SPECTRAL REFLECTANCE OF *Thalassia testudinum* (HYDROCHARITACEAE) SEAGRASS: LOW SALINITY EFFECTS

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Massive anthropogenic changes in estuarine salinities, from manipulations of freshwater flows, are again occurring through governmental projects “correcting” past freshwater alterations. The downstream effects of increased freshwater on seagrass meadows, a major fisheries and ecosystem habitat, are not clear. Spectral responses to low salinities were quantitatively delimited for the important habitat seagrass *Thalassia testudinum* utilizing spectral reflectance measurements for the first time (non-invasive sampling). Over a range of salinities (32–16 parts per thousand sea salts [ppt] for 24 h) and spectra (308–1138 nm), *Thalassia* specimens showed statistically significant differences in spectral values (*P* < 0.05) between treatments at normal (32 ppt) and 50% reduced (16 ppt) seawater. Mature blades yellowed at low salinities. Reflectance changes at 525 nm and 650–680 nm at low salinities suggested changes in xanthophylls and chlorophylls. Four indices were also used to characterize the reflectance spectra to delineate the effect of the salinity changes: (1) The normalized difference vegetation index (NDVI) for mature blades reduced at 16 ppt from that at 32 ppt. (2) The chlorophyll normalized difference index (Chl NDI) suggested chlorophyll content decreases in response to reduced salinity. (3) The structure independent pigment index (SIPI), higher in mature blades at 16 ppt than new blades, indicates a higher carotenoid: chlorophyll ratio in mature blades. (4) The photochemical reflectance index (PRI) suggested a lower photochemical efficiency at lower salinities. The main low-salinity effect on *Thalassia* physiology delineated herein is likely through changes in pigmentation (decreases in chlorophyll and changes in xanthophyll cycle epoxidation).

**Key words:** chlorophyll; chlorophyll normalized difference index; photochemical reflectance index; salinity; seagrasses; spectral reflectance; *Thalassia*.

The seagrass genus *Thalassia* is usually the dominant vegetation in the Atlantic subtropical/tropical submerged marine and estuarine ecosystems and is co-dominant in mixed Pacific seagrass meadows (den Hartog, 1970). *Thalassia testudinum* is associated in the Atlantic with a highly diverse and abundant fauna (Thorhaug et al., 1976; Thorhaug and Roessler, 1978; Livingston, 1984; Livingston et al., 1997) that is the basis of important commercial and sports fisheries. Beyond factors in the marine environment of light penetration (generally a function of depth and water clarity) and soft sediment, the limiting factor for spatial distribution of *Thalassia* in subtropical or tropical estuaries is frequently the salinity regime, usually determined by fresh water inflow. *Thalassia* is generally known to be less tolerant to lowering of oceanic salinity levels by fresh water input than several other seagrass genera, such as *Halodule* (shoal grass), *Halophila* (paddle grass), and *Ruppia* (widgeon grass) from comparative studies by McMillan (1979), Thorhaug and Marcus (1981), and Thorhaug et al. (1985), as well as from numerous field observations but is more tolerant than *Syringodium* (manatee grass). Very few laboratory studies of *Thalassia*’s low salinity responses have been carried out. Testing the ability of seagrass to function over the short term under lowered salinities is of importance for the following reasons: (1) Extreme changes in salinity (inshore waters) are occurring through anthropogenic manipulation of watershed flows through canals, channelization, dams, and other changes of overland and riverine flows around the world. These changes have the potential to alter the amounts and duration of freshwater flow to downstream seagrasses. (2) Governmental agencies are again altering salinities in goals termed “restoration,” designed to remedy past altered-flow mistakes on wetlands (U.S. Army Corps of Engineers, 1999). In the St. Lucie, Florida area, a waterway drainage from leaking flood-control structures has killed a massive amount of seagrass (Graves et al., 2002). The fate of downstream seagrasses from the effects of planned increased fresh water discharges remains unclear. Sustaining seagrasses and their associated food webs and fisheries in the face of these salinity changes are clearly necessary.

