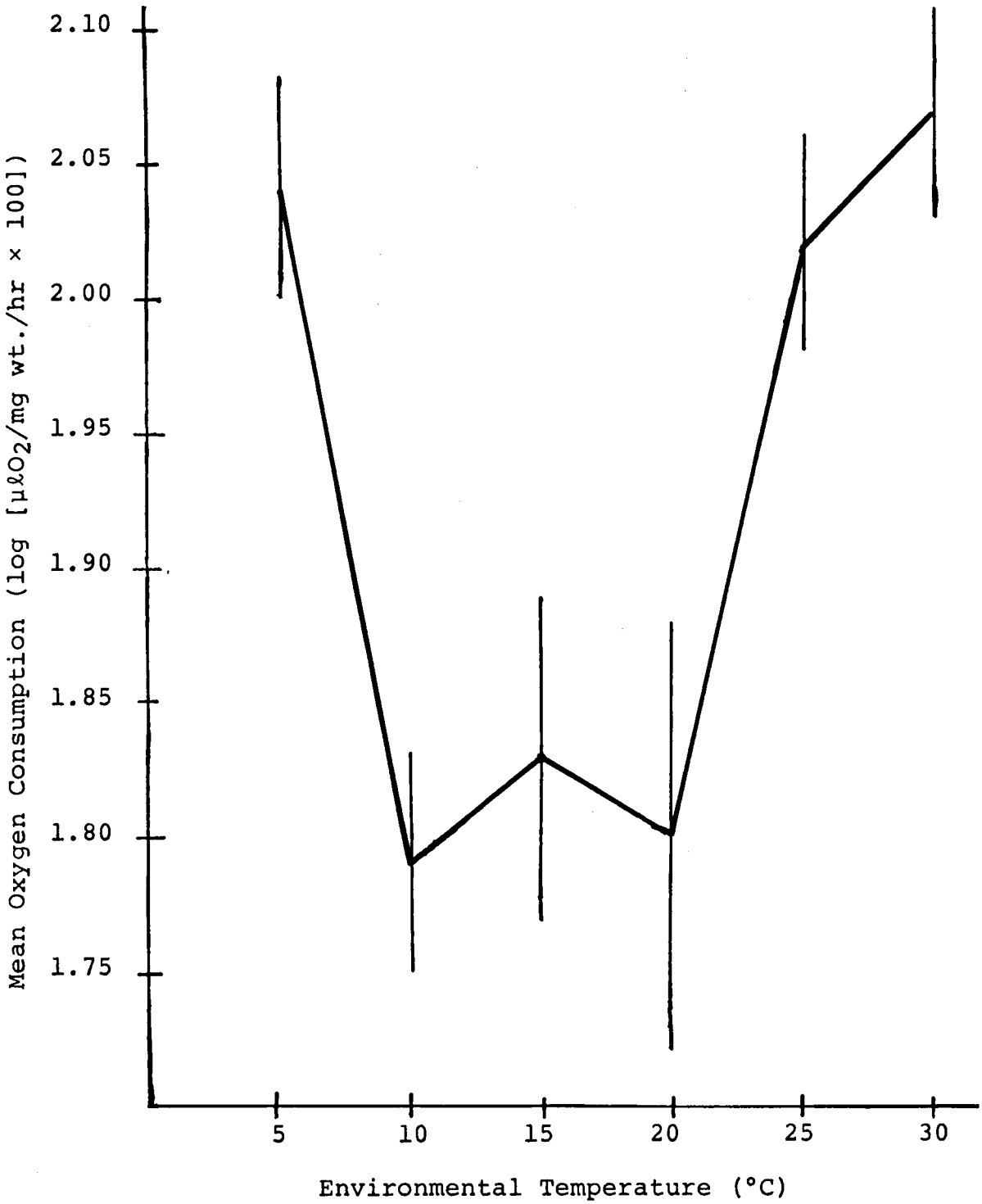


Figure 3

The Effect of Environmental Temperature Upon Oxygen Consumption in Lirceus brachyurus

about each point in the graph represent the standard error associated with each respiration value. The graph is parabolic in shape with oxygen consumption similar (within one standard error) at 10, 15, and 20°C, and at 5, 25, and 30°C.

Tables A-10 through A-15 in the Appendix list mean oxygen consumed per hour over 8 hours for each acclimation-test temperature combination. Acclimation temperatures were pooled for each test temperature and the resulting plot of oxygen consumption with time is shown in Figure 4. This graph indicates that oxygen consumed with time was similar for 10, 15, and 20°C, and for 5, 25, and 30°C.

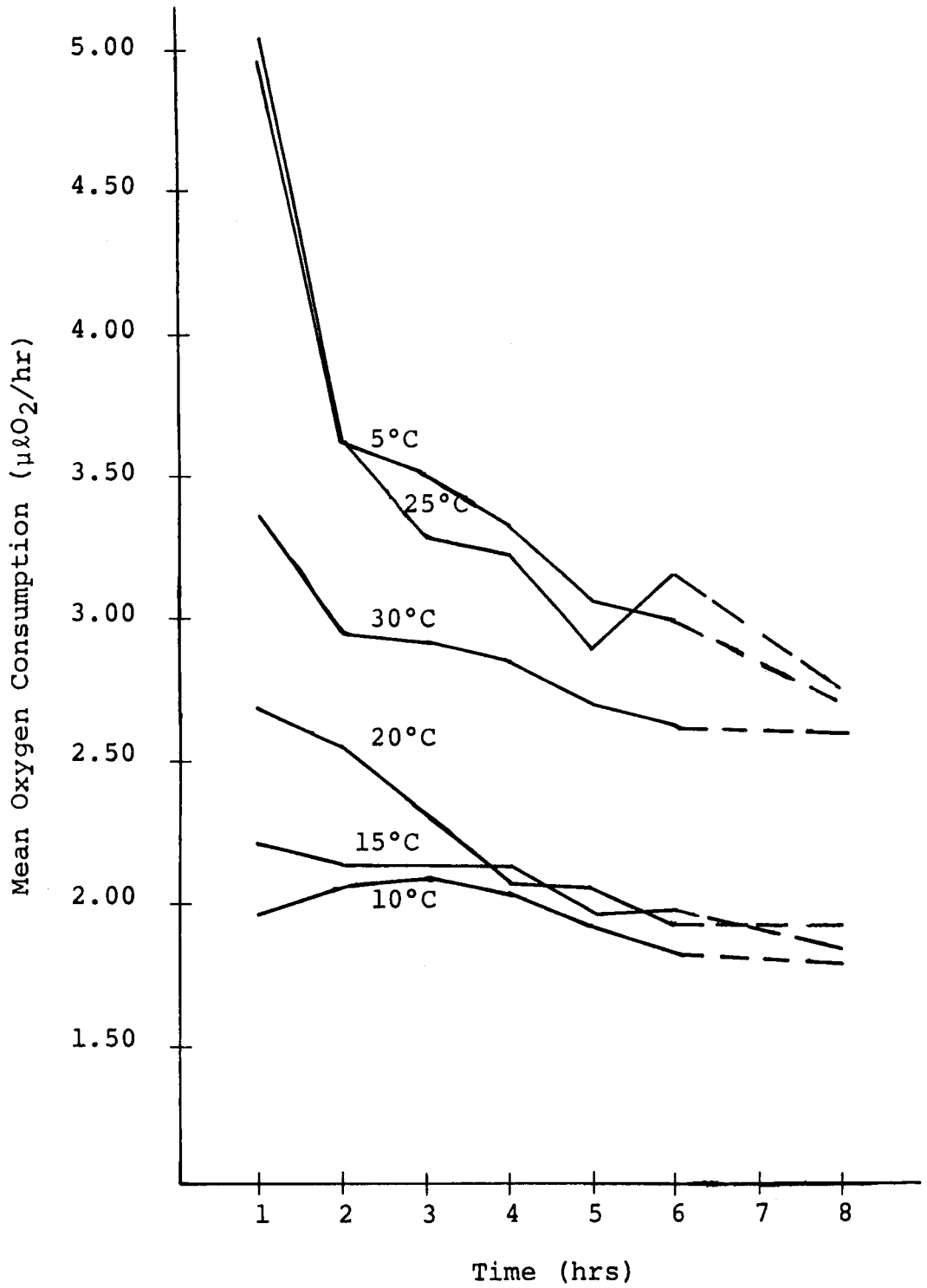


Figure 4

The Effect of Time Spent in The Respirometer Upon Oxygen Consumption in Lirceus brachyurus

CHAPTER V

DISCUSSION

Acclimation and Testing at 25°C

Animals acclimated to 25°C had a much higher natural mortality rate (60 percent) during the period of acclimation than animals acclimated at 5, 10, 15, and 20°C (10 percent). Since mortality was positively related to size, this resulted in the average lengths and weights of animals acclimated at 25°C and tested at the other temperatures being smaller and lighter than animals acclimated at the other temperatures (Table A-1 in the Appendix). In most cases, the larger, heavier animals died during the two week test period. Thus, natural mortality at 25°C affects these animals first, leaving the smaller sized animals as survivors. Sprague (1963) reported that larger sized Asellus intermedius tended to have shorter resistance times to high temperature. Larger size is assumed to be related with older age. However, temperature and food availability can affect an animal's size as well. The variability of these factors prevents the exact relationship between size and age from being adequately determined.

