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ULVA LACTUCA AS A BIOINDICATOR OF COASTAL WATER QUALITY

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ULVA LACTUCA AS A BIOINDICATOR OF COASTAL WATER QUALITY

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by

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ABSTRACT

The use of <u>Ulva</u> tissue disc growth for monitoring nutrient load (N, P) in coastal waters was assessed. Growth patterns obtained from <u>Ulva</u> discs were often irregular and the discs were subject to tissue loss owing to physical and/or biological factors. Position effects among disc replicates within deployment units were evident. Disc growth was influenced by swarmer differentiation and discharge. Disc location within the thallus was also a significant source of growth variation. These complications reduced the feasibility of the <u>Ulva</u> disc monitoring method.

An alternative monitoring approach was developed. Genotypically identical germlings were successfully employed as <u>in situ</u> assay organisms under diverse ecological conditions. The unit of measurement related to germling growth. The ability to characterize growth of <u>Ulva</u> germlings with respect to the physical and chemical parameters operating in the environment was demonstrated.

The use of <u>Ulva</u> as a bioaccumulator of coastal water pollutants was also investigated. Gas chromatograpic analysis of <u>Ulva</u> tissue samples collected from different Massachusetts populations revealed the presence of numerous currently unidentified compounds. To date, PCBs from a New Bedford Harbor Ulva population (197 ppb A1248; 32 ppb

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ABBREVIATIONS USED IN TABLES AND FIGURES

SPORULATION TREATMENTS $\overline{A} = Air$ WT = Wet Towels 1 C1 = Culture Chamber No. C2 = Culture Chamber NO. 2 PD = Petri Dish Culture Conditions MT = Microtiter Culture Conditions THALLUS LOCATION DESIGNATIONS $\overline{A} = Apical$ A-M = Apical-MiddleM = MiddleM-B = Middle-BasalB = BasalP = PeripheralC = CentralULVA SQUARE GROWTH STUDIES $\overline{T} = Top$ M = MiddleB = BottomS = Summation (T+M+B)a = Largest Square b = 2nd Largest Square c = 3rd Largest Square d = 4th Largest Square e = Smallest Square N = No. of Observations s = Standard DeviationP 1-2 = Data Summation, Plants 1-2 P 1-3 = Data Summation, Plants 1-3 $P \ 1-4 = Data$ Summation, Plants 1-4P = 1-5 = Data Summation, Plants 1-5 $P \ 1-6 = Data$ Summation, Plants 1-6

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GERMLING GROWTH STUDIES I = Initial Lengths F = Final Lengths1/1 = Full Salinity (30 ppt)2/3 = 2/3 Full Salinity (20 ppt) 1/2 = 1/2 Full Salinity (15 ppt) 1/3 = 1/3 Full Salinity (10 ppt) O = Distilled Water (O ppt)ppt = Parts Per Thousand T1 = Temperature Treatment 1 T2 = Temperature Treatment 2 T3 = Temperature Treatment 3 T4 = Temperature Treatment 4 A = Raceway AB = Raceway BC = Raceway CIR = Ipswich RiverER = Essex RiverHC = Hodgkins Cove $\bar{\mathbf{x}}$ = Mean HW = High WaterLW = Low Water M1 = First Mid-Water Stage M2 = Second Mid-Water Stage

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I. Ulva AS A BIOINDICATOR

Environmental monitoring of coastal waters has traditionally relied upon the chemical analysis of water samples. A conventional water sample provides information that reflects conditions at the single point in time at which it was collected. These procedures prove inadequate when, as is often the case, the occurrence of a pollutant is unpredictably episodic.

Resident organisms integrate the history of a water mass over prolonged periods of time, and thus can be used as bioindicators of pollution. Water quality assessment by examination of aquatic organisms is hardly new. The value of information derived from freshwater organisms was established more than a quarter century ago (Campbell, 1939; Brinley, 1942; Patrick, 1950; Surber, 1953; Butcher, 1957), however, the development of comparable techniques for coastal waters have lagged behind those for river and lake systems.

Sessile invertebrates in general, and bivalves in particular, have received the most attention as potential indicators of coastal water impurities, but there are obvious shortcomings in relying solely upon animal species. For example, Butler (1974) sites laboratory and field studies demonstrating large individual variations in

bivalve accumulation of organochlorine residue concentrations. Also, most pesticides, other than chlorinated hydrocarbons, do not accummulate in animals (Hayne et al., 1974). In addition, some normally resident organisms, including many shellfish, upon detection of undesirable compounds cease to pump water or they may seek to escape such conditions using various limited methods of motility available to them. Bivalves can also purge themselves of pollutants (depurate) upon restoration of a pollution free environment. Therefore, the use of shellfish as bioindicators must be restricted primarily to the detection of compounds which are more or less continuously present, as opposed to those which may appear in short pulses.

Attached seaweeds, which draw their nourishment directly from the water, are less able to avoid and/or purge pollutants than shellfish. The green alga <u>Ulva</u> <u>lactuca</u> L. (Sea Lettuce) is a benthic seaweed with a ubiquitous distribution in coastal waters. It is found from the polar regions to the equator, from full ocean salinities into river mouths, and in weak or strong currents. <u>U. lactuca</u> is resistant to most types of environmental perturbations encountered in coastal waters, as evidenced by its wide distribution and tolerance of broad habitat diversity. Fluctuating environmental conditions typical of coastal waters and estuaries, and the

various forms of pollution present in these environments result, at times, in conditions too stressful for most organisms, yet <u>U</u>. <u>lactuca</u> occurs in abundance. Estuarine organisms in particular are subjected to a variable environment, and a possible correlation exists between their wide ecological resistance to the natural environment and their tolerance of environments altered by man (Michaels, 1979). Since it is important to identify bioindicators that are capable of tolerating various types (and combinations) of perturbations present in coastal environments (Baker, 1976), the natural hardiness present in U. <u>lactuca</u> is desirable.

One category of bioindicators are the biodetectors, i.e., organisms which respond to different environments in characteristic ways. Biodetection is based on an ability to reflect the integrated effects of the environment through growth and development. Oglesby (1967) noted that size measurements of an organism whose mean dimensions change as some function of distance away from a pollution source could yield a relatively simple measure for evaluating a polluted condition. <u>Ulva</u> growth can be correlated with nutrient concentrations (Cotton, 1911; Sawyer, 1965; Burrows, 1971), and is sensitive to differences in both the water and the mud collected from different locations (Fogg, 1969; Burrows, 1971; Russell, 1973). Accordingly, studies indicate that the growth of

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discs cut from blades of <u>Ulva</u> are indicative of different levels of organic load and associated nutrients (Waite and Gregory, 1969; Cochrane et al., 1970; Burrows, 1971; Rhyne, 1973). These environmentally attuned properties of <u>Ulva</u>, if fully understood, might recommend its use as an indicator for even modest levels of nutrient load in coastal waters.

An alternative way to use Ulva as a biodetector involves measuring the growth and development of early developmental stages of the Ulva plantlet (the germling). The generalized development of Ulva begins with attachment of a naked zooid to the substratum. The first cell division occurs two to three days following settlement. А holdfast differentiates from the proximal cell through repeated cell divisions. A uniseriate germling develops by subsequent divisions of the distal cell. Germlings then become transformed by longitudinal divisions into a hollow tube closed at both ends. The proximal part of the tubular thallus continues to differentiate into the holdfast while the distal part becomes compressed and expands laterally as a double layered membrane, the cells of which eventually differentiate into gametangia or sporangia.

Differentiation and discharge of reproductive cells in <u>Ulva</u> show a marked periodicity. Peaks of discharge are associated with the semi-monthly spring tides. Sexual and asexual reproduction are accomplished through motile

unicells called swarmers. Gametophytic plants give rise to gamete swarmers and sporophytes yield zoospore swarmers. In each case, the fertile regions are most consistently on the upper third of the thalli along the margins.

Our methods enable the acquisition of thousands of genetically identical germlings of precisely the same age from a small segment of the adult thallus. Instead of measuring the growth of <u>Ulva</u> discs, the unit of measurement relates to germling size, i.e., some function of length and morphological development.

Ulva can also be used as a bioaccumulator, i.e., an organism which accumulates pollutants. Analysis of plant tissue in general has found widespread use in evaluating nutrient supplies in soils and in the freshwater environment (Gerloff and Krombholz, 1966). Tissue analyses minimize difficulties associated with obtaining representative samples in aquatic ecosystems. Plants become the sampling device. Seaweeds, which are known to accumulate environmental contaminants (Ware et al., 1968; Sears and Yentsch, 1972; Sikka et al., 1976), can be used in this way. As benthic plants they reflect environmental conditions at one site over time, the paramount criterion for a successful monitoring tool. U. lactuca has been shown to take up metals (North et al., 1972; Imbamba, 1972; Wong and Lau, 1979), hydrocarbons (Youngblood et al., 1971; Youngblood and Blumer, 1973) and pesticides (Sikka et al., 1976) from its environment.

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Rivers, which collect the runoff of entire watersheds, constitute the greatest source of pollutants to inshore Many pollutants (pesticides, PCBs, oils, etc.) waters. are hydrophobic (i.e., only slightly water soluble) and therefore possess high affinities for both particulate matter and living organisms. Compounds associated with particulate matter eventually enter bottom sediments. A chemical equilibrium is established between sediments and the overlying waters which can result in their functioning as a replenishing source for these chemicals (Hayne et al., 1974). Resuspension of sediments by natural forces enhance this exchange. These toxic chemicals can be taken up by benthic organisms, degraded by microbes, or reburied (Hassett, 1975). Ulva is one of the few plants found under the conditions of heavy siltation and fluctuating salinities typical of river mouths.