Field studies have shown that salinity changes resulting from fresh water input alter seagrass species, distribution, abundance, and dependent food web species. In the Gulf of Mexico, Florida, and the Caribbean this alteration is apparent and has been documented as lowering density and distribution of *Thalassia* at the mouths of the man-made drainage canals and in other areas of manipulated flows (Roessler and Tabb, 1974; Thorhaug et al., 1976; Thorhaug et al., 1985; Wang and Cofer-Shabica, 1990; Livingston et al., 1997; Quammen and Onuf, 1993; Onuf, 1993; Lapointe et al., 1994; McKenzie, 1994; Fletcher and Fletcher, 1995; Hillman et al., 1995; Johnson and Johnstone, 1995; Dunton, 1996; Boyer et al., 1999; Kamermans et al., 1999; Tomasko and Hall, 1999; Moore et al., 2000; Forquean et al., 2001; Laboy-Nieves et al., 2001; Irlandi et al., 2002). However, most of the field examples do not precisely delineate the seagrass tol-

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erance limits to low salinity as would laboratory studies, because multiple stresses on the seagrasses usually occur within these field investigations.

One needs to examine salinity alone as a single stress factor on seagrasses as well as in concurrence with multiple stresses. Several multifactorial analyses of seagrasses in the laboratory have included salinity as one factor (Schroeder and Thorhaug, 1979, 1980; Thorhaug and Marcus, 1981; Ralph, 1995; Koch and Erskine, 2001). One study by Thorhaug and Marcus (1981) indicates 20 ppt sustained over 3–4 d caused 100% mortality to *Thalassia testudinum*. At higher temperatures above 29°C, four species of seagrass, including *Thalassia testudinum*, were tolerant to low salinities. Ascendent result of this study, was that at medium temperatures (23–29°C) salinity tolerances occurred to a lethality level at 16 ppt for 3 d in *Thalassia* (the range of salinities being 12 to 37 ppt) (Thorhaug and Marcus, 1981). Schroeder and Thorhaug (1980) found the interaction between heavy metal uptake, salinity, and temperature to be interdependent in *Thalassia* at 23–29°C, with the slope of the rate of metal uptake appearing linearly correlated with increasing temperature.

In terms of physiological mechanisms, some important discoveries will eventually help us isolate the mechanism of low salinity effects. (1) Laboratory studies indicated that the epidermal leaf morphology of seagrass changes with low salinity, included signs of damage (Iyer and Barnabas, 1993). (2) Fukuhara et al. (1996) isolated a putative plasma membrane H⁺-ATP-ase in the plasmalemma of *Zostera marina* during studies of low salinity tolerance effects on this membrane system. They found a amino acid sequence predicted from nucleotide sequence ZHA1 with those encoded by known genes for a plasma membrane H⁺-ATPase strongly expressed in mature blades (specifically epidermal cells), which they hypothesized may be important for salt excretion by the plant. (3) Benjamin et al. (1999) found an unusual double membrane or annulus with blade cells of the euryhaline seagrass *Halophila ovalis*, when incubated at low salinities. (4) In osmotic studies on *Halophila ovalis* by Ralph (1995), strong time dependency effects on health of blade cells and whole plants occurred for salinities of 50% reduction from normal sea water in the 72-h test. The salinity slightly affected measurements of fluorescence after 12 h, with clear effects after 24 h and severe effects at 48 and 72 h (Ralph, 1995).

In our present study, we applied methodology used to quantify stress responses in terrestrial plant physiology (especially changes in pigmentation and consequently photosynthetic light processing) (Peterson et al., 1988; Martin and Aber, 1997; Gamon and Qiu, 1999; Gamon and Surfus, 1999; Ourcival et al., 1999; Richardson et al., 2001; Penuelas et al., 1993; Penuelas and Fillela, 1998) to measure stress responses in marine plants. The methodology of spectral reflectance primarily measures pigment responses in leaves as the amount of reflectance at a range of wavelengths, which can be directly related to the efficiency of the photosynthetic processes of the plant. Characteristics of leaf reflectance are determined by the surface properties, internal structural features, and biochemical components of the leaf. [Richardson et al. (2001), among others, have observed in several species of fir and birch that reflectance in the visible (400–700 nm) part of the spectrum is dominated by pigment content, especially the chlorophylls. The red edge effect occurs as a sharp increase in reflectance around 700 nm. A number of authors have noted that chlorophyll absorbs strongly in red but not in infrared. Scattering of near infrared occurs within the leaf and results in high near infrared reflectance: hence, these cause the red edge to exist. Curran et al. (1990) concluded that the red edge shifts to shorter wavelength that occur under distress or senescence as a product of decrease in chlorophyll.] Excellent technical features are non-invasiveness and nondestructiveness plus the short-term period of measurement (30 s) (Richardson et al., 2002a); additionally the same plants can serve as their own control. Richardson et al. (2001) have pointed out that Penuelas et al. (1995) found both PRI (photochemical response index) of spectral reflectance and maximum yield of photosystem II were correlated with instantaneous gas-exchange and this was based measures of photosynthetic radiation use efficiency (PRUE).

