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**SIMoN Project: Elkhorn Slough Macrophytes
Progress Report – December, 2003**

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Introduction:

Relative to open coast temperate reefs, Elkhorn Slough is inhabited by few species of macrophytes. Some of these, however, such as *Enteromorpha* spp., can be extremely abundant. Unfortunately, other than limited, unpublished distributional information and a species list (Norris 1970, 1998), there is no quantitative information on the distribution and abundance of seaweeds in the Slough, or their temporal variation. Anecdotal information suggests macrophyte distribution and abundance may be changing due to erosion and nutrient loading from the uplands (Zimmerman and Caffrey 2002). The distribution of the only seagrass in the Slough, the eelgrass *Zostera marina*, is better known and does appear to be changing (review in Zimmerman and Caffrey 2002). Macrophytes may be important to other organisms and processes, and may be good indicators of changes in environmental conditions. Thus, monitoring their distribution and abundance will provide a better understanding of the Elkhorn Slough environment and information useful to its management.

Objectives:

The objectives of this study are to develop and test sampling designs to quantitatively determine changes in the distribution and abundance of the major macrophytes in Elkhorn Slough within and among years. This study defines Elkhorn Slough as the area influenced by tidal action east of the Highway 1 bridge to Elkhorn Road, and west of the railroad south of Hudson Landing (Figure 1). Seasonal variation is being determined by dividing the first year into four sampling periods: July and October, 2003, and January and April, 2004. Each of the sampling periods represents a season. These seasonal data will be used to determine and test a within year sampling design that best detects major seasonal changes.

Project Status:

Unanticipated logistical difficulties delayed the first full sampling until July 2003, shifting the first year schedule forward such that the four seasonal samples will not be completed until the end of April, 2004. Sampling designs and approaches have been evaluated with pilot studies, and the first (July 2003) and second (October 2003) seasonal sampling completed. General findings to date, results of the pilot studies, and results of the July 2003 sampling are reported here. The July 2003 data are electronically attached as an Excel file (SIMoN Slough Macrophyte Data). Results of the October 2003 sampling will be available by mid-February, 2004. January 2004 sampling, including aerial photography, has been scheduled.

Descriptions of Major Macrophytes

Enteromorpha spp. (hereafter *Enteromorpha*)

Enteromorpha is generally represented by small (2-20cm long) tubular green thalli (Abbott and Hollenberg 1976), typically unbranched in the Slough. Norris (1998) lists 6 species in Elkhorn Slough. *Enteromorpha clathrata* may be most abundant given it is described by Abbott and Hollenberg (1976) as the species most commonly found free-floating in estuaries and that Norris (1970) found it to be common on intertidal mudflats in the Slough. *Enteromorpha* continues to be most abundant on intertidal mudflats, including mudflat “patches” within the salt marsh. This green alga is also frequently observed drifting in the main channel (especially during high tides) and as mats deposited by the tide on top of *Salicornia*.

During July 2003 the distribution of *Enteromorpha* was as above. The alga was observed throughout the slough mostly on the mudflats between the lower edge of the *Salicornia* marsh (~+1.5 ft. MLLW) and the edge of the water at the lowest tide (~-2.0 MLLW). It was not found subtidally in any area, or above +1.5 ft. in the intertidal except as deposits on *Salicornia*. These deposits die (probably from high light/lack of emersion) and are not included in our biomass estimates.

Ulva spp. (hereafter *Ulva*)

Ulva thalli are commonly broad (10-50cm), flat green sheets that are 2 cells thick (Abbott and Hollenberg 1976). Norris (1970, 1998) reported a total of three species in the Slough, none of which commonly occurred unattached. *Gayralia oxysperma* (= *Monostroma oxyspermum*) looks like *Ulva* but is only one cell thick. It also occurs in the Slough (Norris 1998). Both genera can occur alive but unattached, particularly on mudflats at the landward ends of small tidal channels (M. Foster, pers. obs.). The collection and taxonomic analyses necessary to adequately describe these unattached green blades in the Slough have not been done. Thus, *Ulva* as described and sampled in this project may include one or more species of *Ulva* as well as *G. oxysperma*. Thalli in the Slough are found attached to hard substrata and as drift in the subtidal, and as drift on intertidal mudflats.

