THE EFFECT OF SALINITY, TEMPERATURE, SEASON AND INTERTIDAL HEIGHT ON CALCIUM UPTAKE BY *MYTILUS EDULIS* (LINNAEUS)

by

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Bachelor of Science
(University of British Columbia, 1977)

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
in
THE FACULTY OF GRADUATE STUDIES
(Department of Zoology)

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA
June 1982

Department of ZOOLOGY

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Date 29 JUN 82

ABSTRACT

This study has shown that season, salinity, temperature and intertidal height all affect the rate up calcium uptake by mussels. For summer-adapted mussels, calcium uptake was found to be temperature dependent over the range of acute temperatures measured (1°-23°C). When subjected to a range of salinities over a three week period, summer-adapted mussels showed calcium-uptake rates which were salinity dependent from 25%-75% SW, and which did not show any increase in uptake rate in salinities greater than 75% SW. For winter-adapted mussels, calcium uptake was temperature independent over a temperature range from 5°-17°C. At higher and lower temperatures, uptake was reduced. When subjected to a range of salinities over a three-week period, winter-adapted mussels were also unable to compensate for the lower concentration of calcium in the seawater, and did not show any increase in the uptake rate in salinities greater than 75% SW.

It was found that high and low intertidal mussels had different calcium uptake rates, and that transplantation could alter the uptake rate of transplanted mussels to the uptake rate of untransplanted controls. In the intertidal zone a gradient of shell size was found, which could be associated with the change in uptake range over the intertidal range. Differences in immersion time between the two sites could not explain all of the differences in uptake rate, but high intertidal mussels were found to have less total dry weight of soft parts than low mussels, and correcting for this difference accounted for the

the remainder of the difference in calcium-uptake rate between the two sites.

The soft parts of the mussel were found to become saturated with ^{45}Ca after four hours, while the shell accumulated calcium for the duration of the experiment. The mantle and gill tissue held the same amount of calcium when corrected for differences in weight, while the viscera held a greater pool of calcium. Accounting for real increases in the amount of calcium accumulated by the shell showed that the uptake rates reported in this study are about 59% of the absolute uptake rates.

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ACKNOWLEDGEMENTS

I wish to acknowledge the support of Dr. P.A. Dehnel during the course of this research. In addition to supervising my studies, he has given considerable time and attention to the preparation of this manuscript. I would also like to acknowledge the assistance of Drs. T.H. Carefoot and J.M. Gosline for their advice and criticism during my research, and the valuable help of Dr. David Zittin and the staff of the Biology Data Centre during data analysis and the preparation of this manuscript. I also wish to thank Wendy Hird for her continual support through the course of this study. Finally, I acknowledge the generosity of the Royal Vancouver Yacht Club for allowing me to use their wharf for establishing my intertidal trays, and the Vancouver Public Aquarium for supplying seawater. This research was made possible by a 1980 Summer Graduate Fellowship from the Department of Zoology, and by an NSERC grant (67-7088) to Dr. P.A. Dehnel.

INTRODUCTION

The study of ion transport and ionic and osmotic regulation in freshwater and marine molluscs is well documented (Potts 1954; Little 1965; Pierce 1970; Pierce and Greenberg 1971; Greenaway 1971a, 1971b; Shumway 1977a; Shumway *et al.* 1977). Typically, marine bivalve molluscs maintain their blood in osmotic equilibrium with their surrounding media (Segal and Dehnel 1962), but this does not mean that each blood ion species is in equilibrium with its external counterpart. Significant differences in the internal concentrations of potassium, calcium and carbonate ions are common among marine molluscs (Potts 1954), but other than these three ions, the blood composition is similar to that of seawater. In addition, marine molluscs have been shown to excrete intracellular free amino acids, notably glycine and taurine, in response to osmotic stress (Pierce 1970; Hoyaux *et al.* 1976). This reaction is thought to reduce ion losses by reducing the intracellular osmotic pressure which is contributed by free amino acids, thus, reducing the total osmotic gradient.

Comparisons of the blood ions of marine and freshwater molluscs by Potts (1954) (*Mytilus edulis* and *Anodonta cygnea*) and Chaisemartin *et al.* (1969) (*Margaritifera* and *Lymnaea*) have demonstrated that freshwater molluscs display remarkable differences in blood ion concentrations, when compared to marine molluscs. Blood ions of freshwater molluscs are hyperionic to the corresponding external ions for sodium, potassium, calcium, magnesium, chloride, sulphate, carbon dioxide and phosphate.

Thus, although freshwater molluscs maintain blood ionic and osmotic concentrations lower than marine molluscs, the animals have a demonstrated ability to maintain an ionic gradient between themselves and their environment.

Of the blood ions, calcium is of particular importance with respect to the function of nerve and muscle cells. A low concentration of intracellular calcium is essential to maintain a balance of sodium and potassium ions in squid neurons (Hodgkin and Keynes 1957). Recent studies point to the importance of calcium in the response of bursting pacemaker neurons in Aplysia (Barker and Gainer 1973; Johnston 1976). When intracellular calcium concentrations are reduced experimentally, the cell membrane becomes less able to maintain ionic and electric gradients necessary for normal function. Calcium is also necessary to couple actin and myosin fibrils during striated muscle contraction (Szent-Györgyi 1975). In addition to the other constituents of seawater and hemolymph, calcium plays a part in the process of osmoregulation (Pierce and Greenberg 1971; Shumway 1977a, 1977b; Shumway et al. 1977). Pierce and Greenberg (1971) have shown that when the blood calcium levels of Mytilus are lowered below those found in the blood of mussels in full strength seawater, the mantle tissue becomes less able to withstand osmotic stress. Kirschner (1963) believes that lowered blood calcium concentration causes the mantle of clams (no species given) to become leaky with respect to the transport of sodium and potassium, and interferes with its ability to maintain an electric gradient.

Besides its importance in cellular physiology, calcium is mineralized by animals from every invertebrate phylum (Lowenstam 1981). Among the marine invertebrates there are three major phyla which depend upon calcium carbonate for their exoskeleton: the scleracterian coelenterates (Goreau 1959), the crustacean arthropods (Robertson 1937), and the molluscs (Wilbur and Jodrey 1952). Among the molluscs and coelenterates the precipitation of calcium carbonate is a continuous process which occurs throughout the life of the organism. Crustaceans secrete a new exoskeleton following periodic molting (Travis 1955; McWhinnie *et al*. 1969). Prior to the molt, calcium is dissolved from the integument and held in the blood and hepatopancreas (Travis 1955). Freshwater crayfish hold calcium carbonate stores in gastroliths located in the cardiac stomach (McWhinnie 1962, Chaisemartin 1965). Following molt, these calcium stores are returned to the new integument.

Two recent studies have investigated the kinetics of calcium transport across the molluscan mantle. Greenaway's study (1971b) of changes in the electric potential across the mantle tissue of the aquatic gastropod *Lymnaea stagnalis* indicate that calcium uptake is active when the external calcium concentration ranges between 0.06 and 0.3 mM. Above this concentration, calcium is accumulated by diffusion along an electrical and chemical gradient. In a related study, Greenaway (1971a) has shown that when the external calcium ion concentration falls below the threshold of uptake of the calcium transport system (0.06 mM), calcium is dissolved from the inner surface of the existing shell to make up for efflux losses. Earlier work (Kirschner _et al_. 1960; Kirschner 1963) also has shown the existence of a calcium dependent potential of 20-70 mV across the mantle of clams (no species given).

Calcium may be absorbed from the external environment by a number of tissues, but in molluscs its principal place of deposition is the shell (Wilbur and Jodrey 1952; Jodrey 1953). It has been known for some time that calcium dissolved in the aquatic medium is taken up by the mantle tissues (Schoffeniels 1951a, 1951b). The gill is also known to play a part in uptake in the marine bivalve _Hyriopsis schlegelii_ (Horiguchi 1958) and in the sea mussel _Mytilus californianus_ (Rao and Goldberg 1954). Both gill and foot may function as temporary storage sites in _Viviparus bengalensis_ (Sen Gupta 1977) and the freshwater bivalve _Cristaria plicata_ (Numanoi 1939). The gastropod _Lymnaea_ has been shown to derive about 20% of its calcium requirements from its food (van der Borght and van Puymbroeck 1966).

Because of the predominance of calcium carbonate deposition in the shell in molluscs, there is considerable literature describing this process. Some studies have focussed on the sites of calcium carbonate secretion and upon the relationship between the existing shell, protein matrix, and newly secreted shell. (Kapur and Gibson 1968; Timmermans 1969; Hirata 1953; Bubel 1973; Sminia _et al_. 1977). Molluscan blood is normally saturated or supersaturated with calcium carbonate (Potts 1954), and it is thought that crystalline precipitation is induced by the conformation of the protein matrix of the existing shell

(Kapur and Gibson 1968; Timmermans 1969). Changes in the pH of the blood are also known either to favour precipitation (under alkaline conditions) or dissolution of the shell (under acidic conditions) (Akberali *et al.* 1977). Anaerobic metabolism is known to occur during the tidal emersion of *Venus mercenaria* (Dugal 1939). During these periods of anaerobic metabolism, calcium carbonate is dissolved from the shell in order to buffer pH changes resulting from anaerobic succinate production and ammonia excretion (Dugal 1939; Akberali *et al.* 1977).

Since most of the calcium absorbed from the environment is laid down as new shell, increases in shell dimension have been used commonly as a parameter for the measurement of molluscan growth. Studies measuring growth by calcium uptake or shell deposition have been made by many workers (Orton 1928; Galtsoff 1934; Newcombe 1935; Fox and Coe 1943; Loosanoff and Nomejko 1949; Wagge 1952; Wilbur and Jodrey 1952; Horiguchi *et al.* 1954; Horiguchi 1958; Bonham 1965; Seed 1968; Zischke *et al.* 1970). Some have noted the seasonal effects of temperature on shell growth (Coulthard 1929; Galtsoff 1934; Loosanoff and Nomejko 1949; Orton 1928). Wilbur and Jodrey (1952) have studied the sites of deposition of calcium carbonate on the shell, and found that newly secreted calcium is concentrated at the posterior and ventral margins of the shell. Two studies have assessed calcium budgets of the bivalve *Hyriopsis schlegelii* (Horiguchi *et al.* 1954; Horiguchi 1958), and *Helix aspersa* (Wagge 1952). Bonham (1965) has used ^{90}Sr deposition from the radioactive fallout of nuclear weapons to measure the rate of growth of the bivalve Tridacna gigas. A study by Marbach and Wilbur (1973) has examined the effects of a changing light regime on the daily deposition of calcium carbonate by the limpet Patella rota. Dehnel (1956) has measured the growth of mussels and found significant differences in the rates of growth between southern and northern populations, and between and high and low intertidal populations of Mytilus californianus. His study demonstrated that high intertidal mussels have lower growth rates than low intertidal mussels, and that mussels from northern and southern locations grow at similar rates in spite of temperature differences between the two localities. Newcombe (1935) and Seed (1968) also found that intertidal height and the density of the community affect the rate of shell growth. In spite of the number of studies on growth and calcium metabolism, I have been unable to locate any study which has systematically examined the effect of altered environmental conditions upon calcium uptake in molluscs. There are, however, systematic studies of other parameters as they affect various metabolic functions; for example, the effect of temperature on oxygen consumption (Widdows 1973) and osmoregulation in Mytilus edulis (McLachlan and Erasmus 1974).

The purpose of this study was to examine the effect of salinity, temperature, intertidal height and season upon the uptake rate of calcium by the bay mussel Mytilus edulis (Linnaeus), the genus being common to the intertidal region of coasts throughout the world (Soot-Ryen 1955). Since the Vancouver Harbour study area is subject to seasonal variations in salinity, this study has examined the effect of long-term decreased and increased salinities upon summer- and winter-adapted mussels. This was done to determine whether the calcium-uptake capabilities of mussels were altered with respect to salinity in the summer and winter environment. The temperature of subsurface water (1.0 meter) at the field site in Vancouver harbour was found to vary between 2° and 20°C between January and August, respectively. It seems likely that both acute and seasonal changes in temperature may result in changes in calcium uptake, a finding which Loosanoff and Nomejko (1949) reported for <u>Ostrea edulis</u>, and which Coulthard (1929) reported for <u>Mytilus edulis</u>. The present study determined the effect of acute temperature-changes upon the uptake rate of summer- and winter-adapted mussels. Because of the wide intertidal distribution of <u>Mytilus</u>, this study further examined the calcium-uptake rate of transplanted and untransplanted mussels from the high and low intertidal sites. Ecological factors have been implicated as the cause of intertidal size gradients (Vermeij 1972; Paine 1976; Bertness 1977). It seems likely that differences in the length of immersion would result in an intertidal size gradient, but it is not known whether the reduction of immersion time affects the growth of the shell and the soft parts equally. It is possible that at increased intertidal heights the proportions of the soft parts may differ in order to maximize calcium retrieval from the environment. Differences between high and low intertidal populations are examined in a study of the size distribution of <u>Mytilus edulis</u>

in the intertidal zone. A comparison of the soft part weights and shell weights of high and low intertidal mussels was also made. The final experiment of this study examined the transport of calcium into the soft parts and shell over a 32 hour period. In all of these experiments the calcium-uptake rate is calculated as the mean hourly uptake rate over a 24 hour period. The potential effects of diurnal or tidal rhythms, such as those reported in the limpet Patella rota (Marbach and Wilbur 1973), are removed by this method. Calcium uptake is measured by the use of a radioactive isotope, ^{45}Ca, which is used to label the calcium present in seawater. The use of an isotope in the measurement of calcium uptake requires the estimation of two unknown parameters. The first of these values is the amount of unlabelled calcium which resides in the soft parts, and which does not actually contribute to shell growth. The second unknown value is the length of time required for the ratio of labelled/unlabelled calcium in the seawater (the specific activity) to equilibrate in the calcium pools in the soft tissues. Until there is an equilibrium between the external and internal pools, the actual rate of calcium uptake cannot be determined. These two parameters are estimated in a 32 hour time-course study of the passage of calcium into the mantle, gill, viscera and shell.

A review of studies of calcium-uptake rate and shell growth has shown few which accounted for the size of the experimental animals. It is not known a priori whether the calcium-uptake rate is dependent or independent of weight, or whether the uptake rate shifts between weight dependence and independence. Unless uptake can be expressed on a per gram basis, the foundation for comparison between studies is limited. As Zeuthen (1947) pointed out, the lack of consideration of size can often be ascribed to the small size range of the animals used (Wilbur and Jodrey 1952), but some authors seem to be undecided as to the possible effect of size on uptake rate (Loosanoff and Nomejko 1949). This study, therefore, has used a range of animal sizes, allowing the calculation of the regression line of the rate of calcium uptake as a function of total dry weight. The results of this study contribute to the understanding of the patterns of growth of marine molluscs as they are influenced by different physical factors.

MATERIAL AND METHODS

Collecting site

The mussel *Mytilus edulis* (Linnaeus) was collected from the north jetty of the Royal Vancouver Yacht Club, situated east of Spanish Bank in Vancouver harbour. The tidal datum of the site was found by marking the water line of a piling at successive dates and comparing the time and height with the Canadian Hydrographic Survey prediction for Vancouver tides (Anonymous 1979, 1980). Measurements of intertidal position are accurate to 10 cm relative to datum.

Collection of animals

A column of six rectangular plastic trays was assembled and hung from the jetty. The trays were 30 cm long, 23 cm wide, and 12 cm deep. A 5 cm hole in the floor of each tray allowed water to drain out when it was emersed. The trays were suspended in a vertical line with 0.5 m distance between trays. The lowest tray was 0.2 m above the zero datum point. The corners of each tray were fastened by knots in a 1/4 inch polypropylene rope which passed through the corners of all six of the trays. This assembly was suspended at the top by a 1/4 inch polypropylene rope tied to the four lines at the upper tray, and which passed through a pulley to a cleat attached to one of the jetty's pilings. The trays were held taut at the bottom by a similar line which passed down through a submerged pulley, then back up to fasten to another cleat. The submerged pulley was attached to a heavy concrete block. This movable assembly allowed the trays to be hauled out of the water to retrieve samples. Mussels were obtained by transplantation from the pilings into the trays, and covered the floor of each tray, approximately 300-400 mussels to a tray. Care was taken to insure that the mussels which were transplanted from the pilings into trays remained at the same equivalent intertidal height. Spat fall on the trays was also included in experiments. Subtidal animals were taken from about 50 cm below the waterline of mooring floats which were directly adjacent to the trays.

Field measurements

Temperature measurements were made by means of a Tempscribe recorder, and are accurate to ±1°C. Salinities were determined by means of a Buchler-Cotlove chloridometer. Deep-water seawater samples, obtained from the Vancouver Public Aquarium, indicated that the salinity of harbour water at 60 m remained constant at about 480 mM Cl^-/liter. This salinity, which is equivalent to 31.8 parts/thousand, was established as 100% seawater. The millimolar concentrations of the major ions present in 100% seawater (SW), calculated from Barnes (1954), are Cl^-, 480 mM; Na^+, 412 mM; Mg^{+2}, 47 mM; SO_4^{-2}, 25 mM; K^+, 9 mM; Ca^{+2}, 9mM; and CO_3^{-2}, 2 mM. The 9 mM concentration of calcium in 100% SW corresponds to 0.395 grams calcium/liter seawater.

General laboratory methods

Seawater was supplied by the Vancouver Public Aquarium. Glass distilled water was used to dilute this seawater to the experimental salinities below 100% SW. Higher salinities were made by adding the appropriate inorganic salts to 100% seawater (Barnes 1954).

Calcium uptake was measured by the transfer of ^{45}Ca from seawater into the tissues and shell of the animal. Normal dosage was 50 microcuries. The isotope was supplied by Amersham Corp. as carrier-free aqueous $^{45}CaCl_2$ at pH 5-7. The ratio of labelled/unlabelled calcium (specific activity) in 100% SW was 0.0087.

Animals brought from the field were maintained in an environmental chamber with a light:dark regime of 12 hr:12 hr. Control salinities for the summer and winter seasons were established from measurements of the salinity of the environmental seawater 1 meter below the mooring floats. These measurements were made weekly for one month before the mussels were brought to the laboratory. The calculated mean salinities were 47% and 90% SW for the summer and winter seasons respectively. On the basis of these values, the summer and winter control salinities were established as 50% and 100% SW. The winter and summer control temperatures were established as the mean of a two week continuous measurement (determined every three hours) made 1 meter below the mooring floats. On the basis of these values, the summer control temperatures were established as 15°C for 1979 and 17°C for 1980, while the winter control temperature was 5°C for both years. In the environmental chamber, mussels were held in aerated plastic aquaria identical to the trays used in the field. For experiments which required long-term holding, the water of each aquarium was replaced twice weekly by 5 liters of new water. With the exception of two experiments described later, mussels were not fed.

Before an experiment, each animal was scraped clean and placed in a test dish with 200 ml. of seawater, one mussel to a dish. The sample size for each test was 15 animals, but was made smaller when high mortality occurred within an experimental group. The temperature of the test dishes was maintained by a circulating water bath accurate to ±1°C, and a period of 24 hours was allowed to recover from cleaning and handling. Following the recovery period, the water in each test dish was supplied with 50 microcuries of $^{45}CaCl_2$. The water was stirred, then the mussel was left undisturbed and allowed to take up the isotope for 24 hours. After the 24 hour period, all the mussels were rinsed in clean seawater and frozen. Later, the animals were thawed, the shell and soft parts dissected and dried at 100°C for 24 hours. Following this they were weighed to an accuracy of ±0.1 mg, and the presence of radioactive calcium measured by means of a Nuclear-Chicago planchette proportional counter. Details of the counting procedure are given in the Treatment Of Data And Statistical Analysis section found at the end of the Material and Methods. Contaminated seawater left after each experiment was diluted to prescribed concentrations and disposed of by drain.

1. Salinity experiments

The purpose of this experiment was to determine the calcium-uptake rate of mussels exposed to long-term changes in salinity. Subtidal mussels were removed from the mooring floats during the summer (August 1979) and winter (February 1980) seasons, and maintained as described above. The duration of the experiment was three weeks, and the calcium-uptake rates of groups of mussels were measured at weekly intervals using the methodology described in the General Laboratory Methods section. The experimental salinities used were 25%, 50% (summer control), 75%, 100% (winter control) and 125% SW. The control temperature was 15°C for the summer and 5°C for the winter. Before placing any of the mussels in experimental salinities, a group from the field was placed under control conditions and the initial calcium-uptake rate was measured. This is presented in the Results section as the Day 0 group. During the summer trial, it became apparent that after two days the animals held in the 125% seawater were not opening. This salinity was discarded from the summer trial.

After the experiment was performed, it came to my attention that the Week 0 control salinity (50%) for the August 1979 experiment had been carried out incorrectly. I had erroneously tested the Day 0 group in 100% seawater, instead of 50% seawater. As a result, beginning in December 1980, I began to adapt a group of winter-adapted mussels to summer conditions. This was done by bringing subtidal animals to the laboratory. Their field temperature and salinity were 7°C and 90% at the time they were removed. They were transferred to an environment of 15°C and 50% for 12 days, and maintained on a culture of the diatom Skeletonema costatum at a concentration of 30,000/ml. High mortality after 14 days in these conditions prevented allowing this group a longer period of adaptation. In the Results section, the calcium-uptake rate for the summer-adapted Week 0 control group represents this group of mussels.

2. Acute temperature response experiments

This experiment was designed to determine the acute response of the calcium-uptake rates of summer- and winter-adapted mussels to changes in temperature. Subtidal mussels were removed from the mooring floats during the summer (August 1980) and winter (November 1979) seasons. Mussels were cleaned as described above, then transferred to environmental chambers. The experimental temperatures were 1°, 5° (winter control), 12°, 17° (summer control), and 23°C. While in the environmental chambers, the mussels were held in darkness. The salinities were 100% seawater for the winter and 50% seawater for the summer. Again, it came to my attention in December 1980 that the summer trial had been improperly made. I had erroneously used 100% seawater as the summer control-salinity, instead of 50% seawater. As a result, winter mussels adapted to summer conditions, taken from the Seasonal Salinity Experiment described above, were used to rerun the summer experiment.

3. Intertidal transplant experiment

The purpose of this experiment was to determine the calcium-uptake rates of mussels at different intertidal heights, and to see if the uptake rate was modified by reciprocal transplantation to higher and lower intertidal positions. In October 1979, mussels were removed from the trays in the field located at intertidal heights of 0.2, 1.2 and 2.2 m. Transplants of about 50 mussels were made from each tray into the other two trays: mussels from 0.2 m were transplanted to 1.2 m and 2.2 m; mussels from 1.2 m were transplanted to 0.2 m and 2.2 m; and mussels from 2.2 m were transplanted to 0.2 m and 1.2 m. After 34 days, 15 individuals from each of these nine groups were transported to the laboratory. Three groups were from the untransplanted controls, and six groups were from the transplanted experimental groups. They were cleaned and allowed 24 hours to recover from handling. After the recovery period, the calcium-uptake rate of each of the sample groups was measured according to the methods described in the General Laboratory Methods section.

4. Size gradient study

This study was made to determine whether the size-distribution of Mytilus varied with intertidal height. Tissue weights of mussels taken from the trays at equivalent intertidal heights of 0.2 and 2.2 m were measured to determine whether the proportionate weights of the shell, mantle, gill and viscera remained constant relative to the total dry weight of soft parts between high and low intertidal mussels. The dry weight of the shell and soft parts were determined after 24 hours of drying at 100°C. The volume of the two valves were compared by measuring the length, width and height of individual shells, and then measuring their displacement volume. In this context, shell displacement volume refers to the volume of the shell material of the two valves, and not to the inner shell volume. Shell displacement volume was measured by placing the shell valves in a graduated cylinder, and then measuring the change in volume of butyl alcohol dispensed from a calibrated burette which was required to fill the graduated cylinder. Shells that were too large to fit in the graduated cylinder were broken into pieces. Butyl alcohol was used because of the small miniscus it produced in the graduated cylinder. The volume measurements are accurate to ±0.02 ml.

In January 1979 a study of the vertical distribution of *Mytilus* was made. A 6 cm diameter cable suspended from the jetty and adjacent to the trays was chosen as the collecting site, since it was inaccessible to the mussels' principal predators *Pisaster ochraceous* and *Thais lamellosa*. Beginning at the top of the distribution of *Mytilus* and working down the cable in increments of between 20 and 40 cm vertical distance, all the mussels were removed and returned to the laboratory for measurement. Size measurements accurate to ±0.1 mm were recorded by dial calipers as the greatest distance along the anterior-posterior axis. These values were plotted as histograms at 2 mm increments. Since the mussels were taken from differing lengths of cable, the frequency scale of each histogram was adjusted so that each histogram represented a surface area of 100 square centimeters.

5. Time course uptake study

The purpose of this study was to determine the amount of labelled calcium taken up by the shell, mantle, gill and viscera as a function of time. This permitted the determination of the length of time necessary for the specific activity (ratio of labelled/unlabelled calcium) to equilibrate between the external seawater and the mantle, gill and viscera. It also allowed the calculation of a correction factor between the uptake rates given in this study, and the absolute uptake rates.

In February 1981, 50 subtidal mussels were removed from the mooring floats and transported to the laboratory. They were cleaned and allowed a recovery period as described in the General Laboratory Methods section, and held at winter control conditions (5°C; 100% SW). After the recovery period, all the mussels received a standard dose of isotope. Beginning one hour later, 10 mussels were removed and frozen. After that, at 2, 4, 8, 16 and 32 hours, 10 mussels at each time interval were removed, rinsed in clean seawater and frozen. Later they were thawed, and the shell, mantle, gill and viscera dissected. The shell and tissues were dried and weighed, and the calcium isotope present in the shell and each of the tissues measured.

Data analysis

The measurement of radioactivity, expressed as disintegrations/minute by the proportional counter, was recalculated as microcuries of ^{45}Ca. This was done by measuring the disintegration rate of standards of known activity, and then determining the equation which described the relationship between the real rate of disintegration (determined by the half-life of the isotope), and the less efficient rate which the proportional counter measured. This calculation did not correct for self-absorption by the samples. An attempt was made to spread out the soft parts before drying, rendering them as thin as possible after drying. It was then assumed that the drying of the tissues made them thin enough to reduce the effects of self-absorption. Preliminary experiments showed that after 24 hours, 75-95% of the radioactivity was found in the shell. Since this newly deposited calcium would be present on the inner surface of the shell, the surface which was counted, the effect of self-absorption in the shell would be negligible. Radioactivity measurements underwent further adjustment to compensate for differences in the calcium content of seawater of different salinities, differences in the duration of the experiments, differences in isotope dosage, and isotope decay. After these corrections, the final result was expressed as μg calcium/gram total dry weight/hour. Total dry weight refers to the combined dry weight of the shells and dry weight of soft parts. For each experiment, the regression: $\log_{10}$(μg calcium uptake/gram total dry weight/hour) was plotted as a function of

$\log_{10}$(grams total dry weight). By this method, the slope of the regression line corresponds to a rate constant for each experimental group, and the Y-intercept corresponds to the logarithm of the calcium uptake rate of a 1.0 gram total dry weight mussel. The use of 1.0 gram total dry weight mussels when making comparisons between experiments is arbitrary, and based upon the simplicity of deriving the uptake rate from the Y-intercept of the regression. In those experiments which showed low slopes, the use of this weight made little difference to the uptake rate. However, in those experiments which showed large negative slopes (typically during the summer), small mussels, because of their higher relative surface area, showed higher calcium-uptake rates than large mussels. This peculiarity must be borne in mind when comparing seasonal differences in uptake rate. The null hypothesis for each statistical comparison of experiments was that there was no difference between the slopes or intercepts of the regression lines. Analyses of covariance between experiments were made by a PDP 11/45 computer, and were considered to be significantly different for Alpha less than 0.05. Therefore, in the context of comparisons between experiments, the use of the term significant difference indicates that the analysis of covariance resulted in an F-test value which rejected the null hypothesis when the probability of common variance between sets of compared data was less than 5%. In those instances where the analysis of covariance revealed that the slopes of the lines were not significantly different from one another, comparisons of the Y-intercept were made.