Our hypothesis is that statistically significant changes in spectral reflectance will occur when *Thalassia* is exposed for a sufficient duration to a range of low salinities below those in the natural habitats of *Thalassia* and that those changes in spectral reflectance will be consistent with stress responses in terrestrial species.

MATERIALS AND METHODS

Collection, holding, incubation, and reincubation of *Thalassia*—Whole, unbroken young *Thalassia* plants with seeds attached (3–12 cm blades and 2–10 cm rhizomes) were collected in far southeast Florida, scraped of epibions (in a previously standardized process by Thorhaug [1971]), and handtransported to the experimental site in New Haven, Connecticut, USA. Specimens were three-root young plants with attached seeds gathered from the same area so that in terms of genetics, age, pre-condition, and morphology, they were extremely similar. These seedlings were selected to be complete plants with no torn tissue to expose interior cells or spaces to the changing salinities and resultant osmotic changes. Mature plants with rhizomes, meristems, and intact roots were also used as controls, which were collected, transported, and incubated exactly the same as the other plants. The incubations in various salinities of seawater (from 32 ppt to 16 ppt) were carried out in 80% ambient September/October sunlight at 25°C, in filtered, autoclaved, Florida seawater. Between test incubations, plants were kept in culture for several days in normal Biscayne bay seawater (32 ppt). A 24-h (representing two Atlantic tidal cycles) incubation was based on previous laboratory data showing that a 72-h exposure to lethal factors, including salinity, was a critical period for mortality for *Thalassia* (Thorhaug, 1971, 1974a; Thorhaug and Marcus, 1981; Thorhaug et al., 1989). Incubation light cycles were 12 h light, 12 h dark greenhouse natural daylight. The incubation order of salinities was randomly selected. All plants were incubated at the same salinity simultaneously. Any browning, yellowing, or other morphological changes, such as new leaves, were recorded for plants (numbered with plastic labels) during the entire period. Three days were left between readings to standardize the rest period prior to another test. During incubations, microbions were kept at a minimum by gentle scraping and seawater was changed regularly.

Reflectance measurements on seagrass—Experiments were carried out in low light conditions after a 1-h dark adaptation in their seawater mixture; plants were patted dry and scanned to measure their spectral reflectance using the same experimental procedure for leaves of forest plants in Richardson et al. (2001). After this 30-s dry period for scanning, the plant was immediately reincubated at 32 ppt.

The scanning procedure was as follows: Spectral reflectance was measured using a UNISpec Spectral Analysis System (PP Systems, Haverhill Massachusetts, USA) over the range of 306–1138 nm with a 2.0 mm diameter foreoptic and an internal 6.8-watt halogen lamp (PP systems, Haverhill, Massachusetts, USA). Standard controls were dark and a reflectance white standard, which was obtained with the UNISpec and had been used for all terrestrial studies referred above. Individual blades from a living plant were held in a black PVC clip at a 60° angle relative to the foreoptic. Two blades of the test plants were scanned three times to obtain three spectral reflectance
measurements \( R_{\text{leaf}} = \text{leaf radiance at wavelength } \lambda \text{reflectance standard}

radiance at wavelength } \lambda \). At each selected salinity, 11 plants with two blades each (one new and one mature) were scanned. The three measurements per

blade were averaged. (Therefore, the scans were averaged from 22 entries [11

plants \times 2 blades], each entry the average of three readings per blade or 66

total readings.) Blades were identified as new (just growing from the shoot),
mature, or senescent. A second set of measurements was carried out on partial-

ly or incipiently senescent blades. In general, young plants with attached

seeds shed blades at 15–23-d periods as do adults (Thorhaug, 1971, 1974a, b).

