THERMAL ACCLIMATIZATION AND ACCLIMATION IN THE ECHINOID, STRONGYLOCENTROTUS DROEBACHIENSIS (O. F. MULLER, 1776)

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ABSTRACT

Thermal adaptation in the sea urchin, Strongylocentrotus droebachiensis, is described both in regard to seasonal acclimatization in the natural habitat, and experimental acclimation in the laboratory under summer-like and winter-like thermal regimes. Intact-animal respiration, in vitro tissue respiration, and righting reflex serve as indicators of physiologic performance.

Three thermal compensation coefficients, useful for comparative purposes, are proposed. These permit quantitative description of both the magnitude and specific pattern of a given adaptation response. Assumptions, limitations, and potential difficulties associated with use of the coefficients are discussed.

Regression equations of respiration on weight are presented for summer-and winter-acclimatized urchins. Respiration rates, measured at 0°, 5°, and 10°C., are higher in winter than in summer, indicating substantial acclimatization, but at 15°C. summer and winter rates are similar.

Seasonal acclimatization is also detectable in the respiratory metabolism of several excised tissues, suggesting that increased intact-animal respiration in winter is attributable to augmentation of cellular metabolism, rather than to limitations of oxygen transport in the water vascular system in summer, or to increased efficiency of transport in winter.

An activity coefficient, based on the righting reflex, is defined and shown to be a useful indicator of activity. Acclimatization of metabolism permits urchins to maintain a relatively high rate of activity through the winter. Laboratory studies indicate that acclimation of activity requires 4-5 weeks for completion.

Acclimatization permits gonad development in autumn and winter, in preparation for spawning in early spring.

Food consumption has two maxima: in spring and again in autumn. A decline in food consumption in winter appears to be at least partially offset by an increase in feeding efficiency.

S. droebachiensis exhibits seasonal resistance acclimatization; in summer urchins are able to tolerate short-term exposure to temperature extremes several degrees higher than in winter.

That annual temperature fluctuations are primarily responsible for inducing seasonal acclimatization is indicated by the demonstration of a compensatory adjustment in the respiration of both intact animals and excised tissues of urchins acclimated for 4-6 weeks in the laboratory at summer-like and winter-like temperatures.

Ability of S. droebachiensis to adapt to low temperature is inversely proportional to size. This is demonstrated for metabolism both of intact urchins and of excised tissues. The importance of considering animal size in adaptation studies is stressed.

In general, metabolism of both intact urchins and excised tissues increases with rising temperature in accordance with the Q_{10} rule.

It is concluded that S. droebachiensis exhibits partial adaptation,

involving primarily a translation of rate-temperature relationships; as such the response corresponds to Prosser's (1961) pattern II a.

The water content of stomach tissue increases, and that of intestine decreases on cold-acclimation.

The acclimation of metabolism demonstrable in cell-free intestine homogenates is similar in magnitude and pattern to that observed in relatively undamaged tissue slices.

The hexosemonophosphate shunt enzyme G-6-P dehydrogenase is present in intestine tissue, although the shunt may not be a major metabolic route in this tissue. The activity of G-6-P dehydrogenase increases on cold-acclimation. Cold-acclimation also results in an increase in sensitivity to the glycolysis inhibitor, iodoacetic acid. These results are consistent with the mechanism of adaptation suggested by the adaptation patterns; namely, that the increase in metabolism is primarily brought about by general augmentation of enzyme activity, with little or no change in the relative contributions of alternate metabolic pathways.

The possible adaptive significance of both capacity and resistance adaptation in the life of S. droebachiensis is discussed.

It is suggested that thermal adaptation of S. droebachiensis may be explained by the origin and genetic history of the species.

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INTRODUCTION

Efficient utilization of marine resources requires knowledge of the fundamental bioenergetics of major ecological communities of the sea. Measurement of the metabolism of the plants and animals making up these communities is one important aspect of such bioenergetic studies (Kuenzler, 1969; Pamatmat, 1969). The marked temperature dependence of poikilotherm metabolism, characterized as the Krogh-Arrhenius relationship, demands that in bioenergetic studies involving temperate or boreal marine communities, allowance be made for the influence on metabolism of the considerable, seasonal temperature fluctuations. Frequently, however, such allowance for change in environmental temperature cannot be made on the basis of a single, empirical metabolic rate-temperature relationship, for "nature has learned so to exploit the biochemical situation as to escape from the tyranny of a single application of the Arrhenius equation" (Barcroft, 1934). By means of a compensatory adjustment in metabolism, termed thermal acclimatization, organisms may reduce the inimical effects of temperature change. This permits metabolism and other vital functions to continue at a relatively high level during colder months without pushing these vital functions to wastefully high levels at higher temperatures. The absence of thermal acclimatization to cold is frequently associated with winter torpor and hibernation (Bullock, 1955).

Species differ greatly in their thermal adaptation ability (see Literature Review) requiring that the response be evaluated for each principal species in the community. Adequate information on seasonal acclimatization is available for only a few marine invertebrates, including: the copepods, Diaptomus clavipes, D. pallidus, and D.silicoides (Siefkin and Armitage, 1968); the bivalve, Transennella tantilla (Pamatmat, 1969); the mole crab, Emerita talpoida and the beach flea, Talorchestia megalophthalma (Edwards and Irving, 1943); the American oyster, Crassostrea virginica (Galtsoff, 1964; Percy et al, 1971); and the fiddler crab, Uca pugnax (Vernberg, 1959). The only studies involving echinoderms concern the tropical sea urchins, Eucidaris tribuloides (McPherson, 1968) and Diadema antillarum (Lewis, 1968), and of these, only the former exhibits seasonal acclimatization of respiration. No information is available on seasonal acclimatization of metabolism in echinoderms from boreal or temperate ecosystems, where more extensive seasonal temperature fluctuations are potentially more disruptive of metabolism.

The green sea urchin, Strongylocentrotus droebachiensis (O.F. Müller, 1776)* forms an important component of many arctic-boreal,

* Echinus dröbachiensis O.F. Müller 1776, Prodromus Zoologiae Danicae, p.235. Strongylocentrotus dröbachiensis (O.F. Müller, 1776) ; A. Agassiz, 1872, Revision of the Echini, part 1, p. 162. Strongylocentrotus droebachiensis is a"justified emendation" [The International Code of Zoological Nomenclature, 1958. Art.33(a)(i).]

marine, coastal communities, not only by virtue of its abundance, but also because, as a voracious, benthic herbivore, its feeding activity may play a significant role in determining community structure (Paine and Vadas, 1969). Detailed information on the metabolism of urchins is one of the prerequisites for understanding patterns of energy flow in such arctic-boreal communities.

This study investigates the influence of seasonal temperature fluctuations on the metabolism of S. droebachiensis in a typical boreal, coastal habitat, and presents a detailed description of acclimatization in this species.

Acclimatization resulting from seasonal and geographic temperature differences, has been detected in tissue metabolism measured in vitro (Roberts, 1957; Vernberg and Vernberg, 1965; and Peiss and Field, 1950), and it is significant that it is not always possible, on the basis of the adaptation potential of intact animals, to predict the metabolic responses of their tissues (Kinne, 1964). In fact different tissues from a single organism may exhibit substantially different adaptation responses (Roberts, 1957; Vernberg and Vernberg, 1965; and Percy et al, 1971).

To ascertain whether acclimatization of intact-animal metabolism parallels similar metabolic responses at the tissue level, the metabolic rates of excised tissues of urchins are determined in summer and winter.

Concurrent studies on seasonal changes in activity, feeding rate, reproductive condition and temperature tolerance provide information on the relationship of these factors to acclimatization.

Environmental factors other than temperature, and endogenous physiological rhythms may influence metabolism and it may, therefore, not always be correct to conclude that seasonal metabolic shifts observed in natural populations are primarily attributable to thermal acclimatization. To confirm that seasonal acclimatization is induced chiefly by annual temperature fluctuations, metabolic rates of intact urchins and excised tissues, acclimated to summer-like and winter-like temperatures in the laboratory, were determined.

To facilitate comparison of the adaptation responses measured under different circumstances, I have formulated a series of thermal compensation coefficients, which not only measure the magnitude of adaptation, but also quantitatively describe the type of adaptation in terms of Prosser's (1961) adaptation patterns.

As McWhinnie (1967) points out, there are two widely used approaches to understanding adaptation. The first involves a description of physiological rate functions of organisms exposed to different adaptation temperatures. The second approach considers biochemical modifications accompanying adaptation. The studies outlined above on adaptation in S. droebachiensis are essentially of the first type, and as such provide a "physiological framework within which molecular changes can ..... be assigned" (McWhinnie, 1967). The final aspect of this study concerns the biochemical basis of adaptation with particular reference to Prosser's (1962) concept of alternate metabolic pathways.

ADAPTATION: CONCEPTS AND TERMINOLOGY

Many terms associated with thermal adaptation have been subject to a variety of interpretations. In order to minimize ambiguity I shall, at the outset, delineate the general concept of adaptation upon which this study is based and define associated technical terms.

Adaptation, in the broadest sense, is a biological process which tends to insure physiological homeostasis so that, "given a moderate disturbance that tends to displace the system from its normal values, the parts so react and interact that the harmful effects of the disturbance are much diminished" (Gray, 1961).

Adolph (1956) introduced the terms stressor, designating an environmental disturbance, and adaptate, indicating the subsequent compensatory response induced in a physiological function. The adaptate persists for a variable period following removal of the stressor. Environmental stressors may take a variety of forms, and include changes in salinity, oxygen tension, pH, temperature, pressure, current velocity, humidity, etc. Temperature, largely by virtue of the simplicity of its measurement and control, but also because of its critical biological importance, has received the most intensive study as an adaptation-inducing stressor.

Temperature, the environmental stressor of prime interest in this study, influences the rate at which metabolic and biochemical processes occur. The specific relationship between the rate of the process and the temperature is called here the rate-temperature (R-T) relationship.

The compensatory physiological adjustment induced in a biological system by a change in environmental temperature is termed thermal adaptation. The difference between two adaptation temperatures, defining a given adaptate, will be referred to here as the stress range.

Two distinct types of thermal adaptation are generally recognized (Precht, 1967): capacity adaptation, which involves adjustment of physiological rates to temperature shifts within the normal biological range (non-lethal thermal stressor); and resistance adaptation, which refers to modifications of temperature tolerance at extremes of the biological range (lethal thermal stressor), with death the end point usually measured.

It is important to differentiate clearly between non-genetic (physiological) and genetic thermal adaptation. An individual organism may, during the course of its life, frequently adjust its metabolism to compensate for a change in environmental temperature. However, the range of temperature within which the organism is thus able to adapt physiologically is limited by its genotype. Changes in the genotype may raise or lower the temperature limits within which physiological adaptation may occur, as well as alter the entire rate-temperature relationship of a given rate function. Such genetic adaptations, that may serve to compensate for long-term changes in environmental temperature, are transmissible to succeeding generations. In most cases the genetic or physiological basis of the thermal adaptation response is readily apparent, although, in instances of geographic adaptation (see below) it may be difficult clearly to differentiate.

Application of a stressor may be either intermittent, short-term exposures alternating with periods of stressor withdrawal, or continuous when exposure to the stressor is maintained indefinitely. Little is known about the relative effectiveness of these two forms of stressor application in inducing adaptation. The present study involves only continous thermal stressors.

Stressors may differ also in the swiftness of initial application. Clarke (1967) introduced the engineering terms step-function and ramp-function to describe two basic types. A step-function temperature change is virtually instantaneous and animals do not adapt to intermediate temperatures; in a ramp-function, the change occurs slowly, permitting animals at least partially to adapt to intermediate temperatures. Seasonal adaptation (see below) in the natural habitat is normally induced by an essentially ramp-function temperature change, while laboratory adaptation is usually initiated by a step-function change.

Temperature changes may induce a characteristic sequence of responses in physiological rate functions (Kinne, 1964). Immediately following a temperature shift a fast, direct adjustment in rate occurs to what I shall term a pseudostable level. The initial rate shift to the pseudostable level is completed within minutes to hours. R-T determinations reported in this study are acutely determined (insufficient time for adaptation to occur following transfer to the experimental temperature) and thus represent pseudostable rates.

If the organism is held at the new temperature for an extended period a further change in rate, of a fundamentally different type, may occur. Precht (1958) distinguishes the following five different forms of long-term rate changes (from the initial pseudostable level) that may follow transfer to a lower temperature:

Hypercompensation (Type 1) - the rate rises from the pseudostable level and stabilizes at a level higher than at the original temperature.

Perfect compensation (Type 2) - the rate rises from the pseudostable level and stabilizes at the same level as at the original temperature.

Partial compensation (Type 3) - the rate rises from the pseudostable level and stabilizes at a level below that at the original temperature.

No compensation (Type 4) - the rate remains steady indefinitely at the pseudostable level.

Reverse compensation (Type 5) [Hypoadaptation]* - the rate declines below the pseudostable level.

* "Hypoadaptation" is used in this study, being more consistent with other terms and less confusing than the terms "reverse adaptation", "inverse adaptation", "paradoxical adaptation" and undercompensation", occasionally applied to this type of response.

A comparable series of "mirror image" adjustments may occur following transfer to a higher temperature.

The new rate level attained following adaptation is termed the stabilized rate, and the time required to reach this level the stabilization period (Kinne, 1964). In this study, the term "compensation", used in Precht's scheme, will be replaced by the terms adaptation, acclimatization or acclimation, in accordance with the definitions given below.

Prosser (1961) distinguishes five distinct forms of R-T curve displacement associated with adaptation. A summary of these "patterns of adaptation" is presented elsewhere (Fig. 5).

Thus far, I have considered the nature of physiological response to temperature change, but have said little about the circumstances under which organisms are normally subjected to thermal stressors. Terminology associated with this aspect is particularly confused. In the present study terms are used as follows*: Adaptation is a general term denoting compensation in response to environmental change, regardless of whether the response is genetic or non-genetic in nature. Acclimatization designates a non-genetic adaptation that may be subject to modification by a variable number of environmental or biological factors. This type of response occurs in the natural habitat, either as a consequence of seasonal temperature fluctuations (seasonal acclimatization), or of temperature differences arising from spatial separation (geographic

* Terminology is essentially that employed by Prosser (1961).

acclimatization). The latter may be further divided into macrogeographic acclimatization when temperature differences are associated with latitudinal temperature trends, and microgeographic acclimatization when significant temperature differences occur over relatively short distances, such as intertidally, or vertically in a body of water. Acclimation is employed in a more restricted sense, and refers to non-genetic adaptation in response to change in a single environmental factor. This type of adaptation is normally observed under laboratory conditions. That we distinguish between acclimation and acclimatization does not imply fundamentally different mechanisms, but merely serves to emphasize the more complex nature of the latter. The specific manner in which adaptation is induced, whether by experimental, seasonal or geographic means, will be referred to here as the mode of adaptation.

In the present study, use of the terms warm-acclimated, cold-acclimated, summer-acclimatized, winter-acclimatized, etc. implies that the designated animals have been exposed to the indicated temperature regime for a period at least as long as the stabilization period.

For reasons of economy, expressions such as "cold-adapted rate functions", "warm-adapted Arrhenius curves", etc., will be used in place of the more formally correct "rate functions of cold-adapted urchins", "Arrhenius curves of warm-adapted urchins", etc.

LITERATURE REVIEW

Thermal adaptation has been a subject of continuing biological interest ever since the realization by Krehl and Soetbeer (1899) that poikilotherms are not merely "Spielballe der Umgebung" (Playthings of the environment). The widespread occurrence and biological significance of thermal adaptation is attested to by a voluminous literature, embracing contributions of physiologists, ecologists and biochemists. Because excellent general discussions of thermal adaptation are already available (Prosser and Brown, 1961; Hoar, 1966), and because of the scope of the literature on thermal adaptation, it will be necessary to limit this review primarily to the seasonal aspects of the phenomenon. After briefly considering physiological indicators useful in investigating thermal adaptation, I discuss seasonal capacity-acclimatization, first from the point of view of intact-animal metabolism, then with regard to metabolism of tissues in vitro. The nature of changes in rate-temperature relationships that follow thermal adaptation is considered in a resume of the controversy regarding the precise physiological significance of modifications of temperature characteristics. There is a brief survey of studies concerning mechanisms of adaptation, and a discussion of seasonal resistance-acclimatization.

i) Physiological indicators of adaptation:

The pervasive influence of temperature adaptation on physiological functions is evident from the fact that a wide range of physiological indicators have served as measures of metabolic adaptation. Thermal adaptation has been demonstrated in such diverse processes as: growth rate in molluscs (Dehnel, 1955, 1956); locomotory rate in protozoans (Shortress, 1942); heart rate in molluscs (Takatsuki, 1929; Segal et al, 1953); water propulsion rate in bivalves (Rao, 1953); nerve conduction velocity in fish (Konishi and Hickman, 1964); locomotory pulsation rate in coelenterates (Mayer, 1914); protein synthesis rate (Rao, 1967); ciliary beat rate in bivalves (Lagerspetz and Dubitscher, 1966); and rate of opercular movement in fish (Roberts, 1964). Although each of the above functions provides a valid measure of adaptation within a specific group, for comparative purposes it is the respiration rate that probably offers the most direct and useful estimate. The literature devoted to thermal adaptation of respiration is particularly voluminous. Pertinent reviews include those of McWhinnie (1967), Bullock (1955), and Vernberg (1962).

ii) Seasonal acclimatization in intact animals:

Organisms may respond to seasonal temperature fluctuations by adaptive modifications of metabolic rate. One important aspect of seasonal acclimatization to cold is that the acclimatized animal usually can maintain a high level of general activity even during winter. Schwartz (1884)*, as well as Aderhold (1888)*, for instance, noted that Euglena sp. in summer becomes immobile when the temperature falls below 5°C.; in winter, on the other hand, activity continues down to 0°C. Mean respiration rate of the terrestrial amphipod, Talitrus sylvaticus, over a broad range of temperature is 1.5 times greater in winter than in summer (Clark, 1955), presumably permitting a high level of activity in winter. Additional examples of adaptation in terrestrial creatures have been cited by Bullock (1955).

Many coastal, marine animals in temperate latitudes are also capable of acclimatization. The crustacean, Emerita talpoida, in the Woods Hole region, acclimatizes so that in winter, at 3°C., its metabolic rate is about the same as that in summer at 15°C.; thus, the animal is active the year round (Edwards and Irving, 1943). Oxygen consumption, at a number of temperatures, in the fiddler crab, Uca pugnax, is higher between November and January than between June and August (Vernberg, 1959).

Studies involving seasonal changes in metabolism of a number of intertidal invertebrates clearly demonstrate the need for caution in attributing acclimatization solely to seasonal fluctuations in seawater

* Cited by Behre (1918).

temperature. The bivalve, Transennella tantilla, becomes cold-acclimatized in late autumn, with a metabolic adjustment in the form of a clockwise rotation of the respiratory R-T curve (Prosser pattern III a; see Figure 5), with curves for warm-and cold-acclimatized rates intersecting within the seasonal thermal stress range (Pamatmat, 1969). However, in this instance, acclimatization is not directly related to seasonal changes in seawater temperature, but rather "cold-adaptation was induced by periodic exposure to low temperatures during low tide, while warm-adaptation, or loss of cold-adaptation resulted from periodic exposure to higher temperatures". In other words, intermittent thermal stressors (during air exposure) were of prime importance in inducing acclimatization. A similar modification of the acclimatization response was observed in the crab, Pachygrapsus crassipes. Seasonal acclimatization in this species was practically obliterated as a result of short-term fluctuations in intertidal temperatures (Roberts, 1952). Furthermore, the extent of seasonal acclimatization of heart rate in the limpet, Acmaea limatula, depends, to a large degree, on the location of the animals in the intertidal zone (Segal, 1956). Populations located low in the zone demonstrate a clear, inverse relationship between heart rate and seasonal temperature change, winter and spring rates being higher than summer rates at any given temperature. In populations located high in the intertidal zone on the other hand, seasonal acclimatization was masked by longer periods of air exposure.

Tropical poikilotherms, in general, do not exhibit significant seasonal acclimatization of metabolism, possibly because of the relative thermal constancy of their habitat. Tropical Uca rapax, unlike temperate U. pugnax, referred to earlier, shows no seasonal fluctuations in metabolism (Vernberg, 1962). Likewise, the tropical urchin, Diadema antillarum, collected at Barbados, does not modify its oxygen uptake with the seasons (Lewis, 1968). The sub-tropical urchin, Eucidaris tribuloides, on the other hand, native to Florida, where limited, seasonal temperature fluctuations do occur (winter and summer means: 20° and 30°C., respectively), has a well-defined acclimatory response, with winter rates being higher than summer rates between 15° and 30°C. (McPherson, 1968).

It seems likely that seasonal acclimatization occurs most frequently in animals inhabiting temperate latitudes, because there the fluctuating temperatures are conducive to labile metabolic systems amenable to temperature adaptation. Vernberg (1962) suggests that temperate zone species have a greater "degree of thermal lability which might be associated with a more complex genetic system, especially if most physiological factors are polygenic in nature". Seasonal acclimatization, however, is not universal among temperate zone species, and there are many which become torpid in winter. The metabolic rate, at any given temperature, of the sand flea, Talorchestia megalophthalma, for example, is the same summer and winter and, as a consequence, the animal's activity is severely depressed in winter (Edwards and Irving, 1943). In marked contrast, the crustacean, Emerita talpoida, occurs in the same area, but

is able to acclimatize and thus to remain active throughout the winter. A further example is provided by three species of actinians. Metridium senile, from Massachusetts, is capable of partial acclimation, while Haliplanella luciae and Diadumene leucolena, from Virginia, exhibit hypoacclimation. This latter response is associated with encystment and negative adjustment of metabolic rate (Sassaman and Mangum, 1970). These studies on actinians involve acclimation in the laboratory; whether similar metabolic adjustments occur seasonally is uncertain (see below). Another temperate species, the ant, Formica ulkei, under a thermal regime approximating that of its normal environment, exhibits hypoadaptation, in that summer metabolism, at any given temperature, is higher than that in winter (Dreyer, 1932). Although many fish are capable of adaptation (Fry, 1967; Roberts, 1967), summer and winter respiratory R-T curves of the cunner, Tautogolabrus adspersus, coincide, and the fish become torpid in winter (Haugaard and Irving, 1943).

Wide variations in adaptive ability among different species, similar to those associated with seasonal temperature fluctuations, have also been observed in conjunction with geographic and experimental temperature differences, and, considered together, lead to the conclusion that the occurrence of thermal adaptation in the animal kingdom is "apparently wide, but far from universal" (Bullock, 1955).

iii) Adaptation in excised tissues:

Adaptation of metabolism also occurs in excised tissues and has been correlated with geographic (Vernberg, 1962), seasonal (Hopkins, 1946), and experimental (McWhinnie and O'Connor, 1967) temperature shifts. Prosser (1962) tabulated data on tissue adaptation in poikilothermic vertebrates, and clearly showed that considerable variation exists in the degree and nature of adaptation in different tissues from the same animal. Similar tissue adaptation data for invertebrates are less extensive, and restricted primarily to crustaceans and molluscs. The hepatopancreas from the temperate crayfish, Orconectes virilis, for instance, significantly increases metabolism on cold-acclimation (McWhinnie and O'Connor, 1967). Similarly, the respiration rate of excised muscle of the striped shore crab, Pachygrapsus crassipes, exhibits temperature adaptation (Roberts, 1952). Excised gill, mantle, and adductor muscle of the clam, Mercenaria mercenaria, seasonally acclimatize to cold (Hopkins, 1946). So far, however, no information has been available regarding adaptation of metabolism to temperature in echinoderm tissues.

Tissues do not necessarily show adaptation responses that are quantitatively or qualitatively similar to those of the animal from which they are taken, and, in fact, different tissues from the same animal may have substantially different adaptation patterns. In the fiddler crabs, Uca rapax, U. pugnax, and U. pugilator, there is hypoadaptation of the metabolism of excised esophageal ganglia, and in both U. rapax and U. pugnax heart tissue also exhibits hypoadaptation; in U. pugilator,

however, heart tissue does not adapt (Vernberg and Vernberg, 1965). Goldfish brain brei shows partial adaptation, while muscle tissue exhibits hypoadaptation (Freeman, 1950). The intact sunfish, Lepomis gibbosus, shows evidence of significant metabolic adaptation to cold, whereas its excised brain and liver tissues do not (Roberts, 1967). In the oyster, Crassostrea virginica, gill and mantle exhibit seasonal hypoacclimatization, with summer rates higher than winter rates at similar temperatures, while adductor muscle metabolism partially acclimatizes (Percy et al, 1971).

As Vernberg (1962) points out, studies such as those outlined above suggest that "certain tissues are more important than others in regulating the metabolic response of the organism". However, in drawing conclusions from tissue adaptation studies it is important to keep in mind the observation of Precht (1967) on hormonal mediators: if the hormone induces a metabolic shift that persists in the hormone's absence (after effect) the effect will be detectable in the excised tissues; if the hormonal effect is of a direct type, however, and is abolished in the absence of the hormone, then the excised tissues will not display metabolic adaptation.

iv) Adaptation and temperature characteristics:

After reviewing rate-temperature (R-T) data from numerous species of warm-and cold-adapted poikilotherms, Prosser (1961) concluded that five basic patterns of adaptation exist. These are graphically presented in Figure 5. Patterns are distinguishable on the basis of the nature of the displacement of cold-relative to warm-adapted R-T curves for a particular physiological rate function. The final disposition of warm-and cold-adapted curves may be such that they are either superimposed, translated and/or rotated relative to one another. Superposition indicates a lack of adaptation while translation implies that warm-and cold-adapted R-T curves are essentially parallel and thus, slopes, temperature characteristics (μ)*, and Q_{10} coefficients are similar. Rotation results in differences in slopes, temperature characteristics and Q_{10} coefficients; the direction of rotation determines whether these several parameters are greater in warm-or in cold-adapted systems.

A decrease in slope of an R-T curve (and in associated parameters) following cold-adaptation is conventionally assumed to be of adaptive significance, because metabolic fluctuations resulting from temperature changes tend to diminish as the system becomes less temperature dependent. Examples involving geographic acclimatization include pumping rate in

* Also termed "critical thermal increment" or "apparent activation energy". For a discussion of the interpretation and derivation of temperature characteristics and Q_{10} coefficients refer to Hoar (1966).

the mussel, Mytilus californianus, which has a lower Q_{10} coefficient in colder northern areas than in relatively warm southern waters (Rao, 1953). Similar results have been reported for seasonally acclimatized animals. In the bivalve, Transennella tantilla, for example, respiratory Q_{10} coefficients are lower in autumn, when animals are most cold-adapted, than at other times of the year (Pamatmat, 1969). A number of species of cottid fish have a generally higher rate of respiration and a lower Q_{10} in winter than in summer (Morris, 1961). Respiratory Q_{10} coefficients of the crayfish, Orconectes virilis, are lower in cold-acclimated than in warm-acclimated animals (McWhinnie and O'Connor, 1967). Similarly, in the urchin, S. purpuratus, a decrease in Q_{10} coefficients, measured between 10° and 20°C., follows cold-acclimation, although, between 5° and 10°C. the Q_{10} values of warm and cold-acclimated animals are similar (Farmanfarmaian and Giese, 1963). Bullock (1955) discusses additional examples and concludes that in general cold-adapted poikilotherms have reduced R-T slopes.

However, the occurrence of exactly the opposite rotational trend (R-T slopes of cold-adapted animals greater than the slopes of warm-adapted ones) tends to proscribe use of the concept of adaptation by a decreased temperature dependence of the R-T relationships, as a general principle. For example, several species of the crustacean, Diaptomus, adapt to elevated temperatures with an accompanying reduction in Q_{10} coefficients (Siefkin and Armitage, 1968). Again, respiratory Q_{10} coefficients in the toad, Bufo boreas, are higher in cold-adapted than in

warm-adapted animals (Bishop and Gordon, 1967). A variety of arctic insects, spiders and molluscs have higher Q_{10} coefficients than tropical species (Scholander et al, 1953). The gastropod, Monodonta lineata, from British waters has higher respiratory Q_{10} coefficients than Monodonta turbinata from the Mediterranean (Micallef and Bannister, 1967). Mutchmor (1967), following studies of rate-temperature relationships of apyrase activity in several species of warm-and cold-adapted insects, concludes that higher R-T slopes, frequently noted in cold-adapted insects, might in fact be of adaptive significance, because "small increases in temperature just above coma temperature would increase apyrase activity much above the minimum for muscular activity. Substantial activity at lower temperatures would thereby be permitted."

To further becloud the issue, there are several instances in which adaptation does not appear to involve significant changes in R-T slope. Tashian and Ray (1957), for example, found little difference between respiratory Q_{10} coefficients of a number of species of tropical and temperate amphibians. A similar absence of rotation of respiratory R-T curves characterizes adaptation in a number of species of microorganisms (Christopherson, 1967).

From the foregoing it is clear that defining the adaptive significance of changes in temperature coefficients following thermal adaptation is no straightforward matter. It may indeed be that no broad generalization is possible, and that the adaptive significance of particular patterns of R-T curve displacement will have to be separately assessed in each individual case, with due regard not only to thermal requirements of the animals but also to particular thermal characteristics of their habitat.

v) Mechanisms of capacity adaptation:

As newer experimental approaches provide increasingly detailed information on biochemical changes associated with thermal adaptation, it is becoming clear that "one cannot speak of a single critical mechanism of acclimation" (Prosser, 1967). This might have been anticipated in view of the obvious complexity associated with adaptive compensation in a finely integrated metabolic network with a multitude of ramifications and interrelationships maintained in continual harmony. Furthermore, it may well be that while thermal adaptation in unicellular and other primitive organisms is a direct cellular response, in higher poikilotherms, it is probably a complementary involvement of both direct, primitive, cellular responses and integrated, systemic responses of more recent development (Roberts, 1967). Such an integrated system would probably permit a greater flexibility of metabolic adaptation than direct cellular responses alone.

It thus appears that the biochemical basis for thermal adaptation can be conveniently considered at two distinct levels, provided that the intimate relationship between them is kept clearly in mind. First, from the point of view of biochemical changes occurring in cell metabolism as a consequence of adaptation, and secondly, with regard to the nature of the hormonal and/or nervous integrative mechanisms that permit systemic regulation of metabolic compensation.

Modifications of cell function associated with thermal adaptation are many and varied. Changes in: enzyme activity (Prosser, 1962); metabolic pathways (Hochachka and Hayes, 1962); RNA concentration and protein

synthesis rate (Saroja and Rao, 1965); degree of unsaturation of lipids (Johnson and Roots, 1964); total lipid content (Hoar and Cottle, 1952); isozyme complements (Hochachka, 1965); ionic concentration of body fluids (Saroja and Rao, 1965); and ratio of free and bound water (Precht et al, 1955), suggest a complex and pervasive metabolic response to temperature change. In view of the prominent role of enzymes in regulating cellular metabolism, the changes in enzyme activity are of particular interest.

Probably the single most unifying concept presently available, concerning the biochemical basis of thermal adaptation, is Prosser's (1962) hypothesis linking translation and rotation of physiological rate-temperature relationships during adaptation to an increase in enzyme activity and a shift in metabolic pathway, respectively. Whether the R-T changes can be directly correlated with shifts in metabolic pathways is still a matter of debate. Nevertheless, there is evidence that both quantitative and qualitative changes in enzyme activity occur during adaptation (Hochachka and Hayes, 1962; McWhinnie and O'Connor, 1967).

Metabolic enzymes that increase significantly in activity during cold-adaptation include representatives from all the major functional classes, oxidative, glycolytic, hexose monophosphate shunt, and hydrolytic. Many of the early examples of changes in enzyme activity in poikilothermic vertebrates following adaptation have been tabulated by Prosser (1962), while more recent studies, including a few on enzymes of invertebrates have been reviewed by Rao (1967). It is clear from these reviews that different enzymes from the same source may differ markedly in their response to low-temperature adaptation; some increase, others

decrease, and yet others remain essentially unchanged in activity. Not enough information is available at present to permit firm interspecific generalizations regarding involvement of specific types of enzymes in the adaptation response.

Ekberg (1958) suggests that increased enzyme activity in cold-acclimated animals might be attributable to changes in permeability of the cell membrane to substrate molecules, or to release within the cell of some activating or inhibiting agent, or to inactivation of a chemical inhibitor or activating agent initially present in the cell, or to a change in the intrinsic properties of the enzyme itself. Prosser (1962) is of the opinion that adaptive augmentation of enzyme activity may result from enzyme induction initiated by an accumulation of appropriate metabolic intermediates in the cold.

Prosser's (1962) translation-rotation hypothesis further suggests that in some instances adaptation may be accompanied by a shift in the relative contributions of alternate metabolic pathways. Such adaptive shifts between the glycolytic pathway and the hexose monophosphate shunt during adaptation, have, in fact, been demonstrated, initially by the use of specific inhibitors and by measurement of substrate utilization (Ekberg, 1958), and more recently by the carbon-14 labelled glucose oxidation method (Hochachka and Hayes, 1962; Hochachka, 1967). Such shifts in pathway may occur as a consequence of enzyme induction, with the accumulation of metabolic intermediates in the cold inducing synthesis of enzymes of an alternate metabolic route that have more favorable

temperature characteristics than the original pathway (Prosser, 1962). More recently, Hochachka (1967) suggested that changes in proportions of various isozymal forms of certain metabolic enzymes may play a role in adaptation. He has in addition employed the concept of allosteric modulation to explain metabolic control during adaptation. Considerably more information will be required before it will be possible fully to assess the role in adaptation of either of these mechanisms.

There is increasing evidence that in many higher poikilotherms direct cellular responses are supplemented, and most probably coordinated, by systemic integrative systems. It is difficult, at the present stage of our knowledge, to "assess quantitatively the relative contributions to the whole animal of direct, primitive, cellular compensations to temperature as opposed to more recently evolved, systemic adaptations" (Roberts, 1967). Such systemic integration may be mediated by the nervous system, by endocrine organs, or both.

Adaptive changes occurring in the central nervous system as a consequence of thermal adaptation have been reviewed by Baslow (1967). The involvement of hormones in adaptation has been investigated in only a few species. That hormones might be implicated in adaptation of the earthworm, Lampito mauritii, was first suggested by the observation that, on cold-adaptation, groups of neurosecretory cells increased their activity (Rao and Saroja, 1963). It was also shown that body fluids from cold-acclimated worms stimulated in vitro metabolism of tissues from normally-acclimated worms. A similar metabolic stimulation was observed when nerve extract was used in place of body fluids (Saroja and Rao, 1965).

It was subsequently shown that injection of nerve extract from cold-acclimated worms resulted in biochemical changes in normally-acclimated worms that mimicked, in a number of respects, changes observed during cold-adaptation (Nayeemunnisa, 1966). Hormonal involvement in adaptation has also been shown in several species of fish (Precht, 1964). Rao (1967) feels that such hormonal triggers might be responsible for initiating increased RNA and protein syntheses, activating alternate metabolic pathways, and increasing lipid and oxidative metabolism. It is possible that changes in enzyme activity or in metabolic pathways may be related to hormone-induced changes in isozymal forms (Hochachka, 1967).

In spite of the vast efforts that have been devoted to clarifying the phenomenon of thermal adaptation, it is clear that we are still far from understanding many aspects of the mechanism of the response.

vi) Seasonal resistance adaptation:

In additional to the seasonal changes in metabolic rate (capacity adaptation) referred to above, many poikilotherms exhibit seasonal shifts in their tolerance of high and low temperatures (resistance adaptation). The precise relationship between capacity and resistance adaptation is uncertain, although Precht (1967) asserts that they may be independent of each other. Precht's review further suggests that the relationships between high-and low-temperature resistance adaptations vary from species to species.

Seasonal shifts in thermal tolerance are generally in a direction that has definite adaptive value. For example, the incipient upper lethal temperature for the fish, Perca flavescens, is approximately 3°C. higher in summer than in winter (Hart, 1952). Resistance adaptation has also been studied in many insects, particularly with regard to seasonal changes in low-temperature tolerance (Clarke, 1967; Mutchmor, 1967). One of the few studies on seasonal resistance-adaptation in marine invertebrates reveals that the barnacle, Balanus balanoides, is more tolerant of sub-zero temperatures in winter than in summer (Crisp and Ritz, 1967). On the other hand, the barnacles, Elminius modestus and Balanus crenatus, do not exhibit seasonal changes in cold tolerance (Ritz, 1968).

Temperature tolerance of excised tissues and cells has also been shown to shift with adaptation temperature. For example, warm-acclimation increased the survival time at 44°C. of excised gills of the bivalves, Modiolus demissus and Crassostrea virginica (Vernberg et al, 1963). An

extensive review of the literature pertaining to cell thermostability changes associated with seasonal, geographic, and experimental temperature shifts has been prepared by Ushakov (1964).

Death at temperatures below the freezing point may result from cellular disruption or ionic imbalances accompanying formation of ice crystals (Siminovitch et al, 1967) or from formation of disruptive, intermolecular disulphide bonds among proteins (Levitt, 1967). Death at low temperatures above the freezing point may be attributable to failure of integrative mechanisms.

Despite the fact that heat death has been the subject of intensive study, there is still considerable uncertainty regarding the precise nature of the critical event, or events. The effects of high temperature on a wide variety of cell constituents have been investigated with a view to establishing their involvement in heat death. Pertinent reviews include those concerning temperature effects on: cell water (Ling, 1967), proteins and enzymes (Brandts, 1967), nucleic acids (Szybolski, 1967), and lipids and other membrane constituents (Chapman, 1967).

In view of the uncertainty regarding the precise nature of both cold and heat death it is perhaps understandable that there is, at present, little agreement on mechanisms of adaptational change in thermal tolerance. As Precht (1967) points out, resistance adaptation may be due to a direct influence of temperature on cells and tissues or may be indirectly mediated through hormonal mechanisms.

In many species low-temperature adaptation appears to be correlated with an increase in concentration of cryo-protective solutes such as glycerol. For example, glycerol concentration in the barnacle, Balanus balanoides, is highest in winter when animals are most cold tolerant (Cook and Gabbott, 1970).

Thermal adaptation has been shown to produce changes in protein stability, lipid melting point, concentrations of various ions, and proportions of bound to free water (Hoar, 1966). Each of these correlations has, at one time or another, served as the basis for theories on the mechanisms of high-temperature resistance adaptation.

Ushakov (1964), in his comprehensive review of the literature on adaptation of cell thermostability, concluded that changes in cell heat resistance following adaptation are "the result of changes in the function of endocrine glands". He further suggests that although there frequently occur parallel changes in both intact animal and tissue temperature tolerance following adaptation, the latter does not provide a physiological basis for the former. Thermal death of intact animals is thus thought to be attributable to failure of integrative mechanisms and not to immediate, general tissue death. Ushakov further suggests that parallels reported between resistance adaptation of cells and intact animals are explicable on the grounds that both are related to the same "endocrine rearrangements" in the organism. That the situation may be even more complex is suggested by the rider appended to Ushakov's endocrine hypothesis to the effect that it may be that "in some poikilothermal animals there occur at the cellular level thermal adaptations which

are a consequence of a local effect of high temperature on the organs". Thermal adaptation studies on cells grown in tissue culture would appear to be one method of clarifying this problem.

MATERIALS AND METHODS

i) Habitat; collecting and handling of urchins:

Green sea urchins, Strongylocentrotus droebachiensis (O.F. Muller, 1776), ranging in weight from 5 to 65 grams, were collected at low tide, in 0.6 - 0.9 meters of water, in a small cove on the south-west side of Portugal Cove, Conception Bay, Newfoundland. Physical and biotic characteristics of the collecting area, with particular reference to urchin ecology, have been described in some detail by Himmelman (1970).

Seawater temperatures in the cove, measured every two weeks, at low tide, during daylight hours, fluctuated between approximately 0^{o}C. in winter and approximately 15^{o}C. in summer (Fig.1). These two temperatures were subsequently selected as cold- and warm-acclimation temperatures, respectively, during attempts to reproduce , in the laboratory, metabolic modifications similar to those occurring seasonally in urchins in their natural habitat.

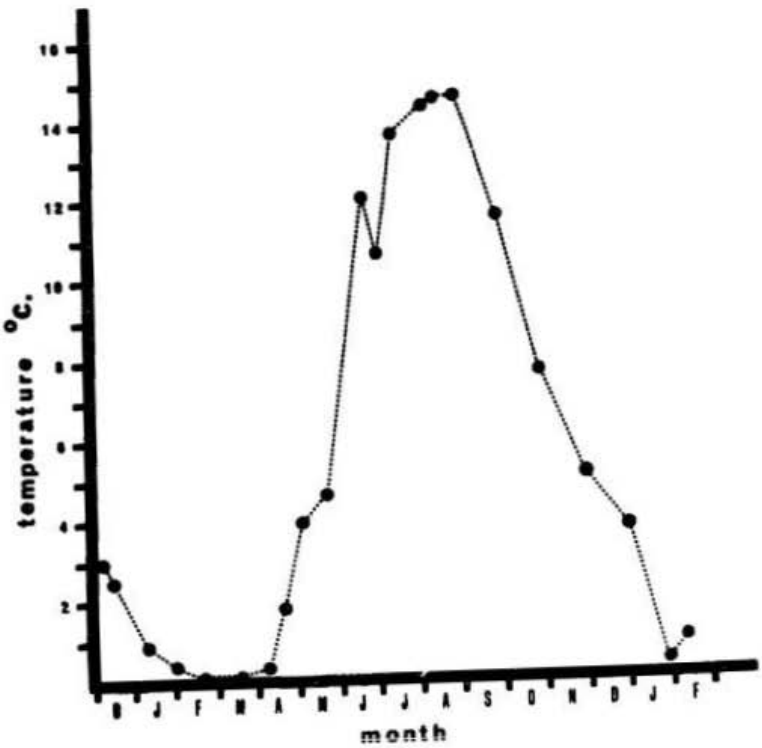
The fact that large numbers of urchins could be collected in the shallow water of the cove throughout the year suggests that little or no seasonal migration to deeper water occurs.

The water at the collection site was shallow and turbulent so that dissolved oxygen must have been at, or near, saturation levels at all times.

The urchins were feeding predominantly on the lower fringes of the dense beds of Ascophyllum nodosum, and Fucus vesiculosus that ringed the cove in the intertidal zone, but masses of urchins were also found

FIGURE 1

Seasonal changes in surface temperature at Portugal Cove during the period of study. (Based on data in appendix I.)


temperature °C.
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on large, detached fragments of a variety of sea weeds, including Ascophyllum nodosum, Fucus vesiculosus, Laminaria sp. and Alaria esculenta. For additional information on feeding in S. droebachiensis the reader is referred to Himmelman (1970).

In order to establish that the urchins in the cove were S. droebachiensis, and that there was no significant admixture of the difficult-to-differentiate S. pallidus (G.O. Sars) 1871, also reported from the Northwest Atlantic (Swan, 1962), two samples of 100 urchins each were collected, one in summer and another in winter, and subjected to Swan's formalin test, according to which, oral spines of S. droebachiensis, exposed to 10% formalin, take on a distinct violet hue, while those of S. pallidus remain cream-colored. All individuals in both samples appeared to be S. droebachiensis on the basis of this test.

The animals were transported to the laboratory, a half-hour from the collection site, in wet sea weed in a chilled, insulated chest. The manner of maintaining urchins in the laboratory varied according to the requirements of the several types of experiments. Stock urchins, and those to be used in studies requiring animals acclimatized in their natural habitat, were held in circulating seawater at temperatures approximating those in their natural environment, and were usually held no longer than 10 days. Laboratory acclimated urchins were, prior to use, held for 4-6 weeks either at a 15°C. "summer" temperature (warm-acclimated urchins) or at a 0°C. "winter" temperature (cold-acclimated urchins), in 10-liter tanks, in continuously aerated seawater with Ascophyllum

nodosum ad libitum. No more than 20 urchins were placed in a single tank. Warm-acclimation tanks were insulated aquaria into which flowed, at a constant rate (100 ml./min.), glass wool-filtered seawater, brought to the desired temperature (15°C.) by a glass heat-exchange coil in a constant temperature bath. In this system, temperature, recorded twice daily, remained constant within ±1°C. Cold-acclimation tanks were kept in a chill room held at 0°±1°C. Water was replaced daily with fresh, temperature-equilibrated seawater.

ii) Intact-urchin respiration:

a) Description of respirometers:

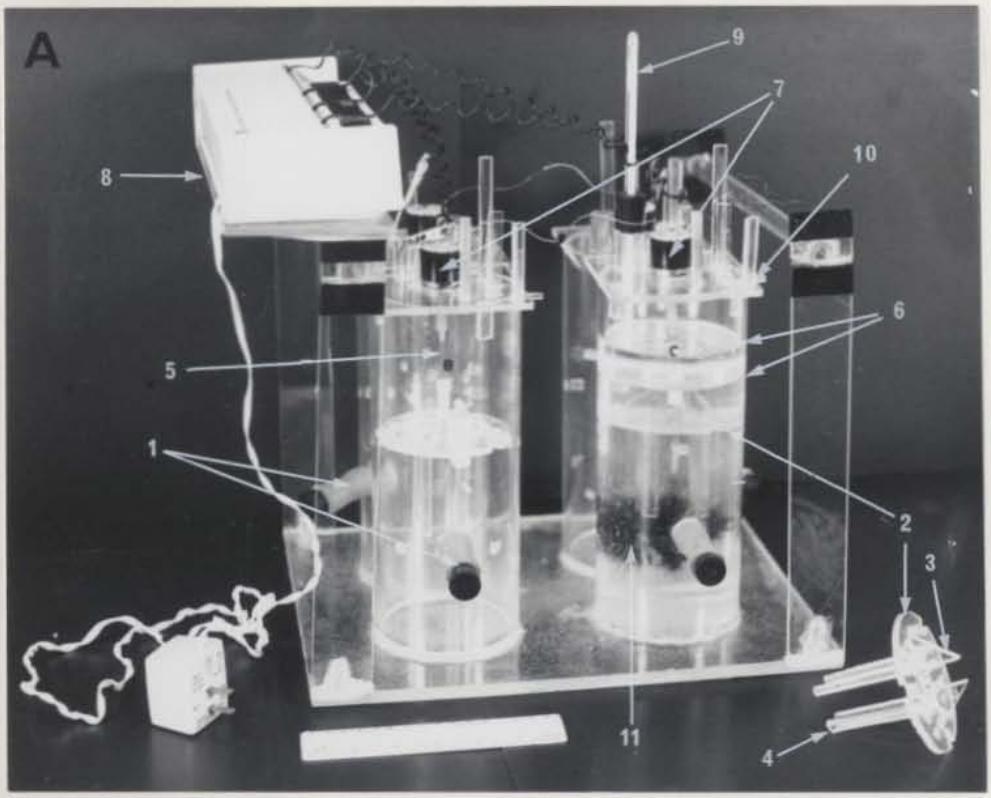
Respirometers used are of specially designed single-unit construction (Fig. 2). The four 24 cm. high respirometer chambers in the unit are constructed of four-inch diameter plexiglass tubing bonded to a bilaminar plexiglass base. Each chamber is equipped with a short side arm for collecting water samples (see below). A circular,perforated, plexiglass restraining disc fits inside each chamber, and is secured in place by lowering over locking-tabs on the chamber wall and rotating. This disc confines the urchin to the lower portion of the chamber. Baffles project from the upper surface of the disc and serve to eliminate the vortex produced by the stirrer. To prevent stratification of water, each chamber is equipped with a stirrer, consisting of a shaft, extending through a hole in the center of the restraining disc, that is fitted with pitched, plastic blades terminally. Four baffle rods project from the bottom of the disc and prevent urchins from approaching the stirrer. The shaft is connected to a 1.5 volt electric motor located on the cover-assembly of each chamber. Motors are powered from a voltage reducing dry cell charger (Dynamic Instrument Corp.). The compact design of the respirometer unit permits easy raising and lowering in a constant temperature bath without disturbing chamber operation. To facilitate handling, the unit is fitted with tubular plexiglass handles. Each chamber cover-assembly is provided with a funnel-thermometer port for oil addition and temperature monitoring.

FIGURE 2

A. Respirometer unit

1. Side arm
2. Restraining disc
3. Vortex baffles
4. Baffle rods
5. Stirrer shaft
6. Oil layer
7. Stirrer motor
8. Battery charger
9. Thermometer
10. Chamber cover-assembly
11. Urchin

B. Sampling syringe


A
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B

b) Preparation and operation of respirometers:

Twelve to 24 hours prior to a run, urchins were deprived of food to minimize the quantity of fecal material deposited in the respirometer chambers. Several hours before a run, the experimental animals (three or four per run) were placed in two-liters of continuously aerated seawater, initially at the same temperature as that from which the urchins were taken, that was gradually allowed to equilibrate at the predetermined test temperature. This adjustment period served to ensure that oxygen equilibrium was established between seawater at the test temperature and the urchin coelomic storage volume (Johansen and Vadas, 1967).

Each respirometer chamber was initially filled with 1025 ml. filtered (double layer of Whatman No. 1 paper) seawater adjusted to a specific gravity of 1.0250. Several hours prior to the commencement of the run, the charged respirometer unit was placed in a constant temperature bath, and the contents aerated and equilibrated at the test temperature. Just prior to the run, aeration was terminated and air bubbles adhering to chamber walls removed by vigorous brushing. Urchins were placed in the chambers (never more than one per chamber) and the restraining discs inserted and secured. The stirrer was started, and a 4 cm. layer of heavy mineral oil was slowly poured onto the water surface through the oiling ports. Water in each of the sealed chambers was stirred for at least 10 minutes before removal of the initial sample. The respirometer unit was raised from the constant temperature bath for

sample collection. Initial 25 ml. samples were taken from each chamber as described below. The time interval between initial and final samples was adjusted so that the oxygen concentration did not fall below the critical tension reported for S. droebachiensis (Johansen and Vadas, 1967). Time intervals used were:

15°C.	2 - 2 1/2 hours.
10°C.	3 hours.
5°C.	4 hours.
0°C.	4 1/2 - 7 1/2 hours.

