

# 4 *Salinity*

## INTRODUCTION

### Canal Impacts

In South Florida and other coastal regions, canals drain low-lying lands for agricultural development and for flood control in urban areas. A gradient of increasing salinity occurs across Biscayne Bay from west to east because of canal inputs of freshwater along the western, mainland coastline. A major factor that may determine ecosystem structure and species composition in Biscayne Bay is the volume, distribution, and timing of freshwater inflow (Alleman 1995). Freshwater discharges from canals are major sources of pollution to the Bay and can periodically create problems in localized areas (Alleman *et al.* 1995).

Historically, freshwater flow originated as rainfall, outflow of surface water from the Everglades through the Atlantic coastal ridge, and as groundwater flow through the porous Biscayne Aquifer system (Alleman *et al.* 1995; Browder and Ogden 1999). Natural water-flows were tied to rainfall events and groundwater flows. Surface runoff occurred throughout the wet season as diffuse groundwater seepage and sheet-flow through coastal marshes and wetlands (Brook 1981; Browder and Ogden 1999). As groundwater levels were historically higher (Cheeseman 1988), subsurface flow to the Bay probably occurred throughout much or all of the dry season (Alleman *et al.* 1995).

Coastal levees and shoreline dredge-and-fill activities have eliminated surface flow of freshwater to the Bay in many areas, so that most of the freshwater enters the system via canal discharges (Alleman *et al.* 1995). The network of canals in South Florida was designed to provide flood-control by moving large volumes of water away from the developed urban and agricultural areas as part of the Central and South Florida Flood

Control Project (C&SF Project) (Light and Dineen 1994; U.S. ACOE 1999). C&SF control structures regulate the flow of water in the canals, control the discharge of excess water during flooding, and detain runoff during periods of drought (Alleman *et al.* 1995; Soleki *et al.* 1999).

These changes have altered the timing of flows, have resulted in more water flowing to the Bay during the wet season, and have decreased the amount of flow that occurs during dry periods (Alleman *et al.* 1995). During the summer rainy season, extreme coastal rainfall events can cause large volumes of water to be released over very short periods of time when the flood gates are opened, which can drastically change salinity at the mouths of canals over very short time intervals, with salinities fluctuating from full seawater to full freshwater and back up again within a matter of a few hours (Fatt and Wang 1987; Cofer-Shabica and Wang 1990). The low salinity, poor-quality water delivered in this manner may not mix well with surrounding higher salinity water and can persist for long periods of time (Alleman *et al.* 1995).

Damage to sensitive communities, such as nearshore seagrass beds in the Bay, can occur from excess amounts of freshwater runoff associated with rainfall events and pulse discharges from canals because dramatic reductions in salinity is associated with reductions in dissolved oxygen, resulting in hypoxic or anoxic conditions in these beds over several days (Jokiel *et al.* 1993; Alleman *et al.* 1995; Lapointe and Matzie 1996). Depending on the hydrodynamics of the area, plumes of low salinity water drift from discharge points and can potentially influence plant and animal populations that are distant from canal mouths (Wang *et al.* 1978; Cofer-Shabica and Wang 1990).

The interaction of the canals with the Bay ecosystem is, therefore, complex. Biscayne Bay provides a variety of estuarine and marine habitats that are necessary to support offshore, near-shore, and Bay-wide fisheries (Zieman *et al.* 1984; Haunert and Startzman 1985; Thayer *et al.* 1987). The survival of many of these organisms depends upon the seasonal availability of low-salinity habitats. Freshwater inflows from canals and surface

runoff transport nutrients and detritus from adjacent marshes and uplands into the Bay (Lugo and Snedaker 1974; Day *et al.* 1982; Alleman 1995). These materials are needed to sustain biological productivity (Snedaker and Brook 1976; Kennish 1986). However, the timing and location of freshwater deliveries must provide conditions that can be tolerated by aquatic communities. During dry periods salinity near the shore increases, and this area is colonized by marine species. Rapid reduction in salinity occurs when freshwater is discharged from the canals and damages the marine communities, and yet these discharges frequently do not continue long enough to provide an estuarine habitat with the appropriate salinity conditions for many marine organisms (Alleman *et al.* 1995).

### **Algal Salinity Responses**

Salinity is an important stressor to estuarine organisms because of the physiological effects of osmotic pressure changes associated with salinity fluctuations. Two aspects of salinity are of biological significance: ion concentrations, and osmotic pressure changes (Gessner and Schramm 1971; Lobban and Harrison 1994). The most consequential effects of salinity on cell physiology are the osmotic consequences of the movement of water molecules along water-potential gradients, and the flow of ions along electrochemical gradients. These two processes take place simultaneously, and are both regulated in part by the semi-permeable membranes that surround cells and organelles (Kirst 1988a; Lüning 1990).

Marine organisms are exposed to an osmotic pressure of about 2 MPa (20 bar) corresponding to a salinity around 35 psu. In order to sustain the turgor within cells required for normal function algae maintain osmotic pressures somewhat higher than ambient, typically in the range of 2.6-3 MPa (Raven and Richardson 1986; Lüning 1990) by maintaining a high internal solute concentration (Lobban and Harrison 1994). The ability of seaweeds to tolerate increased salinity is greater than their tolerance to reductions

in salinity because of this high internal osmotic pressure (Gessner and Schramm 1971; Lobban and Harrison 1994).

A two-phase response to reductions in salinity occurs in algal cells. In the first phase, lasting 1-15 min., rapid water fluxes follow the osmotic gradient and changes in turgor pressure occur (Gessner 1969; Kirst 1988a). When salinity is reduced, the turgor pressure of the cell increases relative to the external conditions, resulting in increased strain on the cell wall, and if the difference in osmotic potential is great enough, the cell can lyse. Therefore, the strength of the cell wall and the ability to make the internal osmotic potential less negative will determine an alga's resistance to low salinity (Lobban and Harrison 1994).

Turgor pressure is restored back to optimal conditions for cell function in the second phase of osmotic regulation (osmo-acclimation), a process that lasts 24-72 hr (Kirst 1990; Lüning 1990). This is achieved mainly by selectively stimulating or inhibiting ion ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ) uptake (Gessner and Hammer 1968; Kirst 1990; Lüning 1990), and to a lesser extent metabolizing low-molecular-weight organic compounds or osmolytes (Russell 1987; Kirst 1988b; Lüning 1990). Organic osmolytes, such as low-molecular-weight carbohydrates, the amino acid proline, and quaternary ammonium compounds and their tertiary sulfonium analogues, are important in the second phase of osmoregulation (Blunden and Gordon 1986; Borowitzka 1986; Lobban and Harrison 1994). These organic osmolytes are useful where higher concentrations of osmolytes are required, because as "compatible solutes" (Borowitzka and Brown 1974; Borowitzka 1986) they do not inhibit cellular metabolism and enzyme activities as much as the inorganic ions do (Raven and Richardson 1986; Edwards 1987; Kirst 1988b; Lüning 1990). They further interact with enzymes to stabilize them against conformational changes caused by water loss (Borowitzka 1986; Raven and Richardson 1986).

The physiological requirement to regulate turgor under varying salinities imposes an energetic cost. The production of organic solutes costs about 1.1-1.3 times as much net

carbon fixation in a “marine” cell as is needed to make such a solute in an otherwise comparable “freshwater” cell (Raven 1984; Raven and Richardson 1986). Many of the plants living in coastal waters have the capacity to vary the quantity of intracellular solutes when the external osmolarity changes. The range of osmolarities over which such regulation can occur and the extent to which regulation can track rapid salinity changes are variable among species (Raven and Richardson 1986).

The dependence of photosynthesis, respiration, and growth on salinity follows an optimal curve (Lüning 1990; Lobban and Harrison 1994). Most subtidal seaweeds have a tolerance range of about 16-50 psu, with an optimum around 30-35 psu (Biebl 1958; Conover 1964; Bird and McLachlan 1986; Lüning 1990). Numerous species of estuarine red algae show maximum growth at salinities less than oceanic (Yarish *et al.* 1979; Yarish and Edwards 1982; Bird and McLachlan 1986; Karsten and Kirst 1989). These euryhaline algae have not shifted the photosynthetic optimum from oceanic salinity; rather, the optimal range is wide so that photosynthesis shows little decline with decreasing salinity (Kirst 1981)

Acclimation of a species to salinity variations may result from genotypic or phenotypic changes. Most estuarine algae have been found to exhibit genotypic variation in response to salinity gradients present, with only some phenotypic capacity (Yarish *et al.* 1979). Lowered salinity often stunts the growth of seaweeds and has variable effects on branching (Gessner and Schramm 1971; Norton *et al.* 1981). In view of the importance of salinity on multiple aspects of algal physiology and growth, and the prevailing management practice of pulsed freshwater discharges from point sources primarily during the wet season, the abiotic variable, salinity, and the algal responses to salinity are important variables in the model (see Chapter 7). The two aims for this chapter were to:

- collect and analyze field data on salinity distribution and seasonal variability within the Bay;

- determine salinity response curves of representatives of the three functional groups under static and dynamically fluctuating salinity conditions in controlled experiments.

## METHODS

### Salinity Regime

Salinity fields vary both temporally and spatially within the Bay, as a function of freshwater input from canal discharge. Mean monthly surface and bottom salinities obtained from DERM's monitoring program (1990-99) and my field sites (1996-99) were plotted along with minimum and maximum values for each salinity regime (see Chapter 3, Table 3 for location of sites and frequency of sampling). Salinity data were collected at my field sites from refractometer measurements on surface-water samples intermittently over the four years. Higher (daily and hourly) frequency fluctuations in conductivity from two stations in each of canal and oceanic regimes were obtained from BNP. I calculated salinity from their conductivity measurements using the formula (Hem 1970):

$$S = K \cdot A$$

where:

$$K = \text{conductivity (mS} \cdot \text{cm}^{-1}\text{)}$$

$$A = \text{slope of regression equation, value used was 0.725, the range of values is 0.54-0.96 (Hem 1970)}$$

$$S = \text{salinity (psu)}$$

Mean daily salinities for the two sites, and minimum and maximum values per regime, were plotted. Hourly salinity variations for the period of record (1996-1999) were also plotted for each site, to show the high frequency fluctuations that occur in canal sites.

A Biscayne Bay circulation model (Wang *et al.* 2001) was used to develop spatial salinity coverages using GIS for Biscayne Bay. Twelve mid-month (approx. day 15 of each month) salinity coverages based on 30 year average simulated salinities in the Bay

were plotted to show the seasonal change in mean salinity fields spatially across the Bay during wet and dry season months.

## **Salinity Responses**

### *1. Static Salinity Experiments*

The salinity response functions for the model presented in Chapter 7 were determined from the responses of the algae under static salinity conditions. I chose salinities of 35 psu to represent the mean oceanic salinity (= control), 20 psu is the mean annual salinity in canal influenced sites, and 10 psu is indicative of a low salinity event seen by benthic algae exposed to a plume of freshwater after a large discharge event. The drift algae were collected from canal sites and the rhizophytic algae were collected from seagrass beds on the oceanic side of the Bay for the quarterly multi-factorial experiments (methods previously described in Chapter 3). Both the drift algae and rhizophytic algae were exposed to the three salinities for one week; initial photosynthesis after 24 hr exposure and final growth after 1 week were recorded. The epiphytes were exposed for 48 hr to a range of salinities from 5 to 55 psu in 10 psu increments, and photosynthesis measured at the end of this exposure period. In another set of experiments, using only the rhizophytic algae *Penicillus* and *Halimeda*, plants were collected from seagrass beds on the eastern (oceanic) side of the Bay in the dry season (Jan. 1998) and wet season (Jul. 1998). Changes in health and photosynthesis in the plants initially, and one week after exposure to 15, 25, 35, 45 psu salinities, were recorded.

Salinities in the microcosm tubes were mixed by dilution of Bay-water with reverse osmosis water (<35 psu) or by the addition of sea salt (Instant Ocean®, Aquarium Systems Inc., Mentor, OH) for salinities greater than 35 psu. The resultant salinity was measured with a refractometer (Sper Scientific, Model 300011 with automatic temperature compensation) and adjusted every few days by adding reverse osmosis water to compensate for evaporation losses in the greenhouse.

