AN ABSTRACT OF THE THESIS OF

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Title: ASPECTS OF RESPIRATION IN VERTICALLY SEPARATED MYTILUS CALIFORNIANUS (CALIFORNIA SEA MUSSEL)

Redacted for privacy

Abstract approved: Dr. Austin W. Pritchard

Mytilus californianus occupies a wide vertical zone in the marine intertidal environment. The breadth of its distribution allows for numerous intraspecific differences to exist in this one species.

Metabolic responses for both high and low-level mussels, as indicated by respiratory rate, critical oxygen tension, capacity for anaerobiosis, weight loss, and oxygen depletion within the mantle cavity fluid were determined. An oxygen macro-electrode in conjunction with a physiological gas analyzer were used to determine changes in oxygen tension within the closed-bottle type respirometer chambers. Lactate was analyzed using a modified Barker and Summerson method, and dissolved oxygen concentrations were determined using a micro-Winkler procedure.

The high-level mussels were found to have a higher metabolic rate during submergence and post-exposure periods. The submerged

oxygen consumption rate was significantly greater in the high as compared to the low-level mussels. The compensatory increase in oxygen consumption following six and twelve hour exposure periods was higher in the high-tide animals, and also was a function of the time of exposure, in the high-tide animals only.

Both the high and low-level mussels are metabolic regulators. The high-level animals show a critical oxygen tension which is slightly lower than the low-level animals.

The levels of tissue lactate were found to be initially low, and the accumulation of this anaerobic end product was not significant after twelve hours of exposure. There is a general tendency for the high-level mussels to accumulate more lactate compared to low-level forms. Each group of mussels was found to show an increased tissue lactate after reimmersion following twelve hours of exposure.

The depletion of oxygen in the mantle cavity fluid was found to decrease initially at a faster rate in the low-level forms, although each group approached zero oxygen concentration in approximately the same time period. Field measurements were found to deviate from "restrained" laboratory mussels, and this difference was discussed in terms of the possible utilization of atmospheric oxygen.

Intershell volume and shell weight were measured at a number of tissue weights and found to be adaptively related to the differences in exposure periods for the high and low-level mussels.

It was concluded that one consequence of high-tide existence was an increase in metabolic functions above that found in low-level animals.

Aspects of Respiration in Vertically Separated *Mytilus californianus* (California Sea Mussel)

by

Thomas William Moon

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Typed by Carolyn Irving for Thomas William Moon

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INTRODUCTION

In the last two decades a number of review articles have discussed the metabolic activity of poikilothermal animals as related to environmental factors--temperature in particular (Prosser, 1955, 1957; Segal, 1956b; Prosser and Brown, 1961; Bullock, 1962; Dill, Adolph and Wilber, 1964). The articles have dealt with criteria which could be used to functionally differentiate animals, either inter- or intraspecifically. The differentiation is based upon testing the animals under controlled stress conditions in the laboratory.

Studies which were undertaken prior to and after these publications have shown that poikilotherms compensate to different environmental conditions by raising or lowering rate functions, such as heart rate and metabolic rate. This has been especially evident in numerous latitudinal studies. Rao (1953a) has found the rate of water propulsion in Mytilus californianus of equal weights to be greater in the more northern forms as compared to the southern forms. A similar relationship was found by Spärck (1936) in M. edulis and numerous other lamellibranchs when oxygen consumption rate was measured. Other physiological functions have been reported to be greater in the more northernly distributed animals: cleavage of sea

urchin eggs (Fox, 1939); larval growth of gastropods (Dehnel, 1955); and heart rate in Mytilus (Pickens, 1965). Dehnel (1955), however, found that larval growth in M. californianus was greater in Southern California populations than larval growth in the same species of southeastern Alaska. Scholander, et al. (1952) have found no substantial differences between the standard metabolic rates of various terrestrial invertebrates and lichens from the arctic as compared to tropic forms, when tested at approximate environmental temperature. He found this to be true also in numerous fish and aquatic invertebrates investigated. The metabolic curves were shifted to the left in arctic forms so they would reach a maximum rate in the neighborhood of 20°C, instead of the maximum at 40°C which was found in the tropical forms (Scholander, et al., 1953).

Other authors have reported little or no rate function adaptation to temperature. Thorson (1951), investigating three mytilid lamellibranchs, found no differences in oxygen consumption in latitudinally separated species. Segal (1961), has summarized the latitudinal investigations by reporting that growth rates, respiration rates, and other rate functions of poikilotherms from different environments do not differ as much as expected from temperature differences between the environments, this being especially true for mollusks.

Similar compensatory responses have been found to exist in poikilotherms inhabiting vertically separated zones of the marine

intertidal. Broekhuysen (1942) has shown in a series of intertidal gastropods that those snails occupying successively higher intertidal positions are more resistant to desiccation, have higher lethal temperatures, can withstand greater salinity variations, and are generally better adapted to exposed conditions than those found at lower levels. Studies of other intertidal invertebrates have shown higher oxygen consumption rates (Sandison, 1966), lower heart rates (Segal, Rao and James, 1953; Segal, 1956b; Pickens, 1965), and greater height and weight of shells (Russell, 1907; Orton, 1929; Segal, 1956a) in successively higher intertidal species.

Lamellibranchs have been used extensively in studies relating environmental factors to zonal distribution. Coulthard (1929) has shown that completely submerged M. edulis populations grow at a greater rate than intermittently exposed groups. This observation was confirmed by Dehnel (1956) who found that lack of food for long periods of time in M. californianus was probably responsible for this growth difference. Thermal tolerances were also found to be greater in lamellibranchs that lived higher in the intertidal zone (Henderson, 1929; Schlieper, 1959; Read, 1964). Pickens (1965) has found that heart rate in M. californianus is greater at all temperatures in the low-level forms.

The intertidal environment has a number of unique features, some of which have only been recently investigated. The most obvious

feature is associated with the periodic rise and fall of the tide. During periods of low tides, the intertidal zone may be exposed for many hours, subjecting its fauna to increased temperature and desiccation and possibly to decreased or increased salinity. Not all organisms have been found to adapt to this period of stress in a similar way.

Respiratory adaptations to exposure have been extensively investigated. Barnes and Barnes (1957) and Barnes, Finlayson and Piatigorsky (1963) found in Balanus balanoides that all mantle water is expelled from the barnacle as the tide recedes. The opercular valves adjust to give body tissues access to atmospheric oxygen. Under these conditions, no lactate is accumulated as would be expected if anaerobic conditions prevailed. The evidence indicates that during prolonged exposure, the barnacle utilizes atmospheric oxygen. In a study on the intertidal barnacles Balanus glandula and Chthamalus sp., Augenfeld (1967) was not able to demonstrate the presence of lactate after six days of exposure at constant temperature, which confirms the previous investigations. Other metabolic end products were not studied, however, and there is now evidence that all invertebrates do not necessarily accumulate lactate as a product of anaerobic glycolysis (Hammen, 1966; Simpson and Awapara, 1966; Awapara and Simpson, 1967).

Studies on other invertebrates have shown that anaerobic metabolism is relied upon during periods of exposure. Von Brand,

Baerstein, and Mehlman (1950), Mehlman and von Brand (1951), von Brand and Mehlman (1953), and von Brand, McMahon, and Nolan (1955) have shown that fresh water snails which are more resistant to anaerobic conditions retain fewer volatile acids in their tissues than less resistant snails. In the more resistant species, a slower rate of carbohydrate utilization occurred and little, if any, lactate was accumulated. The end products of carbohydrate metabolism were not always identified due to their unknown nature, but in many cases they were not lactate.

The intertidal lamellibranchs have the ability to close themselves off from their environment during periods of stress. Although this is an obvious adaptive response to exposure (Galtsoff and Whipple, 1930; Morton, Boney and Corner, 1957; Read, 1964) at the same time it compounds the problem of oxygen availability.

It has been known for many years that various lamellibranchs can withstand an absence of oxygen for considerable periods of time, especially at low temperatures. Dodgson (1928) has reported Mytilus living after twenty-four hours in hot sunlight; and even after forty days of anaerobic existence the adductor muscle retained its contractability. Berkeley (1921) has found the disappearance of glycogen to be greater under anaerobic than aerobic conditions in Saxidomus, although this was not found to be the case in Mya and Paphnia. Mya was found by Collip (1921), to undergo anaerobic oxidative processes

because of a continuous rate of carbon dioxide production throughout an extended exposure period. Similarly, Ostrea was found to be a facultative anaerobe by Galtsoff and Whipple (1930), but no work has been done to determine if vertically separated lamellibranchs differ in their capacity for anaerobiosis.

Mytilus californianus is a common marine lamellibranch which occupies a wide vertical zone within the intertidal. A number of rate functions in connection with vertical distribution have been studied using this animal (Rao, 1953a, b; Segal, Rao, and James, 1953; Pickens, 1965), but to our knowledge oxygen consumption has not been investigated in this regard. In this investigation, the oxygen consumption rate of vertically separated M. californianus was determined as a function of oxygen tension and time of exposure. As a further measure of intraspecific physiological adaptation, weight loss, tissue and mantle fluid lactate accumulation, and oxygen depletion within the mantle cavity were determined during exposure for high and low-level mussels, and discussed in terms of metabolic adaptations.

MATERIALS AND METHODS

Collection and Maintenance of Animals

The location of a suitable collecting site was based upon three requirements: 1. a plentiful supply of similar sized mussels at both the high and low-level marks; 2. a wide vertical difference between high and low-level animals; and 3. ease in reaching the area at normal low tides. An area meeting these requirements is located at Yaquina Head, approximately four miles north of Newport (lat. 44° 39.1'N) on the Oregon coast. Yaquina Head is a typical exposed rocky shore, with basalt rock forming a solid attachment surface for the byssal threads of Mytilus.

The specific collecting site (called Butte Rock) is a westward sloping rock surface, with sharp high and low demarcation lines setting off the Mytilus bed. From survey measurements made at the area, it was found that the Mytilus bed covered a vertical distance of 11.2 feet, from high to low, with the greatest density of high level mussels being 2.9 vertical feet below this extreme point, and the greatest density of low-level animals appearing 8.3 vertical feet below the high-dense level. An attempt was made to correlate these measurements to MLLW (mean lower-low water), but the Yaquina Head area does not have suitable bench markers from which to establish initial vertical distances. It is impossible at this time to

determine percent exposure of the animals at this collecting area.

All animals were collected for experimentation during the months of April, May, and June, 1968. The animals used were found to be sexually mature and gravid as indicated by coloration of the mantle layers. Studies made on Mytilus californianus by Whedon (1936), Whedon and Sommer (1937), and Coe and Fox (1942) have provided no evidence for seasonal changes in metabolism as was reported by Bruce (1926) for M. edulis of the English coast. Spawning can occur at any time of the year, apparently irrespective of temperature or other external stimuli (Whedon, 1936; Coe and Fox, 1942), at least in the California mussel.

An attempt was made at the time of animal collection to standardize the size of the mussels taken for investigations (this was especially true in the respiration studies, to be mentioned later). Animals averaging seven centimeters in length were used in most of the study. In later tests, however, uniformity of size was sacrificed to a degree, and larger animals from the low-level and smaller animals from the high-level zone were used.

Immediately after the animals were removed from the substrate, they were quickly transported to the Oregon State Marine Science Center, Newport, Oregon. The majority of visible organisms were removed from the shell by scraping, rather than by chemical treatment which was found to hamper respiratory movements in Mya

(Collip, 1921). This procedure of scraping the animals was continued throughout, even to those mussels which had relatively little epifauna.

The experimental animals were stored in well aerated fiberglass holding tanks at the Center for one complete tide-cycle before use. No animal was kept longer than three days before testing since laboratory acclimation was not considered desirable for the type of experiments carried out in this study. Segal, Rao, and James (1953), however, found that vertical differences in rate functions of M. californianus were maintained under laboratory conditions for as long as four weeks.