Ulva was observed throughout the Slough during July, but especially attached to shells on the bottom of the main channel, mostly 1-2m deep but occasionally up to 5 m near the Highway 1 bridge. Elsewhere in the Slough it occurred as small, occasional drift patches on mudflats.

Gracilariopsis (= *Gracilaria*) *andersonii*

Gracilariopsis andersonii has numerous erect branches, 6-25cm tall, that are dark red at the base sometimes fading to a lighter straw color (Abbott and Hollenberg 1976). In July 2003, the alga was found in the lower intertidal and subtidally to 4m deep.

A large, persistent bed of *G. andersonii* has been observed in the slough on the intertidal mudflat flat near the Highway 1 bridge (Vierra’s Beach) at least since the late 1970’s (M. Foster, pers. obs.). It can also be abundant between the railroad bridge and Elkhorn Road near Hudson’s Landing (M. Foster, pers. obs.). We observed it in some side channels, but it was generally sparse (<1 branch m⁻²).

Zostera marina

Eelgrass, *Zostera marina*, is a marine angiosperm common in coastal embayments such as estuaries. It occurs from the low intertidal zone to – 15 ft. in the Slough. It appears as multiple shoots arising from the mud. A shoot consists of 1-5 leaves surrounded by a basal sheath. The shoots arise from a rhizome beneath the mud.

As reviewed in Zimmerman and Caffrey (2002), eelgrass is currently found near the entrance to Elkhorn Slough from east of Highway 1 Bridge to Seal Bend (dark areas in Figure 2a,b). The largest continuous bed is located in the northern part of Seal Bend at depths of –3.0 to – 7.0 ft. Patches of *Z. marina* are present on the north and south sides of the Slough in the region west of Seal Bend. Detached leaves are frequently observed floating in the main channel.

Other Macrophytes

The introduced fuclaeen *Sargassum muticum* has been observed in the Slough at least since May 1990 (M. Foster, pers. obs.). In July 2003 it was observed in the subtidal to –15 ft. from the Highway 1 bridge to ~ 300 ft. east. Numerous SCUBA transects across the channel in this region revealed considerable rock and rubble (eg. remains of former oyster culture facilities) inhabited by *S. muticum* and a number of more open coast macrophytes. The large bladed red alga, *Chondracanthus corymbiferus* (= *Gigartina corymbifera*), was most abundant, covering an estimated 80% of the bottom in some areas. The red alga *Pseudogloiophloea confusa* was also abundant, and a juvenile giant kelp, *Macrocystis pyrifera*, was also found. These observations suggest this area may turn into a giant kelp forest. The area may warrant detailed surveys in future monitoring as its size and macrophyte species composition might be a particularly good indicator of the extent of main channel erosion and increased ocean influence.

Pilot Studies and Methods:

Enteromorpha

A pilot study was done to determine if the optical reflectance from *Enteromorpha* mats could be used to infer biomass from remote sensing imagery obtained over the Slough using the Portable Hyperspectral Imager for Low Light Spectroscopy (PHILLS). The biomass estimates from reflectance were, however, highly inaccurate; commonly only 60-70% of the true biomass as measured by drying and weighing. This is no doubt because the relationship between reflectance and biomass is linear only between 0 and 100% cover. Unfortunately, *Enteromorpha* mats exist as multiple overlapping layers in the Slough and their cover commonly exceeds 100%.

Another pilot study was done to determine the optimal quadrat size and number of replicates for sampling *Enteromorpha* biomass. In May 2003 twenty samples from an approximately 100 x 15 m area of abundant *Enteromorpha* were collected at random with 0.0625 and 0.25 m² quadrats. Collections from larger quadrats were difficult to dry. All samples were dried to constant mass. Wet mass did not provide an accurate estimate of biomass because the sponge-like nature of the mats produced high variation in water content. Statistical analyses (power analyses) were used to determine the number of samples needed to detect a particular difference in biomass using each of the two different quadrat sizes. The analyses showed that using the 0.25 m² quadrat, a 20% difference in mean biomass could be detected with around 30 samples. The smaller quadrat required 70 samples to detect the same difference. Therefore, the larger quadrat

was chosen, and at least 30 samples needed to be taken within the Slough to accurately estimate *Enteromorpha* biomass.

