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SALINITY CHANGE AND CELL VOLUME: THE RESPONSE OF TISSUES FROM THE ESTUARINE MUSSEL *GEUKENSIA DEMISSA*

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Summary

The response of cell volume to changes in external salinity was assessed in four tissues (gill, mantle, hemolymph cells and ventricle) of the estuarine mussel *Geukensia demissa* by using one or more of the following three indicators of cell volume response: changes in cell dimensions, cell water space and cell solute content. All three techniques indicated that short-term volume regulation was generally absent from gill tissue. Lateral cell height in gills, measured using differential interference contrast (DIC) microscopy, increased by approximately 20 % after an abrupt exposure to reduced salinity (60 % artificial sea water, ASW). There was significant variability in the observance of a regulatory volume decrease (RVD) subsequent to the initial swelling; cells remained swollen for 1 h after low-salinity exposure in two-thirds of the trials, while there was a return of cell volume towards control values in the remaining one-third of the trials. Lateral cell height increased linearly when salinity was gradually decreased from 100 to 60 % ASW over 135 min. Cell height then returned to control values when the salinity was abruptly returned to 100 % ASW, indicating that an RVD was not elicited by a slow change in salinity of the type normally encountered by estuarine mussels. Cumulative cell water space in gills increased by 47 % after exposure to 60 % ASW and the cells remained swollen for at least 4 h, returning to control values when gills were returned to 100 % ASW. Consistent with the overall lack of an RVD, there was only a small decrease (approximately 5 %) in cumulative osmolyte content (primarily taurine, betaine and K^+) after 4 h in 60 % ASW. Decreases in both cell water space and osmolyte content after 3 weeks of acclimation to 60 % ASW indicated a long-term RVD of approximately 60 %.

Individual cells in the mantle epithelium also generally lacked an RVD in response to lowered salinity. Both abrupt and gradual decreases in salinity caused an increase in mantle cell height to a maximum of 25–30 %, and cell height returned to the control height when salinity was abruptly returned to 100 % ASW. Corresponding with the lack of an RVD in individual mantle cells, there was no change in solute content of the mantle tissue after 4 h of exposure to low salinity. The response of the volume of spherical hemolymph cells to 1 h of abrupt exposure to low salinity, calculated from measured cell diameters, likewise indicated that an RVD is generally lacking in these hemolymph cells. In the ventricle, however, there was a significant decrease in amino acid and betaine content after 4 h of exposure to low salinity, suggesting tissue-specific variability in the cellular response to salinity change.

The consistent lack of a short-term RVD in many tissues may serve to avoid large energetic expenditures associated with repeated volume regulation in the face of the frequent, short-term changes in salinity encountered by estuarine mussels.

Key words: *Geukensia demissa,* cell volume regulation, taurine, betaine, gill, mantle, ventricle.

Introduction

Among the suite of adaptations normally assigned to osmoconforming animals living in estuarine environments is the ability to regulate cell volume. For estuarine bivalves, the hemolymph osmolality typically varies directly with that of the external solution and cells will shrink or swell during a salinity change unless cellular mechanisms are invoked to counteract the change in cell size. There is a large body of literature demonstrating that cells of most tissues studied to date can invoke a regulatory volume decrease (RVD) or regulatory volume increase (RVI) in response to osmotic stress (Chamberlin and Strange, 1989), thus presumably mitigating the functional consequences of a change in cell volume. Unlike many mammalian tissues that are rarely exposed to acute changes in ambient osmolality and yet exhibit a marked ability to regulate cell volume (e.g. proximal renal tubule; Lohr and Grantham, 1986), tissues of estuarine bivalves do encounter large and frequent fluctuations in salinity. In the face of such frequent osmotic challenges, one

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might expect the cells of tissues in estuarine bivalves to have a well-developed ability to regulate their volume by either losing or gaining osmotically active solutes. In fact, there have been many demonstrations of modulation of tissue amino acid content in bivalves in response to long-term (days to weeks) changes in ambient salinity (e.g. Pierce and Greenberg, 1972; Hoyaux *et al.* 1976; Livingstone *et al.* 1979). The response of cell volume in euryhaline bivalves to short-term (minutes to hours) changes in salinity has, however, attracted less attention, although several studies have clearly documented an increased efflux of amino acids from the ventricles of *Geukensia demissa* following acute exposure to dilute sea water (e.g. Pierce and Greenberg, 1972, 1973; Deaton, 1994).

We recently determined the response of cells in gills from two species of euryhaline mussel from the genus *Mytilus* to acute changes in ambient salinity (Neufeld and Wright, 1996). Surprisingly, we observed that an acute regulatory response in lateral ciliated cells of *Mytilus californianus* is most commonly minimal or absent and that, as an organ, the gill of both *M. californianus* and *Mytilus trossolus* demonstrates no discernible regulation of cell water space in response to short-term changes of salinity. While both of these species are euryhaline, *M. californianus* in particular does not normally inhabit environments with fluctuating salinity. In the present work, we extended our study of volume regulation in bivalves to *G. demissa* (formerly *Modiolus demissus*), a euryhaline mussel that normally inhabits estuarine marshes where there are regular fluctuations in salinity and which has served as a model for previous studies of the organismic or tissue-level response to altered salinity (e.g. Pierce, 1970; Pierce and Greenberg, 1972, 1973; Baginski and Pierce, 1977, 1978).

Materials and methods

Animals and tissue

Geukensia demissa Dillwyn were collected at the Whitney Laboratory, St Augustine, FL, USA, and shipped on ice by overnight air-freight to Tucson, AZ, USA. Mussels were maintained unfed in aerated, recirculating aquaria containing artificial sea water (Instant Ocean) held at 20±2 °C. Mussels were acclimated to either 33 ‰ (940 mosmol kg^{-1}; 100 % artificial sea water) or 20 ‰ (60 % artificial sea water), and were used within 2 months of collection. All studies were performed in artificial sea water (ASW) made from the individual salts (in mmol l^{-1}): NaCl, 423; $MgCl_2$, 23; $MgSO_4$, 26; $CaCl_2$, 9; KCl, 9; $NaHCO_3$, 2 (Cavanaugh, 1956). We adjusted the pH of ASW made from the individual salts to between 7.6 and 7.8 with 1 mol l^{-1} NaOH or HCl. All experiments were performed at 20 °C. Prior to experiments, we equilibrated isolated tissue for at least 30 min in ASW of the salinity to which the mussels were acclimated. For trials employing isolated gills, all experiments involving water or solute content were performed on perfused gill tissue; optical measurements were performed on non-perfused gill tissue.

Mantle tissue was isolated from the middle fold of the mantle margin, in the region just posterior to the posterior adductor muscle and near the exhalant siphon (as described for *Mytilus* by White, 1937). A strip of the mantle margin was detached from the shell and cut away from the adjoining mantle tissue. The shell-facing surface of the tissue piece was gently cleared of superficial muscle and connective tissue under a dissecting microscope. The remaining tissue consists of a layer of ciliated epithelial cells that is folded into many grooves; the apical surface of the epithelial cells faces the external seawater solution and the basal surface faces the remaining connective and muscular tissue (Fig. 1). The tissue was mounted in a chamber for microscopic observation with the apical face oriented towards the coverslip. We measured epithelial cell height (the z dimension shown in Fig. 1) by locating grooves and focusing to an area where the epithelial layer was perpendicular to the plane of focus (refer to the micrograph showing plane of focus B in Fig. 1). In order to avoid artifactual changes in the measured cell height caused by changing geometry of the tissue, we were careful to measure epithelial height from those grooves in which the walls remained effectively parallel to each other and perpendicular to the plane of focus throughout the period of the experiments. In some samples, we measured the x–y (side-to-side) dimensions of cells by focusing on an area adjacent to the grooves (i.e. towards the coverslip) so that the polygonal cell boundaries of the epithelial sheet could be visualized (refer to the micrograph showing plane of focus A in Fig. 1). Epithelial cells are rectangular when viewed in the z dimension (apical-to-basal height approximately 15 µm) and polygonal when viewed in the x–y dimension (lateral width approximately 10 µm) (Fig. 1). We estimated changes in the x–y dimension by measuring the change in distance between two points that spanned 3–4 cells.

