INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.
2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.
3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again -- beginning below the first row and continuing on until complete.
4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.
5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

Xerox University Microfilms

300 North Zeeb Road
Ann Arbor, Michigan 48106

7817905

MILLER, KIRK
ACTIVITY METABOLISM IN THE MUDPUPPY, NECTURUS
MACULOSUS.

THE UNIVERSITY OF OKLAHOMA, PH.D., 1978

University
Microfilms
International

300 N. ZEEB ROAD, ANN ARBOR, MI 48106

THE UNIVERSITY OF OKLAHOMA
GRADUATE COLLEGE

ACTIVITY METABOLISM IN THE MUDPUPPY,
NECTURUS MACULOSUS

A DISSERTATION
SUBMITTED TO THE GRADUATE FACULTY
in partial fulfillment of the requirements for the
degree of
DOCTOR OF PHILOSOPHY

BY
KIRK MILLER
Norman, Oklahoma
1978

ACTIVITY METABOLISM IN THE MUDPUPPY,
NECTURUS MACULOSUS

APPROVED BY

Victor H. Heston

Howard Heston

Joseph Boett

Frank J. Heston

ACKNOWLEDGEMENTS

I would like to express sincere appreciation to my major advisor, Dr. Victor H. Hutchison, for his help and encouragement throughout the stages of this work, and to the other members of my dissertation committee, Dr. Howard B. Haines and Dr. Frank J. Sonleitner for their many helpful suggestions. Drs. Gary C. Packard and Martin E. Feder read the manuscript and provided many useful criticisms. Ms. Mildred Fung gave invaluable technical assistance. To all those who provided help and stimulation during the completion of this project I am deeply grateful.

This study was conducted while I held a graduate teaching assistantship in the Department of Zoology, University of Oklahoma and was supported in part by NSF Grant BMS 73-06725 to Dr. Victor Hutchison. This manuscript was prepared in the style for submission to Physiological Zoology.

TABLE OF CONTENTS

	Page
List of Tables and Appendices	v
List of Illustrations	vi
Summary	1
Introduction	3
Materials and Methods	6
Results	10
Discussion	13
Literature Cited	21
Table	27
Figure Legends	28
Figures	31
Appendices	38

LIST OF TABLES AND APPENDICES

Table		Page
I	Total energy budget for 30 min forced activity in the mudpuppy, <u>Necturus maculosus</u>	27
 Appendix		
I	Diurnal variation in spontaneous locomotor activity in the mudpuppy, <u>Necturus maculosus</u>	38
II	Diurnal variation in oxygen consumption	41
III	Diurnal variation in whole body lactate	44
IV	Regression analysis of resting metabolic rate vs body weight in the mudpuppy, <u>Necturus maculosus</u>	46
V	Regression analysis of resting whole body lactate vs body weight in the mudpuppy	48
VI	Post-stimulation whole body lactates	49
VII	Regression analysis of whole body lactate vs hours of recovery in the mudpuppy	51
VIII	Metabolite concentrations before, immediately after and during recovery from 30 min forced activity	52

LIST OF ILLUSTRATIONS

Figure		Page
1	Diurnal cycle of resting oxygen consumption and spontaneous locomotor activity in <u>Necturus maculosus</u> acclimated at 5 C	31
2	Diurnal cycle of resting oxygen consumption and spontaneous locomotor activity in <u>N. maculosus</u> acclimated at 15 C	32
3	Resting oxygen consumption vs body weight in the mudpuppy	33
4	Resting whole body lactate vs body weight	34
5	Diurnal variation of resting whole body lactate and removal of lactate following 30 min forced activity in the mudpuppy	35
6	Blood glucose and muscle and liver glycogen concentrations at rest, immediately following 30 min forced activity and for 24 hours of recovery in the mudpuppy	36
7	Tissue lactate concentrations at rest, immediately following 30 min forced activity and for 24 hours of recovery in the mudpuppy	37

ACTIVITY METABOLISM IN THE MUDPUPPY,
NECTURUS MACULOSUS

SUMMARY

1. The mudpuppy, Necturus maculosus, exhibited significant daily cycles of resting oxygen consumption and spontaneous locomotor activity at 5 C and 15 C with an LD 12:12 photoperiod.
2. Resting whole body lactate levels did not vary with time of day at either acclimation temperature, therefore, high levels of spontaneous locomotor activity are supported entirely aerobically.
3. Lung breathing accounted for half of the oxygen consumed during periods of high resting oxygen consumption and high spontaneous activity at both acclimation temperatures.
4. When mudpuppies were stimulated for 30 min to complete exhaustion during periods of high resting oxygen consumption, no further increase in oxygen consumption was observed.
5. When mudpuppies were stimulated during a period of low resting oxygen consumption, an increase in oxygen consumption attributable entirely to air breathing was observed.
6. Whole body lactate levels increased during stimulation independent of the time of day of stimulation, and apparently independent of acclimation temperature.

7. Mudpuppies did not exhibit an oxygen debt, but rather gradually reduced post-stimulation whole body lactate levels over a recovery period of 24 hours.
8. The prolonged removal of lactate with no accompanying oxygen debt results in a considerable energy savings for this species.
9. The major source of glucose for anaerobic energy production during exhausting activity is liver glycogen.
10. The strong dependence on anaerobiosis for energy production over resting levels coupled with a prolonged recovery period significantly reduces the energetic cost of activity in Necturus and is further evidence for the "low energy evolutionary strategy" of salamanders.

INTRODUCTION

The amount of energy that an animal can expend beyond its maintenance needs, the metabolic capacity for activity, is an index to the animal's ability to perform muscular work and is thus a useful quantity for understanding animal energetics in an ecological context (Bartholomew 1972). Partitioning of metabolic capacity into aerobic and anaerobic fractions can establish the limitations imposed on an animal's activity by its metabolic and respiratory machinery.

Activity metabolism in amphibians has recently received considerable attention due to the phylogenetic importance of this transitional group (Seymour 1973; Bennett and Licht 1973, 1974; Turney and Hutchison 1974; Bennett and Wake 1974; Hutchison and Turney 1975; Weigmann and Altig 1975; Cushman, Packard, and Boardman 1976; Hillman 1976; Hutchison, Turney, and Gratz 1977). Those studies which have measured both aerobic and anaerobic contributions to total energy production conclude that anaerobiosis accounts for a large portion, often most, of the energy produced during sustained activity in amphibians. Various methods have been used to measure the aerobic and anaerobic contributions to burst activity in amphibians with conflicting results.

Diurnal cycles of spontaneous activity (Adler 1969, 1971; Hutchison and Kohl 1971; Demian and Taylor 1977) and of resting metabolism (Guimond and Hutchison 1968; Turney and Hutchison 1974; Hutchison, Turney, and Gratz 1977; and others) occur in anurans and urodeles when they are exposed to a light-dark cycle. Furthermore, the frog Rana pipiens exhibits a distinct

diurnal cycle in resting blood lactate concentration (Hutchison and Turney 1975) indicative of an anaerobic contribution to spontaneous activity. The aerobic component of metabolic capacity and the oxygen debt in Rana pipiens vary with the time of day in which activity is elicited (Turney and Hutchison 1974).

Anaerobic metabolism is strongly temperature dependent in several amphibian species (Bennett and Licht 1974; Hutchison, Turney, and Gratz 1977), but appears to be relatively temperature independent in at least one species (Hutchison, Miller, and Gratz 1978). All of the studies above, however, are based on weight-specific measures of lactate production and depend on a dilution technique (Bennett and Licht 1972) which assumes a constant tissue density. Temperature acclimation may affect water balance in amphibians (Schmidt-Nielsen and Forster 1954; Miller, Standish, and Thurman 1968; Parsons and Alvarado 1968), cold acclimated animals being relatively water loaded compared with warm acclimated animals. Since tissue densities do change with acclimation temperature, direct comparison of anaerobic energy production based on weight-specific lactate concentrations is probably precluded.

