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DIFFUSIONAL WATER PERMEABILITY IN SELECTED MARINE BIVALVES

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The intertidal habitat presents many severe problems to the organisms living in this particular environment. Cyclic fluctuations in salinity, oxygen levels and temperature occur, and these changes in the physical parameters of this environment are amplified with increasing height above the sublittoral zone. Organisms which inhabit the intertidal area have undergone certain adaptations which increase their survival potential in the face of a constantly changing environment.

In crustaceans, the permeability of the body surface to water and ions can be correlated with the animals' particular habitat (Lockwood, 1962; Herreid, 1969a, b). In this case, sublittoral crustaceans are more permeable than littoral species, which in turn are more permeable than estuarine species. Pieces of exoskeleton from crustaceans which are osmoregulators, and for the most part intertidal, are less permeable than osmoconformers, which are generally sublittoral (Gross, 1957). In barnacles, the resistance of the organism to desiccation is a function of its vertical zonation level, *i.e.*, the higher the species in the intertidal zone, the more resistant it is to desiccation (Foster, 1971). Some intertidal crustaceans apparently are capable of controlling the permeability of the exoskeleton and decrease water permeability in response to decreased external salinity (Smith, 1970; Lockwood, Inman and Courtenay, 1973).

Physiological adaptations to some environmental stress situations have also been demonstrated in intertidal molluscs. Resistance to desiccation in certain intertidal gastropods has been correlated with vertical zonation (Brown, 1960), and the sequence of thermal death points in some intertidal gastropods has also been correlated with zonation level (Broekhuysen, 1940). Physiological adaptations at the tissue level have also been demonstrated in intertidal bivalve molluscs (Vernberg, Schlieper and Schneider, 1963). In this case bivalve gill tissue from intertidal animals could withstand a wider range of salinity changes than could sublittoral animals as determined by gill ciliary activity. Behavioral adaptations of intertidal bivalves include closing of the valves during periods of osmotic stress (Krogh, 1939).

A question arises as to whether or not the tissues of intertidal bivalve molluscs display the same adaptive water permeability characteristics as intertidal crustaceans, *i.e.*, a decrease in water permeability with increasing height in the intertidal zone; or whether the distribution of intertidal bivalves is influenced mainly by other environmental factors independent of salinity (Pilgrim, 1953). In addition, can specific intertidal bivalves alter the diffusional water permeability of their tissues in response to an osmotic stress, as is apparently the case in crustaceans? These

problems were investigated in a series of marine bivalves by measuring the rate of movement of tritiated water across the isolated mantle tissue.

Materials and Methods

The diffusional water permeability across isolated mantle tissue was determined in eight different lamellibranch species obtained from different collection sites in New England. Water samples were also collected from each collection site and total salinity determined by potentiometric chloride titration. Specimens of *Placopecten magellenicus* (Gmelin) and *Modiolus modiolus* (L.) were both collected from waters off Mount Desert Island, Maine. *Placopecten* was collected from a depth of 10 m, while *M. modiolus* was found at a depth of 2 to 3 m, in both cases the salinity of the water was approximately 32‰. Specimens of *Spisula solidissima* (Dillwyn) were collected by the Supply Department of the Marine Biological Laboratory, Woods Hole, Massachusetts. *Spisula* was found approximately 2 m deep on a sandy bottom in water with an average salinity of 33‰. This population of *Spisula* was never exposed at low tide. Specimens of *Mercenaria mercenaria* (L.) were collected from Narragansett Bay, Rhode Island, in water 0.5 to 2 m deep with muddy-sand substratum and having a salinity range from 24 to 31‰. Two different groups of *Mytilus edulis* (L.) were collected from the rocky shores off Jamestown, Rhode Island. One group was located low in the intertidal zone (ELWN), while the other group was found sublittorally in approximately 5 m water. The salinity of the water at this site ranges from 26 to 32‰. Specimens of *Modiolus demissus* (Dillwyn), *Crassostrea virginica* (Gmelin) and *Mya arenaria* (L.) were all collected from Sippiwissett salt marsh near Woods Hole and specimens of *Anodonta* sp., also used in this study, were obtained from a local biological supply house. Mantle water permeability, unless indicated otherwise, was measured in freshly collected animals.