Final water samples were collected in the same sequence, and with the same chamber-syringe pairing as the initial samples. Following a run urchins were drained for three minutes on absorbent paper, weighed to the nearest 0.01 gm., and sexed.

c) Collection and analysis of water samples:

Water samples were withdrawn from the respirometer chambers with four 30 ml. syringes (Fig. 2), fitted with holders and adjustable stop-screws (modified from Cummins et al, 1965, and Fox and Wingfield, 1938). With respirometer unit raised from the water bath, the syringe needle was inserted through the rubber-tubing of the side arm and positioned so as to draw water from the center of each chamber. A 4 ml. sample was drawn, and after withdrawing the syringe from the chamber, air bubbles were expelled by forcing water out of the inverted syringe. A 21 ml. sample was then drawn. Just prior to addition of reagents, the stop-screw

was adjusted to bring the syringe volume to 20 ml.

Winkler reagents were prepared according to Strickland and Parsons (1965). Manganous sulphate was drawn into the syringe by one complete turn of the stop-screw, the needle tip rinsed in distilled water, and alkaline iodide drawn in by a further turn. The syringe was shaken three times during a 15 minute oxygen absorption period. Concentrated phosphoric acid was drawn into the syringe by a final turn of the stop-screw, and the syringe shaken until the precipitate dissolved completely. The acidified sample was ejected into a clean dry flask. Two 10 ml. subsamples were drawn with a volumetric pipette and placed in titration tubes (test tubes painted white, with unpainted strip for observing contents). The tubes were illuminated with a microscope lamp and the contents stirred with a magnetic stirrer during titration. Subsamples were titrated with 0.0025 N sodium thiosulphate from a 5 ml. microburette. Starch indicator solution (0.3 ml.) was added during titration. The titer of the thiosulphate solution was standardized frequently against 0.01 N potassium iodate (Fox and Wingfield, 1938). The same standard solution of potassium iodate was used throughout, and stored tightly sealed in a refrigerator.

The rate of oxygen consumption was calculated according to the method of Cummins et al (1965), using a Programma 101 computer to facilitate computation. Unless otherwise stated, results are expressed as µl. O_2/animal/hour.

d) Respirometer controls:

Reproducibility of the syringe sampling technique was ascertained at intervals, using all four syringes to draw water samples from a single, well-stirred chamber. The average range of the individual calculated oxygen concentrations about the mean of the four samples was ± 1.1% (range 0.6% - 1.3%).

Apparent oxygen uptake by the contents of a control chamber, identical to the experimental chambers except lacking an urchin, was determined at intervals. Mean apparent oxygen consumption in the empty chambers, measured at various temperatures, for intervals ranging from 3-23 hours, was 0.005 ml./hr. (range: 0.00 - 0.012 ml./hr.). This was considered negligible.

e) Studies involving intact-urchin respiration:

Respiration rates were determined for intact urchins subjected to two distinct modes of adaptation. One group consisted of freshly collected animals seasonally acclimatized in their natural habitat, while a second group was acclimated at summer-like and winter-like temperatures in the laboratory.

1. Seasonal acclimatization:

Seasonally acclimatized urchins were collected in summer and winter, when habitat temperatures ranged from 12°- 15°C. and 0°- 2°C., respectively. The summer sample consisted of 95 urchins, ranging in weight from 5.66 to 67.80 grams; of these, 54 were male, 40 female and

one undetermined. The winter sample consisted of 84 urchins, ranging in weight from 13.61 to 64.75 grams; of these, 42 were male, 36 female and six undetermined. Respiration rates of both summer-and winter-acclimatized urchins were measured at 0°, 5°, 10° and 15°C. by the method outlined above.

Both exponential regression equations of respiration on wet weight, and exponential multiple regression equations of respiration on weight and temperature were calculated on a Programma 101 computer.

The relationship between urchin wet weight and dry decalcified weight was determined in summer and winter. Decalcification was carried out in nitric acid (Humason, 1967). The urchin test was cut open equatorially, the gut contents rinsed out and discarded, and the lantern apparatus crushed. The entire animal was placed in a beaker of 300 ml. 10% nitric acid. Tests with sodium oxalate indicated that this was adequate for complete decalcification. When bubbling ceased (approximately 72 hours), the contents of the beaker were suction filtered through tared Whatman No. 2 filter paper, dried to constant weight at 105°C., cooled in a dessicator, and weighed to the nearest 0.0001 gm. Regression equations of dry decalcified weight on wet weight were calculated for both summer and winter animals. With the aid of these equations simultaneous regression lines of respiration on both wet and decalcified, dry weights were plotted.

2. Laboratory acclimation:

Animals for laboratory acclimation studies were acclimated for 4-6 weeks at either 0°C. ("winter-like", cold-acclimated) or 15°C. ("summer-like", warm-acclimated) in the manner described earlier. Respiration rates of both warm-and cold-acclimated urchins were measured at 0°, 5°, 10° and 15°C. The warm-acclimated group consisted of 40 urchins, ranging in weight from 24.53 to 34.52 grams (mean = 29.21 grams), and the cold-acclimated group of 40 urchins, ranging in weight from 25.00 to 34.37 grams (mean = 29.16 grams). During each run, respiration rates of both warm-and cold-acclimated individuals were measured simultaneously.

Oxygen consumption was calculated on a per gram wet weight basis. For purposes of comparison with seasonal results, respiration rates of warm and cold-acclimated animals were calculated for an urchin of 30 grams standard weight; the resulting values were used in calculating thermal compensation coefficients.

iii) Excised tissue respiration:

a) Tissues* selected:

Metabolic rates of stomach, intestine, esophagus, tube foot and gonad tissues were determined both in summer and winter, while rates of rectum and coelomic fluid were measured only in summer. Ease of preparation, prior demonstration of acclimatization ability, and availability in adequate quantities were the reasons for restricting tissue-metabolism studies, of warm-and cold-acclimated urchins, to stomach and intestine slices. For work with homogenized tissues, intestine was selected because of its metabolic stability following homogenization.

b) Preparation of tissues:

Urchins, drained on paper towels for three minutes, were weighed to the nearest 0.01 gm. and placed in chilled finger bowls with sufficient seawater to cover them. The extended tube feet were cut off close to the test, and when a sufficient quantity had been severed, a vortex was created in the finger bowl with a pipette, so that the relatively light

* Terminology of Anderson (1966) is used for subdivisions of the digestive tract because this terminology appears to reflect functional differences between the segments. Fell and Pawson (1966) employ the term intestine to designate the entire alimentary tract between esophagus and rectum, while Hyman (1955) subdivided this region into an anterior large and posterior small intestine.

tube feet accumulated centrally while the heavier spine fragments (which were inevitably cut off along with the tube feet) collected peripherally. The tube feet, free from detritus, were then collected and pooled with the tube feet, similarly obtained, from two other urchins to form a single Warburg sample.

For dissection of internal tissues, the animal was inverted in a chilled petri dish and a cut made around the peristomeal membrane. The lantern apparatus was raised, cut free from surrounding muscles and esophagus (at its point of emergence from the lantern mass) and removed. The test was chipped away with forceps, working outwards from the peristomeal opening, while the gut tissue was simultaneously teased from the inner surface of the test with a blunt probe. Removal of half the test permitted ready access to internal tissues.

Esophagus was removed, opened longitudinally, rinsed in chilled seawater and cut into three pieces. Tissue from three urchins was pooled to form a single Warburg sample.

The stomach was then freed from surrounding tissues and cut at approximately the middle of the third festoon; it was transferred to chilled seawater, and cut into 1-2 cm. lengths which were opened longitudinally and rinsed free of stomach contents.

The intestine was cut approximately half a festoon beyond its junction with the stomach, and again at its junction with the rectum. Then the rectum was severed at its junction with the test. Both intestine and rectum were prepared as was stomach tissue.

Small pieces of gonad tissue were teased apart with glass probes, on a glass slide, then transferred to the respirometer flasks.

c) Determination of respiration rate:

Tissue samples of approximately 250 mg. were placed in chilled 15 ml. Warburg flasks containing 2.0 ml. filtered seawater (density 1.0250) in the main compartment and 0.2 ml. of 20% KOH in the center well. Air was used as the gas phase, and the shaking rate was 115 strokes per minute. Flasks equilibrated for 20 minutes before readings were taken, at 20-minute intervals, for two hours. On completion of the run, contents of the flasks were washed into tubes, centrifuged at about 1500 g for 5 minutes, rinsed with distilled water, recentrifuged, transferred to tared weighing pans, dried to constant weight at 105°C., and weighed to the nearest 0.0001 gm.

d) Determination of tissue water content:

The possibility that changes in tissue water content occur in conjunction with adaptation was investigated by comparing water content of stomach and intestine tissues from warm-and cold-acclimated urchins. Each sample consisted of approximately 300 mg. of fresh tissue. This was weighed to the nearest 0.0001 gm., dried for 24 hours at 105°C., and reweighed. Water content is expressed as a percentage of wet weight.

iv) Urchin activity:

a) Activity coefficient:

When an urchin is inverted, it immediately proceeds to right itself, and the rate at which it does so can be used as an indicator of physiological activity. To quantify this reflex urchins are rapidly inverted on to a precisely horizontal, glass plate on the bottom of the aquarium. The time in seconds that each individual takes to raise itself, so that its flattened, oral surface is vertical, is measured. This time interval is the half-righting time, and can be converted into an activity coefficient (A.C.) as follows:

$$\text{A.C.} = \frac{1}{\text{half-righting time (secs.)}} \times 10^3$$

In most instances, results are expressed as mean A.C. $\pm$ standard error of the mean. However, near either extreme of the thermal tolerance range, variable numbers of urchins did not initiate righting within a "reasonable" period (arbitrarily set at 10 minutes). In these instances, the central tendency for the group is described by the median, while the semi-interquartile range (Spiegel, 1961) serves as a measure of dispersion.

b) A.C.: Frequency distribution, reproducibility, and influence of weight:

The A.C. of animals held in ordinary aquaria decreased markedly with repeated trials, probably because, during each transfer from holding to test tank, numbers of tube feet were lost. When the holding tanks

were lined with thin polyethylene, to which the tube feet do not cling so tenaciously as to glass, the difficulty was overcome.

Validation of the A.C. as an acceptable quantitative indicator required the determination of frequency distributions, stability, reproducibility, and variability with weight.

Frequency distributions of A.Cs. were determined with groups of urchins collected in winter and summer. Winter animals were tested only at the prevailing temperature (0°- 1°C.), while summer animals were tested at both summer-(12°- 13°C.) and winter-like (1°- 2°C.) temperatures.

Stability and reproducibility of the A.Cs. were investigated by making measurements daily for 30 days, at 10°C., on a group of 20 urchins.

The influence of animal weight on A.C. was determined for two groups of urchins, ranging in weight from 0.68 to 61.0 grams, at temperatures of 1° and 11°C. (habitat temperatures at time of test). Subsequent righting experiments were conducted on urchins ranging from 25 to 35 grams.

c) Summer and winter R-T relationships:

The R-T relationships for activity were determined, for summer- and winter-acclimatized urchins, over the range 0° to 24°C. Animals were permitted to equilibrate to each test temperature for 15-20 minutes prior to being inverted. Both summer and winter groups (25 and 24 urchins, respectively) were tested at a series of temperatures, starting at the lowest and progressing in sequence to that temperature at which initiation of righting did not take place within 10 minutes in any of the

animals; each group was tested once each day at a single test temperature. All tests were conducted between 10 and 12 a.m. to minimize the influence of diurnal variations in activity. Between tests, urchins were held at approximately natural habitat temperature.

d) Seasonal changes in activity:

The mean A.C. of groups of 15-25 freshly-collected urchins was determined at monthly intervals at prevailing environmental temperatures, 12 to 24 hours following collection.

e) Activity of warm-and cold-acclimated urchins:

Mean A.Cs. were determined, at the respective acclimation temperatures, for groups of 20 urchins each, that had been acclimated in the laboratory for 4-6 weeks at either 0° or 15°C. Three activity trials, at approximately 24 hour intervals were completed for each group.

f) Time course of acclimation of activity:

The time course of the adaptive shift in A.C. at low temperature was investigated. To establish a warm-acclimated baseline, a group of 20 summer urchins was held at 15°C. for 4 weeks, during the last nine days of which the A.C. was determined daily at the holding temperature (15°C.). Then the temperature was permitted to fall, at a rate of approximately 1 1/4°C./hr., to 0°C. The A.C. was determined approximately eight hours after the temperature reached 0°C. and at intervals (usually daily) for 67 days thereafter.

v) Feeding rate and feeding efficiency:

Feeding and fecal production rates during different months were determined for freshly collected groups of 20 urchins (20-30 grams). Animals were held in a 10-liter aquarium into which seawater at the ambient environmental temperature flowed from a pressure-head vessel at a rate of about 1 liter per minute. Laminaria digitata was selected for feeding rate tests because its flat, blade-like form permits rapid, uniform drying with paper towels. The firm, central portion of the blade, cut into 10-20 cm. strips, was used. Animals were provided with excess Laminaria for one week before the recording of data. At the start of the recording period, all feces and old food were removed and approximately 200 grams of fresh, weighed Laminaria added.

During the course of the determinations the Laminaria was towel-dried and weighed every 24 hours; accumulated fecal material was siphoned off, vacuum-filtered through Whatman No. 1 paper, rinsed with distilled water, dried to constant weight at 105°C., and weighed. After weighing, the Laminaria was returned to the feeding tank and sufficient fresh material added to maintain the available food supply at approximately 200 grams. Each determination was continued for 7-14 days.

At the time of each determination, 5 samples of towel-dried Laminaria were weighed, dried to constant weight at 105°C. and reweighed to determine the dry:wet ratio. This ratio was used to convert quantities of food consumed from a wet to a dry weight basis. Feeding rates are

expressed as mg. dry wt. Laminaria per urchin per day. Feeding efficiencies (F.E.) were calculated from the equation:

$$\text{F.E.} = \frac{\text{calculated dry wt. food consumed - dry wt. feces}}{\text{calculated dry wt. food consumed}} \times 100$$

vi) Reproductive cycle:

Monthly determinations of the reproductive condition of S. droebachiensis were made on groups of 20 animals, 4-5 cm. in diameter*, within 48 hours of collection. The urchins were drained for three minutes on paper towels and weighed. The gonads were dissected out, and also drained on filter paper for three minutes and weighed. The gonad index (G.I.) was then calculated according to Boolootian (1966):

$$G.I. = \frac{\text{gonad weight}}{\text{whole urchin weight}} \times 100$$

The animals were sexed macroscopically in winter, when gonads are mature and readily distinguishable as male or female, and microscopically at other times, or in case of doubt.

* Diameter here always refers to diameter of the test, not of the spines.

vii) Seasonal resistance-acclimatization:

Several methods have been employed for detecting adaptation of thermal tolerance in animals. The two most common involve, either subjecting animals to slowly rising temperatures and noting the time of death, or subjecting a group of animals to constant, lethal temperature and measuring the time to death (Hoar, 1966). Another technique involves exposing the animals to a constant, lethal temperature for a predetermined interval and noting survival following return to the normal environmental temperature (Fraenkel, 1960). The first two of these techniques require that the moment of death or, at the very least, the moment of occurrence of fatal, thermal damage, be readily detectable. In S. droebachiensis no unequivocal indicator of thermal death could be established and it was thus necessary to resort to the last of the above methods.

In order to detect seasonal shifts in thermal tolerance, groups of 10 urchins (3-5 cm. in diameter) were exposed once, for one hour, to heat stress, each group to a different temperature between 20° and 30°C. ±0.2°C.). Following exposure, animals were returned to their adaptation temperature, and checked daily over a two-week period, for survival. These data are reported as cumulative mortality.

viii) Homogenate metabolism:

The biochemical changes associated with acclimation were investigated with the help of metabolic inhibitors, for which cell-free tissue homogenates are required. Preliminary studies revealed that in homogenized tissues metabolism remains stable long enough to permit meaningful measurements; other studies revealed that homogenates of acclimated animals demonstrate thermal acclimation. Two metabolic inhibitors were used: iodoacetic acid (IAA) which blocks glycolysis, and 5-bromouracil (5-BU) which, in some animals at least (Hochster, 1961), inhibits the hexosemonophosphate (HMP) shunt.

What one expects here,is that tissues metabolizing primarily via glycolysis will reduce respiration when IAA is added to the reaction vessel. If the main metabolic pathway is via the HMP shunt, on the other hand, 5-BU should produce a marked drop in respiration. By means of these two inhibitors, it is possible to estimate the relative importance of the glycolytic and HMP pathways in tissues from animals acclimated at different temperatures.

Unfortunately, 5-BU does not invariably inhibit the HMP shunt; its activity varies from species to species (Hochster, 1961). Thus, lack of response to the inhibitor might mean, either that the enzyme-to-be-inhibited is not present, i.e. that there is no shunt; or that the enzyme is present and that there may be a shunt in action, but that the enzyme is not sensitive to the inhibitor. To differentiate between these two possibilities, one must determine whether or not the shunt enzyme,

glucose-6-phosphate dehydrogenase, is present.

a) Preparation of homogenates:

The homogenate medium (Peterson and Anderson, 1969) consisted of:

	0.44 M sucrose
100 parts	2.42 gm./1. tris buffer
	0.01 gm./1. $MgSO_4.7H_2O$
4 parts	0.16 M sodium pyruvate
7 parts	0.1 M sodium fumarate
5 parts	0.3 M glucose

The medium adjusted to pH 7.4 with 0.1N HCl, was prepared fresh weekly, and stored in a refrigerator.

Samples consisting of 350 mg. of stomach or intestine tissue, excised as previously described, were touched to a piece of filter paper and placed in an ice-jacketed, glass Potter-Elvehjem homogenizer with 2 ml. of chilled medium. Homogenization was carried out with a motor driven (500-700 r.p.m.) Teflon pestle; 25 up and down strokes of the homogenizer were adequate for virtually complete cellular disruption.

b) Determination of respiration rate:

Homogenates were transferred to chilled, 15 ml. Warburg flasks as in the other experiments. Respiratory determinations were carried out at predetermined temperatures, for one hour; readings were taken every

15 or 20 minutes, and converted to $\mu l.O_2$/gm. dry wt./hr.

To determine the stability of homogenate metabolism at elevated temperatures, and to permit comparison between the metabolic rates of tissue slices and those of homogenates, runs, conducted at 15°C. were continued for an extended period. In view of the metabolic instability of stomach homogenates, only intestine homogenates were used in subsequent studies.

Respiration rates of intestine homogenates from warm-and cold-acclimated urchins (25-35 grams) were determined at 5°, 10°, 15°C. Warm- and cold-acclimated samples were excised and homogenized alternately and respiration rates determined simultaneously.

c) Iodoacetic acid inhibition of respiration:

To determine the concentration of iodoacetic acid required partially to block respiration of intestine homogenates, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} M IAA in buffered sucrose (pH 7.4), and for control, plain buffered sucrose, were used. Tissues were prepared and determinations conducted as outlined.

The effects of iodoacetic acid on the respiration of intestine homogenates from warm-and from cold-acclimated urchins were determined and compared. For this, the inhibitor (final concentration in the reaction vessel: 5×10^{-4} M) was added 40 minutes after the initial reading had been taken. The difference in respiration rate between the initial, 40-minute control interval and a second 40-minute interval is expressed

as a percentage of initial rate. Sucrose controls were prepared for each run using aliquots of the warm-and cold-acclimated homogenates.

d) Activity of glucose-6-phosphate dehydrogenase:

The relative activity of the HMP shunt enzyme (G-6-P dehydrogenase) in intestine homogenates was determined by spectrophotometry [Kornberg (1950) as modified by McWhinnie and Corkill (1964)]. This method is based on the reduction, in the presence of G-6-P dehydrogenase and its substrate, of TPN to TPNH, the accumulation of which is measured by absorption at 340 mu.

A sample of 200 mg. of intestine tissue was divided into two subsamples of 100 mg., one of which was dried to constant weight at 105°C. and weighed to the nearest 0.0001 gm., while the second was homogenized in 2.5 ml. chilled glycylglycine (0.25 M, pH 7.4). Glucose-6-phosphate (0.2 M) was prepared from its barium salt (Nutritional Biochemicals Corp.) and stored refrigerated. TPN solution (Nutritional Biochemicals Corp.) was prepared fresh for each run.

The final reaction cuvette contained:

0.2 ml. $MgCl_2$ (0.1 M)

0.9 ml. glycylglycine (0.25 M, pH 7.4)

1.6 ml. H_2O

0.1 ml. intestine homogenate

0.1 ml. TPN (20 mg./5 ml.)

0.1 ml. G-6-P (0.2 M)

The reaction mixture was incubated at 15°C. during the run. A tissue blank, similar to the reaction flask but lacking G-6-P, was used with each run. The increase in absorption at 340 mu, indicative of TPNH production, was followed at two-minute intervals for 30 minutes using a Spectronic 20 spectrophotometer. Reaction rates are measured and recorded as change in optical density, Δ O.D. (corrected for tissue blank) per mg. dry tissue/30 minutes.

To determine the effect of the G-6-P dehydrogenase inhibitor 5-BU in urchin intestine homogenates, 100 mg. 5-BU were dissolved in 2.5 ml. NaOH (1.0 M) and 1 ml. of this solution was diluted in 19 ml. of glycylglycine (0.25 M, pH 7.8). Each test run consisted of four reaction vessels similar to those described above (p. 57) except that the buffer was adjusted to pH 7.8. Sufficient 5-BU-buffer mixture was added to one of the reaction vessels to make a final 5-BU concentration of 6.9×10^{-4} or 1.4×10^{-3} M. Two vessels without inhibitor served as controls and a fourth vessel served as a tissue blank.

The effect of acclimation on the activity of G-6-P dehydrogenase was investigated. Homogenates from warm-and cold-acclimated urchins were run simultaneously with their corresponding tissue blanks.

THERMAL COMPENSATION COEFFICIENTS

i) Qualitative and quantitative descriptions of adaptation:

A number of attempts have been made to characterize, qualitatively and quantitatively, thermal adaptation responses exhibited by a variety of animals, organs and tissues. Of the qualitative approaches, the most useful are those of Precht (1958) and Prosser (1961).

Precht recognizes five distinct patterns*, according to which, rate-functions may slowly adjust to a change in ambient temperature. His scheme considers only the compensatory shift in rate at the new adaptation temperature relative to the rate at the original temperature.

Prosser's classification of adaptation types (Fig. 5) differs from that of Precht in that it considers the nature of the compensatory shift in a rate-temperature (R-T) relationship consequent upon adaptation. He defines four basic adaptation patterns, with certain of the patterns being further subdivided. According to this scheme, the adaptive adjustment of the R-T curve of a given rate function can be viewed as either a rotation or a translation of the cold-adapted R-T curve relative to the warm-adapted one.

Rigorous comparative studies of thermal adaptation have been hampered by a lack of adequate quantitative methods for assessing the magnitude and form of the response. The acclimatization coefficient proposed by Roberts (1952) represents an attempt to establish "a method of

* Refer to concepts and terminology section for outline of these patterns.

quantitatively measuring potential ability of animals to acclimatize". It is based on the ratio of two slopes derived from R-T relationships of warm-and cold-adapted rate functions. One slope defines the relative magnitude of compensation, while the second serves as a reference slope that defines the sensitivity of the rate function to temperature change. The coefficient has a number of drawbacks and has seen little use (Roberts, 1952; Rao, 1953). One of the principal difficulties, as Roberts himself recognized, is the frequent distortion of the reference slope as a result of differences between warm-and cold-adapted rate functions with respect to the degree of rate depression at elevated temperatures. An additional drawback is that the acclimatization coefficient makes no allowance for the several distinct patterns of adaptation defined by Prosser. It appears doubtful that useful comparisons can be made between such acclimatization coefficients derived from rate functions whose adaptation patterns are fundamentally different.

ii) Thermal compensation coefficients:

It may not be possible to combine, in a single coefficient, the essentials of widespread applicability and facility of computation; the former requisite for comprehensive comparative studies and the latter conducive to general use and ease of interpretation. Some of the difficulties can be surmounted by the use of several related coefficients that describe different aspects of the adaptation response. The proposed scheme, combining the quantitative approach of Roberts with the widespread applicability of Prosser's qualitative approach, provides for three coefficients that define the form and magnitude of an R-T shift

resulting from adaptation.

The three coefficients, together termed thermal compensation coefficients (T.C.C.), are as follows:

1. Coefficient of adaptation (C.A.)
2. Coefficient of rotation (C.R.)
3. Axial coefficient (Ax.)

The coefficient of adaptation measures the compensatory shift in a rate function following a temperature change, and has obvious affinities to Roberts' acclimatization coefficient referred to earlier.

Poikilotherms may compensate for an adverse change in temperature by a quantitative adjustment of the rate, as well as by an alteration of the temperature dependence, of their vital functions. Such a change in temperature dependence is characterized by an increase or decrease in the slope of the R-T relationship, and in the magnitude of the temperature characteristic and Q_{10} coefficients of the given rate function. It is this shift in temperature dependence accompanying adaptation that the coefficient of rotation (C.R.) is designed to measure.

To complete the characterization of the adaptive shift in the rate function it is necessary to determine the intersection point of curves describing the warm-and cold-adapted R-T relationships (i.e. define the hypothetical center about which these curves are considered to rotate as a consequence of adaptation). For a given degree of rotation, the final configuration of the cold-relative to the warm-adapted curve may differ considerably depending upon whether the center of rotation is located at a high, intermediate or low temperature. The

axial coefficient (Ax.) indicates whether this intersection point is located either between the adaptation temperatures (i.e. within the stress range) (Prosser pattern III)*, or at a high (Prosser pattern IV a,b), or low (Prosser pattern IV c,d) temperature (i.e. above or below the stress range, respectively).

iii) Derivation of coefficients:

a) Arrhenius transformation:

Thermal compensation coefficients (T.C.C.) are fundamentally derived from Arrhenius plots** of warm-and cold-adapted R-T data. Arrhenius transformation of biological R-T data generally results in an essentially linear relationship. Such a transformation has a "firm theoretical and experimental basis" (Hoar, 1966), and simplifies the quantitative comparison of different R-T relationships.

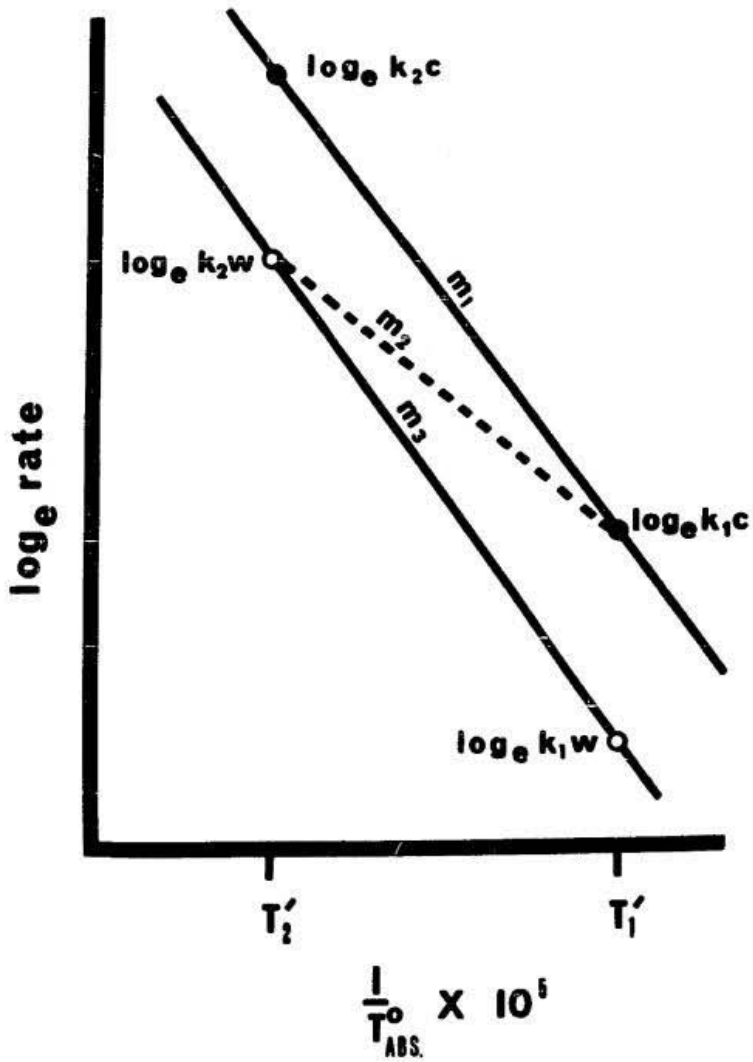
Rate-temperature data for rate functions of animals adapted to low (T_1) and high (T_2) temperatures are converted to an Arrhenius presentation (Fig. 3), by plotting the natural logarithm of the rate against the reciprocal of the absolute temperature multiplied by 10^5. The points T_1' and T_2' represent the transformed cold-(T_1) and warm-(T_2) adaptation temperatures, respectively. Lines with slopes m_1 and m_3 are least square

* Refer to Figure 5 for summary of Prosser patterns.

** See Hoar (1966) for a discussion of theoretical aspects of the Arrhenius transformation.

FIGURE 3

Arrhenius transformation of hypothetical warm-and cold-adapted R-T relationships illustrating derivation of thermal compensation coefficients. (See text for explanation.)


log_e k_2c
log_e k_2w
m_1
m_2
m_3
log_e k_1c
log_e k_1w
log_e rate
T_2'
T_1'
1/T°_ABS. X 10^5

regression lines calculated from Arrhenius plots for the warm-and cold-adapted rate functions, respectively. The points $\log_e K_2c$ and $\log_e K_1c$ are calculated from the regression equation for the cold-adapted rate function, at T_2' and T_1', respectively. Similarly, $\log_e K_2w$ and $\log_e K_1w$ are calculated from the regression equation for the warm-adapted rate function,at T_2' and T_1', respectively. The line with slope m_2 is drawn from the point on the warm-adapted curve at T_2' to the point on the cold-adapted curve at T_1'. The warm-adapted curve, with slope m_3, serves as a reference slope and provides a measure of the temperature sensitivity of the rate function. The line with slope m_2 provides an estimate of the compensatory shift in the cold-adapted R-T curve relative to the warm-adapted one. The three thermal compensation coefficients are calculated* as shown below.

b) Coefficient of adaptation:

The coefficient of adaptation is defined as the ratio of the slope of the line joining the point on the warm-adapted curve at T_2' ($\log_e K_2w$) and the point on the cold-adapted curve at T_1' ($\log_e K_1c$), to the slope of the line joining the point on the warm-adapted curve at T_2' ($\log_e K_2w$) and the point on the warm-adapted curve at T_1' ($\log_e K_1w$) (Fig. 3).

* Refer to appendix II for sample calculation of T.C.C. from actual data.

In other words:

$$C.A. = \frac{m_2}{m_3}$$

and since:

$$m_2 = \frac{\log_e K_2 w - \log_e K_1 c}{T_2' - T_1'}$$

$$m_3 = \frac{\log_e K_2 w - \log_e K_1 w}{T_2' - T_1'}$$

therefore:

$$C.A. = \frac{\log_e K_2 w - \log_e K_1 c}{\log_e K_2 w - \log_e K_1 w}$$

Limits of coefficient of adaptation:

C.A. $>$ 1 (hypoadaptation)

C.A. = 1 (no adaptation)

C.A. $<$ 1, $>$ 0 (partial adaptation)

C.A. = 0 (complete adaptation)

C.A. $<$ 0 (hyperadaptation)

c) Coefficient of rotation:

The coefficient of rotation is defined as the ratio of the slope of the line joining the point on the cold-adapted curve at T_2' ($\log_e K_2 c$) and the point on the cold-adapted curve at T_1' ($\log_e K_1 c$), to the slope

of the line joining the point on the warm-adapted curve at T_2' ($\log_e K_2 w$) and the point on the warm-adapted curve at T_1' ($\log_e K_1 w$).

In other words:

$$C.R. = \frac{m_1}{m_3}$$

and since:

$$m_1 = \frac{\log_e K_2 c - \log_e K_1 c}{T_2' - T_1'}$$

$$m_3 = \frac{\log_e K_2 w - \log_e K_1 w}{T_2' - T_1'}$$

therefore:

$$C.R. = \frac{\log_e K_2 c - \log_e K_1 c}{\log_e K_2 w - \log_e K_1 w}$$

Since the slopes m_1 and m_3 are equal to $\frac{\mu_c}{R}$ and $\frac{\mu_w}{R}$, respectively, where μ_c is the temperature characteristic of the cold-adapted rate function, μ_w that of the warm-adapted rate function, and R is the gas constant, then the coefficient of rotation may also be defined as:

$$C.R. = \frac{\mu_c}{\mu_w}$$

Limits of coefficient of rotation:

C.R. = 1(translation of rate curve on cold-adaptation.)

C.R. $<$ 1(counterclockwise rotation of rate curve on cold-adaptation.)

C.R. $>$ 1(clockwise rotation of rate curve on cold-adaptation.)

d) Axial coefficient:

The axial coefficient is defined as the negative ratio of the distance, measured along the T' axis, between the point of intersection of the warm-and cold-adapted Arrhenius curves (T_i') and the mid-point of the stress range $\left[\frac{T_1' + T_2'}{2}\right]$, to the distance between T_1' and the mid-point of the stress range.

Since: Regression (warm-adapted): $\log_e K_w = a_w + b_w T'$

Regression (cold-adapted): $\log_e K_c = a_c + b_c T'$

then at the intersection point:

$$\log_e K_w = \log_e K_c$$

and:

$$a_c + b_c T_i' = a_w + b_w T_i'$$

$$b_c T_i' - b_w T_i' = a_w - a_c$$

$$T_i'(b_c - b_w) = a_w - a_c$$

$$T_i' = \frac{a_w - a_c}{b_c - b_w}$$

$$Ax. = -\left[\frac{T_i' - \left[\frac{T_1' + T_2'}{2}\right]}{T_1' - \left[\frac{T_1' + T_2'}{2}\right]}\right]$$

Limits of axial coefficient:

Ax. = 0 (intersection at mid-point of stress range)

Ax. > -1, < 1 (intersect within stress range)

Ax. > 1 (high-temperature intersect)

Ax. < -1 (low-temperature intersect)

iv) Convention for presentation of coefficients:

For ease of comparison and interpretation the following convention for presentation of the T.C.C. will be adhered to:

$$\begin{matrix} t_2 \\ t_1 \end{matrix}(C.A.;\ C.R.;\ Ax.)\ D + 1/2\ range$$

where:

t_1 = cold-adaptation temperature

t_2 = warm-adaptation temperature

C.A. = coefficient of adaptation

C.R. = coefficient of rotation

Ax. = axial coefficient

D + 1/2 range = mid-point of adaptation time in days $\pm$ half range

of adaptation time in days.*

v) Interpretations of thermal compensation coefficients:

The interpretations of various numerical values of thermal compensation coefficients are summarized in Figure 4.

The coefficient of adaptation indicates both the direction and magnitude of the compensatory shift in a rate function. The degree of adaptation is inversely proportional to the C.A., with a value of unity indicating no adaptation and a value of zero indicating complete adaptation. Intermediate values are associated with partial adaptation. The terminology associated with this coefficient is essentially that of Precht (1958), with one exception (hypoadaptation) noted previously.

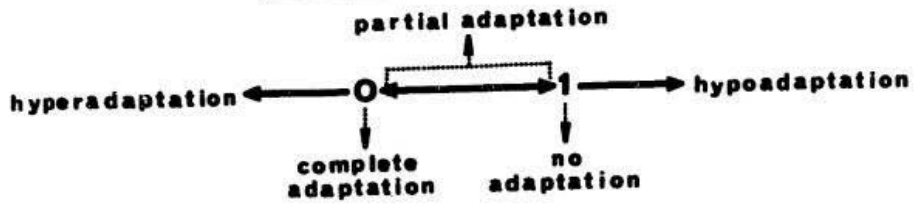
The coefficient of rotation provides information on the nature of the compensatory shift in the R-T relationship induced by a thermal stressor, values close to unity being associated with translation of the curves, and increasing deviations either above or below unity being

* This expression can only be appended to coefficients obtained in situations where reasonably precise information regarding period of exposure to the adaptation temperature is available, and thus will generally only be applicable to studies involving laboratory acclimation. In instances where the T.C.C. are calculated for geographically-or seasonally-adapted populations the expression indicating duration of acclimation may be replaced by G or S, respectively.

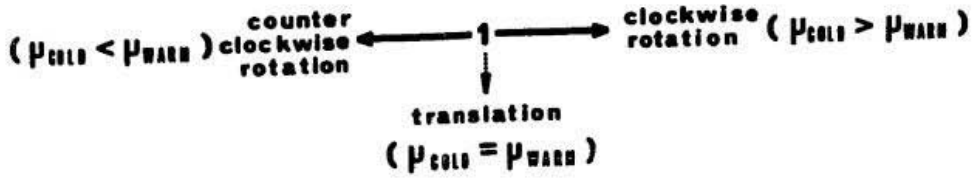
FIGURE 4

Summary of interpretations of thermal compensation coefficients. (Solid arrows indicate numerical range of the coefficients; broken arrows refer to interpretation.)

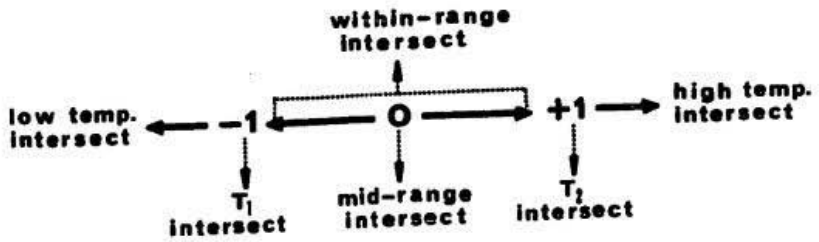
COEFFICIENT OF ADAPTATION



COEFFICIENT OF ROTATION



AXIAL COEFFICIENT



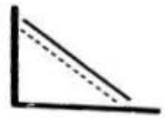
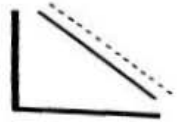
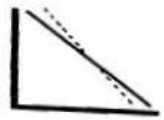
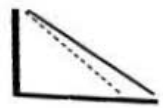
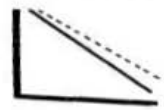
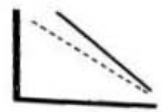
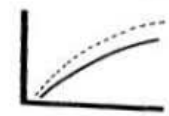
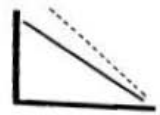
indicative of an increasing degree of clockwise or counterclockwise rotation, respectively, of the R-T curves. The coefficient of rotation also provides information on the relative magnitudes of the temperature characteristics (μ) of the warm-and cold-adapted R-T relationships.

The axial coefficient defines the location of the point of intersection of the warm-and cold-adapted Arrhenius curves by providing a measure of the displacement of the intersection point from the mid-point of the stress range. An Ax. of zero indicates that intersection occurs at the mid-point of the stress range, while increasingly positive or negative values are associated with increasing displacement of the intersection point towards higher or lower temperatures, respectively.

The thermal compensation coefficients quantitatively characterize each of Prosser's adaptation patterns as shown in Figure 5. The different patterns are listed in the first column. Warm-and cold-adapted R-T relationships for each of the patterns are shown in the second column, and the corresponding Arrhenius transformations in the third. The ranges of each of the three coefficients associated with each of the patterns are presented in the last column. The coefficients provide a means of quantitatively comparing adaptation responses that are essentially variations of a single Prosser pattern.

FIGURE 5

Summary of relationships between thermal compensation coefficients and Prosser's (1961) adaptation patterns. (Cold-adapted rate function - solid line; warm-adapted rate function - broken line.)

PROSSER PATTERN	R-T CURVE	ARRHENIUS TRANSFORMATION	THERMAL COMPENSATION COEFFICIENT
PATTERN I no adaptation			C.A. = 1 C.R. = 1 Ax. = ∞
PATTERN IIa translation adaptation			C.A. = <1, >0 C.R. = 1 Ax. = ∞
PATTERN IIb translation hypoadaptation			C.A. = >1 C.R. = 1 Ax. = ∞
PATTERN IIIa rotation adaptation			C.A. = <1, >0 C.R. = <1 Ax. = 0
PATTERN IIIb rotation hypoadaptation			C.A. = >1 C.R. = >1 Ax. = 0
PATTERN IVa trans.-rot. adaptation			C.A. = <1, >0 C.R. = <1, >0 Ax. = > -1
PATTERN IVb trans.-rot. hypoadaptation			C.A. = >1 C.R. = >1 Ax. = > -1
PATTERN IVc trans.-rot. adaptation			C.A. = <1, >0 C.R. = >1 Ax. = >1
PATTERN IVd trans.-rot. hypoadaptation			C.A. = >1 C.R. = <1 Ax. = >1

RESULTS

A. SEASONAL ACCLIMATIZATION

To appreciate fully the significance of temperature adaptation in the life of an organism it is necessary to investigate its metabolic response to thermal stressors as they are routinely encountered in the natural habitat. Demonstration of an ability to adapt to temperature change in an artificial environment does not necessarily imply that the organism normally utilizes such ability in an adaptive manner in the face of natural, cyclic temperature changes. Other factors may significantly modify, or even prevent, the expression of the adaptive response.

This opening phase of the study of thermal adaptation in S. droebachiensis examines seasonal changes in physiological performance of animals in their natural habitat. It attempts to determine whether the urchins metabolically compensate for the considerable annual temperature fluctuations that occur in Newfoundland waters (Fig. 1), and thereby maintain their vital functions at a relatively high level during the winter. It may be that other environmental or endogenous factors preclude such an adjustment and oblige the animals, during colder months, to assume a torpid state in which physiological processes are slowed to a bare minimum. Definitive answers are sought by measuring seasonal changes in respiration rate (both of intact animals and of selected excised tissues), activity, feeding rate, reproductive condition and temperature tolerance.

i) Seasonal changes in intact-urchin respiration:

Respiration, probably the most straightforward, reliable, and widely used estimate of metabolic rate, is used here to characterize seasonal acclimatization of metabolism in S. droebachiensis. In addition to the prime objective of ascertaining whether, and to what extent, cold-induced, metabolic homeostasis occurs, changes in magnitude and temperature relationships with season are also examined.

It has long been known (Zeuthen, 1947) that metabolic rate varies with animal size; specifically, that the metabolism of a unit volume of respiring tissue from a large animal is more sluggish than the respiration rate of an identical volume of respiring tissue from a smaller animal. Bertalanffy (1957) has shown, furthermore, that the rate at which oxygen consumption changes relative to weight (i.e. the regression of metabolic rate on weight) is best described by the exponential equation:

$$M = aW^b$$

where: M = metabolic rate, W = animal weight and a and b constants; a represents the intercept and b the slope, i.e. the actual rate-of-change of the metabolism in respect to weight under the conditions considered. If oxygen consumption increases directly with weight, b will equal 1; a b value below 1 indicates a relative decrement in metabolism with increasing size, and one greater than unity an increase in basal metabolism.

In the present study, this exponential equation proved to be slightly superior (an approximately 5 percent improvement in curve fitting - as determined from the correlation coefficients) to the linear

equation $M = a + bW$ in describing the regression of oxygen consumption on weight in S. droebachiensis. Here, the respiration data are presented as an exponential function of increasing fresh (= wet) weight.

a) Respiration and sex:

No significant difference is demonstrable between regressions of respiration on wet weight for male and female urchins, either in summer or in winter (appendix III c) therefore data from both sexes are pooled in all subsequent analyses.

b) Respiration and weight:

The regressions of oxygen consumption on urchin wet weight at 0°, 5°, 10°, and 15°C. in summer and winter were calculated and the corresponding regression lines plotted (Figs. 6 and 7). For the sake of completeness, and to permit comparisons with certain urchin respiration data in the literature, scales for decalcified dry weights corresponding to selected wet weights were added to the x-axes in Figures 6 and 7 according to the equations (see p. 42):

summer: $W_f = 11.966 + 24.564\ W_d$

winter: $W_f = 8.497 + 23.242\ W_d$

where W_f is urchin wet weight in grams and W_d is decalcified dry weight in grams. For reasons to be considered later, respiration rates are calculated on a per animal, or a per wet weight basis for use in subsequent

FIGURE 6

Regressions of oxygen consumption on weight at 0°, 5°, 10°, and 15°C. for summer-acclimatized S. droebachiensis. (f.w.= wet weight; d.d.w.= decalcified dry weight. Based on data in Table 1.)

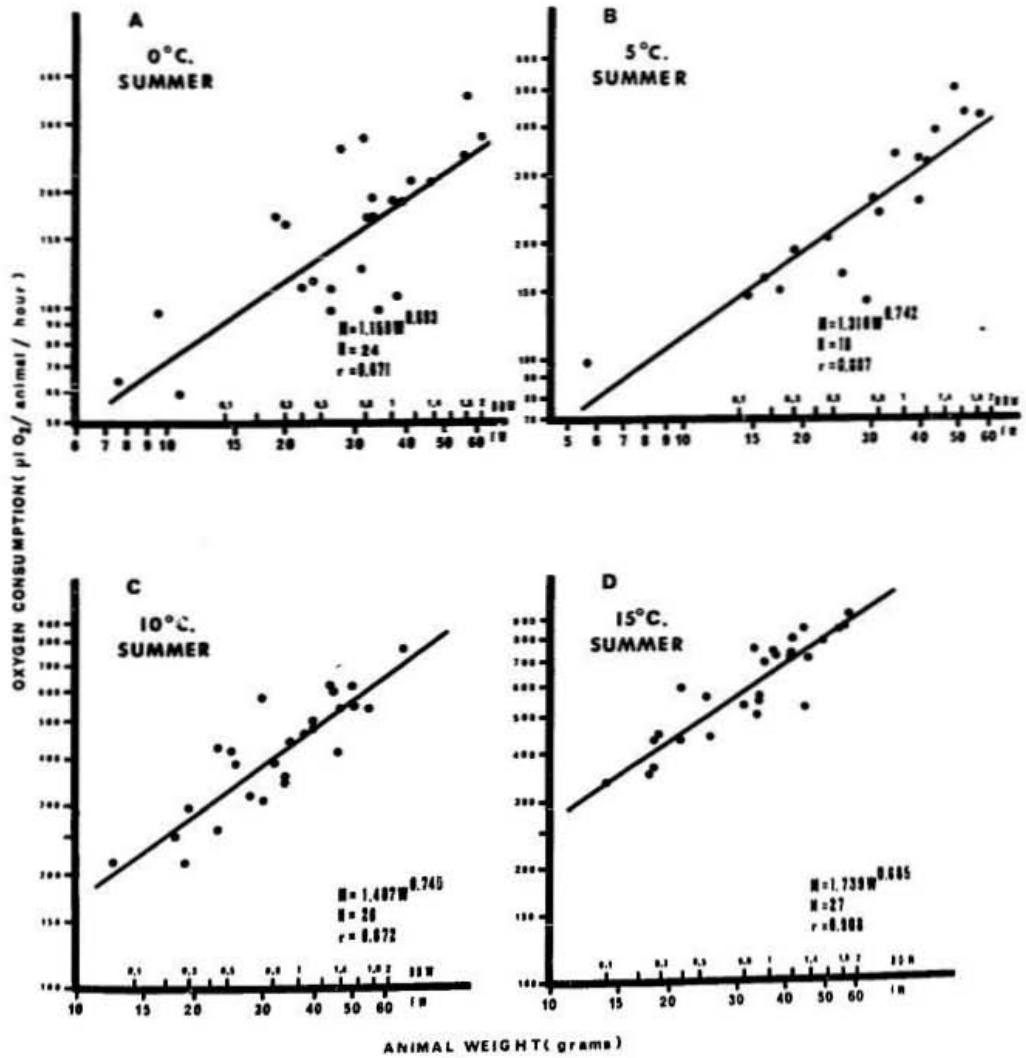
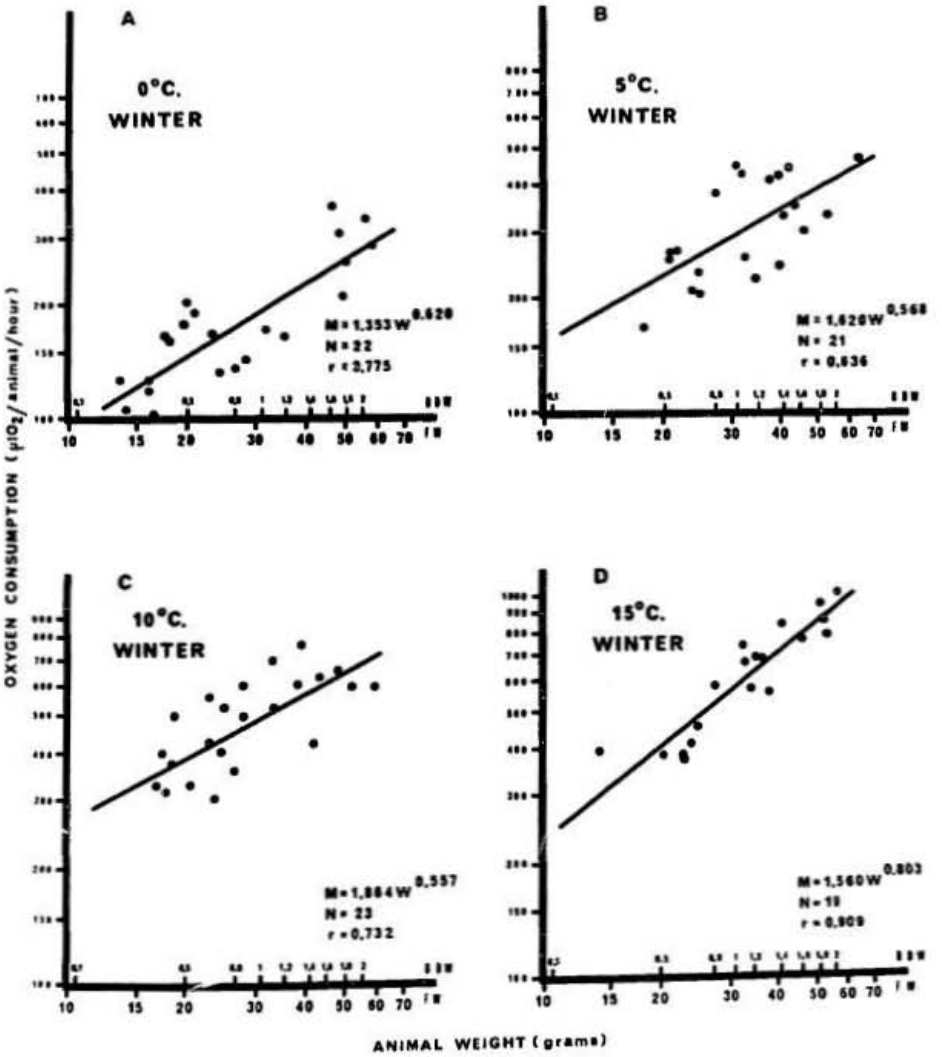

A
0°C.
SUMMER
B
5°C.
SUMMER
C
10°C.
SUMMER
D
15°C.
SUMMER
OXYGEN CONSUMPTION(µl O2/ animal / hour)
ANIMAL WEIGHT(grams)

FIGURE 7

Regressions of oxygen consumption on weight at 0°, 5°, 10°, and 15°C. for winter-acclimatized S. droebachiensis . (f.w.=wet weight; d.d.w.= decalcified dry weight. Based on data in Table 1.)