Partitioning of anaerobic metabolites important in maximal exercise (glycogen, glucose and lactate) into body compartments which are functional during exercise and during recovery from exercise (muscle, liver and blood) provides valuable information on the source and use of anaerobically derived energy (Hutchison and Turney 1975). Such partitioning also provides information concerning the role of these various organs in the recovery from maximal exertion (Gratz and Hutchison 1977).

No work to date has examined the role of substrates other than glucose in amphibian anaerobiosis. Evidence from other vertebrates indicates that the role of amino acid substrates is quantitatively very small during anaerobic energy production (Hochachka, et al. 1975; Johnston 1975; A. Bennett personal communication).

The mudpuppy Necturus maculosus is distributed throughout the Great Lakes region and the Mississippi River system in water of diverse character

(Conant 1975; Bishop 1926). It retains gills in adulthood and is exclusively aquatic, although simple, sac-like lungs are present. At low temperatures all gas exchange occurs aquatically across the cutaneous and branchial surfaces (Guimond and Hutchison 1972). Guimond and Hutchison (1972) postulated that the large volume, small surface area lung serves mainly as a hydrostatic device.

In this study, mudpuppies were used to investigate (1) diurnal cycles of metabolic rate and spontaneous locomotor activity under a controlled photoperiod, (2) aerobic and anaerobic components of metabolic capacity at different times of day, (3) effects of acclimation temperature on the above relationships, and (4) partitioning of anaerobic metabolites into the body compartments which are functional during exercise and recovery.

MATERIALS AND METHODS

Necturus maculosus of mixed sexes and sizes (weight range = 59-178 g; mean weight = 101.9 g) were obtained from a commercial supplier^r (Nasco, Fort Atkinson, Wisconsin). Animals were housed in groups of 10-15 in 20 l plastic containers filled with aerated, aged tap water, and were fed live earthworms and mealworms. Animals were fasted for one week before use. The salamanders were acclimated for at least 2 weeks at either 5 C or 15 C and a LD 12:12 photoperiod with the photophase centered at 1200 CST. Two weeks was probably long enough to elicit the full acclimation response (Hutchison and Rowlan 1975). All animals were maintained at 15 C and either used directly or transferred to 5 C for acclimation at that temperature. Comparison of both resting oxygen consumption and resting whole body lactate revealed no differences between animals which had spent 2 weeks at 15 C and animals held at 15 C for 6 months. All measurements were made at the acclimation temperatures.

Oxygen consumption from air and water was measured in closed system respirometers constructed from 2 l flasks. Oxygen uptake from air (air phase vol. = 500 ml) was measured manometrically, whereas oxygen uptake from water (water phase vol. = 1500 ml) was assessed by withdrawal of a water sample and determination of the decline in oxygen tension with a Beckman oxygen electrode. Air and water phases were separated with mineral oil. Carbon dioxide in the air phase was absorbed with Ascarite^R (Arthur H. Thomas Co.).

Extraction of oxygen from water was independent of P_{O_2} at least to a P_{O_2} of 40 mm Hg. Oxygen tension in the water phase was not allowed to fall below this level.

Animals were weighed and placed individually in respirometers 24 h prior to the beginning of an experiment. During an experiment the respirometers were completely submerged in a water bath maintained at the desired temperature. All gas volumes were corrected to STPD.

Spontaneous locomotor activity of animals was measured by placing them individually in 10 l aquaria inside environmental chambers. Movements were recorded from piezoelectric crystals on styrofoam floats placed on the aquarium water. Movement of the animal through the water resulted in voltage output by the crystal which was amplified and recorded on a Gilson polygraph. Animal movements were summed for each hour and recorded as a percent of the total number of movements in each 24 h period to adjust for variation among transducers.

Salamanders were stimulated to maximum activity with electric shocks delivered by a Grass SM6 stimulator (20 min 50 v followed by 10 min 100 v biphasic stimulation, 20 msec duration, 3 pulses/s). During activity the animals used for the measurement of the anaerobic components of activity metabolism were held in plastic cages which allowed access to air and placed them in the center of an electric field generated by two large aluminum electrodes. This treatment reduced the variation in current flow through the bodies of individual salamanders. The animals used for the measurement of the aerobic components of activity metabolism were shocked in the respirometers with small platinum electrodes. The use of platinum electrodes coupled with biphasic current flow reduced the error in the manometer system due to electrolysis. All animals survived the shocking. No animal was used for more than one period of induced activity.

Measurements of whole body lactate, blood glucose and lactate, liver glycogen and lactate, muscle glycogen and lactate, and heart and kidney lactate were made with techniques identical to those reported in

Gratz and Hutchison (1977) and Hutchison, Turney, and Gratz (1977). Dilutions assume a tissue density of 1.0, thus comparisons between acclimation regimes could be inappropriate due to variation in relative water loading of amphibians at different temperatures.

Lateral muscle masses immediately posterior to the front limbs were used for the determination of lactate and glycogen in skeletal muscle. The heart was rinsed prior to homogenization to remove as much blood as possible.

Energetic equivalents of oxygen consumed and lactate produced in terms of $\mu\text{M ATP g}^{-1}$ and cal g^{-1} were calculated with the conversions cited in Gratz and Hutchison (1977) and Hutchison, Turney, and Gratz (1977).

Analysis of covariance of weight non-specific data was used to identify significant variation among group means for variables which are commonly expressed as a ratio of body weight ($\mu\text{l O}_2 \text{g}^{-1} \text{h}^{-1}$; mg lactate g^{-1}), as well as to allow for the effect of body weight on these variables and to check for variation of body weight among experimental cells (Atchley, Gaskins, and Anderson 1976). Duncan's multiple range test (Duncan 1955) was used to distinguish which cell means were different from others. An arcsin transformation was used to normalize the percentage data generated in the activity experiments; analysis of variance was then used to identify significant variation among group means, and Duncan's multiple range test was used to distinguish which means were different from others. Product-moment correlation coefficients were calculated between oxygen consumption and locomotor activity with the mean of each variable at each hour of the day.

The comparison of resting and stimulated animals is statistically difficult because adjacent values within each group are not completely independent of each other. The goal of the comparison is to determine the length of the recovery period and, in the case of oxygen consumption, the volume of the oxygen debt. At each hour post-stimulation, stimulated animals were compared with resting animals sampled at the same time of day with a one-way analysis of covariance. Statistical significance was judged at the $P < 0.05$ level.

All experiments were randomized as much as possible so as to distribute experimental error equally among experimental cells.

RESULTS

Diurnal cycles

Resting oxygen consumption and spontaneous locomotor activity exhibited significant ($P < 0.0001$ in all cases) diurnal variation at both 5 C and 15 C (figs. 1,2). Animals at 15 C were spontaneously active during the first half of scotophase, and resting oxygen consumption was highest during this period. The majority of the increase in oxygen consumption during the period of activity was the result of air breathing. Salamanders at 5 C were spontaneously active at mid-scotophase and again at mid-photophase, and resting oxygen consumption was highest during these periods. As in 15 C acclimated animals, 5 C acclimated animals increased oxygen consumption during periods of spontaneous activity by breathing air. Oxygen consumption was significantly correlated with locomotor activity at both 5 C and 15 C ($r = 0.54$ and 0.75 respectively, $df = 22$). Resting whole body lactate levels did not vary with time of day at either acclimation temperature ($P > 0.60$ in both cases) (fig. 5).

Body weight

The influence of body weight on resting metabolic rate was evaluated with linear equations relating aerial and aquatic gas exchange to body weight at each acclimation temperature (fig. 3). The line labeled AQUATIC at each acclimation temperature was calculated from data pooled across times of day of exclusively aquatic gas exchange (at 5 C: 0030-0630, 1330-1930; at

15 C: 0030-1730). The line labeled AQUATIC + AERIAL at each acclimation temperature was calculated from data pooled across times of day when animals were supplementing aquatic gas exchange with lung breathing (at 5 C: 0730-1230, 2030-2330; at 15 C: 1830-2330).

Linear equations were calculated to relate resting whole body lactate to body weight at each acclimation temperature (fig. 4). At each acclimation temperature data was pooled across time of day of analysis.

