THE UNIVERSITY OF MANITOBA

ASPECTS IN THERMAL ACCLIMATION IN THE CRAYFISHES *ORCONECTES VIRILIS* (HAGEN) AND *CAMBARUS BARTONI* (FABRICIUS)

by

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ABSTRACT

Oxygen consumption measurements of active and quiet Orconectes virilis acclimated to a variety of temperatures and exposed to either a long day or short day photoperiod regime were carried out, and the results were related to the life history of O. virilis in southern Manitoba.

The results indicated that: (1) the slope of the straight line relationship between oxygen consumption and weight is not affected by activity of the crayfish but it is affected by acclimation temperatures, (2) that the acclimation patterns are not necissarily the same for active and quiet crayfish and (3) the photoperiod may influence the type of acclimation pattern.

Lactic dehydrogenase (LDH) and alpha-glycerophosphate (a-GDH) dehydrogenase specific activities were determined for crude extracts of muscle and hepatopancreas from winter- and spring-acclimatized Cambarus bartoni. The results indicated that in the hepatopancreas of winter-acclimatized crayfish, LDH specific activity decreased and a-GDH specific activity remained constant. In the muscle the specific activities of both LDH and a-GDH increased in winter-acclimatized crayfish.

The changes in specific activities of LDH and a-GDH are discussed in relation to temperature adaptation by C. bartoni.

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INTRODUCTION

Metabolic temperature compensation can be shown to occur in poikilotherms by comparing oxygen consumption rates of these organisms at a given temperature either at various seasons (Edwards and Irving, 1943), at various experimental temperatures (Prosser, 1958), or from varying latitudes (Vernberg, 1962). Oxygen consumption of whole animals is a simple index of the physiological changes occuring in poikilotherms adapted to a new environment. However, it does not explain the cellular adjustments (Bullock, 1955). Another weakness is that the spontaneous activity of the experimental animal is not eliminated so that oxygen consumption at a basal metabolic state is usually not determined. This source of error must be taken into account when determining the oxygen uptake of an organism exposed to an experimental temperature (Newell and Northcroft, 1967).

Using oxygen uptake as their variable, McWhinnie and O'Connor (1967), and Jungreis and Hooper (1968) reported conflicting results on the type of cold temperature adaptation undergone by the crayfish, Orconectes virilis. McWhinnie and O'Connor found that cold-acclimated O. virilis always had a higher metabolism than warm-acclimated crayfish. Jungreis and Hooper found the metabolism of cold-acclimated crayfish was always lower.

The methods used for determining oxygen consumption rates were different. McWhinnie and O'Connor measured oxygen uptake rates of quiet, individual crayfish. On the other hand, Jungreis and Hooper measured the average oxygen consumption of groups of crayfish.

When crowded, crayfish are aggressive, but given the opportunity they will disperse (Bovbjerg, 1956). In other words, associated with crowding there is an increase in motor activity, and therefore an increase in oxygen consumption (Prosser, 1961). My preliminary observations revealed that associated with forced confinement of O. virilis there was an increase in oxygen uptake. Jungreis and Hooper's respirometer was designed to restrict motor activity of the experimental animals. Hence it is possible that the crowding of the crayfish along with the restriction in motor activity were factors causing Jungreis and Hooper's results to be different from those reported by McWhinnie and O'Connor.

It seems likely that McWhinnie and O'Connor determined oxygen uptake under conditions approaching those normally used to determine an index of standard metabolism (resting animals in a post-absorptive state, Fry, 1957). Jungreis and Hooper seem to have determined oxygen uptake under conditions approaching those used to determine an index of active metabolism. Recently Newell and Northcroft (1967) have presented data indicating that the physiological responses during active metabolism are not necessarily the same responses during standard metabolism. Hence if McWhinnie and O'Connor, and Jungreis and Hooper were determining oxygen uptake values for crayfish exposed to different experimen-

tal conditions, it should be possible to resolve the conflict in the data by determining the oxygen consumption rate of crayfish approaching both conditions used to determine active and resting metabolism in the same experiment. This has been one of the objectives of this thesis.

The term "resting metabolism" is used rather than "standard metabolism" for the following reasons. The determination of standard metabolism can only be made by measuring oxygen uptake versus a measure of activity, and then extrapolating back to zero activity (Fry, 1957). There is no satisfactory method for making quantitative measures of crayfish activity. Standard metabolism can not therefore be determined and instead was followed while the animal appeared to be at rest,- an approach to standard metabolic conditions. It is worth noting that McWinnie and O'Connor (1967) also did not measure standard metabolism.

Direct determinations of the physiological adjustments undergone by poikilotherms during cold acclimation have been made by measuring concentrations of end products in the intermediary pathways (Hochachka and Hayes, 1962), or by measuring the activity of enzymes associated with a particular pathway (Prosser, 1962). Recently Hochachka (1967) demonstrated that the activity of the enzyme lactic dehydrogenase (LDH) increased during cold acclimation by the goldfish, Carassius auratus. He suggested that an increase in LDH activity permits a faster conversion of the co-enzyme reduced nicotinamide adenine dinucleotide (NADH) to the oxidized state so that the reaction catalysed by the NAD-dependent enzyme, glyceraldehyde-3-phosphate dehydrogenase would occur

at a higher rate. Hochachka has suggested that such an increase in the catalytic activity would explain the finding that the rate of flux through the Emden-Meyerhof pathway is higher in the liver of cold-acclimated goldfish than in the liver of warm-acclimated goldfish (Hochachka, 1967).

In addition to LDH, a-glycerophosphate dehydrogenase (a-GDH), also converts NADH to NAD. This enzyme catalyses the first reaction of the second form of glycolysis (Mahler and Cordes, 1966). The end product of this pathway is glycerol, a winter-hardening agent in some animals (Prosser, 1961). Hence during cold-acclimation in the crayfish, there may be an increase in the activity of LDH and a-GDH inorder that: (1) there be a faster conversion of NADH to NAD leading to an increase in the rate of flux through glycolysis, and (2) there is an increase in the concentration of glycerol so that the organism can tolerate cold temperatures.

A second objective of this thesis has been to conduct enzyme assays of LDH and a-GDH in the muscle and hepatopancreas of cold- and warm-acclimated crayfish, Cambarus bartoni, in relation to the above hypothesis.

LITERATURE REVIEW

There is considerable literature relating to thermal acclimation in poikilotherms, the methods for examining temperature acclimation, and the physiological changes taking place during thermal acclimation by poikilotherms. The following account is restricted to more recent work related to the present investigation.

1. Acclimation:

Early investigations of thermal acclimation in poikilotherms have been reviewed by Bullock (1955). He considered that the term "acclimation" and "acclimatization" were synonyms. Later, acclimation was defined as "changes produced in whole organisms as a consequence of exposure to a change in the environment". Fisher, 1958 p. 3), and as "the compensatory change in an organism under maintained deviation of a single environmental factor..." (Prosser, 1961 p. 4). Prosser (Ibid.) defined acclimatization as "those compensatory changes in an organism undergoing multiple natural deviations of milieu - climatic, physical and biotic". Prosser's definitions have been used here.

Thermal acclimation by a poikilotherm involves alterations in physiological states. One state is the animal's ability to survive sudden and extreme temperature stress for short periods of time, known as "resistance adaptation" (Fry, 1947; Precht, 1958). The organism's "zone of tolerance" (Fry (1947) to temperature extremes is a function of the acclimation temperature; cold-acclimated poikilotherms are better able to tolerate sudden exposure to lower temperatures than are warm-

acclimated ones, and vice versa. This type of relationship can be graphically displayed as a temperature polygon (Brett, 1944; Fry, 1947; McCleese, 1956; Fisher, 1958).

The other state modified by thermal acclimation is the poikilotherm's metabolism, "processes which supply energy whereby the organism continues to exist and to gain energy with which to respond to its surroundings". (Fry, 1947 p. 6). When a sudden persistent change in ambient temperature occurs, alterations in the poikilotherm's physiology may take place in three phases (Grainger, 1958). Firstly, an increase in motor activity may occur, probably as a result of sensory stimulation, and may last from a few seconds to minutes. This phase gives way to a phase of accommodation; the organism is quiet, the rate of oxygen consumption drops and remains constant, but is still different from that of an organism acclimated to that temperature. The third phase occurs over a period of time during which the metabolism of the poikilotherm adjusts to the new environmental temperature (Grainger, 1958, Prosser, 1958). This metabolic adjustment, known as "capacity adaptation", has been suggested as a physiological process whereby a reasonably constant metabolism is maintained despite long term changes in the ambient temperature (Precht, 1958). Bullock (1955) suggested that capacity adaptation is a mechanism by which poikilotherms extend their ecological, geographical, and seasonal distribution.

2. Measuring Capacity Adaptation and Q_{10}

Two methods have been followed for investigating capacity adaptation. Precht (1958) suggested that the metabolism of the experimental

animal be determined at its acclimation temperature, t_1. Following this determination, the same animal is exposed to a new ambient temperature, t_2, and its metabolism continually determined until it is constant. Precht states that at this new temperature, the metabolism will adjust according to one of five possible ways (Fig. 1):

(1) Type 1; over compensation; the metabolism adjusts so that during heat stress it is lower than the metabolism at the original colder temperature; and during cold stress, the metabolism is higher than it was at the warmer temperature,

(2) Type 2; perfect compensation; the metabolism returns to the original rate in spite of a change in the ambient temperature,

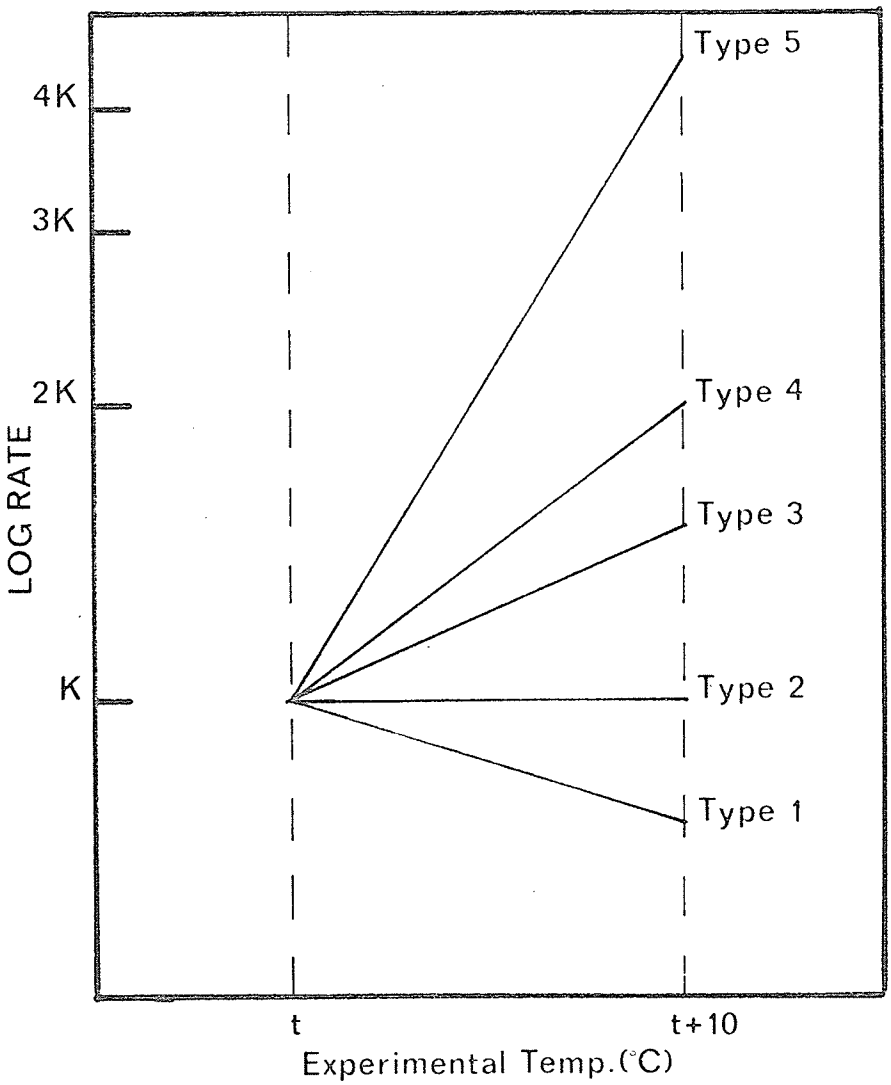
(3) Type 3; partial compensation; the metabolism adjusts almost but not quite, to the original rate,

(4) Type 4; no compensation; the metabolism is a direct function of temperature, follows the van't Hoff relation (Giese, 1962), and remains constant over time at the new experimental temperature,

(5) Type 5; under compensation; the metabolism does not follow the van't Hoff relation; instead it increases over time during heat stress, and decreases over time during cold stress.

If later, the animal is returned to t_1, and its metabolism measured until it is at a constant rate, the experiment will yield four values: (1) metabolism at t_1 when acclimated to t_1, (2) metabolism at t_2 when acclimated to t_1, (3) metabolism at t_2 when acclimated to t_2, and (4) metabolism at t_1 when acclimated to t_2. Using these four values two metabolism-temperature (M-T) curves can be plotted. One curve represents the M-T relationship of an animal adapted to t_1, the other represents the M-T relationship for an animal adapted to t_2 (Fig. 2).

Some insight can be derived as to the nature of the physiological Q_{10} values for metabolism at different acclimation temperatures by comparing the slope of the two M-T curves, as well as their intercepts


Type 5
4K
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K
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t
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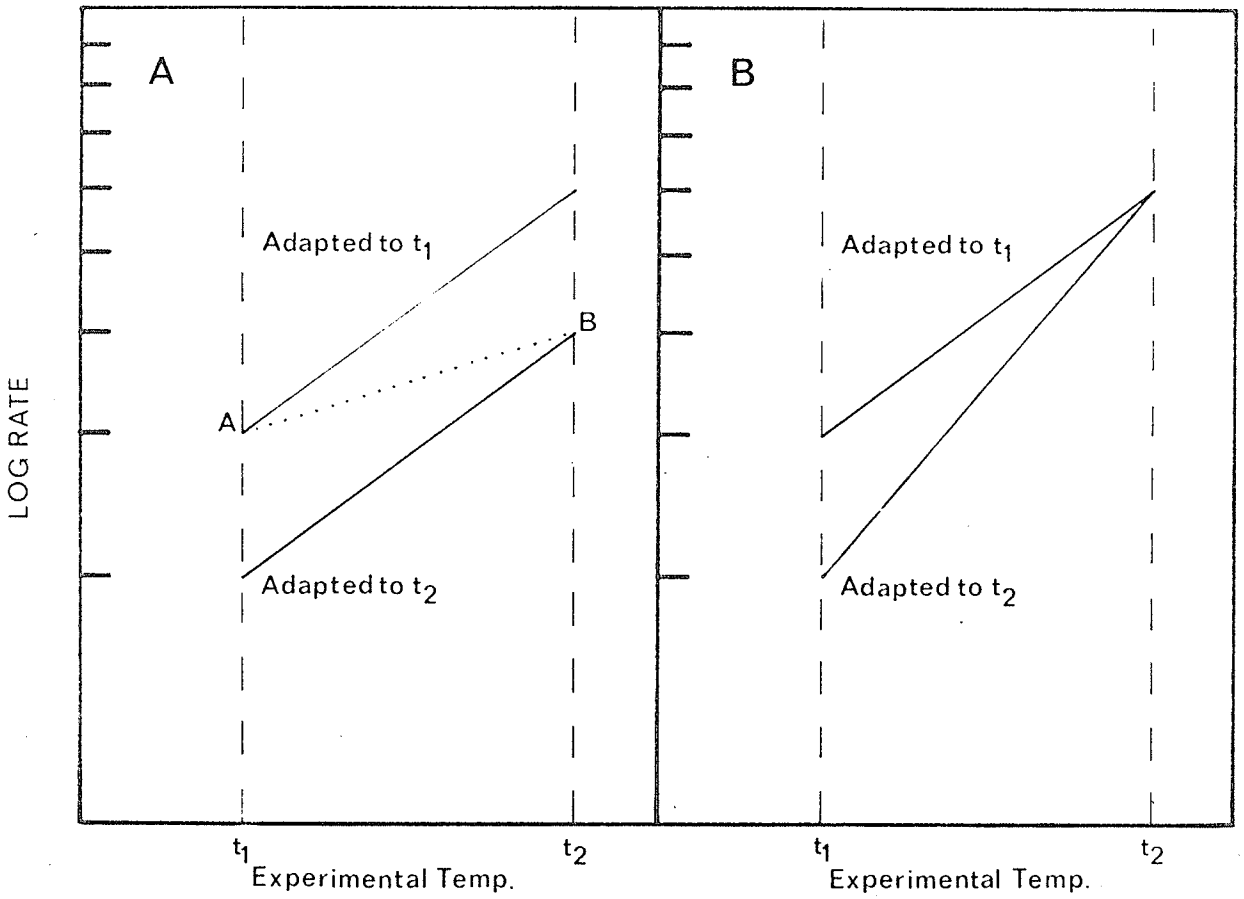
Experimental Temp.(°C)


A
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Adapted to t_1
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LOG RATE
t_1
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Experimental Temp.
Adapted to t_1
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t_1
t_2
Experimental Temp.

at $\underline{t}_1$ and $\underline{t}_2$. The Q_{10} value is defined as the ratio of a rate constant at (t+10)°C to a rate constant at t°C (Giese, 1962; Prosser, 1961; Hoar, 1966). This relationship can be represented by the equation:

$$Q_{10} = \frac{k_{(t+10)}}{k_{(\underline{t})}} ,$$

where $k_{(t)}$ is the rate constant at $\underline{t}$ C and $k_{(t+10)}$ is the rate constant at $\underline{t+10}$ C. Most thermochemical reactions have a Q_{10} of about 2 or 3 (Hoar, 1966), a relation known as the van't Hoff rule (Giese, 1962). The Q_{10} for enzymatic reaction is not constant, but decreases with increasing ambient temperatures. (Giese, 1962; Precht, 1958; Prosser, 1961).

If the Q_{10} is independent of the organism's acclimation state, even though it decreases with increasing experimental temperature, the slopes of the rate-temperature curves of the warm and cold acclimated poikilotherms will be parallel (Precht, 1958). A graphical illustration of this relationship is shown in Fig. 2A. However, if the Q_{10} value depends upon acclimation temperatures as it does in most poikilotherms (Scholander et al 1953a; Rao and Bullock, 1954; Wolvekamp and Waterman, 1960; Bishop and Gordon, 1967), the R-T curves will not be parallel. Because the Q_{10} for rate constants of warm acclimated poikilotherms is usually greater than that of cold acclimated ones, and because the metabolism of cold acclimated animals is higher than that of warm-acclimated ones, the R-T curves will usually intersect at the high experimental temperatures as illustrated in Fig. 2B

(Precht, 1958). It may be worthwhile to point out that there is a relationship between Fig. 2A and Fig. 1. By joining the two points representing the metabolism values of animals tested at their acclimation temperatures (Fig. 2A), a curve is derived which is exactly equivalent to the partial compensation curve illustrated in Fig. 1.

Intersecting curves indicate that the type of adaptation is different for the two acclimation temperatures. Thus Fig. 2B illustrates two points: (1) no compensation (Precht's type 4) occurs when the animal is adapted to t_2, but partial compensation (Precht's type 3) occurs when the animal is adapted to t_1. (2) the Q_{10} is not independent of acclimation temperature (the Q_{10} increases with increasing acclimation temperatures).

Prosser (1958) has suggested that an alternative procedure to Precht's (1958) method would be to measure the metabolism of poikilotherms acclimated and tested over a range of temperatures. After the data were plotted in the typical R-T relationship, the resulting metabolism-temperature curves of the cold and warm acclimated animals could then be compared to determine a number of patterns of thermal acclimation. For cold acclimation, Prosser (1962) listed five possible patterns:

(1) Pattern 1. No acclimation is apparent as the R-T curves of cold- and warm-acclimated poikilotherms coincide.
(2) Pattern 2. Translation occurs; that is the R-T curves of the cold- and warm-acclimated poikilotherms have equal slopes, but the intercept of the R-T curve of cold acclimated poikilotherms is higher than the one for warm acclimated animals.

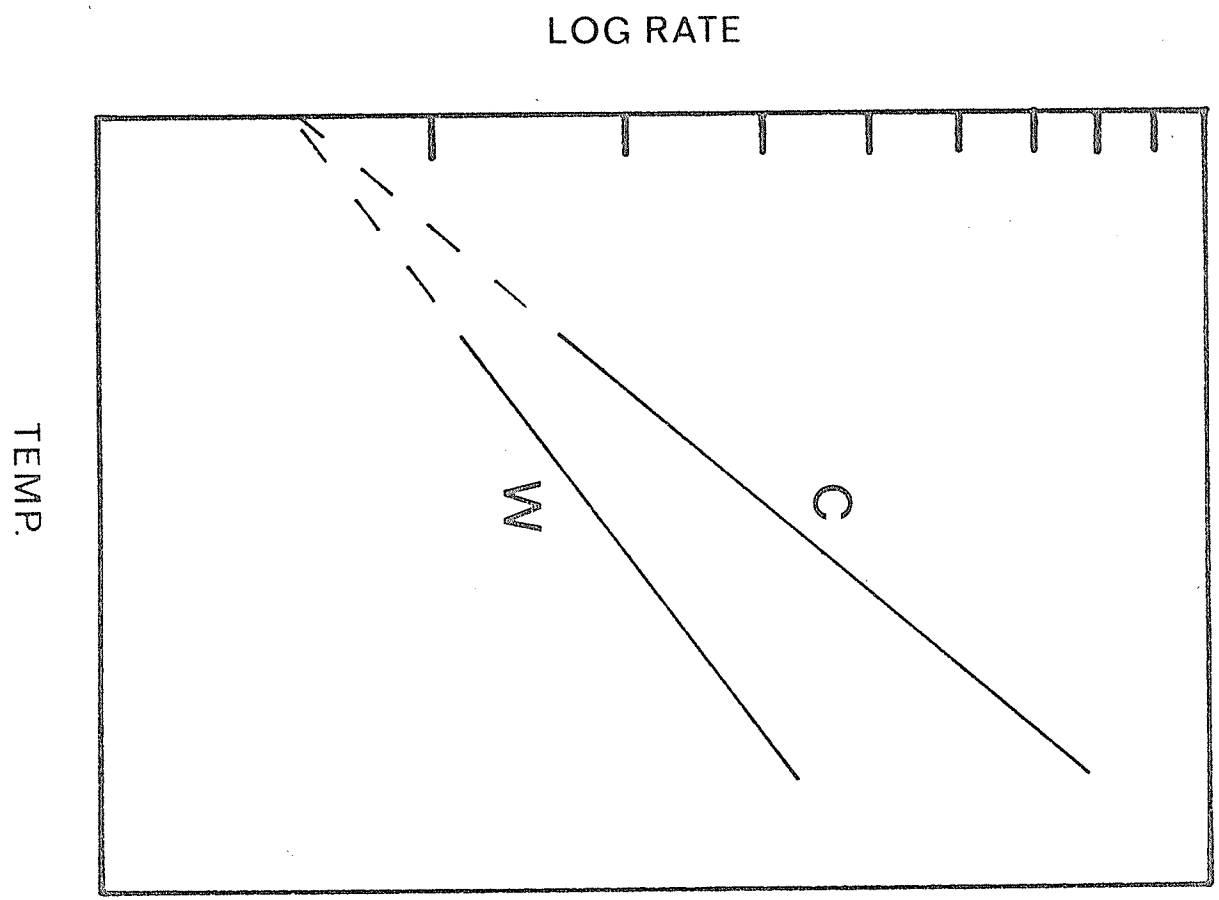
(3) Pattern 3. Rotation is apparent; the slopes of the R-T curves are not equal and intersect near the midpoint of the experimental temperatures.

(4) Pattern 4. Both translation and rotation occur so that the R-T curve of the cold acclimated poikilotherm intersects the R-T curve of the warm acclimated poikilotherm at a high experimental temperature. The Q_{10} increases with <u>increasing</u> acclimation temperatures.

(5) Pattern 5. While both translation and rotation occur, the R-T curve of the cold acclimated poikilotherm intersects at a <u>low</u> experimental temperature. The Q_{10} increases with <u>decreasing</u> acclimation temperatures.

According to Prosser (1962) the most common patterns are Pattern 4 (Fig. 2B) and Pattern 5 (Fig. 3). Figure 2A illustrates Pattern 2.

Figure 3: Rate-Temperature curves of a warm- (W) and cold- (C) acclimated organisms showing Prosser's "Pattern 5". Translation upwards plus anti-clockwise rotation occurs in the rate-temperature curve of the cold-acclimated organism relative to the curve of the warm-acclimated animal. The physiological Q_{10} depends upon the acclimation temperature, increasing with decreasing acclimation temperatures. The two curves intersect beyond the lower lethal temperature of the organism.


LOG RATE
TEMP.
W
C

3. Body Size, Metabolism and Q_{10}

There is some evidence that the magnitude of the change in metabolism when the organism is exposed to a new temperature is related to the body size of the animal. Hence a brief account of the influence of body size on metabolism, and on Q_{10} will be given here.

Extensive reviews on the relationship between body size and metabolism, have been undertaken by Klieber (1947), Zeuthen (1947, 1953), and Hemmingson (1960). Metabolism and body size can be related by the expression:

$$Q_{O_2} = aW^b$$

OR

$$\log Q_{O_2} = \log a + b \log W,$$

where Q_{O_2} is the parameter of metabolism (the organism's oxygen uptake per unit time) (Zeuthen, 1953), and where a and b are coefficients of the expression; a is the value at the Y intercept and b is the value of the slope of the function. Hemmingson concluded that the average value of b for all organisms including beech trees was close to 0.73. Variations in the values among groups of animals at the class level, or at the species level were considered to be oscillations about this slope. For example, Zeuthen (1953) found that for Crustacea, the value of b was close to 0.8 instead of 0.73. In fact, the value for b among various species of Crustacea ranges from 0.67 to 1.0 (Wolvekamp and Waterman, 1960).

When the value of b is 1.0, the metabolism of the organism is considered to be a function of weight, and when the value of b is 0.67,

the metabolism is considered to be a function of the surface area. The net influences of weight and surface area are usually invoked to explain the fact that b has an intermediate value of 0.73. The classical interpretation of the surface area to volume ratio has been modified by Klieber (1947) and Weiser (1966) to include the influence of internal surface areas (respiratory epithelia, blood vessels, intestines, etc.). As the organism increases in size, the proportion of the internal and external surface area increases at a faster rate than does the weight. The efficiency of the absorbing surfaces becomes less efficient with increasing size (Weiser, 1966), so much so that the value of the slope for the metabolism-weight line decreases over the range of the higher weights (Weiser, 1966; Zeuthen, 1953).