The mantle was used in these studies because of its relatively simple structure, an epithelial sheet with two cell layers (Neff, 1972), and the ease with which it can be removed from the animal. The mantle was excised from the animal by cutting the adductor muscles to open the valves and then removing the central portion of the mantle from one of the valves where it was not attached. The mantles were always covered with sea water during dissection and experimental procedure, except for *Anodonta* for which a Ringer's solution was used (Istin and Kirschner, 1968).

The isolated mantle was used to separate a diffusion chamber into two compartments of 10 ml each, the diameter of the exposed tissue being 0.5 cm. The diffusional water permeability (P_d) of the isolated mantle was determined by adding 10 to 50 μCi tritiated water (THO) to one compartment and monitoring its rate of appearance in the other compartment. Both compartments were constantly stirred by bubbling air through the medium. Aliquots (100 μl) of the medium in the second compartment were taken at various time intervals, placed in a scintillation vial with 15 ml Aquasol (New England Nuclear) and counted in a liquid scintillation counter. Each aliquot removed during the course of an experiment was replaced with an equal volume of fresh medium in order to maintain constant volume.

Samples of the medium in the compartment to which THO was initially added were also counted. Since identical solutions are on both sides of the mantle and it is assumed that the concentration of THO added to the first compartment remains constant for the duration of the experiment, P_d can be calculated from the following relationships: first, specific activity (SA) = [THO]/[H_2O]; secondly, tritiated water flux, J_{THO} = slope of the linear portion of the flux curve/mantle surface area; thirdly, water flux, $J_{H_2O} = J_{THO}/SA$; and fourthly, diffusional water permeability, $P_d = J_{H_2O}/[H_2O]$. Mantle thickness was determined by freezing isolated pieces of tissue in an acetone-dry ice bath, then measuring the thickness of broken pieces of the frozen mantle through a microscope with a calibrated ocular micrometer.

Specimens of *Mytilus edulis,* collected by the MBL Supply Department from Lucas Shoal off of Martha's Vineyard at a depth of approximately 9 m, were used in another series of experiments designed to determine whether or not this particular bivalve species is capable of altering its tissue water permeability in response to an induced environmental osmotic stress. Approximately fifty individuals were randomly divided into two groups after the mantle water P_d was initially determined for five individuals. One group of animals was maintained in ten liters of aerated, full-strength sea water, while the other group was maintained in an equal volume of 70% sea water. The water for both groups of animals was changed daily. Mantle