There are three possible causes of this high natural mortality observed at 25°C. One is high light intensity in the incubators used for acclimation and tests. However,

light intensity had been reduced for other studies being conducted in the laboratory (Goss, 1978), and polyethylene was used to cover the culture containers with animals acclimating at 25°C. Consequently, light intensity levels were much lower in this incubator than in the incubators set at the lower acclimation temperatures. Natural mortality was lower in incubators where these measures were not taken, which suggests that light intensity may not have caused this high natural mortality at 25°C. Another possible cause of this mortality is the decaying vegetation, which caused changes in water chemistry parameters (see Table II, p. 24). However, water and vegetation were changed frequently, which should have reduced these changes in water chemistry. Furthermore, an experiment was conducted where animals were acclimated at 25°C in eight dram shell vials (one animal per vial) for two weeks, with Cerophyl but no vegetation added. Excess food was removed daily and the water was changed regularly. Mortality rates were similar to animals acclimated in the culture dishes with vegetation. Finally, the temperature itself (25°C) could be a sub-lethal stress temperature for Lirceus brachyurus. The water temperature of the study creek, even in summer, was never above 18°C. Furthermore, animals acclimated to 5, 10, 15, and 20°C and tested at 25°C, showed mortality rates higher than 10 percent (observed natural mortality) but less than lethal mortality

(50 percent). Animals acclimated at 25°C and tested at lower temperatures showed little or no mortality (10 percent). Thus, 25°C is probably a sub-lethal stress temperature for L. brachyurus, although the other factors mentioned above could contribute to the high mortality rate observed during acclimation.

This high natural mortality observed at 25°C may have affected thermal tolerance tests. Observed mortalities during tests could be due to acclimation at 25°C and not exposure to the other temperatures. As previously mentioned, this problem was not important at test temperatures below 25°C since no LT50 values were recorded. Nor was it a problem at the experimental temperature of 35°C since the animals died fairly quickly (LT50 = 6.34 hours). However, tests conducted at 25°C (the control) and 30°C showed LT50's where the two types of mortality described above could not be separated. Experiments at these temperatures were repeated a number of times, with similar results, hence, mortality for these particular tests were probably due to thermal effects and not the observed natural mortality.

In summary, high natural mortality rates were observed at 25°C. These rates did not appear to effect the results of the tolerance tests, and suggest that 25°C is a sub-lethal stress temperature for Lirceus brachyurus.

Temperature Tolerance

The mean length and weight of animals used in this study were generally similar (see Tables A-2 through A-5 in the Appendix) both within and among collection dates. However, animal survivors acclimated at 25°C tended to be smaller and lighter than animals acclimated at the other temperatures. This observation is related to natural mortality and the sub-lethal effect of 25°C as mentioned above. Minor differences in size of the animals were noted among the collection dates. These differences can be attributed to changes in the population dynamics (life history) of the isopod population. Such changes were noted by Markus (1930) for Mancasellus macrourus (= Lirceus fontinalis), Ellis (1961) for Asellus intermedius, and Steel (1961) for A. aquaticus. For example, animals collected in February, 1978, were smaller than those collected in September, 1977, because animals collected in September had matured since the previous spring and were predominantly males. Very few of the animals collected were reproductive females. The animals collected in February were newly matured adults that had overwintered as juveniles. A fair proportion of this population consisted of gravid females. Edney (1964) found that the size of terrestrial isopods had no effect upon tolerance of lethal temperatures.

There were, in general, no differences in thermal tolerance due to sex. This finding is in agreement with

Sprague (1963). Males tend to be larger than females. This resulted in the skewedness of the sex ratio toward males since common sized animals of about 8 millimeters were desired, and this size class consists mostly of males.

Two methods of exposure to the test temperature were used in this study, gradual and immediate. The results indicate that only at the highest test temperature employed (35°C) did the two methods of exposure differ, with immediately exposed animals succumbing more rapidly. Individual LT50's for significant immediate versus gradual transfers to the test temperature were not performed because of the lack of sufficient data points to perform regression analyses. The data suggested that once the gradually transferred animals reached the test temperature, they died as quickly as the immediately exposed animals. This effect was also obtained for animals acclimated at 5°C and tested at 32.5°C. At the other acclimation-test temperature combinations, there were no significant differences in mode of exposure to the test temperature. These results agree with the work of W. Smith (1970) on Mysis recta but contradict the findings of Bullock (1955) and other authors who indicate that gradually warmed animals have an upper lethal limit 1 to 2°C higher than immediately exposed animals. However, experiments conducted in this study were not designed to detect 1 to 2°C differences in tolerance. Salih et al. (1977)

also found differences in temperature resistance of A. aquaticus acclimated at 20°C and tested at 35°C. However, these authors found that animals gradually raised to 35°C at a rate of 1°C every four minutes were less resistant to 35°C. In conclusion, gradual warming to test temperatures does not increase the thermal resistance of Lirceus brachyurus. Differences in tolerance observed at high temperatures are due to the difference between the test and acclimation temperature (ΔT) and not to additional acclimation during heating.

The effect of increased and decreased heat resistance with acclimation is shown in Figure 1, p. 39. LT50 values are listed in Table III, p. 38. At a test temperature of 35°C, LT50 values are similar for all acclimation temperatures except 25°C, but at 30°C, the LT50 values are similar except for 5°C. At the test temperature of 32.5°C, as the acclimation temperature increased, resistance to heat stress increased. This increase was two to three times higher per five degree increase in acclimation temperature. Only at the acclimation temperature of 25°C, was there reduced tolerance to a test temperature of 0°C. Other authors have demonstrated that acclimation to higher temperatures increases resistance to upper lethal temperatures, while acclimation to lower temperatures increases resistance to lower lethal temperatures (Sprague, 1963, Edney, 1964).

The thermal trapezium for Lirceus brachyurus is shown in Figure 2, p. 40. This figure indicates the upper and lower incipient lethal limits of the isopod, or, in other words, the range of temperatures within which the animal will survive indefinitely. Prosser and Brown (1961) and Prosser (1973) indicate that this type of diagram is fairly characteristic for a species. The diagonal line represents the set of points where the acclimation temperature equals the test temperature. The intersection of this line with the trapezium occurs at approximately 23°C, and indicates the highest temperature to which L. brachyurus can increase its thermal tolerance by acclimation. These upper lethal values are not as high as similar values observed in other isopods. Sprague (1963) recorded that temperatures tolerated by Asellus intermedius ranged from 31 to 33°C, with the highest temperature tolerated equal to 34.6°C. Birstein (1964), and Williams (1962b) found that upper lethal temperatures in A. aquaticus ranged from 28 to 32°C. Edney (1964) found that upper lethal temperatures in Porcellio laevis and Armadillidium vulgare ranged from 37 to 42°C. The low lethal limit for L. brachyurus is 0°C. This is in agreement with other studies done on other isopod species. Birstein (1964) found that A. aquaticus ceased feeding around 4°C. Edney (1964) showed that the range of low temperatures tolerated in P. laevis and A. vulgare were from -3.0 to +6.0°C.