The sea water for the laboratory is drawn directly from the Yaquina Bay estuary and the water characteristics are approximately those of the open coast during spring and early summer. The laboratory salinity ranged from 25 to 32‰ and the temperature ranged from 9 to 14°C during the experimental period. Hydrographic data from the Oregon coast off Newport for the year 1963 (April through June) shows a salinity range from 29.7 to 34.0‰ and a temperature range from 6.6 to 12.0°C (Wyatt and Gilbert, 1967).

The mussels were not fed while retained in the lab. The animals were kept in filtered sea water, and all experimental observations were carried out in this water. The water was prepared by passage through a gradient gravel filter, which filters out particles above 1 to 2μ. The presence of a small amount of feces and

pseudofeces during a three day retention period indicated the mussels were nonetheless deriving some organic material from the water.

Respiration and Oxygen-Tension Measurements

There are two well-documented methods by which the respiration rate of aquatic animals can be determined (Keys, 1930). One is the determination of the rate of change in gas content of a closed vessel containing the experimental animal; and the other is a flow-through system in which a measured amount of water of known gas content is passed through the experimental container in a given period of time, and water samples are collected and analyzed. The latter method eliminates some of the difficulties of the former, primarily accumulation of waste products. With the flow-through method, however, it is more difficult to run a large quantity of animals. In the present study all oxygen consumption studies were carried out using the closed-bottle technique. In such experiments, the Winkler method has generally been used to determine the amount of dissolved oxygen present after prescribed periods of time. The present study utilized a Beckman oxygen macro-electrode together with a Beckman Physiological Gas Analyzer Model 160 to determine the change in oxygen tension within the experimental chamber. This electrode consists of a platinum wire cathode and a silver wire anode electroplated with silver chloride. A polypropylene membrane (0.025 mm thick) is

tightly stretched over the electrode face which has been coated with KCl gel electrolyte to assure electrical contact. The membrane is assumed to be permeable only to oxygen which diffuses from the medium of the experimental animal and contacts the anode, thus initiating a series of events leading to the reading of oxygen tension from the analyzer unit.

Each membrane has its own permeability characteristics, so the electrode must be calibrated prior to use. This procedure consisted of setting both extreme oxygen tension values on the meter. The zero oxygen tension was obtained by placing the probe in an oxygen-free medium (Oxsorbent--Burrel Corp. Catalog No. 39-710) and the saturation level by placing the probe in a well aerated container of sea water and adjusting the meter to a previously calculated reading according to the following formula:

$$\text{Meter setting in mmHg} = \frac{\text{\% of calibrated } O_2 \times \left(\text{Barometric pressure} - \text{partial pressure of } H_2O \text{ vapor at temp.}\right)}{100}$$

A value of 20.95% was used as the amount of oxygen in air. All calibrations were carried out at the experimental temperature in a large volume water bath maintained at $10 \pm 0.2^{\circ}C$. Also, a magnetic stirrer was used in all investigations to prevent either temperature or oxygen gradients from existing within the respiratory chamber. A very slow stirring rate was used so as not to disturb the animals.

There is no direct conversion of oxygen tension (mmHg) to dissolved oxygen in cc/liter of solution. To obtain absolute oxygen values, samples of the water used in the saturation calibration must be analyzed by the Winkler method or some other method of determining oxygen concentrations in water. In previous experiments in our laboratory using concurrent Winkler samples, it has been found that saturation levels are established through relatively short periods (30 mimutes) of aeration of the calibration water. Accordingly, a nomogram developed by Richards and Corwin (1956) which correlates temperature and chlorinity (or salinity) to dissolved oxygen was used to calculate the oxygen concentration in cc/liter from oxygen tension. This method gives comparative rather than absolute values.

All oxygen consumption measurements were recorded in terms of $\mu l O_2$ consumed/gm of dry tissue weight/hour. In metabolic studies, it is not unusual to find either wet or dry weight data. The major reason for the use of dry weight in this study was the difficulty of obtaining a consistent wet weight measurement. Accordingly, all tissue samples were dried to constant weight at $80^{o}C$. Constant weight was achieved in a maximum of three days.

Pickens (1965) suggested that intershell volume is a better metabolic reference than weight of soft-body parts because it is independent of habitat, season, and length of time in the laboratory. Intershell volume was determined by the difference in water

displacement of the whole animal and the valves. This method was also used by Haven (1958) on oysters. Although intershell volume was not used as a metabolic reference point in this study, it was correlated with shell weight in a separate series of tests, reported in the Results section.

Two types of respiratory experiments were carried out, both at 10°C. In one set of experiments the rate of oxygen consumption of pre-exposed, exposed, and post-exposed mussels from both high and low-tide levels was compared. Six and twelve hour periods of exposure were used. These periods correspond approximately to exposure conditions confronted by the mussel in its environment.

Pre-exposure values of oxygen consumption were determined in submerged mussels following a one-half hour acclimation period to experimental conditions. Then each mussel was exposed, except for control animals which were left submerged. To expose the animals, the water was carefully aspirated from the respiratory chamber without disturbing the animal, and the chamber with the experimental animal was left in the temperature bath for the prescribed exposure period. At the end of this period, water at bath temperature was slowly added to the experimental chamber until it was filled (this took approximately three minutes) and the cork with the oxygen probe inserted through it was again put tightly in place. Oxygen consumption measurements were taken immediately and continued until the

rate had returned to pre-exposure levels. Oxygen consumption measurements using the macro-probe were attempted on exposed animals, but no changes in tension could be recorded.

The other set of respiration experiments involved an investigation of the relationship between oxygen consumption rate and oxygen tension. The experimental setup was exactly as before, excepting that a Sargent Linear-Log chart recorder was attached to the gas analyzer and the mussel remained in the respirometer chamber until zero oxygen tension was attained. Thus, a continuous recording of the oxygen tension in the respirometer chamber (time-tension curve) was attained. From this, the oxygen consumption rate at different oxygen concentrations was estimated, as described in more detail in the Results section. When the analyzer indicated zero oxygen tension in the chamber, the animal was removed, and the pH of the water determined with a Beckman Expandomatic pH meter.

All experiments were run under constant light conditions, although no evidence has been found to suspect that this factor may be important in regulating oxygen consumption of Mytilus.

Measurement of Weight Loss during Exposure

Weight loss as a function of time of exposure for the high and low-level populations was determined at two different temperatures. Constant temperatures of 10 and 18°C were maintained in refrigerated

incubator boxes. The relative humidity was maintained at 100% by the use of large, open containers of water. Each mussel was removed from the holding tank, rapidly dried of all excess water, weighed on a Mettler top-loading balance, and placed in the incubators, to be weighed repeatedly during a 48 hour period. According to White (1937), once dried of external water, Mytilus will not gape, thus preventing loss of the trapped mantle chamber water. All animals were kept until the last weighing and then tested for signs of life by reimmerson in a beaker of sea water. All animals were observed to recover in sea water. Weight loss as a function of exposure temperature and intertidal height was then plotted.

Oxygen Content of the Mantle Cavity Water

Oxygen depletion studies of the mantle cavity fluid were carried out using a modified micro-Winkler procedure developed in our laboratory. The main advantage of this method over other micromethods is the direct injection of reagents into the sample-holding syringe. The mussels were "restrained" with a laboratory clamp to prevent gaping during exposure. The temperature ranged from 21 to 24°C during the laboratory exposure periods. Five milliliter samples were withdrawn without bubbles from a hole drilled in one valve of the mussel. The hole was fitted with a round toothpick which served as a standard taper stopper during the exposure period. Duplicate two

milliliter aliquots were titrated with $Na_2S_2O_3$ from a syringe model microtitrator. Results were expressed as ccO_2/liter. An attempt was made to correlate laboratory values and values obtained from animals still attached to the substrate (and generally slightly gaping) at the collecting area.

Lactate Determinations

Lactate determinations were made on both the whole animal tissue and mantle cavity fluid in pre-exposed, exposed and post-exposed mussels. The method used was a modification of the Barker and Summerson method (Barker and Summerson, 1941) developed by Ström (1949), which eliminates the removal of interferring substances and is sensitive to approximately 1μgm.

The following procedures were observed in the collection of lactate samples. All animals were "restrained" as previously described. The mantle cavity fluid was withdrawn by a syringe, diluted 1:1 with 4% perchloric acid, centrifuged if necessary, and the supernatant frozen at -20°C until used. The whole animal was removed from its shell and placed in a preweighed homogenizer containing 5 mls of sea water. Due to the size variation, two mussels were used in all high-level samples, thus allowing measurement of similar tissue weight. The tissue was homogenized in a chilled Virtis Homogenizer and then centrifuged at 12,000 RPM in a Sorvall Refrigerated

Centrifuge at 0°C. The supernatant was diluted 1:1 with 4% perchloric acid, centrifuged, and stored at -20°C until used.

The violet color of the acetaldehyde-p-phenylphenol complex was read at 560 mμ with a Bausch and Lomb Spectronic 20 colorimeter. If any interference was noted, the sample was further diluted or treated with $Ca(OH)_2$ to eliminate the effects of these substances.

RESULTS

Respiration in Pre- and Post-Exposure Periods

The normal (pre-exposure) rate of oxygen consumption in submerged high and low-level M. californianus was determined using the previously described methods. In all cases, it was found that the high-level forms consumed more oxygen per unit time and dry tissue weight than the low-level forms. Values obtained were 347.2 ± 1.85 (8)* $\mu 10_2$/gm/hr and 261.0 ± 3.43 (8) $\mu 10_2$/gm/hr, respectively for high and low-level mussels. These mean values are significantly different at the 1% level ($t = |3.51| \geq t_{0.1} = 2.98$).

A large variation in values was found between individuals. According to several workers (Mitchell, 1912; Collip, 1921; van Dam, 1935) variation in metabolic rate of lamellibranchs could partially be due to the amount of water passed over the gills, which is directly related to the duration of valve opening. The amount of oxygen withdrawn is in most cases very low (van Dam, 1937) so only a slight change in experimental conditions would make a difference. Data was collected only during periods when the shell was open.

The pre-exposure values were used to determine if a compensatory increase in metabolic rate occurred following periods

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* Values are given as: Mean $\pm$ S.E. (N); where S.E. is standard error and N is the number of determinations.

of exposure. The rate of oxygen consumption was plotted as a function of the time after reimmersion following exposure periods of six and twelve hours (Figures 1 and 2). Both figures show consistent increases in oxygen consumption following exposure, with the high-level mussels showing greater increases than the low.

The rise in oxygen consumption following a twelve-hour exposure period was much greater than that following a six-hour period in the high-level forms. Thus, oxygen consumption was 630.3 ± 2.84 (15) $\mu l O_2$/gm/hr after six hours of exposure and 1276.2 ± 1.77 (10) $\mu l O_2$/gm/hr following the twelve hour period. In the low-level mussels the length of exposure does not markedly affect the compensatory rise in oxygen consumption (590.0 ± 3.28 (14) $\mu l O_2$/gm/hr and 673.0 ± 0.84 (8) $\mu l O_2$/gm/hr following six and twelve hours of exposure, respectively). All means differ significantly at the 5% level from those of the controls.

The controls run for each experiment were handled in a manner identical to the experimental animals, except that they were never exposed. During the period of exposure for the experimental group all controls remained submerged in the holding tank at temperatures very close to that which the experimental were exposed. From Figures 1 and 2, it is apparent that controls vary only slightly over the post-exposure period, the greatest variation being at the later time intervals possibly because of the fewer number of readings taken.