Whole Animal Energetics

Salamanders from both acclimation temperatures were stimulated to maximum activity for 30 min at 0700 and 2000 CST. Salamanders reacted to electric stimulation with violent escape movements which diminished in intensity as the shocking period progressed. The stimulation resulted in complete fatigue of the animals as evidenced by their inability to respond to any electric or manual stimulation at the end of the shocking period.

No differences in total gas exchange were observed between resting and stimulated animals at 5 C acclimation temperature at either time of exercise, or in 15 C acclimated animals stimulated at 2000 CST. Increased oxygen consumption in response to electric stimulation was observed only in 15 C acclimated animals stimulated at 0700. These animals increased oxygen consumption above resting levels by breathing air. The duration of elevated oxygen consumption was highly variable among individuals. Individual salamanders consumed oxygen at rates higher than resting for 0.7 to 5.4 h after stimulation. The mean oxygen consumed over resting levels was 970.52 μl (standard error of the mean = 16.52; mean body weight = 101.9 g; $n = 6$) or 10.68 $\mu\text{l O}_2 \text{ g}^{-1}$.

Whole body lactate levels increased in exercised animals at both acclimation temperatures and at both times of stimulation (fig. 5). Time of exercise (AM vs PM) was not a significant source of variation in the data at either acclimation temperature ($P > 0.17$ in both cases), but at 5 C the interaction of time of exercise and hours of recovery was significant ($P = 0.03$). At 24 h

post-stimulation whole body lactate levels were statistically indistinguishable from control levels ($P < 0.001$ in all cases). There was significant heterogeneity of variances among samples of resting and post-stimulation whole body lactate. Linear equations were calculated which predict the decline of whole body lactate with time after stimulation at each acclimation temperature (fig. 5).

Tissue metabolites

Tissue metabolites were only monitored in animals acclimated at 15 C and stimulated to activity at 0700 CST. The pattern of metabolite distribution before, immediately after and during recovery from maximum sustained activity is presented in figures 6 and 7. Since the total muscle weight and the blood volume were unknown, tissue metabolites are presented as weight specific values (mg lactate g^{-1} tissue wet weight), and the error mean square from an analysis of variance on these weight specific data was used in Duncan's multiple range test to determine which means were different from resting. All tissue metabolite concentrations exhibited homogeneity of variances.

Sampling of bladder urine during dissection for metabolite analysis revealed no lactate in the urine; lactate was also absent in the water in which animals were stimulated to activity.

DISCUSSION

Diurnal cycles

Mudpuppies exhibited significant diurnal cycles of resting oxygen consumption and spontaneous locomotor activity when exposed to a photoperiod (figs. 1,2). These two functions were significantly correlated at both acclimation temperatures, although it should be recognized that the correlation coefficients calculated between these two variables are not valid statistical functions because mean values of measurements taken on different groups of animals were used. They do, however, provide some measure of the interrelationship between resting oxygen consumption and spontaneous locomotor activity.

Mudpuppies increased oxygen consumption during periods of high spontaneous activity by breathing air. The lung of this species evidently functions as an accessory respiratory organ as well as a hydrostatic organ (Guimond and Hutchison 1972), and in this regard seems functionally similar to the lung of the Australian lungfish (Grigg 1965; Lenfant, Johansen, and Grigg 1966/67). During periods of highest resting oxygen consumption (1930 CST and 2130 CST at 15 C and 5 C acclimation respectively) aerial gas exchange accounted for just over half of total oxygen consumption (56.9% and 54.6% at 15 C and 5 C, respectively).

Resting whole body lactate did not vary with time of day (fig. 5). The significant variation of resting oxygen consumption over time of day coupled with the lack of variation in resting whole body lactate constitutes

evidence that high levels of spontaneous locomotor activity are supported entirely through aerobic means. This pattern of total aerobic support for spontaneous activity in Necturus stands in contrast to the pattern shown by the frog Rana pipiens (Turney and Hutchison 1974; Hutchison and Turney 1975), but is similar to the pattern exhibited by the terrestrial salamander Ambystoma tigrinum (Hutchison, Turney, and Gratz 1977).

Body weight

The equations relating different types of resting oxygen consumption to body weight (fig. 3) were calculated on a linear scale (rather than a log-log scale) because this was the simplest regression model which fit the available data. The relatively narrow range of body weights made log scale calculations superfluous. Thus the linear equations are tangents to the parabolic curve which would undoubtedly be generated had the range of available body weights been larger and generalizations made from these equations about the effect of body weight on resting metabolic weight in Necturus may not be extended outside the range of body weights used in their calculation. Furthermore, data known to be different were pooled for the calculation of these linear equations. However, there was no significant variation in body weight among times of day at either acclimation temperature and the regression lines should represent the effect of body weight on resting oxygen consumption at an "average" time of day.

Despite the limitations imposed above, it is clear that the percentage contribution of aerial gas exchange during periods of spontaneous activity increases with increasing body weight (Ultsch 1974, 1976). Air breathing by animals at 5 C increases their rate of oxygen consumption to that of a 15 C animal breathing water alone.

The slopes of the lines which relate resting whole body lactate to body weight are less than one (fig. 4). Large animals may be less reliant on anaerobic sources for routine energy production than relatively small animals

of this species. If this is the case, then the increasing reliance on lung ventilation with increasing body size more than compensates for the relative reduction in overall respiratory surface area.

Whole animal energetics

When mudpuppies were stimulated to maximum activity during periods of high spontaneous activity (15 C acclimation stimulated at 2000 CST and 5 C acclimation stimulated at 0700 CST and at 2000 CST) no increase in oxygen consumption over resting levels was observed. This may seem to imply that the method of stimulation interfered with gas exchange. However, when animals were stimulated during a period of low spontaneous activity (15 C acclimation stimulated at 0700 CST) an increase in oxygen consumption entirely due to air breathing was observed, suggesting that electrical stimulation per se does not prevent an increase in oxygen uptake. Evidently, during periods of high spontaneous activity any further increase in energy expenditure must be anaerobically supported. During periods of low spontaneous activity any increase in aerobic energy expenditure must be supported by breathing air, but the majority of the support for maximal exertion is still anaerobic.

Some physiologic control over aquatic gas exchange is indicated by the variation in aquatic oxygen consumption over time of day (figs. 1,2). Yet animals stimulated to maximum activity during periods of low spontaneous activity exhibited no change in aquatic oxygen consumption. The method of stimulation may have interfered with aquatic gas exchange except that levels of aquatic oxygen consumption in stimulated animals were identical with levels in unstimulated animals. That is, aquatic oxygen consumption was high in animals stimulated during periods of high spontaneous activity and low in animals stimulated during periods of low spontaneous activity. Aquatic gas exchange may be under such rigid control that increases in response to the method of stimulation were not possible.

The use of electric shock to stimulate maximum sustained activity may create unwanted artifacts by preventing the development of maximum aerobic power and enlarging the contribution of anaerobiosis to power output through direct muscle stimulation. Nevertheless, electric shocks are quantifiable while manual stimulation in the form of pinching and prodding is not. The method of choice, forcing animals to swim in a water tunnel, either requires non-quantifiable prodding or irregular electric shock to force untrained animals to swim, or some period of training which would probably increase aerobic power output (Baldwin, et al. 1975; Holloszy and Booth 1976).

Whole body lactate levels increased during stimulation to maximum sustained activity independent of the time of day during which activity was stimulated (fig. 5). The variation in whole body lactate following stimulation to maximum sustained activity was significantly greater than that in resting whole body lactate. The large variation in lactate accumulation during sustained activity is in contrast to the much smaller variation in lactate accumulation during burst activity (Bennett and Licht 1973, 1974), an indication of different constraints on burst vs sustained anaerobic energy expenditure. The great differences between individuals in the expenditure of anaerobic energy during maximal exertion probably indicates that these differences exist in the population in nature, rather than differences in treatment or ability to feed in the laboratory. If animals in a variety of physical conditions exist in nature, control of physical condition could be genetically based or simply the result of the previous history of the individual.