The three groups of blades (new, mature and senescent, or blackened with

no chlorophyll) were also compared as a control to indicate whether blade

age and condition were factors in the spectral response of the plant. In ad-

dition, spectral reflectance of mature blades of mature meristems of Thalassia

was measured to test effect of blade morphology on the reflectance. These

tests showed no significant difference at the 0.01 level for spectral reflectance

between mature and seedling blades. The same numbered plants were incu-

bated and their spectral reflectances measured at five different salinities (32,

28, 24, 20, and 16 ppt). Re-emission at 32 ppt occurred between randomly

selected salinity incubations.

Reflectance calculations—We used four indices, based on the reflectance

properties of plant pigments and their biochemical components, to charac-

terize the reflectance spectra in order to delineate the effect of the salinity change.

(1) The photochemical reflectance index (PRI), an indicator of photosyn-

thetic efficiency, was calculated by Gamon et al. (1997) as PRI = \( \frac{(R_{670} - R_{565})}{(R_{565} + R_{670})} \).

(2) A revised version of the normalized difference vegetation index (NDVI) = \( \frac{(R_{705} - R_{670})}{(R_{705} + R_{670})} \) was calculated by Gitelson and

Merzlyak (1994) as (3) chl NDI = \( \frac{(R_{531} - R_{670})}{(R_{531} + R_{670})} \), which is

sensitive to a wide range of chlorophyll \( a \) concentrations and well correlated

to chlorophyll \( a \) and was used in the following studies: Gitelson and Merz-

lyak, 1994, 1996; Gitelson et al., 1996; Richardson et al., 2001. Total chlo-

rophyll content is correlated with the red edge position (Curran et al., 1990),

which is the wavelength \( \lambda \) in nanometers of the maximum slope of the re-

flectance spectrum at wavelengths between 690 and 740 nm. (The first-dif-

ference spectrum measures how much reflectance changes from one wave-

length to the next. It is an approximation of the slope [or the first derivative]

of the raw reflectance spectrum.) (4) The structure-independent pigment index

(SIPI), which is correlated with the carotenoids to chlorophyll \( a \) ratio (Penuelas

and Filella, 1998), was calculated at \( \frac{(R_{660} - R_{540})}{(R_{660} - R_{600})} \).

RESULTS

Low salinity effects on Thalassia—The effects of incubation at lower salinities on Thalassia are clearly seen by comparing reflectance spectra at 32 ppt and 16 ppt (Fig. 1), discussed later (see Low salinity effects on blade senescence). Intermediate salinities give additional interesting information (Fig. 2). At 32 ppt, all Thalassia blades appeared healthy, but after 24 h in 16 ppt, many of the blades had reduced reflectance between 518 and 532 nm, which may indicate changes in xanthophyll-cycle pigmentation (Richardson et al., 2001).

The spectral reflectances of mature Thalassia specimens were not significantly different from the young plants at a given salinity. Between 650 and 680 nm, reflectance was increased following incubation at 16 ppt. This result suggests reduced absorption of light by chlorophyll, which may be a product of stress-reduction in chlorophyll at 16 ppt. At 700 nm, the transition in the near-infrared plateau is much sharper at 32 ppt than at 16 ppt. We have observed a similar response in desiccating leaves of paper birch and mountain ash (Richardson and Berlyn, 2002a). The reflectance difference spectra (reflectance at 32 ppt minus reflectance at 16 ppt) are shown in Fig. 3, where the vertical lines indicate statistically significant differences (\( P < 0.05 \)) between spectra at 32 and 16 ppt on standard \( t \) tests comparing each spectral band at 16 vs. 32 ppt. At 518–532 nm and 650–680 nm, the statistically significant
differences are clear in this treatment. Fig. 4 shows the comparison between the seagrass Thalassia and several terrestrial tree species carried out by Richardson and Berlyn (2002a, b) and Richardson et al. (2003), which functions as a type of methodology control to assure us that the measurements are realistic for seagrass. The terrestrial species have many of the same peaks and troughs as do the seagrasses although at differing values from the seagrass. (One might recall that seagrasses evolved from terrestrial plants (de Hartog, 1970), unlike most marine plant species.