Responses of the algae after the one-week exposure to the static salinity treatments were measured using a variety of metrics developed in Chapter 3: photosynthesis ( $\text{mgO}_2 \cdot \text{g}^{-1} \cdot \text{hr}^{-1}$ ), growth ( $\% \cdot \text{day}^{-1}$ ), and appearance as a qualitative index of health. Growth was found to be a useful metric for drift algae. Rhizophytic algae were found to grow only a negligible amount in one week, so a qualitative health index was developed, based on the descriptions in Thorhaug (1976b).

For the epiphytes, photosynthesis was converted to productivity following the recommendations of Littler and Arnold (1985), using a photosynthetic quotient ( $\text{PQ} = 1$ ) and respiratory quotient ( $\text{RQ} = 1$ ) as advocated by Kinsey (1985) and Rosenberg *et al.* (1989, 1995). Approximate growth of the epiphytes was calculated from this photosynthesis data using a conversion factor of  $0.375 \times \text{mg O}_2 = \text{mg C}$  and then multiplying productivity in  $\text{mgC} \cdot \text{hr}^{-1}$  by 8 hr (dry season) or 9 hr (wet season) to determine approximate daily productivity in the greenhouse microcosms. This productivity value was converted to biomass specific growth ( $\% \cdot \text{day}^{-1}$ ) by taking the ratio of  $\text{gC} \cdot \text{g}^{-1}$  dry weight tissue, where gC is approximately 25-30% of the total tissue content on a dry weight basis (Atkinson and Smith 1983; Duarte 1992).

Growth is an integrated response of physiological processes occurring in response to salinity stress. I also wanted to test the short-term effects occurring soon after the plants were initially exposed to changes in salinity. Measuring photosynthesis using light:dark bottles (as per Chapter 3 methods) less than 24 hr after the initial change in salinity allowed me to look at the osmo-acclimation response in the three functional groups. This is potentially a more sensitive measure than growth of how the algae respond to rapid salinity changes, such as those they experience in the field in near-shore canal-influenced habitats. Photosynthesis of each of the functional groups was plotted across the range of salinities tested in the dry season (Feb.) and wet season (Aug.).

All statistical analyses were performed using JMP version 3.1.5 for Apple Macintosh from the SAS Institute Inc., Cary, NC. Data were tested for normality and

heteroscedasticity before ANOVA tests. For those data sets that did not fulfill the assumptions required for ANOVA, non-parametric Kruskal-Wallis tests were performed. The health index is a discrete, categorical metric, so responses were tested using two-way contingency tables (Sokal and Rohlf 1995; Steel *et al.* 1997) for each species. This was followed by Correspondence Analysis (Greenacre 1984; McGarigal *et al.* 2000) to visualize the results.

## 2. Static Salinity Experiments - Spatial Population Responses

The differences in salinity responses for populations of drift algae and the rhizophytic alga *Penicillus*, which occur in both canal and oceanic regimes, were investigated. The aim of this experiment was to determine whether the same species found in different salinity regimes in the Bay exhibit differences in tolerance to reduced salinities.

Drift algae were collected from FP (canal) and BC (oceanic) in Feb. 1999, and plants from both locations were exposed to 10, 20, and 35 psu salinities at the same time (Table 1). Growth and photosynthesis after the one-week exposure were measured using the same protocols as above. Separate two-way ANOVAs were used to analyze this data.

*Penicillus* was the only rhizophytic genus to occur in abundance on both sides of the Bay. Individuals were collected from FP (canal) and SK (oceanic) in Oct. 1999 and exposed simultaneously to 5, 15, 25, and 35 psu salinities over one week (Table 1). Individuals were “planted” in one liter clear plastic buckets using weathered gravel, and these were kept at ambient temperature by placing them in a flow-through water table. Photosynthesis was measured using a modified light:dark method, whereby plants were incubated *in situ* in the buckets, to minimize stress that could result from moving the plants to and from incubation bottles. Dark buckets were kept covered only during the two-hour incubation period, and for the rest of day the plants were allowed to photosynthesize, like their counterparts in the light buckets. Health and photosynthesis at the end of the one-

week exposure was recorded, and a two-way ANOVA was used to analyze photosynthesis in *Penicillus* on the final day.

Table 1: Abiotic conditions that the algae in the static salinity experiments were exposed to.

<b>Algae - source</b>	<b>Light (<math>\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}</math>)</b>	<b>Temperature (<math>^{\circ}\text{C}</math>)</b>	<b>Salinity (psu)</b>
Drift - Canal	400-500	22-24	10, 20, 35
Drift - Ocean	400-500	22-24	10, 20, 35
<i>Penicillus</i> - Canal	500-600	28-31	10, 20, 35
<i>Penicillus</i> - Ocean	500-600	28-31	10, 20, 35

### 3. Dynamic Salinity Experiments

I manipulated the delivery of freshwater to experimental aquaria to assess the impact of canal discharge events on the growth of *Laurencia*, a common red alga in Biscayne Bay, at the end of the wet season (Oct. 1999). Algae collected from BKP were grown in thirty 30-gallon aquaria and exposed to 4, 6, and 24 hr pulses of diluted low salinity (10 and 20 psu) seawater, before being returned to 35 psu and allowed to grow for one week. Mean daily growth was calculated as per the formula presented in Chapter 3.

Data for the short-term four and six hour pulses were pooled, as these were not found to be significantly different by ANOVA. The short-term exposures (4 and 6 hr pulses) were compared to long-term (24 hr) exposures for the three salinities. A two-way ANOVA was performed on this pooled data set.

## RESULTS

### Salinity Regime

Mean daily discharge volumes into the area where my two canal sites were located are greater during the wet season (Jun.-Nov.) than the dry season months, with volumes during the wettest month (Aug. =  $25.7 \text{ m}^3 \text{ s}^{-1}$ ) almost five times the discharge occurring in the driest months (Apr. & May =  $5.4 \text{ m}^3 \text{ s}^{-1}$ ) (Alleman *et al.* 1995). The discharge of large

volumes of water during the wet season results in reduced salinities around the canal mouths (Fig. 1), with salinity inversely related to discharge volume.

Mean monthly salinity data for the three regimes showed that there was only slight variation between my study sites and the ten-year mean from DERM monitoring stations (Fig. 1). Canal sites had the greatest range in salinity, with minimum salinities near 0 psu year-round (Fig. 1). At the oceanic sites, mean salinity was less variable than canal and sheet-flow sites (Fig. 1). Alleman (1995) previously reported similar mean salinity values for oceanic ( $37 \pm 1.5$  psu), sheet-flow ( $35.9 \pm 4.2$  psu) and canal sites ( $25.4 \pm 9.5$  psu).

Salinities measured in the canal sites at the bottom of the water column (25-30 psu) were higher than those at the surface (10-15 psu) (Fig. 1). Often in summer a definite halocline was observed at the canal sites. Stratification of the water column has previously been reported to occur along the western boundary of Biscayne Bay (Alleman 1995). Canal discharges create a layer of freshwater that covers the surface and forms a stagnant layer of high-salinity water near the bottom (Fatt and Wang 1987).

Measurements from bottom sondes deployed by BNP show low-salinity pulses from canal discharges occur as discrete pulses of freshwater delivery at frequencies of hours to days (Fig. 2). These high-frequency fluctuations were directly related to canal discharge management practices, with salinity fluctuating between 7 to 25 psu on a time scale of a few hours or less (J. Wang, pers. comm.).

The spatial extent of the low-salinity plumes entering the Bay is also of importance in understanding the benthic habitats that are affected, and therefore, the magnitude of impact. Mixing of freshwater from canals and oceanic water within the Bay results in a gradient in salinity, especially during the wet season months (Jun.-Nov.). About 64% of Miami-Dade County rainfall occurs during this five-month wet season (Duever *et al.* 1994; Obeysekera *et al.* 1999). There is about a 30-45 day lag period between rainfall events and discharges from the canals into the Bay. This is evident in the lack of a defined low salinity plume in

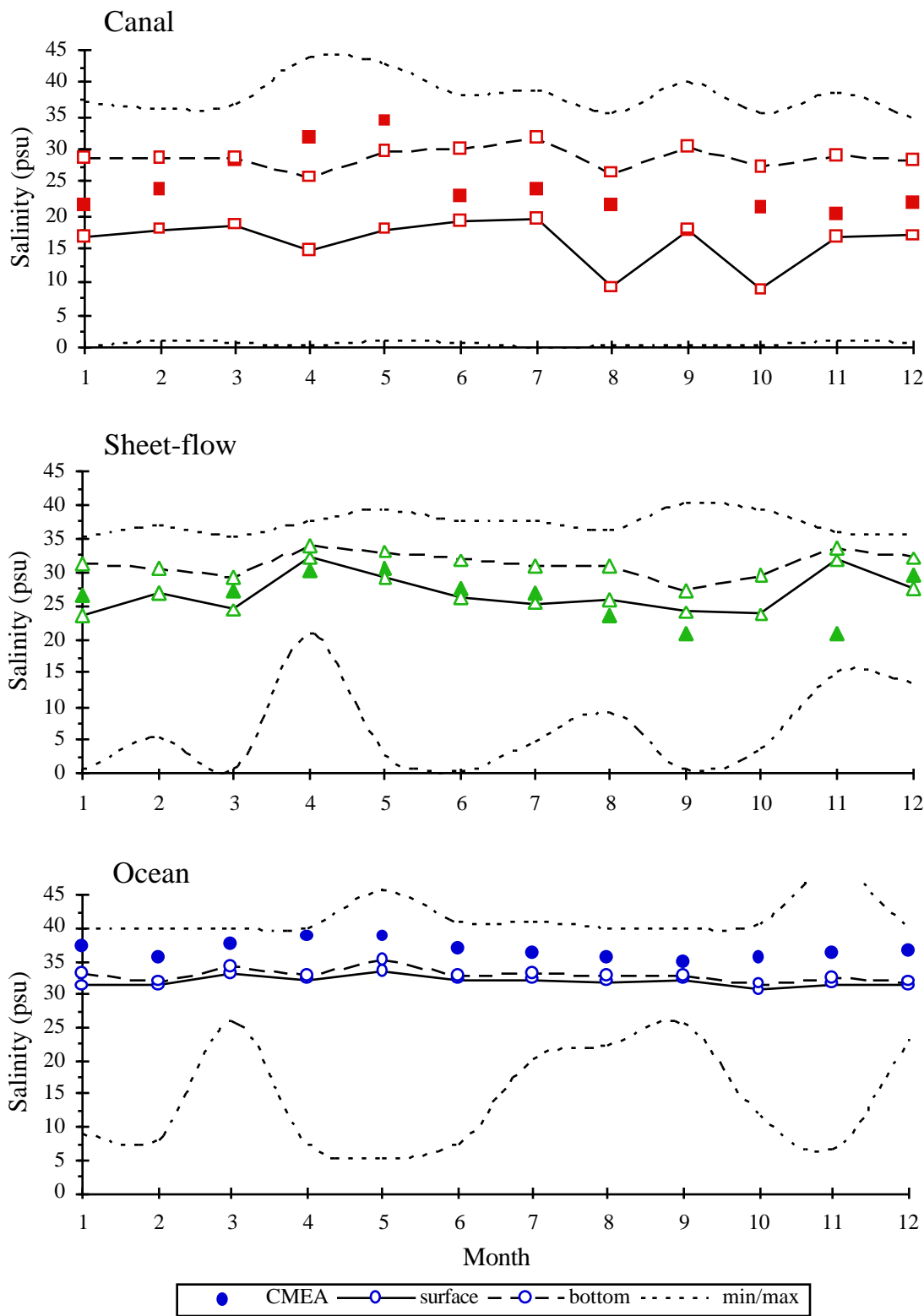


Fig. 1: Mean monthly salinities in the three regimes studied, comparing data measured by DERM from 1990-99 (open symbols, n = 3 sites) with measurements from 1996-99 at my study sites (filled symbols, n = 2 sites).