We collected hemolymph using a syringe from the intermuscular hemolymph sinus, located anterior to the byssal foot and between the anterior byssal retractor muscles (White, 1937). A drop of hemolymph was placed on a coverslip in the perfusion chamber and the cells were allowed to settle for 15 min, during which time some of the free-floating cells attached to the coverslip. Cells that attached could be categorized into two basic morphologies: (1) larger cells (approximately 20 µm diameter) that settled on the coverslip and developed many cellular extensions and (2) smaller cells (10–15 µm diameter) that remained spherical. While most of the smaller cells were washed out of the chamber by the flow of the bathing medium, a few either remained loosely attached to the glass surface or became ‘tethered’ onto cellular extensions from the larger cells. Consequently, representative small cells could be followed through the course of the experiment, and cell volume could be calculated from the measured cell diameter.

Ventricular tissue was isolated as described by Pierce and Greenberg (1972).

Optical measurement of cell size

The dimensions of gill, mantle and hemolymph cells were measured using an optical system described previously (Silva

and Wright, 1994; Neufeld and Wright, 1996). Briefly, we used an Olympus IMT-2 inverted microscope equipped with an ultra-long distance condenser and either an Olympus 40× objective (numerical aperture 0.55) or a Zeiss 63× objective (numerical aperture 1.25) to take optical sections using differential interference contrast (DIC) microscopy. Tissue was held in a flow-through chamber (0.15 ml chamber volume) that allowed superfusion with sea water solutions at a rate of 1 ml min^{-1}. An overlying fine plastic mesh prevented tissue sections from moving and also kept cells within the working distance of the objective. In those experiments that employed a gradual, rather than acute, change in ambient salinity, we used an apparatus similar to that described by Lohr and Grantham (1986). A low-flow pump (Gilson Minipuls 2) removed solution at a rate of 1 ml min^{-1} from a beaker containing 100 % ASW, which in turn received 50 % ASW *via* plastic tubing from another beaker. The result was a linear decrease in salinity occurring over a period of about 2 h (see Fig. 3). In all experiments, the bathing media passed through an insulated cold-water bath before entering the chamber, thereby providing the tissue with a bathing solution of 20 °C. Images were saved and analyzed using Image-1 computer software (Universal Imaging Corp.).

Intracellular water space of gills

Intracellular water space was calculated as the difference between the total water space (calculated by comparing the wet *versus* dry mass) and the extracellular water space (calculated using [^{14}C]polyethylene glycol; [^{14}C]PEG). Prior to an experiment, we catheterized isolated gill tissue (approximately 50 mg wet mass) *via* the efferent vessel, as described by Silva and Wright (1994), and flushed the vasculature with ASW. Gill tissue was first incubated for 5 min in ASW containing 10 μmol l^{-1} 5-hydroxytryptamine (5-HT), which activates the lateral cilia and thereby facilitates the mixing of bathing medium immediately adjacent to the filaments. Gill tissue was then transferred to 20 ml of ASW containing 10 μmol l^{-1} 5-HT and 19 kBq (12.5 μmol l^{-1}) of [^{14}C]PEG (average molecular mass of 4000 Da). The tissue was immediately perfused with 0.75 ml of the ASW solution bathing the external surface; this provided identical specific activities of [^{14}C]PEG in the external and vascular spaces of the gill. The catheter was pulled from the vessel and the gill tissue was allowed to incubate for 6 min in the ASW solution. We cut pieces of gill tissue (approximately 15 mg wet mass) from the area bounded by the suture ties, carefully blotted them on filter paper, and weighed them to the nearest 0.1 mg. Tissues were dried for at least 2 h at 60 °C; a constant tissue mass was achieved after this period and [^{14}C]PEG does not volatilize under these conditions (Neufeld and Wright, 1996). After weighing, the dried sections of gill tissue were extracted overnight in 1 ml of 0.1 mmol l^{-1} HNO_3 before liquid scintillation counting. This method is described in additional detail in Neufeld and Wright (1996).

Because we were not confident that the [^{14}C]PEG space of mantle and ventricle represented an accurate measure of extracellular space, we limited our assessment of water spaces of these tissues to the measurement of total tissue water, i.e. intracellular plus extracellular water. These measurements involved calculating the difference between the wet mass of pieces of these tissues and their respective masses after drying at 60 °C for at least 2 h (i.e. until a constant mass was achieved). There was no difference in hydration of tissues [(wet mass − dry mass)/wet mass] from animals acclimated to 100 % ASW or exposed to 60 % ASW for 4 h: ventricle, 0.823±0.012 (N=4) *versus* 0.808±0.020 ml g^{-1} wet mass (N=4); mantle, 0.815±0.012 (N=5) *versus* 0.807±0.014 ml g^{-1} wet mass

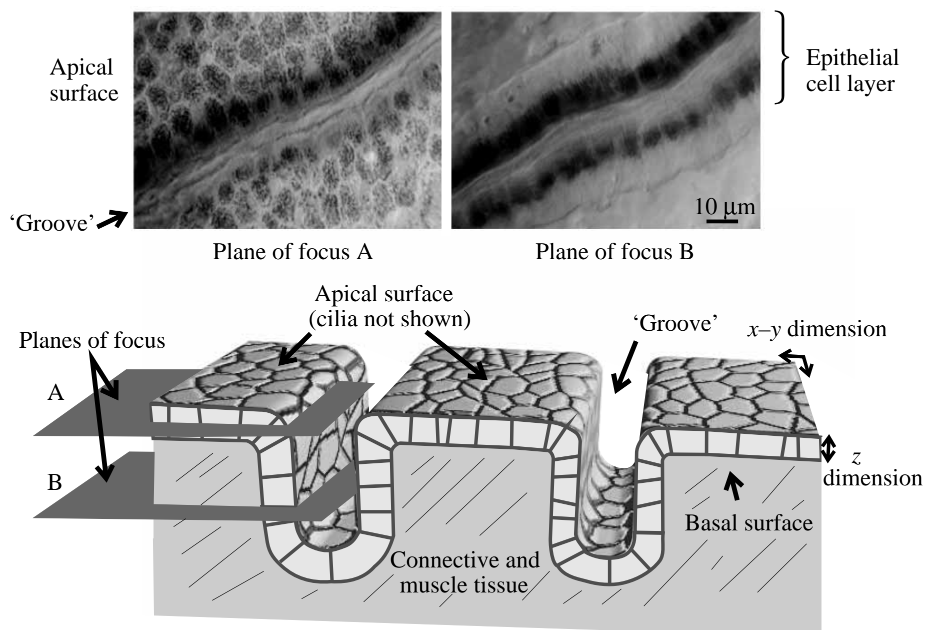

Fig. 1. Lower portion: schematic diagram of a section of the mantle epithelium. The sheet of epithelial cells is folded into grooves, with the apical side containing many cilia and facing the sea water. The basal side of the epithelial cells faces connective and muscular tissue. During microscopy, tissue was oriented with the apical surface resting on the glass coverslip. Cell dimensions were measured by focusing at level A for the lateral width of the cell (*x–y* dimension) or level B for the cell height (*z* dimension). Upper portion: differential interference contrast (DIC) images of cells in the mantle epithelium at these two planes of focus. Plane of focus A shows the cell faces (*x–y* dimension). Plane of focus B shows the cell height of epithelial cells lining a groove.