Lactate removal following exercise to exhaustion in Necturus is linear (fig. 5), in contrast to non-linear lactate removal following burst activity in another species of salamander, Ambystoma tigrinum (Cushman, Packard, and Boardman 1976). The removal of lactate with no accompanying increase in oxygen consumption is not without precedence (Irving, Scholander, and Grinnell 1942) and results in considerable energy savings for the animal. The energy cost of the return to the resting state following activity is often

considerable (Gratz and Hutchison 1977) and by reducing or eliminating this expenditure the animal reduces the total energetic cost of a bout of activity. Presumably, however, high levels of tissue and circulating lactate reduce the animal's potential for a second bout of activity. That is, during a prolonged recovery the animal should have a reduced metabolic capacity and a reduced capacity for activity. Thus, this strategy for energy saving would probably be found only in animals which do not rely on fight or flight for predator avoidance. Salamanders typically rely on noxious skin secretions to avoid predators and indeed, Necturus may have no natural enemies (Bishop 1926). Also, a measure of behavioral recovery is apparently attained before tissue lactate levels have fallen appreciably (Fitts and Holloszy 1976; Olsen and Feder 1978; Putnam 1977). This strategy is in strong contrast to that of another giant aquatic salamander, Amphiuma tridactylum, which returns to the resting state very quickly following a bout of exhausting activity (Preslar and Hutchison 1978), and which probably relies on fight and flight against its only known natural enemy, the mud snake Farancia abacura (Conant 1975). During prolonged recovery in Necturus lactate may be used in preference to other substrates for resting energy production (Hochachka, et al. 1977).

A summary of total energy production during sustained activity to complete exhaustion in Necturus at two acclimation temperatures is given in table 1. This summary is for total energy production and the component of metabolism for maintenance (resting metabolism) is included. This summary is for whole body energy production by an "average" (100 g) salamander weighed at its acclimation temperature and, due to unknown relative water loading at these temperatures, different acclimation temperatures are not strictly comparable to each other. However, every amphibian so far examined becomes relatively water loaded at cooler temperatures (Schmidt-Nielsen and Forster 1954; Miller, Standish, and Thurman 1968; Parsons and Alvarado 1968) and thus estimates of lactate content of 5 C acclimated Necturus based on density would probably be higher than the estimates of lactate content presented here, which are based on weight. Yet every measure of resting and

post-stimulation lactate in 5 C acclimated Necturus is as high as that in 15 C animals, (compare the regression equations for different acclimation temperatures in figs. 4,5) and the anaerobic contribution to exhausting activity is as high in 5 C as in 15 C animals (table 1). Anaerobic energy production appears to be relatively temperature independent in Necturus between 5 C and 15 C.

Tissue metabolites

The major source of glucose for anaerobic energy production in mudpuppies is liver glycogen (fig. 6). The decrease in liver glycogen during stimulation provides enough glucose to account entirely for maximum muscle lactate levels (fig. 7). This situation is similar to that of the tiger salamander at 15 C (Hutchison, Turney, and Gratz 1977) and is in contrast to that of the rainbow trout which apparently utilizes muscle glycogen as an immediate energy source and restores muscle glycogen from liver glycogen during recovery (Black, Robertson, and Parker 1961).

Following the cessation of stimulation, however, liver and muscle glycogen increase to levels which cannot be accounted for entirely by gluconeogenesis from lactate. With known liver weights and an assumed muscle mass of 50% of body weight, at 4 h post-stimulation, liver and muscle together contain 38 mg glucose more than they contained at the initiation of stimulation (fig. 6). At 4 h post-stimulation muscle lactate has fallen significantly but at most could have contributed 24 mg lactate to a total increase of 160 mg glucose in liver and muscle combined. The estimate of muscle mass as 50% of body weight is probably high and results in an over estimation of both total muscle glycogen and total muscle lactate. The muscle lactate values are probably elevated due to the delay of several minutes between dissection and homogenization of tissue. Nevertheless, clearly another and as yet unknown source of substrate for the production of glucose stores is involved.

Tissue metabolites were measured in 15 C Necturus stimulated at 0700 CST, and the measurement of oxygen consumption in animals from that acclimation temperature and stimulated at that time of day revealed an increase in oxygen consumption over resting rates due to air breathing which averaged 971 $\mu\text{l O}_2$. Assuming a P/O ratio = 3.0 this volume of oxygen could have produced 282 $\mu\text{moles ATP}$, enough to convert only 94 μmoles (8.4 mg) lactate to 47 μmoles (8.5 mg) glucose. Thus gluconeogenesis from lactate can account for only a small portion of the muscle lactate which disappears during recovery and the glycogen which appears at the same time. Clearly the vast majority of the lactate formed during anaerobic energy production in the mudpuppy is oxidized in place of other substrates, and very little gluconeogenesis, with lactate as substrate takes place. Phillips and Hird (1977) failed to show significant gluconeogenesis in another species of salamander they termed the axolotl *Sirodon mexicanum*.

Heart and muscle lactate concentrations exhibit similar patterns during recovery from exhaustion (fig. 7) and this provides evidence that these organs are functioning similarly during this period. If so, the role of muscle glycogen in this species may be analogous to that of the anaerobic turtle heart. At low work loads anaerobic turtle hearts utilize glycogen stores for glycolytic substrate, while at high work loads exogenous glucose is the major source of substrate (Reeves 1963). If this analogy is valid, the role of muscle glycogen in Necturus would be to provide an energy source during periods of low environmental oxygen availability.

High kidney lactate concentrations (fig. 7) and the absence of lactate in the urine indicate that the kidney of this species participates significantly in some form of lactate removal (Cohen and Little 1976).

Ecological implications

Mudpuppies routinely produce the energy required for resting needs aerobically. During periods of high spontaneous activity, oxygen uptake is

supplemented by ventilation of the lungs. When a greater energy demand is made of the animal no further increase in oxygen consumption is observed and the entire increase in energy output is through anaerobiosis. The energy production from anaerobic sources is twice that of a terrestrial, lunged salamander Ambystoma tigrinum (Hutchison, Turney, and Gratz 1977) and nearly as high as that of a snake with high anaerobic capacity, Natrix rhombifera (Gratz and Hutchison 1977). Furthermore, Necturus appears to be temperature independent in its anaerobic energy production. Reliance on anaerobiosis is disadvantageous (in an evolutionary sense) because the accumulation of large quantities of lactate in the tissues is physiologically disruptive and prevents further energy production. An animal which relies on anaerobic sources for emergency energy production may fatigue quickly. Also, metabolic homeostasis must be restored following a bout of activity. The ATP produced by the volume of the oxygen debt is part of the true cost of activity. This cost can approach half of total energy production (Hutchison, Turney, and Gratz 1977). Necturus may avoid the latter disadvantage by substituting lactate for other substrates during recovery, and thus avoiding an oxygen debt. Presumably the prolonged elevation of lactate levels is disabling, but the absence of natural predators on Necturus makes the risk negligible.

The evolutionary advantage in the "low energy strategy" of other salamanders (Feder 1976; 1978) is evident in Necturus. In this species energy is saved from the maintenance of a complex respiratory and circulatory system and the energetic cost of reliance on anaerobiosis for emergency energy production is eliminated.

LITERATURE CITED

- Adler, K. 1969. Extraoptic phase shifting of circadian locomotor rhythm in salamanders. *Science* 164: 1290-1292.
- _____. 1971. Pineal end organ: role in extraoptic entrainment of circadian locomotor rhythm in frogs. Pages 342-350 in M. Menaker, ed. *Biochronometry*. National Academy of Science, Washington.
- Atchley, W. R., C. T. Gaskins, and D. Anderson. 1976. Statistical properties of ratios. I. Empirical results. *Systematic Zoology* 25: 137-148.
- Baldwin, K. M., R. H. Fitts, F. W. Booth, W. W. Winder, and J. O. Holloszy. 1975. Depletion of muscle and liver glycogen during exercise. *Pflugers Archive* 354: 203-212.
- Bartholomew, G. A. 1972. Energy metabolism. Pages 44-72 in M. S. Gordon, ed. *Animal physiology: principles and adaptations*. 2d ed. Macmillan, New York.
- Bennett, A. F., and P. Licht. 1972. Anaerobic metabolism during activity in lizards. *Journal of comparative Physiology* 81: 277-288.
- _____. 1973. Relative contributions of anaerobic and aerobic energy production during activity in Amphibia. *Journal of comparative Physiology* 87: 351-360.