These comparisons were also considered significant for Alpha less than 0.05.

RESULTS

Salinity experiments

Summer experiment (August 1979)

The purpose of this experiment was to observe the response of summer-adapted mussels to changes in salinity at a control temperature of 15°C. Measurement of the calcium-uptake rate of mussels held at 50% SW (control salinity) was made initially (Day 0). Thereafter, the calcium-uptake rate was measured in 25%, 50%, 75% and 100% SW at 7, 14 and 21 days. At each weekly interval and at each experimental salinity the regression line of calcium uptake as a function of total dry weight was calculated. The regression lines calculated for the summer experiment are found in Figures 1-3. Figure 1 shows the regression line and data points for the control salinity at Day 0 and Day 7. Figures 2 and 3 show the regression lines for each of the experimental salinities at Day 14 and 21. In Figure 1, the data points for each salinity is shown, while Figures 2 and 3 show only the data points for the control salinity.

When the results of the control group (50% SW) shown in Figures 1-3 are considered, there is no significant change in the intercept value of the regression line of the control group between Figures 1 through 3. That is, there is no significant change in the calcium-uptake rate of a mussel of 1.0 gram total dry weight during the three-week experiment. However, the control group does show a significant change in slope between

Figure 1. Day 7 summer-adapted regression lines of calcium uptake/gram total dry weight/hour as a function of total dry weight at 25%, 50%, 75% and 100% SW. Individual measurements of the salinities are marked as follows: 25% SW, (o); 50% SW, (•); 75% SW, (□); 100% SW, (■). The equations of the lines are:

25% SW; $\log Y = -1.129 \log X + 0.717$ (n=10)
50% SW; $\log Y = -0.916 \log X + 1.124$ (n=15)
75% SW; $\log Y = -1.126 \log X + 1.524$ (n=15)
100% SW; $\log Y = -1.063 \log X + 1.622$ (n=15)

The summer-adapted regression line for the Day 0 control salinity (15°C, 50% SW) is shown for comparison. Individual measurements are indicated by (Δ). The equation of this line is:

50% SW; $\log Y = -0.150 \log X + 0.982$ n=10

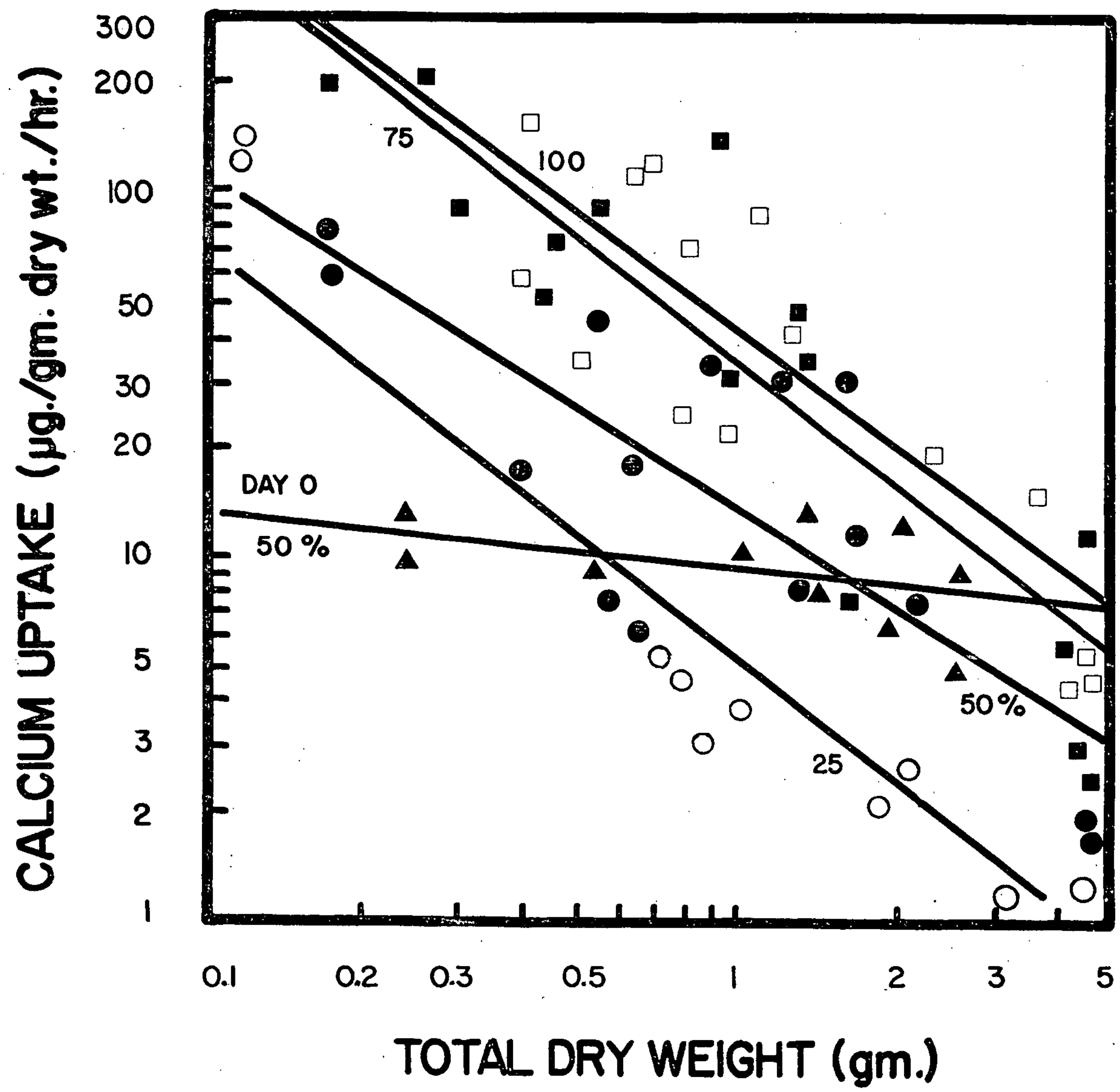

300
200
100
50
30
20
10
5
3
2
1
CALCIUM UPTAKE (µg./gm. dry wt./hr.)
75
100
DAY 0
50 %
50%
25
0.1
0.2
0.3
0.5
1
2
3
5
TOTAL DRY WEIGHT (gm.)

Figure 2. Day 14 summer-adapted regression lines of calcium uptake/gram dry weight/hour as a function of total dry weight at 25%, 50%, 75% and 100% SW. Individual measurements of the control salinity (50% SW) are marked by (•). The summer-adapted regression line for the Day 0 control salinity is included for comparison. The equations of the lines are:

25% SW; $\log Y = -0.928 \log X + 0.622$ n=9
50% SW; $\log Y = -1.101 \log X + 0.901$ n=15
75% SW; $\log Y = -0.812 \log X + 0.966$ n=15
100% SW; $\log Y = -1.063 \log X + 1.506$ n=15

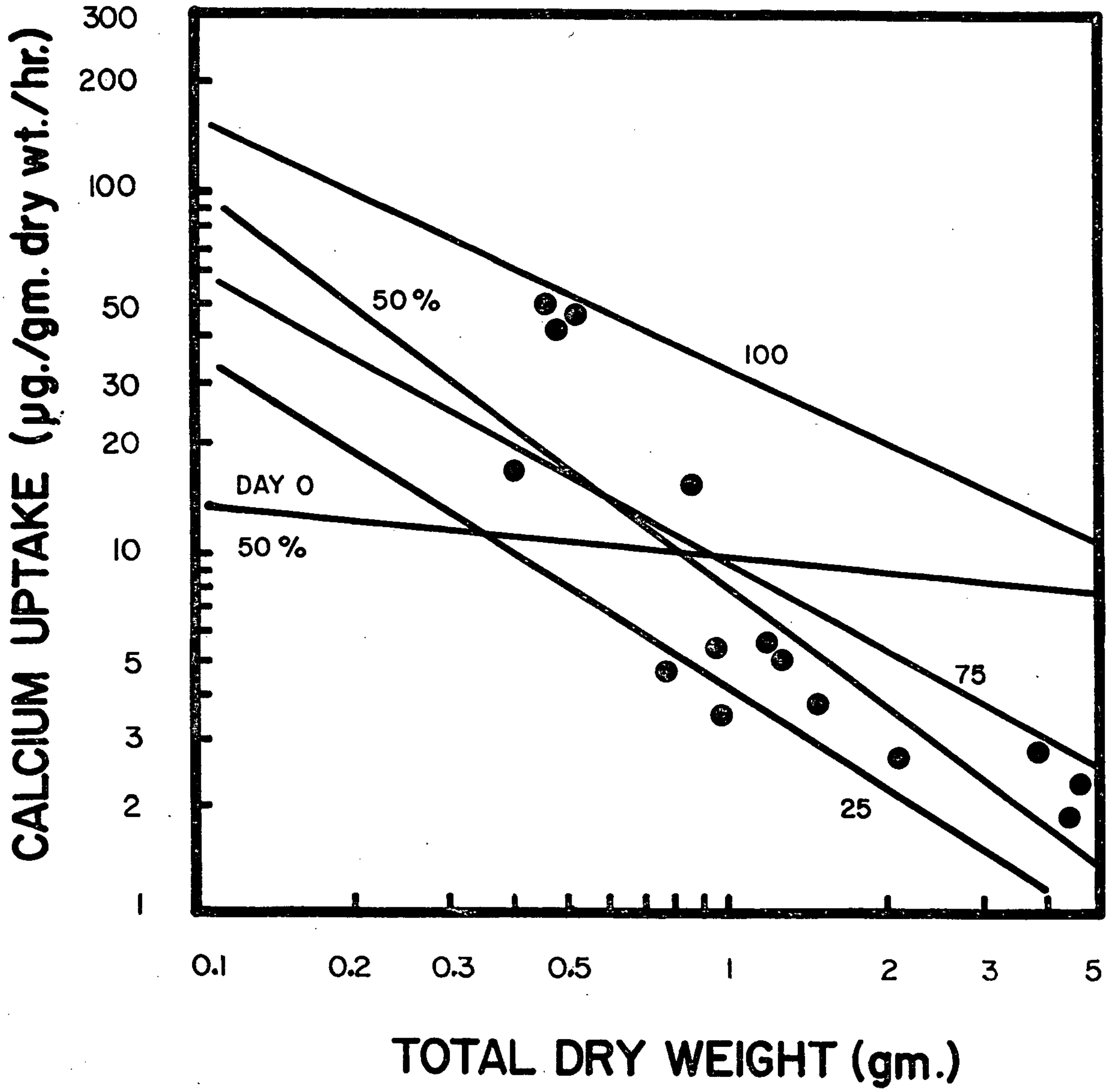
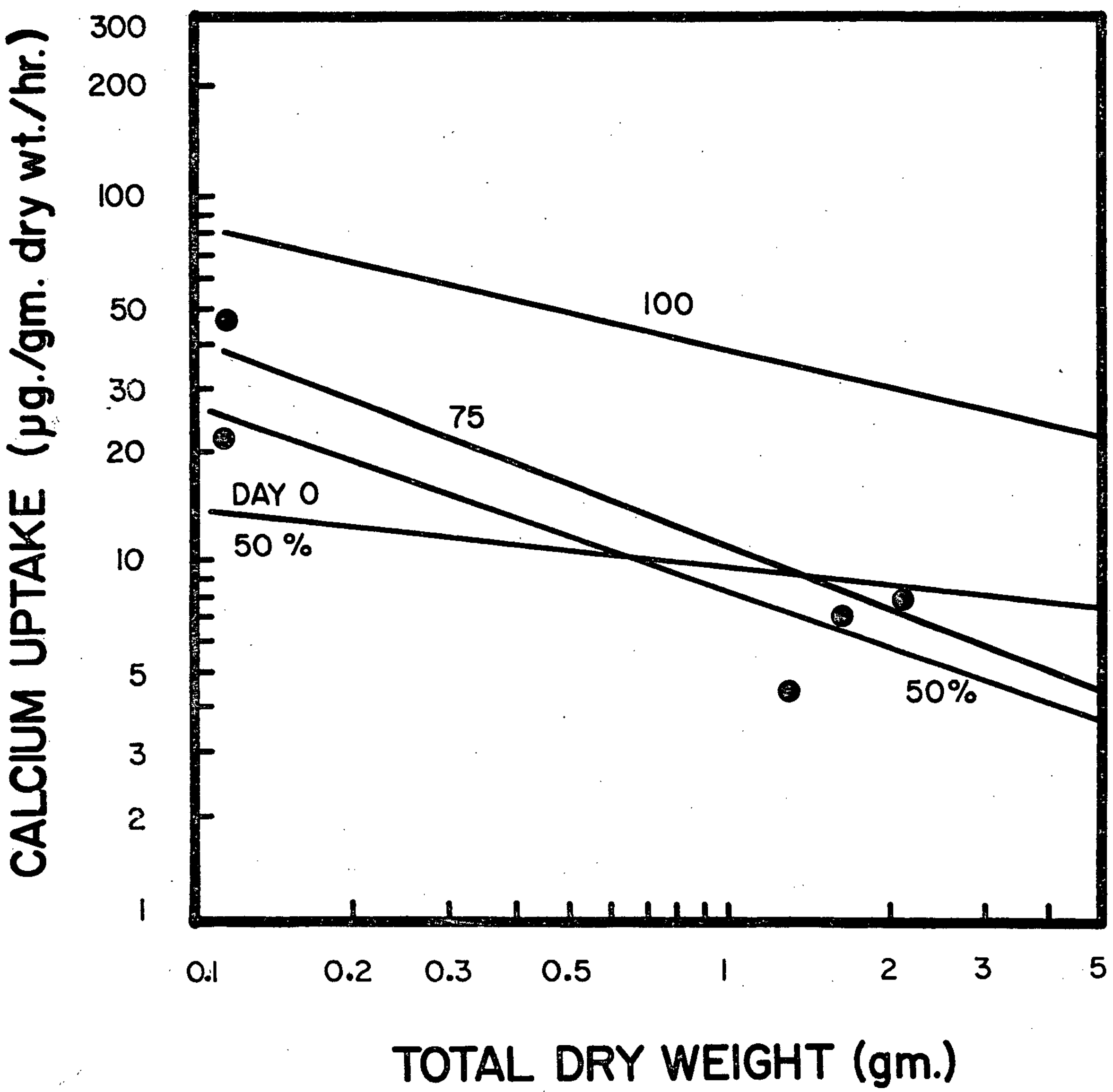

300
200
100
50
30
20
10
5
3
2
1
CALCIUM UPTAKE (µg./gm. dry wt./hr.)
50 %
100
DAY 0
50 %
75
25
0.1
0.2
0.3
0.5
1
2
3
5
TOTAL DRY WEIGHT (gm.)

Figure 3. Day 21 summer-adapted regression lines of calcium uptake/gram dry weight/hour as a function of total dry weight at 50%, 75% and 100% SW. Individual measurements of the control salinity (50%) are marked by (•). The summer adapted regression line for the Day 0 control salinity is included for comparison. The equations of the lines are:

50% SW; $\log Y = -0.524 \log X + 0.912$ n=5
75% SW; $\log Y = -0.579 \log X + 1.032$ n=11
100% SW; $\log Y = -0.349 \log X + 1.578$ n=15


CALCIUM UPTAKE (µg./gm. dry wt./hr.)
300
200
100
50
30
20
10
5
3
2
1
100
75
DAY 0
50 %
50%
0.1
0.2
0.3
0.5
1
2
3
5
TOTAL DRY WEIGHT (gm.)

Day 0 and Day 7. The slope of the line increases from -0.15 to -0.92, as shown in Figure 1. This difference is discussed later.

At Day 7 no significant differences occur among the slopes of the regression lines of the experimental salinities shown in Figure 1. The mean slope of the experimental salinities is -1.06. However, there are significant differences between the intercepts of some of the regression lines. The intercepts of the 25%, 50% and 75% SW groups in Figure 1 are all different from one another, while the intercept of the 100% SW group is not different from the intercept of the 75% SW group. Since the intercepts of the regression lines show differences while the slopes show no such differences, it is apparent that the mechanism of uptake is similar in all cases (hence the same slope), and that the actual rate of calcium uptake (the Y-intercept value) is dependent upon the external salinity. Thus, there is a direct relationship between the external salinity and the uptake rate, as the differences between the 25%, 50% and 75% SW groups show. Since there is no difference between the 75% and 100% SW groups, it may be that the uptake mechanism becomes saturated with respect to calcium in salinities greater than 75% SW.

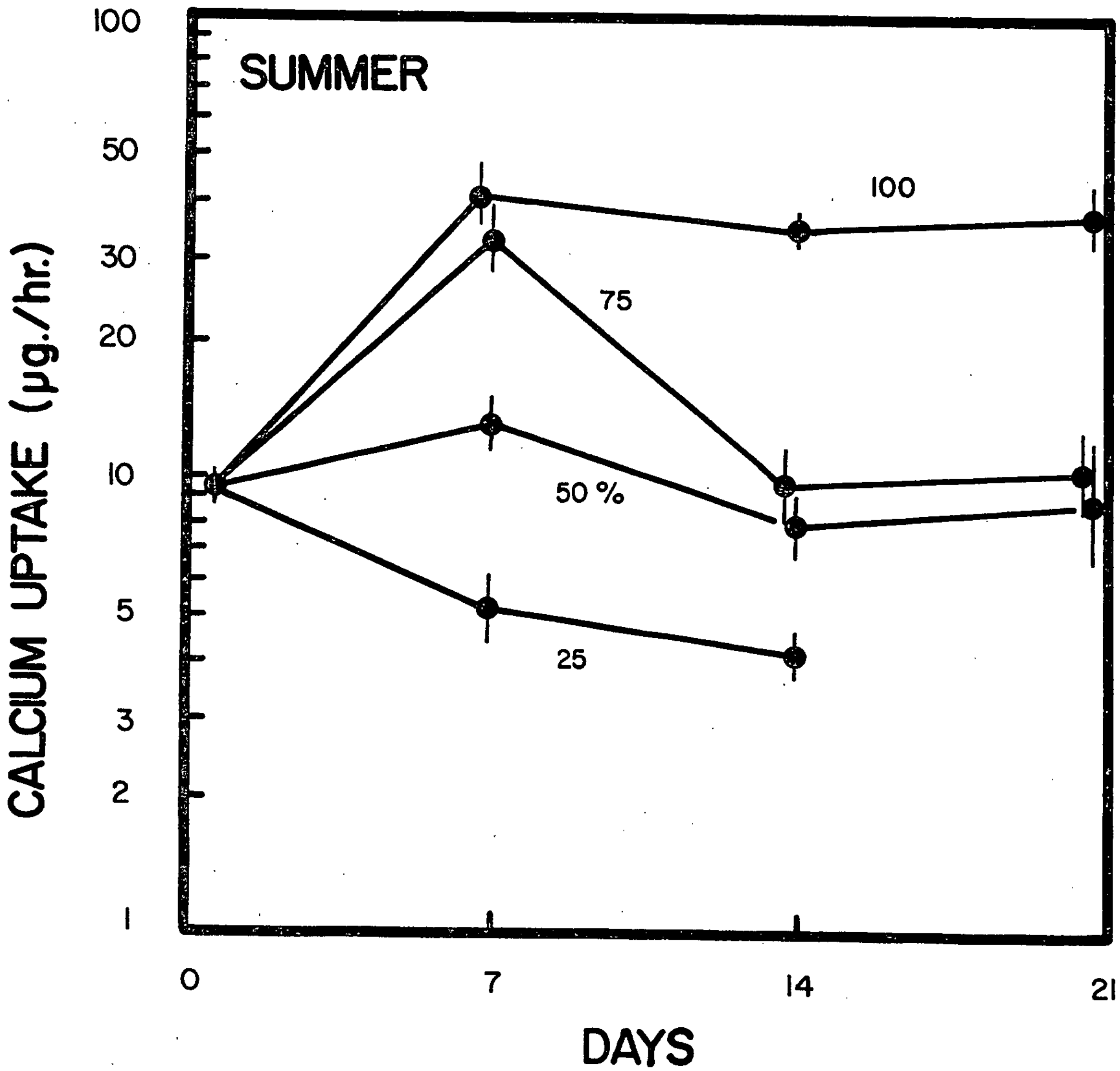
At Day 14 (Fig. 2), mussels in 25%, 50% and 100% SW show no significant change in either the slope or the intercept of the regression line, when compared with experiments made at the same salinity the previous week (Fig. 1). The mean value for the slopes of the regression lines in Figure 2 is -0.87. Mussels in 75% SW show no significant change in slope either, but do show a significant decrease in the intercept, indicating a decrease in the calcium-uptake rate. The decrease in calcium-uptake rate in the 75% SW group could be attributed to continued osmotic stress, which is discussed later.

At Day 21, the remaining experimental groups shown by the regression lines of Figure 3 show no significant change in either slope or intercept, when compared with the experimental groups of the same salinity shown in Figure 2. That is, the 50% and 75% SW groups are not different from one another, while the intercept of the 100% SW group is greater than the control group.

In addition to the results just described, all the mussels in 25% seawater died after Day 14. This was not surprising, since they had shown the highest mortality rate during the first two weeks. In fact, there seemed to be a correlation between the salinity and mortality rate. Although I did not record mortality data, I noticed a continuous high mortality among the 25% SW group, and virtually no mortality among the 100% SW mussels.

The intercepts of the regression lines of each of the experimental salinities shown in Figures 1-3 have been used to plot Figure 4, which shows the calcium-uptake rate of a summer-adapted 1.0 gram total dry weight mussel at each experimental salinity as a function of time. Figure 4 demonstrates that during the summer the calcium-uptake rate is correlated with salinity and that this correlation is evident after 7 days. Figure 4 also shows that summer-adapted mussels are not able to raise their calcium-uptake rates to compensate

Figure 4. The calcium-uptake rate of summer-adapted mussels of 1.0 gram total dry weight expressed as a function of time. The control temperature and salinity are 15°C and 50% SW, experimental salinities are 25%, 75% and 100% SW. Vertical bars on the figure indicate ±1 S.E. about the mean of each point.


SUMMER
100
75
50 %
25
100
50
30
20
10
5
3
2
1
CALCIUM UPTAKE (µg./hr.)
0
7
14
21
DAYS

for lower salinities. Finally, the figure shows that mussels in 75% SW are not able to maintain a calcium-uptake rate intermediate between the 50% and 100% SW groups, since at 14 days the uptake rate decreases to the rate of the control group (50% SW).

Winter experiment (February 1980)

The purpose of this experiment was to observe the response of winter-adapted mussels to changes in salinity at a control temperature of 5°C. Measurement of the calcium-uptake rate of mussels held at the control salinity (100% SW) was made initially (Day 0). Thereafter, the calcium-uptake rate of mussels held at 25%, 50%, 75%, 100% and 125% SW was measured at 7, 14 and 21 days. At each weekly interval and at each experimental salinity the regression line for calcium uptake as a function of total dry weight was calculated. The regression lines calculated for the winter experiment are shown in Figures 5-7. Figure 5 shows the regression line and data points for the control salinity at Day 0 and at Day 7. Figures 6 and 7 show the regression lines for the Day 0 control salinity and for each of the experimental salinities at Day 14 and 21. In Figures 6 and 7 only the data points for the control group (100% SW) are shown.