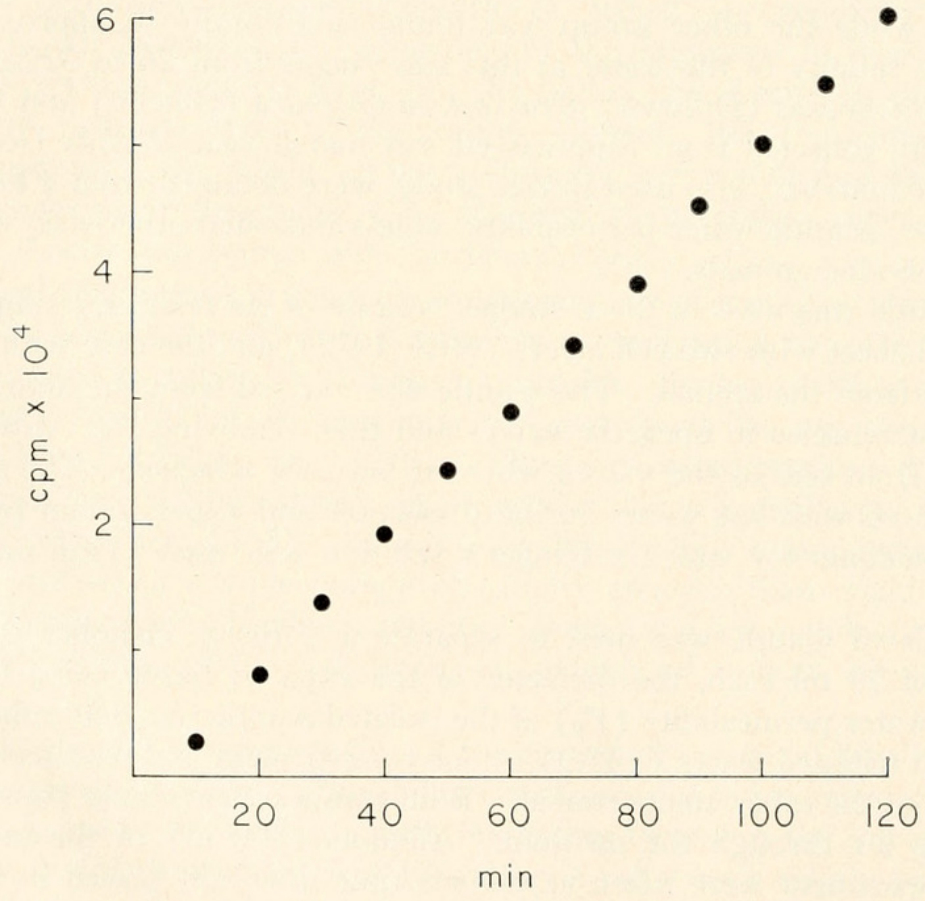

FIGURE 1. Unidirectional, tritiated water movement across an isolated piece of *Mercenaria mercenaria* mantle tissue. The total counts per minute appearing in the bath are presented as a function of time.

water P_d was determined for animals from both groups at various time intervals after the start of the equilibration period.

In all of the experiments in this study, no selection for size was made of the animals used, except that each animal had to be large enough to yield a piece of mantle tissue which would cover the hole between the chambers. All experiments were performed at room temperature, 19 to 20° C. In most instances this temperature was higher than the environmental temperature the animals were collected at, but most likely this temperature change has a minimal effect on the measured mantle water P_d. For example, the diffusion of water between 10 and 20° C is not greatly influenced by an increase in temperature, $Q_{10} = 1.04$. The results are presented as the mean (number of determinations) ± the standard error of the mean.

Results

A representative THO flux curve is shown in Figure 1. THO was added to one side of the flux chamber separated into two compartments by the isoated mantle tissue, and its rate of appearance followed on the other side of the chamber. Since the amount of isotope added to the first compartment is relatively large, and in the time course of these experiments it decreases only slightly (less than 5%), the flux curve is linear after an initial lag period. The observed lag period in these experiments, 3 to 10 minutes, is most likely due to the initial time required for a constant specific activity to be established in the intracellular compartments of the isolated mantle tissue and did not vary in the different species examined in this study.

