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Osmotic adaptation in *Ulva lactuca* under fluctuating salinity regimes

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Abstract. A study has been made of the osmotic responses of the green intertidal alga, *Ulva lactuca*, under two fluctuating salinity regimes; sinusoidal and square-wave fluctuations between 30 and 100% sea water in a 12 h cycle. These regimes closely resemble the tidal fluctuation of salinity encountered by the alga in its natural estuarine habitat. Data on changes in the inorganic ions, potassium, sodium, chloride and sulphate; in the organic solute, dimethylsulphoniopropionate; in the total sugar levels and estimated osmotic and turgor pressures under the two salinity regimes are reported. Significant differences in the solute responses under these different conditions were detected. In general, better control of ion fluxes appeared to be exercised under the sinusoidal conditions which also buffered changes in dimethylsulphoniopropionate levels. Influxes of potassium were highly light-dependent. Chloride levels conspicuously failed to reach the steady-state levels in the 6-h-hyper-osmotic part of either the abrupt or gradual cycle. The possible significance of these data, which may better reflect osmotic changes in the natural environment, and some of the problems encountered, particularly in accounting for charge balance under some conditions, are discussed.

Key words: Dimethylsulphoniopropionate – Osmotic adaptation – Salinity (fluctuating) – *Ulva*.

Introduction

In a previous study (Dickson et al. 1980), we reported on the effects of steady-state, hyper- and hypo-saline stresses on inorganic and organic solute levels in *Ulva lactuca*. Hypo-osmotic conditions decreased tissue K^+, Na^+ and Cl^- concentrations, while hyper-osmotic stress caused a stable accumulation of K^+ and Cl^- but a transient increase in Na^+. Of the organic compounds examined, only β-dimethylsulphoniopropionate (DMSP) responded to external changes in such a manner as to suggest that it might be involved in osmoregulation as a putative cytoplasmic compatible solute (see also Mohsen et al. 1972).

Experiments performed with steady-state saline regimes have attracted criticism in recent years from marine zoologists (e.g. Stickle and Ahokas 1974; Davenport et al. 1975) because they do not reflect field conditions. Littoral zones, especially estuaries, experience large and sometimes rapid fluctuations in salinity due to the interactions of tides, rainfall, fresh water run-off and evaporation. Cawthorne (1979) found that several common organisms of the littoral zone of the Conwy estuary, including *U. lactuca*, encountered tidal salinity fluctuations between 0–33 parts per thousand of salt ($^0/_{00}$S). These could be approximately sinusoidal in character with a frequency of 12 h or, in some instances, more like abrupt, square-wave changes (Fig. 1). With the development of an apparatus for the laboratory simulation of these and other environmental salinity fluctuations (Davenport et al. 1975), the detailed study of the effects of fluctuating salinity upon marine and estuarine organisms has become possible (e.g. Davenport and Cawthorne 1978; Shumway 1976; Cawthorne 1979). Previous work has been restricted to aspects of animal physiology. However we now report on the effects of simulated estuarine conditions on some aspects of the osmotic responses of the macro-alga, *U. lactuca*. It is of particular interest to compare these results with those obtained under conventional, but perhaps less appropriate, steady-state salinities (e.g. Dickson et al. 1980).

