TISSUE RESPIRATION AND SEASONAL ACCLIMATIZATION IN THE AMERICAN OYSTER CRASSOSTREA VIRGINICA (GMELIN)

JONATHAN A. PERCY

15657

TISSUE RESPIRATION AND SEASONAL ACCLIMATIZATION IN THE AMERICAN OYSTER, CRASSOSTREA VIRGINICA (GMELIN).

© Jonathan Arthur Percy B. Sc. (Hons.)

"Submitted in partial fulfilment of the requirements for the degree of Master of Science, Memorial University of Newfoundland.

March 1968

ABSTRACT

The respiration rates of excised gill, mantle and adductor muscle of the American Oyster, Crassostrea virginica, were measured by the Warburg technique, under various conditions of temperature and salinity and at various times during the summer and autumn.

The relative rates of the tissues, when compared to gill respiration as a base, are similar to those reported for a number of other bivalves.

The respiration rate of the mantle is inversely proportional to the Butler "condition" index of the oyster. This is attributed to glycogen accumulation. No significant correlation between "condition" index and either gill or adductor muscle respiration was apparent, a probable consequence of the low, more stable glycogen content of these tissues.

Dilution of the sea water medium to 13°/oo results in stimulation of gill respiration, has no significant effect on mantle respiration, and severely inhibits adductor muscle respiration.

Rate - temperature curves over the range 12° C. - 40° C. are presented for all three tissues. The maximum Q_{10} coefficients for gill and mantle respiration and the

minimum for adductor muscle respiration, occur in the 16° C. - 20° C. range.

The respiration rates of gill and mantle, measured at 20° C. in sea water of density 1.0250, declined by 16.8 percent and 18.4 percent, respectively, during late August - early September, indicating reverse acclimatization. The adductor muscle did not exhibit a corresponding decrease, and in fact its respiration rate may have increased somewhat.

It is concluded that the reverse acclimatization observed in gill, mantle and the intact animal is not attributable to a) seasonal fluctuations in "condition", because autumnal metabolic depression occurred over the whole "condition" range investigated, b) decreased ventilatory efficiency, because ventilation is not limiting under the experimental conditions or c) direct depressent action by the nervous system. The possible roles of intracellular changes and of nutritional and reproductive hormones, in the observed acclimatization effect are also discussed.

The autumnal respiratory depression observed in the intact oyster can be partially accounted for by alterations in the metabolism of the major respiring tissues.

A review of the occurrence of acclimatization in the Phylum Mollusca is presented.

CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

ACKNOWLEDGEMENTS

It is with much gratitude that I acknowledge the field assistance of various members of the Biology Department of Memorial University, particularly Mr. G.C. Lu, Mr. R. Scaplen, Mr. R. Meade and Mr. J. Mercer.

Thanks are extended to the Department of Fisheries for the loan of a small boat for carrying out field work.

Mr. G. Kelland of the Fisheries Research Board, St. John's, kindly agreed to carry out the salinity determinations.

The library staff of the Marine Biological Laboratory, Woods Hole, were especially helpful.

I would also like to thank Dr. T.R. Marcus of the Biology Department of Memorial University for many valuable suggestions and criticisms.

Special thanks are due to my supervisor, Dr. F.A.Aldrich, Director of the Marine Sciences Research Laboratory of Memorial University, for his invaluable guidance, encouragement and helpful criticism.

This work was supported by a grant from the Department of Fisheries to Dr. F.A.Aldrich.

INTRODUCTION

The metabolism of poikilotherms is not necessarily irrevocably linked to fluctuations of their thermal environment. Poikilotherms need not be - in the words of Krehl and Soetbeer (1899), "Spielballe der Umgebung"("playthings of the environment"). Compensation may diminish the accelerating or depressing effect on rate-functions of long term temperature changes.

Coastal, marine organisms of the temperate zones are subject to extensive seasonal temperature fluctuations. Often, these animals are capable of adjusting their metabolic rate so that during extended periods of cold the rate is considerably greater than would be predicted by the Krogh curve. This investigation is an attempt tc determine, by measuring the respiration rates of certain excised tissues, whether the American oyster, Crassostrea virginica, adjusts its metabolic rate to the season.

Two types of metabolic compensation are generally recognized: capacity-compensation, which involves adjustment of a given rate-function to changes in temperature that are within the normal biological range; and resistance-compensation, which refers to modifications of temperature tolerance at the extremes of the biological range, with death being

the end point usually measured. Resistance and capacity compensations usually occur together for any given rate-function, although Precht (1958) asserts that this need not be so.

The terminology associated with these compensatory adjustments is confused; in the present investigation, terms were used as follows: "adaptation" is a general term denoting compensation in response to an environmental change, regardless of whether the observed response is genetic or non-genetic in nature; "acclimatization" applies to compensation under the influence of a number of environmental factors. This type of response is observed under natural conditions, particularly in relation to seasonal and geographic factors; the term "acclimation" is employed in a more restricted sense for non-genetic compensation as a response to change in a single environmental factor. This type of adjustment occurs under controlled, experimental conditions. That we distinguish between acclimation and acclimatization does not imply that their mechanisms are fundamentally different, but merely serves to emphasize the fact that acclimatization is a more complex phenomenon than acclimation simply because the basic compensatory response may be modified by a number of other, often undetermined, factors.

When a metabolic adjustment results in an increase in metabolism, this is positive acclimatization or acclimation; a modification of metabolism in the opposite direction is reverse acclimatization or acclimation.

Adolph (1956) introduced the term "stressor" to designate the environmental change to which an organism is subjected and the term "adaptate" to indicate the compensatory response induced in a given physiological function. It is in the nature of the adaptate that it persists for a variable period of time after removal of the stressor.

According to Kinne (1964) such compensatory adjustments can be quantitatively assessed by measuring, "differences in performance of individuals with different environmental experiences."

Acclimatization and acclimation have been demonstrated in the following rate-functions: growth of molluscs (Dehnel, 1955, 1956), heart beat of molluscs (Takatsuki, 1929; Segal, Rao and James, 1953; Precht, 1958), water-propulsion in bivalves (Rao, 1953), ciliary activity in bivalves (Lagerspetz and Dubitscher, 1966), locomotory pulsation in coelenterates (Mayer, 1914),locomotion in protozoans (Shortress, 1942), enzyme activity in molluscs and amphibians (Mews,1957), and cyanide susceptibility in turbellarians (Behre, 1918). By far the greatest volume of work on thermal acclimatization involves measurement of respiration rate.

Seasonal temperature fluctuations produce a cyclic modification of metabolism in many animals. Terrestrial and fresh-water animals of the temperate zones, subject to extensive, seasonal temperature changes, on the whole have well developed compensatory mechanisms. Seasonal acclimatization to cold often occurs in animals that remain active throughout the winter (Bullock, 1955). Schwartz (1884)* as well as Aderhold (1888)* observed that Euglena sp., during the summer, becomes immobile when the temperature falls below 5°C.; in winter, activity continues down to 0°C. The mean respiration rate of the terrestrial amphipod, Talitrus sylvaticus, over a wide range of experimental temperatures was 1½ times greater in winter than in summer (Clark, 1955). Other examples have been cited by Bullock (1955).

Coastal, marine animals from the temperate latitudes, though subject to less extreme seasonal temperature-fluctuations than terrestrial forms, nevertheless acclimatize. The crustacean, Emerita talpoida, from the Woods Hole region acclimatizes so that in winter at 3°C., its metabolic rate is approximately the same as its rate in summer at 15°C. (Edwards and Irving, 1943). In the fiddler crab, Uca pugnax, oxygen consumption at a number of temperatures was higher between November and January than at the same temperatures between June and August (Vernberg, 1959).

* cited by Behre (1918)

Absence of a seasonal acclimatization response is often associated with winter-torpor and hibernation (Bullock,1955). The metabolic rate, at any given temperature, of the sand flea, Talorchestia megalophthala, for example, is the same summer and winter (Edwards and Irving, 1943), but in winter, the animal's activity becomes severely depressed. Another example is the ant, Formica ulkei. Kept in the laboratory under a thermal regime approximating that of its natural environment, this ant exhibits negative acclimation in that summer metabolism at any given temperature is higher than winter metabolism (Dreyer, 1932).

Bullock (1955) is of the opinion that the distribution of thermal acclimatization in the animal kingdom is "apparently wide, but far from universal".

Instances of seasonal acclimatization in molluscs have been reported (Segal, 1956; Hopkins, 1946). These, along with a number of other cases of geographic acclimatization (Sparck, 1936; Thorson, 1936, 1956; Scholander et al, 1953; Takatsuki, 1929; Rao, 1953; and Dehnel, 1955) and laboratory acclimation (Mews,1957; Kirberger, 1953; Segal, 1956) suggest that acclimatization is widespread among molluscs. Reverse acclimatization of respiration rate has been reported in the gastropod, Ancylus fluviatilis (Berg, 1953).

The oyster, Crassostrea virginica, is a coastal marine form that is subject, in the northern extremities of its range, to a considerable annual temperature range. The rate of oxygen consumption of the intact animal appears to reach a maximum during the summer and then declines in the autumn, suggesting reverse acclimatization (Galtsoff, 1964).

Measurement of oxygen consumption in intact bivalves is beset with technical difficulties. The autumnal decline of respiration in intact oysters may be, not a true metabolic depression initiated at the tissue level, but an effect imposed by decreased efficiency of ventilation. Accumulation of non-respiring storage material (glycogen) could also account for the observed autumnal decline in oxygen uptake per unit tissue weight. On the other hand, it is equally possible that nervous or hormonal mechanisms depress the metabolic rate.

It has been suggested that thermal acclimatization may be a wholly endogenous property of cells (Precht,1958). Positive seasonal acclimatization may occur in the oyster, as in other marine animals, but its expression in the oyster may be masked by opposing factors.

Acclimatization of oxygen consumption, measured in exised tissues, has been correlated with both seasonal

and geographic temperature differences (Roberts, 1957; Vernberg and Vernberg, 1965; Peiss and Field, 1950). In some instances, different tissues from a single organism show substantially different acclimatization responses (Roberts, 1957; Vernberg and Vernberg, 1965). The only reported instance of acclimatization of respiration in excised, molluscan tissues involves gill, mantle, and adductor muscle of the bivalve, Mercenaria mercenaria (Hopkins, 1946).

Could the anomolous response - reverse acclimatization - observed in the whole oyster by Galtsoff be accounted for satisfactorily by the summed responses of individual tissues? In the present study, the respiration rates of excised gill, mantle and adductor muscle of the oyster were measured in summer and in autumn to determine whether an autumnal, metabolic depression occurs. Furthermore, the rate-temperature characteristics of the excised tissues of the oyster were determined because low Q_{10} values may be homeostatic mechanisms, limiting the influence on metabolism of seasonal temperature fluctuations (Scholander et al, 1953).

The rapid valve-closing response of oysters to thermal or osmotic stimuli has made it difficult to obtain data on the short-term influence of these factors on

metabolism. Measurement of the respiration rates of excised tissues over a range of temperatures and salinities has provided a means of approaching these problems.

It is obviously important to have information regarding the physiological state of the experimental oysters. A population study, involving mortality, growth and "condition" determinations, carried out concurrently with the respiration studies as part of a continuing survey of the Broad Lake oysters (Aldrich and Percy, 1967) provided data on the general physiological state of the oyster population from which the samples were collected, and permitted a study of possible relationships between seasonal respiration rates and growth, "condition" and environmental salinity cycles.

MATERIALS AND METHODS

Source of experimental oysters:

The oysters for this study were obtained from an experimental planting of American Oysters, Crassostrea virginica, from Prince Edward Island, made in Broad Lake, Trinity Bay, Newfoundland in May of 1965.

A continuing study of mortality, growth and condition-rating of bottom-planted oysters and of oysters enclosed in submerged or in floating trays, provided information on the general physiological state of the oysters. (Aldrich,1965), (Aldrich and Percy,1967).

Oysters for respiratory studies were collected in approximately 4 feet of water by tonging. Collections were made at approximately two-week intervals during the summer, and less frequently during the autumn. Selected for this study were bottom-planted young oysters about 7 cm. long, with some evidence of recent shell growth. These small animals had a low mortality, showed good growth, and were quite uniformly of fair to good "condition"; adults on the other hand, displayed high mortality, little or no growth and poor "condition" (Aldrich and Percy, 1967). The overall "condition" of the young oysters was essentially the same as that found for similar sized individuals from larger commercial beds

(Aldrich, 1965).

Sampling was discontinued after November because Broad Lake became covered with ice.

Surface-water temperatures at the collecting site were measured frequently. Surface and bottom salinities were determined by standard silver nitrate titrations (Strickland and Parsons, 1960).

Animals were transported to the laboratory in insulated containers, in sea water, and were then maintained in a tank of circulating sea water (density 1.0255-1.0265; temperature 12°± 2°C.) until used. Food was not added but some of the plentiful detritus present may have been utilized as food. In most instances, animals for respiration studies were kept in the tanks less than 8 days even though there was no significant difference between the respiration rates of tissues from oysters held for 1-4 days and those held for 5-8 days.

Preparation of tissues:

Twelve to 24 hours before tissue samples were to be taken, the oysters were cleaned and temperature conditioned. Up to three oysters were scrubbed with a wire brush and placed in a beaker containing 450 ml. of filtered sea water continuously bubble-aerated, maintained at 20°C. ± 1°C. and of standard density 1.0250(31.1‰),

where they remained until actual dissection of the tissues.

Length and width of each oyster were measured to the nearest millimeter with a ruler set into a right-angle board. Length was measured as the greatest antero-posterior dimension and width as the greatest dorso-ventral dimension of the valves (Figure 1).

The oysters' valves were opened by severing the hinge ligament and adductor muscle. Then, the Condition Index (C.I.) was determined using the criterion of tissue translucency established, and ranked numerically, by Dr. P.A. Butler of the U.S. Fish and Wildlife Service according to the following scale:

1. Poor; more than 75% translucent.
2. Poor; less than 75% but more than 25% translucent.
3. Fair; digestive gland visible, less than 25% translucent.
4. Fair; transition between 3 and 5.
5. Fair; digestive gland not visible, less than 25% translucent.
6. Fair; transition between 5 and 7.
7. Good; digestive gland not visible, tissues opaque.
8. Good; transition between 7 and 9.
9. Good; digestive gland not visible, tissues more opaque.
10. Good; entire animal cream coloured and opaque.

Although Butler's scale is based on a visual estimate of tissue opacity, it accurately reflects total tissue-glycogen (i.e. "condition") and permits more reliable and rapid grading of oysters than other methods (e.g. Needler, 1941).

In view of the slow rate of oxygen diffusion through tissue (Umbreit, Burris and Stauffer, 1959) it is essential for gas exchange studies by manometric methods that the organ be, either highly porous, of limited thickness, firm enough for slicing, or amenable to mincing or homogenization. On the basis of these criteria a number of tissues of the oyster were found to be suitable for manometric studies. The adductor muscle was firm enough for slicing, the gill was highly porous and the mantle was of limited thickness.

The gills were excised with scissors (Figure 2), several square pieces of tissue (approximately 5x5x1.5 mm.) being cut from each of the four demibranchs. These samples were then transferred with a blunt-ended glass probe (to minimize trauma) to a watch glass containing sea water of the appropriate experimental density (1.0110, 1.0150 or 1.0250) and temperature(20-23°C.). For each manometric determination five or six tissue blocks from a single oyster were pooled so that at least one sample from each

demibranch was included in the pool.

The mantle was excised with scissors (Figure 1). First the lobulated border was removed and cut into three or four pieces; then the remaining central free portion was cut into three or four pieces, each approximately 5x5x1 mm. Both right and left mantle lobes were used. Six or seven pieces of mantle tissue from a single oyster were placed in each manometer flask. Respiration rates of the mantle border and of the central mantle tissues were determined and compared.

Following excision of the gill and mantle samples, the remaining organs were removed, leaving the adductor muscle attached to the left valve.

The adductor muscle of C. virginica consists of a translucent firm portion and a fibrous white one. Only the vitreous, translucent part of the muscle was firm enough for slicing. It was excised as a single rectangular block by cutting away the white portion and surrounding connective tissue with a scalpel and severing as close to the left valve as possible (Figure 2). The block of tissue thus obtained was then cut into three or four smaller blocks. Slices were cut with a device designed by Deutsch and recommended for slicing muscle tissue by Umbreit, Burris and Stauffer (1959).

As this simple device is not widely known a brief description follows. A frosted glass microscope slide is attached, frosted side up, to a heavy watch glass base by means of paraffin wax. A 2x3 cm. rectangle of hard filter paper, moistened with sea water (density 1.0250), is placed over the frosted section of the slide. The block of muscle tissue is placed on the filter paper, its fibres parallel to the surface of the slide. A second frosted glass slide, moistened with sea water is so positioned that its frosted portion rests on the tissue block. Sectioning is accomplished by inserting a razor blade mounted in a wooden holder, between the slides and drawing it slowly through the tissue parallel, and close to the upper slide. It is possible to adjust the thickness of the slices by finger pressure on the slides.

The slices of muscle tissue thus obtained were rinsed from the glass slide into a watch glass of filtered sea water of the same density as that in the Warburg flask, and held at 20-23° C.

The tissues from three oysters, at most, were prepared at any one time for respiratory studies.

All of the tissue samples were touched to a piece of hard filter paper to remove excess fluid prior to transfer to the Warburg flasks.

Determination of respiration rate:

Tissue respiration studies were carried out by means of Warburg's manometric apparatus (Bronwill Scientific Inc. Circular model) using flasks of approximately 15 ml. capacity.

The flasks and manometers were paired at the outset and calibrated with mercury according to the method outlined by Umbreit, Burris and Stauffer (1959). Following calibration, the flasks were washed in a hot solution of equal parts of concentrated nitric and sulphuric acids in order to remove all traces of the mercury, and then rinsed for several hours in a number of changes of distilled water.

The flask constants obtained by computation (Umbreit, Burris and Stauffer, 1959) were checked by reference to a KO_2 nomogram for Warburg manometers (Dixon, 1943).

Dixon (1943) emphasized the importance of maintaining physiological conditions for the tissues in the Warburg flasks. The medium, made up at intervals as stock solutions, consisted mainly of 1.5 ml. of natural sea water filtered through Whatman No. 2 filter paper. Density of the medium was measured at 15° C. with a Canlab 47-700 model hydrometer and adjusted by the addition of distilled water. The sea water used for

the majority of the experiments was of standard density 1.0250 (31.1°/oo). An additional series of respiratory determinations was made at densities 1.0150 (18.7°/oo) and 1.0100 (13.0°/oo) to determine the effect of dilution of the medium on tissue respiration. pH was checked immediately before use and was consistently in the range 7. 8-8. 0.

Occasionally, samples of the Warburg medium were checked with Hydrion pH paper before and after the respiratory determination. pH changes did not exceed o.6 units during the two hour Warburg run.

The CO_2 generated by the respiring tissues was absorbed in 0.4 ml. of 10% KOH; the absorption rate being increased by the addition of a 2x3 cm. rectangle of loosely rolled filter paper to the centre well. This precaution maintains the effective CO_2 concentration in the flask at a negligible level provided that the CO_2 production does not exceed 1,000 cu. mm. per hour (Dixon and Elliot, 1930).

All respiratory determinations were carried out at a shaking rate of 120 strokes/min. and an amplitude of 7 cm. Air was the gas phase. Runs at 20° C. or higher were carried out in the laboratory, while runs at lower temperatures were conducted in a refrigerated room.

The majority of the respiratory determinations were conducted at 20° C. The effect of temperature on the respiration rates of the various tissues was determined by measuring the O_2 consumption at 4° C. intervals from 12° C. - 40° C. The respiration rate of any given sample, however, was measured at one temperature only. The Warburg water bath was controlled to within 0.1° C.

After the flasks were prepared, attached to the manometer and placed in the water bath the fluid in the manometers was adjusted to a low level (60mm.). Five minutes after placing the flasks in the water bath the stopcocks were closed and the fluid in the left arm of the manometers was adjusted to 150 mm. Thus, the level in the left arm was initially set high, providing not only an extended working range but a rapid indication of leaks. The first readings were made after a 30 minute equilibration period.

Between 45 minutes and 1 hour elapsed between the time that the first oyster was opened and the time that the flasks were immersed in the water bath. Thus, including the half hour equilibration period, a maximum time of 1.5 hours elapsed between excision of the tissues and the first manometer readings. Results are reported with the time of first reading designated as "zero" time.

Because of their delicate nature, the oyster tissues were not weighed until after the manometric determinations were completed. As a result there was some variation from flask to flask in the quantity of tissue; but tissue dry-weights ranged from 20-30 mg. for gill and from 30-40 mg. for mantle and adductor muscle.

Preliminary measurements of respiration rates of gill, mantle and adductor muscle over extended periods of time (see below) demonstrated that oxygen uptake of the tissues continued at a uniform rate for at least 2-3 hours. Consequently, in all subsequent respiratory measurements, manometer readings were made at 20 minute intervals for a two hour period and the results were calculated as the mean rate during this period.

Upon completion of the Warburg run the tissues were removed from the flasks, rinsed in distilled water and placed in 2 gm. shell vials. The tissues were dried in an oven at 60° C. to constant weight. The vials were cooled in a dessicator prior to weighing to the nearest 0.0001 gm.

Respiration rate ($Q0_2$) is expressed throughout as $\mu l.0_2$/gm.dry wt./hr. and was determined according to the formula:

$$\text{rate} = \frac{\text{mm. manometric fluid movement/hr. x } KO_2}{\text{dry weight of tissue in grams}}$$

Significance levels for differences of means were determined by Student's t test.

Standard errors were calculated according to the formula:

$$\text{S. E.} = \frac{\sigma}{\sqrt{N}}$$

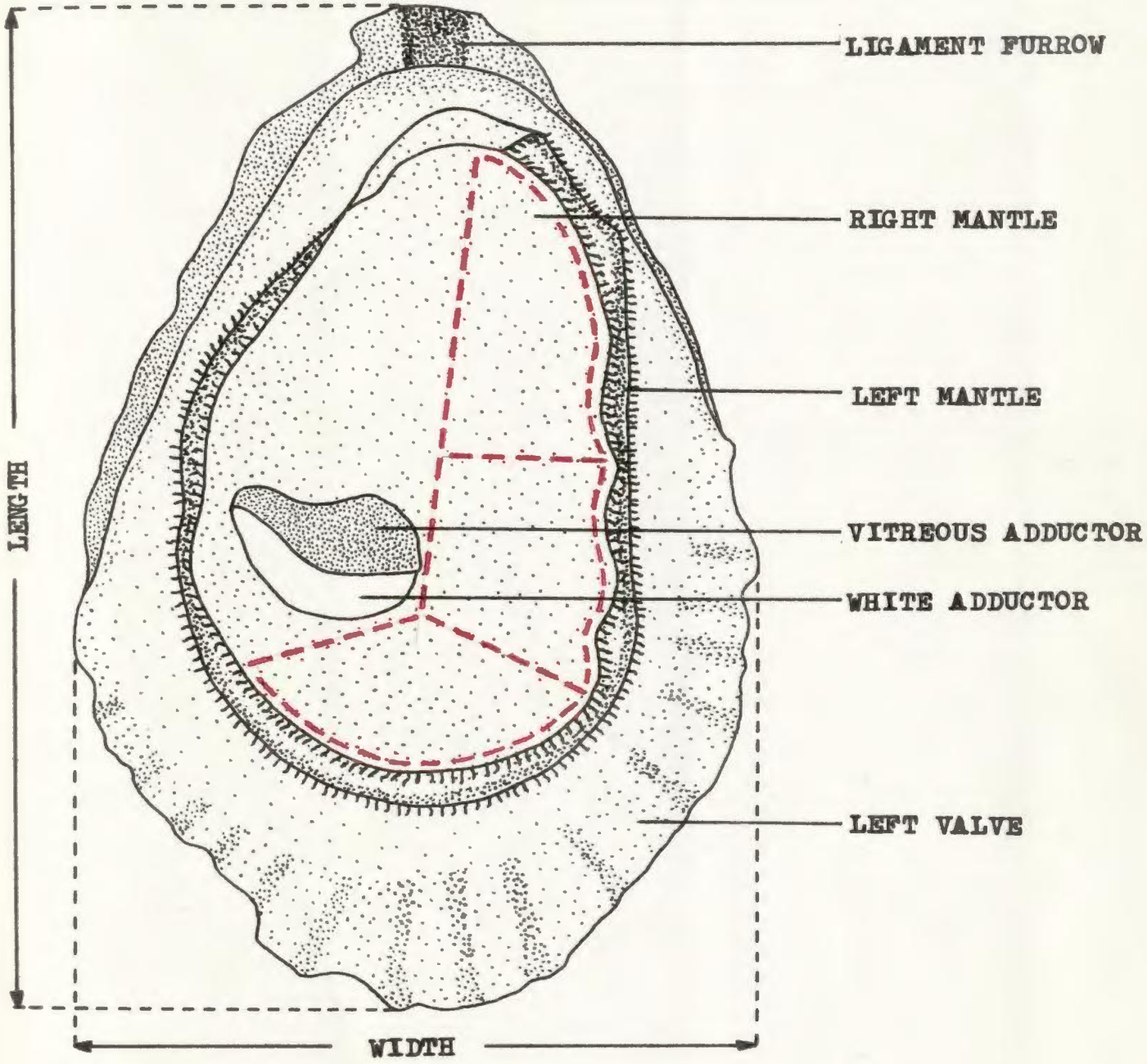

FIGURE 1. Oyster with right valve removed to show mantle tissues (in red) employed for respiration studies. Magnification X 2.