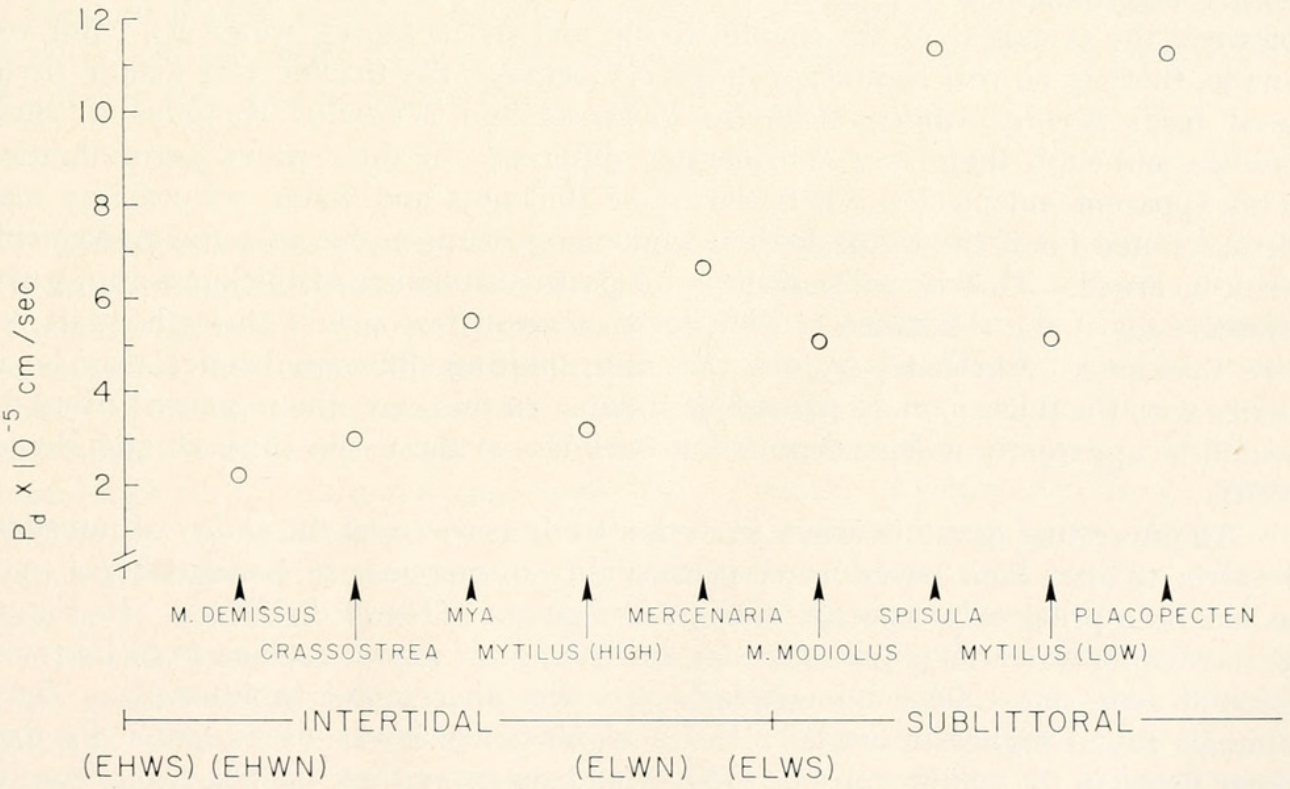

Figure 2. Calculated diffusional water permeabilities (P_d) for isolated mantle tissue from a series of lamellibranch molluscs as a function of their relative distribution to each other.

TABLE I

Mantle thickness in a series of bivalves in which the diffusional water permeabilities were also determined.

	Mantle thickness mm
Modiolus demissus	1.15 ± 0.06 (12)
Mytilus edulis (high)	1.28 ± 0.09 (6)
Mercenaria mercenaria	0.76 ± 0.05 (10)
Modiolus modiolus	1.04 ± 0.07 (7)
Spisula solidissima	0.73 ± 0.02 (6)
Anodonta sp.	0.68 ± 0.05 (7)

Calculated diffusional water permeabilities for eight different marine lamellibranch species are presented in Figure 2 as a function of their distribution in relation to one another. These P_d values are as follows: *Modiolus demissus,* 2.2 ± 0.18 (12) ; *Crassostrea virginica,* 3.01 ± 0.28 (7) ; *Mya arenaria,* 5.51 + 0.19 (9) ; *Mytilus edulis* (high), 3.21 ± 0.61 (6) ; *Mercenaria mercenaria,* 6.69 ± 0.25 (10) ; *Modiolus modiolus,* 5.08 ± 0.24 (7) ; *Spisula solidissima,* 11.40 ± 0.67 (6) ; *Mytilus edulis* (low), 5.18 ± 0.45 (8) ; and *Placopecten magellanicus,* 11.29 ± 0.07 (5) $\times 10^{-5}$ cm/sec. In addition the diffusional water permeability for the isolated mantle tissue of a representative freshwater bivalve, *Anodonta* sp., was found to be 6.17 ± 0.22 (7) $\times 10^{-5}$ cm/sec.