The mussels of the control group (100% SW) show no significant change in either slope or calcium-uptake rate during the three weeks of the experiment. At Day 7 the regression line slopes of the five experimental groups shown in Figure 5 have a mean slope of -0.14, which is not different from the value of

Figure 5. Day 7 winter-adapted regression lines of calcium uptake/gram total dry weight/hour as a function of total dry weight at 25%, 50%, 75%, 100%, and 125% SW. Individual measurements of the control salinity (100% SW) are marked by (•). The equations of the lines are:

25% SW; $\log Y = -0.304 \log X + 0.980$ n=14
50% SW; $\log Y = -0.188 \log X + 1.353$ n=15
75% SW; $\log Y = 0.058 \log X + 1.696$ n=15
100% SW; $\log Y = -0.114 \log X + 1.654$ n=15
125% SW; $\log Y = -0.165 \log X + 1.584$ n=15

the winter-adapted regression line for the Day 0 control salinity (5°C, 100% SW) is shown for comparison. Individual measurements are marked by (Δ). The equation of this line is:

100% SW; $\log Y = -0.026 \log X + 1.897$ n=15

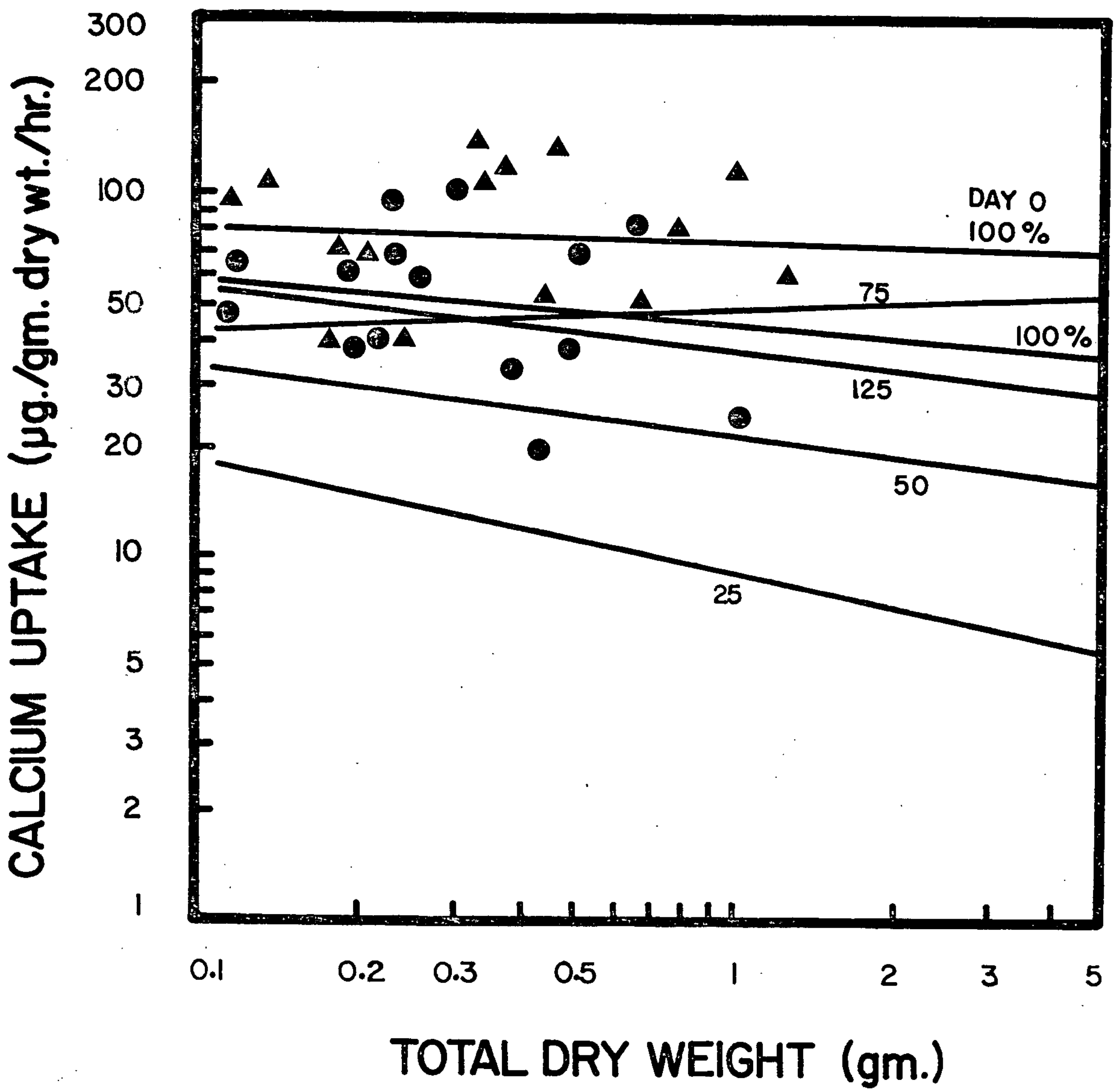
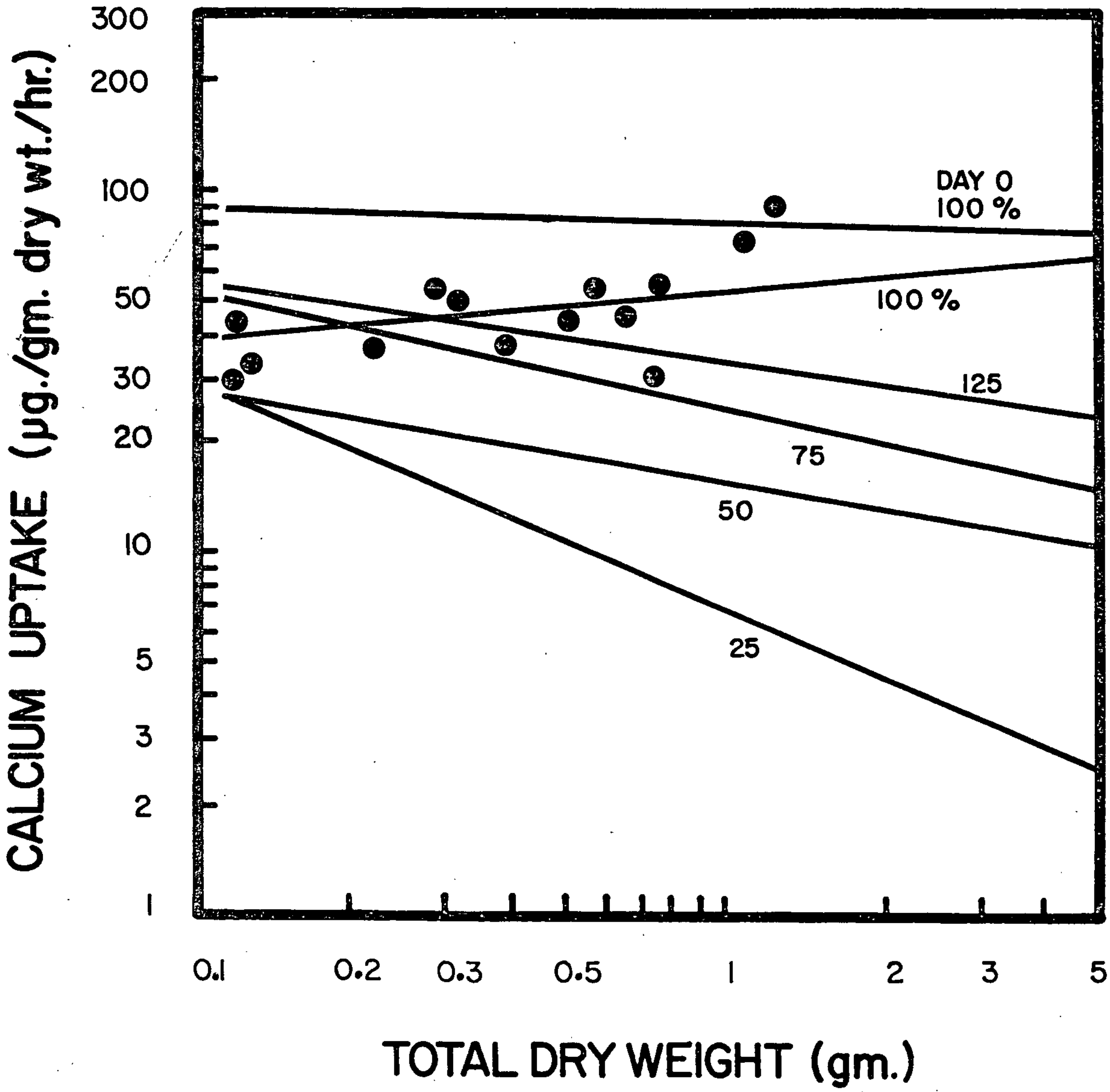

CALCIUM UPTAKE (µg./gm. dry wt./hr.)
300
200
100
50
30
20
10
5
3
2
1
DAY 0
100%
75
100%
125
50
25
0.1
0.2
0.3
0.5
1
2
3
5
TOTAL DRY WEIGHT (gm.)

Figure 6. Day 14 winter-adapted regression lines of calcium uptake/gram dry weight/hour as a function of total dry weight at 25%, 50%, 75%, 100% and 125% SW. Individual measurements of the control salinity (100%) are marked by (•). The winter adapted regression line for the Day 0 control salinity is included for comparison. The equations of the lines are:

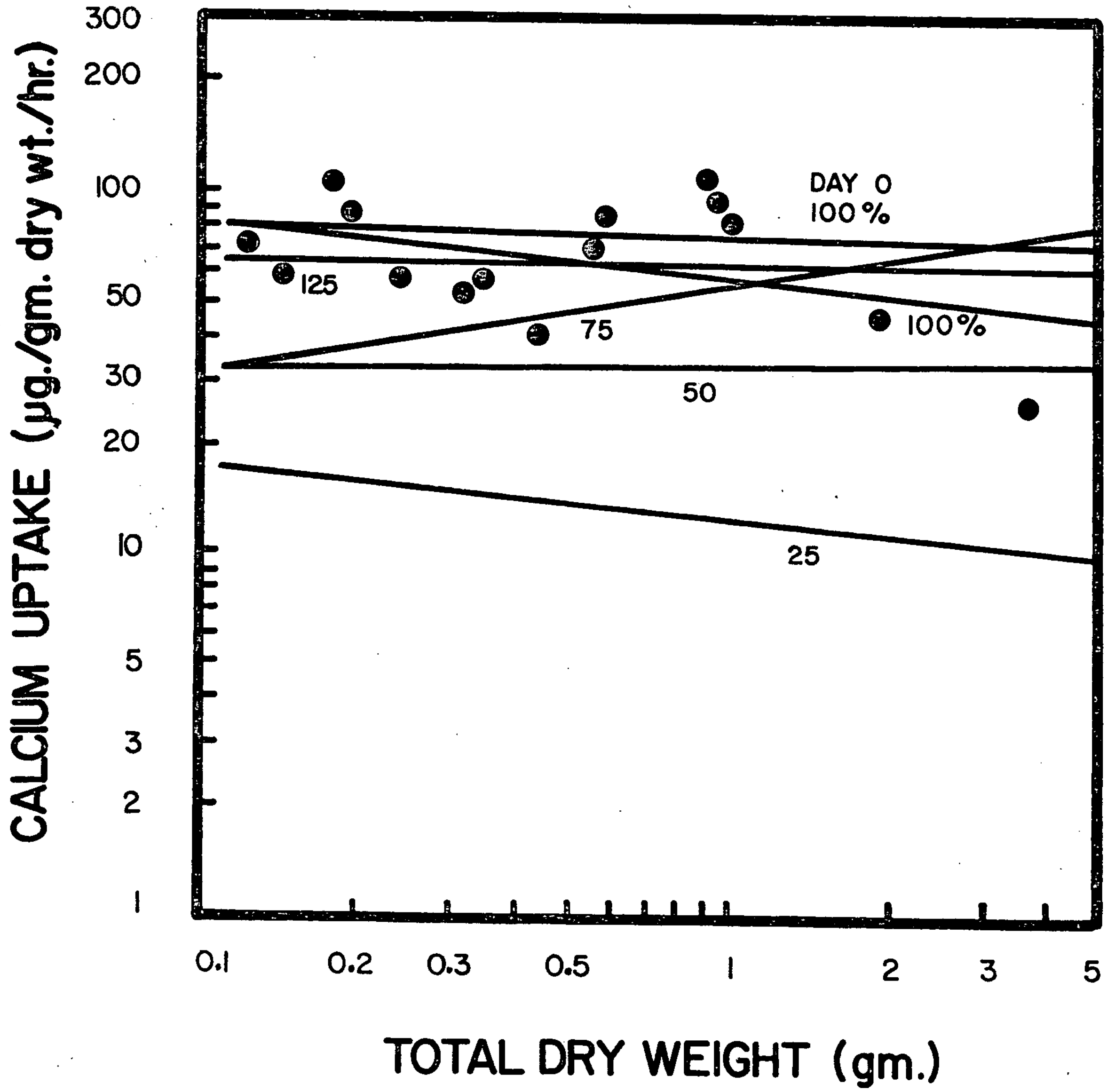
25% SW;	$\log Y = -0.632 \log X + 0.841$	n=15
50% SW;	$\log Y = -0.252 \log X + 1.191$	n=15
75% SW;	$\log Y = -0.318 \log X + 1.397$	n=14
100% SW;	$\log Y = 0.132 \log X + 1.733$	n=14
125% SW;	$\log Y = -0.214 \log X + 1.532$	n=15



CALCIUM UPTAKE (µg./gm. dry wt./hr.)
300
200
100
50
30
20
10
5
3
2
1
DAY 0
100 %
100 %
125
75
50
25
0.1
0.2
0.3
0.5
1
2
3
5
TOTAL DRY WEIGHT (gm.)

Figure 7. Day 21 winter-adapted regression lines of calcium uptake/gram dry weight/hour as a function of total dry weight at 25%, 50%, 75%, 100% and 125% SW. Individual measurements of the control salinity (100% SW) are marked by (•). The winter adapted regression line for the Day 0 control salinity is included for comparison. The equations of the lines are:

25% SW;	$\log Y = -0.148 \log X + 1.095$	n=13
50% SW;	$\log Y = 0.011 \log X + 1.525$	n=15
75% SW;	$\log Y = 0.249 \log X + 1.745$	n=15
100% SW;	$\log Y = -0.158 \log X + 1.757$	n=15
125% SW;	$\log Y = -0.011 \log X + 1.796$	n=15



CALCIUM UPTAKE (μg./gm. dry wt./hr.)
300
200
100
50
30
20
10
5
3
2
1
DAY 0
100%
125
75
100%
50
25
0.1
0.2
0.3
0.5
1
2
3
5
TOTAL DRY WEIGHT (gm.)

-0.03 shown by the control salinity at Day 0. The intercepts of the 75%, 100% and 125% SW groups show no difference from one another or from the intercept of the Day 0 regression, but there are significant differences between the intercepts of the 25%, 50% and the 75% SW groups. This result is similar to that found at Day 7 in summer-adapted mussels. In both cases there is a direct correlation between salinity and uptake rate, and in both cases the rate of calcium uptake reaches a plateau, above which an increase in the external salinity has no significant effect upon the uptake rate.

At 14 days (Fig. 6), the intercepts of the regression lines of mussels held at 75%, 100% and 125% SW show no significant difference from one another or from the intercept values of mussels held at the same experimental salinities at Day 7 (Fig. 5). Mussels in 25% and 50% SW also show no change in calcium-uptake rate when compared to mussels held at the same salinity at Day 7. The slope of the line of the 25% SW group is -0.63, which is significantly different from all the other regression lines shown in Figure 6, which have a mean slope of -0.16.

A comparison of the regression lines at Day 21 (Fig. 7) and Day 14 (Fig. 6) shows that there are no significant changes in any of the intercepts of the regression lines. There is, however, a significant change in the regression slope of the 25% SW group, which changes from -0.63 in Figure 6 to -0.15 in Figure 7.

The intercepts of the regression lines of each of the experimental salinities shown in Figures 5-7 have been used to plot Figure 8. This figure shows the calcium uptake of a winter-adapted 1.0 gram total dry weight mussel at each experimental salinity as a function of time. The decrease in the uptake rate of the control group between Day 0 and Day 7 is not statistically significant, and the increase in uptake rate shown by the 50%, 75% and 125% SW groups between the second and third week is not significant.

A comparison of the calcium-uptake rate response of summer- and winter-adapted mussels to changes in salinity shows that after one week mussels from both seasons show a direct correlation between salinity and the calcium-uptake rate. Mussels from the summer and winter seasons show a plateau in the rate of calcium uptake in seawater above 75% salinity. Neither summer- nor winter-adapted mussels are able to raise their calcium-uptake rate in order to compensate for a reduction in salinity. Under winter conditions in salinities greater than 75% SW, calcium uptake is not limited by external concentration, but rather by the ability of Mytilus to take up calcium. This is shown in Figure 9, which compares calcium uptake rate as a function of external salinity for the summer and winter seasons. The uptake rates shown in this figure are taken from the Day 7 data points of Figures 4 and 8. A plateau in the uptake rates is visible in both seasons, but a constant plateau is not apparent among the summer-adapted mussels, as Figure 4 indicates.

Figure 8. The calcium-uptake rate of winter-adapted mussels of 1.0 gram total dry weight expressed as a function of time. The control temperature and salinity are 5°C and 100% SW, experimental salinities are 25%, 50%, 75% and 125% SW. Vertical bars on the figure indicate ±1 S.E. about the mean of each point.

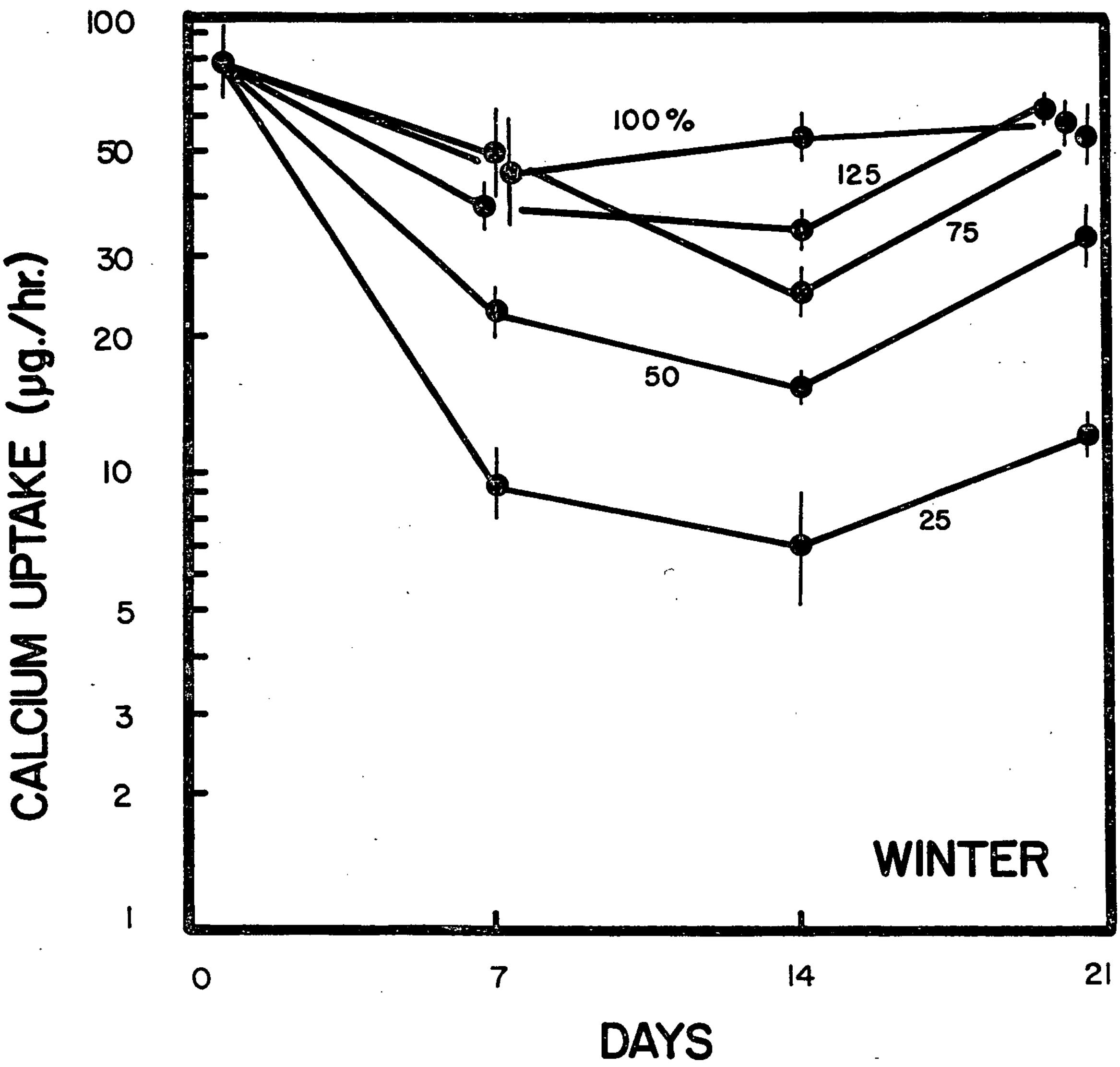
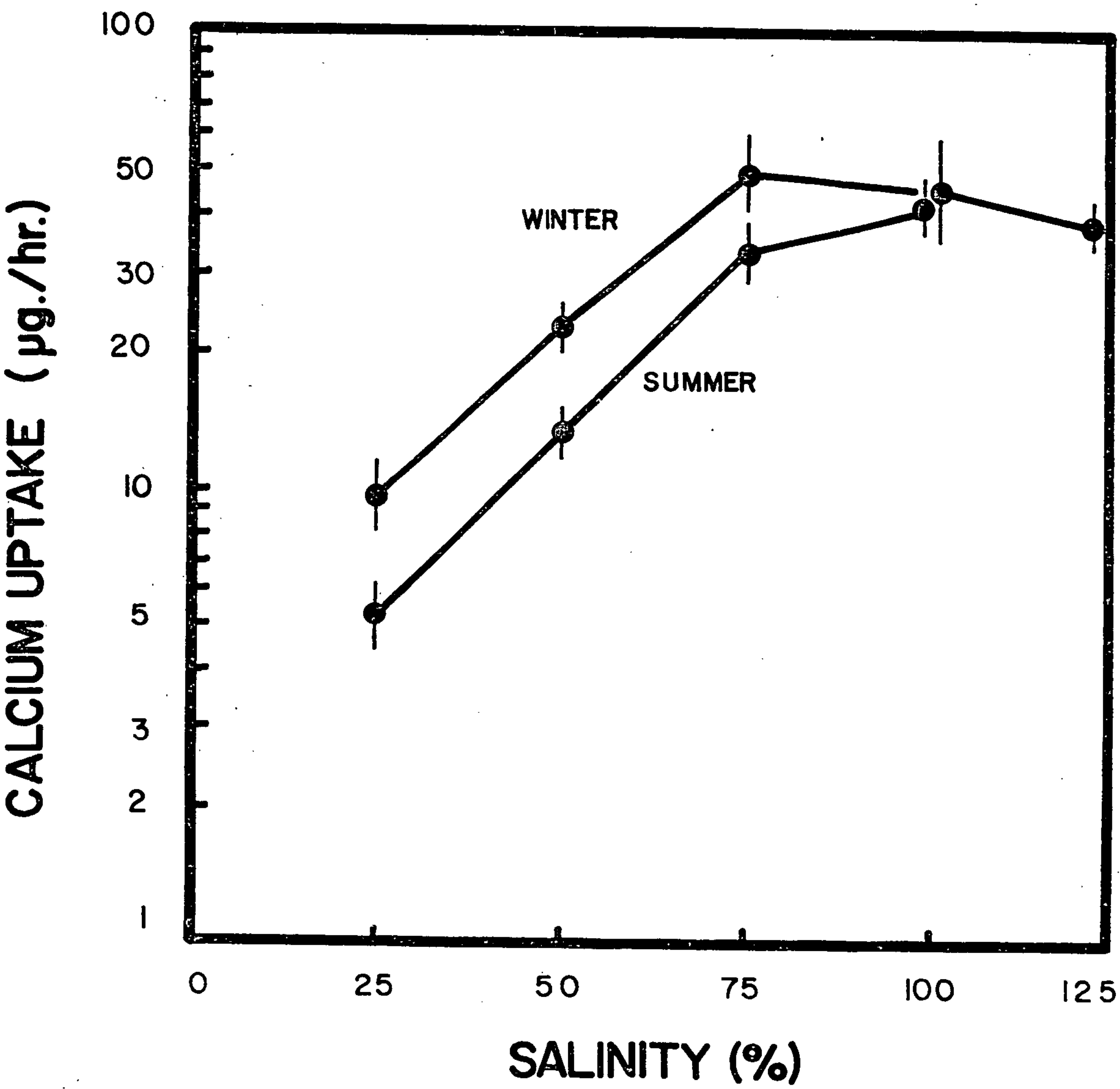

100
50
30
20
10
5
3
2
1
CALCIUM UPTAKE (μg./hr.)
100%
125
75
50
25
WINTER
0
7
14
21
DAYS

Figure 9. Calcium uptake by summer-adapted (15°C, 50% SW) and winter-adapted (5°C, 100% SW) mussels of 1.0 gram total dry weight as a function of salinity. Vertical bars indicate ±1 S.E. about the mean of each point.



100
50
30
20
10
5
3
2
1
CALCIUM UPTAKE (µg./hr.)
WINTER
SUMMER
0
25
50
75
100
125
SALINITY (%)

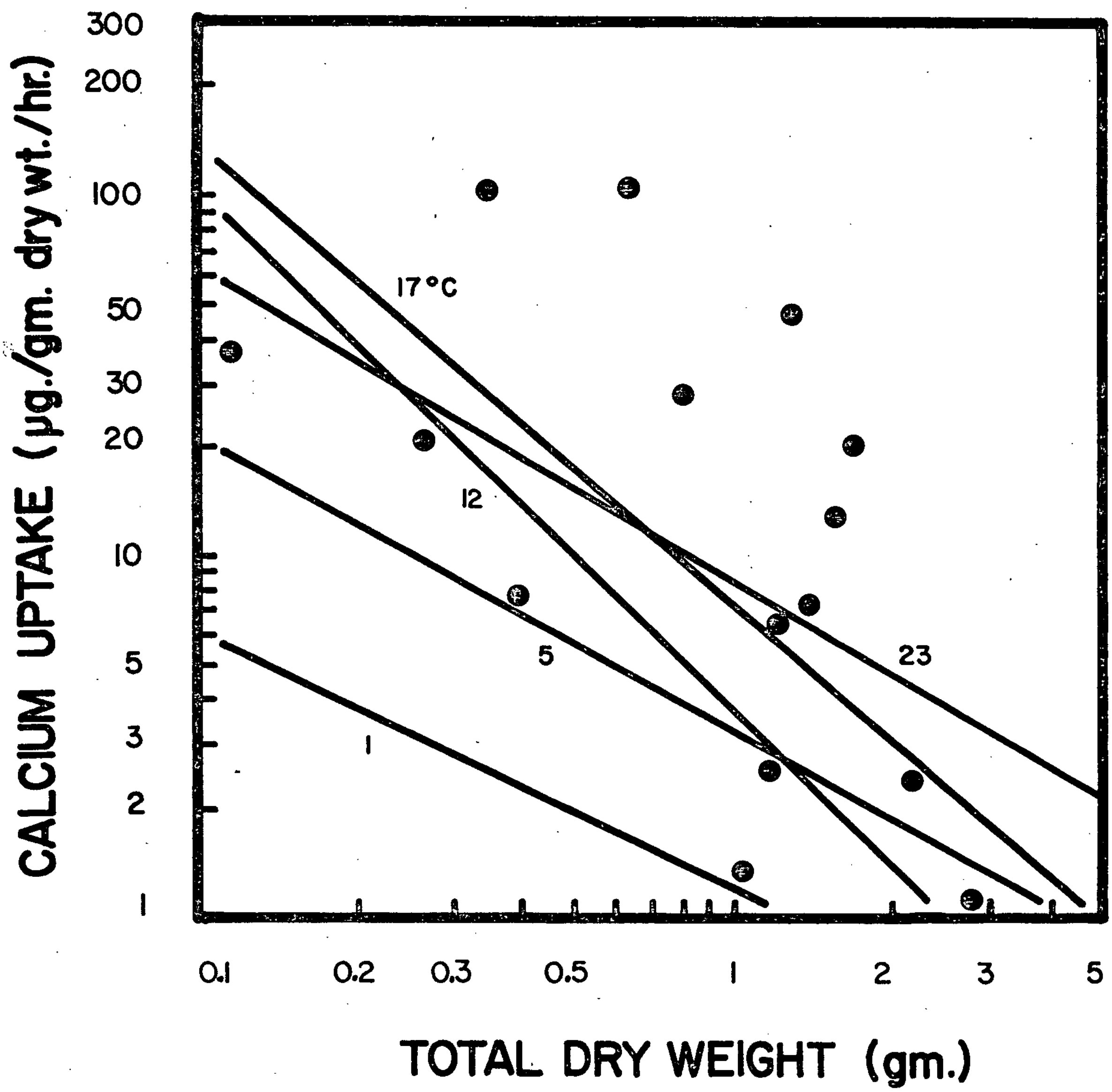
Acute temperature response experiments

Summer experiment (August 1980)

The purpose of this experiment was to determine the calcium-uptake rate of summer-adapted mussels in response to acute changes in temperature. The calcium-uptake rate of mussels in 50% SW was measured at the 1980 summer control temperature, 17°C, and at 1°, 5°, 12°, and 23°C. The regression line of calcium uptake as a function of total dry weight was calculated at each of these temperatures and plotted in Figure 10. In this figure only the data points for the summer control temperature (17°C) are shown. All five of the groups plotted in Figure 10 have regression slopes which show no significant difference from one another. Their mean value is -1.02, which is not significantly different from the slope of -0.92 shown by summer-adapted mussels after one week of holding (Fig. 1). The experimental group at 17°C were taken from winter-adapted mussels which were subsequently adapted to summer conditions in the laboratory. This group shows a slope which is significantly different from those of the Day 0 control group shown in Figure 1, which had a similar history. Possible reasons for this difference are given in the Discussion. In addition, there are no significant differences between the intercepts of the adjacent temperature groups from 5° to 23°C. There is a significant difference between the intercepts of the 12° and 23°C experiments, and between the 1°C group and all the other temperatures.