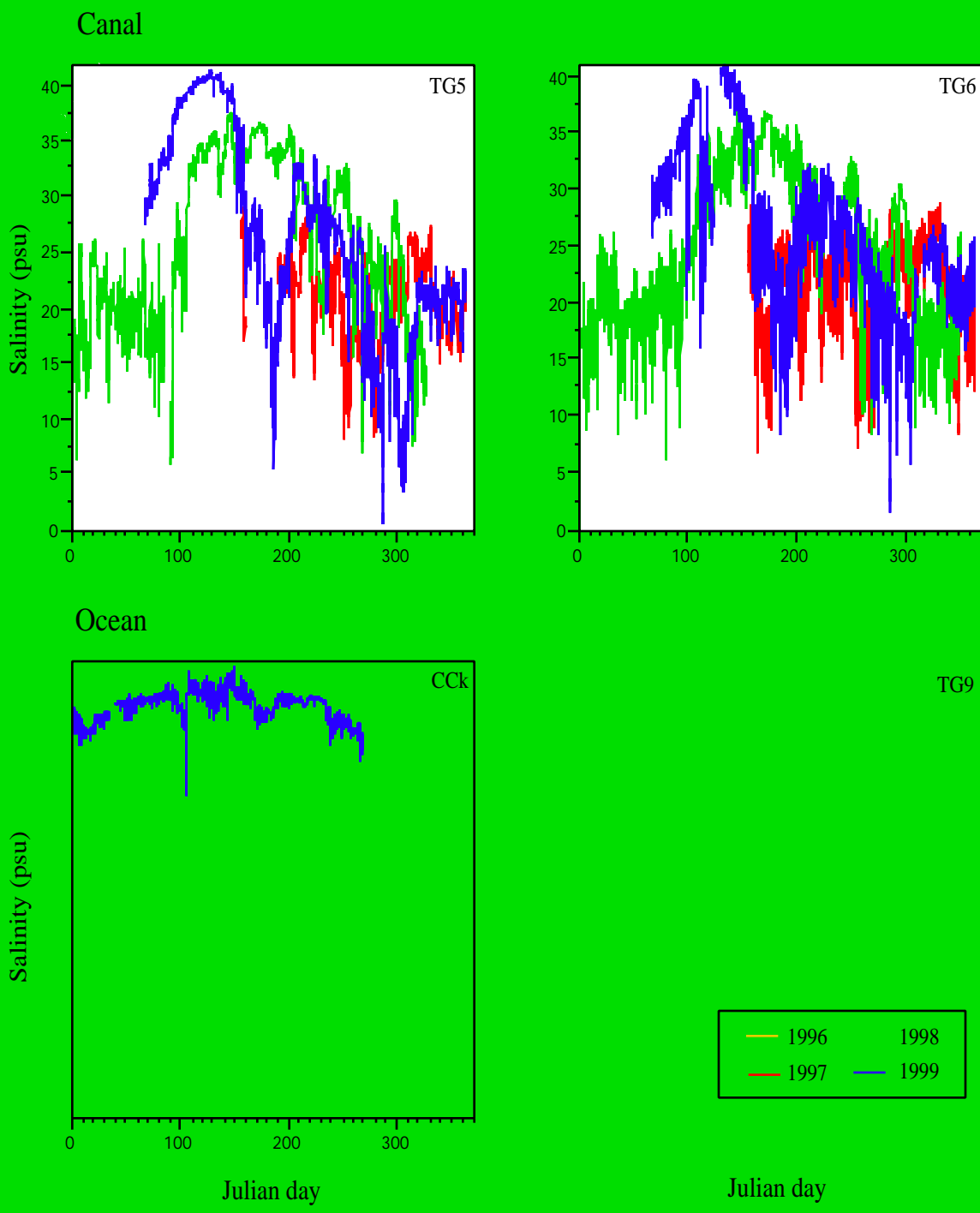


Fig. 2: Hourly salinity fluctuations at two canal (TG5, 6) and two oceanic (CCk, TG9) sites showing bottom salinities. High-frequency, high-magnitude changes in salinity occur at the canal-influenced sites, but not at the oceanic sites.

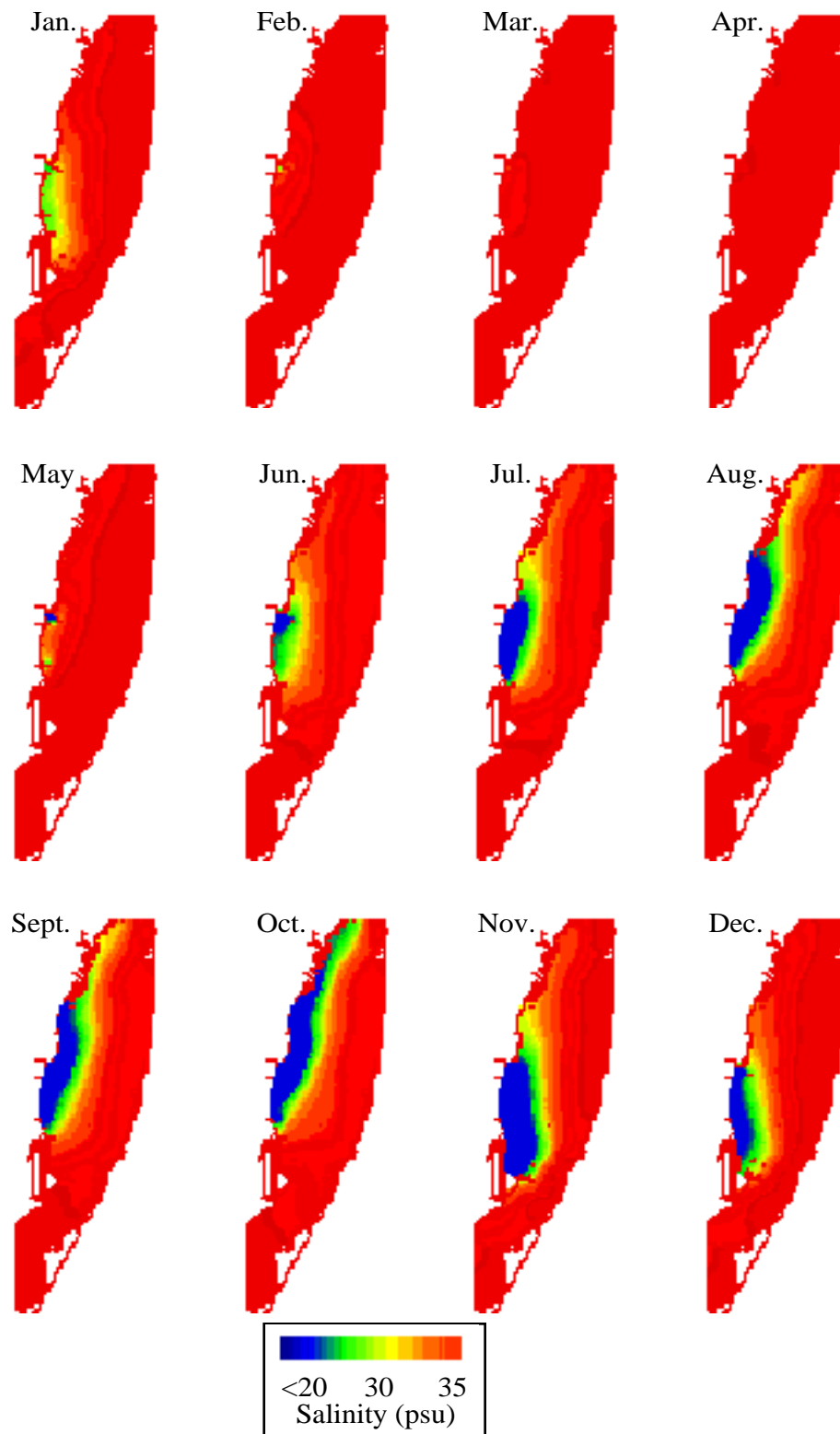


Fig. 3: Mid-monthly snapshots of spatio-temporal distribution of salinities in Biscayne Bay from 30 year averaged output of a hydrodynamic circulation model of Biscayne Bay (Wang *et al.* 2001). Wet season canal discharges result in low salinities from Jun.-Dec.

May and the persistence of low salinities into December, while terrestrial runoff continues after rainfall has abated (Fig. 3). During the six-month period that canals discharge much of the rainfall (Fig. 3) into the Bay, low-salinity plumes extend across to the center of the Bay (e.g., Fig. 3 for Nov.). This contrasts with the relatively negligible salinity gradient occurring during the dry season (e.g., Fig. 3 for Apr.).

## Salinity Responses

### 1. Static Salinity Experiments

#### Photosynthesis

The first metric used to assess salinity response in the three functional groups was gross photosynthesis (Pg), after 24 hours exposure to the salinity treatments. Drift algae and epiphytes showed positive photosynthesis down to salinities of 0-5 psu (Fig. 4). The two rhizophytic algae only had positive Pg above 15 psu (Fig. 4). This indicates a lower ability of the two rhizophytic species to tolerate reduced salinities compared to the drift algae and epiphytes. Drift algae and epiphytes had the highest mean photosynthesis, producing about  $18 \text{ mgO}_2 \cdot \text{g dw}^{-1} \cdot \text{hr}^{-1}$  in the dry season, while the two rhizophytic algae had lower photosynthesis with *Penicillus*  $<4 \text{ mgO}_2 \cdot \text{g dw}^{-1} \cdot \text{hr}^{-1}$ , and *Halimeda*  $<2 \text{ mgO}_2 \cdot \text{g dw}^{-1} \cdot \text{hr}^{-1}$  (Fig. 4).

Seasonal variation in photosynthesis rates were different among functional groups. Seasonality had a significant interaction effect for the epiphytes, but there was no effect of season on photosynthesis in the drift or rhizophytic algae (Table 2A). In the drift algae there was a trend (not significant) and for the epiphytes there was significantly greater photosynthesis in the dry season than the wet season. In *Penicillus* the opposite trend was apparent, while in *Halimeda* there was no difference in photosynthesis between seasons (Fig. 4). In the drift and rhizophytic algae mean photosynthesis was significantly greater at higher salinities than at lower salinities in both seasons (Tables 1A&B). The highest mean photosynthesis in the drift algae and in the two rhizophytes occurred at 35 psu (Fig. 4).

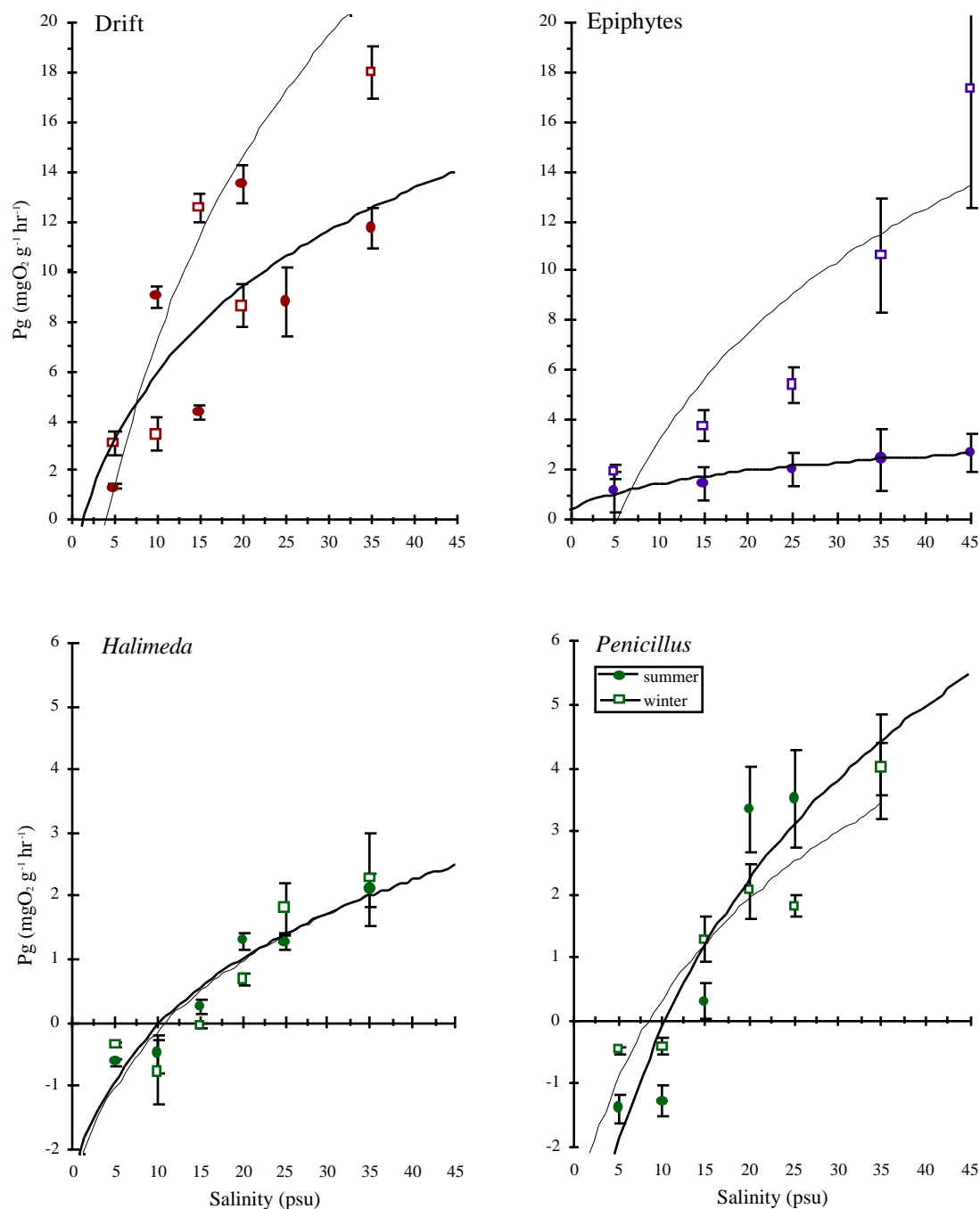


Fig. 4: Photosynthetic response to a range of salinities from 5 to 45 psu measured after <24 hr exposure. Mean ( $\pm$ SD) photosynthesis is plotted for summer and winter populations, light and temperature conditions during the experiment are given in Table 1. Note difference in the Y axis scale for the rhizophyte functional group.