Rao and Bullock (1954) presented data indicating that smaller individuals of crustacean species were more temperature sensitive than were larger individuals, i.e. smaller individuals of the species had higher Q_{10} values than did larger ones. This concept may also be valid for inter-specific comparisons of crustacean species as well. Vernberg (1959) found that smaller species of the genus Uca were more temperature-sensitive than were the larger species.

The concept may apply to other taxa. Barlow (1961) and Wohlschlag et al (1968) found that smaller individuals had higher Q_{10} values than did the large ones of the fish species Gillichthys spp. and Lagodon rhomboides respectively. In the molluscs the data are contradictory. Davies (1966) found that large and small individuals of species of the genus Patella were equally sensitive to temperature. Read (1962) found that smaller individuals of the mussel Mytilus edulis had higher Q_{10}

values than did the larger ones. However, Pickens (1965) found that the larger individuals of the species Mytilus californianus were more temperature-sensitive than were the smaller ones.

These results would therefore indicate that it would be wise to maintain approximately equal weights among the samples of experimental animals when doing temperature acclimation studies.

4. Cellular Mechanisms of Temperature Acclimation:

Recently the research emphasis in temperature acclimation studies has focussed on the cellular mechanisms of thermal acclimation. In this section, aspects of cellular mechanism of thermal acclimation are discussed.

Ekberg (1958) and Prosser (1962) have suggested a number of physiological mechanisms which can account for the phenomenon of thermal acclimation. According to Prosser, translation of the metabolism-temperature curve may be caused by a change in the activity of a multi-enzyme system. Such changes could be due to:

(1) changes in the concentration of a "rate-limiting" enzyme of the system, or
(2) alterations in the ionic strength and intra- and extracellular water content of the cells.

Posser (1962) suggested that rotation of the M-T curve may be caused by factors such as:

(1) a change in the enzyme or some co-factor, or
(2) a shift in the rate of flux through a pathway relative to another.

Ekberg (1958) suggested that other possible factors which could bring about temperature acclimation could be:

(1) a change in the transport mechanism of the cell,
(2) a release of an activating or inhibiting agent from specialized cells, or
(3) an inhibition or activation of an agent already in the cell.

Some of the above hypotheses have been tested, and an account of the findings will now be given here under the following headings:

A. Changes in enzyme concentrations:

B. Changes in water content and electrolyte activity (concentration):

C. Changes in protein (enzyme) structure:

D. Metabolic re-organization in response to changes in ambient temperature:

E. Conclusions:

A. Changes in enzyme activity:

A variety of enzymes has been investigated in order to determine their contribution to the physiology of temperature adaptation. However, the results of these investigations are at times confusing and inconsistent.

Ekberg (1962) has reported that although there was no increase in gill-extract glucose-6-phosphate dehydrogenase (G6PDH) activity of cold acclimated (10°C) carp, Carassius carassius, compared to fish acclimated at 25°C, there was a 134% increase in 6-phosphogluconate dehydrogenase (6PDH) activity. Prosser (1962) cites Murphy (1961) who has reported that in goldfish acclimated to 5°C, the G6PDH activity in the liver dropped to 46% of the activity found for goldfish acclimated at 30°C. The activity of 6PDH remained constant in the liver from cold-

and warm-acclimated fish.

Bishop and Gordon (1967), after examining the influence of temperature on the toad, Bufo boreas, found that in the muscles of cold-acclimated individuals there was no change in G6PDH and 6PDH activity, but there was an increase of 33% in aldolase activity. Bishop and Gordon reported also that no change occurred in cytochrome-c oxidase activity. However, Caldwell (1969) reported that cytochrome-c oxidase activity increased by more than 60% in the extracts from brain, gill, and muscle of cold-acclimated (10°C) goldfish when compared to extracts from warm-acclimated ones. Thus these two investigations show how the same enzyme extracted from the same type of tissue will behave differently in two different species.

Caldwell (1969) investigated the activities of succinate-cytochrome-c reductase and NADH-cytochrome-reductase. He found that these enzymes had activities that were 50% higher in gill mitochondrial preparations from cold acclimated goldfish when compared to the activities in the preparations from warm acclimated fish. On the other hand, cytochrome-c_1 activity dropped by 25%.

The temperature at which the reactions were allowed to occur revealed that relative activities of enzymes were temperature-dependent. For example, liver cytochrome-c oxidase activity was lower in the cold- than in the warm-acclimated goldfish when the determinations were carried out at 10°C. Yet at warmer temperatures, the activity of the enzyme from the cold-acclimated fish was higher when compared to that from warm-acclimated fish. (Caldwell, 1969).

The same enzyme extracted from different tissues of the same species can behave quite differently in response to cold acclimation. Freed (1964) reported that muscle cytochrome oxidase activity from cold-acclimated goldfish was higher than than in warm-acclimated ones. However, Murphy (Prosser, 1962) found that no differences could be detected between warm- and cold-acclimated goldfish livers for cytochrome oxidase.

Murphy reported also that no change occured in succinate dehydrogenase activity. However, Saroja and Rao (1965) have reported that succinate dehydrogenase activity in body wall muscles of the earthworm, Lampito mauritii, increased during cold acclimation.

Murphy found that lactate dehydrogenase activity increased by 63% in livers of cold-acclimated goldfish. Later Hochachka (1965), using electrophoretic techniques, demonstrated that part of the increased activity could be due to activation of the H-type locus for the enzyme. More recently, he has presented results indicating that in cold-acclimated brook trout, Salvelinus fontinalis, activation of liver lactic dehygrogenase isoenzymes was more complex. A group of three loci may be activated (Hochachka, 1967). Vessel and Yielding (1966) reported that cold acclimation of the frog, Rana esculanata, probably causes activation of the H-type locus of lactic dehydrogenase in the liver. Hence this phenomenon may occur widely in poikilothermic vertebrates.

These recent examinations of the relationship between temperature acclimation and enzyme activity have indicated that enzyme activity may alter in response to cold adaptation. However, the relationship is

complex as the same enzyme may behave differently in different tissues of the same species, or may behave differently in the same type of tissues of different species. Therefore, no generalizations can be made about a particular enzyme either among organisms or even among tissues from the same organism.

B. Changes in water content and electrolyte activity:

Changes in the intra- and extracellular water content of a number of phyletically diverse poikilotherms during cold adaptation have been reported by several investigators. Scholander et al (1953b), Precht (1958), and Hoar and Cottle (1962) present results indicating that there is a decrease in the total intracellular water content of poikilotherms acclimated to low temperatures. Results from more recent investigations have indicated that the water content decrease during cold adaptation may occur in specific organs (Hickman et al, 1964; Houston et al, 1968; Jungreis and Hooper, 1968).

Ion concentrations in various tissues of a number of diverse species have been found to change during cold acclimation by the animal (Rao and Ramachandra, 1961; Rao and Venkatareddy, 1962; Saroja and Rao, 1965; Houston et al, 1968; Parvatheswararao, 1967). While the results are often conflicting, their significance can be appreciated in relation to the work reported by Behrisch and Hochachka (1969). They reported that the concentrations of magnesium and manganese influence the kinetics of lungfish liver fructose diphosphatase in an allosteric manner. In addition, the activation energy of fructose diphosphatase is higher with an optimum concentration of manganese than it is

with magnesium. Hence there is the possibility that ion concentrations of the interior milieu may partly control glucose metabolism in poikilotherms. The speculation is not unreasonable as it has been known for quite some time that the ion levels of mammalian tissues are an important control mechanism (Glock and McClean, 1953).

C. Changes in protein(enzyme) structure:

Recent reports have indicated that absolute temperature may alter the tertiary configuration of proteins even within the biokinetic range. For example, Milkman (1967) examined the influence of temperature on Drosophila melanogaster wing formation, and inferred from his results that high temperatures could cause distinct changes in the tertiary configuration of structural proteins. Mutchmor (1967) found that cold adaptation by the insects Periplaneta americana and Musca domestica led to the lowering of the activation energy of the reaction catalysed by the enzyme, adenosine triphosphatase. This would imply that a change has occurred in the enzyme in relation to its substrate, i.e. a change has occurred in the enzyme-substrate complex. Such a change could occur if the tertiary configuration of the enzyme was altered.

Hochachka and Somero (1968) demonstrated that the K_m of LDH isoenzymes from the chars, S. fontinalis and S. namaycush, is a function of temperature. They have also suggested that the K_m of pyruvate kinase is so strongly influenced by the ambient temperature, that this enzyme may be instrumental in shifting glucose metabolism to an alternative pathway (Somero and Hochachka, 1968).

The ambient temperature may influence the kinetics of invertebrate enzymes as well, for Somero and Hochachka (1969) found that the lactic dehydrogenase isoenzymes of three Crustacea had K_m values dependent upon the ambient temperature. Somero (1969) has published an extensive review indicating that this effect occurs in a number of phyletically diverse species. This phenomenon is probably quite important when considered in relation to the Q_{10} value at physiological substrate concentrations. As the temperature rises, it would be expected that the rate of reaction would increase. But at the same time, the K_m of the enzyme also rises. Therefore the enzyme-substrate affinity decreases with the result that the net effect is that the rate of the catalysed reaction does not increase as rapidly as would be expected. Depending on the magnitude of the effect of temperature on the K_m of the enzyme, the net effect of a temperature on the reaction rate may be to increase it slightly, or hold it constant (eg. partial or complete temperature compensation).

These results would seem to support Ekberg's (1958) and Prosser's (1962) speculations that the thermal history of a poikilotherm may cause alterations in the intrinsic nature of some of its enzymes.

D. Metabolic re-organization in response to cold temperature:

Recent reports have been published demonstrating that metabolic re-organization takes place during temperature adaptation by poikilotherms. Usually the re-organization is such that there is an increase in flux through glycolysis or the pentose shunt or both. There may occur as well, increases in fatty acid synthesis and protein synthesis.

There may also be a change in the relative rate of flux through various catabolic and anabolic pathways. Integration of this diverse information has been undertaken by Saroja and Rao (1965) and the contributors to the symposium "Molecular Mechanisms of Temperature Adaptation" held at Berkeley in 1965 (Prosser, 1967).

Ekberg (1958, 1962) after exposing gills from cold- and warm-acclimated cyprinids (C. carassius and C. auratus) to metabolic poisons, found that the pentose shunt was more active in the cold-acclimated fish gills than in the warm ones. Blazka (1958) found that there was an absolute increase in fatty acid production in carp collected during the winter from stagnant ponds compared to carp collected in the summer from the same ponds. He found that lactic acid was not excreted by these fish. He concluded from these results that the lactic acid was being converted to acetate and thence to fatty acids where the energy could be stored for use during the spring. Saroja and Rao (1965) after finding that body wall muscle succinate dehydrogenase activity was higher in the worm, Lampito mauritii, after cold acclimation, suggested that glycolytic activity had increased. McWhinnie and O'Connor (1967) found that there was an increase in the use of the glycolytic pathway as well as a slight increase in the use of the pentose shunt, after the crayfish, O. virilis, was acclimated to cold temperatures. Earlier, Hochachka and Hayes (1962) had used isotopically labelled glucose as a substrate for epaxial muscle metabolism to determine the influence of temperature on the glucose catabolism of the trout, S. fontinalis. Their results indicated that there was an increase in pentose shunt participation when the fish were cold acclimated. Bishop and Gordon

(1967) reported that there was an increase in the activity of the pentose shunt in the muscles of cold acclimated toads (Bufo boreas).

It therefore seems likely that associated with cold acclimation there is an increased participation by the pentose shunt for the catabolism of glucose as well as an increase in the glycolytic pathway activity.

Other reports have revealed that fatty acid synthesis is modified during cold acclimation. For example, Hoar and Cottle (1962) found that not only did the lipid content of the goldfish increase by 1% during cold acclimation, but the iodine value increased from 100 to 120. They concluded from these results that as well as there being an absolute increase in the lipid content there was an increase in the proportion of unsaturated fatty-acids. This would indicate that the enzymes associated with unsaturated fatty acid biosynthesis had increased in activity in relation to the others associated with fatty-acid synthesis. Lewis (1962), using gas-liquid chromatographic techniques for his comparison of lipids extracted from arctic and temperate poikilotherms, found that the species living at cold temperatures had higher proportions of palmitoleic acid (16:1), very low proportions of palmitic acid (16:0), and no stearic acid (18:0) when compared to species living in warm waters. Hochachka and Hayes (1962) found that acetate oxidation was lower and fatty acid incorporation higher in cold-acclimated trout than in warm-acclimated ones. Zandee (1966) undertook an extensive examination of the fatty-acid composition of winter- and summer-acclimatized crayfish (Astacus astacus). He found that crayfish after exposure to winter conditions had higher propor-

tions of poly-unsaturated fatty-acids than had summer crayfish. Johnston and Roots (1964), using the same procedures as Zandee, found that in the brains of cold acclimated goldfish there were higher proportions of stearic,arachidonoic (25:4), and heptadecanoic (17:0) acids than in the brains of warm acclimated fish. Mayeemunisa (in: Rao, 1967) found that there was a higher proportion of fatty-acids in cold acclimated earthworms (L. mauritii) when compared to warm-acclimated ones.

Hochachka (1968) has presented results which provide clues to the possible mechanisms operating to control the flow of carbon through one of a number of pathways of metabolism in tropical air breathing fish. Acetate can either flow through the Krebs cycle or be metabolized by the fatty-acid synthetic pathway. His results suggest that somehow citrate, possibly by acting as a positive allosteric effector of acetyl-CoA carboxylase and the substrate for the citrate cleavage enzyme, activates the enzymes for fatty-acid synthesis. Hochachka (Ibid.) found that the effect of citrate was temperature-dependent. In response to rising temperatures, increasing amounts of acetyl-CoA were converted to fatty acids instead of passing through the TCA cycle.

Thus in addition to the metabolic re-organization that takes place in sugar metabolism, it can be seen that fatty-acid biosynthesis is also influenced by the thermal history of the poikilotherm. It now seems likely that the rates of protein synthesis are also dependent upon the acclimation state of the poikilotherm. Das and Prosser (1967) found that there was an increase in the rate of protein synthesis in the liver, gill, and muscle of goldfish acclimated at 5°C compared to the same organ

of fish acclimated at 20°C. Das (1967) showed also that although there was no change in the amount of DNA or water content of muscle, liver or gill cells from either cold- or warm-acclimated fish, there was an increase of RNA in the microsomal and nuclear fractions during cold acclimation. Dean and Berlin (1969) reported that the level of protein was higher in the liver of cold acclimated rainbow trout when compared to warm acclimated fish. There was, as well, a higher rate of incorporation of isotopically labelled leucine into the liver protein of the cold acclimated fish. Haschemeyer (1968) presented results indicating that after the toadfish, Opsanus tau, was acclimated at 10°C, its rate of liver protein synthesis was 75% higher as compared to protein synthesis in warm acclimated toadfish. Later she reported that the aminoacyl transferase activity was 60% higher in the cold acclimated fish (Haschemeyer, 1969).

While there are few reports available at present, the general trend of the information suggests that metabolic re-organization is a complex physiological process involving alterations in protein, fat and sugar metabolism. (See Rao, 1967 for a figure outlining the possible changes).

E. Conclusions:

The question remains as to whether the metabolic re-organizations of multi-enzyme systems, the alterations of ion levels, the decrease in water content, and the increase in activity of a number of enzymes represent an integrated reponse by a poikilotherm which has been exposed to cold temperatures.

While the evidence at present is scant, there is enough to provide a general scheme indicating that cold acclimation is perhaps a complex physiological phenomenon integrating many physiological mechanisms. Such schemes have already been put forth by Hochachka (1967) and Rao (1967) in their discussions on the influence of temperature on poikilotherm metabolism.

It is known that during cold acclimation, alterations in ion levels of body fluids occur. This could imply that changes in the ion transport mechanism (Giese, 1962) take place in the cells. As a result, it is probably that the ion level of the interieur milieu of the cells changes. Now some ions are co-factors of a number of enzymes (Mahler and Cordes, 1966); more specifically, fructose diphosphatase exhibits allostery in the presence of magnesium and manganese ions (Behrisch and Hochachka 1969), and phosphofructokinase is allosterically influenced by ammonium, phosphate and magnesium ions as well as other factors (Atkinson and Walton, 1965; Lowry and Passoneau, 1966). Since phosphofructokinase may control the activity of the Krebs cycle (Passoneau and Lowry, 1963), then by altering the ion levels within the cell it would be possible to regulate delicately the intermediary metabolism of the organism.

It is known that the K_m of a number of enzymes increases with increasing temperatures (Somero, 1969). Thus at physiological substrate levels, the velocity of a reaction catalyzed by one of these enzymes would remain relatively constant over a certain temperature range; as the ambient temperature decreased, the enzyme- substrate affinity would increase so that the velocity of the reaction would increase. The reverse

would occur if the environmental temperature increased.

Hochachka (1967) has put forward the hypothesis that one mechanism whereby the rate of flux through glycolysis would be increased when the organism was exposed to cold would be to increase the rate of conversion of NADH to NAD , a co-enzyme of the enzyme, glyceraldehyde-3-phosphate dehydrogenase. If the ratio of NAD to NADH increased, then the rate of flux through glycolysis would increase. He has suggested that the increased rate of conversion of NADH to NAD could be accomplished by increasing the activity of lactic dehydrogenase as this enzyme is NADH dependent when pyruvate is the substrate. By converting pyruvate to lactate at a faster rate, NADH conversion to NAD would also occur at a faster rate. Evidence for this hypothesis is the observation that during cold acclimation in the goldfish, an extra isoenzyme of lactic dehydrogenase is produced (Hochachka, 1965).

Amino acid synthesis involves the use of the coenzyme, reduced nicotinamide adenine dinucleotide phosphate (NADPH). Hence it would seem likely that the biological significance of the pentose shunt activation is the increased synthesis of NADPH. Since this shunt also produces the pentoses, the precursors to RNA synthesis, activation of the shunt could be significant from this point of view. Thus it seems likely that activation of the pentose shunt and increased RNA and protein synthesis are related (Rao, 1967).

The results have also indicated that the quantity of fatty-acids increases during cold acclimation, and that the relative proportion of unsaturated fatty acids increases also. These results would therefore

suggest that as well as there being an increase in the enzymes associated with the unsaturation of fatty-acids. The pentose shunt metabolism and fatty-acid biosynthesis seem to be related, as the stimulation of the pentose shunt can produce an increase by thirty times in fatty-acid synthesis in rat liver (Siperstein and Fagen, 1957).

Thus it seems that the pentose shunt activation permits the generation of NADPH and pentoses. The NADPH could then be used either for fatty-acid or for protein synthesis. The increase in pentose would permit an increase in RNA biosynthesis and therefore permit an increase in the synthesis of specific proteins. The proteins synthesized in turn could be the enzymespermitting an increase in flux through the pathways associated with energy metabolism.

While there is still relatively little information at present, there is sufficient to generate hypotheses which can be tested.

MATERIALS AND METHODS

This portion of the investigation will be described under the following headings:

1. Experimental animals
2. Conditions of Acclimation
   a. Temperature control
   b. Photoperiod control
   c. Feeding
   d. Aeration
3. Measurement of Oxygen Consumption During Periods of Activity or Rest.
   a. Determination of an optimum respirometer volume
   b. Detailed description of the respirometer
   c. Measurement of oxygen concentrations
   d. Experimental procedure
4. Electrophoresis
   a. Introduction
   b. General Procedure.
5. Determination of the Specific Activity of Lactic Dehydrogenase and alpha-Glycerophosphate Dehydrogenase
   a. Introduction
   b. Selection of organs
   c. Preparation of crude extracts
   d. Determinations of lactic dehydrogenase specific activity
   e. Determination of a-glycerophosphate dehydrogenase activity
   f. Formula for the calculation of specific activity
6. Statistical Analysis of the Data

1. Experimental Animals:

Crayfish used for thermal acclimation studies were Orconectes virilis (Hagen) and Cambarus bartoni (Fabricius). O. virilis was used in respiration studies. Two groups of O. virilis were collected from the Rat River, near St. Malo, Manitoba , one in June and one in October, 1969.

C. bartoni, obtained from The Lemberger Company, Oshkosh, Wisconsin in March, 1970, was used for the enzyme assays.

2. Conditions of Acclimation:

Groups of O. virilis selected for size were acclimated for three weeks at a variety of temperatures in 15-gal aquaria filled with dechlorinated tap-water. Photoperiod, aeration and feeding were controlled.

a. Temperature control:

For Series I (crayfish collected in June), three acclimation temperatures of 5, 15 and 25°C were used. The lower temperatures were obtained by holding the animals in controlled environment rooms set at 5, and 15°C. The highest temperature was produced by warming an aquarium in the 15°C room with a heating element connected to a mercury plunger relay operating from an adjustable mercury thermoregulator.

For Series II (crayfish collected in October), four acclimation temperatures of 4, 9, 12.5 and 25°C were used. Due to limitations in equipment, the acclimation temperatures used in Series I could not be reproduced. The controlled environment rooms were set at 4, and 12.5°C, providing two of the acclimation temperatures. One aquarium in the

4°C room was heated to 9°C, while another was heated to 25°C in the 12.5°C room.

For the enzyme assays, C. bartoni was acclimated at 9 and 25°C

b. Photoperiod control:

Day-night clock switches controlled a 40-Watt bulb set approximately 30 cm above each aquaria. The photoperiod in Series I was 24 L, 0 D. This regime was imposed to reduce any activity rhythm of the crayfish (Precht, 1958).

In series II, the photoperiod was 8 L, 16 D to simulate the shortest photoperiod of winter.

For the enzyme analysis of C. bartoni, warm-acclimated crayfish were exposed to 12 L, 12 D, while cold-acclimated animals were exposed to 8 L, 16 D. Both photoperiod and temperature were varied inorder to simulate winter and spring conditions.

c. Feeding:

Frozen brine shrimp were given once per week. Respirometry studies were carried out no sooner than 48 hrs after feeding.

d. Aeration:

The aquaria were continually aerated. Regular checks indicated the water was nearly saturated with oxygen. Air was also used to operate filters packed with glass wool to keep the aquaria clear.

3. Measurement of Oxygen Consumption during Periods of Activity or Rest:

Respiration, metabolism and oxygen uptake have been used here with the meaning defined by Zeuthen (1953) as "oxygen uptake per organism per

hour".

Metabolism was determined by measuring the oxygen depletion over time in a respirometer containing one crayfish acclimated at a particular temperature. Metabolism was determined at each experimental temperature, and the animal was then killed and desiccated at 98°C.

a. Determination of an optimum respirometer volume:

Jones (1964) has listed a defect in procedures which use a closed respirometer; the rate of oxygen decrease is both a function of the size of the experimental animal and the volume of water. The change in oxygen concentration per unit time will be too small to detect if the experimental animal is small in relation to the volume of water surrounding it, and the change in oxygen concentration per unit time will be too great if the animal is very large in relation to the volume of water with the result that it may die from anoxia. Jones stated that generally the experimental volume of water used has been 10 to 50 times the volume of the experimental animal.

Preliminary investigations revealed that the volume of O. virilis ranged from 12 to 26 ml. The volume of the respirometer was 492 ml after the oxygen probe was inserted. Thus this vessel fulfilled the requirements of size for the respirometry experiments.

b. Detailed description of the respirometer:

The respirometer was custom made from a Plexiglass cylinder 12 cm long, 7 cm i.d. with a wall thickness of 0.5 cm. This was cemented at one end to a 12 cm square Plexiglass plate 0.6 cm thick. The top of the

respirometer was made from an identical square plate bolted to the bottom one. A tapered hole in the top plate allowed insertion of a BOD-bottle oxygen probe.

c. Measurement of oxygen concentrations:

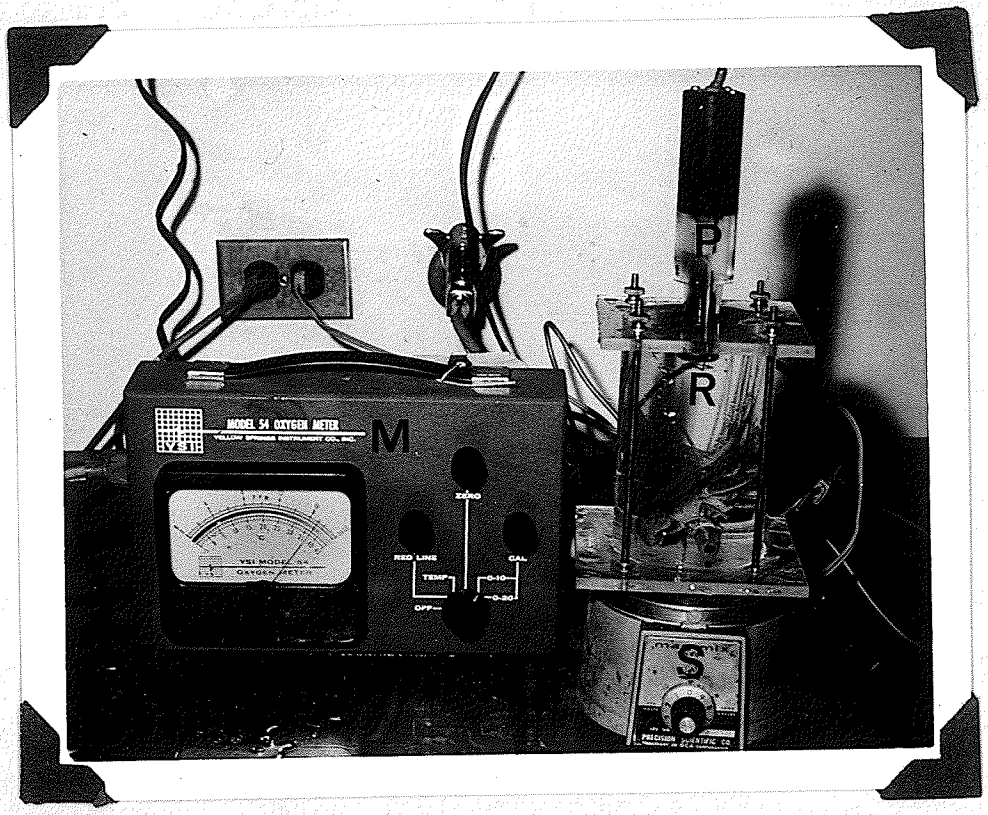
Oxygen concentrations in the respirometer were measured with a YSI model 5420 BOD probe. The probe was connected to a YSI model 54 oxygen meter designed to read oxygen concentrations in parts per million. The accuracy of the instrument with the probe was either $\pm$ 0.1 ppm or $\pm$ 0.2 ppm depending upon whether a short scale or extended scale was used. Plate 1 shows the arrangement of the apparatus for a typical run.

d. Experimental procedure:

At the beginning of each run, the respirometer was filled with water of the desired experimental temperature and placed in an aquarium of that particular temperature. For 10 minutes air was bubbled through the water in the respirometer to insure that the water was completely air-saturated. The oxygen meter was then calibrated.