(N=4) (means ± S.E.M.). To normalize tissue solute contents to the dry masses of these tissues, the data for the two conditions were pooled to calculate the values for dry mass per gram of wet tissue mass: ventricle, 0.182 g dry mass g^{-1} wet mass; mantle, 0.186 g dry mass g^{-1} wet mass.

Solute content

Gill, mantle and ventricle tissues were prepared for amino acid or betaine analysis by extraction with perchloric acid (PCA). Tissue pieces were homogenized in distilled water and allowed to extract overnight at 4 °C in 6 % PCA. Samples were then centrifuged at 50 000 ***g*** for 20 min. The supernatant was titrated to pH 7 with KOH, refrigerated for 2 h, and then centrifuged again at 50 000 ***g*** for 20 min. The resulting supernatant was passed through a Sep-Pak C_{18} cartridge (Millipore Corp.) and a 0.2 μm filter (Acrodisc, Gelman Sciences), then frozen at −70 °C until analyzed.

Levels of free amino acids were measured using ion-exchange chromatography on a Beckman 7300 amino acid analyzer. Samples were diluted with lithium citrate buffer and analyzed using the post-column ninhydrin method. Betaine was estimated using high-performance liquid chromatography (HPLC) (Wolff *et al.* 1989) with a Waters Sugar Pak I column. All gill sections were perfused with ASW prior to solute analysis, flushing out the small amount of organic substances normally present in the hemolymph (free amino acid concentrations approximately 2–10 mmol l^{-1}; Strange and Crowe, 1979*b*). The measurements of free amino acid and betaine levels in gill sections therefore reflect those organic solutes present in the intracellular pool. The vasculature of the mantle and ventricle tissue could not be perfused; solute content therefore reflects both intra- and extracellular content.

K^+ content in gill pieces was measured using a flame photometer (Instrumentation Laboratory). Gill pieces were extracted in 0.1 mmol l^{-1} HNO_3 and subjected to three freeze–thaw cycles before analysis. Since gill sections were perfused with ASW and K^+ concentration was also measured in ASW, the amount of K^+ in the extracellular space could be subtracted from the total K^+ to give a more accurate assessment of intracellular K^+ (see below).

Data treatment

Cell water and solute content of gills are expressed per gram dry mass, as is solute content of mantle and ventricle tissue. Measurements of solute content were combined with measurements of percentage dry mass taken from separate gill pieces to calculate solute content on a dry mass basis. Dry mass was assumed to remain constant during acute exposure to different salinities; changes in water or solute content should therefore reflect accumulation or release of water or solute. In the measurements of K^+ content of gill cells, data were corrected for the quantity of this solute in the extracellular space:

$$\{K^+\}_i = \{K^+\}_t - (V_{ext}[K^+]_e),$$

where $\{K^+\}_i$ and $\{K^+\}_t$ are the intracellular and total K^+ content, V_{ext} is the volume of the extracellular water space, and $[K^+]_e$ is the extracellular K^+ concentration. No correction was necessary for taurine or betaine since these would be flushed from the vasculature during perfusion with ASW.

Statistical procedures used to analyze the data are described by Sokal and Rohlf (1981). Differences were tested for statistical significance using analysis of variance (ANOVA). If ANOVA indicated that significant treatment effects existed, pairwise comparisons among means were made using the Student–Newman–Keuls procedure. Statistical significance was accepted at P<0.05. All data are reported as means ± S.E.M.; N represents the number of mussels tested.

Results

Gill: lateral cell height

The appearance of the lateral ciliated cells ('lateral cells') in *Geukensia demissa* gill was very similar to that of lateral cells in *Mytilus californianus* gill (see Silva and Wright, 1994; Neufeld and Wright, 1996). The apical and basal boundaries of lateral cells in the gill were easily delineated and the cilia arising from the row of lateral cells could be reproducibly located, allowing the height of these cells to be followed throughout an experiment. Moreover, because the lateral dimensions (x and y cell axes) of these epithelial cells change minimally during acute exposure to 60 % ASW (see Silva and Wright, 1994), changes in the height of lateral cells (the z dimension) represent a reasonable estimate of changes in relative cell volume.

A rapid increase in lateral cell height (and, therefore, cell

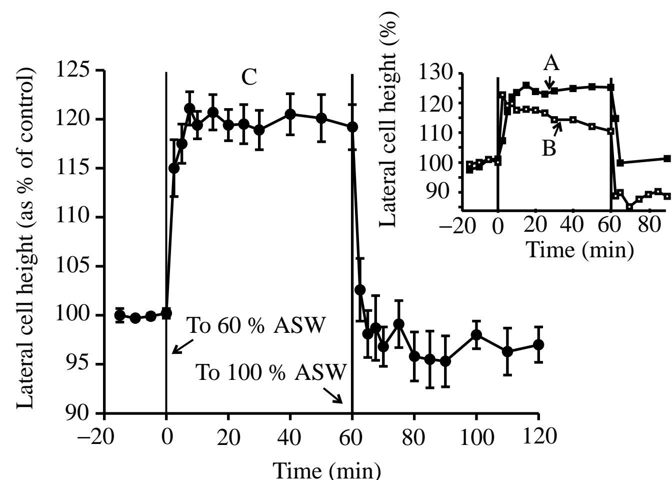

Fig. 2. The mean height of individual lateral cells from *Geukensia demissa* gill acclimated to 100 % ASW, abruptly exposed for 1 h to 60 % ASW and subsequently returned to 100 % ASW. Trace A in the inset shows a representative trace from the 10 trials in which there was no significant decrease in cell height after the initial swelling (as determined by linear regression, see Results for explanation). Trace B in the inset shows a representative trace from the six trials in which cell height after the initial swelling decreased significantly with time. Pooled data from all optical measurements are shown in the main panel (trace C; N=16). See Results for statistics. Values are means ± 1 S.E.M.

volume) followed abrupt exposure to 60 % ASW; cell height increased by 19.4±1.5 % (*N*=16) within 10 min of the low-salinity exposure (Fig. 2). Over a 1 h period following cell swelling, we observed individual variation in lateral cell height that could be roughly classified into two categories. Cell height of most cells remained at or near their maximum values for the entire 1 h period, then returned to the control height when the gill section was returned to 100 % ASW (trace A in Fig. 2 inset). We interpreted this profile as being indicative of a lack of a significant regulatory volume decrease in these cells. In some cases, however, an RVD was evident: cell height gradually declined during the 1 h exposure to low salinity (trace B in Fig. 2 inset) and then dropped below the control value upon return to 100 % ASW, consistent with a net loss from the cells of osmotically active solutes (presumably associated with the regulatory loss of cell volume). The presence of two distinct profiles of lateral cell response to an acute hypotonic challenge was similar to that observed for lateral cells in the gill of *Mytilus californianus* (Silva and Wright, 1994; Neufeld and Wright, 1996). To estimate the percentage of cells displaying an RVD in response to low-salinity exposure, we performed a linear regression on each trial from the point of maximum increase in cell height to the value at the maximum time in 60 % ASW (see Neufeld and Wright, 1996). The calculated regression slope indicated a statistically negative correlation (*P*<0.05) between time and cell height in six of 16 trials, consistent with the presence of an RVD in 38 % of the trials. Cell height did not decrease with time in the remaining 10 trials, indicating that an RVD was absent or minimal in 62 % of the trials.

We pooled the results for all gills tested (C in Fig. 2) and made a statistical comparison between five time points: 100 % ASW control, 10 min of exposure to 60 % ASW, 1 h of exposure to 60 % ASW, 10 min after returning to 100 % ASW and 30 min after returning to 100 % ASW. Cell height after 10 min and 1 h of exposure to 60 % ASW was significantly greater than cell height in 100 % ASW (*P*<0.001). There was no significant change in the mean height of lateral cells between the 10 min and 1 h following 60 % ASW exposure (*P*>0.1). Cell height 10 min after re-exposure to 100 % ASW was equivalent (*P*>0.1) to the control height (height before 60 % ASW exposure), but after 30 min of re-exposure cell height was significantly lower than the control height (*P*<0.05).