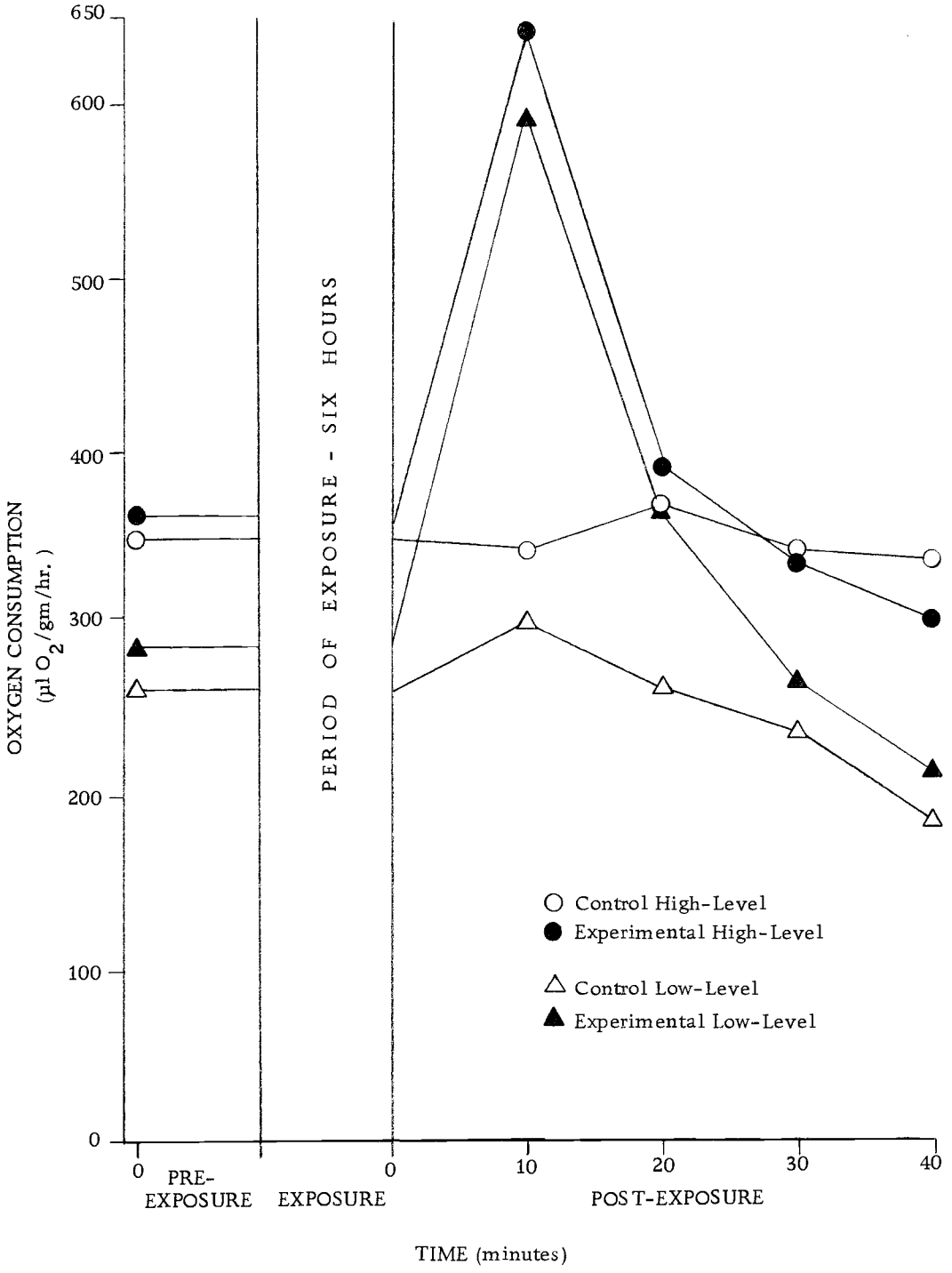

Figure 1. Oxygen consumption at 10°C following a six hour exposure period (maintained at 10°C during this period) in vertically separated M. californianus

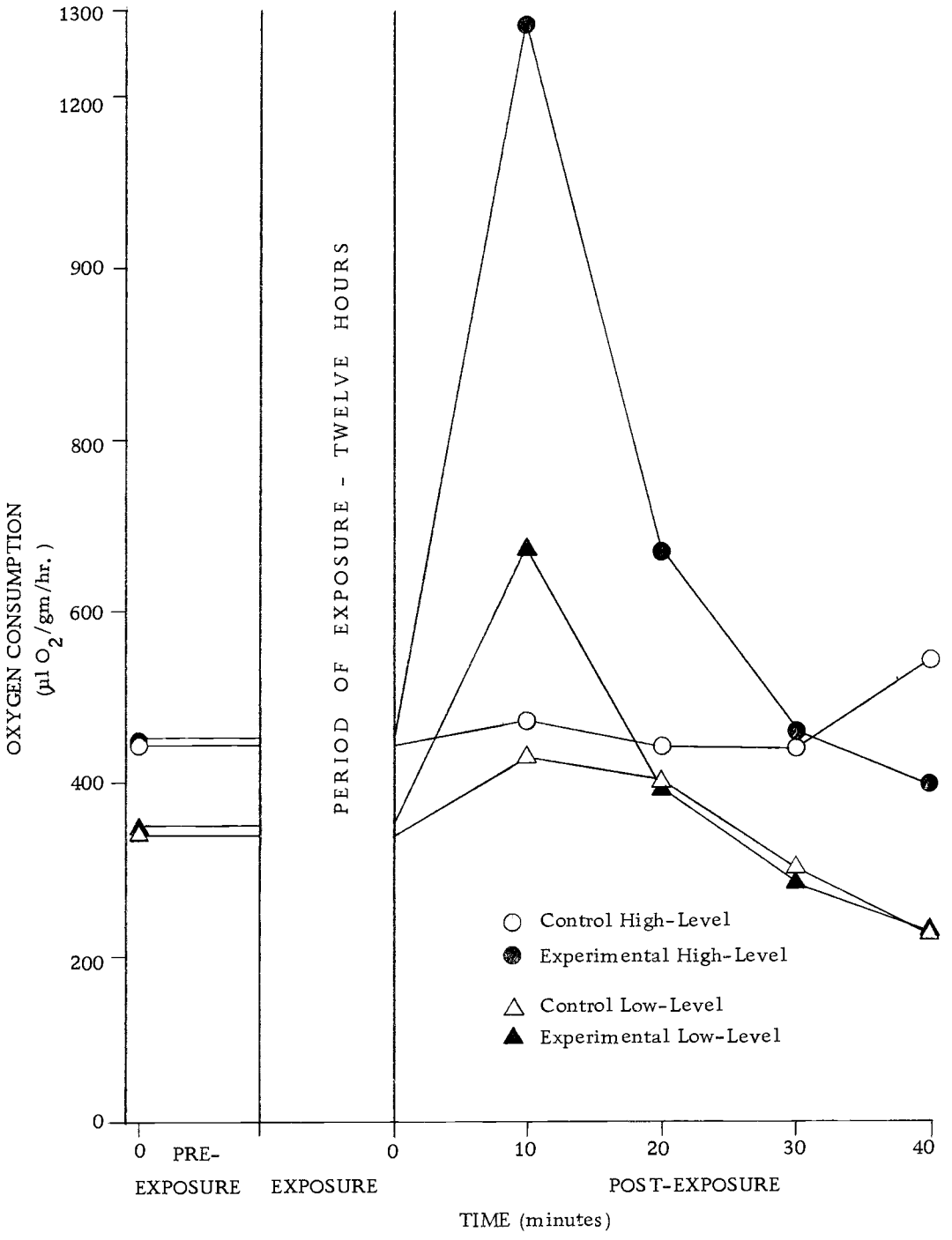

Figure 2. Oxygen consumption at 10°C following a twelve hour exposure period (maintained at 10°C during this period) in vertically separated M. californianus

The time to recover after exposure was not dependent upon the period of time exposed. Figures 1 and 2 demonstrate little difference in recovery times, which is approximately 30 to 35 minutes in all cases. This recovery period also does not differ between high and low-level animals.

As noted previously, respiratory measurements were attempted in a number of animals during the period of exposure. No change in oxygen tension could be detected by the oxygen electrode, which might result from the large volume of the respirometer chamber (340 to 380 mls), or the absence of stirring to eliminate oxygen gradients.

Oxygen Consumption as a Function of Oxygen Tension

Oxygen tension in the respirometer chamber was plotted as a function of time in both high and low-level M. californianus (Figures 3 and 4). The high-level mussels show a nearly linear relationship between time and oxygen tension at all but very low tensions. One mussel (1.09 gm), in fact, demonstrates a completely linear relationship at all tensions (Figure 3). The low-level forms behave in a similar manner (Figure 4), with a near linear relationship at high tensions, but the deviation from linearity occurs at a higher tension than for the high-level mussels.

The rate of oxygen consumption was calculated from these

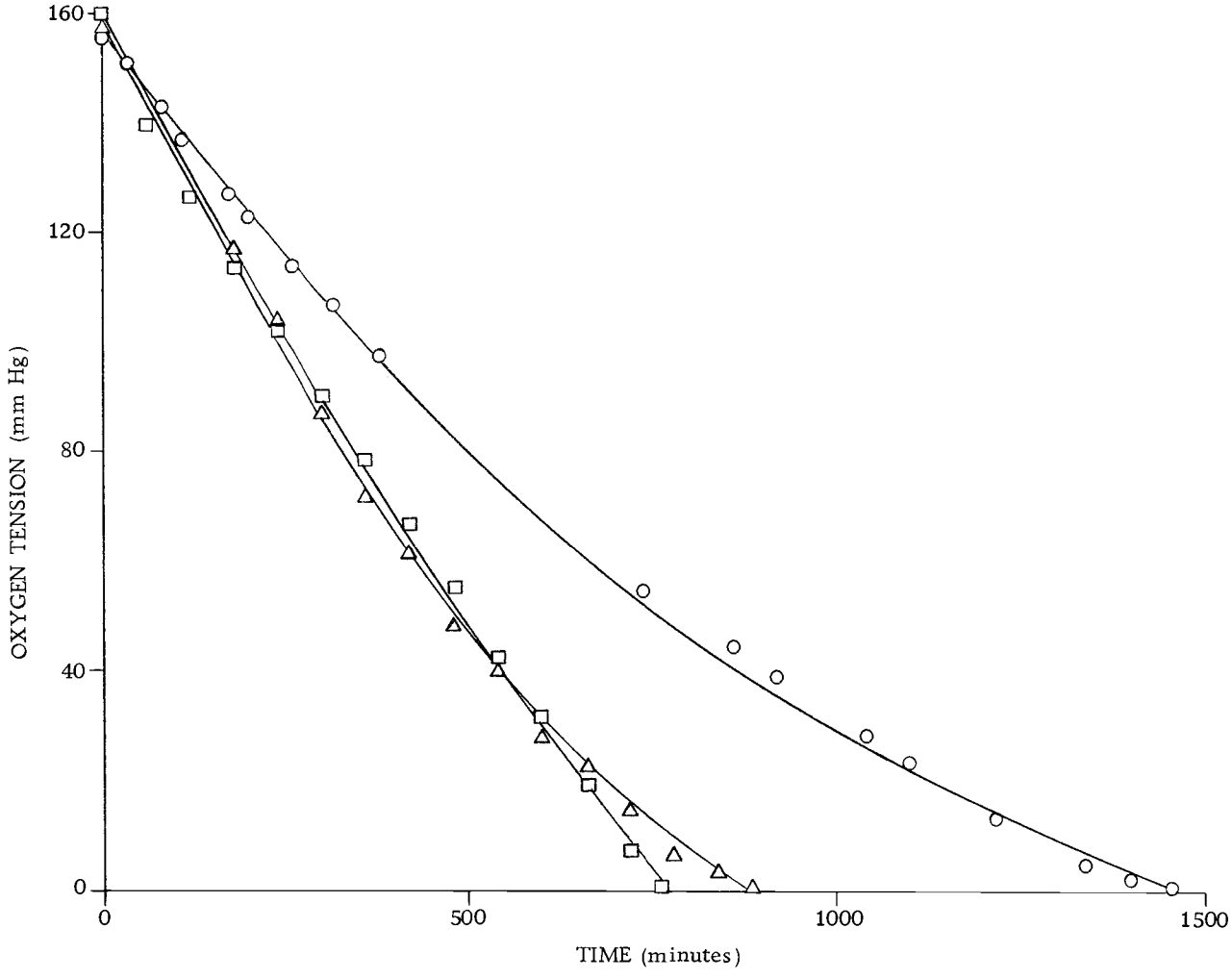

Figure 3. The rate of oxygen depletion of high level M. californianus (○ .78 gms., △ 1.02 gms., and □ 1.09 gms. dry weight animal)