Next, the experimental animal plus a magnetic stirring bar 30 mm long and 6 mm wide and teflon coated, were put in the respirometer. The top was bolted on and the probe was inserted. Special care was taken to make sure that no air was trapped in the vessel. After 15 minutes of accommodation, the respirometer was removed from the aquarium and placed on an electric stirrer. The stirrer was started causing the bar inside the respirometer to spin. The experimental animal in every case became very active and swam or showed other motor activity.

Plate 1: Apparatus used to measure oxygen uptake. M, Oxygen meter; P, BOD probe, R, Respirometer; S, magnetic stirrer.


MODEL 54 OXYGEN METER
YSI
M
ZERO
RED LINE
TEMP
CAL
OFF
0-10
0-20
P
R
S

Oxygen concentration was measured at the beginning of the trial and after 3 minutes on the stirrer. The difference was recorded as the oxygen consumption under active metabolic conditions.

Following this procedure, the respirometer was returned to the aquarium. The crayfish was then allowed to remain undisturbed for 30 minutes. Oxygen concentration readings were then taken at 3 minute intervals (6 minute intervals in the case of oxygen readings at very cold experimental temperatures) until the rate of oxygen decrease in the respirometer was constant. This rate was recorded as the oxygen consumption under resting metabolic conditions.

If the crayfish moved its body, oxygen consumption readings were retaken. As a result, only oxygen consumption at rest was determined.

After the trial, the volume of water in the respirometer was measured. The crayfish was marked on its carapace with nail polish and returned to its original acclimation aquarium. This procedure was repeated for all acclimated crayfish.

It was later suggested that the oxygen consumption of the crayfish at rest should be measured before active metabolism as initial activity of the animal may influence its resting metabolism. Thus in Series II, the animal was allowed to accommodate to the new surroundings for 30 minutes. The oxygen consumption for the resting metabolism was then determined, as previously described, followed by the determination of oxygen consumption under active conditions.

In all trials only crayfish in the intermoult state (Passano, 1960) were used. Sometimes it was not obvious until the animal was killed that

it was in premoult. In these cases, the results from the trials with these animals were rejected.

No trial was used if the concentration of oxygen dropped more than 25% from the saturation level. If this occurred the trial was repeated the next day.

4. Electrophoresis:

a. Introduction:

An excellent review of the theory of electrophoretic methods is given in Smith (1960). Basically electrophoresis depends on the zwitterion property of proteins (Giese, 1962). Hence by adjusting the pH of the solution of proteins either the acidic or basic groups will be neutralized so that the proteins will have a net charge and therfore move in an electric field.

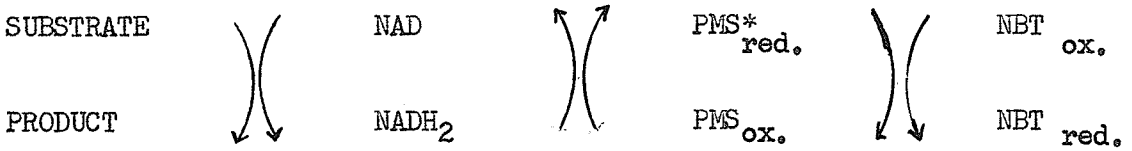
Because proteins vary in the number of constituent amino acids they vary in size. Thus by controlling the concentration of the support material holding the electrolyte solution as well as controlling the pH of the electrolyte, proteins can be separated on the basis of size and charge through an electric field.

Of the numerous support materials used for electrophoresis, polyacrylamide gel provides excellent resolution of proteins (Smith, 1960; Nerenberg, 1966). It was used as the support medium throughout the whole experiment. This material is easy to prepare, handle, store and can be poured into tubes to make cylindrical gels. The sample to be electrophoresed is poured on the top of a vertically arranged gel.

During electrophoresis, the proteins will separate in the form of "discs", giving rise to the term "disc electrophoresis". Disc electrophoresis permits the use of sample volumes larger than those in other methods, as the sample spreads on the top of the gel in a thin layer. As a result, there is little tailing; the end of one disc does not blend into the beginning of another.

Gels and buffers were prepared according to the proportions in Appendix Table B I.

Localization of activity and hence staining of oxido-reductive enzymes is indirectly determined by the reduction of the soluble yellow dye, nitro blue tetrazolium (NBT), to a purple precipitate according to the following scheme:



* PMS: Phenazine methosulphate. NBT can not be directly reduced by $NADH_2$ so that the intermediate compound, PMS is used. Both enzymes assayed here are NAD-dependent

The proportions of compounds used to make the staining solution are given in Appendix Table B II.

b. General procedure:

An acclimated crayfish was killed. The carapace was removed, the hepatopancreas and heart were extracted and placed in chilled tris buffer (0.3M, adjusted to pH 8.5 with conc. HCl). The abdominal muscle was then removed and also placed in buffer. The organs were minced with

scissors in the buffer three times the volume of the material and homogenized with a pyrex handle tissue-mill. The homogenates were then centrifuged in either an International micro-capillary centrifuge Model MB fitted with a head for spinning centrifuge tubes of 1 ml capacity, or in a Coleman micro-centrifuge. Centrifuge tubes used in the Coleman micro-centrifuge were plastic and held a 1 ml solution. The supernatant fluid was removed and added to an equal volume of a chilled 50% sucrose solution containing bromophenol blue. The stain was used to mark protein migration through the gel.

Fifty microliters of the crude extract were pipetted onto the top of the already chilled gels set up in an electrophoretic cell. Electrophoresis of the extract was continued until the bromophenol blue had reached the bottom of the gel. The electrophoresis was carried out in either a refrigerator or a controlled environment room set at 4°C. Afterwards the gels were removed from the cell and stained to locate LDH or a-GDH.

The specific conditions of the trial are listed with the figure presented in the Results and Discussion section.

5. Determination of the Specific Activity of Lactic Dehydrogenase and alpha-Glycerophosphate Dehydrogenase:

a. Introduction:

Procedures for the determination of the specific activities of these enzymes are described in Colowick and Kaplan (1955) and Bergemeyer (1963). The basis for determining the specific activity of NAD-dependent enzymes is that the co-enzymes exhibit an absorption peak at 340 nm in

the reduced state which is not present in the oxidized state. Because the absorbance of a solute is a function of its concentration (Dawes, 1969), and because the extinction coefficient of $NADH_2$ at 340 nm is 6.22×10^6 cm^2/mole, the change in absorbance per minute at 340 nm will indicate the amount of NAD reduced or the amount of $NADH_2$ that is oxidized per minute in a given volume of the reaction solution. For the purpose of this investigation, the specific activity is defined as the amount in micromoles of NAD reduced or $NADH_2$ oxidized per minute per mg protein of crude extract in the reaction volume.

b. Selection of organs:

Muscle and hepatopancreas were selected as the source of the enzymes LDH and a-GDH, because it was suspected that the acclimation temperature might influence the specific activities of these two enzymes in these organs.

c. Preparation of the crude extract:

Preliminary trials indicated that the rates of reactions with crude extracts were adequate to record a change in absorbance accurately. Hence no further purification was attempted.

The crude extracts were prepared as previously described for the electrophoretic procedures. The only modifications made were that: (1) the tissues were homogenized with an electric tissue-mill using a glass mortar and teflon pestle, and (2) the homogenates were centrifuged in an International Refrigerated Centrifuge Model B20 firstly at 8,000 x

g and 2°C for 30 minutes and secondly after removing the residue at 15,000 x g and 2°C for 60 minutes. This procedure provided a clear crude extract solution, and thus avoided violation of Beer's Law (Dawes, 1969).

Of the many methods available for determination of protein concentrations (Clark, 1964), the Biuret test was selected because of its simplicity. As a standard, bovine serum albumin grade V* was used.

d. Determination of lactic dehydrogenase specific activity:

The assay was based on the procedure described by Bergemeyer (1963). The reactants plus concentrations and volumes used in the assay are listed in Appendix Table B III.

The assay was performed at 25°C and the change in absorbance at 340 nm was followed using a Unicam SP 800 spectrophotometer fitted with the Unicam SP820 fixed wavelength accessory and quartz cuvettes of 1 cm depth.

The tissue extract was added to the reactants minus the NAD in a cuvette which was subsequently inserted into the spectrophotometer. After the materials had equilibrated for 5 minutes, the NAD was added. Less than 10 seconds later, recordings of absorbance were made. The reaction was allowed to proceed until the velocity of the reaction began to decrease.

The reference cuvette in the muscle assay was filled with buffer.

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* Sigma Chemical Co., St. Louis, Mo., U.S.A.

In the hepatopancreas assays, the cuvette had equal volumes and final concentrations of the extract solution. This was necessary as the optical density of the crude extract solution of the hepatopancreas was high before the start of the reaction.

3. Determination of a-GDH activity:

The assay was based on the procedure described by Marquardt and Brosemer (1966). In this assay procedure the oxidation of $NADH_2$ was followed because the equilibrium of the reaction is such that the formation of a-glycerophosphate from dihydroxyacetone phosphate is very much favoured rather than the reverse (Baranowski, 1963).

The reactants plus concentrations and volumes used in the assay are listed in Appendix Table B IV.

Except for starting the reaction with $NADH_2$ proceudres described for the LDH assay were used.

f. Formulat for the calculation of specific activity:

The specific activity of an enzyme can be determined by using the equation:*

$$S \ \left(\frac{\text{micro-moles}}{\text{min x mg}}\right) = \frac{\Delta E \times V \times 10^6}{\epsilon \times v \times p \times t \times d}$$

where:
S = specific activity (micro-moles of product formed per minute per mg protein of crude extract)
ΔE = the change in optical density
V = volume of solution in the cuvette (ml)
ϵ = extinction coefficient of the light absorbing substance (cm^2/mole)
v = volume of the extract solution taken for the determination (ml)
p = concentration of protein in the extract solution (mg/ml)
t = time taken for the reaction to produce E (min)
d = light path of the cuvette (cm)

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* See Appendix Table C1

6. Statistical Analysis of the Data:

Since the metabolism of an organism is a function of its weight raised to some power (Literature Review), logarithmic transformation of oxygen uptake values and weights was undertaken. The transformation of the data permitted the use of linear regression analysis (Li, 1964) for determining the value of the exponent for the weight.

Within Series I and II, there are a number of combinations of acclimation temperatures and experimental temperatures. Within each combination, there are two regressions - one regression for the relationship between log oxygen uptake during activity versus log body weight, and one regression for the relationship between log oxygen uptake at rest versus log body weight. Covariant analysis of each pair of regressions was undertaken to determine whether the lines had equal slopes and intercepts.

Among the combinations of acclimation and experimental temperatures, there are at least six regression lines of log oxygen uptake versus log body-weight, half of which are for active metabolism and half for resting metabolism. Therefore, inorder to determine whether the acclimation temperature influences the slopes and intercepts of the regressions at each experimental temperature, multiple covariant analyses of at least three regressions were undertaken. The results of the multiple covariant analysis were used to ascertain the influence of acclimation temperature on the metabolism-weight regressions at each experimental temperature.

Bartlett's test (Li, 1964) was used to determine whether significant differences occurred among the variances of groups of regressions.

Paired *t* tests were undertaken on the data collected during the LDH and a-GDH specific activities determinations. The results of the tests would indicate whether there was a significant different between values for winter- and spring-acclimatized crayfish.

RESULTS AND DISCUSSION

Even though results collected during the project are related, each aspect of temperature acclimation is discussed separately, because of the diverse nature of the results. A resume of the Results and Discussion is presented in the section titled "Summary and Conclusions".

The following apsects of temperature acclimation will be discussed:

(1) The influence of acclimation temperature on the slopes and intercepts of the metabolism-weight regression lines for active and resting metabolism,

(2) Determination of patterns of acclimation during active and resting metabolism by O. virilis of a selected weight, and exposed to different photo-periods,

(3) Determination of patterns of acclimation in the manner as described by Jungreis and Hooper (1968),

(4) Determination of Precht-types, and discussion of Precht-types and acclimation patterns in relation to the life history of O. virilis, and

(5) Determination of specific activities of LDH and a-GDH in muscle and hepatopancreas, and discussion of results in relation to metabolic re-organization in response to cold by C. bartoni.

These aspects will be discussed under the following headings:

1. The influence of temperature on metabolism-weight regressions:

    a. A comparison between slopes of regressions for active and resting metabolism.

    b. A comparison between intercepts of regressions for active and resting metabolism

2. The influence of acclimation temperature and photoperiod on active and resting metabolism of O. virilis:

    a. Subjective interpretation of acclimation patterns

b. Interpretation of acclimation patterns after statistical analysis of oxygen uptake data

c. Acclimation patterns produced in the manner as described by Jungreis and Hooper (1968)

3. Determination of Precht-types:

4. The relation of the results to the life history of O. virilis:

5. Electrophoresis of LDH and a-GDH crude extracts from O. virilis:

a. Significance of results

6. Specific activities of LDH and a-GDH in crude extracts from C. bartoni

a. Significance of results

Figure 4: Metabolism-weight regressions for the active and resting metabolism of Series I Orconectes virilis acclimated to 5°C and exposed to 5, 15 and 25°C. Upper regression represents the active metabolism (AM)- weight relationship. Lower regression represents the resting metabolism (RM)-weight relationship. Crayfish were collected in June, 1969 and exposed to a 24 L, 0 D photoperiod. Each point along the regression represents one animal.

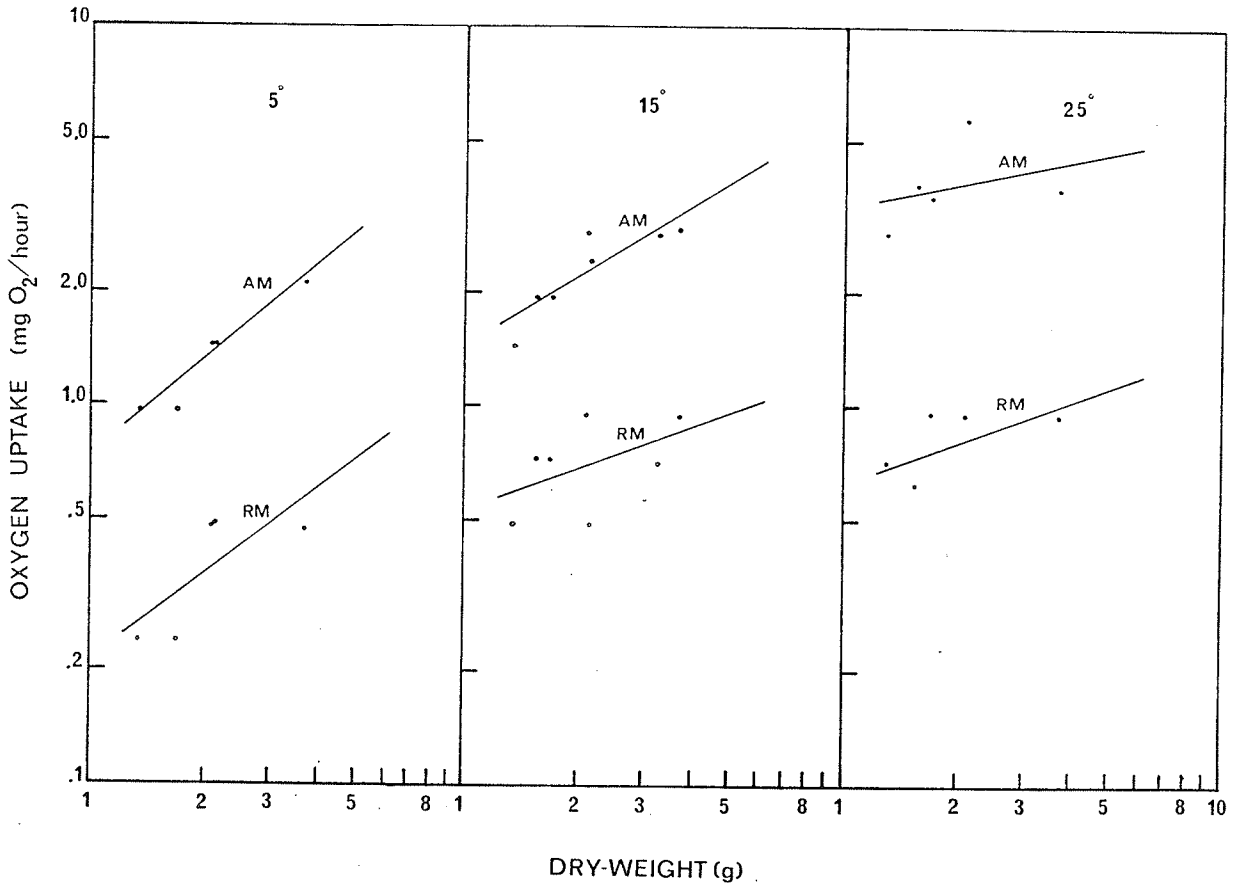

10
5.0
2.0
1.0
.5
.2
.1
OXYGEN UPTAKE (mg O_2/hour)
5°
15°
25°
AM
RM
AM
RM
AM
RM
1
2
3
5
8
1
2
3
5
8
1
2
3
5
8
10
DRY-WEIGHT (g)

TABLE I

Summarized statistical data of Orconectes virilis acclimated to 5°C and exposed to experimental temperature (ET) of 5, 15 and 25°C.

Experimental conditions for the samples	Equation of the regression line Y = A + bX	$S_{Y.X}$	d.f. (n-2)	Chi^2	F-test slopes	F-test intercepts
ET = 5°C						
Active metabolism	Y = 1.86 + 0.85X	0.0021	3	2.22	0.0689 d.f. = 1:6	89.66**
Resting metabolism	Y = 1.33 + 0.75X	0.0154	3			
ET = 15°C						
Active metabolism	Y = 2.16 + 0.85X	0.0039	5	1.61	0.626 d.f. = 1:10	107.9**
Resting metabolism	Y = 1.73 x 0.36X	0.0133	5			
ET = 25°C						
Active metabolism	Y = 2.53 + 0.20X	0.0138	3	0.01	0.114 d.f. = 1:6	90.58**
Resting metabolism	Y = 1.80 + 0.36X	0.0120	3			
Active Metabolism						
ET = 5°C	Y = 1.86 + 0.85X	0.0021	3	2.65	1.20 d.f. = 2:11	43.15**
ET = 15°C	Y = 2.16 + 0.85X	0.0039	5			
ET = 25°C	Y = 2.53 + 0.20X	0.0138	3			
Resting Metabolism						
ET = 5°C	Y = 1.33 + 0.75X	0.0154	3	0.01	0.836 d.f. = 2:11	13.23**
ET = 15°C	Y = 1.73 + 0.36X	0.0133	5			
ET = 25°C	Y = 1.80 + 0.36X	0.0120	3			

Figure 5: Metabolism-weight regressions for the active and resting metabolism of Series I *Orconectes virilis* acclimated to 15° and exposed to 5, 15 and 25°C. Other conditions are as described in Figure 4.

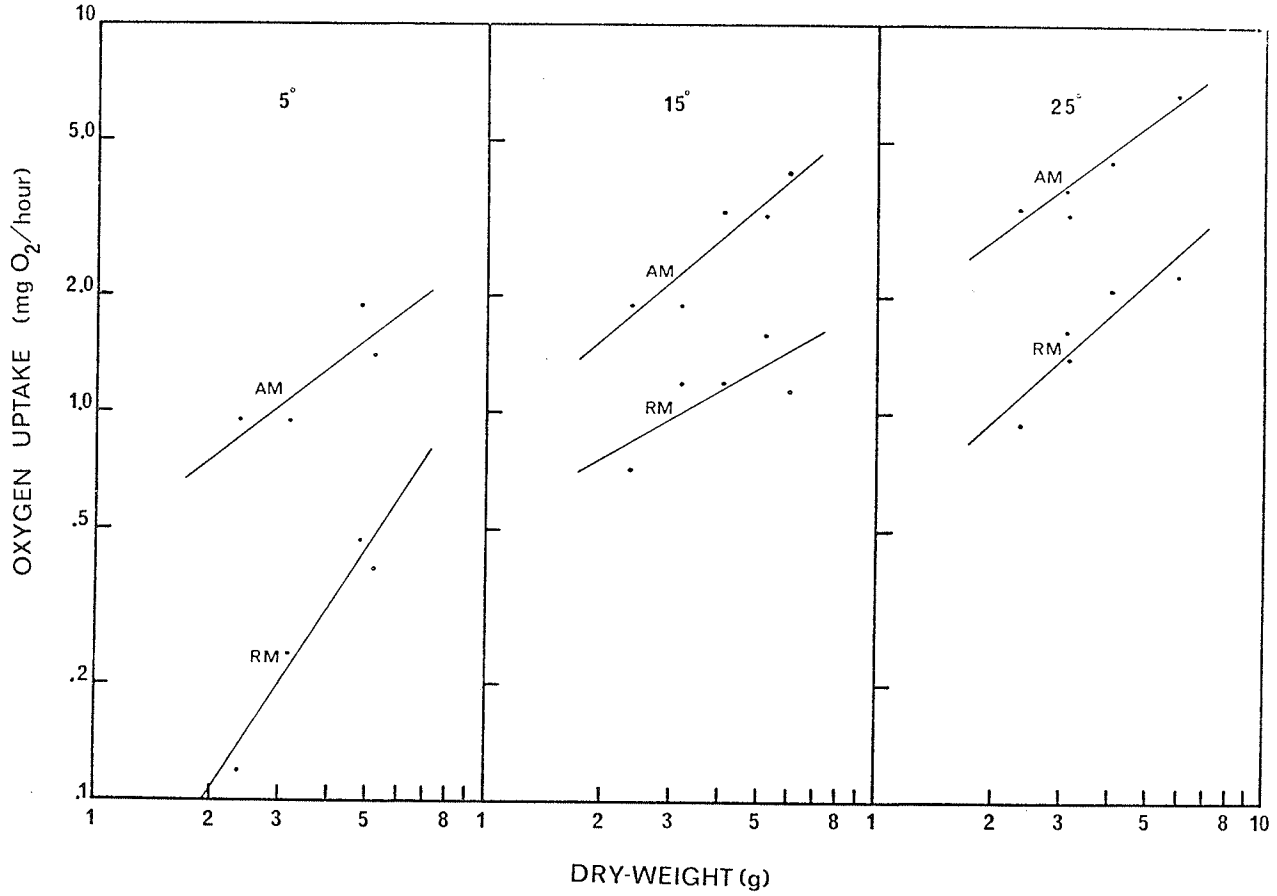

10
5,0
2,0
1,0
.5
.2
.1
OXYGEN UPTAKE (mg O_2/hour)
5°
15°
25°
AM
RM
AM
RM
AM
RM
1
2
3
5
8
1
2
3
5
8
1
2
3
5
8
10
DRY-WEIGHT (g)

TABLE II

Summarized statistical data of Series I Orconectes virilis acclimated to 15°C and exposed to experimental temperatures (ET) of 5, 15 and 25°C.

Experimental conditions for the samples	Equation of the regression line Y = A + bX	$S_{Y.X}$	d.f. (n-2)	Chi^2	F-test slopes	F-test intercepts
ET = 5°C						
Active metabolism	Y = 1.64 + 0.78X	0.0070	3	0.04	4.00 d.f. = 1:6	170.0**
Resting metabolism	Y = 0.56 + 1.56X	0.0054	3			
ET = 15°C						
Active metabolism	Y = 1.93 + 0.86X	0.0041	3	0.43	0.667 d.f. = 1:6	54.91**
Resting metabolism	Y = 1.71 + 0.57X	0.0094	3			
ET = 25°C						
Active metabolism	Y = 2.22 + 0.75X	0.0028	3	0.24	0.307 d.f. = 1:6	117.1**
Resting metabolism	Y = 1.70 + 0.92X	0.0052	3			
Active metabolism						
ET = 5°C	Y = 1.64 + 0.78X	0.0070	3	0.55	0.075 d.f. = 2:9	85.54**
ET = 15°C	Y = 1.93 + 0.86X	0.0041	3			
ET = 25°C	Y = 2.22 + 0.75X	0.0028	3			
Resting Metabolism						
ET = 5°C	Y = 0.56 + 1.56X	0.0054	3	0.29	3.406 d.f. = 2:9	122.6**
ET = 15°C	Y = 1.71 + 0.57X	0.0094	3			
ET = 25°C	Y = 1.70 + 0.92X	0.0052	3			

Figure 6: Metabolism-weight regressions for the active and resting metabolism of the Series I Orconectes virilis acclimated to 25°C and exposed to 5, 15 and 25°C. Other conditions are as described in Figure 4.

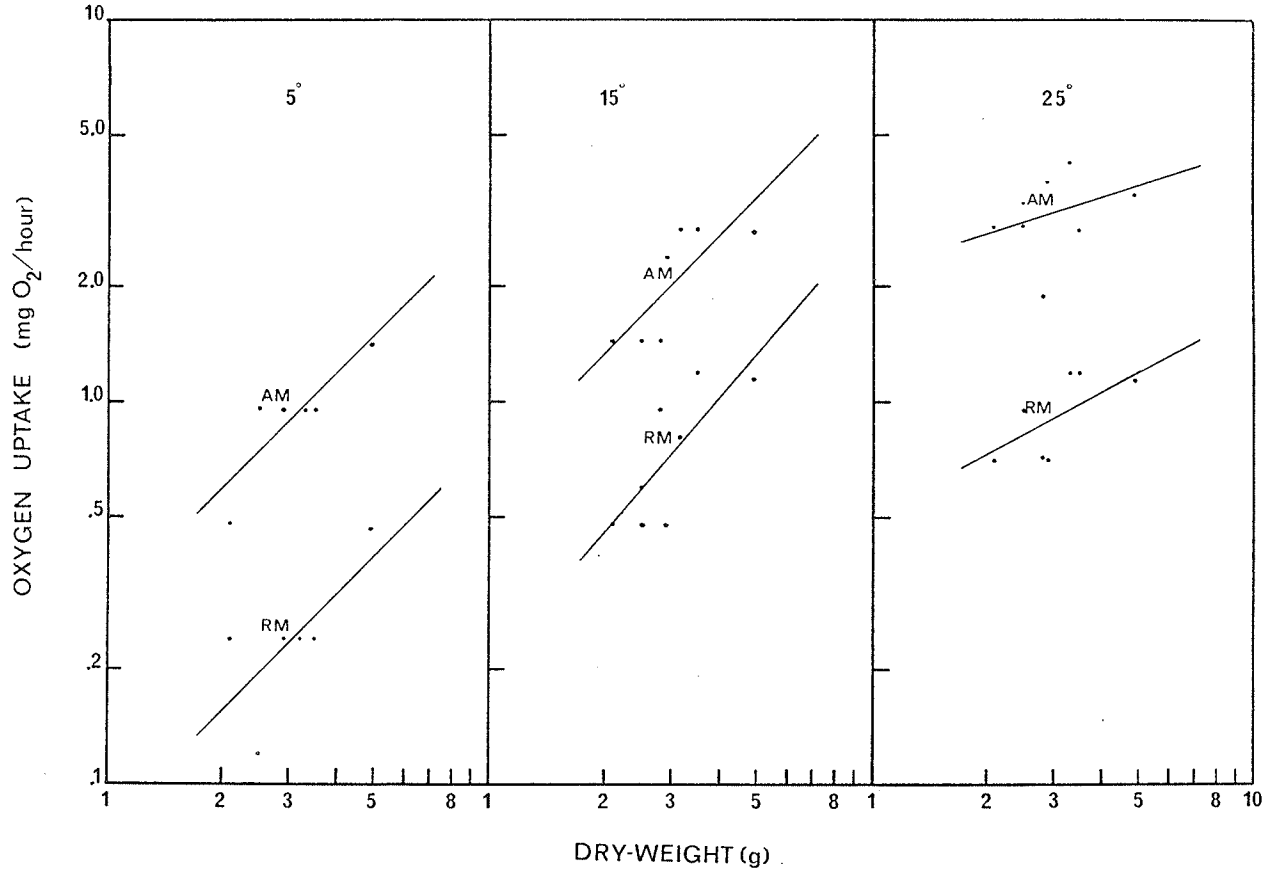

10
5.0
2.0
1.0
.5
.2
.1
OXYGEN UPTAKE (mg O_2/hour)
5°
15°
25°
AM
RM
AM
RM
AM
RM
1
2
3
5
8
1
2
3
5
8
1
2
3
5
8
10
DRY-WEIGHT (g)

TABLE III

Summarized statistical data of Series I Orconectes virilis acclimated to 25°C and exposed to experimental temperatures (ET) of 5, 15 and 25°C.