An objective of this study is to estimate seasonal changes in total *Enteromorpha* biomass in the Slough. To do this quadrat estimates must be combined with an estimate of *Enteromorpha* distribution within the Slough when the quadrats are sampled. Given the size of the Slough and difficulty walking over mudflats, is logistically very difficult to obtain an estimate of distribution from the ground. Use of a hovercraft was proposed and evaluated. Even though it allowed easy access to the mudflats without the substrate disturbance caused by boats and walking, the small hovercraft was judged by some to be inappropriate due to putative noise problems. Aerial images taken at ~ 0.0 MLLW were evaluated, and found to well-show *Enteromorpha* mats. Moreover, the ability to detect *Enteromorpha* is thought to be consistent as long as tidal levels are similar and images are obtained around mid-day (R. VanWagenen, pers. com.). Mats appear most clearly in the images when mats are 100% or greater cover on the ground, and ground observations showed that while areas of < 100% cover occur, they are rare; the alga typically occurs in thick mats. While quadrats combined with aerial images probably under estimate total biomass, the inaccuracy is unlikely to be ecologically unimportant. A set of images was obtained in July and October 2003, and will continue to be obtained to estimate distribution at each sampling.

Aerial images were used to divide the slough into five sections that contained *Enteromorpha* and were of similar size (Figure 1). This division was done to insure samples for this most abundant macrophyte were distributed throughout the Slough. These sections were further subdivided into “intertidal” and “subtidal.” Intertidal was defined as areas above 0.0 MLLW and subtidal as areas below that. This was done so that aerial images could be taken of the “intertidal” during all months of the year.

Based on the pilot study, the July 2003 seasonal sampling consisted of taking 20 samples from section 1, and 10 samples from each of the other sections for a total of 60 samples. More samples were taken in section 1 because prior aerial photos indicated *Enteromorpha* covered a larger portion of this section than any other. Sample locations were selected within Section 1 in the field by picking random sampling points from GPS coordinates that covered the entire section. This method resulted in numerous points without, or with vary sparse, *Enteromorpha*. Visiting enough points to locate 20 with sufficient *Enteromorpha* (defined as at least a 1 m² mat of mostly 100% cover because anything less would not show up in an aerial image – see below) was very time consuming. Sampling was therefore modified for subsequent sections. The modified sampling consisted of walking through the entire section and noting the presence of suitable mats. A subset of these was then picked haphazardly from throughout the section for sampling, the number depending on the number of samples needed. A single quadrat was then sampled in the center of each of these mats. Samples were rinsed with seawater and then freshwater, dried to constant mass (24-48 hrs), and recorded to the nearest gram. A one-way Model 1 ANOVA was used to determine significant differences in biomass among sections. Data were tested for equal variances with Cochran’s C.

Biomass estimates derived from the 0.25m² quadrats were used in conjunction with aerial images (taken by R. VanWagenen, EcoScan Co.) using a digital camera to estimate total biomass per section and total biomass in the slough. An image analysis program was developed using MatLab v6.1 to analyze the images for the area covered by

Enteromorpha. The routine counted the number of pixels ($\sim 0.25 \text{ m}^2$ each) that contained the alga based on each of the RGB values of the pixel (Fig. 2). Each layer of the picture (Red, Green, Blue) was searched individually for the range of values that represented *Enteromorpha* spp. The range of RGB values was determined by selecting pixels within the picture and setting the upper and lower constraints to these values: red (110-217), green (166 – 255), and blue (109-181). After the program found all pixels that were within the given range for each layer it then found all pixels that were found in all three layers. From this the final count of pixels with *Enteromorpha* spp. was reported. Exact pixel size was referenced to a known length within the image.

Ulva

General surveys throughout the intertidal zone of the Slough showed that *Ulva* was sparse (widely distributed patches less than 20 cm in dia.) to absent except for a large patch on the intertidal mudflat near Hudson Landing between the railroad bridge and Elkhorn Road (Fig. 3). Twelve samples were taken from this patch at random points along a 200 m long transect through the center of the patch parallel to Elkhorn Road. Samples were processed as for *Enteromorpha* above.