In the experiments on cell water space of intact gill tissue, discussed below, 5-HT was used to activate the cilia of lateral cells. 5-HT has, however, been shown to influence an apparent volume regulatory response in ventricular tissue from the clam *Mercenaria mercenaria*: tissue hydration rapidly increases after exposure to low salinity and the subsequent return towards the control value is more rapid in the presence of 5-HT (Deaton, 1990). Therefore, we tested whether the presence of 5-HT could potentiate a volume regulatory decrease in lateral cells by including 10 μmol l^{-1} 5-HT in the bathing solution and recording the response of cell height for 30 min after exposure to 60 % ASW. Since, in this set of experiments, the basal membrane could not be clearly delineated in all cases, we measured the total filament width (apical-to-apical distance) rather than cell height. Although filament width includes both the acellular basement matrix on which the gill epithelium rests and the underlying hemolymph space (see Silva and Wright, 1994), changes in filament width were correlated with cell height (data not shown) and were used as an estimate of cell volume response. The percentage increase of filament width in gills exposed to 5-HT was not significantly different (*P*>0.1) at 10 min (14.1±3.3 %) and 30 min (15.4±1.8 %) after exposure to 60 % ASW. In addition, this swelling was not significantly different (*P*>0.1) from that of gills exposed to 60 % ASW for 10 min (12.7±2.2 %) or 30 min (12.3±1.0 %) in the absence of 5-HT. Likewise, exposure to a lower-salinity shock (40 % ASW) did not elicit an RVD; the increases in filament width after 10 min (42.4±8.3 %) and 30 min (38.6±14.7 %) exposures to 40 % ASW were equivalent to each other (*P*>0.1), but greater than the increase in filament width after 60 % ASW exposure (*P*<0.001). Therefore, neither 5-HT nor a lower-salinity shock elicited a discernible RVD over a 30 min period.

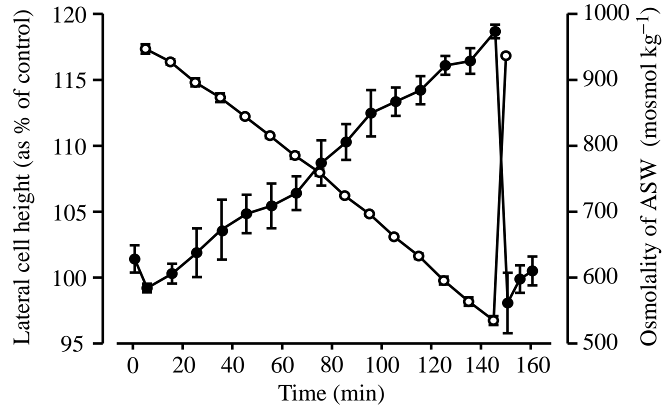

Fig. 3. Response of individual lateral cells (filled symbols) in *Geukensia demissa* gills to a gradual decrease in salinity from 100 % ASW to 60 % ASW, followed by an abrupt return to 100 % ASW (*N*=6). See Results for statistics. Values are means ± 1 S.E.M. Open symbols, osmolality.

Since estuarine mussels *in situ* are not likely to be exposed to an abrupt (i.e. step) change in ambient osmolality, we also measured the response of lateral cell height to a gradual decrease in salinity. When salinity was linearly decreased from 100 % to 60 % ASW over a 135 min period, lateral cell height increased linearly (Fig. 3), reaching a maximum increase (18.7±0.6 %, *N*=6) that was not significantly different (*P*>0.1) from the level noted after a 10 min acute exposure to 60 % ASW (19.4±1.5 %, Fig. 2). In addition, when these gills were abruptly reintroduced to 100 % ASW, lateral cell height did not fall below but, rather, returned to the control value (*P*>0.1), indicating that there had not been a significant loss of cell solutes over the period of exposure to dilute sea water. There was, therefore, no evidence of an RVD in response to a gradual change in salinity.

Gill: cell water space

Cell water space, measured as the difference between total water space and extracellular water space, was

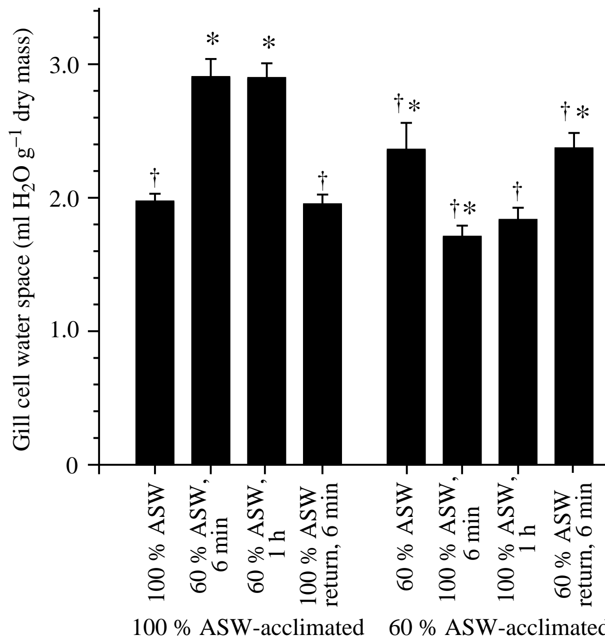

Fig. 4. Cell water space in *Geukensia demissa* gills during acute exposure to ASW that was either hypo- or hyperosmotic to the acclimation salinity, followed by a return to the acclimation salinity (*N*=9 for mussels acclimated to 100 % ASW; *N*=5 for mussels acclimated to 60 % ASW). *Cell water space was significantly different (*P*<0.05) from cell water space in gills of control mussels acclimated to 100 % ASW. †Cell water space was significantly different (*P*<0.05) from cell water space in 100 % ASW-acclimated mussels after 6 min in 60 % ASW. Values are means + 1 S.E.M.

1.97±0.06 ml g^{-1} dry mass in gills from animals acclimated to 100 % ASW. When gills were exposed to 60 % ASW for 6 min, the cell water space increased by 47 % compared with cell water space in 100 % ASW (Fig. 4). The cell water space of gills was still elevated by 47 % after 1 h in 60 % ASW, and returned to 1.95±0.08 ml g^{-1} dry mass when gills were transferred back to 100 % ASW for 6 min, indicating the overall lack of an RVD in gill cells (Fig. 4). In a separate experiment, we observed that cell water space increased by 46 % and 53 %, respectively, after 1 and 4 h exposures to low salinity and that cell water space returned to the control value (2.10±0.15 ml g^{-1} dry mass; *P*<0.05) when gills were returned to 100 % ASW after a 4 h exposure to 60 % ASW. Therefore, over a period of at least 4 h of exposure to 60 % ASW, there was no significant regulation of cell water space in gill tissue.

A prolonged exposure to 60 % ASW did, however, result in an apparent regulatory decrease in the cell water space of gill tissue. When mussels were acclimated for 3 weeks to 60 % ASW, the intracellular water space of gills was 2.36± 0.20 ml g^{-1} dry mass. This value, although significantly greater (*P*<0.005) than the intracellular water space of mussels acclimated to 100 % sea water, was significantly less (*P*<0.01) than that of gills acutely exposed to low salinity (Fig. 4), indicating that a degree of volume regulation had occurred. Assuming that dry mass was unchanged from that of mussels acclimated to 100 % ASW, this represents a 58 % regulation of cell volume over the 3 week exposure to the low salinity. Also, when the tissues that had been acclimated to 60 % ASW were abruptly reintroduced to 100 % ASW, cell water space decreased by 28 % to a value that was significantly (*P*<0.05) below the cell water space of tissues acclimated to 100 % ASW (Fig. 4), suggesting that there was a loss of osmotically active solutes during the acclimation period. Following this shrinkage of cell water space, there was no evidence of an acute RVI; after 1 h, cell water space was not different from the value measured after 6 min in 100 % ASW, and then returned to 2.37±10.12 ml g^{-1} dry mass when gills were returned to 60 % ASW.