Table 2A: Two-way Sheirer-Ray-Hare extension of non-parametric Kruskal-Wallis tests on photosynthesis by season and salinity (both fixed factors) for the three functional groups presented in Figure 4. The appropriate chi-square test is  $SS/MS_{\text{total}}$  (Sokal and Rohlf 1995). Significant results at  $\alpha = 0.05$  are highlighted in bold.

<b>Algae</b>	<b>Source</b>	<b>df</b>	<b>SS</b>	<b>MS</b>	$\chi^2$	<b>P</b>
<b>Drift</b>	Season (Se)	1	2330.00	2330.00	0.8039	<0.50
	Salinity (Sal)	5	242180.85	48436.17	83.5538	<b>&lt;0.01</b>
	Se x Sal	5	150551.06	30110.21	51.9410	<0.10
	Error	174	107396.95	617.22		
	Total	185	536222.50	2898.50		
<b>Epiphytes</b>	Season	1	270.40	270.40	0.8866	<0.50
	Salinity	5	9358.70	1871.74	30.6843	<b>&lt;0.001</b>
	Se x Sal	5	4270.40	854.08	14.0013	<b>&lt;0.025</b>
	Error	48	1149.60	23.95		
	Total	59	17995.00	305.00		
<b><i>Penicillus</i></b>	Season	1	4997.16	4997.16	1.1480	<0.50
	Salinity	5	200534.52	40106.90	46.0698	<b>&lt;0.001</b>
	Se x Sal	5	27318.20	5463.64	6.2759	<0.50
	Error	208	387722.54	1864.05		
	Total	219	953272.23	4352.84		
<b><i>Halimeda</i></b>	Season	1	3432.14	3432.14	0.8627	<0.50
	Salinity	5	145774.04	29154.81	36.6405	<b>&lt;0.001</b>
	Se x Sal	5	30927.76	6185.55	7.7737	<0.25
	Error	206	414654.40	2012.89		
	Total	217	863334.50	3978.5		

For drift algae the ANOVA  $r^2=0.7997$ ,  $n=10-20$ ; epiphytes the  $r^2=0.9361$ ,  $n=5$ ; *Penicillus* the  $r^2=0.5933$ ,  $n=12-29$ ; *Halimeda* the  $r^2=0.5197$ ,  $n=14-20$ .

Table 2B: Tukey's post-hoc comparisons of significant results in Table 2A indicate groups with like means by the same letter, and significantly higher photosynthesis is highlighted.

Source	Tukey's					
<b>Drift</b>						
Salinity (Sal)	5psu	10psu	15psu	20psu	25psu	35psu
Tukey's	<b>D</b>	<b>C</b>	<b>BC</b>	<b>B</b>	<b>A</b>	<b>A</b>
<b>Epiphytes</b>						
Se x Sal	5psu	15psu	25psu	35psu	45psu	
winter dry	<b>C</b>	<b>BC</b>	<b>BC</b>	<b>AB</b>	<b>AB</b>	
summer wet			<b>n.s.</b>			
	winter dry	summer wet				
5 psu		<b>n.s.</b>				
15 psu	<b>A</b>	<b>B</b>				
25 psu	<b>A</b>	<b>B</b>				
35 psu	<b>A</b>	<b>B</b>				
45 psu	<b>A</b>	<b>B</b>				
<b>Penicillus</b>						
Salinity	5psu	10psu	15psu	20psu	25psu	35psu
Tukey's	<b>B</b>	<b>B</b>	<b>B</b>	<b>A</b>	<b>A</b>	<b>A</b>
<b>Halimeda</b>						
Salinity	5psu	10psu	15psu	20psu	25psu	35psu
Tukey's	<b>B</b>	<b>B</b>	<b>B</b>	<b>A</b>	<b>A</b>	<b>A</b>

For the epiphytes there was a significant season x salinity interaction effect (Tables 1A&B). Photosynthesis in the epiphytes did not vary significantly among salinity levels in the wet season, but was significantly greater at 35 and 45 psu than at 5 psu (Table 2B). Also in this functional group photosynthesis was significantly greater in the dry season than the wet season in all salinities, except 5 psu (Table 2B).

### Growth

Growth provides an integrated measure of salinity response, and is potentially the most useful of the three metrics used, as it provides data in the same units as required in the model. Mean daily growth of the drift algae and epiphytes was greater than of the two rhizophytes in most salinities tested (Fig. 5). There were significant seasonal differences in growth in the drift algae, and a significant interaction effect in the epiphytes and *Penicillus* (Table 3A).

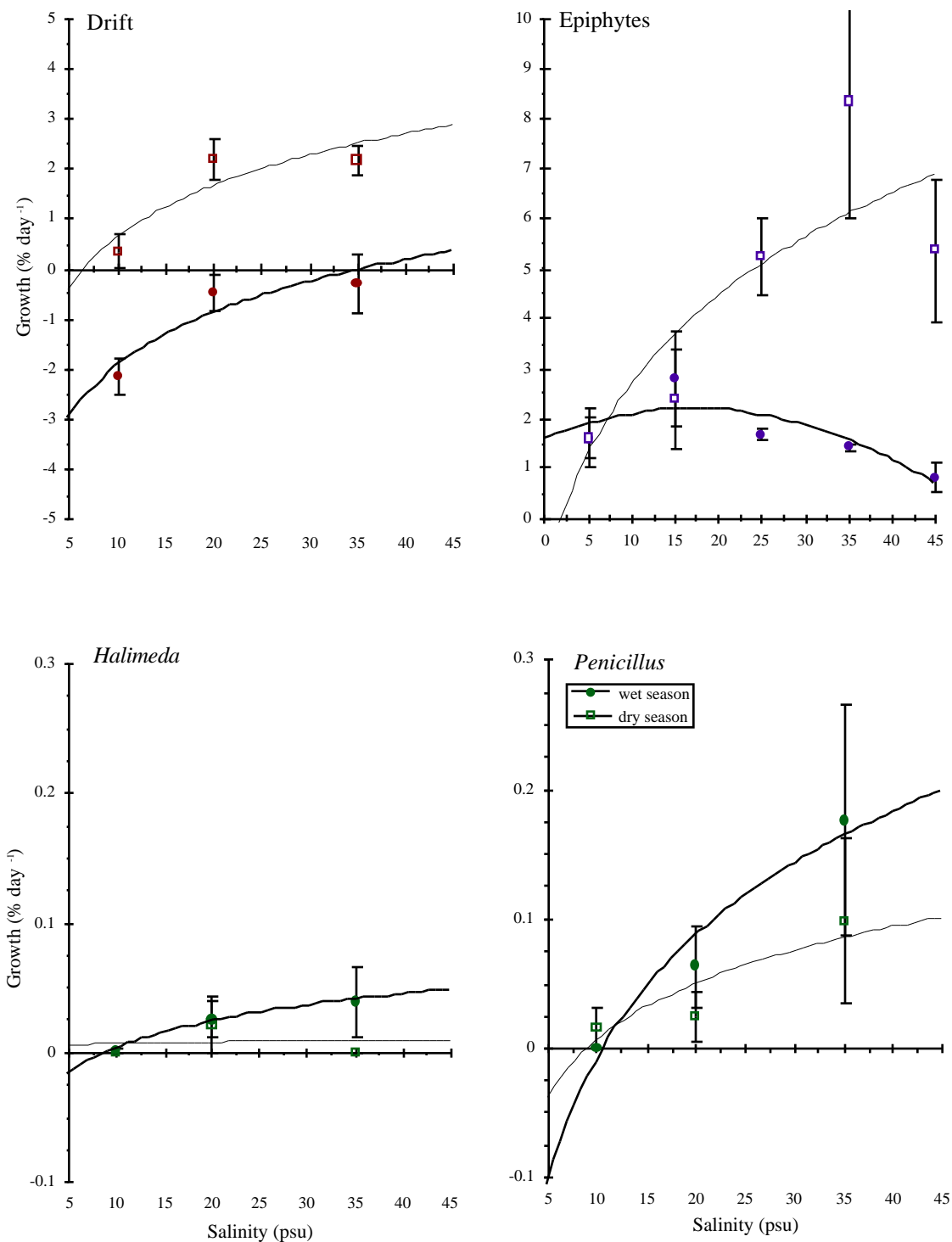


Fig. 5: Mean daily growth ( $\pm$ SD) of the three functional groups of algae exposed to a range of salinities for one week. Trend-lines denote wet season (summer) and dry season (winter) responses. Note different scales on Y axes among functional groups.

Table 3A: Two-way Sheirer-Ray-Hare extension of non-parametric Kruskal-Wallis tests on growth by season and salinity (both fixed factors) for the three functional groups presented in Figure 5. The appropriate chi-square test is  $SS/MS_{total}$  (Sokal and Rohlf 1995). Significant results at  $\alpha = 0.05$  are highlighted in bold.

<b>Algae</b>	<b>Source</b>	<b>df</b>	<b>SS</b>	<b>MS</b>	$\chi^2$	<b>P</b>
<b>Drift</b>	Season (Se)	1	9796.36	9796.36	16.0083	< <b>0.001</b>
	Salinity (Sal)	2	6835.54	3417.77	11.1700	< <b>0.005</b>
	Se x Sal	2	78.58	39.29	0.1284	<0.75
	Error	98	46320.99	472.66		
	Total	103	63031.47	611.96		
<b>Epiphytes</b>	Season	1	384.40	384.40	1.1360	<0.50
	Salinity	4	2450.17	612.54	7.240	< <b>0.05</b>
	Se x Sal	4	3373.15	843.29	9.967	< <b>0.01</b>
	Error	48	9764.40	203.43		
	Total	53	17936.98	338.43		
<b>Penicillus</b>	Season	1	61.97	61.965	3.0337	<0.10
	Salinity	2	37.75	18.875	1.8482	<0.50
	Se x Sal	2	148.24	74.120	7.2576	< <b>0.05</b>
	Error	69	1263.52	18.312		
	Total	74	1511.47	20.425		
<b>Halimeda</b>	Season	1	36.44	36.440	3.7701	<0.10
	Salinity	2	8.33	4.165	0.8618	<0.50
	Se x Sal	2	36.51	18.255	3.7773	<0.25
	Error	69	633.97	9.188		
	Total	74	715.25	9.666		

For drift algae the ANOVA  $r^2=0.5058$ ,  $n=16-18$ ; epiphytes the  $r^2=0.9361$ ,  $n=5$ ; *Penicillus* the  $r^2=0.1421$ ,  $n=8-17$ ; *Halimeda* the  $r^2=0.1053$ ,  $n=8-17$ .

Table 3B: Tukey's post-hoc comparisons of significant results in Table 3A indicate groups with like means by the same letter, and significantly higher growth is highlighted.