Experimental conditions for the samples	Equation of the regression line Y = A + bX	$S_{Y.X}$	d.f. (n-2)	Chi^2	F-test slopes	F-test intercepts
ET = 5°C						
Active metabolism	Y = 1.47 + 1.01X	0.0073	4	0.93	0.000 d.f. = 1:8	75.23**
Resting metabolism	Y = 0.88 + 1.02X	0.0207	4			
ET = 15°C						
Active metabolism	Y = 1.81 + 1.03X	0.0101	6	0.12	0.075 d.f. = 1:12	65.28**
Resting metabolism	Y = 1.30 + 1.17X	0.0135	6			
ET = 25°C						
Active metabolism	Y = 2.35 + 0.31X	0.0144	7	0.23	0.267 d.f. = 1:14	110.8**
Resting metabolism	Y = 1.70 + 0.55X	0.0078	7			
Active Metabolism						
ET = 5°C	Y = 1.47 + 1.01X	0.0073	4	0.21	1.480 d.f. = 2:17	56.12**
ET = 15°C	Y = 1.81 + 1.03X	0.0101	6			
ET = 25°C	Y = 2.35 + 0.31X	0.0114	7			
Resting Metabolism						
ET = 5°C	Y = 0.88 + 1.02X	0.0207	4	1.18	0.734 d.f. = 2:17	56.12**
ET = 15°C	Y = 1.30 + 1.17X	0.0135	6			
ET = 25°C	Y = 1.70 + 0.55X	0.0078	7			

Figure 7: Metabolism-weight regressions for the active and resting metabolism of Series II Orconectes virilis acclimated to 4°C and exposed to 4, 9, 12.5 and 25°C. Upper regression represents the active metabolism (AM)-weight relationship. Lower regression represents the resting metabolism (RM)-weight relationship. Crayfish were collected in October, 1969 and exposed to an 8 L, 16 D photoperiod. Each point along the regression represents an animal.

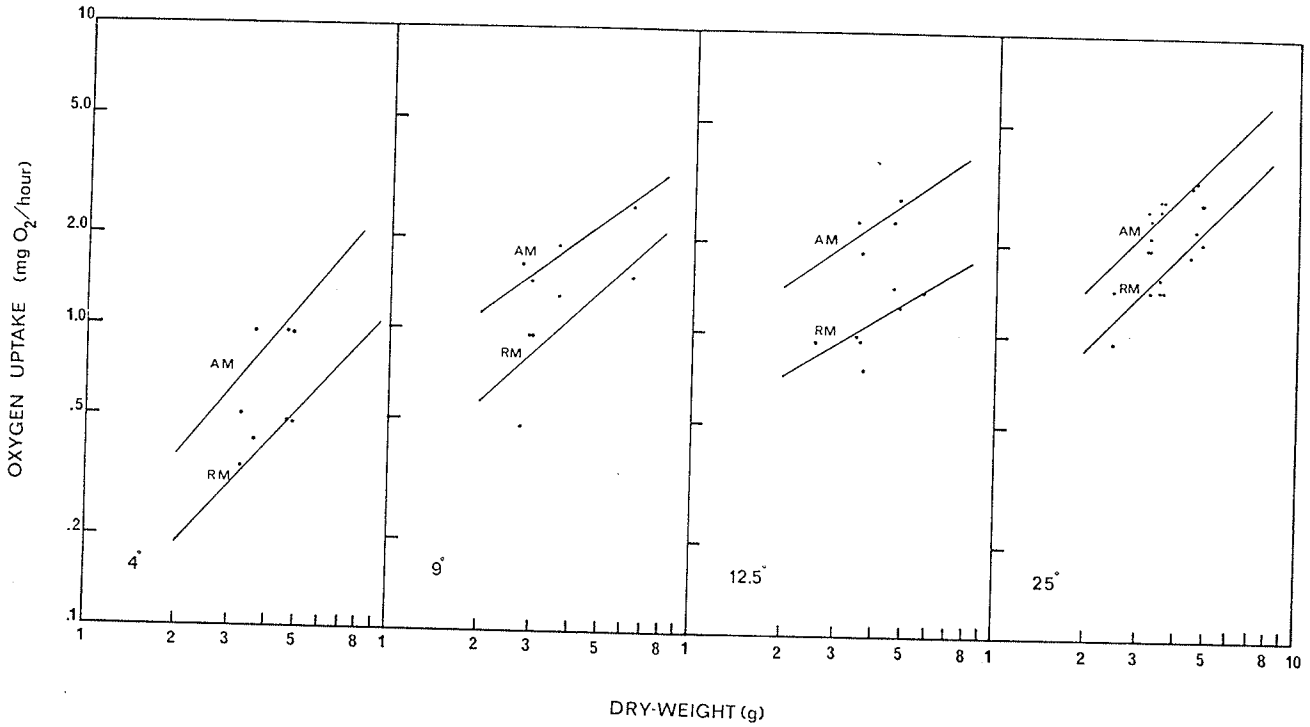

OXYGEN UPTAKE (mg O_2/hour)
10
5.0
2.0
1.0
.5
.2
.1
AM
RM
4°
9°
12.5°
25°
1
2
3
5
8
10
DRY-WEIGHT (g)

TABLE IV

Summarized statistical data of Series II Orconectes virilis acclimated to 4°C and exposed to experimental temperatures (ET) of 4, 9, 12.5 and 25°C

Experimental conditions for the samples	Equation of the regression line Y = A + bX	$S_{Y.X}$	d.f. (n-2)	Chi^2	F-test slopes	F-test intercepts
ET = 4°C						
Active metabolism	Y = 1.19 + 1.20X	0.0135	2	1.64	0.039 d.f. = 1:4	26.60**
Resting metabolism	Y = 0.94 + 1.09X	0.0015	2			
ET = 9°C						
Active metabolism	Y = 1.76 + 0.82X	0.0124	3	0.21	0.037 d.f. = 1:6	6.95**
Resting metabolism	Y = 1.47 + 0.94X	0.0220	3			
ET = 12.5°C						
Active metabolism	Y = 1.94 + 0.71X	0.0035	2	0.05	0.020 d.f. = 1:7	54.00**
Resting metabolism	Y = 1.65 + 0.99X	0.0057	5			
ET = 25°C						
Active metabolism	Y = 1.87 + 1.00X	0.0034	8	0.38	0.000 d.f. = 1:16	41.76**
Resting metabolism	Y = 1.67 + 0.99X	0.0054	8			
Active Metabolism						
ET = 4°C	Y = 1.19 + 1.20X	0.0035	2	2.73	0.179 d.f. = 3:15	40.99**
ET = 9°C	Y = 1.76 + 0.82X	0.0124	3			
ET = 12.5°C	Y = 1.94 + 0.71X	0.0035	2			
ET = 25°C	Y = 1.87 + 1.00X	0.0034	8			
Resting Metabolism						
ET = 4°C	Y = 1.19 + 1.20X	0.0035	2	2.73	0.179 d.f. = 3:15	40.99**
ET = 9°C	Y = 1.76 + 0.82X	0.0124	3			
ET = 12.5°C	Y = 1.94 + 0.71X	0.0035	2			
ET = 25°C	Y = 1.87 + 1.00X	0.0034	8			

Figure 8: Metabolism-weight regressions for the active and standard metabolism of the Series II Orconectes virilis acclimated to 9°C and exposed to 9, 12.5 and 25°C. Other conditions are described in Figure 7.

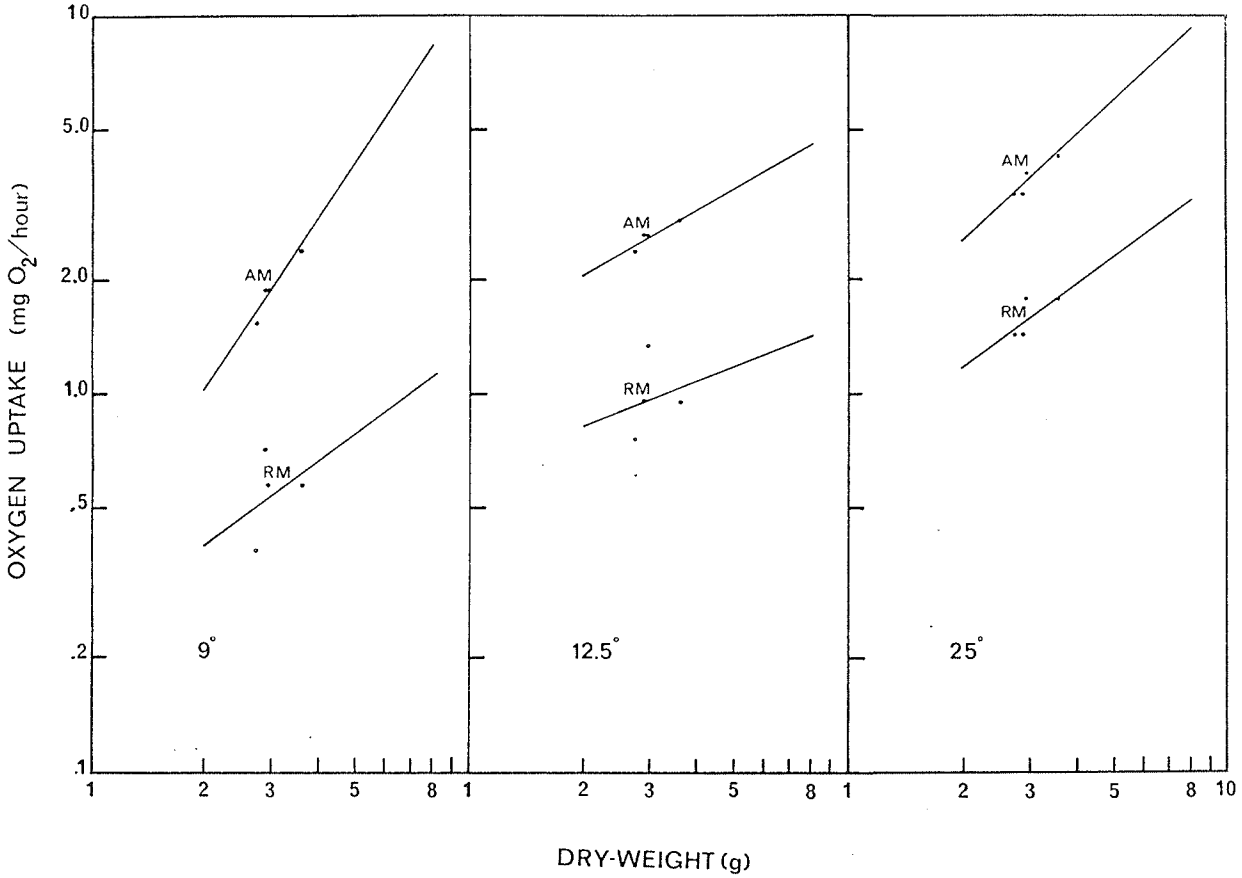

OXYGEN UPTAKE (mg O_2/hour)
10
5.0
2.0
1.0
.5
.2
.1
AM
RM
9°
12.5°
25°
1
2
3
5
8
10
DRY-WEIGHT (g)

TABLE V

Summarized statistical data of Series II Orconectes virilis acclimated to 9°C and exposed to experimental temperatures (ET) of 9, 12.5 and 25°C.

Experimental conditions of the samples	Equation of the regression line Y = A + bX	$S_{Y.X}$	d.f. (n-2)	Chi^2	F-test slopes	F-test intercepts
ET = 9°C						
Active metabolism	Y = 1.87 + 0.66X	0.0009	2	2.67	0.019	45.44**
Resting metabolism	Y = 1.39 + 0.73X	0.0172	2		d.f. = 1:4	
ET = 12.5°C						
Active metabolism	Y = 2.15 + 0.57X	0.0002	2	4.81	0.035	48.00**
Resting metabolism	Y = 1.80 + 0.39X	0.0158	2		d.f. = 1:4	
ET = 25°C						
Active metabolism	Y = 2.12 + 0.93X	0.0007	2	0.460	0.377	185.0**
Resting metabolism	Y = 1.86 + 0.72X	0.0021	2		d.f. = 1:4	
Active Metabolism						
ET = 9°C	Y = 1.87 + 0.66X	0.0009	2	1.24	2.59	116.8**
ET = 12.5°C	Y = 2.15 + 0.57X	0.0002	2		d.f. = 2:6	
ET = 25°C	Y = 2.12 + 0.93X	0.0007	2			
Resting Metabolism						
ET = 9°C	Y = 1.39 + 0.73X	0.0172	2	1.69	0.033	18.40**
ET = 12.5°C	Y = 1.80 + 0.39X	0.0158	2		d.f. = 2:6	
ET = 25°C	Y = 1.86 + 0.72X	0.0021	2			

Figure 9: Metabolism-weight regressions for the active and standard metabolism of the Series II *Orconectes virilis* acclimated to 12.5°C and exposed to 4, 12.5 and 25°C. Other conditions are as described in Figure 7.

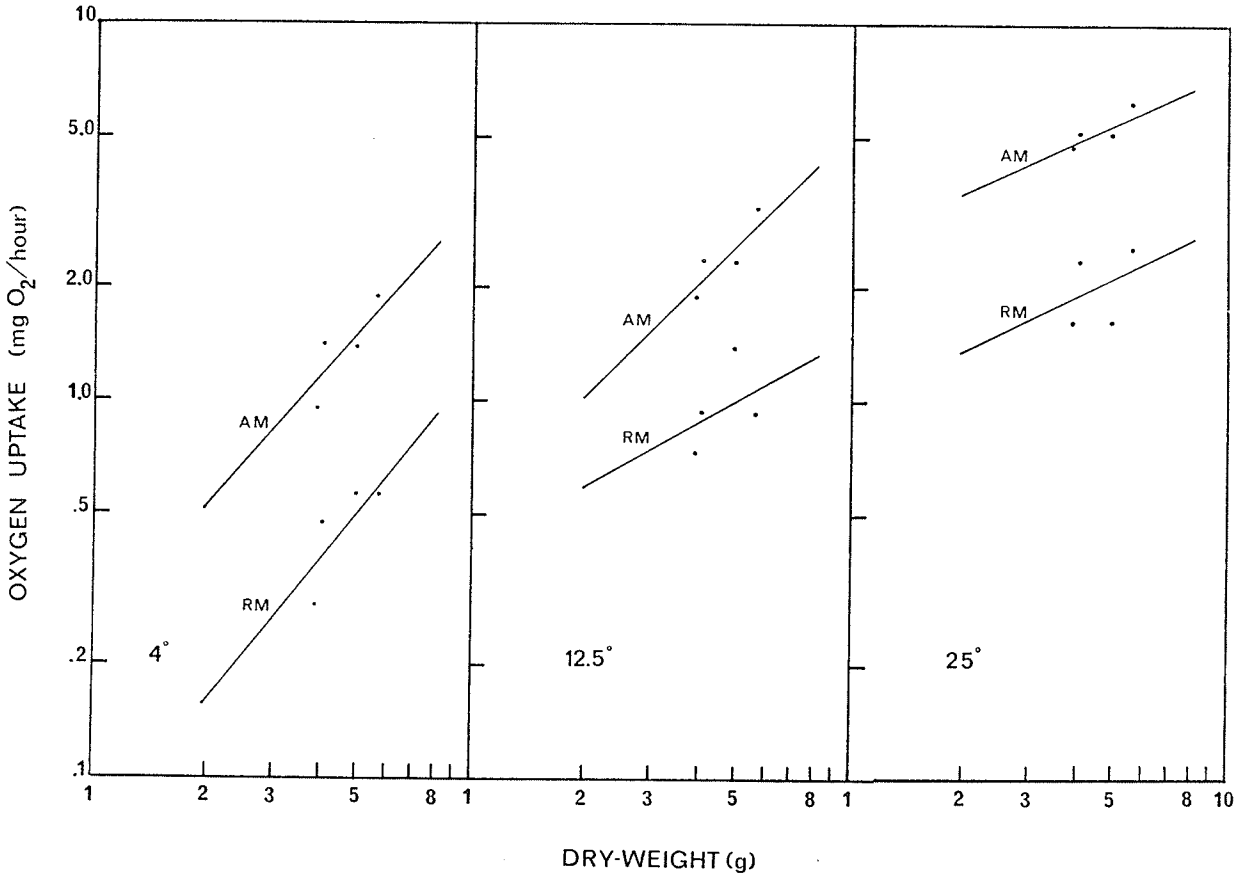

OXYGEN UPTAKE (mg O_2/hour)
10
5.0
2.0
1.0
.5
.2
.1
AM
RM
4°
12.5°
25°
1
2
3
5
8
10
DRY-WEIGHT (g)

TABLE VI

Summarized statistical data of Series II Orconectes virilis acclimated to 12.5°C and exposed to experimental temperatures (ET) of 4, 12.5 and 25°C.

Experimental conditions of the samples	Equation of the regression line Y = A + bX	$S_{Y.X}$	d.f. (n-2)	Chi^2	F-test slopes	F-test intercepts
ET = 4°C						
Active metabolism	Y = 1.37 + 1.15X	0.0064	2	0.18	0.018 d.f. = 1:4	47.31**
Resting metabolism	Y = 0.82 + 1.27X	0.0187	2			
ET = 12.5°C						
Active metabolism	Y = 1.72 + 1.00X	0.0027	2	1.31	0.225 d.f. = 1:4	29.60**
Resting metabolism	Y = 1.62 + 0.56X	0.0187	2			
ET = 25°C						
Active metabolism	Y = 2.42 + 0.46X	0.0006	2	2.79	0.006 d.f. = 1:4	53.90**
Resting metabolism	Y = 1.96 + 0.52X	0.0124	2			
Active Metabolism						
ET = 4°C	Y = 1.37 + 1.15X	0.0064	2	1.93	1.910 d.f. = 2:6	108.0**
ET = 12.5°C	Y = 1.72 + 1.00X	0.0027	2			
ET = 25°C	Y = 2.42 + 0.46X	0.0006	2			
Resting Metabolism						
ET = 4°C	Y = 0.82 + 1.27X	0.0187	2	0.09	0.582 d.f. = 2:6	28.14**
ET = 12.5°C	Y = 1.62 + 0.56X	0.0187	2			
ET = 25°C	Y = 1.96 + 0.52X	0.0124	2			

Figure 10: Metabolism-weight regressions for the active and standard metabolism of the Series II Orconectes virilis acclimated to 25°C and exposed to 4, 9, 12.5 and 25°C. Other conditions are as described in Figure 7.

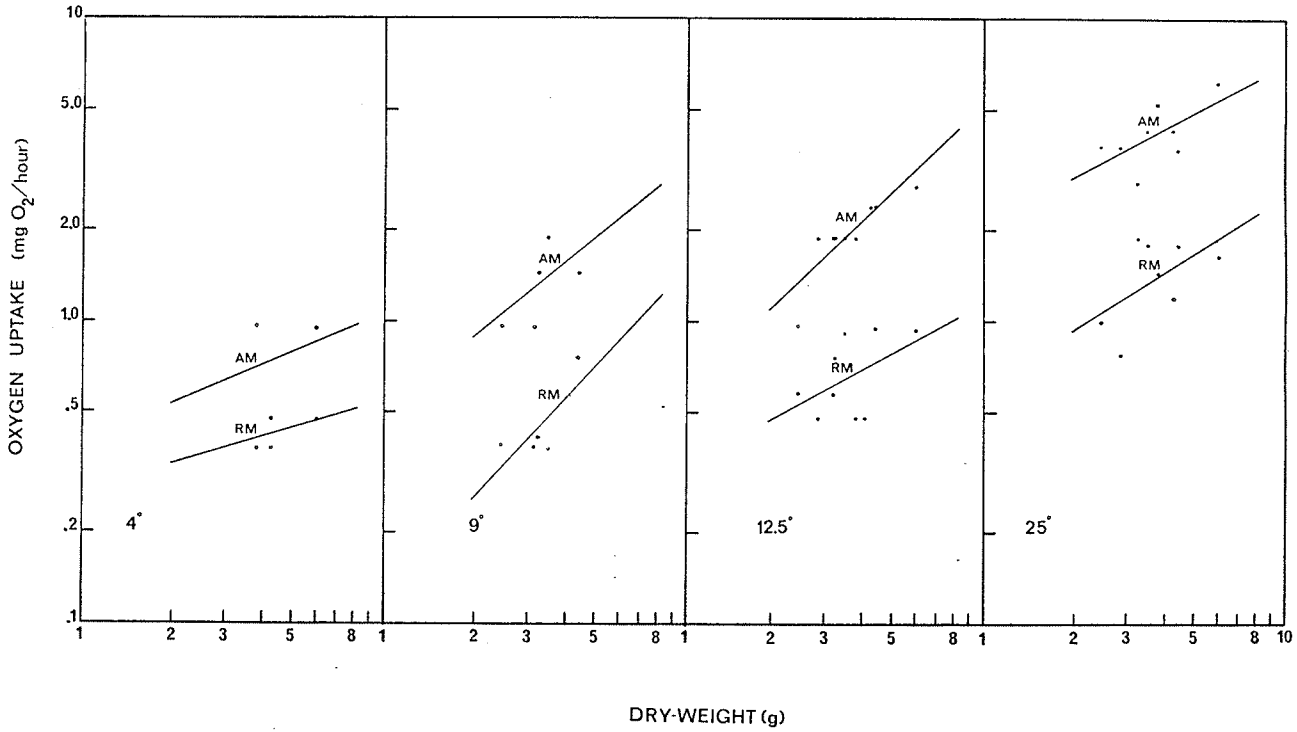

OXYGEN UPTAKE (mg O_2/hour)
10
5.0
2.0
1.0
.5
.2
.1
AM
RM
4°
9°
12.5°
25°
1
2
3
5
8
10
DRY-WEIGHT (g)

TABLE VII

Summarized statistical data of Series II Orconectes virilis acclimated to 25°C and exposed to experimental temperatures (ET) of 4, 9, 12.5 and 25°C.

Experimental conditions of the samples	Equation of the regression line Y = A + bX	$S_{Y.X}$	d.f. (n-2)	Chi^2	F-test slopes	F-test intercepts
ET = 4°C						
Active metabolism	Y = 1.60 + 0.42X	0.0562	1	1.23	0.007	3.13
					d.f. = 1:2	
Resting metabolism	Y = 1.44 + 0.29X	0.0024	1			N.S.
ET = 9°C						
Active metabolism	Y = 1.68 + 0.84X	0.0143	3	0.130	0.042	44.99**
					d.f. = 1:6	
Resting metabolism	Y = 1.10 + 1.08X	0.0091	3			
ET = 12.5°C						
Active metabolism	Y = 1.75 + 0.96X	0.0053	7	1.98	0.880	90.90**
					d.f. = 1:14	
Resting metabolism	Y = 1.52 + 0.53X	0.0164	7			
ET = 25°C						
Active metabolism	Y = 2.33 + 0.52X	0.0067	6	1.10	0.051	10.20**
					d.f. = 1:12	
Resting metabolism	Y = 1.79 + 0.63X	0.0167	6			
Active Metabolism						
ET = 4°C	Y = 1.60 + 0.42X	0.0562	1	3.97	0.377	11.42**
ET = 9°C	Y = 1.68 + 0.84X	0.0143	3		d.f. = 3:17	
ET = 12.5°C	Y = 1.75 + 0.96X	0.0053	7			
ET = 25°C	Y = 2.33 + 0.52X	0.0164	6			
Resting Metabolism						
ET = 4°C	Y = 1.44 + 0.29X	0.0024	1	1.14	0.227	17.94**
ET = 9°C	Y = 1.10 + 1.08X	0.0091	3		d.f. = 3:17	
ET = 12.5°C	Y = 1.52 + 0.53X	0.0164	7			
ET = 25°C	Y = 1.79 + 0.63X	0.0167	6			

1. The Influence of Temperature on Metabolism-Weight Regressions.

The relationship between metabolism and the weight of whole crayfish acclimated at and exposed to various temperatures is shown in Figures 4 - 10. Figures 4 - 6 show the relationship for crayfish collected in June, (Series I), exposed to a 24 hour light photoperiod, and acclimated at 5 (Fig. 4), 15 (Fig. 5) and 25°C (Fig. 6). Figures 7 - 10 show the relationship for crayfish collected in October (Series II), exposed to a photoperiod of 8 hours light and 16 hours darkness, and acclimated at 4 (Fig. 7),9 (Fig. 8), 12.5 (Fig. 9) and 25°C (Fig. 10). The data shown by the upper set of points in Figures 4 - 10 represent oxygen consumption when the crayfish were active. The data represented by the lower set of points in the same figures represent oxygen consumption when the crayfish were quiet. Regression lines for the upper and lower set of points in Figures 4 - 10 have been fitted by the method of least squares (Li, 1964). The equation of the regression lines shown in Figures 4 - 10 are listed in Tables I - VII respectively. The regression lines in Figures 4 - 10 have been extrapolated beyond the available data. However, in no case were values along these regression lines outside the available data used in any analysis.