A
0°C.
WINTER
M = 1,353W^0,620
N = 22
r = 0,775
B
5°C.
WINTER
M = 1,626W^0,568
N = 21
r = 0,636
C
10°C.
WINTER
M = 1,884W^0,557
N = 23
r = 0,732
D
15°C.
WINTER
M = 1,360W^0,803
N = 19
r = 0,909
OXYGEN CONSUMPTION (µlO2/animal/hour)
ANIMAL WEIGHT (grams)

calculations and comparisons.

A summary of the exponential regression analysis of respiration on wet weight in summer-and winter-acclimatized urchins is presented in Table 1.

The exponential regression equations for summer-acclimatized animals are:

(0°C.) $M = 1.159\ W^{0.693}$

(5°C.) $M = 1.310\ W^{0.742}$

(10°C.) $M = 1.487\ W^{0.745}$

(15°C.) $M = 1.739\ W^{0.685}$

where M is respiration rate ($\mu l.O_2$/urchin/hr.), and W is urchin wet weight (grams).

For winter-acclimatized animals the corresponding regression equations are:

(0°C.) $M = 1.353\ W^{0.620}$

(5°C.) $M = 1.620\ W^{0.568}$

(10°C.) $M = 1.864\ W^{0.557}$

(15°C.) $M = 1.560\ W^{0.803}$

The regression coefficient b ranges from 0.685 to 0.745 (mean: 0.716) in summer. In contrast, in winter, between 0° and 10°C., b ranges from 0.557 to 0.620 (mean: 0.582), considerably below summer

Table 1. Exponential regression analyses of respiration on weight of summer-and winter-acclimatized S. droebachiensis. (Based on data in appendix III a and b.)

season	temp. (°C.)	N	r	$\bar{X}$*	$\bar{Y}$ ± **	b^+	log a^+	p
summer	0°	24	0.671	28.5	147.6 ± 20.5	0.693	1.159	<0.10
winter	0°	22	0.775	25.6	168.5 ± 25.2	0.620	1.353	
summer	5°	18	0.887	27.8	240.1 ± 52.3	0.742	1.310	<0.05
winter	5°	21	0.636	32.8	302.8 ± 38.6	0.568	1.620	
summer	10°	26	0.872	32.9	414.3 ± 52.8	0.745	1.487	<0.05
winter	10°	22	0.732	29.0	476.8 ± 52.6	0.557	1.864	
summer	15°	27	0.908	32.4	593.4 ± 59.8	0.685	1.739	N.S.
winter	15°	19	0.909	32.9	601.3 ± 86.0	0.803	1.560	

* Mean weight of urchins (grams wet weight).

** Mean respiration rate ($\mu l O_2$/animal/hr.) ± 95% confidence interval.

\+ Coefficients of the exponential estimating equation

$\log M = \log a + b \log \text{weight}$.

values over the same temperature range. Thus, in summer animals, the change in metabolic rate per unit weight is more pronounced than in winter. The high b value (0.803) in winter at 15°C. is a reflection of the size-dependent respiration R-T slope depression between 10° and 15°C. (see below).

That the respiration rate per unit wet weight decreases with increasing urchin weight is indicated by the fact that b values are below unity in all instances.

c) Respiration and temperature:

Summer regression lines of respiration on wet weight are translated regularly upwards with increasing temperature between 0° to 15°C., and remain essentially parallel (Fig. 8). On the other hand while the corresponding winter regression lines are translated uniformly upwards with increasing temperature between 0° and 10°C., the 15°C. regression line is considerably depressed in the lower weight range. The nature of this depression can be more readily seen in rate-temperature (R-T) curves calculated from summer and winter exponential regression equations for urchins of different standard weights (Fig. 9). There is a distinct difference between summer and winter R-T relationships. In summer, respiration rates of all urchins, regardless of size, increases regularly with increasing temperature, with no evidence of an abnormal decline in the respiratory R-T slope at temperatures approaching 15°C. In contrast, in winter, the R-T slope remains relatively uniform with increasing temperature

FIGURE 8

Effect of temperature on regressions of oxygen consumption on wet weight for summer-and winter-acclimatized S. droebachiensis. (Based on data in Table 1).

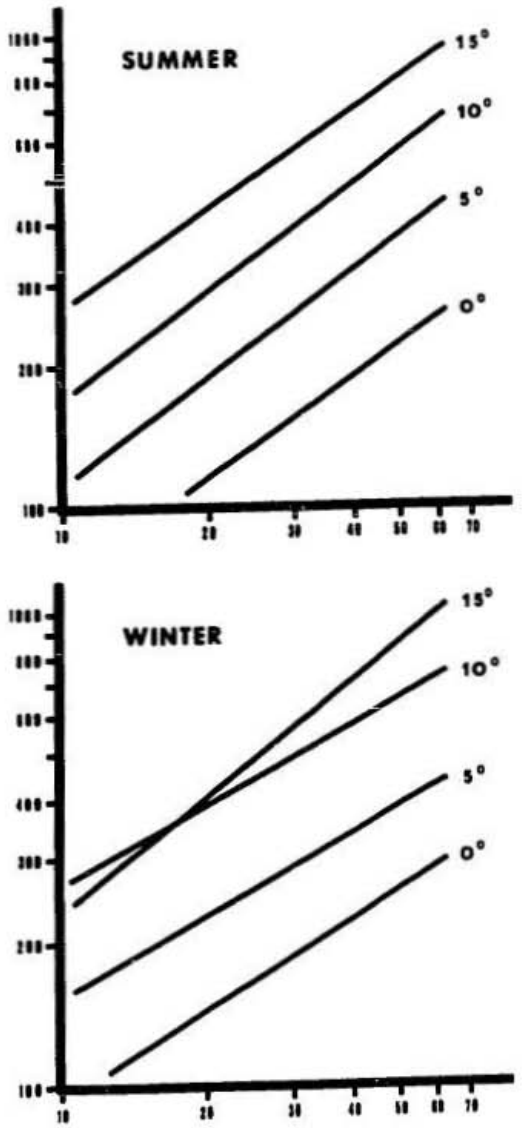
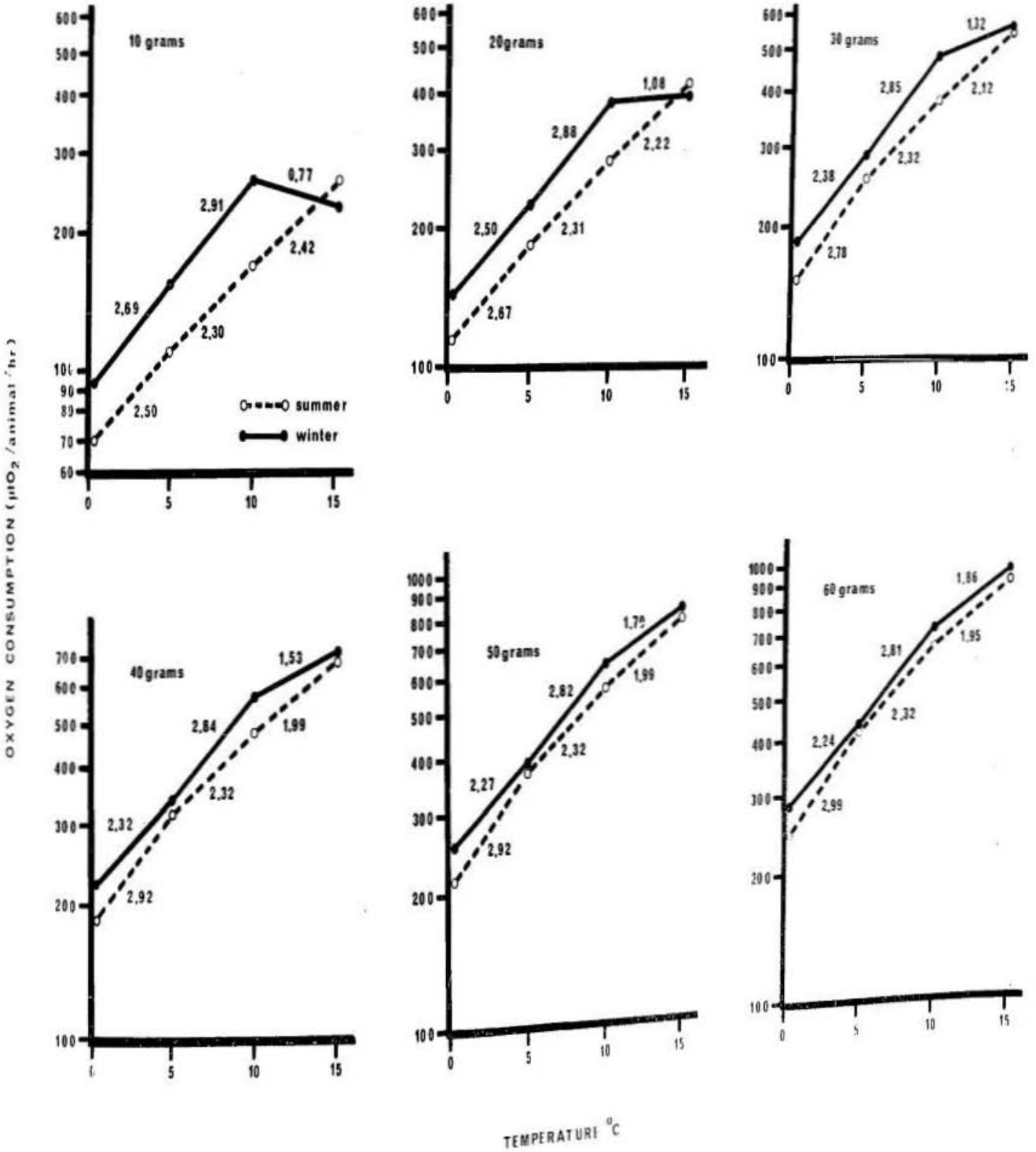

SUMMER
15°
10°
5°
0°
WINTER
15°
10°
5°
0°
OXYGEN CONSUMPTION µlO2/urchin/hr.

FIGURE 9

Rate-temperature relationships for respiration of summer-and winter-acclimatized S. droebachiensis of different standard weights. (Respiration rates calculated from exponential regression equations in Figures 6 and 7. Q_{10} values indicated for each temperature range.)


10 grams
20grams
30 grams
40grams
50grams
60 grams
summer
winter
2,69
2,91
0,77
2,42
2,30
2,50
2,50
2,88
1,08
2,22
2,31
2,67
2,38
2,85
1,32
2,12
2,32
2,78
2,32
2,84
1,53
1,99
2,32
2,92
2,27
2,82
1,75
1,99
2,32
2,92
2,24
2,81
1,86
1,95
2,32
2,99
OXYGEN CONSUMPTION (µlO2 /animal /hr)
TEMPERATURE °C

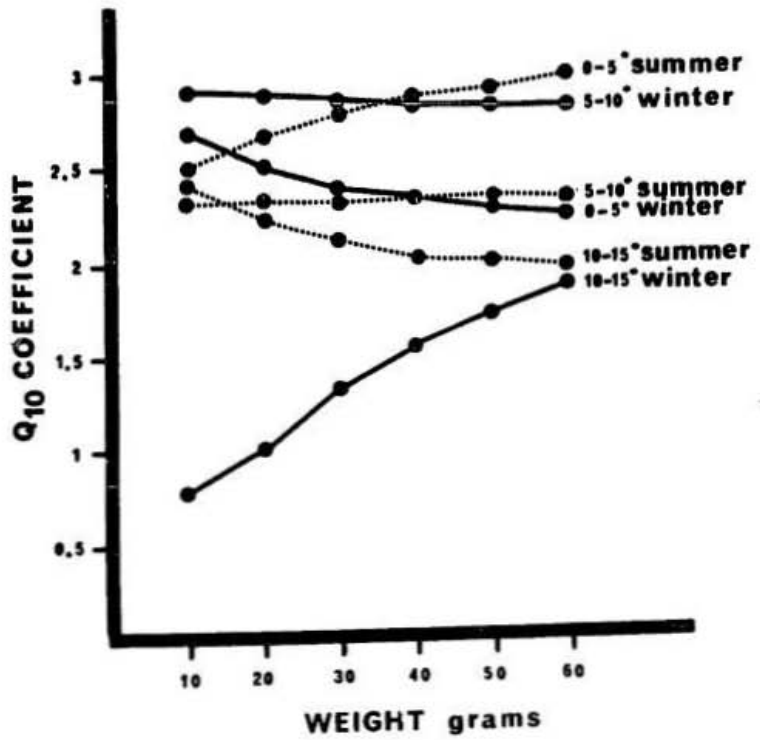
up to 10°C., followed by a pronounced decrease at 15°C. This decline in R-T slope is greatest in small urchins and diminishes with increasing body weight, indicating that the metabolism of large,cold-acclimatized urchins is less adversely affected by elevated temperatures than is that of small, cold-acclimatized animals. This observation may have particular importance when considered in conjunction with the phenomenon of size-dependence of adaptation ability (see below). The significance of the upward translation to the left of winter R-T curves relative to the summer ones in Figure 9 will be discussed later.

The influence of temperature on respiration is more fully characterized by Q_{10} coefficients calculated over 5°C. intervals, for animals of different standard weights (Fig. 9). Q_{10} values ranged from 0.77 to 2.99, with the majority between 2 and 3, and thus in general accord with the Q_{10} rule.

Trends in Q_{10} coefficients with respect to temperature, animal size, and season are evident (Fig. 10). Q_{10} values tend to decline with increasing temperature, albeit rather irregularly, particularly in winter, when values below 1.0 occur at higher temperatures. The relationship between Q_{10} and animal weight is complex and appears to be related not only to the temperature range over which the coefficient is measured, but also to the acclimatization temperature. Within the 5°- 10°C. range, midway between acclimatization temperatures, there is little change in Q_{10} with increasing urchin weight, either in summer or in winter. Over the temperature range near the acclimatization temperature, i.e. 0°- 5°C.

FIGURE 10

Influence of weight, season, and temperature range on Q_{10} coefficients for respiration of S. droebachiensis. (Q_{10} coefficients from Figure 9.)


Q10 COEFFICIENT
3
2,5
2
1,5
1
0,5
10
20
30
40
50
60
WEIGHT grams
0-5° summer
5-10° winter
5-10° summer
0-5° winter
10-15° summer
10-15° winter

in winter and 10°- 15°C. in summer, Q_{10} decreases with increasing weight. In contrast, over the temperature range remote from the acclimatization temperature, i.e. 10°- 15°C. in winter and 0°- 5°C. in summer, Q_{10} increases with increasing urchin weight. To illustrate these relationships more clearly, summer and winter Q_{10} coefficients were converted to RQ_{10} ratios such that:

$$RQ_{10} = \frac{Q_{10} \text{ cold-acclimatized animals}}{Q_{10} \text{ warm-acclimatized animals}} \times 100$$

The resulting RQ_{10} values, plotted against urchin weight (Fig. 11), indicate that at low temperatures (0°- 5°C.) the effect of temperature on respiration of cold-adapted relative to that of warm-adapted urchins decreases with increasing weight. At high temperatures (10°- 15°C.) this ratio increases with increasing weight. In the intermediate range (5°- 10°C.) the relationship between cold and warm-acclimatized Q_{10} remains essentially constant with increasing weight. The reasons for these complex relationships are not yet clear.

Temperature characteristics (μ) for summer and winter metabolism were calculated from the slope of the regression lines of Arrhenius plots (Fig. 14) according to the relationship:

$$\mu = \text{slope} \times R \qquad \text{(Hoar, 1966)}$$

where R is the gas constant (1.987 cal./degree/mole). Temperature characteristics in winter are only slightly greater than in summer, with the

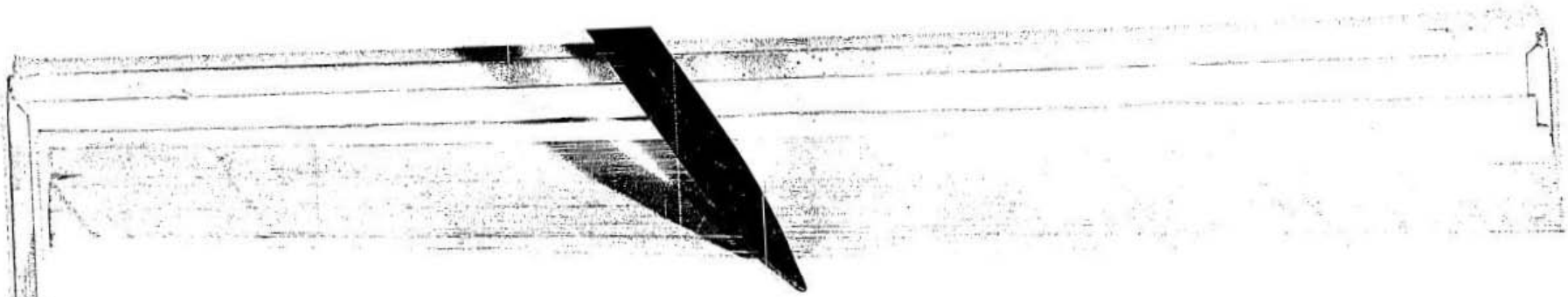
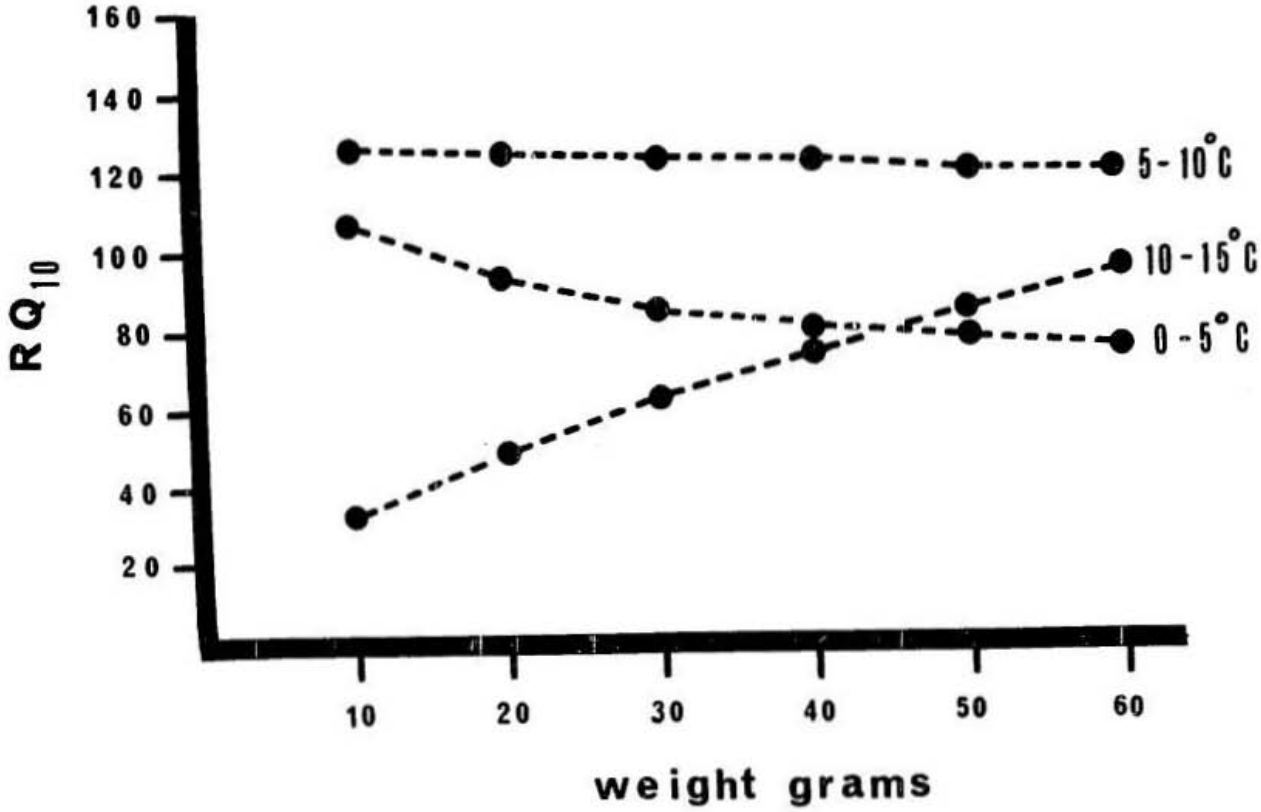


FIGURE 11

Ratio of winter to summer Q_{10} coefficients for respiration of S. droebachiensis, and the influence of temperature and animal weight. (Ratios calculated from Q_{10} coefficients in Figure 9.)


160
140
120
100
80
60
40
20
RQ10
5-10°C
10-15°C
0-5°C
10
20
30
40
50
60
weight grams

difference decreasing with increasing urchin weight. There is little change in μ with increasing size in summer, while in winter μ declines from 15,900 in 10 gram animals to 14,100 in 60 gram animals.

d) Respiration and season:

Comparing the relation between respiration rate and animal weight, in winter and summer, by use of the regression of respiration on wet weight (Fig. 12), revealed, at 0°, 5° and 10°C., by a distinct upward displacement of winter regression lines, a substantial augmentation of metabolism on cold-acclimatization. Analysis of covariance (Dixon and Massey, 1957) of the data in appendix III a and b indicates that the summer and winter regression lines are significantly different ($p < 0.05$) at 5° and 10°C. The lack of statistical significance at 0°C. may be attributable to the considerable individual variation in respiration rates measured at this low temperature (Figs. 6 and 7). Summer and winter regression lines, at 15°C., however, virtually coincide.

The precise character of this seasonal shift in metabolism is perhaps more readily apparent from summer and winter rate-temperature curves, determined for urchins of different standard weights (Fig. 9). The winter R-T curves are displaced upwards and to the left relative to summer ones, indicating augmentation of metabolism in the cold. The compensatory shift is greatest in small urchins and decreases with increasing weight. This is more clearly shown by means of RQO_2 ratios, where:

$$RQO_2 = \frac{\text{cold-acclimatized respiration rate}}{\text{warm-acclimatized respiration rate}} \times 100$$

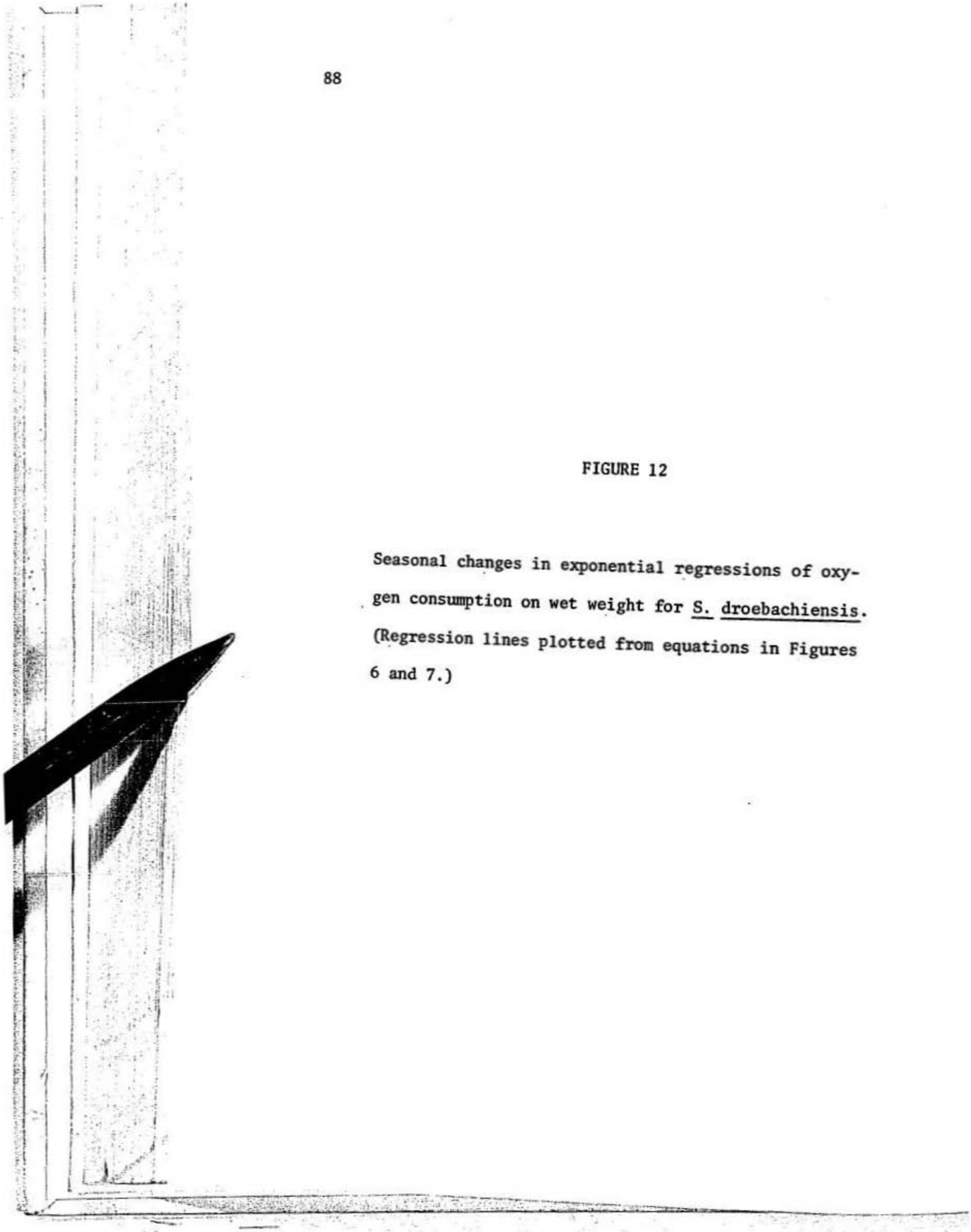
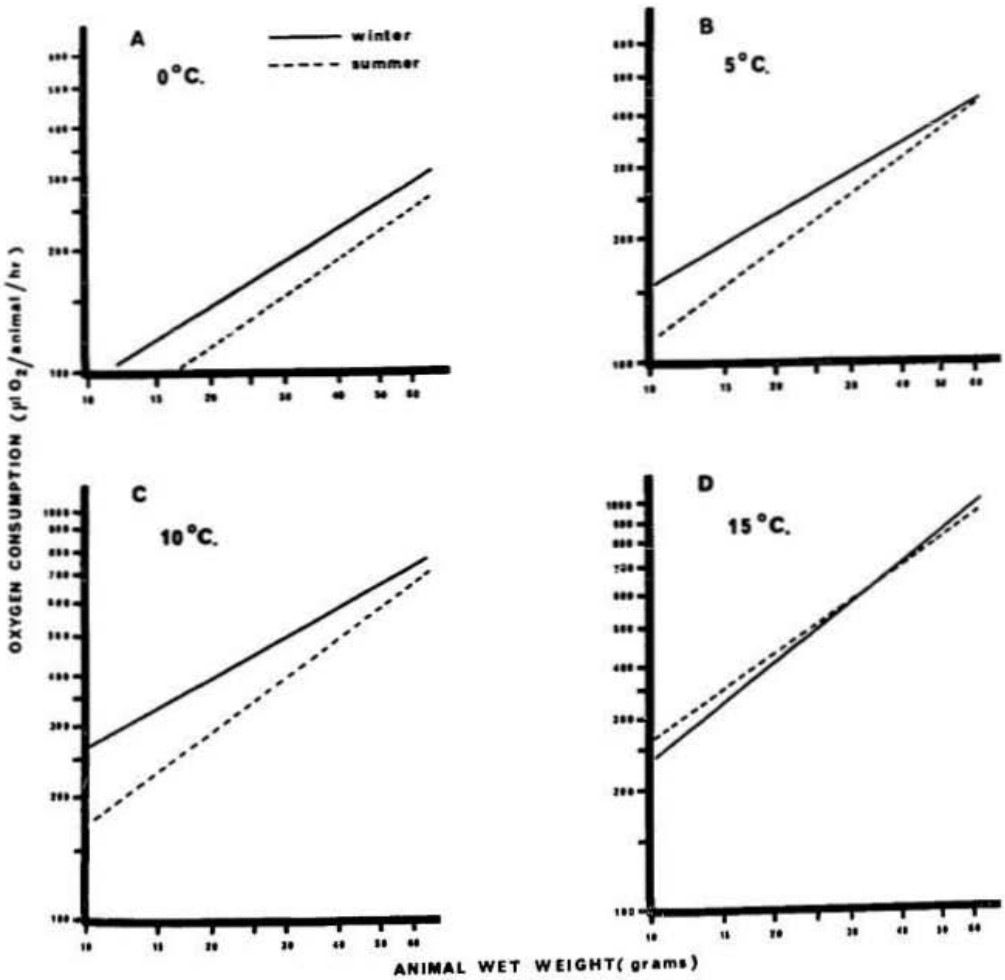


FIGURE 12

Seasonal changes in exponential regressions of oxygen consumption on wet weight for S. droebachiensis. (Regression lines plotted from equations in Figures 6 and 7.)


A
0°C.
winter
summer
B
5°C.
C
10°C.
D
15°C.
OXYGEN CONSUMPTION (µl O2/animal/hr)
ANIMAL WET WEIGHT(grams)

This ratio provides a relative measure of the cold-induced increase in respiration at any given temperature. An $RQO_2 > 100$ indicates that at a given temperature, respiration in winter is greater than that in summer. At 0°, 5°, and 10°C. the graph (Fig. 13) shows that, as the animal gets larger, the difference between the winter and summer respiration rates becomes progressively less. At 15°C., on the other hand, winter respiration is much depressed in small animals, progressively less depressed in larger ones.

e) Thermal compensation coefficients (T.C.C.):

For computation of T.C.C., R-T curves for respiration of urchins of different standard weights (Fig. 9) were converted to Arrhenius plots as described earlier. Regression equations were calculated for the transformed summer and winter data, and the corresponding least-square regression lines plotted (Fig. 14). Arrhenius transformation of summer R-T data results in linear relationships with high coefficients of correlation ($r > 0.99$). However, in the transformed winter R-T curve, the 15°C. point is considerably displaced(indicated by a fine dotted line in Figure 14) from the regression line drawn through points at 0°, 5°, and 10°C. This is a consequence of the high-temperature respiratory depression described earlier. The displacement decreases with increasing urchin weight. To minimize distortion of the T.C.C., the depressed 15°C. points are excluded from the calculation of regression equations for the transformed R-T data of winter-acclimatized urchins.

FIGURE 13

Ratios of winter to summer respiration rates (RQO_2) for S. droebachiensis of different standard weights, and at different temperatures. (Ratios calculated from respiration rates derived from exponential regression equations in figures 6 and 7.)

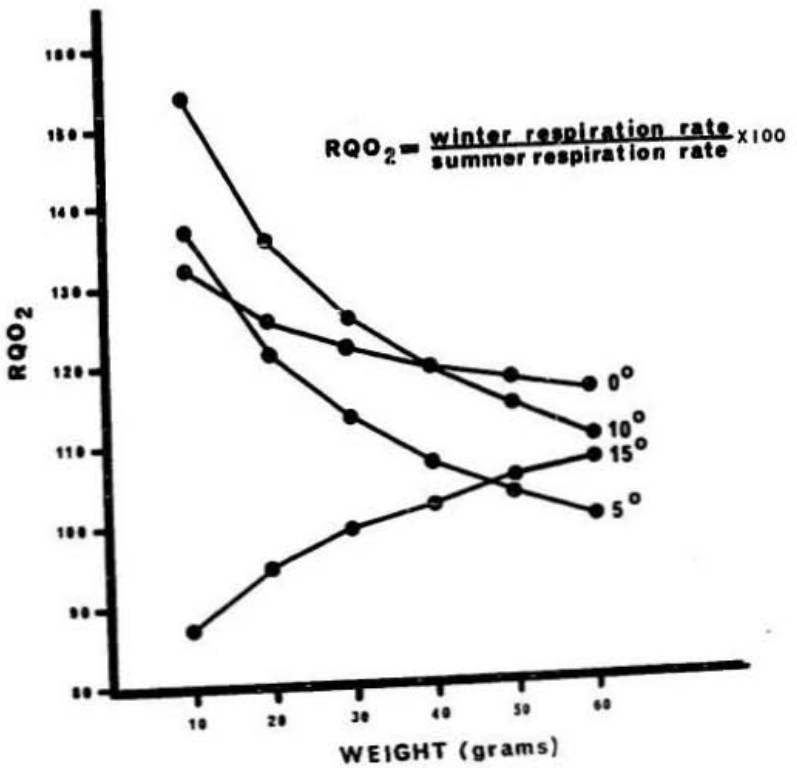
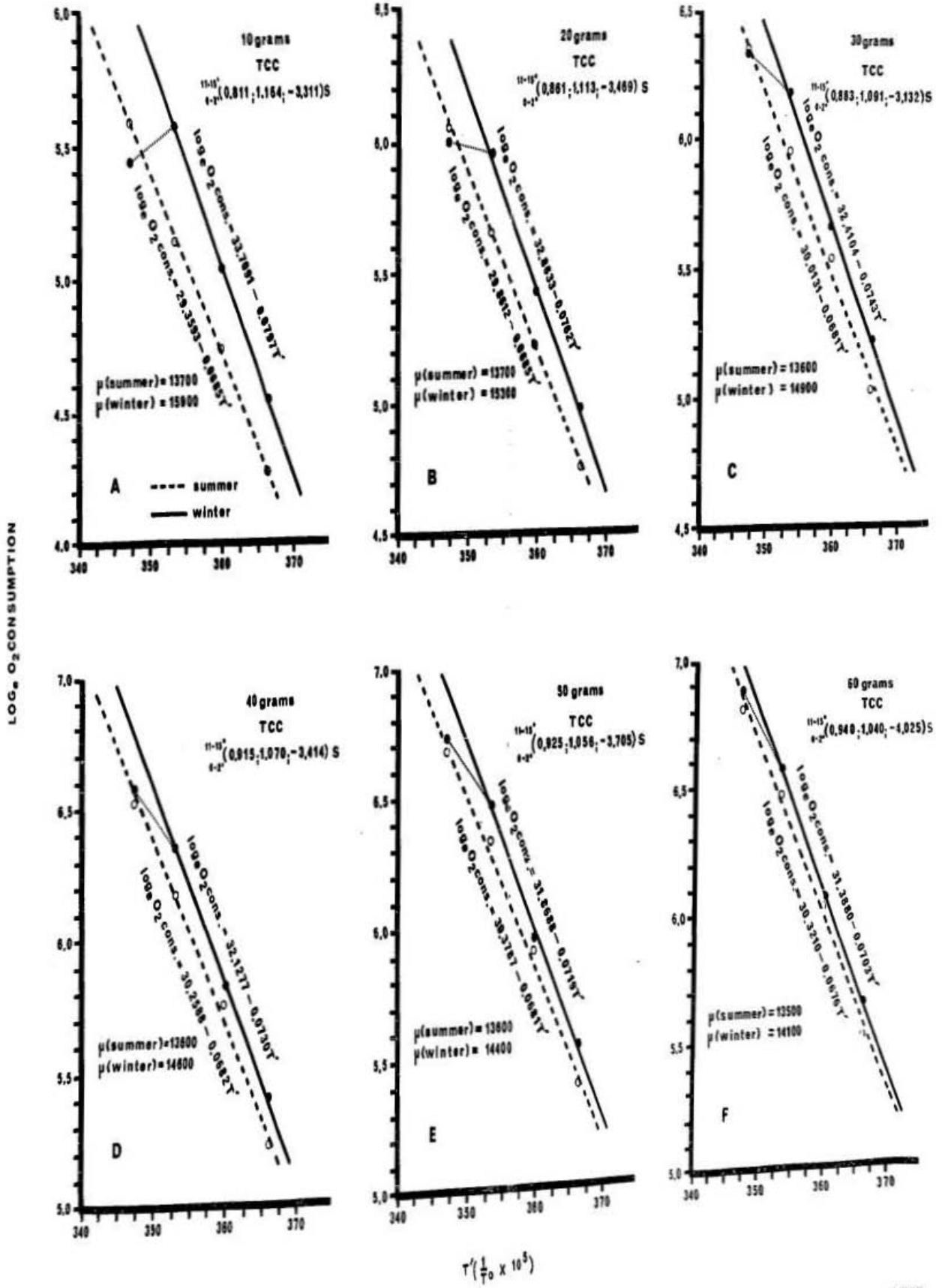

$RQO_2 = \frac{\text{winter respiration rate}}{\text{summer respiration rate}} \times 100$
RQO_2
0°
10°
15°
5°
10
20
30
40
50
60
WEIGHT (grams)

FIGURE 14

Arrhenius transformation of R-T relationships (Figure 9) for respiration of summer-(open circles) and winter-(closed circles) acclimatized S. droebachiensis of different standard weights.(Regression equations for Arrhenius relationships adjacent to corresponding regression lines.) (Finely dotted line indicates displacement of 15°C. data point.)


10 grams
TCC
20 grams
TCC
30 grams
TCC
40 grams
TCC
50 grams
TCC
60 grams
TCC
μ(summer) = 13700
μ(winter) = 15900
μ(summer) = 13700
μ(winter) = 15300
μ(summer) = 13600
μ(winter) = 14900
μ(summer) = 13600
μ(winter) = 14600
μ(summer) = 13600
μ(winter) = 14400
μ(summer) = 13500
μ(winter) = 14100
A
B
C
D
E
F
summer
winter
LOG e O2 CONSUMPTION
T'(1/T0 x 10-3)

T.C.C. for respiration of urchins of different standard weights are presented in Figure 14. Coefficients of adaptation range from 0.810 for 10 gram urchins to 0.939 for 60 gram animals, indicating a partial acclimatization response, and a decline in adaptation ability with increasing animal weight. Coefficients of rotation, ranging from 1.040 to 1.155 indicate that low-temperature acclimatization is characterized by translation of the cold-relative to the warm-acclimatized R-T relationship, with little, if any, rotation. Furthermore, axial coefficients of approximately -3 to -4 indicate that the intersection of the warm-and cold-acclimatized Arrhenius curves occurs at a low temperature, outside the stress range. These values of the C.R. and Ax. suggest a quantitative (mass effect) rather than a qualitative change in the mechanisms underlying acclimatization.

f) Multiple regression of respiration on weight and temperature:

It is possible to relate respiration rate to the two independent variables, weight and temperature, simultaneously, by multiple regression analysis (Snedecor, 1956). The result provides an equation that permits calculation of the oxygen consumption from wet weight and temperature, only. The general formula is:

$$\log M = B_0 + B_1 \log W + B_2 \log T$$

where M is respiration rate ($\mu l.O_2$/urchin/hour), W is fresh weight of the urchin (grams), T is temperature (°C.) and B_0, B_1, B_2 are exponential regression coefficients.

Summer and winter urchin respiration data were analyzed in this manner. A summary of the multiple regression analysis is presented in Table 2. The respiration rate, between 0° and 15°C., of summer-acclimatized S. droebachiensis may be estimated from the equation:

$$\log M = 1.281 + 0.749 \log W + 0.227 \log T \quad .$$

For winter animals for which only the 0°, 5°, and 10°C. data were used (because of the respiratory R-T slope depression at 15°C.) the equation becomes:

$$\log M = 1.668 + 0.515 \log W + 0.180 \log T$$

A nomogram (Fig. 15) based on these equations has been constructed and provides a concise summary of seasonal changes in respiration of S. droebachiensis. The nomogram also provides researchers with a rapid means of estimating respiration rates of S. droebachiensis from the readily obtainable measures of size and ambient temperature. This aide may prove useful in future studies on the role of this species in ecological bioenergetics of North Atlantic coastal, marine communities.

Table 2. Exponential multiple regression analysis of respiration ($\mu l.O_2$/ animal/ hr.) on weight and temperature of summer-and winter-acclimatized S. droebachiensis.

(Based on data in appendix III a. and b.)

season	temperature range	B_o +	B_1 +	B_2 +	S_{B_1} *	S_{B_2} *	B_1 **	B_2 **	R ++
summer	0°- 15°C.	1.281	0.749	0.227	0.050	0.011	±0.097	±0.022	0.792
winter	0°- 10°C.	1.668	0.515	0.180	0.085	0.017	±0.168	±0.033	0.738

+ Coefficients of multiple regression equation of the form :

$$\log M = B_o + B_1 \log(\text{wet weight}) + B_2 \log (\text{temperature } °C.)$$

++ Coefficient of multiple determination.

* Standard errors of regression coefficients.

** 95% confidence limits for regression coefficients.

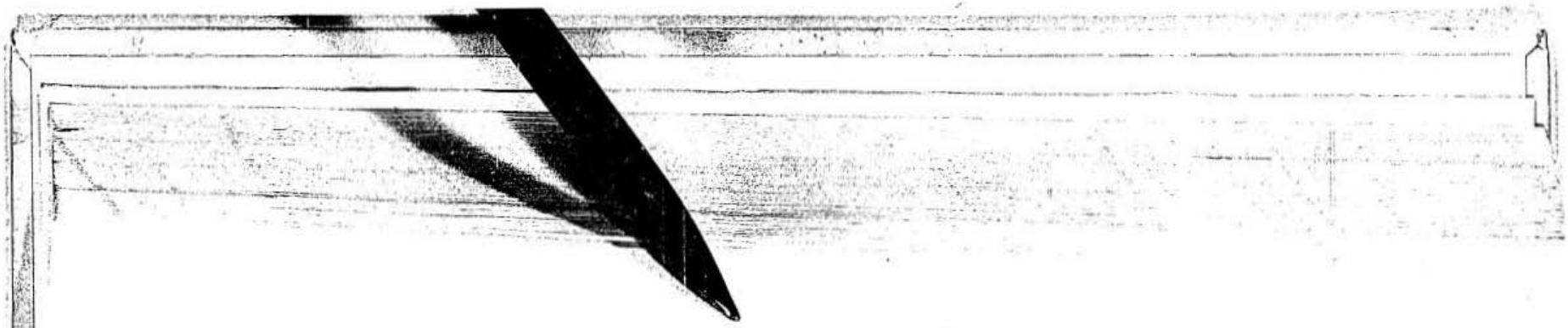
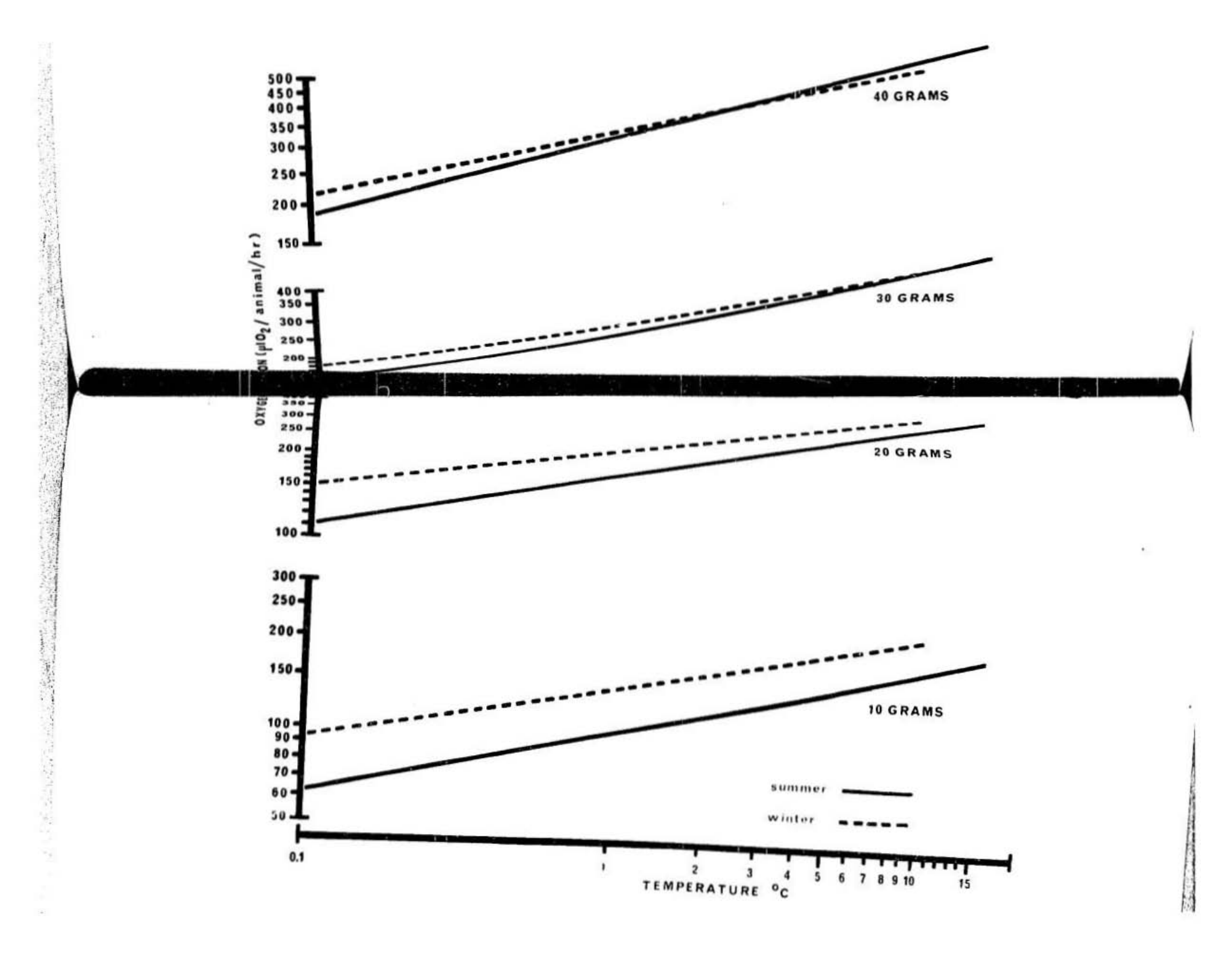


FIGURE 15

Nomograms for oxygen consumption of S. droebachiensis in summer and winter. (Lines plotted from indicated multiple regression equations; refer to Table 2.)


500
450
400
350
300
250
200
150
40 GRAMS
400
350
300
250
200
30 GRAMS
350
300
250
200
150
100
20 GRAMS
300
250
200
150
100
90
80
70
60
50
10 GRAMS
summer
winter
0.1
1
2
3
4
5
6
7
8
9
10
15
TEMPERATURE °C

ii) Seasonal changes in excised tissue respiration:

To ascertain whether the seasonal respiratory adaptation of intact urchins is paralleled by similar adaptive shifts in the metabolism of the urchins' tissues, respiration of stomach, intestine, esophagus, rectum, tube feet, and male and female gonad tissues was measured, in vitro, in summer and winter*, at 5°C. intervals between 5° and 25°C. (Tables 3 to 8), and respiratory R-T curves plotted (Figs. 16, 17).

a) Relative tissue respiration rates:

Summer R-T curves for tissue respiration (Fig. 16) reveal that in the digestive tract, respiration increases from anterior to posterior: esophagus < stomach < intestine < rectum. This relationship is clearly shown when the absolute respiration rates of digestive tract tissues are expressed as percentages of the esophagus rate (Table 9).

Winter R-T curves (Fig. 17), on the other hand, fail to show such a relationship between the various segments of the digestive tract (Table 9). Different degrees of acclimatization (see below) in stomach and intestine may account for the absence of a comparable metabolic gradient in winter-acclimatized urchins.

Tube foot respiration is considerably lower than that of stomach and intestine tissue during both summer and winter (Figs. 16, 17). Tube foot respiration rate in summer is greater than, and in winter less than, that of esophagus tissue, a possible reflection of an apparent absence

* No winter data for rectum.

Table 3. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of excised stomach from summer-and winter-acclimatized S. droebachiensis. (Based on data in appendix V a.)

	SUMMER			WINTER			
temperature (°C.)	N	O_2 cons.	S.E.	N	O_2 cons.	S.E.	p
5°	8	231.3	16.4	9	358.2	23.3	<0.001
10°	15	411.2	21.2	9	580.1	21.8	<0.001
15°	10	546.3	14.5	9	932.5	27.8	<0.001
20°	11	914.0	59.1	11	1263.7	71.3	<0.005
25°	8	1610.2	184.7	12	1834.2	56.0	<0.250

Table 4. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of excised intestine from summer- and winter-acclimatized S. droebachiensis. (Based on data in appendix V b.)

	SUMMER			WINTER			
temperature (°C.)	N	O_2 cons.	S.E.	N	O_2 cons.	S.E.	p
5°	9	305.0	26.5	9	398.3	23.0	< 0.010
10°	14	441.7	19.6	10	541.6	24.5	< 0.005
15°	12	683.9	25.5	9	799.3	32.5	< 0.010
20°	11	971.0	62.7	12	925.0	81.3	< 0.400
25°	8	1281.8	89.3	12	1515.0	70.5	< 0.050

Table 5. Respiration rates ($\mu l.O_2$/gm.dry wt./hr.) of excised esophagus from summer-and winter-acclimatized S. droebachiensis. (Based on data in appendix V c.)