The distromatic structure of <u>Ulva</u> (Plate Ib) favors its functioning as a bioaccumulator in two ways. First, virtually every cell within the organism is in direct contact with the water. Extracts made from the entire plant are therefore more meaningful than would be the case if only localized segments functioned as the primary sites of accumulation. Secondly, <u>Ulva</u> has a large surface area to volume ratio, enhancing the efficiency of the alga as a sampling device. The macroscopic size of <u>Ulva</u> permits the collection of tissue samples in excess of 100-200 grams,

quantities often necessary for chemical analyses. <u>Ulva</u> can be harvested from environments favoring bioaccumulation, as in the mouths of rivers where there is constant water movement, and where chemical pollutants are commonplace. Water movement is crucial as algae accumulate environmental contaminants in greater concentrations in flowing waters than from still waters (Rose and McIntine, 1970; Sears and Yentsch, 1972; Butler, 1977). Uptake is clearly a function of the total volume of water that comes into contact with the surface of the alga.

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Our goal was to develop methods which capitalize on the capacity of <u>Ulva</u> to function as a bioindicator for coastal waters. We have performed preliminary studies evaluating the growth of <u>Ulva</u> discs and germlings as they relate to physical parameters operating in the environment. Investigations on the suitability of <u>Ulva</u> as a bioaccumulator have also been initiated.

II. TISSUE DEPLOYMENT STUDIES

INTRODUCTION

Although genetically uniform discs can be obtained from one <u>Ulva</u> blade, the physiological state of tissue discs may differ as a function of position within the thallus. Therefore, the first question to be addressed was what variation in growth patterns can be attributed to positional effects within the thallus? This question can be further divided into effects of swarmer differentiation on disc growth and the variability of disc growth in the absence of swarmer differentiation.

The next question addressed was whether the discs could be used to characterize the nutrient load of a simulated quiet water, silty embayment. This study examines the ability of disc growth to reflect environmental heterogeneities (i.e., variations in light and nutrients) within a relatively simple system. Of particular interest was whether sediments function as a source of nutrients for the plants.

SPORULATION METHODOLOGY

Preliminary studies employed <u>Ulva</u> discs obtained using a number 1 disc borer, which produced 0.4 cm diameter

discs. Later, two razor blades separated by a 0.4 cm spacer were used with more accuracy and facility. The razor blade technique produced squares (actually parallelograms) having more clearly defined edges than the cork borer-generated discs, which often had jagged edges from the tearing action of the borer. Both types of discs were subject to irregular growth and some loss of tissue due to physical and/or biological factors.

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Plants used for these studies came from an <u>Ulva</u> population attached to a floating dock at the UMASS Marine Station in Gloucester (Plate Id). Owing to attachment onto the floating dock, the experimental material was maintained constantly within 5 cm of the water surface. These plants were relatively uniform in size, shape and temporal background. The use of plants from this one relatively homogenous population was an attempt to eliminate as many sources of growth variation as possible.

Four plants were collected from the floating dock <u>Ulva</u> population on 7/28/77, two days prior to a spring tide. An additional plant was collected from the floating dock <u>Ulva</u> population on 3/1/77, two days following the spring tide. The rational for the additional collection and subsequent laboratory efforts was simply to compare the degree of swarmer discharge from plants collected prior to and following a spring tide (see later discussion of Rhyne's observations). Care was taken to select plants which were as uniform as possible.

Each pre-spring tide plant underwent an overnight presporulation treatment. One plant was left overnight in a tray without water at room temperature (treatment A). Two plants were left in dry trays in culture chambers at 22 degrees C (C1) and at 14 degrees C (C2). An additional thallus was sandwiched between seawater dampened paper towels (treatment WT). After sixteen hours, all seaweeds were cut into five sections, starting at the blade tip and progressing downwird to the rhizoids (Figure 1). Eight 0.4 cm discs were obtained from each of these sections, two within each of four subsections: (1) peripheral (a), (2)central (a), (3) central (b), and (4) peripheral (b). No disc contained noticable (upon visual examination) fertile regions or blemishes. Of the discs obtained from the air treated plant, half were placed in a series of 35 X 10 mm petri dishes, each containing 5 ml 0.45 um filtered seawater (1 disc/dish); the other half were placed in the individual wells of a plastic microtiter unit (0.2 ml/well). A similar procedure was followed for the other experimental plants (WT, C1, C2). Petri dishes and microtiters were then placed in a culture chamber (12-16 degrees C, 12:12 Light: Dark). Additions of distilled water maintained water levels in both regimes. To measure the degree of swarmer discharge from the discs, a discharge index was established where O denoted a completely green vegetative disc with no evidence of discharge; 1

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represented discs possessing a slight degree of discharge; 2 represented discs which had mostly discharged; 3 denoted a disc whose cells had completely discharged swarmers. Discs were scored for swarmer discharge on 8/1/77 (t1) and 8/26/77 (t2).



Figure 1 Disc Site Identity Within Ulva Thallus.

DEPLOYMENT METHODOLOGY

A sediment tank (150 cm X 120 cm X 30 cm) with a flow regulator was established at the UMASS Marine Station, Gloucester, MA (Plate Ia). Prior to this work, the

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sediment within the tank (average depth - 10 cm) had been in place for greater than two years, and was complete with the found characteristic of silty embayments. The rate of seawater flowing through the system was maintained at approximately 3.5 liters/minute. The metered serwater passed through three trays containing dense growths of <u>Ulva</u> prior to introduction into the tank. This pretreatment was to lower the ambient concentration of compounds used by <u>Ulva</u> during growth. In this way, any effect of sediments as a nutrient source for Ulva would be more apparent.

Deployment units were placed within the sediment tank. Each unit consisted of a polypropylene line moored by a metal bolt and maintained vertically in the water column by a styrofoam float. Fiberglass net units were attached to these lines in three places, five cm from the surface, in the middle and 3 cm from the sediments. All materials were leached in running serwater for seven days prior to deployment. Five <u>Ulva</u> squares (0.4 cm X 0.4 cm) were placed within each net unit. All <u>Ulva</u> squares were taken from central locations within the lower third of the thalli to minimize position effects as a source of variation in disc growth. Initially, monofilament was used to sew close the net units; later, they were heat scaled using a soldering iron.

Six series of sediment tank deployments were made, each using a different plant from the floating dock <u>Ulva</u>

population as a source for <u>Ulva</u> squares. On 3/10, plant no. 1 yielded enough squares to establish ten deployment units. Plants no. 2-5, deployed on 3/11, 8/13, 8/16 and 3/17 respectively, also provided discs for the establishment of ten deployment units each. Plant no. 6 (deployed on 8/19) provided squares for twelve units.

SPORULATION RESULTS

The mean discharge index (see above) for each of the four pretreatments at t1 followed the same order (WT>A>C1>C2) in both petri dish and microtiter data sets (Figures 2a and 2b). Overall discharge means at t2 have C2 last under both microtiter and petri dish conditions. In the microtiter set, WT treated discs are again those most likely to release swarmers, while in the petri dish set, WT, A and C1 are all clustered at a high level of discharge (2.60-2.75).

The two most distal sections (A and A-M) had an average sporulation index of 2.50-3.00 for the WT and A pretreated plants (Table 1).

Values for discharge in C1 A and A-M sections at t1 are 1.50-2.25, and 2.25-3.00 at t2. Values for discharge in C2 A and A-M sections at t1 were 0.75-1.50, and 1.50-3.00 at t2.

In the three lower thalli sections (M, M-B, B), mean

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Figure 2

TABLE 1: SWARMER DISCHARGE UNDER VARYING CONDITIONS

	MICRO	TITER	DISCHAR	GE: t1				
	AIR		WET T	OW ELS	CHAMB	ER 1	CHAMB	ER 2
	PCCP	MEAN	PCCP	MEAN	PCCP	MEAN	PCCP	MEAN
A	3333	3.00	3322	2.50	2103	1.50	2001	0.75
AM	3333	3.00	3333	3.00	3303	2.25	1101	0.75
M	3102	1.50	3023	2.00	1332	2.25	2002	1.00
м-в	3003	1.50	2222	2.00	3001	1.00	2012	1.25
R	1300	1.00	2122	1.75	2103	1.50	2001	0.75
MEAN		2.00		2.25	-	1.70		0.90

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	PETRI	DISH	DISCHAR	GE: t1				
	AIR		WET T	OW ELS	CHAMB	ER 1	CHAMB	ER 2
	PCCP	MEAN	PCCP	M E AN	PCCP	MEAN	PCCP	MEAN
A	3333	3.00	3332	2.75	3103	1.75	3100	1.00
A-M	3333	3.00	3233	2.75	3330	2.25	2112	1.50
М	1001	0.50	0103	1.00	1022	1.25	2002	1.00
M-B	1002	0.75	2112	1.50	2013	1.50	2011	1.00
В	3103	1.75	3313	2.50	0221	1.25	2000	0.50
MEAN		1.80		2.10		1.60		1.00

	MICRO	TITER	DISCHAR	GE: t2				
	AIR		WET T	OWELS	СНАМВ	ER 1	CHAMB	ER 2
	PCCP	MEAN	PCCP	MEAN	PCCP	MEAN	PCCP	MEAN
A	3333	3.00	3333	3.00	3223	2.50	2103	1.50
A-M	3333	3,00	3333	3.00	3313	2.50	3103	1.75
М	3302	2.00	3123	2.25	3333	3.00	3202	1.75
M-B	3003	1.50	3 3 3 3	3.00	3001	1.00	3112	1.75
В	1300	1.00	3233	2.75	3123	2.25	3212	2.00
MEAN		2.10		2.80		2.25		1.75

	PETRI	DISH	DISCHAR	GE: t2				
	AIR		WET T	OW ELS	СНАМВ	ER 1	CHAMB	ER 2
	PCCP	MEAN	PCCP	MEAN	PCCP	MEAN	PCCP	MEAN
Α	3333	3.00	3333	3.00	3123	2.25	3203	2.00
A-M	3333	3.00	3333	3.00	3333	3.00	3333	3.00
М	3223	2,50	0203	1.25	3333	3.00	3222	2.25
М-В	3333	3.00	3333	.3.00	2033	2.00	3033	2.25
В	3213	2.25	3323	2.75	3333	3.00	3302	2.00
MEAN		2.75		2.60		2.65		2.30

TABLE 2: DISC GROWTH IN THE ABSENCE OF SWARMER DISCHARGE

Anioni	Peripheral	Central	Central	Peripheral *
Aprear	0.29	0.29	8.00	
Apical-Mid	*	7.00	7.25	6.25
Middle	7.25	8.00	7.00	6.50
Mid-Basal	7.25	8.50	9.25	*
Basal	7.75	9.25	9.00	8.50

* Only growth of those discs with no evidence of discharge were measured. Units = millimeters \mathbf{U}

microtiter disc values for all pretreatments ranged between 0.75-2.25, while petri dish disc values at t1 were 0.50-2.25. The discharge index values for the M, M-B and B subsections at t2 ranged between 1.25-3.00 for microtiter discs and between 0.50-3.00 for those discs kept under petri dish conditions. Means for each plant section are plotted in Figures 3a and 3b.