Abbreviations: DSMP = 3-(dimethylsulphonio)propionate; FW = fresh weight

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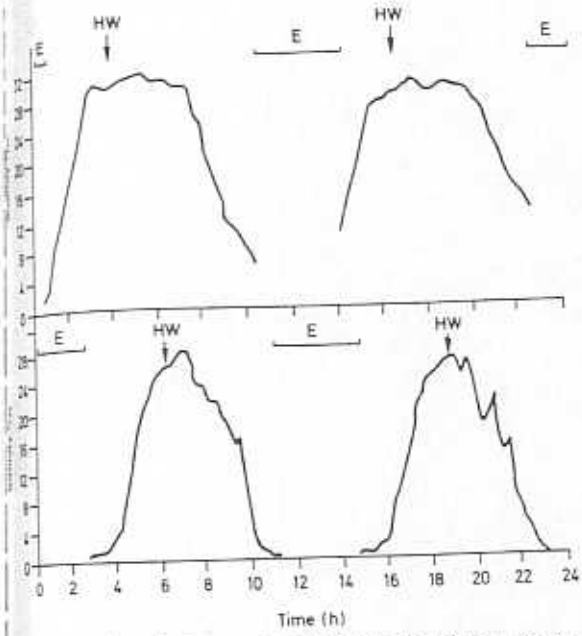

Fig. 1. Examples of changes in salinity in the Conwy estuary during the tidal cycle. HW, Time of high water; E, period of ebb tide when the site of sampling was exposed (from Cawthorne 1979); ‰, parts per thousand of salt

Material and methods

Actively growing *Ulva lactuca* plants were collected from the shore of Ynys Tysilo (Church Island) in the Menai near Porthaethwy (Menai Bridge) and maintained in aquaria at 15° C and 33‰ S with a 12 h light/dark photoperiod (130 μE m^{-2} s^{-1}). Plants kept in this way maintained high K^+/Na^+ ratios for weeks. However, in no case were plants kept longer than 7 d before experimental use.

The apparatus used to provide seawater of fluctuating salinity has been described by Davenport et al. (1975). Fronds of *U. lactuca* were placed in this experimental apparatus and acclimated to 100% seawater (33‰ S) at 15° C either under constant illumination (400 μE m^{-2} s^{-1}; between 400–700 nm) or in darkness for 12 h prior to the initiation of a fluctuating salinity cycle. Mixing of the seawater and its circulation was ensured by bubbling compressed air into the boxes at the point of water entry. Flow rates were maintained at about 500 ml min^{-1}. Fronds were subjected to either sinusoidal or abrupt salinity regimes as shown in Fig. 2. These regimes were chosen to resemble the changes found in the natural environment (compare with Fig. 1). All experiments were performed at 15° C, the mean summer temperature of the water of the Menai.

Algal tissue was taken every hour and washed in $Ca(NO_3)_2$ iosmotic with seawater for 30 s. Separate experiments showed that the further loss of ions from the tissue after the 30-s wash was very small. The ions lost during the 30-s wash were considered to be the free-space component (West and Pitman 1967). Fronds were blotted dry between sheets of tissue paper prior to weighing for the fresh-weight determination. Tissue was then frozen at −20° C prior to organic and inorganic analyses and determination of dry weight.

Organic and inorganic analyses. All analyses were carried out by the methods already published (see Dickson et al. 1980 and references therein) except for sulphate which was measured as described by Jackson and McCandless (1978).

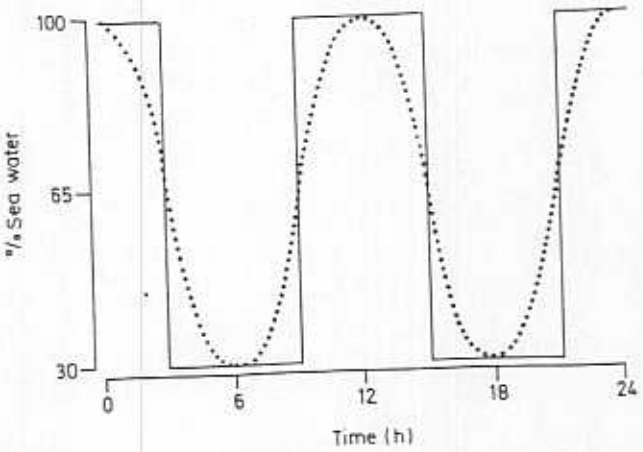

Fig. 2. Fluctuating salinities regimes to which the *U. lactuca* was exposed. ——, abrupt, square-wave regime; ·····, sinusoidal regime