Source		Tukey's				
<b>Drift</b>						
Season (Se)	winter dry	summer wet				
Tukey's	<b>A</b>	<b>B</b>				
Salinity (Sal)	10psu	20psu	35psu			
Tukey's	<b>B</b>	<b>A</b>	<b>A</b>			
<b>Epiphytes</b>						
Se x Sal	5psu	15psu	25psu	35psu	45psu	
winter dry	<b>C</b>	<b>BC</b>	<b>AB</b>	<b>A</b>	<b>AB</b>	
summer wet			<b>n.s.</b>			
	winter dry	summer wet				
5 psu		<b>n.s.</b>				
15 psu		<b>n.s.</b>				
25 psu	<b>A</b>	<b>B</b>				
35 psu	<b>A</b>	<b>B</b>				
45 psu	<b>A</b>	<b>B</b>				
<b>Penicillus</b>						
Se x Sal	10psu	20psu	35psu			
winter dry		<b>n.s.</b>				
summer wet		<b>n.s.</b>				
	winter dry	summer wet				
10 psu	<b>A</b>	<b>B</b>				
20 psu		<b>n.s.</b>				
35 psu		<b>n.s.</b>				

For the drift algae growth was positive during the dry season in all salinities tested, compared to loss of biomass occurring during the wet season (Fig. 5). Mean daily growth in the dry season ( $1.6\% \text{ day}^{-1}$ ) was significantly greater than in the wet season ( $-1\% \text{ day}^{-1}$ ) (Table 3B). Growth decreased significantly with salinity, with sharply reduced growth at 10 psu ( $-0.85\% \text{ day}^{-1}$ ), compared to 20 and 35 psu ( $0.91\text{-}1.02\% \text{ day}^{-1}$ ) (Table 3B).

There was a significant season by salinity interaction effect on growth for the epiphytes (Table 3A). Growth during the wet season was not significantly different among salinity levels, however, in the dry season growth at 35 psu ( $8.3\% \text{ day}^{-1}$ ) was significantly greater than at 5 and 15 psu ( $1.6\text{-}2.4\% \text{ day}^{-1}$ ) with 25 and 45 psu levels having intermediate growth rates (Table 3B). Growth rates compared across seasons within a salinity level

showed no significant seasonal differences at 5 and 15 psu, while above 15 psu growth was significantly greater in the dry season than the wet season (Table 3B).

Mean daily growth rates for the two rhizophytic species, *Penicillus* ( $<0.2\% \text{ day}^{-1}$ ) and *Halimeda* ( $<0.05\% \text{ day}^{-1}$ ) were much less than in the drift algae or epiphyte functional groups (Fig. 5). In *Penicillus* there was a significant season by salinity interaction effect on growth (Table 3A). Growth at 10 psu was significantly greater in the dry season ( $0.016\% \text{ day}^{-1}$ ) than the wet season ( $0\% \text{ day}^{-1}$ ) (Table 3B). In *Halimeda* no significant season or salinity effects were evident (Table 3A).

### Health

A health index (Table 4) was developed because growth, measured as change in weight over time, was found to be a poor method to determine short-term responses (1 week) to salinity because of the significant  $\text{CaCO}_3$  content of the thallus in the rhizophytic algae. With increasing stress *Halimeda* changes color from deep green, to pastel green, to pale yellow, and finally white (Table 4). All these colors may exist in small sections of a completely healthy specimen, but when the terminal segments are dramatically lighter than proximal ones, death is indicated. Individual segments crack easily, and separation of the segments occurs on contact. Loss of turgor is the final stage, indicated by a rubbery

Table 4: Health index for rhizophytic algae, based on morphological and visible criteria reported in Thorhaug (1976b). Better health results in a higher rank.

<b>Rank</b>	<b>Health</b>	<b>Characteristics</b>
1	bad, dead	bleached, flaccid thallus; no green color; starting to decay.
2	poor	mostly bleached; <i>Halimeda</i> some green segments, <i>Penicillus</i> brush bleached, stalks green.
3	normal	partly bleached; <i>Halimeda</i> most segments green, <i>Penicillus</i> part of brush bleached, may be somewhat flaccid.
4	good	no bleaching evident; entire thallus bright green and turgid.

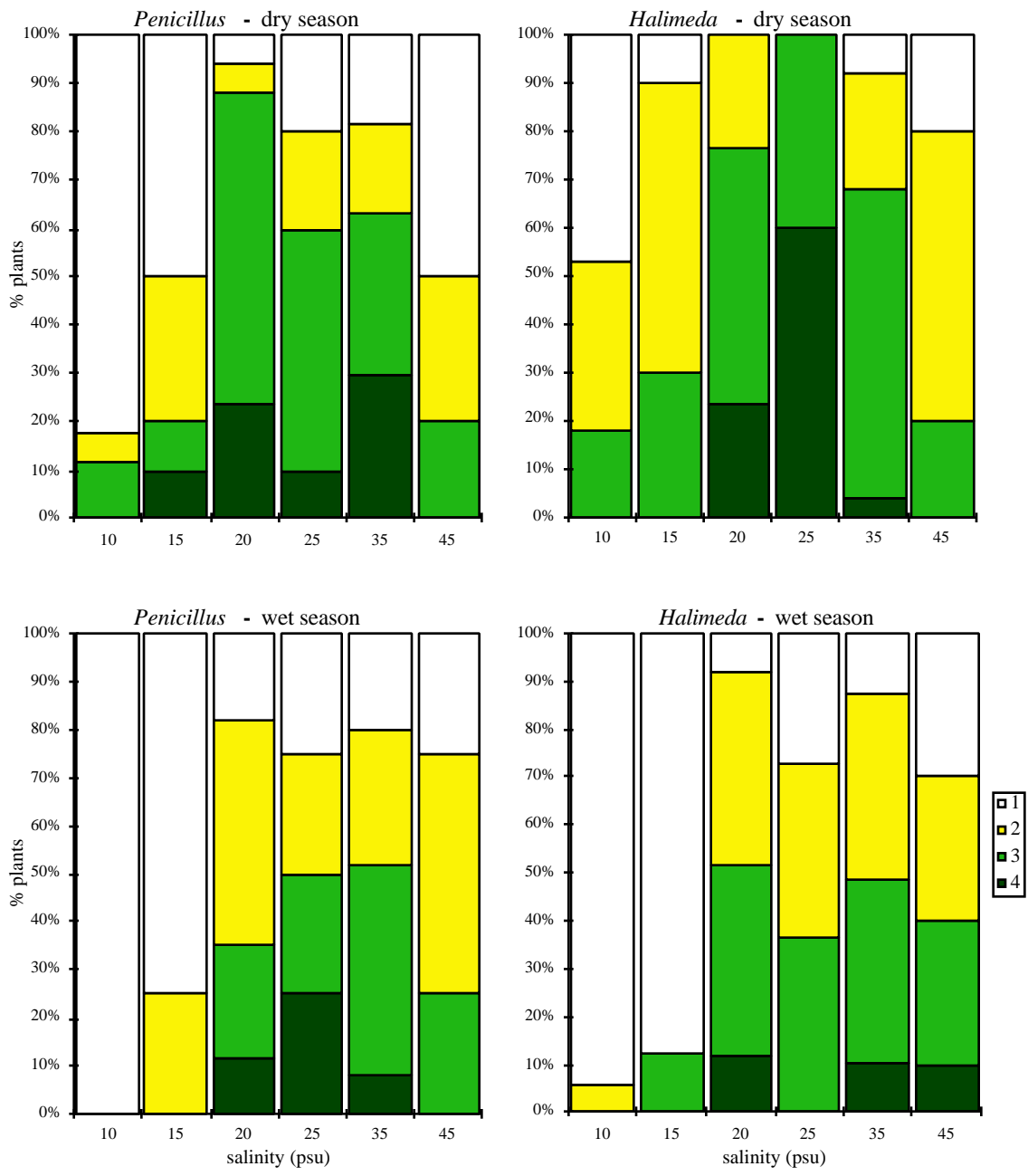


Fig. 6: Percentage of plants within each health category (see Table 4) as a measure of salinity tolerance after one week exposure to the salinity levels shown for the two species of rhizophytic algae in two seasons.

Table 5: Categorical tests of final health in experiments presented in Fig. 6. <sup>1</sup>G statistic (Likelihood ratio) and <sup>2</sup>Pearson statistic from separate two-way contingency table analyses. Significant results at  $\alpha = 0.05$  are highlighted in bold.

Algae	Source	df	LogLikelihood	$\chi^2$	P
<i>Penicillus</i>					
dry season	Health	15	23.2390	46.478 <sup>1</sup>	<b>&lt;0.0001</b>
	Error	73	96.7422	42.950 <sup>2</sup>	<b>0.0002</b>
	Total	88	119.9812		
wet season	Health	15	27.2665	54.533 <sup>1</sup>	<b>&lt;0.0001</b>
	Error	65	76.2488	47.477 <sup>2</sup>	<b>&lt;0.0001</b>
	Total	80	103.5153		
<i>Halimeda</i>					
dry season	Health	15	28.8939	57.788 <sup>1</sup>	<b>&lt;0.0001</b>
	Error	71	83.9679	59.885 <sup>2</sup>	<b>&lt;0.0001</b>
	Total	86	112.8618		
wet season	Health	15	25.0014	50.003 <sup>1</sup>	<b>&lt;0.0001</b>
	Error	65	69.9849	40.991 <sup>2</sup>	<b>0.0003</b>
	Total	80	94.9863		

flexibility to branches, basal stalks, and the entire plant. Soon after this the thallus starts to disintegrate.

In *Penicillus* similar color occur from a healthy dark green, to pale green, to yellow green, and then white, especially in the terminal filaments (Table 4). Loss of turgor occurs in the filaments, and the stalk becomes rubbery, and then brittle. Decay of the plant occurs with filaments decaying first, then the interior of the stipe. Again the final stage is disintegration of the thallus.

For both species health varied significantly across salinity in both the wet and dry seasons (Table 5). Seasonal responses were evident, with plants in the dry season having better health at lower salinities than the same species in the wet season, indicating better low-salinity tolerance in plants during the cooler months (Fig. 6).

*Penicillus* showed poor health (>50% of plants with a health rank of one) at salinities of 15 psu or less in both the dry and wet seasons. Similarly in *Halimeda*, health at low salinities was poor. Health of *Halimeda* was worse in the wet season than the dry season at a given salinity (Fig. 6).

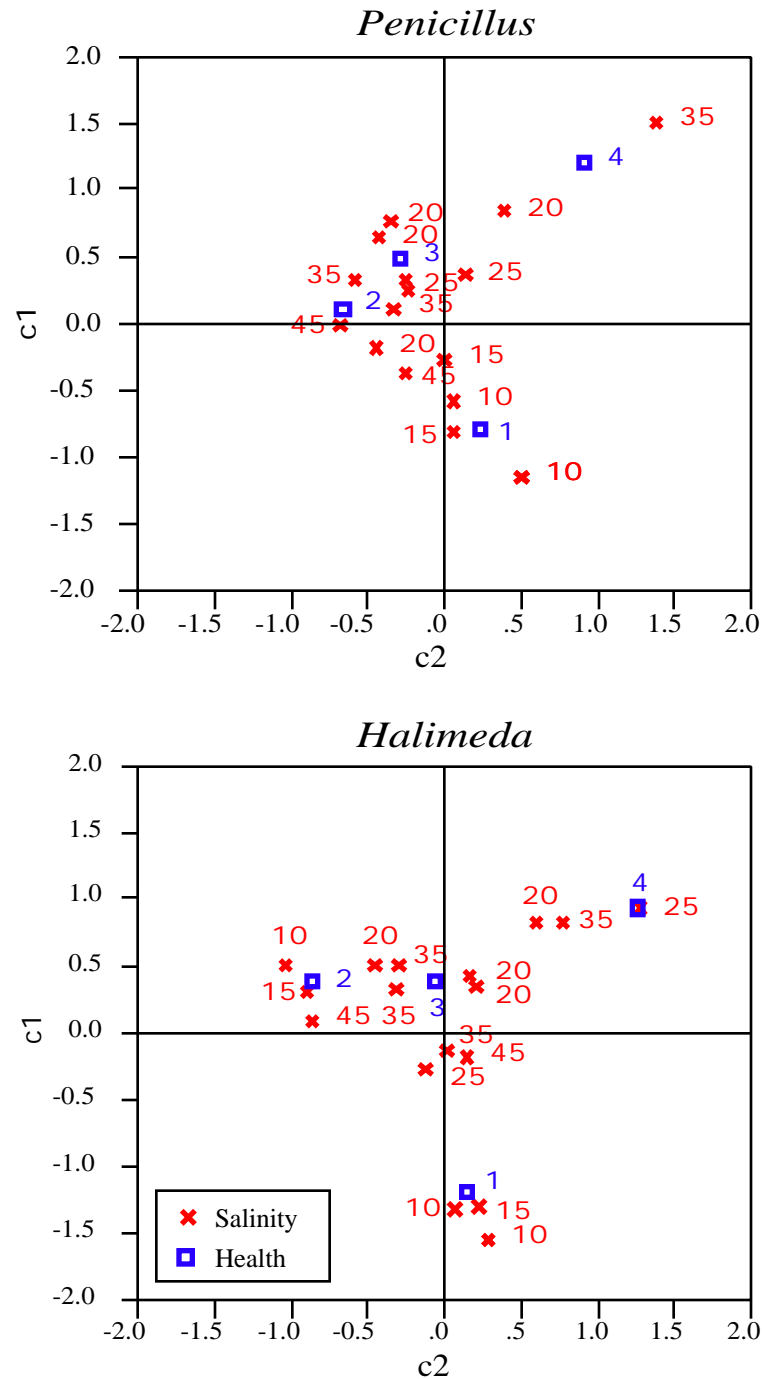


Fig. 7. Results of Correspondence Analyses for health of rhizophytic algae after one-week exposure to the range of salinities shown in Fig. 6.