- _____. 1974. Anaerobic metabolism during activity in amphibians. *Comparative Biochemistry and Physiology* 48A: 319-327.
- Bennett, A. F., and M. H. Wake. 1974. Metabolic correlates of activity in the caecilian Geotrypetes seraphini. *Copeia* 1974: 764-769.
- Bishop, S. C. 1926. Notes on the habits and development of the mudpuppy Necturus maculosus (Rafinesque). *New York State Museum Bulletin* 268, 60 pp.
- Black, E. C., A. C. Robertson, and R. R. Parker. 1961. Some aspects of carbohydrate metabolism in fish. Pages 89-124 in A. W. Martin, ed. *Comparative physiology of carbohydrate metabolism in heterothermic animals*. University of Washington, Seattle.
- Cohen, J. J., and J. R. Little. 1976. Lactate metabolism in the isolated perfused rat kidney: relations to renal function and gluconeogenesis. *Journal of Physiology* 255: 399-414.
- Conant, R. 1975. *A field guide to reptiles and amphibians of eastern and central North America*. Houghton Mifflin, Boston. 429 pp.
- Cushman, J. R., G. C. Packard, and T. J. Boardman. 1976. Concentrations of lactic acid in neotenic and transformed tiger salamanders (Ambystoma tigrinum) before and after activity. *Journal of comparative Physiology* 112: 273-281.
- Demian, J. J., and D. H. Taylor. 1977. Photoreception and locomotor rhythm entrainment by the pineal body of the newt, Notophthalmus viridescens (Amphibia, Urodela, Salamandridae). *Journal of Herpetology* 11: 131-139.

- Duncan, D. 1955. Multiple range and multiple F - tests. *Biometrics* 11: 1-6.
- Feder, M. E. 1976. Lunglessness, body size, and metabolic rate in salamanders. *Physiological Zoology* 49: 398-406.
- _____. 1978. Effect of temperature on post-activity oxygen consumption in lunged and lungless salamanders. Submitted to *Journal of Experimental Zoology*.
- Fitts, R. H., and J. O. Holloszy. 1976. Lactate and contractile force in frog muscle during development of fatigue and recovery. *American Journal of Physiology* 231: 430-433.
- Gratz, R. K., and V. H. Hutchison. 1977. Energetics for activity in the diamondback water snake, Natrix rhombifera. *Physiological Zoology* 50: 99-114.
- Grigg, G. C. 1965. Studies on the Queensland lungfish, Neoceratodus forsteri (Krefft) III. Aerial respiration in relation to habits. *Australian Journal of Zoology* 13: 413-421.
- Guimond, R. W., and V. H. Hutchison. 1968. The effect of temperature and photoperiod on gas exchange in the leopard frog, Rana pipiens. *Comparative Biochemistry and Physiology* 27: 177-195.
- _____. 1972. Pulmonary, branchial and cutaneous gas exchange in the mud puppy, Necturus maculosus maculosus (Rafinesque). *Comparative Biochemistry and Physiology* 42A: 367-392.
- Hillman, S. S. 1976. Cardiovascular correlates of maximal oxygen consumption rates in anuran amphibians. *Journal of comparative Physiology* 109: 199-207.

- Hochachka, P. W., G. C. Liggins, J. Qvist, R. Schneider, M. Y. Snider, T. R. Wonders, and W. M. Zapol. 1977. Pulmonary metabolism during diving: conditioning blood for the brain. *Science* 198: 831-834.
- Hochachka, P. W., T. G. Owen, J. F. Allen, and G. C. Whittow. 1975. Multiple end products of anaerobiosis in diving vertebrates. *Comparative Biochemistry and Physiology* 50B: 17-22.
- Holloszy, J. O., and F. W. Booth. 1976. Biochemical adaptations to endurance exercise in muscle. *Annual Reviews of Physiology* 38: 273-291.
- Hutchison, V. H., and M. A. Kohl. 1971. The effect of photoperiod on daily rhythms of oxygen consumption in the tropical toad, Bufo marinus. *Zeitschrift vergleichende Physiologie* 75: 367-382.
- Hutchison, V. H., and L. D. Turney. 1975. Glucose and lactate concentrations during activity in the leopard frog, Rana pipiens. *Journal of comparative Physiology* 99: 287-295.
- Hutchison, V. H., and S. D. Rowan. 1975. Thermal acclimation and tolerance in the mudpuppy, Necturus maculosus. *Journal of Herpetology* 9: 367-368.
- Hutchison, V. H., L. D. Turney, and R. K. Gratz. 1977. Aerobic and anaerobic metabolism during activity in the salamander Ambystoma tigrinum. *Physiological Zoology* 50: 189-202.
- Hutchison, V. H., K. Miller, and R. K. Gratz. 1978. Aerobic and anaerobic contributions to sustained activity in the giant toad Bufo marinus. Submitted to *Journal of comparative Physiology*.

- Irving, L., P. F. Scholander, and S. W. Grinnell. 1942. Experimental studies of the respiration of sloths. *Journal of Cellular and Comparative Physiology* 20: 189-210.
- Johnston, I. A. 1975. Anaerobic metabolism in the carp (Carassius carassius L.) *Comparative Biochemistry and Physiology* 51B: 235-241.
- Lenfant, C., K. Johansen, and G. C. Grigg. 1966/67. Respiratory properties of the blood and pattern of gas exchange in the lungfish Neoceratodus forsteri (Krefft). *Respiration Physiology* 2: 1-21.
- Miller, D. A., M. L. Standish, and A. E. Thurman, Jr. 1968. Effects of temperature on water and electrolyte balance in the frog. *Physiological Zoology* 41: 500-506.
- Olsen, L. E., and M. E. Feder. 1978. Behavioral and physiological correlates of recovery from exhaustion in the lungless salamander Batrachoseps attenuatus (Amphibia: Plethodontidae). Submitted to *Journal of comparative Physiology*.
- Parsons, R. H., and R. H. Alvarado. 1968. Effect of temperature on water and ion balance in larval Ambystoma gracile. *Comparative Biochemistry and Physiology* 24: 61-72.
- Phillips, J. W., and F. J. R. Hird. 1977. Gluconeogenesis in vertebrate livers. *Comparative Biochemistry and Physiology* 57B: 127-131.
- Preslar, A. J., III, and V. H. Hutchison. 1978. Energetics for activity in the salamander Amphiuma tridactylum. Submitted to *Journal of comparative Physiology*.
- Putnam, R. W. 1977. Relationship between fatigue and changes of lactate and glycogen in muscles of anuran amphibians. *American Zoologist* 17: 893.

- Reeves, R. B. 1963. Control of glycogen utilization and glucose uptake in the anaerobic turtle heart. *American Journal of Physiology* 205: 23-29.
- Schmidt-Nielsen, B., and R. P. Forster. 1954. The effect of dehydration and low temperature on renal function in the bullfrog. *Journal of Cellular and Comparative Physiology* 44: 233-246.
- Seymour, R. S. 1973. Physiological correlates of forced activity and burrowing in the spadefoot toad, Scaphiopus hammondi. *Copeia* 1973: 103-115.
- Turney, L. D., and V. H. Hutchison. 1974. Metabolic scope, oxygen debt and the diurnal oxygen consumption cycle of the leopard frog, Rana pipiens. *Comparative Biochemistry and Physiology* 49A: 583-601.
- Utsch, G. R. 1974. Gas exchange and metabolism in the Sirenidae (Amphibia: Caudata)--I. Oxygen consumption of submerged sirenids as a function of body size and respiratory surface area. *Comparative Biochemistry and Physiology* 47A: 485-498.
- _____. 1976. Respiratory surface area as a factor controlling the standard rate of O₂ consumption of aquatic salamanders. *Respiration Physiology* 26: 357-369.
- Weigmann, D. L., and R. Altig. 1975. Anaerobic glycolysis in two larval amphibians. *Journal of Herpetology* 9: 355-357.