Results

Inorganic ion contents. Ulva lactuca subjected to both the abrupt, square-wave and gradual sinusoidal fluctuations in salinity showed substantial changes in its internal contents of K^+ and Na^+ (Fig. 3) and Cl^- and SO_4^{2-} (Fig. 4). Clear differences emerged in the ion contents of the tissue exposed to the two experimental regimes and to light and dark. Data in Fig. 3 and 4 are expressed on a tissue-water basis to allow for changes in hydration. These can be re-expressed on a fresh-weight (FW) basis by the use of the tissue-water content and free-space component (see Dickson et al. 1980). While the exact conversion factor varies with salinity, fresh-weight values are approximately half of the ion concentrations expressed on a tissue-water basis.

In the sinusoidal light regime, cellular K^+ closely followed the external salinity but in the dark K^+ accumulation during the hyper-osmotic portion of the cycle was severely restricted. Tissue Na^+ and SO_4^{2-} levels also showed near-sinusoidal changes but with little light dependence. In the case of Na^+, there was a tendency, particularly in the light, for changes in response to the hyper-osmotic stress to lag behind the external salinity (Fig. 3). Chloride changes were also unaffected by light and were approximately sinusoidal in character but during the hyper-osmotic cycles the chloride concentration of the tissue consistently failed to attain the level (about 400 μmol ml^{-1}) found in the tissue equilibrated for several days with 100% salinity.

As might be anticipated, changes in ionic concentrations are much more irregular in tissue subjected to the abrupt regime (Figs. 3c, d; 4c, d).

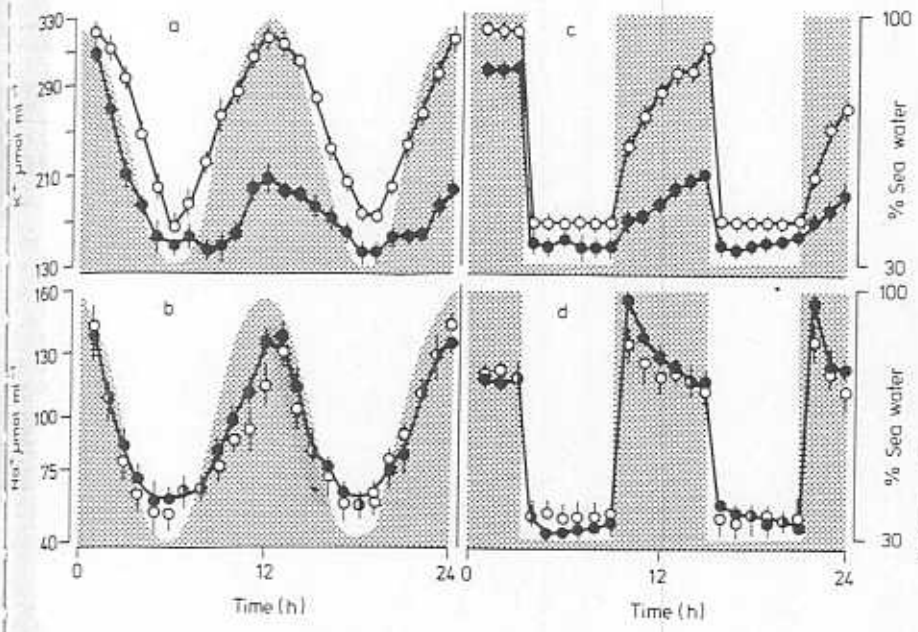

Fig. 3a–d. Changes in tissue K^+ and Na^+ ion contents in *U. lactuca* fronds exposed to the sinusoidal (**a, b**) and square-wave (**c, d**) regimes. Background speckling indicates the salinity regime. Ion concentrations of K^+ (**a, c**) and Na^+ (**b, d**) are expressed on a tissue-water basis. Error bars give 95% confidence limits. o, Tissue in the light; • tissue in the dark