To determine the abundance of *Ulva* in the subtidal zone of the slough (below 0.0 ft. MLLW), 4 locations were surveyed with SCUBA across the main channel at low tide (Fig. 3). Surveys were done by swimming a zig-zag pattern across the channel, covering $\sim 1000 \text{ m}$ of the channel in area 1, 300 in area 2, 300 in area 3 and 100 in area 4. The only *Ulva* spp. observed was in area 1 within 500 m east of the Highway 1 bridge. Five 0.25 m^2 samples were collected from this subarea at random (random kicks along SCUBA transects), processed as for *Enteromorpha*, and scaled to 1 m^2 .

Gracilariopsis andersonii

Intertidal and subtidal surveys of the entire Slough were done as for *Ulva* above. In the intertidal zone, large, dense ($> 1 \text{ m}^2$ with 75% or higher cover) patches (beds) of *Gracilariopsis andersonii* were found only at Vierra's Beach (Fig. 3). Small patches were found throughout the Slough, primarily in side channels, but were not large enough to sample effectively. Four 0.25 m^2 quadrats were sampled at random within the $\sim 150 \times 50 \text{ m}$ bed at Vierra's Beach. This red alga was also surveyed in the same 4 subtidal areas surveyed for *Ulva*. It was only observed in the same subarea where subtidal *Ulva* was found, and was similarly sampled (5 quadrats; 0.25 m^2 scaled to 1 m^2).

Zostera marina

Previous descriptions of *Zostera marina* bed distribution have noted eelgrass only in the low intertidal to shallow subtidal zone ($- 1.0$ to 12 ft MLLW) in the northern section of Seal Bend and the north side of the slough from the Highway 1 Bridge to Seal Bend (Zimmerman and Caffrey 2002). Our general surveys found both of these beds as well as some previously undescribed patches on the southern side of the main channel between the old power plant outfall and Seal Bend. The latter were sparse relative to those at Seal Bend and the North Side, and were more patchy. Neither the area (too sparse to see in aerial images) nor the shoot densities were sampled in these newly found patches.

The two large eelgrass beds at Seal Bend and North Side were sampled. Their areas were determined from the aerial images (SCUBA surveys verified that the entire beds were detectable in these images). A central point was chosen within a representative area (qualitative eelgrass cover similar to most of the bed). Samples ($n=12/\text{bed}$) were chosen at random compass headings and distances (random kicks for a maximum distance of ~ 10 m) away from the central point. Sampling was done by placing a 0.25 m^2 quadrat on the bottom over the shoots and counting all shoots that originated within the quadrat. One representative shoot from each quadrat was collected at Seal Bend. The length of all leaves in these shoots was measured, and average used as a single replicate for the quadrat. Since all the leaves are in the same plane and are of equal width just above the sheath, the width of the entire group of leaves was measured at this point. The dry mass of the entire shoot was determined after rinsing with fresh water and drying for 36-48 hrs. Leaf Area Index (LAI) was calculated from leaf lengths and widths by determining the total area of the leaves in each shoot and multiplying by the number of shoots per quadrat, giving $n=12$ replicates of LAI for Seal Bend. All data were scaled to 1 m^2 .

Results and Discussion

Enteromorpha

Fifty-six samples were used to estimate biomass. One sample each from sections 1 and 2, and two samples from section 5 contained clay that could not be removed. These samples were not used. Variances were not significantly different, and there was no significant difference in biomass among sections as estimated by the 0.25 m^2 quadrats (Table 1). Total biomass among sections was similar except for section 4 which had the smallest total area (Table 2). Combining the area covered by *Enteromorpha* from aerial images and the overall estimate of biomass from the quadrats ($110 \text{ gDW}/\text{m}^2$), the total dry biomass of *Enteromorpha* in the Slough was 12,382 kg in July 2003 (Table 2).

Ulva

The estimated biomass of drift intertidal *Ulva* sampled near Hudson's Landing was 132.6 ± 20.7 (SE) gDW m^{-2} ($n=12$). The biomass of the subtidal, attached population of *Ulva* found near just east of the Highway 1 bridge was 2.3 ± 1.1 (SE) gDW m^{-2} ($n=5$). The total biomass of this macrophyte in the slough was not calculated given the near impossibility of sampling its generally sparse populations and that it was not detectable in aerial images. This estimate will be made in future surveys if biomass increases to the point where it is detectable in aerial images. Since *Ulva*, unlike *Enteromorpha*, only floats if it forms a mat that traps air (T. Schaadt, pers. obs.), it is likely exported from the slough near the bottom of the main channel and thus not easily detectable in our surveys. A large concentration of drift *Ulva* was observed from -1 to -5 ft. MLLW at the NW corner of Seal Bend in November, 2003.