Low salinity effects on blade senescence—Low salinity affected the color of blades. Browning blades included about 30% (mature blades at 16 ppt after 24 h) have some chlorophyll and appear yellow with brown spots and a greenish tint), but most blades are yellow-brown and translucent. Those blades which have become black blades at 16 ppt have lost all pigments and have become an opaque, dark brownish-black color with no yellows or greens. This is an irreversible condition for the blade. They are expected to consist mostly of cell wall material. The blades on small test specimens remain intact on the plant for some period in this condition. In nature, eventually a wind event detaches black blades from the plant. These phases have been recognized in many toxicity tests by others and by us and are like the steps of blade senescence that occur every 15–22 d, even in young, immature plants. We have found by incubation with various toxicants over various time periods that blade yellowing and browning precedes death of the plant (Thorhaug, 1971; Thorhaug et al., 1989). Here, browning and necrosis resulted in a clear change in the shape of reflectance spectra for Thalassia blades (Fig. 1). The normal “green” reflectance peak was shifted to a longer wavelength (620 nm) in brown blades and was nonexistent in necrotic black blades. Reflectance at 680 nm increased to about 5% in brown blades (compared to 2–3% in healthy blades), but was again lower (c. 2–3%) in black blades. Neither brown nor black blades exhibited the sharp “red edge” transition region around 700 nm, and in fact, the shape of both spectra in this region was dramatically different from that of healthy blades. We observed that the slope of the reflectance spectra in the neighborhood of 750 nm was the steepest for black blades, positive but not very steep in 16-ppt blades, and slightly negative in 32-ppt blades. This suggested to us that $D_{750}$ (the first derivative of reflectance at 750 nm) might be a suitable stress index for Thalassia.

Salinity effects on reflectance indices—Several reflectance indices (Fig. 5) demonstrated the effects of changes in salinity from 32 to 16 ppt on Thalassia. Reduced salinity effects were most pronounced at 16 ppt, and thus this discussion focuses on the difference between incubation at 32 vs. 16 ppt (Fig. 5). We also compare the differences in index values between new and mature blades at 16 and 32 ppt in seedlings. The normalized difference vegetation index (NDVI) was similar at 32 ppt for both new and mature blades, but a slight increase in NDVI for the new blades was seen at 16 ppt. Similarly, chlorophyll NDI was similar for both blade types at 32 ppt, but increased in new blades and decreased in old blades at 16 ppt. Both NDVI and chl NDI results suggest that in response to the reduced salinity, the chlorophyll content of new blades increases slightly and that of mature blades decreases sharply. Our hypothesis for this result is that over 24 h the new blades’ expansion may have slowed, which would increase the chlorophyll content, while older blades were stressed into a senescence-like response, whereupon they lost chlorophyll. This hypothesis clearly needs further investigation. The structure-independent pigment index (SIP) was similar in both mature and new blades at 32 ppt, but at 16 ppt was higher in mature blades than in new blades. A higher value of SIP for mature blades at 16 ppt suggests a higher carotenoid to chlorophyll ratio. It is not clear, however, whether these changes are the result of changes in both carotenoids and chlorophylls or just chlorophylls. The observed changes in SIP between 32 and 16 ppt are consistent with an increase in chlorophyll in new blades and a decrease in chlorophyll in mature blades. At 32 ppt, the photochemical reflectance index (PRI) was lower in mature blades than in new blades, suggesting a lower photochemical efficiency in the mature blades. Both new and mature blades showed reductions in PRI at 16 ppt. In our past work (Richardson et al., 2001), PRI was a more reliable indicator of plant stress than most chlorophyll-based indices. This may be due to the fact that changes in xanthophyll-cycle pigmentation are known to occur very rapidly, whereas chlorophyll is degraded more slowly. The slope of the reflectance spectra at 750 nm, $D_{750}$, was similar in new and mature blades at 32 ppt, was somewhat higher in new blades at 16 ppt, but was dramatically higher in old blades at 16 ppt. Both PRI and $D_{750}$ thus suggest that reduced salinity has a somewhat larger effect on mature blades than on new blades.

Across the range of salinities studied (32, 28, 24, 20, 16 ppt), both PRI and $D_{750}$ decreased as salinity decreased (Fig. 2). For PRI and $D_{750}$, the response can be divided into three levels: (1) normal seawater response (in the range of seawater down to about 28 ppt); (2) a range of responses different from normal seawater response (28 to 24); and (3) sharply reduced photochemical efficiency (below 20 to 16 ppt), which apparently may not be viable for time periods over three or more days. The slope of the reflectance spectra at 750 nm, $D_{750}$, shows this more distinctly in mature blades. At present, further experimentation would be necessary to delimit which of these response levels occurs at salinities between 24 and 20 ppt.