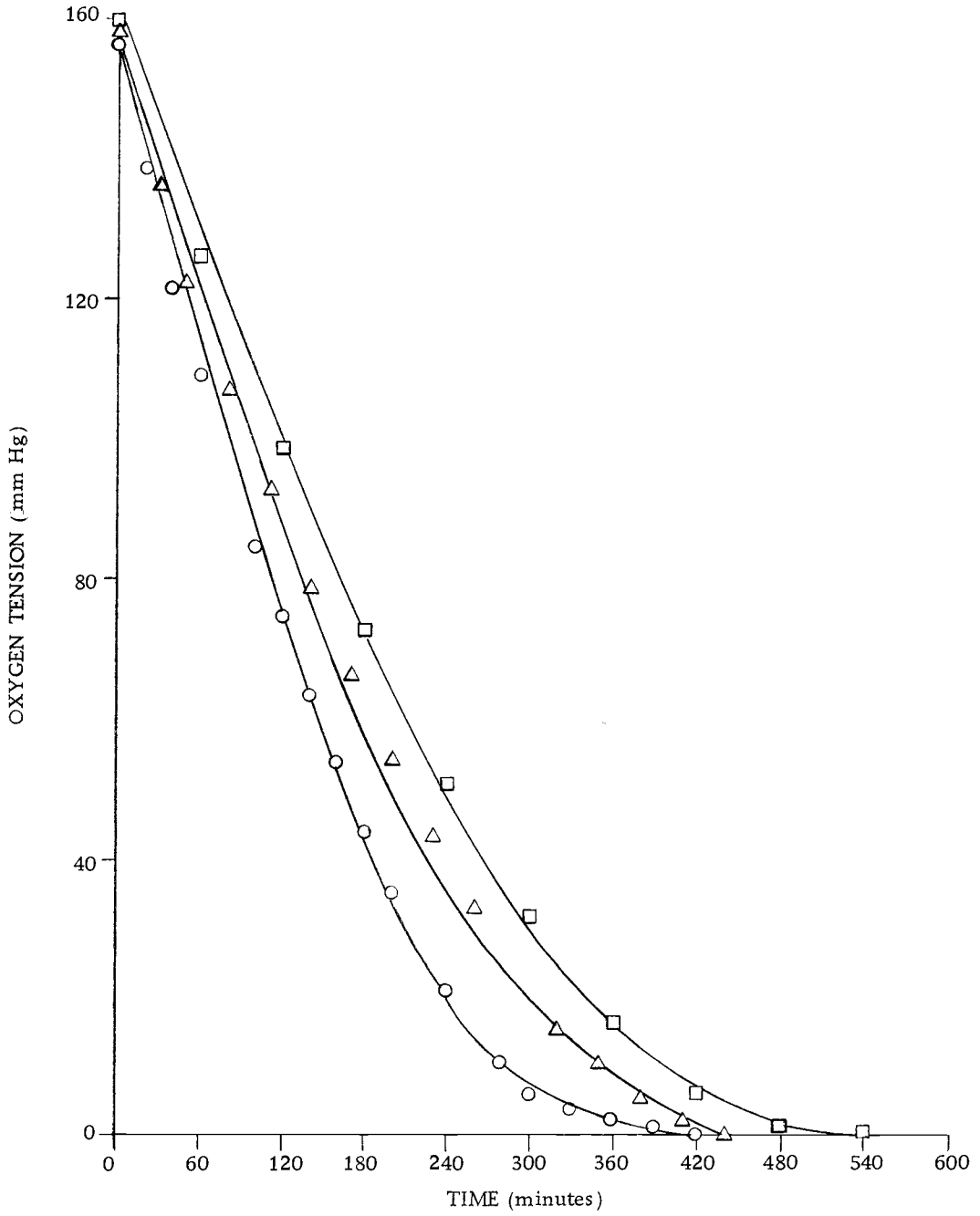

Figure 4. The rate of oxygen depletion of low level M. californianus (O 2.48 gm., △ 1.67 gm., and □ 1.65 gm. dry weight animal)

figures, using 10 to 15 minute intervals depending on the linearity of the graph. Rates were calculated in a manner explained in the previous section, by use of a nomogram and the dry tissue weights of each animal. Rates differ considerably between individuals so a separate curve was drawn for each animal.

In Figures 5 and 6, oxygen consumption was plotted as a function of oxygen tension. To avoid elimination of significant points, lines were drawn by eye to best fit all points. The figures appear similar in over-all pattern. In each case there is a narrow zone of Respiratory Independence, and on either side are zones of Respiratory Dependence (Prosser, 1955). The range of oxygen tensions where respiration rate changes from being independent to being dependent upon the oxygen tension has been termed the critical oxygen tension, or T_c (Prosser, 1955). For the high-level mussels, T_c was 24 to 34 mmHg, and for the low-level mussels this value was 38 to 53 mmHg.

Normal pH values for sea water initially sampled in the time-tension experiments was 7.7. At the completion of a few experiments, the water was again sampled. The values after testing appear to be different, but too few samples were taken to show any significance. The water from the low-level mussel chambers had an average pH of 6.92 after six hours, and water from the high-level containers an average pH of 7.22 after eight hours.

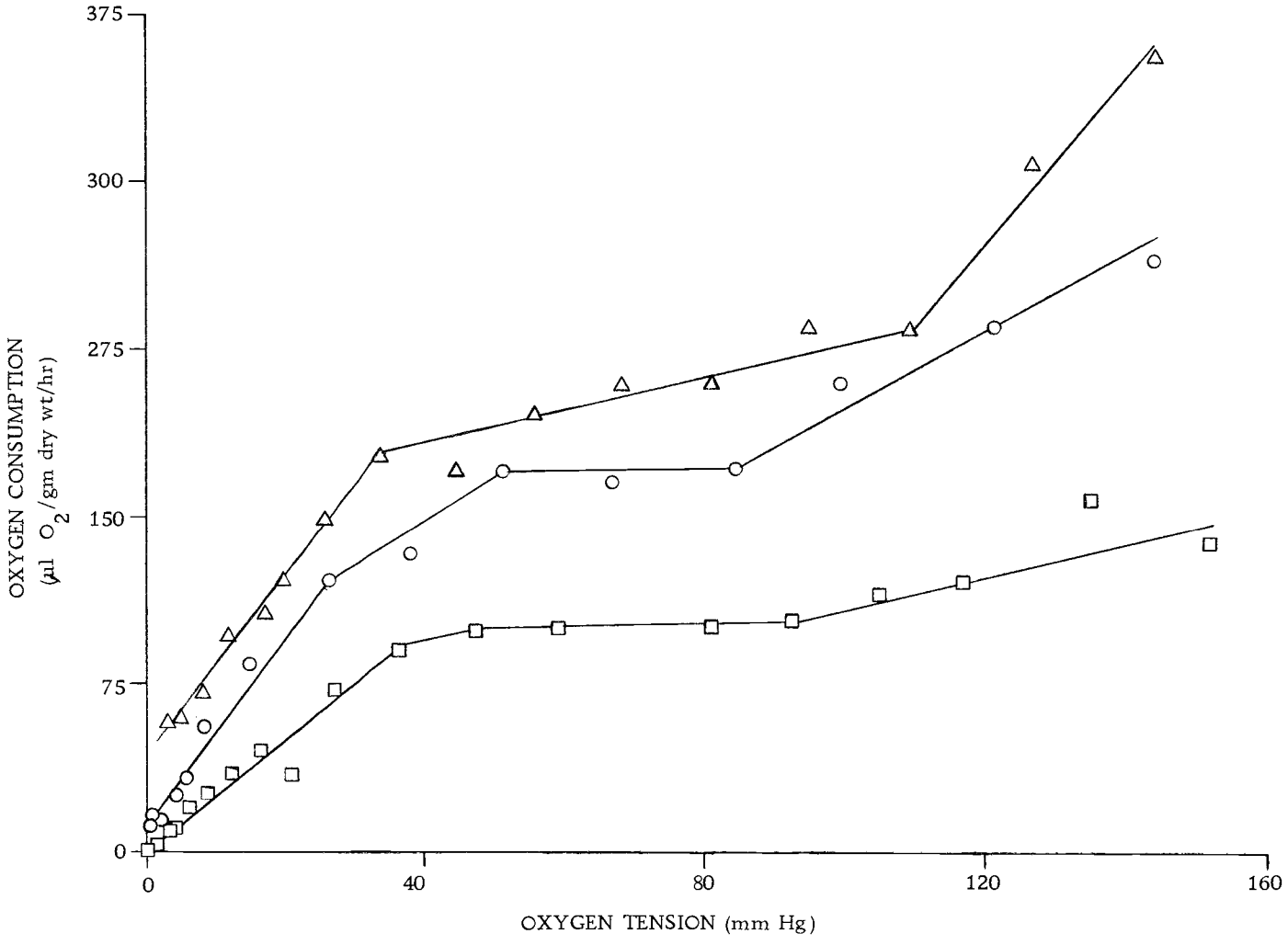

Figure 5. Oxygen consumption of low-level M. californianus as a function of oxygen tension (O 2.48 gm., △ 1.67 gms., and □ 1.65 gms. dry weight animal)