The relationship between metabolism and the weight of a whole crayfish can be expressed in the equation

$$QO_2 = aW^{\underline{b}},$$

where QO_2 is the oxygen consumption per unit time of a crayfish, where W is its weight, and where $\underline{a}$ and $\underline{b}$ are coefficients in the equation

(Zeuthen, 1953).

Equation 1 can be expressed in the logarithmic form

$$Y = A + bX,$$

where Y is log QO_2, A is log 100a, X is log W, and where b is equal to the exponent b in equation 1. Therefore, the values for the slopes of the regressions listed in Tables I - VII are estimates of the exponents in the equations expressing the relationship between metabolism of an organism and its weight.

After inspecting Figures 4 - 10 it becomes apparent that: (1) at any one test and acclimation temperature, metabolism increases with weight - both for active and resting metabolism, (2) the relationship between log metabolism and log weight can be represented by a straight line, (3) oxygen uptake during activity is greater than oxygen uptake at rest, (4) the slopes of the regressions for active and resting metabolism do not always appear to be parallel at every experimental temperature, and (5) at any one acclimation temperature, both active and resting metabolism increase as the experimental temperature and weight increases.

a. A comparison between slopes of regressions for active and resting metabolism:

Results from Bartlett's test for a comparison of the variances of the regressions of active and resting metabolism at every experimental temperature were not significant at the 5% level of confidence. These results are listed in Tables I - VII along with the variances of the regressions.

Results from covariant analyses of the regressions of active and resting metabolism (Tables I - VII), indicate that at every experimental temperature the slopes of the regressions, and therefore the exponent b are not significantly different at the 5% confidence interval. These results indicate, therefore, that the relationship between metabolism of a crayfish and its weight is independent of its condition of activity.

This conclusion is in agreement with the one presented by Hemmingson (1960), who concluded from his analysis on the relationship between metabolism of an organism and its weight that the value of the exponent b was independent of the activity of organisms as phyletically diverse as dogs and flies.

Covariant analyses of the regressions of active metabolism for O. virilis acclimated at 5, 15, 25°C (Figs. 4 - 6 respectively, Tables I - III respectively) and 4, 9, 12.5 and 25°C (Figs. 7 - 10 and Tables IV - VII respectively) indicate that these is no significant change in the value of the exponent b when O. virilis is exposed to different experimental temperatures. Similar covariant analyses of the regressions for resting metabolism also indicated that there was no change in b. However, many of the figures, especially Figures 4, 6 and 9 show that the slopes of the curves become less steep with increasing experimental temperatures. This phenomenon is quite well known, and has been reported to occur in other crustacea (Rao and Bullock, 1954), bivalves (Read, 1962), gastropods (Davies, 1966) and fish (Barlow, 1961; Wohlschlag et al, 1968). The explanation is that the smaller individuals of the species are more temperature sensitive (have higher Q_{10} values) than are larger individuals.

For example, the Q_{10} of active metabolism between 5 and 15°C, and 15 and 25°C of a 1.25 g crayfish acclimated to 5°C (Fig. 4) is approximately 2, while the Q_{10} under the same experimental conditions of a 3.5 g crayfish is approximately 1.5. Possibly if larger sample sizes were used and the scatter of the points about the metabolism-weight regressions was decreased then significant differences between slopes might become apparent.

The value of the exponent b of the regressions for active and resting metabolism at all acclimation temperatures ranged from a low of 0.31 (Table III) to a high of 1.20 (Table IV). The average value of b was found to be 0.73. Davison (1956) found that the value of the exponent for Procambarus alleni acclimated to 25°C was 0.76. The value found for O. virilis acclimated to 25°C ranged from 0.31 to 0.63. Probably the value of b for O. virilis acclimated to 25°C was under estimated in the project because the range of weight was too small to calculate an accurate estimate (Zeuthen, 1953).

b. A comparison between Y intercepts of regressions for active and resting metabolism:

In all cases but one, covariant analyses of the regressions indicated that the intercepts of the metabolism-weight lines for active and resting metabolism (Figs. 4 - 10) are significantly different. The exceptional case, results of crayfish acclimated at 25°C, exposed to an eight hour light period and to a 4°C experimental temperature (Fig. 10, Table VII) may be due to a very small sample size (n = 6). In all other trials, there was a significant difference at the 5% level

of confidence between the intercepts of regressions for both active and resting metabolism (Tables I - VII).

Since there is a difference between active and resting metabolism at all experimental temperatures but one, the influence of acclimation temperature on oxygen uptake during activity and rest of O. virilis can be treated separately. The outlying case already mentioned will be treated as though there is a difference between the intercepts of active and resting metabolism-weight regressions for the sake of convenience.

2. Influence of Acclimation Temperature and Photoperiod on Active and Resting Metabolism.

The results have so far indicated that (1) the value of the exponent b probably decreases with increasing experimental temperatures, and (2) the value of the Y intercept is different for the regressions of active and resting metabolism. The question remains as to whether acclimation temperature influences the value of the Y intercepts of the regressions for either active or resting metabolism. One method for visualizing the data to determine if acclimation temperature influences the value of the Y intercepts at various experimental temperatures is to select from each regression, representing the relationship between active or resting metabolism and weight, the oxygen uptake values of an organism of an arbitrarily selected weight, acclimated at a particular temperature and exposed to a series of experimental temperatures. These values could then be used to plot a metabolism-temperature (M-T) curve of an animal acclimated at a particular temperature and tested

at a series of others (Prosser, 1958; Newell and Northcroft, 1967). For the purpose of this project the selected weight for the determination of the oxygen uptake values of Series I crayfish is the mean weight of that series, 3.04 g. The mean weight and hence selected weight for the determination of the oxygen uptake values for Series II crayfish is 3.88 g.

The oxygen uptake values of O. virilis collected in June and October and exposed to the previously mentioned experimental conditions are listed in Tables VIII and IX respectively. The metabolism-temperature curves based on the oxygen uptake values of Table VIII are shown in Figure 11. The M-T curves based on the oxygen uptake values of Table IX are shown in Figures 12 and 13 for active and resting metabolism respectively.

a. Subjective interpretation of the acclimation patterns for active and resting metabolism.

For the purposes of this presentation the direction of translation and rotation of M-T curves will be made in reference to the curve representing oxygen uptake versus temperature of the organism acclimated to the highest temperature. With this point in mind, the following interpretations of the M-T curves can be made.

The patterns of acclimation of the crayfish collected in June and exposed to a 24 hour light photoperiod will be discussed first. The M-T curve of active metabolism of crayfish acclimated to 5°C (Fig. 11) displays translation up at all three test temperatures. Clockwise rotation of the curve occurs between 5 and 15°C, but not between

TABLE VIII

Oxygen uptake values (mg O_2/ hr per organism) during active metabolism (AM) and resting metabolism (RM) of Orconectes virilis in Series I weighing 3.04 g (dry-weight). Values are derived from equations listed in Tables IV - VI .

Acclimation Temp. (°C)	Experimental Temperature (°C) 5		15		25	
	AM	RM	AM	RM	AM	RM
5	1.84	0.487	2.86	0.796	4.26	0.949
15	1.04	0.206	2.22	0.980	3.81	1.40
25	0.899	0.238	2.04	0.723	3.16	0.963

TABLE IX

Oxygen uptake values (mg O_2/ hr per organism) during active metabolism resting metabolism (RM) of Orconectes virilis in Series II weighing 3.88 g (dry-weight. Values are derived from equations listed in Tables VII - X.

Acclimation Temp. (°C)	Experimental Temperature (°C) 4		9		12.5		25	
	AM	RM	AM	RM	AM	RM	AM	RM
4	0.791	0.383	1.75	1.07	2.29	1.07	2.83	2.78
9			1.83	0.659	3.04	1.06	4.64	1.91
12.5	0.983	0.368			2.04	0.883	4.89	1.85
25	0.704	0.399	1.49	0.542	2.08	0.686	4.30	1.44

Figure 11: Metabolism-Temperature curves for active (solid points) and resting (open points) of Orconectes virilis in Series I. Acclimation temperatures are 5, 15, and 25°C. Crayfish were exposed to temperatures of 5, 15 and 25°C. Weights of the crayfish are 3.04 g. Values of oxygen uptake are listed in Table XVIII.

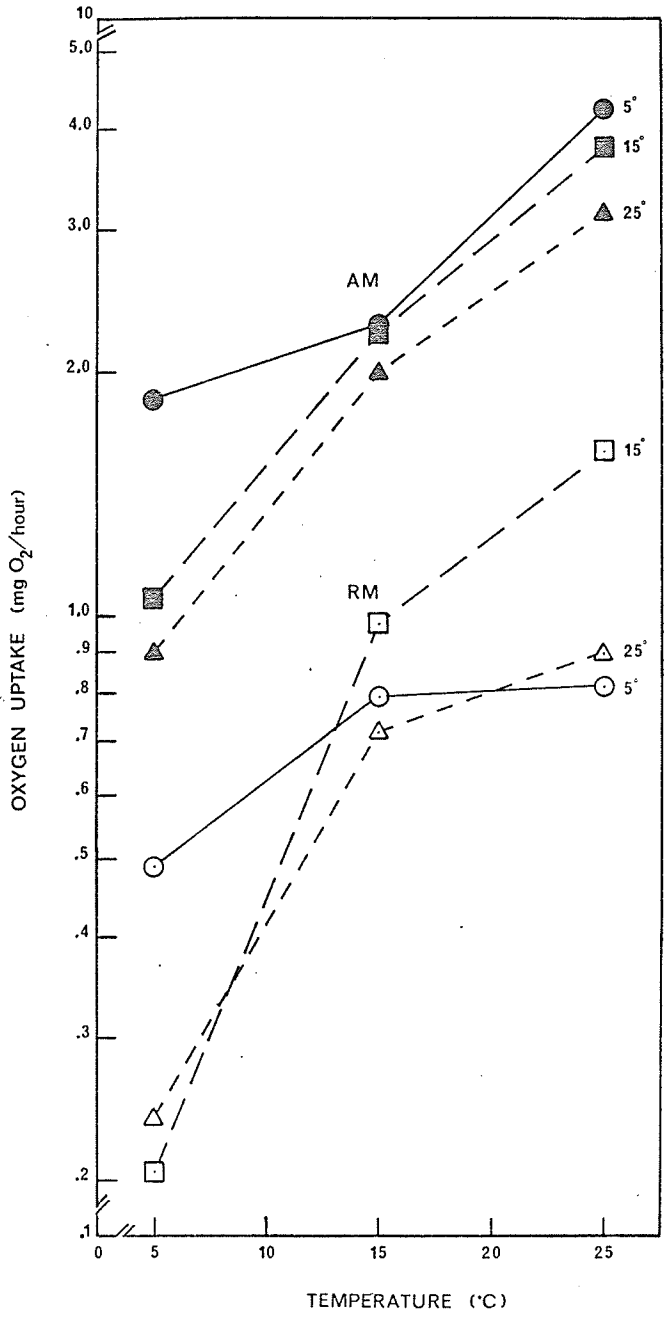

10
5.0
4.0
3.0
2.0
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
5
10
15
20
25
OXYGEN UPTAKE (mg O_2/hour)
TEMPERATURE (°C)
AM
RM
5°
15°
25°
15°
25°
5°

TABLE X

Summarized statistical data derived from multiple covariant analyses of metabolism-weight regressions of Series I Orconectes virilis. Acclimation temperatures (AT) are 5, 15 and 25°C and experimental temperatures are (ET) of 5, 15 and 25°C.

Experimental conditions of the samples	Equation of the regression line Y = A + bX	$S_{Y.X}$	d.f. (n-2)	Chi^2	F-test slopes	F-test intercepts
Active Metabolism						
ET = 5°C						
AT = 5°C	Y = 1.86 + 0.85X	0.0021	3	1.15	0.196	15.89**
AT = 15°C	Y = 1.64 + 0.78X	0.0070	3		d.f. = 2:10	
AT = 25°C	Y = 1.47 + 1.01X	0.0073	4			
ET = 15°C						
AT = 5°C	Y = 2.16 + 0.62X	0.0039	5	1.36	0.744	5.25*
AT = 15°C	Y = 1.93 + 0.86X	0.0041	13		d.f. = 2:14	
AT = 25°C	Y = 1.81 + 1.03X	0.0101	6			
ET = 25°C						
AT = 5°C	Y = 2.53 + 0.20X	0.0138	3	1.68	0.900	1.81
AT = 15°C	Y = 2.22 + 0.75X	0.0028	3		d.f. = 2:13	
AT = 25°C	Y = 2.35 + 0.31X	0.0114	7			N.S.
Resting Metabolism						
ET = 5°C						
AT = 5°C	Y = 1.32 + 0.75X	0.0154	3	1.21	1.05	8.94**
AT = 15°C	Y = 0.56 + 1.56X	0.0054	3)1		d.f. = 2:10	
AT = 25°C	Y = 0.88 + 1.02	0.0207	4)1			
ET = 15°C						
AT = 5°C	Y = 1.73 + 0.36X	0.0133	5	0.13	1.50	1.71
AT = 15°C	Y = 1.71 + 0.57X	0.0094	3		d.f. = 2:14	
AT = 25°C	Y = 1.30 + 1.17X	0.0135	6			N.S.
ET = 25°C						
AT = 5°C	Y = 1.803 + 0.36X	0.0120	3)1	0.46	0.980	4.15*
AT = 15°C	Y = 1.703 + 0.92X	0.0052	3		d.f. = 2:13	
AT = 25°C	Y = 1.696 + 0.55X	0.0078	7)			

*

1: There is no significant difference between the intercepts of these pair of regressions. F-equals 1.43 (d.f. = 1:7) and 2.17 (d.f. = 1:10) respectively.

Figure 12: Metabolism-Temperature curves for active Orconectes virilis in Series II. Acclimation temperatures are; 4, 9, 12.5 and 25°C. Weights of the crayfish are 3.88 g. Values of oxygen uptake are listed in Table IX.

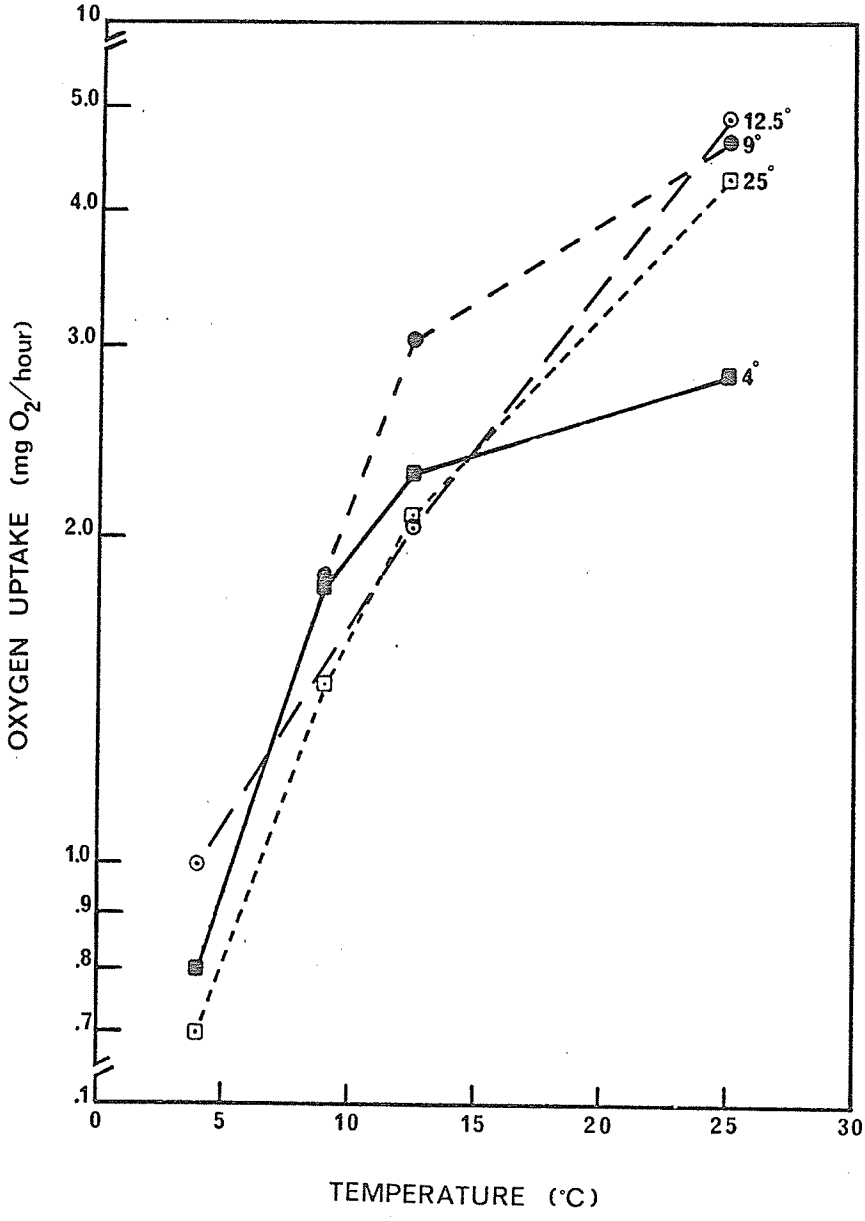

10
5.0
4.0
3.0
2.0
1.0
.9
.8
.7
.1
0
5
10
15
20
25
30
OXYGEN UPTAKE (mg O_2/hour)
TEMPERATURE (°C)
12.5°
9°
25°
4°

TABLE XI

Summarized statistical data derived from multiple covariant analyses of active metabolism-weight regressions of Series II Orconectes virilis. Acclimated temperatures (AT) and experimental temperatures (ET) are listed.

Experimental conditions of the samples	Equation of the regression line Y = A + bX	$S_{Y.X}$	d.f. (n-2)	Chi^2	F-test slopes	F-test intercepts
ET = 4°C						
AT = 4°C	Y = 1.19 + 1.20X	0.0124	2	1.41	0.215	2.46
AT = 12.5°C	Y = 1.37 + 1.15X	0.0064	2		d.f. = 2:5	
AT = 25°C	Y = 1.60 + 0.42X	0.0562	1			N.S.
ET = 9°C						
AT = 4°C	Y = 1.76 + 0.82X	0.0124	3	2.79	0.015	1.05
AT = 9°C	Y = 1.87 + 0.66X	0.0009	2		d.f. = 2:8	
AT = 25°C	Y = 1.68 + 0.84X	0.0143	3			N.S.
ET = 12.5°C						
AT = 4°C	Y = 1.94 + 0.71X	0.0035	2	3.84	0.152	2.03
AT = 9°C	Y = 2.15 + 0.57X	0.0002	2		d.f. = 3:13	
AT = 12.5°C	Y = 1.72 + 1.00X	0.0027	2			N.S.
AT = 25°C	Y = 1.75 + 0.96X	0.0053	7			
ET = 25°C						
AT = 4°C	Y = 1.87 + 1.00X	0.0034	8	4.34	0.936	18:21**
AT = 9°C	Y = 2.12 + 0.93X	0.0007	2)		d.f. = 3:18	
AT = 12.5°C	Y = 2.42 + 0.46X	0.0006	2)*1			
AT = 25°C	Y = 2.33 + 0.52X	0.0067	6)			

*

1. When multiple covariant analysis is applied to these regressions values are: F slopes = 0.1612 with 2:10 d.f.; F intercepts = 0.053 with 2:10 indicating that these three regressions are not significantly different.

Figure 13: Metabolism-Temperature curves for resting Orconectes virilis in Series II. Acclimation temperatures are; 4, 9, 12.5 and 25°C. Crayfish were exposed to temperatures of 4, 9, 12.5 and 25°C. Weights of the crayfish are 3.88 g. Values of oxygen uptake are listed in Table IX.

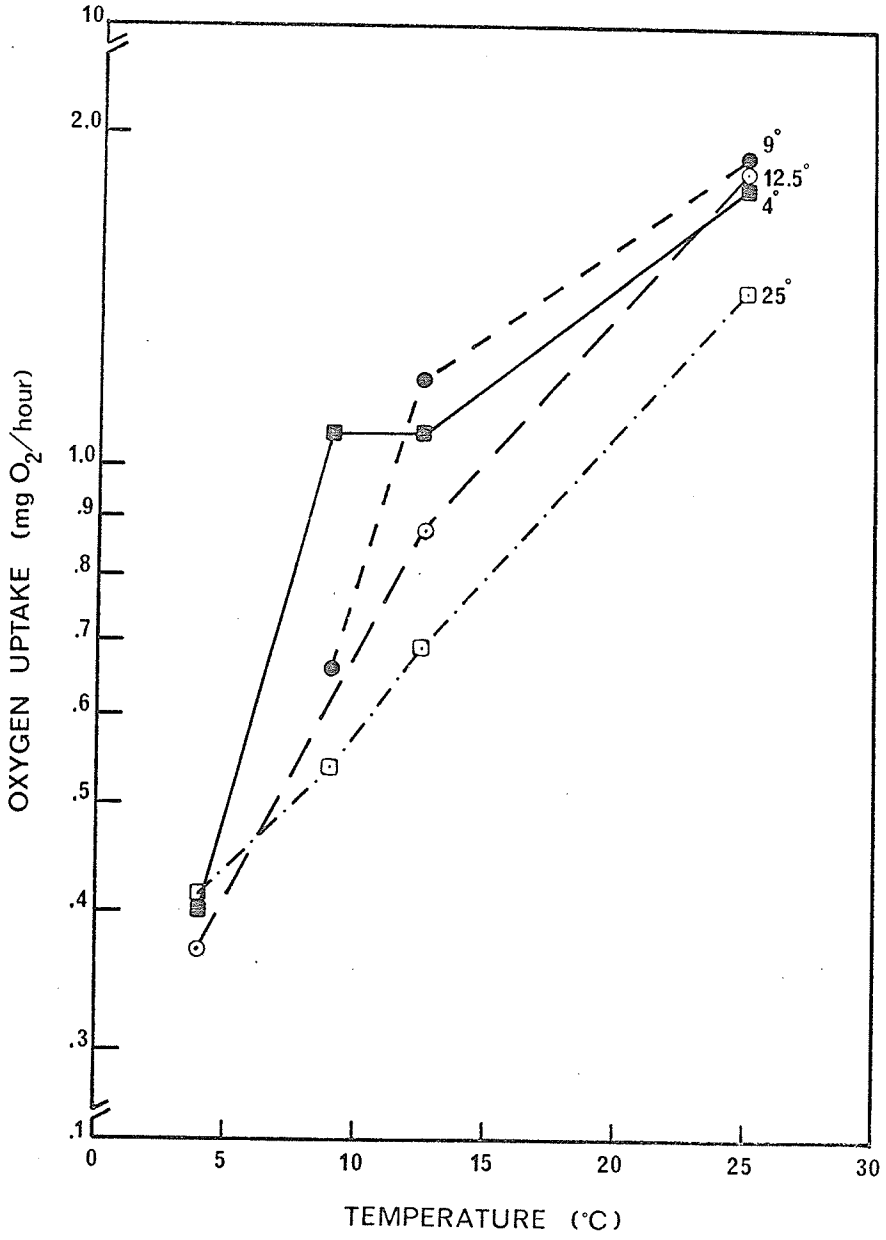

10
2.0
1.0
.9
.8
.7
.6
.5
.4
.3
.1
0
5
10
15
20
25
30
OXYGEN UPTAKE (mg O_2/hour)
TEMPERATURE (°C)
9°
12.5°
4°
25°

TABLE XII

Summarized statistical data derived from multiple covariant analyses of resting metabolism-weight regressions of Series II Orconectes virilis held at various acclimation temperatures (AT) and exposed to various experimental temperatures (ET).

Experimental conditions of the samples	Equation of the regression line Y = A + bX	$S_{Y.X}$	d.f. (n-2)	Chi^2	F-test slopes	F-test intercepts
ET = 4°C						
AT = 4°C	Y = 0.94 + 1.09X	0.0015	2	1.77	1.013	0.031
AT = 12.5°C	Y = 0.82 + 1.27X	0.0126	2		d.f. = 2:5	
AT = 25°C	Y = 1.44 + 0.29X	0.0024	1			N.S.
ET = 9°C						
AT = 4°C	Y = 1.47 + 0.94X	0.0220	3	0.49	0.025	7.149*
AT = 9°C	Y = 1.39 + 0.73X	0.0172	2		d.f. = 2:8	
AT = 25°C	Y = 1.10 + 1.08X	0.0091	3			
ET = 12.5°C						
AT = 4°C	Y = 1.65 + 0.64X	0.0057	5	1.48	0.019	3.769*
AT = 9°C	Y = 1.80 + 0.39X	0.0158	2		d.f. = 3:16	
AT = 12.5°C	Y = 1.62 + 0.56X	0.0187	2			
AT = 25°C	Y = 1.52 + 0.53X	0.0164	7			
ET = 25°C						
AT = 4°C	Y = 1.67 + 0.99X	0.0054	8	3.78	0.236	4.748*
AT = 9°C	Y = 1.86 + 0.72X	0.0021	2		d.f. = 3:18	
AT = 12.5°C	Y = 1.96 + 0.52X	0.0124	2			
AT = 25°C	Y = 1.79 + 0.63X	0.0166	6			

15 and 25°C. The M-T curve of crayfish acclimated at 15°C displays only upward translation.

The M-T curves for resting metabolism of the Series I crayfish are more difficult to interpret. The M-T curve of crayfish acclimated to 5°C displays clockwise rotation plus translation upwards, while the M-T curve of the 15°C acclimated group displays anticlockwise rotation plus translation upwards (Fig. 11).

The M-T curves for active metabolism of O. virilis collected in October are shown in Figure 12. From 4 to 12.5°C, the M-T curve of 4°C acclimated crayfish displays translation upwards, while from 12.5°C to 25°C the curve displays clockwise rotation. The M-T curve of crayfish acclimated to 9°C displays translation up plus clockwise rotation from 9 to 25°C. The M-T curve of the animals acclimated at 12.5°C seems to display possible translation upwards without any rotation.

The M-T curves for resting metabolism of O. virilis collected in October are shown in Figure 13. From 4 to 25°C, the M-T curve of crayfish acclimated to 4°C displays translation up plus anticlockwise rotation. The M-T curves of crayfish acclimated at 9°C and 12.5°C also display translation up plus anticlockwise rotation.

While it is possible at this time to discuss the physiological significance of these various patterns of thermal acclimation, an important point would be over looked. The possibility exists that the patterns of acclimation displayed in Figures 11 - 13 are due to chance alone. The values obtained at any experimental temperature

may possibly be variations about a mean, in other words, the values of oxygen uptake for crayfish acclimated at various temperatures and exposed to a particular temperature may not be significantly different. At the same time, the M-T curve of a cold acclimated organism could display translation in relation to the curve of the warm acclimated organism by chance alone. As a result, a gross misinterpretation of data could occur. This possibility has been unnoticed in many thermal acclimation studies.

b. Interpretation of acclimation patterns after statistical analyses of the oxygen uptake data.