DISCUSSION

Low salinity effects on Thalassia testudinum spectral reflectance measurements and indices—Our hypothesis of sig-

![Graph](image-url)
Fig. 5. Effects on new and mature Thalassia blades incubated at two salinity levels (in parts per thousand, ppt) as measured by various spectral indices (means ± SE): the normalized difference vegetation index (NDVI); the chlorophyll difference index (Chl NDI); the structure-independent pigment index (SIPI); the photochemical reflectance index (PRI); and the first derivative of the raw reflectance spectrum at 750 nm (D750/106).

significant change in spectral reflectance caused by lowered salinities was confirmed. In addition, the seagrass spectral reflectance changes at low salinities were comparable to changes measured in birch and fir under stress. The data herein suggest that the physiological processes of Thalassia are stressed by and sensitive to salinities lowered from oceanic or estuarine seawater to near 16 ppt. Changes in the reflectance spectra between 32 and 16 ppt were significant ($P < 0.05$) (Fig. 3) for wavelengths associated with carotenoids (445–550 nm) and chlorophylls (650–680 nm). Changes in spectral reflectance from normal reflectances at salinities of 20 ppt and upward to 32 ppt did not significantly differ. The reflectance indices NDVI, Chl NDI, SIPI, PRI, and $D_{750}$ (Fig. 5) also indicated significant physiological changes between normal and the low salinity at 16 ppt. These spectral results at 16 ppt for Thalassia are consistent with the stress responses noted by others for terrestrial plants (Carter, 1993; Richardson et al., 2001; Stylinski et al., 2002; Sims and Gamon, 2002). In Richardson et al. (2002), they compare spectral reflectance indices to chemical methods determining chlorophyll concentrations. They find some indices highly correlated to chemical-analysis values. The non-invasive technique of spectral reflectance for seagrass does not differ in use from the terrestrial investigators’ method. Chemically measured levels of seagrass chlorophyll are in the same ranges found with our spectral reflectance methods (Dennison, 1990). There is also stress at 16 ppt indicated in whole-plant, lethal-limit experiments discussed later. The similarity between stress in terrestrial plants and seagrass measured by spectral reflectance deserves further investigation. There also appears, from Fig. 2 to be a different level of spec-
tral reflectance between 28 ppt and 24 ppt when seagrasses are incubated at these reduced salinities for 24 h. A third reduced-photosynthesis physiological level occurs in the lower salinity range of 20 ppt and 16 ppt over a 24-h incubation period, which apparently is not stable if protracted. (These spectral data should be compared to the mortality of Thalassia after 72 h at 20 ppt and lower [Thorhaug and Marcus, 1981] and to Ralph’s [1995] analysis of the seagrass Halophila after various times [12, 24, 48, and 72 h] at reduced salinities.) Sixteen parts per thousand is a very low salinity for a marine environment, but is encountered regularly in shallow water during hurricane or typhoon seasons for very short durations (probably much less than 24 h, dependent upon tidal cycles and land run-off) or in the rainy season in very shallow areas with heavy rain over 24 h. Also, 16 ppt could be encountered at Thalassia bed edges toward river run-off areas in estuaries. Dieback of Thalassia in Florida Bay plus the Indian River Lagoon (St. Lucie by Graves et al., 2002) may be examined in light of these data. Adjacent to Florida Bay is Biscayne Bay with the same weather patterns and similar rains, but a modified, lesser freshwater drainage flow (Wang and Cofer-Shabica, 1990). In the 1990s Biscayne Bay did not have a concurrent Thalassia die-back, while Thalassia in Florida Bay was dying. Biscayne Bay’s salinity is presently not greatly affected by Everglades runoff, while large extents of Florida Bay’s salinities can be temporarily altered by Everglades freshwater run-off. (Keep in mind, however, that in situ freshwater runoff is frequently accompanied by herbicides, metals, and other compounds, which we have not included in these preliminary laboratory tests [see the discussion of general salinity considerations in the introduction].) Sudden freshwater entry (by leaking dikes) into the St. Lucie Waterway caused large-scale Thalassia dieoff (Graves et al., 2002).