	SUMMER			WINTER			
temperature (°C.)	N	O_2 cons.	S.E.	N	O_2 cons.	S.E.	p
5°	5	163.3	29.5	3	255.0	27.7	<0.050
10°	5	271.7	12.7	5	414.1	22.7	<0.0005
15°	5	462.5	41.8	4	597.3	24.9	<0.025
20°	5	582.8	44.2	4	849.9	85.7	<0.025
25°	3	1008.3	117.8	6	1283.8	118.4	<0.250

Table 6. Respiration rates ($\mu l.O_2$/gm.dry wt./hr.) of excised tube feet from summer-and winter-acclimatized S. droebachiensis. (Based on data in appendix V d.)

	SUMMER			WINTER			
temperature (°C.)	N	O_2 cons.	S.E.	N	O_2 cons.	S.E.	p
5°	3	151.6	32.6	6	223.8	26.8	<0.250
10°	3	374.3	49.2	5	347.5	42.1	<0.400
15°	3	540.5	95.9	5	653.4	56.8	<0.250
20°	3	810.5	43.2	4	834.8	40.5	<0.400
25°	3	1087.0	158.0	6	1212.0	151.6	<0.300

Table 7. Respiration rates ($\mu l.O_2/gm.$ dry wt./hr.) of excised male and female gonads from summer-and winter-acclimatized S. droebachiensis. (Based on data in appendix V e.)

		SUMMER			WINTER		
	temperature (°C.)	N	O_2 cons.	S.E.	N	O_2 cons.	S.E.
male	5°	7	134.5	21.5	-	-	-
	10°	2	123.1	104.7	6	2742.6	327.2
	15°	4	109.6	7.8	3	2084.9	165.0
	20°	6	137.1	15.8	3	2066.8	229.9
	25°	-	-	-	5	2710.3	638.8
female	5°	2	11.4	0.1	-	-	-
	10°	6	21.2	1.7	3	443.0	112.3
	15°	4	36.5	12.8	4	401.7	87.6
	20°	2	18.2	6.0	4	504.2	105.3
	25°	-	-	-	3	704.5	199.7

Table 8. Respiration rates (µl. O_2/gm. dry wt./hr.) of excised rectum from summer-acclimatized S. droebachiensis. (Based on data in appendix V f.)

temperature (°C.)	N	O_2 cons.	S.E.
5°	3	421.2	117.9
10°	2	549.7	30.9
15°	3	682.0	145.5
20°	3	985.3	61.1
25°	3	1708.5	234.0

FIGURE 16

R-T relationships for respiration of excised tissues of summer-acclimatized S. droebachiensis. (Based on data in Tables 3, 4, 5, 6, 7, and 8.)

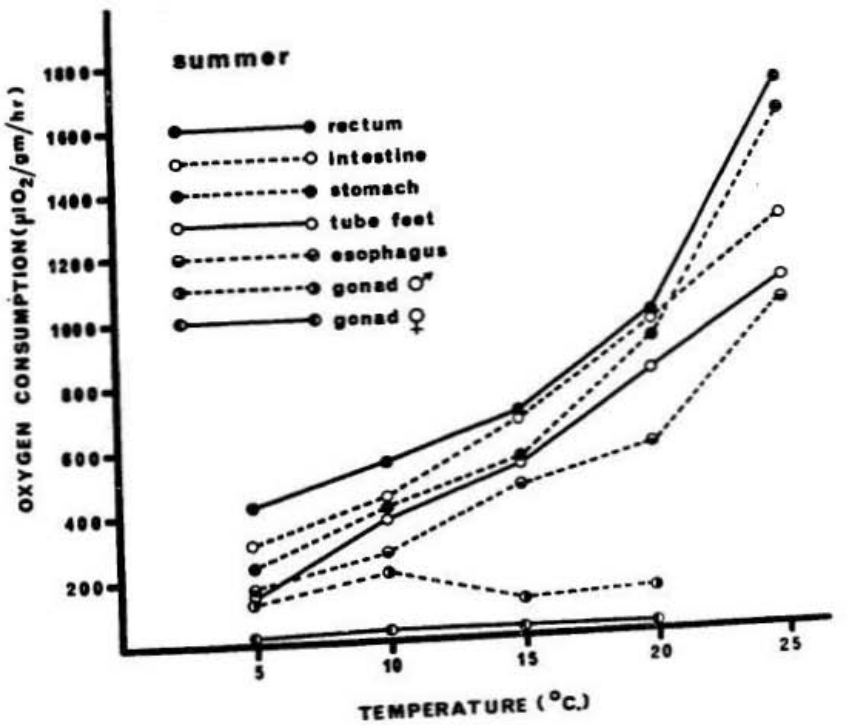

summer
rectum
intestine
stomach
tube feet
esophagus
gonad ♂
gonad ♀
OXYGEN CONSUMPTION(μlO2/gm/hr)
1800
1600
1400
1200
1000
800
600
400
200
5
10
15
20
25
TEMPERATURE (°C.)

FIGURE 17

R-T relationships for respiration of excised tissues of winter-acclimatized S. droebachiensis. (Symbols as in Figure 16. Based on data in Tables 3,4, 5, 6, and 7.)

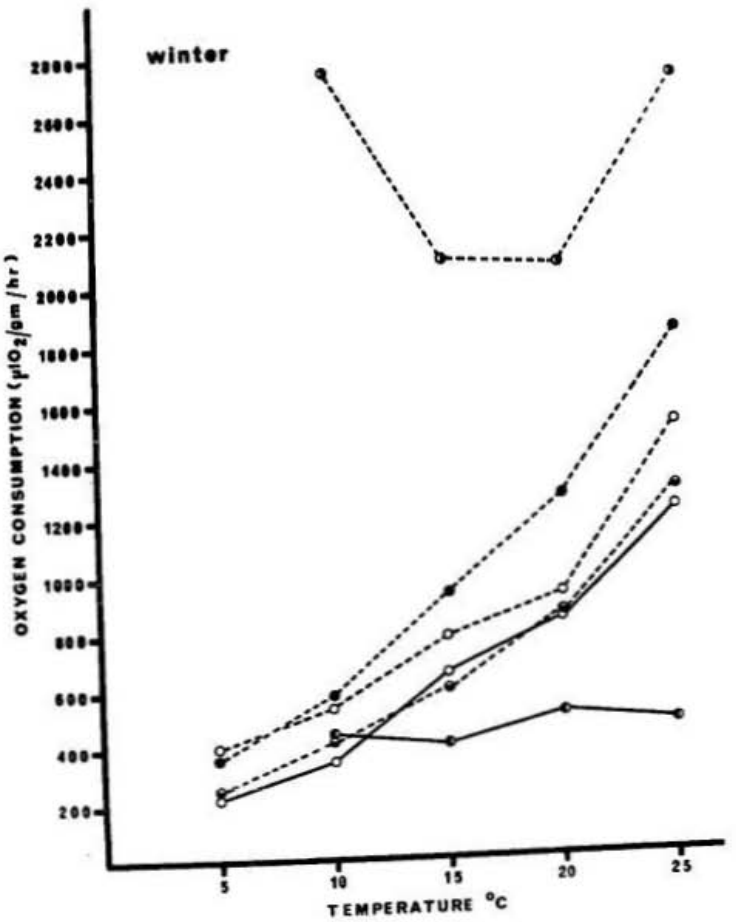

winter
OXYGEN CONSUMPTION (µlO2/gm/hr)
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
5
10
15
20
25
TEMPERATURE °C

Table 9. Relative respiration rates of excised tissues from summer- and winter-acclimatized S. droebachiensis. (Rates expressed as a percentage of the esophagus rate. Based on data in Tables 3, 4, 5, 6, 7, and 8.)

	TEMPERATURE (°C.)				
	5°	10°	15°	20°	25°
SUMMER					
Esophagus	100.0	100.0	100.0	100.0	100.0
Stomach	141.6	151.4	118.1	156.9	159.7
Intestine	186.9	162.6	147.9	166.7	127.1
Rectum	257.9	202.3	147.5	169.1	169.4
Tube feet	92.8	137.8	116.9	139.1	107.8
Gonad (male)	82.3	45.3	23.7	23.5	-
Gonad (female)	7.0	7.8	7.9	3.1	-
WINTER					
Esophagus	100.0	100.0	100.0	100.0	100.0
Stomach	140.5	140.1	156.1	148.7	142.9
Intestine	156.2	130.8	133.8	108.8	118.0
Tube feet	87.8	83.9	109.4	98.2	94.4
Gonad (male)	-	662.3	349.1	243.2	211.1
Gonad (female)	-	107.0	67.2	59.3	54.9

of significant adaptation to low temperature in tube feet (see below).

Female gonads, in summer, have the lowest in vitro respiration rate of any of the tissues examined (Fig. 16), and although in winter the respiration increases considerably (Table 9), it still remains lower than that of other tissues (Fig. 17).

The in vitro respiration rate of male gonads is consistently higher than that of female gonads, particularly during the winter (Figs. 16, 17), when testis respiration increases approximately twenty-fold over its summer rate. Testis samples show considerable individual variation in respiration rate (appendix V e). One must assume that the measurement of "testis respiration" in reality is a measure of metabolism of two separate components, testicular tissue and active spermatazoa. Differences in the quantity and maturity of the latter may account for the great variation in rates. Spermatazoa in the respirometer vessels are extremely active, possibly explaining the high respiration rates recorded for testis.

Respiration of coelomic fluid was not investigated in detail. A single sample in summer had a respiration rate of 528.9 $\mu l.O_2$/gm. dry wt./hr. at 15°C., indicating that, on a dry weight basis, coelomic fluid respiration rate is similar in magnitude to that of alimentary tract tissues.

b) Respiration and temperature:

There is no evidence, at elevated temperatures, of a decline in the R-T slopes for tissue respiration, in either summer (Fig. 16) or winter (Fig. 17), comparable to that occurring in intact-urchin respiration, between 10° and 15°C., in winter (Fig. 9).

The precise interpretation to be placed upon the apparent failure of both male and female gonad respiration to increase consistently with rising temperature (Figs. 16, 17) is uncertain.

Q_{10} coefficients of respiration in summer and winter were calculated over 5°C. intervals for each of the tissues from data in Tables 3, 4, 5, 6. In general the tissues adhere closely to the Q_{10} rule, with most respiration rates increasing 2-3 times for each 10°C. rise in temperature (Table 10). The unusually high Q_{10} of 6.09 for tube foot respiration in summer is probably attributable to small sample size rather than to an inherent physiological peculiarity. There is no evidence of a consistent increase or decrease in Q_{10} with rising temperature, between 5° and 25°C. A seasonal trend is evident, in that approximately 70% of the seasonally paired coefficients in Table 10 have a Q_{10} slightly higher in summer than in winter.

Temperature characteristics (μ) of stomach and intestine tissues are derived from the Arrhenius plots (Fig. 19). In winter, μ values for both stomach and intestine (15,300 and 13,800, respectively) are higher than in summer (11,100 and 10,000, respectively).

c) Respiration and season:

In winter, respiration rates of stomach, intestine, esophagus and male and female gonads are significantly higher than in summer at the same temperatures (Tables 4, 5, 6, 8) (Fig. 18). The seasonal differences, highly significant at low and intermediate temperatures, are

Table 10. Q_{10} coefficients for respiration of excised tissues from summer- and winter-acclimatized S. droebachiensis.

temperature range (°C.)	stomach		intestine		esophagus		tube feet	
	summer	winter	summer	winter	summer	winter	summer	winter
5-10°	3.16	2.62	2.10	1.85	2.77	2.64	6.09	2.41
10-15°	1.77	2.58	2.40	2.18	2.90	2.08	2.09	3.54
15-20°	2.80	1.84	2.02	1.34	1.59	2.03	2.25	1.63
20-25°	3.11	2.11	1.74	2.68	3.00	2.28	1.80	2.11

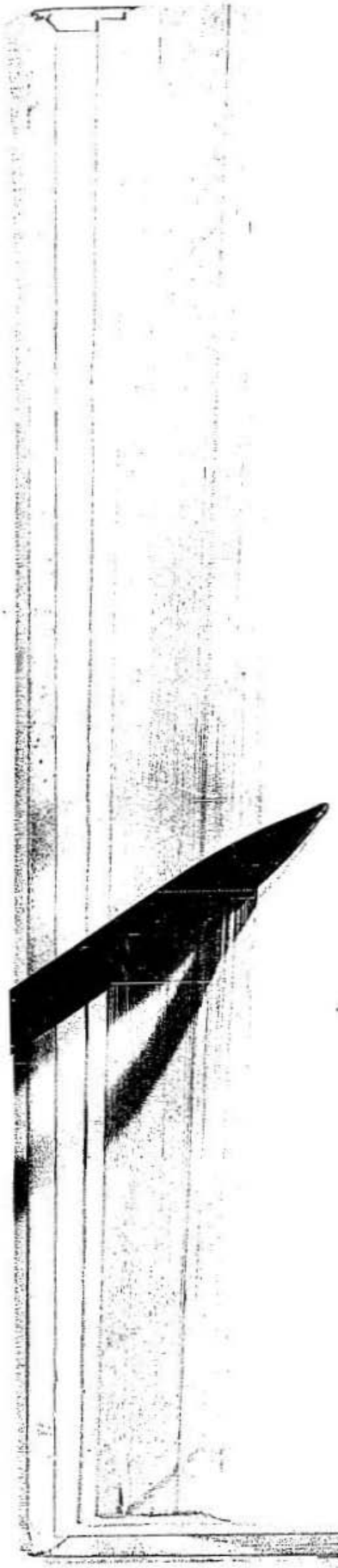
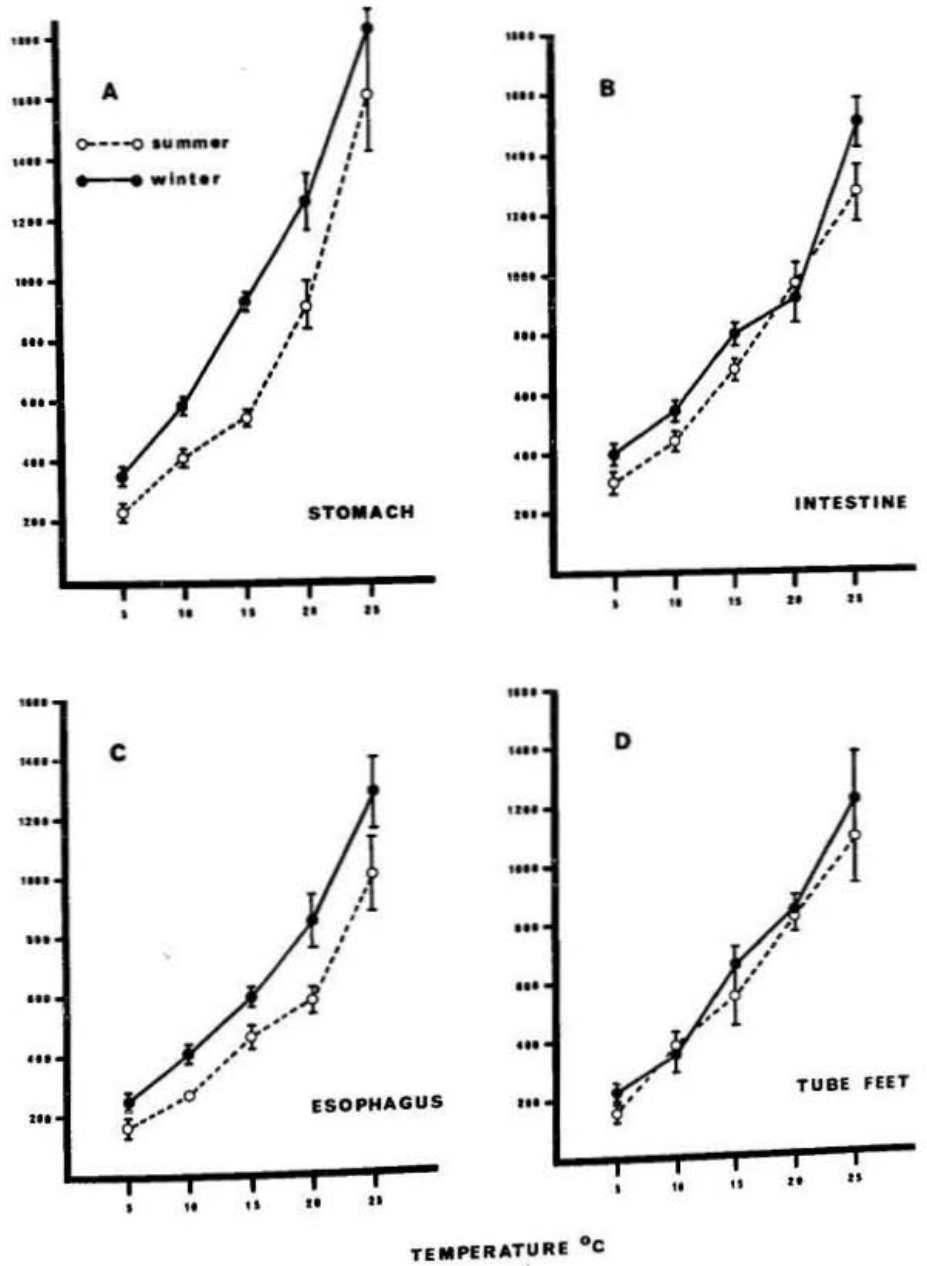


FIGURE 18

R-T relationships for respiration of stomach, intestine, esophagus, and tube foot tissues of summer-and winter-acclimatized S. droebachiensis. (Based on data in Tables 3, 4, 5, and 6.)


A
summer
winter
STOMACH
B
INTESTINE
C
ESOPHAGUS
D
TUBE FEET
TEMPERATURE °C

less so at elevated temperatures (except for gonads; see below). This may be partly attributable to the considerable increase in individual variation at higher temperatures, as indicated by the increasing standard errors, and partly to an actual decrease in the relative difference between the seasonal rates with rising temperature. This latter point is particularly evident from comparison of RQO_2 coefficients (ratio of winter to summer respiration expressed as a percentage) at different temperatures (Table 11). The unusually high RQO_2 values for male and female gonads do not decline at elevated temperatures. The seasonal changes in gonad respiration are, most probably, primarily attributable to changes in reproductive condition, rather than to metabolic adjustments associated with thermal adaptation.

The respiration rates of tube foot tissue appear to be somewhat higher in winter than in summer, although the differences are not statistically significant. Small sample sizes, combined with the highly contracted state of tube feet following excision (p. 187) are probably responsible for the absence of a clear-cut adaptation response.

d) Thermal compensation coefficients:

Thermal compensation coefficients for stomach and intestine tissues are derived from Arrhenius plots (Fig. 19) based on seasonal R-T curves (Fig. 18). It was not deemed feasible to calculate reasonably accurate T.C.C. for esophagus and tube foot tissues because of the small sample sizes (resulting from the necessity of using pooled samples).

Table 11. Seasonal RQO_2 coefficients $\frac{\text{winter rate}}{\text{summer rate}} \times 100$ for respiration of excised tissues of S. droebachiensis. (Based on data in Tables 3, 4, 5, 6, and 7.)

temperature (°C.)	stomach RQO_2	intestine RQO_2	esophagus RQO_2	tube feet RQO_2	male gonad RQO_2	female gonad RQO_2
5°	210.2	145.9	156.1	147.6	-	-
10°	128.9	116.5	152.4	92.8	1292.8	2089.6
15°	159.0	108.9	129.2	120.9	1902.5	1100.3
20°	138.3	95.3	145.9	103.0	1507.9	2770.3
25°	113.9	118.2	127.3	111.5	-	-

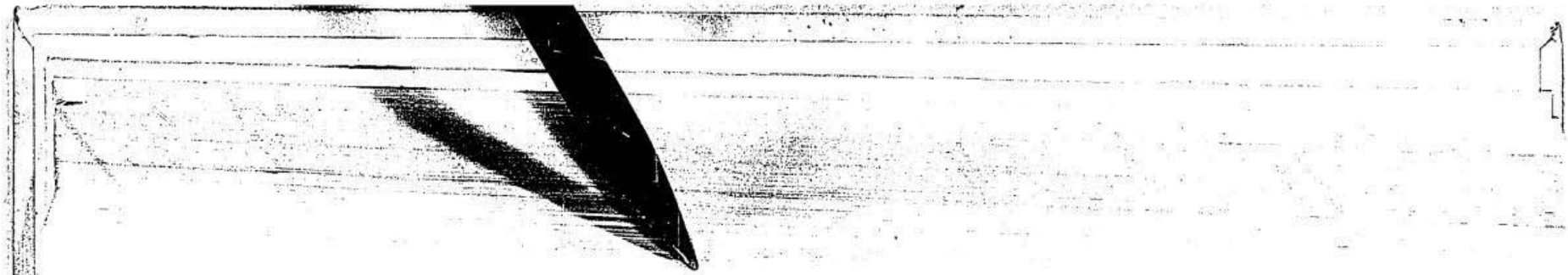
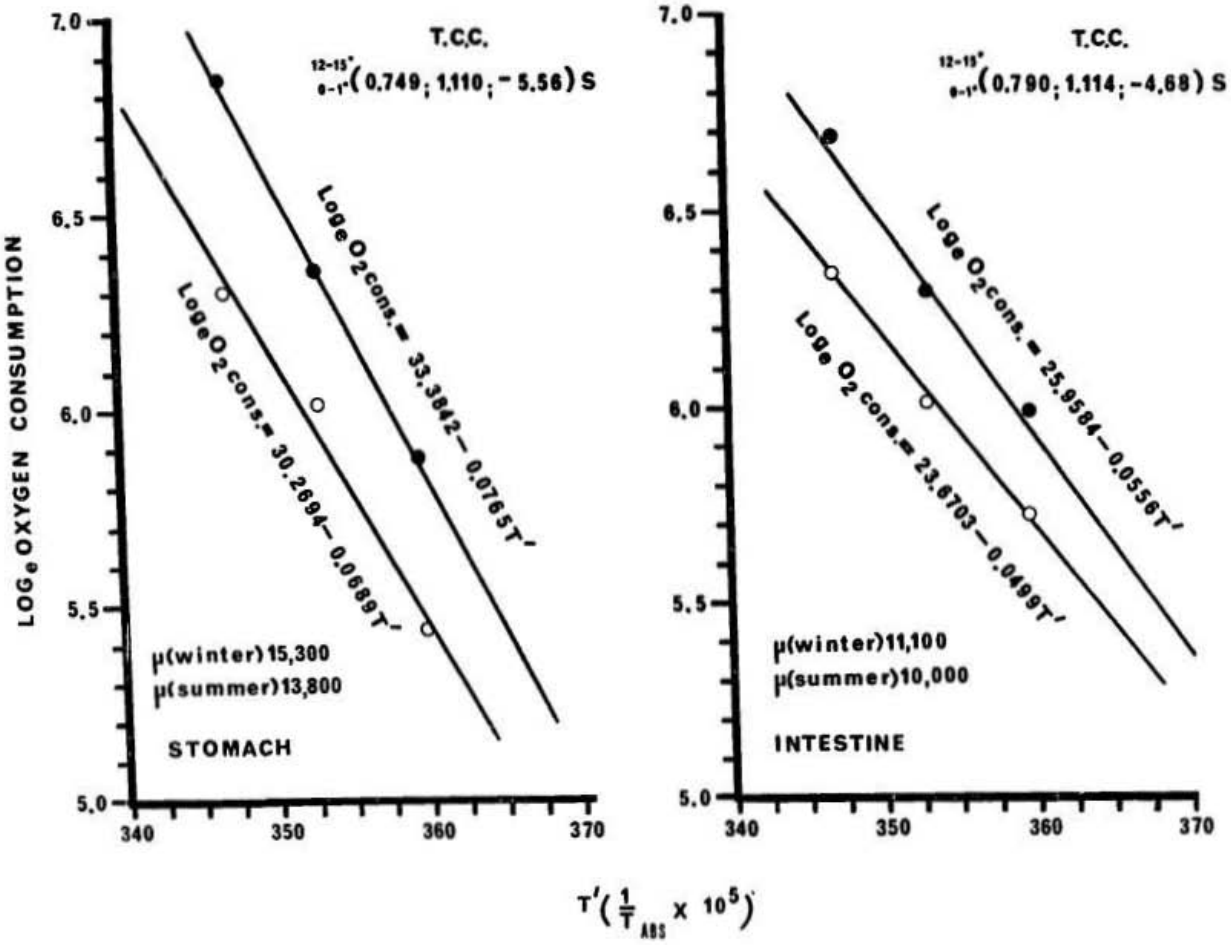


FIGURE 19

Arrhenius transformation of R-T relationships (Figures 18 a and b) for respiration of stomach and intestine tissues from summer-and winter-acclimatized S. droebachiensis.


T.C.C.
12-15°
θ-1°(0.749; 1.110; -5.56) S
Loge O2 cons. = 33.3842 - 0.0765T'
Loge O2 cons. = 30.2694 - 0.0689T'
μ(winter) 15,300
μ(summer) 13,800
STOMACH
T.C.C.
12-15°
θ-1°(0.790; 1.114; -4.68) S
Loge O2 cons. = 25.9584 - 0.0556T'
Loge O2 cons. = 23.6703 - 0.0499T'
μ(winter) 11,100
μ(summer) 10,000
INTESTINE
LOGe OXYGEN CONSUMPTION
7.0
6.5
6.0
5.5
5.0
340
350
360
370
T'(1/T ABS x 10^5)

Stomach tissue has a C.A. of 0.748, indicating an approximately 25% compensatory adjustment in respiratory metabolism. A C.R. of 1.110 suggests that adaptation involves essentially a relative translation of R-T curves with little or no rotation.

Intestine tissue has a C.A. of 0.790, indicating an approximately 20% compensatory adjustment in metabolism; slightly less than that of stomach. A C.R. of 1.114 indicates that, as was the case with stomach tissue, intestine respiratory acclimatization occurs primarily by a relative translation of winter-and summer-acclimatized R-T curves, with a minimum of rotation.

Axial coefficients of -5.56 and -4.68, for stomach and intestine, respectively, show that the intersections of the warm-and cold-acclimatized Arrhenius curves in both instances occur outside the stress range, at low temperatures.

iii) Seasonal changes in urchin activity:

The preceding sections have clearly shown that S. droebachiensis significantly alters its respiratory metabolism in a manner that partially compensates for low winter temperatures. Does such acclimatization of metabolism enable the urchins to maintain a relatively high level of activity during colder months? Before attempting to answer this question it is necessary to consider several aspects of the method selected for measuring activity.

a) Activity coefficient:

A direct measure of urchin activity was obtained by converting half-righting time to an activity coefficient (A.C.). The relationship between the two is illustrated in Figure 20 a; the mean winter and summer A.Cs. are indicated.

Frequency distributions (Fig. 20 b) reveal that A.Cs. measured at prevailing habitat temperatures are generally normally distributed, but that test temperature and season markedly influence the frequency distribution.

Relative stability, over an extended period, of the mean A.C. of a group of 20 urchins is demonstrated in Figure 20 c. The mean A.C. ranges from 10.18 to 15.24, with a mean of 13.65 and a standard error of 0.304. There is no evidence of either a significant increase or decrease in mean A.C. following repeated trials at approximately 24 hour intervals [provided urchins are held in polyethylene-lined tanks (p. 47)].

FIGURE 20

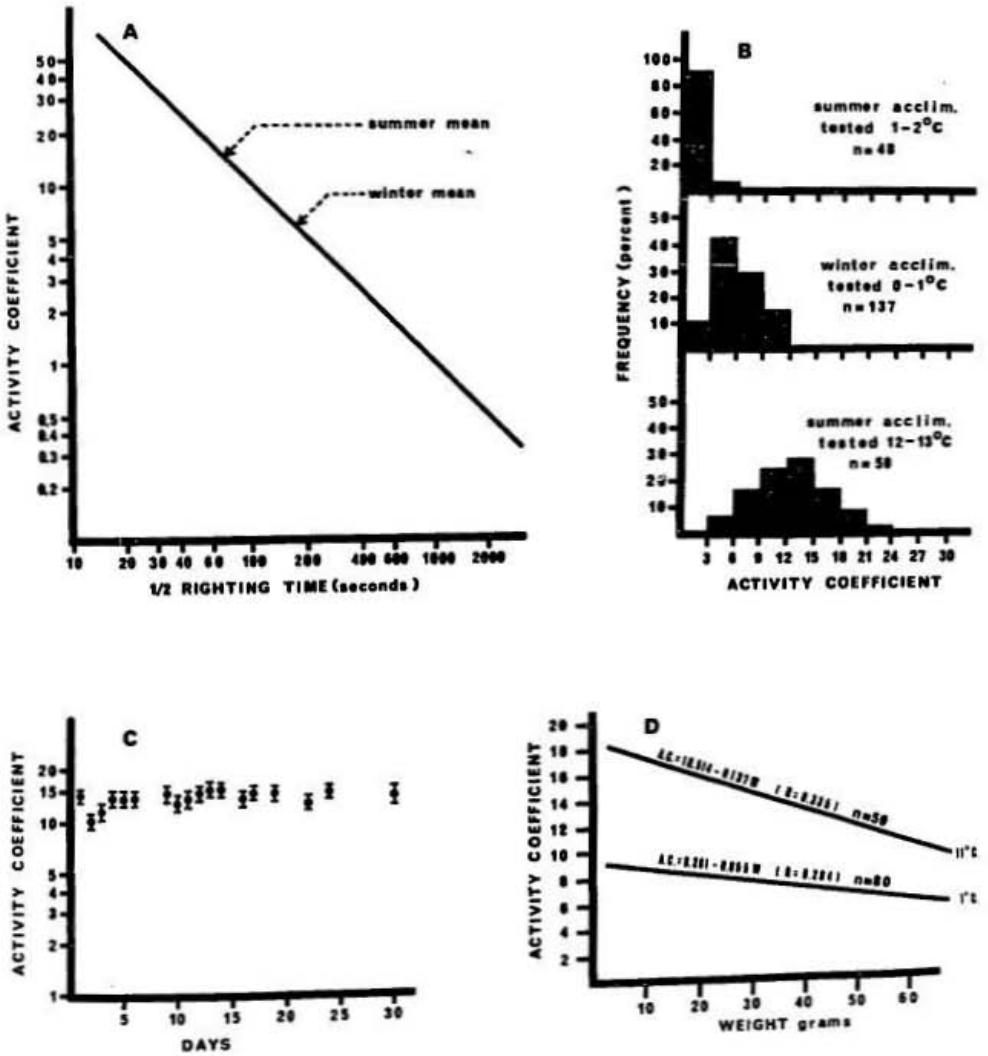
Characterization of activity coefficient (A.C.).

a. Relationship between A.C. and half-righting time.

b. Frequency distribution of A.C. in summer and winter.

c. Stability of A.C. over an extended period. (Based on data in appendix VIII a.)

d. Influence of urchin size on A.C.


A
summer mean
winter mean
ACTIVITY COEFFICIENT
1/2 RIGHTING TIME (seconds)
B
summer acclim.
tested 1-2°C
n=48
winter acclim.
tested 0-1°C
n=137
summer acclim.
tested 12-13°C
n=58
FREQUENCY (percent)
ACTIVITY COEFFICIENT
C
ACTIVITY COEFFICIENT
DAYS
D
ACTIVITY COEFFICIENT
WEIGHT grams

The A.C. decreases with increasing animal weight at both high and low temperatures although, low correlation coefficients suggest that the relationship is not particularly rigid (Fig. 20 d). To avoid the possibility of size complications, subsequent activity studies are conducted on 25-35 gram urchins.

These preliminary studies, designed to characterize the righting reflex and activity coefficient, indicate that, used with caution, the A.C. is indeed an indicator of urchin activity suitable for present purposes.

b) Activity R-T relationship and season:

Rate-temperature curves for activity coefficients of summer-and winter-acclimatized urchins are distinctly different (Fig. 21). Righting of summer animals is extremely slow at low temperatures (A.C. = 0.56 at 1°C.). In contrast, an A.C. in winter, at 1°C., of approximately 4.4, indicates a considerable degree of acclimatization to low temperature. In summer, optimum activity occurred at 15°C. (A.C. = 15.0), whereas in winter maximum activity was observed at 5°C. (A.C. = 6.5). Righting data for the tropical urchin Lythechinus variegatus (Kleitman, 1941), converted to A.C., are plotted beside the S. droebachiensis results (Fig. 21) to illustrate the phenomenon of relative, geographic adaptation of different species.

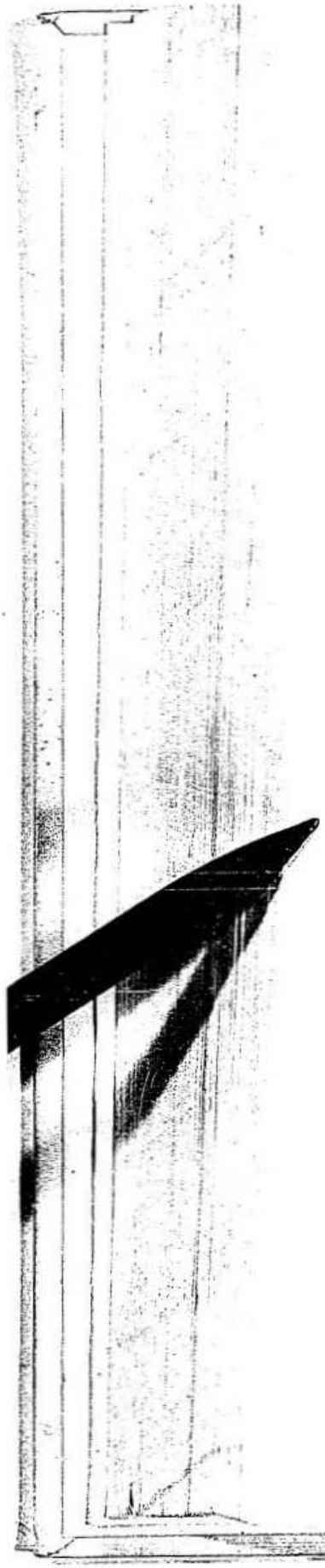
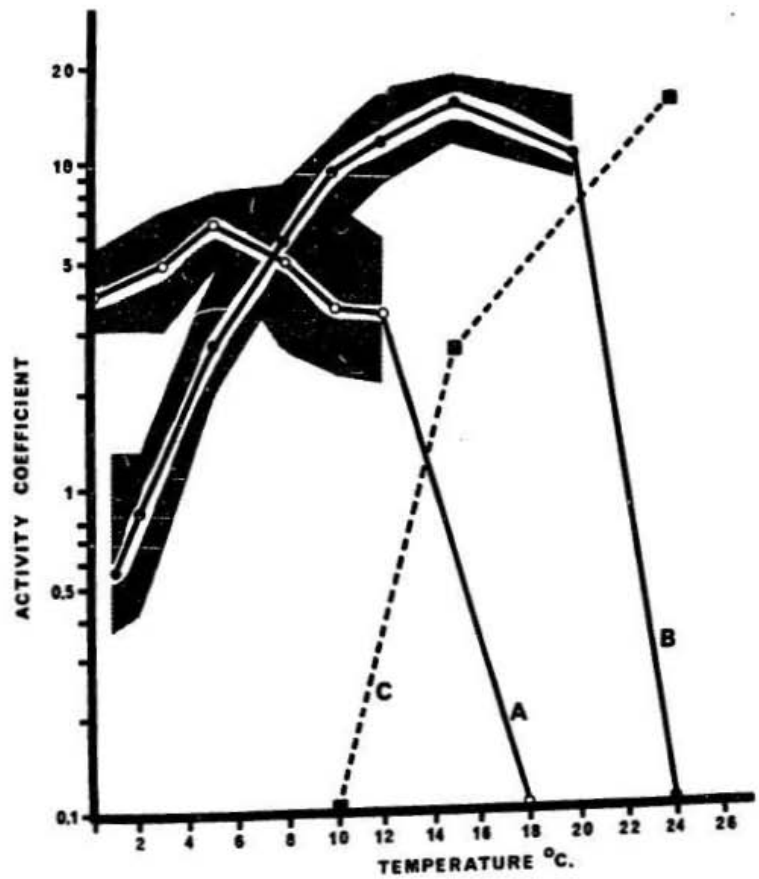


FIGURE 21

R-T relationships for activity of winter(A) and summer (B) acclimatized S. droebachiensis. [Based on data in appendix VIII b. Data for Lythechinus variegatus (C) from Kleitman (1941).]


ACTIVITY COEFFICIENT
20
10
5
1
0.5
0.1
2
4
6
8
10
12
14
16
18
20
22
24
26
TEMPERATURE °C.
A
B
C

c) Activity temperature-limits and season:

An urchin exposed in the laboratory to a temperature far different from the one to which it is adapted, may fail to right itself, or even to initiate the righting reflex*. The exact inhibition temperature varies individually, so that with increasing divergence from the adaptation temperature increasing numbers of animals become inhibited.

When one calculates, from the summer and winter rate-temperature data upon which Figure 21 is based, the percentage of animals that do not initiate righting within an arbitrary period (in this instance 10 minutes) at each test temperature, there is evidence of a seasonal shift in the thermal limits of the righting response (Fig. 22). The compensatory shift occurs at both high-and low-temperature extremes**.

At temperatures near 0°C., righting of summer animals is virtually inhibited, while at similar temperatures winter animals are active and right themselves readily.

* At least within time-limits dictated by practical considerations; there is no reason to assume that the "failure to initiate a righting reflex" as understood here, represents a phenomenon qualitatively distinct from a normal, cold-induced prolongation of righting time.

** It is important clearly to differentiate between high-and low-temperature inhibition of activity since the nature of the inhibitory process is undoubtedly different in each case.

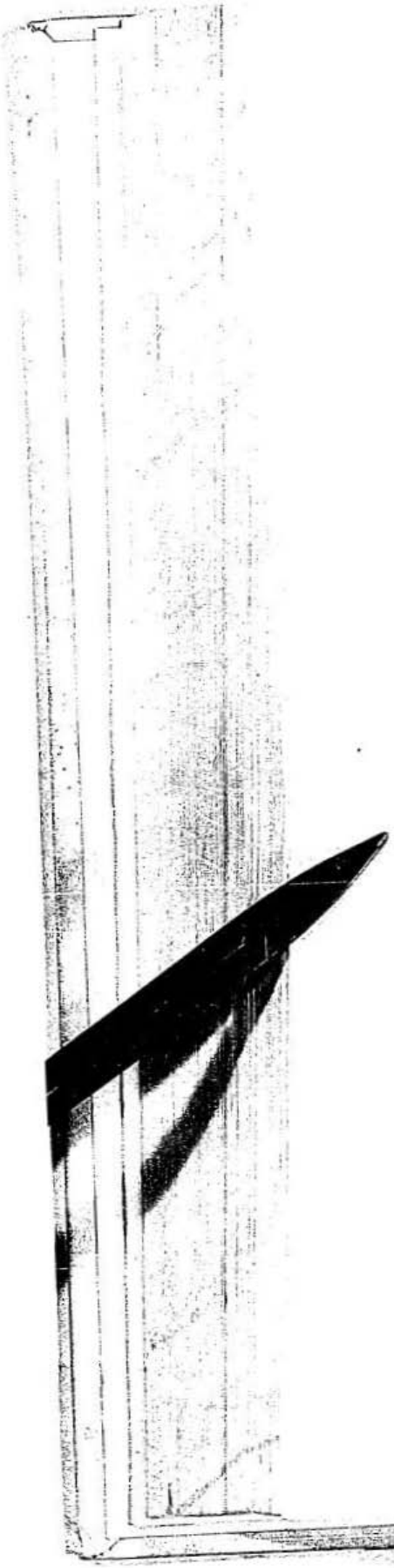
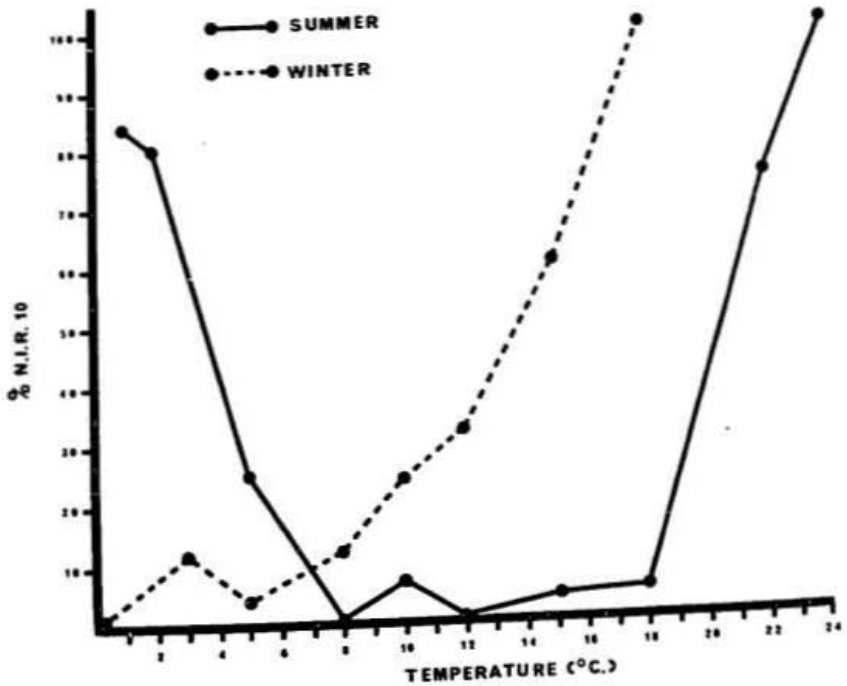


FIGURE 22

Seasonal changes in temperature limits of activity of S. droebachiensis (% n.i.r. 10= percentage of animals not initiating righting within 10 minutes. Derived from data obtained for R-T relationships of summer-and winter-acclimatized animals: appendix VIII b.).


SUMMER
WINTER
% N.I.R. 10
TEMPERATURE (°C.)

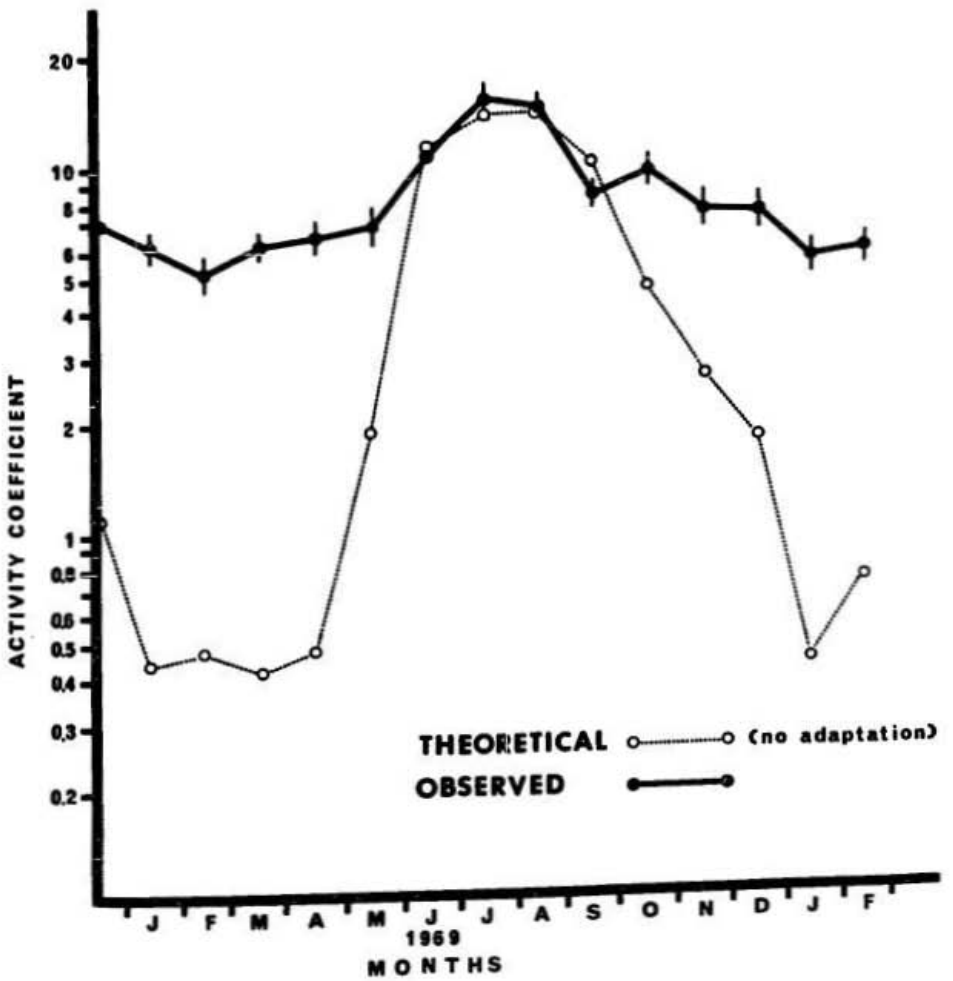
In winter, heat-induced inhibition of righting increases rapidly above 8°C., becoming complete at 15°- 18°C. In contrast, righting of summer animals is not seriously inhibited at temperatures up to 18°C., and complete inhibition of the response does not occur until 22°- 24°C. Comparing the 50 percent-inhibition levels of the two groups at elevated temperatures, it is apparent that the temperature-limit of the righting response is elevated by approximately 7°C. in animals acclimatized to 15°C. relative to those acclimatized to 0°C.

d) Activity coefficients and season:

Mean A.Cs. of groups of 15-25 urchins were determined monthly at prevailing environmental temperatures. In summer, the A.C. rises to a maximum of 15-16, before it begins to decline gradually to a winter minimum of 5-6 (Fig. 23). Theoretical A.Cs., anticipated from strict adherence to the Krogh-Arrhenius temperature relationship (i.e. complete absence of acclimatization), are derived from the summer activity R-T relationship determined earlier (Fig. 21), at temperatures corresponding to the habitat temperatures during different months. While observed and theoretical A.Cs. are in close agreement in summer, as soon as the temperature begins to decline in the autumn the two become increasingly divergent, and as a result winter A.Cs. are considerably greater than would be the case if thermal acclimatization did not occur. Thus, on the basis of the rate-temperature relationship of righting of urchins in summer, one would anticipate that the winter A.Cs. would be approximately 2.5% of that in summer, instead of the 33% evident in Figure 23. This suggests a compensatory adjustment in A.C. in winter of about 30%.

FIGURE 23

Observed and theoretical A.C. of S. droebachiensis during different months at ambient temperatures. (Vertical lines indicate ± S.E. Based on data in appendix VIII c.)


ACTIVITY COEFFICIENT
20
10
8
6
5
4
3
2
1
0.8
0.6
0.5
0.4
0.3
0.2
THEORETICAL (no adaptation)
OBSERVED
J F M A M J J A S O N D J F
1969
MONTHS

iv) Seasonal changes in feeding rate:

In view of the demonstrated seasonal acclimatization of respiratory metabolism and activity in S. droebachiensis, it is of considerable interest to determine if these metabolic adjustments are accompanied by seasonal changes in total food consumption or in efficiency of food utilization by the urchins.

Feeding rate and feeding efficiency were measured in the laboratory at prevailing environmental temperatures during different months (Table 12). Rate of consumption of Laminaria sp. by urchins fluctuates considerably during the year (Fig. 24). From a February minimum of approximately 400 mg. dry Laminaria/urchin*/day the feeding rate increases to a May maximum in excess of 1100 mg., an almost threefold increase. There follows a depression in feeding rate during summer to about 600 mg. and a subsequent autumnal increase to 900 mg. by November.

In additional to these seasonal fluctuations in the total quantity of food ingested, there also occurs a considerable seasonal shift in feeding efficiency (Fig.25). Although the limited data in Table 12 are not adequate to characterize completely the seasonal shift in efficiency, they do suggest that feeding efficiency is maximal (60-70%) during the colder months of the year and minimal during the summer. The feeding rate and feeding efficiency data together indicate that the amount of food actually used by the urchins is highest in late spring and again in autumn.

* Animals of 20-30 gms. wet weight.

Table 12. Food consumption (mg. dry Laminaria/urchin/day) and feeding efficiency of S. droebachiensis during different months.

month	N*	food consumption	S.E.	feeding efficiency
February	15	351.8	8.4	71.2%
May	7	1123.9	30.0	-
July	13	895.6	45.1	58.9%
August	14	608.8	34.7	42.1%
November	13	955.6	61.8	65.1%

* Number of daily measurements of food consumption made on each monthly group.

FIGURE 24

Feeding rate of S. droebachiensis during different months. (Vertical lines indicate ± S.E. Based on data in Table 12. Open circles from Himmelman, 1970.)