Becker et al. (1977) also noted that crayfish (Pacifastacus lenuisculus) tolerated 0°C. During the course of this study, it was noted that animals would survive at 0°C or below as long as there was a pocket of unfrozen water, but did not survive complete ice mineralization of water. The thermal tolerance of L. brachyurus does not appear to agree with the findings of Styron (1967, 1968) on L. fontinalis. Styron showed that a creek population of L. fontinalis tolerated 40°C for 10 days. Styron's results are difficult to interpret because his study was part of a factorial experiment involving resistance to temperature, drought, and radiation in different populations of L. fontinalis. His methodology was much different than the one employed in this study. Furthermore, the creek from which Styron collected L. fontinalis had water temperatures 2 to 5°C higher than the creek used in this study. This suggests that temperature tolerances of different isopod species will tend to be similar but the specific upper and lower lethal limits will be modified by local environmental conditions.

The zone of tolerance described for this population of L. brachyurus corresponds well with natural temperatures recorded in the creek where these animals were collected. The minimum winter temperature was 2°C while the maximum summer temperature was approximately 18°C. Thus, the temperature tolerance of L. brachyurus correlates with the

natural temperature range to which the animals are exposed. Also, the upper lethal limit of tolerance exceeds the highest natural temperature experienced by the animal. This difference represents a "safety zone" between normal activity and the onset of thermal death. Within this zone, animals can adjust for temperature changes by acclimatization or migration. This result has been observed in numerous other aquatic animals from both marine and freshwater habitats.

Speakman and Krenkel (1972) describe conditions where lethal temperatures may be encountered in power plant operations. The results of this study suggest that L. brachyurus would be more severely affected by hot water discharges (above 25°C) while cold water discharges during initiation of power plant output would not affect isopod populations.

Summarizing, the thermal tolerance of L. brachyurus is similar to thermal tolerances of other isopod species, except that the upper lethal temperature tolerated is lower than was anticipated. Thermal tolerance was related to naturally occurring environmental temperatures, thus, local conditions must be considered when describing temperature tolerance.

An interesting sidelight of this study is the tolerance of Lirceus to polluted waters. Hynes (1960) characterizes isopods as members of the polluted water fauna, along with

chironomids. Ellis (1961) found Asellus intermedius most abundant in areas near a sewer outfall. However, Williams (1972) indicates that North American isopods are not mentioned in the fauna of polluted waters. Styron (1967, 1968) indicated that a creek population of L. fontinalis barely survived an application of herbicide to a nearby tennis court. The indications from this study are that L. brachyurus is not tolerant of polluted streams. Its lower upper lethal limit and high natural mortality at high acclimation temperatures suggest that it too would not survive in polluted waters.

Fluctuating Temperature

The results of the fluctuating temperature experiments indicate that L. brachyurus can tolerate changes in temperature (ΔT) of up to 10°C for 16 hours within the range of temperatures examined (5 to 30°C). This correlates with the findings of the temperature tolerance experiments since LT50 values (if obtained) were longer than the time animals spent at the elevated/reduced temperature in these experiments. Diurnal temperature cycles have not been studied in aquatic invertebrates. Most studies have concentrated on the effects of fluctuating temperatures during acclimation (Edney, 1964) or in establishing exposure temperatures which will kill 50 percent of the animals in a set period of time, i.e. 96 hours (W. Smith, 1973). Research by Ginn et al. (1974, 1976) approximates the type of experiments performed in this study

on the aspect of diurnal cycles. These authors measured survival and reproduction of Gammarus spp. at elevated temperatures for short periods of time to evaluate the effects of power plant effluents. Their results are somewhat similar to those in this study in that a ΔT of 8.3°C above 25.5°C and 16.7°C above 11.7°C were not fatal to the amphipods but a ΔT of 11.1°C above 26.5°C produced significant mortality after exposures of 30 and 60 minutes to the elevated temperature.

The results of this study imply that isopods will survive temperatures that might otherwise kill them if they are exposed to a diurnal temperature cycle. This can be of great ecological significance to an animal near power plants where ejected condenser water may vary widely in temperature (Speakman and Krenkel, 1972). The aforementioned authors also indicate that the growth of the power industry will result in more intensified waste heat discharges, which could prove deleterious to the survival of aquatic life. Although no LT50 values were measured in this study, fluctuations in temperature between 20 and 30°C killed about 42 percent of the animals. Consequently, any increase in ΔT above 10°C near upper lethal limits could prove fatal to L. brachyurus populations.

Photoperiod

The results of the photoperiod experiments indicated that tolerance of an elevated temperature was similar for animals exposed to different photoperiod regimes during the acclimatory process. The photoperiod regimes were selected to examine if long-, intermediate-, or short-day light cycles increased temperature tolerance. It was also observed that LT50's, irrespective of photoperiod, increased as the temperature increased. This result was expected, and agrees with the results of the thermal tolerance experiments previously described.

Photoperiod has been shown to affect numerous facets of the biology of aquatic animals. For example, Allee (1926), Sther (1931), and Warburg (1964, 1968) show that aquatic and terrestrial isopods tend to avoid light, especially at high temperatures. Cloudsley-Thompson (1952) and Enright (1976) indicate that locomotory rhythms in terrestrial and intertidal isopods are correlated with alternating light and darkness. They suggest that these light-dark cycles are related to the normal activity patterns of these animals, such as feeding. Molt cycles have been shown to be influenced by photoperiod. Most of the work on the interrelationship between photoperiod and molting has been conducted on macruran crustaceans (Travis, 1954, Stephens, 1955, and Armitage et al., 1973). The latter authors indicate that molting rates are higher

under long-day photoperiod regimes. Lirceus brachyurus possesses an unusual molt cycle. The posterior half of the cuticle is shed approximately 24 hours before the anterior half is shed (Hawkes and Schraer, 1973). In the present study, no unusual observations were noticed with respect to molting except that intermolt periods decreased with increasing temperature. Finally, photoperiod has been shown to affect reproduction and incubation periods in isopods and amphipods (Steel, 1961, Kruschwitz, 1971, and DeMarch, 1977). These authors point out that photoperiod is the main cue inducing and terminating reproduction. However, continued or discontinued reproduction is influenced by temperature. In the present study, there was no noticable increase or decrease of reproduction under any photoperiod regime at any acclimation temperature.

Although temperature tolerance was not significantly different at any of the three photoperiod regimes investigated in this study, a pattern was observed for most acclimation temperatures. Survival times were longest at the long-day photoperiod regime (22L:02D). Sther (1931) indicated that Asellus aquaticus and A. communis increase their activity when subjected to light. Thus, animals acclimated to 22 hours of light for two weeks would be predisposed to be more active than animals acclimated to intermediate or short periods of light. This increased activity could be channeled into

metabolic reactions which would counteract the effects of the elevated temperature. In other words, it is possible that L. brachyurus, acclimated to a long-day photoperiod, attempts to reduce heat stress by increased respiration or other metabolic methods. Thus, the data suggest (but do not prove) that increased activity due to long-day photoperiod regime may reduce the effects of heat stress.

Respiration

Preliminary analyses were conducted on the respiration data to examine the variation in oxygen consumption among replicates per test, among animals acclimated at various temperatures and tested at the same experimental temperature, and the relationship between respiration and body weight.

There was much variation among replicates for any one acclimation-test temperature combination. Consequently, mean oxygen consumption was determined for each combination of temperatures and the values are shown in Table A-8 in the Appendix. Both Newell et al. (1976) for the isopod Ligia and Laird et al. (1976) for the crab Callinectes observed variation in oxygen consumption among individuals at any one set of experimental conditions. Also, some variation in respiration values was observed among animals acclimated at varying temperatures and tested at one specific test temperature. Most of the values obtained were not significant (see Table A-9 in the Appendix), yet there did exist some

significant variation between extreme values within one test temperature. This was also observed by Newell et al. (1974, 1976). Finally, there was not a clear cut relationship between oxygen consumption and body weight. Individual regressions of the form

$$\log Y = a + b \log X$$

where Y is oxygen consumption per hour, and X is body weight, were run on each acclimation-test temperature combination. The slopes ranged from -.74 to .86, indicating no constant relationship between these two variables. Newell et al. (1976) also observed a wide range of slopes (-.48 to 2.01) in Ligia oceanica respiration, using the same regression equation.