The data suggest the need to consider low-salinity effects on seagrasses, a critical fisheries habitat, in plans for introducing greater freshwater inflow into Florida estuaries.

Low salinity effects on seagrasses—Seagrasses originated on land, migrated to fresh or brackish water and are the only group of higher plants to live wholly submerged in the marine environment (den Hartog, 1970). Salinity tolerance is a major evolutionary factor in physiological adaptation separating seagrasses from their freshwater relatives. An important evolutionary question in seagrass physiology is “Did seagrasses retain the ability to carry out their physiological functions when their ambient salinity was lowered from full seawater to far lesser salinities?” Ecological data on seagrass distribution (den Hartog, 1970) indicate that the important habitat of Thalassia meadows is not found in saline lagoons (such as the Laguna Madre in the Gulf of Mexico in its original saline state) or in high-salinity bodies of water (Arabian Gulf). In addition, Thalassia is not found in portions of estuaries where salinities normally fall below 16 ppt (den Hartog, 1970; McMillan, 1979) or at river mouths. This distributional data and laboratory survival studies (Thorhaug and Marcus, 1981) indicate that for Thalassia testudinum the long-term tolerance to low salinity is not as great as certain other seagrass species, such as species of Halophila, Halodule wrightii, or Ruppia maritime. The latter are found in areas of greatly lowered salinity from that of seawater (den Hartog, 1970). On the other hand, distributional field data do not indicate whether Thalassia has the ability to cope with short-term exposures to lowered salinities such as rain runoff from land or typhoons and tropical storms during which several days of intensive rain may occur, and so forth. Our experiments to determine the effect of short-term lowering of salinity on the spectral reflectance properties of Thalassia lead to the larger questions of seagrass photosynthetic/low-salinity responses. Resolving the total underlying issues of seagrass and low salinity will require studies on membrane transport, osmotic pressure change effects as well as investigations of potential changes in other important systems, particularly photosynthetic processes and structural and chemical changes. We have some preliminary answers. Benjamín et al. (1999) found a double membrane only with blade cells of Halophila ovalis incubated at a series of low salinities. Fukuhara et al. (1996) reported putative plasma membrane H+-ATP-ase changes with low salinities. Iyer and Barnabas (1993) described ultrastructural changes in Zostera capensis occurring in mitochondria, chloroplasts, cell wall, and vacuoles as a response to low salinity.

The present experiments, showing significance in reflectance indices, demonstrate that important physiological processes are affected by lower salinities, that is, 16 ppt for 24 h, a moderately short period. (Twenty-four hours is longer than a normal daily tropical rain event, but less than a hurricane-related rain event.) Ralph (1995), using a salinity-tolerant species, Halophila ovalis, described the initial effects of low salinity stress on photosynthetic processes after 12 h, clear effects after 24 h, and severe effects after 48 h. Marcus and Thorhaug (1981), examining the combined low salinity, temperature, and light relations of four species of seagrasses including Thalassia, found mortality in Thalassia occurred at 20 ppt after 72–96 h.

Conclusions—The reflectance spectra of the seagrass Thalassia testudinum shows that the physiological processes in Thalassia are strongly and significantly affected by short-term 24-h exposures to salinities at 40% and 50% below seawater salinity, thus confirming our hypothesis. The main effect on physiological processes, seen in reflectance indices, is suggested to be due to changes in pigmentation (notably increases in chlorophyll and changes in xanthophyll cycle epoxidation). These measured spectral effects appear to be similar to results of spectral reflectance measurements of stress in several terrestrial species (Carter, 1993; Demming-Adams and Adams, 1996; Fillella et al., 1996, 1999; Gamon and Surfas, 1999; Richardson et al., 2001, 2002a, b; Styinski et al., 2002). Accumulated field and laboratory evidence reviewed for both medium and low salinities also demonstrates the inability of Thalassia, a dominant subtropical and tropical seagrass, to adjust to salinities of 20 to 16 ppt and below for long time periods (48 h or more). Previous laboratory studies of Thalassia have shown mortality at 20 ppt after 72 to 96 h (Thorhaug and Marcus, 1981).

LITERATURE CITED