Gill: osmolyte content

In mussels acclimated to either 100 % ASW or 60 % ASW, the gill contents of K^+, betaine and taurine were much higher

Table 1. *Content of* Geukensia demissa *gill amino compounds (µmol g^{-1} dry mass) during hypo- and hyperosmotic exposure, measured using ion-exchange chromatography*

	100 % ASW-acclimated			60 % ASW-acclimated	
	100 % ASW Control	60 % ASW 1 h	60 % ASW 4 h	60 % ASW Control	100 % ASW 1 h
Taurine	234.5±18.1†	229.8±20†	258.9±8.8†	127.4±18.7*	154.5±22.9*
Glycine	36.4±5.9†	18.4±2.8*,†	20.4±17.2	7.7±1.6*	10.4±2.0*
Alanine	31.2±6.2†	22.1±3.4	22.8±14.1	14.1±3.6*	15.1±9.1*
Aspartate	19.4±4.5†	18.4±5.0†	14.6±4.3†	5.4±2.0*	9.4±3.3*
Glutamate	15.6±3.1†	9.8±3.6	9.0±7.9	6.7±2.7*	8.6±3.1*
Ornithine	4.8±1.2†	4.3±0.8†	4.2±1.9†	2.3±0.2*	2.2±0.8*
Serine	4.0±0.9†	6.9±1.1*,†	5.8±3.6†	1.5±0.3*	2.3±0.4*,†
GABA	3.4±1.6	4.8±2.3	9.3±9.0	1.7±1.4	1.5±1.5
Phosphoserine	1.6±0.4	1.8±0.4	2.5±0.2†	1.6±0.3	1.0±0.6
Threonine	1.2±0.4	1.1±0.3	0.8±0.7	0.9±0.3	1.8±0.3†

*Significantly different from control content in animals acclimated to 100 % ASW.
†Significantly different from control content in animals acclimated to 60 % ASW.
Values are means ± 1 S.E.M., *N*=3–8.

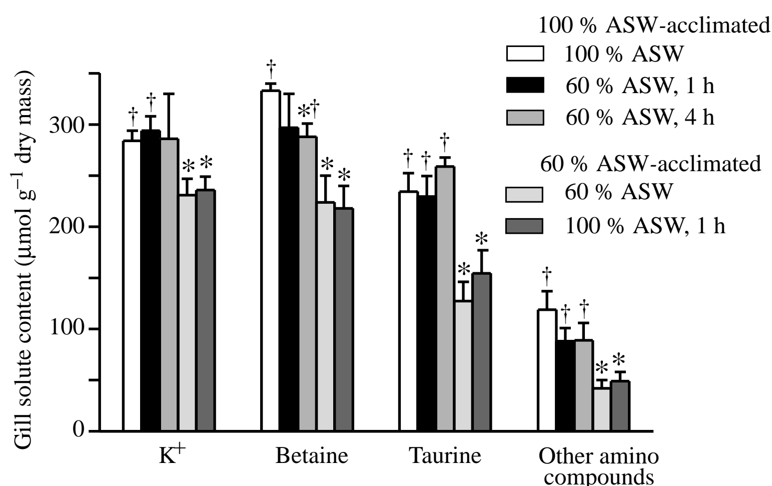

Fig. 5. Levels of the major solutes in gills of *Geukensia demissa* following acute exposure to ASW either hypo- or hyperosmotic to the acclimation salinity (*N*=3–8). Individual levels of the other amino compounds are listed in Table 1. *Solute content was significantly different ($P<0.05$) from solute content in control mussels acclimated to 100 % ASW. †Solute content was significantly different ($P<0.05$) from solute content in mussels acclimated to 60 % ASW. Values are means + 1 S.E.M.

than the contents of other solutes measured (Fig. 5). K^+, betaine and taurine were 284±10, 333±7 and 235±18 μmol g^{-1} dry mass, respectively, in animals acclimated to 100 % ASW. The contents of glycine, alanine, aspartate and glutamate were all between 15 and 40 μmol g^{-1} dry mass (Table 1). Phosphoserine, threonine, serine, γ-aminobutyric acid (GABA) and ornithine concentrations were all less than 5 μmol g^{-1} dry mass. The calculated concentrations of K^+, betaine and taurine (based on a cell water space of 1.97 ml g^{-1} dry mass) were 144, 169 and 119 mmol l^{-1}, respectively, together constituting approximately 45 % of the osmotically active solute expected in the cell water of gills from mussels acclimated to 100 % sea water (940 mosmol l^{-1}). The remaining amino compounds together accounted for an additional 60 mmol l^{-1} in mussels acclimated to 100 % sea water.

As expected from the absence of a significant volume regulatory response by gill cells during an acute exposure to dilute sea water, there was no significant change in tissue content of K^+, betaine or taurine compared with control levels after 1 h of hypo- or hyperosmotic exposure (Fig. 5). After 4 h of exposure to 60 % ASW, K^+ and taurine contents were unchanged but the betaine content decreased slightly ($P<0.001$). There was a significant decrease in glycine ($P<0.005$) and increase in serine ($P<0.05$) content after 1 h of hypo-osmotic exposure, but the combined content of the minor amino compounds was unchanged with 1 h or 4 h of hypo-osmotic exposure. After 4 h in 60 % ASW, levels of combined solutes decreased from 969 to 922 μmol g^{-1} dry mass, representing a net loss of only about 5 % in the measured solute content of gill tissue.

A 3 week acclimation to 60 % sea water did, however, result in significant decreases in content of the major osmolytes, K^+, betaine and taurine (Fig. 5), as well as decreases of many of the minor amino compounds (Table 1). Indeed, the decrease in concentration of osmolytes from gills during this acclimation to 60 % ASW correlated quantitatively with the observed decrease in cell water space over the same period: the loss of some 345 μmol g^{-1} dry mass of osmolytes can account for a decrease of 0.61 ml cell water g^{-1} dry mass, comparable to the decrease of 0.54 ml cell water g^{-1} dry mass actually observed after acclimation to 60 % sea water. An acute 1 h exposure to 100 % ASW of gills from animals acclimated to 60 % ASW did not result in an increase in levels of any of the major gill osmolytes (Fig. 5; Table 1); threonine and serine were the only minor amino compounds that increased in content ($P<0.05$).

Mantle: epithelial cell height

The structure of the mantle epithelium allowed us to use two planes of focus to estimate the changes in both the *x*–*y* dimensions and *z* dimensions of these cells (Fig. 1). Lateral (*x*–*y*) dimensions were not significantly different ($P>0.1$; $N=4$ for each) from the control value 15 min and 60 min after an abrupt exposure to 60 % ASW (changes in width relative to control were 2.5±3.9 % and 3.5±3.1 %, respectively), or after 10 min of re-exposure to 100 % ASW (change in width relative to control was −2.7±1.5 %). The lateral dimensions of cells were, therefore, not substantially affected by changes in salinity, and changes in cell volume should be reflected by changes in cell height.