Figures 4a and 4b show the relationship between discs taken from the central vs. peripheral regions of the <u>Ulva</u> thallus. There is a consistent tendency for greater swarmer release from the peripheral regions. Figures 5a and 5b present the discharge means for blade regions in VT and A pretreatments for both microtiter and petri dish data sets at t1 and t2, and for both peripheral and central subsections.

The post-spring tide plant collected from the floating dock <u>Ulva</u> population on 3/1/77 was exposed to the A pretreatment as before, yet when scored at t1' (3-4-77) there was no evidence of discharge (i.e., the overall discharge mean was 0), and at t2' (9-10-77) only three discs showed any evidence of swarmer release. This set of discs, which experienced no significant swarmer release, was measured for the longest undisturbed dimension per disc. The overall mean was 7.39 (s = 0.84) for the peripheral discs, and 3.15 (s = 0.87) for the centrally isolated discs (Table 2). Within the central disc



Figure 3. Effects of Pretreatments on <u>Ulva</u> Sporulation: Sectional Pretreatment Means.



Figure 4



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Petri Dish Data



grouping, discs isolated from basal regions generally exhibited greater growth than those more distally isolated discs. These data were analyzed using the nonparametric Wilcoxon Rank Sum Statistic. Growth of peripheral discs (which showed no evidence of discharge) was compared to centrally isolated discs. Peripheral disc growth was significantly lower (p = 0.05). A subsequent test was made comparing those central discs taken from the two top sections of the plant (A & A-M) and central discs taken from the two bottom sections of the plant (M-B & B). Growth was significantly higher in the basal regions.

DEPLOYMENT RESULTS

The data are assigned to five post deployment size categories (a,b,c,d,e) with the largest squares per net unit assigned to the "a" category, the next largest to "b", then "c", "d" and "e" (Table 3). In this way comparisons could be made between the data as a whole, or subdivided into those squares which grew under the best conditions within each net unit.

Of the <u>Ulva</u> squares deployed, 39-75% (per plant) were unsuitable for measurement, i.e., they were either lost or possessed no dimension undisrupted during deployment. Figure 6 summarizes the data on the degree of square disruption incurred during deployment. In all cases, mean

TABLE 3: ULVA GROWTH (SQUARES): SEDIMENT TANK, PLANTS #1-6

	SQUAF TOP	RE GRO	O₩TH:	Plant	: No.	1 MIDDI	LE			•	BOTT	MC						
	8	Ъ	С	d	е	a	Ъ	с	đ	е	a	Ъ	с	d	e	MEAN	N	S
1.1	6.00					4.75	4.25								-	5.00	3	0.90
1.2	5.00					5.00	4.75	4.25	4.00		4.25					4.54	6	0.43
1.3	4.75	4.75	4.25	4.25		5.00	4.75	-			4.75					4.64	7	0.28
1.4	4.75	4.50				6.00	5.25	5.00	5.00		6.25	4.75				5.19	8	0.62
1.5	6.00	5.75	5.00			4.75	4.75	4-75			6.00	-				5.29	7	0.60
1.6	5.50	4.50									5.75	5.00				5.19	4	0.55
1.7	5.00	5.00	4.75			5.00	5.00	4.75			7.25	-				5.25	7	0.89
1.8	5.00	4.75				5.25	5.00	5.00	5.00		5.00					5.00	7	0.14
1.9	5.75	5.00	5.00	5.00		5.25		•	•		5.50	5.50				5.29	7	0.30
1.10	5.00	5.00	5.00	5.00	5.00	5.75	5.25				6.00	5.50	4.75	4.50		5.16	11	0.44
MEAN	5.28	4 - 91	4.80	4.75	5.00	5.19	4.88	4.75	4.67		5.64	5.19	4.75	4.50		-		
N	10	8	5	3	1	9	8	5	3	0	9	4	1	1	0		67	
9	0.49	0.40	0.33	0.43		0.43	0.33	0.31	0.58		0.89	0.38						

	SQUA	RE GE	OW TH:	Plan	t No.	2												
	TOP					MIDDLE					BOTTOM							
	a	Ъ	с	d	е	a	Ъ	С	đ	е	a	ъ	с	đ	е	MEAN	N	S
2.1	5.00	4.25														4.63	2	0.53
2.2	5.25	5.00														5.13	2	0.19
2.3	4.75										5.00	5.00				4.92	3	0.14
2.4	5.75	5.25	5.00			5.25	5.00	4.75			5.75	5.00				5.22	8	0.36
2.5						4.75	4.00									4.38	2	0.53
2.6	4.75					5.25					5.50					5.17	3	0.38
2.1	5.00										• • •					5.00	1	
2.0	2-50	5.00	5.00	5.25		2.00	5.00	4.75	4.75		6.00	4.75				5.10	10	0.39
2.9	5.00	5 25	F 00			5.00	4 • 12				2.25	5.00	4.25			4.88	6	0.34
MEAN	5.19	1 05	5.00	5 25		5 08	1 75	4 75	4 75		5.00	4.00	4 25			5.04	1	0.55
N	9	5	3	1	0	6	5	2 2	4+75	0	5.42	4.17	4.27	0	0			
9	ó. 39	0.41	ó	•	v	0.20	0.43	ō		v	0.41	0.43	•	0	U		44	
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Table 3 (cont.)

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	SQUARE GROWTH: TOP	Plant No.	3 MIDDLE				ጉ ሰ ጥጥ ሰላ						
3.1 3.2 3.3	a b c 4.75 4.50 4.50 5.00 6.25 5.25	d e 4.25	a 5.00 4.50 5.00 4.75	с 4.25	đ	е	a b	с	đ	e	MEAN 4.54 4.75	N 7 4	s 0.27 0.35
3.4 3.5 3.6 3.7	5.00 5.00 5.00 4.75 4.75 4.50	4.25 4.50	4.75 4.50 5.00 5.00 5.00 5.00	5.00 4.25			4.75 5.00 4.75	4.75			5.75 4.75 4.92 4.68	2 7 6 7	0.71 0.29 0.13 0.28
3.8 3.9	5.00 4.50		4.50 4.50								4.67	3	0.29
3.10 MEAN	5.25 5.25 5.00 5.06 4.95 4.75	4.33	5.00 5.00 4.89 4.75	4.00 4.38			5.00 4.75 4.75	4.75			4.93	7	0.43
9 9	8 5 4 0.53 0.33 0.29	30.14	7 7 0.20 0.25	4 0.43	0	0	4 1 0.35	1	0	0		44	
	SQUARE GROWTH:	Plant No.	4										
	a b c	d e	MIDDLE a b	~	A	<u>م</u>	BOTTOM	~	a	•	MEAN	N	~
4.1 4.2	4.50 4.50 4.00	4.00	5.00 4.25	4.25	4.00	0	5.00 4.50	4.25	4.25	4.25	4.37	13	0.33
4.3 4.4 4.5	5.00 4.50 4.50 4.75 4.50 4.00 5.50 5.00 4.50	4.25 4.25	5.25 5.00 4.75 4.50 5.00	4.90 5.00 4.25	4.25		4.50 4.25 5.50 5.25	5.00	4.50		4.55 4.81 4.46	11 13 5	0.29 0.41 0.29
4.6 4.7 4.8	5.00 5.00 6.25 5.75 5.25	5.00 5.00	5.25 4.75	5 50	5 00		4.75 5.50 5.00	5.00	4.75		4.88	2 7	0.18
4.9 4.10	6.00 5.00 5.00	5.00 4.75	5.00 4.75	4.75	4.50		7.00 6.00	5.00	4.50		5.17	13	0.72
	2.20 2.00 2.25	4.75	5.25 5.00				5.50 5.00	4.25			5.06	9	0.39
MEAN N	5.25 4.88 4.63	4.75	5.25 5.00	4.71	4.45	_	5.38 5.00	4.25	4.50	4.25	5.06	9	0.39
Table 3 (cont.)