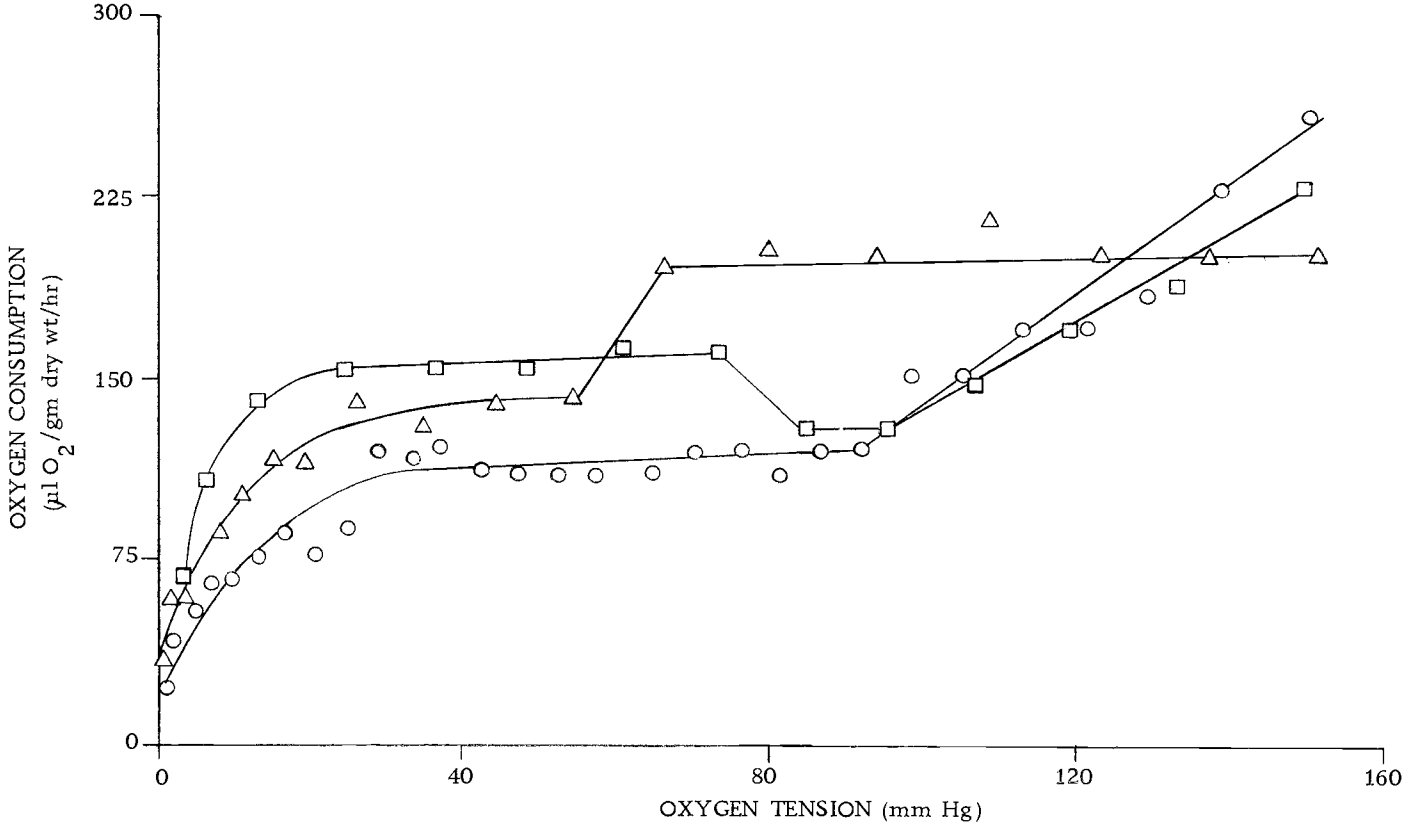

Figure 6. Oxygen consumption of high level M. californianus as a function of oxygen tension (○ .78 gm., △ 1.02 gm., and □ 1.09 gm. dry weight animal)

Oxygen Depletion from the Mantle Cavity

The concentration of dissolved oxygen in the mantle cavity was plotted as a function of exposure time for "restrained" high and low-level M. californianus (Figure 7). The curves are hyperbolic, demonstrating an initially large decrease in dissolved oxygen content, followed by a relatively slow decline to approximately zero concentration. Both curves are similar in shape. The high-level mussels, however, maintain a slightly higher concentration of oxygen at all points except the final time interval. This difference reflects the initially faster depletion of oxygen by the low-level forms.

No mussel was found to maintain a mantle fluid oxygen concentration similar to that of the sea water surrounding them. This may reflect the insensitivity of the micro-Winkler method, or the sampling technique used. To sample the mantle fluid while the animal was submerged, the mussel was quickly removed from the holding tank and 5 mls of fluid was withdrawn using a 10 ml glass syringe. This fluid was analyzed and the dissolved oxygen content was plotted as time zero on Figure 7. The possibility remains that the water sample had previously been used by the mussel for respiratory purposes, although the figure given by van Dam (1937) of 5-10% utilization of oxygen from water in lamellibranchs would thereby be in error for M. californianus.

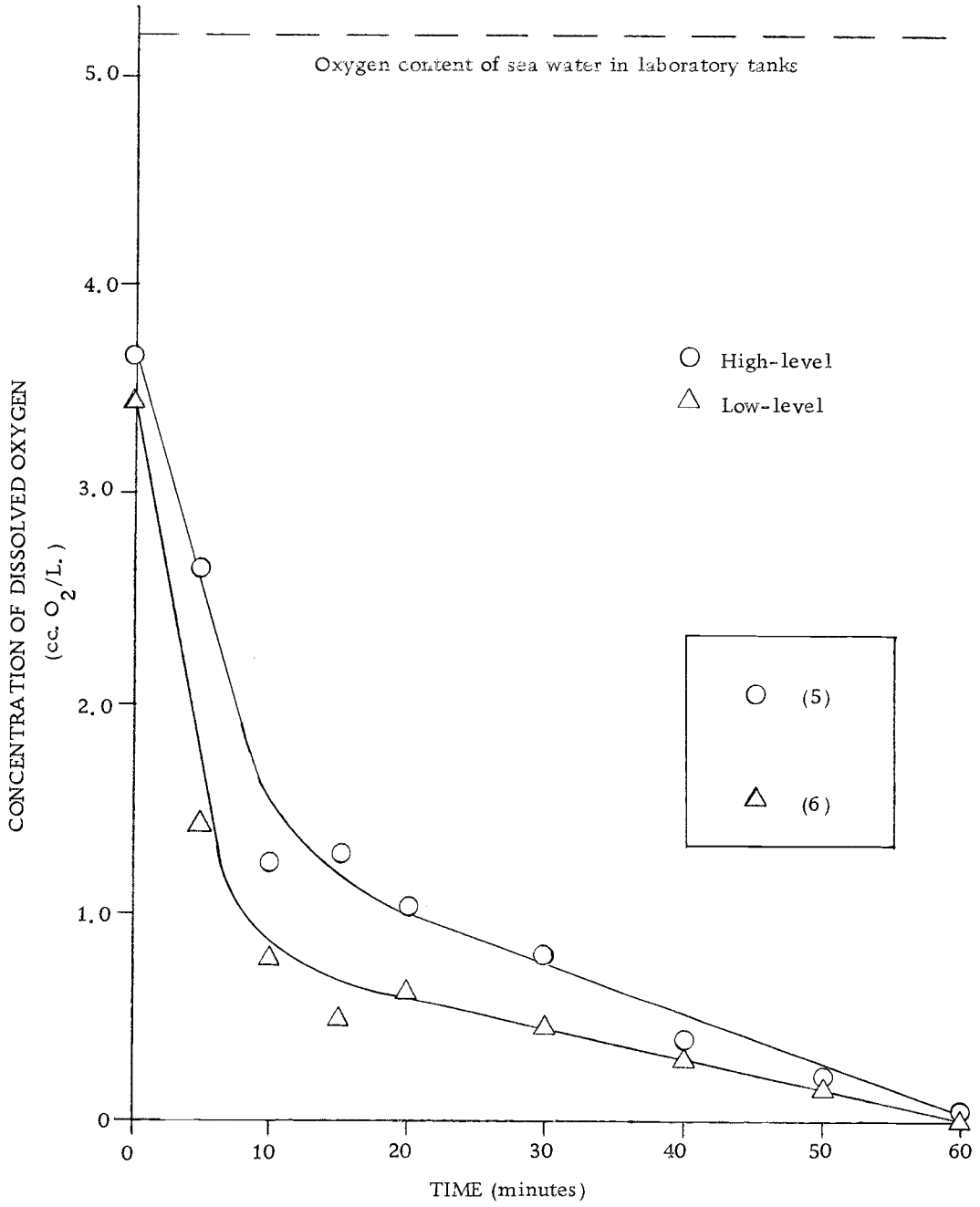

Figure 7. Comparative oxygen depletion curves for the mantle cavity fluid in vertically separated M. californianus as a function of time exposed under laboratory conditions (temperature of approximately 22°C). Insert indicates oxygen values obtained from a few mussels occurring naturally in the collecting area after an exposure of at least three hours. (Numbers represent samples taken.)

Many times it was difficult, if not impossible, to withdraw fluid from the mantle cavity. This was true in mussels from both levels and even in very large animals which were run in preliminary tests. When all the water had been lost, the mussel was found to gape immediately and emit air bubbles upon reimmersion in the holding tank, thus indicating filling of the cavity.

Few mussels (approximately 15) were also sampled at the collecting area and the concentration of dissolved oxygen in the mantle fluid determined. These values are plotted as an insert to Figure 7. Some mussels were found that did not retain water, but these animals were not necessarily opened to the atmosphere. In all the field samples, none were found that contained zero dissolved oxygen concentration, even after an exposure period of at least three hours. The particular day that sampling was carried out, the weather was partly sunny and the air temperature was about 19 or 20^{o}C.

Mussels have been observed in the field to gape temporarily and release small droplets of water from between the mantle layers. This may be a form of evaporative cooling as found by Lewis (1963) for a number of gastropod mollusks during exposure. During periods of high environmental temperatures, however, internal mussel temperatures above 30^{o}C have been recorded in the collecting area of this study (personal communication, Gonor and Barnes). This is higher than the thermal death point in M. edulis as determined by Read (1967).

The large variation in dissolved oxygen concentration precluded any meaningful statistical analysis of the data.

Weight Loss vs. Exposure

The percent weight loss of high and low-level M. californianus was plotted as a function of exposure time at two different environmental temperatures (Figure 8). The decrease in weight was assumed to be due to mantle cavity and tissue water loss, as a result of evaporation either through a poorly sealed shell or due to gaping of the mussel. The amount of water lost from the shell was taken to be insignificant. The 10 and 18°C temperatures were chosen arbitrarily, although 18°C would approximate an average environmental air temperature on an overcast day when the animals are exposed to the atmosphere.

Each curve indicates an initially large decrease in weight, followed by a slow tapering off of the weight-loss. There is a large scattering of points, but the tendency for the curves to depend both on temperature and intertidal position is apparent. The high-level mussels at each temperature tend to lose more total weight and at a faster rate than the low-level forms. There appears, however, to be no difference in the 10 and 18°C curves for the high-level mussels. The low-level mussels, on the other hand, show a decreased weight loss with increased temperature, which may be of adaptive significance