Figure 10. Summer-adapted regression lines of calcium uptake/gram total dry weight/hour as a function of total dry weight, acutely tested at 1°, 5°, 12°, 17° and 23°C in 50% SW. Individual measurements at the summer control temperature (17°C) are marked by (•). The equations of the lines are:

1°C; $\log Y = -0.714 \log X + 0.079$ n=13
5°C; $\log Y = -0.822 \log X + 0.519$ n=14
12°C; $\log Y = -1.439 \log X + 0.580$ n=15
17°C; $\log Y = -1.272 \log X + 0.881$ n=15
23°C; $\log Y = -0.870 \log X + 0.935$ n=13



CALCIUM UPTAKE (μg./gm. dry wt./hr.)
300
200
100
50
30
20
10
5
3
2
1
17°C
12
5
1
23
0.1
0.2
0.3
0.5
1
2
3
5
TOTAL DRY WEIGHT (gm.)

The intercepts of the regression lines of each of the experimental temperatures shown in Figure 10 have been used to plot Figure 11. This figure shows the calcium-uptake rate of summer-adapted 1.0 gram total dry weight mussels at each of the experimental temperatures. These results suggest that the uptake rate is temperature dependent between 1° and 23°C.

Winter experiment (November 1979)

The purpose of this experiment was to determine the calcium-uptake rate of winter-adapted mussels in response to acute changes in temperature. The calcium-uptake rate of mussels in 100% SW was measured at the winter control temperature, 5°C, and at 1°, 12°, 17° and 23°C. The regression line of calcium uptake as a function of total dry weight was calculated at each of these temperatures and plotted in Figure 12. In this figure only the data points for the control temperature (5°C) are shown. The regression lines from Figure 12 can be divided into two groups on the basis of the slope of the regression lines. One group, comprising the 1° and 23°C experimental groups, has a mean slope of -0.54. The other group, composed of the 5°, 12° and 17°C groups, has a mean slope of +0.08. This indicates that calcium uptake by winter-adapted mussels is independent of weight between 5° and 17°C, but weight dependent above and below those limits. This is shown in Figure 13, which plots the calcium-uptake rate of winter-adapted 1.0 gram total dry weight mussels at each of the experimental temperatures. Figure 13

Figure 11. The calcium-uptake rate of summer-adapted mussels of 1.0 gram total dry weight expressed as a function of acute temperature. The salinity is 50% SW. Experimental temperatures are 1°, 5°, 12°, 17° and 23°C. Vertical bars on the figure indicate ±1 S.E. about the mean of each point.

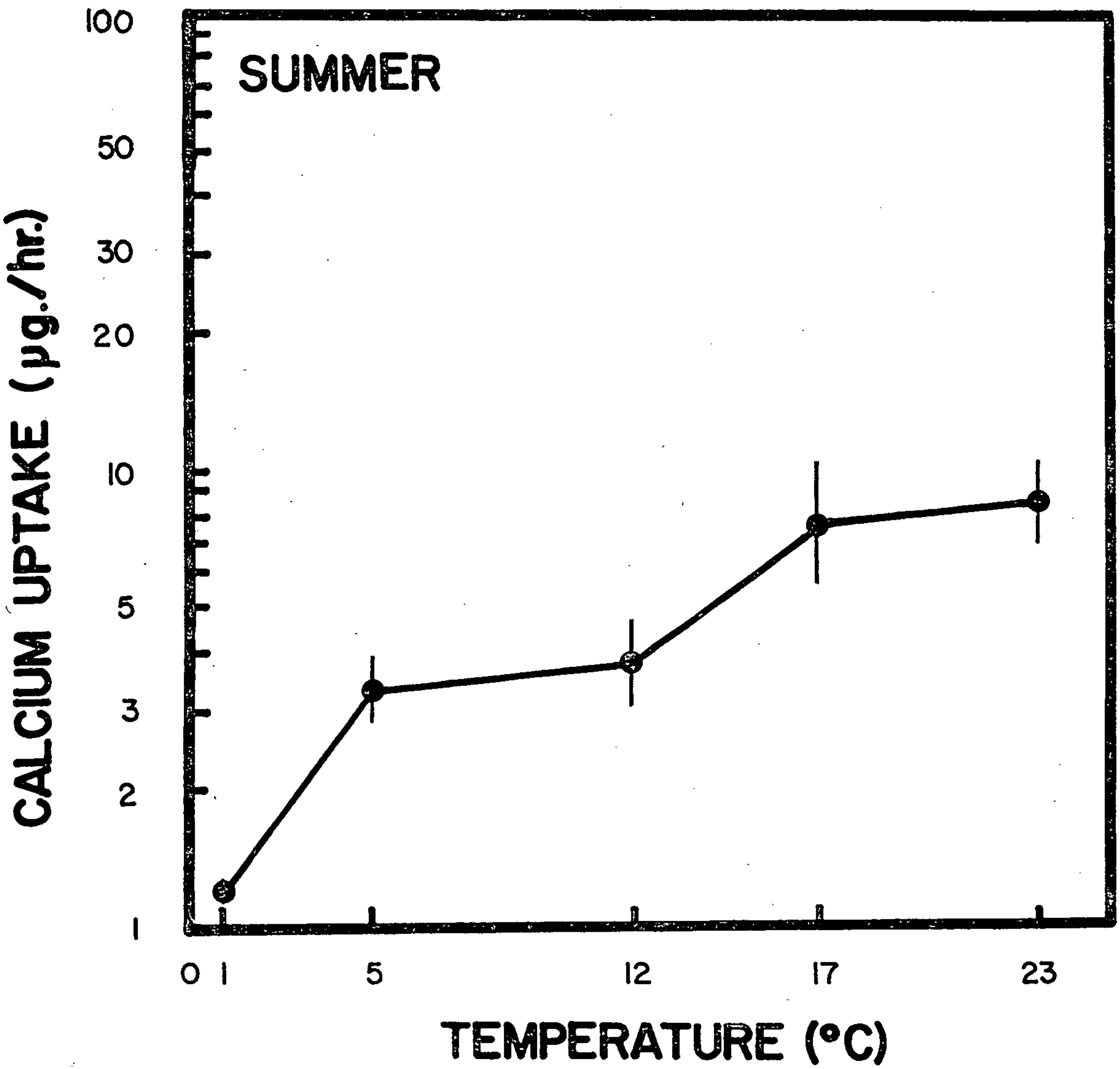

SUMMER
CALCIUM UPTAKE (µg./hr.)
100
50
30
20
10
5
3
2
1
0 1
5
12
17
23
TEMPERATURE (°C)

Figure 12. Winter-adapted regression lines of calcium uptake/gram total dry weight/hour as a function of total dry weight, acutely tested at 1°, 5°, 12°, 17° and 23°C in 100% SW. Individual measurements of the winter control temperature (5°C) are marked by (•). The equations of the lines are:

1°C;	$\log Y = -0.601 \log X + 1.004$	n=14
5°C;	$\log Y = -0.114 \log X + 1.654$	n=15
12°C;	$\log Y = -0.054 \log X + 1.595$	n=14
17°C;	$\log Y = 0.166 \log X + 1.498$	n=15
23°C;	$\log Y = -0.477 \log X + 0.852$	n=15

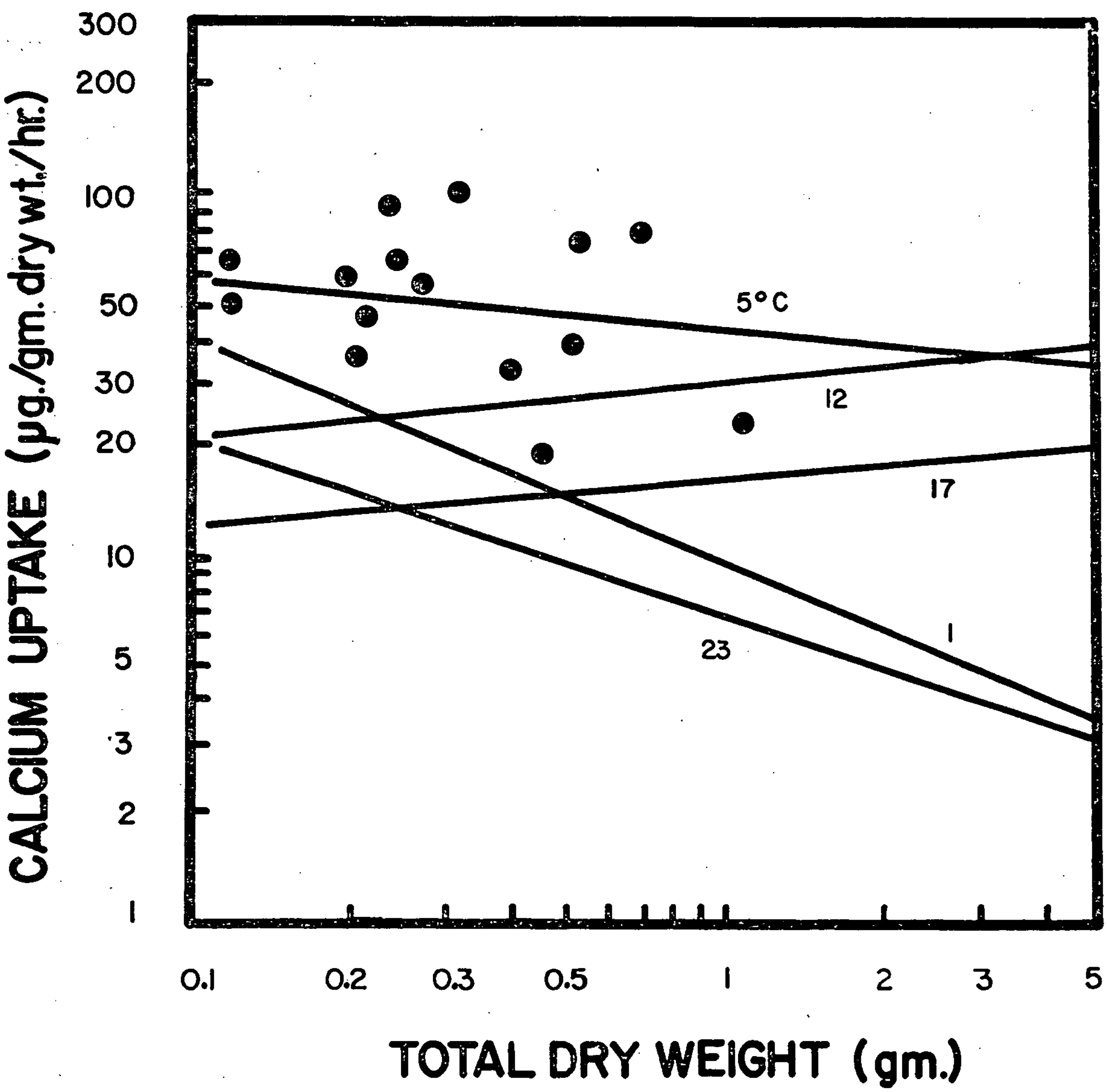
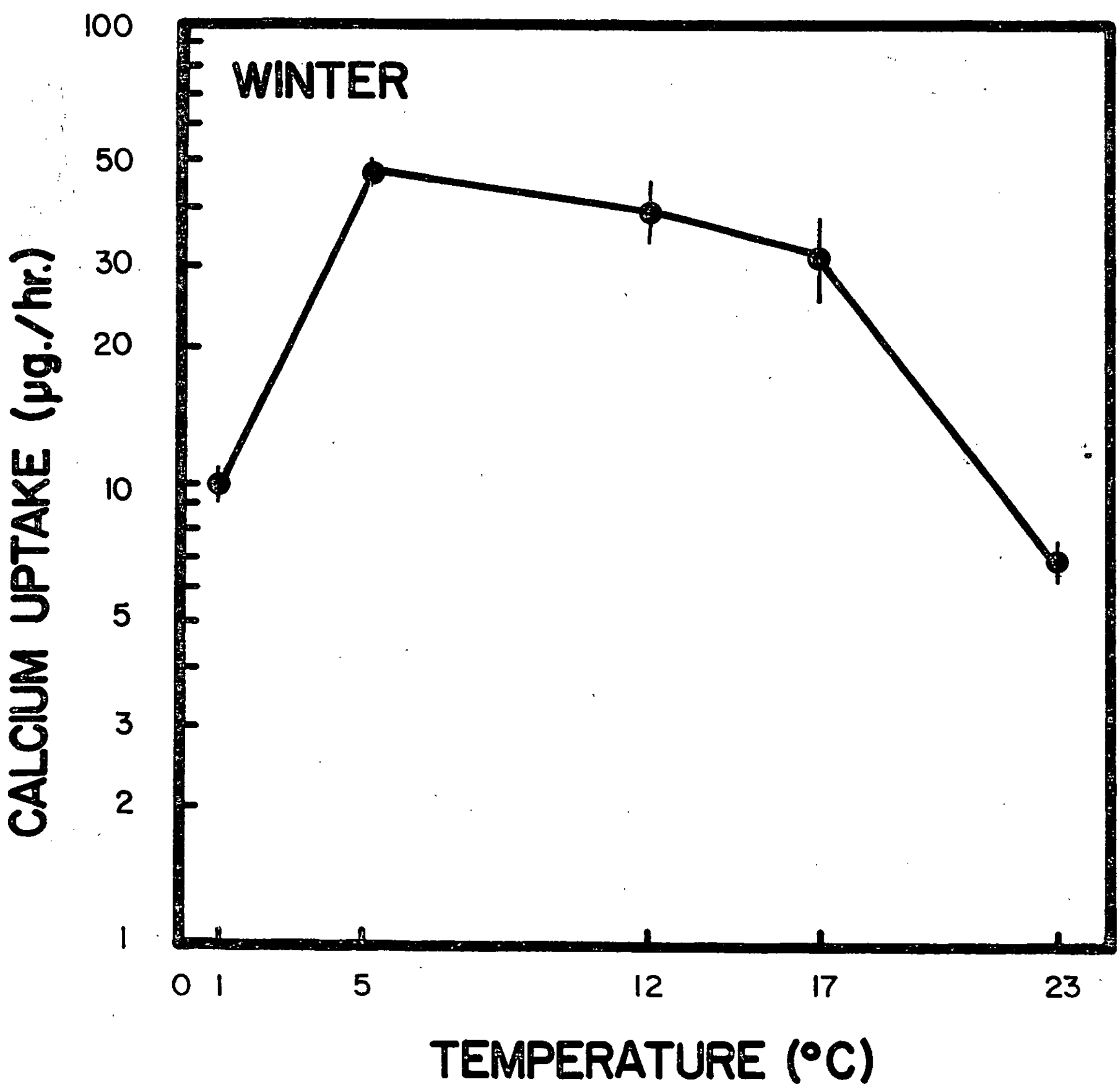

300
200
100
50
30
20
10
5
3
2
1
CALCIUM UPTAKE (µg./gm. dry wt./hr.)
5°C
12
17
1
23
0.1
0.2
0.3
0.5
1
2
3
5
TOTAL DRY WEIGHT (gm.)

Figure 13. The calcium-uptake rate of winter-adapted mussels of 1.0 gram total dry weight expressed as a function of acute temperature. The salinity is 100% SW. Experimental temperatures are 1°, 5°, 12°, 17° and 23°C. Vertical bars on the figure indicate ±1 S.E. about the mean of each point.


WINTER
CALCIUM UPTAKE (µg./hr.)
100
50
30
20
10
5
3
2
1
0
1
5
12
17
23
TEMPERATURE (°C)

shows a plateau in calcium-uptake rates between 5° and 17°C, and a reduction in uptake at temperatures about these points. The differences in uptake rate among the experimental groups at 5°, 12° and 17°C are not statistically significant, and the calcium uptake rate within this range is, therefore, independent of temperature.

Intertidal transplant experiment

The purpose of this experiment was twofold: to determine whether intertidal height affected the rate of calcium uptake in mussels; and to see if the rate of calcium uptake could be altered by changing the intertidal height of mussels, by comparing the uptake rates of transplanted mussels with those of untransplanted control mussels. Mussels from the trays located at 2.2, 1.2 and 0.2 m above datum were transplanted reciprocally from 2.2 m to 0.2 and 1.2 m; from 1.2 m to 2.2 and 0.2 m; and from 0.2 m to 2.2 and 1.2 m. One month after transplantation, the calcium-uptake rates of the transplanted and the untransplanted mussels were measured in the laboratory, employing 1979 summer control conditions (15°C, 50% SW). The regression lines of calcium uptake as a function of total dry weight were calculated for the experimental and control groups located at 2.2, 1.2 and 0.2 m, and plotted in Figures 14, 15 and 16 respectively. In each of the figures, only the data points of the untransplanted control groups are shown.

A comparison of the slopes of the regression lines in Figure 14 shows that there are no significant differences

Figure 14. Reciprocal transplant regression lines of calcium uptake/gram total dry weight/hour as a function of total dry weight. Individual measurements of the untransplanted controls (2.2 m equivalent intertidal height) are marked by (•). The equations of the lines are:

2.2 m; $\log Y = -0.171 \log X + 1.388$ $n=10$
1.2 m; $\log Y = -0.693 \log X + 0.997$ $n=11$
0.2 m; $\log Y = -0.290 \log X + 1.254$ $n=10$

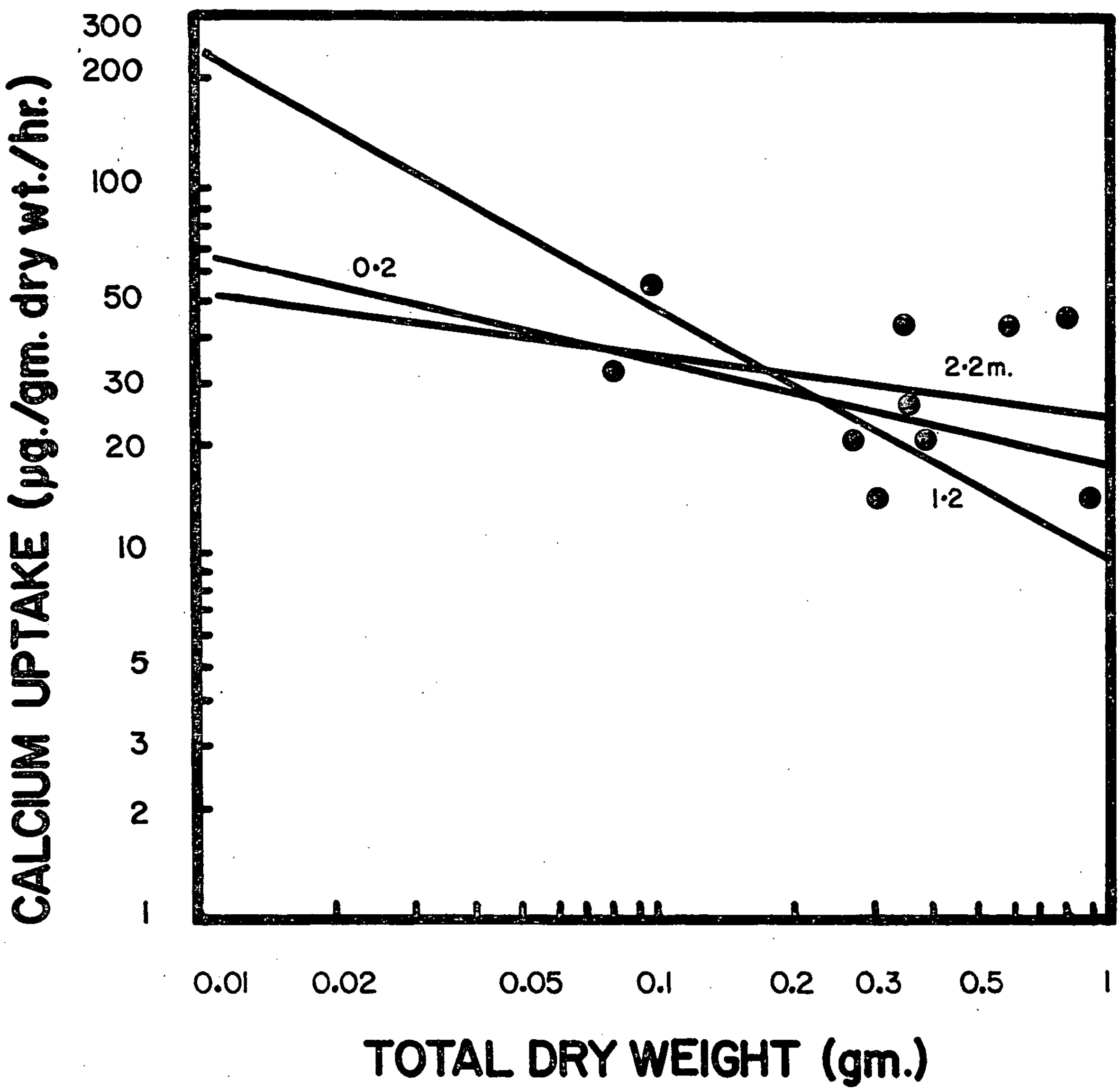

300
200
100
50
30
20
10
5
3
2
1
CALCIUM UPTAKE (µg./gm. dry wt./hr.)
0·2
2·2m.
1·2
0.01
0.02
0.05
0.1
0.2
0.3
0.5
1
TOTAL DRY WEIGHT (gm.)

Figure 15. Reciprocal transplant regression lines of calcium uptake/gram total dry weight/hour as a function of total dry weight. Individual measurements of the untransplanted controls (1.2 m equivalent intertidal height) are marked by (•). The equations of the lines are:

2.2 m; $\log Y = -0.142 \log X + 1.562$ n=10
1.2 m; $\log Y = -0.210 \log X + 1.468$ n=12
2.2 m; $\log Y = 0.006 \log X + 1.552$ n=10

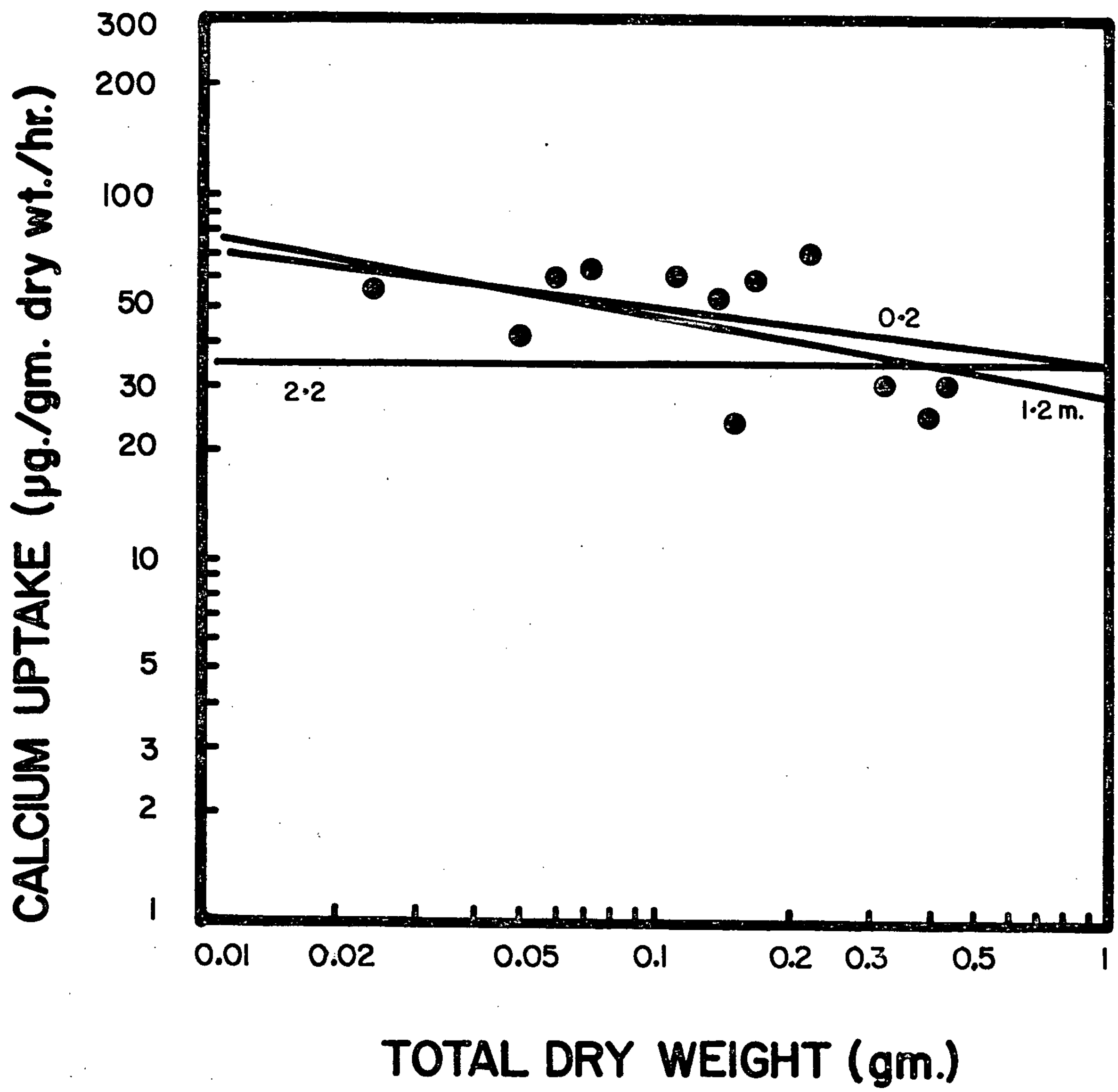
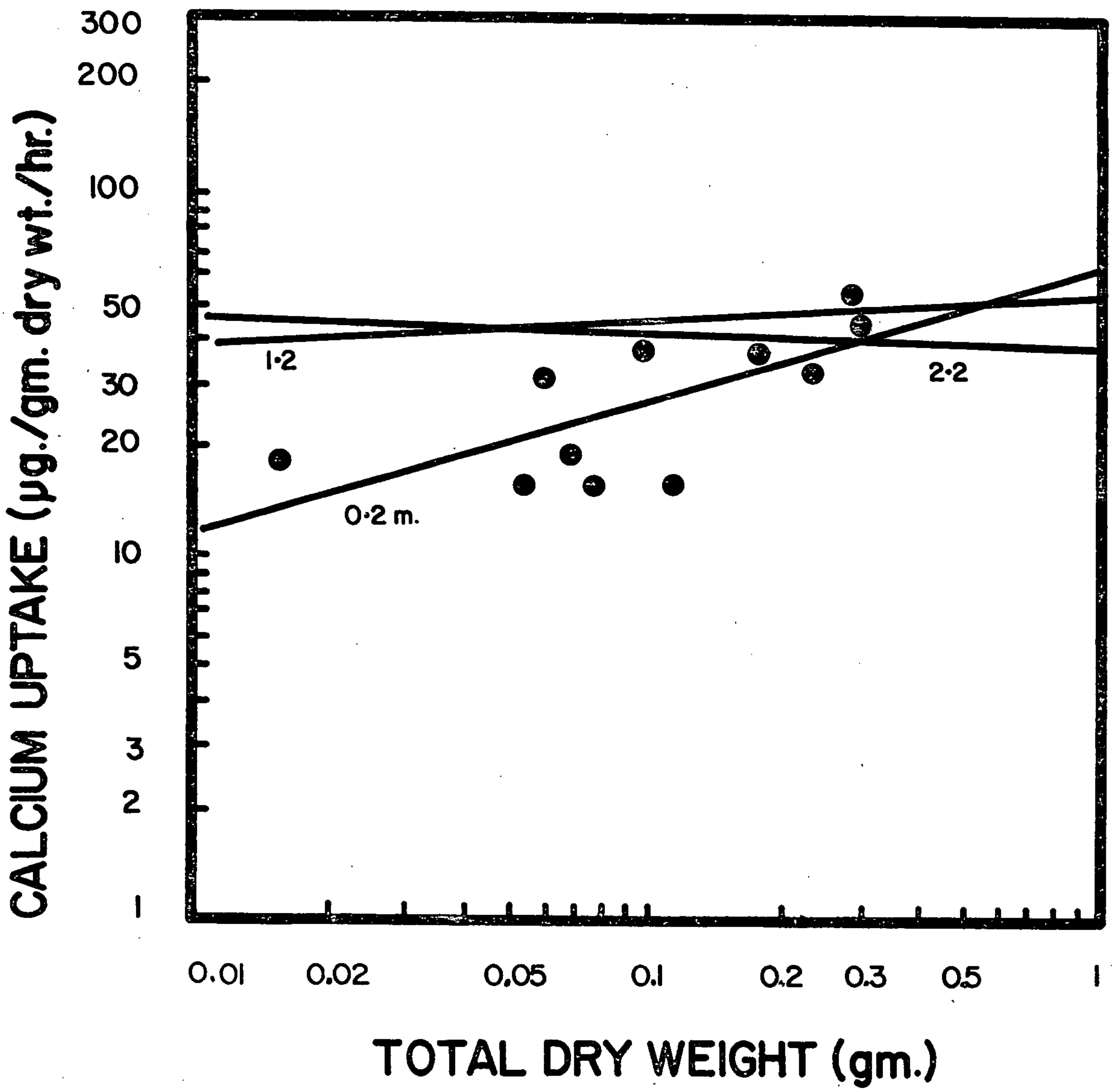

CALCIUM UPTAKE (µg./gm. dry wt./hr.)
300
200
100
50
30
20
10
5
3
2
1
0·2
2·2
1·2 m.
0.01
0.02
0.05
0.1
0.2
0.3
0.5
1
TOTAL DRY WEIGHT (gm.)