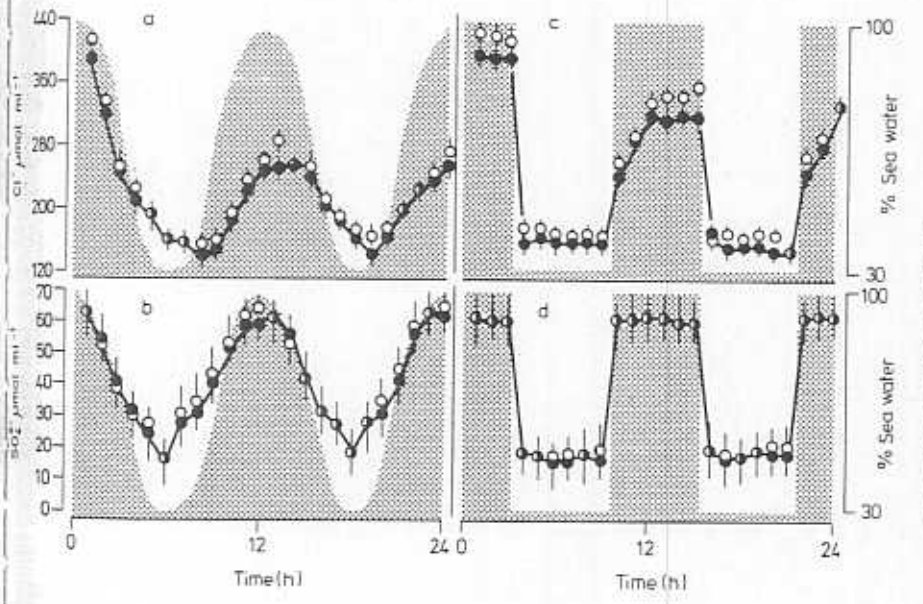

Fig. 4a–d. Changes in tissue Cl^- and SO_4^{2-} ion contents in *U. lactuca* fronds exposed to the sinusoidal (**a, b**) and square-wave (**c, d**) regimes. Background speckling indicates the salinity regime. Ion concentrations of Cl^- (**a, c**) and SO_4^{2-} (**b, d**) are expressed on a tissue-water basis. Error bars give 95% confidence limits. o Tissue in the light; •, tissue in the dark

Changes in K^+ concentration are again markedly light-dependent and lagged significantly behind the external salinity changes during the hyper- but not hypo-osmotic shock (Fig. 3c). Following the 30- to 100%-seawater hyper-osmotic shock, tissue K^+ concentrations only reached the usual equilibrium, 'sea-water' value after 6 h. The increase in the intracellular K^+ concentration over the first hour approximated to 80 µmol K^+ ml^{-1} of tissue water, equivalent to an uptake rate of 40 µmol K^+ g^{-1} FW h^{-1}. After the first hour the rate fell to approx. 24 µmol K^+ ml^{-1} h^{-1} (equivalent to an uptake of 12 µmol K^+ g^{-1} FW h^{-1}), while in the dark, influx rates were only about 14 µmol K^+ ml^{-1} h^{-1} (equivalent to an uptake of 7 µmol K^+ g^{-1} FW h^{-1}).

In marked contrast to K^+, Na^+ flooded rapidly into the tissue after hyper-osmotic shock reaching levels approaching 160 µmol ml^{-1} but then gradually falling back to 130–140 µmol ml^{-1}. In this case the initial influx was greater in the dark than in the light (Fig. 3d). The behaviour of Cl^- closely resembled that of K^+ except that the light-dependence of hyper-osmotic recovery was much less pronounced. Sulphate concentration showed a square-wave function independent of light and closely tuned into the external salinity changes. The internal Mg^{2+} content was measured (data