Since each point in Figures 11 - 13 is derived from a particular regression (Fig. 4 - 10), then inorder to determine whether there is a significant difference between the values of two points, covariant analyses of the regressions, from which the values of these two points were derived, must be undertaken. If the intercepts of the two regressions are significantly different, then the derived values are also significantly different. Therefore multiple covariant analyses of the regressions represented by oxygen uptake values of crayfish acclimated at the various temperatures and exposed to a particular temperature provided the data to make objective interpretations of the acclimation patterns shown in Figures 11 - 13. The results for the objective evaluation of the oxygen uptake values of Figures 11 - 13 are listed in Tables X - XII respectively.

The results from the multiple covariant analyses used to determine if there were any differences in oxygen uptake at any one particular

temperature will be discussed first for the Series I crayfish collected in June (Fig.11) then for the Series II crayfish collected in October (Figs. 12, 13).

During the active metabolism of the crayfish collected in June, the intercepts of the metabolism-weight regressions of the animals acclimated at 5, 15 and 25°C and exposed to 5°C are significantly different (Table X). Therefore the oxygen uptake values of active metabolism of the June collected O. virilis acclimated at 5, 15 and 25°C and exposed to 5°C are also significantly different (Fig. 11). Since the intercepts of the active metabolism-weight regressions of the same collection of crayfish exposed to the 15°C test temperature are also significantly different (Table X), then the oxygen uptake values shown for active metabolism of the 5, 15 and 25°C acclimated crayfish exposed to 15°C are also significantly different. However, the intercepts of the active metabolism-weight regressions of the June collected crayfish exposed to the 25°C temperature are not significantly different (Table X) indicating that the oxygen uptake values shown for active metabolism of the 5, 15 and 25°C acclimated crayfish exposed to 25°C are not significantly different.

The results from the multiple covariant analyses of the regressions (Figs. 4-6) represented by the oxygen uptake values in Figure 11 indicate that at 5 and 15°C there is an increase in active metabolism during cold acclimation, and that the magnitude of the increase increases with decreasing acclimation temperatures down to 5°C. This magnitude of increase can be expressed as the percent increase in oxygen uptake

after acclimation has taken place. The oxygen uptake of a 15°C-acclimated crayfish exposed to 15°C is 2.25 mg O_2/hr, while the oxygen uptake of a 25°C-acclimated crayfish is 2.00 mg O_2/hr. Therefore the present increase is (2.25 - 2.00) x 100/ 2.00 = 12.5%. The percent increase in oxygen uptake of a 5°C-acclimated crayfish is:

(1.85 - .90) x 100/ .90 = 106%.

Since the 5°, 15° and 25°C-acclimated crayfish have no significantly different oxygen uptake values at 25°C, it may be concluded that the pattern of temperature adaptation during active metabolism is one of translation upwards and clockwise rotation with the M-T curves intersecting near 25°C. The magnitude of rotation seems to increase with decreasing acclimation temperatures. This pattern is described by Prosser (1961) as IV A, the Q_{10} increases with increasing acclimation temperatures. Kanungo and Prosser (1959) have shown that the same pattern is displayed by goldfish acclimated to 10 and 30°C.

During resting metabolism of Series I crayfish the intercepts of the metabolism-weight regressions of the animals acclimated at 5, 15 and 25°C and exposed to 5°C are significantly different (Table X). However, covariant analyses of only the 15 and 25°C acclimated groups exposed to 5°C indicates that the intercepts of these two curves are not significantly different. These results would imply therefore that animals acclimated at 15 and 25°C exhibit the same responses when exposed to 5°C. The results also indicate that capacity adaptation

for resting metabolism occurs in O. virilis acclimated at 5°C as the oxygen uptake for this group is more than twice the values shown by the 15 and 25°C acclimated groups.

The results of the analyses of the metabolism-weight regressions of the three acclimated groups of O. virilis exposed to 15°C indicate that there is no significant different among the oxygen uptake values at 15°C (Table X ; Fig. 11). The results would thus indicate that from 5 to 15°C, crayfish acclimated to 15 and 25°C show the same response to low temperatures. On the other hand, the cold acclimated group shows capacity adaptation at 5 but not at 15°C.

While the results of analyses of the metabolism-weight regressions of the three acclimated groups of crayfish exposed to 25°C indicated that there is a significant difference among the oxygen uptake values at 25°C (Table X ; Fig.11) this is only due to the significant difference between the intercepts of the regressions for the 15°C and 25°C-acclimated groups, (Table X). The intercepts of the regression for the 5°C acclimated group of crayfish is not significantly different from either of the other intercepts of the regressions for the 15 and 25°C acclimated groups, probably because the variance about the regression line of the 5°C acclimated group is higher than the variance about the other two regressions (Table X).

The results from the analyses of the regressions for resting metabolism, represented by the data in Figure 11 , indicate that O. virilis acclimated at 15°C has a higher metabolism than crayfish acclimated at 25°C. The question remains as to why metabolism of

the 5°C-acclimated crayfish tested at 25°C is not higher than that of crayfish acclimated to 15°C.

If 25°C is assumed to be the upper lethal limit of O. virilis acclimated at 5°C, then it is possible to explain the results exhibited by the crayfish in relation to what is known about the effect of lethal temperatures. Prosser (1961) has reviewed a number of investigations indicating that the physiological effect of lethal temperatures is caused by a combination of the magnitude of the temperature and the length of time an organism is exposed to that temperature. Since the oxygen consumption values during resting metabolism of 5°C acclimated crayfish were determined at least 30 minutes after the oxygen consumption values during active metabolism, it is possible that the length of exposure to 25°C was sufficient to affect the physiology of the animals while exposure during active metabolism was sufficiently short not to have had an affect. As this argument is based on an assumption, it must remain tentative.

However, the results have raised another more interesting question. Suppose that 25°C is only a sub-lethal temperature. In this case the same physiological responses to heat may be exhibited to a less degree than if the temperature were higher, i.e. a slow but steady drop in the oxygen uptake over time. Therefore, at what time should the experimeter record the metabolism inorder to determine the effect of exposure to high temperatures on the organism? This problem might deserve some study.

McWhinnie and O'Connor (1967) acclimated O. virilis to 5°C and 18°C

as well as exposing them to a 12L, 12D photoperiod. The pattern of acclimation between 5° and 15°C for O. virilis after acclimation for two weeks was one of translation upwards only.

By neglecting for the moment the oxygen uptake values of the 25°C acclimated group in Figure 11 the pattern of acclimation from 5° to 15°C during active or resting metabolism of the crayfish used in this investigation would be one of translation upwards plus clockwise rotation. This type of pattern was displayed by the crayfish in McWhinnie and O'Connor's results after one week of acclimation.

The results of oxygen uptake studies on O. virilis collected in October and exposed to the 8L, 16D are presented in Figures 12 and 13 and Tables XI and XII for active and resting metabolism respectively. Because the determination of oxygen uptake during active metabolism was made directly after the determination of oxygen consumption during resting metabolism, the influence of time as a factor producing different responses for active and resting metabolism in the cold-acclimated crayfish at extreme temperatures will be negligible. As a result, the differences in the patterns of acclimation between active and resting metabolism can be more easily interpreted for this series of experiments than for the previous one.

Multiple covariant analyses of the active metabolism-weight regressions determined for crayfish in the following trails: (1) acclimated at 4, 12.5 and 25°C and exposed to 4°C; (2) acclimated at 4, 9, and 25°C and exposed to 9°C; and (3) acclimated at 4, 9, 12.5 and 25°C and exposed to 12.5° (Table XI), indicate that

there is no significant difference among the regressions for each set of trials. Therefore, there is no significant difference among the oxygen uptake values representing the regressions at the experimental temperatures of 4, 9 and 12.5°C for active metabolism (Fig. 12). There is a significant difference among the intercepts of the regressions for animals acclimated at 4, 9, 12.5 and 25°C. Therefore the oxygen uptake values representing these regressions in Figure 12 are significantly different. However, when the regression for the 4°C-acclimated group is left out, there is no significant difference among the remaining regressions.

Multiple covariant analysis of the resting metabolism-weight regressions determined for crayfish acclimated at 4, 12.5 and 25°C and exposed to 4°C indicates that there is no significant difference among the intercepts (Table XII). Therefore, the oxygen consumption values representing these regressions (Fig. 13) at 4°C are not significantly different either.

Results from using the same statistical procedure as above indicate that there is a significant difference among the oxygen uptake values of crayfish exposed to 9, 12.5 and 25°C. Therefore, the pattern of acclimation can be defined as Pattern IV-C; the M-T curve is for cold acclimation translated upwards and rotated anti-clockwise indicating that Q_{10} increases with decreasing acclimation temperatures (Prosser, 1961).

The results of the analyses would therefore suggest that for O. virilis acclimated from 4 to 25°C and exposed to a 8L, 16D

photoperiod, there is no significant acclimation of the active metabolism. The "no acclimation" pattern (Pattern I, Prosser, 1961) of active metabolism and Pattern IV-C of resting metabolism of O. virilis do not resemble the patterns obtained for the same species by McWhinnie and O'Connor when they exposed their experimental animals to a 12L, 12D photoperiod.

A comparison of the results obtained in this investigation with those obtained by McWhinnie and O'Connor would suggest that photoperiod may have a fundamental role in the physiology of thermal adaptation of O. virilis.

It is worthwhile at this point to summarize briefly the conclusions concerning the acclimation patterns obtained from Series I and II experiments. In Series I, active crayfish acclimated at 5°C yield an M-T curve which is translated upwards and rotated clockwise between 5 and 25°C (Fig. 11). Active crayfish acclimated at 15°C display only translation upwards between 5 and 25°C. When the M-T curve of the 25°C-acclimated crayfish is ignored and the temperature range from 5 to 15°C is considered, then the M-T curve of the 5°C-acclimated crayfish is translated upwards and rotated clockwise. This acclimation pattern is the same as the one reported for O. virilis by McWhinnie and O'Connor after they acclimated the animals for one week.

In Series I (Fig. 11) resting crayfish acclimated at 5°C yield an M-T curve which is translated upwards and rotated clockwise, while resting crayfish acclimated at 15°C yield a curve which is translated

upwards but rotated anti-clockwise. The difference in the two patterns may be due to the procedure in which oxygen uptake for active and resting crayfish was determined. When the M-T curve of the 25°C-acclimated crayfish is ignored and the temperature range from 5 to 15°C is considered, then the M-T curve of the 5°C-acclimated crayfish is translated upwards and rotated clockwise. This pattern was also evident during active metabolism of the crayfish.

In Series II, active crayfish (Fig. 12) acclimated from 4 to 25°C and exposed to a temperature range from 4 to 12.5°C yielded M-T curves which indicated that no acclimation had taken place. 4°C-acclimated crayfish exposed to 25°C consumed less oxygen than did the warmer-acclimated crayfish. Resting crayfish (Fig. 13) acclimated between 4 and 25°C yielded acclimation patterns between 4 and 25°C which indicated that cold-acclimated crayfish had higher Q_{10} values than did warm-acclimated ones.

Neither pattern for active or resting metabolism was similar to the one reported by McWhinnie and O'Connor.

c. Acclimation patterns produced in the manner as described by Jungreis and Hooper (1968):

Metabolism-temperature curves can be derived by pooling the experimental data collected for each group of crayfish acclimated to a particular temperature. In this case the groups of O. virilis used in the two series for each acclimation temperature are considered as samples. Subsamples of these groups are used to determine the

oxygen consumption at the various experimental temperatures. Neither the variances of the weights nor the differences in mean weight among the samples were significantly different at the 5% level of confidence (Tables XIII, XIV). Thus the data for each acclimated group of O. virilis can be fitted to the best linear regression line by the method of least squares, the two variables being oxygen uptake and experimental temperature. The derived equations are in the form:

$$Y = A + b\underline{x} ,$$

where Y is the log oxygen uptake in mg O_2 per hour at the experimental temperature $\underline{x}$, A is the log oxygen uptake x 100 at 0°C, and b is the slope of the equation (Jungreis and Hooper, 1968). The equation of of the Metabolism-temperature regressions are listed in Table XVIII.

Within the June and October series, the mean weights (Table XV) among the acclimated groups of O. virilis are not significantly different except for one case (Tables XVI, XVII). As a result of these exceptions any apparent translation of the M-T curves will be due to two factors operating: the physiological response of cold and the differences in weight among the samples. This limitation is born in mind when the patterns of acclimation in Figures 14 and 15 are discussed.

Figure 14 displays the M-T curves for active and resting metabolism of Series I O. virilis. The M-T curves of the 5°C and 15°C acclimated crayfish during active metabolism display translation upwards. Results from covariant analyses of the regressions are significantly

Figure 14: Metabolism-Temperature curves from straight line regression analysis for the active (AM) and resting (RM) metabolism of Series Orconectes virilis.

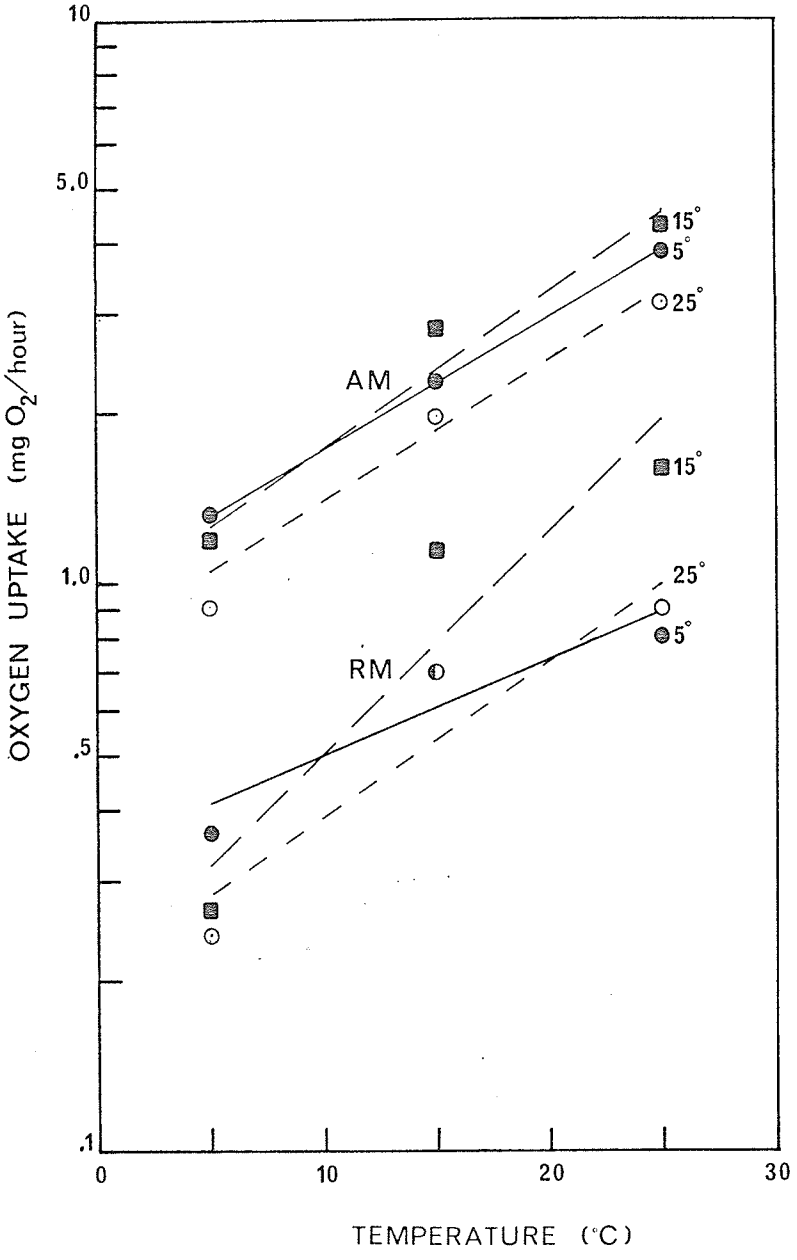

10
5.0
1.0
.5
.1
0
10
20
30
OXYGEN UPTAKE (mg O_2/hour)
TEMPERATURE (°C)
AM
RM
15°
5°
25°
15°
25°
5°

Figure 15: Metabolism-Temperature curves from straight line regression analysis for the active (AM) and resting (RM) metabolism of Series II Orconectes virilis.

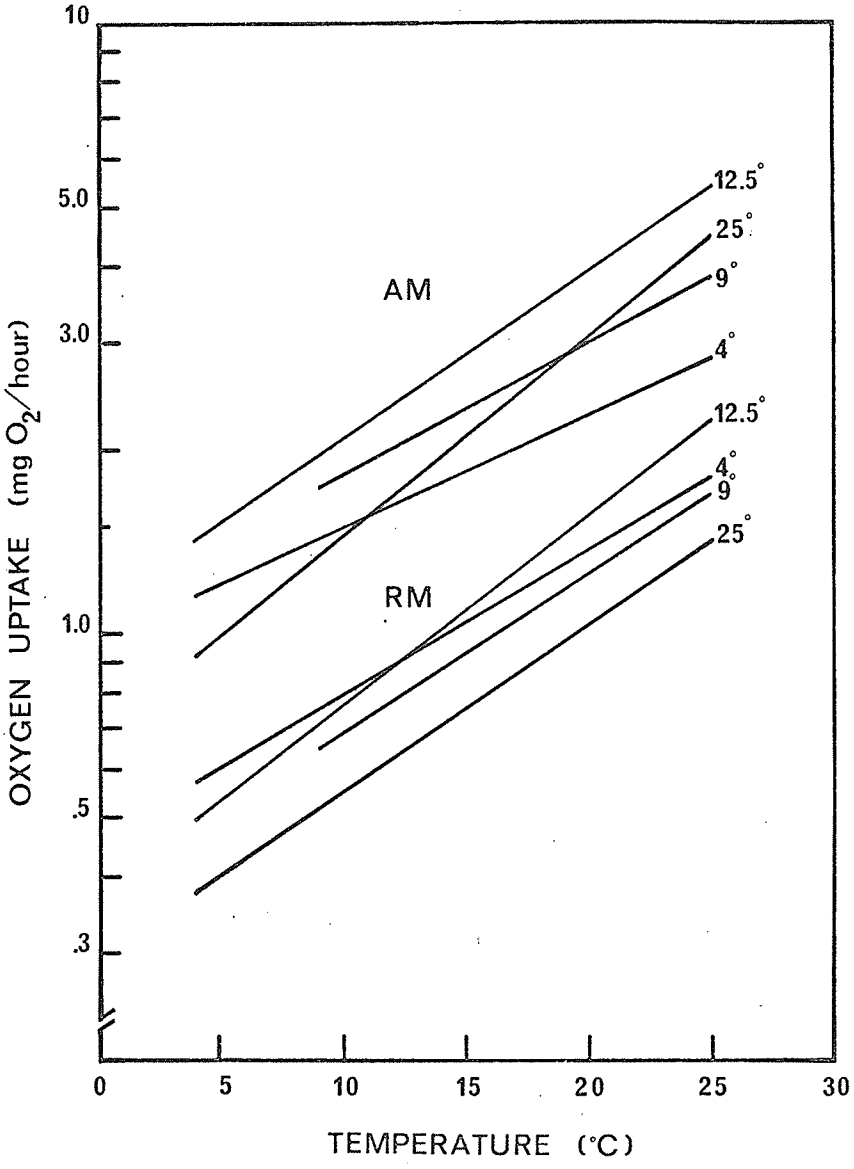

10
5.0
3.0
1.0
.5
.3
OXYGEN UPTAKE (mg O_2/hour)
AM
RM
12.5°
25°
9°
4°
12.5°
4°
9°
25°
0
5
10
15
20
25
30
TEMPERATURE (°C)

TABLE XIII

Statistical data of subsamples for each acclimation temperature of Series I Orconectes virilis.

Acclimation Temperatures (°C)		Experimental Temperatures (°C) 5	15	25
5	No. of observations	5	7	5
	Mean dry-weight	2.20g	2.25g	2.08g
	Variance of sample	0.399	0.335	0.421
	Chi^2 of variances: 0.066 with 2 d.f.			
	F ratio of means: 0.057 with 2 and 14 d.f.			
15	No. of observations	5	5	5
	Mean dry-weight	3.72g	4.15g	3.73g
	Variance of sample	0.540	0.662	0.625
	Chi^2 of variances: 0.039 with 2 d.f.			
	F ratio of means: 0.158 with 2 and 14 d.f.			
25	No. of observations	6	8	9
	Mean dry-weight	3.18g	3.03g	2.97g
	Variance of sample	0.401	0.306	0.201
	Chi^2 of variances: 0.734 with 2 d.f.			
	F ratio of means: 0.126 with 2 and 22 d.f.			

TABLE XIV

Statistical data of subsamples for each acclimation temperature of Series II Orconectes virilis

Acclimation Temperatures (°C)		Experimental Temperatures 4	9	12.5	25
4	No. of observations	4	5	7 (4)*	10
	Mean dry-weight	4.04g	3.70g	4.07g	3.64g
	Variance of sample	0.380	0.654	0.392	0.230
	Chi^2 of variances: 1.54 with 3 d.f. F ratio of means: 0.34 with 3 and 25 d.f.				
9	No. of observations		4	4	4
	Mean dry-weight		3.06g	3.06g	3.06g
	Variance of samples		0.1	0.1	0.1
12.5	No. of observations	4		4	4
	Mean dry-weight	4.63g		4.63g	4.63g
	Variance of samples	0.397		0.397	0.397
25	No. of observations	3	5	8	8
	Mean dry-weight	4.68g	3.35g	3.81g	3.81g
	Variance of samples	0.684	0.313	0.392	0.392
	Chi^2 of variances: 0.416 with 3 d.f. F ratio of means: 1.01 with 3 and 23 d.f.				

TABLE XV

Statistical data of samples of Orconectes virilis used for the Series I and II investigations.

Acclimation Temperatures (°C)	Number of Observations	Mean Dry-weight	Variance of Sample
5	17	2.19g	0.206
15	15	3.87g	0.331
25	23	3.05g	0.161
	Chi^2 of variances: 2.29 with 2 d.f.		
	Error MS: 0.9166		
	F ratio of means: 12.32** with 2 and 53 d.f.		
4	23	3.83g	0.185
9	12	2.83g	0.098
12.5	12	4.63g	0.207
25	24	3.82g	0.214
	Chi^2 of variances: 2.08 with 3 d.f.		
	Error MS: 0.7817		
	F ratio of means: 6.25** with 3 and 7 d.f.		

TABLE XVI

Duncan's New Multiple Range test on sample means of Series I Orconectes virilis. Notations and values are from Steel and Torrie (1960). Bracketted numbers are acclimation temperatures.

	A(15°C)	B(25°C)	C(5°C)
$\bar{x}$ =	3.87	3.05	2.19
$S_{\bar{x}}$ =	$\frac{\text{Error MS}}{r}$	$\frac{0.9166}{3}$	0.553

d.f. = 40

Value of P:	2	3
SSR :	2.86	3.01
LSR :	1.58	1.66

			Conclusions
A - C =	1.68	1.66	Significant
A - B =	0.82	1.58	Not Significant
B - C =	0.86	1.56	" "

Summary of Results:

A (15°C)	B (25°C)	C (5°C)

TABLE XVII

Duncan's New Multiple Range test on Sample means of Series II *Orconectes virilis*. Notations and values are from Steel and Torrie (1960). Bracketted numbers are acclimation temperatures.

	A(12.5°C	B(4°C)	C(25°C)	D(9°C)
$\bar{x}$	4.63	3.83	3.82	2.83
$S_{\bar{x}}$	$\frac{\text{Error MS}}{r}$	$\frac{0.7817}{4}$	= 0.442	

d.f. = 70

Value of P:	2	3	4
SSR :	2.83	2.98	3.08
LSR :	1.25	1.32	1.36

			Conclusion
A - D =	1.80	1.36	Significant
A - C =	0.81	1.32	Not Significant
A - B =	0.08	1.25	" "
B - D =	1.00	1.32	" "
B - C =	0.01	1.25	" "
C - D =	0.99	1.25	" "

Summary of Results

A (12.5°)	B (4°)	C (25°)	D (9°)

TABLE XVIII

Summarized statistical data derived from multiple covariant analyses of Metabolism-Temperature regressions for each acclimation temperature (AT). Regressions are illustrated in Figures 14 (Series I), 15 and 16 (Series II).

Experimental conditions of the samples	Equation of the regression line Y = A + bX	$S_{Y.X}$	d.f. (n-2)	Chi^2	F-test slopes	F-test intercepts
SERIES I						
Active Metabolism						
AT = 5°C	Y = 2.01 + 0.023X	0.0138	15	0.225	0.175	26.08**
AT = 15°C	Y = 1.96 + 0.028X	0.0194	13		d.f. = 2:49	
AT = 25°C	Y = 1.86 + 0.036X	0.0180	21			
Resting Metabolism						
AT = 5°C	Y = 1.52 + 0.017X	0.0195	15	2.13	3.954*	5.604**
AT = 15°C	Y = 1.30 + 0.039X	0.0436	13		d.f. = 2:49	
AT = 25°C	Y = 1.32 + 0.028X	0.0297	21			
SERIES II						
Active Metabolism						
AT = 4°C	Y = 1.98 + 0.019X	0.0225	21	10.15*	3.594*	6.720**
AT = 9°C	Y = 2.07 + 0.020X	0.0070	10		d.f. = 3:64	
AT = 12.5°C	Y = 2.03 + 0.028X	0.0078	10			
AT = 25°C	Y = 1.83 + 0.033X	0.0064	23			
Resting Metabolism						
AT = 4°C	Y = 1.67 + 0.023X	0.0248	24	1.96	0.415	10.77**
AT = 9°C	Y = 1.58 + 0.026X	0.0128	10		d.f. = 3:67	
AT = 12.5°C	Y = 1.57 + 0.030X	0.0150	10			
AT = 25°C	Y = 1.83 + 0.027X	0.0163	23			

different (Table XVIII) and therefore confirm the above speculation. Probably the M-T curve of the 5°C-acclimated group is lower than the one for the 15°C-acclimated group because the mean weight for the 5°C sample is very much lower than the one for the 15°C sample. (Table XVI).

The M-T curves during resting metabolism of the Series I crayfish display an acclimation pattern unlike that displayed for the M-T curves during active metabolism. Firstly the slopes of the three curves are significantly different at the 5% confidence interval (Table XVIII). The value of the Y intercept for the 5°C acclimated sample is much higher than the values for the other two samples, suggesting that translation upwards occurs during adaptation to cold. The apparent rotation of the curve has previously been discussed. It is possible that a 30-minute exposure of cold-acclimated crayfish to 25°C is sufficient time to cause sub-lethal effects at this temperature.

The anticlockwise rotations displayed by both the 5 and 15°C acclimated samples suggest that the Q_{10} increases with decreasing temperature to at least as low as 5°C.