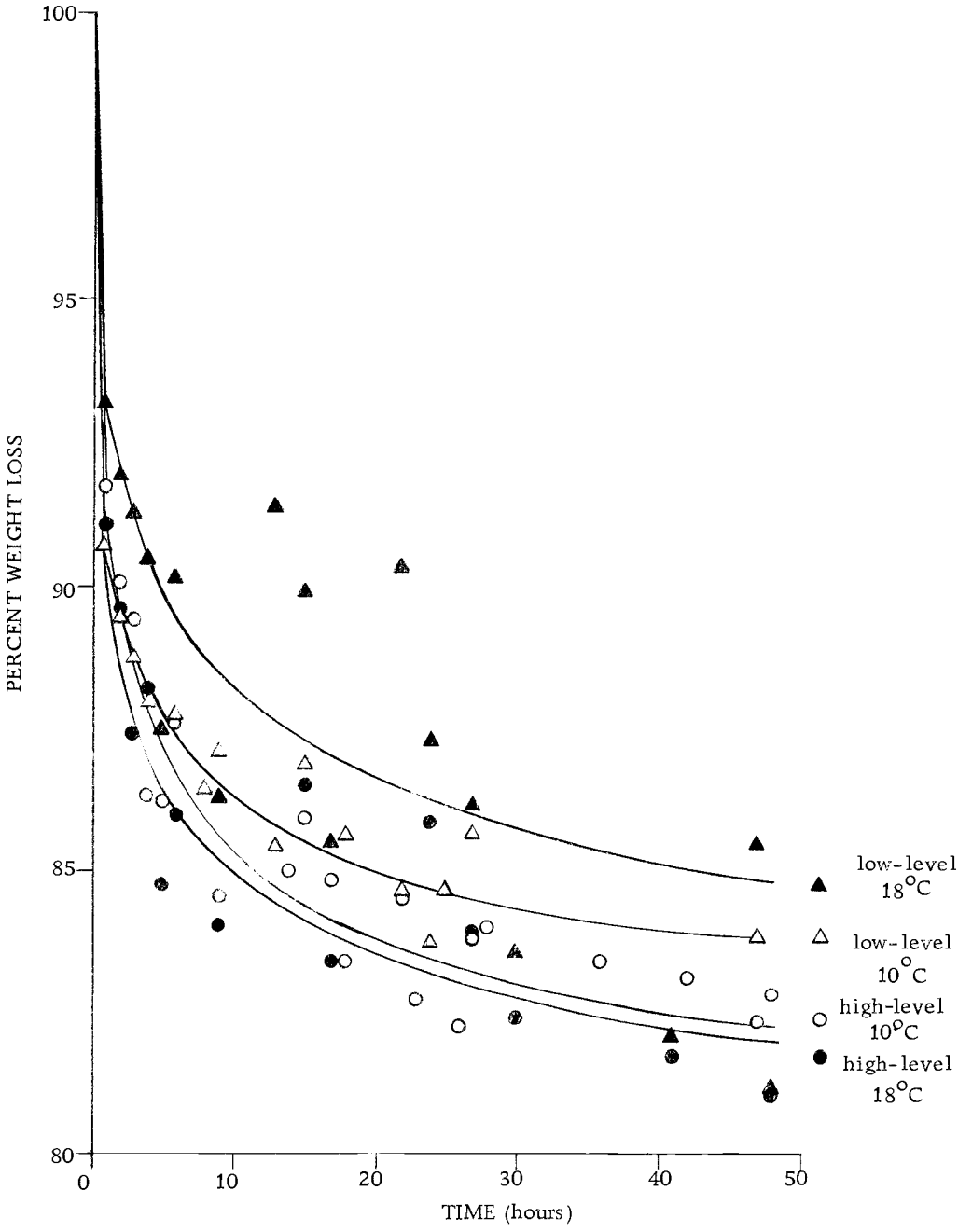

Figure 8. Weight loss as a function of temperature (10 and 18°C) and vertical distribution in M. californianus (Relative humidity = 100%)

as discussed later.

Lactate Concentrations

The concentration of lactate in whole animal homogenates and mantle cavity fluid samples was determined as a function of the length of exposure. The values showed considerable individual variation. Tissue lactate in the high-level mussels rose from a pre-exposure level of 29.9 μgm/gm tissue weight to 43.5 μgm/gm tissue weight after twelve hours exposure. The low-level mussels show no net lactate accumulation within the tissues, even after twelve hours of exposure (32.1 μgm/gm tissue in pre-exposed animals compared to 32.6 μgm/gm tissue after twelve hours exposure). A large increase in lactate, however, was found in the mantle fluid after six hours of exposure, perhaps indicating elimination of the acid from the tissues. This accumulation is not due to contamination of glassware, because similar values were obtained after a number of determinations. A similar increase in mantle fluid lactate was also seen in the high-level mussels; however, the rise is not as significant as in the low forms, which may be related to the higher lactate concentration found in the soft parts of the higher level animals.

Lactate was determined after an arbitrary two hour reimmersion period following the twelve hour exposure period. The data indicates an even higher concentration of lactate in the tissues, which

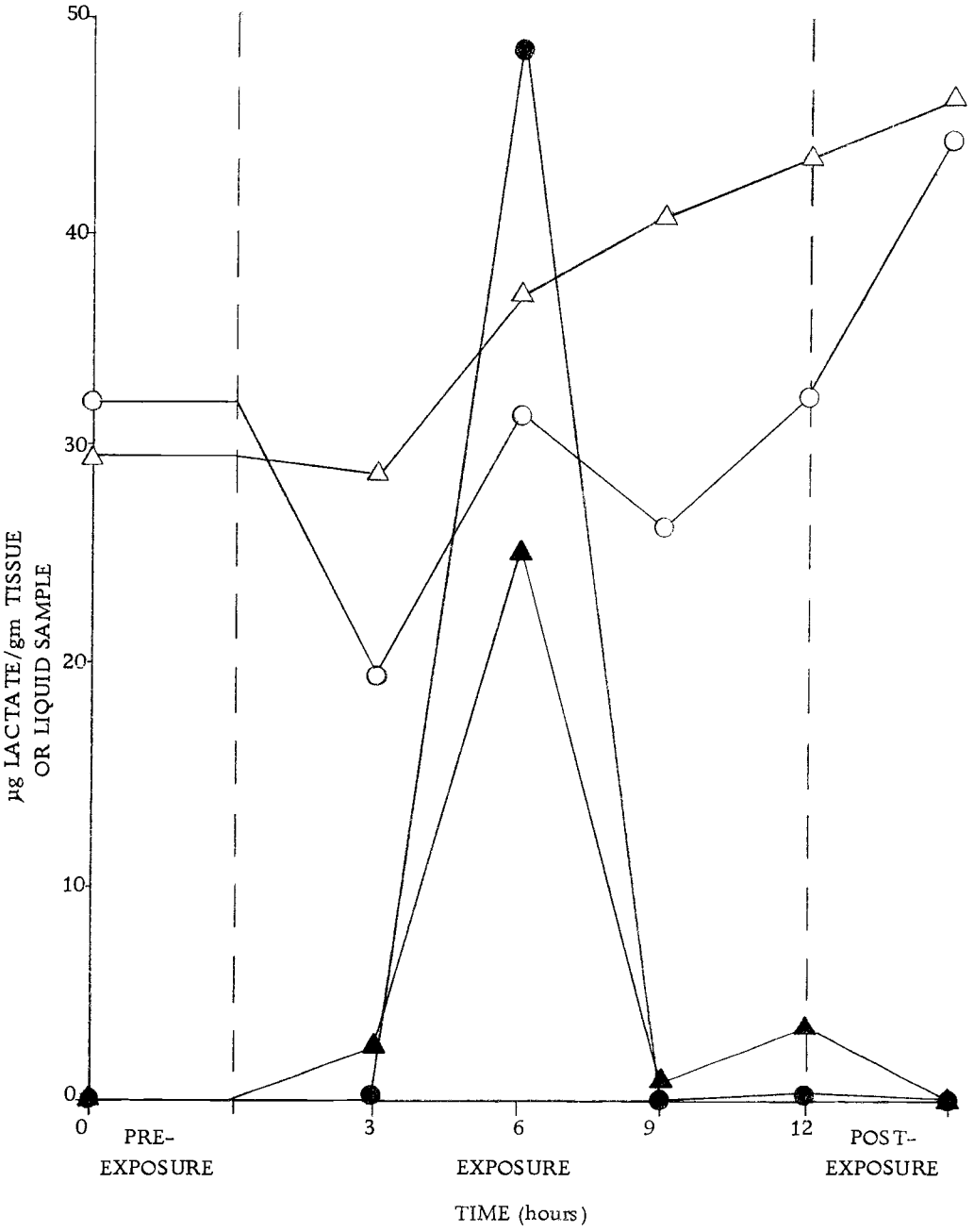

Figure 9. The rate of lactate accumulation in high and low M. californianus as a function of exposure as well as measurements taken two hours after submergence following twelve hour exposure for both whole animal homogenates and mantle chamber fluid. (homogenates: ○ low, and △ high; mantle fluid: ● low, and ▲ high)

may reflect the release of lactate from some bound state. In this free state it may be re-oxidized.

Samples were also taken from the sea water surrounding the submerged mussel, and no lactate was detected, either because there was none present or because it was diluted by the large volume of water used in submerging the animals. The volume was 160 to 211 mls, depending upon the size of the mussel, which would not be a dilution in excess of that used in the tissue sample determinations.

Since the variation was quite large, no statistical analysis was carried out. The experiment was intended to be preliminary in nature, but the general trends established indicate that additional experiments would be worthwhile.

Intershell Volume and Shell Weight

The volume of the intershell space was plotted by the use of linear regression as a function of dry tissue weight in high and low-level M. californianus. From regression analysis, the equations for the lines were determined as:

High-level animals: $y = 11.22x + 4.04$ (1a)

Low-level animals: $y = 9.35x + 2.63$ (1b)

These equations and Figure 10 indicate that at all dry tissue weights determined, the high-level animals have a greater intershell volume and, consequently, the ability to retain more water than the low-level

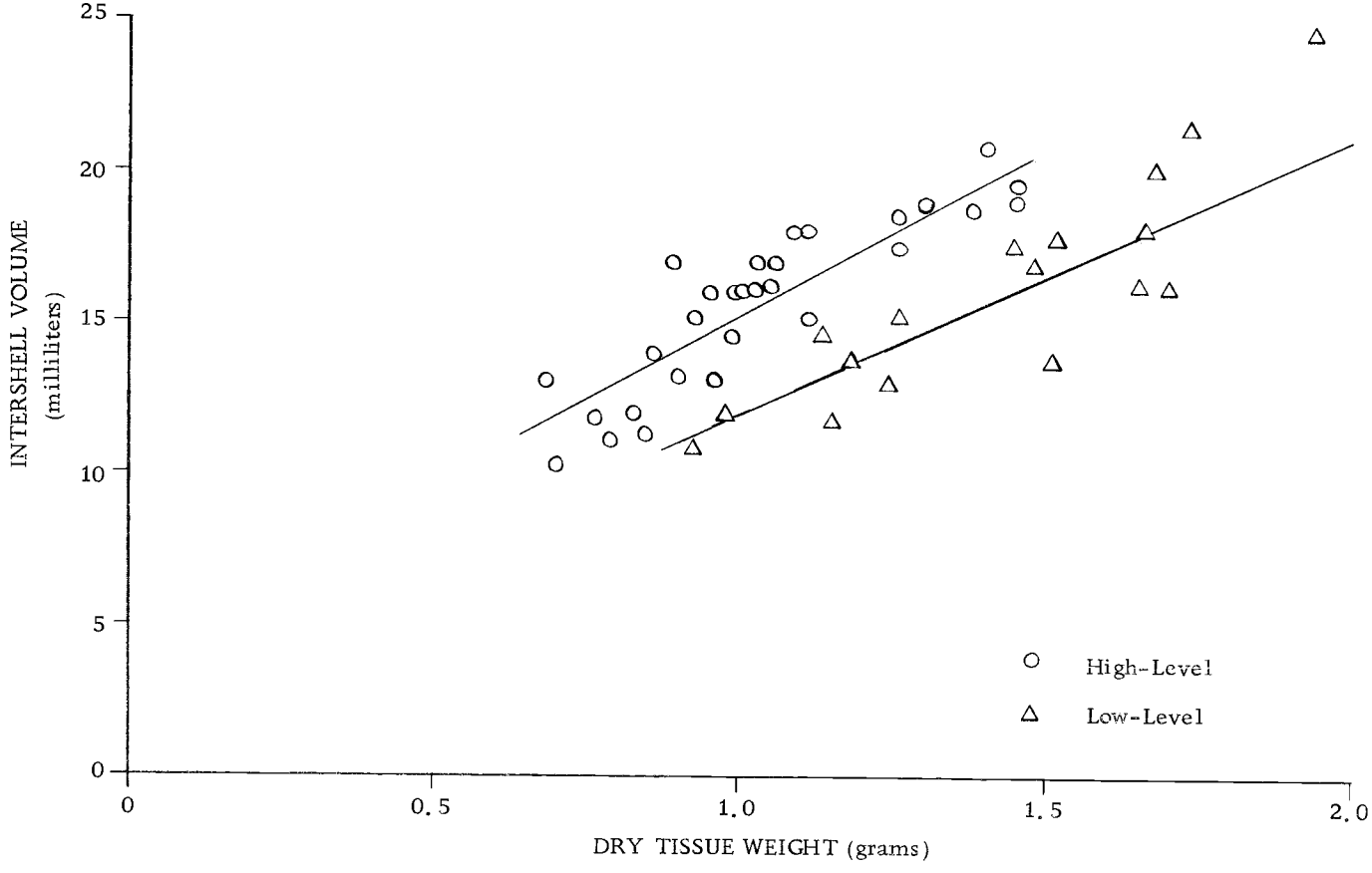

Figure 10. Intershell volume as a function of dry tissue weight and intertidal separation in M. californianus

forms.