Plants with the highest health rank (4) could indicate the most preferred salinities. For *Penicillus* this corresponds to a range of preferred salinities from 15-35 psu in the dry season, and 20-35 psu in the wet season (Fig. 6). In *Halimeda* the preferred salinities are slightly higher, ranging from 20-35 psu in the dry season, and 20 and 35-45 psu in the wet season (Fig. 6).

Correspondence analysis is a graphical technique derived from Principle Components Analysis (McGarigal *et al.* 2000), which can be used to visualize pattern within a large contingency table (Anon. 1994). Results of the Correspondence Analyses (Fig. 7) for *Penicillus* and *Halimeda* showed that low health values of 1 (= bad, dead) were associated with low salinity exposures, while a high rating of 4 (= good) was found in salinities of 25-35 psu. Salinities of 45 psu were already above optimal (Figs. 6&7), as less healthy plants were associated with these high salinities.

## 2. Static Salinity Experiments - Spatial Population Responses

The responses evident in the previous experiments suggested that algae may be acclimating to the seasonal fluctuations in their environment. I was interested, therefore, in determining if spatial differences also existed in algae occurring in different salinity regimes within the Bay. Results from experiments with drift algae and the rhizophytic algae, *Penicillus*, suggest that this may be so.

Drift algae: (primarily *Laurencia poiteaui*) from a canal-influenced site (FP), had significantly higher photosynthesis rates after one-week exposure to reduced salinities compared to those from the oceanic site (BC) (Table 6A, Fig. 8). Mean photosynthesis increased significantly with salinity in plants from both canal and ocean sites (Table 6A). Drift algae from FP grew significantly more ( $<2\% \text{ day}^{-1}$ ) than the same species collected from an area with constant oceanic salinity ( $<0.5\% \text{ day}^{-1}$ ), when the two populations were exposed to the same salinity treatment (Table 6A, Fig. 8). Mean daily growth in plants from FP

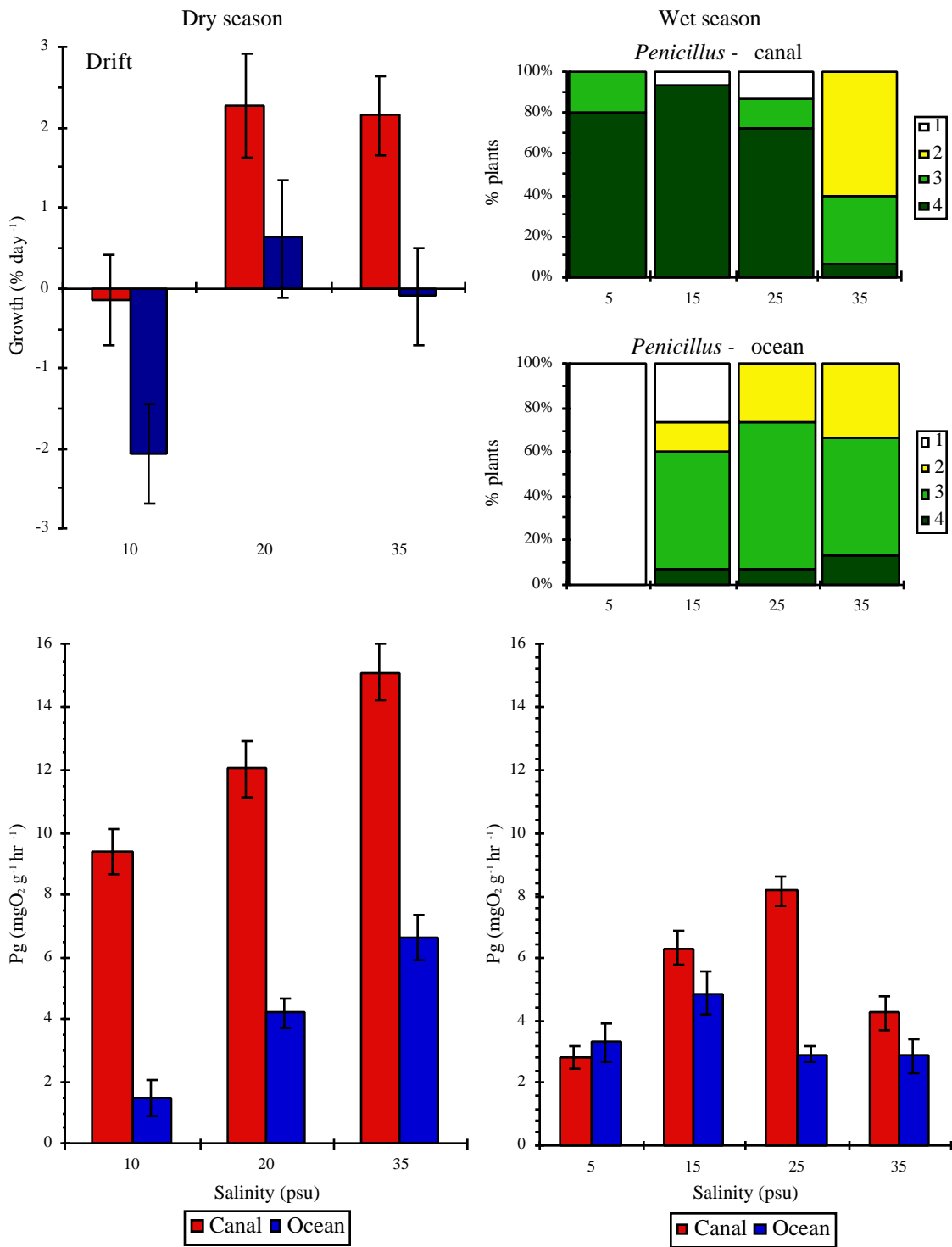


Fig. 8: Mean ( $\pm$ SE) growth and photosynthetic response of drift algae in winter and the rhizopytic alga, *Penicillus*, in summer collected from oceanic (BC, SK) and canal sites (FP) to a range of salinities occurring in Biscayne Bay. Health index shows % of plants from the canal and oceanic sites in each health ranking in each salinity.

Table 6A: Two-way ANOVAs on photosynthesis by location (fixed factor) and salinity (fixed factor) in two species of algae collected from a canal and an oceanic site (see Fig. 8). Significant results at  $\alpha = 0.05$  are highlighted in bold. Tukey's post-hoc comparisons indicate groups with like means by the same letter.

Experiment	Source	df	SS	MS	F	P
<sup>a</sup> Drift	Location (L)	1	249.313	249.313	57.6224	< <b>0.0001</b>
	Salinity (S)	2	132.184	66.092	15.2755	< <b>0.0001</b>
	L x S	2	1.109	0.555	0.1281	0.8800
	error	42	181.720	4.327		
<sup>b</sup> <i>Penicillus</i>	Location	1	0.015	0.015	0.0810	0.7768
	Salinity	3	8.096	2.699	14.4851	< <b>0.0001</b>
	L x S	3	3.889	1.296	6.9580	<b>0.0004</b>
	error	64	11.924	0.186		
Drift	<u>Location</u>	<u>canal</u>	<u>ocean</u>			
	Tukey's:	<b>A</b>	<b>B</b>			
<i>Penicillus</i>	<u>Salinity</u>	<u>10psu</u>	<u>20psu</u>	<u>35psu</u>		
	Tukey's:	<b>B</b>	<b>AB</b>	<b>A</b>		
<i>Penicillus</i>	<u>L x S</u>	<u>5psu</u>	<u>15psu</u>	<u>25psu</u>	<u>35 psu</u>	
	Canal	<b>B</b>	<b>B</b>	<b>A</b>	<b>B</b>	
	Ocean			<b>n.s.</b>		
		<u>Canal</u>	<u>Ocean</u>			
		5 psu	<b>n.s.</b>			
		15 psu	<b>n.s.</b>			
	25 psu	<b>A</b>	<b>B</b>			
	35 psu		<b>n.s.</b>			

<sup>a</sup>For drift algae data are normal (Shapiro-Wilk's  $W=0.9535$ ,  $P<0.0924$ ), and homoscedastic (Bartlett's  $F=0.7009$ ,  $P<0.6227$ ), with  $r^2=0.8489$  for the ANOVA,  $n=8$ . <sup>b</sup>For *Penicillus* data are normal (Shapiro-Wilk's  $W=0.9924$ ,  $P<0.9911$ ), and homoscedastic (Bartlett's  $F=1.4842$ ,  $P<0.1675$ ), with  $r^2=0.5419$  for the ANOVA,  $n=9$ .

declined significantly at salinities less than 20 psu (Table 6B). In contrast, mean daily growth in plants from BC declined both at lower and higher salinities than 20 psu (Fig. 8). The drift algae from the canal site had better tolerance to lower salinity than did the same species collected from an oceanic site, indicating probable differences in algal population responses depending on their provenance.

Table 6B: Two-way ANOVA on growth by location (fixed factor) and salinity (fixed factor) in drift algae collected from a canal and an oceanic site (see Fig. 8). Data are normal (Shapiro-Wilk's  $W=0.9683$ ,  $P<0.2983$ ), and homoscedastic (Bartlett's  $F=0.2651$ ,  $P<0.9323$ ), with  $r^2=0.4231$  for the ANOVA,  $n=9$ . Significant results at  $\alpha = 0.05$  are highlighted in bold. Tukey's post-hoc comparisons indicate groups with like means by the same letter.

Source	df	SS	MS	F	P
Location (L)	1	16.627	16.627	4.874	<b>0.0321</b>
Salinity (S)	2	33.871	16.936	4.964	<b>0.011</b>
L x S	2	0.822	0.411	0.121	0.8867
error	48	163.76	3.412		

Location	canal	ocean
Tukey's:	<b>A</b>	<b>B</b>

Salinity	10psu	20psu	35psu
Tukey's:	<b>B</b>	<b>A</b>	<b>A</b>

Table 6C: Categorical test on final health in *Penicillus* presented in Fig. 8. <sup>1</sup>G statistic (Likelihood ratio) and <sup>2</sup>Pearson statistic from separate two-way contingency table analyses. Significant results at  $\alpha = 0.05$  are highlighted in bold.