6.12 MEAN

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5.00 4.50 4.50 4.46 4.18 4.15 4.00 11 7 5 2 0.39 0.24 0.22 0

0

	SQUARE GROWTH: TOP	Plant No.	5 MIDDLE										
5.1 5.2	a b c 4.75 4.25 4.25 4.00	d e 4.00 4.00	a b 4.50 4.25 4.25	с 4.25	đ	e	a b 4.25 4.25	c 4.00	₫ 4.00	e	MEAN 4-23	N 2	8 0.23
5.3 5.4 5.5 5.6 5.7 5.8 5.10 MEAN N S	$\begin{array}{r} 4.00 \ 4.00 \ 4.00 \\ 4.25 \ 4.00 \ 4.00 \\ 5.00 \ 5.00 \ 5.00 \\ 5.00 \ 5.00 \ 5.00 \\ 5.00 \ 4.25 \ 4.00 \\ 5.00 \ 5.00 \ 4.25 \\ 4.00 \ 4.00 \\ 5.25 \ 4.75 \\ 4.73 \ 4.47 \ 4.36 \\ 10 \ 9 \ 7 \\ 0.66 \ 0.46 \ 0.45 \end{array}$	$\begin{array}{c} 4.00 & 4.00 \\ 4.00 & 4.00 \\ 5.00 & 5.00 \\ 4.00 & 4.00 \\ 4.00 & 4.00 \\ 4.00 & 4.00 \\ 4.14 & 4.17 \\ 7 & 6 \\ 0.38 & 0.41 \end{array}$	4,75 4.50 4.75 4.50 4.75 4.75 5.75 5.50 4.25 4.25 5.00 4.75 4.50 4.00 4.25 4.00 4.25 4.00 4.68 4.50 10 9 0.46 0.47	4.00 4.00 5.00 4.25 4.00 4.25 4.00 4.22 9 0.34	4.00 4.25 5.00 4.00 4.00 4.00 4.00 4.18 7 0.37	4.00 5.00 4.00 4.33 0.58	$\begin{array}{c} 4.00\\ 4.00\\ 4.25\\ 6.00\\ 5.00\\ 4.25\\ 4.25\\ 5.25\\ 4.50\\ 4.50\\ 4.56\\ 4.46\\ 8\\ 0.70\\ 0.37\\ \end{array}$	5.00 4.25 4.00 4.31 4 0.47	4.00 1	0	4.13 4.13 4.55 5.25 4.23 4.50 4.08 4.36	2 9 12 10 13 10 13 6 9 96	0.18 0.28 0.25 0.40 0.58 0.30 0.47 0.20 0.44
	SQUARE GROWTH: TOP	Plant No.	6 MIDDLE				BOMMON						
6.1 6.2 6.3	a b c 4.00 4.00 4.00 4.00 4.25	d e	a b 4.00	c	đ	8	a b	c	đ	e	MEAN 4.00 4.00	N 3 2	s 0 0
6.4 5.5 6.6	4.50 4.50 4.00 4.75 4.25 4.25	4.00 4.00	4.25 4.00 5.00 4.25	4.25	4.25		5.00 4.25				4.25 4.31 4.38	1 8 8	0.35 0.33
6.7 6.8 6.9 6.10 6.11	4.00 4.25 4.00 4.00 4.00 4.25 4.00 4.00 5.00		4.00 4.50 4.00 4.00 4.00 4.75				4.00				4.00 4.38 4.00 4.05	2 2 5 5 2	0 0.18 0 0.11
6.12 MEAN	5.00 4.50 4.50 4.50 4.46 4.18 4.15	4.00	4.50 4.25	1 25	4 25		4.00				4.46	6	0.33

 $\begin{array}{c} 4.00 \\ 4.75 \\ 4.50 \\ 4.25 \\ 4.33 \\ 4.13 \\ 4.25 \\ 4.25 \\ 9 \\ 4 \\ 1 \\ 1 \\ 0 \\ 0.38 \\ 0.14 \end{array}$

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4.00 4.33 4.25 3 1 0.58

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Figure 6 Number Of Measurable <u>Ulva</u> Squares: Sediment Tank Environment.

values for the number of top, medium and bottom squares which survived the deployment with at least one dimension undisrupted (and therefore measurable) followed the pattern of having highest survivorship within the top units, and the least within bottom units.

Light varied as a function of position within the sediment tank. Light intensity decreased from the net units at the surface to those at the bottom for the unshaded units, but increased slightly with depth for shaded units (Table 4) due to diffuse light input from adjacent unshaded regions.

Plants no. 1-3 each established ten deployment units which were arranged as three rows situated towards one end of the sediment tank. Those rows derived from plants no. 4-6 were more centrally located and therefore subject to some degree of shading from the flow regulator and associated <u>Ulva</u> trays mounted over the central portion of the tank.

The operation of both light and nutrient factors can be seen in Figures 7a-7f. These graphs present means of the longest disc dimensions for top, middle and bottom deployed <u>Ulva</u> squares. In Figure 7a, <u>Ulva</u> squares maintained at the surface grew better than those at the middle depth, but not as well as those near the bottom. In 7b, surface squares had the greatest growth and middle squares the least. The same pattern is seen in 7c although

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TABLE 4: SEDIMENT TANK LIGHT INTENSITIES

DEPTH

	0 cm	5 cm	10 cm	15 cm	25 cm
Sun	1.23	1.19	1.09	1.06	1.02
Shade	0.21	0.23	0.24	0.24	0.27

Measurements taken between 9:45-10:00, 7/21/77. A LI-185 Quantum Radiometer Photometer was employed. Units = microeinsteins/meter square/sec

TABLE 5: DATES OF Ulva GROWTH STUDIES

	DEPLOYMENT	RETRIEVAL
SEDIMENT TANK STUDY		
Plant No. 1	8/10/77	8/30/77
Plant No. 2	8/11/77	8/30/77
Plant No. 3	8/13/77	8/31/77
Plant No. 4	8/16/77	9/10/77
Plant No. 5	8/17/77	9/10/77
Plant No. 6	8/19/77	10/1/77
GERMLING STUDIES		
Salinity	10/14/79	10/27/79
Temperature	10/14/79	10/27/79
Water Velocity	10/14/79	10/27/79
Deployment I	10/14/79	10/27/79
Deployment II	10/27/79	11/18/79



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Figure 7

the bottom units are closer to the middle units in overall growth. In Figures 7d, 7e and 7f, representing squares centrally located in the tank and consequently shaded, bottom squares outgrew both middle and top squares. Growth of top and middle positioned discs were similar for plants no. 4-6.

Figure Sa presents graphs resulting from the progressive summation of the complete data sets of plants 1-6. There was a consistent pattern of the greatest no. growth from bottom squares and the least growth from the middle squares. The fewer bottom squares measured (attributable to increased square disruption at that depth) could be argued to have skewed results toward the more successful surviving squares. This objection is negated by performing the same plots using the "a" data sets only. The total number of squares processed are now comparable for each level (top, middle, bottom). The overall pattern remains the same (Figure 8b). When all of the sediment tank data is analyzed by analysis of variance (ANOVA) procedures, significant depth and row effects are obtained. Due to the within plant nested effects, it is more appropriate to test for sources of variation using the data restricted to one plant at a time. When this is done, statistically significant depth effects are restricted to plant no. 1, and significant row effects limited to plants 3-6. This is not surprising since plants no. 3-6 in no.



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Figure 8 Progressive Summation of <u>Ulva</u> Square Growth Data: Sediment Tank Environment.

general, and plants no. 4 & 5 in particular, were those which gave rise to deployment units placed within the shaded central regions of the sediment tank. Rows 1-4 for both plants no. 4 & 5 were directly under the flow regulator and pretreatment trays while rows 5-10 were not shaded. The Wilcoxon Rank Sum Test shows a significant decrease in square growth in those units deployed in rows 1-4 from plants no. 4 & 5 when compared to those in rows 5-10. This result is obtained whether the entire data set is analyzed or only those squares grown in "top" units (i.e., those most influenced by shading) were used. It also holds with the use of the "a" data set only.

DISCUSSION

The process of swarmer differentiation and discharge critically alters the capacity of <u>Ulva</u> discs to grow vegetatively. The data reveal patterns of discharge interspersed with irregularities. There is a greater tendency for swarmer differentiation and discharge in the upper regions of thalli, reflecting the <u>in situ</u> response, but there are no regions which completely fail to evidence discharge once isolated as discs. Peripheral tissue displays greater discharge tendencies than centrally isolated tissue, yet even discs taken from central regions will, to a lesser extent, yield swarmers.

The data indicate that the WT treatment is the best for sporulation induction, followed by A, C1 and C2. The culture chamber pretreatments (C1 and C2) resulted in lower degrees of discharge and greater variability in the spatial arrangement of discharge. We speculate that this is due to a damping effect of the culture chambers on whatever natural rhythms are operating in the <u>Ulva</u> sporulation process. This may be a function of temperature, environmental constancy, or both. The WT and A pretreated discs are taken as the more realistic approximation of the sporulation process exhibited in situ.

These results suggest that the culture chamber pretreatments are not condusive to sporulation induction in the short run (t1), although given time (t2) a moderate to high degree of discharge can be obtained. Considering both culture chamber treatments, C2 had the most pronounced damping action and the lowest recovery rates over time. We assume the lower temperature (14 vs 22 degrees C) was the principal responsible factor.

Microtiter conditions resulted in greater sporulation than that observed under petri dish conditions. The greater degree of stress imposed by the reduced volume of culture medium may be responsible for the increased escape response within the microtiter cultured discs.

The original four <u>Ulva</u> plants used in these studies to provide disc material were collected on 7/28/77. The peak

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in tidal amplitude was achieved on 7/30/77, corresponding with the full moon. A second Ulva plant collection was made two days after the spring tide. In accordance with Rhyne's (1973) documentation of swarmer discharge being synchronized with the spring tides, discharge was obtained in the first set of plants collected, but not with the latter (post-spring tide) plants. If it could be ascertained at what point the plants cease to be influenced by tidal rhythms, deployment periods could be chosen to minimize swarmer discharge occurring during deployment intervals. However, the observed irregularity in discharge patterns may indicate a physiological heterogeneity between vegetative cells from different parts of the thallus. This heterogeneity may reflect different disc growth capabilities, complicating the interpretation of deployment results.

Disc growth, as influenced by thallus position in the absence of swarmer discharge, was analyzed by measuring discs obtained from the plant collected on 8/1/77 and grown in petri dishes. These admittedly limited data indicate that even in the absence of sporulation there may be different growth capacities between peripherally and centrally isolated <u>Ulva</u> discs. There was also a greater growth capacity in discs isolated from the basal region of the plant than from more apically oriented discs. This differential growth capacity of Ulva discs obtained from

one thallus greatly restricts the quantity of tissue available for disc procurement within any one plant.

The five squares placed within each net unit within the sediment tank were originally intended to function as replicates. Owing to a tendency for the thallus squares to overlap one another after deployment, resulting in more favorable positions for some discs relative to light and nutrient sources, they actually functioned as competitors for these resources. This effect was circumvented during analysis by the "a"-"e" (<u>Ulva</u> square size categories) designations, which enabled the comparison of squares having the most favorable positions within the net units.