The determination of shell weight was more critical than that of shell volume. Due to the large number of encrusting organisms found attached to the valves of the mussels, it was difficult to determine to which layer of the shell these organisms should be removed.

After a drying period of one hour at 80°C, all mussel shells were scraped to eliminate any material which was loosened. Most of the periostracum flaked off with this treatment and was subsequently removed.

Figure 11 indicates the correlation between shell weight and dry tissue weight. Equations for the straight lines were determined from linear regression as follows:

High-level animals: $y = 18.58x + 3.86$ (2a)

Low-level animals: $y = 8.19x + 4.96$ (2b)

Again, the high-level mussels had a greater shell weight at all tissue weights measured, although the lines do intersect at low tissue weights as can be seen from the graph and equations. The significance of this difference will be discussed.

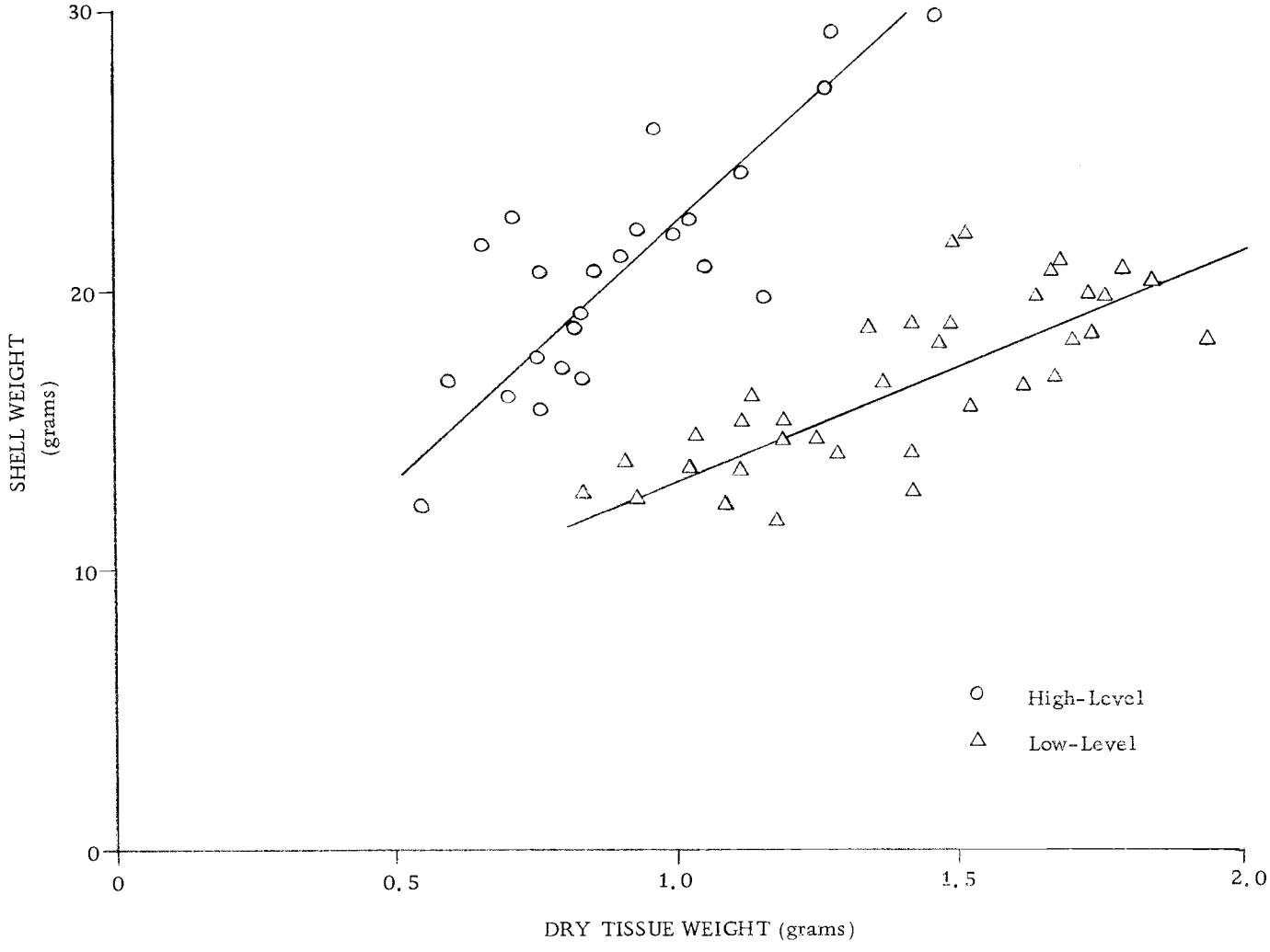

Figure 11. Shell weight as a function of dry tissue weight and intertidal separation in M. californianus

DISCUSSION

Respiration Studies

Oxygen consumption values for Mytilus sp. have been found to range from 22.0 to 55.0 $\mu l O_2$/gm wet wt/hour (National Academy of Sciences, 1958; Rotthauwe, 1958). In this investigation the respiration rate for submerged M. californianus ranged from 261.0 to 347.2 $\mu l O_2$/gm dry wt/hour. These values, corrected to wet weight using a dry weight:wet weight ratio of 0.2, compare favorably with the reported values. Although care was taken to control as many factors as possible that are known to affect metabolism--in particular temperature and nutritional state--two factors, gonadal condition and body size, require additional comment. It was not possible to determine gonadal condition until the animals were sacrificed at the end of the experiments. Since the experiments were performed over a relatively short period (May through June) all animals were essentially in the same condition; that is, all were gravid. No evidence could be found in the literature indicating sex differences affect metabolic rate in mussels. It is well known that small organisms of a species tend to have a higher oxygen consumption per unit body mass than large ones (see reviews by Zeuthen, 1953 and Hemmingsen, 1960). Although care was taken to use similar sized animals in all respiration experiments, tissue weights were slightly larger in the

lower level animals. It was judged that the size difference was not great enough to justify linear regression analysis to correct for a possible size effect.

Few investigations have related oxygen consumption to intertidal position. Morton, Boney, and Corner (1957) found that high-level Lasaea rubra (a small lamellibranch) consumed oxygen at a rate 50% higher than the lower level forms. These high-level forms of Lasaea were also found to have a greater filtration rate of water through the ctenidia than the low-level species. Read (1962) found the rate of oxygen consumption of Brachidontes demissus increased with temperature up to 35.2°C while that of M. edulis reached a plateau at 20°C. Equal sized individuals of the two species, however, were found to have approximately equal rates of oxygen uptake at their respective environmental temperatures. Read related this to the ecology of the species since B. demissus, a higher intertidal form, is exposed more often than M. edulis.

The present study has demonstrated that high-level M. californianus have a significantly greater rate of oxygen consumption at 10°C than the corresponding low-level forms. These results are similar to those obtained by Morton, et al. (1957), but are not consistent with the results of other rate functions studied using M. californianus. Previous studies have shown that heart rate (Pickens, 1965), water propulsion (Segal, Rao, and James, 1953), rate of linear

growth (Dehnel, 1956) and total shell weight (Rao, 1953a), are greater in mussels from lower intertidal levels. The significance of a higher respiratory rate in the high-level mussels may be the result of a generally higher metabolic activity. This feature will be discussed in light of other data collected in the present investigation.

The effects of oxygen tension on oxygen consumption or metabolic rate have been studied in a number of organisms. From such investigations, the classical idea of aquatic poikilothermic animals being either "conformers" or "regulators" with respect to external oxygen has emerged (Prosser, 1955; Prosser and Brown, 1961). A conformer is an animal whose rate of oxygen consumption is in strict linear relationship to oxygen tension; if a critical oxygen tension exists, it is well above the level of saturation (150 mmHg). When the oxygen consumption is independent of oxygen tension over a certain range, then the animal is designated as a regulator. At the extremes of environmental tolerances, homeostatic mechanisms presumably breakdown and regulation fails (Prosser, 1955).

In general, mollusks have been found to be regulators. Prosser and Brown (1961, p. 166) give a list of molluskan regulators that include the clam, Mya, oysters, two fresh-water snails (Ancylus and Australabrus), and Mytilus sp. Maloeuf (1937), however, found that the rate of oxygen consumption in M. edulis was linear with oxygen tension at all temperatures. The graphs plotted by Maloeuf

were done with very few points, and the points were so arranged that either a linear or sigmoidal line could be drawn; that is, not enough determinations were made at the intermediate oxygen tensions to substantiate his claims. Another Mytilidea, M. perna, was found by Bayne (1967) to be a regulator, but the degree of regulation depended upon animal size. This mussel was found to increase its ventilation rate as the tension decreased from 100 to 50% saturation, and below this level, the ventilation rate quickly declined. Over this same range, the efficiency of oxygen withdrawal remained constant.

M. californianus has been found in this study to be a regulator. Oxygen consumption was independent of oxygen tension over a narrow range and this was, in most cases, enclosed by areas of oxygen dependent respiration. The significance of a dependent relationship at high oxygen tensions is not understood although it appears to confirm the theoretical curves given by Prosser (1955), indicating homeostatic failure at extreme oxygen tensions.

The data indicates that the T_c (critical oxygen tension) values for the high-level species are lower than those for the low-level mussels (average of 29 mmHg compared to 45 mmHg). The difference in T_c values does not necessarily indicate that the high-level mussels are able to tolerate a lack of oxygen more effectively than the low-level forms. A low T_c may be significant in allowing for a higher rate of respiration to continue in the high-level mussels as the oxygen

concentration in the mantle fluid decreases during exposure. This is discussed later in connection with measurements of mantle fluid oxygen concentration in exposed animals.

Respiratory activities during periods of exposure have been examined in a variety of intertidal invertebrates. The barnacle, Balanus balanoides, has been found to adjust its opercular valves to give body tissues access to atmospheric oxygen (Barnes and Barnes, 1957; Barnes, Finlayson and Piatigorsky, 1963). Lent (1968) has observed that the ribbed mussel, Modiolus demissus, has the ability to utilize atmospheric oxygen under conditions of exposure. This activity has been seen in a variety of intertidal gastropods (Sandison, 1967, 1968; Baker, 1968) as well as Lasaea rubra (Wieser, 1963), although this latter case has been denied by other workers (Morton, et al., 1957).

The quiescent period associated with decreased metabolic functions, presumably related to reduced availability of oxygen during exposure periods, is found to be a common feature of intertidal bivalves (Newell, 1966). Numerous authors have reported a decrease in heart rate (Koehring, 1937; Hisock, 1953; Schlieper, 1955; Helm and Trueman, 1967) and respiration rate (Hiscock, 1953; Newell, 1966) upon valve closure. In this investigation, no uptake of oxygen from the air in the respiratory chamber could be detected even though the experiments were run for periods up to four hours. Under

the conditions of this investigation we would be forced to conclude that during periods of laboratory exposure M. californianus does not utilize atmospheric oxygen, even though its valves do gape during this time. A similar conclusion was reached by Mitchell (1912) in a study of metabolic responses to exposure in oysters. In M. californianus the amount of atmospheric respiration might have been so minute as to not be detected with the instrumentation used. Also, our finding does not preclude the possibility that in its natural environment, where exposure conditions are quite different than those used in the laboratory, M. californianus may consume measurable amounts of oxygen when exposed.