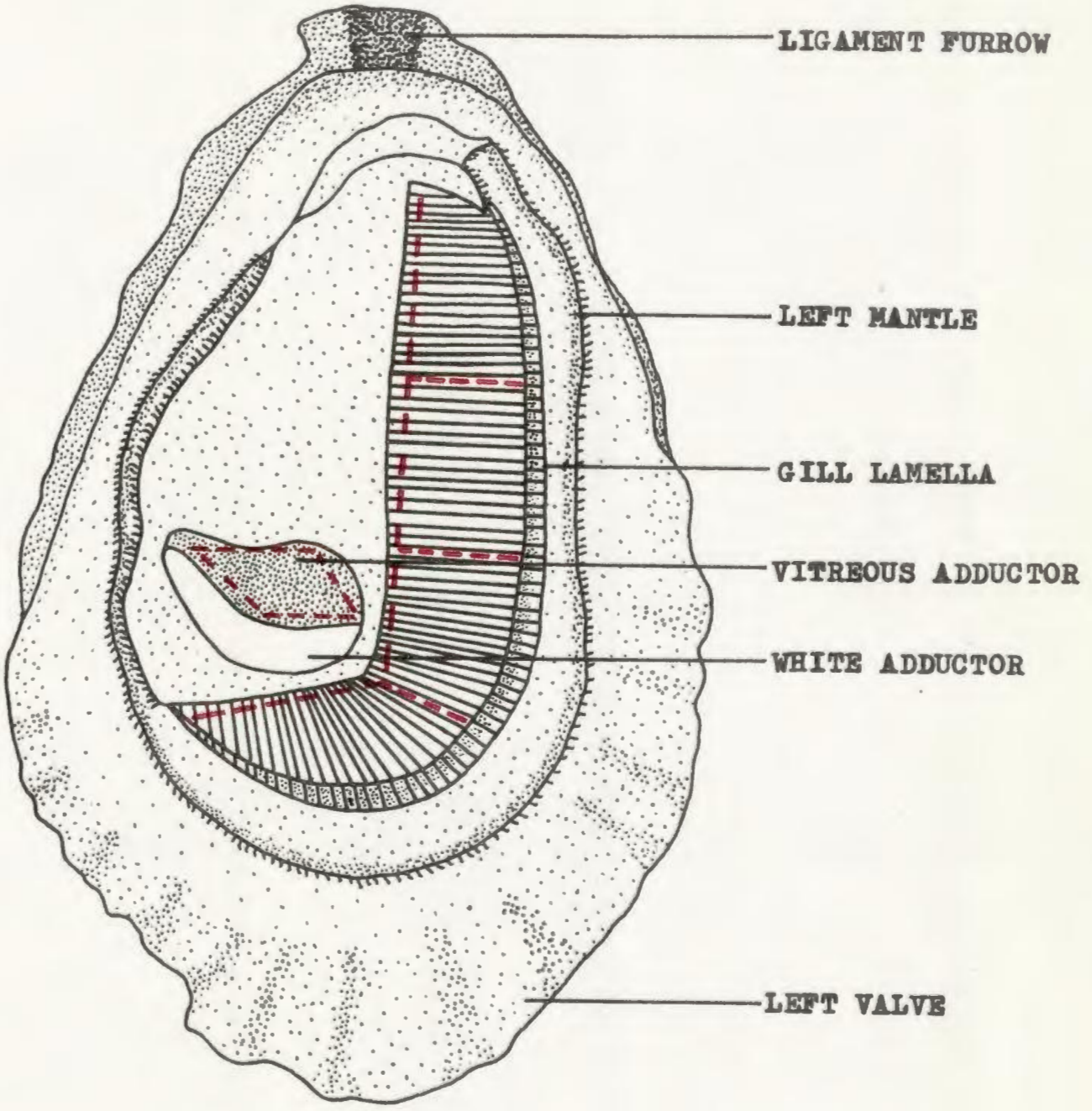

FIGURE 2. Oyster with right valve and portion of right mantle removed to show gill and adductor muscle tissues employed for respiration studies (in red). Magnification X 2.

Description of Tissue:

Tissues suitable for manometric respiratory studies were, according to the criteria previously described (vide supra), gill, mantle and adductor muscle. The digestive gland, though it has been used in previous manometric studies (Chapheau, 1932), was not used in the present work because of difficulties in isolating the glandular tissue from the surrounding connective tissue and excessive breakage of the fine tubules during shaking in the Warburg flasks. For the convenience of the reader a brief description of the tissues (based largely on the work of Galtsoff, 1964) follows:

Gill Tissue (Ctenidia)

The gills of the oyster function as highly developed feeding structures which filter particulate material from the water circulated by the ciliary tracts (Nelson, 1938) and also play an important role in respiration. During the spawning season, the gills are instrumental in the shedding of gametes.

In C. virginica, the gills consist of four folds of tissue, or demibranchs located two on either side of the visceral mass from which they are suspended. Each demibranch is composed of a linear series of conjoined tubular filaments, reflected back upon themselves distally

to form a V-shaped, bilamellar structure. These hairpin-like filaments are so conjoined that the descending elements form one lamella, the ascending elements a second lamella. The lamellae are pleated to form a series of parallel vertical folds. In each of the two lamellae, connective tissue junctions firmly link adjacent filaments. Connective tissue junctions also join ascending and descending arms of some of the filaments to form a series of parallel, vertical water tubes within the demi-branchs, that open into a dorsal epibranchial chamber. Oval openings, the ostia, on the surface of the gill between adjacent filaments, permit passage of water from the mantle cavity into the water tubes.

Both the external surfaces of the gills and the walls of the water tubes are densely covered with cilia that sweep a steady current of water through the gill structure (Kellogg, 1915; Atkins, 1937, 1938).

Large cells, secreting copious quantities of mucus onto the gill surface, are abundant in the gill epithelium.

The muscular system of the gills is well developed and consists of fibres oriented in three planes; longi-tudinal fibres extending from proximal to distal ends of the filaments; transverse fibres extending from side to side of the interlamellar junctions in a plane parallel to

the gill surface; and tangential fibres running in a plane at right angles to the gill surface joining the lamellae across the interlamellar junction. Upon excision, the gill muscles contract vigorously thus reducing the apparent area of the gill.

The structure of the gills, as indicated in the preceeding description, is sufficiently porous to permit adequate gas exchange with the surrounding medium without the necessity of employing slicing or mincing techniques. In the words of Ghiretti (1966), "There is clearly a great superabundance of respiratory surface."

Mantle (Pallium):

The mantle functions principally in the formation and calcification of the valves. It also serves as a respiratory organ of some importance. In fact, Pedersen (1947) believes that, in the oyster, the mantle has become the principal respiratory organ, the gills having taken on primarily a feeding function.

The mantle of C. virginica consists of two thin sheets of tissue, attached dorsally, which pass down on either side of the visceral mass and continue over the surface of the gills as free folds. The dorso-lateral portion of the left mantle lobe is fused to the lateral surface of the visceral mass; the dorso-lateral portion

of the right lobe is separated from the visceral mass by the promyal chamber.

The mantle is a relatively simple structure, consisting of a sheet of connective tissue with muscles,blood vessels and nerves. Both surfaces of the mantle are covered with epithelium, the inner one ciliated, the outer one non-ciliated and involved in shell-secretion. Especially powerful cilia are located along the mantle border (Menzel,1955).

The periphery of each mantle fold is developed into thick muscular marginal lobes. The outer lobe, nearest the shell,is primarily a secretory organ. The middle lobe bears many tentacles and is chiefly a sensory structure. The inner lobe, which also bears numerous tentacles, is highly muscular and is both a sensory organ and a"pallial curtain" regulating the flow of water into the mantle cavity.

Adductor Muscle:

Crassostrea virginica, having lost the anterior adductor muscle during larval development, is an anisomyarian bivalve. The single, remaining adductor muscle accounts for 20-40% of the total tissue weight of the animal and consists of two distinct portions,one translucent, the other opaque.

Two thirds of the adductor muscle is firm and translucent and is responsible for rapid closure of the valves. The fibres of the translucent portion are thin and obliquely striated, and are oriented at right angles to the surfaces of the valves. The remaining one third of the adductor is milky white, opaque, and composed of tough thick, parallel, smooth-muscle fibres that are chiefly responsible for maintaining valve closure over extended periods. A connective tissue layer separates the two regions. The fibres of both the translucent and the white portions are supported in a connective tissue matrix.

RESULTS

Environmental temperature and salinity.

Surface and bottom water temperatures and salinities at the collecting site were checked regularly (Appendix: Table 38). As the shallow water(3 - 6 feet) over the oyster plants was subject to considerable mixing by wind and wave action, there was little difference between bottom and surface temperatures and salinities.

Salinity remained reasonably uniform after the middle of June, neither exceeding 30 °/oo, nor falling below 28 °/oo (Figure 3). The melting of the ice-cover considerably reduced the salinity in the spring, as the low May salinity indicates.

The difference between surface and bottom temperatures and salinities never exceeded 0.2°C. and 0.15 °/oo, respectively.

Oysters collected during July and August had been exposed to temperatures in excess of 15°C. (15°C. - 19°C.), while those collected in the autumn were subject to temperatures lower than 15°C. (7°C. - 15°C.) (Figure 4).

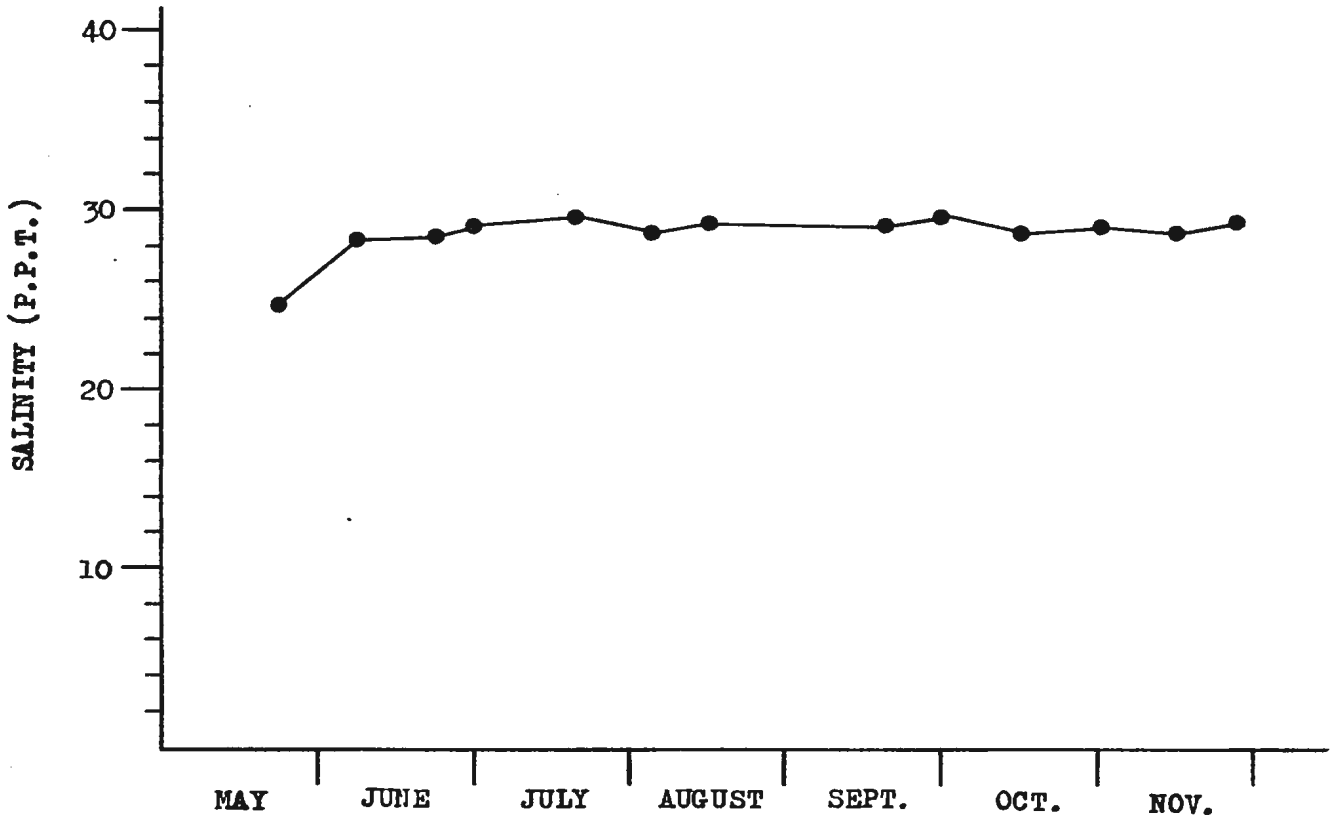

FIGURE 3. Variations in surface salinity at the collecting site during the period of the study.

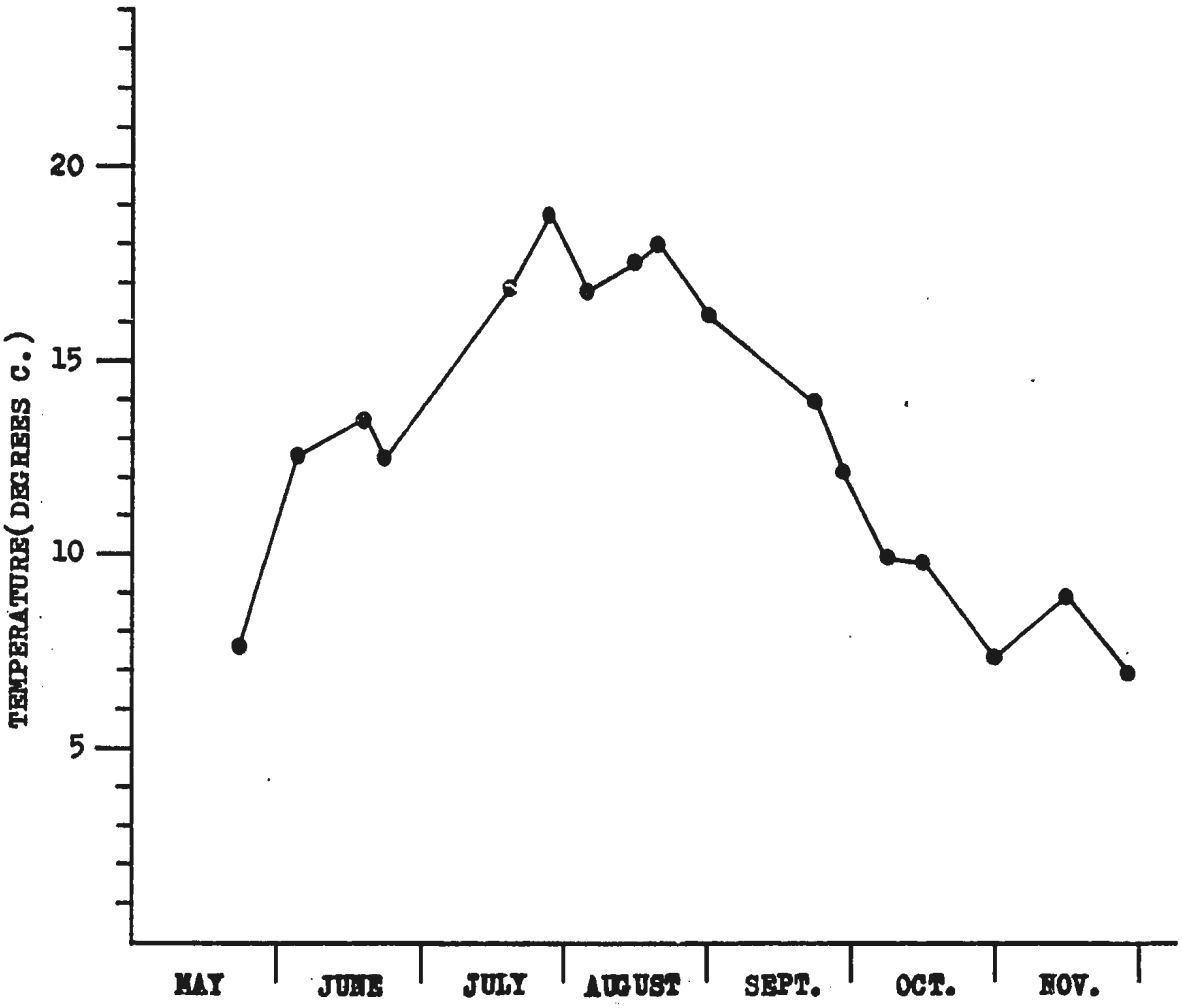

FIGURE 4. Variations in surface water temperature at collecting site during the period of study.

Preliminary experiments:

Preliminary experiments were conducted to establish:

1. the period - if any - during which oxygen consumption rate of the excised tissue would be uniform;

2. the mean respiration rates of different regions of the mantle;

3. the relative respiration rates of gill, mantle and adductor muscle;

4. the variability in respiration rate among gill tissue samples; and

5. the relationship between "condition" index and dry weight of the oyster.

Uniformity of oxygen consumption over extended periods:

The results of the long term (6 hours) respiration studies on gill, mantle and adductor muscle are presented in Tables 1 - 3.

At 25° C. the mean gill respiration rate during the first two hours was 2989 $\mu l.O_2$/gm./hr. This was 3.1% lower than the mean rate calculated for the first hour. By the sixth hour, the rate had decreased 17.4% to 2469 $\mu l.O_2$/gm./hr. At 22° C. the mean gill respiration rate during the first two hours was 1739 $\mu l.O_2$/gm./hr. This was 4.3% lower than the rate calculated for the first hour. During the sixth hour the respiration rate was 1171 $\mu l.O_2$/gm./hr.; a decline of 32.7%.

At 25° C. the mean mantle respiration rate during the first two hours was 1248 $\mu l.O_2/gm./hr.$ This was 2.6% lower than the rate calculated during the first hour. By the sixth hour the rate had fallen to 858 $\mu l.O_2/gm./hr.$; a decline of 31.3%. At 22° C. the mean mantle respiration rate was 921 $\mu l.O_2/gm./hr.$ during the first two hours. This was 1.4% lower than the rate calculated for the first hour. The rate during the sixth hour was 643 $\mu l.O_2/gm./hr.$; a decrease of 30.2%.

At 25° C. the mean adductor muscle respiration rate showed little change during the first six hours of the Warburg run, rising from 196 $\mu l.O_2/gm./hr.$ during the first hour to 205 $\mu l.O_2/gm./hr.$ during the sixth hour.

In all three tissues, the rate of oxygen consumption remained uniform for at least two or three hours, after which gill and mantle respiration gradually decreased.

The variations in measured oxygen consumption among each of the 20 minute sampling intervals for gill and mantle respiration were small during the first two hours and then increased progressively with time (Figures 7 and 8).

In view of these results, the tissue respiration rates in all subsequent determinations are presented as

the mean rates during the initial two hours of the Warburg run.

The temperature effects apparent in figures 5 and 6 will be considered below.

TABLE I

Cumulative long-term oxygen consumption of excised gill (Experimental: medium density 1.0250; temperature as indicated; specimen no. in parentheses)

Time(mins)	$\mu l.O_2/gm.$ 25° C. (#5-13)	$\mu l.O_2/gm.$ 25° C. (#5-14)	$\mu l.O_2/gm.$ 25° C. (#6-45)	mean $\mu l.O_2/gm.$
0	0	0	0	0
20	1058	1214	953	1075
40	1970	2318	1794	2027
60	2918	3587	2747	3084
80	3830	4746	3644	4073
100	4815	5739	4429	4994
120	5654	6843	5438	5978
140	6547	7836	6223	6869
160	7495	8829	7176	7833
180	8334	9767	8017	8706
200	9209	10705	8802	9572
220	10230	11753	9643	10542
240	10959	12746	10484	11396
260	11798	13684	11381	12288
280	12618	14567	12054	13080
300	13420	15560	12839	13940
320	14076	16664	13624	14788

TABLE I (Cont'd)

Time(mins)	µl.O_2/gm. 25° C. (#5-13)	µl.O_2/gm. 25° C. (#5-14)	µl.O_2/gm. 25° C. (#6-45)	mean µl.O_2/gm.
340	14988	17712	14353	15684
360	15717	18595	14914	16409

TABLE 1(a)

Time(mins.)	$\mu l.O_2/gm.$ 22° C. (#6-49)	$\mu l.O_2/gm.$ 22° C. (#6-48)	Mean $\mu l.O_2/gm.$
0	0	0	0
20	603	638	621
40	1206	1146	1176
60	1852	1784	1818
80	2498	2263	2381
100	2972	2742	2857
120	3618	3340	3479
140	4178	3858	4018
160	4652	4297	4475
180	5169	4935	5052
200	5557	5374	5466
220	6117	5892	6005
240	6591	6450	6521
260	7065	6849	6957
280	7453	7248	7351
300	7755	7607	7681
320	8186	8125	8156
340	8315	8604	8459
360	8660	9043	8852

TABLE 2

Cumulative long-term oxygen consumption of excised mantle (Experimental: medium density 1.0250; temperature as indicated; specimen no. in parentheses)

Time(mins.)	$\mu l.O_2/gm.$ 25° C. (#5-13)	$\mu l.O_2/gm.$ 25° C. (#5-14)	Mean $\mu l.O_2/gm.$
0	0	0	0
20	473	399	436
40	893	851	872
60	1313	1250	1282
80	1733	1675	1704
100	2179	1967	2073
120	2599	2392	2496
140	2967	2658	2813
160	3413	3030	3222
180	3754	3349	3552
200	4174	3562	3864
220	4620	3987	4302
240	4909	4253	4581
260	5277	4439	4858
280	5645	4731	5188
300	6013	5023	5518
320	6223	5289	5756
340	6669	5541	6105
360	6958	5793	6376

TABLE 2(a)

Time(mins.)	µl.O_2/gm. 22°C. (#6-48)	µl.O_2/gm. 22°C. (#6-46)	µl.O_2/gm. 22°C. (#6-49)	Mean µl.O_2/gm.
0	0	0	0	0
20	357	309	276	314
40	674	549	552	592
60	1071	858	874	934
80	1388	1098	1288	1258
100	1626	1304	1426	1452
120	2023	1613	1886	1841
140	2301	1853	2116	2090
160	2579	2127	2346	2351
180	2936	2401	2714	2684
200	3174	2607	2944	2908
220	3491	2916	3220	3209
240	3848	3190	3496	3511
260	4046	3430	3726	3734
280	4324	3602	3864	3930
300	4483	3911	4186	4193
320	4642	4117	4416	4392
340	4761	4357	4600	4573
360	4920	4666	4922	4836

TABLE 3

Cumulative long term oxygen consumption of excised adductor muscle. (Experimental; medium density 1.0250; temperature as indicated; specimen no. in parentheses)

Time(mins.)	$\mu l.O_2/gm.$ 25° C. (#5-13)	$\mu l.O_2/gm.$ 25° C. (#5-14)	Mean $\mu l.O_2/gm.$
0	0	0	0
60	122	269	196
120	274	423	349
180	426	615	522
240	578	769	674
300	760	961	862
360	942	1191	1067
420	1124	1383	1254

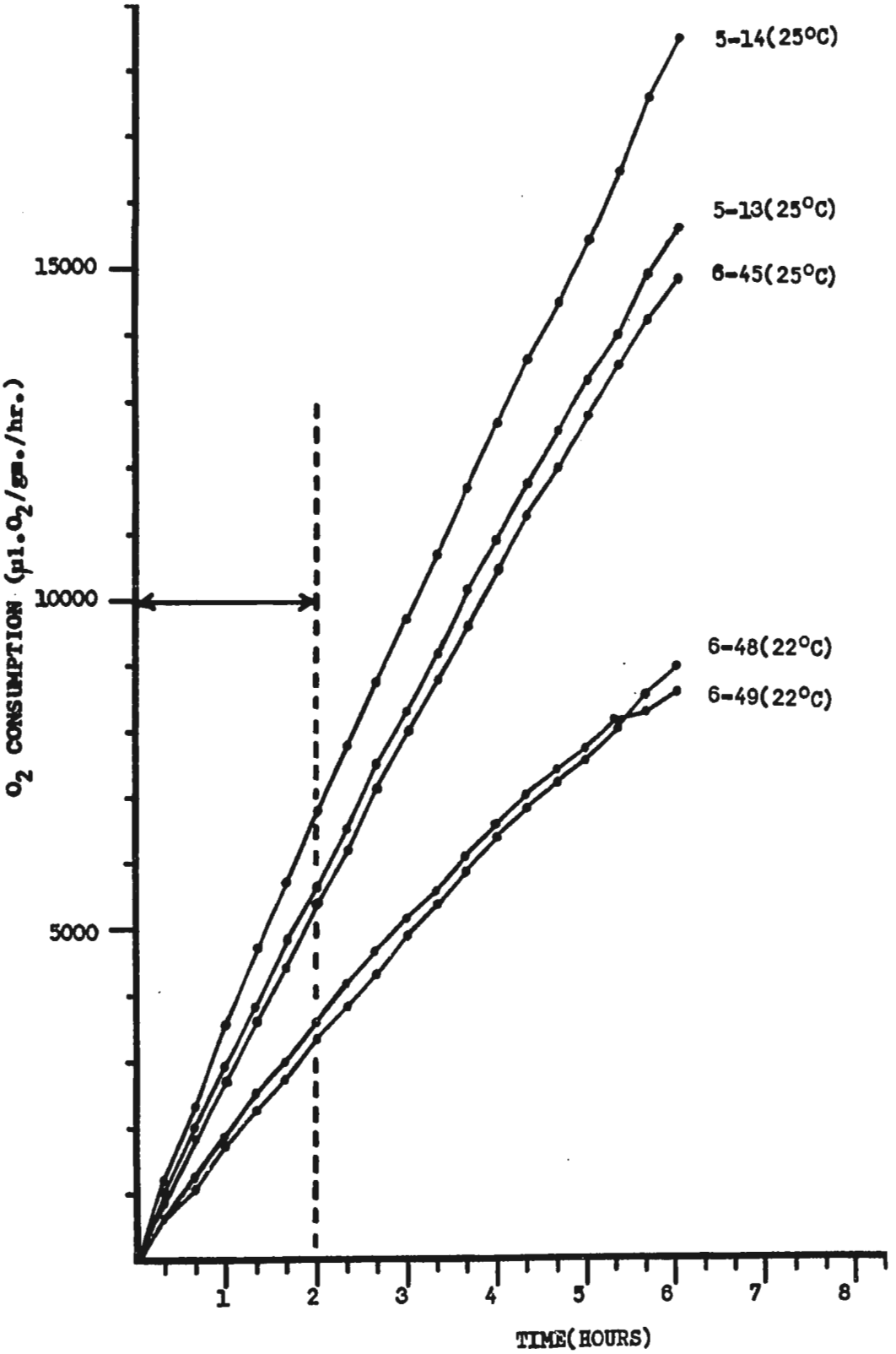

FIGURE 5. Respiration of excised gills over an extended period.(Sea water density 1.0250 ; temperature as indicated) . "Zero" time approximately 1 1/2 hours following excision. Arrow indicates standard measuring interval.