Measurements of the thickness of the mantles used in some of these permeability determinations are given in Table I. There is apparently no correlation between the thickness of the mantle tissue and its measured water P_d. For example, there is no real significant difference between the thickness of mantle tissue used from *Mytilus edulis, Modiolus demissus* and *Modiolus modiolus* in these studies, although there is a considerable difference in their water permeabilities. This apparent independence between tissue thickness and water permeability may be accounted for if the tissue itself is nonhomogeneous as far as water movements are concerned. That is, permeability is a positive function of thickness in a given system only if the resistance to diffusive movement is constant throughout its entire thickness. Alternatively, one thin rate limiting diffusion barrier may be in series with the thicker, more permeable tissue. In this case, the measured tissue P_d would be apparently independent of the thickness of the tissue (Prusch and Benos, 1976).

An interesting question arises from this study concerning the ability of intertidal bivalves to alter their tissue water permeability in response to osmotic stress, such as has been previously reported in some crustaceans (Herreid, 1969a). In Figure 2, the mantle water P_d is presented for two groups of *Mytilus edulis* from the same general area, one collected intertidally and the other found sublittorally. Those animals found higher intertidally had a significantly lower tissue water P_d than those found in the sublittoral zone. This would suggest either that the two groups of mussels represent different physiological races or that this animal is capable of altering its tissue permeability in response to an osmotic or desiccation stress situa-

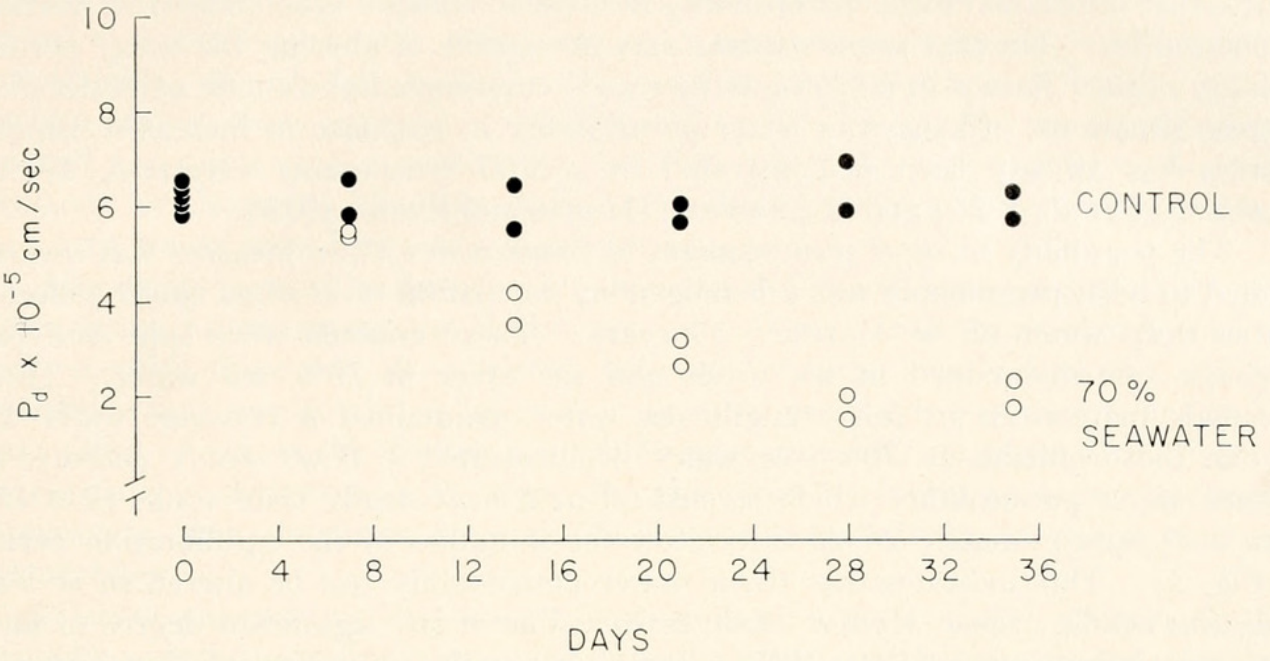

FIGURE 3. Diffusional water permeability as a function of time in *Mytilus edulis;* solid points represent animals maintained in full strength sea water; and open points; animals maintained in 70% sea water.

tion. In experiments with a different population of *Mytilus edulis* in the Woods Hole area, it was found that these animals could indeed decrease their tissue permeability in response to an altered osmotic environment (70% sea water) as is shown in Figure 3. Those animals maintained in full-strength sea water maintained a constant mantle water P_d for the duration of the experiment, 35 days, at about 6×10^{-5} cm/sec, while those in 70% sea water slowly decreased their mantle water P_d during this time period to about 2×10^{-5} cm/sec.