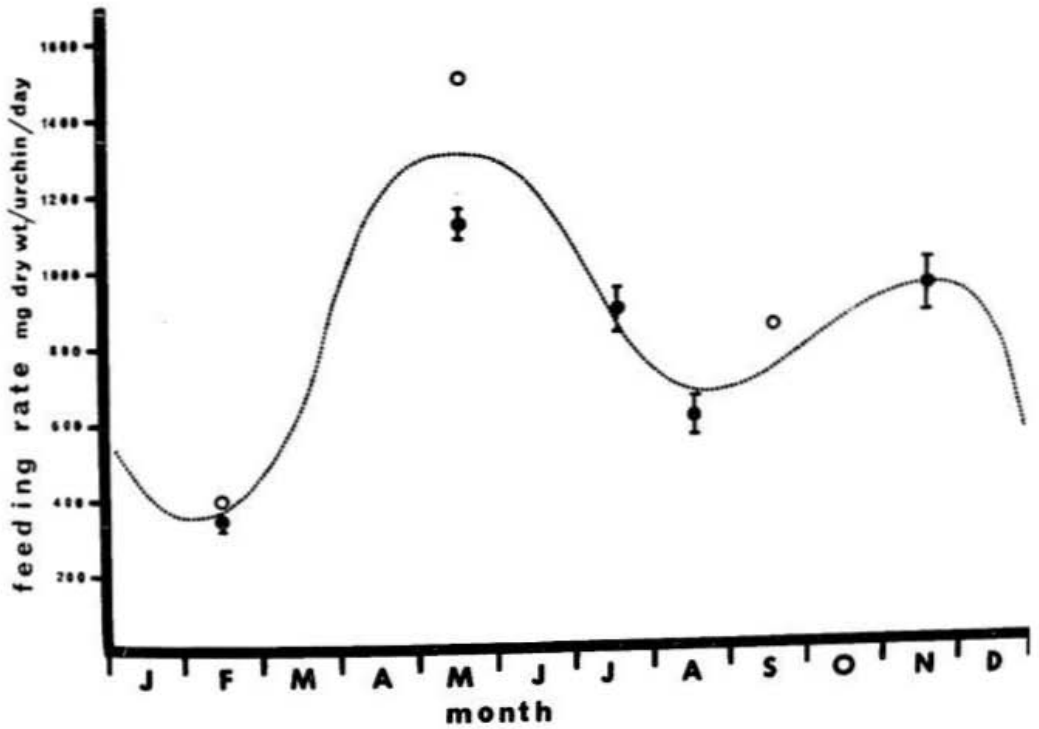
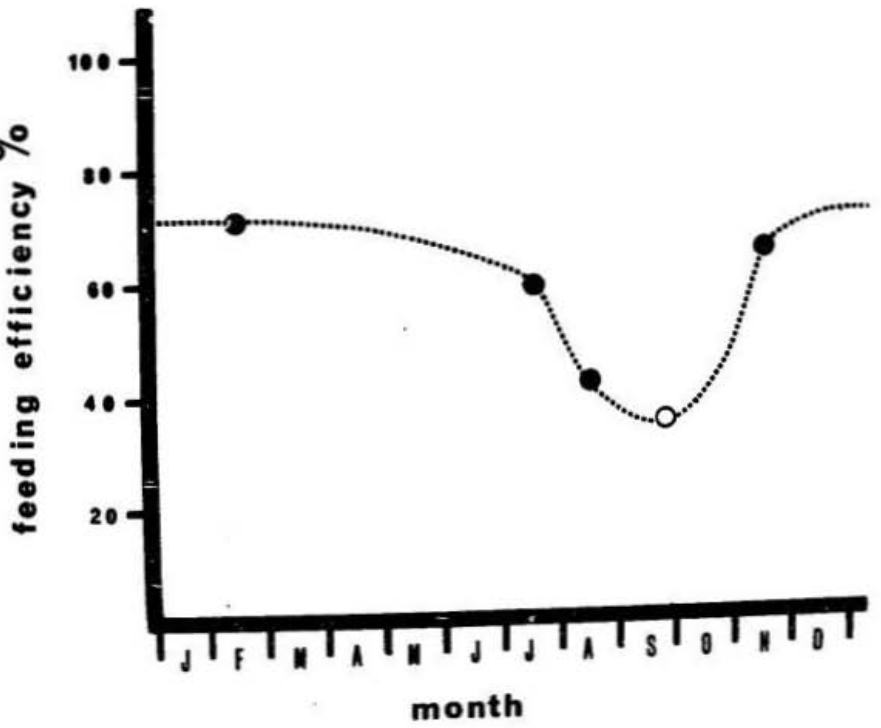

feeding rate mg dry wt/urchin/day
1600
1400
1200
1000
800
600
400
200
J
F
M
A
M
J
J
A
S
O
N
D
month

FIGURE 25

Feeding efficiency of S. droebachiensis during different months. (Based on data in Table 12. Open circles from Himmelman, 1970.)


feeding efficiency %
100
80
60
40
20
J
F
M
A
M
J
J
A
S
O
N
D
month

v) Seasonal changes in reproductive condition :

In urchins, as in most animals, a major, seasonally fluctuating, physiological condition with considerable potential influence on the animal's basal metabolic rate is associated with reproduction. Thus it was necessary to establish each month what stage in the reproductive cycle the animal had reached. For this, a monthly gonad index (G.I.) was obtained.

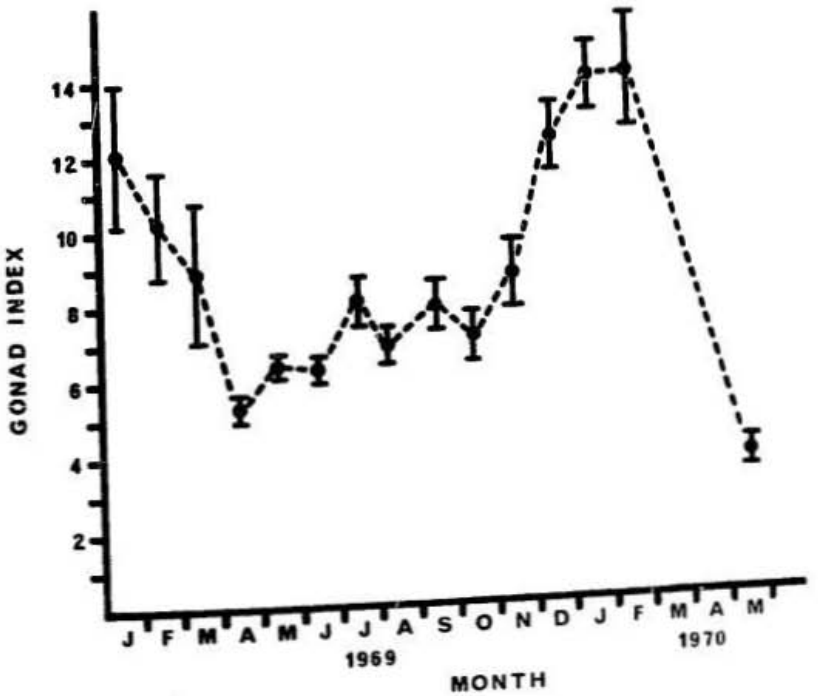
The G.I. is uniformly low during the summer, then rapidly increases in October - November (Fig. 26). Maximum gonad development, representing an approximately 50% increase in G.I. over the mean summer value, is reached in December - January. Thereafter, the G.I. declines again, indicating spawning, from January to March or April. It thus appears that in S. droebachiensis the greater part of gonad development takes place at that time when habitat temperatures are either falling rapidly, or at a minimum. An additional, less pronounced, increase in G.I. occurs in late spring - early summer, immediately following spawning, possibly indicating a gonadal "recovery" phase just prior to the summer quiescent phase.

Table 13. Gonad index of S. droebachiensis during different months.

month	N	gonad index	S.E.
Jan.	14	12.15	1.77
Feb.	13	10.20	1.24
Mar.	14	8.92	1.75
Apr.	15	5.31	0.26
May.	20	6.42	0.30
Jun.	20	6.36	0.34
Jul.	20	8.13	0.59
Aug.	20	6.69	0.49
Sep.	20	7.87	0.60
Oct.	20	7.11	0.60
Nov.	20	8.75	0.82
Dec.	20	12.40	0.96
Jan.	20	13.94	0.93
Feb.	20	14.02	1.53
May.	15	3.72	0.35

FIGURE 26

Seasonal changes in gonad index of S. droebachiensis.
(Vertical lines indicate ± S.E. Based on data in Table 13.)


GONAD INDEX
14
12
10
8
6
4
2
J F M A M J J A S O N D J F M A M
1969
1970
MONTH

vi) Seasonal changes in temperature tolerance:

In addition to compensating for seasonal temperature changes by metabolic adjustments of the type reported above (capacity acclimatization), animals may also seasonally alter their ability to tolerate temperature extremes (resistance acclimatization). To determine if urchins are capable of such resistance acclimatization, the heat tolerances of summer-and winter-acclimatized animals were measured and compared.

a) Heat stress and thermal death:

Attempts were first made to establish an effective indicator of thermal death, or incipient thermal death. Results, presented here in some detail, illustrate characteristic responses of heat-stressed urchins.

When urchins, acclimatized to 13°C., were placed in water at 8°C. which was then gradually warmed (1°C./min.), the first detectable sign of distress - a j-shaped flexion, or drooping, of the tips of some tube feet - appeared at about 17°C., when it affected some 10% of the tube feet in a given urchin. As the temperature increased, more and more tube feet, first drooped, then contracted, until all tube feet were contracted at 27.5°C. At that temperature,however, the spine reflex (a rapid inclination of surrounding spines towards a surface stimulus such as a light touch with a blunt probe) was still relatively strong. At 34°C. even the spine reflex was abolished. However, provided exposure had been short, i.e. animals were returned to their 13°C. holding tanks immediately

after the spine reflex had disappeared, recovery was complete within one hour. Thus, none of the readily detectable responses provided an adequate indication of immediate thermal death.

b) High-temperature tolerance:

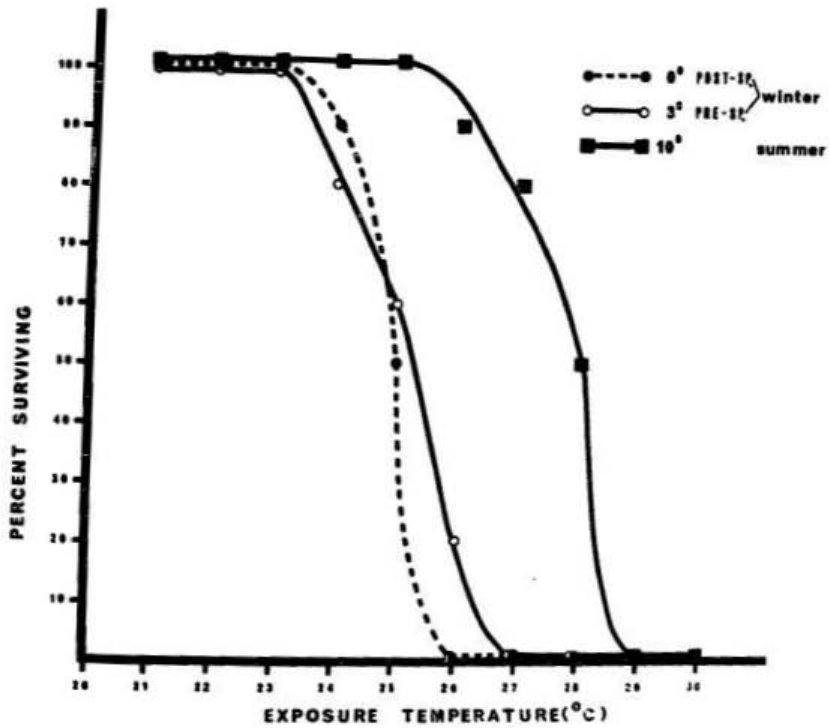
A distinct, seasonal shift in thermal tolerance was apparent (Fig. 27). In two groups of winter-acclimatized urchins (0° and 3°C. environmental temperature) the first deaths (p. 53) occurred following exposure to 24°C. In contrast, no deaths occurred in summer-acclimatized animals (10°C. environmental temperature) until exposure to 26°C. In winter and summer animals, 50 percent mortality occurred at temperatures of approximately 25° and 28°C., respectively. The temperature at which 100 percent mortality was first observed is similarly related to adaptation temperature; in the 0°, 3° and 10°C. adapted groups 100 percent mortality occurred at 26°, 27° and 29°C., respectively. It is clear that summer animals have a temperature tolerance that is at least 2°- 3°C. higher than that of winter animals.

The range of individual variability in temperature tolerance is limited, with a matter of 3 or 4 degrees being the difference between 100 percent survival and 100 percent mortality of the group.

Temperature tolerance does not appear to be related to gonad development. The two winter groups had similar temperature tolerances even though the animals acclimatized to 3°C. were in a pre-spawning condition with a high gonad index, while those acclimatized to 0°C. had already

FIGURE 27

Short-term temperature tolerance of winter-(pre- and post-spawning) and summer-acclimatized S. droebachiensis. (Groups of 10 urchins held at indicated exposure temperatures for one hour.)


PERCENT SURVIVING
EXPOSURE TEMPERATURE(°C)
winter
summer

spawned and thus had a low gonad index.

Though I did not specifically study long-term tolerance of extreme temperatures, I did note in passing that summer as well as winter animals tolerate, even adapted to 15°C., and that summer animals were not adversely affected by a four-week exposure to 18°C.

c) Low-temperature tolerance:

Both summer and winter urchins readily tolerate exposure to 0°C.; no summer animals died during the 4-6 weeks acclimation at 0°C. Summer animals exposed to subzero temperatures (-1.5°C. for 72 hours), although inactive and motionless during the exposure period, rapidly recovered when returned to warmer water.

B. LABORATORY ACCLIMATION

Organisms in their natural habitat are subject to a wide range of interacting environmental and endogenous factors. It need not be correct, therefore, to conclude that seasonal metabolic adjustments, such as those observed in freshly collected urchins, are primarily induced by temperature fluctuations. Temperature may be more definitely implicated if it can be demonstrated that metabolic adjustments, similar to those occurring seasonally, are induced in an experimental situation in which temperature alone is the factor that differs significantly.

In this second phase of the study, an attempt is made to evaluate performance of urchins after extended laboratory acclimation to summer-like and winter-like temperatures. In addition to examining adaptive shifts in the respiratory metabolism of intact animals and of excised tissues, differences in the levels of activity of warm-and cold-acclimated urchins are considered.

i) Intact-urchin respiration:

To determine whether a metabolic adjustment, comparable to the seasonal one, could be induced in the laboratory, urchins were acclimated at winter-like (0°C.) and summer-like (15°C.) temperatures, their respiration rates measured at a series of temperatures (Table 14), and compared. Respiration rates in Table 14 are expressed on a per gram fresh weight basis. The mean weights of warm-and cold-acclimated urchins are 29.21 and 29.16 grams, respectively. To permit direct comparisons with seasonally acclimatized animals, respiration rates are calculated for urchins of 30 grams standard weight, and the resulting values used in plotting the R-T curve in Figure 28 and in computing thermal compensation coefficients.

a) Respiration and acclimation:

The respiration rate of a cold-acclimated urchin is significantly greater than that of a warm-acclimated one at 0°, 5°, and 10°C., but it is identical to the latter at 15°C. (Fig. 28). This coincidence of the respiratory rates at 15°C. is probably due to a depression of respiration in the cold-acclimated animal and is essentially similar to the depression previously observed in winter-acclimatized animals of similar size (Fig. 9).

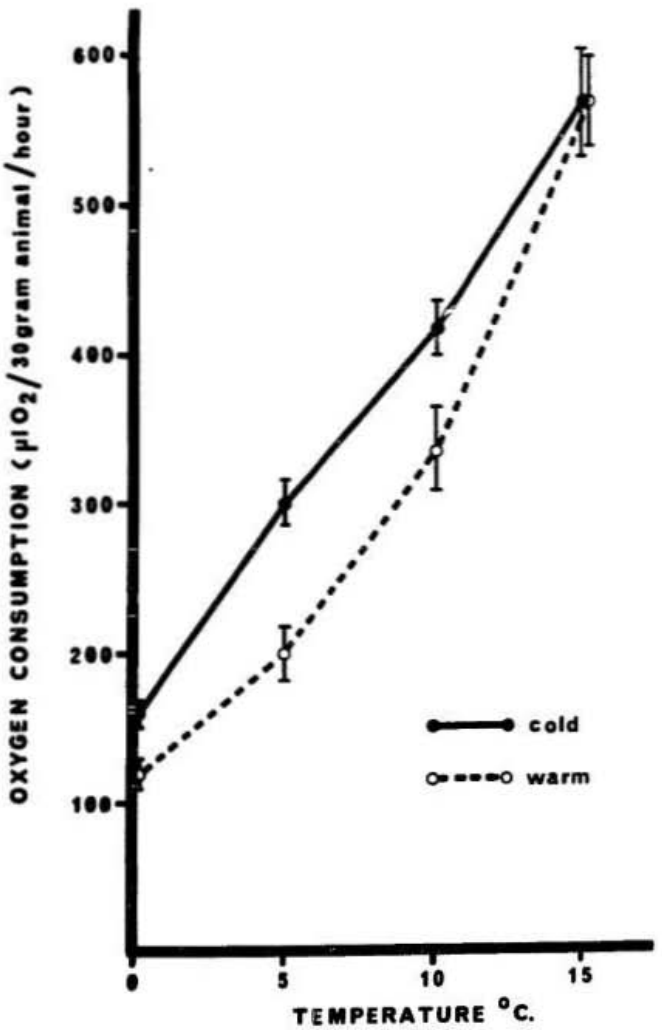
The absolute respiration rates of warm-and of cold-acclimated urchins (Fig. 28) are comparable to those of the seasonally acclimatized animals of 30 grams standard weight (Fig. 9). Similarly, the RQO_2

Table 14. Respiration rates ($\mu l.O_2$/gm. wet wt./hr.) of warm-and cold-acclimated S. droebachiensis. (Based on data in appendix IV a and b.)

test temperature (°C.)	acclimation temperature (°C.)	N	mean urchin weight	S.E.	O_2 cons.	S.E.	t	p
0°	0°	10	28.83	0.86	5.36	0.28	2.84	< 0.01
	15°	10	29.65	0.81	4.09	0.36		
5°	0°	9	29.07	1.11	9.96	0.47	4.34	< 0.0005
	15°	10	29.00	0.87	6.66	0.60		
10°	0°	11	28.96	0.99	13.88	0.59	2.48	< 0.025
	15°	11	29.52	1.02	11.12	0.94		
15°	0°	10	29.80	0.99	18.80	1.20	0.00	n.s.
	15°	9	28.68	1.01	18.80	1.00		

FIGURE 28

R-T relationships for respiration of warm-and cold-acclimated S. droebachiensis. (Vertical lines indicate ± S.E. Based on data in Table 16, for urchins of 30 grams standard weight.)


OXYGEN CONSUMPTION ($\mu l O_2$ / 30 gram animal / hour)
600
500
400
300
200
100
0
5
10
15
TEMPERATURE °C.
cold
warm

coefficients of these two groups of animals are of like magnitude:

	laboratory acclimated	seasonally acclimatized*
0°C. =	131.3	122.0
5°C. =	149.6	113.2
10°C. =	124.9	125.6
15°C. =	100.0	99.2

b) Respiration and temperature:

Q_{10} coefficients for the respiratory rate of laboratory acclimated urchins, ranging from 1.835 to 3.453 (Table 15), indicate that metabolism increases approximately 2-3 times for each 10°C. rise in temperature, just as observed earlier for seasonally acclimatized animals (Fig. 9).

Q_{10} coefficients for warm- and cold-acclimated urchin respiration exhibit striking differences. In cold-acclimated urchins, Q_{10} decreases with increasing temperature, indicating a decline in temperature sensitivity at high temperatures. A similar decline is evident in the Q_{10} values of winter-acclimatized, 30 gram urchins; in the 0°-5°C. range Q_{10} = 2.38 and in the 10°-15°C. range Q_{10} = 1.32. In contrast, in warm-acclimated urchins the Q_{10} remains relatively stable over the range 0°-15°C. Q_{10} coefficients of summer-acclimatized urchins, on the other

* Figure 13.

Table 15. Q_{10} coefficients for respiration of warm-and cold-acclimated *S. droebachiensis*. (Based on data in Table 14.)

temperature range(°C.)	Q_{10} cold-acclimated	Q_{10} warm-acclimated
0 - 5°	3.453	2.657
5 - 10°	1.941	2.786
10 - 15°	1.835	2.863

hand, decrease with increasing temperature (Fig. 9).

The temperature characteristics (μ) of 14,700 and 16,000 for cold-and warm-acclimated animals, respectively, are derived from Arrhenius regression lines (Fig. 29). These values are of comparable magnitude to the μ values for the respiratory metabolism of seasonally acclimatized urchins (winter: 14,900; summer: 13,600; from Figure 14). Neither in laboratory acclimated, nor in seasonally acclimatized urchins is the difference between μ values of warm-and cold-adapted animals particularly great, indicating that warm-and cold-adapted Arrhenius slopes are essentially parallel.

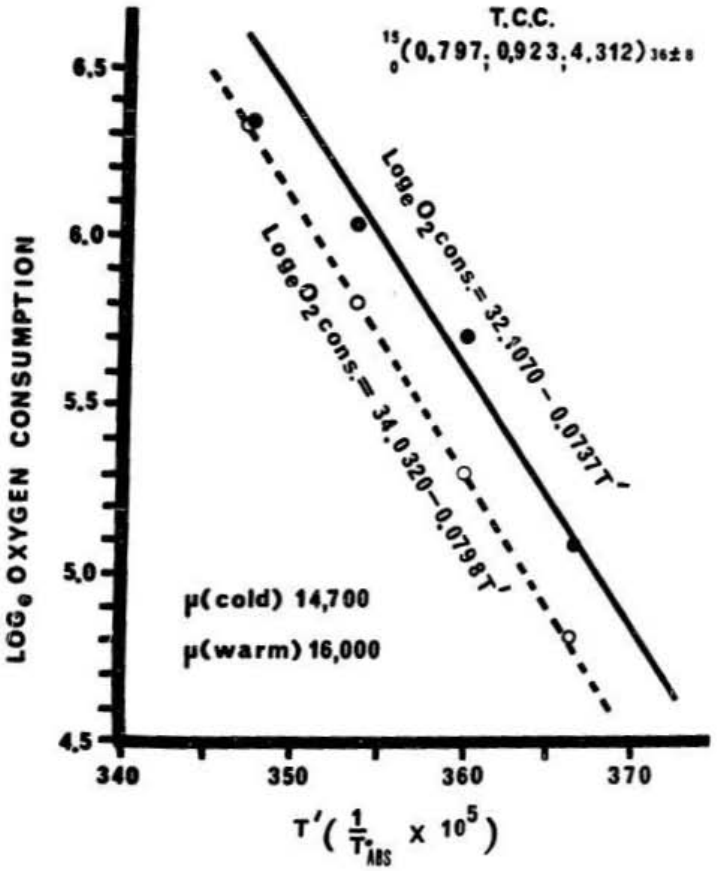
c) Thermal compensation coefficients:

The mean respiration rate, at 15°C., of cold-acclimated urchins was excluded from the calculation of the Arrhenius regression lines (Fig. 29) for reasons previously outlined (p. 89).

The coefficient of adaptation is 0.797, indicating an approximately 20% compensation in metabolism. The calculated coefficient of rotation was 0.923, and the axial coefficient 4.312. These coefficients indicate that there is little, if any, difference in slope between warm-and cold-acclimated Arrhenius curves, and that acclimation essentially involves translation of one curve relative to the other.

FIGURE 29

Arrhenius transformation of R-T relationships (Figure 28) for respiration of warm-and cold-acclimated S. droebachiensis.


T.C.C.
15°(0,797; 0,923; 4,312) 36±8
Loge O2 cons.= 32,1070 – 0,0737T'
Loge O2 cons.= 34,0320–0,0798T'
μ(cold) 14,700
μ(warm) 16,000
LOGe OXYGEN CONSUMPTION
6,5
6,0
5,5
5,0
4,5
340
350
360
370
T'(1/T°ABS x 10^5)

ii) Excised tissue respiration:

a) Respiration and size:

Studies were conducted to determine the effect of laboratory acclimation of from 4-6 weeks duration at 0°C. and at 15°C., on the respiration rates of excised stomach and intestine tissues from large ($\overline{X}$ = 48.9 gms.; range: 41.1-73.8 gms.) and small ($\overline{X}$ = 30.6 gms.; range: 26.5-34.7 gms.) urchins (Tables 16, 17, 18, 19).

Both stomach and intestine tissues from small urchins have consistently higher metabolic rates than the same tissues from large animals over the temperature range investigated (Fig. 30). This parallels the earlier observation (p. 80) that the respiration rate of whole urchins, expressed on a unit weight basis, decreases with increasing urchin weight.

b) Respiration and acclimation:

The size-adaptation relationship previously observed in seasonal,whole-animal metabolism is again evident in the adaptation response of excised tissues. Respiration rates of stomach and intestine tissues from small, cold-acclimated urchins are considerably higher than rates for the same tissues from similarly sized, warm-acclimated urchins between 5° and 15°C. (Fig. 30). In contrast, there is little, if any, difference between respiration rates of both stomach and intestine tissues from warm-and cold-acclimated, large urchins at lower

Table 16. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of excised stomach from warm-and cold-acclimated, large S. droebachiensis. (Based on data in appendix VI a.)

test temperature (°C.)	acclimation temperature (°C.)	N	O_2 cons.	S.E.	t	p
5°	0°	11	294.8	26.0		
					0.14	n.s.
5°	15°	12	299.8	22.3		
10°	0°	9	449.5	30.9		
					0.82	< 0.25
10°	15°	9	412.8	31.7		
15°	0°	9	621.3	44.4		
					1.88	< 0.025
15°	15°	9	512.1	37.0		
20°	0°	6	956.2	43.4		
					1.26	n.s.
20°	15°	5	869.7	52.8		

Table 17. Respiration rates ($\mu l.O_2/gm.$ dry wt./hr.) of excised stomach from warm- and cold-acclimated, small S. droebachiensis. (Based on data in appendix VI b.)

test temperature (°C.)	acclimation temperature (°C.)	N	O_2 cons.	S.E.	t	p
5°	0°	6	377.9	20.8		
					2.622	< 0.025
5°	15°	6	314.9	12.0		
10°	0°	6	715.3	40.5		
					3.407	< 0.005
10°	15°	6	514.9	42.7		
15°	0°	6	924.5	91.8		
					1.369	< 0.100
15°	15°	6	773.4	61.2		

Table 18. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of excised intestine from warm-and cold-acclimated, large S. droebachiensis. (Based on data in appendix VI c.)

test temperature (°C.)	acclimation temperature (°C.)	N	O_2 cons.	S.E.	t	p
5°	0°	12	346.9	21.9	0.99	< 0.250
5°	15°	11	314.6	24.2		
10°	0°	8	532.2	51.6	0.16	N.S.
10°	15°	8	543.5	41.9		
15°	0°	9	654.1	28.8	2.21	< 0.025
15°	15°	8	557.4	32.9		
20°	0°	6	987.1	34.5	3.65	< 0.005
20°	15°	5	787.8	42.2		

Table 19. Respiration rates ($\mu l.O_2/gm.$ dry wt./hr.) of excised intestine from warm-and cold-acclimated, small S. droebachiensis. (Based on data in appendix VI d.)

test temperature (°C.)	acclimation temperature (°C.)	N	O_2 cons.	S.E.	t	p
5°	0°	6	415.4	26.3		
					1.979	< 0.05
5°	15°	6	348.4	21.3		
10°	0°	6	833.8	49.8		
					3.815	< 0.005
10°	15°	6	572.3	47.2		
15°	0°	6	926.0	79.5		
					1.786	< 0.100
15°	15°	6	745.2	62.7		

FIGURE 30

R-T relationships for respiration of stomach (A) and intestine (B) tissues from warm-and cold-acclimated, large and small S. droebachiensis. (Vertical lines indicate ± S.E. Based on data in Tables 16, 17, 18, and 19.)

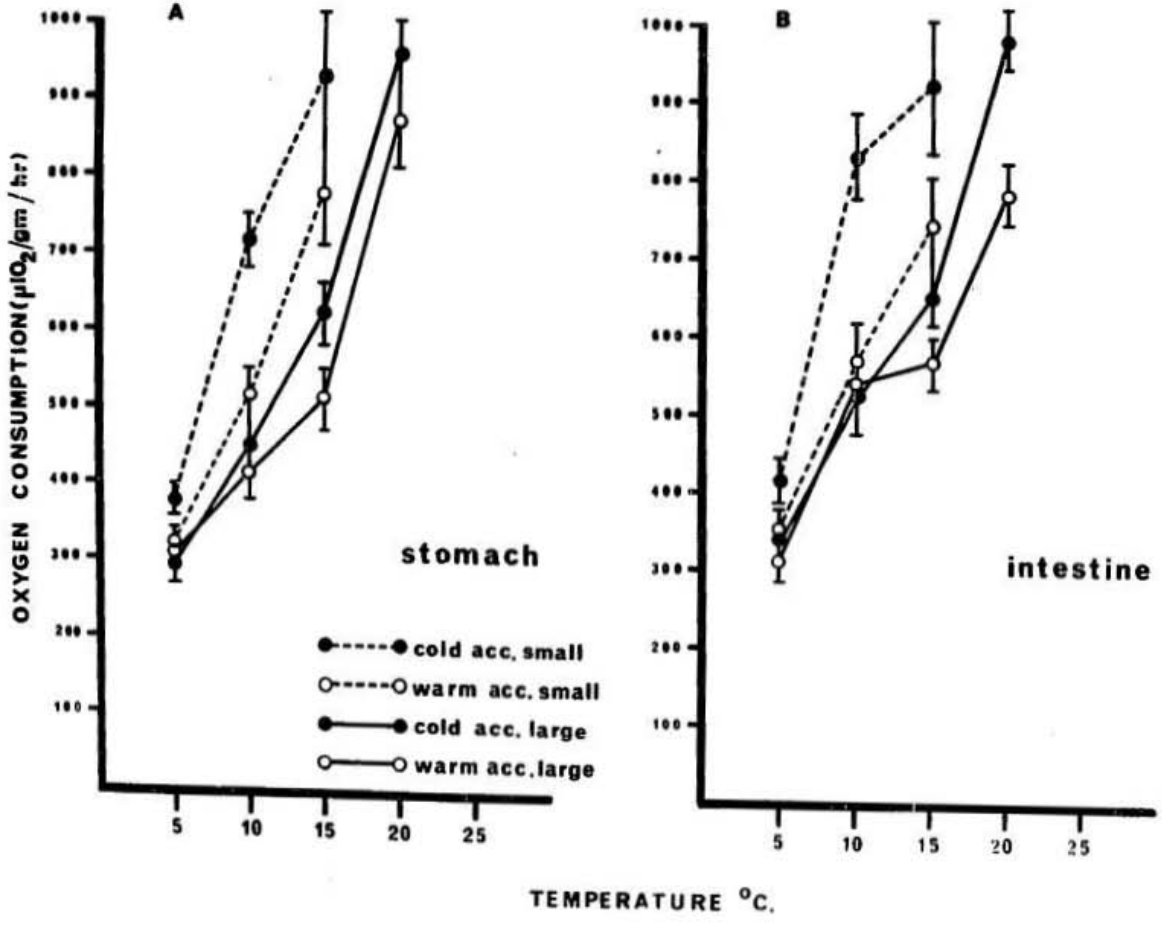

A
B
stomach
intestine
cold acc. small
warm acc. small
cold acc. large
warm acc. large
OXYGEN CONSUMPTION(μlO2/gm/hr)
TEMPERATURE °C.
5
10
15
20
25

Table 20. Q_{10} coefficients of respiration of excised stomach and intestine from warm-and cold-acclimated, large and small S. droebachiensis. (Based on data in Tables 16, 17, 18, and 19.)

tissue	acclimation temperature (°C.)	TEMPERATURE RANGE(°C.) 5-10°	10-15°	15-20°
LARGE URCHINS				
stomach	15°	1.897	1.539	2.884
stomach	0°	2.325	1.910	2.368
intestine	15°	2.985	1.052	1.996
intestine	0°	2.924	1.216	2.276
SMALL URCHINS				
stomach	15°	2.673	2.256	-
stomach	0°	3.584	1.672	-
intestine	15°	2.669	1.694	-
intestine	0°	4.027	1.234	-

temperatures. At higher temperatures (15° and 20°C.) the respiration rates of both stomach and intestine tissues from cold-acclimated, large urchins are significantly higher (Tables 16, 18) than rates for the same tissues from warm-acclimated large urchins.

c) Respiration and temperature:

Q_{10} coefficients for respiration of excised stomach and intestine tissues from warm-and cold-acclimated urchins range from 1.100 to 4.027 (Table 20). At low temperatures (5°-10°C.) Q_{10} coefficients for tissues from cold-acclimated animals are generally higher than those from warm-acclimated ones. Also, at low temperatures respiratory Q_{10} values for tissues of small urchins are generally higher than those for tissues of large animals. At higher temperatures these Q_{10} relationships become less clear.

Of particular interest is the considerable decline in Q_{10} coefficients in the 10°-15°C. range relative to those at both higher and lower temperatures. The data are not adequate to determine if the Q_{10} values for tissues of small urchins return to a high level in the 15°-20°C. range as is the case with metabolism of tissues of large urchins. The reduction in Q_{10} in the 10°-15°C. range indicates a slight decline in the temperature sensitivity of the metabolic system over this range. Complete temperature independence ($Q_{10} = 1$) is not attained in any of the tissues.

d) Thermal compensation coefficients:

To determine thermal compensation coefficients for the various tissues the rate-temperature curves of Figure 30 are transformed into Arrhenius plots (Fig. 31). Regression lines for warm-and cold-acclimated metabolism are, in each case, drawn through data points between 5° and 15°C.

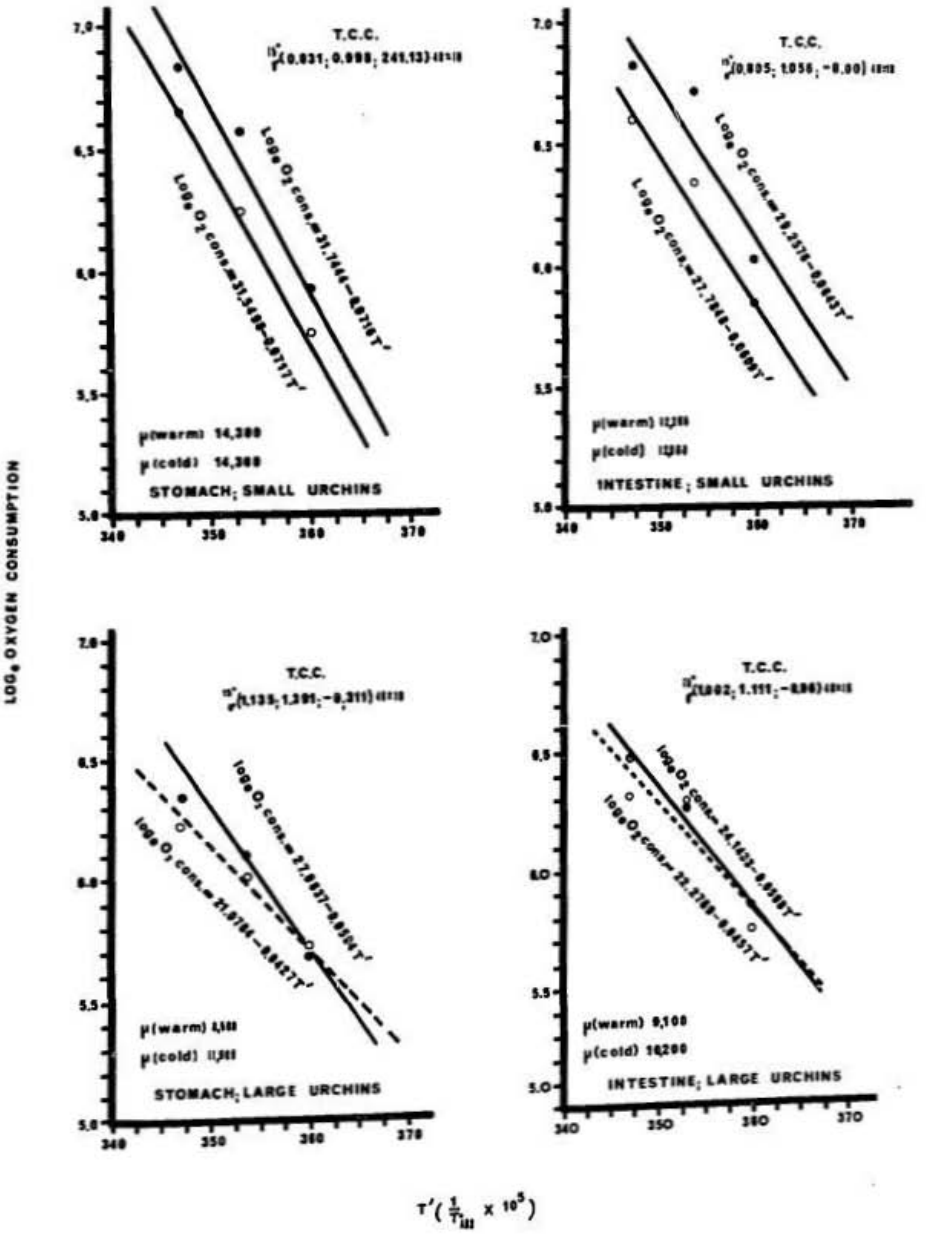
For small urchins the coefficients of acclimation (C.A.) are 0.831 and 0.804, for stomach and intestine tissues, respectively, indicating a compensatory shift of approximately 20% in both instances. In contrast, the C.A. for stomach and intestine tissues from large urchins are 1.134 and 1.002, respectively, indicating a slight hypo-acclimation in the former and a complete absence of an acclimation response in the latter.

Coefficients of rotation (C.R.) near unity and axial coefficients far removed from zero suggest that the metabolic shift in stomach and intestine tissue of small urchins is primarily of a translatory nature. In contrast, stomach and intestine tissues from large urchins have C.R. greater than 1 and axial coefficients between zero and -1, indicating a clockwise rotation of the warm-and cold-acclimated Arrhenius curves with intersection occurring within the stress range.

Temperature characteristics (μ) were essentially similar for tissues from warm-and cold-acclimated small urchins (Fig. 31). In large urchins μ values were higher for cold-acclimated tissues than for warm-acclimated ones.

FIGURE 31

Arrhenius transformation of R-T relationships (Figure 30) for respiration of stomach and intestine tissues from warm- and cold-acclimated, large and small S. droebachiensis.


T.C.C.
STOMACH; SMALL URCHINS
INTESTINE; SMALL URCHINS
STOMACH; LARGE URCHINS
INTESTINE; LARGE URCHINS
LOG$_e$ OXYGEN CONSUMPTION
T'($\frac{1}{T_{abs}}$ x 10^5)

e) Acclimation and tissue water content:

The effect of acclimation on the tissue water content differs considerably in stomach and intestine (Table 21). Stomach tissue from warm-acclimated urchins had a slightly higher water content ($p < 0.05$) than that from cold-acclimated ones. In contrast, the water content of intestine tissues from cold-acclimated urchins is significantly greater ($p < 0.001$) than that from warm-acclimated ones.

Table 21. Influence of acclimation temperature on water content of stomach and intestine tissues of S. droebachiensis. (Water content expressed as percent of wet weight.)

tissue	acclimation temperature (°C.)	N	water content	S	S.E.	t	p
stomach	0°	24	76.76%	2.89	0.60		
						1.941	< 0.05
stomach	15°	24	78.26%	2.34	0.49		
intestine	0°	24	79.24%	2.49	0.52		
						4.594	< 0.001
intestine	15°	23	76.24%	1.87	0.40		

iii) Activity:

a) Activity of acclimated urchins:

To ascertain that levels of activity and degrees of activity adaptation of urchins acclimated for extended periods in the laboratory are comparable to those of seasonally-acclimatized urchins, the activity coefficients (A.C.) of warm-and cold-acclimated urchins were measured at the respective adaptation temperatures (Table 22).

The mean A.C. of warm-acclimated urchins at 15°C. (15.47) is essentially the same as that observed in summer animals (15.71 in July). The mean A.C. of cold-acclimated urchins at 0°C. (4.95) is similar to that previously recorded for winter animals (5.26 in February). The A.C. of cold-acclimated urchins at 0°C. is approximately 32% that of warm-acclimated ones at 15°C. This is comparable to the 33% difference observed earlier between summer and winter A.Cs. (Fig. 23). These results indicate that confinement under the laboratory conditions described earlier has no apparent adverse effect on urchin performance.

b) Time course of acclimation of activity:

To estimate the time required for urchins to adapt their activity to low temperature, warm-acclimated animals were transferred to 0°C., following the determination of a 15°C. reference A.C. The median A.C. at 15°C. during the final 9 days of warm-acclimation was 14.71 (range: 12.66 - 18.52). Following transfer of the animals to 0°C. the median

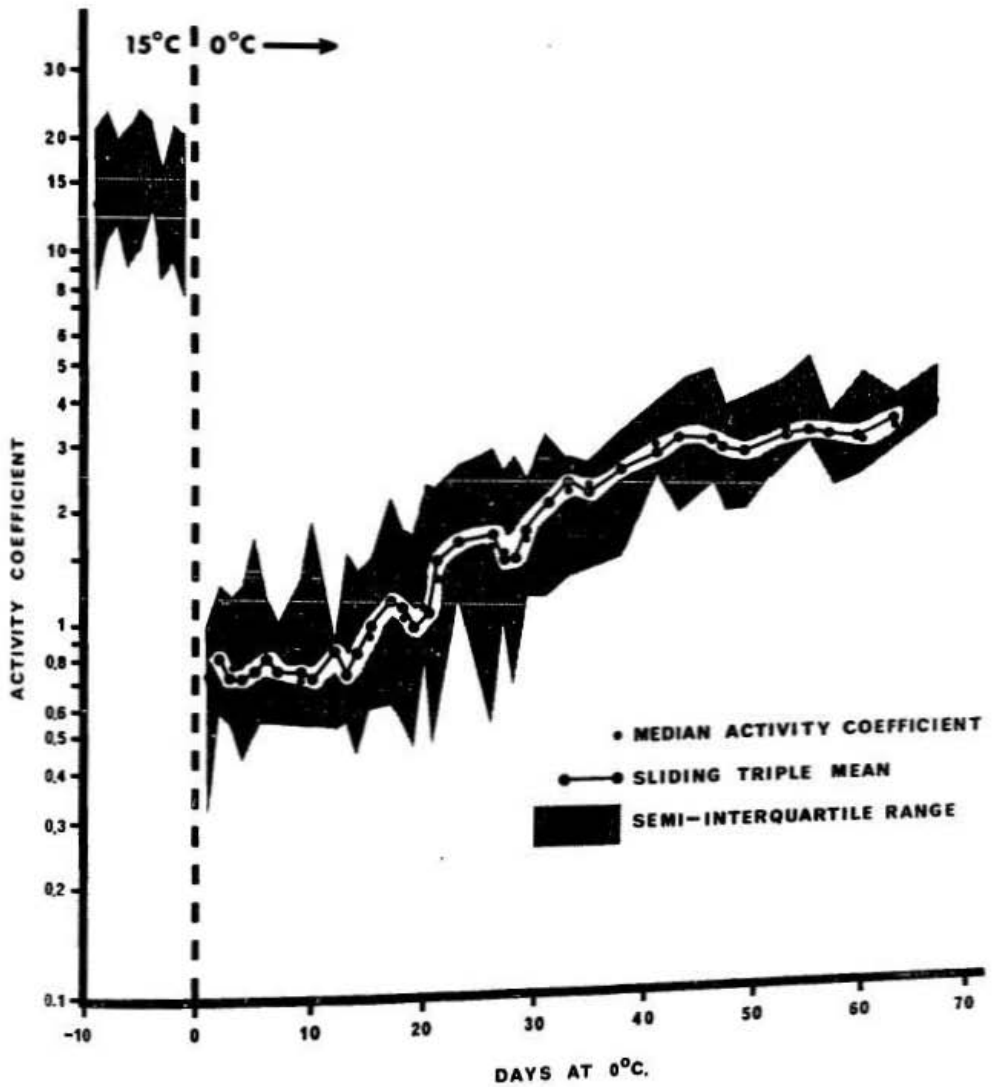
Table 22. Activity coefficients of warm(15°C.) and cold(0°C.) acclimated S. droebachiensis. (Measured at 15°C. and 0°C., respectively. N = 20 in each group.)

COLD-ACCLIMATED			WARM-ACCLIMATED		
trial	A.C.	S.E.	trial	A.C.	S.E.
A	5.07	0.42	A	14.88	1.53
B	4.73	0.46	B	15.40	1.30
C	5.04	0.54	C	16.14	1.89
mean	4.95		mean	15.47	

$$\frac{\text{cold-acclimated A.C.}}{\text{warm-acclimated A.C.}} \times 100 = 31.9$$

FIGURE 32

Time course of acclimation of activity of S. droebachiensis following transfer to 0°C. (Based on data in appendix VIII d.)


15°C
0°C
ACTIVITY COEFFICIENT
30
20
15
10
8
6
5
4
3
2
1
0,8
0,6
0,5
0,4
0,3
0,2
0,1
-10
0
10
20
30
40
50
60
70
DAYS AT 0°C.
• MEDIAN ACTIVITY COEFFICIENT
SLIDING TRIPLE MEAN
SEMI-INTERQUARTILE RANGE

A.C. was sharply reduced (Fig. 32). For almost two weeks following the temperature change there was no detectable shift in the depressed A.C. (mean A.C. = 0.66; range: 0.51 - 1.12). With continued exposure to 0°C., however, the A.C. began to increase at a rate of approximately 0.08 units/day, until after 40 days it levelled off at approximately 3.5, a compensatory increase of about 20% relative to the original, 15°C., reference A.C. The reason for the failure to achieve compensation of about 30%, similar to that observed earlier with laboratory acclimated (Table 22) and seasonally acclimatized (Fig. 23) urchins, is uncertain. There is evidence that a further slow rise in A.C. occurs when low temperature exposure is continued beyond 40 days. Nevertheless, it seems as if the major part of the acclimation response takes place within 30-40 days.

C. HOMOGENATE METABOLISM

The final phase of this study was designed to elucidate what, if any, biochemical changes might be associated with thermal adaptation, and to determine the extent to which such changes, if observed, are consistent with the mechanism of adaptation suggested by the physiological profile developed in the previous sections.

The experiments here require cell-free preparations or homogenates, and it was therefore necessary, first to examine the stability and magnitude of respiratory metabolism in such homogenates; and second to ascertain whether the adaptive response is still in evidence in such preparations.

i) Stability of tissue slice and homogenate respiration:

Experiments were conducted to determine the level and relative stability of respiration of essentially cell-free homogenates of stomach and intestine. Parallel studies of organized tissues, in the form of slices, were made for purposes of comparison. The Warburg runs at 15°C., in either seawater or buffered sucrose, lasted 2 1/2 hours; readings were taken every 15 minutes.

Respiration of stomach and intestine slices in seawater continues at a uniform rate for at least 2 1/2 hours (Fig. 33; curves D and E, respectively). There appears to be little difference in rates between the two tissues under these conditions. In buffered sucrose medium metabolic rates of both stomach and intestine tissues increase by approximately 50 percent over those in seawater (Fig. 33; curves B and A, respectively). As in seawater medium, metabolic rates remain reasonably uniform for at least 2 1/2 hours and again, there is little or no difference between stomach and intestine rates.

Homogenization reduces the metabolic rate of intestine in sucrose medium (Fig. 33; curve C); however, the respiration rate remains reasonably uniform for at least 2 1/2 hours. In contrast, homogenization of stomach tissue not only sharply reduces its metabolic rate, but also adversely affects the stability of its respiration (Fig. 33; curve F). The respiration rate of stomach homogenate declines continuously during the Warburg run so that after 2 1/2 hours the rate is about 60 percent of that of intestine homogenate. In view of this metabolic instability

FIGURE 33

Stability of respiration of stomach and intestine slices and homogenates from S. droebachiensis. (Based on data in appendix VII a.)

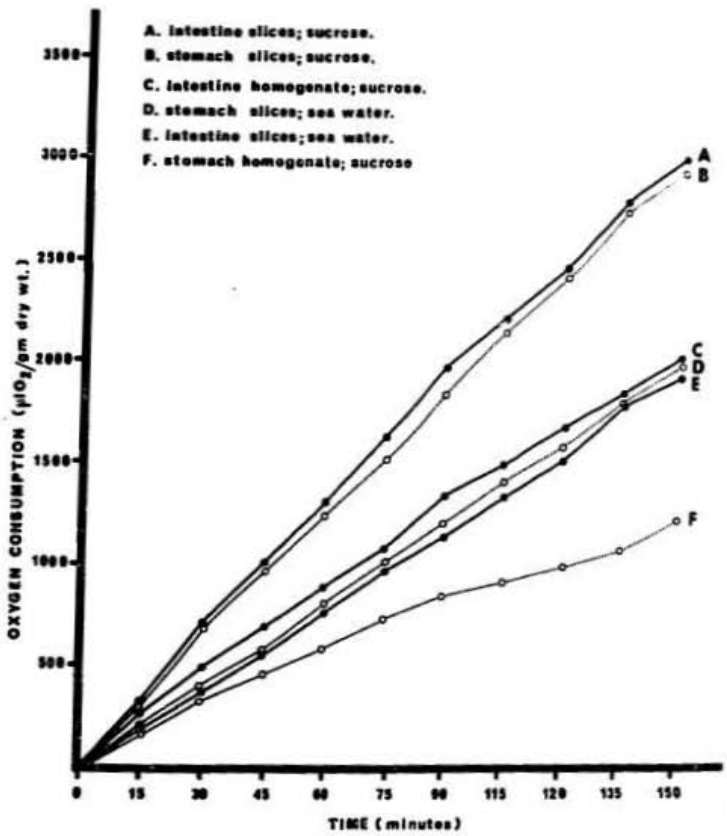

A. Intestine slices; sucrose.
B. stomach slices; sucrose.
C. Intestine homogenate; sucrose.
D. stomach slices; sea water.
E. Intestine slices; sea water.
F. stomach homogenate; sucrose
OXYGEN CONSUMPTION (µlO2/gm dry wt.)
3500
3000
2500
2000
1500
1000
500
0
15
30
45
60
75
90
105
120
135
150
TIME (minutes)
A
B
C
D
E
F

Table 23. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of intestine homogenates from warm-and cold-acclimated S. droebachiensis. (Buffered sucrose medium, pH 7.4. Based on data in appendix VII b.)

test temperature (°C.)	acclimation temperature (°C.)	N	O_2 cons.	S.E.	t	p
5°	0°	6	461.3	32.9		
					2.401	< 0.025
5°	15°	6	372.4	17.1		
10°	0°	6	679.7	36.2		
					2.223	< 0.025
10°	15°	6	559.7	40.0		
15°	0°	6	946.5	46.6		
					3.001	< 0.01
15°	15°	6	741.8	49.8		

of stomach tissue, subsequent homogenate studies focussed on intestine tissue alone.

ii) Temperature and homogenate respiration:

Q_{10} coefficients for metabolism of intestine homogenates from warm-and cold-acclimated urchins are:

5°-10°C.: warm-acclimated = 2.86; cold-acclimated = 2.17

10°-15°C.: warm-acclimated = 1.76; cold-acclimated = 1.94

The temperature characteristics (μ) for respiration of intestine homogenates from cold-and warm-acclimated animals are, for all practical purposes, the same (11,500 and 11,000, respectively).

iii) Acclimation of homogenate respiration:

Augmentation of metabolism on cold-acclimation, observed earlier in intact urchins and in tissue slices, is also detectable in intestine homogenates from small (25-30 gms.) laboratory-acclimated urchins (Table 23). Metabolic rates at 5°, 10° and 15°C. of intestine homogenates from cold-(0°C.) acclimated urchins are significantly greater than were rates of homogenates from warm-(15°C.) acclimated animals at the same temperatures (Fig. 34).

iv) Thermal compensation coefficients:

Rate-temperature data in Table 23 were transformed according to the Arrhenius relationship for computation of thermal compensation coefficients (Fig. 35). The coefficient of acclimation of 0.809 indicates

FIGURE 34.