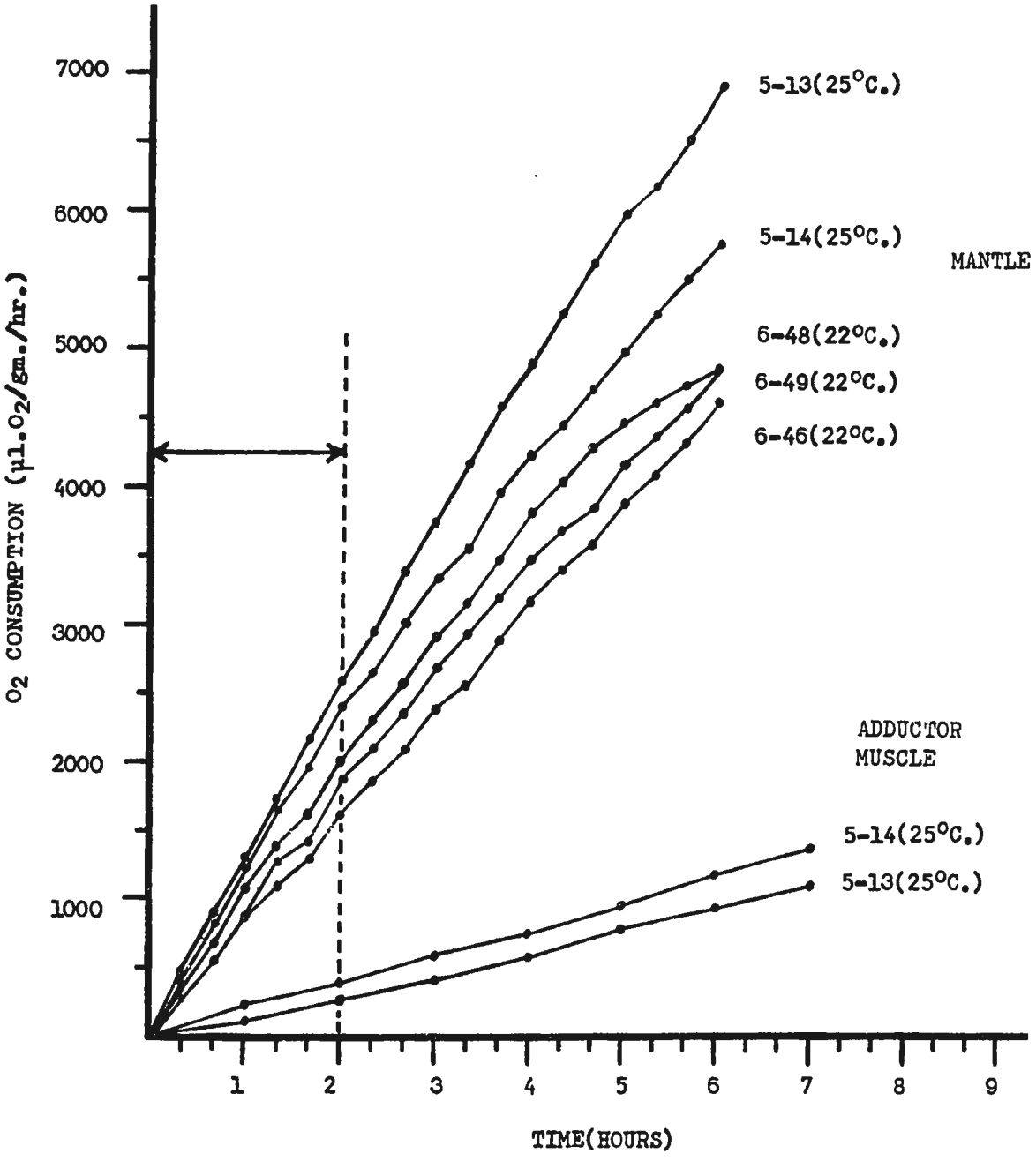

FIGURE 6. Respiration of excised mantle and adductor muscle of C. virginica over an extended period. Sea water density 1.0250; temperature as indicated. "Zero" time approximately 1 1/2 hours following excision. Arrow indicates standard measuring interval.

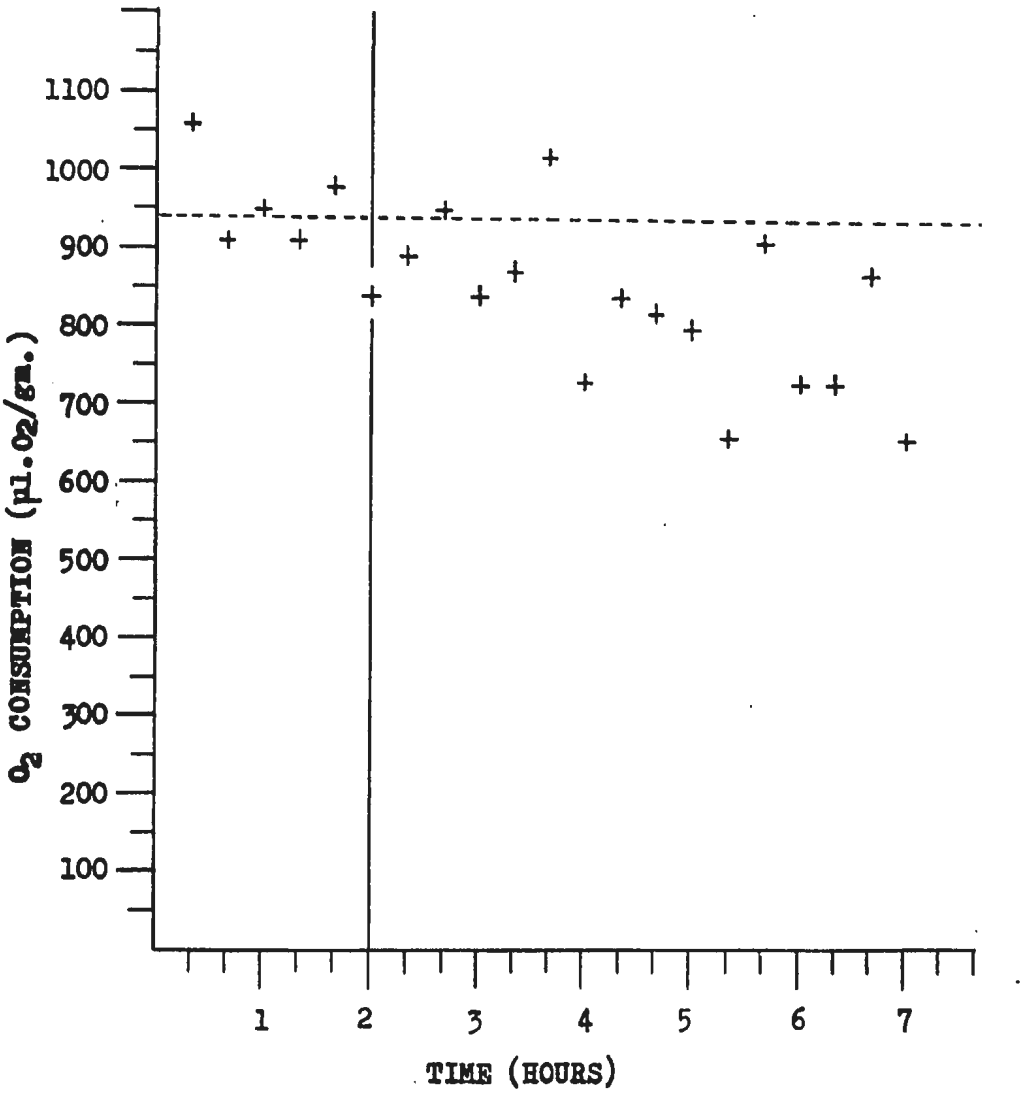

FIGURE 7. Representative sample of respiration rates (at 20 minute intervals) of excised oyster gill. Broken line indicates mean rate during initial two hours of the Warburg run. Sea water density 1.0250 ; temperature 25°C. Oyster no. 5 - 13 .

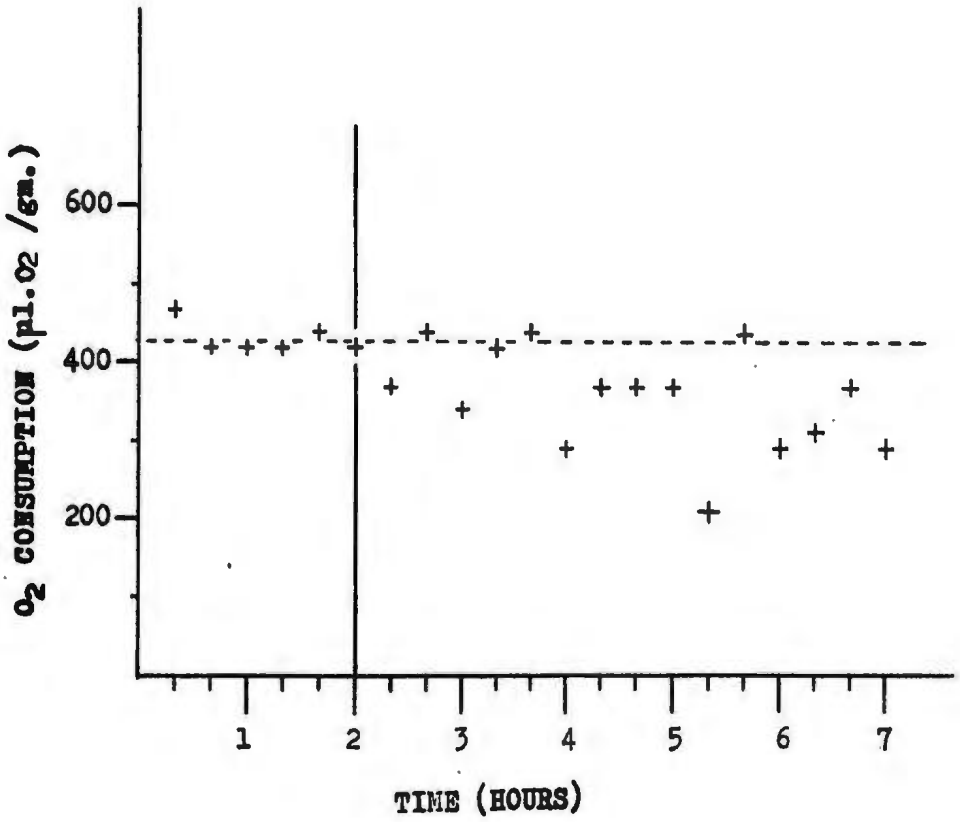

FIGURE 8. Representative sample of respiration rates (at 20 minute intervals) of excised oyster mantle. Broken line indicates mean rate during initial two hours of the Warburg run. Sea water density 1.0250 ; temperature 25° C. Oyster no. 5 - 13 .

Zonation of mantle respiration

The thickened border of the mantle had a significantly greater respiration rate than the thinner, central portion (Figure 9). The mean difference between the two rates was approximately 20% (Table 5).

In view of this difference, the mantle border was removed (Figure 1) for all subsequent respiratory determinations. Thus, the term mantle, as employed in this study, refers only to the thin, central portion of the structure.

Relative respiration rates of tissues:

The average respiration rates at 20° C. of excised gill, mantle and adductor muscle from oysters collected during the month of July are presented in the appendix (Table 25). These results may be summarized as follows:

TABLE 4

Tissue	n	QO_2 $\mu l.O_2/gm../hr. \pm S.E.$	range $\mu l.O_2/gm./hr.$
Gill	23	1853±57	1457-2370
Mantle	20	1084±43	795-1501
Adductor	19	208±15	83-359

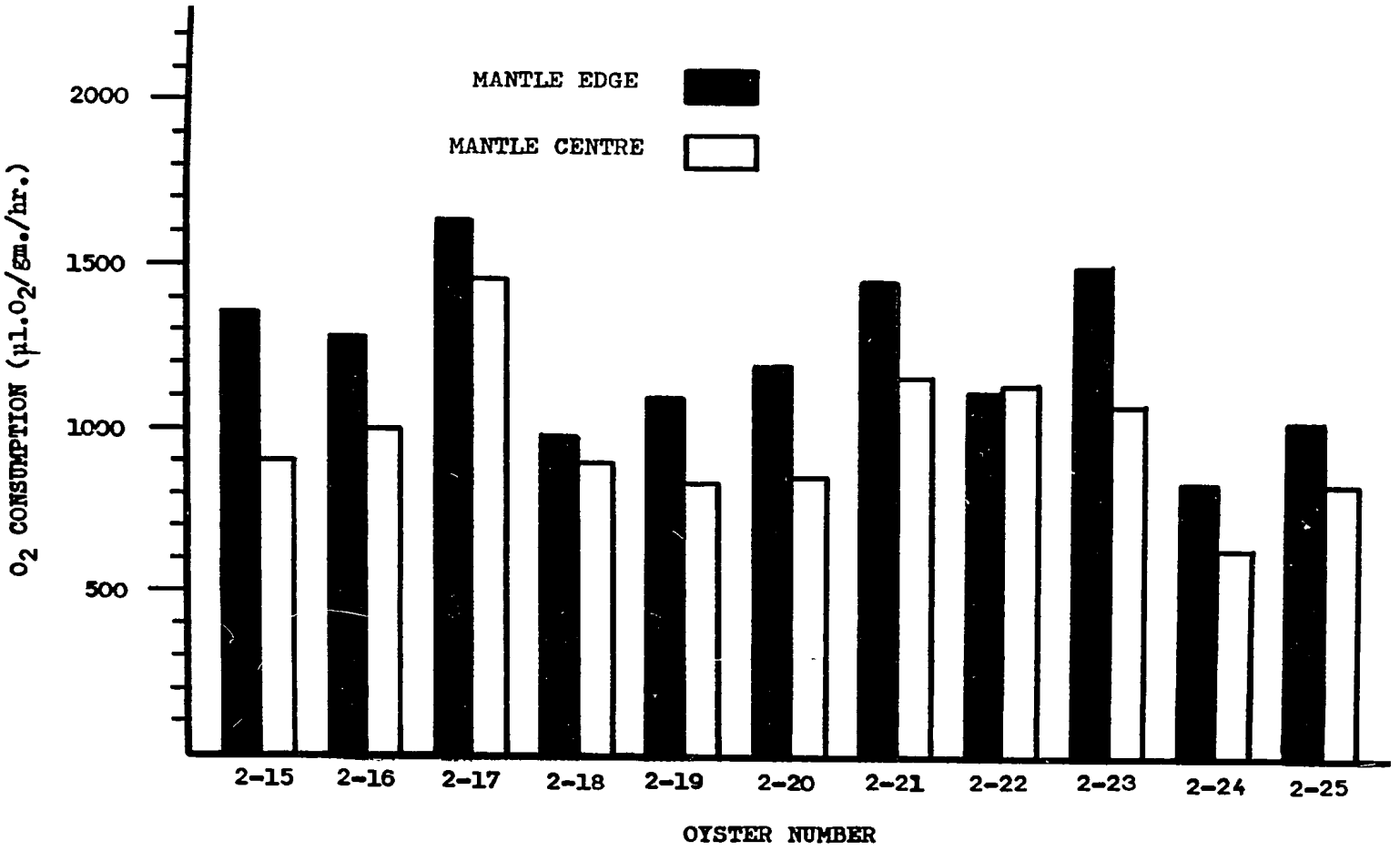

FIGURE 9. Comparison of respiration rates of tissues from central region of the mantle and from the mantle edge. Temperature 20° C.; Sea water density 1.0250 .

TABLE 5

Comparison of respiration rates of excised mantle border and mantle centre tissues of C. virginica. The rates of the two tissues from each oyster determined simultaneously at 20° C. in filtered sea water density 1.0250.

Specimen No.	Mantle edge QO_2 ($\mu l.O_2/gm./hr.$)	Mantle centre QO_2 ($\mu l.O_2/gm./hr.$)	% Difference
(#2-15)	1364	904	-33.8
(#2-16)	1287	1004	-22.0
(#2-17)	1647	1469	-10.8
(#2-18)	989	903	-8.7
(#2-19)	1099	839	-23.7
(#2-20)	1198	857	-28.5
(#2-21)	1452	1167	-19.6
(#2-22)	1116	1143	+2.4*
(#2-23)	1492	1084	-27.4
(#2-24)	834	646	-22.5
(#2-25)	1019	844	-17.2
MEAN ± S.E.	1227 ± 71	987 ± 64	-19.6

* This result not included in calculation of the mean.

When the oxygen consumption of gill tissue was used as the primary standard (=100%), then the mantle rate was about one half (58.5%) of the gill rate, while the adductor muscle rate was a little more than one tenth (11.2%) that of the gill rate.

Variability of respiration rates.

The wide range in respiration rates among tissue samples from different oysters (Table 6) might be due either to physiological differences among individuals or to errors arising from the experimental technique. An attempt was therefore made to determine the approximate magnitude of the metabolic variation directly attributable to the experimental technique, by measuring simultaneously the oxygen consumption of two or more samples of the same tissue taken from a single individual. Because of the limited quantities of tissues available, it was possible to carry out the analysis on gill tissue only.

The mean difference between maximum and minimum gill respiration rates (calculated as a percentage of the mean rate) for multiple samples from a single individual was approximately 7.7% (Table 6). As can be clearly seen in Figure 10, this is a small difference when compared with differences in oxygen consumption between individual animals and indicates that the manometric

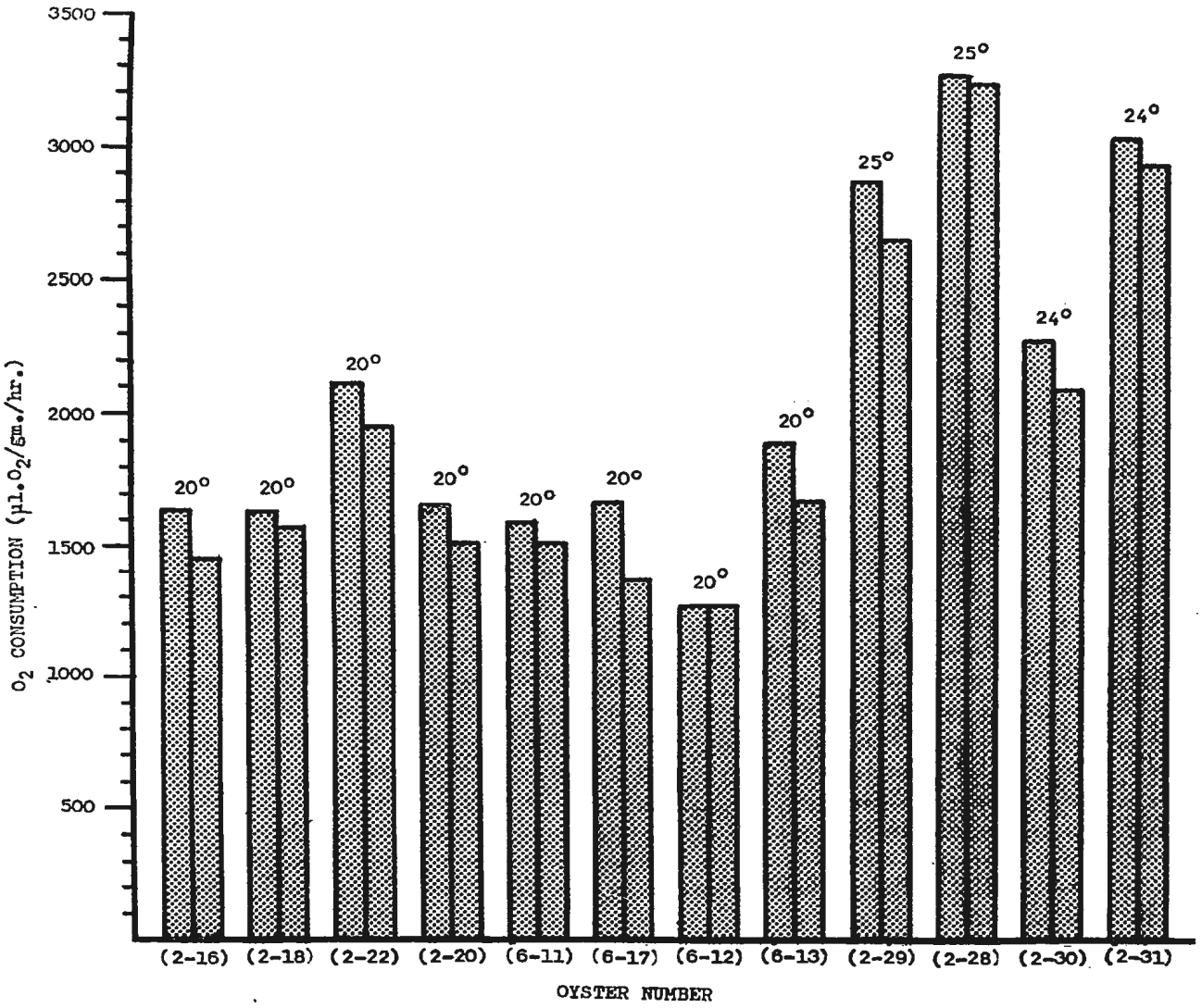

FIGURE 10. Maximum and minimum respiration rates of multiple gill samples from single oysters. (Sea water density 1.0250 ; temperature as indicated) .

TABLE 6

Experimental variations in respiration rates of multiple gill tissue samples from single oysters. (in sea water density 1.0250 at the indicated temperatures).

Specimen No.	Maximum rate ($\mu l.O_2$/ gm./hr.)	Minimum rate ($\mu l.O_2$/ gm./hr.)	difference % of mean	experimental temperature
(#2-16)	1638	1465	11.1	20° C.
(#2-18)	1654	1581	4.5	20° C.
(#2-22)	2113	1965	7.3	20° C.
(#2-20)	1661	1512	9.4	20° C.
(#6-11)	1609	1515	6.0	20° C.
(#6-12)	1693	1392	19.5	20° C.
(#6-13)	1288	1275	1.0	20° C.
(#6-17)	1894	1682	11.8	20° C.
(#2-29)	2870	2653	7.8	25° C.
(#2-28)	3285	3236	1.5	25° C.
(#2-30)	2288	2108	8.1	24° C.
(#2-31)	3056	2936	4.0	24° C.

Mean difference: 7.7%

technique is sufficiently sensitive for the purposes of the present study.

Relationship between "condition" index and dry weight of oysters.

If the oyster's "condition" index is indeed a function of the quantity of glycogen stored within the tissues, as previously suggested, then the dry weights of the animals should increase with increasing "condition" rating.

The dry body weights (excluding shell) of the animals used for respiratory studies were grouped according to "condition" index. The mean length of the oysters used was 7.5 cm. (range: 6.2-9.6 cm.) and the mean width was 4.6 cm. (range: 3.7-5.9 cm.). There was no apparent correlation between animal size and "condition" index over the limited size range used.

The total dry body weight increased with increasing "condition" index (Figure 11) in animals of approximately the same overall size. The mean dry weight of oysters of "condition" 2 was less than half (46.9%) that of oysters of "condition" 7 (Table 7).

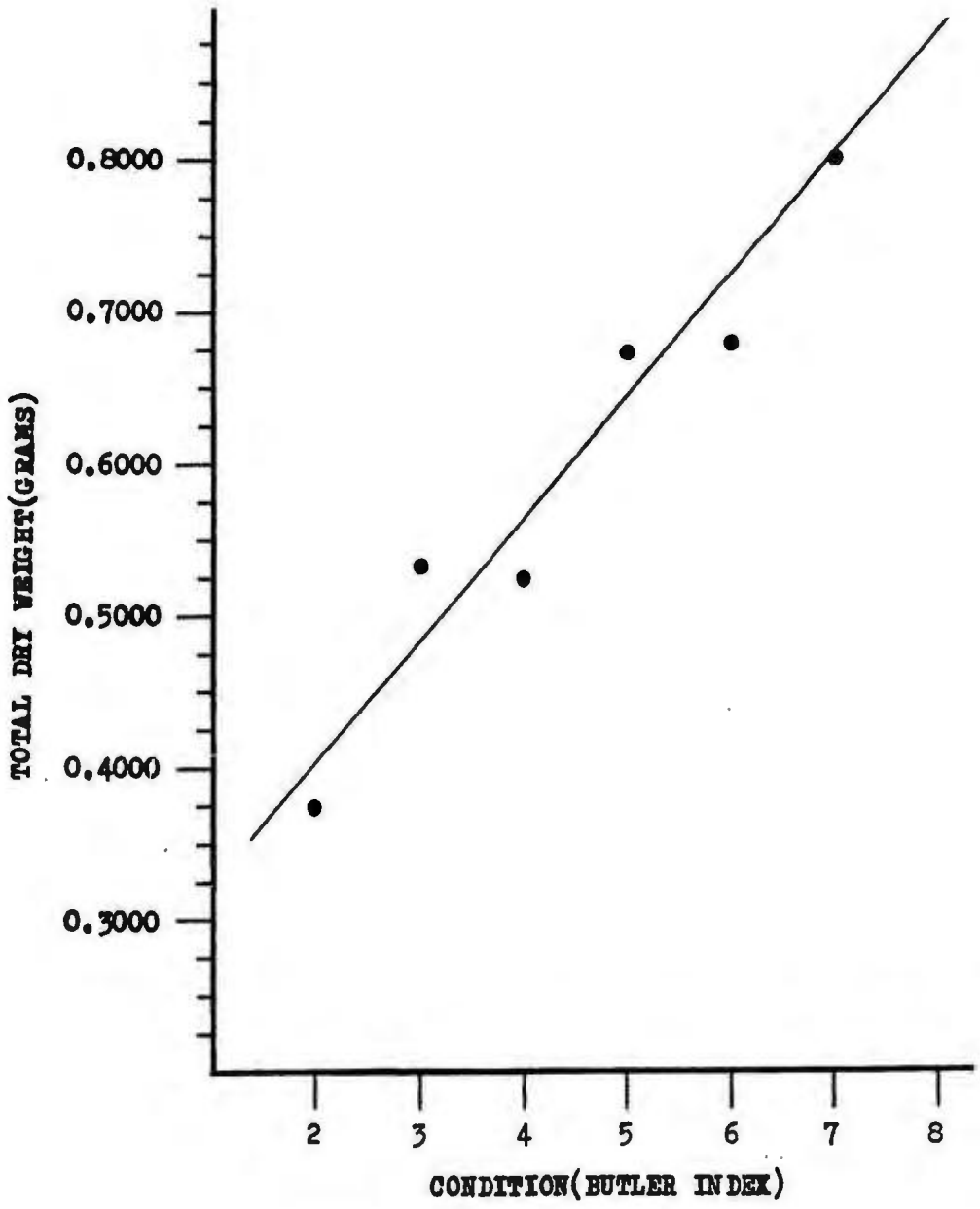

FIGURE 11. Correlation between "condition" of the oyster and dry body weight (excluding shell) .