The height (*z* dimension) of mantle epithelial cells increased by 22.9±3.6 % ($N=6$) at 10 min after an abrupt change to 60 % ASW (Fig. 6A). While we did not test enough animals to establish clearly the presence of substantial variation in the response of individual cells to a 1 h exposure to 60 % ASW of the type noted for lateral cells in gills, the mean response of the mantle epithelium clearly indicated that an RVD was usually absent or minimal (Fig. 6A). Cell height after 1 h of exposure to 60 % ASW was not significantly different from cell height after 10 min of exposure to 60 % ASW ($P>0.1$), and the cell height after 10 min of re-exposure to 100 % ASW was equivalent ($P>0.1$) to the cell height prior to the low-salinity exposure. When salinity was linearly decreased from 100 % to 60 % ASW over a 2 h period, epithelial cell height slowly increased to a maximum value (30.1±3.0 %, Fig. 6B) not significantly different ($P>0.1$) from the maximum noted for

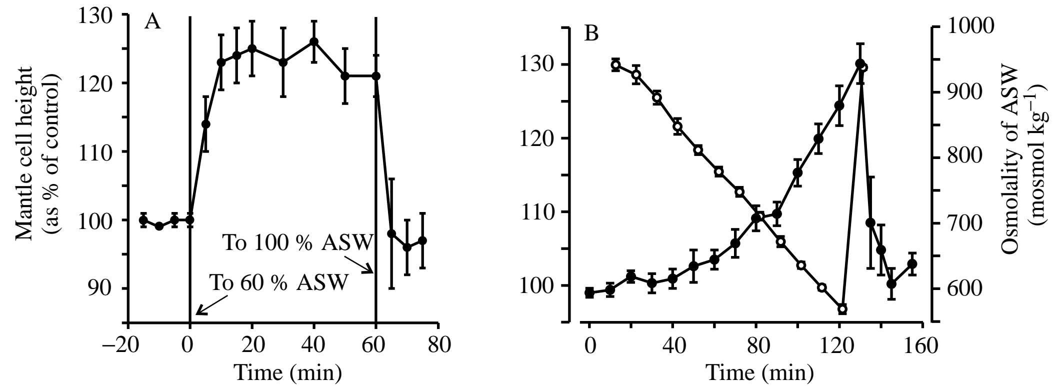

Fig. 6. (A) Response of height of individual mantle cells from *Geukensia demissa* acclimated to 100 % ASW, abruptly exposed for 1 h to 60 % ASW and subsequently returned to 100 % ASW (N=5–6). (B) Response of height of individual mantle cells in *G. demissa* (filled symbols) to a gradual decrease in osmolality (open symbols), from 100 % ASW to 60 % ASW, followed by an abrupt return to 100 % ASW (N=6–8). See Results for statistics. Values are means ± 1 S.E.M.

cells exposed to an abrupt drop in salinity (Fig. 6A). An abrupt return to 100 % ASW after exposure to the gradual decrease in salinity caused cell height to shrink back to within 0.2±2.1 % of the cell height measured prior to the salinity decrease (P>0.1). Thus, there was no evidence of substantial volume regulation in individual epithelial cells of the mantle caused by either abrupt or gradual exposure to 60 % ASW.

Mantle: osmolyte content

The primary nitrogenous organic solutes in mantle tissue from mussels acclimated 100 % ASW were betaine (169±24 μmol g^{-1} dry mass) and taurine (108±13 μmol g^{-1} dry mass; N=5) (Fig. 7). Levels of alanine, glycine, GABA, aspartate, cystine, glutamate, ornithine and serine were all 5.0 μmol g^{-1} dry mass or less and together constituted an additional 53±9 μmol g^{-1} dry mass in the mantle tissue. Glycine was the only solute that significantly decreased in content after 4 h of exposure to 60 % ASW, but the change (9 μmol g^{-1} dry mass) was small compared with the total organic osmolyte content (354 μmol g^{-1} dry mass in 100 % ASW), making it unlikely that there was a significant solute-coupled loss of cell water in mantle tissue. Significant changes in solute content were evident, however, after a longer-term exposure to low salinity; taurine, glycine and alanine contents were decreased (P<0.05 for each) after 3 weeks of acclimation to 60 % ASW, with a total decrease of 106 μmol g^{-1} dry mass, representing a loss of approximately 30 % of the organic osmolyte pool.

Ventricle: solute content

As in both the gill and mantle tissues, betaine and taurine were the organic solutes present at the highest levels (602±106 and 279±34 μmol g^{-1} dry mass, respectively) in the ventricles from mussels acclimated to 100 % ASW (Fig. 8). Glycine and alanine contents were also relatively high (96±21 and 83±6 μmol g^{-1} dry mass, respectively). Glutamate and aspartate together accounted for an additional 42 μmol g^{-1} dry mass of solute. On a dry mass basis, a 4 h exposure to 60 % ASW caused significant decreases (P<0.05 for each) in the content of each of these organic osmolytes: in μmol g^{-1} dry mass, betaine decreased to 405±42; taurine to 165.9±3.8; glycine to 35.2±6.0; alanine to 56.6±7.1; glutamate to 12.1±3.3; and aspartate to 3.8±1.6. Taken together, a 4 h exposure of isolated ventricles to 60 % ASW decreased the total organic osmolyte pool by 38 %, from 1102 to 679 μmol g^{-1} dry mass.

Hemolymph cells: cell volume

Although hemolymph cells from *G. demissa* represent a small fraction of the total tissue mass of the intact animal, and the function of these cells is not certain (Cheng, 1981), they are a non-epithelial tissue and, therefore, represent a cell type quite different (structurally, at least) from the epithelial cells of the gill and mantle. Thus, it was interesting to note that the

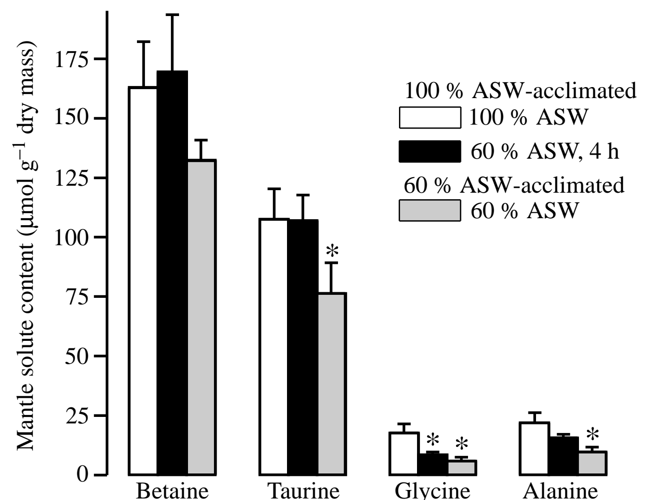

Fig. 7. Content in mantle tissue of the major nitrogenous solutes (taurine, betaine) and minor nitrogenous solutes (glycine, alanine) that changed significantly upon exposure of tissue from *Geukensia demissa* acclimated to 100 % ASW to 60 % ASW for 4 h or 3 weeks (N=5–6). *Solute content was significantly different (P<0.05) from solute content in mussels acclimated to 100 % ASW. Values are means + 1 S.E.M.

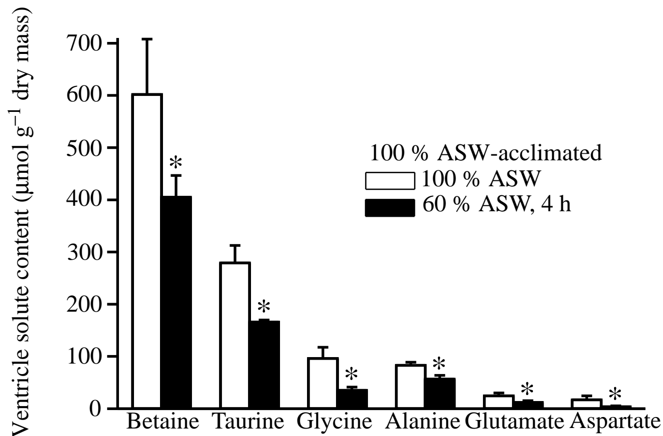

Fig. 8. Solute content in the ventricle of 100 % ASW-acclimated *Geukensia demissa* after 4 h of exposure to 60 % ASW (N=5). *Solute content was significantly different (P<0.05) from solute content in mussels acclimated to 100 % ASW. Values are means ± 1 S.E.M.