Gracilariopsis andersonii

Like *Ulva*, *G. andersonii* was sparse, with only one large concentration noted (Vierra's Beach) in July 2003. Surprisingly, even this dense bed was not visible in aerial images, suggesting that this macrophyte cannot be sampled with aerial images even if it

were abundant throughout the Slough. Other wavelengths such as IR might detect it, but would need to be tested. The biomass of the intertidal population of *G. andersonii* was 303.5 ± 166.3 (SE) gDW m⁻² (n=4), and the subtidal population 47.1 ± 37.9 (SE) gDW m⁻² (n=5). The large SE for the latter is indicative of the patchiness of the subtidal population.

Zostera marina

The areas of the eelgrass beds at Seal Bend and North Side, as estimated from aerial images were 18,409 and 12,686 m² respectively. Characteristics of these two beds are given in Table 3. These estimates are ~ 5X less than those in Zimmerman and Caffrey (2002), but the difference may be due simply to how the areas were estimated. The latter estimates were based on a rough calculation of total area potentially available, not actual measurement of the area occupied (R. Zimmerman, pers. comm.). The most surprising finding was the new, albeit sparse eelgrass found between the old power plant outfall and the beginning of Seal Bend (Fig. 3). This bed may reflect changes related to erosion and/or decreased turbidity near the entrance to the Slough, and will be monitored carefully for change in future surveys.

Acknowledgements:

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Literature Cited:

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Norris, J.N. 1970. Studies on the taxonomy and distribution of some Ulvales from Elkhorn Slough, California. MA thesis, San Francisco State University, San Francisco. 102 pp.

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Zimmerman, R.C. and Caffrey, J.M. 2002. Primary producers. In: Caffrey, J., Brown, M., Tyler, W.B. and Silberstein, M. (eds.), Changes in a California Estuary. Elkhorn Slough Foundation, Moss Landing. p. 117-133.

Table 1. ANOVA for differences in biomass of *Enteromorpha* among sections based on samples from 0.25 m² quadrats.

Source	df	F-ratio	P
Section	4	1.943	0.117
Error	51		

Data by section in g DW 0.25 m⁻², SE, n:

Sec. 1: 31.84, 4.5, 19

Sec. 2: 17.01, 3.7, 9

Sec. 3: 19.38, 7.3, 10

Sec. 4: 40.14, 8.9, 10

Sec. 5: 23.30, 9.1, 8

Table 2. Biomass estimates for *Enteromorpha* in Elkhorn Slough. Calculated using estimated biomass from all quadrats pooled (110 g DW m⁻², SE 12.1) multiplied by the area of *Enteromorpha* within sections determined from aerial images.

	Area of <i>Enteromorpha</i> (m ²)	Total Biomass in Section (kg)
Sec. 1	23815	2620
Sec. 2	30275	3330
Sec. 3	22713	2498
Sec. 4	15481	1703
Sec. 5	20278	2231
	Total Biomass for Slough:	12382

Table 3. Characteristics of the Seal Bend and North Side *Zostera marina* beds. () = SE. Samples scaled to m². * no samples taken (see text).

	Seal Bend	North Side
n	12	12
# of shoots/ m²	103 (8.5)	162 (17.7)
Blade Length (cm)	46 (2.9)	*
Blade Width (cm)	1.1 (0.05)	*
Shoot Biomass (gDW)	1.31(0.21)	*
Leaf Area Index	2.0 (0.2)	*