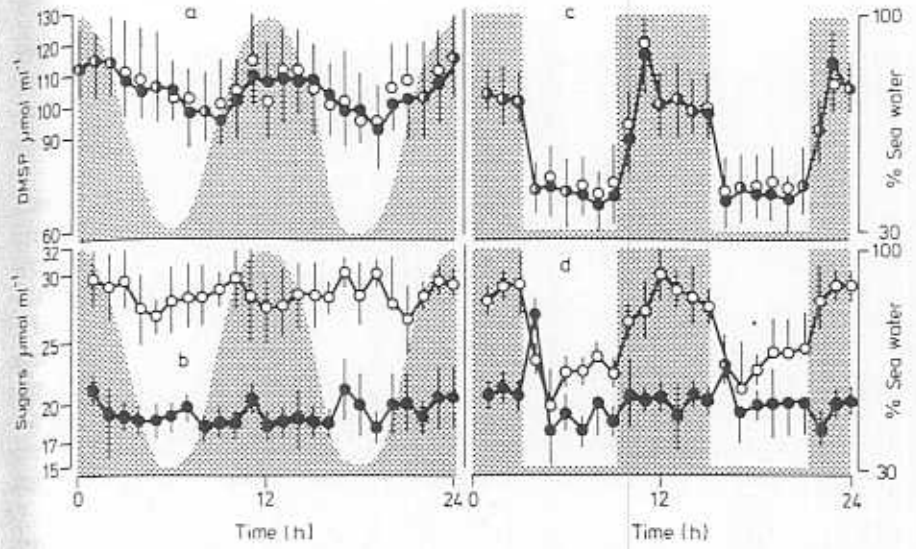

Fig. 5a–d. Changes in the dimethylsulphoniopropionate (**a, c**) and sugar (**b, d**) levels in *U. lactuca* exposed to the sinusoidal (**a, b**) and square-wave (**c, d**) salinity regimes. Error bars give 95% confidence limits. o, Tissue in the light; • tissue in the dark

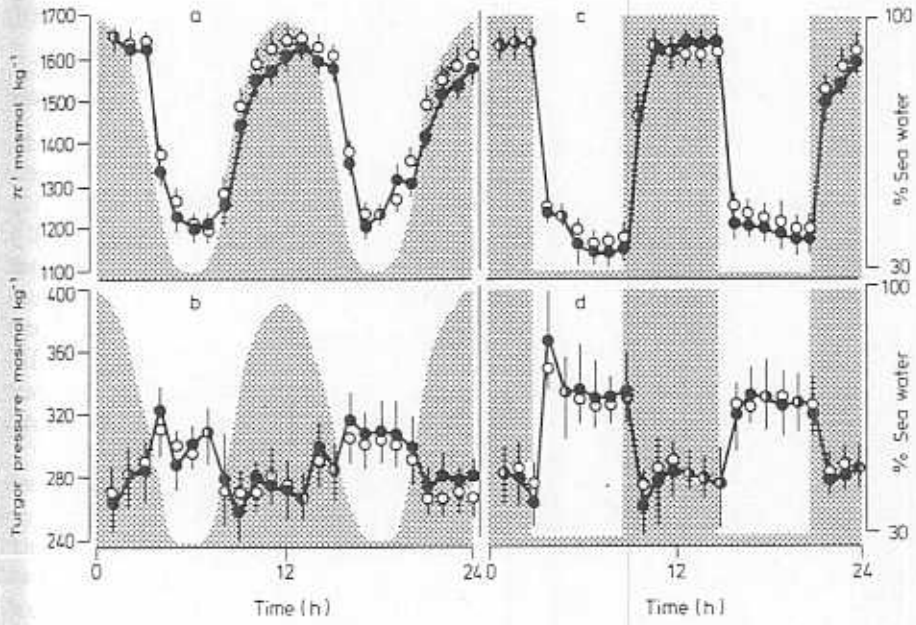

Fig. 6a–d. Changes in the tissue osmolality (**a, c**) and apparent turgor pressure (**b, d**) and square-wave (**c, d**) salinity regimes. Error bars give 95% confidence limits. o, Tissue in the light; •, tissue in the dark.

not presented) and, at about 240 μmol g^{-1} FW, did not change under either salinity regime.