Two sources of variation were observed in the present study which were not a source of concern in other isopod studies. The first one involves differences in methodology. Newell et al. (1974, 1976) exposed the same set of isopods, acclimated at one temperature, to all test temperatures in one experimental run. I was unable to duplicate this method, consequently, a different set of animals was used for each acclimation-test temperature combination. Thus, an extra source of variation is introduced into these respiration studies due to variation among animals within one test temperature for all acclimation temperatures examined. Also,

the weight of animals was determined in different ways. The studies of Newell et al., and others, measured weight after drying at a constant temperature for a set period of time. In this study, weight was measured after lyophilization. Thus, the method of obtaining weights may cause variation in observed respiration values between this and other studies.

The second, and probably more important, source of variation involves the movement of animals within the experimental chambers during a test. Although sufficient time (two hours) was allowed before readings were taken, it was impossible to control movement of animals within the test flasks. Frequent observation during experiments indicated that animals tended to remain quiescent, but movements were also observed. Thus, the effect of locomotion upon respiration can not be adequately ascertained. Consequently, oxygen consumption values varied in part due to the inability to control movement. Other studies have used terrestrial or intertidal animals (Newell et al., 1974, 1976) which remained quiescent during tests, or aquatic animals which must constantly move in the water column, as fairy shrimp (Knight et al., 1974), or Daphnia (Goss, 1978). Future studies with aquatic animals similar to Lirceus should employ techniques to eliminate this source of variation in oxygen consumption. In brief, the sources of variation described above may have affected the results of the

respiration experiments and must be taken into account in their interpretation.

The plot of oxygen consumption versus experimental temperature (rate-temperature curve) is shown in Figure 3, p. 45. Acclimation temperatures were combined for each test temperature since McFarland et al., (1965), and Newell et al. (1974, 1976) demonstrated that thermal acclimation was relatively unimportant in the determination of oxygen consumption. Also, respiration values were not significantly different among one another except at extremes (Table A-9 in the Appendix). The high respiration observed at 5°C could be due to the fact that this temperature is close to the lower limit of metabolic activity (4°C, Birstein, 1964). Consequently, animals may have been trying to adjust to this temperature by increasing respiration. In other words, 5°C represents a "stress" level with respect to activity and the isopods cope with this stress by increasing metabolic rates. Few studies have looked at oxygen consumption at this temperature. Knight et al. (1974) examined respiration of the anostracan Chirocephalopsis bundyi at 5°C and found oxygen consumption values much lower than the ones in the present study. However, this can be attributed to the differences in morphology, life history, and type of habitat in these two groups of animals.

Oxygen consumption tended to increase with temperature (10 to 30°C) except for 20°C. The slight decrease indicated

at 20°C may be due however to the extremely low respiration value of animals acclimated at 25°C (1.45µlO_2/mg body weight/hour). If this value is omitted, the mean oxygen consumption for the test temperature of 20°C equals 2.01µlO_2/mg body weight/hour. Thus, there is a tendency for oxygen consumption to increase with experimental temperature. This has been demonstrated by numerous other authors (McFarland et al., 1965, Newell et al. 1974, 1976, and Laird et al., 1976).

However, the data can be analyzed in another manner. Looking at the standard errors plotted on Figure 3, p. 45, it is observed that respiration values are similar at extreme test temperatures (5, 25, and 30°C) and at intermediate test temperatures (10, 15, and 20°C). If a new mean oxygen consumption value is calculated for 20°C, its standard error would overlap with 5 and 25°C. This indicates that Lirceus brachyurus may regulate its oxygen consumption at intermediate temperatures, and respond in a similar manner to increased/decreased temperatures. Thus, it is possible that this isopod may maintain its metabolism at certain temperatures without changes in energy expenditures. Newell et al. (1976) also observed areas of reduced temperature sensitivity in the middle part of the rate-temperature curves for Ligia oceanica. Furthermore, similar results have been found by Knight et al. (1974) in fairy shrimp, and by Laird et al. (1976) in blue crabs. Vernberg and Vernberg (1970) also indicate that

metabolic compensation appears to be the predominant mechanism by which aquatic invertebrates adjust for changes in temperature.

Figure 4, p. 47, shows the effect of time spent by the animals in the respirometer. The data used to draw this figure are found in Tables A-10 through A-15 in the Appendix. In this figure, time in hours is plotted against oxygen consumption per hour. Again, acclimation temperatures were combined for each test temperature. Acclimation temperatures did not seem to affect changes in respiration per hour, since all acclimation-test temperature combinations showed similar decreases in oxygen consumed as time spent in the respirometer increased. The effect of time upon oxygen consumption was investigated because of the study of Newell et al. (1974) on Porcellio scaber. These authors indicate that as time spent in the respirometer increased, the oxygen consumption of the animals changed from an "excited" to a "standard" rate.

The results obtained in this experiment generally agree with Newell et al. (1974) except that differences between exposure temperatures are not as well defined as in their study. Again, differences in methodology and uncontrollable movements in the test flasks could account for some of this observed variation. In all cases, the variation in oxygen consumption was much higher at the end of the first hour than

at the end of the eighth hour. Also, variation in respiration values tended to decrease with time. In addition, animals tested at 5 and 25°C showed much higher respiration values at hour one as opposed to hour eight. This is probably due to the fact that an animal's respiration increased between the various acclimation temperatures and these test temperatures. This implies that the greater the difference between test and acclimation temperature (ΔT), the greater the variation in oxygen consumption due to transference to the respirometer. It is interesting to note however, that mean respiration values were similar for 10 and 15°C, and to an extent, 20°C, during the entire time spent in the respirometer. Again, this suggests that there may be some regulation of oxygen consumption at intermediate environmental temperatures. Also, for 5, 25, and 30°C, readings were again similar suggesting that changes in metabolism to both high and low temperatures are equivalent. Animals respond to either an increase or decrease in temperature by raising oxygen consumption rates to approximately the same level.

Summarizing, oxygen consumption appears to be similar for high and low temperatures, and for intermediate temperatures. Variation in oxygen consumption decreases with time toward a standard (constant) rate, regardless of temperature. However, mean respiration rates do not decrease as much at intermediate temperatures as at higher

or lower temperatures. This implies that metabolic compensation is occurring within this temperature range.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to examine the effects of temperature upon biological processes in a freshwater isopod.

The temperature tolerance of Lirceus brachyurus was determined, and incipient lethal limits ranged from 0 to 23°C. The thermal tolerance of L. brachyurus is similar to that of other aquatic invertebrates. The range of temperatures tolerated generally correspond to naturally occurring environmental temperatures in the animal's habitat. The specific temperatures tolerated were lower than those recorded for other isopod species. Thus, the response to temperature may be modified by local environmental conditions. Related species, and other aquatic forms, also show a relationship between temperatures tolerated and the range of naturally occurring temperatures in their habitats. This suggests that thermal tolerance may be determined by the environment as well as by the genetic and physiological capabilities of the animals themselves.

The results of this study imply that L. brachyurus would be severely affected by heated effluents or stationary plumes of power plants at temperatures of greater than or equal to

25°C. These isopods would not be affected by the release of cold water after a period of inoperation by the plant.

Lirceus brachyurus does not appear to be tolerant of polluted waters. Other creek populations of isopods of the genus Lirceus are also intolerant of eutrophic waters, as opposed to Asellus which generally tolerates polluted waters.

Dirunal cycles in exposure temperatures of ± 10°C were not deleterious to animals within the temperature range of 5 to 30°C. The exposure of animals to cycles of temperature fluctuation after acclimation has not been examined in aquatic invertebrates. This type of experimentation should be further investigated since these fluctuations resulted in increased survival at temperatures that might otherwise kill the organisms. Increased tolerance of higher temperatures due to cyclic fluctuations in temperature implies that isopods would not be seriously affected by power plant discharges 10 degrees higher or lower than ambient, but would be affected by ΔT's greater than 10°C, especially near lethal limits.

Photoperiod does not appear to increase the tolerance of isopods to an elevated temperature. However, animals acclimated to a long-day photoperiod may be predisposed to reduce heat stress metabolically due to increased activity.

The respiration of L. brachyurus is quite variable within any acclimation-test temperature combination. This is partly due to uncontrollable movements by animals in the

test flasks during experiments. Also, the use of different animals for each test adds to the observed variation in respiration values. Respiration tends to increase with experimental temperature regardless of acclimation (except at 5°C). Oxygen consumption per hour decreases with time spent in the respirometer. However, mean oxygen consumption values remained similar at intermediate, and high and low experimental temperatures. This suggests that L. brachyurus may regulate its metabolism at intermediate temperatures and responds to elevated or reduced temperatures in a similar fashion. This observation has also been recorded in other aquatic invertebrates.