Figure 16. Reciprocal transplant regression lines of calcium uptake/gram total dry weight/hour as a function of total dry weight. Individual measurements of the untransplanted controls (0.2 m equivalent intertidal height) are marked by (•). The equations of the lines are:

2.2 m; $\log Y = -0.042 \log X + 1.573$ n=12
1.2 m; $\log Y = 0.060 \log X + 1.708$ n=12
0.2 m; $\log Y = 0.356 \log X + 1.777$ n=11


CALCIUM UPTAKE (µg./gm. dry wt./hr.)
300
200
100
50
30
20
10
5
3
2
1
1·2
2·2
0·2 m.
0.01
0.02
0.05
0.1
0.2
0.3
0.5
1
TOTAL DRY WEIGHT (gm.)

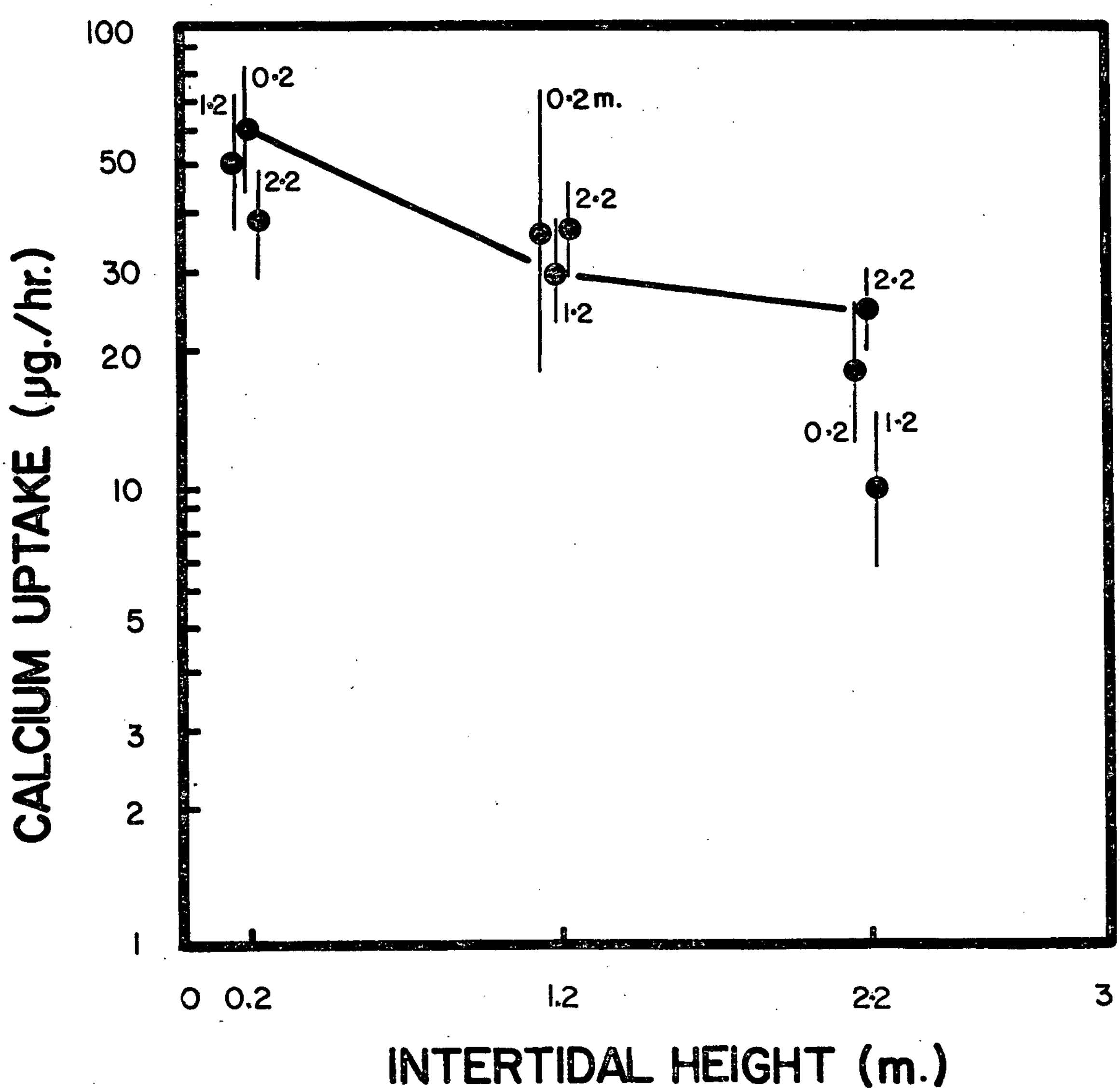
between any of the experimental groups. The mean slope of the three groups is -0.38. There are no significant differences between any of the intercepts in this figure.

Figure 15 shows the regression lines of the experimental groups at 1.2 m. As in Figure 14, there are no significant differences between the slopes of the regression lines of any of the experimental groups. The mean value of the slope is -0.12. In addition, there are no significant differences between any of the intercepts of any of the experimental groups.

The regression lines of the experimental groups at 0.2 m are shown in Figure 16. As in Figures 14 and 15, there are no significant differences between either the slopes or intercepts of the regression lines of the experimental groups. The mean value of the slope is +0.12.

A comparison of the regression lines from Figures 14, 15 and 16 shows no significant difference between the slopes of the regression lines. There is a significant difference between the uptake rates of mussels at the upper and lower extremes, although this difference is obscured by the large scatter found in some of the experiments. This difference is illustrated in Figure 17, which shows the calcium-uptake rate of 1.0 gram total dry weight mussels of transplanted and untransplanted groups from each of the intertidal trays. In addition, there is a trend in the slopes of the regression lines; -0.38 at 2.2 m to +0.12 at 0.2 m. These values are the mean slopes of the controls and transplants at the upper and lower trays.

Figure 17. The calcium-uptake rate of untransplanted and reciprocally transplanted intertidal mussels of 1.0 gram total dry weight expressed as a function of intertidal height. The line between intertidal heights connects untransplanted controls. Original intertidal heights of transplants are marked above the individual measurements. Vertical bars on the figure indicate ±1 S.E. about the mean of each point.


CALCIUM UPTAKE (µg./hr.)
100
50
30
20
10
5
3
2
1
0 0.2
1.2
2.2
3
INTERTIDAL HEIGHT (m.)
1·2
0·2
2·2
0·2 m.
2·2
1·2
2·2
0·2
1·2

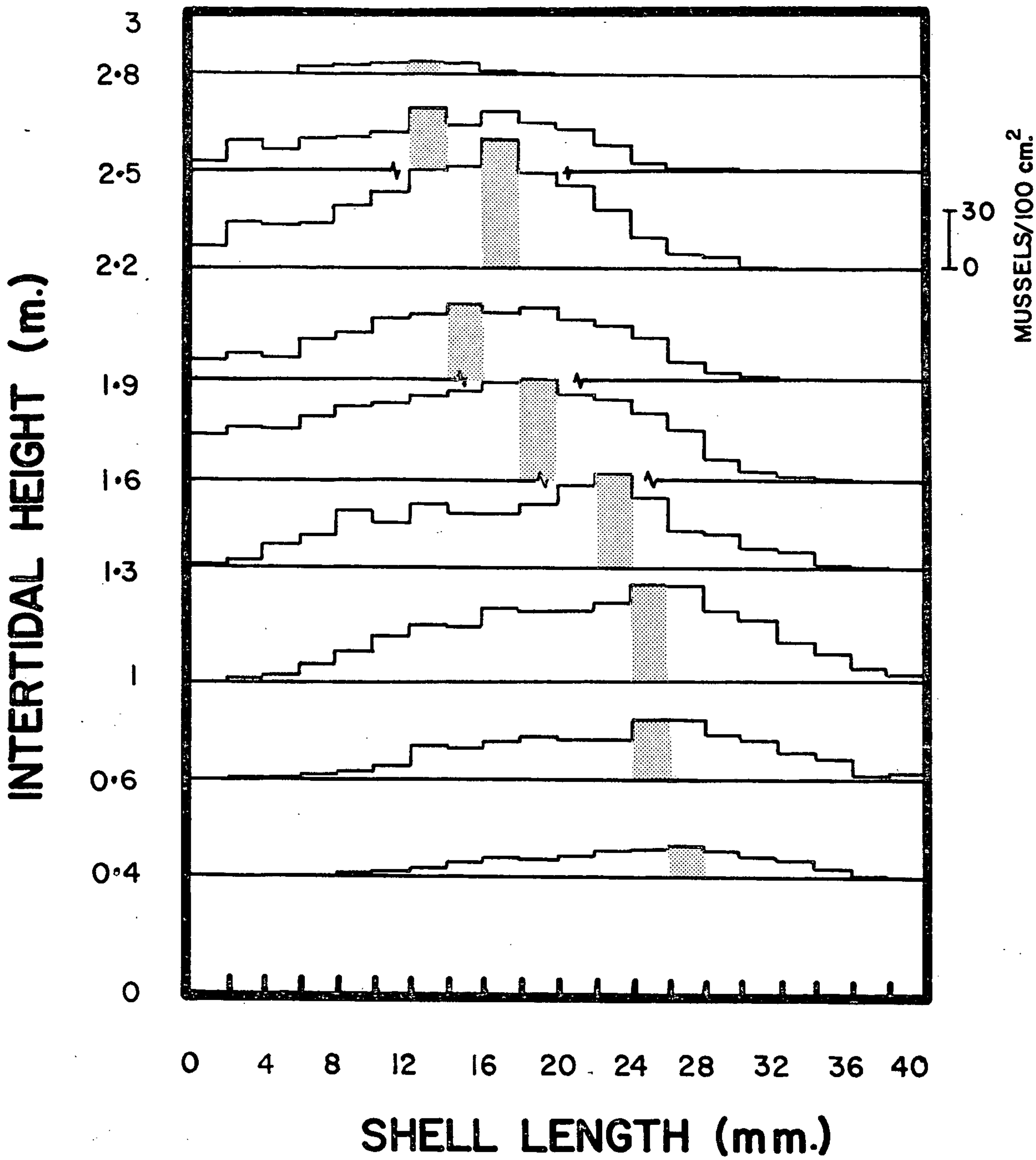
Size gradient study

The purpose of this study was threefold: to see whether an intertidal size gradient existed among mussels; to see whether there were differences in the dry weight of the mantle, gill or viscera of high and low intertidal mussels; and to see whether there were differences in the size, shape or displacement of the shells of high and low intertidal mussels. Differences between high (2.2 m) and low (0.2 m) mussels with respect to any of these physical characteristics could yield information useful to the interpretation of calcium-uptake differences between the two heights.

The size measurements obtained from the mussels removed from the cable were plotted as histograms in Figure 18. The ordinate of Figure 18 indicates the intertidal height from which the mussels used in each histogram were taken, and the abscissa indicates the shell length size intervals of the mussels in each histogram. An examination of the histograms shows that the modal shell length (indicated by the shaded interval of each histogram in Figure 18) decreases steadily with increasing intertidal height. Since the site was inaccessible to its major predators (as described in the Materials and Methods), this suggests that physical factors are able to produce a size gradient. This is consistent with the results of the reciprocal transplant experiment, which showed that high intertidal mussels had lower rates of calcium uptake, and, presumably, lower rates of shell growth, when compared to low intertidal mussels.

Mussels from the 0.2 and 2.2 m trays were compared for

Figure 18. The size-frequency distribution of mussels as a function of intertidal height. The intersection of each histogram with the ordinate marks the intertidal height from which the shell length data for that histogram were collected. The abscissa marks the size increments for the histograms. The density of mussels in each histogram is given by the legend bar marked 0 to 30 at the 2.2 m histogram. The modal size interval is indicated by shading.


INTERTIDAL HEIGHT (m.)
3
2·8
2·5
2·2
1·9
1·6
1·3
1
0·6
0·4
0
30
0
MUSSELS/100 cm.^{2}
0
4
8
12
16
20
24
28
32
36
40
SHELL LENGTH (mm.)

differences in the relationship of the total dry dry weight of all soft parts to the dry weight of the shell, mantle, gill and viscera. The dry weights of the shell, mantle, gill and viscera are plotted as a function of the total dry weight of soft parts in Figures 19-22. Figure 19 demonstrates that there is a significant difference in the weight of the shells of high and low mussels. High mussels have dry shell weights 710% of the total dry weight of soft parts, while low shells have weights 230% of the total dry weight of soft parts; a difference of approximately three times. There is no significant difference between the two Y-intercepts in Figure 19. Figures 20, 21 and 22 indicate that there are no significant differences between the dry weight of mantle, gill or visceral tissue as a function of the total dry weight of soft parts between high (2.2 m) and low (0.2 m) mussels. Expressed as a percentage of the total dry weight of soft parts, these are 39% for mantle, 8% for gill and 53% for viscera.

The difference in shell weights between high and low populations may be attributed to differences in the total dry weight of the soft parts, or to differences in the amount of calcium carbonate in shells of similar shape, or to a combination of these two factors. Figures 23 and 24 show the relationship between shell height and length, and shell width and length, respectively. There are no significant differences between the high and low groups in either figure. This demonstrates that the shells are of similar shape. Figure 25 shows that there is no significant difference in the density of

Figure 19. The regression lines of dry weight of shell as a function of total dry weight soft parts of mussels from 2.2 m (o) and 0.2 m (•). The equations of the lines are:

2.2 m; $Y = 7.126 X - 0.063$ n=44
0.2 m; $Y = 2.285 X + 0.266$ n=45

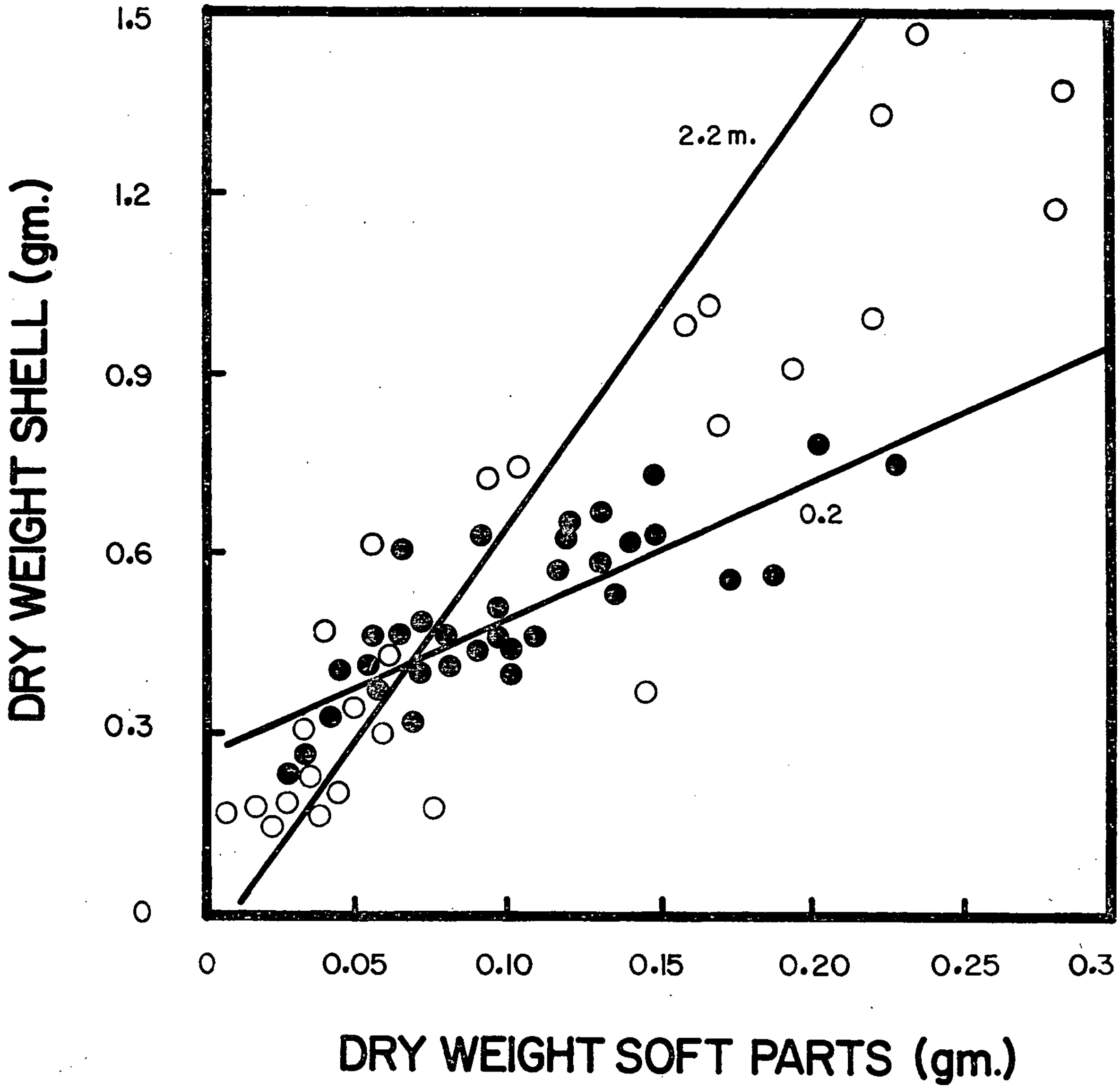

1.5
1.2
0.9
0.6
0.3
0
2.2 m.
0.2
0
0.05
0.10
0.15
0.20
0.25
0.3
DRY WEIGHT SHELL (gm.)
DRY WEIGHT SOFT PARTS (gm.)

Figure 20. The regression lines of dry weight of mantle as a function of total dry weight of soft parts of mussels from 2.2 m (o) and 0.2 m (•). The equations of the lines are:

2.2 m; $Y = 0.389\ X - 0.043$ n=44
0.2 m; $Y = 0.407\ X - 0.073$ n=45

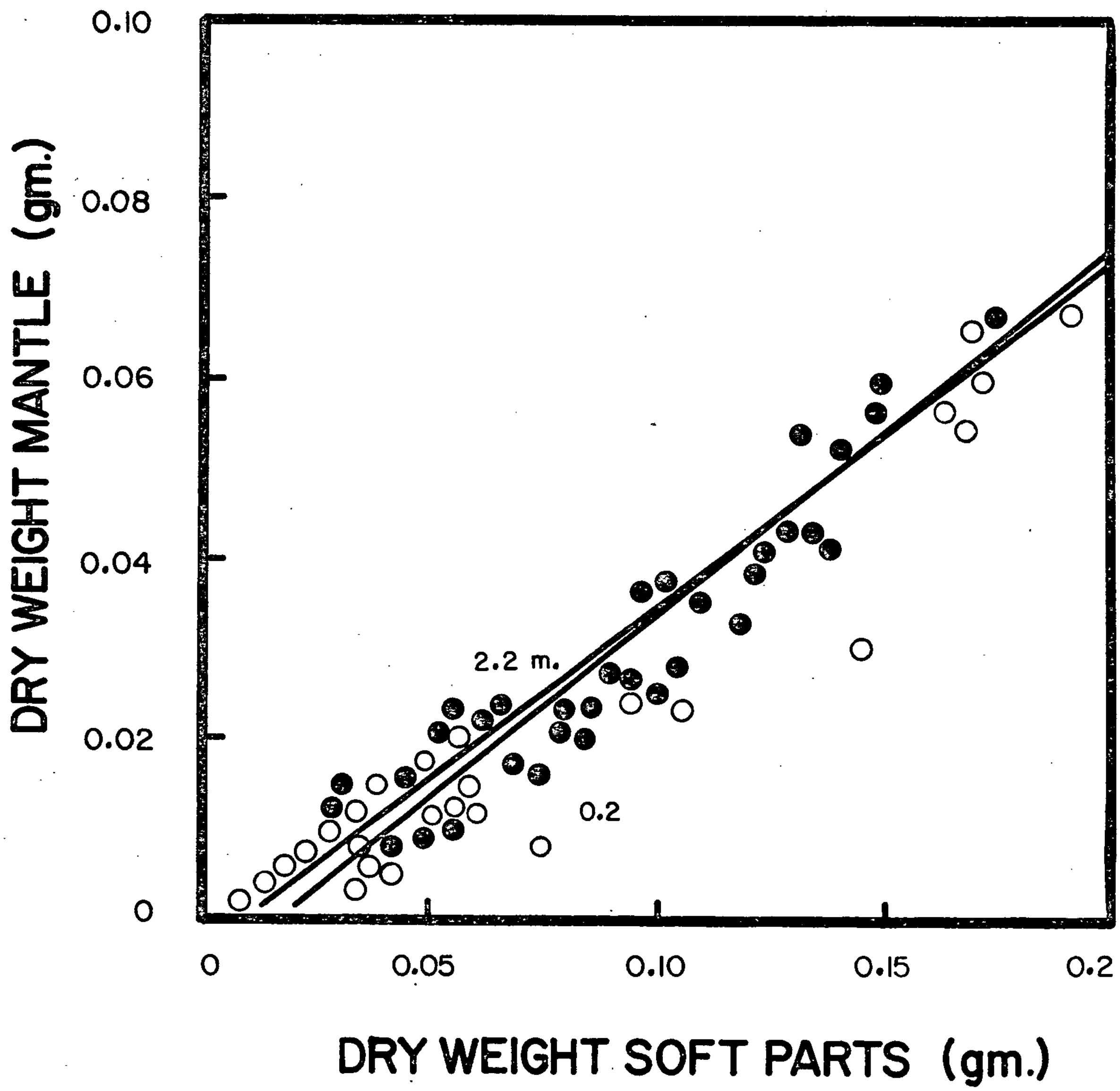

0.10
0.08
0.06
0.04
0.02
0
DRY WEIGHT MANTLE (gm.)
2.2 m.
0.2
0
0.05
0.10
0.15
0.2
DRY WEIGHT SOFT PARTS (gm.)