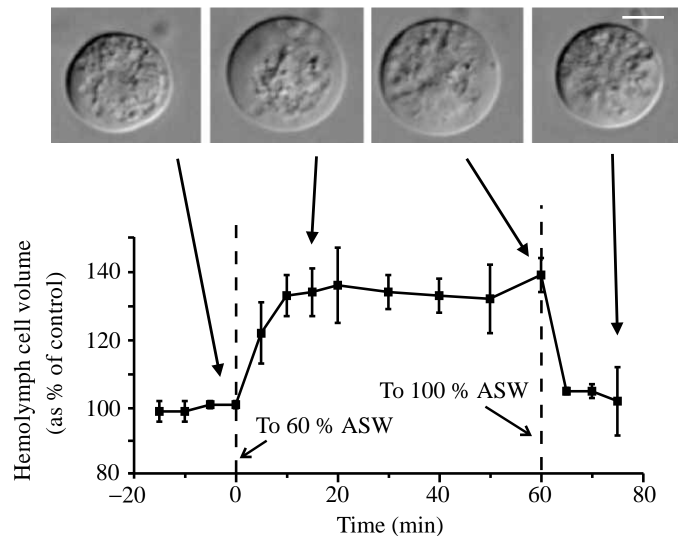

Fig. 9. Volume of spherical hemolymph cells from *Geukensia demissa* acclimated to 100 % ASW that were abruptly exposed for 1 h to 60 % ASW and subsequently returned to 100 % ASW. The photographs (all to the same scale) show the response of a typical cell. Scale bar, 5 μm. Cell volume was calculated from measurements of cell diameter. The data points represent the average results of cells from 6–9 separate animals. Values are means ± 1 S.E.M. See Results for statistics.

response of these spherical hemolymph cells was consistent with that of the cells of the gill and mantle in that an RVD was typically not observed during acute exposure to low salinity (Fig. 9). The volume of hemolymph cells increased within 10 min following exposure to 60 % ASW to 135±6 % of the control volume and was still 138±8 % (not significantly different from the volume at 10 min, P>0.1) after 1 h. Hemolymph cell volume returned to the control value (103±9 %) when they were re-exposed to 100 % ASW.

Discussion

The ribbed mussel *Geukensia demissa* survives in a wide range of salinities (3–48 ‰; Pierce, 1970) and commonly inhabits estuarine marshes, where it is likely to be exposed to salinity changes on a regular basis. This mussel normally remains with the valves gaped open and the mantle cavity actively perfused unless the ambient salinity drops below about 60 % sea water (Shumway and Youngson, 1979; Strange and Crowe, 1979*a*), thereby avoiding the physical isolation caused by valve closure that would severely restrict the amount of time available for feeding and respiration. Since *G. demissa* is an osmoconformer, hemolymph osmolality closely parallels changes in salinity under these conditions (Shumway and Youngson, 1979; Strange and Crowe, 1979*a*), and cells of the animal are therefore exposed to changes in external salinity. Given the capability of a wide variety of animal cells to regulate volume after an osmotic shock (Chamberlin and Strange, 1989; Hoffman and Simonsen, 1989), the cells of tissues in *G. demissa* might be expected to exhibit a well-developed ability to regulate cell volume.

The observations contained in the present report support conclusions concerning three separate issues pertaining to cellular volume regulation in *G. demissa.* First, our results confirm and extend previous studies with *G. demissa* showing that a short-term exposure (1–4 h) to reduced salinity does result in a loss of organic osmolytes from ventricular tissue consistent with a regulatory reduction in ventricular cell volume. Second, and in marked contrast with the former observation, we found that cells from several other tissues (gill, mantle and hemolymph) neither decrease their solute content nor regulate their volume in response to a short-term exposure to reduced salinity. Third, a long-term (3 week) acclimation of intact animals to reduced salinity does result in a significant loss of solutes from gill and mantle cells, suggesting that exposure of intact animals to reduced salinity ultimately leads to regulatory volume decreases in all cells, but that the rate of such responses varies markedly in different tissues. Each of these subjects warrants discussion.

There is a substantial literature showing the response to osmotic stress of ventricular tissue from *G. demissa*. In two seminal studies, Pierce and Greenberg (1972, 1973) showed that ventricles exposed acutely to 50 % sea water display a significant loss of amino acids that is complete within 2 h, an observation subsequently confirmed and quantified by Deaton (1994). This loss of osmotically active solutes appears to be under cellular control and is influenced by the ambient concentration of Ca^{2+} (Pierce and Greenberg, 1973) and by phorbol esters (Deaton, 1994). Indeed, this suite of observations formed the basis for suggestions that molluscan tissues deal with exposure to hypo-osmotic sea water by means of a cellular RVD that is accomplished by a regulated efflux of specific amino acids (e.g. Pierce, 1982). In the present study, we also noted that exposure of ventricles to dilute (60 %) sea water results in a net decrease in the tissue content of taurine, glycine and alanine that amounted to some

200 μmol g^{-1} dry mass, a value similar to the osmotically sensitive efflux of these amino acids from ventricles measured by Deaton (1994; 114 μmol g^{-1} dry mass). Betaine proved to be both the largest constituent of the organic osmolyte pool in ventricles and the osmolyte displaying the largest absolute decrease (approximately 200 μmol g^{-1} dry mass) following 4 h in 60 % ASW. We can conclude that an acute 400–500 mosmol l^{-1} reduction in ambient osmolality results in a rapid loss from ventricular cells of as much as 40 % of their total organic osmolyte pool, i.e. about 100 mosmol l^{-1} total tissue water (intra- plus extracellular water). A decrease in solute content of this size would be expected to have a substantial effect on cell volume.

The loss of organic osmolytes from ventricular cells during a short-term exposure to reduced salinity contrasted sharply with the failure to observe any significant loss of solute from gill tissue or to observe directly a routine regulatory decrease in volume of gill cells. The pattern of response of cells from the gill of *G. demissa* was, in fact, consistent with that observed recently for gills from the mussel *M. californianus* (Neufeld and Wright, 1996). Although a distinct regulatory response was noted in approximately 30 % of lateral cells from the gills of both species, the combined results of direct optical assessment of cell volume and indirect measurements of cell water and cytoplasmic solute content support the conclusion that neither an abrupt nor a gradual exposure to dilute sea water results in a significant short-term volume regulatory response by the cells of mussel gills. While it is unclear what causes the variability in the lateral cell RVD observed in *M. californianus* and *G. demissa*, we believe that the responses observed for these mussels are indicative of a routine degree of variability in the cellular response of lateral cells to hypo-osmotic stress, since the presence of an RVD could not be correlated with factors such as the experimental temperature, the length of laboratory acclimation, the collection site, the feeding status, the presence of 5-HT, the magnitude of the salinity shock or the rate of salinity change (D. S. Neufeld, unpublished observations).

It should be noted that mussel gills have been shown to lose amino acids when exposed acutely to dilute sea water. Deaton (1994), for example, reported that exposure of gill tissue isolated from *G. demissa* to 50 % ASW for 2 h results in a loss of approximately 30 μmol g^{-1} dry mass of amino acids (i.e. taurine, glycine and alanine) , similar to the loss of taurine from isolated *M. californianus* produced by acute exposure to 60 % ASW (Wright *et al.* 1987). However, although such losses of solute are clearly related to exposure of gills to acute hypotonic stress, the absolute loss of solute is small, less than 10 % of the amino acid pool of the tissue (Table 1; see also Wright *et al.* 1987), and less than 5 % of the total organic osmolyte pool (i.e. including betaine).