Light and nutrient availability are the two primary factors believed to be controlling the growth of <u>Ulva</u> squares deployed in the sediment tank. While the growth pattern for all plants is that of increased growth for those discs closest to the sediment, a secondary pattern is evident of somewhat greater growth in unshaded discs deployed near the top of the tank. The disc growth of plants no. 1-3 reflected the operation of these factors. Growth of top and middle positioned discs were similar for plants no. 4-6. This is attributed to the altered light distribution pattern mentioned above for shaded units. Shading may partly explain the decreased growth rates generally obtained from discs of plants no. 4-6. Other factors, however, were also operating. For example, each

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set of deployment units from each plant was deployed at a different time and for different intervals. The use of different plants could also be influential in growth differences.

The primary cause of the increased disruption of bottom squares is thought to be predation. This was a function of proximity to the sediment associated fauna, which were frequently observed to be on and about the net units. III. GERMLING DEPLOYMENT STUDIES

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INTRODUCTION

Growth of <u>Ulva</u> germlings as an <u>in situ</u> assay for nutrient load in coastal waters requires delimitation of variation in germling growth attributable to physical factors operating in these environments. Only after this has been accomplished can nutrient effects be factored out. Studies were therefore initiated on the effects of temperature, salinity and water velocity on the growth of Ulva germlings.

GERMLING PROCUREMENT METHODOLOGY

Male gametophytes of <u>Ulva lactuca</u> were collected from a Salem Harbor population on 7/7/79, placed in plastic bags and transported back to the laboratory on ice. Fertile margins were trimmed from the plants, placed between seawater soaked paper towels, and kept at 12 degrees C for 30-36 hours. Reproductive material was subsequently diced, transferred to Erlenmeyer flasks containing sterilized seawater, and kept suspended by magnetic stirrers for 1-3 hours. Following sufficient discharge of swarmers, as indicated by the suspension's yellow tinge, the diced tissue bits were filtered out through 300 um mesh Nitex

netting.

Two types of regimes were employed for swarmer The first involved the placement of numerous settlement. water-logged applicator sticks (15 cm long X 2 mm wide) on the bottom of a pyrex baking dish (20 cm X 20 cm). The sticks could either be immersed in the swarmer suspension or in a mixture of sterilized seawater and the swarmer suspension, depending upon the discharge densities achieved and the settlement densities desired. Light tight covers were placed around the pyrex dishes such that only bottom illumination was received. The light source was a bank of fluorescent tubes situated 30 cm below the settlement Saran wrap was used to cover the pyrex dish tops. dishes. Alternatively, settlement on applicator sticks was obtained using test tubes fitted with one or two holed stoppers. Sticks were maintained vertically within the test tubes by inserting them into the stopper holes which were then sealed on the top. Test tubes were exposed to diffuse fluorescent illumination from all sides. As in the settlement regime described above, the applicator sticks could be completely submerged in either the swarmer suspension or in some dilution of it. The pyrex dish method permitted relatively easy procurement of a large number of sticks with germlings distributed unilaterally. The test tube method yielded more evenly colonized sticks and permitted transporting and handling of settled germlings with less effort.

Four media mixtures were tested for sporulation and settlement suitability: (1) Erdschreiber soil extract, (2) Erdschreiber soil extract + vitamin supplement, (3) Erdschreiber soil extract + vitamin supplement + germanium dioxide, and (4) sterilized Hodgkins Cove seawater. The vitamin supplement used was that of Guillard and Ryther (1962). Germanium dioxide inhibits growth of diatoms, the most common contaminant in macroalgal cultures. Sterilized seawater, which was subsequently used for all sporulation studies, was unsurpassed in supporting germling growth. Aeration was unnecessary for germling growth and only increased the probability of cultures becoming contaminated.

GERMLING DEPLOYMENT METHODOLOGY

Applicator sticks containing germlings were cut into two centimeter lengths. Germling samples were taken from each applicator stick prior to deployment and mounted in 30% Karo for initial size determinations using a recticle equipped compound microscope. Germling removal was accomplished by slicing off wood slivers with a scalpel under low power microscopic observation. The same technique was employed to remove and preserve germlings after deployment.

Germlings were bathed for 30 minutes in saturated.

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solutions of Calcofluor White (in sterilized seawater) prior to deployment. Calcofluor White is a fluorescent "brightener" which is selectively absorbed by cell walls and is non-toxic to plants. It is stable, intensely fluorescent within the pH range of 5.0-8.5, and is transported to growing regions within the plant (Cole. 1964). The Calcofluor White treatment permits positive identification of germlings initially deployed. Other germlings which may have colonized the applicator sticks during the deployment period are easily distinguished under a dissection scope using a Black-Ray UVL-21 Ultra Violet lamp emmitting radiation at 360 nm. Upon retrieval of the applicator sticks, all segments were gently cleaned with a camel hair brush, mounted in 30% Karo, and screened for the ten largest deployed (i.e., fluorescent) germlings, whose lengths were measured.



Figure 9 Diagram of In Situ Deployment Unit

Germlings on applicator stick segments were deployed <u>in situ</u> between the turns of polypropylene lines which were moored with cynder blocks and kept vertical in the water column with a surface float (Figure 9). Germlings were maintained at relatively constant distances from the surface through the tidal cycle using weights attached to the lines below the float (Ramus et al., 1976). This technique minimized the effect of light variation between sites. Care was taken to limit physical contact to only the median regions of the applicator sticks which were essentially devoid of germlings since the germlings from those regions were taken previously for initial size classing. Dates and times for all deployments and retrievals are given in Table 5.

In Deployment I, germling containing applicator stick segments were inserted into the deployment units at two depths. Depth 1 was approximately 25 cm below the surface, and depth 2 was approximately 25 cm above the bottom. The absolute depths for depth 2 germlings over the tidal cycle varied from 2.5-7.0 meters according to the tidal stage. In Deployment II, segments were only inserted at depth 1. Water samples were taken for determination of salinity, pH and subsequent nutrient analysis. Spectrophotometric determinations of total nitrogen and total phosphorus were performed by 0.T. Zajicek of the Chemistry Department, University of Massachusetts, Amherst. The procedures

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TT d followed (total Kjeldahl N and the single reagent method for determining total P) were based on those described in the EPA's manuel entitled "Methods for Chemical Analysis of Water and Wastes". Temperatures were measured at the sites.

Of the six segments deployed on 10/14, those at depth 1 for all three sites (Ipswich River, Essex River, Hodgkins Cove) were from one applicator stick settled with gametes from one plant. Those segments deployed at depth 2 at the three sites were taken from another applicator stick which was also settled by gametes of the same plant. Therefore, the genotypes of all germlings deployed on 10/14 were theoretically identical, although there were slight initial size differences between the two sets of plantlets. Segment samples were prepared and the initial germling size determinations made on the night of 10/13. Segment samples were also prepared at this time for the temperature and salinity study which paralleled Deployment I in duration.

Four temperature treatments were tested. Test tubes containing applicator segments with germlings in 15 ml filtered seawater were placed in culture chambers (12:12 Light:Dark) at 22 degrees C (T1), 16 degrees C (T2), and 9 degrees C (T3). Another test tube containing germlings was placed in flowing seawater adjacent to the laboratory (T4), the temperature of which ranged between 6-9 degrees C during the experiment.

Five salinity treatments were evaluated for germling growth suitability. Temperature was held constant at 22 degrees C (12:12 Light/Dark). Hodgkins Cove seawater (30 ppt) was filtered for treatment 1, and diluted with distilled water to achieve the four remaining treatments: 2/3 full salinity (20 ppt), 1/2 full salinity (15 ppt), 1/3 full salinity (10 ppt) and distilled water.

Applicator sticks with attached germlings were also placed at this time in a flow-through raceway apparatus (Plate Ic) under three different flow regimes. The programmed water flow rates for raceways A, B, and C were 6400-6700 ml/min, 2500-2800 ml/min, and 2000-2300 ml/min, respectively. Due to a seawater system malfunction, flows were irregular for the last four days of the experiment, ending on 10/27. During these final days raceway C received reduced flow rates, and A was without flowing water for at least part of the time (up to three days). Only raceway B received the programmed water flow uninterrupted:

Deployment I segments were retrieved on 10/27, except for the Ipswich River depth 1 segment which was missing. Additional segments were put out at this time (Deployment II) at the three sites, but only at depth 1. These segments originated from one applicator stick settled at the same time as those in Deployment I but from a different plant. Deployment II units were harvested on 11/18. At

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, (12 this time it was discovered that the Essex River depth 1 segment was not always at a constant distance from the water surface over the entire tidal cycle. This was due to the water depth being greatest on the incoming tide, as opposed to the high tide when they were deployed. As a result, there were times during the mid-tidal stages bracketing the spring tides when these units were slightly greater than 25 cm below the surface.