Site	Source	df	LogLikelihood	$\chi^2$	P
Canal	Health	9	25.886	51.772 <sup>1</sup>	<b>&lt;0.0001</b>
	Error	48	35.450	46.498 <sup>2</sup>	<b>&lt;0.0001</b>
	Total	57	61.336		
Ocean	Health	9	29.429	58.857 <sup>1</sup>	<b>&lt;0.0001</b>
	Error	48	43.655	48.477 <sup>2</sup>	<b>&lt;0.0001</b>
	Total	57	73.084		

Rhizophytic algae: *Penicillus* was the only species of rhizophytic algae that occurred in any abundance in canal influenced sites (see Chapter 2). There was a significant interaction effect on photosynthesis measured at the end of the experiment (Table 6A). There was no significant difference in photosynthesis among salinity levels in plants from the ocean site (Table 6A), but in plants from the canal site photosynthesis was significantly enhanced at 25 psu, compared to the other salinities tested (Table 6A). Mean Pg was highest at 25 psu in the plants from the canal site (FP), and declined at both higher and lower salinities (Fig. 8). Examining for site effects at each salinity level, found no differences between sites except at 25 psu, where photosynthesis was significantly higher in plants from the canal

site than those from the ocean site (Table 6A). In the salinity range of 5-15 psu, photosynthesis reduced more rapidly in canal-influenced plants than in individuals from the ocean site (Fig. 8). The individuals collected from FP had higher health at low salinities (5-25 psu) compared to their counterparts from SK, but slightly lower health at 35 psu (Fig. 8). Plants from the canal site were healthier at low salinities (5-15 psu) than those collected from the oceanic site (Fig. 8). Health of the canal plants tended to decrease with increasing salinity, while health increased in plants from the oceanic site (Fig. 8).

To summarize this set of experiments, both drift and rhizophytic algae exhibited better tolerance of low salinities (<20 psu) in plants from canal sites compared to oceanic sites. These results indicate that there are probably physiologically distinct populations with respect to salinity of these species occurring within Biscayne Bay.

### *3. Dynamic Salinity Experiments*

Static salinity exposures are ecologically unrealistic for populations of algae from Biscayne Bay, as salinity fluctuations occur in the form of short-term periodic pulses of reduced salinities. The static condition represents the extreme for an exposure, and was used to determine the species-response curve in a fashion analogous to the LD<sub>50</sub> approach used ubiquitously in toxicity testing (Rand 1995).

Dynamic salinity exposures are a more realistic method to test the ecological effects of canal discharges on algal growth. A single-pulse exposure followed by one-week recovery at 35 psu was tested. Pulses were either short duration (4-6 hr) or long duration (24 hr) (Fig. 9A). No significant differences in growth rates with pulse duration were found (Table 7). Nevertheless, there was a trend for greater growth in algae that were exposed only to the shorter pulses (Fig. 9B). Salinity had a significant effect on mean daily growth (Table 7), with algae exposed to 10 psu growing significantly less than at 20 and 35 psu (Fig. 9B). Algae in both 4 hr and 24 hr exposures grew less as salinity decreased to 10 psu (Fig. 9B). At the mean salinity found in canal sites (20 psu) the greatest difference occurred in mean

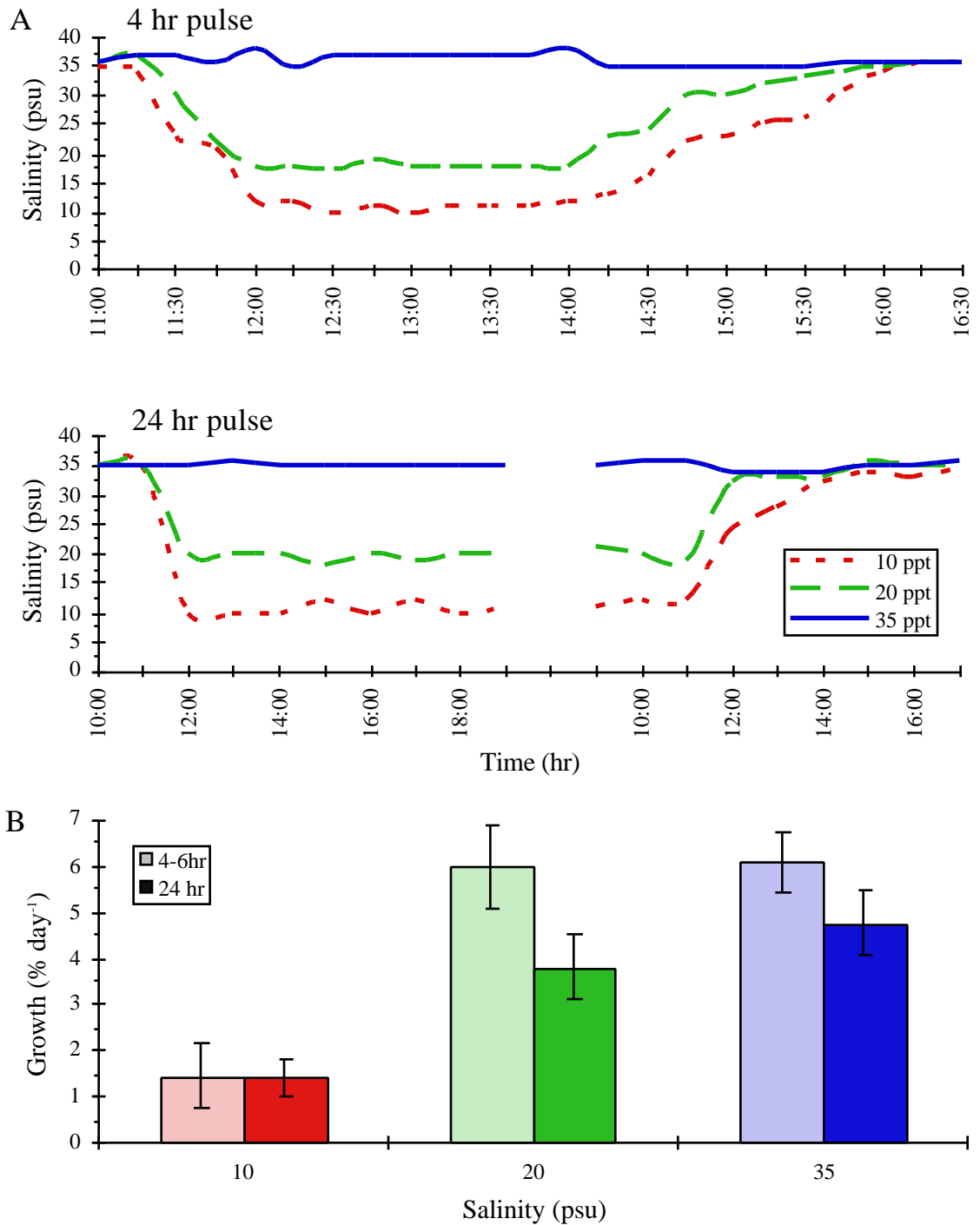


Fig. 9A: Salinities measured in dynamic salinity-pulse experiments. Two types of pulse durations were simulated: short-term releases (4 hr and 6 hr) and long-duration releases of floodwaters from canals (24 hr). Two low salinities were tested: surface salinities (10 psu) and bottom salinities (20 psu), and compared to algae in controls (35 psu). Fig. 9B: Mean ( $\pm$ SE) daily growth of the drift algae *Laurencia* exposed to the various combinations of low-salinity pulses shown in Fig. 9A.

growth between short and long pulses of low salinity water, possibly indicating that short-duration pulses may be less sedulous than longer pulses for long-term growth and survival of algae near canals. The mean growth at 10 psu was almost the same for the two pulse durations, which was probably caused by the negative influence of the low salinity in this exposure over-riding any effect from pulse duration.

Table 7: Two-way ANOVA on growth by pulse duration (fixed factor) and salinity (fixed factor) for drift algae shown in Fig. 9A. Data are normal (Shapiro-Wilk's  $W=0.9752$ ,  $P<0.1195$ ), and homoscedastic (Bartlett's  $F=1.2676$ ,  $P<0.2747$ ), with  $r^2=0.2250$  for the ANOVA,  $n=19-47$ . Significant results at  $\alpha = 0.05$  are highlighted in bold. Tukey's post-hoc comparisons indicate groups with like means by the same letter.

Source	df	SS	MS	F	P
Pulse (P)	1	0.0792	0.0792	0.2262	0.6350
Salinity (S)	2	7.7997	3.8999	11.1370	<b>&lt;0.0001</b>
P x S	2	0.7562	0.3781	1.0797	0.3419
error	180	63.0315	0.3502		
<u>Salinity</u>	<u>10psu</u>	<u>20psu</u>	<u>35psu</u>		
Tukey's:	<b>B</b>	<b>A</b>	<b>A</b>		

The more ecologically realistic simulation of multiple exposures with only a short recovery period was not tested because of resource limitations (lack of sufficient reverse osmosis water to create low salinities). Nonetheless, this type of exposure regime would be the most appropriate for testing the effects of canal discharges. The potential inability of the algae to recovery sufficiently from one pulse before the next is what probably limits the distribution of algae near canal mouths, before adequate mixing of the low-salinity freshwater with bay-waters reduces the impact to less stressful conditions.

## DISCUSSION

### Canal Impacts

Biscayne Bay is a shallow subtropical bay that receives freshwater from rainfall, surface runoff, a series of drainage canals along its western shore, and groundwater seepage (Alleman *et al.* 1995). Exchange with the ocean occurs via numerous openings (= cuts) including Bear Cut, the Safety Valve, Angelfish-, Broad-, and Caesar's Creeks (see

Chapter 6, Fig. 3). From the output of a Biscayne Bay hydrodynamic circulation model (Wang *et al.* 2001) and previous studies (Alleman *et al.* 1995) it is known that salinity contours run in a north-south direction, parallel to the western shoreline. Stratification of the water column can occur in this region because of the large quantities of freshwater input during the rainy season (Fatt and Wang 1987).

In this dissertation I investigated the community-level impacts to benthic macrophytes of freshwater inputs from canal discharges. The area I concentrated on, South Biscayne Bay, receives about 40% of the total discharge into the Bay from canals, and probably the vast majority of sheet-flow runoff, on account of development around the mid and upper basins (Alleman 1995). Canal discharges into this region can vary greatly among years because of annual variances in rainfall, and therefore the amount of head behind the control structures in place to manage water-levels in the canals (Cheeseman 1990, Winsberg 1990).

The low salinity water entering the Bay from canal-discharge may not mix well with the surrounding higher salinity bay-waters (Alleman *et al.* 1995). This can result in an observable vertical stratification of the water column with respect to salinity, i.e., an estuarine salt-wedge formation (Kennish 1986) with the salinity at the bottom about 10 psu greater than at the surface. Therefore, benthic organisms that are not directly in the freshwater plume coming out of the canal mouth are unlikely to experience mean low salinity values (<10 psu) for prolonged periods of time (>24 hr), and are actually less impacted by low salinity than may be expected from surface measurements. For this reason, knowledge of the daily discharge and monitored salinities are only an indication of the actual salinity conditions that benthic macroalgae experience during a discharge event.

Salinity near canals does not exhibit typical estuarine dynamics, however, because of the pulsed nature of the discharges. Rather than salinity declining/increasing gradually with seasonal changes in rainfall as it does at the sheet-flow sites, pulse-discharges from canals cause rapid, severe drops in salinity. Canal discharges tend to be short (1-3 hr) in duration

and frequent (once to many times day<sup>-1</sup>) (Cofer-Shabica and Wang 1990). From my analysis of the DERM and BNP data it is apparent that low salinity values of around 10 psu at the sediment-water column interface occur approximately once a month during the wet season, and are less frequent during the dry season. Mean salinities of 20 psu can occur for longer time periods (1-2 weeks) during the wet season. Even though the mean benthic salinity is between 10 to 20 psu in the wet season, the surface salinity values are observed to fluctuate ( $\pm 10$ -20 psu) rapidly on a time scale of a few hours. This is evident in the BNP data, and corresponds directly to canal discharge events (Fatt and Wang 1987; Cofer-Shabica and Wang 1990).

The geography of South Biscayne Bay into which the canal discharges occur is important in determining the spatial extent and residence time of the low-salinity plume and the degree of mixing that occurs in the water column. The dominant forcing mechanisms for mixing and transport within the Bay are tide and wind (Lee 1975). Low salinity plumes were simulated to occur from July through December (Wang *et al.* 2001). These plumes lag seasonal rainfall by about 1-1.5 months, the time it takes water to travel through the canal-system and be discharged into the Bay. Low salinity plumes (<25 psu) can extend greater than halfway across the Bay during periods of heavy discharge (e.g. November). In the area between Black Point and Turkey Point most of the canals open into a region of reasonably good tidal flushing, and the residence time of the low salinity plume is likely to be similar to that of Biscayne Bay in general (approx. 1.0 - 1.5 months), whereas Barnes and Card Sound (sheet-flow sites) have longer residence times (2-4 months or greater) because of limited tidal exchange (Lee 1975; Fatt and Wang 1989). This can result in longer low-salinity events in these latter two basins during the wet season (Alleman *et al.* 1995).