Neither one of the two acclimation patterns for active and resting metabolism obtained in Series I resembles the pattern obtained by Jungreis and Hooper (1968) in their investigation, even though they exposed their cold-acclimated (1 - 2°C) and warm-acclimated (21°C) O. virilis to a 24L, OD photoperiod.

Figure 15 shows the acclimation patterns obtained from the investigation of oxygen uptake during active metabolism of the Series II

crayfish. It is evident that except for the 12.5°C-acclimated group of O. virilis, translation of the M-T curves during active metabolism is down. Results from multiple covariant analysis of the regressions (Table XVIII) of the four acclimated groups indicate that the slopes are significantly different.

The pattern of acclimation seems to be divided into two phases, the division occuring between 9 and 12.5°C. At 12.5°C the pattern is one of translation upwards(Pattern II A; Prosser, 1961) while below 12.5°C the pattern is one of rotation clockwise (Pattern III; Prosser, 1961). The results would thus imply that there is partial compensation at the lower acclimation temperatures by O. virilis, but that these low temperature acclimated animals are not able to tolerate the warmer experimental temperature during active metabolism. The crayfish acclimated at 4°C have a lower oxygen consumption at 25°C than do the crayfish acclimated at 9°C (Figure 15).

However, during resting metabolism, translation of the M-T curves is very apparent for the four acclimated groups of O. virilis (Figure 15, Table XVIII). The pattern of acclimation though, is still such that there is an indication that the low temperature acclimated groups are not able to tolerate exposure of 25°C as well as the crayfish acclimated to 12.5°C. The results therefore suggest that not only is Figure 15 representing the pattern for capacity adaptation, but it is also representing a pattern for resistance adaptation (Precht, 1958), i.e. crayfish acclimated to 4°C are not able to tolerate exposure to 25°C as well as crayfish acclimated to 9°C, these crayfish

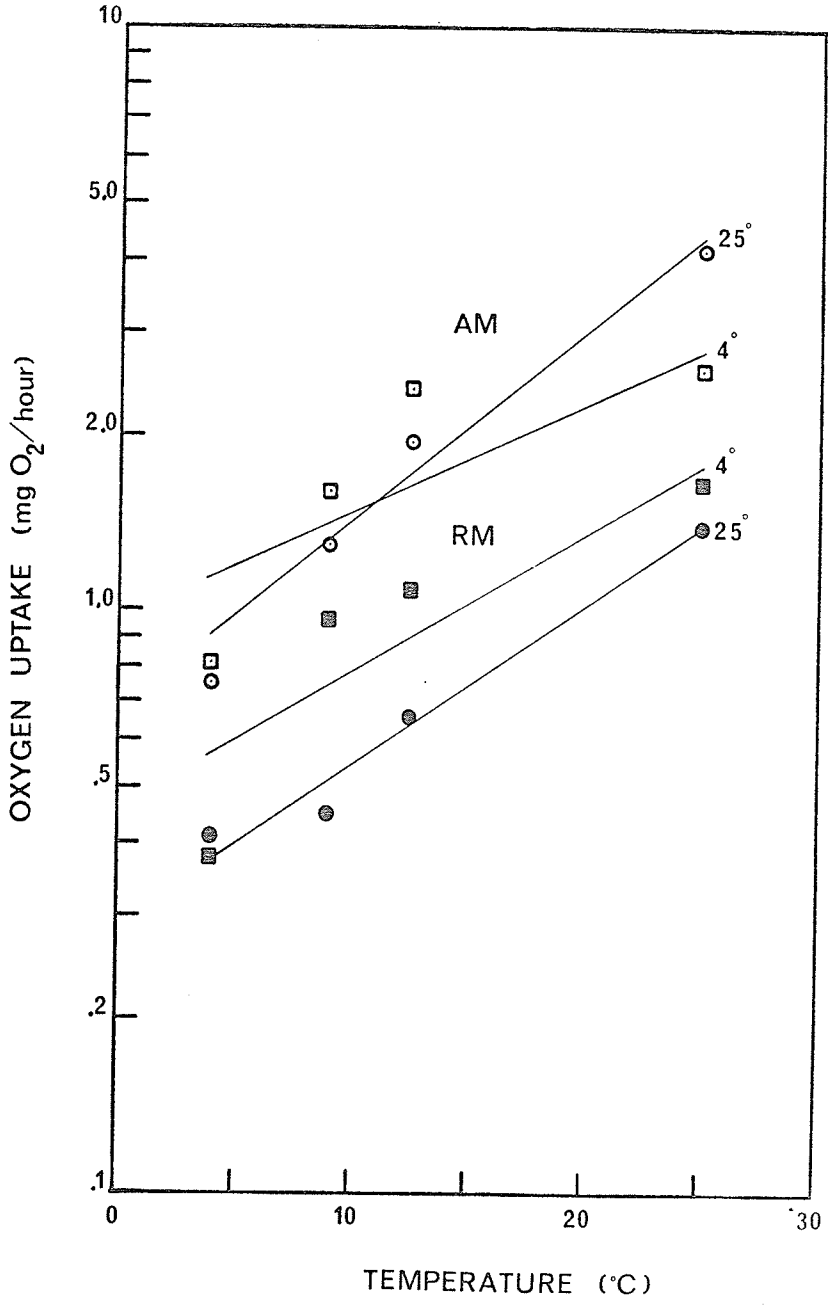
in turn are not able to tolerate exposure to 25°C as well as the ones acclimated to 12.5°C.

In Figure 15 there is some bias in the intercepts brought about by unequal mean weights among the samples. (Table XVII). However, there is no significant difference between the mean weights of the 4 and 25°C acclimated groups. The regressions plus the mean oxygen uptake values of these two groups have been plotted in Figure 16. In this figure, it is very apparent that the clockwise rotation during active metabolism is entirely due to the oxygen uptake values recorded at 25°C, for the mean oxygen uptake values of the 4°C group are higher than the ones for the 25°C group at every other experimental temperature. Prosser (1961; 1958) has stated that the pattern shown in Figure 16 (Pattern IV D) is rare, and that it may be due to another limiting factor such as low oxygen tensions influencing the results. Hence the results may indicate that low oxygen tensions plus high temperature contributed so much stress to 4°C acclimated crayfish that the oxygen uptake values at 25°C were depressed.

The pattern for resting metabolism is one of translation upwards, and would seem to indicate that high temperatures plus low oxygen tensions can be tolerated by O. virilis only during resting metabolism.

The pattern shown for active metabolism in Figure 16 closely resembles the one obtained by Jungreis and Hooper (1968). However, when their units were converted to the ones used in this project,

Figure 16: Metabolism-Temperature curves for the active (AM) and resting (RM) metabolism of Series II Orconectes virilis acclimated to 4 and 25°C, with the mean oxygen consumption values. Round symbols are mean values for the crayfish acclimated to 25°C. Square symbols are mean values for crayfish acclimated to 4°C. Open symbols are for active metabolism, solid symbols are for resting metabolism. Mean values caluclated from values listed in Appendix Table II.


10
5.0
2.0
1.0
.5
.2
.1
0
10
20
30
OXYGEN UPTAKE (mg O_2/hour)
TEMPERATURE (°C)
AM
RM
25°
4°
4°
25°

their values of oxygen uptake were found to be closer to the values obtained for crayfish under resting metabolic conditions.

The results of the analyses just discussed are summarized in Table XIX. A comparison of the results based on the two methods used for analyzing the influence of temperature on metabolism indicates that the methods of analyzing the data may influence the final conclusions. Since metabolism-temperature regressions are not usually straight lines, the Q_{10} decreasing with increasing experimental temperature (Precht, 1958), then the statistical procedure from which straight line M-T curves are derived is a simplification of the real situation. Probably the method to be used for this type of analysis may be dependent upon how much one is ready to deviate knowingly from the real world.

3. Determination of Precht Types.

Precht type determinations are useful as they provide means of extropolating data from the laboratory to the environment. Figure 17 illustrates the types of temperature adaptation according to Precht (1958) and for the active and resting metabolism of the Series I crayfish. The oxygen consumption of a 3:04 g crayfish is represented in Figure 17A. Figure 17B represents the average oxygen consumption of each sample of crayfish. The solid lines join values of oxygen uptake for the three acclimation temperatures. Table XX lists the Q_{10} values based on calculations derived from results represented in Figure 17A. The range of Q_{10} values in Figure 17A is from approximately

TABLE XIX

Summary of the acclimcation patterns using the two different methods described in the test.

	Acclimation Patterns	
Experimental Conditions	Metabolism-Temperature curves based on oxygen uptake values derived from Metabolism-Weight relationships	Metabolism-Temperature curves ignoring the Metabolism-weight relationship
SERIES I	Active Metabolism: Curves are translated upwards and rotated clockwise.	Active Metabolism: Curves are translated upwards.
	Resting Metabolism: Curve of crayfish acclimated to 5°C is translated upwards and rotated clockwise, curve of crayfish acclimated to 15°C is translated upwards and rotated anti-clockwise.	Resting Metabolism: Curve of crayfish acclimated to 5°C is translated upwards and rotated clockwise, curve of crayfish acclimated to 15°C is translated upwards and rotated anti-clockwise.
SERIES II	Active Metabolism: No acclimation	Active Metabolism: Curve for crayfish acclimated to 12.5°C is translated upwards. Curves for crayfish acclimated to 4 and 9°C is translated down and rotated anti-clockwise
	Resting Metabolism: Curves are translated upwards and rotated anti-clockwise.	Resting Metabolism: Curves are translated upwards.

Figure 17: Types of Acclimation according to Precht (1958) for the Series I Orconectes virilis. Solid lines join oxygen uptake values for both active metabolism (AM) and resting metabolism (RM). Broken lines join values for the scopes of activity at these same acclimation temperatures.

Figure 17A. The oxygen uptake values are based on the data listed in Table VIII and plotted in Figure 11.

Figure 17B. The oxygen uptake values are based on the mean weight of crayfish used for the trial.

The difference in oxygen uptake values for each acclimation temperature gives an estimate of the bias due to weight in Figure 14.

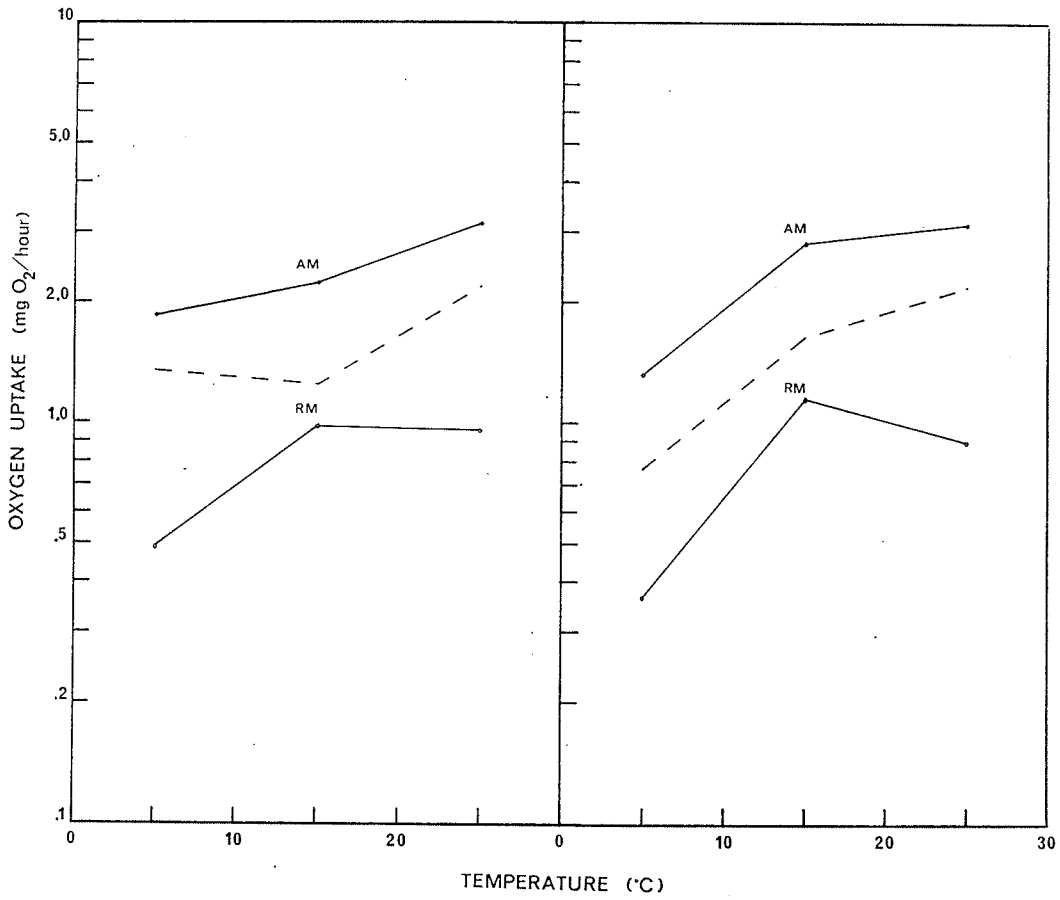

10
5,0
2,0
1,0
.5
.2
.1
OXYGEN UPTAKE (mg O_2/hour)
AM
RM
AM
RM
0
10
20
0
10
20
30
TEMPERATURE (°C)

TABLE XX

The change in oxygen uptake in Series I and II Orconectes virilis after acclimation expressed as Q_{10}.*

	Active Metabolism	Resting Metabolism
SERIES I		
Acclimation Temperature Interval		
5 - 15°C	1.21	2.01
15 - 25°C	1.42	0.98
SERIES II		
Acclimation Temperature Interval		
4 - 9°C	5.35	2.96
9 - 12.5°C	1.46	2.81
12.5 - 25°C	1.82	1.48

* Where $Q_{10} = \left(\frac{\text{Rate at } t_1}{\text{Rate at } t_2}\right)^{\frac{10}{t_1 - t_2}}$

1 to 2. These values suggest that between 5 and 15°C, oxygen uptake during active metabolism of a 3:04 g crayfish is partially compensated (Q_{10} = 1.21). During resting metabolism there is no compensation (Q_{10} = 2.01). At the higher range of temperatures, partial compensation seems evident during active metabolism (Q_{10} = 1.42) and perfect compensation during resting metabolism (Q_{10} = 0.98).

A comparison of the oxygen uptake values between Figures 17A and 17B provides an indication of how the bias due to unequal mean weights among the three groups of crayfish affects the estimate of metabolism when the average oxygen uptake values of groups of crayfish are used instead of determining the oxygen consumption of a 3:04g crayfish. The values of oxygen uptake of the 15°C-acclimated group of crayfish are slightly over estimated, while the values of oxygen uptake of the 5 and 25°C-acclimated groups are slightly underestimated if average values of oxygen consumption are used to plot M-T curves as in Figure 14. This is to be expected as the mean weights of the three acclimated groups of crayfish are significantly different (Table XV).

The broken line in Figure 17A joins values of the scope for activity (Fry, 1947) of the crayfish acclimated to the three temperatures. These results would suggest that the scope for activity increases with temperature from 15 to 25°C. Below 15°C in Figure 17A the scope for activity seems to remain constant.

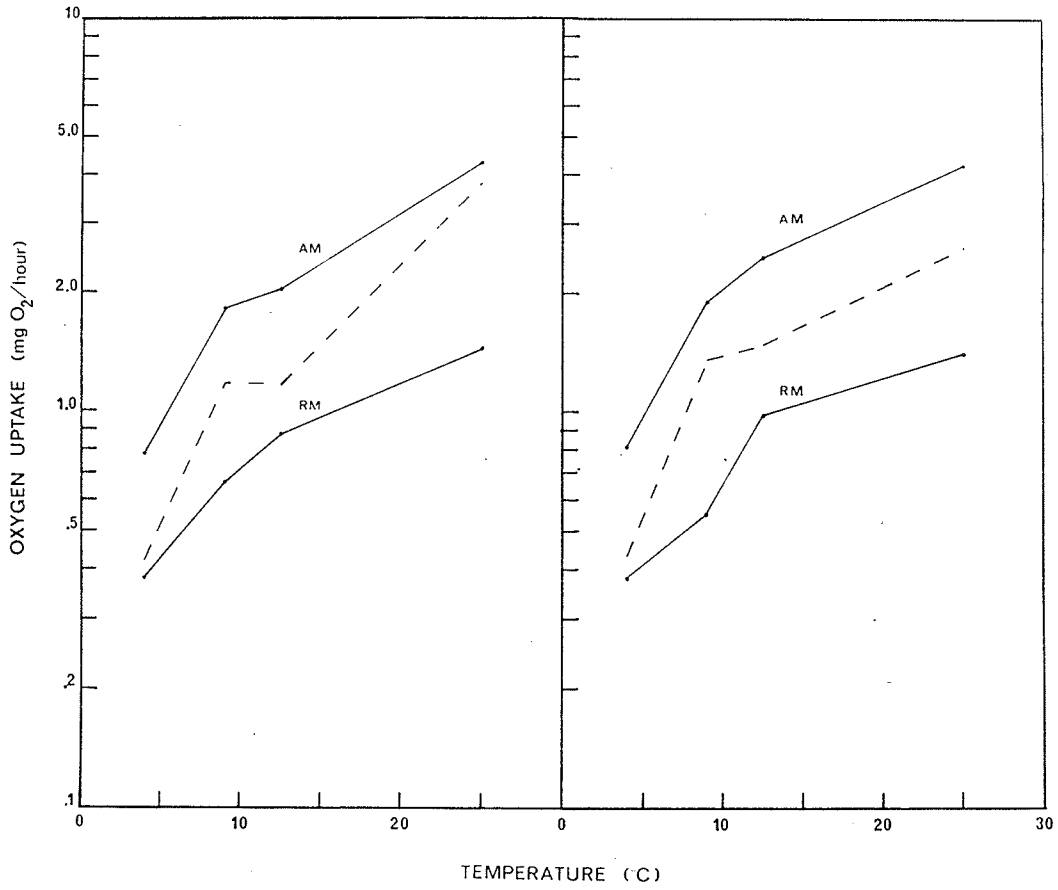
Figure 18 illustrates the types of temperature adaptation according to Precht (1958) for the active and resting metabolism of Series II

Figure 18: Types of acclimation according to Precht (1958) for the Series II Orconectes virilis. Solid lines join oxygen uptake values for both active metabolism (AM) and resting metabolism (RM). Broken lines join values for the scopes of activity at these same acclimation temperatures.

Figure 18A. The oxygen uptake values are based on the data listed in Table IX and plotted in Figures 12 and 13.

Figure 18B. The oxygen uptake values are based on the mean weight of crayfish used for the trial.

The difference in oxygen uptake values for each acclimation temperature gives an estimate of the bias due to weight in Figure 15.


OXYGEN UPTAKE (mg O_2/hour)
10
5.0
2.0
1.0
.5
.2
.1
AM
RM
AM
RM
0
10
20
0
10
20
30
TEMPERATURE (°C)

crayfish. The oxygen consumption of a 3.88 g crayfish is represented in Figure 18A. Figure 18B represents the average oxygen consumption of a sample of crayfish exposed to the experimental temperatures. The solid lines join values of oxygen uptake for the four acclimation temperatures. Table XX lists the Q_{10} values based on caluclations derived from the results presented in Figure 18. The range of the Q_{10} values is from approximately 1.5 to 5.4. Between 4 and 9°C the Q_{10} value for oxygen uptake during metabolism is 5.35 indicating that there is under compensation. During resting metabolism there is no compensation (Q_{10} = 2.96) within the same temperature range. Between 9 and 12.5°C and between 12.5 and 25°C, partial compensation of oxygen uptake during active metabolism seems to occur as the Q_{10} values for the two temperature ranges are 1.46 and 1.82 respectively. The Q_{10} value for oxygen consumption during resting metabolism between 9 and 12.5°C is 2.81 implying that there is no compensation. However, between 12.5 and 25°C there is partial compensation as the Q_{10} value is 1.48.

A comparison of the results shown in Figures 18A and 18B indicates that the bias of having unequal mean weights among the four samples of acclimation would not drastically influence the M-T curves for the various acclimation temperatures in Figure 15.

The broken line in Figure 18A joins values of the scope of activity at each acclimation temperature. The line indicates that the scope for activity increases with increasing acclimation temperatures.

4. Relation of the Results to the Life History of O. virilis:

Rather than just present a summary of the data and conclusions given in the previous sections, an attempt will be made to relate the findings of this project to the life history of O. virilis in Manitoba.

From the findings in their investigation, McWhinnie and O'Connor (1967) concluded that O. virilis can not survive more than a few weeks at low temperatures. On the other hand, Jungreis and Hooper (1968) demonstrated that the acclimation pattern of O. virilis was one of translation down with clockwise rotation. They also found that starved O. virilis do not utilize stored glycogen and break down instead fats or portions of proteins or both. Jungreis and Hooper suggested that by depressing the metabolism during cold acclimation the crayfish would need to utilize less stored materials.

The findings in this project seem to indicate that thermal acclimiation in O. virilis is a function of photoperiod as well as temperature. Case (1970) has shown that during the winter, the water temperature of the Rat River ranges from 2 to 3°C for at least two months. Therefore the crayfish in Manitoba must be able to survive the low temperatures. When Figures 17A and 18A are compared, it can be seen that associated with a short photoperiod is a reduction in oxygen uptake at low acclimation temperatures. In fact, no acclimation occurs for either the resting or the active metabolism (Figures 12-13). However, the temperature of the Rat River rises above 6°C in May, the same time at which O. virilis begin to copulate (Crocker and

and Barr, 1968). Associated with the increase in temperature is the increase in day-length. From Figures 17A and 17B it can be seen that a longer day-length elevates the metabolism in spite of low temperatures.

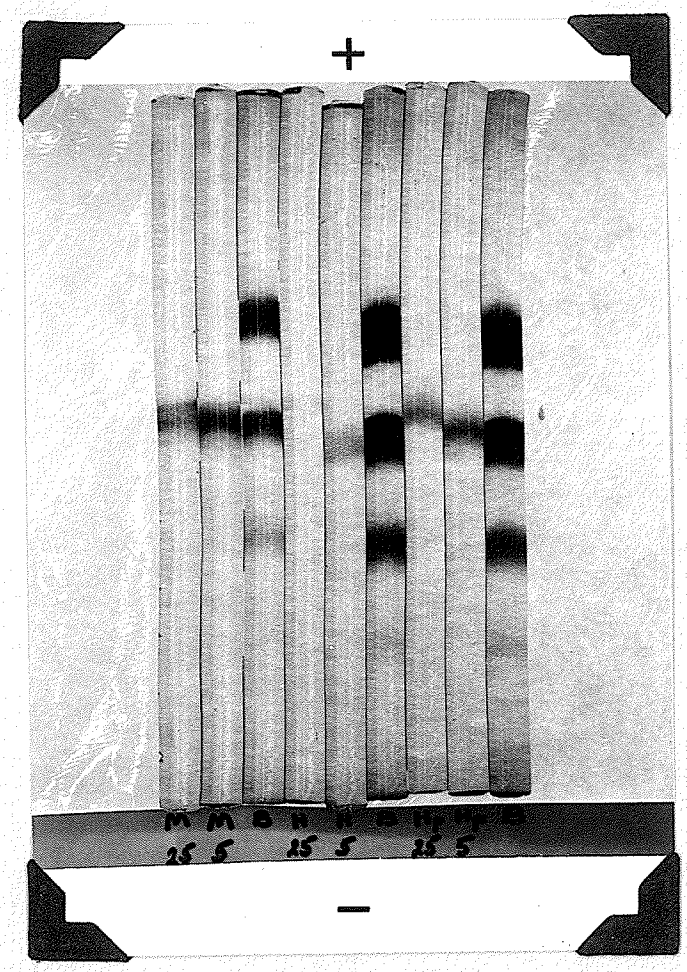
For example, the oxygen uptake values for active and resting metabolism of a Series I crayfish acclimated to 5°C and weighing 3.04g are 1.85 and 0.49 mg O_2 per hour respectively, whilst the corresponding oxygen uptake values of a Series II crayfish acclimated at 5°C weighing 3.88g would be approximately 0.90 and 0.43 mg O_2 per hour respectively.

Therefore the findings in this investigation would indicate that the pattern of acclimation of O. virilis is dependent upon the photoperiod to which it is exposed. It is possible that dependence upon the photoperiod is selectively advantageous to O. virilis as it may permit a cue for indicating a level of metabolism the species should obtain; low metabolism during the winter, and a high metabolism during the spring and summer.

5. Electrophoresis of LDH and a-GDH Crude Extracts from O. virilis:

The properties of LDH extracted from O. limosus have been described by Urban (1969) who found only one electrophoretic band for several tissue extracts including heart, muscle and hepatopancreas. Plate 2 shows electrophoretograms of tissue extracts of heart, muscle and hepatopancreas extirpated from 5- and 25°C-acclimated O. virilis and stained for LDH activity. Rabbit blood was simultaneously electrophoresed for comparison. LDH from O. virilis corresponds in migration

Plate 2: Electrophoretogram of LDH from Orconectes virilis and the rabbit, Lapins albino. Acclimation temperatures of the crayfish were 25°C. M. crayfish abdominal muscle; B. rabbit blood; H. crayfish heart; Hp. hepatopancreas. Conditions: Gel concentration - 4%; Gel buffer - 0.050 M Tris/HCl pH 8.8; Bridge buffer 0.3M Tris/HCl - pH 9.4; Running time - 4 hours at 4 milliamperes per gel and variable voltage.


+
M M B N N B N N B
25 5 15 5 25 5
−

to rabbit LDH 4. Results from Urban's extensive research indicate that other properties such as molecular weight and inhibition by pyruvate are similar in crayfish and mammals as well.

Electrophoresis of a-GDH crude extracts of muscle and hepatopancreas followed by activity staining produced only one band.

a. Significance of results:

These results suggest that O. virilis does not have the genetic ability to yield various combinations of bands of LDH or a-GDH in response to cold temperatures. Thus the mechanisms of thermal acclimation in O. virilis may be different than those in goldfish and trout (Hochachka, 1967).

6. Specific Activities of LDH and a-GDH in Crude Extracts from C. bartoni:

Homogenates of abdominal muscle and hepatopancreas from cold (9°C)- and warm (25°C)- acclimated C. bartoni were assayed for LDH and a-GDH. As indicated in Tables XXI and XXII, the activity of LDH was higher in the muscle extracts and lower in the hepatopancreas extracts from cold-acclimated crayfish when compared to the activities for warm-acclimated crayfish.

Tables XXIII and XXIV list the results of the assays for a-GDH. The assays indicate that no significant change in the activity of this enzyme from muscle and hepatopancreas occurs during cold acclimation. On the other hand, the value obtained from the t-test of the results on muscle is close to the value of the 5% level of confidence. If

TABLE XXI

Lactic dehydrogenase specific activity in the abdominal muscle of cold- and warm-acclimated Cambarus bartoni.