TABLE 1

Total energy budget for 30 min forced activity to complete exhaustion at two acclimation temperatures in the mudpuppy, Necturus maculosus.

Acclimation Temperature (C)	source	$\mu\text{l O}_2$	mg lactate	$\mu\text{moles ATP}$	calories	% contribution
5	aerobic	918.6		266.4	4.69	12.5
	anaerobic		111.7	1865.4	29.1	87.5
	total			2131.8	33.79	
15	aerobic	1100.6		319.2	5.62	15.9
	anaerobic		100.8	1683.4	26.3	84.1
	total			2002.6	31.92	

Note.--Values represent net O_2 consumption and net lactate production by animals with a mean body weight of approximately 100 g during 1/2 hour of stimulated activity.

FIGURE LEGENDS

Fig. 1.--Diurnal cycle of resting oxygen consumption (circles) and of spontaneous locomotor activity (triangles) in Necturus maculosus acclimated at 5 C. The period of scotophase is indicated by the black bar on the abscissa. Locomotor activity, n = 12; oxygen consumption, n = 7 to 28. The stippled area between the open and closed circles on the oxygen consumption curve is the increment due to air breathing. Oxygen consumption data are given as $\mu\text{l g}^{-1} \text{h}^{-1}$ to allow comparison with other studies, although analysis was performed on weight non-specific data ($\mu\text{l h}^{-1}$) with analysis of covariance using body weight as the covariate.

Fig. 2.--Diurnal cycle of resting oxygen consumption (circles) and of spontaneous locomotor activity (triangles) in Necturus acclimated at 15 C. The manner of presentation is the same as in figure 1. Locomotor activity, n = 12; oxygen consumption, n = 7 to 27.

Fig. 3.--Least squares linear regression lines relating resting oxygen consumption to body weight at two acclimation temperatures in Necturus. See text for methods used to calculate the equations. For 5 C acclimation:

$$\dot{V}_{\text{O}_2} \text{ AQUATIC } (\mu\text{l h}^{-1}) = 2.59 \text{ body weight} + 354.6$$

$$\dot{V}_{\text{O}_2} \text{ AERIAL} + \text{AQUATIC } (\mu\text{l h}^{-1}) = 7.33 \text{ body weight} + 728.3$$

For 15 C acclimation:

$$\dot{V}_{\text{O}_2} \text{ AQUATIC } (\mu\text{l h}^{-1}) = 11.09 \text{ body weight} + 286.2$$

$$\dot{V}_{\text{O}_2} \text{ AERIAL} + \text{AQUATIC } (\mu\text{l h}^{-1}) = 28.50 \text{ body weight} - 303.0$$

Fig. 4.--Least squares linear regression lines relating resting whole body lactate pooled across time of day of determination to body weight in Necturus acclimated at 5 C (crosses) and 15 C (circles). For 5 C acclimation:

$$\text{mg lactate} = 0.242 \text{ body weight} - 2.22$$

For 15 C acclimation:

$$\text{mg lactate} = 0.258 \text{ body weight} - 4.85$$

Fig. 5.--Diurnal variation of resting whole body lactate (lower curves and lower scale) and removal of lactate following 30 min stimulation to complete exhaustion (upper curves and upper scale) in Necturus acclimated at 5 C (solid circles) and 15 C (open circles). Each point for resting animals represents the mean of 4 observations. There is no significant variation across time of day for resting whole body lactate. Each point for post-stimulation animals represents the mean of 12 observations pooled across time of day of stimulation. The vertical bars are one standard error on either side of the mean.

For 5 C acclimated post-stimulation animals:

$$\text{mg lactate} = -3.824 \text{ hrs of recovery} + 110.5$$

For 15 C acclimated post-stimulation animals:

$$\text{mg lactate} = -3.050 \text{ hrs of recovery} + 94.6$$

Fig. 6.--Blood glucose concentration and muscle and liver glycogen concentrations at rest, immediately following stimulation to maximum sustained activity and for 24 h of recovery in Necturus. The shaded box on the abscissa represents the time of stimulation. Each point is the mean of 5 to 11 observations.

Fig. 7.--Tissue lactate concentrations at rest, immediately following stimulation to maximum sustained activity and for 24 h of recovery in Necturus. The shaded box on the abscissa represents the time of stimulation. Each point represents the mean of 4 to 22 observations.

APPENDIX I

Diurnal variation in spontaneous locomotor activity in the mudpuppy, Necturus maculosus. Values are presented as percent of total activity and correspond to those in figures 1,2. The table of values is followed by the results of an analysis of variance performed on the arcsin transformed data and the results of a Duncan's multiple range test, also performed on the arcsin transformed data.

Time (CST)	Acclimation Temperature			
	5 C n = 12		15 C n = 12	
	Mean %	Standard error	Mean %	Standard error
0030	6.9	0.74	7.9	1.42
0130	5.7	0.64	6.6	1.37
0230	5.0	0.94	4.3	1.15
0330	3.6	0.60	3.4	1.09
0430	3.0	0.64	1.9	0.67
0530	2.2	0.48	1.8	1.20
0630	3.2	0.52	1.8	0.64
0730	3.5	0.88	1.5	0.71
0830	3.3	0.45	1.5	0.89
0930	3.8	0.62	0.7	0.42
1030	4.1	1.20	0.4	0.20
1130	3.0	0.58	0.8	0.65
1230	3.1	0.69	0.2	0.14
1330	5.5	1.71	0.2	0.11
1430	3.5	0.82	0.2	0.15
1530	2.7	0.42	0.2	0.12
1630	2.0	0.44	0.4	0.23
1730	2.0	0.53	1.9	0.91
1830	2.9	0.48	6.2	1.43
1930	4.0	0.87	12.1	2.05
2030	5.3	0.95	14.8	2.24
2130	6.8	0.82	12.2	2.75
2230	7.0	0.61	7.9	1.05
2330	7.9	1.02	10.8	3.01

5 C Acclimation

Source of variation	DF	Mean Square	F	P
Animals	5	10.11	0.75	0.59
Time of Day	23	76.15	5.68	< 0.0001
Animals X Time of Day	115	18.66	1.39	0.03
Error	144	13.40		

15 C Acclimation

Source of variation	DF	Mean Square	F	P
Animals	11	26.44	0.82	0.62
Time of Day	23	565.29	17.57	< 0.0001
Error	253	32.17		

Summary of the results of Duncan's multiple range test applied to the spontaneous locomotor activity data. To find if the mean activity at two times differ significantly, find the point of intersection of the two times, if a 1 or a 5 appears, the means differ significantly at the 1% or the 5% level, respectively.

5 C Acclimation

	2330	2230	2130	2030	1930	1830	1730	1630	1530	1430	1330	1230	1130	1030	0930	0830	0730	0630	0530	0430	0330	0230	0130	0030	
0030					1	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1			.	
0130						5	1	1	1	5		5	1			5	5	5	1	1			.		
0230	5					1	1												1	5		.		5	
0330	1	1	1																		.		5	1	
0430	1	1	1	5							5									.			1	1	
0530	1	1	1	1							1								.			5	1	1	
0630	1	1	1																				1	1	
0730	1	1	1															.				5	1	1	
0830	1	1	1													.						5	1	1	
0930	1	1	5			5								.									1	1	1
1030	1	1	1											.								5	1	1	1
1130	1	1	1	5									.									5	1	1	1
1230	1	1	1	5								.										5	1	1	1
1330	5					1	1	5			.											5	1	1	1
1430	1	1	1							.												5	1	1	1
1530	1	1	1	5					.													5	1	1	1
1630	1	1	1	1				.														5	1	1	1
1730	1	1	1	1		.																	1	1	
1830	1	1	1			.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5		
1930	1	1	1		.	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	
2030	5		.			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	
2130		.				5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	
2230		.	5			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5		
2330	.					1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		

15 C Acclimation

APPENDIX II

Diurnal variation in oxygen consumption in the mudpuppy, Necturus maculosus. Gas volumes are presented as $\mu\text{l h}^{-1}$ STPD. Data correspond to those in figures 1,2. Each table is followed by the results of a one-way analysis of covariance using body weight as the covariate.