Damage to biological communities in the Bay can occur from excessive amounts of freshwater, but is generally limited to near the vicinity of the actual inflow points (Brook 1981; Szmant 1987; Alleman 1995). Salinity is an important abiotic variable that controls

growth of marine plants, which can have important ecological implications for system structure and function (Irlandi *et al.* 1997; Martins *et al.* 1999; Kamer and Fong 2000). For instance, Thorhaug (1976a) observed that species diversity and abundance of numerous species of fish, mollusks, crustaceans, and algae decreased in the vicinity of a canal during periods of freshwater discharges. In another study by Brook (1981), a severe freshwater discharge event caused long-term changes in the adjacent seagrass community, which had not recovered to pre-stress conditions 18 months later. However, this may depend on the proximity to the canal-mouth. We have found that seagrass communities in the canal-influenced region of the Bay, but not directly adjacent to the canal mouth, were not significantly adversely affected by the low-salinity water (Irlandi *et al.* 2002).

In areas with widely fluctuating salinities adjacent to canals, biomass is generally low and species dominance can change frequently (Brook 1981; Montague and Ley 1993). High variances around a mean salinity have previously been shown to be stressful and deleterious to marine macrophytes (Gessner and Schramm 1971), with an order of magnitude decline in total benthic plant biomass measured for every 3 psu increase in standard deviation of salinity (Montague and Ley 1993). Because of the pulsed nature of canal discharges, salinity may not remain within the range of tolerance of any one plant species for long enough to allow the development of a substantially vegetated community. Gaining control over salinity fluctuation through changes in canal discharge management protocols may be a major key to improving near-shore estuarine seagrass habitats in South Florida (Montague and Ley 1993).

## **Algal Salinity Responses**

### *Salinity Tolerance Inferred from Distribution Patterns*

Often investigators in ecological studies attempt to correlate distribution and abundance of macroalgae with environmental gradients, such as salinity. Early investigators reported that numerous taxa of macroalgae occurred in estuarine conditions along the Florida

peninsula (Phillips 1960; Hamm and Humm 1976; Josselyn 1977; Dawes *et al.* 1978). Many taxa were reported having euryhaline distributions; nonetheless, the number of species decreased as salinity decreased, indicating physiological tolerance limits to low salinity (Phillips 1960; Gessner and Schramm 1971; Kendrick *et al.* 1988; Middleboe *et al.* 1998). Drifting rhodophytes, which are common in Florida estuaries, were reported to tolerate lower salinities than attached algae, such as the rhizophytes in Texas lagoons (Conover 1964). In the course of dissertation research I observed distributions of the functional groups (see Chapter 2) which lend further support to this reported pattern. Drift and epiphytes appear to have euryhaline distributions, occurring in oceanic, sheet-flow, and canal conditions. In contrast, the rhizophytic species appear to be stenohaline, as they were mostly restricted in distribution to locations with near-oceanic salinities.

#### *Salinity Tolerance Measured under Laboratory Conditions*

From the three metrics (photosynthesis, growth, health) reported for the three functional groups in this chapter, it is evident that the drift algae and epiphytes were more tolerant of low-salinity stress than were the two common rhizophytic genera, *Halimeda* and *Penicillus*. Drift algae and epiphytes performed best (by these three metrics) over a range of salinities from 10-35 psu, while the two rhizophytes did not do as well at salinities below 15-20 psu as they did at salinities above this range. However, even within these functional groups there are plants that are better adapted to a wide range of salinity, and others that are able to tolerate only a narrow salinity range, potentially even within the same species (Conover 1964; Bäck *et al.* 1992a, 1992b), making the distinction between euryhaline and stenohaline characteristics less clear than at first may seem.

Photosynthesis: For the three functional groups, photosynthetic production measured after 24 hr exposure to the salinity treatment, exhibited similar responses as did growth rate measured after a week-long exposure. This indicates that future studies could focus on the rapid assessment of salinity response using short-term incubations. Growth was the main

variable of interest to me, however, because this was the metric used later to parameterize the model (see Chapter 7). Also, stress-responses measured by photosynthetic productivity do not necessarily translate directly to growth (Gessner and Schramm 1971; Markager and Sand-Jensen 1994).

For instance, in a short-term (<48 hr) study transient decreases in photosynthesis and increases in respiration followed hypo-osmotic shock from 26 to 16 psu salinity in the red alga *Gracilaria tikvahiae* (Lapointe *et al.* 1984). Photosynthetic productivity decreased about 50% when measured after four hours exposure to the 16 psu salinity, but recovered within less than 48 hr to previous levels even though the algae remained in the 16 psu water. This rapid physiological acclimation demonstrates how quickly algae are able to adapt to altered salinity conditions. Only by measuring growth over a longer time period can the integrated effects of low-salinity stress be assessed.

Another problem with the photosynthesis method can arise from CO<sub>2</sub>-limitation in low-salinity treatments. In laboratory studies most investigators obtain reduced salinities by simply diluting seawater with distilled water, but this is not typical of many natural situations (Hammer 1968; Dawes and McIntosh 1981). As far as photosynthesis is concerned, the total CO<sub>2</sub> content of the seawater is probably of greater importance than the absolute concentration of sea salts or the osmotic pressure (Ogata and Matsui 1965; Gessner and Schramm 1971; Yarish *et al.* 1979). The potentially deleterious effects of diluting natural seawater with distilled water in these studies could largely be relieved with the addition of extra HCO<sub>3</sub><sup>-</sup> (Hammer 1968; Lobban and Harrison 1994).

Growth: In agreement with the results obtained for the drift algae it has previously been reported that many common Florida rhodophyte taxa exhibit euryhaline tolerances in short-term (<1 week) laboratory exposures (Mathieson and Burns 1971; Mathieson and Dawes 1974; Dawes *et al.* 1976; Dawes *et al.* 1978; Dawes and Kovach 1992). However, these results notwithstanding, a one week exposure may be too short a time period for the effects of salinity stress to translate into measurable changes in growth. In a study by Floreto *et*

*al.* (1993), it took more than two weeks before the effects of exposure to a reduced salinity of 20 psu became evident as reductions in the specific growth rate of the alga *Ulva*, a cosmopolitan estuarine genus.

The rhizophytic algae exhibit more stenohaline tolerances, with better growth, health, and photosynthetic production at higher salinities; they exhibited poor tolerance to low salinity. This may be in part a result of the ultrastructure of these algae. O'Neal and Prince (1988) studied the response of *Caulerpa paspaloides* to reductions in salinity and proposed that the thin cell wall of *Caulerpa* in the apical region rendered it susceptible to damage caused by hypotonic stress. Moreover, other members of this genus were found to be unable to equilibrate to hypotonic conditions, and rupturing of the cell walls could result (Tosteson *et al.* 1971). These experimental findings, along with the observed distribution of this functional group, suggests that low salinities may be partly responsible for the exclusion of many rhizophytic algae from the canal-influenced region of western Biscayne Bay.

#### *Spatial and Temporal Differences in Salinity Tolerance*

Photosynthetic productivity and growth of macroalgae from any particular location also depends on a plant's physiological adaptation to past and extant conditions (Gessner and Schramm 1971; Josselyn 1977). The generation time of the algae studied is on the order of months (Stockman *et al.* 1967; Thorhaug 1976a; Josselyn 1977), so I hypothesized that the salinity response of these algae may change seasonally and differs spatially. The drift algae in particular were investigated because they occur over a wide range of salinity conditions, and were further found to have significant seasonal differences in growth and photosynthesis in Chapter 3.

Acclimation to seasonal salinity changes was exhibited by the algae I studied. Drift algae and epiphytes had higher gross photosynthesis and growth rates in winter than the summer, while the opposite seasonal trend was observed in the rhizophytes. Previous

laboratory studies in Florida on two genera of estuarine red algae, *Eucheuma* and *Hypnea*, support these observations on the drift algae. Both genera had higher photosynthetic rates in colder water temperatures typical of winter conditions compared to summer temperatures (Mathieson and Dawes 1974; Dawes *et al.* 1976). It has also been observed that there are more species of drift algae present in the winter (dry season) (Hamm and Humm 1976). These authors suggest sensitivity to salinity fluctuations and higher water temperatures during the summer and fall (wet season) as possible explanations for their observation.

Similarly, for the rhizophytic algae seasonal variations in the standing stock biomass have been recorded (Bach 1979; Wefer 1980), with higher biomass in summer than winter. This may be because the summer-time conditions are more favorable for growth in species like *Penicillus* and *Halimeda*, while the cooler winter months often induce a state of dormancy (Bach 1979; Wefer 1980). Reduced salinity from canal-inputs or sheet-flow run-off in summer is likely to affect actively growing rhizophytic algae that are intolerant of low-salinity stress more so than during the winter when they are in a dormant phase. This has been observed in a number of species of estuarine algae where low salinities appeared less detrimental in colder winter months when they were dormant (Doty and Newhouse 1954; Conover 1964; Kjeldsen and Phinney 1972).

Spatial differences in salinity regimes occur in the Bay because of the canal inputs and sheet-flow run-off along the western shoreline. Drift algae and the rhizophytic algae *Penicillus* were collected from oceanic and canal regimes and exposed to the same salinities. In both groups there was a significantly lower ability of algae from the oceanic site to tolerate reduced salinities typical of canal-influenced areas of the Bay. The drift algae from the canal site were able to tolerate the lower salinities better than *Penicillus*, even though both occur in this regime. Also, *Penicillus* from FP were smaller than their counterparts from oceanic areas of the Bay (see Chapter 2), which may in part be because of the stress posed by the low-salinity conditions present in the canal sites throughout much of the year (Gessner and Schramm 1971). Furthermore, the ubiquitous abundance of drift

algae at the canal sites, and the paucity of rhizophytic algae indicates inherent differences in the ability of these two groups to tolerate the low salinities present in summer.

#### *Salinity Tolerance Under Pulsed vs. Static Salinity Stress*

Growth of drift algae exposed to a pulsed salinity treatment was found to be influenced by the salinity more than the duration of the pulse. At salinities of 10 psu pulse duration was not a significant factor, as the stress posed by this low salinity was greater than any effect arising from pulse-duration. At 20 psu, however, drift algae grew significantly less when exposed to a long pulse, than when exposed to a shorter pulse. This is important, because the average wet-season salinity experienced by drift algae in canal-influenced regions of Biscayne Bay is around 20 psu. Subjecting drift algae to more frequent pulses of low-salinity water, analogous to frequent daily discharge events would be a way to test the cumulative effect of pulse-duration and salinity. The results obtained from the single-pulse exposure suggest that frequent discharge events, which reduce the regional salinity to 20 psu or less, could have deleterious effects on the ability of drift algae to persist under these conditions. This scenario would then be similar to the static exposures, where drift algae were found to photosynthesize and grow at a reduced amount, compared to controls in 35 psu water. The reduction in drift biomass at the canal-sites in summer may, therefore, be due, in part, as much to the low salinities present, as the higher water temperatures determined to be important in Chapter 3 in determining seasonal biomass dynamics of this functional group.

#### **SUMMARY**

Analysis of the environmental variable salinity, for later modeling efforts, highlights the importance of understanding landscape-level dynamics and the concomitant temporal resolution required to sample the data adequately. As the salinity extremes, rather than the mean, are the major determining influence on the structure and function of the communities

within canal-discharge plumes, there is a need for high-resolution, long-term data sets to understand the dynamic salinity regime that the benthic algae are exposed to.

The responses of the algae to a range of salinities commonly encountered in Biscayne Bay showed that there were seasonal and spatial differences in their tolerances to salinity. Drift and epiphytes tolerated lower salinities than did the rhizophytic algae. Drift and epiphytic red algae are euryhaline and tolerate low salinities down to 10 psu, which is the lower mean salinity during the wet season at canal sites. Conversely, the green rhizophytic algae are stenohaline. As such they exhibit poor tolerances to reduced salinities, with adverse effects evident at 15-20 psu. For this reason it is likely that salinity reductions from canal discharges are in large part responsible for excluding this latter group of algae from canal-influenced portions of the Bay.