TABLE 7

Relation between oyster "condition" index and total dry tissue weight (excluding shell). (Dried at 60° C. to constant weight).

	"condition" index					
	2	3	4	5	6	7
dry weight(gm.)	0.3766	0.5362	0.5237	0.6680	0.6820	0.8072
sample size.	4	8	24	45	21	6
stand. error.	±0.0311	±0.0445	±0.0220	±0.0200	±0.0349	±0.0474

Relationship between "condition" index and tissue oxygen consumption.

The mean respiration rates of gill, mantle and adductor muscle from oysters grouped according to "condition" indices are presented in tables 8, 9 and 10. In these tables results from animals collected in summer and in autumn are grouped separately. Table 11 shows the respiration rates (expressed as percent of respiration rate at C.I. 3) of summer and autumn samples of oysters ranging in "condition" index from 2 to 7.

The mantle respiration rate showed marked inverse correlation with the overall "condition" index of the oysters in that the rate declined steadily with increasing "condition" index (Table 11). This correlation was evident in oysters collected in both summer and autumn (Figure 13). During the summer the mantle from oysters of "condition" 7 had a 38% lower mean respiration rate than similarly sized oysters of "condition" 3. During the autumn the difference in mantle respiration rates of oysters of "condition" 3 and 7 was approximately 31%, with the lowest "condition" oysters again exhibiting the greatest respiration rate.

There appeared to be no significant correlation between the respiration rates of excised gills or adductor muscle and the "condition" index of the oysters (Figures 12 and 14) comparable to that exhibited by the mantle.

TABLE 8

The relation between "condition" index of oyster and respiration rate of excised gill tissue (medium: sea water density 1.0250, temperature 20° C.).

Gill (July and August)

"condition" index	n	$QO_2 \pm$ S.E. $\mu l.O_2/gm./hr.$
3	2	1834 ± 198
4	6	1666 ± 128
5	24	1852 ± 63
6	8	1851 ± 52
7	2	1929 ± 192

Gill (September, October, November)

"condition" index	n	$QO_2 \pm$ S.E. $\mu l.O_2/gm./hr.$
2	4	1524 ± 240
3	6	1619 ± 104
4	18	1550 ± 48
5	21	1516 ± 40
6	12	1489 ± 92
7	5	1397 ± 98

TABLE 9

Relation between "condition" index of oyster and respiration rate of excised mantle tissue of C. virginica (medium: sea water, density 1.0250, temperature 20° C.).

Mantle (July and August)

"condition" index	n	$QO_2 \pm$ S.E. $\mu l.O_2/gm./hr.$
3	2	1203± 88
4	6	1097± 92
5	24	1019± 37
6	7	920± 45
7	2	749± 12

Mantle (September, October and November)

"condition" index	n	$QO_2 \pm$ S.E. $\mu l.O_2/gm./hr.$
2	4	1037± 21
3	6	995± 59
4	18	923± 34
5	22	774± 23
6	13	822± 48
7	4	683± 37

TABLE 10

Relation between "condition" index of oyster and respiration rate of excised adductor muscle of C. virginica (medium: sea water, density 1.0250, temperature 20° C.).

Adductor Muscle (July and August)

"condition" index	n	$QO_2 \pm$ S.E. $\mu l.O_2/gm./hr.$
3	2	179 ± 27
4	5	196 ± 21
5	19	213 ± 14
6	7	221 ± 28
7	2	198 ± 8

Adductor muscle (September, October and November)

"condition" index	n	$QO_2 \pm$ S.E. $\mu l.O_2/gm./hr.$
2	3	233 ± 49
3	3	251 ± 46
4	12	240 ± 23
5	19	235 ± 18
6	10	234 ± 16
7	2	181 ± 10

TABLE 11

Mean respiration rates (expressed as percentage of rate of tissues from oysters of "condition" 3) of gill, mantle and adductor muscle in summer and autumn in relation to "condition" index.

"condition" index (C.I.)	gill rate % of C.I.3		mantle rate % of C.I.3		adductor rate % of C.I. 3	
	summer	autumn	summer	autumn	summer	autumn
2	-	94%	-	104%	-	93%
3	100%	100%	100%	100%	100%	100%
4	91%	96%	91%	93%	109%	96%
5	101%	94%	85%	78%	119%	94%
6	101%	92%	76%	83%	123%	93%
7	105%	86%	62%	69%	111%	72%

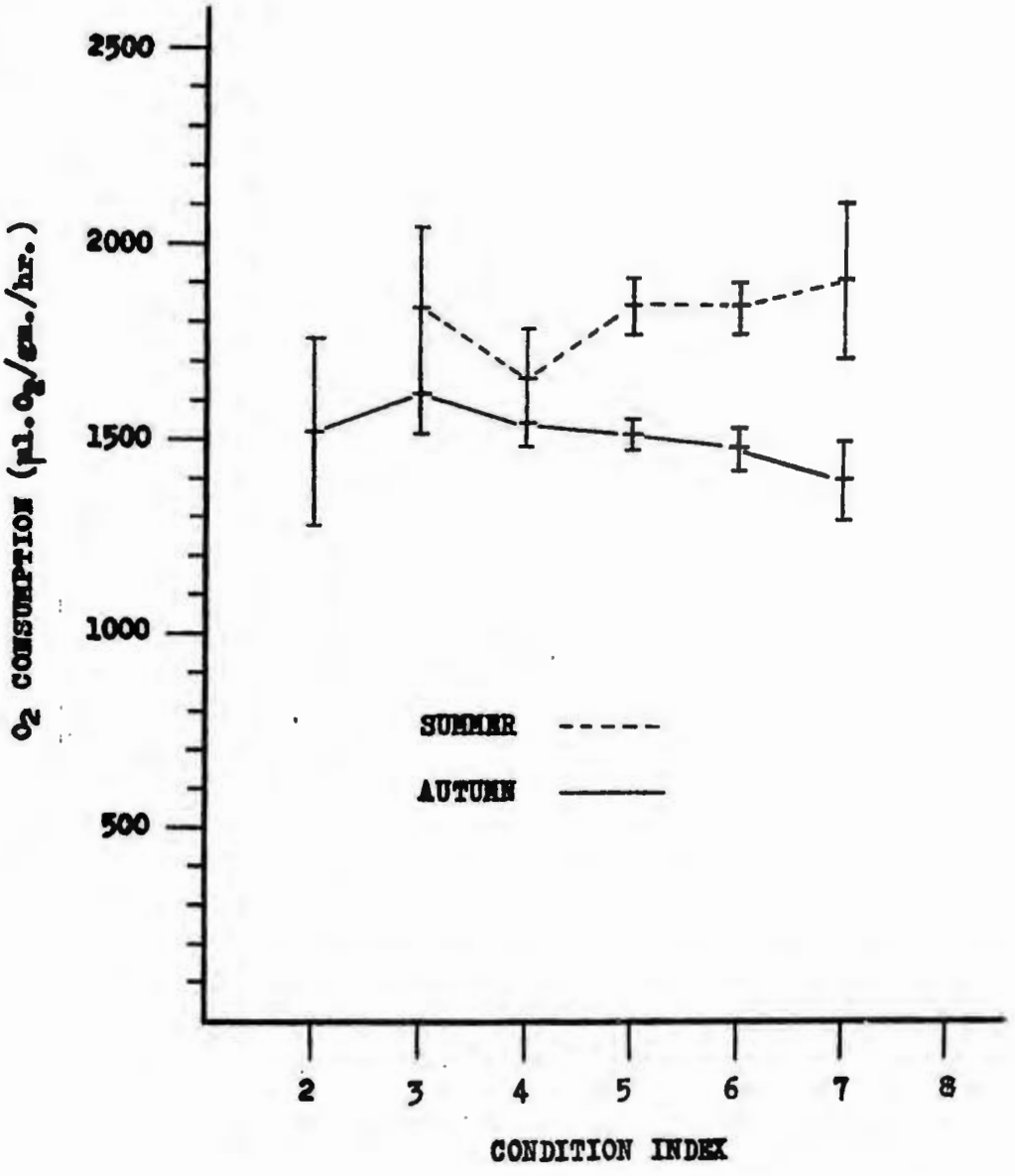

FIGURE 12. Respiration rates of excised gill during summer and autumn as a function of "condition" rating of the oyster. Sea water density 1.0250 ; temperature 20° C.

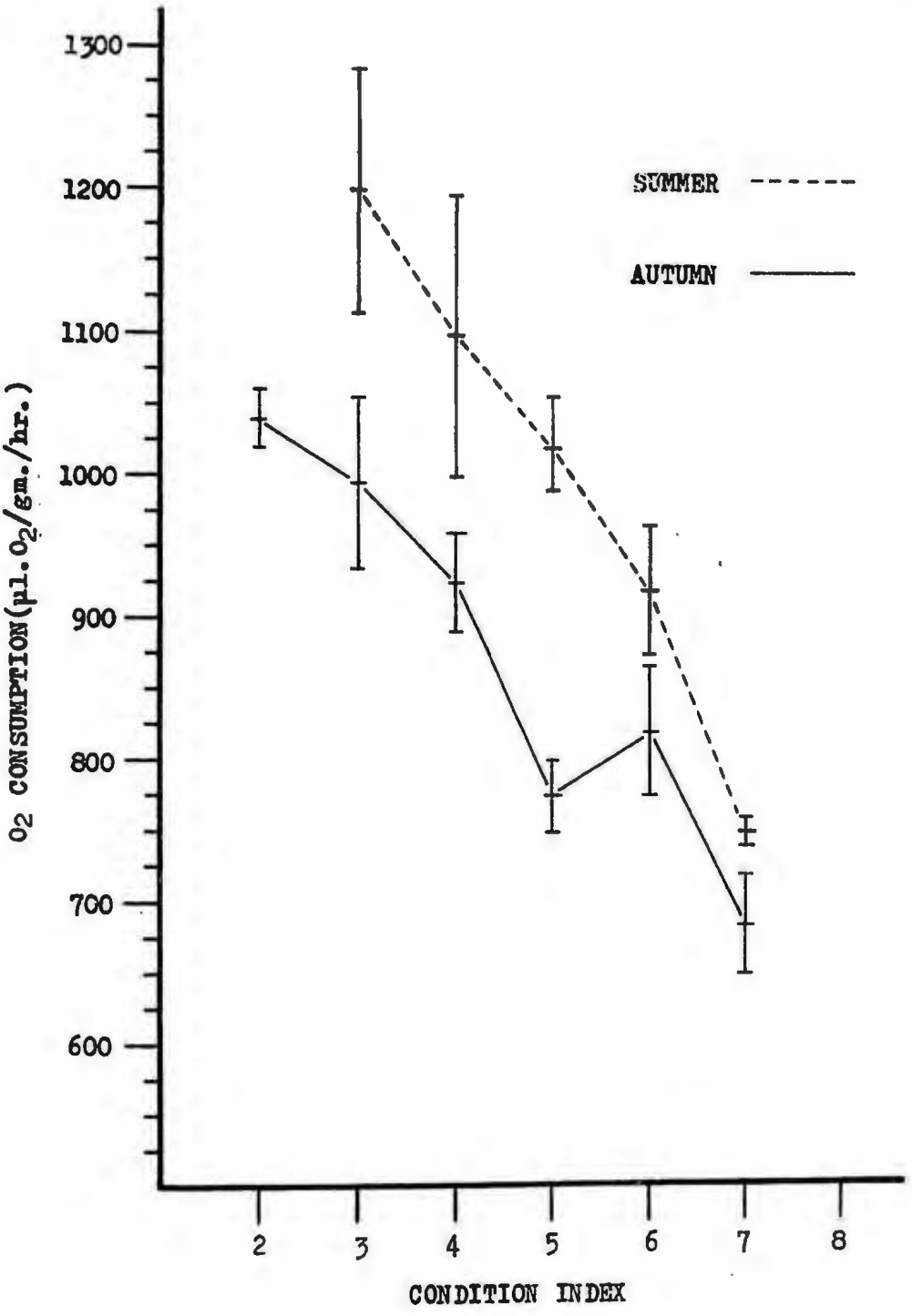

FIGURE 13. Respiration rates of excised mantle during summer and autumn as a function of "condition" rating of the oyster. (Sea water density 1.0250 ; temperature 20° C.)

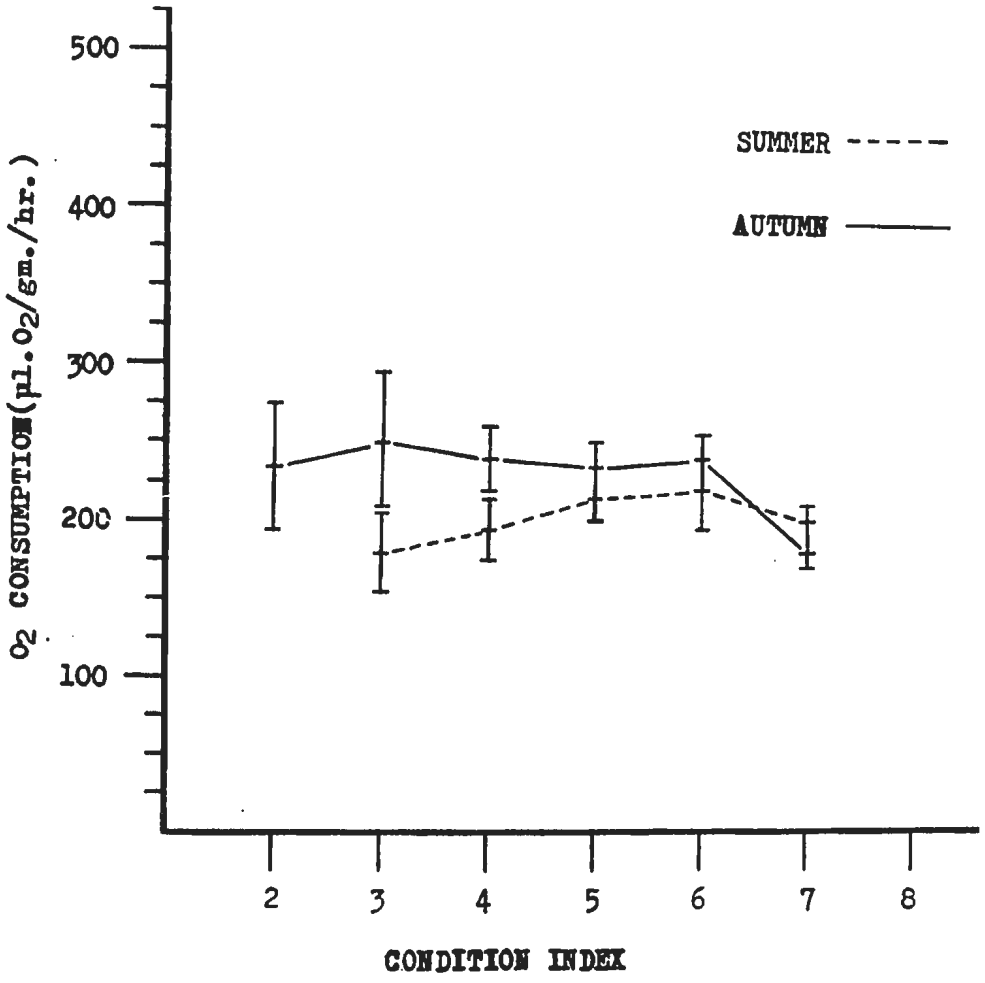

FIGURE 14. Respiration rates of excised adductor muscle during summer and autumn as a function of "condition" rating of the oyster . (Sea water density 1.0250 ; temperature 20 ° C.

Influence of salinity on respiration of excised tissues:

Excised gill, mantle and adductor muscle differed considerably in their metabolic response to dilution of the sea water medium (Table 12).

Gill respiration increased upon dilution of the medium (Figure 15). In sea water of standard density (1.0250) the mean oxygen consumption was 1853 $\mu l.O_2/gm./hr.$ In sea water diluted to densities of 1.0150 and 1.0100 the mean respiration rates were 8.5% (2010 $\mu l.O_2/gm./hr.$) and 28.6% (2383 $\mu l.O_2/gm./hr.$) greater, respectively, than in sea water of standard density (1.0250).

Dilution of the sea water medium inhibited the respiration rate of excised mantle. In sea water of density 1.0250 the mean rate was 1084 $\mu l.O_2/gm./hr.$ In sea water diluted to densities of 1.0150 and 1.0100 the respiration rates were 0.6% (1078 $\mu l.O_2/gm./hr.$) and 13.4% (939 $\mu l.O_2/gm./hr.$) lower, respectively, than in sea water of density 1.0250.

Extensive dilution of the medium resulted in a marked overall decline in adductor muscle respiration. In sea water of standard density (1.0250) the rate was 208 $\mu l.O_2/gm./hr.$ In sea water diluted to densities of 1.0150 and 1.0100 the rates were 220 $\mu l.O_2/gm./hr.$ and 98 $\mu l.O_2/gm./hr.$, respectively; the latter representing a 52.9% decrease from the rate in standard density (1.0250) sea water.

The mean respiration rates of excised gill at densities of 1.0150 and 1.0250 were not significantly different at the 0.05 level of confidence. However, the difference between the rates of oxygen consumption in sea water of densities 1.0250 and 1.0100 was highly significant (0.01 level of confidence).

There was no significant difference (at 0.05 level of confidence) between the mean respiration rates of mantle slices in sea water of densities 1.0250 and 1.0100. The apparent depression of mantle respiration in dilute sea water is thus of questionable significance.

There was no significant difference between the rates of oxygen consumption in sea water of densities 1.0250 and 1.0150 (0.05 level of confidence). However, the difference between the rates in sea water of densities 1.0250 and 1.0100 was highly significant (0.01 level of confidence).

TABLE 12

Respiration rates of excised gill, mantle and adductor muscle of C. virginica in sea water of various densities (Temperature 20° C.).

Tissue	Density	n	QO_2 $\mu l.O_2/gm./hr. \pm S.E.$
GILL	1.0100	8	2383 ± 86
	1.0150	7	2010 ± 120
	1.0250	23	1853 ± 57
MANTLE	1.0100	11	939 ± 96
	1.0150	6	1087 ± 83
	1.0250	20	1084 ± 43
ADDUCTOR	1.0100	6	98 ± 7
	1.0150	5	220 ± 30
	1.0250	19	208 ± 15

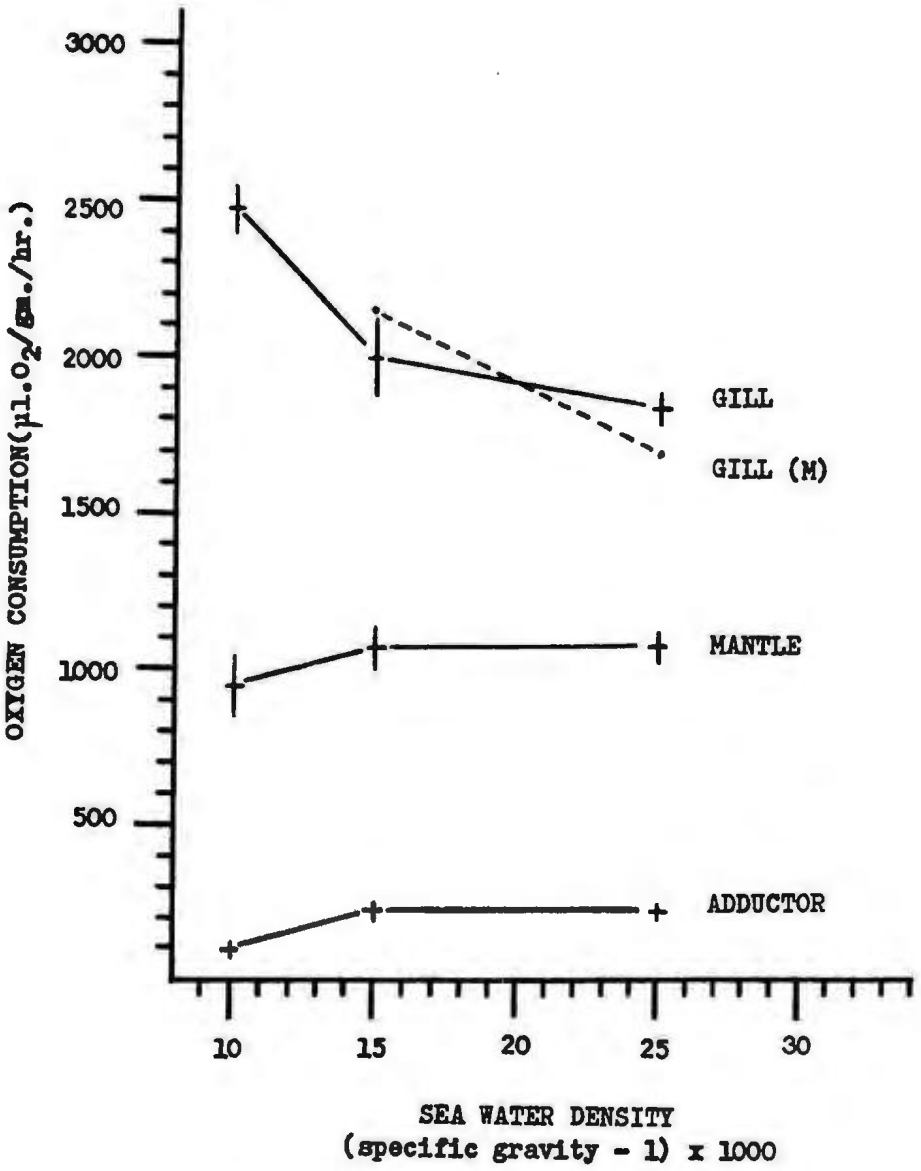

FIGURE 15. Influence of sea water density on the respiration rates of excised gill, mantle and adductor muscle of C. virginica and gill (M) of M. mercenaria (Hopkins, 1946). Temperature 20° C.

Influence of temperature on tissue respiration.

Respiration rates of gill, mantle and adductor muscle were determined at 4°C. intervals from 12°C. to 40°C. without prior acclimation of the animal to the experimental temperatures. Rates measured in this manner are said to be acutely determined. The respiration of any one tissue sample was measured at only one temperature.

All three tissues exhibited an increase in oxygen consumption with rising temperature (Tables 13,14,15). Both the mantle and adductor muscle had respiratory maxima in the vicinity of 32°C. while the gill respiration rate increased continuously up to 40°C., with the rate of increase in the latter tissue falling off considerably above 28°C. (Figure 16).

The maximum Q_{10} coefficients for gill and mantle were 4.353 and 4.648, respectively, and both occurred in the 16°C. - 20°C. range (Table 16). In contrast, the Q_{10} coefficient of the adductor muscle respiration was minimal (1.000) in the 16°C. - 20°C. range, indicating a plateau of relative temperature insensitivity in the rate curve (Figure 17).

TABLE 13

Influence of temperature on respiration rate of excised gill of C. virginica (August sample. Medium: sea water density 1.0250).

Temperature	n	$Q0_2$ $\mu l.0_2/gm./hr. \pm S.E.$
12° C.	6	639 ± 54
16° C.	5	1008 ± 38
20° C.	21	1815 ± 67
24° C.	5	2761 ± 98
28° C.	4	3564 ± 111
32° C.	5	3881 ± 246
36° C.	5	4307 ± 462
40° C.	6	4418 ± 522

TABLE 14

Influence of temperature on respiration rate of excised mantle of C. virginica (August sample. Medium: sea water, density 1.0250).

temperature	n	Q_{O_2} $\mu l.O_2/gm./hr. \pm S.E.$
12° C.	5	421 ± 30
16° C.	5	531 ± 57
20° C.	20	982 ± 29
24° C.	6	1266 ± 113
28° C.	6	1695 ± 132
32° C.	6	1951 ± 103
36° C.	5	1613 ± 115
40° C.	6	1081 ± 179

TABLE 15

Influence of temperature on respiration rate of excised adductor muscle of *C. virginica* (August sample. Medium: sea water, density 1.0250).

temperature	n	QO_2 $\mu l.O_2/gm./hr. \pm S.E.$
12° C.	4	80 ± 11
16° C.	2	213 ± 8
20° C.	17	212 ± 16
24° C.	4	238 ± 25
28° C.	5	388 ± 54
32° C.	5	485 ± 72
36° C.	4	350 ± 24
40° C.	3	165 ± 9

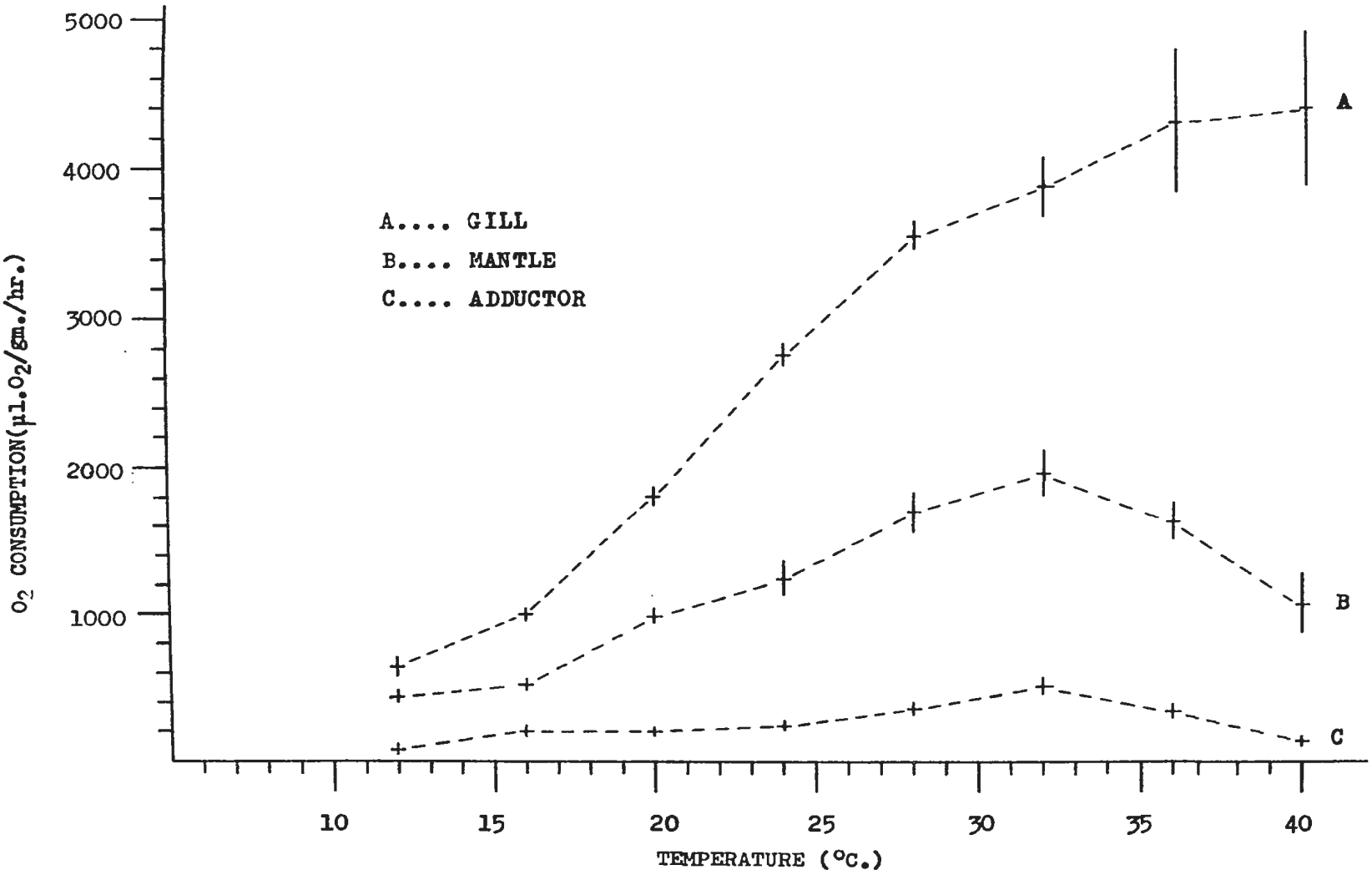

FIGURE 16. Acutely determined rate - temperature curves of respiration of excised gill, mantle and adductor muscle. Sea water density 1.0250 ; August sample.