R-T relationships for respiration of intestine homogenates from warm-and cold-acclimated S. droebachiensis. (Vertical lines indicate ± S.E. Based on data in Table 23.)

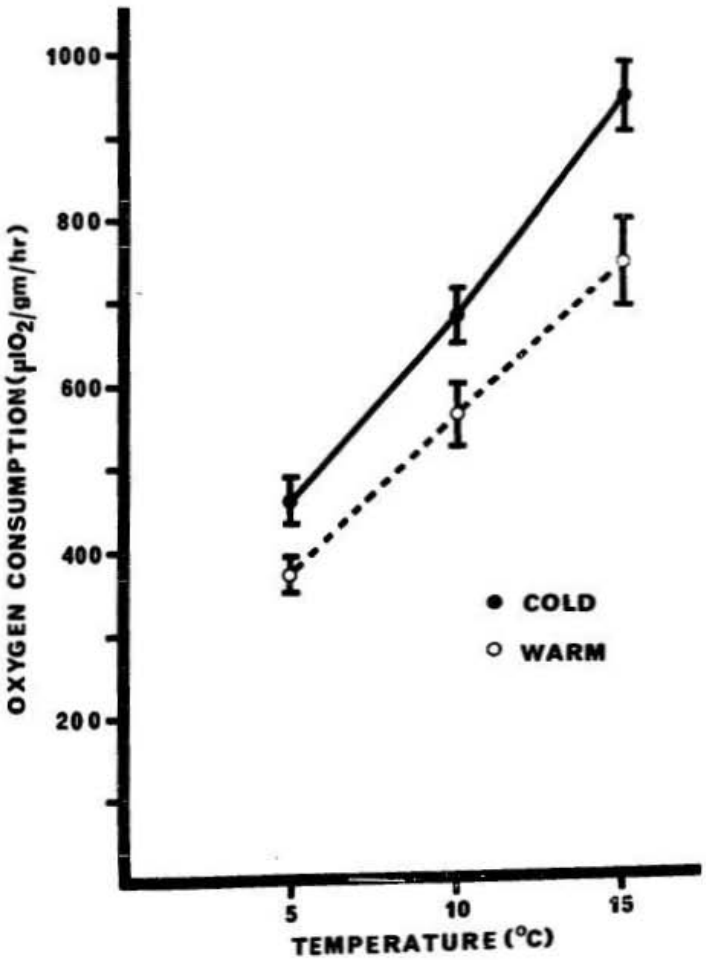
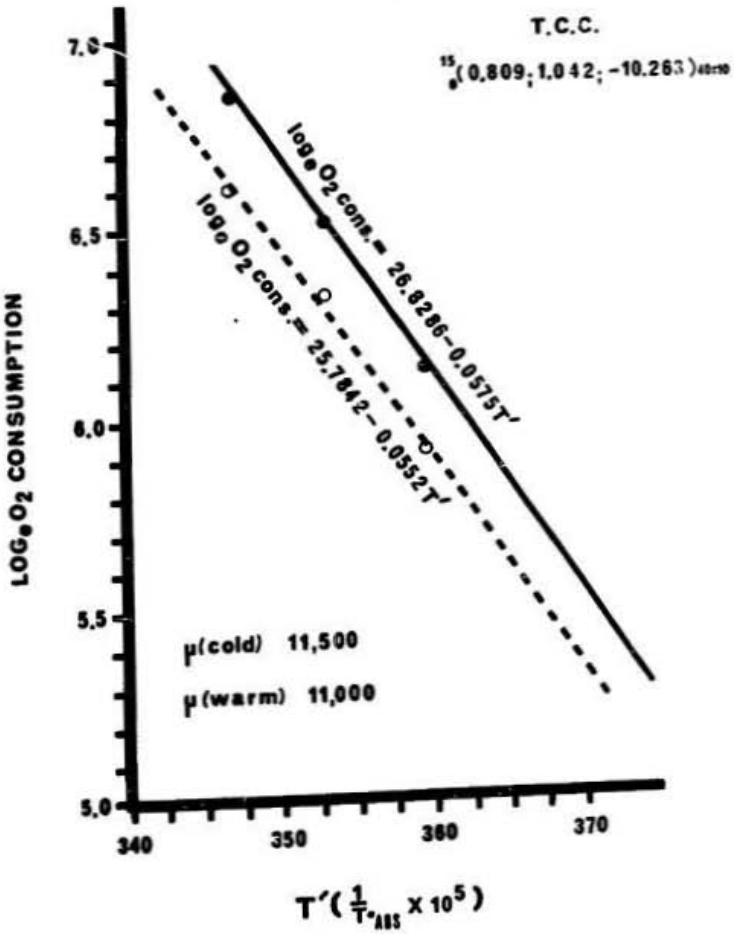

1000
800
600
400
200
OXYGEN CONSUMPTION(μlO2/gm/hr)
COLD
WARM
5
10
15
TEMPERATURE (°C)

FIGURE 35.

Arrhenius transformation of R-T relationships (Figure 34) for respiration of intestine homogenates from warm-and cold-acclimated S. droebachiensis.


T.C.C.
$^{15}_{0}$(0.809; 1.042; -10.263)40:10
7.0
6.5
6.0
5.5
5.0
LOG$_e$ O$_2$ CONSUMPTION
log$_e$ O$_2$ cons.= 26.8286-0.0575T'
log$_e$ O$_2$ cons.= 25.7842-0.0552T'
μ(cold) 11,500
μ(warm) 11,000
340
350
360
370
T'($\frac{1}{T°_{ABS}}$ X 10^5)

an approximately 21% augmentation of metabolism on cold-acclimation. A coefficient of rotation of 1.042 and an axial coefficient of -10.26 suggest that the compensatory shift involves primarily a translation of the cold-relative to the warm-acclimated Arrhenius curve.

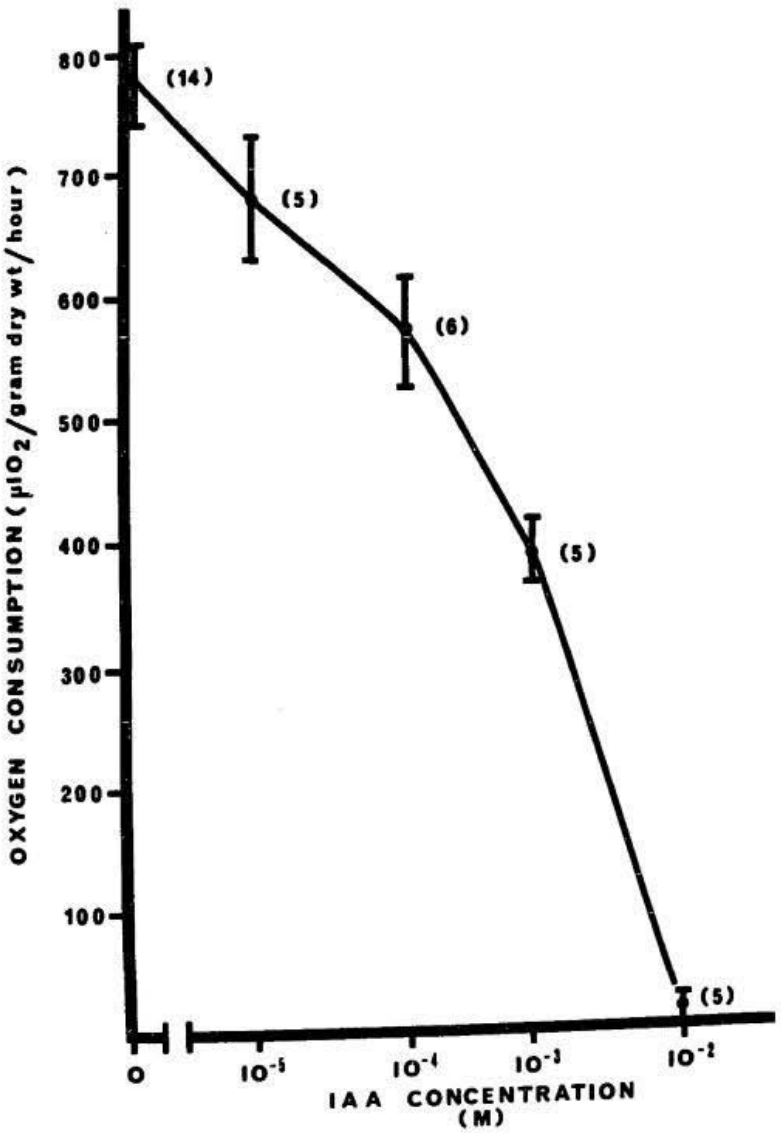
v) Iodoacetic acid inhibition:

The metabolic inhibitor iodoacetic acid was used to gain information on possible shifts in metabolic pathways associated with thermal acclimation. Iodoacetic acid effectively blocks the glycolytic pathway by inhibiting glyceraldehyde-3-phosphate dehydrogenase (Fruton and Simmonds, 1958).

To determine the iodoacetic acid concentration necessary partially to inhibit respiration, intestine homogenate respiration rates in a sucrose medium were measured in the presence of different concentrations of the inhibitor. Increasing the concentration above 10^{-5}M markedly reduces respiration until, at a concentration of 10^{-2}M, it is virtually arrested (Fig. 36). An inhibitor concentration of 5×10^{-4}M was selected for differential inhibition studies on warm-and cold-acclimated intestine homogenates. The respiratory rate, in buffered sucrose, was measured at 15°C. First the basal rate was determined during an initial, 40 minute, control period (period I). Then iodoacetic acid was added from the side arm. After a 5 minute re-equilibration period, respiration was measured for a further 40 minutes (period II).

FIGURE 36

Influence of iodoacetic acid concentration on respiration of intestine homogenates from S. droebachiensis. (Vertical lines indicate ± S.E. Based on data in appendix VII c.)


OXYGEN CONSUMPTION (µlO2/gram dry wt/hour)
800
700
600
500
400
300
200
100
(14)
(5)
(6)
(5)
(5)
0
10-5
10-4
10-3
10-2
IAA CONCENTRATION (M)

To rule out the possibility that the respiration rates of warm- and cold-acclimated homogenates are not equally stable under these experimental conditions, a preliminary series of control samples were run (Table 24). These were identical to those above, except that buffered sucrose was added in place of iodoacetic acid at the end of period I. Neither warm-nor cold-acclimated homogenate respiration declined significantly during the course of the experiment (-0.6% and -2.5%, respectively; significance of difference between warm and cold controls: $p < 0.30$).

The sensitivity of respiration to iodoacetic acid differed markedly in warm-and cold-acclimated homogenates (Table 24). The mean respiration rate of cold-acclimated tissue was inhibited by 24.5%, while respiration of warm-acclimated homogenates was reduced by only 11.1%. The magnitude of the inhibition in the two groups was significantly different ($t = 2.908$, $p < 0.01$).

vi) Acclimation and pentose shunt activity:

Activity of the pentose shunt enzyme glucose-6-phosphate (G-6-P) dehydrogenase in crude bacterial extracts is effectively inhibited by 5-bromouracil (5-BU) (Hochster, 1961). G-6-P dehydrogenase from several other sources, however, appears to be inhibited more variably and to a lesser extent by 5-BU. Despite this variability in inhibitory effect and considerable uncertainty regarding the precise mode of inhibition and the active ionic form of the inhibitor, 5-BU has been employed to

Table 24. Influence of iodoacetic acid on respiration ($\mu l.O_2$/gm. dry wt./hr.) of intestine homogenates from warm-and cold-acclimated S. droebachiensis. (Temperature 15°C. Buffered sucrose medium pH 7.4. Based on data in appendix VII d and c.)

	acclim. temp. (°C.)	N	O_2 cons. period I	S.E.	O_2 cons. period II	S.E.	% diff.	t	p
control	0°	6	864.0	34.0	858.9	31.8	-0.6		
								0.663	< 0.30
control	15°	6	730.0	30.6	711.9	47.7	-2.5		
+IAA*	0°	6	773.1	19.3	583.5	13.0	-24.5		
								2.908	< 0.01
+IAA*	15°	6	693.4	45.0	616.8	42.9	-11.1		

* 5.0×10^{-4} M.

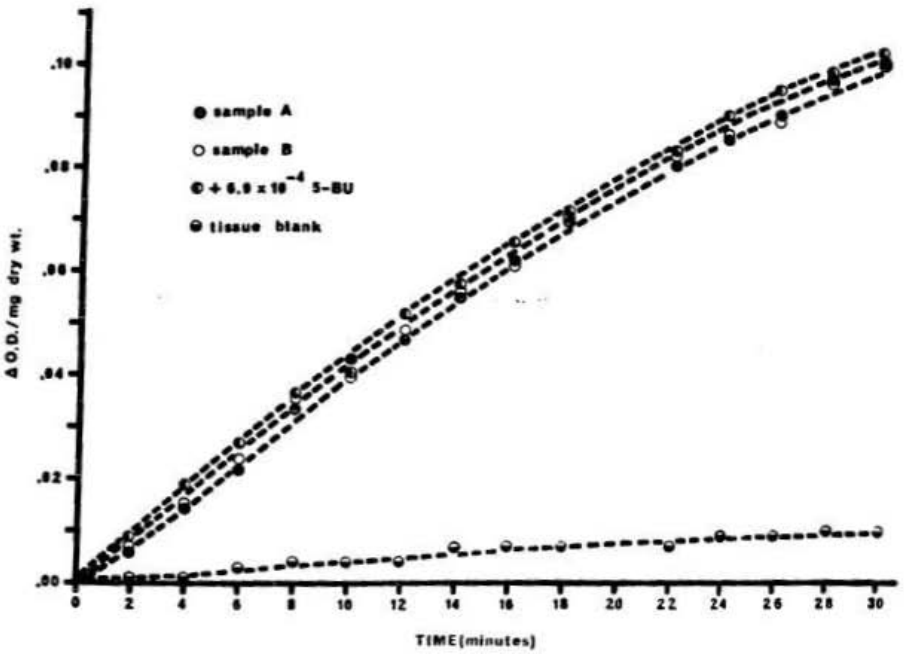
assess changes in the relative magnitude of the pentose shunt in various tissues under different conditions (McWhinnie, 1964; McWhinnie and Corkill, 1964; McWhinnie and O'Connor, 1967).

Preliminary studies were undertaken to investigate a) the occurrence G-6-P dehydrogenase in urchin intestine tissue and b) the inhibitory effect of 5-BU on the activity of the enzyme. It is clear from Figure 37 that addition of the substrate G-6-P to the reaction vessel substantially increases the rate of TPN degradation above that in the tissue blank, indicating that G-6-P dehydrogenase is indeed present in urchin intestine tissue. It is further clear that 6.9×10^{-4} 5-BU has no detectable inhibitory effect on the enzyme (Fig. 37). Increasing the 5-BU concentration to 1.5×10^{-3}M still does not result in inhibition. Furthermore, use of tris buffer in place of glycylglycine yields essentially similar results. Thus although it appears that G-6-P dehydrogenase (and presumably also the pentose shunt) is present in urchin tissues 5-BU cannot be used for differential respiratory inhibition studies.

To determine whether cold-acclimation has any significant effect on the G-6-P dehydrogenase activity of urchin tissue, tests were conducted on homogenates prepared from animals (mean weight = 28.1 grams) acclimated at 0°C. and 15°C. for at least four weeks. Homogenates were prepared, as outlined previously, in glycylglycine buffer pH 7.4. The mean cumulative changes in optical density per mg. dry wt. for the two groups (N = 6 in each group) were determined at 2-minute intervals

FIGURE 37

Activity of G-6-P dehydrogenase in intestine homogenates of S. droebachiensis, and the effect of 5-bromouracil. (Based on data in appendix VII f.)


● sample A
○ sample B
◐ + 6.8 x 10⁻⁴ 5-BU
⊖ tissue blank
Δ O.D./mg dry wt.
.10
.08
.06
.04
.02
.00
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
TIME (minutes)

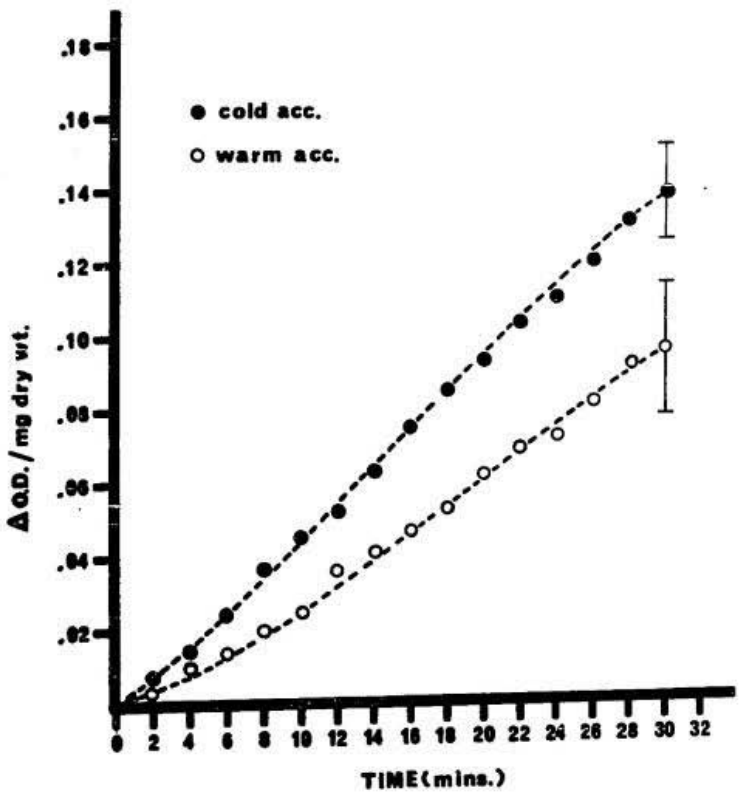
(Fig. 38). Activity of the cold-acclimated tissue is higher than that of the warm. For statistical analysis the cumulative changes in optical density/mg. dry wt. at the end of 30 minutes were determined for each sample (Table 25). The mean rate for cold-acclimated samples is 0.139/ mg. dry wt./30 minutes and for warm-acclimated samples 0.096/mg. dry wt./30 minutes, an increase of approximately 45% on cold-acclimation ($p < 0.05$).

Table 25. G-6-P dehydrogenase activity (Δ O.D./mg. dry wt./30 mins.) in intestine homogenates from warm-and cold-acclimated S. droebachiensis.

sample	cold-acclimated activity	warm-acclimated activity
a	0.181	0.023
b	0.108	0.163
c	0.104	0.079
d	0.165	0.114
e	0.137	0.101
f	0.139	0.096
N =	6	6
$\overline{X}$ =	0.139	0.096
S.E. =	0.012	0.018
t =	1.94	
p =	< 0.05	

FIGURE 38

Activity of G-6-P dehydrogenase in intestine homogenates from warm-and cold-acclimated S. droebachiensis. (Vertical lines indicate ± S.E. N = 6 for each group.)


● cold acc.
○ warm acc.
ΔO.D./mg dry wt.
.18
.16
.14
.12
.10
.08
.06
.04
.02
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
TIME(mins.)

DISCUSSION

The common green sea urchin, Strongylocentrotus droebachiensis, has a circumpolar distribution with southward extensions of its range into both North Atlantic and North Pacific Oceans. In more southerly reaches of its range, seasonal temperature fluctuations of 15°C. or more may be encountered, constituting a potentially debilitating thermal stress, unless effectively countered by a homeostatic response such as thermal adaptation. This study is an attempt to elucidate the magnitude and character of seasonal, thermal adaptation in S. droebachiensis.

i) Rate functions; general considerations:

Adaptation can be readily detected by comparing "differences in performance of individuals with different environmental experiences" (Kinne, 1964). Here, intact-animal-respiration, in vitro tissue respiration, and righting activity serve as indicators of urchin performance. In this discussion, I will consider first several fundamental aspects of these physiological indicators, and then discuss thermal adaptation in S. droebachiensis in particular.

a) Intact-urchin respiration:

Respiration rate, being a direct expression of normal metabolic activity, provides probably the most meaningful, convenient and widely used indicator of metabolic adaptation. However, with urchins, as with many invertebrates, it is difficult either to determine basal metabolic rates, or adequately to quantify activity levels. Consequently, as Newell (1969) points out, "in organisms which show an enormous range of movements there is an almost continuous series of rates of O_2 consumption ranging from a minimal rate observed during quiescence to a maximal rate during full pumping, crawling or swimming activity". The individual variation in respiration rates observed here may be, in part, attributable to such differences in levels of activity.

Respiration rates of intact S. droebachiensis determined in this study are similar in magnitude to those reported for S. purpuratus of comparable size at the same temperatures (Farmanfarmaian and Giese, 1963).

Comparison of metabolic rates of boreal S. droebachiensis and a subtropical urchin provides an example of interspecific geographic adaptation. Eucidaris tribuloides, from Florida, in summer, at 30°C. (mean, summer temperature for the region), has a respiration rate of about 590 $\mu l.O_2$/gm. decalcified dry wt./hr. (McPherson, 1968). S. droebachiensis in Newfoundland waters, in summer, has a similar rate (620 $\mu l.O_2$/gm. decalcified dry wt./hr.; from Fig. 6 D) at its mean, summer temperature of 15°C. Observations by Mayer (1914) on pulsation rates of the jellyfish Aurelia from Nova Scotia and Florida revealed that animals from both areas have similar optimum pulsation rates, but the optimum occurred at a much lower temperature in northern animals. Several other such examples of both interspecific and intraspecific, geographic adaptation of rate functions are discussed in a review by Vernberg (1962).

The total oxygen consumption of S. droebachiensis increases with increasing body weight, although when expressed on a unit weight basis the rate is higher in smaller animals. This size-rate relationship, frequently observed in metabolic studies, has been critically reviewed by Bertalanffy (1957). As a consequence of this relationship, metabolic rate may best be expressed as an exponential function of weight in the form:

$$M = aW^b$$

where "M" is the total oxygen consumption per unit time, "W" is body weight, "b" the slope of a logarithmic plot of oxygen consumption against weight and "a" the point at which this plot intercepts the ordinate.

Bertalanffy (1957) contends that three basic values of the exponent "b" commonly occur, corresponding to three distinct metabolic types. Values close to unity suggest that metabolism is proportional to body weight, while values around 0.66 are considered more nearly proportional to surface area. Values intermediate to these two also occur. The "b" values for S. droebachiensis are close to 0.66 and as such fall in the second of the above categories. The tropical urchin, Eucidaris tribuloides, has a similar respiration-weight relationship with "b" values of 0.65 to 0.75 (McPherson, 1968). Both these species have "b" values that are approximately the same as those of the majority of "gill breathing" poikilotherms tabulated by Bertalanffy (1957). An exceptionally low "b" value of 0.31 for the starfish, Asterias rubens (Koller and Meyer, 1933), and high values of 0.73 - 0.87 for the sea cucumber, Stichopus japonicus (Choe, 1962), are puzzling. The marked seasonal change in the slope of the respiration-weight relationship observed in S. droebachiensis will be considered below.

No significant difference was found between respiration rates of male and female S. droebachiensis, either in summer or in winter. A similar lack of sexual difference in rate is found in the tropical urchin, Eucidaris tribuloides (McPherson, 1968). This absence of a sexual difference in respiration rates, even though there are present relatively large masses of ovary and testis tissue with very different in vitro respiration rates, indicates that in situ oxygen consumption rates of ovary and testis are comparable. In this connection it is interesting

to note that in both S. purpuratus (Giese et al, 1966) and in Eucidaris tribuloides (McPherson, 1968) the rate of respiration per unit wet weight is approximately the same regardless of gonad index. However, respiration rates expressed on a dry weight or total protein basis decline considerably with increasing gonad index. Giese et al (1966) attribute this situation to the fact that an increase in gonadal mass has no effect on animal wet weight because it is compensated for by a decrease in coelomic fluid having a specific gravity similar to that of gonad tissue. In addition, O_2 supply to the gonad tissues is thought to be limited, so that with increasing gonad mass the O_2 uptake per unit volume of tissue declines while total uptake remains constant. This lack of correlation between respiration rate and gonad index will be of considerable importance in the later consideration of seasonal changes in oxygen uptake.

b) Excised tissue respiration:

Oxygen uptake in vitro by both tissue slices and cell-free homogenates were used as indicators of adaptation. Metabolic rates of excised tissues undoubtedly differ from those in situ. Such differences may be attributable to disruption of integrative mechanisms or to modification of physiological conditions. One assumption implicit in many tissue respiration studies is that although the absolute respiration rate of an excised tissue may bear little resemblance to its in situ rate, nevertheless, the former closely reflects progressive changes (such as those accompanying thermal adaptation) occurring in the latter.

Information about metabolic rates of echinoderm body components is meager. Intestine and branchial tree tissues from the holothurian, Isostichopus badionotus (Stichopus mobii), have respiration rates (Robie, 1949) less than half those of digestive tract tissues of S. droebachiensis. Digestive tract tissue of the purple sea urchin, S. purpuratus, has a respiration rate at 13°C. of 102.8 $\mu l.O_2$/gm. wet wt./hr. (Giese et al, 1966). Assuming that gut tissue in this species has a water content similar to that for stomach and intestine tissue of S. droebachiensis (approximately 78%; Table 21), then the respiration rate, expressed on a dry weight basis, is 467.3 $\mu l.O_2$/gm./hr. This is intermediate between values observed for various subdivisions of the digestive tract of S. droebachiensis (Fig. 16).

The physiological significance of the gradient in metabolic rate observed in the alimentary tract of S. droebachiensis is not known.

Respiration rates are lower, per unit of weight, in stomach and intestine excised from large animals than in tissues from small animals (Fig. 30). This corresponds well with the data from whole-animal respiration, which also show that smaller animals are more active metabolically. These observations indicate that the size-related, metabolic differences noted in whole animals must be referred to metabolic differences at the cellular level, and not to differences in whole-animal gas transport physiology. Bertalanffy (1957) suggests that such a clear relationship is not always apparent.

Oxygen uptake by stomach and intestine slices continues at a

uniform rate for several hours in either buffered sucrose or seawater (Fig. 33). Similarly, respiration of intestine homogenate is stable for at least two hours. In marked contrast, respiration of stomach tissue declines continuously following homogenization. Autodigestion of stomach, but not of intestine, homogenates may account for the difference. The little that is known concerning digestion in echinoids supports this interpretation. Lipases, carbohydrases, and proteases have been detected in gut extracts from various echinoids (Anderson, 1966). Numerous secretory cells of a type found only in the stomach lining contain abundant granular material thought to be a source of digestive enzymes (Anderson, 1966). Furthermore, Farmanfarmaian and Phillips (1962) detected an enzyme in stomach extracts of S. purpuratus that liberates reducing sugars from algal fragments; intestine homogenates were without effect. Destruction of respiratory proteins and other associated macromolecules could be expected following liberation of such digestive enzymes by homogenization.

c. Urchin activity:

The righting reflex provides a useful measure of the activity of sea urchins. Literature on righting in urchins and other echinoderms has been extensively reviewed by Reese (1966). The righting response is essentially a "forced" activity, and as such may bear little direct relationship to actual spontaneous locomotory activity of the animal. It does, however, provide a measure of the potential for

activity under given conditions.

An urchin spends about 80% of the full righting-time in raising itself through the first 90 degrees. Turning through the final 90 degrees is accomplished either by a gradual lowering or by a release of the supporting tube feet and a rapid fall into the normal position. Variation in activity coefficient is minimized somewhat by disregarding the descending phase of the response, and measuring only the half-righting time.

Measurement of half-righting time began as soon as the urchin was inverted. It is now apparent that this may have contributed to individual variability in righting rate because a slight but variable delay occurs between the actual inversion of the animal and initiation of righting. A similar phenomenon has been observed in inverted starfish which may at first seem to hesitate and then "suddenly this unified impulse appears and all opposition and incoordination is gone" (Jennings, 1907). In the great majority of urchins examined the period of "indecision" is negligible and a "unified impulse" occurs within seconds of inversion. In a very few individuals a delay lasting up to a minute or more may occur. Onset of the "unified impulse" is quite distinct in both urchins and starfish. In future studies on echinoid activity, variability of response can be further minimized by timing from the moment of initiation of the "unified impulse".

The few studies that have been reported on the temperature relations of echinoid righting clearly illustrate the well known phenomenon of interspecific, geographic, thermal adaptation (Vernberg, 1962).

Both the tropical urchin, Lythechinus variegatus (Kleitman, 1941), and the boreal S. droebachiensis, have optimal activity coefficients of about 15. However, in S. droebachiensis this optimum occurs at 15°C. while in L. variegatus it occurs at 24°-26°C. (Fig. 21). At 15°C. the A.C. of the tropical urchin is only 2.56, considerably lower than that of S. droebachiensis in winter at 0°C. Furthermore, whereas S. droebachiensis is able to right at 0°C., righting of the tropical species does not occur below 10°-11°C. In S. purpuratus, a form with a more southerly range than S. droebachiensis and a more northerly one than L. variegatus, righting is inhibited below 5°C. (Farmanfarmaian and Giese, 1963).

Reference was made earlier to non-initiation of righting at both high and low temperatures. The causes of righting failure in the two cases are undoubtedly different. At low temperature extremes the tube feet remain extended, move slowly and retain their ability to adhere to the substrate. Inability to right under such conditions may be due to cold-induced failure of nervous coordination required for initiation of the "unified impulse", or to inadequate energy production by tube feet. At elevated temperature righting failure appears to be associated with heat narcosis of tube feet, as they initially become limp, lose their ability to adhere to the substratum and ultimately contract.

ii) Acclimatization and acclimation:

Thermal adaptation, as described earlier, can be considered to have two basic forms, distinguished as acclimatization and acclimation. The latter implies an adaptive response induced under controlled conditions (usually in the laboratory), with all environmental factors, except temperature, essentially unchanged or stabilized. The former refers to an adaptive response occurring in animals in their natural habitat, a response in which temperature may play a predominant role, but in which changes in a variety of other environmental or endogenous factors may be involved as well, or even exclusively.

Under natural conditions, temperature changes normally occur slowly and somewhat erratically, in marked contrast to the "step" or "steep ramp" function temperature change associated with acclimation. Acclimatization thus occurs gradually in response to successive small temperature shifts, while acclimation is induced by a relatively massive thermal shift over an interval too short to permit adaptation to intermediate temperatures. Temperatures in these two situations also may differ in stability, with acclimation temperatures remaining relatively constant, while habitat temperatures usually fluctuate considerably about a mean.

Thus, it may not always be correct to deduce from metabolic changes induced by acclimation that adjustments in the natural habitat will be similar in quality, quantity or etiology. A majority of adaptation studies deals with either acclimation or acclimatization (seasonal or

geographic), rarely with both, and failure to consider the differences between these two experimental modalities may lead to incorrect assumptions concerning the cause or occurrence in the natural habitat of a metabolic adjustment.

Demonstration of a metabolic adjustment during a particular season need not imply an adaptation to temperature; confirmatory acclimation studies are required. For example, a number of species of cottid fish have higher metabolic rates in winter than in summer, measured at a given temperature. However, comparable adjustments of metabolism and lethal temperature have not been detected in fish acclimated in the laboratory (Morris, 1961). It is not clear whether this failure to acclimate is attributable to insufficient acclimation time or to suppression of the response in the artificial laboratory environment. It may also be, that acclimatization in these fish is not a direct response to a temperature change, but is associated with seasonal, endogenous metabolic rhythms. Such a situation occurs in goldfish, which even though kept at a constant temperature in the laboratory, still increase their heat resistance in summer and their cold resistance in winter (Hoar, 1955).

Similarly, it may not be correct to deduce from metabolic changes induced by laboratory acclimation that similar adjustments take place in the natural habitat. Behavioral, endocrine, nutritional or other factors may suppress the potential metabolic adjustment. Confirmatory acclimatization studies are required to assess the natural occurrence

and ecological significance of the response.

In the present study this potential pitfall has been avoided by a two-pronged approach using both seasonally-acclimatized and laboratory-acclimated urchins.

iii) Seasonal acclimatization:

The urchins of Newfoundland coastal waters exhibit marked seasonal adjustment in metabolism. A metabolic shift, evident in respiration of both intact animals and excised tissues, is reflected also in urchin activity. Acclimatization causes winter animals to have higher metabolic rates, at any given temperature, than summer ones. The metabolic adjustment primarily involves an upward translation of winter rate-temperature curves relative to summer ones, with little or no apparent rotation. The magnitude of this adaptive shift is inversely proportional to animal weight. A fuller discussion of this interesting phenomenon is presented below.

Seasonal acclimatization has also been demonstrated for respiration of the tropical urchin, Eucidaris tribuloides, collected in Florida, although in this instance summer and winter temperatures are approximately 30° and 20°C., respectively (McPherson, 1968). Acclimatization in this species involves clockwise rotation of winter relative to summer rate-temperature curves. In contrast, the tropical urchin, Diadema antillarum, collected at Barbados where there is little seasonal temperature fluctuation, exhibits no significant seasonal differences in oxygen uptake (Lewis, 1968).

The regression coefficient "b", described earlier, exhibits a number of seasonal differences. Increasing temperature appears to affect "b" differently in summer and winter. In winter, "b" increases with rising temperature up to 10°C. then decreases slightly at 15°C. In

summer, on the other hand, "b" declines as the temperature rises to 10°C. and then rises sharply at 15°C. The significance of these changes is unclear, although McPherson (1968) points out that "in some echinoderms 'b' has been shown to decrease with increasing temperature, indicating that larger animals consume proportionally less oxygen than smaller animals as the temperature increases. This may be related to decreased ambient O_2 or increased O_2 demand but insufficient supply." The sharp rise in "b" at 15°C. during the winter may be attributable to a limiting effect on intact-animal respiration at high temperatures that has a more pronounced effect on small urchins than on large ones (see below).

Between 0° and 10°C. summer "b" values are about 20% higher than in winter (means = 0.716 and 0.582, respectively). This indicates that winter animals consume proportionally less oxygen with increasing weight than do summer animals. This may be a reflection of the decline in the degree of winter-acclimatization with increasing animal weight (see section: Adaptation ability and size).

That the seasonal acclimatization observed in urchin respiration reflects a true metabolic adjustment mediated at the tissue level, and is not simply attributable to a marked increase in gonad mass during the winter, is evident from the facts that urchin respiration is independent of gonad index (Giese et al, 1966) and of sex, and that similar adaptive shifts are detectable in the respiration of excised tissues.

Esophagus, stomach, and intestine tissue all exhibit well developed seasonal acclimatization of respiration. This has not previously been reported for echinoderm tissues. In each of these tissues the relative magnitude of the adaptive shift (as indicated by the coefficients of adaptation) is similar to that of intact-urchin respiration. Furthermore, acclimatization of the tissues involves essentially a translation of rate-temperature curves (as indicated by the coefficients of rotation), just as in the case of intact-urchin respiration. Results for excised tube feet are less clear, possibly because tube feet, unlike the other tissues, contract strongly upon excision and become compact, immobile cylinders, far different from their normal in situ form. The differences in respiration rates between summer-and winter-adapted tube feet are probably considerably less in vitro than in situ. Support for this view comes from the observation that urchin activity, a function in which tube feet play a major role, exhibits considerable seasonal acclimatization.

Acclimatization of urchin activity is such that, during winter the mean half righting time at 0°C. is approximately 3 minutes, instead of the more than 30 minutes required if no acclimatization had occurred. In contrast during summer, at temperatures between 12° and 15°C., the mean half righting time is approximately one minute. In addition to this evident capacity acclimatization there occurs also a distinct seasonal adjustment in the maximum temperature at which righting is possible. Whereas righting in winter is effectively inhibited

above 12°-15°C., in summer urchins right readily at 20°C.

In discussing seasonal shifts in urchin metabolism it may be relevant to consider seasonal changes in two other important physiological processes; namely, reproduction and feeding. Each is intimately involved in urchin metabolism and each may influence, or be influenced by, seasonal metabolic adjustments.

S. droebachiensis, in Newfoundland waters, has a well defined reproductive cycle characterized by an increase in gonad index during autumn and winter, followed by spawning in early spring (Fig. 26). This pattern is essentially similar to that reported earlier for S. droebachiensis from Newfoundland (Himmelman, 1970), Maine (Cocanour and Allen, 1967), and Norway (Vasseur, 1952). The relationship between various environmental factors, particularly temperature, and initiation of gonad development and stimulation of spawning has not yet been satisfactorily clarified (Boolootian, 1966).

It is clear that the major part of gonad development occurs at a time either when environmental temperature is falling rapidly or when it is near its annual minimum. Confronted with such adverse thermal conditions the urchin can continue gonad development only if it has either adequate nutritional reserves or a mechanism for so counteracting the cold-induced metabolic depression that feeding continues. But echinoids lack special nutritive storage organs; limited reserves may accumulate in the walls of the digestive tract, and in the gonads themselves (Anderson, 1966). In fact, the gonads may function as relatively major depots, particularly in summer when food is plentiful. At that

time the gonads of Echinus esculentus reach maximum size, while in winter, they shrink as the urchin draws upon their nutritive reserves, until they enlarge again just prior to the breeding season (Moore, 1937). Obviously, because of the timing of its gonad development, S. droebachiensis is not in a position similarly to utilize gonadal reserves to carry it through the winter. Instead, S. droebachiensis adapts its metabolism to the falling temperature and is thus able to remain active and feeding throughout the winter. Feeding continues even when environmental temperature falls to 0°C. At such temperatures, the rate of food consumption is only half that in summer (Fig. 24). However, in summer, feeding efficiency is only 35-40 percent, whereas in winter efficiency rises markedly to approximately 70 percent. An opposite but still seasonal change in feeding efficiency is also reported in S. intermedius, in which efficiency ranges from 70 percent in summer to 50 percent in winter (Fuji, 1962). It may be significant here, that while gonad development in S. droebachiensis occurs in autumn and winter with spring spawning, in S. intermedius gonad development occurs in the summer and spawning in late summer or autumn (Boolootian, 1966).

Although the total food intake of S. droebachiensis differs considerably in summer and winter, it appears that the actual quantities absorbed are essentially similar. It can be reasoned that since metabolic requirements for maintenance and activity are lower in the winter at 0°C. than in summer at 15°C., the excess nutritive intake during winter is primarily utilized for gonadal development.

It may be that the considerable increase in food consumption in May is related to the limited increase in gonad index following spawning. This relatively rapid gonad development, evident in Figure 26, may result in a slight secondary maximum in the annual gonad index cycle. Gonad growth ceases in early summer and the G.I. remains essentially constant for the remainder of the summer. A similar secondary peak in gonad index in late spring is evident in the figure of Cocanour and Allen (1967) for the reproductive cycle of S. droebachiensis in Maine. Himmelman (1970) also observed such a secondary peak and suggested that it might be associated with a second spawning. It seems more reasonable to attribute it to a rapid accumulation of nutritive reserves in the gonads following the acute depletion associated with spawning. Boolootian (1966) reports that in many echinoids, gonad growth occurs prior to gametogenesis, with the gonads functioning as storage organs. Such accumulation of reserves in S. droebachiensis may accompany the increase in food consumption following spawning. It may be that once an adequate level of nutritive reserves is attained no further increase in gonad mass occurs for the remainder of the summer and food consumption falls accordingly. In the autumn, gonadal development begins and food consumption again rises. However, as environmental temperature approaches the winter minimum, urchin activity and feeding are somewhat impaired. The nutritive requirements for gonad development are, however, probably met by a compensatory increase in feeding efficiency. This scheme, though somewhat speculative, is consistent with the present nutritional,

reproductive and metabolic data and provides a framework for further nutritional studies.

It is anticipated that some of the present findings may eventually find practical applications in bioenergetic analyses of boreal coastal ecosystems of the North Atlantic. To this end I have heeded the plea of Pamatmat (1969) that physiologists present data on respiration in "a form more useful to ecologists working on the problems of energetics of a community".

It is clear that urchin respiration varies with animal weight, experimental temperature, and season. The latter factor may assume importance in bioenergetic studies conducted in regions with pronounced seasonal temperature fluctuations. In such instances, occurrence of acclimatization precludes the use of a single rate-temperature relationship to describe metabolism at different seasons. An additional complication arises as a consequence of the dependence of adaptation on the size of the animal.

Summer and winter, multiple regression equations describing the relationship between oxygen consumption, temperature, and weight permit a ready approximation to total annual metabolism of an urchin population of known weight-class composition. Clearly, summer and winter equations represent only the two extremes of acclimatization; intermediate states, characterized by different rate-temperature relationships, probably occur at intermediate environmental temperatures. For extremely refined studies it may ultimately become necessary to compute monthly regression

equations, similar to those reported here. However, use of summer and winter equations will probably provide sufficient accuracy for the majority of bioenergetic studies, particularly in cases where temperature changes in spring and autumn are relatively rapid. The nomogram (Fig. 15), presented here, is based on summer and winter multiple regression equations, and not only permits rapid estimation of metabolic rates in different circumstances but also provides a concise visual summary of seasonal acclimatization of S. droebachiensis.

iv) Laboratory acclimation:

Urchins maintained in the laboratory for 4-6 weeks under a summer-like thermal regime (15°C.) displayed differences in activity and metabolism from animals similarly kept in a winter-like (0°C.) state, and these differences were similar to those noted between freshly collected summer and winter (i.e. seasonally acclimatized) urchins.

Mean A.Cs. of 15.47 and 4.95 for warm-and cold-acclimated urchins, respectively, compare well with mean A.Cs. of 15.71 (July) and 5.26 (February) for acclimatized animals. In both summer-acclimatized and warm-acclimated urchins activity was severely depressed (A.Cs. approximately 0.5 and 0.7, respectively) immediately after transfer to 0°C. Between 30 and 40 days elapsed before the A.Cs. of animals held at 0°C. levelled-off at a value approximating that of fully cold-adapted urchins. According to Farmanfarmaian and Giese (1963), S. purpuratus, transferred from 14°- 19°C. to 5°C. shows evidence of acclimation of activity after 15 days; after 35 days "acclimation is fairly complete". It is difficult rigorously to compare these, largely qualitative, observations with the present results, nevertheless, both sets of data indicate that in urchins complete adaptation (of activity, at least) requires approximately 5 weeks; only further studies will establish whether metabolism, per se, measured directly (e.g. by respiration), exhibits a comparable time course of acclimation. A similar period (29 days) was required for acclimation of heart rate in the limpet, Acmaea limatula (Segal, 1956), while acclimation of metabolism in the cockroach, Periplaneta americana,

took 1-3 weeks (Dehnel and Segal, 1956). Resistance acclimation in the American lobster, Homarus americanus, was complete after 22 days (McLeese, 1956).

In general, it seems that the higher invertebrate metazoans require somewhere between 3 and 5 weeks in order fully to acclimate. If acclimation is primarily a basic, cell-physiologic phenomenon - as the tissue studies seem to suggest - then it is not unreasonable to expect a basic similarity in the responses of animals as different in phylogeny and in size and life-styles as a limpet, a lobster, and a cockroach. If acclimatization furthermore, is primarily an adaptation to natural temperature changes, the 3-5 week period appears reasonable.

It must also be mentioned, however, that in 1918, Behre showed in the planarian, Planaria dorotocephala, significant thermal acclimation of metabolism occurred within 3 days. It is not clear whether the acclimation, observed in this instance, was complete; furthermore, planarians are not "higher invertebrate metazoans". In any case, additional studies are required on the influence of such factors as species, life cycle stage, age, rate function tested, and extent of temperature change on the time course of thermal adaptation.

Following transfer to 0°C., a 12-14 day pseudostable interval occurred before the activity of S. droebachiensis began to increase. A similar delay was noted in the acquistion of resistance acclimation in the American lobster, H. americanus, where 8-10 days elapsed before the resistance curve began to rise slowly (McLeese, 1956). This particular

aspect of thermal adaptation has received little consideration, and additional comparative studies at both the physiological and biochemical levels are necessary to assess the frequency of occurrence and significance of the phenomenon.

Acclimation of intact-animal respiration in the laboratory is not only similar in magnitude to seasonal acclimatization (C.A. 0.797 and 0.847 - 0.883*, respectively) but also in form. In both instances, there is translation of R-T curves, with little or no rotation (C.R. 0.923 for acclimation and 1.090 - 1.112 for acclimatization). Only two other studies on acclimation of echinoids have been reported. S. purpuratus of the Northern Pacific has a higher respiration rate, at any given temperature, when acclimated at 5°C. than when held at 14°- 19°C. (Farmanfarmaian and Giese, 1963). In contrast, Arbacia punctulata, the "Atlantic coast counterpart" of S. purpuratus, does not adapt to low temperatures and may, in fact, exhibit hypoadaptation, because the respiration rates, over a range of temperatures, of urchins acclimated at 3-5°C. were significantly lower than those of animals kept at 20°C. (Boolootian and Cantor, 1965). The activity and respiratory metabolism of A. punctulata at different seasons would be interesting to compare to those of S. droebachiensis, because both, in certain areas of their ranges, experience similar seasonal temperature fluctuations.

* Coefficients for urchins of 20 and 30 grams standard weight.

The slope of the respiratory R-T curve of cold-acclimated urchins decreases markedly between 10° and 15°C. (Fig. 28) in a manner strikingly similar to that of winter-acclimatized animals (Fig. 9). In contrast, respiratory R-T slopes of neither summer-acclimatized nor warm-acclimated urchins decrease significantly in the 10°- 15°C. range. A possible explanation for this interesting difference is presented in the section on adaptation of thermal tolerance.

Differences in metabolic rates between excised tissues from warm- and cold-acclimated, small urchins are essentially similar to those between summer- and winter-acclimatized urchins of similar size. C.A. of 0.790 and 0.805 for intestine tissue of acclimatized and acclimated animals, respectively, suggest similar degrees of adaptation in the two situations. The metabolism of stomach tissue of acclimatized animals, however, seems to have adapted to a greater degree (C.A. 0.749) than the metabolism of acclimated ones (C.A. 0.831).

Coefficients of rotation of both stomach and intestine tissues of acclimatized and acclimated urchins are close to unity (C.R. stomach: acclimatized = 1.110; acclimated = 0.998; intestine: acclimatized = 1.114; acclimated = 1.056) indicating that these adaptive shifts are primarily translatory. In this regard, adaptation of excised tissues is similar to that of intact urchins, raising the possibility that the metabolic shift in intact urchins may be a direct expression of a metabolic shift in the urchins' tissues. Further support for this comes from the fact that a similar adaptive response (C.A. 0.809; C.R. 1.042) is

evident in cell-free homogenates (Fig. 35). These results support the contention of Dehnel and Segal (1956) that "compensatory responses to environmental stresses are inherent components of protoplasmic systems".

v) Adaptation and temperature characteristics:

Although their biochemical interpretation remains in some doubt, temperature characteristics (μ) of physiological rate functions may, nevertheless, prove empirically useful in comparing rate-temperature relationships. Interesting trends are apparent in the μ values for respiration of S. droebachiensis with respect to tissue type, animal size, and adaptation state.

The μ value for intestine tissues from both acclimatized and acclimated urchins are consistently lower than those of corresponding stomach tissues, (Table 26). The difference may be attributable to differences in metabolic pathways in the two tissues.

Temperature characteristics also appear to vary consistently with animal size. Values of μ for both stomach and intestine tissues are higher in smaller animals. A similar trend is evident in intact-urchin respiration, with smaller animals again exhibiting higher temperature characteristics. This situation may be attributable to relative shifts in metabolic pathways with increasing age although, the complexities of underlying processes rule out the possibility of an unequivocal explanation at the present time.

Temperature characteristics of urchin respiration vary with adaptation temperature. Despite differences in organizational level (intact animals; tissue slices; cell-free homogenates) of the respiring systems and in the mode of adaptation (seasonally acclimatized; laboratory acclimated), in virtually all instances cold-adapted μ values are greater

Table 26. Influence of summer- and winter-acclimatization and warm and cold-acclimation on temperature characteristics (μ) for respiration of intact S. droebachiensis and of excised stomach and intestine.

sample	warm μ	cold μ	$\frac{\text{cold } \mu}{\text{warm } \mu}$
acclimatized intact (10 grams)	13,700	15,900	1.16
acclimatized intact (20 grams)	13,700	15,300	1.12
acclimatized intact (30 grams)	13,600	14,900	1.10
acclimatized intact (40 grams)	13,600	14,600	1.07
acclimatized intact (50 grams)	13,600	14,400	1.06
acclimatized intact (60 grams)	13,500	14,100	1.04
acclimated intact	16,000	14,700	0.92
acclimatized stomach	13,800	15,300	1.11
acclimatized intestine	10,000	11,100	1.11
acclimated stomach, large	8,500	11,900	1.40
acclimated stomach, small	14,300	14,300	1.00
acclimated intestine, large	9,100	10,200	1.12
acclimated intestine, small	12,200	12,900	1.06
acclimated intestine homogenate	11,000	11,500	1.05

than warm-adapted ones (Table 26). Ratios of cold-adapted relative to warm-adapted μ values ranged from 0.92 to 1.40 with the great majority lying between 1.00 and 1.16.