Figure 21. The regression lines of dry weight of gill as a function of total dry weight of soft parts of mussels from 2.2 m (o) and 0.2 m (•). The equations of the lines are:

2.2 m; $Y = 0.077 X + 0.003$ n=44
0.2 m; $Y = 0.074 X + 0.005$ n=45

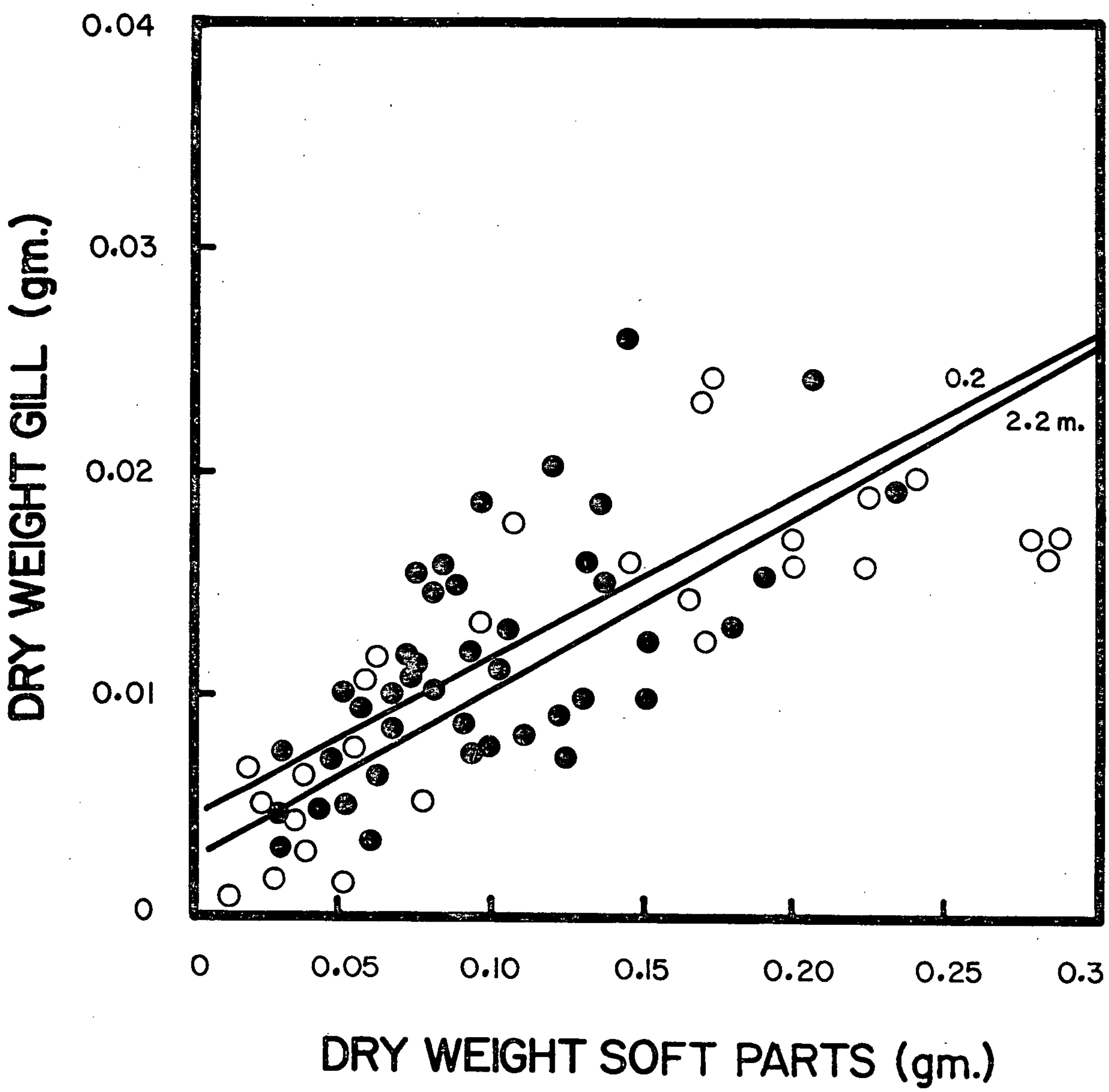
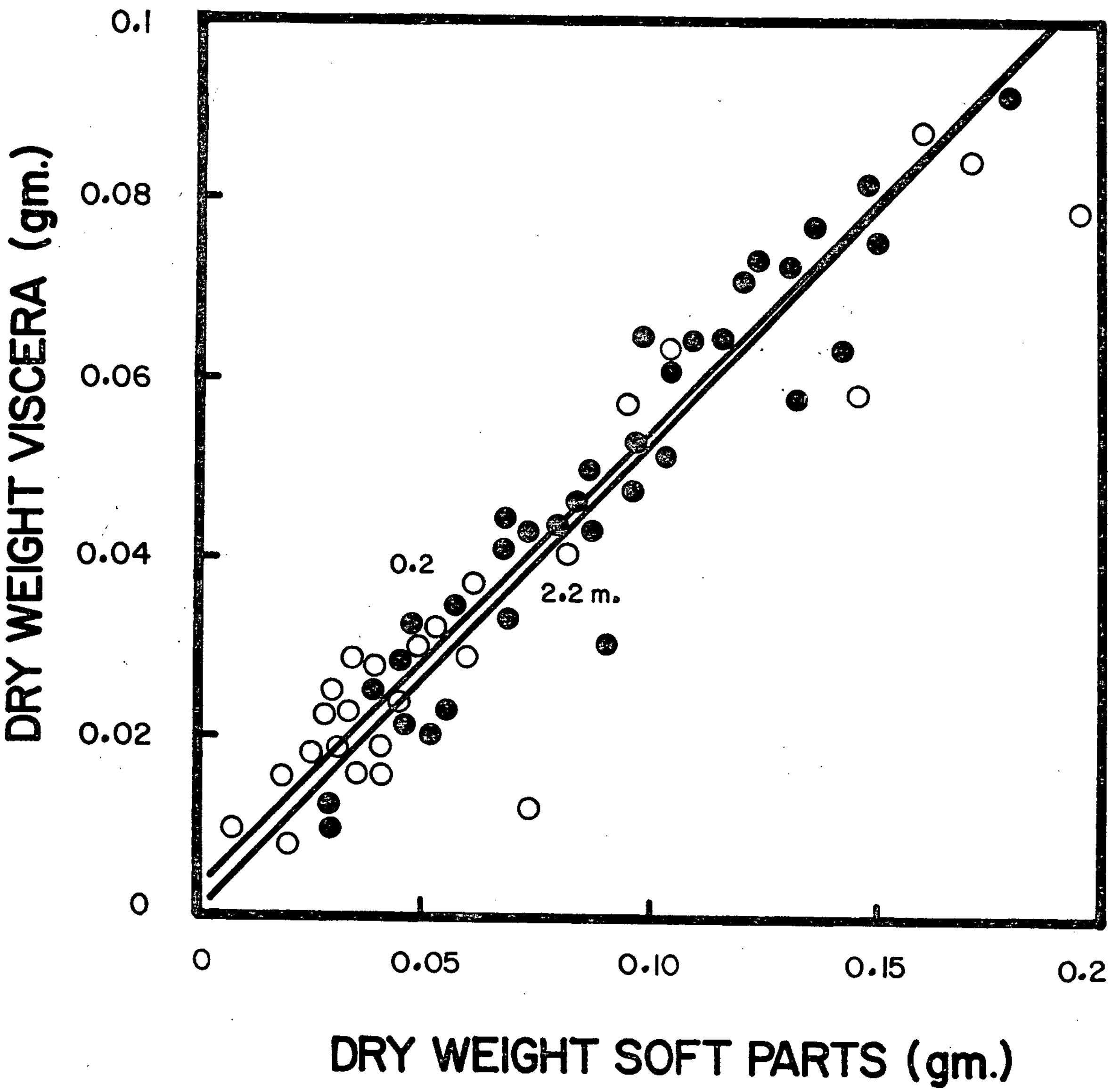

0.04
0.03
0.02
0.01
0
DRY WEIGHT GILL (gm.)
0.2
2.2 m.
0
0.05
0.10
0.15
0.20
0.25
0.3
DRY WEIGHT SOFT PARTS (gm.)

Figure 22. The regression lines of dry weight of viscera as a function of total dry weight of soft parts of mussels from 2.2 m (o) and 0.2 m (•). The equations of the lines are:

2.2 m; $Y = 0.530\ X + 0.001$ n=44
0.2 m; $Y = 0.520\ X + 0.002$ n=45



DRY WEIGHT VISCERA (gm.)
0.1
0.08
0.06
0.04
0.02
0
0.2
2.2 m.
0
0.05
0.10
0.15
0.2
DRY WEIGHT SOFT PARTS (gm.)

Figure 23. The regression lines of shell height as a function of shell length in mussels from 2.2 m (o) and 0.2 m (•). The equations of the lines are:

2.2 m; $Y = 0.518\ X + 0.860$ n=36
0.2 m; $Y = 0.507\ X + 0.716$ n=36

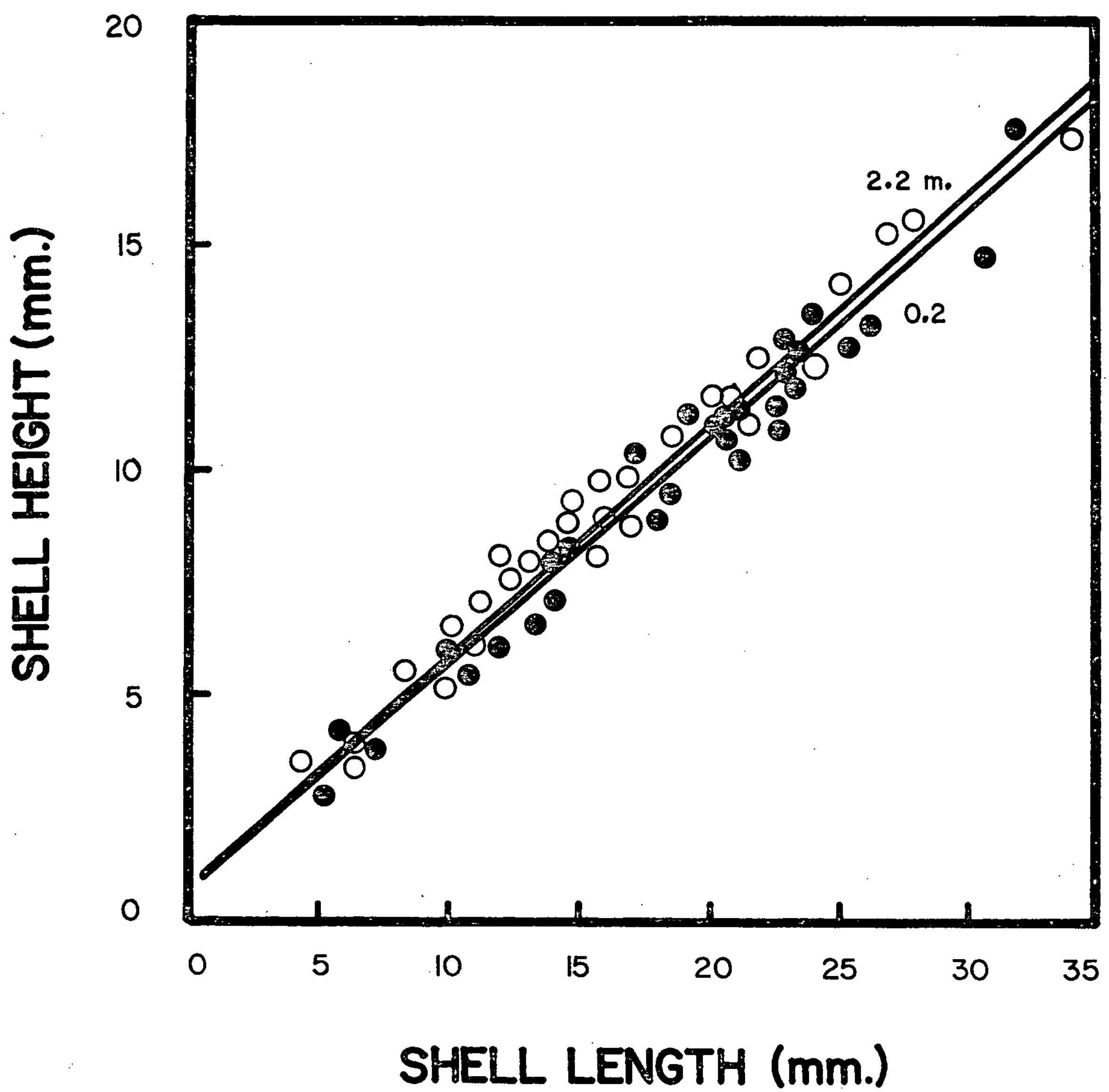
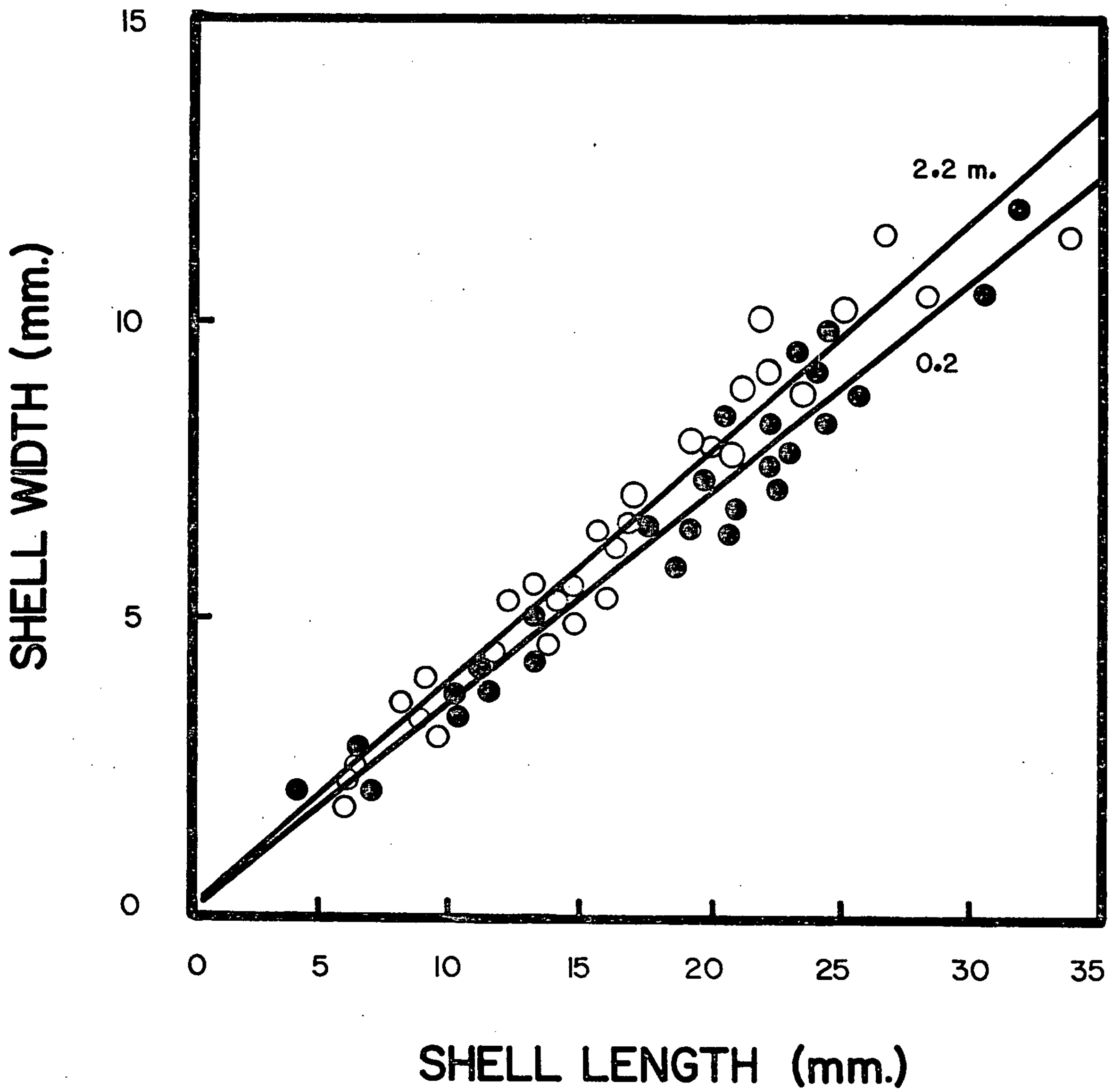

20
15
10
5
0
SHELL HEIGHT (mm.)
2.2 m.
0.2
0
5
10
15
20
25
30
35
SHELL LENGTH (mm.)

Figure 24. The regression lines of shell width as a function of shell length in mussels from 2.2 m (o) and 0.2 m (•). The equations of the lines are:

2.2 m; $Y = 0.382\ X + 0.175$ n=36
0.2 m; $Y = 0.352\ X + 0.098$ n=36



15
10
5
0
0
5
10
15
20
25
30
35
SHELL WIDTH (mm.)
SHELL LENGTH (mm.)
2.2 m.
0.2

the shell valves from high and low sites. The mean of the two slopes of the regression lines is 0.40 ml./gm. The inverse of this value, 2.51 gm./ml., approximates the published value for the density of calcite, 2.7-2.9 grams/ml. (Weast, 1974). Figure 26 shows that there are no significant differences between high and low mussels with respect to the volume of the shell valves as a function of shell length. In summary, Figures 23-26 indicate that the shells of high and low mussels show no significant difference with respect to shape, density or valve displacement. Thus, the differences in shell dry weight as a function of the total dry weight of soft parts (Fig. 19) is due to a difference in the dry weights of the soft parts between high and low mussels.

Time course uptake study

The purpose of this study was to follow the movement of labelled calcium from the seawater to the shell, mantle, gill and viscera over 32 hours. The experiment was performed under winter control conditions (5°C, 100% SW). Initially, 60 mussels received a dose of ^{45}Ca. After intervals of 1, 2, 4, 8, 16 and 32 hours, ten mussels were removed from the seawater. The mussels were dissected into shell, mantle, gill and viscera, and the radioactivity of the shell and each of the tissues measured. From the shell and from each tissue, the regression line for calcium uptake as a function of total dry weight was calculated at each of the six time intervals. These regression lines are plotted in Figures 27-30 for the shell, mantle, gill and

Figure 25. The regression lines of shell valve displacement as a function of total dry weight shell in mussels from 2.2 m (o) and 0.2 m (•). The equations of the lines are:

2.2 m; $Y = 0.381\ X + 0.015$ n=36
0.2 m; $Y = 0.415\ X + 0.001$ n=36

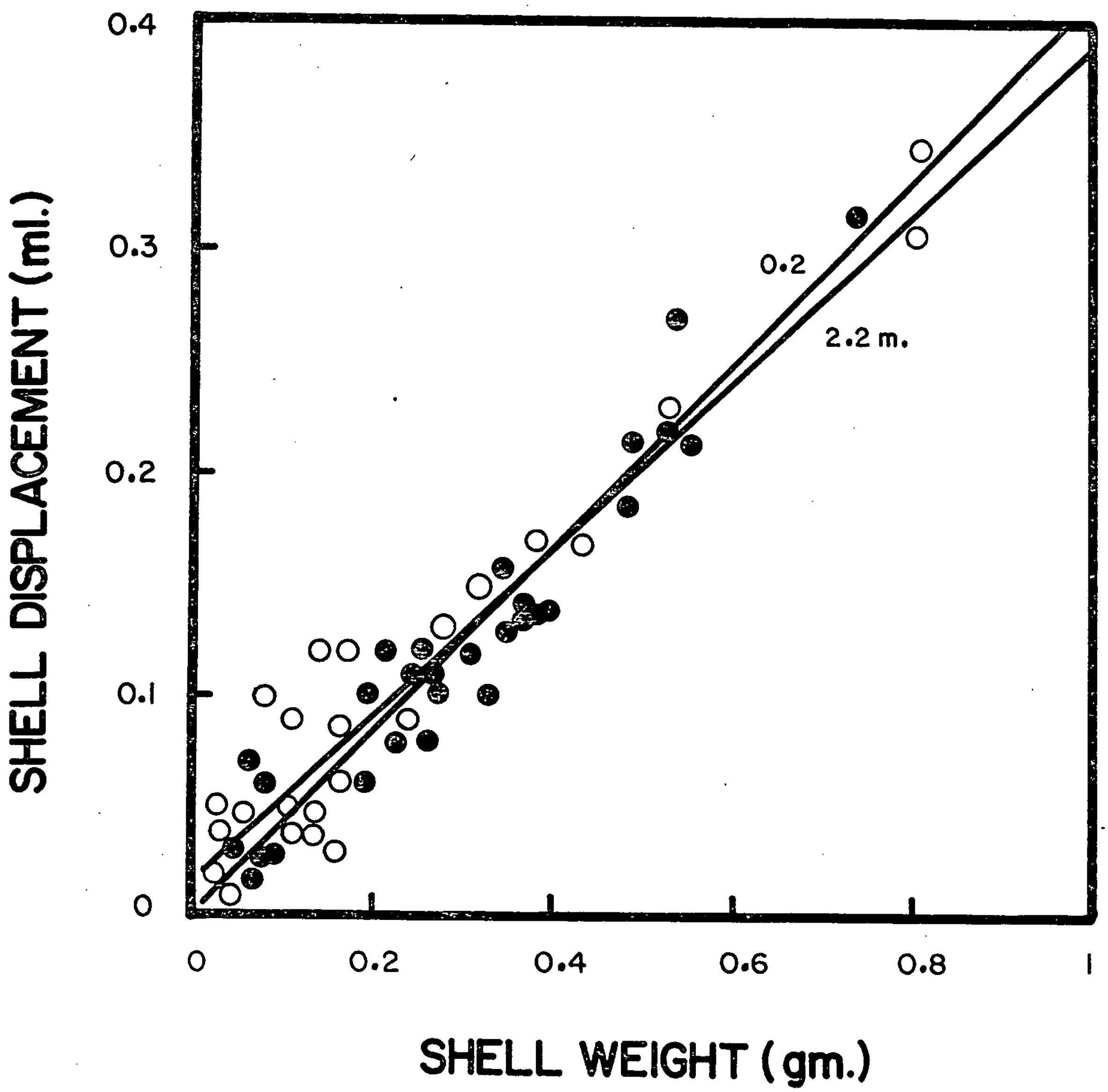
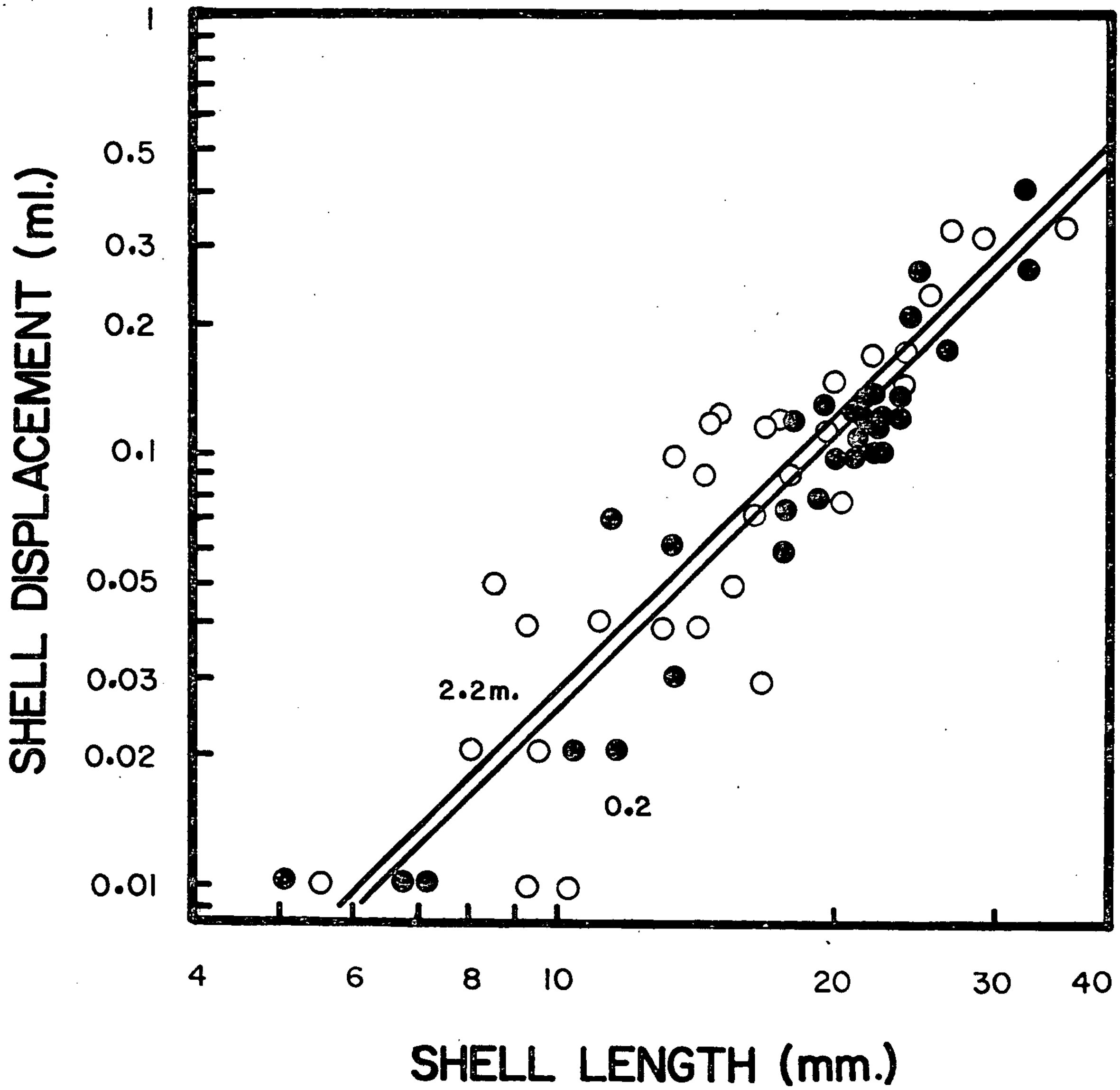

0.4
0.3
0.2
0.1
0
SHELL DISPLACEMENT (ml.)
0.2
2.2 m.
0
0.2
0.4
0.6
0.8
1
SHELL WEIGHT (gm.)

Figure 26. The regression lines of shell valve displacement as a function of shell length in mussels from 2.2 m (o) and 0.2 m (•). The equations of the lines are:

2.2 m; $\log Y = 2.136 \log X - 3.685$ n=36
0.2 m; $\log Y = 2.118 \log X - 3.705$ n=36


SHELL DISPLACEMENT (ml.)
1
0.5
0.3
0.2
0.1
0.05
0.03
0.02
0.01
2.2m.
0.2
4
6
8
10
20
30
40
SHELL LENGTH (mm.)

viscera, respectively. In each of the figures, only the data points from the final interval (32 hours) are shown. Differences between intercepts in the Figures 27-30 are artifacts produced when the regression lines are calculated as an hourly uptake rate. Therefore, these differences are of no real signifigance, and are not discussed further.

Figure 27 shows the calculated regression lines for calcium uptake by the shell as a function of dry shell weight. The slopes of the regression lines show no significant differences, and have a mean value of -0.09. Figure 28 shows the regression lines for calcium uptake by the mantle as function of the dry weight of mantle. The slopes of the regression lines show no significant differences, with a mean slope of -0.01. Figure 29 shows the regression lines for calcium uptake by the gill as a function of the dry weight of gill. The slopes of the regression show no significant differences and have a mean value of +0.23. Finally, Figure 30 shows the regression lines for calcium uptake by the viscera as a function of the dry weight of the viscera. Again, the slopes of the regression show no significant differences, and have a mean value of -0.11.