TABLE 16

Q_{10} values of respiration rates of excised gill, mantle, and adductor muscle of C. virginica (August sample).

Temperature range	Gill Q_{10}	Mantle Q_{10}	Adductor Q_{10}
12° C. - 16° C.	3.125	1.785	11.570
16° C. - 20° C.	4.353	4.648	1.000
20° C. - 24° C.	2.853	1.887	1.336
24° C. - 28° C.	1.893	2.075	3.392
28° C. - 32° C.	1.238	1.422	1.747
32° C. - 36° C.	1.298	-	-
36° C. - 40° C.	1.065	-	-

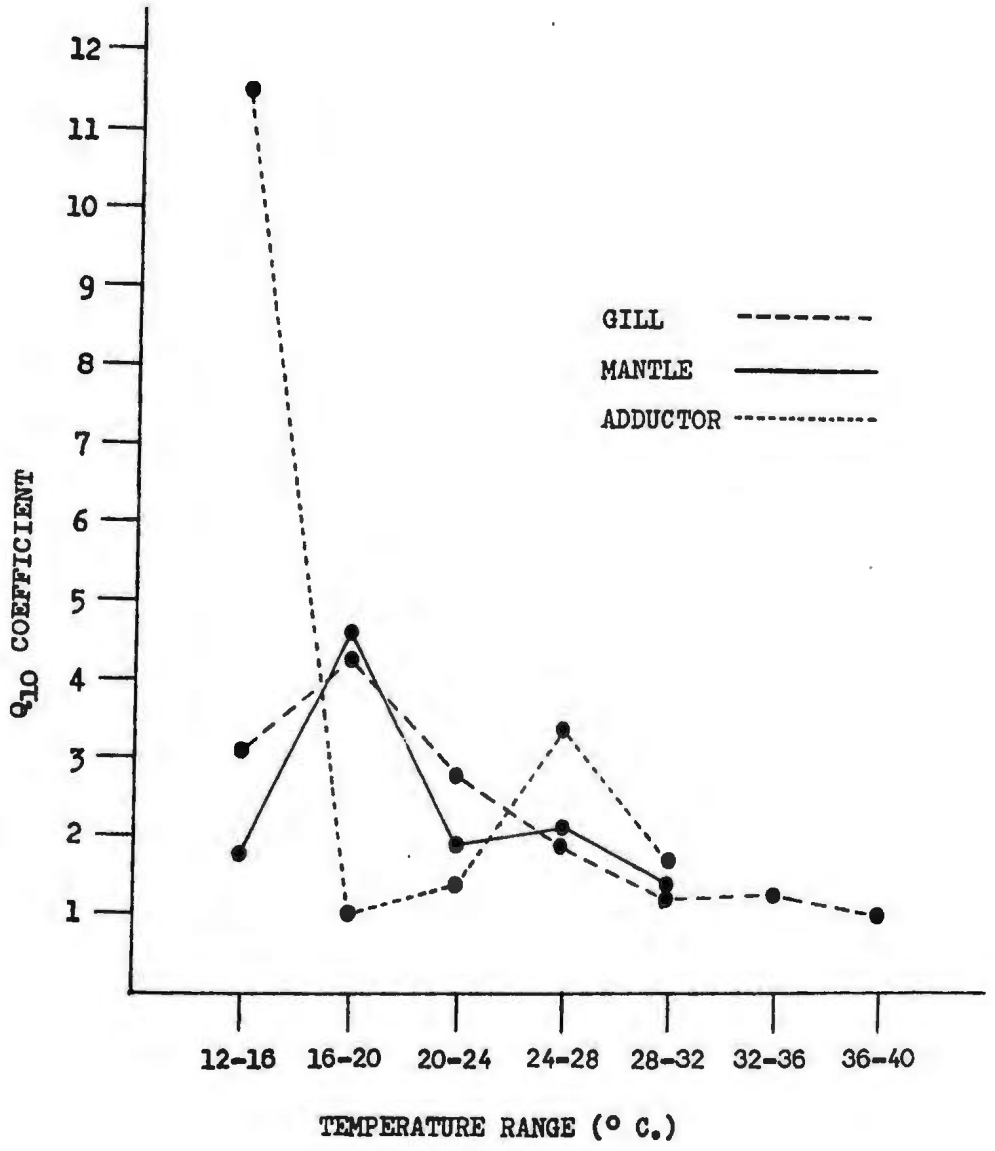

FIGURE 17. Q_{10} coefficients of respiration of excised gill, mantle and adductor muscle of C. virginica .

Respiration rates of excised tissues in summer and autumn.

A marked difference in respiration rate was evident between gill, mantle and adductor muscle from animals collected during the summer (July and August) and similar tissues from animals collected during the autumn (September, October and November).

TABLE 17

Respiration rates of excised gill, mantle and adductor muscle of C. virginica in summer and autumn. (Sea water density, 1.0250, temperature 20° C.).

	Mean summer QO_2		Mean autumn QO_2	
Tissue	n	$\mu l.O_2/gm./hr. \pm$ S.E.	n	$\mu l.O_2/gm./hr. \pm$ S.E.
Gill	44	1835 ± 44	53	1524 ± 28
Mantle	40	1033 ± 27	54	833 ± 22
Adductor	36	210 ± 10	42	237 ± 12

Gill tissue from animals collected in the summer had a mean QO_2 of 1835 $\mu l.O_2/gm./hr.$ at 20° C., while in the autumn at the same temperature, the mean respiration rate of excised gill tissue was 1524 $\mu l.O_2/gm./hr.$; an overall decline of 16.8% (Table 17). This difference was highly significant (0.001 level). Tables 18 and 19 indicate that the sharp fall in gill metabolism occurred during the late August - early September period. In August the mean gill respiration rate was 1815 $\mu l.O_2/gm./hr.$ and in September it fell to 1508 $\mu l.O_2/gm./hr.$ and remained at approximately the same level during the succeeding two months (Tables 18 and 19).

At 20° C. the mean respiration rate of excised mantle during the summer was 1033 $\mu l.O_2/gm./hr.$. The mean respiration rate during September, October and November was 833 $\mu l.O_2/gm./hr.$, a decline of 18.4% from the summer rate (Table 17). The difference between summer and autumn mantle respiration was highly significant (0.001 level). As in the case of the gill respiration, the major part of the decline in mantle respiration occurred during the late August - early September period (Tables 18 and 19). In August the mantle rate was 982 $\mu l.O_2/gm./hr.$ and in September it was 831 $\mu l.O_2/gm./hr.$

During the summer at 20° C., the respiration rate of excised adductor muscle was 210 $\mu l.O_2/gm./hr.$ In the autumn the rate increased by 13.8% to 237 $\mu l.O_2/gm./hr.$ This response is exactly opposite to that observed in the gill and mantle. As in the case of the gill and mantle, the major portion of the metabolic shift occurred during the late August - early September period. In August, the mean rate was 212 $\mu l.O_2/gm./hr.$ while during September it rose to 236 $\mu l.O_2/gm./hr.$ and remained at approximately the same level during the two succeeding months. The mean difference between adductor muscle respiration in summer and autumn was not significant at the 0.05 level.

Measurements of oxygen consumption of gill, mantle and adductor muscle at 28° C. during August and again during November revealed shifts in rates similar to those observed at 20° C. (Table 20). At 28° C. the gill respiration fell from 3564 $\mu l.O_2/gm./hr.$ in August to 3489 $\mu l.O_2/gm./hr.$ in November. At the same temperature the respiration rate of excised mantle declined from 1695 $\mu l.O_2/gm./hr.$ in August to 1548 $\mu l.O_2/gm./hr.$ in November. The adductor muscle respiration again exhibited the opposite effect, rising from 388 $\mu l.O_2/gm./hr.$ in August to 463 $\mu l.O_2/gm./hr.$ in November.

Figures 18, 19 and 20 which show respiration rates at 20° C. and 28° C. of gill, mantle and adductor muscle, respectively, during the summer and autumn demonstrate clearly that during the autumn, over this limited temperature range, the rate-temperature curves for excised gill and mantle are shifted to the right relative to the summer rate -temperature curve. For the adductor muscle, the autumn R-T curve is shifted to the left relative to the summer R-T curve.

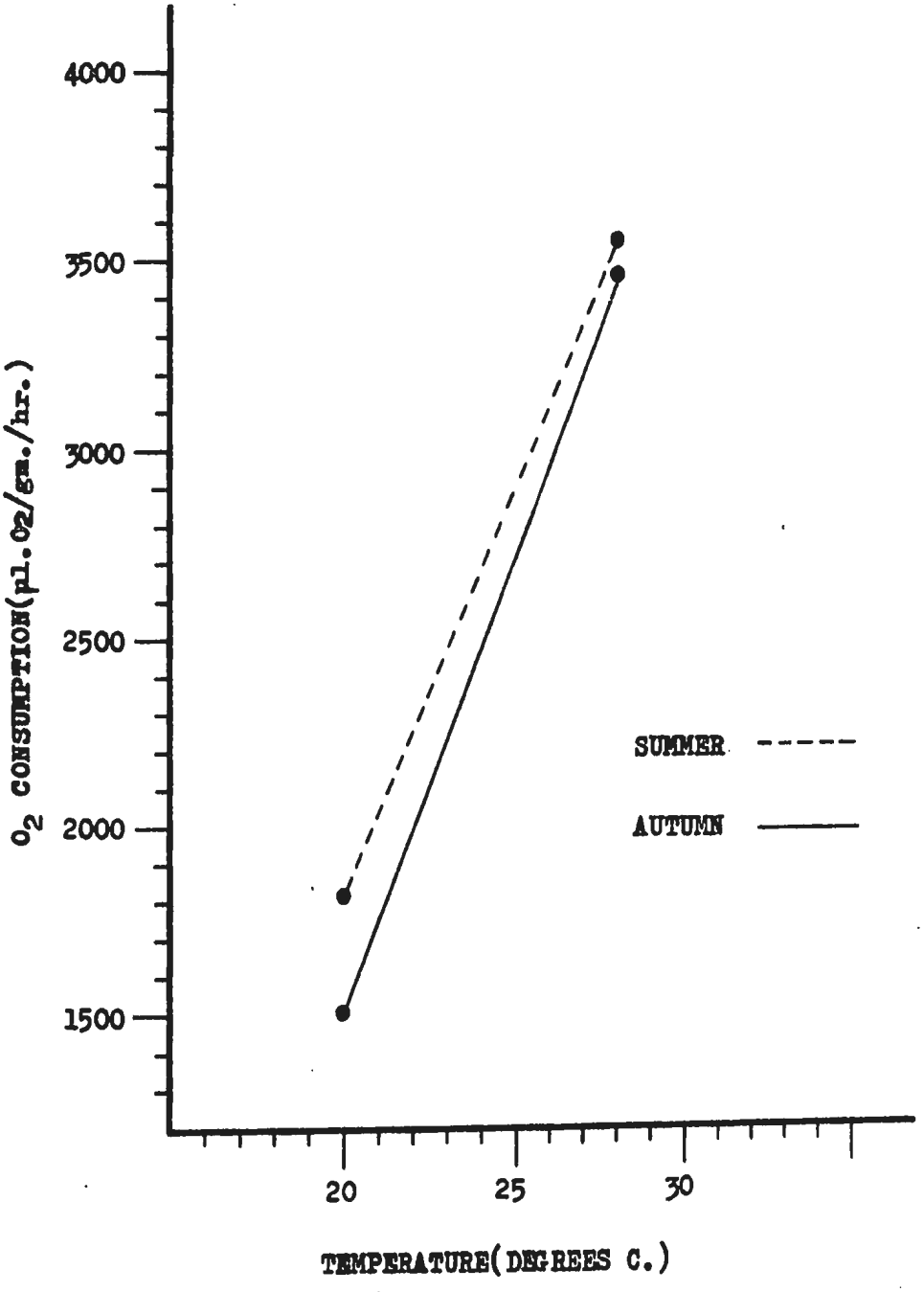

FIGURE 18. Rate - temperature curves for excised gill during summer and autumn . (Sea water density 1.0250).

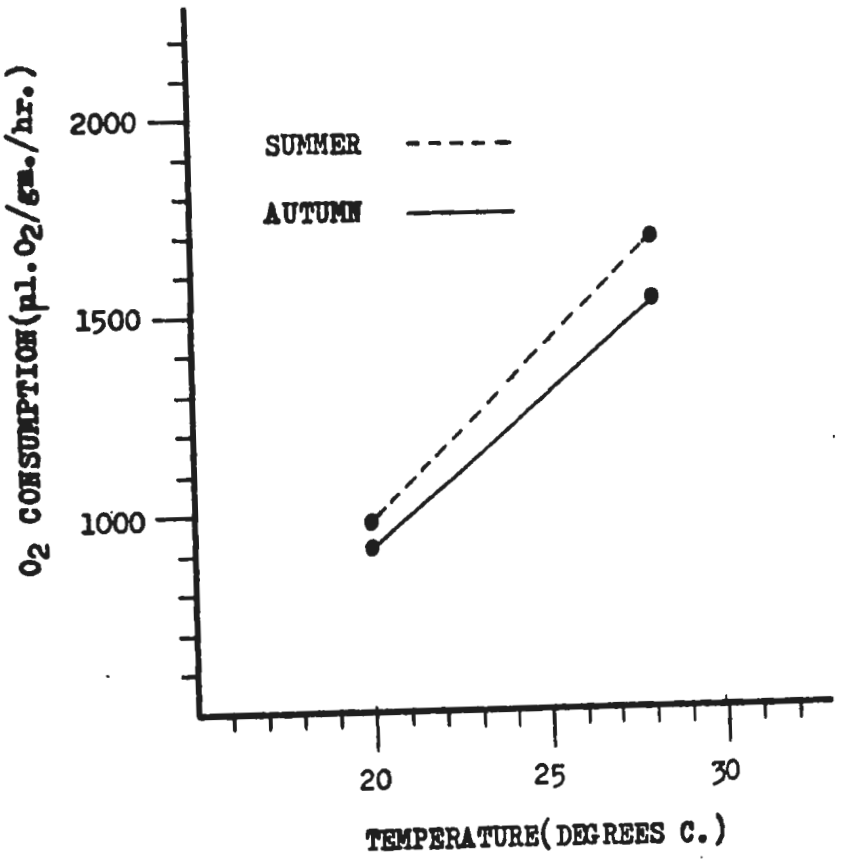

FIGURE 19. Rate - temperature curves for excised mantle during summer and autumn. (Sea water density 1.0250).

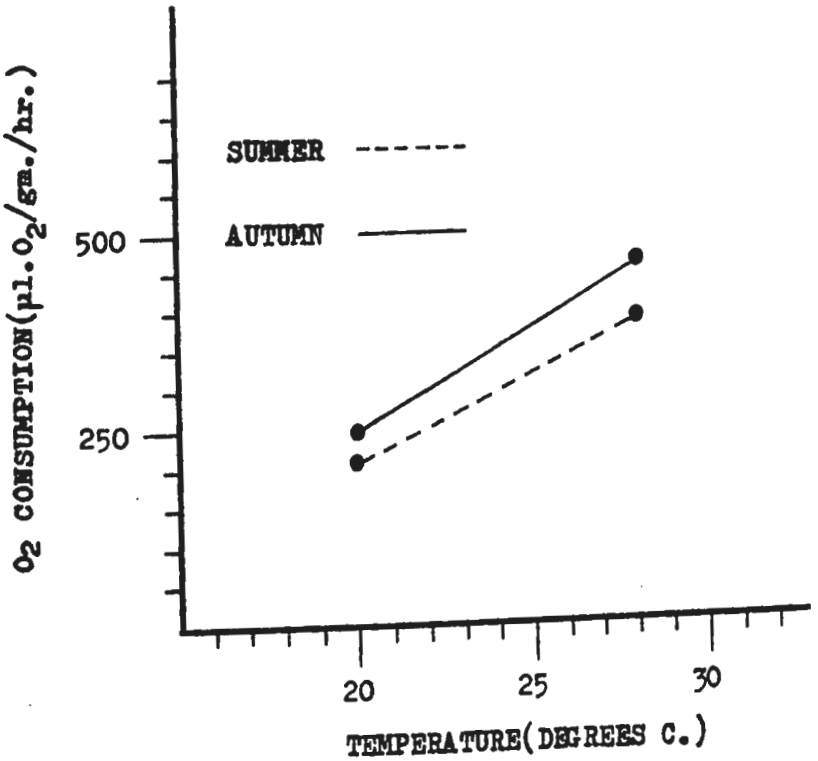

FIGURE 20. Rate - temperature curves for excised adductor muscle during summer and autumn.(Sea water density

TABLE 18

Respiration rates ($\mu l.O_2/gm.$ dry wt./hr.) of excised gill, mantle and adductor muscle of C. virginica during July and August. (At 20° C. in sea water density 1.0250).

Month	Tissue	n	QO_2 $\mu l.O_2/gm./hr.$ ± S.E.
July	GILL	23	1853 ± 57
	MANTLE	20	1084 ± 43
	ADDUCTOR	19	208 ± 15
August	GILL	21	1815 ± 67
	MANTLE	20	982 ± 29
	ADDUCTOR	17	212 ± 16

TABLE 19

Respiration rates ($\mu l.O_2/gm.$ dry wt./hr.) of excised gill, mantle and adductor muscle of C. virginica. during September, October and November. (At 20° C. in sea water density 1.0250).

Month	Tissue	n	QO_2 $\mu l.O_2/gm./hr. \pm$ S.E.
September	GILL	18	1508 ± 50
	MANTLE	18	831 ± 37
	ADDUCTOR	14	236 ± 21
October	GILL	20	1561 ± 30
	MANTLE	23	782 ± 22
	ADDUCTOR	17	234 ± 17
November	GILL	16	1509 ± 59
	MANTLE	16	915 ± 44
	ADDUCTOR	11	246 ± 20

TABLE 20

Summer and autumn respiration rates at 28° C. of gill, mantle and adductor muscle tissue of C. virginica. (Sea water density 1.0250).

Month		n	Q_{O_2} $\mu l.O_2/gm./hr. \pm$ S.E.
August sample 28°C.	GILL	4	3564 ± 111
	MANTLE	6	1695 ± 132
	ADDUCTOR	5	388 ± 54
November sample 28°C.	GILL	8	3489 ± 112
	MANTLE	8	1548 ± 83
	ADDUCTOR	6	463 ± 58

DISCUSSION

The American Oyster, a sedentary, coastal marine animal of the north temperate zone-ranging from the Gulf of St. Lawrence to the Gulf of Mexico and the West Indies- may be subject to considerable seasonal fluctuations in temperature and salinity. The work of Galtsoff(1964) indicates that the intact oyster exhibits reverse acclimatization in response to seasonal temperature changes and compensatory acclimatization in response to long-term salinity changes. It is evident from my work that characteristic immediate and long-term responses to changes in temperature and salinity are evident also in excised tissues. However, it is not always possible to predict, on the basis of the responses of the intact animal, the metabolic responses of any given tissue. The results obtained in the present study indicate that gill, mantle and adductor muscle of the oyster differ considerably from one another in both their immediate and long-term responses to changes in these environmental factors.

Critique of tissue respiration measurement: The metabolism of excised tissue undoubtedly differs from that of the same tissue in situ. For examplo, the activity of the gill cilia of the freshwater bivalve, Anodonta cygnea, is greater in the excised gill than in that still attached to the animal (Lagerspetz and Dubitscher,1966). Furthermore, the oxygen uptake of excised tissues varies according to the particular measuring technique employed (Chapheau, 1932). However,

although the respiration rate of excised tissue may bear little resemblance to the absolute respiration rate of the tissue *in situ*, it is quite generally used as an indicator of progressive changes, such as acclimatization, occurring in the respiratory metabolism of the tissue *in situ*.

It is obviously important to maintain as nearly physiological conditions as possible in the Warburg flasks. The lower - than - natural CO_2 tension in the flasks may have affected the respiration rates. The work of Umbreit, Burris and Stauffer (1959), however, suggests that the direct effect of low CO_2 tension is insignificant. Furthermore, a potential indirect effect, the inactivation of the natural bicarbonate buffer system resulting from low CO_2 tension in the sea water medium, was considered unimportant in the present study; the change in hydrogen ion concentration from the beginning to the end of the Warburg run was less than pH 0.6 . Robbie (1949), working with excised tissues of marine invertebrates, reported that the respiratory CO_2 production was sufficient to prevent excessive pH shifts in the medium; Hopkins (1946) furthermore, using excised gills of the clam, *Mercenaria mercenaria*, found the change in hydrogen ion concentration to be less than pH 0.4.

Survival of excised tissues: In view of the overall low respiration rates of the excised tissues it was desirable to extend the respiratory determinations over as long a period

as possible. Consequently it was necessary to establish the maximum period which oxygen consumption remained reasonably constant.

Excised bivalve tissues, in general, appear to be able to survive without special treatment for a considerable period. In the freshwater mussel, Dreissensia sp.*, the ciliary activity and oxygen consumption of the gill continued at a fairly uniform level for at least 24 hours after excision (Wernstedt,1944; Bielawski, 1961). The isolated gills of the freshwater clam, Anodonta cygnea, survived in tap water for several days at 21°C. and for several weeks at 5°C. (Lagerspetz and Dubitscher, 1966), while the gills of the scallop, Pecten sp., exhibited great sensitivity and muscular response for two or three days after separation from the body (Setna, 1930). However, none of these studies involved confinement of the tissues in Warburg flasks where there is constant shaking in a small volume of medium.

In the present study it was found that excised gill, mantle and adductor muscle tissues of the oyster utilized oxygen while in the Warburg flasks for periods up to 3 or 4 hours, and ciliary activity in gill was observed after a 7 hour Warburg run. Generally after 2 -3 hours the respiration rate gradually began to decline. Factors which may have reduced the duration of respiratory constancy include tissue injury resulting from prolonged shaking in the Warburg flasks and the gradual accumulation of toxic metabolic wastes in

* Preferably Dreissena ; probably the zebra mussel, D. polymorpha.

the medium. Furthermore, the respiring tissues gradually reduced the partial pressure of oxygen in the closed vessels, and this may have contributed to the decline in the rate of oxygen uptake over an extended period. However, my results (Figures 7 and 8), which show increasing fluctuations in oxygen uptake by excised tissues with time, suggest that much of the respiratory decline over an extended period results from a gradual disorganization and loss of control of metabolic processes.

Peiss and Field (1950) demonstrated that in the excised brain and liver tissues of the polar cod, Boreogadus saida,* as the experimental temperature was increased, the duration of respiratory constancy decreased. In this study I observed that high temperature respiratory rates were lower during the second hour than during the first, suggesting that the duration of respiratory constancy decreased with increasing temperature, presumably resulting from rapid denaturation of metabolic enzymes at higher temperatures. However, no long-term respiratory determinations were conducted at high temperatures.

Variation in respiration rate: There was much variation of the respiration rates in the same tissues of oysters of the same size measured under the same experimental conditions (Table 6). This was anticipated because Zeuthen (1947) had

*Boreogadus saida is the arctic cod. The polar cod is Arctogadus glacialis (Amer. Fish.Soc.Spec. Publ. 1960).

already pointed out that high scattering of data - often as high as 200-300 percent - is characteristic of rate-functions of marine invertebrates. According to Ghiretti (1966), bivalves are particularly prone to wide variations in metabolic rate, and Galtsoff (1964) noted, in intact adult oysters, a considerable variation in oxygen consumption which he attributed to variations in muscular activity and fluctuations in ventilation rate.

Under the conditions of my experiments, ventilation does not play a limiting role in the oxygen uptake and similar volumes of oxygen are available to the tissues in each of the Warburg flasks. Furthermore, muscular activity in excised tissues is probably much less than in tissues in situ and, under the experimental conditions, the degree of muscular activity in the same tissue from different animals would tend to be similar. Consequently, observed rate variations among tissue samples from different individuals are a combination of basic metabolic differences among individuals and/or errors resulting from the experimental technique. Figure 10, which presents respiration rates of two or more samples of gill tissues taken from individual oysters, indicates that intrinsic physiological differences among individuals result in greater variations in respiration than do differences arising from the experimental technique.

The contribution of fluctuations in glycogen content to the variation in respiration rates among individuals will be considered below.

Zonation of mantle respiration: The high metabolic rate of tissues from the edge of the mantle compared to the rate of tissues from the more central region of the structure (Figure 9) requires explanation.

As previously described, the mantle border consists of three lobes. The middle and inner of these lobes are equipped with numerous extensible tentacles. Menzel (1955) reported intense ciliary activity along the periphery of the mantle in C. virginica, and Galtsoff (1964) also noted that the cilia on the mantle edge are "especially powerful". In the groove between the inner and middle lobes lies the periostracal gland that secretes the outer conchiolin layer of the shell. Calcium salts are deposited both by mantle edge tissues and by those from the more central regions. The rate of calcium deposition by the mantle of C. virginica is greatest in the thin band of tissue, known as the pallial zone, immediately central to the lobulated margin of the mantle. (Wilbur and Jodrey, 1952).