Organic solute levels. Previous work had shown that the pool of free amino acids was relatively small in this alga and unlikely to be involved in osmotic regulation (Dickson et al. 1980). This was confirmed in this study as the levels did not change under either regime (data not presented). However, changes were observed in both the free-sugar and DMSP levels (Fig. 5a–d); the latter being identified as the putative compatible solute in this species (Dickson et al. 1980). In the sinusoidal regime a high DMSP concentration was maintained throughout with only a slight indication of a fall under low-salinity conditions (Fig. 5a). These solute concentrations were independent of the light regime. However when exposed to the abrupt regime, the tissue DMSP changed substantially, falling from 110–120 μmol ml^{-1} to approx. 80 μmol ml^{-1} after the hypo-osmotic shock and then recovering in about 2 h after the hyper-osmotic shock. Both accumulation and loss of DMSP were light-independent. Similarly sugar concentration changes are insignificant in the sinusoidal regime although light levels always exceed those found in dark-grown tissue as previously noted (Dickson et al. 1980). In the abrupt regime rather complex changes in free-sugar concentrations were observed. In the light the sugar concentration fol-

lowed the salinity changes, while in the dark this pattern was absent, although there was an indication of a significant increase in sugar immediately after the hypo-osmotic shock.

Osmotic and turgor pressure. To substantiate the data on solute fluctuations, tissue osmotic pressures were measured by vapour pressure osmometry on freeze-thawed discs and an assessment of turgor pressure made from the difference in equilibrium vapour pressure of undamaged and freeze-thawed discs (see Dickson et al. 1980) (Fig. 6a–d). During the sinusoidal regime, the apparent turgor pressure changed only slightly in the range 270–310 mosmol kg^{-1} (equivalent to about 0.7 MPa) although the tissue osmotic pressure changed markedly from 1,650 to 1,200 mosmol kg^{-1}. The external salinity changed from approx. 1,000 to 300 mosmol kg^{-1} during this period.

Discussion

The major purpose of this study was to compare osmotic adaptation in *U. lactuca* exposed to quasi-steady-state changes in external salinity with that in tissue exposed to fluctuating salinity regimes bearing at least some resemblence to the salinity changes found in local estuaries (Cawthorne 1979). Interesting differences were detected both between the fluctuating regimes and the steady-state experiments (Dickson et al. 1980) and between the two fluctuating regimes.

The major difference appeared between regimes, which involved abrupt hypo- or hyper-osmotic *shocks*, that is both the quasi-steady-state regime of Dickson et al. (1980) and the square-wave regime used in this paper, and the gradual changes in salinity stress used in the sinusoidal regime.

In the sinusoidal light regime the K^+ content as well as being highly light-dependent was highly correlated ($r=0.9$; $P<0.01$) with external salinity and external K^+ content. Tissue Na^+ content was similarly highly correlated ($r=0.9$; $P<0.01$) with salinity but largely light-independent. Correlations of salinity with Cl^- content were not examined because the tissue level consistently failed to recover its equilibrium level after the hyper-osmotic stress.