RESULTS

Results of the salinity study are presented in Figure 10a (and Table 6). No significant difference was evident between those germlings grown under treatments 1 and 2 (corresponding to salinities of 30 ppt and 20 ppt respectively). Further salinity reductions resulted in significantly diminished germling growth. Growth occurred under treatments 3 and 4 (salinities 15 ppt and 10 ppt respectively), while germlings placed under treatment 5 (distilled water) bleached and showed no growth. There was a highly significant (p = .01) difference between all salinity treatments with the exception of salinities 1 & 2, which did not differ significantly. This result was obtained by analysis of variance (ANOVA) procedures. Subsequently, it was determined that the data failed to fit a normal distribution (based on Bartlette's Test for



Figure 10

10 Germling Growth: Influence of Physical Parameters

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TABLE 6: GERMLING GROWTH AS A FUNCTION OF GALINITY

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	Salin Trt.	nity 1	Salin Trt.	nity 2	Silii Trt.	nity 3	Salir Trt.	ity 4	Silir Trt.	ity 5
	I I	ייעק ד	(20 J	F	I	F F	I I	יייקי ק	(O PI	F
1	13.9	35.7	10.4	47.5	15.4	28.2	12.2	29.2	18.2	14.2
2	13.0	30.7	9.7	44.2	14.4	20.1	11.1	16.0	18.0	9.6
3	12.3	30.7	9.7	32.7	12.6	19.8	11.0	15.9	15.4	9.2
4	10.6	30.2	9.5	29.3	12.5	19.3	10.6	15.1	14.8	8.3
5	10.2	28.9	9.4	26.8	12.5	19.2	10.3	14.9	14.4	8.3
6	9.7	28.8	9.3	26.1	11.0	19.1	10.0	14.3	14.0	7.5
7	9.6	28.0	8.6	23.3	11.0	17.8	9.9	13.3	14.0	7.3
8	9.2	26.8	8.5	22.4	10.8	17.3	9.8	12.9	12.6	7.1
9	8.9	26.4	8.3	22.1	10.2	16.9	9.7	12.8	12.2	6.1
10	8.8	25.9	7.6	21.7	10.0	15.8	9.6	12.3	11.9	6.0
MEAN	10.6	29.2	9.1	29.6	12.0	19.4	10.4	15.7	14.6	8.4
3	1.8	2.9	0.8	9.3	1.8	3.4	0.8	4.9	2.2	2.4

TABLE 7: GERMLING GROWTH AS A FUNCTION OF TEMPERATURE

	TEMP 1		TEMP 2		ΤE	MP 3	TEMP 4		
	22 C		1	16 C		С	6-	6-9 C	
	I	F	I	F	I	F	I	F	
1	13.9	35.7	18.5	53.1	24.1	20.3	21.2	30.4	
2	13.0	30.7	18.2	38.5	18.5	20.1	17.3	23.6	
· 3	12.3	30.7	17.3	38.3	16.1	19.7	15.8	23.3	
4	10.6	30.2	16.0	31.7	14.4	19.0	15.0	20.7	
5	10.2	28.9	15.4	25.9	13.4	18.9	13.8	20.3	
6	9.7	28.8	12.2	24.7	12.9	18.4	12.7	19.9	
7	9.6	28.0	9.5	24.4	12.1	17.0	10.7	19.7	
8	9.2	26.8	9.2	24.3	12.1	16.7	10.2	19.1	
9	8.9	26.4	8.9	24.2	11.4	16.3	9.1	18.9	
10	8.8	25.9	8.8	23.7	9.9	16.2	9.0	17.3	
MEAN	10.6	29.2	13.4	30.9	14.5	18.3	13.5	21.3	
9	1.8	2.9	4.1	9.7	4.2	1.6	4.0	3.7	

TABLE 8: GERMLING GROWTH AS A FUNCTION
OF WATER VELOCITY

	Racev	vay A	Racev	vay B	Race	жау С
	I	F	I	F	I	F
1	12.7	30.0	10.0	37.2		20.5
2	11.0	26.5	9.2	27.0		19.5
3	10.2	25.4	8.7	24.8		19.3
4	9.8	25.1	8.2	24.2		17.5
5	9.4	24.2	7.2	23.4	\mathbf{L}	17.0
6	9.3	23.5	6.8	23.3	0	16.5
7	9.0	21.8	6.7	23.3	S	16.2
8	8.8	20.6	6.7	22.5	Т	16.2
9	8.4	20.6	6.6	22.4		15.5
10	8.2	20.0	6.2	21.4		15.4
MEAN	9.7	23.8	7.6	25.0		17.4
	1 4	3 2	1 3	1 6		1 8

which possessed sufficient normality to permit ANOVA procedures, displayed no significant differences between raceways A and B. Raceway C was statistically distinct from both of the higher flow treatments (p = .01).

Data from Deployments I and II are presented in Figures 11a and 11b (and Table 9). The Essex River germlings deployed at depth 1 during Deployment I outgrew those deployed at Hodgkins Cove. Analysis of variance, which was applicable for all in situ deployment data, revealed a highly significant difference (p = .01) between germling growth at these two sites. Of the Deployment I depth 2 segments, those at the Ipswich River grew best, followed by Hodgkins Cove and Essex River germlings. There was no statistically significant difference between germling growth at Hodgkins Cove and Essex River sites at depth 2, but there was a highly significant difference between final lengths attained at either of these locations when compared to the Ipswich River germlings. The Deployment II segments were limited to depth 1. Greatest growth occurred at Hodgkins Cove followed by Ipswich River and Essex River germlings. There was a highly significant difference between germling growth at Hodgkins Cove and either the Ipswich River or Essex River sites, which were themselves statistically equivalent.

To interpret these data, reference must be made to the chemical and physical parameter determinations made at the

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Homogeneity) necessitating use of the nonparametric Wilcoxon Rank Sum Test for determining significance between treatments. The results were the same as those derived from ANOVA.

Results of the temperature study are presented in Figure 10b (and Table 7). Final germling lengths were slightly greater at 16 degrees C than at 22 degrees C. Germling growth was reduced under the T3 treatment (9 degrees C). Germlings grown under T4 (6-9 degrees C) grew at a rate intermediate between T1 and T2. Both ANOVA and the Wilcoxon Rank Sum Test revealed all temperature treatments to have highly significant differences from each other with the exception of treatments 1 & 2.

The effects of different water velocities on germling growth were also studied (Figure 10c and Table 8). Mean initial values for the ten largest germlings from applicator sticks deployed in raceways A and B (the data from raceway C being lost) ranged between 8-10 um. Final mean values for the ten largest germlings from each applicator stick deployed in the raceways were 23.8 um (s = 3.2), 25.0 um (s = 4.6) and 17.4 um (s = 1.8) for raceways A, B and C respectively. Raceway C, which received the slowest flow rate, had the lowest growth increase. Raceway B, the only raceway to receive the programmed water flow uninterrupted, had the largest germling growth increase, with those in raceway A slightly lower. The raceway data,



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TABLE 9: In situ GERMLING GROWTH VALUES

		DEPLOYMEN	ΓI	DEPLOYMENT	II
		DEPTH 1	DEPTH 2	DEPTH 1	
		I F	I F	I F	
Hodgkins	1	15.8 20.8	18.5 19.1	15.5 51.2	
Cove	2	12.4 17.4	18.0 17.3	14.2 35.2	
	3	10.5 15.3	11.1 16.9	12.8 34.6	
	4	10.1 15.0	9.5 15.3	12.3 33.7	
	5	10.0 14.8	9.1 13.5	12.0 32.4	
	6	9.6 14.5	9.0 12.2	11.4 31.1	
	7	9.6 14.1	8.8 12.2	10.8 30.5	
•	8	9.4 13.9	8.4 11.5	10.8 29.8	
	9	9.3 12.4	8.2 11.5	10.7 29.8	
	10	9.2 12.3	8.2 11.2	10.6 29.3	
	х	10.6 15.1	10.9 14.1	12.1 33.8	
	S	2.1 2.5	4.0 2.9	1.7 6.5	
Essex	1	37.1	27.0	15.7 32.4	
River	2	<u> 56.8</u>	14.6	14.4 29.0	
	3	55·1	13.9	12.5 24.0	
	4	22.5	9.9	12.2 27.2	
	5	21.7		11.2 21.2	
	6	20.9	6.2	11.2 21.2	
	7	29.8		10.7 20.2	
	8	20.9		10.7 20.0	
	10	20.1	2.2	0719.4	
	10	21.0	5•2 10 1	9.7 10.9	
	x	21.9	10.1	1 0 1 5	
Inquitab	5 1	J• J	25 0	13 5 33 5	
River	2		24.0	12.2 29.2	
MI VCI	3		24.0	11.2 27.2	
	Á		20.0	10.8 25.3	
	5	\mathbf{L}	19.2	10.3 25.2	
	6	Ō	18.9	10.0 24.3	
	7	Š	10.8	9.4 23.8	
	8	Ť	17.5	9.3 21.8	
	9	-	17.2	9.3 21.8	
	10		16.4	9.2 21.5	
	x		20.2	10.5 25.3	
	9		3.3	1.4 3.8	

TABLE 10: ENVIRONMENTAL PARAMETERS AT DEPLOYMENT SITES

SITE	DEPLOYMENT	TIDE	DEPTH	TIME	TEMP (C)	SAL (ppt)	nН	ΤΟΤΔΤ. Ν	
Ipswich	I	HW	1	11:45	13.0	29.0	$\frac{1}{7.8}$	1.90	104 - 1
River	I	НW	2	11:45	13.0	29.5	7.8	1.33	0.29
	I	$\mathbf{L}\mathbf{W}$	1	18:00	12.5	19.0	7.2	0.56	0.21
	I	\mathbf{LW}	2	18:20	12.5	18.5	7.2	0.36	0.03
	II	M 1	1	6:35	5.7	24.4	7.9	0.16	0.03
	II	HW	1	9:45	7.1	26.2	7.8	0.22	0.03
	II	M2	1	12:45	7.9	22.0	7.9	0.18	0.03
	II	LW	1	16:15	6.8	16.2	7.6	0.30	0.05
Essex	I	Η'A	1	12:30	12.5	29.0	7 5	0.84	
River	I	ΗW	2	12:30	12.5	LOST	LOST	0.48	0.05
	I	$\mathbf{L}\mathbf{M}$	1	16:50	13.0	28.0	7.8	LOST	0.03
	I	L₩	2	16:50	13.0	28.0	7.8	0.60	0.21
	II	M 1	1	7:10	6.7	25.5	7.9	0.71	0.04
	II	ΗW	1	10:30	7.1	27.2	7.8	0.30	0.05
	II	M2	1	13:30	8.0	27.5	7.9	0.27	0.03
	II	LW	1	15:45	6.7	25.1	7.9	0.15	0.04
Hodgkins	I	MW	1	14:45	12.0	28.0	7.8	0.80	0.14
Cove	I	MW	2	14:45	11.0	29.5	7.8	0.80	0.14
	II	M 1	1 .	7:40	6.9	26.3	8.0	0.16	0.29
	II	НW	1	11:00	7.8	27.8	8.0	0.25	0.04
	II	M2	1	13:50	8.1	27.8	8.0	0.14	0.03
	II	\mathbf{LW}	1	17:00	7.5	28.1	8.0	0.21	0.04

Nitrogen and Phosphorus Units = mg per liter

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times of deployment (Table 10). Both the Ipswich River and Essex River sites are high water flow, completely mixed environments as evidenced by the temperature, salinity, pH data, and observations made while scuba diving. Temperature and salinity values indicate some degree of stratification at the Hodgkins Cove site. This was expected since tidal forces are the sole source of water exchange within Hodgkins Cove. The Ipswich River site experienced the greatest salinity fluctuation, with values obtained ranging between 16-30 ppt. Fluctuations in Ipswich River pH values also revealed the freshwater influence, with low water values down to 7.2, while high water values were 7.8. The Essex River site, which possesses a considerably smaller drainage basin than the Ipswich River, had a much reduced tidal influence, with salinities ranging between 25-29 ppt. Hodgkins Cove, with no major freshwater input, had salinities ranging between 26-30 ppt.