Tsuji and Isono (1963) found that in the mantle of the freshwater bivalve, Cristaria plicata, both the marginal and

pallial zones, considered separately, had higher respiration rates than the thin central region of the mantle.

It would thus appear that the high respiration rate of the mantle edge is attributable to a combination of intense muscular, ciliary, and secretory activities.

Relative respiration rates: It is difficult to compare the absolute respiration rates of bivalves, as reported in the literature, because of wide variations in environment, experimental conditions, and methods of reporting.

Although in many instances we have no way of comparing absolute rates it is possible to compare relative rates of the various tissues of different animals by setting the gill respiration rate equal to 100 percent in each case. On this basis, the relative respiration rates of excised gill (100%), mantle (58.5% of gill rate), and adductor muscle (11.2% of gill rate) of C. virginica appear to be essentially similar to those observed in several other species of bivalves (Figure 21). The one notable exception is the pearl oyster Pinctada martensii*, in which the mantle respiration rate is only 31 percent that of the gill rate (Kawai, 1959). It would be interesting to see if this low value is a result of a greater accumulation of non-respiring glycogen reserves in the mantle of P. martensi than in that of the other bivalves.

*Should be Pinctada martensi (Dunker).

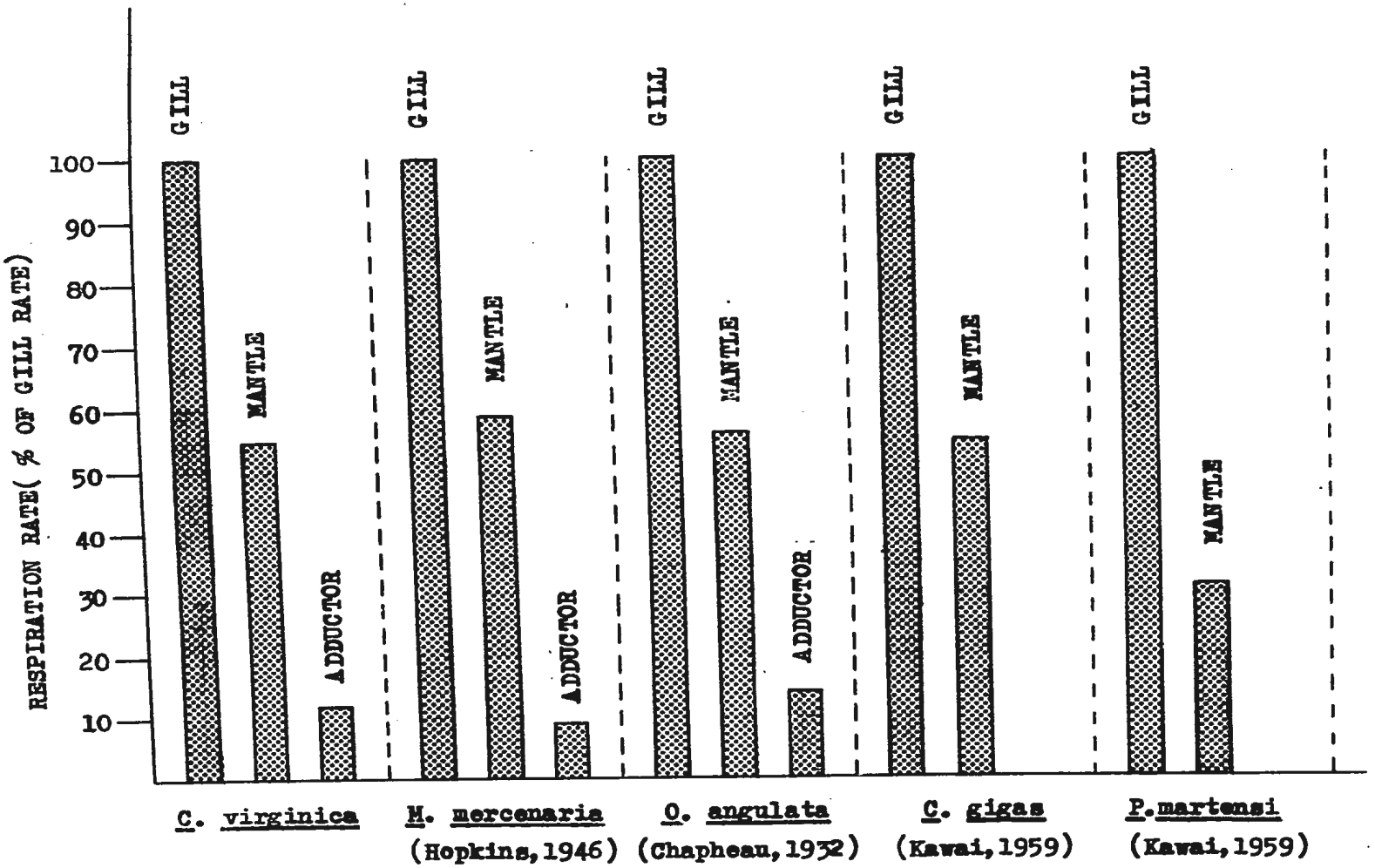

FIGURE 21 . Comparative respiration rates of excised tissues of the bivalves Crassostrea virginica , Mercenaria mercenaria , Ostrea angulata , Crassostrea gigas and Pinctada martensi . Gill rate expressed as 100 percent in each case.

It is interesting to compare the respiration rates of excised tissues of C. virginica obtained in the present study with those of Hopkins (1930, 1946) for the same tissues in the clam, Mercenaria mercenaria. In one series of determinations Hopkins (1930) found that the respiration rate of adductor muscle from the oyster was 17 per cent greater than that of the clam. It is not possible to compare these absolute rates with my results since the measuring techniques were quite different. However, in a later series of experiments, Hopkins (1946) measured the respiration rates of excised tissues of M. mercenaria by a technique similar to that used in the present study. The difference between the respiration rate of clam adductor muscle and my results for oyster adductor muscle is similar (21.9 per cent) to the difference found by Hopkins in his earlier study (17 per cent), and suggests that a valid comparison can be made between my absolute rates for tissues of the oyster and those of Hopkins (1946) for the clam. This comparison will be considered in more detail below.

My results, furthermore, confirm Hopkins' observation that the respiration rate of excised adductor muscle of the oyster is greater than that of the excised adductor muscle of the clam. This result was unforseen by Hopkins

in view of the fact that the oyster is a more sedentary species than the clam. It is possible that the relatively high metabolic rate of the oyster adductor muscle is somehow related to its function as the principal defense mechanism of the animal.

Chapheau (1932) reported respiration rates of several tissues of the Portuguese oyster, Ostrea angulata, which were lower than those obtained for similar tissues of C.virginica. It is, however, difficult to make a worthwhile comparison because the Portuguese oysters were apparently obtained from estuarine waters of very low salinity, and no indication was given of the absolute experimental salinity.

Gill tissue has the highest respiration rate of the three tissues studied. Clearly this high rate of gill tissue is a reflection of the endogenous metabolic rate of the excised gill and is in no way related to the respiratory function of gills whereby O_2 is taken up by circulating fluids. Whether oxygen destined for other tissues is absorbed through the gill in sufficient quantities to warrant the appelation "major respiratory organ" is still a matter of some debate. In fact, the high endogenous respiration of gill tissue would probably tend to reduce considerably its efficiency as a respiratory prgan,

compared to the mantle tissue which has a somewhat lower endogenous respiration rate.

Relationship between "condition" index and respiration rate:

It is important to consider the relationship between "condition" index of the oyster and tissue respiration since seasonal fluctuations in "condition" may result in fluctuations in tissue respiration.

Aldrich (Pers. comm.) and others have clearly established that "condition" index is a reasonably reliable measure of the overall glycogen content of the oyster. Aldrich (1965) reported that he and Swabey, while calibrating the Butler scale in terms of glycogen content, found that glycogen ranged from less than 1 per cent in oysters of "condition" 2 to over 4% in those of "condition" 8. Presumably the greater part of the variation in dry body weight with "condition" rating noted in the present study (Figure 11) can be attributed to differences in the quantity of stored glycogen.

Glycogen, the principal storage product of the oyster is concentrated primarily in the Leydig cells which are abundant in the glycogen bearing tissues. Bargeton (1941) reported that cells rich in glycogen were approximately 120 x 20 μ. while depleted cells were 30 x 10 μ.

Galtsoff (1964) analyzed the glycogen content of various tissues of oysters of good condition collected in January, with the following results:

Mantle: 3.37% glycogen;
Body (visceral mass?): 3.96% glycogen;
Gills: 1.53% glycogen;
Adductor muscle: 1.40% glycogen.

Thus of the three tissues studied, the mantle had the highest glycogen content, with considerably less being stored in the gill and adductor muscle. In Ostrea circumpicta, a slightly higher percentage of glycogen occurs in the white adductor muscle than in the translucent portion (Kobayashi. 1929). Galtsoff (1964) feels that an essentially similar distribution occurs in the adductor muscle of C. virginica. It is not at present clear how much of the glycogen present in the adductor muscle is actually stored in the tissue and how much is part of the muscular mechanism. The present study indicates that the glycogen present in the adductor muscle and gill is less readily mobilized than that deposited in the mantle tissues.

It seems likely that the correlation between mantle respiration rate expressed on a weight basis, and oyster "condition" rating can be attributed largely to the storage of quantities of non-respiring glycogen in the connective tissues of the mantle.

Chapheau (1932) noted that in the Portuguese oyster, O. angulata, "fatness" of oysters was usually associated with low respiration rate. He suggested that this respiratory depression resulted from the accumulation of non-respiring material.

The lack of significant correlation between the condition rating of the oyster and the respiration rates of excised gill and adductor muscle reflects the lower glycogen stores in these tissues and suggests that their glycogen content does not fluctuate as greatly as that of the mantle.

The correlation between mantle respiration rate and the quantity of stored glycogen suggests that the autumnal decline in respiration rate of the intact animal (Galtsoff, 1964) might be attributable to accumulation of glycogen. Further consideration will be given to this point in the discussion of seasonal acclimatization.

It is clear from these results that in all studies of intact bivalve respiration consideration should be given to the glycogen content of the experimental animals, as variations in this factor may modify the observed respiration rate considerably.

Influence of Salinity on respiration: Marine invertebrates vary considerably in their metabolic response to sudden changes in environmental salinity. Upon transfer to dilute sea water, some species exhibit increased oxygen consumption: Gunda ulvae (Beadle, 1931), Carcinus mediterraneus (King, 1965); others decreased oxygen consumption; Mytilus edulis, Asterias forbesii (Maloef, 1938), Maja verrucosa (King, 1965); and yet others no change whatsoever: Eriocheir sinensis (Schwabe, 1933), Limnoria lignorum (Eltringham, 1965).

Analysis of the short-term effect of reduced salinity on the respiration rate of intact bivalves in complicated by the ability of these animals to close their valves rapidly following extensive alteration of the osmotic enviroment. Sealed off from the surrounding sea water in this manner they are able to maintain the intervalvular fluid undiluted for a considerable period. Maloef (1938) suggested that a closing response is the principal osmo-regulatory mechanism employed by many lamellibranchs. Only after the animal reopens its valves can adaptation to the changed salinity occur. The use of excised tissues permitted a study of the short-term effect on metabolism of sudden dilution of the medium.

The present study indicated that dilution of the medium stimulated the respiration of gill tissues, had little or no effect on the respiration rate of mantle tissues and depressed the respiration of adductor muscle at high dilution. Both gill (Figure 15) and mantle tissues of the hard clam, Mercenaria mercenaria, showed an increased respiration rate on dilution of the medium, while the respiration rate of adductor muscle tissue was depressed. In contrast, the addition of 20% fresh water to a sea water medium had no apparent effect on the respiration rates of gill, mantle and adductor muscle tissues of the European oyster, Ostrea angulata (Chapheau, 1932). The addition of 50% fresh water, however, resulted in a decrease in respiration rate of as much as 15% in all tissues. It is difficult to reconcile this finding with the present results for C. virginica or with the results obtained from other bivalves. It is possible that the low environmental salinity of the European oysters resulted in a modified response to dilution. It is perhaps also pertinent to recall the statement of Korringa (1952) to the effect that "there is a fairly wide gap in many respects between these two groups of oysters (Crassostrea and Ostrea) and that we need not worry about finding serious discrepancies in their biological behavior".

The stimulating effect of dilution of the medium on the respiration rate of the major respiring tissues of C. virginica is interesting in view of the finding of Galtsoff (1964) that in the intact oyster, no respiratory stimulation was apparent in dilute sea water to which the oyster had been allowed to adjust for three days. This suggests that the stimulating influence of a hypoosmotic medium is relatively transitory. This parallels the finding of Schlieper (1929) that in Mytilus edulis the increased gill respiration rate is only temporary, and the rate gradually returns to normal. In contrast, in M. mercenaria the stimulating effect on gill respiration of dilution of the medium was still in evidence in animals that had been maintained at the lowered salinity for several days (Hopkins, 1946).

Ghiretti (1966) makes the point that isolated tissues do not necessarily exhibit the same respiratory responses to salinity as the intact animal. This is clearly demonstrated by Mytilus edulis which responded to either an increase or decrease in sea water concentration by a decrease in oxygen consumption (Maloef, 1938). The excised gills on the contrary, had a higher respiration rate in brackish water than in full strength sea water (Schlieper, 1929). King (1965) also demonstrated that in various species of

crustaceans the respiratory responses of isolated gill tissues to dilution of the medium did not consistently parallel the responses of the intact animal.

A comparison of the influence of salinity on the respiration of the intact animal and on the oxygen uptake in isolated tissues is complicated by the fact that the respiring tissues in the intact organism are frequently bathed in a body fluid that may or may not have the same osmotic concentration or ionic composition as the external medium. Oysters are believed to possess only limited powers of osmoregulation (Galtsoff, 1964).

The gills of C. virginica are characterized by the presence of considerable ciliated epithelium. In view of the heightened oxygen consumption with increasing ciliary rate (Gray, 1924) the increased respiration rate observed in the present instance could conceivably be due to stimulated ciliary activity in the dilute medium. However, such an explanation appears unlikely since Vernberg et al (1963) demonstrated that C. virginica exhibits normal ciliary activity down to a dilution of 12°/oo. At greater dilutions inhibition of ciliary activity occurred. The ciliary activity of the gills of M. mercenaria was depressed in sea water diluted to 60% despite the fact that a significant increase in gill respiration was observed (Hopkins,1949).

It appears more likely that the increased respiration rate is a response to the disturbance of the osmotic equilibrium existing between the cell fluid and the external medium.

Schlieper (1929) suggested that the response to reduced salinity is a result of the extra energy required to maintain an osmotic difference between the interior and exterior of the cell. In the words of Maloef (1938),

> "animals which are capable of osmoregulation are not thus affected (decreased O_2 consumption in hypotonic medium) because the density of their body fluids is not allowed to attain that of the external medium by virtue of an increased labour in excretion against an osmotic force, the energy of which is apparently furnished by oxidative metabolism."

Hopkins (1949) attempted to explain the increase in respiration rates of gill and mantle tissue and the decrease in adductor muscle of M. mercenaria on dilution of the medium on the grounds that gill and mantle tissue being primarily epithelial tissues, are functionally adapted to resist water entrance, presumably employing metabolic energy in the process. Potts and Parry (1964) conclude , however, that the metabolic response to changed salinity is too great to be attributable simply to osmotic requirements. They argue that the transport efficiency of a direct respiration-osmotic work mechanism is far lower than the demonstrated efficiency of ion transport systems.

Hydration of the tissues with subsequent alteration of enzyme activity has been suggested as a possible cause of increased respiration in dilute media (Remane and Schlieper, 1958).

Beadle (1931) suggested the possibility that the increased respiration rate in a dilute medium results from increased muscle tension. The adductor muscle results obtained in the present study appear to contradict such an interpretation.

Influence of temperature on respiration: The accelerating effect of temperature on tissue metabolism has been noted by a great many workers. Krogh (1914) and Belehradek (1930) reviewed the most widely employed quantitative approaches to the problem. The effect is usually represented as a rate-temperature (R-T) curve.

Bullock (1955) distinguished two distinct types of R-T curves; an acclimated R-T curve which involves acclimating the animals to each of the successive experimental temperatures for a considerable period prior to measuring the respiration rate; and the more commonly employed, "acutely" determined R-T curve which is obtained by measuring respiration rates at a variety of temperatures without prior acclimation of the animal to the experimental temperature. The acutely determined R-T curve consequently

represents a sequence of pseudostable metabolic rate levels. In the present study, all the R-T curves obtained were of the acutely determined variety.

All three tissues demonstrated an increase in respiration rate with rising temperature. Figure 16 indicates that the respiratory maxima of mantle and adductor tissue occur at a lower temperature than the maximum for gill tissue. Galtsoff (1928) observed that the frontal cilia of C. virginica exhibited a maximum rate of beat at 25°C.- 30°C. At higher temperatures the beats became irregular, and at 37°C.- 38°C. they stopped. In my study the respiration rate of the gill tissue began to decrease above 28°C. (Figure 16) which corresponds to the temperature at which ciliary activity is at a maximum (Galtsoff, 1928). It appears that above 28°C. the rate-temperature curve is a resultant of two opposing tendencies; the direct effect of temperature on metabolism tending to increase the rate of respiration and heat inactivation of the cilia tending to reduce the rate.

If, as the above seems to indicate, ciliary activity does account for a significant part of the oxygen consumption of the gill tissue, then it is possible to view the reflex ciliary inhibition during valve closure described by Galtsoff (1964) as an oxygen conserving mechanism.

The maximum Q_{10} s for both gill and mantle tissues occur in the 16°C - 20°C. range. In this range the metabolism of the tissues undergoes a maximum rate change for a given temperature change. It is interesting to note that this range of maximum instability with respect to temperature occurs within the range of the normal summer temperature. Thus, the tissue metabolism is sensitive to slight temperature variations about the mean environmental summer temperature, but with increasing thermal deviation from the normal temperature the degree of sensitivity declines, considerably extending the range within which metabolism can occur at a reasonable rate. It has been suggested that low Q_{10} values serve to a certain extent as homeostatic mechanisms at temperature extremes (Scholander et al, 1953; Precht, 1958).

In contrast to the situation observed in gill and mantle tissues the Q_{10} coefficient of the adductor muscle is minimal in the 16°C. - 20°C. range, indicating a plateau of relative temperature insensitivity in the rate curve. Figure 17 which represents a log plot of the adductor R-T curve shows this plateau clearly.

Such metabolic stability over a restricted temperature range appears to be a fairly common phenomenon. Vernberg (1959) reported that the respiration rate of the crab, Uca pugilator was temperature insensitive between 12°C. and 17°C. Furthermore, the oxygen consumption of

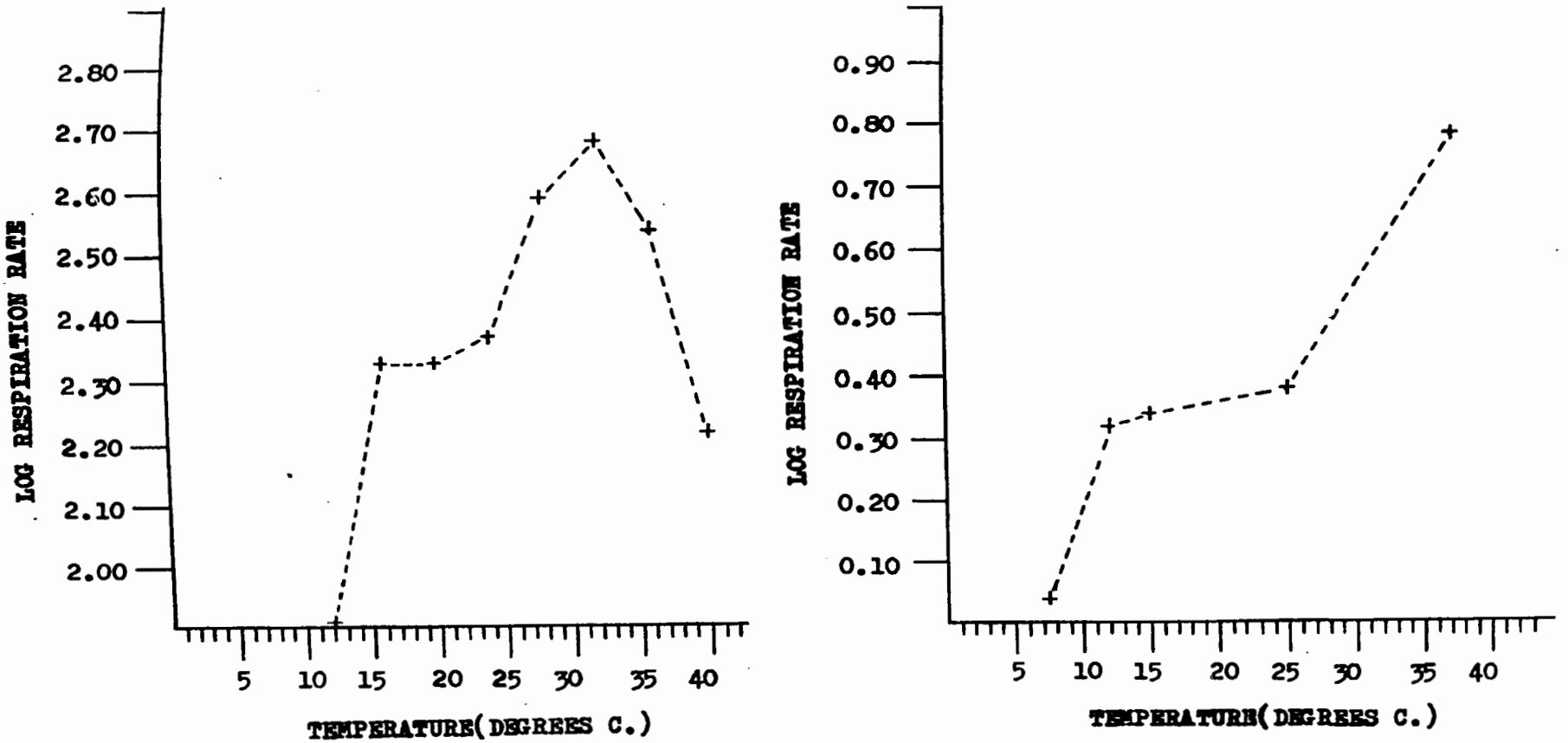

FIGURE 22. Comparison of rate - temperature curves of respiration of excised adductor muscle of C. virginica (left) and foot retractory muscle of Mytilus edulis, (Glaister and Kerly,1936) .

the crab Uca minax, was found to be the same at 11.1°C. as at 15.9°C.(Teal, 1959). Bullock (1955) reported that Vernon (1897) studied carbon dioxide formation as a function of temperature and observed rate plateaus, sometimes as great as 12°C., in a number of species, including the earthworm, Lumbricus sp.

Among the mollusca, temperature insensitive regions for heart rate were recorded in the oysters, Ostrea circumpicta, and O. dendata (Takatsuki,1929). Glaister and Kerly (1936) demonstrated a rather broad plateau in the respiration rate of the excised foot retractor muscle of Mytilus edulis similar to that noted for the adductor muscle of C. virginica (Figure 22). It remains to be seen whether or not such regions of distinct temperature insensitivity are characteristic of molluscan muscle in general. In all cases the plateau phenomenon acts as a homeostatic mechanism over a limited temperature range.

It is possible that the plateau results from the fact that the metabolic equilibrium is such that slight thermal deviations from the normal environmental temperature cause rapid compensation and thus the rate appears to be insensitive to temperature change. With greater fluctuations on either side of the environmental temperature the compensation response would be increasingly slow. Bullock (1955)

was of the opinion that observed instances of temperature insensitivity are in reality cases of extremely rapid compensation. Thus he felt that apparent insensitivity to temperature and gradual compensation are simply grades of the same process. The extremely rapid compensation is probably masked by the initial experimental "blind spot" - that period required for the equilibration of the apparatus - inherent in most respiration measuring techniques.

Seasonal variations in respiration: During the period of this study the Broad Lake environment underwent a thermal change that permitted experimental utilization of oysters derived from two distinct thermal regimes (Figure 4). Oysters collected during the months of July and August (Summer) were subjected to environmental temperatures that ranged from 15-19°C. (warm acclimatized) and oysters collected during September, October and November (Autumn) were exposed to environmental temperatures ranging from 7-15°C. (cold acclimatized).

Kinne (1964) in reviewing the literature pointed out that when a poikilotherm is transferred from one temperature to another, three distinct types of immediate responses may occur in various rate functions. The first is a shock type response, noted by Behre (1918) and Grainger (1956) in various rate functions following transfer of an animal to

a higher or a lower temperature, in which the rate function exhibits an initial overshoot prior to stabilizing at a new rate level which we have designated the pseudostable level because after a period of time at the new temperature the rate may rise or fall from this level. Other organisms exhibit a fast, direct adjustment of rate to the pseudostable level without overshoot (Belehradek, 1930; Grainger, 1958; Sumner and Lanham, 1942). The third type of immediate response is characterized by a more gradual adjustment to the pseudostable state (Schlieper, 1950)*.

The initial change to the pseudostable rate is of relatively short duration and is completed within minutes to hours following an alteration of temperature. The precise level attained depends upon the previous acclimation state of the animal. It is this temporarily stabilized rate that is usually measured for acutely-determined rate-temperature curves.

If this temperature change persists following the attainment of the pseudostable rate, a different type of adjustment in the rate level may occur over an extended period of time. Prosser and Brown (1961) state that Precht (1958) distinguished five types of gradual adjustments that may occur following transfer from a lower to a higher temperature:

* Cited in Kinne (1964).

Hypercompensation: (Type 1) the rate curve rises from the pseudostable level and stabilizes at a new level that is higher than the rate level recorded at the initial temperature.

Perfect compensation: (Type II) the rate level rises gradually from the pseudostable level and stabilizes at a level below that observed at the initial temperature.

Partial compensation: (Type III) the rate level rises somewhat from the pseudostable level and stabilizes at a level below that observed at the initial temperature.

No compensation: (Type IV) the rate remains steady indefinitely at the pseudostable level.

Inverse compensation: (Type V) the rate declines below the pseudostable rate.

A comparable series of adjustments - in the opposite direction - may occur when an animal is transferred from a lower to a higher temperature.

In the case of seasonal acclimatization it is obvious that the temperature change is not abrupt, but is instead a gradual shift, with the modification of metabolism occurring correspondingly slowly over a period of time.