Using information from the Size Gradient Study, it was possible to calculate the amount of isotope taken up by the shell and tissues of a subtidal mussel of 1.0 gram total dry weight. The shell and tissue weights were calculated to be 0.695 grams shell; 0.104 grams mantle; 0.024 grams gill; 0.177 grams viscera. Using the equations of the regression lines in Figures 27-30 given in the legends of each figure, the uptake rate of

Figure 27. The regression lines of shell calcium uptake/gram dry weight shell/hour as a function of dry weight shell at 1, 2, 4, 8, 16 and 32 hours. Individual measurements of the 32 hour experiment are marked by (•). The equations of the lines are:

1 hr; $\log Y = -0.160 \log X + 2.822$ n=10
2 hr; $\log Y = -0.054 \log X + 2.382$ n=10
4 hr; $\log Y = 0.120 \log X + 2.494$ n=10
8 hr; $\log Y = -0.376 \log X + 1.895$ n=9
16 hr; $\log Y = -0.475 \log X + 1.636$ n=9
32 hr; $\log Y = 0.388 \log X + 1.864$ n=9

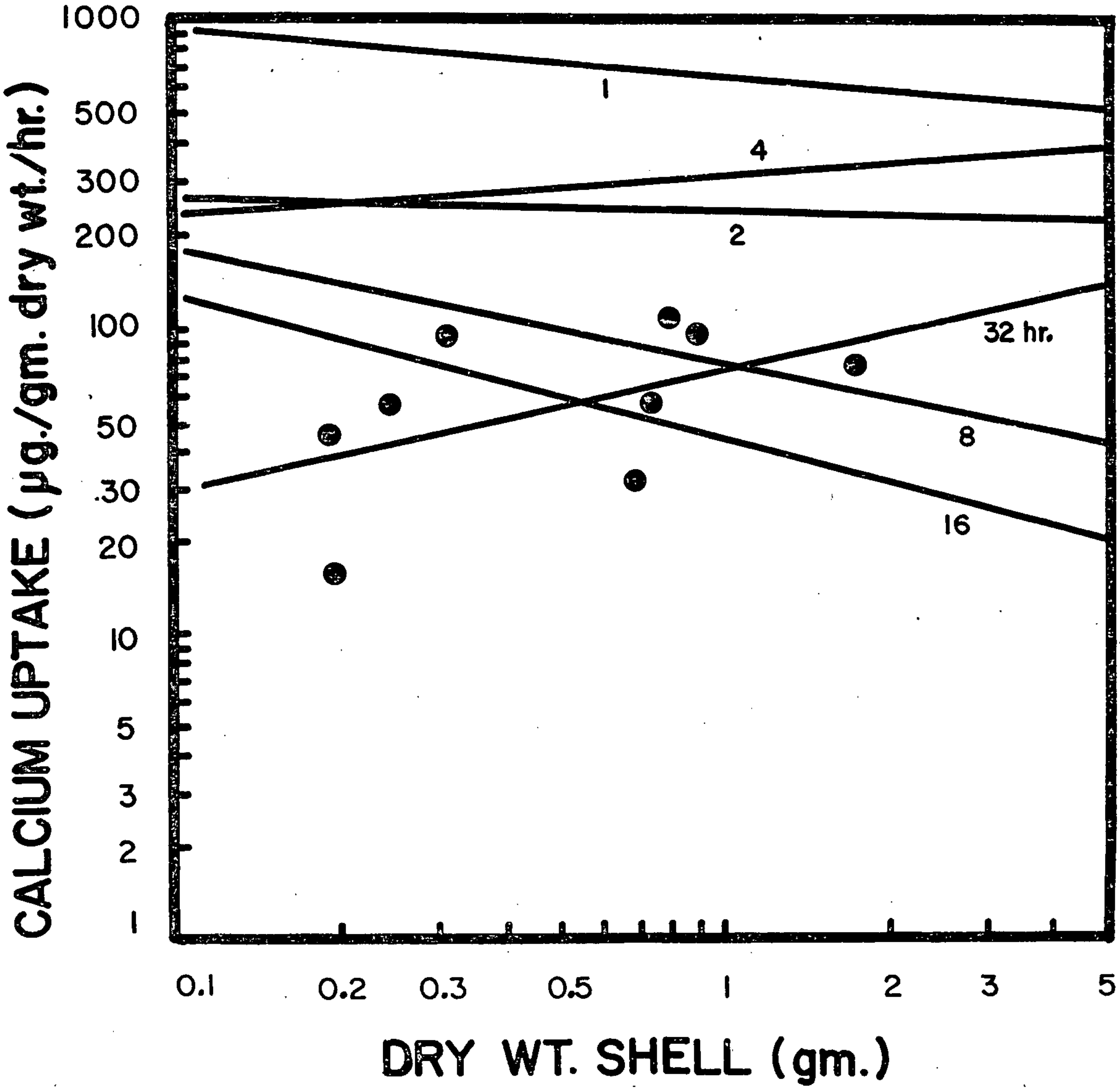

1000
500
300
200
100
50
.30
20
10
5
3
2
1
CALCIUM UPTAKE (μg./gm. dry wt./hr.)
1
4
2
32 hr.
8
16
0.1
0.2
0.3
0.5
1
2
3
5
DRY WT. SHELL (gm.)

Figure 28. The regression lines of mantle calcium uptake/gram dry weight mantle/hour as a function of dry weight mantle at 1, 2, 4, 8, 16 and 32 hours. Individual measurements of the 32 hour experiment are marked by (•). The equations of the lines are:

1 hr;	$\log Y = -0.068 \log X + 2.421$	n=9
2 hr;	$\log Y = 0.056 \log X + 2.544$	n=10
4 hr;	$\log Y = 0.332 \log X + 2.587$	n=9
8 hr;	$\log Y = -0.052 \log X + 1.686$	n=9
16 hr;	$\log Y = -0.254 \log X + 1.414$	n=9
32 hr;	$\log Y = -0.097 \log X + 1.060$	n=8

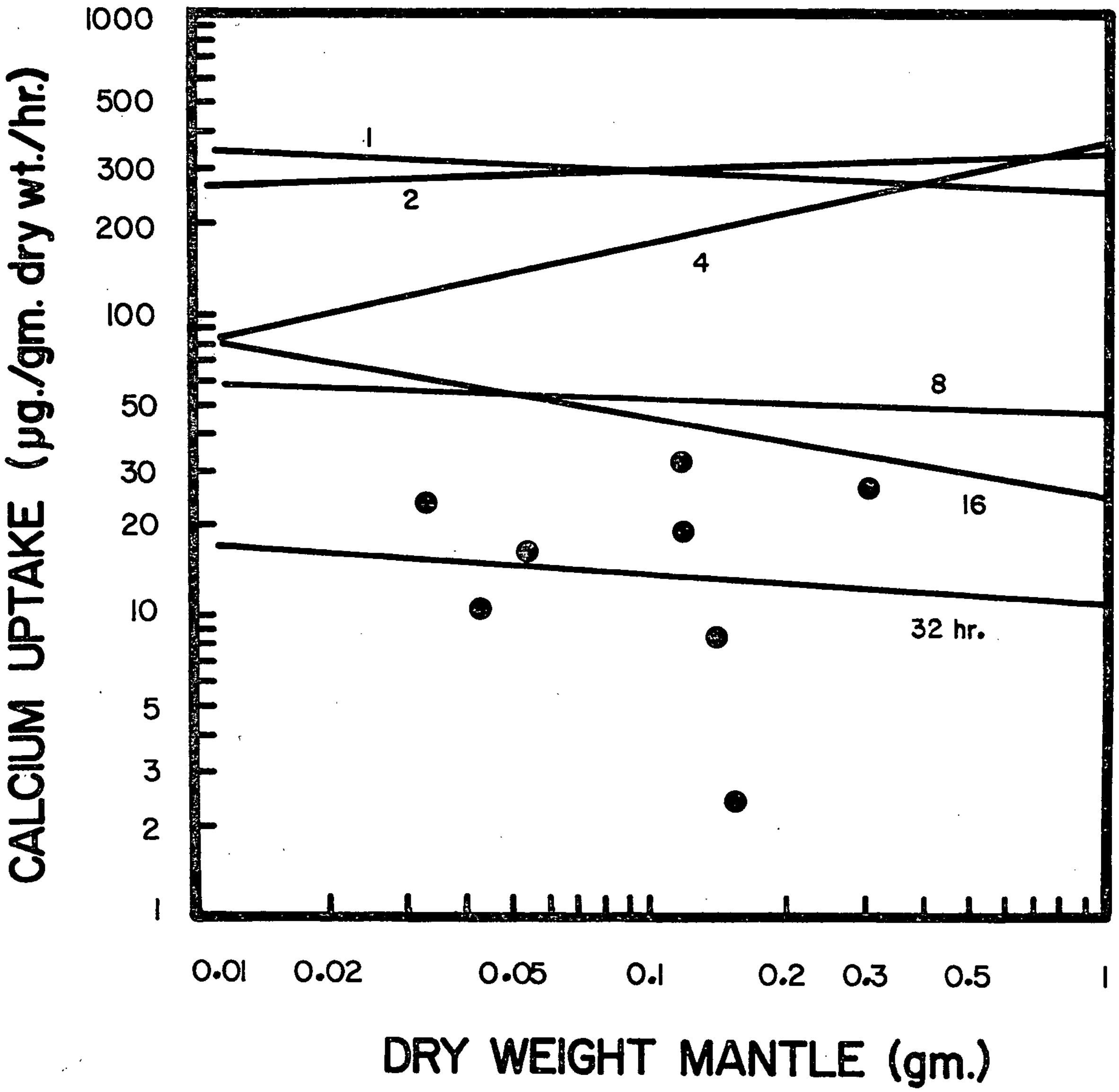

CALCIUM UPTAKE (μg./gm. dry wt./hr.)
1000
500
300
200
100
50
30
20
10
5
3
2
1
1
2
4
8
16
32 hr.
0.01
0.02
0.05
0.1
0.2
0.3
0.5
1
DRY WEIGHT MANTLE (gm.)

Figure 29. The regression lines of gill calcium uptake/gram dry weight gill/hour as a function of dry weight gill at 1, 2, 4, 8, 16 and 32 hours. Individual measurements of the 32 hour experiment are marked by (•). The equations of the lines are:

1 hr; $\log Y = 0.347 \log X + 3.463$ n=7
2 hr; $\log Y = 0.109 \log X + 2.852$ n=7
4 hr; $\log Y = 0.474 \log X + 3.206$ n=8
8 hr; $\log Y = -0.135 \log X + 1.612$ n=5
16 hr; $\log Y = 0.118 \log X + 1.295$ n=9
32 hr; $\log Y = 0.488 \log X + 2.042$ n=9

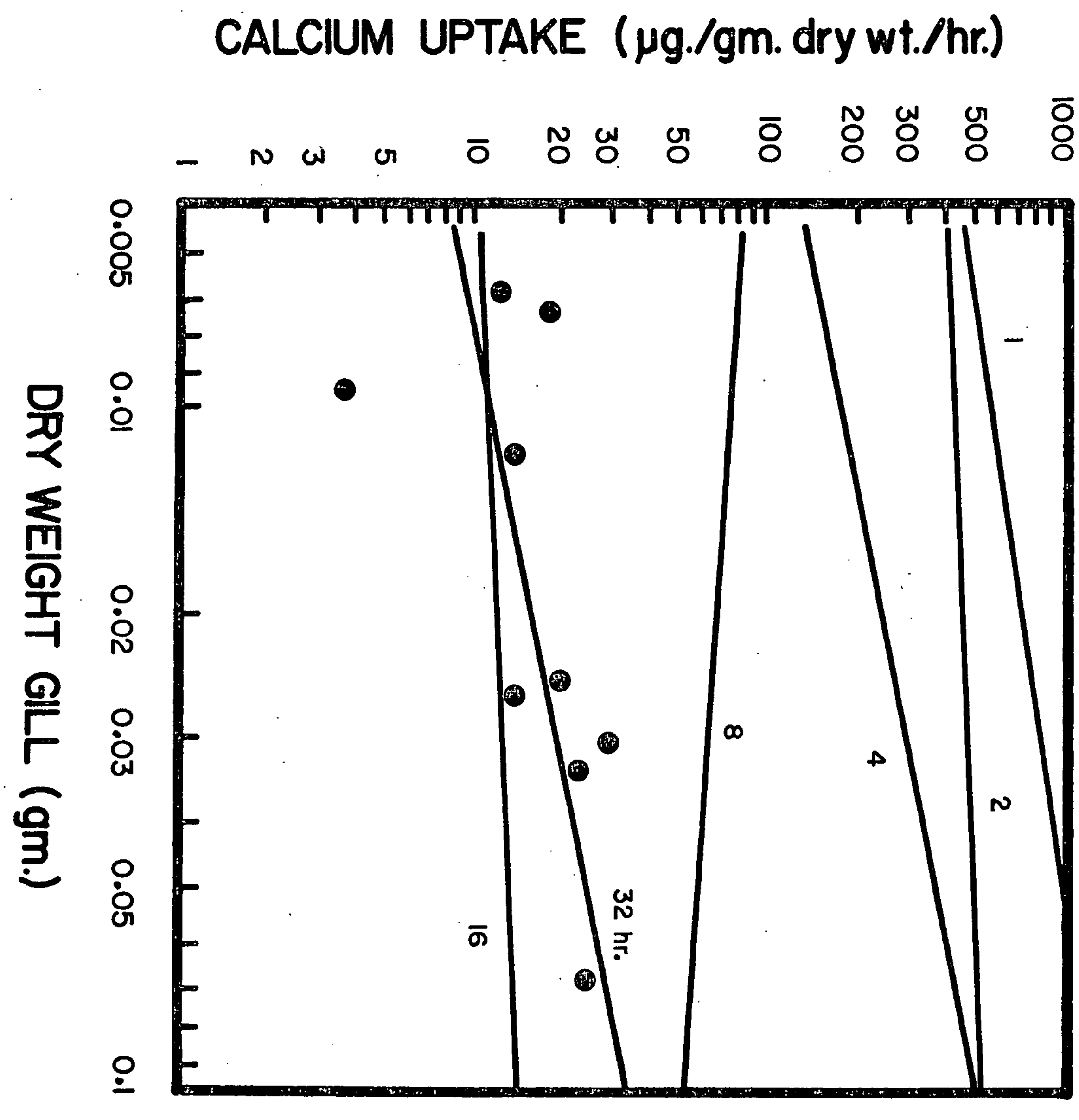
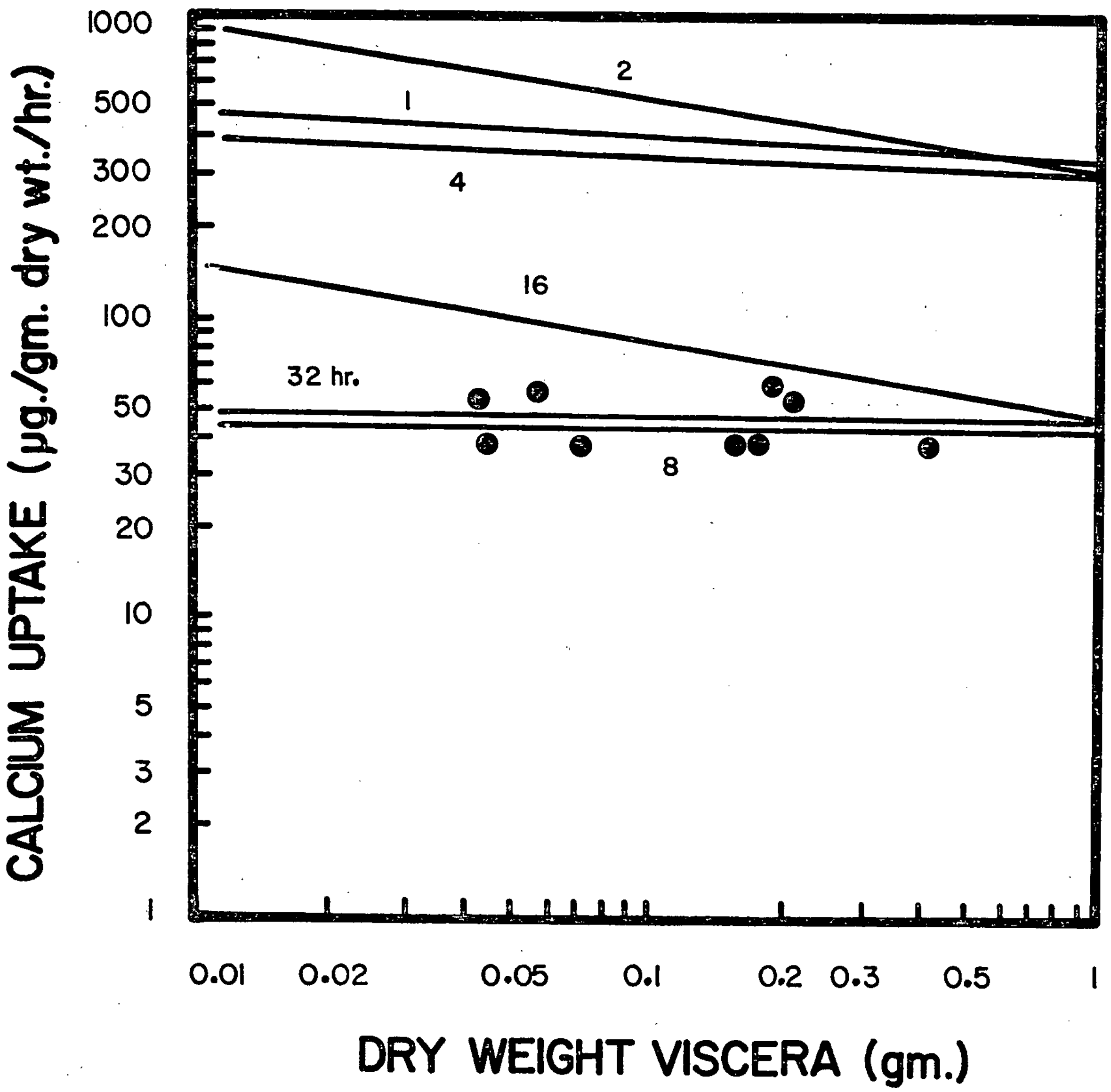

CALCIUM UPTAKE (µg./gm. dry wt./hr.)
1
2
3
5
10
20
30
50
100
200
300
500
1000
DRY WEIGHT GILL (gm.)
0.005
0.01
0.02
0.03
0.05
0.1
1
2
4
8
16
32 hr.

Figure 30. The regression lines of visceral calcium uptake/gram dry weight viscera/hour as a function of dry weight viscera at 1, 2, 4, 8, 16 and 32 hours. Individual measurements of the 32 hour experiment are marked by (•). The equations of the lines are:

1 hr;	$\log Y = -0.074 \log X + 2.525$	n=9
2 hr;	$\log Y = -0.238 \log X + 2.501$	n=10
4 hr;	$\log Y = -0.064 \log X + 2.490$	n=9
8 hr;	$\log Y = 0.026 \log X + 1.726$	n=8
16 hr;	$\log Y = -0.265 \log X + 1.669$	n=10
32 hr;	$\log Y = 0.165 \log X + 1.728$	n=9

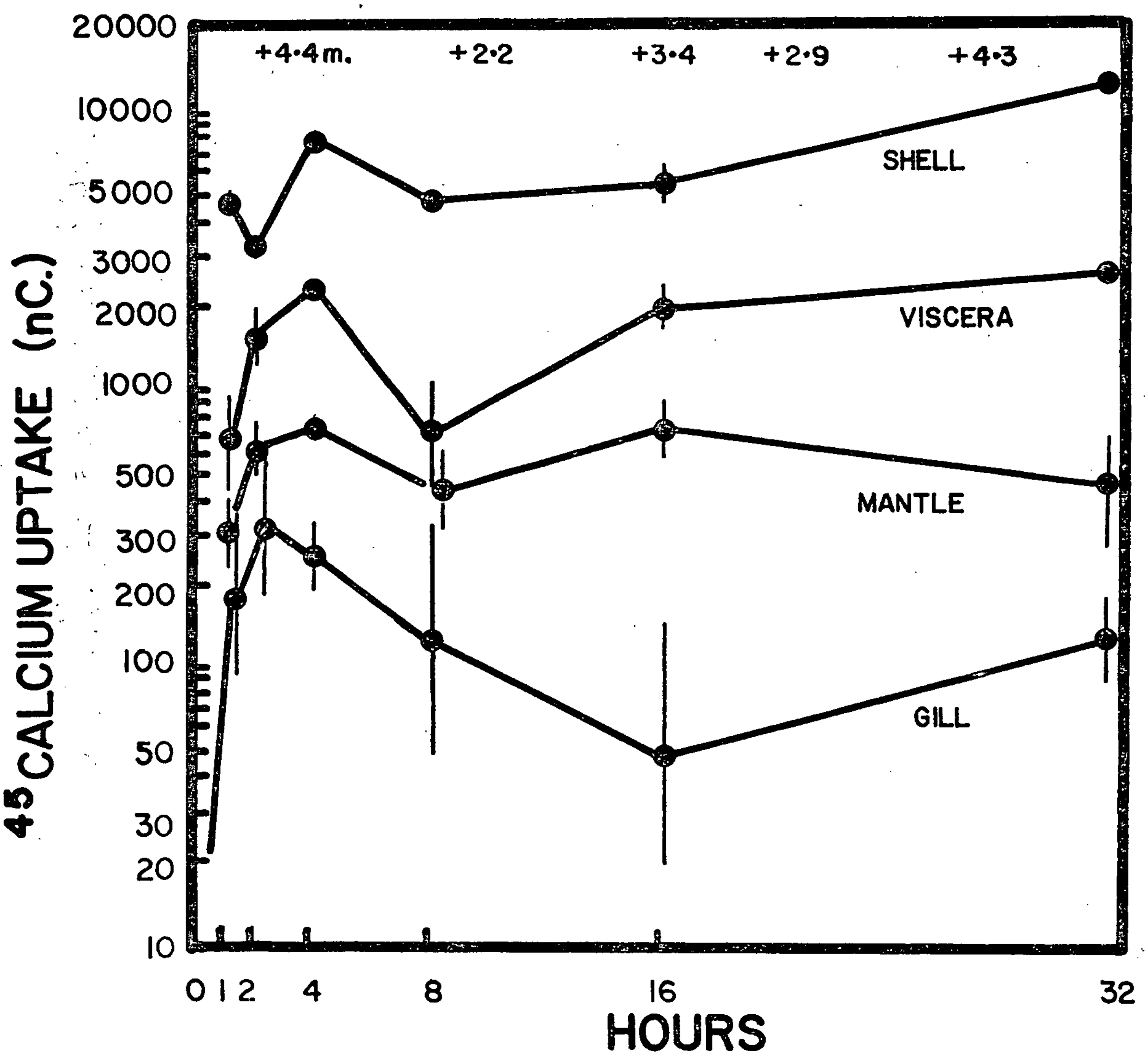

1000
500
300
200
100
50
30
20
10
5
3
2
1
CALCIUM UPTAKE (µg./gm. dry wt./hr.)
2
1
4
16
32 hr.
8
0.01
0.02
0.05
0.1
0.2
0.3
0.5
1
DRY WEIGHT VISCERA (gm.)

each tissue was calculated. Finally, each hourly uptake rate was multiplied by the duration of the experiment and the specific activity of the isotope, 0.0087, to give the total amount of isotope present in the shell and each of the tissues for each time interval of the experiment. This information is displayed in Figure 31, and shows the amount of ^{45}Ca present in the shell, mantle, gill and viscera of a mussel of 1.0 gram total dry weight, measured at intervals over a period of 32 hours. In this figure, the lines connecting the origin of the graph with the mantle, viscera and shell have been omitted for clarity, leaving only the line connecting the origin to the gill at 1 hour. It was assumed that equilibration of the specific activity of the soft tissues and the seawater occurred at the fourth hour, and that it was possible to calculate the amount of calcium in the tissues, based upon the amount of ^{45}Ca present. This assumption is defended in the Discussion.

The calcium taken up by the mantle does not increase after 1 hour, and remains at a low level throughout the experiment. This indicates that the unlabelled calcium in the mantle tissue equilibrates with the ^{45}Ca in the seawater very quickly, and accumulates no net calcium over the course of the experiment. The calcium in the gill shows a similar response at 1 hour, and shows no significant change throughout the experiment. The viscera show no significant change after 4 hours, except for a decrease at 8 hours, followed by an increase at 16 hours to the amount of calcium taken up at 4 hours. The shell removes calcium from the seawater during the course of the experiment, but shows

Figure 31. ^{45}Ca uptake by the shell, mantle, gill and viscera of a 1.0 gram total dry weight winter-adapted mussel (5°C, 100% SW) as a function of time. The dry shell and dry tissue weights are 0.695 grams shell, 0.104 grams mantle, 0.024 grams gill, and 0.177 grams viscera. Vertical bars on the figure represent ±1 S.E. about the mean of each point, the marks along the top of the figure indicate tidal maxima and minima (measured in meters above datum) which occurred during the course of the experiment, and which may explain some of the variation between data points at 4 and 8 hours.

NOTE: These data were log-transformed to make possible their presentation in one figure. Therefore, care must be taken in interpreting differences along the ordinate.



+4·4m.
+2·2
+3·4
+2·9
+4·3
20000
10000
5000
3000
2000
1000
500
300
200
100
50
30
20
10
45 CALCIUM UPTAKE (nC.)
SHELL
VISCERA
MANTLE
GILL
0 1 2 4 8 16 32
HOURS

wide fluctuations in the amount of calcium taken up, especially at 1 hour and 4 hours. These fluctuations may be caused by tidal rhythms, and are dealt with in the Discussion.

In summary, the ^{45}Ca in seawater reaches equilibrium with the calcium of the mantle and gill at 1 hour, and with the viscera at 4 hours. After this equilibration period, the shell accumulates calcium from the seawater. In this experiment, a sizeable fraction of the radioactive calcium taken from the seawater is found in the soft tissues. If the time course of this experiment had been longer, the relative amount of calcium in the soft parts would have been lower compared to the amount of calcium taken into the shell, and, therefore, most of the labelled calcium taken up would represent shell growth. In addition, much of the calcium taken up by the shell occurred in the process of equilibration, and does not represent true calcium uptake. In order to estimate the true uptake rate, the slope of the shell-uptake line was estimated between the 4 and 32 hour intervals. This line yielded an average uptake rate of 32 μg Ca/hr, compared to an estimate of 55 μg Ca/hr which is calculated from the amount of calcium in the shell and soft parts, determined at 24 hours in Figure 31. Therefore, the values of calcium-uptake rate presented in Figures 4, 8, 11, 13 and 17 are overestimated by a factor of 55/32, or 1.7. The presence of a correction factor, while reducing the absolute values of calcium-uptake rate, does not affect the differences between uptake rates which have been shown in these figures. It should also be noted that this correction factor could be different for summer-adapted mussels, since they show weight-dependent regression slopes, and may therefore be taking up calcium by a different mechanism.

DISCUSSION

Salinity experiments

A comparison of the summer and winter experiments shows that the summer regression-lines have slopes which are typically steeper than -0.5, while the winter regression-lines show slopes which are often near zero. This difference is probably not a calcium-specific response, and likely represents a change in the overall metabolism of summer- and winter-adapted mussels -- summer-adapted mussels being less active metabolically than winter-adapted ones. When the results of the three-week experiments shown in Figures 4 and 8 are pooled to give an average uptake rate for the 100% SW experimental groups from each season, winter mussels of 1.0 gram total dry weight show maximum calcium-uptake rates about 1.5 times greater than summer mussels in the same salinity: 59 μg Ca/hr compared to 38 μg Ca/hr. A comparison of the control salinity uptake rates reveals an even greater contrast: 10 μg Ca/hr for the summer control group, and 59 μg Ca/hr for the winter control group. In 25% SW, winter mussels show calcium-uptake rates twice those of summer mussels: 10 μg Ca/hr compared to 5 μg Ca/hr. Further, there is a consistent relationship between the seawater salinity and the rate of calcium uptake during both seasons. The uptake rates of the control groups, as shown in Figures 4 and Figure 8, show no significant change between Days 0, 7, 14 and 21. A reduction in the salinity causes a decrease in calcium-uptake rate when the salinity is below 75% SW, while in most cases an increase in the salinity above 75% SW causes no increase in the uptake rate. During both seasons, mussels were not able to increase their calcium uptake rate in response to extended periods in dilute seawater. However, during the summer experiment shown in Figure 4, the experimental group in 75% SW showed a response between the first and second week of the experiment which could be interpreted as regulation of the uptake rate.

In the case of the summer-adapted mussels (Fig. 4), the 75% seawater group showed an increase at Day 7 compared with the Day 0 control salinity, but returned to the Day 0 control level at Day 14. This change cannot be attributed to starvation, since it would have presumably affected the 100% seawater group similarly. Differences of slope would indicate differences in the metabolic states of the experimental groups, but, with the exception of the Day 0 control, there are no differences among any of the regression slopes in Figures 1-3. Mussels are known to be poor osmoregulators in dilute seawater, and show higher mortality in low salinities than in high ones (Potts 1954; McLachlan & Erasmus 1974; Hoyaux _et al._ 1976). It follows that summer-adapted mussels are subject to osmotic stress which is not lethal, but for which they do not compensate their blood ion composition. Because of this prolonged stress, summer-adapted mussels may have a limited ability to respond to increases in salinity by increasing their calcium-uptake rate, and thus show only a temporary increase in uptake rate when maintained in 75% SW. This interpretation is speculative, but may be supported by the fact that summer regression-lines (Fig. 1-3) are usually quite steep. Although the slopes of the regressions must be interpreted with caution, steep slopes probably indicate low metabolic activity. If low metabolic activity is characteristic of the summer-adapted group, it could predispose them to a reduced ability to respond to the increases in salinity desribed previously. If this is true, it further suggests that the Day 0 summer-adapted control group, which represented the summer-adapted winter mussels referred to in the Material and Methods, showed a low regression slope because they had not acclimated to summer conditions in the time allowed, and that a longer period of adaptation might have resulted in slopes similar to those shown by the Week 1 regression lines (Fig. 1).