Cells from at least two other tissues (mantle and hemolymph) from *G. demissa* displayed a response to acute exposure to reduced salinity similar to that of gill, rather than ventricular, cells. Direct optical measurement of cell dimensions of mantle and hemolymph cells failed to resolve an RVD during a 1–2 h exposure to 60 % ASW. Consistent with the absence of a short-term reduction in the volume of mantle cells was the absence of a significant net decrease in organic osmolyte content of mantle tissue during a 4 h exposure to 60 % ASW, the same period of exposure that resulted in a significant reduction in osmolyte content in ventricular tissue.

These observations indicate that cells from different tissues in *G. demissa* respond to a short-term (up to 4 h) exposure to reduced salinity in a different manner: whereas ventricular cells respond with a decrease in osmolyte content consistent with a substantial regulatory decrease in volume, cells from at least three other tissues fail either to regulate their volume or to decrease their osmolyte content over a period of up to 4 h. Interestingly, we did observe a significant reduction in osmolyte content in gill and mantle tissue from animals exposed for a prolonged period (3 weeks) to 60 % ASW. In gills (where such measurements were possible), we also noted that the increase in cell water space that occurred during a short-term exposure to 60 % ASW was reduced by 58 % following 3 weeks of acclimation to the dilute medium, a loss of volume that was quantitatively correlated with the loss of solutes noted above.

We can speculate as to the possible adaptive basis for the observed variability in the cellular response to hypo-osmotic stress. It may lie in the trade-off that must exist between the functional consequences of a change in cell volume and the energetic cost of preventing or limiting that change. The energetic cost to an intact mussel of volume regulation during tidal cycles can be large when considered in the context of the metabolic rates of these animals. The regulation of cell volume during the transient but extreme fluctuations in ambient osmolality associated with estuarine habitats must involve the cyclic removal from and replacement into cell cytoplasm of substantial amounts of osmotically active solutes. Several models have been proposed to account for the loss or gain of solute during osmotic stress (e.g. Baginski and Pierce, 1975; Bartberger and Pierce, 1976; Pierce, 1982; Deaton, 1987). In general, these models involve the downhill efflux from cells of solutes (e.g. amino acids) during an RVD, followed by the accumulation of solutes, *via* transport from the hemolymph, or the intracellular production of solutes through metabolism or degradation of macromolecules, during the ensuing RVI. Of these different ‘strategies’, the most energetically conservative is a model that involves an RVD that is the result of transmembrane solute efflux, followed by an RVI that is the result of transmembrane solute influx. In this model, the energetic cost of regulation occurs at the ‘uphill’ influx step. It is possible, therefore, to make an estimate of the minimal cost for organismic regulation of cell volume from the known stoichiometric link between the active flux of solute and the hydrolysis of ATP. Indeed, the most conservative model would entail the cyclic transmembrane flux of K^+ (and a counteranion) with the active step involving the hydrolysis of 1 ATP during accumulation of 2 K^+. In the case of a 12 h sinusoidal shift between 100 % and 50 %, and back to 100 % sea water, the cumulative cost of solute uptake for volume

regulation in all cells of a mussel (i.e. *Mytilus* sp.) would be at least 30 % of the standard metabolic rate (see Neufeld and Wright, 1996). To the extent that fluxes of organic osmolytes (e.g. taurine or betaine) or intracellular synthetic steps are involved instead of, or in addition to, the simple transport model outlined above, the cost of volume regulation would be proportionally greater. Therefore, for an estuarine organism that is exposed to large and frequent swings in ambient osmolality, the reduction or elimination of cell volume regulation during short-term exposures to reduced salinity would represent a substantial saving of metabolic energy.

Allowing cell volume to respond in a largely passive manner to short-term changes in salinity is not likely to be an equally adaptive strategy for all tissues. The excitable cells in an organ such as the ventricle, for example, might be expected to suffer from a serious decrement in function when the cytoplasmic concentrations of inorganic ions are substantially perturbed or the shape of the cell is changed (e.g. by swelling). In fact, the contractile activity of *G. demissa* ventricles has been shown to cease after acute exposure to dilute sea water, and to recover with a time course that correlates closely with the loss of amino acids noted earlier (Pierce and Greenberg, 1972). The metabolic cost of repeated transport of osmotically active solutes may be small compared with the benefit to the organism of maintaining critical aspects of cellular function of an organ the activity of which is central to the maintenance of organismic homeostasis. However, it is not clear that there is a substantial 'functional cost to pay' if the cellular volume of other tissues is perturbed, at least over the short term. In fact, there are few data available on the short-term influence of acute, short-term changes in salinity on the cellular physiology of tissues from euryhaline bivalves. The limited data that are available suggest that, for the gill at least, the functional cost of not regulating volume may be modest: the activity of frontal cilia in isolated *G. demissa* gills is decreased by less than 15 % following an acute shift from sea water of 30 ‰ to sea water of 20 ‰ (Van Winkle, 1972). Thus, the functional consequences of a transient change in size for gill and mantle cells may be insufficient to warrant the expenditure of energy associated with regulation of cellular volume.

When gill and mantle cells are exposed to reduced salinity for a prolonged period, i.e. much longer than typically associated with tidal cycles, a substantial modulation of cellular osmolyte content and, hence, a regulation of cell volume does occur. Thus, it appears that the functional consequences of a long-term perturbation of cell size and/or cytoplasmic composition do warrant a regulatory response. The time frame of an RVD response of these tissues is generally such that the degree of regulation (i.e. solute flux) occurring during the hypo-osmotic period of a normal tidal cycle (approximately 6 h) is small, thus leaving the animal with a concomitantly small energetic expenditure (through membrane transport) during re-exposure to 100 % sea water conditions.

The comparatively slow decrease in the osmolyte content of selected tissues in hypo-osmotically stressed bivalves does agree with several reports in the literature (e.g. Baginski and Pierce, 1977; Livingstone *et al.*1979; Hawkins and Hilbish, 1992). In *G. demissa* gills, as noted earlier, there is a short-term increase in the rate of amino acid efflux after low-salinity exposure (Deaton, 1994), but this rate of efflux (approximately 30 μmol g^{-1} dry mass 2 h^{-1}), even if sustained, would account for a decrease of 0.1 ml g^{-1} dry mass in 4 h, which is equivalent to only 11 % of the swelling (0.93 ml g^{-1} dry mass) observed after low-salinity exposure in the present study. This rate of osmolyte loss, although slow compared with that observed in ventricular tissue, could be representative of the mechanism(s) that leads to the more gradual changes in tissue solute content arising from prolonged exposure to reduced salinity that appear to be characteristic of gill and other tissues.

In conclusion, our evidence on the effect of hypo-osmotic stress on the regulation of cell volume now includes data from three species of euryhaline bivalve, *G. demissa* (present study), *M. californianus* and *M. trossolus* (Silva and Wright, 1994; Neufeld and Wright, 1996), employing animals that were both laboratory-acclimated and freshly collected, and tissues that were exposed to both abrupt and gradual changes in salinity. These data consistently indicate that cell volume regulation is absent or minimal in gill and mantle tissue, an adaptation that may have arisen because of the substantial energetic expenditure required for the regulation of cell volume during the repeated exposure of these animals to the fluctuating salinity of the estuarine habitat. However, ventricular tissue from the only species tested (*G. demissa*) does display a substantial loss of organic osmolytes during a short-term exposure to dilute sea water, suggesting that the functional consequences of cell swelling in this tissue outweigh the energetic advantages arising from the more limited or delayed regulatory response of cells from gill, mantle and hemolymph. These observations indicate that there is a significant difference in the response strategies employed by cells of different tissues during exposure to altered salinity. The 'organismic response' to altered salinity is, therefore, a combination of those of its several tissues and represents a balance between the functional consequences of unregulated cell swelling and the substantial energetic costs of cell volume regulation.

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