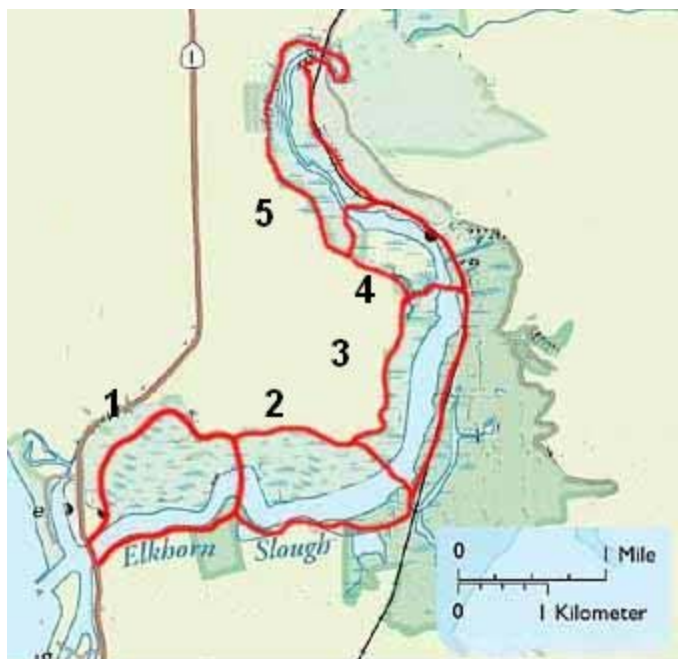


Figure 1. Sections within Elkhorn Slough used for sampling *Enteromorpha*.

Figure 2. Sections from aerial photos on July 2, 2003. *Enteromorpha* distribution in red based on MatLab processing (see Methods). Scale bar in lower right of each figure = 0.5 km.