The biochemical basis for the concept of temperature characteristics is firmly founded in reaction-rate theory (Hoar, 1966). However, complications occur when an attempt is made to extrapolate from the biochemical level to the level of physiological rate functions. It is generally conceded that Crozier's (1925) suggestion that the μ value of a given physiological rate function is essentially a reflection of the μ value of a "master" or "rate-limiting" reaction, and thus characteristic of a unique enzyme system, is an oversimplification. Difficulties arise because physiological rate functions are not simply expressions of catenary series of chemical reactions, but instead are the product of a complex inter-relationship of biochemical and physical events. Though it may be impossible uniquely to identify a given μ value with a specific enzyme system, nonetheless, μ values may "serve as the basis for inferences leading to fruitful experimentation by other procedures" (Giese, 1962).

One such attempt at drawing inferences from temperature characteristics is that of Prosser (1961). According to his formulation (Fig. 5) adaptation may occur with or without significant change in slope of rate-temperature curves (i.e. with or without changes in μ value). A change in μ following adaptation implies a relative rotation of warm- and cold-adapted R-T curves, while an unchanging μ indicates translation

of the curves. These two distinct types of adaptation may reflect different underlying mechanisms. Prosser (1967) suggests that translation is consistent with an increase in concentration or activity of certain enzyme systems, with no essential change in relative contributions of different metabolic pathways. Rotation, on the other hand, is consistent with a shift in relative activity of alternate metabolic routes with different enzyme and substrate systems which have their individual temperature characteristics. Each of these ideas concerning the mechanisms of thermal adaptation has received considerable experimental support (see Literature Review) and it is clear that they need not be mutually exclusive.

According to Prosser's hypothesis, the slight increase in the μ value of urchin respiration on cold-adaptation indicates that the process involves primarily an increase in enzyme concentration or activity; shifts in biochemical pathways probably play a minor role.

The precise significance of a modification of temperature characteristics during adaptation is still a matter of conjecture. Bullock (1955) reported on a number of cold-adapted poikilotherms that had lower temperature characteristics for various rate functions than warm-adapted ones. A number of species of cottid fish have a generally higher rate of respiration and a lower Q_{10} in winter than in summer (Morris, 1961). It is conventionally assumed that such a decrease in temperature dependence in the cold is adaptive; a decline in environmental temperature would result in less of a metabolic rate change in cold-adapted

(with shallower R-T slope) than in warm-adapted animals. However, this interpretation may be too simplistic, for numerous exceptions have been reported (see Literature Review). It has even been argued that precisely the opposite to the conventional interpretation (i.e. an increase in μ on cold-adaptation) may be adaptive because in the cold a slight increase in temperature would result in a greater augmentation of metabolism (and thus a greater level of activity) in systems with steeper rate-temperature slopes. Changes in μ values of respiration of S. droebachiensis on cold-adaptation are probably not of sufficient magnitude to serve any real adaptive purpose on the basis of either of the above interpretations.

vi) Thermal adaptation and animal size:

One aspect of thermal adaptation which has been neglected to a large extent is the relationship between animal size and ability to adapt. It is clear from both intact-animal (Fig. 9) and excised tissue (Fig. 30) studies that in S. droebachiensis degree of adaptation is inversely proportional to animal size. The fact that this inverse relationship is demonstrable even in the tissues taken from small and large animals suggests that the phenomenon is attributable, neither to changes in whole-animal surface-volume relationships nor to animal size-related limitations of oxygen transport, but to processes at the cellular or biochemical levels; this suggests that the ability to adapt may be age, rather than size, related. It is conceivable that the decline in adaptive ability may be related to the well-documented (Zeuthen, 1947; Bertalanffy, 1957) fact that intensity of metabolism per unit of tissue decreases with increasing animal size. Studies on the relationship between animal size and augmentation of enzyme activity consequent upon cold-adaptation would be of considerable interest in this regard.

Further evidence that the phenomenon is not attributable to some peculiarity of urchin morphology or to respiratory mechanics comes from the observation that similar size-adaptation relationships have been recognized in forms as divergent as cockroach and mussel. In the mussel, Mytilus edulis, in which water-pumping rate at different latitudes exhibits geographic acclimatization, Rao (1953) found that "smaller animals compensate better than larger animals". Similarly, adults and

nymphs of the American cockroach, Periplaneta americana, are able to adapt respiratory metabolism to temperature, but "smaller adults are... ...doing a better job of acclimating than large adults and all sizes of nymphs are doing a better job than all sizes of adults" (Dehnel and Segal, 1956). Clearly, many more such studies will be required before it can be unequivocably stated that thermal adaptation ability is always inversely proportional to size, but these results from such diverse sources, and obtained under such different modes of adaptation, are certainly suggestive.

Neglect of this size-adaptation relationship can lead to significant errors. The majority of studies on laboratory adaptation involve groups of uniformly-sized animals. However, as Rao (1953) points out, in many geographic acclimatization studies no allowance is made for the, frequently considerable, differences in mean animal sizes among the populations being compared.

It is interesting to reconsider at this point the size-related respiratory depression at 15°C. referred to earlier (Fig. 9). In winter, respiratory depression at 15°C. is most pronounced in small urchins, and virtually non-existent in larger ones; but in summer, at 15°C., no such depression occurs in any animal, large or small. That the high-temperature depression effect is detectable in winter but not in summer might be viewed as a resistance adaptation, i.e. summer animals being warm-adapted require a higher temperature for thermal depression of metabolism than cold-adapted, winter urchins. If capacity and resistance adaptations are functionally interrelated, the size-adaptation relation-

ships may also have a common basis. If, indeed, such a relationship does exist, it may be admissible here to make a prediction, a prediction which - one hopes - may stimulate further research into this somewhat uncertain terrain. The hypothesis is that small urchins in adapting well to low temperature may simultaneously reduce high-temperature resistance. Large urchins, on the other hand, because of their diminished ability to adapt to cold do not reduce their high-temperature resistance as much as small animals and can thus stand greater extremes of thermal regimes. If such were in fact the case, then it is probable that larger, cold-adapted urchins also have a higher thermal-death temperature than smaller ones.

vii) Thermal compensation coefficients; assumptions and limitations:

Thermal compensation coefficients (T.C.C.), which provide a quantitative description of the magnitude and form of thermal adaptation, are particularly suited to comparative studies. In the present work, T.C.C. are used to compare adaptation ability in relation to size, adaptation under natural and laboratory conditions, and also to determine whether the thermal adaptation of respiration, demonstrable in intact animals, is similar to the metabolic adaptation that is demonstrable in isolated tissues. Determination of the T.C.C. of large numbers of species under relatively standard conditions may eventually permit answers to the largely neglected question of the extent of occurrence of thermal adaptation ability in the biosphere and to related questions regarding phyletic and geographic correlates of the presence or absence of thermo-adaptive ability in different spec es.

Thermal compensation coefficients should only be used after careful consideration of the underlying assumptions and limitations of the method. Despite obvious affinities to Prosser's (1961) adaptation patterns, the T.C.C. are not intended to be infallible indicators of biochemical mechanisms of adaptation. They should only be regarded as concise descriptions of empirical changes in R-T relationships of physiological rate functions, intended primarily for comparative purposes. Rotation and translation of R-T curves, following a temperature change, may indeed be expressions of different adaptation mechanisms (see Literature Review), but until the biochemical basis for temperature characteristics (μ) of physiological rate functions has been adequately clarified

it would be inadvisable rigidly to associate particular values of the T.C.C. with specific biochemical mechanisms. Similarly, the terms translation and rotation used in connection with adaptation patterns have no implied biochemical or biophysical significance, but are merely descriptive of the displacement of cold-relative to warm-adapted Arrhenius curves.

One fundamental assumption underlying the T.C.C. concept is that Arrhenius transformation of the rate-temperature data yields an essentially linear relationship. Most biological functions adequately fulfil this condition over a certain temperature range (Prosser, 1961). Occasional exceptions, however, may cause marked distortion of the T.C.C. and,unless appropriate adjustments are possible, may preclude their use.

Significant departure from linearity of the Arrhenius curve occurs at elevated temperatures. Characteristically, at some "critical temperature" the Arrhenius slope changes abruptly. Normally this sudden break occurs near the high, lethal temperature (Mutchmor, 1967) although there are exceptions (see below). This type of distortion of the T.C.C. can be avoided by restricting the Arrhenius plot to temperatures within the predetermined stress range, which in turn should be well below the thermal tolerance limits of the species.

Low-temperature adaptation may be accompanied by a decrease in tolerance of high temperature (resistance shift). If, as a result, the "critical temperature" falls within the stress range, the Arrhenius plot may be non-linear. Such a situation was encountered in the present study.

In both summer-acclimatized and warm-acclimated urchins the Arrhenius plot for intact-animal respiration was linear over the entire stress range (0° - 15°C.). In contrast, in both winter-acclimatized and cold-acclimated urchins the Arrhenius plot, although linear between 0° and 10°C. suddenly "broke" between 10° and 15°C. (Figs. 14, 29). A likely physiological explanation for this break has already been discussed (p. 186). In this case, to avoid distortion of the T.C.C., data from temperatures above the "critical temperature" have been excluded from calculation of the Arrhenius plot regression equation.

Departure from linearity may occasionally take the form of "plateaus" of relative temperature independence in the rate-temperature curve (Newell, 1969; Percy and Aldrich, 1971). If relatively broad "plateaus" occur within the stress range it may prove impossible to compute valid thermal compensation coefficients, particularly as the "plateaus" may shift with adaptation temperature. Such "plateaus" of temperature independence may represent yet another form of metabolic compensation, having particular adaptive significance in habitats characterized by frequent, rapid temperature fluctuations (e.g. intertidal).

The occurrence of such peculiarities in rate-temperature relationships emphasizes the desirability of plotting regression lines of Arrhenius-transformed rate-temperature data and assessing linearity, either visually or by correlation coefficients, prior to calculating the T.C.C.

viii) Mechanisms of adaptation:

Stomach and intestine tissues of S. droebachiensis undergo significant changes in water content on cold-adaptation (Table 21). It is surprising, however, that these tissues, both of which acclimate readily to low temperature, alter their water content in precisely opposite directions; that of stomach increasing and that of intestine decreasing in the cold.

Previous attempts to demonstrate relationships between acclimation and tissue water-content also reveal a lack of consistency. It is clear that tissue water-content may or may not change upon acclimation. For example, muscle water-content in the amphibians Rana pipiens and Bufo boreas does not change on cold-acclimation (Bishop and Gordon, 1967), while a significant change does occur in muscle tissue of R. esculenta (Stangenberg, 1955). Furthermore, in several instances where acclimation has been shown significantly to alter tissue water-content, the direction of change has not been consistent: in both liver and muscle of the goldfish, tissue water-content is directly proportional to acclimation temperature (Hoar and Cottle, 1952); and in the earthworm Lampito mauritii muscle water decreases on cold-acclimation (Saroja and Rao, 1965); in contrast, muscle water-content of the frog, R. esculenta, increases on cold-acclimation (Stangenberg, 1955).

An hypothesis has been proposed which explains thermal acclimation in poikilotherms in terms of changes in proportion of free to bound water (Precht et al, 1955). Changes in cell water-content, coupled with

changes in cellular protein concentration on acclimation could be expected to lead to changes in the proportion of free to bound water (Saroja and Rao, 1965). However, variability in the relationship between tissue water-content and acclimation, demonstrated both in the examples cited above and in the results obtained here with tissues of S. droebachiensis, cast doubt on the free vs. bound water hypothesis of the mechanism of adaptation as a concept of general validity.

Cold-acclimation gives rise to increased respiration of cell-free intestine homogenates. A number of studies indicates that such compensatory increases in metabolism may be attributable to shifts in the relative contributions to metabolism of glycolytic and hexosemonophosphate pathways. Cold-acclimation generally results in an increase in the latter (McWhinnie and O'Connor, 1967; Ekberg, 1962). The conventional method of detecting such a qualitative, metabolic shift involves comparing the relative sensitivities of respiratory metabolisms of warm- and cold-acclimated homogenates to the glycolytic inhibitor iodoacetic acid (inhibits glyceraldehyde-3-phosphate dehydrogenase), and to the hexosemonophosphate (HMP) shunt inhibitor 5-bromouracil (5-BU) (inhibits glucose-6-phosphate dehydrogenase) (McWhinnie and O'Connor, 1967).

The importance of the HMP pathway as an alternate route to glycolysis varies considerably from tissue to tissue. In some, the HMP shunt is virtually non-existent, while in others it may be of greater significance than glycolysis (Fruton and Simmonds, 1958).

Attempts at inhibiting respiration of urchin intestine homogenates with 5-BU were unsuccessful, suggesting perhaps that the HMP route is non-existent in this tissue. However, direct spectrophotometric assay for G-6-P dehydrogenase revealed that the enzyme is present, but that it is not subject to inhibition by 5-BU. In this connection it must be borne in mind that it has been shown that the inhibition of G-6-P dehydrogenase by 5-BU varies markedly depending upon the enzyme's source (Hochster, 1961).

The presence of G-6-P dehydrogenase suggests that the HMP shunt may occur in urchin intestine. The shunt is also active in digestive tract tissue (hepatopancreas) of the crayfish Orconectes virilis (McWhinnie and Corkill, 1964). It is estimated that in crayfish tissue 10% of the available substrate is oxidized via the HMP route. In urchin intestine it seems probable that the shunt plays an even less significant role in metabolism. While the metabolic rate of urchin intestine is approximately 60% that of O. virilis hepatopancreas (Altman and Dittmer, 1968), the G-6-P activity of the former is less than 15% that of the latter.

While G-6-P dehydrogenase activity did increase on cold-acclimation in the urchins, its apparently small contribution to total metabolism makes it doubtful that an increase in HMP shunt activity accounts for the considerable increase in respiratory metabolism which accompanies adaptation to cold. This view is supported by the respiration inhibition studies with IAA. If a substantial shift to the HMP shunt did occur on

cold-acclimation then it would be anticipated that respiration of cold-acclimated tissue would be less sensitive to glycolytic IAA inhibition than warm-acclimated tissue, as is, in fact, the case in the hepatopancreas of crayfish (McWhinnie and O'Connor, 1967). In urchins on the other hand cold-acclimated tissue is more sensitive to iodoacetic acid inhibition than warm-acclimated tissue, as shown by the fact that IAA abolishes the low-temperature induced augmentation of respiration, so that metabolic rates of warm- and cold-acclimated tissues become similar. Thus, here, the increase in metabolism on cold-acclimation is accompanied by a simultaneous increase in IAA sensitivity.

If pentose shunt augmentation is not adequate to account for the increased metabolism on cold-acclimation, then the most reasonable alternative, barring the possible involvement of other , unknown, shunts, is that acclimation primarily involves a quantitative increase in existing pathways in which glycolysis plays the leading role. The increase of HMP shunt activity can then be viewed as part of a general, quantitative increase in metabolism, without significant alteration in the relative contributions of the various enzyme systems.

It has been suggested that cold-acclimation may be accompanied by conformational changes in particular enzymes (Prosser, 1967). It may be that a conformational change in the glycolysis enzyme glyceraldehyde-3-phosphate dehydrogenase, rendering it more susceptible to IAA inhibition, occurs in conjunction with a cold-induced increase in activity of this enzyme in urchin intestine.

While these studies do not permit a conclusive explanation of the biochemical basis of acclimation in S. droebachiensis tissue, they do suggest important differences from the mechanism proposed for acclimation in crayfish tissue. More intensive biochemical studies will be required fully to characterize the exact nature of these differences.

ix) Adaptation of thermal tolerance:

The importance of considering the length of exposure in determining lethal temperatures has often been stressed (Fraenkel, 1960; Read, 1967). The time element makes it difficult to extrapolate from an experimentally determined, short-term, lethal temperature to maximum tolerable habitat temperature of indefinite duration. Short-term lethal temperature is generally higher than maximum tolerable habitat temperature (Read, 1967). Thus, although the short-term lethal temperature of S. droebachiensis is relatively low (100% mortality between 26° - 29°C., depending upon thermal adaptation state), maximum tolerable habitat temperature is undoubtedly lower still. Determining maximum habitat temperature more closely is complicated by seasonal resistance-adaptation. Summer urchins tolerate short-term exposure to high temperature better than winter animals (Fig. 27). The levels of both short-term, lethal and maximum tolerable habitat temperatures usually depend upon the environmental temperature (Read, 1967). It is, therefore reasonable to assume that seasonal adaptation of short-term lethal temperature in urchins is paralleled by a similar adaptation in maximum tolerable habitat temperature.

Gradual adaptation of urchins to temperatures somewhat higher than the summer norm may effectively raise the maximum tolerable habitat temperature yet higher. That S. droebachiensis is capable of a more extensive adaptive shift in short-term lethal temperature than I observed is suggested by Read's (1969) observation that in Maine tide pools,

where the summer water temperatures are higher than in Newfoundland, urchins were unaffected by several hours exposure to 28°C., whereas in my own observation in a tide pool at Portugal Cove (see below), urchins were adversely affected - motionless, with tube feet semi-contracted - at 23°- 24°C., even during the summer. At present there is no way of telling whether this difference in high-temperature tolerance reflects physiological or genetic adaptation. A further interesting observation by Read is that urchins compensate behaviorally for high temperatures; migrating, in a warming tide pool, from the warm shallows (30°- 32°C.) to the cooler bottom (28°C.).

Biochemical and biophysical events associated with heat death remain essentially unknown. It is generally conceded that critical events may be associated with a "weak link" in the constitution of the organism. This is supported by the observations that thermal tolerance limits of isolated tissues are generally greater than those of intact animals, and that death of individual tissues is sequential, different tissues succumbing at different temperatures (Prosser, 1961). Interest is currently focussed on the nervous system as the potential "weak link" and there is a growing consensus that temperature tolerance is probably "limited by the ability of the central system to hold the numerous reactions occurring in the body in some sort of harmony" (Clarke, 1967).

Specific damage to the "weak link" might be associated with enzyme inactivation, changes in cellular lipids, structural alterations in cell membranes, liberation of toxins by heated tissues or a variety of other

factors.

It is possible that the lethal effect of elevated temperature on urchins is not directly attributable to heat death of tissues as outlined above. Tube foot function is adversely affected by relatively low temperature (17°C. for initial stages of contraction in summer) suggesting that a phenomenon analagous to temperature-induced-failure of ventilation in fish (Precht, 1967) may be implicated. Tube feet provide the only significant route for oxygen transport to internal tissues (Farmanfarmaian, 1966) and are most effective in this role when fully extended and actively moving. It seems likely therefore, that the decrease in oxygen transport that must surely accompany tube foot contraction, occurring, as it does, at a time when tissue oxygen demand is rapidly increasing (in accordance with the Q_{10} rule) will result in anoxic conditions within the perivisceral cavity. Death or degeneration of a tissue, or tissues, particularly susceptible to anoxia could ultimately prove fatal. Measurement of oxygen tension in coelomic fluid of heat stressed urchins would be of interest in this regard. If "heat death" is indeed attributable to anoxia then seasonal shifts in thermal tolerance may, in fact, be a direct result of the adaptive shift in the upper temperature limit of tube foot function suggested in the activity studies (Fig. 22). Other factors may be involved in adaptation of thermal tolerance. Changes in tissue water-content, formation of protective substances and special molecular linkages have all been suggested (Precht, 1967).

A comprehensive review (Ushakov, 1964) of factors influencing tissue thermostability suggests that seasonal changes in temperature tolerance may be associated with reproduction. A number of papers cited support the contention that thermal tolerance changes in a wide spectrum of tissues are associated with fluctuations in concentration of gonadal hormones. The present data for S. droebachiensis are not adequate to assess fully the relationship between the urchins' heat tolerance and their reproductive cycle. The similarity of thermal tolerance of pre- and postspawning urchins does, however, indicate that the marked increase in gonad mass in winter is not, in itself, sufficient to account for the seasonal differences in high-temperature tolerance.

Seasonal shifts in high-temperature tolerance may be adaptive, for during the summer, urchins in tide pools frequently encounter temperatures considerably higher than those to which animals in the open water of the cove are exposed. In a small tide pool adjacent to the collecting site, temperature, on warm, sunny afternoons, occasionally rose to 23°- 24°C. during the 2-3 hours of tidal withdrawal; even higher temperatures may have been attained at times. Yet the pool always held large numbers (> 300) of urchins, and there was no evidence at any time of abnormal mortality although, as reported earlier, activity was curtailed at the elevated temperatures. Had no increase in high-temperature tolerance occurred in summer, then a noticeable number of urchins would have succumbed following even a one hour exposure to 24°C. If one assumes that only limited migrations of urchins into and out of the tide

pools occur, then it is conceivable that, in summer, such tide pool urchins become, as a consequence of repeated exposure to elevated temperature (intermittent stressor), physiologically adapted to higher temperatures than do urchins inhabiting the cove (microgeographic acclimatization). This represents an interesting area for further study, particularly as little is known concerning the relative effectiveness in inducing capacity and resistance adaptation of continuous and intermittent stressors.

Little information is available regarding adaptation of low lethal-temperatures in urchins. My results clearly indicate that S. droebachiensis readily withstands, and even adapts to, 0°C. In contrast, S. purpuratus, which has a more southerly range than S. droebachiensis, becomes limp, unresponsive, and dies within 24 hours at 0°C. (Farmanfarmaian and Giese, 1963).

x) Adaptive significance of thermal adaptation:

It has been said that thermal adaptation in poikilotherms to a limited extent "accomplishes the same results as homeothermism accomplishes for warm blooded animals" (Dehnel and Segal, 1956). Many poikilotherms, by means of adaptation, tend to maintain their vital functions and energy requirements at a relatively constant level despite considerable changes in body temperature. Such a capacity for metabolic homeostasis has particular adaptive value in the case of organisms, such as S. droebachiensis, that are exposed to extensive seasonal temperature fluctuations. The poikilotherm's only alternatives to adaptation are migration, hibernation (estivation) or death.

The enhanced metabolism of S. droebachiensis in winter permits a greater degree of activity than would otherwise be possible. This may have survival value, particularly in urchins living along coasts frequently subject to storms. Not only does a greater activity potential permit more forceful adhesion to the substrate, but it also increases the chance of reattachment should animals be dislodged by turbulence.

A late winter - early spring breeding season in S. droebachiensis necessitates that gonad growth and development continue throughout the winter. As indicated earlier, echinoids have relatively limited extragonadal nutritive reserves, and for normal gonad development to occur it is essential that feeding continue during the autumn and winter. When S. purpuratus was starved it failed to reproduce (Giese, 1959). Without the substantial increase in metabolism and activity associated

with low-temperature acclimatization in S. droebachiensis food consumption would probably be negligible and gonad development severely impaired. S. purpuratus, which has a winter breeding season, is also capable of adapting to low temperature (Farmanfarmaian and Giese, 1963). In contrast, Arbacia punctulata which is unable to adapt to low temperature (Boolootian and Cantor, 1965) breeds in summer (Harvey, 1956). Adaptation to low temperature thus permits some species to breed at times of the year that may be unsuitable for other species. As Cocanour and Allen (1967) point out with reference to S. droebachiensis and Echinarachnius parma, such differences in spawning time provide effective genetic isolation of two hybridizable species inhabiting the same area. In addition such an arrangement would eliminate much interspecific competition for food among larval stages.

While adaptation to low temperature permits urchins to maintain a relatively high metabolic rate during the winter, the reverse process, adaptation to high temperature, permits the organism to reduce its metabolic rate to a more efficient level during summer and thus diminish its food requirements.

An increase in maximum tolerable temperature of activity and survival during the summer has obvious adaptive significance. It not only permits the animal to function effectively and survive indefinitely at normal summer temperatures, but in addition enables it to tolerate greater short-term temperature increases, a factor of definite survival value during tide pool entrapment in summer.

In addition to permitting S. droebachiensis to function efficiently in the face of seasonal temperature fluctuations, thermal adaptation also permits the species to function efficiently over a wide geographic range. S. droebachiensis occurs in subzero arctic waters (Grainger, 1955) and has also succeeded in extending its intertidal range as far south as Cape Cod on the Atlantic coast and Friday Harbor, Washington, on the Pacific coast of North America (Swan, pers. comm.).

xi) Thermal adaptation and the origin of S. droebachiensis:

Bullock (1955) remarked that occurrence of thermal acclimatization ability among animals is "apparently wide but far from universal". Frequently, species living in close proximity and subject to similar seasonal temperature fluctuations differ markedly in their ability to adapt to low temperature. An excellent example, involving the crustaceans Talorchestia megalophthalma and Emerita talpoida (see Literature Review), was reported by Edwards and Irving (1943). In addition, Strongylocentrotus purpuratus of the Pacific coast of North America adapts readily to 5°C. (Farmanfarmaian and Giese, 1963) while its Atlantic coast counterpart, Arbacia punctulata does not adapt to low temperature (Boolootian and Cantor, 1965). Even a cursory review of the literature suffices to confirm the observation of Pamatmat (1969) that there is "no apparent phylogenetic trend in the phenomenon". Such apparent randomness in occurrence has been a continuing puzzle to physiologists.

A possible solution is suggested by the speculation of Armitage (1962) that the occurrence in some circumpolar antarctic species of a potential for capacity adaptation over a temperature range far greater than that presently encountered may be a reflection of a genetic history related to an earlier habitat with greater temperature variation. I would carry this argument yet further by suggesting that the presence of the ability to adapt in a given species, may reflect changing thermal regimes experienced by the species during its history.

According to this view, the temperature limits of the adaptive capability of a species, in general, reflect the long term maximum and minimum habitat temperatures experienced during a great expanse of its history. During the gradual change from some relatively stable, original habitat-temperature the species may gradually evolve biochemical modifications that permit more efficient metabolism at the slightly altered temperature, while at the same time retaining the genetic potential for re-establishing the original biochemical state that permitted maximum metabolic efficiency at the original temperature. By such a stepwise process the species could eventually acquire the genetic potential for rapidly modifying its metabolism to permit maximum metabolic efficiency over a relatively wide temperature range. Current uncertainty regarding many biochemical aspects of adaptation precludes speculation as to the exact nature of these stepwise modifications.

S. droebachiensis provides a possible example of just such a relationship between the genetic history of a species and its thermo-adaptive ability. To establish the nature of this relationship we must consider first, the origin and dispersion of S. droebachiensis and secondly, the character of past long-term temperature trends in associated geographic regions.

Ekman (1967) in reviewing the zoogeographic relationship between North Atlantic and North Pacific fauna concludes that available evidence supports the view that "a considerable part of the North Atlantic boreal fauna and the Polar Sea and arctic fauna is derived from the North

Pacific". The genus Strongylocentrotus is one of several cited as evidence. According to this interpretation,of the nine Strongylocentrotus species found in the North Pacific only one*, S. droebachiensis, has succeeded in invading the Polar Sea and spreading into the North Atlantic.

It is clear that any such migration must have occurred by way of the Bering Strait, a marine channel that has opened and closed intermittently over the past 100 million years. The seaway was open to marine migration prior to the paleocene. Additional openings occurred in the late miocene, late pliocene and during several of the interglacial phases of the pleistocene (Briggs, 1970). It may be that elements of the Strongylocentrotus genus extending into the relatively warm Polar Sea (see below) during one of the early seaway openings became reproductively isolated from Pacific elements by reformation of the Bering land bridge, a situation conducive to speciation (Kendeigh, 1961). Such a Polar Sea species with metabolic machinery designed for efficient functioning at temperate or even subtropical temperatures would have been exposed to a general cooling trend as shown by the following:

* Ekman apparently did not differentiate between S. droebachiensis and S. pallidus.

Period	Epoch	Polar Sea temperature*
Quaternary	Pleistocene	-1.9°- 0°C.
	Pliocene	2°C.
	Miocene	7°C.
Tertiary	Oligocene	10°C.
	Eocene	
	Paleocene	
Cretaceous (Upper)		16°- 17°C.

Regardless of the precise time of its extension into the Polar Sea it is clear from the above that the North Pacific - Polar Sea Strongylocentrotus complex has been subject to an overall long-term declining temperature. It should be possible to distinguish within this complex two distinct thermo-adaptive groups, on the basis of minimal temperature limits of adaptation capability. One group, including all the wholly Pacific elements of the complex, although exposed over long periods to a generally declining temperature regime and thus capable of adapting over a fairly wide temperature range, were never subject to the extreme minimum temperatures experienced by the Polar Sea group. The latter group, which has extended its adaptive capability to temperatures

* From Emiliani (1961) as cited by Briggs (1970).

of zero and probably lower, is represented by S. droebachiensis (and possibly S. pallidus). S. purpuratus, a representative of the former group adapts to 5°C., but not to lower temperatures (Farmanfarmaian and Giese, 1963). Additional information is required concerning lower adaptive limits of the other Strongylocentrotus species that have been restricted to the Pacific.

At a later date, S. droebachiensis presumably extended south into both Atlantic and Pacific to assume its present distribution, its southward extension possibly being limited by the maximum thermal adaptation limits established earlier in its genetic history.

Extending a similar line of reasoning to other marine species we can predict that in the North Atlantic, those species which exhibit an ability to adapt to low temperature are either indigenous or have migrated from the North Pacific or Polar Sea. On the other hand, species that are unable to adapt to low temperature and become torpid during colder months will generally be those that have extended into the North Atlantic from more southern regions. A similar scheme can, no doubt, be formulated for other geographic areas.

I fully appreciate the tentative nature of this scheme, based as it is upon considerable biochemical speculation and many zoogeographical assumptions. It may nevertheless serve to promote discussion, stimulate research and thus ultimately clarify an aspect of adaptation that has been long ignored.

SUMMARY AND CONCLUSIONS

This study concerns the influence of seasonal temperature fluctuations encountered in a typical boreal, coastal marine habitat on the performance of the sea urchin, S. droebachiensis. Intact-animal respiration, in vitro tissue respiration, and righting activity serve as indicators of performance. A detailed description of thermal adaptation in this species is presented, from the point of view of both acclimatization in the natural habitat and acclimation in the laboratory.

A series of three thermal compensation coefficients are proposed. These quantitatively describe the character and magnitude of adaptation responses, using Prosser's adaptation patterns as a basis. Here, the T.C.C. are used to compare thermal adaptation ability of animals of different size, acclimatization and acclimation, and adaptation in the intact animal and in its component tissues. Assumptions and limitations associated with use of the T.C.C. are discussed. It is hoped that these coefficients will permit the presentation of adaptation data in a systematic form suitable for comparative studies and thereby lead to a better understanding of the occurrence of adaptation ability in different species and of the associated phyletic and geographic correlates.

Regression equations of respiration on weight, at a series of temperatures, of urchins collected in summer and winter are presented. Respiration rates are higher in winter than in summer at 0°, 5°, and 10°C. At 15°C. summer and winter rates are similar, possibly a consequence of the fact that impairment of tube foot function may occur at

lower temperatures in winter than in summer, which would result in a decrease in oxygen transport by the water vascular system in winter, at elevated temperatures.

The regression coefficient "b" is greater in summer than in winter, indicating that in winter urchins consume proportionally less oxygen with increasing weight than in summer.

The increase in urchin respiration in winter is attributable to augmentation of cellular metabolism, rather than to modifications in functioning of the water vascular oxygen transporting system. This is demonstrated by the fact that a parallel increase occurs in the *in vitro* metabolism of excised stomach, intestine and esophagus tissue. It is suggested that the small acclimatization response observed for excised tube feet is not an accurate reflection of the magnitude of normal *in vivo* adaptation.

In gonad tissue, adaptation could not be demonstrated because any change in metabolism induced by thermal acclimatization is overshadowed by the increase in *in vitro* respiration in winter resulting from changes in reproductive condition.

An activity coefficient based on the urchin's righting reflex is defined, and shown to be a useful indicator of overall physiologic activity. As a consequence of metabolic acclimatization, urchins are able to maintain a relatively high rate of activity during the coldest months. In addition, optimum, maximum and minimum temperatures for righting are higher in summer than in winter.

Adaptation of metabolism permits S. droebachiensis to continue gonad development during autumn and winter and to spawn early in spring.

Maximum food consumption occurs in spring and autumn. It may be that the increase in feeding at these two times is associated with replenishment of gonad reserves following spawning, and with rapid gonad development preceding spawning, respectively. A decline in food consumption in winter appears to be compensated for by a considerable increase in feeding efficiency.

In addition to the evident seasonal capacity-acclimatization, S. droebachiensis also exhibits a marked seasonal resistance-acclimatization, detectable as an increase in short-term high-temperature tolerance in summer.

That the annual temperature fluctuation is the principal factor involved in inducing seasonal compensatory adjustments in metabolism is suggested by the fact that an adaptation response, similar in extent and character to that observed in urchins in their natural habitat, occurs in animals acclimated for 4-6 weeks in the laboratory at summer-like and winter-like temperatures. The effects of acclimation are demonstrable in metabolism of both intact urchins and excised stomach and intestine tissues.

Activity coefficients of warm-and cold-acclimated urchins are similar to those of freshly-collected urchins in summer and winter, respectively. Acclimation of activity to low temperature requires approximately 4-5 weeks for completion.

The ability to adapt to low temperature is inversely proportional to animal size (age?). This is shown not only in seasonal acclimatization of intact-urchin respiration, but also in acclimation of metabolism of stomach and intestine tissues. The importance of taking size into consideration in adaptation studies is stressed.

In general, the metabolism of both intact urchins and excised tissues adhere closely to the Q_{10} rule, increasing two to three times for each 10°C. rise in temperature.

Temperature characteristics (μ) of respiration, measured in both intact urchins and excised tissues under conditions of acclimatization and acclimation increase only slightly, if at all, on cold-adaptation. The possible adaptive significance of a change or lack of change in temperature coefficients is discussed.

Coefficients of adaptation are generally in the vicinity of 0.800, indicative of partial adaptation. Coefficients of rotation in most instances are close to unity suggesting that adaptation primarily involves translation of rate-temperature curves. Such an adaptation response corresponds essentially to Prosser's pattern II a.

Thermal acclimation is demonstrable in the respiratory metabolism of cell-free intestine homogenates. The magnitude and pattern of the response is comparable to that detectable in the respiration of intestine slices.

Biochemical changes associated with thermal acclimation are investigated. The water content of stomach tissue decreases significantly, while that of intestine increases significantly, on cold-acclimation.

An attempt is made to determine whether adaptation in S. droebachiensis occurs as a result of a shift in metabolism from glycolysis to hexose-monophosphate shunt. The HMP shunt enzyme glucose-6-phosphate dehydrogenase is present in intestine, and it does increase substantially on cold-acclimation. However, the level of G-6-P dehydrogenase activity is considered to be too low adequately to account for the metabolic increase on acclimation. Such a view is consistent with the additional observation that cold-acclimated respiration is more sensitive to the glycolytic inhibitor iodoacetic acid than is warm-acclimated. It is suggested that adaptation may involve an increase in glycolysis coupled with conformational changes in specific enzymes that render them more sensitive to iodoacetic acid inhibition. Such a quantitative increase in metabolism would be consistent with the observation that adaptation takes place primarily by a translation of rate-temperature relationships.

The adaptive significance of both capacity adaptation and high-temperature resistance adaptation in the life of S. droebachiensis is discussed.

It is suggested that the present thermal adaptation ability of S. droebachiensis may reflect the origin and genetic history of the species.

LITERATURE CITED

* ADERHOLD, R. 1888. Beitrag zur kenntniss Richtender Krafte bei der Bewegung niederer Organismen. Jena. Zeitschr., 22:310-342.

ADOLPH, E.F. 1956. General and specific characteristics of physiological adaptations. Amer. J. Physiol., 184:18-28.

ALTMAN, P.L. and D.S. Dittmer. 1968. Metabolism. Biological Handbooks. Fed. Amer. Soc. Exptl. Biol., Bethesda, Maryland, pp. 737.

ANDERSON, J.M. 1966. Aspects of nutritional physiology. In: Physiology of the Echinoderms. (R.A. Boolootian, ed.). Interscience, New York, pp. 329-357.

ARMITAGE, K.B. 1962. Temperature and oxygen consumption of Orchomonella chilensis (Heller), (Amphipoda: Gammeroidea). Biol. Bull. (Woods Hole), 123:225-232.

BARCROFT, J. 1934. Features of the Architecture of Physiological Function. Cambridge University Press, London, pp. 368.

BASLOW, M.H. 1967. Temperature adaptation and the central nervous system of fish. In: Molecular Mechanisms of Temperature Adaptation. (C.L. Prosser, ed.). Publ. 84. Amer. Assoc. Adv. Sci., Wash., D.C., pp. 205-226.

BEHRE, E.H. 1918. An experimental study of acclimation to temperature in Planaria dorotocephala. Biol. Bull. (Woods Hole), 35: 277-317.

BERTALANFFY, L. VON. 1957. Quantitative laws in metabolism and growth. Quart. Rev. Biol., 32(3):217-231.

BISHOP, L.G. and M.S. Gordon. 1967. Thermal adaptation of metabolism in anuran amphibians. In: Molecular Mechanisms of Temperature Adaptation. (C.L. Prosser, ed.). Publ. 84. Amer. Assoc. Adv. Sci. Wash., D.C., pp. 263-280.

BOOLOOTIAN, R.A. 1966. Reproductive physiology. In: Physiology of Echinodermata. (R.A. Boolootian, ed.). Interscience Publ., New York, pp. 561-613.

_____ and H. Cantor. 1965. Preliminary report on respiration, nutrition, and behavior of Arbacia punctulata. Life Sci., 4:1567-1571.

BRANDTS, J.F. 1967. Heat effects on proteins and enzymes. In: Thermobiology. (A.H. Rose, ed.). Academic Press, London, pp. 25-72.

BRIGGS, J.C. 1970. A faunal history of the North Atlantic Ocean. Systematic Zool., 19(1):19-34.

BULLOCK, T.H. 1955. Compensation for temperature in metabolism and activity of poikilotherms. Biol. Revs. (Cambridge), 30:311-342.

CHAPMAN, D. 1967. The effect of heat on membranes and membrane constituents. In: Thermobiology. (A.H. Rose, ed.). Academic Press, London, pp. 123-146.

* CHOE, S. 1962. Biology of the Japanese common sea cucumber, Stichopus japonicus Selenka. Pusan Fisheries College, Pusan National University, pp. 226.

CHRISTOPHERSON, J. 1967. Adaptive temperature responses of microorganisms. In: Molecular Mechanisms of Temperature Adaptation. (C.L. Prosser, ed.). Publ. 84. Amer. Assoc. Adv. Sci., Wash., D.C., pp. 327-348.

CLARK, D.P. 1955. Influence of body weight, temperature, and season upon the rate of oxygen consumption of the terrestrial amphipod, Talitrus sylvaticus (Haswell). Biol. Bull. (Woods Hole), 108:253-257.

CLARKE, K.U. 1967. Insects and temperature. In: Thermobiology. (A.H. Rose, ed.). Academic Press, London, pp. 293-352.

COCANOUR, B. and K. Allen. 1967. The breeding cycles of a sand dollar and a sea urchin. Comp. Biochem. Physiol., 20:327-331.

COOK, P.A. and P.A. Gabbott. 1970. Seasonal changes in the free glycerol level in the body parts of the adult barnacle, Balanus balanoides. Mar. Biol., 7(1):11-13.

CRISP, D.J. and D.A. Ritz. 1967. Changes in temperature tolerance of Balanus balanoides during its life cycle. Helgolaender Wiss. Meeresuntersuch, 15:98-115.

CROZIER, W.J. 1925. On biological oxidations as function of temperature. J.Gen Physiol., 7:571-579.

CUMMINS, K.W., L.D. Miller, N.A. Smith, and R.M. Fox. 1965. Experimental Entomology. Reinhold Publ. Corp., New York, pp.176.

DEHNEL, P.A. 1955. Rates of growth of gastropods as a function of latitude. Physiol. Zool., 28:115-144.

_____ 1956. Growth rates in latitudinally and vertically separated populations of Mytilus californianus. Biol. Bull. (Woods Hole), 110:43-53.

_____ and E. Segal. 1956. Acclimation of oxygen consumption to temperature in the American cockroach (Periplaneta americana). Biol. Bull. (Woods Hole), 11:53-61.

DIXON,W.J. and F.J. Massey. 1957. Introduction to Statistical Analysis. 2nd. edition. McGraw Hill, New York, pp.488.

DREYER,W.A. 1932. The effect of hibernation and seasonal variation of temperature on the respiratory exchange of Formica ulkei. Physiol. Zool. 5:301-331.

EDWARDS, G.A. and L. Irving. 1943. Temperature and seasonal metabolism of the sand crab, Emerita. J.Cell.Comp. Physiol., 21: 169-181.

EKBERG, D.R. 1958. Respiration in tissues of goldfish adapted to high and low temperatures. Biol. Bull. (Woods Hole), 114:308-316.

_____ 1962. Anaerobic and aerobic metabolism in gills of the crucian carp adapted to high and low temperatures. Comp. Biochem. Physiol., 5:123-128.

EKMAN, S. 1967. Zoogeography of the Sea. Sidgwick and Jackson, London, pp. 417.

* EMILIANI, C. 1961. The temperature decrease of surface seawater in high latitudes and of abyssalhadal water in open oceanic basins during the past 75 million years. Deep Sea Res., 8:144-147.

FARMANFARMAIAN, A. 1966. The respiratory physiology of echinoderms. In: Physiology of Echinodermata. (R.A. Boolootian, ed.). Interscience Publ., New York, pp. 245-265.

_____ and A.C. Giese. 1963. Thermal tolerance and acclimation in the western purple sea urchin, Strongylocentrotus purpuratus. Physiol. Zool., 36(3):237-243.

_____ and J.H. Phillips. 1962. Digestion, storage, and translocation of nutrients in the purple sea urchin (Strongylocentrotus purpuratus). Biol. Bull. (Woods Hole), 123:105-120.

FELL, H.B. and D.L. Pawson. 1966. General biology of echinoderms. In: Physiology of Echinodermata. (R.A. Boolootian, ed.). Interscience Publ., New York, pp. 1-48.

FOX, H.M. and C.A. Wingfield. 1938. A portable apparatus for the determination of oxygen dissolved in a small volume of water. J. Exptl. Biol., 15:437-445.

FRAENKEL, G. 1968. The heat resistance of intertidal snails at Bimini, Bahamas; Ocean Springs, Mississippi, and Woods Hole, Massachusetts. Physiol. Zool., 41(1):1-13.

FREEMAN, J.A. 1950. Oxygen consumption, brain metabolism, and respiratory movements of goldfish during temperature acclimatization, with special reference toward lowered temperatures. Biol. Bull. (Woods Hole), 99:416-424.

FRUTON, J.S. and S. Simmonds. General Biochemistry. 2nd. ed. John Wiley, New York, pp. 1077.

FRY, F.E.J. 1967. Responses of vertebrate poikilotherms to temperature. In: Thermobiology. (A.H. Rose, ed.). Academic Press, New York, pp. 375-409.

FUJI, A. 1962. Studies on the biology of the sea urchin. V. Food consumption of Strongylocentrotus intermedius. Jap. J. Ecol., 12:181-186.

GALTSOFF, P.S. 1964. The American Oyster, Crassostrea virginica, Gmelin. Fishery Bull. 64. Fish and Wildlife Service, Bur. Comm. Fisheries, pp. 480.

GIESE, A.C. 1959. Comparative physiology: annual reproductive cycles of marine invertebrates. Ann. Rev. Physiol., 21:547-576.

_____ 1962. Cell Physiology. 2nd. ed. Saunders, Philadelphia, pp. 592.

_____, A. Farmanfarmaian, S. Hilden, and P. Doezema. 1966. Respiration during the reproductive cycle in the sea urchin, Strongylocentrotus purpuratus. Biol. Bull. (Woods Hole), 130:192-201.

GRAINGER, E.H. 1955. Echinoderms of Ungava Bay, Hudson Strait, Frobisher Bay, and Cumberland Sound. "Calanus" Series, No. 9. J. Fish. Res. Bd. Can., 12(6):899-916.

GRAY, P. 1961. The Encyclopedia of the Biological Sciences. Reinhold, New York, pp. 1119.

HART, J.S. 1952. Geographical variations of some physiological and morphological characters in certain fresh-water fish. Univ. Toronto Biol. Ser. 60, Publ. Ontario Fisheries Res. Lab., 72:1-79.

HARVEY, E.B. 1956. The American Arbacia and Other Sea Urchins. Princeton University Press, Princeton, pp. 298.

HAUGAARD, N. and L. Irving. 1943. The influence of temperature upon the oxygen consumption of the cunner, Tautogolabrus adspersus, in summer and winter. J. Cell. Comp. Physiol., 21:19-26.

HIMMELMAN, J.H. 1970. Some aspects of the ecology of Strongylocentrotus droebachiensis in eastern Newfoundland. M.Sc. Thesis, Memorial University of Newfoundland.

HOAR, W.S. 1955. Seasonal variations in the resistance of goldfish to temperature. Trans. Roy. Soc. Canada, 49:25-34.

_____ 1966. General and Comparative Physiology. Prentice-Hall, Englewood Cliffs, N.J., pp. 815.

_____ and M.K. Cottle. 1952. Some effects of temperature acclimation on the chemical constitution of goldfish tissues. Can. J. Zool., 30:49-54.

HOCHACHKA, P.W. 1965. Isoenzymes in metabolic adaptation of a poikilotherm: subunit relationships in lactic dehydrogenases of goldfish. Arch. Biochem. Biophys., 111:96-103.

_____ 1967. Organization of metabolism during temperature compensation. In: Molecular Mechanisms of Temperature Adaptation. (C.L. Prosser, ed.). Publ. 84. Amer. Assoc. Adv. Sci., Wash., D.C., pp. 177-203.

_____ and F.R. Hayes. 1962. The effect of temperature acclimation on the pathways of glucose metabolism in the trout. Can. J. Zool., 40:261-270.

HOCHSTER, R.M. 1961. 5-bromouracil: a new inhibitor of glucose-6-phosphate dehydrogenase. Biochem. Biophys. Res. Comm., 6(4):289-292.

HOPKINS, H.S. 1946. The influence of season, concentration of sea water, and environmental temperature upon the oxygen consumption of tissues in Venus mercenaria. J. Exptl. Zool., 102(2):143-158.

HUMASON, G.L. 1967. Animal Tissue Techniques. 2nd. ed. W.H. Freeman, San Francisco, pp. 569.

HYMAN, L.H. 1955. The Invertebrates. Echinodermata. The Coelomate Bilateria. McGraw-Hill, New York, pp. 763.

JENNINGS, H.S. 1907. Behavior of the starfish, Asterias forreri de Loriol. Univ. Calif. Publ. in Zool., 4(2):53-185.

JOHANSEN, K. and R.L. Vadas. 1967. Oxygen uptake and responses to respiratory stress in sea urchins. Biol. Bull. (Woods Hole), 132(1):16-22.

JOHNSON, P.V. and B.I. Roots. 1964. Brain lipid fatty acids and temperature acclimation. Comp. Biochem. Physiol., 11:303-309.

KENDEIGH, S.C. 1961. Animal Ecology. Prentice-Hall, Englewood Cliffs, N.J., pp. 468.

KINNE, O. 1964. Non-genetic adaptation to temperature and salinity. Helgolaender Wiss. Meeresuntersuch, 9(1):433-458.

KLEITMAN, N. 1941. The effect of temperature on the righting of echinoderms. Biol. Bull. (Woods Hole), 80:292-298.

* KOLLER, G. and H. Meyer. 1933. Versuche über die Atmung der Echinodermen (Asterias rubens und Echinus miliaris). Biol. Zbl., 53:655-661.

KONISHI, J. and C.P. Hickman, Jr. 1964. Temperature acclimation in the central nervous system of rainbow trout, Salmo gairdnerii. Comp. Biochem. Physiol., 13:433-442.

KORNBERG, A. 1950. Enzymatic synthesis of TPN. J. Biol. Chem., 182:805-813.

* KREHL, L. and F. Soetbeer. 1899. Untersuchungen uber die Warmokonomie der Poikilothermen Wirbeltiere. Pflug. Arch. Ges. Physiol., 77:61.

KUENZLER, E.J. 1961. Structure and energy flow of a mussel population in a Georgia salt marsh. Limnol. Oceanog., 6:191-204.

LAGERSPETZ, K.Y.H. and I. Dubitscher. 1966. Temperature acclimation of the ciliary activity in the gills of Anodonta. Comp. Biochem. Physiol., 17:665-671.

LEVITT, J. 1967. Status of the sulfhydryl hypothesis of freezing injury and resistance. In: Molecular Mechanisms of Temperature Adaptation. (C.L. Prosser, ed.). Publ. 84. Amer. Assoc. Adv. Sci., Wash., D.C., pp. 41-51.

LEWIS, J.B. 1968. Respiration of the tropical sea urchin, Diadema antillarum Philippi. Physiol. Zool., 41(4):476-480.

LING, G.N. 1967. Effects of temperature on the state of water in the living cell. In: Thermobiology. (A.H. Rose, ed.). Academic Press, London, pp. 5-24.

MAYER, A.G. 1914. The effects of temperature upon tropical marine animals. Papers from Tortugas Lab., Carnegie Inst., 6:3-24.

MCLEESE, D.W. 1956. Effects of temperature, salinity, and oxygen on the survival of the American lobster. J. Fish. Res. Bd. Can., 13(2):247-272.

MCPHERSON, B.F. 1968. Feeding and oxygen uptake of the tropical sea urchin, Eucidaris tribuloides (Lamarck). Biol. Bull. (Woods Hole), 135(2):308-321.

MCWHINNIE, M.A. 1964. Temperature responses and tissue respiration in antarctic crustacea with particular reference to the krill, Euphausia superba. In: Biol. Antarctic Seas. Vol. I, Antarctic Res. Ser., Natl. Acad. Sci., Natl. Res. Counc., Publ. 1190, pp. 63-72.