Trial No.	Acclimation Temp. (C)	Cuvette volume (ml)	Protein Conc. (mg/ml)	Specific activity (micro-moles NAD reduced/min./mg protein)
1	9	2.35	32.0	0.02832
	25	2.35	8.0	0.01888
2	9	2.35	22.6	0.03342
	25	2.35	14.6	0.02844
3	9	2.35	31.8	0.05700
	25	2.35	35.0	0.01789
4	9	2.35	32.8	0.04845
	25	2.35	32.8	0.04156
5	9	2.35	33.0	0.04126
	25	2.35	45.2	0.04008
6	9	2.35	62.0	0.00363
	25	2.35	29.2	0.00387
7	9	2.75	21.0	0.00926
	25	2.75	17.0	0.00312
8	9	2.45	40.5	0.04095
	25	2.45	31.0	0.01652

Hypothesis: The differences between the specific activities of cold and warm acclimated crayfish muscle LDH is equal to zero.

$$t = \frac{0.01148}{0.00469} = 2.45 \text{ with } 7 \text{ d.f. } -1.90 < t < 1.90$$

Conclusion: The hypothesis is rejected; the LDH activity of cold-acclimated crayfish muscle is higher than that of warm-acclimated crayfish muscle.

TABLE XXII

Lactic dehydrogenase specific activity in the hepatopancreas of cold- and warm-acclimated Cambarus bartoni.

Trial No.	Acclimation Temp. (C)	Cuvette volume	Protein Conc. (mg/ml)	Specific activity (micro-moles NAD reduced/min/mg protein)
1	9	2.55	38.0	0.00043
	25	2.55	47.0	0.00080
2	9	2.45	35.0	0.00074
	25	2.45	24.6	0.00160
3	9	2.35	49.2	0.00076
	25	2.35	24.0	0.00262
4	9	2.45	74.0	0.00086
	25	2.35	35.0	0.00131
5	9	2.55	35.0	0.00112
	25	2.55	33.0	0.00124
6	9	2.45	33.3	0.00156
	25	2.45	58.0	0.00130
7	9	2.55	43.0	0.00095
	25	2.55	21.0	0.00130
8	9	2.35	29.0	0.00130
	25	2.45	31.0	0.00191

Hypothesis: The difference between the specific activites of cold and warm-acclimated crayfish hepatopancreas LDH is equal to zero.

$$t = \frac{-0.000434}{0.000224} = -1.94 \text{ with } 7 \text{ d.f.}$$

critical region: $-1.90 < t < 1.90$ for P = 5% with 7 d.f.

Conclusion: The hypothesis is rejected.

TABLE XXIII

Alpha-glycerolphosphate dehydrogenase specific activity in the abdominal muscle of cold- and warm-acclimated Cambarus bartoni.

Trial No.	Acclimation Temp. (C)	Cuvette volume (ml)	Protein Conc. (mg/ml)	Specific Activity (micro-moles NAD reduced/min/mg protein)
1	9	2.22	22.6	0.0489
	25	2.22	14.6	0.0342
2	9	2.22	31.8	0.00785)*
	25	2.22	35.0	0.01631)*
3	9	2.22	32.8	0.0174
	25	2.22	32.8	0.0044
4	9	2.22	33.0	0.01081
	25	2.22	45.2	0.00750
5	9	2.22	62.0	0.00086
	25	2.22	29.2	0.00122
6	9	2.35	21.0	0.00359
	25	2.35	17.0	0.00177
7	9	2.35	40.0	0.0283
	25	2.35	31.0	0.0183
8	9	2.525	20.0	0.00355
	25	2.525	17.0	0.00235
9	9	2.35	81.0	0.01072
	25	2.35	54.0	0.00979

Hypothesis: The difference between the specific activities of cold and warm acclimated muscle a-GPDH is equal to zero.

$$t = \frac{0.00403}{0.00249} = 1.61 \text{ with } 8 \text{ d.f.}$$

critical region; $-1.86 < t < 1.86$ for P = 5% with 8 d.f.

Conclusion: The hypothesis is accepted.

* When these observations are dropped, t = 2.62
critical region: $-2.36 < t < 2.37$ for P = 2.5% with 7 d.f.
In this case the hypothesis would be rejected.

TABLE XXIV

Alpha-glycerolphosphate dehydrogenase specific activity in the hepatopancreas of cold and warm-acclimated Cambarus bartoni.

Trial No.	Acclimation Temp. (C)	Cuvette volume (ml)	Protein Conc. (mg/ml)	Specific Activity (micro-moles NADH oxidized/min/mg protein)
1	9	2.22	35.0	0.00127
	25	2.22	24.6	0.00180
2	9	2.32	49.2	0.00151
	25	2.32	24.0	0.00310
3	9	2.32	74.0	0.00109
	25	2.32	35.0	0.00106
4	9	2.42	35.0	0.00222
	25	2.42	33.0	0.00353
5	9	2.22	33.3	0.00107
	25	2.22	58.0	0.00130
6	9	2.65	35.0	0.00146
	25	2.65	31.0	0.00082
7	9	2.55	29.0	0.00141
	25	2.55	31.0	0.00198
8	9	2.35	81.0	0.00060
	25	2.45	63.0	0.00093
9	9	2.325	51.0	0.00183
	25	2.325	29.0	0.00128

Hypothesis: The difference between the specific activities of cold and warm-acclimated hepatopancreas a-GPDH is equal to zero.

$$t = \frac{-0.00019}{0.00028} = -0.66 \text{ with } 7 \text{ d.f.}$$

Conclusion: The hypothesis is accepted.

one pair of the observations is excluded (No. 2, Table XXIII) the value of t is such that it would be concluded that there is a higher activity of a-GDH from cold-acclimated crayfish muscle when compared to warm-acclimated crayfish muscle. There is no empirical evidence to justify the rejection of this pair of observations. However, the difference between these observations is so much greater than the differences between the other pairs of observations there is some doubt about the validity of this set of experiments.

The value of t obtained from the t-test for observations of the hepatopancreas extracts is so low that there is no doubt about the validity of the conclusions.

a. Significance of the Results:

Crustacean intermediary metabolic pathways are qualitatively similar to those found in the majority of organisms. The Embden-Meyerhof and hexose monophosphate pathways plus the citric acid cycle occur in the lobster Homarus americanus (Hochachka et al, 1962), in the crab Hemigrapsus nudus (Hu, 1958; Bergreen et al, 1961), and in the crayfish O. virilis (McWhinnie and Kirchenberg, 1962; McWhinnie and Corkill, 1964; McWhinnie and O'Connor, 1967) and other Crustacea (Vonk, 1960; Boulton and Huggins, 1970).

Aspects of lipid metabolism in the Crustacea have been investigated by Zandee, (1966), Brockerhoff and Hoyle (1967) and O'Connor (1968) and O'Connor and Gilbert (1969). These investigations indicate that: (1) little glucose is converted into lipid, (2) dietary lipid is readily incorporated into fat reserves with little alteration, and (3) ace-

tate can be used for fatty acid synthesis.

Evidence provided by Huggins (1966) and Jungreis (1967) indicates that the main energy reserve in decapods may be protein, especially collagen, which had a high proportion of proline, a precursor of glutamic acid.

Activities of LDH in tissue extracts from the crayfish O. limosus and a-GDH in tissue extracts from the isopod Oniscus asellus have been reported by Urban (1969) and Hartenstein (1964a, 1964b) respectively. Urban found that the specific activity of LDH in a crude extract of abdominal muscle of O. limosus was 0.175 micro-moles of NAD per minute per mg protein. However, when the formation of NADH was followed, Urban found that the specific activity of the enzyme of this reverse reaction was lower by a factor of 0.37. This would indicate therefore that if lactate had been used for the LDH assay of a crude extract of muscle from O. limosus, the specific activity would have been reported by Urban to be approximately 0.065 units per mg protein. Urban's reported value of LDH specific activity in O. limosus is twice as high as the average values reported for crude extracts of muscle LDH for C. bartoni in this project (Table XXI)

Hartenstein (1964a) has reported that the specific activity of a-GDH in the body wall muscle is 0.0141 units per mg protein of muscle extract. There was no reaction when the hepatopancreas extract was used. Hartenstein's value for muscle a-GDH is close to the average value obtained for muscle extracts from C. bartoni.

Table XXV shows the percentage change in specific activity of LDH

and a-GDH crude extracts from muscle and hepatopancreas during temperature acclimation. The Table also shows a-GDH specific activity as a percentage of LDH specific activity.

Assuming that *in vitro* temperature does not alter molecular configuration of either LDH, a-GDH or both, and therefore does not alter the K_m values of these enzymes, there will be no difference in the ratio of a-GDH specific activity to LDH specific activity when the assays are carried out either at 25 or 9°C. In other words, if it is assumed that during cold acclimation in the crayfish there is neither a change in enzyme structure, nor a change in the amount of soluble protein (Jungreis and Hooper, 1968) but only a change in the enzyme concentration, then the ratio of a-GDH specific activity to LDH specific activity, when measured at 25°C is useful in indicating changes in flux through metabolic pathways.

Following the reaction catalyzed by aldolase the two products can follow one of two pathways, or follow both pathways with a certain proportion of the resulting end products being produced in each pathway. Glucose anaerobic catabolism results predominantly in the formation of lactic acid in vertebrate muscle. However, in insect muscle, the anaerobic metabolism of glucose results in the formation of a-glycerophosphate (Gilmour, 1961). The a-glycerophosphate is then transported to the mitochondria and oxidized to yield ATP (Baranowski, 1963; Estabrook and Sactor, 1958; Weis-Fogh, 1961; Sactor and Dick, 1962).

Which pathway is predominantly followed may be dependent upon the

relative specific activities of LDH, a-GDH, and glyceraldehyde phosphate dehydrogenase (GPDH). Evidence that one of the rate-limiting reactions of glycolysis is catalyzed by GPDH has been presented by Velick and Furfine (1963) and Hartenstein (1964b). This enzyme is NAD-dependent (Conn and Stumpf, 1963). If the amount of NAD and NADH remains constant, then by increasing the specific activity of either LDH or a-GDH, the rate of recycling NAD will increase with the result there will be an increase in the rate of flux through glycolysis (Hochachka, 1967). If there were no LDH, then the formation of NAD must be dependent upon the reaction catalyzed by a-GDH, whilst if there were no a-GDH, then the formation of NAD must occur during the reaction catalyzed by LDH. In the coxal muscle of the insect, Periplaneta americana, the activity of LDH is 0.31% of the activity of a-GDH (Chefurka 1958). In man, the activity of LDH in the skeletal muscle is approximately 100% greater than that of a-GDH (Schmidt and Schmidt, 1960: In: Bergemeyer, 1963, p 659).

The formation of lactate has been defined as the first form of glycolysis while the formation of a-glycerophosphate has been defined as the second form (Neuberg and Reinfurth, 1910; in Mahler and Cordes, 1966, p 433). The percentage values in the bottom line of Table XXV provides a rough estimation of the relative proportions of the two forms of glycolyis in the hepatopancreas and muscle of C. bartoni. A comparison of the values between the two tissues indicates that relatively more a-glycerophosphate is being produced in the hepatopancreas than in the muscle at both acclimation temperatures. However,

the absolute amount of a-glycerophosphate being produced in the hepatopancreas is probably much less than the absolute amount being produced in the muscle. The specific activity of a-GDH in hepatopancreas crude extracts is much lower than the specific activity in the muscle crude extracts. Because the specific activity of LDH is also much lower in hepatopancreas than in the muscle, it would seem that any lactate produced in the muscle would not be transported to the hepatopancreas (liver) as in man (Guyton, 1966), but rather the lactate is probably reconverted to glycogen in the muscle as in the frog (Baldwin, 1953). The low specific activity values for the two enzymes in the hepatopancreas would indicate that glycolysis is not an important pathway for energy production. Zandee (1966) and Huggins (1966) have demonstrated that glucose is not an important source of acetate in the hepatopancreas of the crayfish and isopod respectively. Jungreis (1967) has demonstrated that glycogen is not an important energy reserve in the crayfish. Thus the above interpretation of the results seems valid. The accumulated a-glycerophosphate in the hepatopancreas may be used as a substrate for lipid synthesis (Kennedy, 1961; Kornberg and Pricer, 1953), or possibly it may be transported to the mitochondria as in insects (see page 114). This speculation could easily be tested.

Cold-acclimation seems to influence the relative proportions of flux through the first and second forms of glycolysis in the hepatopancreas. The specific activity of LDH drops during cold accomation by 31% while the activity of a-GDH remains constant (Table XXV). This

TABLE XXV

The average specific activities (micro-moles/min per mg protein) of LDH and a-GDH from the muscle and hepatopancreas of Cambarus bartoni acclimated to 9°C and 8L, 16D, and 25°C and 12L, 12D. Numbers in brackets are the numbers of observations.

	MUSCLES 9°C	MUSCLES 25°C	percent change in specific activity in muscle during cold acclimation
LDH	0.0328 (8)	0.0214 (8)	53.3%
a-GDH	0.0147 (9)	0.0106 (9)	+39%
	0.0155 (8)	0.0099 (8)	+57%
$\frac{\text{a-GDH}}{\text{LDH}} \times 100$	16.6% (17.5%)[1]	18.3% (17.1%)[1]	

	HEPATOPANCREAS 9°C	HEPATOPANCREAS 25°C	percent change in specific activity in hepatopancreas during cold acclimation
LDH	0.0097 (8)	0.00140 (8)	-30.7%
a-GDH	0.00142 (9)	0.00161 (9)	-21.8% (N.S.)
$\frac{\text{a-GDH}}{\text{LDH}} \times 100$	54.2%	42.6%	

1. When the pair of observations in trial 2 of Table XXIII are rejected the difference between the means is significant.

suggests that more a-glycerophosphate is being produced during cold acclimation. Again, such a hypothesis could be easily tested.

The anaerobic metabolism of glucose in crayfish muscle is also altered during cold-acclimation. In the muscle there is a 50% increase in LDH specific activity as opposed to the decrease found in the hepatopancreas. The results for a-GDH are more difficult to interpret. If all pairs of observations are included (Table XXIII), then the results of the t-test indicate that 39% increase in specific activity is not significant at the 5% level of confidence. Hence the results suggest that during cold-acclimation there is an absolute increase in the rate of glycolysis, with the increase in flux passing through the first form.

However, if the values of one pair of observations are dropped (No. 2), then a different interpretation of the results would be made. While there is no objective justification for rejecting this pair of observations, it seems reasonable because the difference between the two specific activity values is so unlike any of the other values obtained. Rejecting the values would yield averages of specific activities of a-GDH for warm- and cold-acclimated C. bartoni which indicates that there is a 57% increase in a-GDH specific activity of a crude extract of muscle during cold acclimation. However the relative flux through the first and second forms would remain constant.

The results therefore would suggest that during winter-acclimatization of C. bartoni there is a change in the specific activity of LDH in muscle and hepatopancreas, plus a change in the specific activity

of a-GDH in the muscle. After certain assumptions are made, it could be concluded that in the hepatopancreas there is a decrease in the rate of flux through glycolysis, while in the muscle there is an increase in the rate.

SUMMARY AND CONCLUSIONS

1. The relationship between oxygen uptake (mg O_2/hr per organism) and weight was shown to be a straight line when log oxygen uptake was plotted against log dry weight for the crayfish Orconectes virilis.

2. There was no significant difference at the 5% level of confidence between slopes of the regressions for active and resting metabolism at any combination of acclimation temperatures and experimental temperature. This indicated that activity of crayfish did not influence the rate of change of oxygen uptake with changing weight.

3. The intercepts of the active metabolism-weight regressions were significantly higher than the intercepts of the resting metabolism-weight regressions.

4. The slopes of the metabolism-weight curves seem to decrease with either increasing acclimation temperatures or increasing experimental temperatures. The change in slopes may be due to the difference in temperature sensitivity of large and small crayfish. Small crayfish seem to be more sensitive to a change in temperature (higher Q_{10}) values than are large crayfish.

5. Metabolism-Temperature curves were derived using the metabolism-weight regressions and linear regression analysis on the relationship between temperature and oxygen uptake. The results from both methods did not yield the same patterns of acclimation.

6. A comparison of the metabolism temperature curves revealed that the patterns of thermal acclimation may be dependent upon the photoperiod. Active cold-acclimated crayfish held under a photoperiod regime of 8L, 16D yielded a "no acclimation" pattern while active cold-acclimated crayfish held under a photoperiod regime of 24L, 0D yielded curves which were translated upwards.

7. Precht-types were determined for the Series I and II crayfish, and the results were discussed in relation to the life history of Orconectes virilis in southern Manitoba.

8. The specific activities of LDH and a-GDH in the muscle and hepatopancreas may vary according to the acclimatization state of Cambarus bartoni. Abdominal muscles of crayfish exposed to 9°C and to a photoperiod of 8L, 16D may have a higher specific activity of LDH, and possibly a-GDH, than do muscles of crayfish exposed to 25°C and a photoperiod of 12L, 12D. In the hepatopancreas, the LDH specific activity may decrease while the a-GDH specific activity probably remains constant in the cold-acclimated, short photoperiod crayfish. The results are discussed in relation to temperature adaptation at the cellular level.

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APPENDIX TABLE I

Summary of data of Series I

Acclimation Temp.(C)	Experimental Temp.(C)	Dry Body Weight(g)	Rate of Oxygen Consumption (mg O_2/hour x 100) Active Metabolism	Routine Metabolism
5	5	3.69	212.	47.2
		2.15	146.	48.5
		2.09	144.	48.2
		1.70	96.8	24.3
		1.36	97.2	24.3
5	15	3.69	294.	94.4
		3.25	285.	71.3
		2.15	243.	48.5
		2.09	289.	96.4
		1.70	193.	72.6
		1.54	194.	72.8
		1.36	146.	48.6
5	25	3.69	378.	94.4
		2.09	578.	96.4
		1.70	368.	96.8
		1.54	388.	53.4
		1.36	292.	72.9
15	5	5.20	139.	39.8
		4.77	187.	46.8
		3.14	95.0	23.8
		3.12	95.2	23.8
		2.36	96.0	12.0
15	15	5.99	413.	115.
		5.20	325.	162.
		4.06	329.	118.
		3.12	190.	119.
		2.36	192.	72.0
15	25	5.99	665.	230.
		4.06	447.	212.
		3.14	333.	142.
		3.12	428.	166.
		2.36	336.	95.2

(cont'd)

Acclimation Temp.(C)	Experimental Temp.(C)	Dry Body Weight(g)	Rate of Oxygen Consumption (mg O_2/hour x 100) Active Metabolism	Routine Metabolism
25	5	4.90	140.	46.5
		3.47	94.8	23.7
		3.29	95.0	23.7
		2.87	95.4	23.9
		2.48	95.8	12.0
		2.09	48.2	24.1
25	15	4.90	279.	114.
		3.47	284.	119.
		3.14	284.	71.1
		2.87	239.	47.7
		2.81	144.	95.8
		2.51	144.	47.9
		2.48	144.	59.9
		2.09	145.	48.2
25	25	4.09	349.	116.
		3.47	284.	119.
		3.29	427.	119.
		3.14	426.	71.1
		2.87	382.	71.6
		2.81	192.	71.9
		2.51	335.	95.8
		2.48	287.	95.8
		2.09	289.	72.3

APPENDIX TABLE II

Summary of data of Series II

Acclimation Temp.(C)	Experimental Temp.(C)	Dry Body Weight	Rate of Oxygen Consumption (mg O_2/hour x 100) Active Metabolism	Routine Metabolism
4	4	4.78	94.8	47.7
		4.59	95.6	47.5
		3.56	95.8	31.6
		3.23	50.6	33.6
4	9	6.25	253.	150.
		3.61	187.	129.
		2.96	144.	96.2
		2.92	96.2	96.2
		2.76	164.	48.3
4	12.5	5.75		141.
		4.78	284.	123.
		4.59	238.	143.
		3.68		76.5
		3.56	192.	95.8
		3.51	239.	101.
		2.61		96.4
4	25	4.78	284.	209.
		4.59	333.	230.
		4.40	324.	191.
		3.56	287.	144.
		3.51	268.	163.
		3.48	287.	144.
		3.25	250.	202.
		3.23	221.	144.
		3.17	269.	192.
		2.46	145.	96.8
9	9	3.61	173.	57.5
		2.96	166.	57.7
		2.92	144.	72.2
		2.76	144.	38.6

(cont'd)

Acclimation Temp.(C)	Experimental Temp.(C)	Dry Body Weight	Rate of Oxygen Consumption (mg O_2/hour x 100) Active Metabolism	Routine Metabolism
9	12.5	3.61	288.	95.8
		2.96	265.	135
		2.92	265.	86.6
		2.76	242.	77.3
9	25	3.61	431.	178.
		2.96	385.	178.
		2.92	336.	144.
		2.76	338.	145.
12.5	4	5.60	188.	59.5
		4.94	142.	56.9
		4.05	143.	47.7
		3.91	95.6	28.7
12.5	12.5	5.60	330.	94.2
		4.94	237.	142.
		4.05	239.	95.4
		3.91	191.	71.7
12.5	25	5.60	613.	258.
		4.94	521.	166.
		4.05	525.	239.
		3.91	478.	166.
25	4	6.02	94.4	47.0
		4.25	47.6	38.1
		3.77	95.6	38.2
25	9	4.40	143.	76.3
		3.48	192.	38.3
		3.25	144.	43.2
		3.17	96.2	38.5
		2.46	96.8	38.7
25	12.5	6.02	282.	94.0
		4.40	239.	95.4
		4.25	239.	47.8
		3.77	189.	47.6
		3.48	192.	95.8
		3.25	192.	76.8
		2.85	193.	48.2
		2.46	96.8	57.7

(cont'd)

Acclimation Temp.(C)	Experimental Temp.(C)	Dry Body Weight(g)	Rate of Oxygen Consumption (mg O_2/hour x 100) Active Metabolism	Routine Metabolism
25	25	6.02	611.	166.
		4.40	382.	181.
		4.25	430.	120.
		3.77	524.	144.
		3.48	431.	182.
		3.25	288.	188.
		2.85	377.	76.9
		2.46	387.	101.

APPENDIX TABLE B I

Reagents used for the preparation of gels, gel buffers and electrode buffers. (After Nerenberg, 1966).

REAGENTS:

1.	8% (W/V) Acrylamide monomer (Eastman #5521)[1] solution	75 ml
2.	2% N,N' methylenebisacrylamide (Eastman #8383) solution	13 ml
3.	0.28% N_4-tetramethylenediamine (Eastman #8178) solution	19 ml
4.	12% (W/V) Ammonium persulfate	0.8 ml
5.	Gel Buffer (pH 8.8) 0.05M Tris (Sigma)[2]/HCl	19 ml
6.	Electrode buffer (pH 9.4) 0.03M Tris/citric acid, sodium salt (Sigma)	1.0 l
7.	H_2O	23 ml

The final volume of the polyacrylamide solution will be 149.8 ml.

1 Eastman Organic Chemicals, Rochester 3, New York.

2 Sigma Chemical Co., St. Louis, Missouri.

APPENDIX TABLE B II

Composition of staining solution for lactic dehydrogenase and a-glycerophosphate dehydrogenase.

REAGENTS:

1.	Substrate	
	1M Solution lactate (Sigma)	20 ml
	or	
	1M a-Glycerophosphate	20 ml
2.	NAD (nicatinamide adenine dinucleotide) (Sigma, Grade III)	100 mg
3.	PMS (phenazine methosulfate) (Sigma, Grade C)	4 mg
4.	0.5M Tris/ HCl pH 7.5 (Sigma)	20 ml
5.	0.1M NaCN	10 ml
6.	H_2O	140 ml

Stain the gels in the dark.

APPENDIX TABLE B III

Concentrations and volumes of reactants used for the determination of lactic dehydrogenase activity.

REACTANTS	CONCENTRATION	VOLUME (ml)
Crude extract solution pH 8.4		0.2 - 0.4
Sodium lactate pH 7.0	1 M	1.0
NAD	36 mM	0.15
Buffer 0.1M Tris/HCl pH 8.4		1.0

APPENDIX TABLE B IV

Concentrations and volumes of reactants used for the determination of a-glycerophosphate dehydrogenase activity.

REACTANTS	CONCENTRATION	VOLUME (ml)
Crude extract solution pH 8.4		0.2 - 0.4
Dihydroxyacetone phosphate	32 mM	0.1
$NADH_2$	12 mM 5 mM	0.02 0.05
Buffer 0.1M Tris/ HCl pH 8.4		2.0

APPENDIX TABLE C I

Derivation of the formula for Specific Activity.

Lambert's Law states:

$$I = I_o \, 10^{-Kl} \qquad \text{Eq (1)}$$

"where I_o and I are the intensities of the incident and transmitted light respectively, l is the thickness of the obsorbing material and K is the extinction coefficient, defined as the reciprical of the thickness of medium (in cm) required to weaken the light to one tenth of its incident intensity" (Dawes, 1969).

$$Kl = E \qquad \text{Eq (2)}$$

which is known as the optical density.
E is dimensionless as:

$$E = \frac{1}{cm} \times cm \quad \text{or} \quad \log (I_o/I) \qquad \text{Eq (3)}$$

Beer's Law states:

$$I = I_o \, 10^{-\epsilon cl}$$

"where c is the molar concentration of the substance, l the depth of the solution, and ϵ is the molar extinction coefficient of the solute for the particular wavelength in question." (Dawes, 1969).

$$\text{Now } I_o \, 10^{-\epsilon cl} = I_o \, 10^{-Kl}$$
$$\text{Therefore } \epsilon cl = Kl$$
$$\text{Therefore } \epsilon = K/c \text{ or } \frac{1}{cm} \times \frac{cm^3}{mole} \text{ or } cm^2/mole.$$

$$\text{Since } Kl = E \text{ (Eq 2)}$$
$$\text{Then } \epsilon cl = E$$
$$\text{Therefore } c = \frac{E}{\epsilon \times l}$$
$$\text{and the dimensions are: moles/ml} = \frac{1}{cm^2/mole \times (cm)}$$

cont'd

Therefore moles formed in the cuvette =

$$\frac{1 \times \text{Vol of solution } (cm^3)}{cm^2/\text{mole} \times (cm)}$$

Therefore moles/mg $\cdot min^{-1}$ =

$$\frac{1 \times V}{cm^2 \times \text{mole}^{-1} \times cm \times mg \times min}$$

$$= \frac{1 \times V}{\times\ l \times (v \times mg/ml) \times t\ (min)}$$

where (v x mg/ml) is the amount of protein added to the cuvette.

Therefore the Specific Activity (micro-moles/mg protein $\cdot$ min^{-1})

$$= \frac{\Delta E \times V \times 10^6}{\epsilon \times l \times v \times p \times t}$$