DISCUSSION

Diffusional water permeabilities in a series of lamellibranchs, as measured across the isolated mantle tissue, can be correlated with the habitat of the animal (Fig. 2). The animals used in this study can be divided into several groups: high intertidal or estuarine, low intertidal, and sublittoral. In general, the diffusional water permeability of the mantle decreases with increasing osmotic or desiccation stress to which the animal is exposed. That is, *Modiolus demissus* and *C. virginica,* which are found high in the salt marsh, are much less permeable to water than are *P. magellanicus* and *S. solidissima,* which are located in relatively deep water. Organisms which are exposed intermittently or for brief periods of time, such as *Mya, Mercenaria* or *M. modiolus,* have intermediate diffusional water permeabilities.

In this particular study, two different groups of *Mytilus edulis* were used. One group was distributed on rocks low in the intertidal zone, approximately ELWN, while another group at the same site (Jamestown, Rhode Island) was found sublittorally. As can be seen in Figure 2, water P_d in the isolated mantle tissue from these two groups of animals is significantly different, with animals from the higher-zoned area having the lower water permeability. This not only indi-

cates that diffusional water permeability in bivalve molluscs is influenced by vertical zonation level, but that some bivalves may be capable of altering the water permeability of their tissues in response to increased environmental osmotic or desiccation stress situations. Changes in water permeability in response to increased osmotic stress has already been demonstrated in several crustaceans (Herreid, 1969a; Lockwood *et al.*, 1973) and in *Limulus* (Hannan and Evans, 1973).

The possibility of short term changes in tissue water P_d in bivalves was investigated in what presumably was a homogenous population of *Mytilus edulis* collected from deep water off of Martha's Vineyard. These animals were split into two groups, one maintained in sea water and the other in 70% sea water. Those animals maintained in full-strength sea water maintained a constant water P_d, while those animals in 70% sea water demonstrated a slow, steady decrease in tissue water permeability which leveled off at a new steady state value (2×10^{-5} cm/sec) approximately 24–28 days after the initiation of the equilibration period (Fig. 3). This indicates that tissue water permeability can be altered in at least *Mytilus edulis*. Since *Mytilus edulis* is incapable of any significant degree of ionic or osmotic regulation (Potts, 1954), then what is the physiological significance of this permeability change? It may simply be that those specimens of *Mytilus*, which are located in areas subjected to alterations in the osmotic and ionic environment, decrease the rate of tissue equilibration in response to these environmental alterations by decreasing their tissue permeability.

Anodonta, a freshwater lamellibranch, has a diffusional water permeability which is also intermediate between high and subtidal species. In a similar study with crustaceans, Rudy (1967) found that *Astacus*, a freshwater crayfish, had the lowest water permeability found in a series of decapod crustaceans ranging from marine to freshwater species. Why this is not the case in a similar series of lamellibranchs used in this study is not known, but may be related to the maintenance of an extremely low osmolality of the hemolymph in these organisms (Potts, 1954). Since these animals maintain themselves only slightly hyperosmotic to their environment in comparison with other freshwater invertebrates, their osmotic problems are correspondingly decreased, and they may not need to reduce their tissue water permeability further in order to maintain their osmotic equilibrium.