_____ 1967. The heat responses of invertebrates (exclusive of insects). In: Thermobiology. (A.H. Rose, ed.). Academic Press, New York, pp. 353-373.

_____ and A.J. Corkill. 1964. The hexosemonophosphate pathway and its variation in the intermolt cycle in crayfish. Comp. Biochem. Physiol., 12:81-93.

_____ and J.D. O'Connor. 1967. Metabolism and low temperature acclimation in the temperate crayfish, Orconectes virilis. Comp. Biochem. Physiol., 20:131-145.

MICALLEF, H. and W.H. Bannister. 1967. Aerial and aquatic oxygen consumption of Monodonta turbinata (Mollusca: Gastropoda). J. Zool. (London), 151:479-482.

MOORE, H.B. 1937. A comparison of the biology of Echinus esculentus in different habitats. Part III. J. Mar. Biol. Assoc. U.K., 21:711-719.

MORRIS, R.W. 1961. Distribution and temperature sensitivity of some eastern Pacific cottid fishes. Physiol. Zool., 34:217-227.

MUTCHMOR, J.A. 1967. Temperature adaptation in insects. In: Molecular Mechanisms of Temperature Adaptation. (C.L. Prosser, ed.). Publ. 84. Amer. Assoc. Adv. Sci., Wash., D.C., pp. 165-176.

* NAYEEMUNNISA, K. 1966. Lipid metabolism in relation to thermal acclimation in earthworms. Ph.D. Thesis, Sri Venkateswara University, Tirupati, A.P., India.

NEWELL, R.C. 1969. Effect of fluctuations in temperature on the metabolism of intertidal invertebrates. Amer. Zoologist, 9:293-307.

PAINE, R.T. and R.L. Vadas. 1969. The effects of grazing by sea urchins, Strongylocentrotus spp. on benthic algal populations. Limnol. Oceanog., 14(5):710-719.

PAMATMAT, M.M. 1969. Seasonal respiration of Transennella tantilla Gould. Amer. Zoologist, 9:418-426.

PEISS, C.N. and J. Field. 1950. Tissue metabolism of fish from different latitudes. Biol. Bull. (Woods Hole), 99:213-224.

PERCY, J.A., F.A. Aldrich and T.R. Marcus. 1971. Influence of environmental factors on respiration of excised tissues of American oysters, Crassostrea virginica (Gmelin). Can. J. Zool.: in press.

_____ and F.A. Aldrich. 1971. Temperature independent metabolic rate in mollusc tissue. Nature: in press.

PETERSON, R.H. and J.M. Anderson. 1969. Effects of temperature on brain tissue oxygen consumption in salmonid fishes. Can. J. Zool., 47:1345-1353.

PRECHT, H. 1958. Concepts of the temperature adaptation of unchanging reaction systems of cold blooded animals. In: Physiological Adaptation. (C.L. Prosser, ed.). Amer. Physiol. Soc., Wash., D.C., pp. 50-78.

_____ 1964. Uber die Bedeutung des Blutes für die Temperaturadaptation von Fischen. Zool. Jahrb. Abt. Allgem Zool. Physiol. tiere, 71:313-327.

_____ 1967. A survey of experiments on resistance adaptation. In: The Cell and Environmental Temperature. (A.S. Troshin, ed.). Pergamon Press, New York, pp. 307-321.

* _____, J. Christophersen, and H. Hensel. 1955. Temperatur und Leben. Springer Verlag, Berlin, pp. 514.

PROSSER, C.L. 1962. Acclimation of poikilothermic vertebrates to low temperatures. In: Proceedings, Symposia on Arctic Biology and Medicine. II. Comparative Physiology of Temperature Regulation. (J.P. Hannon and E. Viereck, eds.). Arctic Aeromedical Lab., Fort Wainwright, Alaska, pp. 1-44.

_____ and F.A. Brown. 1961. Comparative Animal Physiology. 2nd. edition. W.B. Saunders, Philadelphia, pp. 688.

RAO, K.P. 1953. Rate of water propulsion of Mytilus californianus as a function of latitude. Biol. Bull. (Woods Hole), 104:171-181.

_____ 1967. Biochemical correlates of temperature acclimation. In: Molecular Mechanisms of Temperature Adaptation. (C.L. Prosser, ed.). Publ. 84. Amer. Assoc. Adv. Sci., Wash., D.C., pp. 227-244.

_____ and K. Saroja. 1963. Physiology of low temperature acclimation in tropical poikilotherms. V. Changes in the activity of neurosecretory cells in the earthworm, Lampito mauritii, and evidence for a humoral agent influencing metabolism. Proc. Indian Acad. Sci., Sect. B, 58:14-18.

READ, K.R.H. 1967. Thermostability of proteins in poikilotherms. In: Mechanisms of Temperature Adaptation. (C.L. Prosser, ed.). Publ. 84. Amer. Assoc. Adv. Sci., Wash., D.C., pp. 93-106.

_____ 1969. Thermal tolerance of the bivalve mollusc, Modiolus modiolus L. Amer. Zoologist, 9(2):279-282.

REESE, E.S. 1966. The complex behavior of echinoderms. In: Physiology of Echinodermata. (R.A. Boolootian, ed.). Interscience, New York, pp. 157-218.

RITZ, D.A. 1968. The effects of temperature on activity and survival of barnacles. Ph.D. Thesis, University of Wales.

ROBBIE, W.A. 1949. Respiration of the tissues of some invertebrates and its inhibition by cyanide. J. Gen. Physiol., 32:655-670.

ROBERTS, J.L. 1952. Studies on acclimatization of respiration to temperature in the lined shore crab, Pachygrapsus crassipes Randall. Ph.D. Thesis, University of California, Los Angeles.

_____ 1957. Thermal acclimation of metabolism in the crab, Pachygrapsus crassipes. II. Mechanisms and the influence of season and latitude. Physiol. Zool., 30:242-255.

_____ 1964. Metabolic responses of fresh-water sunfish to seasonal photoperiods and temperature. Helgolaender Wiss. Meeresuntersuch, 9:459-473.

_____ 1967. Metabolic compensations for temperature in sunfish. In: Molecular Mechanisms of Temperature Adaptation. (C.L. Prosser, ed.). Publ. 84. Amer. Assoc. Adv. Sci., Wash., D.C., pp. 245-262.

SAROJA, K. and K.P. Rao. 1965. Some aspects of the mechanism of thermal acclimation in the earthworm, Lampito mauritii. Zeits. fur vergl. Physiol., 50:35-54.

SASSAMAN, C. and C.P. Mangum. 1970. Patterns of temperature adaptation in North American Atlantic coastal actinians. Mar.Biol.,7(2): 123-130.

SCHOLANDER,P.F., W. Flagg, V. Walters, and L. Irving. 1953. Climatic adaptation in arctic and tropical poikilotherms. Physiol. Zool., 26:67-92.

* SCHWARTZ, F. 1884. Der Einfluss der Schwerkraft auf die Richtungsbewegungen der Chlamydomonaden und Euglenen. Ber. Dtsch. Bot. Ges. 2:51-72.

SEGAL, E. 1956. Microgeographic variation as thermal acclimation in an intertidal mollusc. Biol. Bull. (Woods Hole), 111: 129-152.

_____, K.P. Rao, and T.W. James. 1953. Rate of activity as a function of intertidal height within populations of some littoral molluscs. Nature, 172: 1108-1111.

SHORTRESS, G.S. 1942. Adaptation of rate of locomotion of Peranema. Physiol. Zool., 15:184-195.

SIEFKIN, M. and K.B. Armitage. 1968. Seasonal variation in metabolism and organic nutrients in three Diaptomus (Crustacea:Copepoda). Comp. Biochem. Physiol., 24:591-609.

SIMINOVITCH, D., B. Rheaume, and R. Sachar. 1967. Seasonal increase in protoplasm and metabolic capacity in tree cells during adaptation to freezing. In: Molecular Mechanisms of Temperature Adaptation. (C.L. Prosser, ed.). Publ. 84. Amer. Assoc. Adv. Sci., Wash., D.C., pp. 3-40.

SNEDECOR, G.W. 1956. Statistical Methods Applied to Experiments in Agriculture and Biology. 5th. ed. Iowa State Univ. Press., Ames, Iowa, pp. 534.

SPIEGEL, M.R. 1961. Theory and Problems of Statistics. Schaum, New York, pp. 359.

STANGENBERG, G. 1955. Der Temperatureinfluss auf Lebensprozesse und der Cytochrom-c-Gehalt beim Wasserfrosch. Arch. Ges. Physiol., 260:320-332.

STRICKLAND, J.D.H. and T.R. Parsons. 1965. A Manual of Sea Water Analyses. 2nd. ed. Bull. Fish. Res. Bd. Can., 125, pp. 203.

SWAN, E.F. 1962. Evidence suggesting the existence of two species of Strongylocentrotus (Echinoidea) in the Northwest Atlantic. Can. J. Zool., 40:1211-1222.

SZYBALSKI, W. 1967. Effects of elevated temperatures on DNA and on some polynucleotides: denaturation, renaturation and cleavage of glycosidic and phosphate ester bonds. In: Thermobiology. (A.H. Rose, ed.). Academic Press, London, pp. 73-122.

TAKATSUKI, S. 1929. The heart pulsation of oysters in tropical seas compared with that of those living in seas of the temperate zone. Records of Oceanographic Works in Japan, 1:102-112.

TASHIAN, R.E. and C. Ray. 1957. The relation of oxygen consumption to temperature in some tropical, temperate, and boreal anuran amphibians. Zoologica, 42:63-68.

USHAKOV, B. 1964. Thermostability of cells and proteins of poikilotherms and its significance in speciation. Physiol. Revs. (Cambridge), 44:518-560.

VASSEUR, E. 1952. Geographic variation in the Norwegian sea urchins Strongylocentrotus droebachiensis and S. pallidus. Evolution, 6:87-100.

VERNBERG, F.J. 1959. Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus Uca. II. Oxygen consumption of whole organisms. Biol. Bull. (Woods Hole), 117:163-184.

_____ 1962. Comparative physiology: latitudinal effects on physiological properties of animal populations. Amer. Rev. Physiol., 24:517-546.

_____, C. Schlieper, and D.E. Schneider. 1963. The influence of temperature and salinity on ciliary activity of excised gill tissues of molluscs from North Carolina. Comp. Biochem. Physiol., 8:271-285.

VERNBERG, W.B. and F.J. Vernberg. 1965. Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus Uca. V. Effect of temperature on tissue respiration. Comp. Biochem. Physiol., 17:363-374.

ZEUTHEN, E. 1947. Body size and metabolic rate in the animal kingdom with special regard to the marine micro-fauna. Compt. Rend. Lab. Carlsberg (Ser. Chim.), 26(3):17-161.

* Not seen by author.

APPENDICES

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Appendix I. Surface temperatures at Portugal Cove during period of study.

Date		Temperature (°C.)
1968	Dec. 4	3.0
	Dec. 14	2.5
1969	Jan. 10	0.9
	Jan. 29	0.4
	Feb. 24	0.0
	Mar. 18	0.0
	Apr. 8	0.3
	Apr. 19	1.8
	May. 4	3.9
	May. 21	4.6
	Jun. 22	12.0
	Jul. 1	10.6
	Jul. 13	13.6
	Aug. 4	14.3
	Aug. 12	14.5
	Aug. 30	14.5
	Sep. 29	11.5
	Oct. 27	7.6
	Nov. 29	5.0
	Dec. 29	3.7
1970	Jan. 28	0.3
	Feb. 9	0.9
	Mar. 8	1.7
	Mar. 20	1.7
	Apr. 5	1.0
	May. 15	3.8
	May. 29	5.4
	Jun. 24	10.0

Appendix II. Sample calculation of thermal compensation coefficients.

Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of excised intestine from warm-and cold-acclimated, small S. droebachiensis are:

COLD-ACCLIMATED		WARM-ACCLIMATED	
temperature (°C.)	O_2 consumption	temperature (°C.)	O_2 consumption
5°	415.4	5°	348.4
10°	833.8	10°	572.3
15°	926.0	15°	745.2

By converting temperature to the reciprocal of the absolute temperature and multiplying by 10^5, and converting the respiration rates to natural logarithms we obtain:

T'	$\log_e O_2$ consumption	T'	$\log_e O_2$ consumption
359.7	6.028	359.7	5.852
353.4	6.726	353.4	6.349
347.2	6.831	347.2	6.613

Calculating the regression of $\log_e O_2$ consumption on T' for warm-and cold-acclimated groups we obtain equations in the form:

$$\log_e O_2 \text{ consumption(cold)} = a_c + b_c T'$$

$$\log_e O_2 \text{ consumption(warm)} = a_w + b_w T'$$

For the data given above the regression equations are:

$$\log_e O_2 \text{ consumption(cold)} = 29.2576 - 0.0643T'$$

$$\log_e O_2 \text{ consumption(warm)} = 27.7848 - 0.0609T'$$

From these regression equations we calculate $\log_e O_2$ consumption values at T_1' (corresponding to the 0°C. acclimation temperature) and T_2' (corresponding to the 15°C. acclimation temperature) for both warm-and cold-acclimated groups.

since: $T_1' = 366.3$

and: $T_2' = 347.2$

then:

cold-acclimated: $\log_e O_2 \text{ consumption}(T_1') = \log_e k_1c = 5.7045$

$\log_e O_2 \text{ consumption}(T_2') = \log_e k_2c = 6.9326$

warm-acclimated: $\log_e O_2 \text{ consumption}(T_1') = \log_e k_1w = 5.4771$

$\log_e O_2 \text{ consumption}(T_2') = \log_e k_2w = 6.6403$

From these relationships the thermal compensation coefficients are calculated as follows:

COEFFICIENT OF ADAPTATION

$$C.A. = \frac{\log_e k_2w - \log_e k_1c}{\log_e k_2w - \log_e k_1w}$$

$$C.A. = \frac{6.6403 - 5.7045}{6.6403 - 5.4771}$$

$$C.A. = \frac{0.9358}{0.1632}$$

$$C.A. = 0.805$$

COEFFICIENT OF ROTATION

$$C.R. = \frac{\log_e k_{2c} - \log_e k_{1c}}{\log_e k_{2w} - \log_e k_{1w}}$$

$$C.R. = \frac{6.9326 - 5.7045}{6.6403 - 5.4771}$$

$$C.R. = \frac{1.2281}{1.1632}$$

$$C.R. = 1.056$$

AXIAL COEFFICIENT

The T' value at the intersection point is:

$$T_i' = \frac{a_w - a_c}{b_c - b_w}$$

$$T_i' = \frac{27.7848 - 29.2576}{(-0.0643) - (-0.0609)}$$

$$T_i' = \frac{-1.4728}{-0.0034}$$

$$\overline{T_i'} = 433.2$$

and:

$$Ax. = - \left[\frac{T_i' - \left(\frac{T_1' + T_2'}{2} \right)}{T_1' - \left(\frac{T_1' + T_2'}{2} \right)} \right]$$

$$T_1' = 366.3$$

$$T_2' = 347.2$$

therefore:

$$Ax. = - \left[\frac{433.2 - 356.8}{366.3 - 356.8} \right]$$

$$Ax. = - \left[\frac{76.4}{9.5} \right]$$

$$Ax. = - 8.04$$

Thus:

$$T.C.C. = {}^{15}_{0}(0.805;\ 1.056;\ -8.0)$$

Appendix III a. Respiration rates ($\mu l.O_2$/animal/hr.) of summer-acclimatized S. droebachiensis.

TEMPERATURE												
0°C.			5°C.			10°C.			15°C.		
wt. (gms.)	sex	O_2 cons.	wt. (gms.)	sex	O_2 cons.	wt. (gms.)	sex	O_2 cons.	wt. (gms.)	sex	O_2 cons.
28.45	M	278.79	25.95	M	163.44	30.31	M	583.35	33.77	M	563.67
19.01	F	170.82	14.47	F	146.47	23.19	F	259.11	34.39	M	540.26
55.94	F	369.03	34.00	F	343.84	40.12	M	477.81	25.72	F	442.29
32.32	M	307.80	23.94	M	206.47	30.34	M	311.41	34.71	F	689.37
11.69	M	37.21	30.22	F	263.14	18.20	F	252.93	41.56	M	791.59
33.47	M	192.51	51.13	F	443.16	25.54	M	394.61	37.64	F	711.72
62.51	F	273.39	18.96	M	190.55	47.33	F	541.99	21.70	M	432.21
26.00	F	97.71	28.73	F	139.74	51.26	F	546.27	33.52	F	500.64
41.05	M	212.89	16.12	F	161.51	23.42	M	435.97	44.73	F	700.18
22.99	M	110.00	38.93	F	335.73	34.36	F	357.05	22.94	M	595.87
32.95	F	171.48	56.38	M	431.18	19.63	M	299.05	56.58	M	849.44
9.56	M	96.42	39.55	F	255.88	67.80	M	770.67	43.77	M	523.84
23.57	M	114.21	43.18	M	397.43	28.73	M	323.29	48.73	M	786.62
38.93	M	103.53	17.67	M	149.90	55.94	M	537.24	19.29	M	452.96
56.32	F	245.89	41.40	F	312.14	24.92	F	422.18	31.89	M	531.91
7.56	M	64.71	5.66	F	98.00	44.04	M	628.04	41.41	M	718.62
39.78	-	63.74	48.32	F	509.94	18.40	F	213.47	14.04	F	335.97
31.59	M	123.59	30.92	F	240.27	45.90	F	603.80	53.99	M	829.27
39.05	M	183.45				35.27	M	445.95	41.61	F	711.71
46.20	M	207.07				49.70	M	620.44	18.56	M	354.99
33.11	M	171.48				40.89	F	504.43	56.90	M	910.52
26.34	M	109.03				32.13	F	391.75	18.74	F	433.36
20.00	F	163.39				37.99	M	465.45	44.40	M	839.64
37.91	M	187.65				34.09	M	346.59	37.65	M	734.76
						12.82	M	216.32	18.58	F	367.67
						46.90	F	416.00	25.29	M	559.57
									33.50	F	755.50

*				
N	24	18	26	27
log a	1.159	1.310	1.487	1.739
b	0.693	0.742	0.745	0.685
r	0.671	0.887	0.872	0.908
$S_{y.x}$	0.174	0.092	0.071	0.052

* Exponential regression analyses; for complete summary refer to Table 1.

Appendix III b. Respiration rates ($\mu l.O_2$/animal/hr.) of winter-acclimatized S. droebachiensis.

TEMPERATURE

0°C.			5°C.			10°C.			15°C.		
wt. (gms.)	sex	O_2 cons.	wt. (gms.)	sex	O_2 cons.	wt. (gms.)	sex	O_2 cons.	wt. (gms.)	sex	O_2 cons.
19.43	F	178.20	44.17	M	349.51	24.89	F	525.05	22.85	M	383.20
31.53	M	170.17	17.86	M	167.97	47.87	F	651.20	46.25	M	754.50
17.40	M	166.64	27.62	M	379.89	24.52	M	402.24	14.04	F	396.05
57.87	F	281.59	21.20	M	266.24	43.05	F	627.40	32.88	M	666.43
28.43	M	142.14	64.75	F	467.95	39.40	M	759.26	34.61	-	677.58
23.26	M	167.15	38.72	M	409.84	33.52	F	523.63	51.07	F	939.47
13.61	M	126.88	22.01	M	268.07	17.28	F	327.50	32.54	M	740.48
20.84	F	191.27	40.44	M	421.70	17.81	F	316.56	24.27	F	407.69
56.06	M	329.03	30.81	F	448.51	23.36	F	302.27	34.17	F	563.22
48.40	F	301.92	25.43	F	234.08	38.62	F	602.17	51.72	M	849.12
18.19	F	160.18	42.18	M	443.15	20.97	-	329.88	23.03	F	368.81
19.68	M	202.89	32.76	M	434.21	42.68	M	423.66	41.26	M	835.97
16.27	F	126.80	21.10	-	256.60	17.59	M	397.48	25.19	M	451.15
45.96	M	359.88	25.13	M	204.42	27.64	M	600.74	38.08	F	547.78
15.96	F	120.22	46.50	F	298.41	26.71	F	360.35	20.55	M	383.68
14.03	M	105.80	24.00	M	207.28	51.97	-	592.65	36.64	F	677.58
16.47	M	103.30	41.63	-	328.79	18.47	F	373.68	52.86	M	780.51
23.80	M	131.77	35.31	F	224.43	28.03	M	497.44	27.34	F	583.24
26.35	M	102.75	32.87	-	256.60	32.47	M	697.37	55.73	F	1003.51
27.35	F	134.21	53.35	F	328.79	22.82	M	423.66			
36.14	F	161.50	40.54	M	243.55	23.34	F	561.71			
48.68	M	207.95				59.48	F	592.65			

*				
N	22	21	22	19
log a	1.353	1.620	1.864	1.560
b	0.620	0.568	0.557	0.803
r	0.775	0.636	0.732	0.909
$S_{y.x}$	0.098	0.099	0.081	0.057

* Exponential regression analyses; for complete summary refer to Table 1.

Appendix III c. Statistical summary of exponential regression of respiration on weight of male and female, summer- and winter-acclimatized S. droebachiensis.

season	temperature	sex	N	r	$\bar{X}$*	$\bar{Y}$**	b+	log a+	p
summer	0°C.	F	7	0.756	35.0	197.2	0.640	1.306	N.S.
summer	0°C.	M	14	0.732	27.1	143.2	0.551	1.367	
winter	0°C.	F	9	0.805	25.9	174.8	0.549	1.467	N.S.
winter	0°C.	M	13	0.760	25.3	164.3	0.659	1.291	
summer	5°C.	F	12	0.879	27.5	243.1	0.697	1.382	N.S.
summer	5°C.	M	6	0.937	28.2	234.3	0.923	1.030	
winter	5°C.	F	6	0.503	40.6	320.4	0.444	1.791	N.S.
winter	5°C.	M	12	0.723	30.0	300.7	0.780	1.325	
summer	10°C.	F	11	0.901	32.7	389.1	0.810	1.363	N.S.
summer	10°C.	M	15	0.856	33.0	433.8	0.691	1.588	
winter	10°C.	F	12	0.804	28.8	462.2	0.578	1.821	N.S.
winter	10°C.	M	8	0.545	28.3	509.4	0.488	1.999	
summer	15°C.	F	10	0.918	28.4	542.0	0.723	1.683	N.S.
summer	15°C.	M	17	0.877	35.0	626.1	0.651	1.791	
winter	15°C.	F	9	0.891	31.3	574.8	0.750	1.638	N.S.
winter	15°C.	M	9	0.925	34.3	620.7	0.865	1.465	

* Mean wet wt. (grams).

** Mean respiration rate ($\mu l O_2$/animal/hr.)

\+ Coefficients of exponential regression equation $\log M = \log a + b \log(wt.)$

Appendix IV a. Respiration rates ($\mu l.O_2$/gm. wet wt./hr.) of warm-acclimated S. droebachiensis.

	TEMPERATURE							
	0°C.		5°C.		10°C.		15°C.	
	wt. (gms.)	O_2 cons.	wt. (gms.)	O_2 cons.	wt. (gms.)	O_2 cons.	wt. (gms.)	O_2 cons.
	28.26	5.65	32.06	4.33	29.83	14.23	29.91	21.33
	28.94	2.99	27.53	7.23	34.44	13.49	24.53	20.52
	25.47	2.89	31.61	6.13	30.89	12.67	32.84	15.10
	31.92	3.49	34.19	5.05	26.64	5.69	30.43	18.26
	25.84	3.89	26.27	6.61	26.73	9.55	28.11	22.12
	32.61	5.09	26.23	10.81	27.08	14.58	28.27	21.45
	31.96	3.67	29.44	6.61	28.97	9.97	24.88	16.51
	31.12	6.12	26.87	5.32	34.52	10.37	32.55	20.08
	29.11	3.75	28.33	5.81	25.38	9.77	26.59	13.86
	31.24	3.31	27.44	8.70	33.60	6.92		
					26.66	14.99		
N	10	10	10	10	11	11	9	9
$\bar{X}$	29.65	4.09	29.00	6.66	29.52	11.12	28.68	18.80
$\bar{X}_{30}$*		122.70		199.80		333.60		564.00
S	2.42	1.07	2.61	1.80	3.24	2.98	2.86	2.84
S.E.	0.81	0.36	0.87	0.60	1.02	0.94	1.01	1.00

* Mean respiration rate ($\mu l.O_2$/animal/hr.) of urchins of 30 grams standard weight.

Appendix IV b. Respiration rates ($\mu l.O_2$/gm. wet wt./hr.) of cold-acclimated S. droebachiensis.

TEMPERATURE								
0°C.		5°C.		10°C.		15°C.	
wt. (gms.)	O_2 cons.	wt. (gms.)	O_2 cons.	wt. (gms.)	O_2 cons.	wt. (gms.)	O_2 cons.
30.03	5.92	31.76	9.17	25.00	17.76	34.37	18.52
30.44	5.06	27.20	8.07	31.19	14.66	26.91	23.43
33.87	4.07	28.31	10.87	35.61	12.94	28.24	20.04
26.86	4.49	33.40	10.03	28.49	15.13	33.57	16.60
25.00	5.50	33.17	10.46	26.17	15.42	29.08	19.34
29.12	6.36	25.96	8.06	30.53	13.79	25.80	26.30
26.27	6.39	25.19	11.15	27.54	12.21	31.87	19.06
31.18	5.67	31.18	9.59	32.79	12.63	25.86	14.77
29.08	4.14	25.46	12.28	28.89	14.80	30.27	14.53
26.43	6.04			25.70	10.40	32.06	15.43
				26.63	12.93		
N	10	10	9	9	11	11	10	10
$\bar{X}$	28.83	5.36	29.07	9.96	28.96	13.88	29.80	18.80
$\bar{X}_{30}$*		160.80		298.80		416.40		564.00
S	2.57	0.83	3.15	1.13	3.13	1.88	2.96	3.61
S.E.	0.86	0.28	1.11	0.47	0.99	0.59	0.99	1.20

* Mean respiration rate ($\mu l.O_2$/animal/hr.) of urchins of 30 grams standard weight.

Appendix V a. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of excised stomach from summer-and winter-acclimatized S. droebachiensis.

TEMPERATURE									
5°C.		10°C.		15°C.		20°C.		25°C.	
summer	winter	summer	winter	summer	winter	summer	winter	summer	winter
233.93	428.31	372.04	564.47	601.33	832.11	750.66	1457.83	1206.17	1836.69
321.63	391.78	518.36	537.40	548.77	966.13	1122.63	934.78	1119.69	2149.44
247.75	377.14	498.15	702.56	446.76	1008.44	507.09	1447.20	1109.79	1625.53
175.69	371.14	437.81	525.94	570.43	820.18	764.82	1575.10	1106.46	1914.30
188.56	300.05	381.24	550.27	569.40	1014.59	893.37	1547.33	2103.16	1865.46
202.09	342.60	516.70	566.92	564.87	1053.95	947.00	1053.42	2286.85	2004.73
221.99	472.75	372.22	679.02	513.58	876.84	1187.60	1115.48	2110.26	2149.50
258.56	260.02	488.56	522.52	506.98	895.96	811.24	992.58	1839.31	1785.69
	279.73	382.56	571.66	591.14	923.95	1050.58	1460.36		1747.52
		299.15		549.55		1046.79	1188.85		1569.44
		301.19				972.37	1128.14		1742.49
		327.64							1619.20
		427.19							
		524.64							
		320.23							
N	8	9	15	9	10	9	11	11	8	12
$\bar{X}$	231.3	358.2	411.2	580.1	546.3	932.5	914.0	1263.7	1610.2	1834.2
S	43.4	65.9	79.4	61.7	43.6	78.6	187.0	225.5	488.8	185.6
S.E.	16.4	23.3	21.2	21.8	14.5	27.8	59.13	71.30	184.7	56.0
t	4.450		5.549		12.313		3.770		1.160	
p	< 0.001		< 0.001		< 0.001		< 0.005		< 0.250	

Appendix V b. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of excised intestine from summer-and winter-acclimatized S. droebachiensis.

TEMPERATURE									
5°C.		10°C.		15°C.		20°C.		25°C.	
summer	winter	summer	winter	summer	winter	summer	winter	summer	winter
334.80	437.14	345.13	532.70	745.71	864.83	945.65	640.31	1336.70	1761.52
395.69	443.55	310.56	689.96	540.82	958.80	1202.94	425.03	1506.23	2157.67
420.85	395.89	496.16	636.85	767.55	742.28	849.11	712.54	1054.23	1353.36
258.12	420.92	461.33	427.72	776.98	780.54	840.71	1204.43	978.46	1359.33
347.64	361.79	562.13	490.95	644.54	884.56	1079.68	1376.01	1744.66	1347.21
334.72	228.41	509.47	462.46	715.84	663.80	1329.12	1078.16	1100.46	1274.03
205.86	446.81	455.45	527.76	724.69	832.07	1190.65	863.90	1293.09	1459.35
226.25	418.46	409.77	530.16	751.25	793.15	951.24	708.26	1240.58	1390.39
221.10	431.76	382.57	561.09	753.45	673.43	724.83	1000.87		1463.69
		422.08	556.92	521.59		669.51	1269.55		1416.89
		523.48		621.64		897.00	819.36		1587.47
		461.01		642.39			1001.08		1608.94
		359.87							
		484.41							
N	9	9	14	10	12	9	11	12	8	12
$\bar{X}$	305.0	398.3	441.7	541.7	683.9	799.3	970.9	925.0	1281.8	1515.0
S	74.9	65.1	70.5	73.4	84.4	91.8	198.2	269.8	236.2	233.7
S.E.	26.5	23.0	19.6	24.5	25.5	32.5	62.7	81.3	89.3	70.5
t	2.658		3.191		2.797		0.44		2.05	
p	< 0.01		< 0.005		< 0.01		< 0.40		< 0.05	

Appendix V c. Respiration rates ($\mu l.O_2/gm.$ dry wt./hr.) of excised esophagus from summer-and winter-acclimatized S. droebachiensis.

TEMPERATURE									
5°C.		10°C.		15°C.		20°C.		25°C.	
summer	winter	summer	winter	summer	winter	summer	winter	summer	winter
172.0	284.4	225.4	472.9	466.1	594.7	750.2	227.2*	921.7	1288.4
100.9	281.0	289.8	447.8	561.4	660.9	560.9	903.1	862.0	1704.6
115.6	199.5	298.4	384.5	539.4	539.2	525.7	653.9	1241.3	1034.7
159.3		276.6	344.9	412.5	594.6	497.7	785.8		1493.0
268.9		268.3	420.4	333.2	1019.5*	579.3	1056.7		1268.7
									913.3
N	5	3	5	5	5	4	5	4	3	6
$\bar{X}$	163.3	255.0	271.7	414.1	462.5	597.3	582.6	849.9	1008.3	1283.8
S	59.0	39.2	25.4	45.4	83.6	43.1	88.5	148.4	166.6	264.7
S.E.	29.5	27.7	12.7	22.7	41.8	24.9	44.2	85.7	117.8	118.4
t	2.262		5.479		2.771		2.770		1.640	
p	< 0.050		< 0.0005		< 0.025		< 0.025		< 0.250	

* Not included in calculation of mean.

Appendix V d. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of excised tube feet from summer-and winter-acclimatized S. droebachiensis.

	TEMPERATURE									
	5°C.		10°C.		15°C.		20°C.		25°C.	
	summer	winter	summer	winter	summer	winter	summer	winter	summer	winter
	106.0	289.2	341.4	490.7	402.3	654.6	896.0	799.8	1003.1	1143.8
	214.8	178.7	471.0	329.7	494.5	717.9	777.6	737.8	865.1	1042.1
	134.2	222.0	310.5	348.3	724.7	825.3	757.8	887.4	1392.7	969.8
		317.4		342.2		498.2		914.1		1674.8
		156.9		226.6		571.1				783.0
		178.5								1658.3
N	3	6	3	5	3	5	3	4	3	6
$\bar{X}$	151.6	223.8	374.3	347.5	540.5	653.4	810.5	834.8	1087.0	1212.0
S	46.1	60.0	69.5	84.2	135.6	113.6	61.0	70.2	223.4	339.0
S.E.	32.6	26.8	49.2	42.1	95.9	56.8	43.2	40.5	158.0	151.6
t	1.708		0.413		1.012		0.400		0.570	
p	< 0.25		< 0.40		< 0.25		< 0.40		< 0.40	

Appendix V e. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of excised gonads from summer-and winter-acclimatized S. droebachiensis.

	TEMPERATURE									
	5°C.				10°C.				15°C.	
	male		female		male		female		male	
	winter	summer	winter	summer	winter	summer	winter	summer	winter	summer
		215.0		11.0	3316.6	212.1	175.1	30.0	2090.7	92.8
		73.6		11.8	2491.7	34.2	525.0	17.5	2431.7	134.7
		227.0			4133.5		629.1	20.6	1732.5	108.3
		97.8			2775.1			21.4		102.7
		118.3			1908.7			19.1		
		88.5			1830.1			18.7		
		121.0								
N		7		2	6	2	3	6	3	4
$\bar{X}$		134.5		11.4	2742.6	123.1	443.0	21.2	2084.9	109.6
S.E.		21.5		0.1	327.2	104.7	112.3	1.7	165.0	7.8

TEMPERATURE									
15°C.		20°C.				25°C.			
female		male		female		male		female	
winter	summer	winter	summer	winter	summer	winter	summer	winter	summer
610.4	22.4	1607.8	149.7	287.8	9.7	946.9		1193.1	
520.6	31.1	2014.9	151.3	601.7	26.7	3768.7		459.3	
15.6*	13.4	2577.8	108.5	324.9		1096.3		461.2	
162.2	79.3		107.9	802.6		4466.6			
313.4			95.7			3273.0			
			209.4						
4	4	3	6	4	2	5		3	
401.7	36.5	2066.8	137.1	504.2	18.2	2710.3		704.5	
87.6	12.8	229.9	15.8	105.3	6.0	638.8		199.7	

* Not included in calculation of mean.

Appendix V f. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of excised rectum from summer-acclimatized S. droebachiensis.

	5°C.	10°C.	15°C.	20°C.	25°C.
	614.8	580.6	489.5	1073.0	1607.0
	440.8	518.8	967.3	867.8	1363.6
	208.0		589.2	1015.1	2154.8
N=	3	2	3	3	3
$\bar{X}$=	421.2	549.7	682.0	985.3	1708.5
S.E.=	117.9	30.9	145.5	61.1	234.0

Appendix VI a. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of excised stomach from warm-and cold-acclimated, large S. droebachiensis.

TEMPERATURE

5°C.		10°C.		15°C.		20°C.	
warm	cold	warm	cold	warm	cold	warm	cold
303.5	306.1	413.9	381.0	378.1	532.6	944.2	899.4
296.7	257.1	249.0	443.6	451.3	720.5	716.6	1007.0
295.3	228.1	429.1	467.6	680.9	363.5	899.9	821.0
339.7	277.2	492.4	553.6	455.7	799.2	783.0	1133.1
240.5	339.0	569.2	639.2	506.8	653.8	1004.7	955.1
387.8	497.1	407.4	361.3	566.4	561.3		921.6
296.4	266.3	406.5	384.7	545.8	567.4		
139.7	163.5	298.0	432.1		756.2		
308.8	358.7	449.4	382.1		636.8		
203.0	307.0						
395.0	242.3						
391.7							
N 12	11	9	9	7	9	5	6
$\bar{X}$ 299.8	294.8	412.8	449.5	512.1	621.3	869.7	956.2
S 73.9	82.1	89.7	87.4	90.6	125.5	105.5	97.1
S.E. 22.3	26.0	31.7	30.9	37.0	44.4	52.7	43.4
t	0.14	0.82		1.88		1.26	
p	N.S.	< 0.250		< 0.025		N.S.	

Appendix VI b. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of excised stomach from warm-and cold-acclimated, small S. droebachiensis.

TEMPERATURE

5°C.		10°C.		15°C.	
warm	cold	warm	cold	warm	cold
310.7	434.9	411.5	867.6	818.4	1203.9
300.4	421.2	603.1	585.0	653.7	991.1
372.9	365.5	678.2	766.0	1042.5	1102.2
289.6	353.2	429.4	683.0	770.5	907.7
307.5	396.6	492.1	742.9	627.2	742.0
308.4	295.9	475.1	647.1	728.3	600.1
N	6	6	6	6	6	6
$\bar{X}$	314.9	377.9	514.9	715.3	773.4	924.5
S	26.9	46.5	95.4	90.6	136.7	205.3
S.E.	12.0	20.8	42.7	40.5	61.2	91.8
t	2.622		3.407		1.369	
p	< 0.025		< 0.005		< 0.100	

Appendix VI c. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of excised intestine from warm-and cold-acclimated, large S. droebachiensis.

TEMPERATURE							
5°C.		10°C.		15°C.		20°C.	
warm	cold	warm	cold	warm	cold	warm	cold
219.9	273.5	591.7	537.1	431.2	625.1	742.0	920.6
246.1	268.6	778.0	313.8	518.1	653.0	824.1	1001.2
352.8	207.0	576.5	531.8	523.3	688.6	782.5	857.2
337.4	368.7	592.5	1018.9*	550.3	771.6	921.6	1036.2
448.2	315.8	495.7	776.5	477.6	595.7	668.7	1015.5
406.4	343.2	441.3	478.7	697.8	730.5		1091.6
191.1	419.5	405.7	567.2	681.4	695.6		
273.6	423.6	466.2	520.6	579.6	653.6		
289.9	324.9				472.9		
384.3	463.8						
310.4	334.4						
	419.5						
N	11	12	8	7	8	9	5	6
$\bar{X}$	314.6	346.9	543.5	532.2	557.4	654.1	787.8	987.1
S	76.5	72.6	111.0	126.3	87.1	81.4	84.3	77.1
S.E.	24.2	21.9	41.9	51.6	32.9	28.8	42.1	34.5
t	0.99		0.16		2.21		3.66	
p	< 0.250		N.S.		< 0.025		< 0.005	

* Not included in calculation of mean.

Appendix VI d. Respiration rate ($\mu l.O_2$/gm. dry wt./hr.) of excised intestine from warm-and cold-acclimated, small S. droebachiensis.

	TEMPERATURE					
	5°C.		10°C.		15°C.	
	warm	cold	warm	cold	warm	cold
	361.1	463.1	539.7	987.8	807.8	1256.5
	326.6	482.1	578.9	696.0	641.5	735.1
	443.7	425.4	735.0	979.3	740.4	977.5
	340.8	333.1	665.4	778.3	843.4	862.7
	289.7	338.3	502.1	752.0	933.8	742.0
	328.3	450.5	412.5	809.4	504.4	982.3
N	6	6	6	6	6	6
$\bar{X}$	348.4	415.4	572.3	833.8	745.2	926.0
S	47.7	58.8	105.5	111.2	140.2	177.7
S.E.	21.3	26.3	47.2	49.8	62.7	79.4
t	1.979		3.815		1.786	
p	< 0.050		< 0.005		< 0.100	

Appendix VII a. Stability of respiration ($\mu l.O_2$/gm. dry wt.; expressed as cumulative O_2 consumption) of stomach and intestine slices and homogenates from S. droebachiensis (temperature 15°C.; medium either filtered seawater or buffered sucrose, pH 7.4).

	seawater		sucrose		sucrose	
Time	intact stomach	intact intestine	intact stomach	intact intestine	stomach homogenate	intestine homogenate
15	205.6(3)*	170.0(3)	273.8(6)	321.5(6)	186.5(3)	274.2(3)
30	389.6	369.1	702.3	710.3	325.5	489.8
45	564.7	538.5	973.8	1002.4	452.2	681.0
60	798.9	752.9	1235.8	1299.2	573.1	882.9
75	983.0	959.6	1516.3	1625.8	718.1	1070.0
90	1201.8	1128.9	1832.8	1957.7	839.1	1320.3
105	1405.9	1328.7	2137.2	2201.7	893.4	1479.7
120	1565.5	1505.0	2399.9	2446.5	978.0	1661.2
135	1778.1	1777.8	2734.8	2775.8	1056.5	1835.8
150	1983.6	1910.3	2934.9	2990.0	1201.4	2001.7

* Number of samples.

Appendix VII b. Respiration rates ($\mu l.O_2$/gm. dry wt./hr.) of intestine homogenates from warm-and cold-acclimated S. droebachiensis.

	TEMPERATURE					
	5°C.		10°C.		15°C.	
	warm	cold	warm	cold	warm	cold
	388.9	360.8	467.8	595.6	841.9	803.2
	381.9	469.8	417.4	589.4	564.4	841.8
	338.8	395.4	624.4	655.0	836.7	913.1
	369.1	588.0	661.8	830.4	719.9	1053.3
	437.7	451.1	628.9	691.8	852.3	1087.5
	317.8	502.8	557.8	713.2	635.5	979.8
N	6	6	6	6	6	6
$\bar{X}$	372.4	461.3	559.7	679.2	741.8	946.5
S	38.2	73.5	89.5	81.0	111.4	104.1
S.E.	17.1	32.9	40.0	36.2	49.8	46.6
t	2.401		2.223		3.001	
p	< 0.025		< 0.025		< 0.010	

Appendix VII c. Influence of iodoacetic acid concentration on respiration ($\mu l.O_2$/gm. dry wt./hr.) of intestine homogenates from S. droebachiensis (temperature 15°C.; sucrose medium, pH 7.4).

IODOACETIC ACID CONCENTRATION (M.)				
10^{-2}	10^{-3}	10^{-4}	10^{-5}	control
25.8	363.4	526.6	763.9	725.9
0.0	446.6	618.6	508.5	703.4
17.2	335.4	415.2	684.9	925.1
0.0	380.1	534.9	713.7	716.7
17.2	409.9	717.2	737.5	729.5
		601.8		779.1
				691.9
				588.1
				746.9
				1008.9
				969.2
				771.0
				713.7
				837.5
N	5	5	6	5	14
$\bar{X}$	12.0	387.1	569.1	681.7	779.1
S.E.	5.2	19.2	41.7	45.2	31.3

Appendix VII d. Stability of respiration ($\mu l O_2$/gm. dry wt./hr.) of intestine homogenates from warm-and cold-acclimated S. droebachiensis (temperature 15°C.; sucrose medium,pH 7.4).

WARM-ACCLIMATED				COLD-ACCLIMATED			
period I*	period II**	diff.	percent change	period I*	period II**	diff.	percent change
711.8	645.2	- 66.6	- 9.4	773.4	816.5	+43.1	+5.6
675.6	567.6	-108.0	-16.0	986.1	962.0	-24.1	-2.4
849.5	871.8	+ 22.3	+ 2.6	909.1	909.1	0.0	0.0
776.5	820.2	+ 43.7	+ 5.6	815.7	792.2	-23.5	-2.9
728.2	728.2	0.0	0.0	907.8	907.8	0.0	0.0
638.2	638.2	0.0	0.0	791.7	766.0	-25.7	-3.2
		N = 6				N = 6	
		$\bar{X}$ = -2.9%		t = 0.663		$\bar{X}$ = -0.5%	
		S.E.= 3.3		p < 0.300		S.E.= 1.3	

* Initial 40 minutes of run.

**Final 40 minutes of run.

Appendix VII e. Iodoacetic acid inhibition of respiration ($\mu l.O_2$/gm. dry wt./hr.) of intestine homogenates from warm-and cold-acclimated S. droebachiensis (temperature 15°C.; sucrose medium, pH 7.4).

WARM-ACCLIMATED				COLD-ACCLIMATED			
period I* (control)	period II** (+ IAA***)	diff.	percent change	period I* (control)	period II** (+ IAA***)	diff.	percent change
601.6	495.2	-106.4	-17.7	823.1	598.6	-224.5	-27.3
881.8	789.6	- 92.2	-10.5	834.3	613.3	-221.0	-26.5
701.6	657.2	- 44.4	- 6.3	710.2	573.1	-137.1	-19.3
567.3	587.7	+ 20.4	+ 3.6	747.4	618.0	-129.4	-17.3
685.2	640.5	- 44.7	- 6.5	768.6	538.0	-230.6	-30.0
723.1	530.7	-192.4	-26.6	755.2	560.1	-195.1	-25.8
		N = 6				N = 6	
		$\overline{X}$ = -10.7%		t = 2.908		$\overline{X}$ = -24.4%	
		S.E.= 4.3		p < 0.025		S.E.= 2.0	

* Initial 40 minutes of run.

** Final 40 minutes of run.

*** 5.0×10^{-4} M.

Appendix VII f. Activity (Δ O.D./ mg. dry tissue) of G-6-P dehydrogenase in intestine homogenates of S. droebachiensis, and the effect of 5-bromouracil (Temperature 15°C.; glycylglycine buffer, pH 7.8).

time (mins.)	control A	control B	+ 5-BU*	tissue blank
2	0.007	0.006	0.009	0.001
4	0.015	0.015	0.019	0.001
6	0.024	0.022	0.027	0.003
8	0.035	0.034	0.035	0.004
10	0.041	0.043	0.041	0.004
12	0.049	0.047	0.052	0.004
14	0.056	0.056	0.058	0.007
16	0.062	0.062	0.066	0.007
18	0.071	0.071	0.072	0.007
22	0.083	0.081	0.084	0.007
24	0.087	0.087	0.091	0.009
26	0.090	0.091	0.096	0.009
28	0.100	0.099	0.099	0.010
30	0.102	0.102	0.103	0.010

* 6.94×10^{-4}M.

Appendix VIII a. Stability of activity coefficient of S. droebachiensis over an extended period. (Temperature = 10°C.; N=20)

Day	Mean A.C.	S.E.
1	14.37	1.23
2	10.18	1.06
3	11.35	1.12
4	13.54	1.15
5	13.42	0.93
6	13.62	1.24
9	14.49	1.33
10	12.83	0.81
11	13.45	0.96
12	14.60	1.17
13	15.23	0.98
15	15.24	1.07
16	13.38	1.12
17	14.43	0.88
19	14.23	1.08
22	12.78	0.90
24	14.82	0.92
30	13.81	1.38

Appendix VIII b. R-T relationships for activity coefficients of summer- and winter-acclimatized S. droebachiensis.

Temperature (°C.)	Summer N	Summer A.C.	Summer Q.D.*	Winter N	Winter A.C.	Winter Q.D.*
0°	-	-	-	25	3.94	1.27
1°	24	0.56	0.50	-	-	-
2°	24	0.85	0.45	-	-	-
3°	-	-	-	25	4.81	2.15
5°	24	2.77	1.15	25	6.41	1.88
8°	24	5.78	2.09	25	4.90	3.07
10°	24	9.35	3.56	25	3.55	2.24
12°	24	11.49	4.17	25	3.69	1.86
15°	24	15.15	3.75	25	n.i.r.	-
18°	-	-	-	25	n.i.r.	-
20°	24	10.64	3.87	25	n.i.r.	-
22°	24	1.67	-	25	n.i.r.	-
24°	24	n.i.r.**	-	25	n.i.r.	-

* Quartile deviation.

** No initiation of righting within 10 minutes.

Appendix VIII c. Seasonal shifts in activity coefficients of S. droebachiensis. [Theoretical A.C. derived from summer R-T curve (Fig. 21).]

Month	Habitat Temp. (°C.)*	Test Temp. (°C.)	N	Observed A.C.	S.E.	Theoretical A.C.
Dec.	2.5°	2.8°	25	7.21	0.77	1.20
Jan.	0.8°	0.4°	25	6.29	0.46	0.44
Feb.	0.0°	0.5°	15	5.26	0.62	0.48
Mar.	0.0°	0.3°	24	6.29	0.47	0.42
Apr.	0.3°	0.6°	23	6.63	0.68	0.48
May.	4.0°	3.0°	25	7.00	0.91	1.90
Jun.	12.0°	8.5°	25	11.31	1.18	11.50
Jul.	14.3°	12.2°	25	15.71	1.37	14.00
Aug.	14.5°	11.7°	23	14.91	1.13	14.80
Sep.	11.5°	9.6°	25	8.50	0.65	10.50
Oct.	7.6°	7.0°	25	10.18	1.09	4.80
Nov.	5.0°	4.0°	25	7.82	0.90	2.70
Dec.	3.7°	3.6°	25	7.70	0.93	1.80
Jan.	0.3°	0.8°	25	5.78	0.47	0.44
Feb.	1.7°	1.0°	25	6.03	0.53	0.74
Mar.	1.7°	1.4°	25	6.73	0.43	0.74

$\frac{\text{Winter A.C.}}{\text{Summer A.C.}} \times 100 = 33.5\%$

$\frac{\text{Theoretical Winter A.C.}}{\text{Theoretical Summer A.C.}} \times 100 = 3.4\%$

* From Figure 1.

Appendix VIII d. Time course of low-temperature acclimation of activity in S. droebachiensis. (A.C= activity coefficient.)

day	median A.C.	Q.D.*	day	median A.C.	Q.D.	day	median A.C.	Q.D.
-9	12.99	6.85	7	0.67	0.25	29	1.72	0.62
-8	18.52	6.88	9	0.72	0.40	31	2.41	1.06
-7	16.29	3.48	10	0.90	0.67	33	2.34	0.75
-6	12.66	6.14	12	0.58	0.16	35	2.38	0.69
-5	12.99	6.99	13	1.12	0.51	38	2.36	0.92
-4	17.24	4.79	14	0.51	0.43	41	3.08	0.72
-3	13.51	4.24	15	0.95	0.46	43	2.33	1.18
-2	13.89	6.35	17	1.55	0.82	46	3.44	1.26
-1	14.29	6.27	18	1.04	0.65	47	2.71	0.92
0**	-	-	19	0.83	0.65	49	2.62	1.00
1	0.75	0.38	20	1.12	0.78	53	3.36	0.80
2	0.95	0.35	21	1.36	0.93	55	3.77	1.03
3	0.78	0.32	23	2.05	0.76	57	2.86	0.64
4	0.49	0.38	26	1.65	1.22	60	3.13	1.13
5	0.94	0.58	27	1.55	1.54	63	3.40	0.58
6	0.88	0.34	28	1.30	1.11	67	3.98	0.70

* Quartile deviation (Semi-interquartile range).

** Change from 15°C. to 0°C.