It is hoped that this study can be used as a basis for field investigations to provide a better understanding of the influence of temperature upon biological parameters of aquatic invertebrates, as well as increase our knowledge of the effects of an environmental factor upon aquatic ecosystems.

LITERATURE CITED

LITERATURE CITED

Al-Habbib, O. A. M., and J. M. R. Grainger. 1977. The effect of constant and changing temperatures on the thermal resistance of Lymnaea peregra (Müller). J. Therm. Biol. 2: 191-195.

Allee, W. C. 1926. Studies in animal aggregations: causes and effects of bunching in land isopods. J. Exp. Zool. 45: 255-277.

Armitage, K. A., A. Buikema, and N. Willems. 1973. The effect of photoperiod on organic constituents and molting of the crayfish Orconectes nais (Faxon). Comp. Biochem. and Physiol. 44A: 431-456.

Beatty, J. A. 1974. Key to the terrestrial and fresh water isopods of Illinois. (unpublished). 19 pp.

Beck, S. D. 1963. Animal Photoperiodism. Holt, Rinehart, and Winston, Inc., New York. 124 pp.

Becker, C. D., R. G. Genoway, and M. J. Schneider. 1977. Comparative cold resistance of three Columbia River organisms. Trans. Amer. Fish Soc. 106: 178-184.

Berg, K., and K. Ockelmann. 1959. The respiration of freshwater snails. J. Exp. Biol. 36: 690-708.

Birstein, Y. A. 1964. Fauna of USSR. Crustacea. Freshwater Isopods (Asellota). Vol. VII. No. 5. Translated from Rusian by Israel Program for Scientific Translations. 148 pp.

Bregassi, P. K. 1972. The effects of low temperature upon the locomotor activity rhythm of Talitrus saltator Crustacea:Amphipoda. J. Exp. Biol. 57: 393-399.

Bullock, T. 1955. Compensation for temperature in the metabolism and activity of poikilotherms. Bio. Reviews. 30: 311-342.

Bunting, D. L. 1974. Zooplankton: thermal regulation and stress. In B. J. Gallagher (ed.), Proc. Sym. Energy Production and Thermal Effects. Limnetics Inc. Milwaukee, September 1973. pp. 50-55.

Bursell, F. 1974. Environmental aspects- Temperature. In M. Rockstein (ed.), The Physiology of Insecta. Vol. II. pp. 1-41.

Calow, P. 1975. The respiratory strategies of two species of freshwater gastropods (Ancylus fluviatilis Müll and Planorbis contortus Limm) in relation to temperature, oxygen concentration, body size, and season. Phys. Zool. 48: 114-129.

Cloudsley-Thompson, J. L. 1952. Studies in diurnal rhythms. II. Changes in the physiological responses of the wood-louse Oniscus asellus to environmental stimuli. J. Exp. Biol. 29: 295-303.

Comita, G. 1965. Oxygen consumption in Diaptomus siciloides. Limnol. and Oceanogr. 10: 466-468.

Culver, D., and T. Poulson. 1971. Oxygen consumption and activity in closely related amphipod populations from cave and surface habitats. Amer. Mid. Nat. 85: 74-84.

DeMarch, B. G. E. 1977. The effect of photoperiod and temperature on the induction and termination of reproductive resting stage in the freshwater amphipod Hyalella azteca (Saussure). Can. J. Zool. 55: 1595-1600.

Edney, E. B. 1964. Acclimation to temperature in terrestrial isopods. I. Lethal temperatures. Phys. Zool. 37: 364-377.

Edwards, R. W., and M. Learner. 1960. Some factors affecting the oxygen consumption of Asellus. J. Exp. Biol. 37: 706-718.

Ellis, R. J. 1961. A life history study of Asellus intermedius Forbes. Trans. Amer. Micros. Soc. 80: 80-102.

Ellis, R. J. 1971. Notes on the biology of the isopod Asellus tomalensis in an intermittent pond. Trans. Amer. Micros. Soc. 90: 51-61.

Ellis, R. J., and H. Gowing. 1957. Relationship between food supply and condition of wild brown trout, Salmo trutta Limnaeus, in a Michigan stream. Limnol. and Oceanogr. 2: 299-308.

Eltringham, S. K. 1967. The effect of temperature on the development of Limnoria eggs (Isopoda:Crustacea). J. Appl. Ecol. 4: 521-529.

Enright, J. T. 1976. Plasticity in an isopod's clockworks: Shaking shapes form and affects phase and frequency. J. Comp. Physiol. 107: 13-37.

Finney, D. J. 1962. Probit Analysis. Second edition. Cambridge University Press. xiv + 318 pp.

Fleming, L. 1972. The evolution of the eastern North American Isopods of the genus Asellus (Crustacea: Asellidae). Part I. Int. J. Speleol. 4: 221-256.

Fleming, L. 1973. The evolution of the eastern North American Isopods of the genus Asellus (Crustacea: Asellidae). Part II. Int. J. Speleol. 5: 283-310.

Frier, J. O. 1976. Oxygen consumption and osmoregulation in the isopods Sphaeroma hookeri Leach and S. rigicauda Leach. Ophelia 15: 193-203.

Ginn, T. C., W. T. Waller, and G. Lauer. 1974. The effects of power plant condenser cooling water entrainment on the amphipod Gammarus spp. Water Res. 8: 937-945.

Ginn. T. C., W. T. Waller, and G. Lauer. 1976. Survival and reproduction of Gammarus spp. (Amphipoda) following short-term exposure to elevated temperatures. Cheaspeake Sci. 17: 8-14.

Goss, L. B. 1978. The effect of temperature on Daphnia pulex Leydig and Daphnia magna Straus. Doctoral Dissertation, University of Tennessee, Knoxville. 123 pp.

Goss, L. B., and D. L. Bunting. 1976. Thermal tolerance of zooplankton. Water Res. 10: 387-398.

Hatchett, S. P. 1947. Biology of the Isopoda of Michigan. Ecol. Monog. 17: 47-79.

Hawkes, J. W., and H. Schraer. 1973. Mineralization during the molt cycle in Lirceus brachyurus (Isopoda:Crustacea). Calcif. Tissue Res. 12: 125-136.

Hubricht L., and J. G. Mackin. 1949. The freshwater Isopoda of the genus Lirceus (Asellota:Asellidae). Amer. Mid. Nat. 24: 334-349.

Hynes, H. B. N. 1960. The Biology of Polluted Waters. Liverpool U.P. xiv + 202 pp.

Hynes, H. B. N., and W. D. Williams. 1965. Experiments on competition between two Asellus species (Isopoda: Crustacea). Hydrobiologia 26: 203-210.

Jones, M. B. 1974. Survival and oxygen consumption in various salinities of three species of Idotea (Crustacea: Isopoda) from different habitats. Comp. Biochem. Physiol. 48A: 501-506.

Keister, M., and J. Buck. 1974. Respiration: Some exogenous and endogenous effects on rate of respiration. In M. Rockstein (ed.), The Physiology of Insecta. Vol. VI. pp. 469-509.

Knight, A., R. Lippson, and M. Simmons. 1974. The effect of temperature on the oxygen consumption of two species of fairy shrimp. Amer. Mid. Nat. 94: 236-240.

Kring, R. L., and J. O'Brien. 1976. Effect of varying oxygen concentrations on the filtering rate of Daphnia pulex. Ecology 57: 808-814.

Krog, J. 1954. The influence of seasonal environmental changes upon the metabolism, lethal temperature, and rate of heart beat of Gammarus limnaeus (Smith) taken from an Alaskan lake. Biol. Bull. 107: 397-410.

Kruschwitz, L. G. 1971. Environmental control of reproduction in the amphipod Hyalella azteca. Amer. Zool. 11: 693.

Laird, C. E., and P. A. Haefner Jr. 1976. Effects of intrinsic and environmental factors on oxygen consumption of the blue crab, Callinectes sapidus Rathburn. J. Exp. Mar. Biol. Ecol. 22: 171-178.

Lawton, J. H., and J. Richards. 1970. Comparability of Cartesian Diver, Gilson, Warburg, and Winkler methods of measuring the respiratory rates of aquatic invertebrates in ecological studies. Oecologia. 4: 319-324.

