ADAPTATIONS OF THE SURFGRASS *PHYLLOSPADIX* TO HARD MARINE

SUBSTRATES: TESTS OF ANATOMICAL DIFFERENTIATION

AND

CARBON ISOTOPE FRACTIONATION HYPOTHESES

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By

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ABSTRACT

The study examined adaptation in the seagrass genus *Phyllospadix* to rocky substrates, habitats not generally exploited by seagrasses. One hypothesis tested whether the genus exhibits anatomical features distinguishing it from other seagrasses. A corollary predicted that individual *Phyllospadix* species show additional specialization, based on observations that three species are distinctly zoned where they occur together.

A second hypothesis tested a model of carbon assimilation that predicts that submerged aquatic plants growing on hard substrates, such as *Phyllospadix* species and most marine algae, experience less transport resistances to inorganic carbon uptake than rooted and rhizoidal plants. As a consequence, it was predicted that *Phyllospadix* species would show enzymatic discrimination against carbon-13 similar to marine algae and dissimilar to other seagrasses.

Carbon isotopic variability in *Phyllospadix serrulatus* and *Phyllospadix torreyi* was compared with that of the algae *Egregia menziesii* and *Halosaccion americanum* growing at the same location. Carbon isotopic variability in eelgrass, *Zostera marina*, was also examined to provide a basis of comparison to sediment rooted seagrasses.

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Comparison with *Z. marina* was useful in defining anatomical features in *Phyllospadix* that are adaptations to rocky littoral environments. These features include greater hypodermal fiber and roothair development, thickened rhizomes, and smaller lacunae. Comparison among *Phyllospadix* spp. for microhabitat adaptations was less fruitful.

Phyllospadix spp. show carbon isotopic discriminatory patterns distinct from *Z. marina* and marine algae. Although marine algae and *Phyllospadix* spp. overlapped isotopically, only the seagrasses became isotopically lighter with increasing intertidal height, probably through atmospheric carbon dioxide incorporation. Carbon isotope ratios in submerged seagrasses did not appear to be affected by water motion, as predicted by boundary layer considerations. An observed correlation between leaf thickness and leaf isotopic ratios also indicated complications to simple models of carbon assimilation in submerged aquatic plants.

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Chapter 1: Introduction

Phyllospadix is a seagrass genus of five species restricted to exposed rocky littoral and sublittoral environments in the northern Pacific. The five species are anomalies among the approximately fifty seagrasses because they grow on hard substrates. Den Hartog (1970) reported that both a tropical Indo-Pacific species, ***Thalassodendron ciliatum*** (Forsk.) den Hartog, and an Austral temperate species, ***Amphibolis antarctica*** (Labill.) Sonder et Aschers., will also sometimes grow on rocks, coral reefs, or hard packed clays. However, *Phyllospadix* is the only flowering plant genus that grows predominately on hard substrates in the ocean. This propensity to grow not only where there is no sediment, but also on exposed shoreline, (Ricketts, et al., 1985) and in competition with macroalgae (Turner, 1985) suggests that the plant is ecologically distinct from other seagrasses. This implies a divergence from evolutionary and ecological patterns of succession, nutrient cycling, and community stability that have been used to distinguish soft substrate communities dominated by seagrasses from those dominated by marine algae (McRoy and Lloyd, 1981). Also implicit is a prediction that *Phyllospadix* must have adapted in ways analogous to how macroalgae adapt to hard rocky habitats.

These adaptions could have an anatomical, physiological, ecological, or other biological form, but previous studies of *Phyllospadix* do not provide immediate examples. There are only a few studies specifically centered on *Phyllospadix* (Dudley, 1894; Gibbs, 1902; Miki, 1933; Drysdale and Barbour, 1975; Barbour and Radosevich, 1979; Phillips, 1979; McMillan and Phillips, 1981; Turner, 1983a, 1983b, 1985; Turner

1

and Lucas, 1985). Much of this work either does not address the habitat divergence of *Phyllospadix* from sediment rooted seagrasses or indicates that analogous ecological processes are present irrespective of substrate. For instance, Turner (1985) concluded that *P. scouleri* Hook. has a high persistence stability and responds slowly to disturbance. This is similar to successional models of climax seagrass communities growing in soft sediment (McRoy and Lloyd, 1981). In addition, while recruitment of *P. scouleri* seedlings is physically facilitated by several rocky intertidal algae (Gibbs, 1902; Turner, 1983), the rhizoidal marine green alga *Caulerpa cuppressoides* (West) C. Agardh nutritionally facilitates recruitment by Caribbean sediment based seagrasses (Williams, 1981), so comparable systems are operating in both hard and soft substrates.