Without regard to the actual mechanism by which water moves across the isolated mantle, but assuming the mechanism of water movement across the mantle tissue is the same in the different bivalves used in this study, reduction of tissue water permeability could be brought about in one of several different means. These would include reduction of exposed, permeable surfaces, increasing tissue thickness or changes in the chemical composition of the tissue, among other possibilities. An organism could reduce the total surface area of permeable tissues, thereby reducing its overall permeability. This mechanism has been utilized by some crustaceans in which it has been noted that there is a reduction in gill area per unit weight going from subtidal to intertidal species, the gill being the most permeable structure in crustaceans (Gray, 1957). This noted reduction in crustacean gill area may also be dependent in part upon the availability of oxygen. That is, proceeding from the subtidal to the estuarine and terrestrial environments there is an increase in the availability of oxygen, and therefore an animal could carry out its respiratory

functions with less gill surface area. Intertidal bivalves have most likely not resorted to a reduction in gill surface area as a means to decrease their total surface permeability, even though the gills make a major contribution to the total exposed surface area in these animals. The gill in these animals is used for filter-feeding, as well as maintaining a respiratory function, and with a reduction in the time available for this type of feeding higher in the intertidal area, reduction in gill surface area would most likely be counter-productive.

Alternatively, an organism could reduce the diffusional water permeability of a given tissue by increasing the thickness of the tissue. In a series of different bivalve mantles, there was no correlation between measured mantle thickness and diffusional water permeability (Table I). In addition, there was no change in lag time (Fig. 1) across the mantles with different permeabilities, which is what could be expected if decreases in P_d were brought about by increased tissue thickness. Apparently then, molluscs have not utilized this possibility to decrease tissue permeability.

Without changes in the physical dimensions of a given tissue, changes in diffusional water permeability could be brought about by changes in the chemical composition of the tissue. For example, the water permeability of artificial bilayer membranes is influenced by lipid composition (Cass and Finkelstein, 1967; Granziani and Livne, 1972). Exposure of the blue crab *Callinectes* to decreased osmolality results in an increase in lipid synthesis in the gill (Whitney, 1974). *Callinectes* is an estuarine organism and is capable of withstanding large changes in external salinity, accomplished in part apparently by decreasing its water permeability. The spider crab *Libinia* on the other hand is a sublittoral animal incapable of any great degree of osmoregulation. When this animal was exposed to lowered salinities, there was no change in gill lipid synthesis. Differences in diffusional water permeability across the mantle tissue of bivalves from different habitats may then reflect differences in the lipid composition of the tissue. That is, there may be an increase in the lipid/protein ratio in the mantle tissue with increasing exposure to osmotic stress resulting in decreased diffusional water permeability.

Adaptations to water problems in other intertidal molluscs include structural, behavioral, and physiological processes. The structure of the shell in the European limpet *Patella* is correlated with their intertidal distribution (Davies, 1969). Higher zoned animals have higher shells with a smaller circumference than lower zoned animals. This effectively reduces the surface area of the higher zoned animals. Davies also suggested that there may be differences in the water permeability of the mantle tissue of these limpets. The false limpet *Siphonaria pectinata* has no ability to osmoregulate but can tolerate salinities between 20 and 40‰. Salinity variations outside of the tolerance range cause the animal to contract the foot musculature creating a seal between the shell and substrate, effectively shutting out the external environment (McAlister and Fisher, 1968). Wolcott (1973) claims that the most important adaptation of *Acmaea digitalis* to environmental stress situations is the secretion of a mucous sheet between the shell margin and substratum, again sealing off the external environment, a situation analogous to the secretion of the epiphragm in terrestrial snails (Machin, 1968).

Although biotic factors, such as competition, behavior, predation, etc., probably

play a major role in the vertical distribution of intertidal animals (Wolcott, 1973), abiotic factors also influence the intertidal distribution of these animals (Newell, 1970). This present study suggests that the ability of certain groups of animals to adapt physiologically to specific environmental stress situations may also influence their distribution.

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Summary

1. The diffusional water permeability of the isolated mantle tissue from a series of marine, and one freshwater, species of lamellibranch molluscs was determined.

2. The water permeability of the mantle tissue was generally correlated with the habitat of the organism, permeability decreasing with increasing height above the sublittoral zone.

3. Evidence is presented that a given intertidal lamellibranch species, *Mytilus edulis,* is capable of altering its tissue water permeability when presented with changes in external osmolality.

4. The observed differences in tissue water permeability from different animals are not due to change in the physical dimensions of the tissue, but may be the result of changes in the chemical composition of the tissue.

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