Hypo-osmotic shock led to a rapid loss of tissue K^+, Na^+ Cl^- and SO_4^{2-} (but not Mg^{2+}, data not presented), confirming earlier observations on *U. lactuca* transferred to 30% and 60% seawater (Dickson et al. 1980). Recovery of K^+ after such hypo-osmotic shocks is highly light-dependent and ^{42}K fluxes in tissue equilibrated with 100% seawater were sufficient to account for the initial increase of the intracellular K^+ concentration of about 80 µmol ml^{-1} (Dickson 1981). Flux rates were calculated from measurements of the exchange of appropriate radioactive tracer ions ($^{42}K^+$, $^{22}Na^+$ and $^{36}Cl^-$; obtained from the Radiochemical Centre, Amersham, U.K.) between 100% seawater and discs of *U. lactuca*, using the method of Black and Weeks (1972). Accumulation of Na^+ after the hyper-osmotic shock indicated a large initial influx, possibly greater in the dark, followed by an efflux of the ion. The initial fall in the intracellular concentration of Na^+ in the dark (about 28 µmol ml^{-1}; equivalent to 14 µmol g^{-1} FW h^{-1}) exceeded the efflux rate (namely, 11.8 ± 0.7 µmol g^{-1} FW h^{-1}, value for five separate determinations – see Dickson 1981) estimated using $^{22}Na^+$ in tissue equilibrated with 100% seawater. The higher intracellular Na^+ content induced by the hyper-osmotic shock may have stimulated the Na^+ efflux pump as reported in other species (Saddler 1970; Barber and Shieh 1973; Tromballa 1974).

The Cl^- accumulation in the hyper-osmotic phase of the abrupt regime was particularly interesting and difficult to interpret. There was initially a rapid light-dependent increase in the tissue intracellular Cl^- concentration of about 96 µmol ml^{-1} in the first hour; this is equivalent to an uptake rate of 45 µmol g^{-1} FW h^{-1}). This rate decreased after 1 h to approx. 35 µmol ml^{-1} (equivalent to an uptake of 18 µmol g^{-1} FW h^{-1}), and further diminished after 3 h to approx. 3.6 µmol g^{-1} FW h^{-1}. Thus after 6 h the Cl^- content failed to reach the equilibrium level. The initial phase of Cl^- uptake (namely, 125 µmol ml^{-1}; equivalent to an uptake rate of 60 µmol g^{-1} FW in about 2 h) may reflect the amount accumulated in the cytoplasm since the plasmalemma flux, measured independently using $^{36}Cl^-$ in the light, was found to be about 19.8 ± 0.9 µmol g^{-1} FW h^{-1} (value is for five separate determinations – see Dickson 1981) in tissue bathed in 100% seawater. The level of cytoplasmic Cl^- which would result from an influx of 60 µmol g^{-1} FW in 2 h is higher than that estimated by flux analysis to be present in the cytoplasm of tissue equilibrated with 100% seawater (40 ± 2.3 µmol g^{-1} FW; Dickson, 1981).

This high cytoplasmic Cl^- content may lead to the inhibition of further Cl^- influx (see also *Codium* and *Halicystis*, Gutknecht et al. 1978). The final rate of Cl^- influx between 4–6 h (3.6 µmol g^{-1} FW h^{-1}) was very similar to tonoplast flux in both light and dark. If, under these conditions, the Cl^- tonoplast flux is rate limiting this could

account for the inability of tissue Cl^- to reach the equilibrium level in 6 h. The rapid initial Cl^- influx found in tissues subjected to hyper-osmotic shock (45 μmol g^{-1} FW h^{-1}) might be due to a transient increase in the Cl^- permeability of the plasmalemma following this harsh treatment. A very similar explanation, except for the absence of a transient response to the hyper-osmotic shock, may be offered for the inability of Cl^- to reequilibrate in the sinusoidal regime.

No statistically significant changes in DMSP, sugars or free amino acids (data not presented) were found in tissue subjected to the sinusoidal regime. In marked contrast the DMSP level fell immediately after hypo-osmotic shock and took about 2 h to recover after the hyper-osmotic phase. Rather similar changes were noted in free sugar levels but on a smaller scale.

The changes in turgor and, to a lesser extent, osmotic pressures were also heavily buffered in the sinusoidal regime in comparison with the abrupt regime.

Since the fall in external osmotic pressure ($\Delta\pi = 700$ mosmol kg^{-1}) exceeded the fall in tissue osmotic pressure ($\Delta\pi_i$ approx. 450 mosmol kg^{-1}) in the sinusoidal regime, a greater increase in turgor pressure than that found in Fig. 6b might be expected.