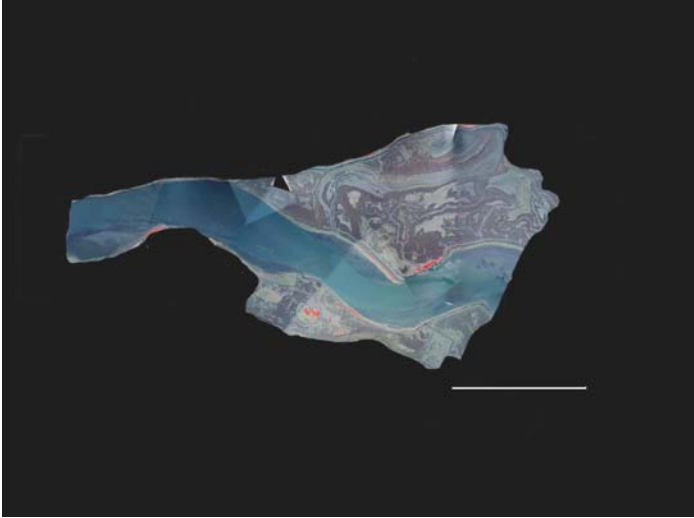


Figure 2a. Section 1

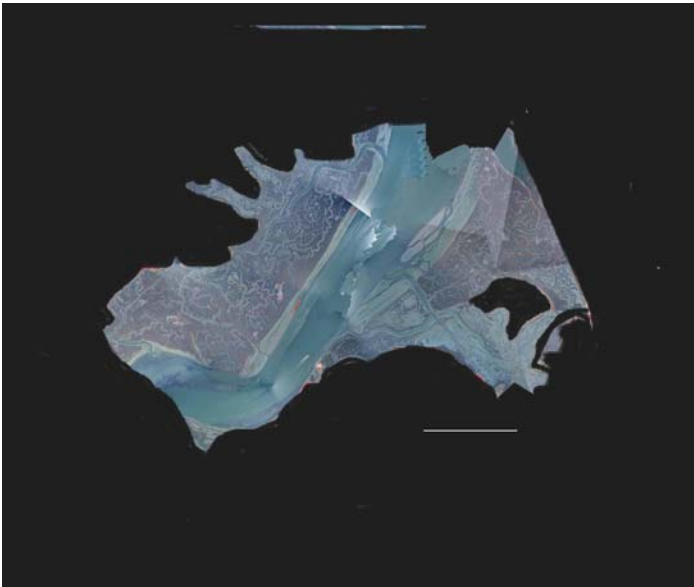


Figure 2b. Section 2



Figure 2c. Section 3

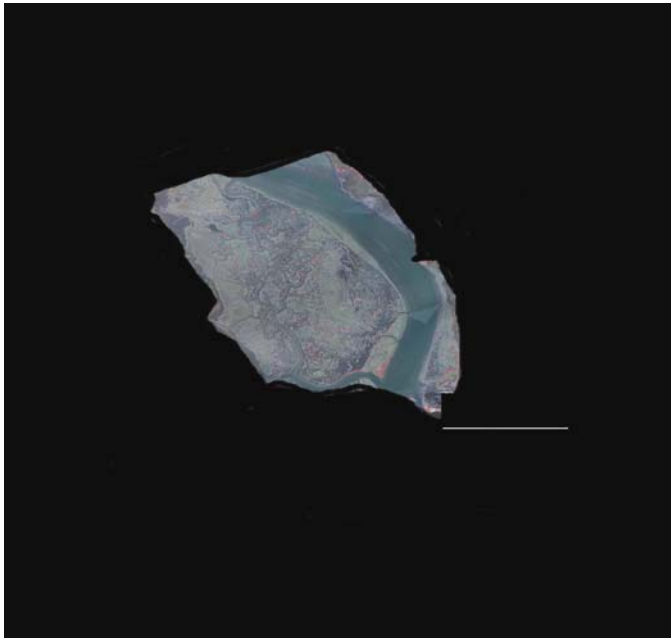


Figure 2d. Section 4

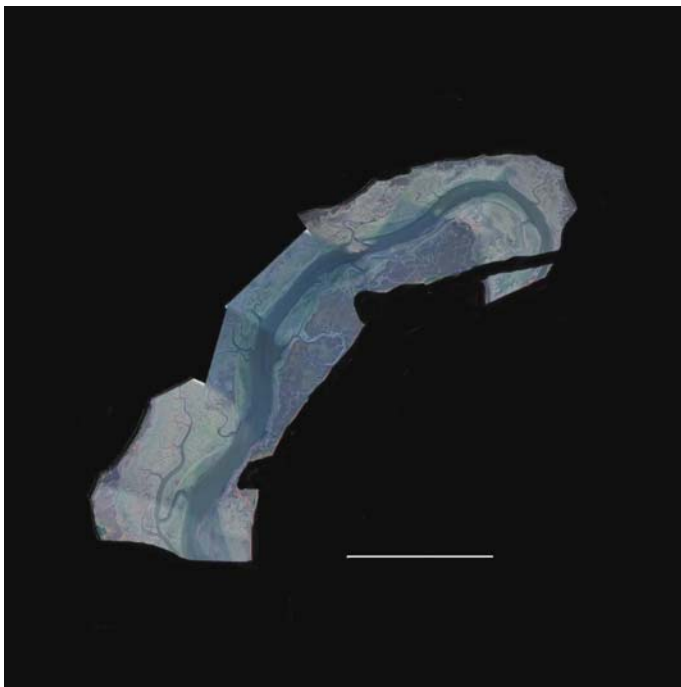


Figure 2e. Section 5

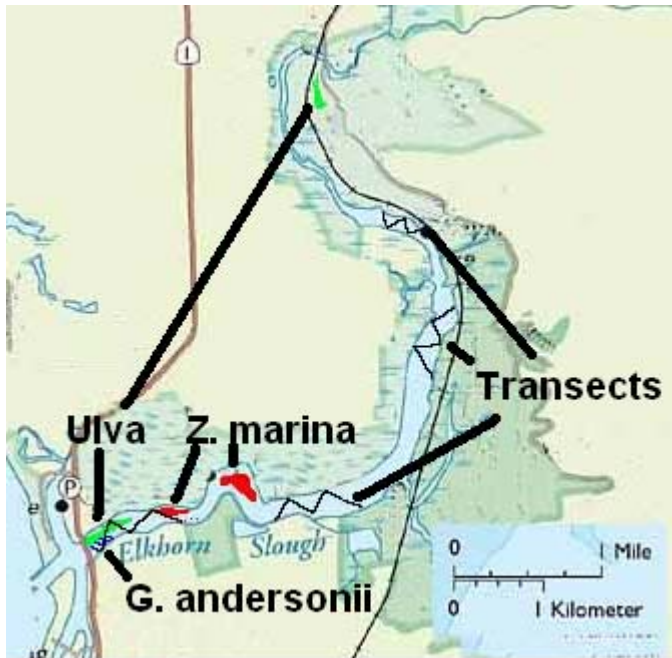


Figure 3. Macrophyte concentrations (other than *Enteromorpha*) found in July 2003. Red indicates *Zostera marina*, green indicates subtidal *Ulva* and region where open coast macrophytes found growing on rock/rubble, and blue hashed area indicates *Gracilariopsis andersonii*. Subtidal transects shown by black zig-zag lines crossing the main channel.