Total nitrogen, considered to be the limiting factor for algal growth in coastal waters (Ryther and Dunstan, 1971), reached 1.90 mg/l at the Ipswich River site, 0.84 mg/l at the Essex River location, and 0.80 mg/l at Hodgkins Cove. These data reflect the general nutrient condition where the Ipswich River is the richest site in terms of nutrient load. Also of significance in the interpretation of germling growth data are the bottom characteristics of

the experiment sites. Both the Ipswich River and Hodgkins Cove locations had sediment deposits, while the Essex River site was completely scoured.

DISCUSSION

Germling growth under salinity treatments 1 & 2 was essentially equivalent, while diminished values were obtained under treatments 3 & 4. This wide tolerance to salinity variation reflects the euryhaline character of <u>Ulva</u>. The data suggest that germlings should be deployed at sites exposed to salinities of 20 ppt and greater if salinity is to be removed as a major source of variation in growth. For the most part, the experimental sites met this criterion, although germling growth may have been reduced slightly as a result of temporary low salinities at the Ipswich River site.

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Temperature treatments 1, 2 and 3 were all conducted under identical light regimes. The results are ambiguous as to whether the <u>Ulva</u> germlings grow better at 16 or 22 degrees C since overall growth was greatest at 16 degrees C but the difference between initial and final lengths was greatest at 22 degrees C. There was, however, a clearly reduced growth rate at 9 degrees C (T3). The temperature at which treatment 4 plants grew fluctuated between 6-9 degrees C. yet their mean length exceeded the T3 (9

degrees C) grown plants. This was attributed to the ambient light regime they received.

We believe the difference in germling growth between raceways B and C reflects a velocity effect. It is unclear as to why growth in raceway B exceeded that in raceway A. Conceivably, there is an optimal flow rate above which growth slows to some degree. Alternatively, the effect of raceway A being without flowing water for as long as the final four days of the experiment could have been detrimental.

Further studies are required to clarify the relationship between germling growth and the physical factors encountered <u>in situ</u>. These initial results, however, demonstrate the feasibility of calibrating the growth of <u>Ulva</u> germlings as influenced by environmental parameters.

Given the relatively equal nutrient, temperature and salinity regimes between depth 1 sites at the Essex River and Hodgkins Cove sites, the enhanced growth of Essex River germlings over those at Hodgkins Cove during Deployment I can be attributed to the difference in water velocity at the two sites. The increased growth of depth 2 germlings at Ipswich River over Essex River may be explained on the basis of the higher nutrient load at the former since the opposite would be expected from the effects of salinity and flow. A greater growth disparity could be expected if the

salinity regimes were more comparable (salinities down to 16 ppt being recorded at the Ipswich River compared to a low of 25 ppt at the Essex River). In attempting to explain the higher growth of Hodgkins Cove depth 2 germlings over those at the Essex River site we resort to the presence of sediments at Hodgkins Cove and their absence at the Essex River. As indicated by our sediment tank studies, sediments are a significant source of nutrients, and may be a major factor controlling germling growth.

Retrieved germlings exhibited a continuous range of morphologies from uniseriate to tubular to blade-like plantlets. Germlings gave rise to blade-like morphologies (Plate IIIc) only within depth 1 and raceway regimes. Tubular germlings were also obtained under these conditions (Plate IIIb), however, no blades were obtained either at depth 2 sites or in culture chamber grown germlings. It would therefore appear that both high light intensities and rapid flow rates are required for blade development in <u>Ulva</u> germlings (at least over the time span of these experiments). A variety of rhizoid types were also obtained (Plate III).

On occasion, the initial uniseriate germlings failed to undergo longitudinal divisions near the apex, but gave rise to multiseriate blades some distance below the apex (Plate IIId). Also observed was the occasional occurrence

of bubble-like protuberances from the apical cell (Plate IIa-c), the nature of which is not understood.

Concerning the Deployment II results, we speculate that at this time of year (Oct-Nov) water movement is detrimental to <u>Ulva</u> germling growth. Although <u>Ulva</u> can be found year-round in protected habitats, in other localities it is essentially an annual plant which is initiated in the spring, flourishes in the summer and disappears in late autumn. This may be responsible for the germlings at the relatively protected Hodgkins Cove site outgrowing germlings from both river sites. Greater growth of the Ipswich River germlings over those at the Essex River site is again attributed to the enhanced nutrient availability within the Ipswich River.

This investigation has only begun to define the effects of physical parameters operating in the environment on <u>Ulva</u> germling growth. Both technical and procedural protocols need refinement before a more complete understanding of the processes controlling growth of <u>Ulva</u> germlings is achieved.

IV. TISSUE ANALYSIS STUDIES

INTRODUCTION

Algae can be used as continuous sampling monitors for pollutants in coastal waters. Harding and Phillips (1978) demonstrated transfer of hydrophobic compounds from microparticulates to phytoplankton. Chemicals available for accumulation by algae therefore exceed those concentrations dissolved in the water. There is generally a rapid and virtually unlimited uptake of these compounds by phytoplankton, with concentrations often exceeding 100-1000 times ambient levels (Cox, 1972; Keil et al., 1971; Södengren, 1968; Södengren, 1971; Butler, 1977; Vance and Drummond, 1969; Harding and Phillips, 1978; Rice and Sikka, 1973).

Inhibitory effects of pesticides and PCBs on phytoplankton growth are well documented (Biggs et al., 1979; Mosser et al., 1972; Moore and Harris, 1972; Harding, 1976; Cole and Plapp, 1974; Ware and Roan, 1970; Butler, 1977; Södengren, 1968). These studies demonstrate how algae vary both qualitatively and quantitatively in their response to such man-made substances. Typically, pesticide and PCB concentrations in the parts per million (ppm) range are toxic to most algal species. Various nonlethal effects occur in the parts per billion (ppb) range. These can be

ecologically significant since different degrees of susceptibility can result in community composition changes. For example, PCB concentrations as low as 0.13 ppb have resulted in substantial community structure changes in mixed cultures of natural phytoplankton (Fisher et al., 1974).

<u>Ulva lactuca</u> is admirably suited as a bioaccumulator owing to its high resistance to environmental perturbations, sessile nature, and advantageous structural features. To evaluate the use of <u>Ulva</u> as a bioaccumulator in coastal waters, we have performed tissue analyses on plants collected from Massachusetts populations.

METHODOLOGY

<u>Ulva lactuca</u> samples were collected during the periods of June 23-24, July 21-22 and August 5-6, 1979. Specimens were wrapped in foil, sealed within plastic bags and transported to the laboratory on ice. All plants were hand cleaned of sediments, epiphytes and associated invertebrates (to a reasonable degree) before being stored at 0-4 degrees C. Extractions were made during the period of August 14-31, 1979 at the UMASS Cranberry Experimental Station, Wareham, Massachusetts. Laboratory procedures were designed to detect chlorinated pesticides, phosphorus containing pesticides and PCBs (Hammarstrand, 1976).

Approximately 100 grams (wet weight) of Ulva was homogenized at high speed in a blender for two minutes (longer when required) with 200 ml acetonitrile and 10 grams celite. The slurry was filtered and the filtrate volume measured and transferred to a one liter separatory funnel. This raw extract was then subjected to "cleaning up" procedures to remove interferring substances and to concentrate compounds of interest. Petroleum ether (100 ml) was added and the mixture shaken vigorously for two minutes, followed by the addition of 10 ml saturated sodium chloride solution and 600 ml distilled water. The contents of the separatory funnel were gently mixed and allowed to separate. The bottom phase was discarded and the upper petroleum ether phase washed twice with 100 ml distilled Subsequently, the volume of extract was measured, water. residual water removed with anhydrous sodium sulfate, and the extract concentrated by evaporation down to 10 ml. Α column (22 mm i.d. X 20 cm long) was then packed with florisil to a height of 10 cm, followed by 2 cm sodium sulfate, and the extract added to the column. The compounds of interest were eluted as two fractions with 6% diethyl ether and 15% diethyl ether (in petroleum ether). Both the petroleum ether and the diethyl ether solvents were redistilled prior to use. The column retained algal pigments and polar lipids in the florisil adsorbent, while yielding a relatively purified pesticide extract. The 6%

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RESULTS

Sites from which Ulva was collected for tissue analysis are shown in Figure 12. With the possible exception of a trace of DDT from the Weweantic River Ulva population, none of the pesticides screened for were detected. Numerous compounds were, however, found. Of these, only PCBs from New Bedford Harbor Ulva (197 ppb A1248; 32 ppb A1254) and saturated hydrocarbons (C17H36, C25H52, C27H56, C29H60, and C33H68) from Hingham Harbor have been identified to date. Quantitation of PCBs was accomplished by comparing PCB peak area measurements with those from standardized PCB solutions of known concentrations. Identification of hydrocarbons was by mass spectrometer analysis performed by E.J. Guzik of the UMASS Chemistry Dept. We are currently following up on these results. Representative samples of the tissue analysis data are presented below.