Lees, A. D. 1968. Photoperiodism in insects. In A. C. Giese (ed.), Photophysiology. Vol. IV. pp. 47-137.

Markus, H. 1930. Studies on the morphology and life history of the isopod, Mancasellus macrourus. Trans. Amer. Micros. Soc. 49: 220-237.

Martien, R. F. and A. C. Benke. 1977. Distribution and production of two crustaceans in a wetland pond. Amer. Mid. Nat. 98: 162-175.

McFarland, W. N., and P. E. Pickens. 1965. The effects of season, temperature, and salinity on standard and active oxygen consumption of the grass shrimp, Palaemonetes vulgaris (Say). Can. J. Zool. 43: 571-585.

McMahon, R. 1975. Effects of artificially elevated water temperatures on the growth, reproduction, and life cycle of a natural population of Physa virgata Gould. Ecology 56: 1167-1175.

McMahon, R. 1976. Effluent-induced interpopulation variation in the thermal tolerance of Physa virgata Gould. Comp. Biochem. Physiol. 55A: 23-28.

Mill, P. J. 1974. Respiration: Aquatic insects. In M. Rockstein (ed.), The Physiology of Insecta. Vol. VI. pp. 403-467.

Moon, H. P. 1957. The distribution of Asellus in Windermere. J. Anim. Ecol. 26: 113-123.

Naylor, E. 1965. Effect of heated effluents upon marine and estuarine organisms. Adv. Mar. Biol. 3: 63-103.

Newell, R. C. 1973. Environmental factors affecting the acclimatory responses of ectotherms. In W. Wieser (ed.), Effects of Temperature on Ectothermic Organisms. pp. 151-164.

Newell, R. C., W. Wieser, and V. I. Pye. 1974. Factors affecting the oxygen consumption in the woodlouse Porcellio scaber Latr. Oecologia 16: 31-51.

Newell, R. C., A. Roy, and K. B. Armitage. 1976. An analysis of factors affecting the oxygen consumption of the isopod Ligia oceania. Phys. Zool. 49: 109-137.

Økland, K. 1978. Life history and growth of Asellus aquaticus (L.) in relation to environment in an eutrophic lake in Norway. Hydrobiologia 59: 243-259.

Pardi, L., and F. Papi. 1961. Kinetic and tactic responses. In T. Waterman (ed.), The Physiology of the Crustacea. Vol. II. pp. 365-399.

Pennak, R. W. 1978. Fresh-Water Invertebrates of the United States. Second edition. John Wiley and Sons, Inc., New York. xv + 803 pp.

Precht, H., J. Christopherson, H. Hensel, and W. Larcher. 1973. Temperature and Life. Springer-Verlog., Berlin, Heidelberg, New York. xix + 779 pp.

Prosser, C. L. (ed.). 1973. Comparative Animal Physiology. Third edition. Vol. I. W. B. Saunders Co., Philadelphia. xxii + 456 pp.

Prosser, C. L., and F. Brown. 1961. Comparative Animal Physiology. W. B. Saunders Co., Philadelphia. 688 pp.

Rockstein, M., and J. Miquel. 1973. Aging in insects. In M. Rockstein (ed.), The Physiology of Insecta. Vol. I. pp. 371-478.

Salih, T. M. and J. R. N. Grainger. 1978. The effect of the rate of change of temperature on the resistance of Asellus aquaticus (L.) to high lethal temperatures. J. Therm. Biol. 3: 121-123.

Sherberger, F., E. Benfield, K. Dickson, and J. Cairns Jr. 1977. Effects of thermal shocks on drifting aquatic insects: a laboratory simulation. J. Fish. Res. Board. Can. 34:529-536.

Smith, A. U. 1970. Current Trends in Cryobiology. Plenum Press., New York, London. xi + 252 pp.

Smith, W. 1970. Tolerance of Mysis recta to thermal shock and light. Trans. Amer. Fish. Soc. 99: 418-422.

Smith, W. 1972. Culture, reproduction, and temperature tolerance of Pontoporeia affinis in the laboratory. Trans. Amer. Fish. Soc. 101: 253-256.

Smith, W. 1973. Thermal tolerance of two species of Gammarus. Trans. Amer. Fish. Soc. 102: 431-433.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco. xxi + 776 pp.

Speakman, J. N., and P. A. Krenkel. 1972. Quantification of the effects of rate of temperature change on aquatic biota. Water Res. 6: 1283-1290.

Sprague, J. B. 1963. Resistance of four freshwater crustaceans to lethal high temperature and low oxygen. J. Fish. Res. Board Can. 20: 378-415.

Stanley, J. 1963. The Essence of Biometry. McGill University Press., Montreal. xiii + 147 pp.

Steel, A. E. 1961. Some observations on the life history of Asellus aquaticus (L.) and Asellus meridianus Racovitza (Crustacea:Isopoda). Proc. Zool. Soc. London. 137: 71-87.

Steele, R. D. G., and J. H. Torrie. 1960. Principles and Procedures in Statistics. McGraw-Hill, New York. 481 pp.

Stephens, G. C. 1955. Induction of molting in the crayfish, Cambarus by modification of daily photoperiod. Biol. Bull. 108: 235-241.

Sther, W. 1931. The activating influence of light upon certain aquatic arthropods. J. Exp. Zool. 59: 297-335.

Styron, C. 1968. Ecology of two populations of an aquatic isopod, Lirceus fontinalis, Raf. Ecology 49: 629-636.

Styron, C. 1969. Taxonomy of the two populations of an aquatic isopod, Lirceus fontinalis, Raf. Amer. Mid. Nat. 82: 402-416.

Styron, C., and W. D. Burbanck. 1967. Ecology of an aquatic isopod, Lirceus fontinalis, Raf., emphasizing radiation effects. Amer. Mid. Nat. 78: 384-415.

Theede, H. 1973. Resistance adaptations of marine invertebrates and fish to cold. In W. Wieser (ed.), Effects of Temperature on Ectothermic Organisms. pp. 249-269.

Travis, D. 1954. The molting cycle of the spiny lobster, Panulirius argus Latreille. I. Molting and growth in laboratory maintained individuals. Biol. Bull. 107: 433-450.

Vernberg, F. J., and W. B. Vernberg. 1970. Aquatic invertebrates. In G. C. Whittow (ed.), Comparative Physiology of Thermoregulation. Vol. I. Invertebrates and Nonmammalian Vertebrates. pp. 1-44.

Vlasblom, A. G., S. J. Graafsma, and J. T. A. Verhoeven. 1977. Survival, osmoregulatory ability, and respiration of Idotea chelipes (Crustacea:Isopoda) from Lake Veere in different salinities and temperatures. Hydrobiologia 52: 33-38.

Warburg, M. R. 1964. The response of isopods toward temperature, humidity, and light. Anim. Behav. 12: 175-186.

Warburg, M. R. 1968. Behavioral adaptations in terrestrial isopods. Amer. Zool. 8: 545-560.

Ward, H. B., and G. C. Whipple. 1959. Fresh-Water Biology. Vol. II. (W. T. Edmondson, ed.), John Wiley and Sons, Inc., New York. xx + 1248 pp.

Williams, W. D. 1962a. The geographical distribution of the isopods, Asellus aquaticus (L.) and A. meridianus Rac. Proc. Zool. Soc. London 139: 75-96.

Williams, W. D. 1962b. Notes on the ecological similarities of Asellus aquaticus (L.) and A. meridianus Rac. (Crustacea:Isopoda). Hydrobiologia 20: 1-30.

Williams, W. D. 1963. The ecological relationships of isopod crustaceans Asellus aquaticus (L.) and A. meridianus Rac. Proc. Zool. Soc. London. 140: 661-679.

Williams, W. D. 1970. A revision of North American epigean species of Asellus (Crustacea:Isopoda). Smithsonian Contributions to Zoology. 49: 1-80.

Williams, W. D. 1972. Freshwater isopods (Asellidae) of North America. Biota of Freshwater Ecosystems. Identification Manual No. 7. Environmental Protection Agency. ix + 45 pp.

Wolff, W. J. 1973. The distribution of Asellus aquaticus (L.) and Proasellus meridianus (Rac.) in the southwestern part of the Netherlands. Hydrobiologia. 42: 381-392.

Wolvekamp, H. P., and T. H. Waterman. 1960. Respiration. In T. H. Waterman (ed.), The Physiology of the Crustacea. Vol. I. pp. 35-100.