Since metabolic functions decline but obviously do not cease, M. californianus must accumulate an "oxygen debt" while exposed. The data from this investigation indicates that the debt is repaid rapidly after reimmersion in sea water by a greater than normal rate of oxygen consumption (Figures 1 and 2). In Mya arenaria, a compensatory increase in oxygen consumption was found if the species was exposed for several days under anoxic conditions (Collip, 1921). This increase in rate gradually declined over a period of as long as three days in some cases. Van Dam (1935) found an increased utilization coefficient and ventilation rate after a low-tide period of about twenty hours in Mya arenaria, which gradually returned to normal in three to four hours following reimmersion. The increase in

utilization coefficient was found to be directly related to the period of exposure. Increased rates of respiration and circulation as a result of anoxia also have been found in M. edulis (Schlieper, 1957), Anodonta (Culbreth, 1941), and the American oyster (Mitchell, 1912). Helm and Trueman (1967) have observed heart rate acceleration in M. californianus following exposure of three hours. Heart rates returned to submergence values in the relatively short period of ten minutes. All of the authors have related the compensatory rate increase to the relative absence of oxygen during an exposure period. Morton, et al. (1957), however, has found that Lasaea resumed normal oxygen consumption rates immediately following reimmersion.

The greater compensatory response in oxygen consumption found in the high-level mussels (Figures 1 and 2) probably indicates a greater total oxygen deficit. Since a higher rate of metabolism (compared to low-level mussels) was observed in the pre-exposure respiratory values for the high-level animals, this is not altogether surprising. The accumulation of lactate was also somewhat greater in the tissues of high-level animals.

As the tide recedes mussels have the ability to trap water in the mantle cavity. This water may serve as a store of oxygen for respiratory activities during periods of exposure. The oxygen store, however, would not suffice to allow the animal even minimal respiratory activities during prolonged periods of exposure. Maloeuf

(1937a, b) indicated that the amount of oxygen enclosed within the valves of M. edulis would be utilized in an hour or less, although no data was given. Similar results were reported by Lent (1968) in Modiolus but no time periods were cited.

The results of this investigation indicated that the high-level mussels maintained a higher oxygen concentration within the mantle cavity fluid, compared to the low-level forms (Figure 7). From the few samples taken from mussels in the collecting area, the high-level mussels again showed a higher content of dissolved oxygen than did the low-level animals (see insert to Figure 7). Tentatively, then, we suggest there is a lower rate of oxygen withdrawal from the mantle fluid in the high-level mussels, which is also consistent with the greater compensatory rise following exposure seen in these animals.

The comparison of dissolved oxygen contents of laboratory and habitat exposed animals shows a rather large discrepancy. It has been observed that mussels attached in the intertidal area periodically separate their valves. The significance of this behavior in M. californianus was recently compared with the activities of Balanus balanoides by Helm and Trueman (1967). They have also observed the persistence of a film of water covering the folds of the mantle and periostracum for more than three hours under conditions of laboratory exposure. This would be analogous to the micropylar opening of the opercular valves in B. balanoides, which is known to utilize atmospheric

oxygen (Barnes, Finlayson, and Piatigorsky, 1963). Lent (1968) also reported that the oxygen tension in the mantle chamber did not fall below 10 mmHg in Modiolus when exposed for prolonged periods (no time periods were given).

The author has seen similar behavior of mussels at the collecting site. Temporary gaping may allow for diffusion of atmospheric oxygen into the mantle fluid thus at least partially explaining the oxygen concentration differences found in the field and in the laboratory studies where the valves were kept tightly closed.

Studies on anaerobiosis in lamellibranchs initially concerned the determination of the amount of carbon dioxide produced and only recently have there been attempts to identify the end products of anaerobic metabolism. Early experiments indicated that carbon dioxide was accumulated but buffered by alkaline reserves represented mainly by the calcareous shell (Collip, 1920, 1921; Maloeuf, 1937a; Culbreth, 1941). Studies undertaken recently, particularly on oyster mantle tissue (Hammen, 1959, 1966; Simpson and Awapara, 1966) have shown an accumulation of succinate rather than lactate during anaerobiosis. The apparent mechanism for this conversion of glucose to succinate has been reviewed by Simpson and Awapara (1966) and Awapara and Simpson (1967).

The results of the present study indicate relatively little accumulation of lactate in whole animal homogenates of M. californianus

during exposure. Von Brand, et al. (1950, 1953, 1955) recorded much higher values in freshwater snails following anoxic stress. The high-level animals accumulate more lactate in the tissues than the low-level forms (Figure 9) which is consistent with the hypothesis that the high-level forms show a greater capacity for anaerobiosis during exposure. The difference between the two groups is not nearly as great as that shown for vertically separated populations of intertidal barnacles (Barnes, et al., 1957, 1963; Augenfeld, 1967). Two hours after reimmersion the low-level mussels exhibited a rise in tissue lactate to a level greater than that seen following twelve hours of exposure (Figure 9). This was also seen in the high-level mussels although the increase was not as great. It is suggested that this may indicate the rapid oxidation of some anaerobic end product to lactate, thus freeing the coenzyme to another biochemical pathway. A similar condition to my knowledge has not been reported, nor is there sufficient evidence in this investigation to comment on what the end product might be or on the eventual fate of the lactate.

The accumulation of lactate in the mantle cavity fluid after six hours of exposure (Figure 9) cannot be easily explained. For the acid to be detected in this fluid, it must presumably have been eliminated by the tissues. If this were the case, the amount should have remained high in the mantle cavity fluid as the exposure period lengthened. A comparatively high concentration of lactate was

detected in the fluid, and it seems doubtful that a reserve could buffer this quantity of acid so rapidly. Dugal and Irving (1937) found that Venus produced lactate which was buffered by calcium salts from the shell, but no data was presented to indicate the fate of this bound acid.

Weight Loss as a Function of Exposure

If percent weight loss is expressed as a function of time, the rate of weight loss was greatest at time zero and decreased logarithmically with time (Figure 8). Lent (1968) found this relationship in Modiolus to be dependent upon both temperature and relative humidity, with the higher temperature, lower humidity conditions resulting in less percent weight loss. It has been observed that the low-tide animals are less resistant to conditions of desiccation than the high-tide animals (Broekhuysen, 1942; Morton, et al., 1957), as one would expect.

The present investigation has demonstrated a greater percent weight loss in the high level as compared to the low-level mussels, at all temperatures measured. Apparently, in contrast to previous studies, the low-level animals have at least as good, if not better, ability to close their valves tightly. With the tightly closed valves, the animal is again subjected to lack of oxygen. This paradoxical situation may result in the high-level forms regulating their respiratory activities by gaping and allowing desiccation to occur down to a

critical weight loss. After this point, the valves may close to prevent further dehydration in spite of a decreased availability of oxygen. M. californianus has been shown by Helm and Trueman (1967) to decrease its heart rate upon exposure but remain opened for periods of up to three hours. After this time, the valves are closed and the heart rate drops drastically.

The low-level mussels demonstrated a reduced rate of weight loss at higher temperatures. This was not shown in the high-level forms, where the two weight loss curves essentially overlapped. At 18°C which corresponds to an overcast, exposed day in the intertidal zone, the weight loss in low-level forms was considerably less than at 10°C. This may be of adaptive significance because it would allow the mussel to maintain more tissue and mantle cavity fluid during exposure. With more fluid available, the diffusion of oxygen is more likely to take place.

Shell Weight and Intershell Volume

The valves of the mussel serve as a barrier between the soft-body tissues and the desiccating environment outside. A number of workers have correlated the ability to resist desiccation and the weight or volume of the shell. Early work by Russell (1907) and Orton (1929) found that the limpet, Patella vulgata, had thicker, heavier, and higher spired shells the higher in the intertidal zone

they were collected. Segal (1956a) found this to be true in vertically separated Acamaea limatula, although the higher limpets had smaller shell volumes. Rao (1953b) working with high, low, and sub-littoral M. californianus and M. edulis, found that the heaviest shells for any given weight of soft tissues occurred in the lower mussels. This deviation from previous studies was explained by Rao as based on the total time of submergence; the lower level mussels were submerged for longer periods of time so they could accumulate more calcium necessary for increasing shell weight. Baird and Drinnan (1957), studying M. edulis, however, have found a higher shell-to-meat ratio for higher intertidal mussels.

The results obtained in this investigation (Figure 10) are similar to those reported by Baird and Drinnan (1957). Heavier shell weights are adaptive to greater desiccation resistance which is necessary for those animals exposed for greater time periods. The factors that affect shell thickness are light, wave action, and exposure to air (Baird and Drinnan, 1957) all of which are more prevalent in the higher level mussels.

Our results also indicated a greater intershell volume in the high-level mussels (Figure 11). A greater shell volume would enable the mussel to retain a greater quantity of mantle cavity fluid, and this might partially explain the higher levels of dissolved oxygen maintained by the high-level mussels; that is, if equal amounts of

oxygen are retained and consumed by a mussel, the mussel with the largest volume of fluid would show the slower rate of oxygen depletion.

Conclusions

In conclusion, the results of this investigation indicate that one consequence of high-tide existence in M. californianus as compared to low-tide, is the maintenance of a greater metabolic activity. Baird and Drinnan (1957) concluded that mussels which are exposed to air to the greatest extent are obligated to expend more energy to maintain basal metabolism while not feeding. This is felt to be one of the limiting factors for Mytilus and may be responsible for the small animal size in the extreme high edge of their intertidal zone. A higher rate of respiration while submerged was demonstrated for the high-tide forms, as well as a greater accumulation of lactate during exposure. The high-level forms also showed a greater compensatory respiration rate following exposure. These findings suggest a greater capacity for anaerobiosis in the high-level mussels.

SUMMARY

1. The respiratory responses of vertically separated Mytilus californianus from Yaquina Head, Newport, Oregon were measured.
2. Pre-exposed (submerged) rates of oxygen consumption indicated that the high-level mussels maintained a significantly higher respiratory rate as compared to the low-level forms. Values of 347.2 μlO_2/gm dry wt/hour were obtained for the high and 261.0 μlO_2/gm dry wt/hour for the low-tide mussels, at $10^{o}C$.
3. Determination of the influence of oxygen tension on oxygen consumption revealed that this species is a metabolic regulator. The critical oxygen tension for the high-level mussels was 24 to 34 mmHg and for the low-level mussels 38 to 52 mmHg.
4. Post-exposure rates of oxygen consumption following six and twelve hour exposure periods indicated that the high-level mussels compensate to a greater extent than do the low tide forms. The degree of compensation was directly related to the time of exposure only for the high-level animals. The period of compensation was 30 to 35 minutes following both exposure periods.
5. The oxygen depletion from the mantle cavity fluid was reduced in the high-level mussels as compared to low, but this difference was not significant. Large discrepencies in values between

laboratory and field exposure periods were found, and the possible utilization of atmospheric oxygen by animals in the field was discussed.

6. Tissue lactate levels were found to be relatively low and there was relatively little accumulation during twelve hours of exposure. High-level mussels accumulated more than low-level. Both groups of mussels showed a higher tissue lactate content following reimmersion than following twelve hours of exposure, and the possible significance of this is discussed.

7. Percent weight loss as a function of exposure time was found to depend upon intertidal height and temperature.

8. Intershell volume and shell weight were found to be greater in the high-level animals at all tissue weights measured. The adaptive significance of this was correlated with the vertical separation of the mussels.

9. An attempt was made to correlate intertidal distribution with the respiratory responses of M. californianus. There is an indication that one of the consequences of high intertidal existence is a higher metabolism during periods of submergence and a greater capacity for anaerobiosis during exposure.

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