Kinne (1964) claims that,

> "little is known about what these different types and patterns imply in terms of the underlying physiological mechanisms".

Both gill and mantle tissues displayed Type V acclimatization. At 20°C. gill and mantle during the autumn had 16.8% and 18.4%, respectively, lower rate of oxygen consumption than during the summer.

Adductor muscle appears to respond differently than gill or mantle tissues to long-term temperature changes. In the autumn the rate at 20°C. was 13.8% higher than during the summer. Unfortunately, the data in this case was non-significant and only future experiments will be able to tell whether the adductor muscle does in fact show positive acclimatization. It is,however, reasonable to assume from the data that adductor muscle respiration does not decline in the autumn in a manner similar to gill and mantle respiration. The fact that adductor muscle does not exhibit reverse acclimatization may be of considerable survival importance to the animal. Although the gill and mantle enter an evident state of hibernation in the autumn, the adductor muscle, being the principal defense mechanism of the oyster cannot be thus seriously impaired in its activity without affecting the ability of the animal to

respond effectively to the sudden onset of adverse environmental conditions.

The classification of acclimation types devised by Prosser and Brown (1961) unfortunately could not be used in this study because my data do not cover a sufficient temperature range.

Clearly, the seasonal responses of excised gill and mantle tissues are qualitatively the same as the seasonal response of the intact animal, in that the metabolic rate declines sharply in the autumn. Berg (1953) observed a similar type of **reverse** acclimatization in the gastropod, Ancylus fluviatilis, collected from different environments. Animals from a warm environment had a higher respiration rate, at any given temperature, than animals from a cold environment.

Reverse acclimation has also been demonstrated in a number of excised tissues and it is apparent that there may be puzzling differences in the same animal. In the fiddler crabs, Uca rapax, U. pugnax and U. pugilator, there was reverse acclimation in the oxygen uptake of the suboesophageal ganglia, and in both U. rapax and U. pugnax the heart tissues also showed reverse acclimation, while U. pugilator heart tissue showed no acclimation (Vernberg and Vernberg, 1965). In the goldfish, Freeman (1950) found

that, while brain brei showed a positive compensation, muscle tissues exhibited reverse acclimation.

As previously mentioned the excised adductor muscle probably does not respond to low temperature in the same manner as either the excised gill and mantle tissues or the intact animal. This need not be surprising since Precht in 1958 already commented that in some cases tissues adapt in a manner that is just the reverse of that demonstrated by the intact animal, and other similar cases have been reported (Vernberg, 1956; Roberts, 1957). It is also obvious that the high rate of oxygen uptake by gill and mantle tissue will effectively mask the relatively small opposite contribution of the adductor muscle to the total change in the intact animal.

The variations in metabolic rate observed in my experiments are really cases of seasonal acclimatization rather than strict thermal acclimation; consequently, the interpretation of the results is complicated by a wide range of interacting factors. Knowledge of the metabolic physiology of molluscs is so scanty that perhaps the best that one is able to do is to suggest factors that could account for the observed results. The uncertainty that surrounds the mechanism of acclimation serves to cloud the issue still further.

Many studies suggest an intracellular mechanism for acclimation. The apparently increased respiration rate of adductor muscle of C. virginica on cold acclimatization could be attributed to an increased concentration of a key metabolic enzyme as a consequence of enzyme induction. Precht (1958) noted that certain krebs cycle-enzymes increased on cold acclimation and decreased on warm acclimation, while Saroja (cited in Saroja and Rao, 1965) reported an increased activity of succinic dehydrogenase on cold acclimation.

The reverse acclimatization response noted in the gill and mantle tissues could also be accounted for on an intracellular basis by assuming the presence of alternate metabolic pathways. According to McWhinnie and O'Connor (1967), "negative translation (of the R-T curves) to the right could occur when an enzyme decreases as there is an increase in an alternate enzyme or pathway consequent to low temperature treatment".

Factors other than changes in metabolic enzymes may account for the observed acclimatization responses. The possibility of a cause and effect relationship between tissue glycogen and respiration requires consideration because both my observations and those of others have demonstrated regular seasonal fluctuations in glycogen

content which could conceivably account for the observed seasonal change in respiration rate.

The population from which the oysters were collected showed seasonal change in "condition" rating: from a May high, "condition" steadily declined to a July-August minimum and then increased again in September (Aldrich and Percy, 1967). This seasonal cycle is essentially similar to cycles observed in other populations of C. virginica (Galtsoff et al, 1947; Medcof, 1961). According to Goddard and Martin (1966) the annual glycogen cycle results from a "complicated interaction of seasonal, nutritional and breeding factors". Long ago Mitchell (1917) suggested that the increased utilization of stored glycogen which occurred in summer might be the result of an increased oxidative metabolism concomitant with higher temperatures. This may also account for the observation of Saroja and Rao (1965) that the glycogen content of cold acclimated worms, Lampito mauritii, was four times greater than in warm acclimated animals, although it is also possible that glycogen accumulation is enhanced in the cold.

Can we causally connect these fluctuations of glycogen content with the metabolic changes that I observed in excised tissues, and that Galtsoff (1964) observed in the intact animal?

As previously demonstrated, the respiration of the excised mantle varies inversely with the glycogen content of the animal (Figure 13). The autumnal accumulation of glycogen would therefore result in a reduction of the mantle respiration when calculated on a weight basis i.e. including a large amount of metabolically inactive glycogen per unit of respiring cytoplasmic mass. The respiration rate of the intact animal would be affected in a similar manner.

However, seasonal fluctuations in "condition" rating do not fully account for the observed seasonal shifts in respiration rate of excised gill and mantle tissues. If glycogen accumulation alone was responsible for the depression, then animals of the same "condition" should have the same respiration rate in summer and autumn, however, this was not found to be the case. The depressed respiration rates of gill and mantle tissues of animals collected during the autumn are evident even when tissue respiration rates of groups of animals of the same "condition" index collected during the summer and autumn are compared (Figures 12 and 13). Over the whole "condition" range studied, the QO_2 curves for gill and mantle tissues from animals collected in the autumn are shifted to the left relative to the QO_2 curves for the same tissues

from animals collected during the summer. On the other hand, the QO_2 curve for adductor muscle tissue from autumn-collected animals is shifted to the right relative to the summer QO_2 curve over the whole "condition" range investigated (Figure 14).

Seasonal changes in glycogen content are, thus, not sufficient by themselves to account for the marked autumnal decline in gill, mantle and intact-animal respiration in C. virginica, and additional possible causal factors must be considered.

Hormones may play a role in acclimatization. Support for this hypothesis comes from the demonstration of increased neurosecretory activity of the suboesophageal ganglia of cold acclimated earthworms (Rao and Saroja, 1963). In addition, they reported that Habibulla (1962)* found a similar phenomenon in cold acclimated scorpions. Saroja and Rao (1965) further demonstrated that body fluids extracted from cold acclimated earthworms had an immediate in vitro stimulating effect on the respiration rate of tissues from normal worms. These authors suggested that glycogen accumulation observed on cold acclimation is under hormonal regulation and it was pointed out that previous workers had demonstrated that vertebrate glycogen formation and metabolism are usually under the control of a hormone such as insulin. Small

* Cited in Rao and Saroja (1963).

quantities of insulin result in an increased rate of glycogen formation in rat diaphragm muscle (Fruton and Simmonds, 1961). Possibly some similar regulating system controls the accumulation of glycogen in bivalves.

Collip (1923) extracted an insulin-like substance from the clam, Mya arenaria, and predicted that "wherever glycogen occurs there would be insulin not far distant". Hopkins (1946) reported that Kumagai and Shikanami (1929) found an insulin-like substance in the Japanese oyster, Ostrea gigas, that had a greater activity during the summer than during the winter. He suggested that such a hormone, having a stimulating effect on tissue metabolism, might be present in the tissues of M. mercenaria.

In view of the recent rapid advances in insulin research, it would be desirable to repeat, employing more rigorous assay procedures, some of these early experiments indicating the presence of an insulin-like material in bivalves. Additional experiments should also be carried out to determine the effect, if any, of insulin on the respiratory metabolism of bivalves. Collip (1925) noted a sharp rise in oxygen consumption after injection of insulin into fish. Without any firm evidence for the presence or absence of a cyclical glycogen regulating hormone in the oyster and, if present, its effect on the

respiratory metabolism of the tissues, it is clearly impossible to reach any conclusions concerning the possible contribution of such a hormone to the observed seasonal fluctuations in respiration.

In addition, one must consider the possibility that reproductive hormones might be responsible for the observed cyclic metabolic effect. It was previously indicated that the overall tissue respiration rates of C. virginica and M. mercenaria at 20° C. and in sea water of density 1.0250 were similar. However, the two forms appear to be seasonally out of phase, in so far as the respiration rates of gill and mantle tissues are concerned.

TABLE 21

Comparison of summer and autumn respiration rates of gill, mantle and adductor muscle of *C. virginica* and *M. mercenaria* (sea water density 1.0250; temperature 20°C.).

	M. mercenaria QO_2 ($\mu l.O_2/gm./hr. \pm$ P.E.*)	*C. virginica* QO_2 ($\mu l.O_2/gm./hr. \pm$ S.E.)
SUMMER		
Gill	1661 ± 71	1835 ± 44
Mantle	912 ± 28	1033 ± 27
Adductor	163.9	210 ± 10
AUTUMN		
Gill	1802	1524 ± 28
Mantle	1627	833 ± 22
Adductor	181.7	237 ± 12

*Probable error.

The respiration rates of gill and mantle tissues of the oyster are higher than those of the clam during the summer, while in the autumn and winter the reverse is true. These results suggest that the gill and mantle tissues of the clam exhibit positive seasonal acclimatization while those of the oyster exhibit reverse Acclimatization. The adductor-muscle behaviour suggests positive cold acclimatization in this tissue for both species.

These comparative results are interesting when considered in conjunction with the observations that in M. mercenaria, the most active gametogenesis occurs during the autumn and early winter period, ripe spermatozoa and ova being retained in the animal during the winter. In C. virginica on the other hand, early stages of gametogenesis occur in late summer and the products are stored. The gonads remain in a resting state during the autumn and winter with the major part of the gametogenesis occurring in the spring and early summer (Loosanoff, 1942). It is possible that these gonadal changes are regulated by a cyclical hormone that has an effect on the metabolism of tissues other than the specific target tissues.

My work indicates that oysters have a seasonal growth cycle that appears to parallel the seasonal respiration cycle of the animal.

The population studies (Aldrich and Percy, 1967) indicated that the most active growth occurs when the water temperature rises above approximately 15° C. during the months of July and August. This temperature-growth correlation is in agreement with the statement of Medcof (1961) that although slight growth may occur at temperatures in excess of 10° C., maximal growth only occurs when the temperature rises above 15° C. Is it possible to causally connect these variations in growth with the metabolic changes I observed in excised tissues?

Since the mantle is the organ directly involved in shell secretion, one would anticipate that a decline in growth in the autumn would be accompanied by a decline in mantle metabolism. The gills, however, play no role in shell secretion and yet they exhibit an autumnal metabolic depression of approximately the same magnitude as the mantle. This suggests that perhaps the general metabolic depression resulting from some other factor results in the inability of the mantle to continue shell secretion. Results from a study of seasonal respiration of the excised mantle edge would perhaps have been

enlightening in this regard since the mantle edge is the major secretory region concerned with increases in length and width of the valves, while the central mantle region secretes calcium carbonate to thicken the valves.

During the period of the study the salinity at the collecting site varied very little (Figure 3); consequently, observed autumnal changes in respiration rates cannot be attributed to seasonal fluctuations in salinity.

The decline in metabolism of gill and mantle tissues in the autumn parallels that previously noted in the whole animal. The depression of respiration, since it can be detected in excised tissues, cannot be attributed to failure of ventilatory mechanisms at low temperatures or to a direct depressant action on metabolism by the central nervous system, but most probably results from a modification of metabolism occuring at the tissue level.

In many cases where seasonal acclimatization has been demonstrated it has been correlated with a continuation of activity on the part of the animal through the colder months of the year (Edwards and Irving, 1943). On the other hand, absence of positive seasonal acclimatization is often associated with winter torpor (Bullock, 1955). It is obvious that oyster metabolism falls in this latter category, with the major respiring tissues

exhibiting a clear hibernation effect characterized by a distinct metabolic depression during the colder months of the year. The adductor muscle, on the other hand, appears to show no such metabolic depression and, in fact, may positively compensate to a limited extent during extended periods of cold.

Molluscan acclimatization; The reverse acclimatization response exhibited by C. virginica appears to be the exception rather than the rule among molluscs. Studies of various rate functions at both inter - and intra-specific levels, indicate that molluscs have a well developed ability to acclimatize positively in response to temperature shifts.

An interspecific comparison of respiration rates of Arctic and Mediterranean bivalves (Pecten, Cardium and Mytilus) led Thorson (1936) to conclude that " species with a northerly distribution have a higher metabolism than southerly distributed species of the same genus at the same temperature." He further demonstrated (1956) that "an arctic Macoma community at 0° C. shows roughly a similar metabolic rate, a similar rate of growth and similar feeding habits as a boreal Macoma community at 8° C. or a Mediterranean community at about 12° C. or a tropical community at a still higher temperature."

Furthermore, Arctic snails and slugs from Alaska had higher respiration rates at all temperatures below 30° C. than tropical snails (Scholander et al, 1953).

Takatsuki (1929) reported a compensatory adjustment to temperature of heart rate in oysters. Ostrea circumpicta, from the temperate Japanese waters, had a heart rate that was consistently higher, over a wide range of temperatures, than that of the tropical Ostrea dendata from Palau, Western Carolines.

Several intraspecific studies have been conducted. Kirberger (1953) demonstrated positive acclimation in the rate of oxygen uptake by the Gastropod, Helix pomatia, while Mews (1957) noted that the proteolytic activity of the stomach juice of the same species varied with the acclimation temperature, presumably as a result of variations in the rate of enzyme secretion. The rate at which specimens of Mytilus californianus pump water through their gills shows a distinct north-south trend. Northern animals at 6.5° C. had the same rate of pumping as more southerly animals at 10° C. and as even more southerly animals at 12° C. Furthermore within a single macrogeographic population there were animals within localized temperature zones that exhibited micro-geographical acclimatization (Rao, 1953).

Studies by Dehnel (1956) suggest that the growth rate of Mytilus californianus in Southern California is approximately the same as that in South-eastern Alaska. However, the conclusion is somewhat uncertain as the results are complicated by a number of other environmental factors that necessitate the employment of several extrapolations. Dehnel (1955) also compared the growth rates of three species of gastropod larvae from Alaska and California and found that the northern larvae grew more rapidly at all experimental temperatures, compared to southern larvae of the same species.

It frequently happens that areas in close proximity are characterized by persistent thermal differences. In a number of instances it has been demonstrated that groups of animals have become acclimatized to these localized or microgeographic, thermal environments. Microgeographic acclimatization in Mytilus californianus (Rao, 1953) has already been considered above. Segal, Rao and James (1953), in addition, observed that limpets from the low intertidal zone had a more rapid heart rate than those from a high intertidal level.

A difficulty in evaluating many acclimatization studies rigorously, arises when the animals are taken from different macro - or micro-geographic environments because

one cannot rule out completely the possibility of genetic differences. In some instances transplantation experiments, designed to investigate the likelihood of a genetic basis for the observed acclimatization effect, have been conducted. Transplantation of limpets between the two intertidal zones, referred to above, reversed the observed difference in heart rate (Segal, 1956). Animals transplanted to the low tidal level responded in a manner that suggested positive cold acclimatization. Measurements of temperatures in the two zones showed that the low-level forms were exposed to a lower mean temperature than high-level individuals. Adjustment of heart rate in a manner suggestive of positive thermal acclimation also occurred in individuals maintained in the laboratory.

Ciliated epithelial and muscular tissues of a number of gastropods of the genus Nerita, and bivalves of the genus Donax, exhibited thermal resistance acclimatization adjustment of the thermal tolerance limits that correlated closely with the various littoral, thermal microclimates that are inhabited by these animals (Zhirmunski and Tsu, 1964).

The evidence accumulated thus far suggests that molluscs, on the whole, are capable of compensatory, thermal acclimatization in response to low temperatures,

although as indicated in this study certain species, such as the American Oyster, Crassostrea virginica, exhibit non-compensatory reverse acclimatization on exposure to cold.

SUMMARY

The three tissues studied showed a considerable difference in respiration rate relative to one another. If the mean gill respiration rate is considered to be 100 percent, then mantle respiration was 58.5 percent, and adductor muscle 11.2 percent, that of the gill. These proportions are similar to those reported for tissue respiration in a number of other bivalves. The respiration rate of the mantle edge was approximately 20 percent greater than that of the thinner central region of the mantle; probably a consequence of the intense muscular, ciliary and secretory activities of the mantle periphery.

The respiration rate of the excised mantle, calculated on a dry weight basis, was inversely proportional to the Butler "condition" index of the oyster. This was attributed to the increasing accumulation of non-respiring glycogen in the mantle tissues with improving "condition". No significant correlation was found between gill or adductor muscle respiration and "condition" index, probably because glycogen is present in the gills and adductor muscle in much lower concentrations than in the mantle.

Dilution of the sea water medium was found to affect the respiration of each of the tissues in a different manner. The excised gills exhibited increased oxygen

consumption in sea water diluted to a density of 1.0150 and an even greater increase in sea water of density 1.0100. The respiration rate of excised mantle did not appear to be altered significantly even in sea water of density 1.0100. At high dilutions (density 1.0100) the respiration of the excised adductor muscle was severely depressed.

Acutely determined rate - temperature curves were obtained for each of the tissues over the range from 12° C. to 40° C. Both the mantle and adductor muscle had respiratory maxima in the vicinity of 32° C., while the gill rate increased continuosly up to 40° C. The maximum Q_{10} coefficients for both gill and mantle occurred in the 16° C. - 20° C. range. In contrast, the Q_{10} coefficient for adductor muscle respiration was minimal in the 16° C. - 20° C. range, indicating a plateau of relative temperature insensitivity in the rate curve. Although similar plateaus have been observed in the metabolism of a number of intact animals and excised tissues, their precise physiological significance is uncertain.

The mean respiration rates of gill, mantle and adductor muscle at 20° C. in sea water of density 1.0250 were determined during July, August, September, October

and November. Animals collected during July and August had been exposed to an enviromental temperature ranging from 15° C. - 20° C., while those collected during the three autumn months had been exposed to environmental temperatures ranging from 7° C. - 15° C. The respiration rates of excised gill and mantle declined by 16.8 percent and 18.4 percent, respectively, during late August - early September. The respiration of the adductor muscle did not show a corresponding decline, and in fact may have increased.

The autumnal metabolic depression observed in gill and mantle indicates reverse acclimatization, a response that appears to parallel that observed by Galtsoff (1964) in the intact oyster. The absence of a reverse acclimatization response in the adductor muscle may be of survival value to the oyster.

The autumnal metabolic depression was not a result of the accumulation of glycogen, because a decline in metabolism was observed even when the mean summer and autumn tissue respiration rates of oysters of the same "condition" categories were compared.

Since the metabolic depression was detectable in excised tissues the depression previously observed in the intact animal cannot be attributed to failure of

ventilatory mechanisms at low temperature. Nutritional or reproductive hormones may play a role in the seasonal respiration cycle. Alteration of metabolic pathways following a temperature change may also account for the observed acclimatization.

The autumnal respiratory depression observed in the intact animal is essentially a hibernation response and can be at least partially accounted for by a metabolic depression occurring within certain of the tissues, although all tissues do not necessarily exhibit the same response.

REFERENCES

* 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. Am. Journal Physiol., 184:18-28.

ALDRICH, F.A. 1957. Statistical studies of oysters and associated organisms. Acad. Nat. Sci. Philadelphia, 209 pp. (Restricted circulation)

——— 1965. Investigations on oysters, Crassostrea virginica (Gmelin) planted at Broad Lake, Bellevue, Trinity Bay. Special report to the Canada Department of Fisheries, Fish Culture Branch, 53 pp. (Mimeographed)

———and J.A. Percy. 1967. Investigations on oysters Crassostrea virginica (Gmelin) planted at Broad Lake, Bellevue, Trinity Bay. Special Report to the Canada Department of Fisheries, Fish Culture Branch. Report No. 2, 61 pp. (Mimeographed)

ATKINS, D. 1937. On the ciliary mechanisms and interrelationships of lamellibranchs. Part II. Sorting devices on the gills. Quart. Jour. Microsc. Sci., 79(315): 339-373.

——— 1938. On the ciliary mechanisms and interrelationships of lamellibranchs. Part VII. Laterofrontal cilia of the gill filaments and their phylogenetic value. Quart. Jour. Microso. Sci., 80(318): 345-436.

BARGETON, M. 1941. Note sur les "cellules vesiculeuses a glycogène." Etude de la distribution du glycogène chez les Lombriciens et chez les Ostréides. Bull. Mus. Hist. Nat. Paris., 13:119-125.

——— 1942. Les variations saisonières du tissue conjonctif vesiculeux de l'huitre. Bull. Biol., 76:175-191.

BEADLE, L.C. 1931. The effects of salinity changes on the water content and respiration of marine invertebrates. Jour. Exp. Biol., 8:211-227.

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

BELEHRADEK, J. 1930. Temperature coefficients in biology. Biol. Rev., 5:30-58.

———— 1957. Physiological aspects of heat and cold. Ann. Rev. Physiol. 19:59-82.

BERG, K. 1952. Oxygen consumption by freshwater snails. Hydrobiologia., 4:225-267.

———— 1953. The problem of respiratory acclimation. Hydrobiologia., 5:331-350.

BIELAWSKI, J. 1961. The influence of the salinity of the medium on respiration in the isolated gills of the clam Dreissensia polymorpha (Pall). Comp. Biochem. Physiol., 3:250-260.

* BOUXIN, H. 1931. Influence des variations rapides de la salinité sur la consomation d'oxygène chez Mytilus edulis var. Galloprovincialis. Bull. Inst. Oceanogr., Monaco, 569:1-11.

BRUCE, J.R. 1926. Respiration of Mytilus. Biochem. Jour., 20:829-846.

BULLOCK, T.H. 1955. Compensation for temperature in the metabolism and activity of poikilotherms. Biol. Rev., 30:311-342.

CHAPHEAU, M. 1932. Recherches sur le metabolisme cellulaire de quelques invertèbrés marins. Bull. Sta. Biol. Arcachon., 29:85-152.

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., 108:253-257.

COLLIP, J.B. 1921. A further study of the respiratory processes in Mya arenaria and other marine mollusca. Jour. Biol. Chem., 49:297-310.

———— 1923. The demonstration of an insulin like substance in the tissues of the clam (Mya arenaria). Jour. Biol. Chem.(ABS.), 55: XXXIX.

———— 1925. The effect of insulin on the oxygen consumption of certain marine fish and invertebrates. Am. Jour. Physiol., 72(1): 181-182.

CROZIER, W.J.,and T.B. STIER. 1926. On the modification of temperature characteristics. J. Gen. Physiol. 9:547-559.

DAVIES, P.S. 1965. Environmental acclimation in the limpet Patella vulgata (L.). Nature, 4974:924.

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., 110:43-53.

DIXON, M. 1943. Manometric Methods as Applied to the Measurement of Cell Respiration and Other Processes. 2nd. ed. Macmillan Co. New York. pp. 165.

——— and K.A.C. Elliot. 1930. The use of the Barcroft apparatus for the measurement of tissue respiration. Biochem. Jour., 24:820-833.

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

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

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

ELTRINGHAM, S.K. 1965. The respiration of Limnoria in relation to salinity. Jour. Mar. Biol. Assoc., U.K., 45(1):145-152.

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

FIELD, J., H.S. Belding, and A.W. Martin. 1939. Relation between tissue and basal metabolism. Jour. Cell. Comp. Physiol., 14:143-157.

FOX, H.M. 1936. The activity and metabolism of poikilothermal animals in different latitudes. Proc. Zool. Soc. Lond., 2:945-955.

FREEMAN, J.A. 1950. Brain metabolism, temperature adaptation, fish. Biol. Bull., 99:416-424.

FROMM, P.O. 1955. The respiratory metabolism of frogs as related to season. Jour. Cell. Comp. Physiol., 45:343-359.

FRUTON, J.S., and S. Simmonds. 1961. General Biochemistry. 2nd. ed. John Wiley and Sons Inc., N.Y., pp. 1077.

FRY, F.E.J. 1947. Theory of temperature adaptation. Publ. Ontario Fish. Res. Lab., 68:1-62.

———— 1958. Temperature compensation. Ann. Rev. Physiol., 20:207-224.

GALTSOFF, P.S. 1928. Effect of temperature on the mechanical activity of the gill of the oyster. Jour. Gen. Physiol., 11:415-431.

———— and W.A. Chipman, Jr., J.B. Engle, and H.N. Calderwood. 1947. Ecological and physiological studies of the effects of sulphate pulp mill wastes on oysters in the York River, Va. U.S. Fish and Wildlife Service, Fish. Bull., 51:59-186. (Fisheries Bulletin No. 43).

———— 1955. Recent advances in the studies of the structure and formation of the shell of Crassostrea virginica. Proc. Nat. Shellfisheries Assoc., 45:116-135.

———— 1964. The American Oyster. Crassostrea virginica, Gmelin. Fishery Bull. 64. Fish and Wildlife Service, Bureau of Commercial Fisheries, pp. 480.

GHIRETTI,F. 1966. Respiration. In: Physiology of Mollusca vol. 2. (K.M. Wilbur and C.M. Yonge, eds.). Academic Press, N.Y., pp. 175-208.

GLAISTER, D. and M. Kerly. 1936. The oxygen consumption and carbohydrate metabolism of the retractor muscle of the foot of Mytilus edulis. Jour. Physiol., 87:56-66.

GODDARD, C.K. and A.W. Martin, 1966. Carbohydrate metabolism. In Physiology of the Mollusca. vol. 2(K.M. Wilbur and C.M. Yonge, eds.). Academic Press, N.Y., pp. 275-309.

GRAINGER, J.N.R. 1956. Effects of changes of temperature on the respiration of certain crustacea. Nature, Lond., 178:930-931.

———— 1958. First stages in the adaptation of poikilotherms to temperature change. In: Physiological Adaptations. (C.L. Prosser, ed) Am. Physiol. Soc., Washington, D.C., pp.79-91.

GRAY, J. 1924. The mechanism of ciliary activity. IV. The relation of ciliary activity to oxygen consumption. Proc. Roy. Soc. Lond., B. 96:95-114.