Wright, E., and A. A. Wright. 1976. The respiratory quotient of Gammarus pulex L. Comp. Biochem. Physiol. 53A: 45-46.

APPENDIX

TABLE A-1

RESULTS OF SIGNIFICANT T-TESTS APPLIED TO LENGTHS AND WEIGHTS OF DEAD VERSUS ALIVE ANIMALS USED IN THERMAL TOLERANCE EXPERIMENTS

Collection Date	Acclimation Temp. (°C)	Test Temp. (°C)	t-Value
		Length	
July, 1977	5	10	53.44[a]
July, 1977	25	25	54.70[b]
July, 1977	25	20	3.50
July, 1977	25	30	2.66
		Weight	
July, 1977	25	10	11.30
July, 1977	25	25	3.53
July, 1977	25	25	2.42

[a] t-value (.05) = 2.069.

[b] t-value (.05) = 2.074.

TABLE A-2

RESULTS OF MODIFIED DUNCAN'S MULTIPLE RANGE TEST APPLIED TO MEAN LENGTH AND WEIGHT OF ANIMALS COLLECTED JULY, 1977

Acclimation Temp. (°C)	Test Temp. (°C)	Mean Length	Range of Means	Acclimation Temp. (°C)	Test Temp. (°C)	Mean Weight	Range of Means
25	25	1.77[a]		25	25	1.000[b]	
5	25	1.80		5	25	1.012	
5	30	1.83		5	20	1.105	
5	35	1.85		5	30	1.150	
5	20	1.85		25	20	1.206	
25	15	1.85		5	15	1.221	
25	20	1.85		5	35	1.236	
25	35	1.85		25	15	1.258	
25	10	1.87		25	35	1.263	
5	15	1.88		5	10	1.279	
25	5	1.88		15	15	1.341	
25	30	1.88		5	5	1.342	
5	10	1.89		25	30	1.352	
15	25	1.90		15	20	1.356	
15	20	1.90		15	25	1.375	
5	5	1.91		25	10	1.377	
15	30	1.92		25	5	1.391	
15	15	1.92		15	35	1.416	
15	5	1.93		15	30	1.424	
15	10	1.93		15	10	1.429	
15	35	1.94		15	5	1.443	

[a]Values are log [length(mm) × 10].

[b]Values are log [weight(mg) × 10].

TABLE A-3

RESULTS OF MODIFIED DUNCAN'S MULTIPLE RANGE TEST APPLIED TO MEAN LENGTH AND WEIGHT OF ANIMALS COLLECTED SEPTEMBER, 1977

Acclimation Temp. (°C)	Test Temp. (°C)	Test Length	Range of Means	Acclimation Temp. (°C)	Test Temp. (°C)	Mean Weight	Range of Means
25	25	1.85[a]		25	25	1.157[b]	
20	10	1.91		20	25	1.354	
20	25	1.92		20	10	1.354	
20	20	1.93		20	15	1.361	
20	15	1.93		20	20	1.362	
20	5	1.93		20	35	1.391	
20	35	1.94		20	5	1.398	
5	32.5	1.94		5	5	1.453	
5	5	1.94		15	15	1.475	
15	32.5	1.96		5	32.5	1.497	
15	15	1.97		15	32.5	1.518	

[a]Values are log [length(mm) × 10].

[b]Values are log [weight(mg) × 10].

TABLE A-4

RESULTS OF MODIFIED DUNCAN'S MULTIPLE RANGE TEST APPLIED TO MEAN LENGTH AND WEIGHT OF ANIMALS COLLECTED NOVEMBER, 1977

Acclimation Temp. (°C)	Test Temp. (°C)	Test Length	Range of Means	Acclimation Temp. (°C)	Test Temp. (°C)	Mean Weight	Range of Means
25	25	1.82[a]		25	25	1.086[b]	
10	0	1.83		20	30	1.215	
20	30	1.87		10	0	1.269	
25	0	1.89		5	0	1.326	
5	0	1.89		25	0	1.360	
15	0	1.89		10	10	1.364	
20	25	1.90		20	25	1.370	
20	0	1.91		15	0	1.407	
10	10	1.92		10	30	1.409	
10	30	1.93		20	0	1.441	

[a]Values are log [length(mm) × 10].

[b]Values are log [weight(mg) × 10].

TABLE A-5

RESULTS OF MODIFIED DUNCAN'S MULTIPLE RANGE TEST APPLIED TO MEAN LENGTH AND WEIGHT OF ANIMALS COLLECTED FEBRUARY AND JULY, 1978

Acclimation Temp. (°C)	Test Temp. (°C)	Mean Length	Range of Means	Acclimation Temp. (°C)	Test Temp. (°C)	Mean Weight	Range of Means
			February				
10	32.5	1.86[a]		20	20	1.286[b]	
20	32.5	1.89		10	32.5	1.290	
20	20	1.90		20	32.5	1.326	
			July				
25	32.5	1.81		25	32.5	1.115	
25	30	1.84		25	25	1.231	
25	25	1.86		25	30	1.233	
20	25	1.88		20	25	1.314	
10	35	1.90		10	35	1.405	

[a]Values are log [length(mm) × 10].

[b]Values are log [weight(mg) × 10].

TABLE A-6

RESULTS OF MODIFIED DUNCAN'S MULTIPLE RANGE TEST APPLIED TO SIGNIFICANT MEAN LENGTHS OF ANIMALS USED IN PHOTOPERIOD EXPERIMENTS

Acclimation Temp. (°C)	Light-Dark Regime (L:D)	Mean Length (mm)	Range of Means
5	14L:10D	8.65	|
	22L:02D	6.90	|
	02L:22D	6.75	|
15	14L:10D	9.19	|
	22L:02D	7.88	|
	02L:22D	7.83	|
20	14L:10D	7.83	|
	02L:22D	7.07	|
	22L:02D	6.43	|

TABLE A-7

RESULTS OF MODIFIED DUNCAN'S MULTIPLE RANGE TEST APPLIED TO SIGNIFICANT MEAN WEIGHTS OF ANIMALS USED IN PHOTOPERIOD EXPERIMENTS

Acclimation Temp. (°C)	Light-Dark Regime (L:D)	Mean Weight (mg)	Range of Means
5	14L:10D	3.202	|
	02L:22D	1.965	|
	22L:02D	1.827	|
15	14L:10D	3.429	|
	02L:22D	2.406	|
	22L:02D	2.403	|
20	14L:10D	2.328	|
	02L:22D	1.882	|
	22L:02D	1.580	|

TABLE A-8

MEAN AND STANDARD ERROR OF OXYGEN CONSUMPTION FOR EACH ACCLIMATION-TEST TEMPERATURE COMBINATION

Temp. (°C)	Acclimation Temp. (°C)	Sample Size (n)	Mean Oxygen Consumed $\log[(\mu l O_2/mg/hour) \times 100]$	Standard Error
5	5	5	2.00	.069
	10	8	2.12	.055
	15	5	2.14	.156
	20	8	2.24	.077
	25	8	1.84	.092
10	5	4	1.95	.110
	10	8	1.91	.056
	15	8	1.80	.104
	20	10	1.81	.062
	25	11	1.62	.105
15	5	8	2.08	.089
	10	4	1.95	.115
	15	5	1.84	.132
	20	11	1.63	.124
	25	5	1.74	.146
20	5	9	1.94	.097
	10	7	2.02	.079
	15	4	2.06	.189
	20	5	2.09	.100
	25	9	1.45	.142
25	5	7	2.22	.051
	10	8	2.00	.069
	15	9	1.96	.010
	20	5	1.78	.086
	25	7	2.07	.073
30	5	5	2.33	.082
	10	5	2.09	.070
	15	5	2.11	.057
	20	5	1.97	.075
	25	8	1.94	.087

TABLE A-9

RESULTS OF MODIFIED DUNCAN'S MULTIPLE RANGE TEST APPLIED TO MEAN OXYGEN CONSUMPTION OF ANIMALS USED IN RESPIRATION EXPERIMENTS