When the calcium-uptake rates reported in these experiments are compared with published reports of calcium-uptake rates, it is usually necessary to refer to studies which have measured shell growth rate over monthly, or longer, intervals. Fortunately, since most calcium is used for shell growth, calcium uptake measurements and shell growth rates are comparable. Although shell growth and growth of the soft parts are not synonymous, shell size is commonly used as an indicator of growth. Galtsoff (1934) for example, found that shell growth in <u>Crassostrea</u> <u>virginica</u> continued after low-temperature fasting had begun, and that the weight of the soft parts did not increase during periods of fasting at low temperatures. In his study, Galtsoff found that the shell weight of <u>C</u>. <u>Virginica</u> increased from 60 to 130 grams over 11 months. Assuming that the calcium-uptake rate was independent of weight (as my study has found it to be under most conditions), this indicates an average uptake rate of 39 μg Ca/gram/hr. In their study of the sea mussel Mytilus californianus, Fox and Coe (1943) reported an increase in shell weight from 15 grams to 105 grams over a period of 30 months. This corresponds to an average calcium-uptake rate of 30 μg Ca/gram/hr. When one considers that these two values are annual averages and do not account for seasonal differences in salinity or temperature, the reported values are in general agreement with the uptake rates found in this study, namely 59 μg Ca/hr during the winter season and 38 μg Ca/hr during the summer season (based on a 1.0 gram total dry weight mussel in 100% SW). The agreement is even closer if the correction factor of 1.7 (referred to previously) is applied to my data, giving winter and summer uptake rates of 30 and 22 μg Ca/hr. In a study of shell growth in Crassostrea (Ostrea) virginica, Wilbur and Jodrey (1952) found shell growth to be 0.92 grams/month, determined at 22°C and 35 parts/thousand seawater. However, Wilbur and Jodrey reported only the length of the shells used in their study: 8-9 cm. In an attempt to estimate the weight of shells of this length, in order to compare Wilbur and Jodrey's data, I used two valves of C. gigas from the U.B.C. Invertebrate Museum collection, and calculated the weight of a valve of 9 cm as being 36 grams. Table 2 of Quayle (1969) indicates that oysters with shells of this weight would have a total dry weight of approximately 80 grams. Using these assumptions, the uptake rate of the oysters was 7 μg Ca/gram/hr. This is a very low uptake rate (probably due to the dissection technique), and does not agree with Galtsoff's measurement of shell growth based on 11 months growth: 39 μg Ca/gram/hr. The large difference between these two values underscores the necessity to validate growth measurements based on isotope uptake against actual measurements of increase in shell weight.

In conclusion, a survey of shell growth studies indicates that Mytilus californianus and Crassostrea virginica show similar calcium-uptake rates compared to those reported here for Mytilus edulis. This lends credence to the results given here, since I was concerned with recording uptake rates in short term experiments, while the experiments of Fox and Coe and Galtsoff were concerned with long-term shell growth.

Acute temperature response experiments

The temperature experiments summarized in Figures 11 and 13 display distinct seasonal differences in the response to acute changes in temperature. During the summer, calcium uptake is temperature dependent from 1° to 23°C, although the uptake rates of adjacent experimental temperatures are not usually significantly different. During the winter season calcium uptake is temperature-independent between 5° and 17°C, as shown by Figure 13. The mean regression slope for these temperatures is +0.08 (Fig. 12). The mean slopes of the upper and lower temperatures, 1° and 23°C, are -0.54. Referring to the summer experiment, it should be noted that significant differences were found between the slopes of the two groups of summer-adapted winter mussels: the Day 0 summer control group from the Seasonal Salinity Experiments and the summer Acute Temperature Response Experiment. These two groups had identical previous histories, and were not separated until the experiments were performed. The only difference between the experimental conditions was the absence of light in the case of the acute-temperature response experiment. It may be, then, that the lighting conditions caused this difference.

According to Galtsoff (1934), fasting occurred at temperatures below 4°C in Crassostrea virginica, but he indicated that shell growth was not reduced at temperatures lower than this. Loosanoff and Nomejko (1949) reported fasting in C. virginica at temperatures below 5°C, along with reduced growth in shell weight. They also reported that shell growth was greatest between 15° and 20°C. Richards (1935) has reported that shell growth of Mytilus edulis was reduced at temperatures above 20° and below 5°C, limits which agree with the results of the experiment on winter-adapted mussels in this study. Seed (1968) reported that the shell of Mytilus edulis in England showed little linear increase during the period from October to April, and 90% of their annual shell growth during the remaining interval. Quayle (1969) reported that both the shell and soft parts of Crassostreas gigas ceased growing between November and April, when the water temperature was less than 10°C. In the present study, however, winter-adapted Mytilus edulis continued to take up calcium independently of temperature as low as 5°C. If this abrupt reduction in uptake rate is a general feature of marine bivalves at low temperatures, it helps to explain the reduction of shell growth at low temperatures reported by Richards (1935), Loosanoff and Nomejko (1949) and Seed (1968), and may explain some of the apparent contradictions between studies which report winter growth and those which report no winter growth. It does not explain the basis for the change in uptake rate, but the fasting at low temperatures as reported by Galtsoff (1934) may be responsible for the cessation of a number of metabolic activities, including calcium uptake. The reduction of uptake rate among winter-adapted mussels at high temperatures was noted by Coe and Fox (1942) in Mytilus californianus (season not given) at temperatures above 20°C. This temperature may represent an upper physiological limit which Mytilus californianus is capable of tolerating.

On a seasonal basis, the difference between the regression slopes of summer- and winter-adapted mussels may be attributed to seasonal differences in salinity and food resources between the two seasons. Quayle (1969) reported that winter shell-growth rates in Crassostrea gigas were low in the absence of high phytoplankton concentrations, while soft-part growth, shell growth and the accumulation of glycogen stores (=fattening) were correlated with seasonal increases in phytoplankton concentrations. Quayle also reported a high increase in shell weight in August in the absence of high phytoplankton concentrations, but I suspect that this single observation was not representative of the long-term availability of phytoplankton.

The correlation between phytoplankton concentration, glycogen reserves, and shell growth is obscured by temperature and salinity effects which cannot be separated in field studies. This study has shown that starvation has no effect upon the calcium uptake rates of summer- and winter-adapted mussels measured over a three week period in the laboratory. This conclusion is based upon the fact that the uptake rates of summer- and winter-control mussels did not change significantly during the salinity experiments. However, I think that the reason for seasonal differences in the metabolic states of the mussels lies in the long-term osmotic stress to which summer-adapted mussels are subjected. If this is true, the annual pattern of winter and summer temperature and salinity conditions limits the calcium uptake of mussels in the following way. During winter, mussels experience little low salinity stress, and except at temperatures below 5°C, are able to take up calcium faster than at any other time of year. However,the phytoplankton which they require for their metabolism are typically in very low concentration during the winter (Fox & Coe 1943). In addition, winter temperatures are likely to fall below 5°C occasionally. During the summer the opposite condition exists. Mussels are under osmotic stress, and, therefore, unable to take up much calcium. This occurs at a time when phytoplankton concentrations are high. Since calcium uptake is only one of the metabolic activities of mussels, it is reasonable to expect that during the summer they make use of high phytoplankton concentrations for the growth of soft parts, for gonadal development, and for glycogen stores. This has been shown to take place in Ostrea edulis (Orton 1928), in Mytilus californianus (Fox & Coe 1943), and in Crassostrea virginica (Galtsoff 1934; Loosanoff and Nomejko 1949). Thus, although summer-adapted Mytilus are not well-adapted to take up calcium, they may be expected to feed and increase the weight of their soft parts and accumulate energy reserves. Winter-adapted Mytilus may show reduced growth of soft parts, but given temperatures greater than 5°C are able to continue shell growth in the absence of high phytoplankton concentrations by drawing upon their glycogen reserves. Orton (1928) and Coe and Fox (1942) suggest that an antagonistic mechanism exists which inhibits calcium deposition during periods when glycogen is being synthesized (Orton 1928; Coe and Fox 1942), but this suggestion is untested and may simply be a reflection of changes in shell growth rate as a function of seasonal changes in salinity.

It may be suggested that gonadal development could interfere with calcium uptake and shell deposition by competing for glycogen stores. At the collecting site at Spanish Bank, I noticed spawning mussels from about March until September, and developing gonadal tissue invading the mantle tissue at all times of the year. Since gonadal development occurs throughout most of the year, it is not possible to answer this question based on my data. However, Loosanoff and Nomejko (1949) have found that spawning did not affect the rate of shell growth in Crassostrea virginica. Thus, the relationship between gonadal development and calcium uptake remains unresolved.

In conclusion, the pattern of calcium uptake shown by summer-adapted mussels is much different from that of winter-adapted ones. These differences are evidenced by the differences in the slopes of the regression lines, and by differences in the uptake rates themselves (Figs. 4 and 8). I believe that these differences are caused by the persistence of summer control conditions, namely low salinity and high temperature, which reduce the ability of summer-adapted *Mytilus* to take up calcium independently of weight at any temperature. This is also reflected in the effect of acute changes in temperature upon calcium uptake by summer-adapted mussels.

Intertidal transplant experiment

The results of the reciprocal transplant experiment are shown in Figure 17. These data show that mussels which were transplanted to new intertidal heights had calcium-uptake rates similar to those of untransplanted mussels at the same intertidal height. The variances are large among mussels transplanted from 0.2 m compared with mussels from 2.2 m, and this suggests that there is less variation in the uptake rates of high intertidal mussels. Figure 17 also suggests that these differences in variation persist for at least one month, since mussels originating at 0.2 m generally had uptake rates with large standard errors, while those originating at 2.2 m had uptake rates with small standard errors which persisted at 1.2 and 0.2 m. There is a change in the mean value of the regression slopes, from -0.38 at 2.2 m to +0.12 at 0.2 m, and although these differences are not significant, they demonstrate a trend in the regression slopes which I believe is real, and which suggests that mussels high in the intertidal zone are less metabolically active than mussels low in the intertidal zone. This interpretation is supported by the work of Segal *et al.* (1953), and Rao (1954) who found that the water-pumping rates of high intertidal *Mytilus californianus* were more weight-dependent than their low intertidal counterparts.

Although the reciprocal transplant experiment of the present study demonstrates that both the uptake rate and the regression slopes of mussels within the intertidal zone can be altered by transplantation to different heights, it does not demonstrate how this occurs. Rao (1954) has shown that pumping rates of *Mytilus edulis* and *Mytilus californianus* are synchronous with tidal rhythms, and can be altered by transplantation. His study also showed that tidal pumping rhythms were present in subtidal mussels which were not subject to tidal emersion. It is, therefore, not difficult to imagine that the activity rhythms of intertidal mussels could be reset by the occurrence of new immersion/emersion patterns. Tidal emersion would clearly limit the amount of time available for filtering, gas exchange, and other activities. At the the Spanish Bank collecting site, the average amount of time emersed each day at each of the intertidal positions are as follows: 0.2 m, 0 hours; 1.2 m, 2.6 hours; 2.2 m, 7.0 hours. These emersion times are taken from Quayle (1969), who based his calculations upon annual Canadian Hydrographic Survey tidal records for Point Atkinson. Even when the uptake rate of mussels at 2.2 m are adjusted to account for only 17 hours immersion (by multiplying the uptake rate by 24/17), they still have uptake rates much lower than those at 0.2 m. Assuming, for the sake of comparison, that the duration and rates of pumping of mussels at the two heights were the same, the uptake rate at 2.2 m would only change from 18 μg Ca/hr to 25 μg Ca/hr, still much lower than the uptake rate of 49 μg Ca/hr shown by mussels at 0.2 m. Since the soft parts comprise the sites of calcium uptake before deposition on the shell edge and inner surface, it seems reasonable to presume that the difference in calcium-uptake rates may be correlated with the differences in the weight of soft parts between the high and low mussels. The results of the Size Gradient Study show that mussels from low intertidal heights have greater total dry weight of soft parts than those from high intertidal heights. Using the regressions given in the legends of Figure 21, it can be shown that mussels from the upper and lower heights having a total dry weight of 1.0 gram have a total dry weight of soft parts equalling 0.123 and 0.241 grams, respectively. Assuming, for the moment, that this difference somehow affects the calcium-uptake rate, then the difference between calcium-uptake rates of high and low mussels is removed when these rates are calculated based on the total dry weight of soft parts, and both groups show identical uptake rates: 203 μg Ca/gram total dry weight soft parts/hr if this were true, then mussels transplanted from 0.2 m to 2.2 m might have been expected to show higher uptake rates than the 0.2 m controls. Since this did not occur after one month, the mussels transplanted from 0.2 to 2.2 m may have reduced their weight of soft parts.

Segal (1956a) has shown that the limpet *Acmaea limatula* can resorb gonadal tissue after one month of transplantion from low intertidal to high intertidal sites. So it is possible that the weight of the soft parts did actually decrease. Rao (1953a) found that high intertidal *Mytilus californianus* had less soft parts than low intertidal mussels. In this study, he also showed that high intertidal mussels had lower water pumping rates than low intertidal mussels from the same location, even when measured as a function of the wet weight of the soft parts. So in the case cited by Rao, differences in weight could not be pointed to as the sole cause for the difference in water pumping rates at different intertidal heights. Although other factors may be involved, this leads me to suggest that differences in the weight of soft parts between high and low intertidal mussels are the principal reason for differences in measurements of calcium-uptake rate between these two groups. However, the underlying causes for the difference in the weight of the soft parts of high and low mussels remains unresolved, since it cannot be explained simply by differences in immersion time.

Size gradient study

The present study is not alone in noting differences in growth rates between vertically separated intertidal bivalves. Newcombe (1935) recorded the length of Mytilus edulis taken from 3 feet (1 m) above the mean of the lower low waters (MLLW) in England. The annual growth of these mussels was from 9.2 to 16.0 mm. Assuming, for the sake of comparison, that the shell and soft parts relationships are the same as those described in this study, this indicates a change in shell weight of 0.121 grams and an average calcium uptake rate of 37 μg Ca/gram/hr at about 1 m above datum, compared to 34 μg Ca/gram/hr measured at 1.2 m in this study. This comparison shows that the two uptake rates are approximately equal, but since the tidal patterns at Newcombe's site are unknown, and since there is no assurance that mussels from different locations have the same shell and soft parts morphology (Fox & Coe 1943), the agreement may be fortuitous.

Seed (1968) has also recorded shell length data of vertically and geographically separated populations of Mytilus edulis in England. In this study, Seed calculated the age of mussels on the basis of incremental growth rings. At one site he recorded the length of 9 year old mussels from 'high shore' and 'low shore' sites as 5.5 and 4.0 cm respectively. At another site he recorded lengths from high shore and low shore mussels as 4.0 and 2.0 cm. Employing the same assumptions as the previous example, the uptake rates from mussels at all of these sites are about 7 μg Ca/gram/hr, indicating that the calcium-uptake rates are identical among vertically separated mussels from these sites. This is a very low uptake rate when compared with those rates found in this study, and also when compared with those found for another population of Mytilus edulis from England, 37 μg Ca/gram/hr (Newcombe 1935). However, the mussels used in Seed's study were all presumably very old, and neither the present study nor Newcombe's dealt with animals that old. These low values do raise doubts, though, about the age estimates which Seed made in his study.

In a study of the growth rate of intertidally and latitudinally separated populations of Mytilus californianus, Dehnel (1956) calculated growth rates of southern California mussels from 0.3 m and 1.0 m above MLLW. He recorded the absolute growth of mussels over 30 days from these intertidal heights, and found shell length increases of 2.3 mm and 0.96 mm from animals from 0.3 and 1 m respectively. From these measurements, instantaneous growth rates of 0.002 and 0.0008 were calculated. Using these length increase measurements and growth rates, the total length of the mussels can be calculated to be 38.3 and 40.0 mm for the mussels from 0.3 and 1 m. For the sake of comparison, I have used regression equations given by Coe and Fox (1942) which calculate the weight of the shell and the dry weight of the soft parts of Mytilus californianus, in order to calculate the calcium-uptake rates for these two intertidally separated populations. Using these regressions, Mytilus californianus of 38.3 and 40.0 mm length have calculated total dry weights of 3.64 and 4.08 grams. Mussels of this size show shell weight increases of 0.562 grams and 0.245 grams at 0.3 and 1 m respectively, and these increases correspond to calcium-uptake rates of 79 μg Ca/gram/hr at 0.3 m, and 32 μg Ca/gram/hr at 1 m, without correcting for differences in immersion time. Therefore, it can be shown from Dehnel (1956) that Mytilus californianus which are vertically separated in the intertidal zone also show differences in calcium uptake rate which are similar to those shown by the present study of Mytilus edulis.

Judging from the shell growth studies referred to above, differences in the calcium-uptake rate at different intertidal heights may be common to bivalves. The results shown by Figure 18 indicate that the differences in calcium-uptake rates discussed above may contribute to the gradient of shell lengths which occurs within the intertidal distribution of Mytilus edulis. The collection site had been free from Pisaster or Thais predation for the life of the mussels at the site, and this lends support the interpretation that physical factors are the cause for the size gradient. A size gradient might also have been produced by differential settling of spat at lower intertidal heights, followed by higher settling during later spat falls. It does seem unlikely, though, that subsequent spat fall would have been at sequentially higher intertidal heights. It seems most likely that the size gradient shown in Figure 18 is the result of longer emersion times at higher intertidal heights, and as a consequence, reduced feeding time. However, if differences in feeding time were the only limitation, it would be expected that mussels would simply grow more slowly. Although this may be the case, Figure 19 demonstrates that they also show differential growth of the shell with respect to the total dry weight of the soft parts. This is similar to the findings of Segal (1956b), who reported that the soft parts of high intertidal Acmaea limatula weighed less than low intertidal limpets. However, in the case of A. limatula, high intertidal limpets were also found to have thicker shells. If shell growth requires little metabolic energy, then it is conceivable that mussels may expend little energy growing larger shells. Segal (1956b) and Segal and Dehnel (1962), have reported that the limpet A. limatula living high in the intertidal retains more water in the mantle cavity than its counterparts low in the intertidal zone. They have suggested that during emersed periods and high temperatures, high intertidal limpets allow this water to evaporate, and escape overheating by evaporative cooling of the mantle water. Besides this heat buffering effect, they suggest that the retention of a larger volume of mantle water would also buffer the osmotic stress caused by water loss from the soft parts during long periods of emersion. This hypothesis may explain how mussels living high in the intertidal zone adapt to their location, since they have a relatively larger mantle volume than low intertidal mussels. In another study of mussels, Rao (1953b) found that the weight of soft parts varied as a function of intertidal height for both Mytilus edulis and Mytilus californianus. In the case of Mytilus edulis, he found that low intertidal mussels had greater shell weights for the same wet weight of soft parts when compared with high intertidal mussels, in contradiction to the results shown in Figure 19 of this study. In Rao's report, he compared <u>Mytilus edulis</u> underneath floating wharves with mussels growing on pilings at 0.5-0.6 m and found that for 10 grams wet weight of soft parts, the subtidal mussels had shells weighing 26 grams, while the intertidal mussels had shells weighing 17.5 grams. From these data, he concluded that the weight of the shell was dependent upon the immersion time. These results are difficult to explain, since they conflict with his earlier (1953a) report, with the results of this study, and with the results of Segal (1956b) for <u>A. limatula</u>.

<u>Time course uptake study</u>

The results of the time-course study indicate that the soft parts become saturated with ^{45}Ca within the first four hours of immersion in labelled seawater, and with some variations, maintain a constant level of isotope after that period. In a similar study by Wilbur & Jodrey (1952), the mantle of <u>Crassostrea virginica</u> required only 30 minutes to reach isotope saturation. The weight of the soft part tissue appears to be correlated with the length of time required for saturation. For example, in a mussel of 1.0 gram total dry weight, the gill, with a dry weight of 0.024 grams, was saturated after one hour, the mantle, weighing 0.104 grams, was saturated after two hours, and the viscera, weighing 0.177 grams, became saturated after four hours. There is also a correlation between the dry weight of the tissue and calcium-pool size. The calcium pools of the mantle, gill and viscera in a mussel of 1.0 grams total dry weight were determined by the mean level of calcium in each tissue after reaching saturation with ^{45}Ca, and found to contain 62, 19 and 249 micrograms respectively. When adjusted for differences in weight, however, it becomes apparent that the mantle and gill behave similarly in the amount of calcium they carry in their tissues. The gill carries about 770 μg Ca/gm, and the mantle about 600 μg Ca/gm. By comparison, the viscera carry the highest concentration of calcium, 1400 μg Ca/gm. Since calcium is always associated with muscular activity (Szent-Györgyi 1975), this difference may be attributed to the presence of muscle tissue in the viscera, compared with its absence in the gill and the small amounts found in the mantle. When the soft parts are considered in total, the average concentration of calcium is 0.12%, which is in agreement with a chemical determination made by Fox and Coe (1943), who reported a concentration of 0.15% calcium in the soft parts of *Mytilus californianus*. This supports the conclusion that the soft parts are saturated with ^{45}Ca at 4 hours, and that the unlabelled calcium can be determined from the isotope activity. In comparison to the soft parts, the shell, after the four hour equilibration, continues to accumulate calcium for the remainder of the experiment, and shows a real uptake rate of about 32 μg Ca/hr.

In addition to the results discussed above, there are some noteworthy variations in tissue calcium levels. The gill reaches a plateau in calcium level after one hour, and thereafter shows variations which are obscured by the large variances found throughout the course of the experiment. The reason for the high variability in the gill calcium level is unknown, but has been noted by Chaisemartin *et al.* (1969) in a study of the freshwater bivalve *Margaritifera* (no species given) in a study of the marine bivalves *Pteria martensii* and *Hyriopsis schlegelii*, Horiguchi (1958) has reported that the gill is active in calcium uptake and storage, but that the calcium found in the gill is extremely labile, and may be turned over in as little as 10 minutes. This is in agreement with a report by Rao and Goldberg (1954), who, in a autoradiographic study, showed that the gill of *Mytilus californianus* is the first tissue to take up calcium. They also found that the mucus which coated the gills adsorbed calcium rapidly, and they concluded that most calcium uptake took place by adsorption onto the mucous sheet, followed by ingestion into the gut. A similar study by Fretter (1953), using ^{90}Sr, reached the same conclusion. The shell shows a significant variation in calcium at the second hour. However, since the soft parts did not reach equilibrium until the fourth hour, changes in the the specific activity of ^{45}Ca in the shell may have produced this artifact during the equilibration of the specific activity in the whole animal. A more serious question is raised by the combined variation of the shell and viscera at 8 hours. This variation indicates a drop in the shell calcium from 825 to 500 μg and a decrease in visceral calcium from 240 to 70 μg; a combined loss of almost 500 μg calcium which represents a 54% decrease in the total amount of calcium present at the fourth hour. It is possible that this loss of calcium is due to a change in water pumping rhythms or to closing of the valves for a period of time. This is consistent with Rao's report (1954) that pumping rates of subtidal *Mytilus californianus* are synchronous with their intertidal counterparts, that water pumping rates are highest at high tide, and lowest at low tide, and that the synchrony of pumping is maintained for long periods when *Mytilus californianus* is held in the laboratory. Thus, during the course of this experiment, the mussels would have maintained tidal rhythms synchronous with those in the field. The Canadian Hydrographic Survey tidal predictions (Anonymous 1981) for the day of the experiment (Hour 0 = 0830 27 January 1981) have provided the tidal information shown in Figure 31. These tidal records indicate that the tide was high from Hour 2 until Hour 4, but at its lowest point around Hour 8. If the valves were closed for a period around the time of low tide, then it is possible that the mussels were subject to anaerobic conditions. Under these conditions bivalves have been shown to dissolve calcium from the shell in order to buffer pH changes in the blood (Akberali *et al*. 1977). For the time periods after Hour 8, tidal effects would not be detected because of the long intervals between samplings.

In conclusion, the soft parts of *Mytilus edulis* become saturated with labelled calcium within four hours of the introduction of the label. The shell continues to accumulate calcium, and is at all times the largest pool of calcium. The dynamics of calcium transport between tissue compartments are not well understood, but may be affected by variations in activity that are synchronous with the tides.

SUMMARY

1. The calcium-uptake rate of Mytilus edulis (Linnaeus) was examined under different seasonal, salinity, temperature and intertidal height conditions. The relationship between the total dry weight of soft parts and the weight of shell was studied at two intertidal heights.

2. There were significant differences between summer- and winter-adapted mussels subjected to three weeks immersed in seawater varying in salinity from 25% to 125% SW (100% SW = 480 mEq Cl^-/liter). The calcium-uptake rate was directly correlated with salinity in both seasons, but showed no increase in uptake rate in salinities above 75% SW. Summer-adapted mussels had lower uptake rates at all salinities when compared to winter-adapted mussels. For example, the calcium-uptake rates of summer and winter-adapted mussels of 1.0 gram total dry weight were 38 μg Ca/hr and 59 μg Ca/hr in 100% SW.

3. There were differences between summer- and winter-adapted mussels subjected to acute changes in temperature. Summer-adapted mussels were more temperature dependent than winter-adapted mussels, which took up calcium independently of temperature between 5° and 17°C.

4. There were significant differences in the calcium-uptake rates of intertidal mussels from 0.2 and 2.2 m above datum.

These differences were shown to be interchangeable by reciprocal transplantation. Mussels from the high site (2.2 m) had lower calcium-uptake rates than mussels from the low site (0.2 m); 18 μg Ca/hr versus 49 μg Ca/hr. Differences in the immersion times at the two sites were not sufficient to account for all of this difference. If the uptake rates of mussels from the high and low intertidal sites were based upon the total dry weight of soft parts, instead of the total dry weight, then it was found that the difference in uptake rate between the two sites disappeared. It was shown that there was an intertidal size-gradient in the shells of mussels. This gradient may be related to the reduction in the calcium-uptake rate among mussels higher in the intertidal zone.

5. Mussels from 0.2 and 2.2 m were found to have similar dry weights of mantle, gill and viscera compared to the total dry weight of soft parts, but mussels at 2.2 m had less total dry weight of soft parts in proportion to the total dry weight, compared to mussels at 0.2 m.

6. A time-course uptake study showed that the soft parts became saturated with ^{45}Ca within four hours, but that significant fluctuations took place between sampling times. It was shown that these fluctutions might be explained by tidal opening or pumping rhythms of the mussels. After the equilibration period, the soft parts did not accumulate any calcium, while the shell showed a net accumulation of calcium

throughout the experiment.

7. A correction factor was established, based on the net uptake of the shell after the equilibration of the shell and soft parts. It was found that absolute uptake rates were about 59% of the uptake rates measured here.

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