In the abrupt regime, the overall changes in π_i were similar to those observed in tissues exposed to gradual changes in salinity ($\max\Delta\pi_i = 450$–500 mosmol kg^{-1}) but the turgor pressure changes were greater and more significant (approx. 70 mosmol kg^{-1}), so that the change in external osmotic pressure (about 700 mosmol kg^{-1}) is approximately balanced by the sum of the changes in tissue osmotic and turgor pressure (520–600 mosmol kg^{-1}). As noted previously, tissue-water content was also recorded throughout both regimes, and while the water content was greater at low external salinity and in dark-growth tissue (data not presented), the absolute changes were not large, maximum differences being from 66.5 to 71% tissue-water content.

A major problem revealed by these data is that of charge balance during the changes in salinities. The levels of the measured inorganic ions in *U. lactuca* equilibrated with 100% sea water indicate an excess cationic charge of approx. 50–60 m equivalents (Table 1). The failure of Cl^- to reach the equilibrated tissue value in 6 h resulted in the light, abrupt, hyper-osmotic regime in this gap increasing to 107 m equivalents. However the situation was even worse in the hyper-osmotic, gradual-stress condition where the greater control of Na^+ influx increased the apparent charge imbalance to 166 m equivalents. It is quite apparent that such a charge imbalance cannot possibly exist but at the moment we are not aware of the explanation for these discrepancies. In the dark regimes the charge imbalance is less marked. However, it is likely that these solutes are not evenly distributed between the cytoplasm and vacuole (Dickson 1981; personal communication by Pitman and Wyn Jones) and any attemt to assess charge balance on a compartmental basis leads to even greater problems.

Although a number of problems are raised from these data, and these require further detailed

Table 1. The levels of inorganic ions in the tissue of *U. lactuca* equilibrated with 100% seawater in the light (400 μE m^{-2} s^{-1}) and in the dark, and after abrupt or sinusoidal hyper-osmotic changes of salinity. Values are expressed as m-equivalents l^{-1} and are the means for three separate determinations

Salinity	Cation m-equiv. l^{-1}		Anion m-equiv. l^{-1}	
100% steady-state salinity (light)	K^+	366	SO_4^{2-}	124
	Na^+	144	Cl^-	428
	Mg^{2+}	102		
	Σzj	612	Σzj	552
Σzj (cation) − Σzj (anion) = 60 m-equiv.				
100% steady-state salinity (dark)	K^+	327	SO_4^{2-}	124
	Na^+	141	Cl^-	400
	Mg^{2+}	104		
	Σzj	572	Σzj	524
Σzj (cation) − Σzj (anion) = 48 m-equiv.				
After hyper-osmotic abrupt change of salinity (light)	K^+	345	SO_4^{2-}	126
	Na^+	150	Cl^-	364
	Mg^{2+}	102		
	Σzj	597	Σzj	490
Σzj (cation) − Σzj (anion) = 107 m-equiv.				
After hyper-osmotic abrupt change of salinity (dark)	K^+	219	SO_4^{2-}	132
	Na^+	135	Cl^-	290
	Mg^{2+}	102		
	Σzj	456	Σzj	453
Σzj (cation) − Σzj (anion) = 3 m-equiv.				
After hyper-osmotic gradual change of salinity (light)	K^+	332	SO_4^{2-}	124
	Na^+	144	Cl^-	290
	Mg^{2+}	104		
	Σzj	580	Σzj	414
Σzj (cation) − Σzj (anion) = 166 m-equiv.				

study, it is apparent that metabolic responses in *U. lactuca* are highly buffered in the sinusoidal regime in comparison with the abrupt square-wave salinity regime. In that such gradual changes in salinity are typical of many estuarine and intertidal habits, this is probably an important aspect of osmoregulation which has not been detected by the conventional quasi-steady-state experiments employed by most investigators.

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