5 C Acclimation

Time (CST)	Mean $\dot{V}_{\text{O}_2}$	N	Standard error
0030	830.50	10	81.37
0130	814.85	10	181.61
0230	754.20	10	142.45
0330	683.14	10	135.05
0430	423.27	10	180.12
0530	542.93	10	149.11
0630	1044.31	21	86.44
0730	1918.46	26	143.37
0830	1034.27	28	95.04
0930	1499.54	18	107.82
1030	1655.42	18	113.82
1130	889.81	18	63.62
1230	1624.25	18	159.34
1330	781.45	18	160.66
1430	608.04	18	118.40
1530	681.60	18	142.59
1630	452.50	18	92.03
1730	483.08	18	67.77
1830	613.79	7	134.77
1930	933.94	10	102.93
2030	1756.03	10	175.65
2130	2315.54	10	244.49
2230	2198.69	10	110.05
2330	1346.28	10	224.50

overall mean body weight = 112.05 g

Source of variation	DF	Mean Square	F	P
Time of Day	23	4596526.452	18.413	< 0.0001
Error	329	249638.239		

15 C Acclimation

Time (CST)	Mean $\dot{V}_{O_2}$	N	Standard error
0030	1808.40	21	187.00
0130	1309.20	20	125.95
0230	1417.10	18	133.54
0330	1509.66	18	163.23
0430	1671.93	18	139.19
0530	1360.67	18	96.63
0630	1496.06	18	154.19
0730	1511.34	26	394.83
0830	1567.02	12	149.32
0930	1617.35	19	125.42
1030	1634.80	19	87.39
1130	1587.86	18	146.53
1230	1532.74	18	127.54
1330	1443.24	13	121.65
1430	1155.92	11	105.43
1530	909.66	8	114.43
1630	966.95	7	154.83
1730	1590.00	16	156.47
1830	2890.95	21	188.49
1930	4003.22	27	239.50
2030	2602.11	27	178.85
2130	2653.77	27	202.65
2230	3144.57	27	188.30
2330	2011.36	24	149.47

overall mean body weight = 110.13 g

Source of variation	DF	Mean Square	F	P
Time of Day	23	10937660.28	25.198	< 0.0001
Error	426	434071.69		

APPENDIX III

Diurnal variation in whole body lactate in the mudpuppy, Necturus maculosus. Lactates are presented as mg in the whole body of the animal. Data correspond to those in figure 5. Each table is followed by the results of a one-way analysis of covariance performed on the data with body weight as the covariate.

5 C Acclimation

Time (CST)	Mean	N	Standard error
0030	30.25	4	11.16
0230	25.25	4	4.91
0430	22.67	4	2.32
0630	17.96	4	2.46
0730	24.36	4	2.11
0830	20.43	4	4.90
0930	24.43	4	3.82
1030	28.90	4	8.52
1130	12.59	4	2.55
1330	17.98	4	3.35
1530	15.78	4	3.16
1730	17.26	4	3.53
2030	17.93	4	3.77
2130	29.91	4	12.55
2230	16.34	4	3.52
2330	26.64	4	5.93

overall mean body weight = 99.04 g

Source of variation	DF	Mean Square	F	P
Time of Day	15	98.177	0.871	0.5986
Error	47	112.680		

15 C Acclimation

Time (CST)	Mean	N	Standard error
0030	23.69	4	9.12
0230	21.95	4	6.25
0430	16.79	4	1.33
0630	18.78	4	2.58
0730	26.81	4	1.60
0830	17.72	4	0.55
0930	19.49	4	3.87
1030	29.14	4	20.25
1130	17.34	4	4.99
1330	21.25	4	4.98
1530	29.02	4	5.14
1730	17.85	4	2.73
2030	19.57	4	5.65
2130	16.50	4	4.76
2230	14.29	4	5.34
2330	21.94	4	3.08

overall mean body weight = 99.38 g

Source of variation	DF	Mean Square	F	P
Time of Day	15	83.938	0.532	0.9087
Error	47	157.649		

APPENDIX IV

Regression analysis of resting metabolic rate during times of day of exclusively aquatic gas exchange vs body weight in the mudpuppy, Necturus maculosus .

5 C Acclimation

$$\dot{V}_{O_2} (\mu l h^{-1}) = 2.5863 \text{ body weight} + 354.56$$

Source of variation	DF	Mean Square	F	P
Explained	1	813079.0994	3.827	0.0519
Unexplained	186	212431.9595		

15 C Acclimation

$$\dot{V}_{O_2} (\mu l h^{-1}) = 11.0907 \text{ body weight} + 286.24$$

Source of variation	DF	Mean Square	F	P
Explained	1	12289260.05	39.261	< 0.0001
Unexplained	296	313016.6147		

Regression analysis of resting metabolic rate during times of day of
aerial gas exchange vs body weight in the mudpuppy, N. maculosus .

5 C Acclimation

$$\dot{V}_{O_2} (\mu l h^{-1}) = 7.3269 \text{ body weight} + 728.31$$

Source of variation	DF	Mean Square	F	P
Explained	1	5683466.652	12.447	0.0005
Unexplained	164	456629.575		

15 C Acclimation

$$\dot{V}_{O_2} (\mu l h^{-1}) = 28.487 \text{ body weight} - 302.96$$

Source of variation	DF	Mean Square	F	P
Explained	1	50359182.58	50.426	< 0.0001
Unexplained	151	998676.44		

APPENDIX V

Regression analysis of resting whole body lactate vs body weight in
the mudpuppy, Necturus maculosus.

5 C Acclimation

$$\text{lactate (mg)} = 0.2424 \text{ body weight} - 2.216$$

Source of variation	DF	Mean Square	F	P
Explained	1	1471.5381	13.479	0.0005
Unexplained	62	109.1737		

15 C Acclimation

$$\text{lactate (mg)} = 0.2577 \text{ body weight} - 4.854$$

Source of variation	DF	Mean Square	F	P
Explained	1	1502.4404	10.746	0.0017
Unexplained	62	139.8155		

APPENDIX VI

Post-stimulation whole body lactates immediately after and during recovery from 30 min forced activity in the mudpuppy, Necturus maculosus. N = 6 for each group. For each acclimation temperature, the data summary is followed by the results of a two-way analysis of covariance performed on the data with body weight as the covariate.

5 C Acclimation

	Stimulated at 0700		Stimulated at 2000	
	Mean mg	Standard error	Mean mg	Standard error
Immediately after	132.56	41.61	90.74	24.05
1 hour of recovery	91.13	23.03	98.15	29.44
2 hours of recovery	82.59	12.47	149.62	21.68
3 hours of recovery	92.34	16.22	112.24	11.06
4 hours of recovery	100.31	7.87	113.48	27.70
6 hours of recovery	64.90	12.48	82.35	8.18
8 hours of recovery	73.51	13.43	84.33	17.74
10 hours of recovery	61.61	12.15	72.72	13.22
24 hours of recovery	24.92	2.45	18.70	2.47

Source of variation	DF	Mean Square	F	P
Time of stimulation	1	3316.2013	1.9037	0.1716
Hours of recovery	7	4587.3395	2.6334	0.0170
Interaction	7	4126.6049	2.3689	0.0300
Error	79	1741.9754		

15 C Acclimation

	Stimulated at 0700		Stimulated at 2000	
	Mean mg	Standard error	Mean mg	Standard error
Immediately after	94.46	12.86	107.18	31.59
1 hour of recovery	44.72	11.34	135.35	39.09
2 hours of recovery	49.34	9.49	99.91	24.59
3 hours of recovery	56.03	10.93	88.09	15.56
4 hours of recovery	125.31	40.73	117.77	19.40
6 hours of recovery	54.30	12.53	55.48	11.40
8 hours of recovery	87.32	18.68	66.45	8.17
10 hours of recovery	54.91	16.73	67.79	16.31
24 hours of recovery	23.82	2.17	20.05	2.52

Source of variation	DF	Mean Square	F	P
Time of stimulation	1	1897.4656	0.966	0.329
Hours of recovery	7	4189.5024	2.132	0.0497
Interaction	7	1510.6660	0.769	0.615
Error	79	1964.7190		

APPENDIX VII

Regression analysis of whole body lactate vs hours of recovery from 30 min forced activity in the mudpuppy, Necturus maculosus. Data from different times of stimulation were pooled for this analysis.