Figure 13 presents four chromatograms generated by passing 6% extract fractions through the phosphorus-detecting GC. Each graph begins with a large peak which reflects the detection of the extract solvent. The Westport River graph (Figure 13a) is an example of a clean extract. The usual pattern is exhibited by the New Bedford run (Figure 13b), in which one additional peak occurs. We suspect this peak represents some naturally

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Legend for Figure #12

- 1. Merrimack River
- 2. Plum Island Sound
- Plum Island Sound -Ipswich River
- 4. Ipswich River
- 5. Castle Neck River
- 6. Essex River
- 7. Hodgkins Cove
- University of Massachusetts Marine Station (Point of Jetty)
- University of Massachusetts Marine Station (Outfall Side of Jetty)
- 10. Plum Cove
- 11. Folly Cove
- 12. Halibut Point
- 13. Beverly Harbor
- 14. Salem Harbor
- 15. Nahant (Nahant Bay Side)
- 16. Nahant (Broad Bay Side)
- 17. Pines River
- 18. Neponset River
- 19. Hull
- 20. Hingham
- 21. North River
- 22. Sandwich Marsh
- 23. Weweantic River

- 24. New Bedford Harbor
- 25. Slocums River
- 26. Westport River

	Legend for Figure #12	
1.	Merrimack River 24.	
2.	Plum Island Sound 25.	
3.	Plum Island Sound - 26.	1
	Ipswich River	
4.	Ipswich River	
5.	Castle Neck River	
6.	Essex River	
7.	Hodgkins Cove	
8.	University of Massachusetts Marine Station (Point of Jetty)	
9.	University of Massachusetts Marine	
	Station (Outfall Side of Jetty)	
10.	Plum Cove	
11.	Folly Cove	
12.	Halibut Point	
13.	Beverly Harbor	
14.	Salem Harbor	
15.	Nahant (Nahant Bay Side)	
16.	Nahant (Broad Bay Side)	
17.	Pines River	
18.	Neponset River	
19.	Hull	
20.	Hingham	
21.	North River	
22.	Sandwich Marsh	
23.	Weweantic River	

- 24. New Bedford Harbor
- 25. Slocums River
- 26. Westport River



Figure 12 Map of Coastal Massachusetts Showing Sites of <u>Ulva</u> Populations Sampled



Figure 13 Tissue Analyses: Organophosphorous 6%

occurring phosphorus metabolic (or storage) product within <u>Ulva</u> since it occurred to different degrees in most of the phosphorus detecting analyses. The Slocums River graphs (Figures 13c and 13d) show peaks that represent accumulation of unknown compounds.

Figure 14 presents four additional 6% fractions passed through the phosphorus detecting GC. All three Pines River extracts, which were made from the same <u>Ulva</u> population sampled in June, July and August, 1979, contain a series of four peaks, two larger peaks bracketing two smaller peaks. This particular pattern was not found elsewhere with the possible exception of the Nahant extract. The Nahant sample displays a similar pattern but at presumably lower concentrations. If the source of these compounds was indeed the Pines River area, transport to the nearby Nahant site would cause some dilution and result in the lower concentrations observed.

Analysis of the more complex organochlorine GC peak patterns within and between stations revealed that some peaks were represented in virtually all samples, others were present to different degrees, and some restricted to one or two sites. The common peaks have been assigned numbers for ease of recognition. We emphasize the subjective character of our interpretations to date.

Figure 15 presents four 6% fractions passed through the halogen detecting GC. All Hingham extracts, made from





June, July and August 1979 collections from the same <u>Ulva</u> population, display similar peak patterns. Hull, a closely situated site, shows the same general pattern as seen at Hingham. Relative constancy of the basic peak pattern is evident between other sites as well (Figure 16). Some extracts showed significant departures from the basic pattern. The Merrimack River extract had an unusual beginning peak pattern (Figure 17a) while the Beverly (Figure 17c) and Slocums River (Figure 17d) extracts had enigmatic middle components.

DISCUSSION

There is widespread concern over the ability of aquatic organisms to accumulate and transfer toxic compounds to higher trophic levels. This capacity has been documented for both estuarine (Petrocelli et al., 1975) and open ocean food chains (Ware and Roan, 1970; Butler, 1977). Accordingly, there has been a shift from the less biodegradable organochlorine pesticides to more water soluble alternatives such as the organophosphorus pesticides which are not accumulated by members of the aquatic biota to the same extent. Our failure to detect any organophosphorus pesticides may reflect the success of this approach. We cannot, however, rule out the possibility that the operating procedures followed failed







Tissue Analyses: Organohalide 6%









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to preserve these compounds. Refinements in collection and extraction techniques are planned for subsequent attempts.

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Our detection of PCB concentrations within <u>Ulva</u> on the order of 0.2 ppm more closely matches the lowest values found in New Bedford Harbor sediments, 0.5-620 ppm (Mass. Dept. of Env. Quality Eng., unpublished data) and in hard shelled clams (<u>Mercenaria mercenaria</u>) collected from New Bedford Harbor, 0.2-1.6 ppm A1242 and 0.2-1.5 ppm A1254 (Hatch et al., in preparation) but they do not match those of New Bedford mussels (<u>Mytilus edulis</u>), which have been found to concentrate up to 110 ppm (Risebrough, in preparation). Since only one New Bedford Harbor <u>Ulva</u> collection was made, conclusions concerning the PCB uptake capacity of Ulva would be premature.

Filter feeding bivalves accumulate many pollutants to levels above those found in seaweeds; this primarily reflects accumulation of particulate matter within the bivalve gastrointestinal tract. Those organisms which accumulate pollutants to the greatest degree may appear to be the best candidates for bioaccumulation monitoring species. However, the ability of shellfish to cease pumping at certain times, and to depurate upon restoration of a pollution-free environment argues against their use for episoidal pollutants.

The detection of C17-C33 alkanes may merely reflect natural constituents of Ulva since hydrocarbons naturally

found in higher organisms are usually in the C15-C30 range, and odd-carbon compounds predominate (Goldberg et al., 1978). The alkanes may, however, reflect uptake of oils present in coastal waters as a result of man. Although Youngblood et al. (1971, 1973) have provided some data on hydrocarbon constituents within <u>Ulva</u>, further work is required before the origin of hydrocarbons found within Ulva can be determined.

We believe the presently unidentified peaks obtained represent combinations of natural constituents and other compounds accumulated by Ulva. Goldberg et al., (1978), reporting on results of the EPA "Mussel Watch" program for monitoring coastal waters, also obtained numerous unidentified peaks in their gas chromatograms. The possibility that the peaks represent ubiquitous pollutants cannot be ruled out. Conceivably, a generalized peak pattern could be established for Ulva, against which patterns obtained by analyzing field samples would be compared. Anomalous peaks would merit further investigation. Unknown peaks could be characterized with respect to their position relative to known peaks, eventually building up a library of non-algal compounds, enabling rapid identification of substances previously identified.

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V. CONCLUSIONS

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The <u>Ulva</u> tissue disc method for monitoring organic load in constal waters was demonstrated to be marginally effective. Several factors, both biological and environmental, reduced the reliability of this approach. Differentiation and discharge of reproductive cells is a major factor affecting the growth capacity of <u>Ulva</u> discs. Also, in the absence of reproduction, disc growth potential was found to vary in relation to disc origin within the thallus. Physical and biological factors result in significant disc disruption, necessitating the use of replicates, yet competitive interactions exist between replicates. Owing to the lack of reliability, and reproducibility, the <u>Ulva</u> tissue disc method was abandoned.

We have found, however, that growth of parthenogenetically developed germlings has considerable promise in assessing environmental differences. Single thalli can be induced to yield genetically identical germlings for <u>in situ</u> deployments. Our procedures provide a level of reproducibility unmatched by organisms lacking asexual propagation.

Use of <u>Ulva</u> as an <u>in situ</u> sampling device also demonstrated appreciable success. A generalized peak pattern of natural constituents within <u>Ulva</u> must be established against which patterns obtained by analyzing field samples can be compared. Anomalous peaks, representing putative pollutants, would then become evident, and possibly merit further investigation. Such peaks could be easily characterized by their position relative to known peaks. This approach would also enable rapid recognition of compounds previously identified.

Areas for future development of the use of <u>Ulva</u> as a bioindicator fall into five categories;

- (1) to improve procedures for gamete settlement,
- (2) to refine the characterization of the germling growth response as influenced by physical factors operating in the environment.
- (3) to characterize the natural constituents of <u>Ulva</u> as revealed by gas chromatographic analyses,
- (4) to develop the use of parthenogenetically developed germlings for batch and/or continuous flow bioassay systems, and
- (5) to define the capacity for accumulation and depuration of pollutants by Ulva.

In summary, <u>Ulva</u> is ideally suited as a bioindicator for coastal waters due to its structure, habitat diversity, nardiness and capacity for asexual reproduction. Although the technique we have developed needs further refinement before it is ready for general application, it appears to hold great promise for monitoring episodic or chronic but low levels of environmental pollutants.

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Plate I

- a. Sediment tank with flow regulator and <u>Ulva</u> pretreatment trays.
- b. Ulva blade, X-section (400 x).
- c. Raceway apparatus used to control flow rate.
- d. Floating dock Ulva population
- e. Ulva blade, vegetative cells (1000 x).
- f. <u>Ulva</u> blade, reproductive cells (1000 x).



Plate II

a-c Post-deployment germlings with apical bubble
without developed rhizoidal cells (400 x,
 b-c phase contrast).

- d Post-deployment germling with non-elongated rhizoidal cells (400 x, phase contrast).
- e-f Post-deployment germlings with elongated rhizoidal cells (400 x, phase contrast).

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Plate III

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and the sector

- a. Germlings prior to deployment attached to wooden substratum (40 x).
- b. Post-deployment germlings without blade formation, attached to wooden substratum (100 x).
- c. Post-deployment germlings with blade formation, attached to wooden substratum (100 x).
- d. Post-deployment blade with uniseriate remnant of initial germling (200 x).

WATER QUALITY