Test Temp. (°C)	Acclimation Temp. (°C)	Mean Oxygen Consumption $\log[(\mu l O_2/mg/hour) \times 100]$	Range of Means
5	25	1.84	
	5	2.00	
	10	2.12	
	15	2.14	
	20	2.24	
15	20	1.60	
	25	1.74	
	15	1.84	
	10	1.95	
	5	2.08	
20	25	1.45	
	5	1.94	
	10	2.02	
	15	2.06	
	20	2.09	
25	20	1.78	
	15	1.96	
	10	2.00	
	25	2.07	
	5	2.22	
30	25	1.94	
	20	1.97	
	10	2.09	
	15	2.11	
	5	2.33	

TABLE A-10

MEAN AND STANDARD ERROR OF ANIMALS IN RESPIRATION EXPERIMENTS ACCLIMATED AT VARYING TEMPERATURES AND TESTED AT 5°C

Acclimation Temp. (°C)	Hours							Mean Weight (mg)	Sample Size (n)
	1	2	3	4	5	6	8		
5	2.89[a]	3.03	2.88	2.56	2.40	2.30	2.20	2.137	5
	1.094[b]	.666	.535	.483	.441	.454	.378	.2596	
10	5.85	4.19	4.18	3.97	3.81	3.70	3.35	2.498	8
	1.096	.483	.504	.404	.434	.419	.418	.1868	
15	5.80	4.92	4.57	4.15	3.75	3.59	3.29	3.695	5
	1.190	1.055	.751	.685	.639	.564	.479	.3032	
20	7.57	4.26	4.22	3.90	3.56	3.50	3.01	1.742	8
	1.441	.507	.408	.332	.310	.265	.261	.1924	
25	3.05	1.93	1.85	2.03	1.77	1.76	1.60	1.982	8
	.496	.388	.377	.340	.371	.325	.339	.1377	

[a]Mean $\mu l O_2$ consumed/hour.

[b]Standard error.

TABLE A-11

MEAN AND STANDARD ERROR OF ANIMALS IN RESPIRATION EXPERIMENTS ACCLIMATED AT VARYING TEMPERATURES AND TESTED AT 10°C

Acclimation Temp. (°C)	Hours							Mean Weight (mg)	Sample Size (n)
	1	2	3	4	5	6	8		
5	3.28[a]	3.25	2.83	2.57	2.52	2.39	2.28	2.490	4
	.896[b]	.679	.764	.641	.649	.593	.540	.4504	
10	2.52	2.54	2.56	2.53	2.32	2.30	2.13	2.886	8
	.401	.377	.336	.279	.230	.201	.173	.2448	
15	2.16	2.28	2.35	2.42	2.16	2.05	1.97	2.471	8
	.418	.442	.431	.367	.370	.358	.319	.2471	
20	1.94	1.97	1.80	1.98	1.90	1.75	1.70	2.419	10
	.447	.457	.360	.355	.313	.303	.279	.1857	
25	1.26	1.26	1.59	1.21	1.27	1.06	.99	1.786	11
	.352	.212	.272	.272	.222	.230	.215	.1008	

[a]Mean $\mu l O_2$ consumed/hour.

[b]Standard error.

TABLE A-12

MEAN AND STANDARD ERROR OF ANIMALS IN RESPIRATION EXPERIMENTS ACCLIMATED AT VARYING TEMPERATURES AND TESTED AT 15°C

Acclimation Temp.(°C)	Hours							Mean Weight(mg)	Sample Size(n)
	1	2	3	4	5	6	8		
5	2.07[a]	2.15	-----	2.14	1.91	1.84	1.75	1.331	8
	.566[b]	.427	-----	.402	.344	.335	.315	.1166	
10	3.08	2.59	-----	2.98	2.82	2.70	2.63	2.823	4
	.474	.454	-----	.672	.435	.575	.477	.2891	
15	3.44	3.53	-----	3.40	3.15	3.06	2.87	3.511	5
	.995	.847	-----	.792	.778	.738	.699	.1263	
20	1.50	1.45	1.41	1.31	1.18	1.18	1.14	2.072	11
	.262	.316	.290	.292	.246	.244	.231	.2075	
25	2.01	1.93	1.87	1.93	1.80	1.93	1.83	2.706	5
	1.057	.707	.660	.668	.596	.629	.615	.5376	

[a]Mean $\mu l O_2$ consumed/hour.

[b]Standard error.

TABLE A-13

MEAN AND STANDARD ERROR OF ANIMALS IN RESPIRATION EXPERIMENTS ACCLIMATED TO VARYING TEMPERATURES AND TESTED AT 20°C

Acclimation Temp. (°C)	Hours							Mean Weight (mg)	Sample Size (n)
	1	2	3	4	5	6	8		
5	3.45[a]	3.31	2.76	2.44	2.28	2.24	2.16	2.462	9
	.629[b]	.599	.469	.409	.342	.338	.298	.3169	
10	3.19	2.92	2.66	2.44	2.52	2.37	2.40	2.184	7
	.608	.382	.293	.329	.318	.322	.363	.1683	
15	2.93	2.53	2.39	2.16	2.11	1.98	2.04	1.393	4
	1.182	1.043	.875	.863	.746	.642	.758	.0943	
20	3.48	3.07	3.05	2.71	2.68	2.50	2.29	1.748	5
	1.022	.874	.887	.678	.644	.561	.486	.2126	
25	1.03	1.35	1.07	1.11	1.01	.92	.96	2.380	9
	.383	.335	.309	.298	.313	.300	.301	.1948	

[a]Mean $\mu l O_2$ consumed/hour.

[b]Standard error.

TABLE A-14

MEAN AND STANDARD ERROR OF ANIMALS IN RESPIRATION EXPERIMENTS ACCLIMATED TO VARYING TEMPERATURES AND TESTED AT 25°C

Acclimation Temp. (°C)	Hours							Mean Weight (mg)	Sample Size (n)
	1	2	3	4	5	6	8		
5	6.62[a]	4.02	3.63	3.79	-----	3.22	3.03	1.866	7
	1.043[b]	.251	.305	.384	-----	.208	.193	.2097	
10	4.83	3.44	3.25	3.50	-----	2.72	2.65	2.383	8
	1.032	.664	.731	.697	-----	.503	.472	.1481	
15	4.55	3.51	3.26	3.03	3.09	3.02	2.92	2.898	9
	.613	.512	.507	.489	.516	.674	.448	.2820	
20	2.03	1.56	1.35	1.33	1.31	-----	1.37	2.153	5
	.282	.274	.300	.231	.278	-----	.245	.2826	
25	6.13	4.99	4.34	3.90	3.71	3.62	3.20	2.635	7
	.668	.530	.517	.447	.515	.444	.421	.1246	

[a]Mean $\mu l O_2$ consumed/hour.

[b]Standard error.

TABLE A-15

MEAN AND STANDARD ERROR OF ANIMALS IN RESPIRATION EXPERIMENTS ACCLIMATED TO VARYING TEMPERATURES AND TESTED AT 30°C

Acclimation Temp. (°C)	Hours							Mean Weight (mg)	Sample Size (n)
	1	2	3	4	5	6	8		
5	5.47[a]	5.20	5.26	4.98	4.55	4.64	4.19	1.941	5
	.977[b]	.873	.761	.661	.639	.567	.497	.1779	
10	3.21	2.59	2.93	2.83	2.57	2.80	2.68	2.060	5
	.793	.776	.596	.653	.608	.538	.493	.1370	
15	3.24	2.17	2.21	2.52	2.43	2.39	2.54	2.011	5
	.258	.374	.219	.433	.384	.379	.526	.4844	
20	3.04	2.04	2.26	2.13	2.08	1.86	1.88	1.933	5
	.514	.436	.469	.459	.384	.317	.321	.2873	
25	2.43	2.82	2.28	2.18	2.18	1.82	2.02	2.199	8
	.468	.410	.374	.270	.291	.243	.255	.1733	

[a]Mean $\mu l O_2$ consumed/hour.

[b]Standard error.

VITA

Nicholas Joseph Cheper was born in Louisville, Kentucky on January 22, 1949. He attended elementary schools in that city, Stamford, Connecticut, and Larchmont, New York. He was graduated from Fordham Preparatory School in June, 1967. The following September, he entered the University of Notre Dame and received a Bachelor of Science degree in Biology in June, 1971. In the fall of 1971, he entered the State University of New York at Stony Brook, and in August, 1974, he received a Master of Arts in Ecology. He entered the University of Tennessee in the Fall of 1974, and received the Doctor of Philosophy degree with a major in Zoology in June, 1979.