5 C Acclimation

$$\text{lactate (mg)} = -3.8239 \text{ hrs} + 110.54$$

Source of variation	DF	Mean Square	F	P
Groups	8	10529.7049	4.779	< 0.0001
linear regression	1	75839.1311	34.422	< 0.0001
deviation	7	1199.7869	0.5446	0.7987
Error	100	2203.1898		

15 C Acclimation

$$\text{lactate (mg)} = -3.0504 \text{ hrs} + 94.5618$$

Source of variation	DF	Mean Square	F	P
Groups	8	9716.0372	3.840	0.0006
linear regression	1	48263.0381	19.072	< 0.0001
deviations	7	4209.3228	1.663	0.1268
Error	100	2530.5453		

APPENDIX VIII

Metabolite concentrations before, immediately after and during recovery from 30 min forced activity at 15 C, 0700 CST in the mudpuppy, Necturus maculosus. For each metabolite, the data summary is followed by the results of an analysis of variance and a Duncan's multiple range test performed on the data. See page 40 for directions for use of the multiple range test tables.

Blood Glucose

	Mean (mg ml ⁻¹)	Sample size	Standard error
Control (C)	0.17	11	0.02
Immediately after (I)	0.46	11	0.08
1 hour of recovery	0.27	9	0.01
2 hours of recovery	0.45	11	0.08
4 hours of recovery	0.41	11	0.08
6 hours of recovery	0.36	5	0.05
8 hours of recovery	0.37	5	0.05
10 hours of recovery	0.25	5	0.05
24 hours of recovery	0.17	5	0.03

Source of variation	DF	Mean Square	F	P
Between samples	8	0.1108	3.131	0.0048
Within samples	64	0.0354		

Summary of the results of Duncan's multiple range test applied to the blood glucose data.

Time	24	10	8	6	4	2	1	1	C
C					5	1		1	
1	5								
1									
2	5								
4	5								
6									
8									
10									
24									

Liver Glycogen

	Mean (mg g ⁻¹)	Sample size	Standard error
Control (C)	45.27	5	0.81
Immediately after (I)	16.81	5	0.80
1 hour of recovery	23.43	5	0.79
2 hours of recovery	26.82	5	1.03
4 hours of recovery	36.61	5	1.24
6 hours of recovery	37.01	5	1.32
8 hours of recovery	38.42	5	1.38
10 hours of recovery	38.49	5	1.44
24 hours of recovery	38.30	5	1.53

Source of variation	DF	Mean Square	F	P
Between Samples	8	410.7468	58.7380	< 0.0001
Within Samples	36	6.9928		

Time	24	10	8	6	4	2	1	I	C
C	1	1	1	1	1	1	1	1	
I	1	1	1	1	1	1	1		
1	1	1	1	1	1				
2	1	1	1	1	1				
4									
6									
8									
10									
24									

Muscle Glycogen

	Mean (mg g ⁻¹)	Sample size	Standard error
Control (C)	2.40	10	0.35
Immediately after (I)	2.33	6	0.38
1 hour of recovery	2.13	6	0.16
2 hours of recovery	2.39	8	0.27
4 hours of recovery	3.80	8	0.74
6 hours of recovery	3.61	5	0.44
8 hours of recovery	3.96	5	0.40
10 hours of recovery	3.42	5	0.40
24 hours of recovery	3.36	5	0.33

Source of variation	DF	Mean Square	F	P
Between samples	8	3.3583	2.6580	0.0166
Within samples	49	1.2635		

Time	24	10	8	6	4	2	1	I	C
C			5		5				
I			5		5				
1			5		5				
2			5		5				
4									
6									
8									
10									
24									

Blood Lactate

	Mean (mg ml ⁻¹)	Sample size	Standard error
Control (C)	0.03	22	0.01
Immediately after (I)	0.30	18	0.02
1 hour of recovery	0.40	10	0.02
2 hours of recovery	0.67	12	0.07
4 hours of recovery	0.87	5	0.04
6 hours of recovery	0.74	5	0.03
8 hours of recovery	0.72	5	0.03
10 hours of recovery	0.42	5	0.03
24 hours of recovery	0.06	5	0.01

Source of variation	DF	Mean Square	F	P
Between samples	8	0.84175	74.8573	< 0.0001
Within samples	78	0.01124		

Time	24	10	8	6	4	2	1	I	C
C		1	1	1	1	1	1	1	
I	1	5	1	1	1	1	5		
1	1		1	1	1	1			
2	1	1			1				
4	1	1	5						
6	1	1							
8	1	1							
10	1								
24									

Kidney Lactate

	Mean (mg g ⁻¹)	Sample size	Standard error
Control (C)	0.88	8	0.17
Immediately after (I)	0.49	5	0.09
1 hour of recovery	1.23	4	0.10
2 hours of recovery	1.26	4	0.28
4 hours of recovery	1.28	4	0.12
6 hours of recovery	1.23	4	0.13
8 hours of recovery	1.24	4	0.12
10 hours of recovery	0.88	4	0.13
24 hours of recovery	0.75	4	0.09

Source of variation	DF	Mean Square	F	P
Between samples	8	0.3711	3.1054	0.0104
Within samples	32	0.1195		

Time	24	10	8	6	4	2	1	I	C
C			1	1	1	1	1		
I									
1									
2									
4									
6									
8									
10									
24									

Heart Lactate

	Mean (mg g ⁻¹)	Sample size	Standard error
Control (C)	1.01	4	0.22
Immediately after (I)	1.21	3	0.27
1 hour of recovery	2.57	2	0.18
2 hours of recovery	1.47	2	0.28
4 hours of recovery	1.63	4	0.18
6 hours of recovery	1.10	4	0.14
8 hours of recovery	1.17	4	0.16
10 hours of recovery	1.13	4	0.16
24 hours of recovery	1.09	4	0.12

Source of variation	DF	Mean Square	F	P
Between samples	8	0.5775	4.9120	0.0014
Within samples	22	0.1176		

Time	24	10	8	6	4	2	1	I	C
C					5		1		
I							1		
1	1	1	1	1	1	1			
2									
4									
6									
8									
10									
24									

Liver Lactate

	Mean (mg g ⁻¹)	Sample size	Standard error
Control (C)	0.30	12	0.03
Immediately after (I)	0.38	8	0.06
1 hour of recovery	0.81	6	0.06
2 hours of recovery	0.67	6	0.04
4 hours of recovery	0.79	5	0.04
6 hours of recovery	0.79	5	0.03
8 hours of recovery	0.41	5	0.03
10 hours of recovery	0.36	5	0.03
24 hours of recovery	0.34	5	0.03

Source of variation	DF	Mean Square	F	P
Between samples	8	0.3063	25.0631	< 0.0001
Within samples	48	0.0122		

Time	24	10	8	6	4	2	1	I	C
C				1	1	1	1		
I				1	1	1	1		
1	1	1	1						
2	1	1	1						
4	1	1	1						
6	1	1	1						
8									
10									
24									

Muscle Lactate

	Mean (mg g ⁻¹)	Sample size	Standard error
Control (C)	0.77	16	0.10
Immediately after (I)	1.73	10	0.25
1 hour of recovery	2.59	8	0.22
2 hours of recovery	1.71	8	0.10
4 hours of recovery	2.12	5	0.09
6 hours of recovery	1.46	5	0.08
8 hours of recovery	1.55	5	0.08
10 hours of recovery	1.15	5	0.08
24 hours of recovery	0.91	5	0.08

Source of variation	DF	Mean Square	F	P
Between samples	8	2.8921	14.0701	< 0.0001
Within samples	58	0.2056		

Time	24	10	8	6	4	2	1	I	C
C			1	1	1	1	1	1	
I	1	5					1		
1	1	1	1	1		1			
2	1								
4	1	1		5					
6									
8	5								
10									
24									