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

HOPKINS,H.S. 1924. Respiration in the tissues of molluscs in relation to age. (ABS.) Anat. Rec., 29:91.

———— 1930. Age differences and respiration in muscle tissues of molluscs. Jour. Exptl. Zool., 56:209-239.

———— 1939. Seasonal differences in the oxygen exchange and glycolysis of tissues in the clam, Venus mercenaria. (ABS.) Anat. Rec., 75:133.

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

———— 1949. Metabolic reactions of clam's tissues to changes in salinity. Physiol. Zool., 22:295-308.

ISHIDA, S. 1935. On the oxygen consumption in the oyster, Ostrea gigas (Thunberg), under various conditions. Sci. Repts. Tohuku Imp. Univ., Ser. 4, 10:619-638.

KASINATHAN, S. 1963. Effect of insulin on carbohydrate metabolism of the bivalve mollusc Meretrix casta (Chemnitz) Proc. Indian Acad. Sci., Ser. B, 58:367-374.

KAWAI, K. 1959. The cytochrome system in marine lamellibranch tissues. Biol. Bull. 117: 125-132.

KELLOGG, J.L. 1915. Ciliary mechanisms of lamellibranchs with descriptions of anatomy. Jour. Morph., 26(4):625-701.

KING, N. 1965. The oxygen consumption of intact crabs and excised gills as a function of decreased salinity. Comp. Biochem. Physiol., 15:93-102.

KINNE, O. 1962. Irreversible non-genetic adaptation. Comp. Biochem. Physiol., 5:265-282.

———— 1964. Non-genetic adaptation to temperature and salinity. Helgol. Wiss. Meeresunters, 9(1) 433-458.

* KIRBERGER, C. 1953. Untersuchungen uber die Temperaturabhangigkeit von Lebensprozessen bei verschiedenen Wirbellosen. Z.vergl.Physiol., 35: 175 - 198.

KOBAYASHI, S.1929. Lactic acid and glycogen in the adductor muscles of the oyster, Ostrea circumpicta (Pils.). Sci. Repts. Tohuku Imp. Univ., Ser. 4(1), fasc. 2:193-205

KORRINGA, P. 1952. Metabolism of oysters. Quart. Rev., Biol., 27:266-308.

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

KROGH, A. 1914. The quantitative relation between temperature and standard metabolism in animals. Int. Z. Phys. Chem. Biol., 1:491 -508.

* KUMAGAI, T. and Y. Shikinami. 1929. Uber die Hypoglykamie Erzeugende Substanz aus Austern. (Ostrea gigas). Tohuku Jour. Exp. Med., 12:425-432.

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.

LOOSANOFF,V.L.1942. Seasonal gonadal changed in the adult oysters, Ostrea virginica, of Long Island Sound. Biol. Bull., 82(2): 195-206.

———and C.A. Nomejko. 1949. Growth of oysters Ostrea virginica during different months. Biol. Bull., 97(1): 82-94.

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

* MALOEF, N.S. 1938. Studies on the respiration (and osmo-regulation) of animals. Z. vergl. Physiol., 25:1-42.

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

MEDCOF, J.C. 1944. Structure, deposition and quality of oyster shell. (Ostrea virginica Gmelin). Jour. Fish. Res. Bd. Canada, 6:209-216.

———— 1961. Oyster farming in the maritimes. Jour. Fish. Res. Bd. Canada, Bulletin, 131, pp. 158.

——— and A.W.H. Needler. 1941. The influence of temperature and salinity on the condition of the oyster, Ostrea virginica. Jour. Fish. Res. Bd. Canada. 5(3):253-257.

MELLANBY,K. 1940. Temperature coefficients and acclimatization. Nature, Lond., 146:165.

MENZEL, R.W. 1955. Some phases of the biology of Ostrea equestris Say. and a comparison with Crassostrea virginica (Gmelin). Publications of the Institute of Marine Science, University of Texas, 4(1):69-153.

MEWS, H.H. 1957. Uber die temperaturadaptation der eiweisspaltenden und sythetisierenden zellfermente von froschen. Z. Vergleich. Physiol. 40:356-362.

MITCHELL,P.H.1917. Nutrition of oysters:glycogen formation and storage. Bull. U.S. Bur. Fish. (1915-1916), 35:151-161.

NEEDLER,A.W.H.1941. The influence of temperature and salinity on the condition of oysters (Ostrea virginica). Jour. Fish. Res. Bd. Canada. 5(3):253-257.

NELSON,T.C. 1938. The feeding mechanism of the oyster.I. On the pallium and branchial chambers of Ostrea virginica, O. edulis, and O. angulata with comparisons with other species of the genus. Jour. Morph., 63(1):1-61

OKAZAKI,K., and S. Kobayashi. 1929. The seasonal variation of the glycogen content of the oyster Ostrea circumpicta. Sci. Repts. Tohuku Imp. Univ., Ser. D, 4:183-191.

* PEDERSEN, E. 1947. Ostersens respirasjon. Undersokelser Utfort ved. Statens Utklekningsansstalt Flodevigen. Fiskeridir Skr. Havundersok, 8:1-51.

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

POTTS,W.T.W.and G. Parry. 1964. Osmotic and Ionic Regulation in Animals. Pergamon Press, London, pp. 423.

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

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

PROSSER,C.L. 1958. The nature of physiological adaptation. In Physiological Adaptation. (C.L. Prosser, ed.) Ronald Press, N.Y., pp.67-180.

————and F.A. Brown, 1961. Comparative animal physiology. 2nd. ed. W.B. Saunders and Co., Philadelphia, pp. 688.

RANSON, G. 1949. Quelques observations sur la biologie de Gryphaea angulata (Lmk.). Bull. Mus. Hist. Nat. Paris, 21:574-579.

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

———— 1954. Tidal rhythmicity of rate of water propulsion in Mytilus,and its modifiability by transplantation. Biol. Bull., 106: 353-359.

——— 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. Ind. Acad. Sci., 58:14-18.

* REMANE, A. and C. Schlieper. 1958. Die Biologie Des Brackwassers. In: Die Binnengewasser (A. Thienmann, ed.). Schweizerbart, Stuttgart, pp. 348.

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

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

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

* SCHLIEPER, C. 1929. Uber die Einwirkung niederer Salzkonzentrationem auf marine organismen. Z. vergl. Physiol., 9:478-514.

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

* SCHWABE, E. 1933. Uber die osmoregulation verschiedener Krebse (Malacostracen). Z. vergl. Physiol., 19:183-236.

* SCHWARTZ, F. 1884. Der Einfluss der Schwerkraft auf die Bewegungsrichtung von Chlamidomonas und Euglena. Ber. Bot. Ges. II:51-72.

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

——— and 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.

SETNA, S.B. 1930. The neuromuscular mechanism of the gill of Pecten sp. Quart. Jour. Microsc. Sci., 73:365-391.

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

SPARCK, R. 1936. Metabolism of lamellibranchs from different latitudes. K. Danske Videnske. Selsk. Skr. Biol. Med., 13:1-27.

STRICKLAND, J.D.H. and T.R. Parsons. 1960. A Manual of Sea Water Analysis. Bull. Fish. Res. Bd. Canada. Bull., No. 125, pp. 185.

SUMNER, F.B. and V.N. Lanham. 1942. Metabolism and tolerance of fish from warm and cold springs. Biol. Bull., 82:313-327.

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.

TEAL, J. M. 1959. Respiration of crabs in Georgia salt marshes and its relation to their ecology. Physiol. Zool., 32:1-14

THORSON, G. 1936. The larval developement, growth and metabolism of arctic marine bottom invertebrates as compared with those of other seas. Medd. om.Gronland, 100:1-155.

———— 1956. Marine level bottom communities of recent seas, their temperature adaptation and their balance between predators and food animals. Trans. N.Y. Acad. Sci., 18: 693-700.

TSUJI, T. and O. Isono. 1963. Studies on the mechanism of shell and pearl formation. VII. The respiratory metabolism of the mantle in the normal and regenerating shell formation. Jour. Fac. of Fisheries. Prefectural Univ. Mie., 5(3): 371-377.

UMBREIT, W.W., R.H. Burris, and J.F. Stauffer. 1959. Manometric Techniques. 2nd ed. Burgess Publishing Co., Minneapolis, pp.338.

USUKI, I., and S. Koizumi. 1954. Seasonal variations of the ciliary activity of the gills and some chemical components in the oyster Ostrea gigas. Sci. Repts. Tohuku Univ., 4th. Ser., 20:304-317

VERNBERG, F.J. and I.E. Gray. 1953. A comparitive study of the respiratory metabolism of excised brain tissue of marine teleosts. Biol. Bull., 104:445.

VERNBERG, F.J. 1956. Study of the oxygen consumption of excised tissues of certain marine decapod crustacea in relation to habitat. Physiol. Zool., 29:227-234

———— 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., 117:163-184.

———— and 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.

* VERNON, H.M. 1897. The relation of respiratory exchange of cold blooded animals to temperature. Jour. Physiol., 21:443.

VON BRAND, T., M.O. Nolan, and E.R. Mann. 1948. Observations on the respiration of Australorbis glabratus and some other aquatic snails. Biol. Bull., 95:119-213.

WERNSTEDT, C. 1944. Metabolism of gill epithelium of a freshwater mussel. Nature, London, 154:463.

WILBUR, K.M., and L.H. Jodrey, 1952. Studies on shell formation. I. Measurement of the rate of shell formation using Ca.45. Biol. Bull., 103:269-276.

ZEUTHEN, E. 1947. Body size and metabolic rate in the animal kingdom. C.R. Lab. Carlsberg, (Ser. Chim.), 26:17-162.

* ZHIRMUNSKI, A.V. and Li Ts'ung Ts'u. 1964. Prisoposoblenie tropicheskikh mollyuskov Nerita i Donax k temperaturnym usloviyam obitanii Zh. Biol. No.9029(trans).

* Not seen by author

APPENDICES

APPENDIX TABLES

PAGE

PAGE

APPENDIX A

TABLE 22

Temperatures and salinities at collecting site(Broad Lake,1966).

Date	Temperature (°C.)	Salinity (o/oo) Surface	Bottom
May 20	7.6	24.83	24.85
June 3	12.6	-	-
June 8	-	28.62	-
June 16	13.5	-	-
June 20	12.5	28.44	28.55
June 29	-	29.13	-
July 17	16.9	29.74	29.74
July 28	18.8	-	-
Aug.4	16.8	28.80	-
Aug.14	17.6	29.23	29.29
Aug.18	18.0	-	-
Aug.27	16.2	-	-
Sept. 2	15.5	-	-
Sept. 19	14.0	29.38	29.45
Sept. 28	12.2	29.70	-
Oct. 7	10.0	-	-
Oct. 15	9.9	28.89	-
Oct. 31	7.4	29.16	-
Nov. 13	9.0	28.86	-
Nov. 28	7.0	29.33	-

APPENDIX B

TABLE 23

Relationship between "condition" of the oyster and the respiration ($\mu l.O_2/gm./hr.$) of excised gill.(Summer sample; sea water density 1.0250 ; temperature 20 ° C.)

"Condition" index				
3	4	5	6	7
1554	2080	1792	2022	2200
2114	1791	1539	2017	1658
	1957	1649	1846	
	1287	2324	1704	
	1623	1528	1820	
	1260	1740	1985	
		1724	1584	
		1519	1832	
		2356		
		1968		
		1457		
		1687		
		2372		
		2137		
		1921		
		1660		
		1293		
		2050		

TABLE 23 (continued)

	"Condition" index				
	3	4	5	6	7
			2218		
			1970		
			1694		
			1510		
			2142		
			2196		
Mean:	1834	1666	1852	1851	1929
n:	2	6	24	8	2
S.E.:	±198	±128	±63	±52	±192

APPENDIX B

TABLE 24

Relationship between "condition" of the oyster and the respiration ($\mu l.O_2/gm./hr.$) of excised gill. (Autumn sample; sea water density 1.0250; temperature 20° C.)

"Condition" index					
2	3	4	5	6	7
1234	1334	1357	1435	1554	1373
1716	1284	1687	1549	1743	1213
1561	1951	1542	1294	1216	1830
1588	1923	1572	1473	1557	1286
	1628	1679	1170	1721	1281
	1591	1494	1604	1323	
		1503	1568	1422	
		1291	1732	1349	
		1739	1719	1384	
		1447	1651	1558	
		1783	1495	1661	
		1273	1664	1379	
		2076	1733		
		1594	1526		
		1495	1717		
		1261	1375		
		1738	1452		
		1363	1344		
			1823		

TABLE 24 (continued)

"condition" index

	2	3	4	5	6	7
				1201		
				1320		
Mean:	1525	1619	1550	1516	1489	1397
n:	4	6	18	21	12	5
S.E.:	±240	±104	±48	±40	±46	±98

APPENDIX B

TABLE 25

Relationship between "condition" of the oyster and the respiration ($\mu l.O_2/gm./hr.$) of excised mantle. (Summer sample; sea water density 1.0250 ; temperature 20 ° C.)

"Condition" index				
3	4	5	6	7
1328	1501	1327	1113	716
1078	809	1065	795	781
	1050	1152	847	
	1150	986	1041	
	1193	1279	801	
	877	1377	836	
		914	1005	
		1087		
		1188		
		856		
		686		
		647		
		960		
		786		
		920		
		1038		
		958		
		1034		
		817		

TABLE 25(continued)

"condition" index

	3	4	5	6	7
			1006		
			968		
			1003		
			1162		
			1235		
Mean:	1203	1097	1019	920	749
n:	2	6	24	7	2
S.E.:	±88	±92	±37	±45	±12

APPENDIX B

TABLE 26

Relationship between "condition" of the oyster and the respiration ($\mu l.O_2/gm./hr.$) of excised mantle.(Autumn sample;sea water density 1.0250; temperature 20 ° C.)

"Condition" index					
2	3	4	5	6	7
1016	1222	967	840	518	605
1036	949	1052	636	1030	625
1044	926	982	721	1000	776
1051	1155	857	700	865	727
	809	737	718	633	
	909	804	840	882	
		784	947	916	
		837	740	703	
		776	576	581	
		828	780	751	
		938	692	786	
		1023	891	918	
		1343	824	1105	
		924	1006		
		1036	718		
		778	758		
		1062	735		

TABLE 26 (continued)

"Condition" index

	2	3	4	5	6	7
			882	738		
				538		
				924		
				865		
				830		
Mean:	1037	995	923	774	822	683
n:	4	6	18	22	13	4
S.E.:	±21	±59	±34	±23	±48	±37

APPENDIX B

TABLE 27

Relationship between "condition" of the oyster and the respiration ($\mu l.O_2/gm./hr.$) of excised adductor muscle. (Summer sample ; sea water density 1.0250 ; temperature 20 ° C.)

"Condition" index				
3	4	5	6	7
219	269	216	216	209
138	145	125	152	187
	206	220	134	
	142	284	178	
	217	225	196	
		174	325	
		344	343	
		243		
		161		
		195		
		167		
		128		
		262		
		213		
		146		
		167		
		238		

TABLE 27 (continued)

"Condition" index

	3	4	5	6	7
			227		
			317		
Mean:	179	196	213	221	198
n:	2	5	19	7	2
S.E.:	±27	±21	±14	±28	±8

APPENDIX B

TABLE 28

Relationship between "condition" of the oyster and the respiration ($\mu l.O_2/gm./hr.$) of excised adductor muscle.(Autumn sample ; sea water density 1.0250 ; temperature 20 ° C.)

"Condition" index					
2	3	4	5	6	7
123	175	163	124	242	184
330	221	200	148	250	177
246	358	166	173	277	
		219	162	240	
		408	325	169	
		103	351	253	
		250	385	147	
		320	236	332	
		195	217	187	
		265	222	240	
		320	199		
		272	168		
			330		
			292		
			229		
			196		
			123		

TABLE 28 (continued)

"Condition" index

	2	3	4	5	6	7
				367		
				217		
Mean:	233	251	240	235	234	181
n:	3	3	12	19	10	2
S.E.:	±49	±46	±23	±18	±16	±10

APPENDIX C

TABLE 29

Influence of salinity on the respiration($\mu l.O_2/gm./hr.$) of excised gill.(temperature 20 ° C.)

Sea water density		
1.0100	1.0150	1.0250
2155	2195	1792
2308	2569	1539
2378	2239	1649
2449	1848	2324
2889	1924	1528
2572	1596	2080
2253	1700	1740
2059		1792
		1957
		1724
		2022
		1519
		2356
		1968
		2017
		1457
		1687
		1620
		2234
		1826

TABLE 29 (continued)

Sea water density

1.0100	1.0150	1.0250
		1777
		1647
		2370
Mean: 2383	2010	1853
n: 8	7	23
S.E.: ±86	±120	±57

APPENDIX C

TABLE 30

Influence of salinity on the respiration ($\mu l.O_2/gm./hr.$) of excised mantle. (Temperature 20 ° C.)

Sea water density		
1.0100	1.0150	1.0250
1370	937	1327
966	1384	1065
1690	991	1152
966	1368	986
741	783	1501
558	1002	1279
834		809
639		1050
847		1377
1027		1113
689		914
		1087
		1188
		795
		856
		1066
		809
		1222
		973
		1125

c.1

TABLE 30 (continued)

	Sea water density		
	1.0100	1.0150	1.0250
Mean:	939	1078	1084
n:	11	6	20
S.E.:	±96	±83	±43

APPENDIX C

TABLE 31

Influence of salinity on the respiration ($\mu l.O_2/gm./hr.$) of excised adductor muscle. (Temperature 20 ° C.)

Sea water density		
1.0100	1.0150	1.0250
97	284	216
133	174	125
83	151	220
102	313	284
90	179	225
82		269
		174
		145
		344
		216
		243
		161
		195
		152
		359
		177
		151
		206
		83
Mean: 98	220	208
n: 6	5	19
S.E.: ±7	±30	±15

APPENDIX D

TABLE 32

Influence of temperature on the respiration ($\mu l.O_2/gm./hr.$) of excised gill (Summer sample ; sea water density 1.0250) .

	Temperature							
	12°C.	16°C.	20°C.	24°C.	28°C.	32°C.	36°C.	40°C.
	843	1070		2830	3532	3842	4686	5347
	740	890		2807	3520	2942	6040	4791
	715	1107	August	3083	3300	3822	4136	6573
	544	1062	seasonal	2694	3905	4186	2967	3513
	512	909	data	2389		4608	3708	3345
	477							2939
Mean:	639	1008	1815	2761	3564	3881	4307	4418
n:	6	5	21	5	4	5	5	6
S.E.:	±54	±38	±67	±98	±111	±246	±462	±522

APPENDIX D

TABLE 33

Influence of temperature on the respiration ($\mu l.O_2/gm./hr.$) of excised mantle (Summer sample ; sea water density 1.0250).

Temperature							
12°C.	16°C.	20°C.	24°C.	28°C.	32°C.	36°C.	40°C.
467	723		1159	1936	1669	1631	1309
339	526	August	1527	1181	1889	1795	1581
411	596	seasonal	1183	2106	1779	1631	1617
487	347	data	1199	1819	2218	1132	508
401	465		844	1364	1772	1876	776
			1692	1763	2376		698
Mean: 421	531	982	1266	1695	1951	1613	1081
n: 5	5	20	6	6	6	5	6
S.E.: ±30	±57	±29	±113	±132	±103	±115	±179

APPENDIX D

TABLE 34

Influence of temperature on the respiration ($\mu l.O_2/gm./hr.$) of excised adductor muscle (Summer sample ;sea water density 1.0250).

	Temperature						
12°C.	16°C.	20°C.	24°C.	28°C.	32°C.	36°C.	40°C.
78	209		312	291	442	371	163
46	216	August	260	241	596	253	169
114		seasonal	192	256	547	286	162
80		data	186	594	435	464	
				561	466		
Mean: 80	213	212	238	388	485	350	165
n: 4	2	17	4	5	5	4	3
S.E.: ±11	±8	±16	±25	±54	±72	±24	±9

APPENDIX E

TABLE 35

Respiration rates of excised gill, mantle and adductor muscle during July (Oysters of C.I. 4,5 and 6 ; temperature 20 ° C. ; sea water density 1.0250) .

Oyster number	Gill QO_2 ($\mu l.O_2/gm./hr.$)	Mantle QO_2 ($\mu l.O_2/gm./hr.$)	Adductor QO_2 ($\mu l.O_2/gm./hr.$)
7 - 1	1792	1327	216
7 - 2	1539	1065	125
7 - 3	1649	-	220
7 - 7	2324	1152	284
7 - 8	1528	986	225
7 - 9	2080	1501	269
7 - 11	1740	1279	174
7 - 12	1792	809	145
7 - 36	1957	1050	-
7 - 38	1724	1377	344
7 - 39	2022	1113	216
7 - 14	1519	914	243
7 - 15	2356	1087	161
7 - 46	1968	1188	195
7 - 44	2017	795	152
7 - 51	1457	856	-
7 - 53	1687	-	-
7 - 6	1620	1066	359

c.1

TABLE 35 (continued)

Oyster number	Gill QO_2 ($\mu l.O_2/gm./hr.$)	Mantle QO_2 ($\mu l.O_2/gm./hr.$)	Adductor QO_2 ($\mu l.O_2/gm./hr.$)
7 - 18	2234	-	177
7 - 13	1826	809	151
7 - 22	1777	1222	206
7 - 27	1647	973	-
7 - 47	2370	1125	83
Mean :	1853	1084	208
n:	23	20	19
S.E.:	±57	±43	±15

APPENDIX E

TABLE 36

Respiration rates of excised gill, mantle and adductor muscle during August (Oysters of C.I. 4,5 and 6 ; temperature 20 ° C. ; sea water density 1.0250) .

Oyster number	Gill QO_2 ($\mu l.O_2/gm./hr.$)	Mantle QO_2 ($\mu l.O_2/gm./hr.$)	Adductor QO_2 ($\mu l.O_2/gm./hr.$)
8 - 1	1287	1150	206
8 - 2	2372	960	167
8 - 3	1846	847	134
8 - 10	1623	1193	142
8 - 11	2137	786	-
8 - 12	1921	920	128
8 - 13	1660	1038	262
8 - 14	1293	958	213
8 - 15	1704	1041	-
8 - 16	2050	1034	146
8 - 17	2218	817	167
8 - 181	1970	1006	238
8 - 182	1694	968	227
8 - 19	1510	1003	317
8 - 20	1260	877	217
8 - 24	1820	801	178

c.1

TABLE 36 (continued)

Oyster number	Gill QO_2 ($\mu l.O_2/gm./hr.$)	Mantle QO_2 ($\mu l.O_2/gm./hr.$)	Adductor QO_2 ($\mu l.O_2/gm./hr.$)
8 - 25	2142	1162	-
8 - 26	1985	836	196
8 - 30	1584	-	325
8 - 31	2196	1235	-
8 - 32	1832	1005	343
Mean :	1815	982	212
n :	21	20	17
S.E. :	±67	±29	±16

APPENDIX E

TABLE 37

Respiration rates of excised gill, mantle and adductor muscle during September (Oysters of C.I. 4, 5, and 6 ; temperature 20 ° C. ; sea water density 1.0250) .

Oyster number	Gill QO_2 ($\mu l.O_2/gm./hr.$)	Mantle QO_2 ($\mu l.O_2/gm./hr.$)	Adductor QO_2 ($\mu l.O_2/gm./hr.$)
9 - 1	1357	967	163
9 - 2	1435	840	124
9 - 3	1549	636	148
9 - 4	1687	1052	-
9 - 5	1554	518	242
9 - 7	1743	1030	250
9 - 9	1964	655	291
9 - 10	1216	1000	277
9 - 11	1294	721	173
9 - 12	1557	865	-
9 - 19	1213	633	240
9 - 21	1721	882	-
9 - 25	1473	700	162
9 - 26	1323	916	169
9 - 28	1170	718	325
9 - 31	1604	840	351
9 - 33	1568	947	385
Mean :	1496	819	236
n :	17	17	14
S.E.:	±50	637	±21

APPENDIX E

TABLE 38

Respiration rates of excised gill, mantle and adductor muscle during October (Oysters of C.I. 4, 5 and 6 ; temperature 20 ° C. ; sea water density 1.0250) .

Oyster number	Gill QO_2 ($\mu l.O_2/gm./hr.$)	Mantle QO_2 ($\mu l.O_2/gm./hr.$)	Adductor QO_2 ($\mu l.O_2/gm./hr.$)
10 - 2	-	740	236
10 - 3	1732	576	217
10 - 4	1422	703	253
10 - 6	1542	982	200
10 - 7	1572	857	166
10 - 8	1679	737	219
10 - 9	1719	780	222
10 - 10	1651	692	199
10 - 11	1349	581	147
10 - 12	1384	751	-
10 - 16	1494	804	408
10 - 17	1503	784	103
10 - 18	1291	837	250
10 - 19	1495	891	168
10 - 20	1664	824	330
10 - 21	1558	786	332

c.1

TABLE 38 (continued)

Oyster number	Gill QO_2 ($\mu l.O_2/gm./hr.$)	Mantle QO_2 ($\mu l.O_2/gm./hr.$)	Adductor QO_2 ($\mu l.O_2/gm./hr.$)
10 - 22	1733	1006	-
10 - 23	1739	776	-
10 - 25	1447	828	-
10 - 26	1526	718	292
10 - 27	1717	758	229
Mean :	1561	782	234
n :	20	21	17
S.E. :	±30	±22	±17

APPENDIX E

TABLE 39

Respiration rates of excised gill, mantle and adductor muscle during November (Oysters of C.I. 4, 5, and 6 ; temperature 20 ° C. ; sea water density 1.0250).

Oyster number	Gill QO_2 ($\mu l.O_2/gm./hr.$)	Mantle QO_2 ($\mu l.O_2/gm./hr.$)	Adductor QO_2 ($\mu l.O_2/gm./hr.$)
11 - 3	1375	735	196
11 - 5	1452	738	123
11 - 7	1344	538	-
11 - 8	1783	938	320
11 - 9	1273	1023	-
11 - 10	2076	1343	-
11 - 11	1823	924	367
11 - 12	1201	865	-
11 - 13	1594	924	195
11 - 14	1495	1036	265
11 - 15	1261	778	-
11 - 17	1661	918	187
11 - 20	1738	1062	320
11 - 21	1363	882	272
11 - 22	1320	830	217
11 - 23	1379	1105	240
Mean :	1509	915	246
n :	16	16	11
S.E. :	±59	±44	±20

c.1