I tested two predictions about *Phyllospadix* made by Phillips (1979) and Raven (1981) concerning niche differentiation and carbon assimilation, respectively. The niche differentiation prediction was the basis for an anatomical study of the differences among *Phyllospadix* species and in contrast to the sediment rooted seagrass *Zostera marina* L. The carbon assimilation prediction was the basis for a study of stable carbon isotopic variability within *Phyllospadix*. For comparison I also studied carbon isotopic variability in two marine algae growing alongside *Phyllospadix* spp. and in eelgrass, *Zostera marina* L. Both portions of the study had as a goal testing the hypothesis that *Phyllospadix* has adapted to life in rocky marine environments in ways analogous to marine macroalgae and divergent from other seagrasses.

Phillips' Prediction

Phillips (1979) observed that the three northeastern Pacific species, *P. scouleri*, *P. serrulaturs* Rupr. ex Aschers., and *P. torreyi* S. Watson, are distinctly zoned where they grow at the same location. He predicted that adaptations in the genus would permit each species to succeed in the habitat that it dominates. For example, it could be expected that leaves and other plant organs of *P. serrulatus* might be less robust than those of *P. torreyi* and *P. scouleri* because *P. serrulatus* grows highest intertidally when all three species co-occur. Between the genera *Phyllospadix* and *Zostera*, differences could be related to the different substrates or degrees of exposure the plants are adapted to. Anatomical comparisons within northeastern Pacific *Phyllospadix* and in contrast to *Z. marina* form the basis for Chapter 2.

Raven's Prediction

Raven (1981), in a discussion of nutritional strategies of submerged plants, categorized *Phyllospadix* as a haptophyte, a plant not penetrating into the substrate. Raven categorized haptophytes as plants that must obtain all of their nutrients from the bulk water phase, as opposed to the sediments or pore waters within sediments. In addition to *Phyllospadix*, this definition of haptophytes includes most benthic algae, all aquatic lichens, most aquatic mosses and liverworts, coelenterates and lamellibranchs with symbiotic microalgae, and the freshwater angiosperms in the Podostemaceae. He differentiated these plants from rhizophytes, plants that do penetrate the substrate and can obtain some of the nutrients from the sediment. In categorizing *Phyllospadix* as a haptophyte, Raven also made a prediction that the plant acquired nutrients in ways similar

to most marine algae. He suggested that a plant $\delta^{13}C$ value[1], a standard means of expressing carbon isotope ratios, could be used to measure the extent of "carbon transport resistances". He predicted that diffusion limitations through unstirred boundary layers and during active transport of bicarbonate at the plasmalemma or chloroplast envelope could influence carbon isotope ratios of aquatic plants.

Raven (1981) suggested that acquisition of carbon (C), nitrogen (N), and phosphorus (P) would be fundamentally different in haptophytes and rhizophytes. He predicted that there would be greater uptake resistances for C assimilation in rhizophytes because these plants can obtain N and P from the sediments. Thus the relative scarcity of N and P with respect to C would be diminished in aquatic rhizophytes and transport resistances during carboxylation were predicted to be higher and more apparent. Raven used carbon isotope discrimination data to evaluate these transport resistances and observed that rhizophytes often were heavier isotopically (exhibit less negative $\delta^{13}C$ values) than haptophytes.

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[1]

$$\delta^{13}C = \left[\frac{R_{sample} - R_{standard}}{R_{standard}} \right] \times 1000$$

R=Mass 45 CO_2 : Mass 44 CO_2. Standard is Peedee Belemnite from the Peedee Formation. South Carolina, given an arbitrary value of 0. A correction is made for the contribution of ^{17}O to CO_2 of mass 45 as per Craig (1957).

Published $\delta^{13}C$ values for **Phyllospadix** spp. tend to verify Raven's hypothesis. As a genus, **Phyllospadix** was reported to have the most negative mean $\delta^{13}C$ value of all 12 genera of seagrasses (McMillan, et al., 1980). Within the genus, species mean $\delta^{13}C$ values ranged from -12.4 to -16.8. By comparison, rhizophytic seagrass mean $\delta^{13}C$ values were as heavy as -4.9, -5.0, and -5.8 [**Syringodium isoetifolium** (Aschers.) Dancy, **Syringodium filiforme** Kutz., and **Enhalus acoroides** (L.f.) Royle, respectively]. Even the closely related rhizophytic eelgrass, **Z. marina** (species mean: -9.9), was heavier than all **Phyllospadix** species. Nevertheless at least a couple of other explanations are possible for the relatively light $\delta^{13}C$ range of **Phyllospadix**. Raven (1970; 1981) and Smith and Walker (1980) predicted that $\delta^{13}C$ values would be more negative in aquatic plants growing in fast-flowing water with small boundary layers around leaves. In these situations, inorganic carbon diffusion would be enhanced and carbon isotope discrimination would be closer to that expected solely from enzymatic discrimination. (The initial carboxylating enzyme in the Calvin cycle, ribulose-1,5 bisphosphate carboxylase, discriminates against ^{13}C, so all photosynthetic carbon is slightly depleted in ^{13}C relative to the universal average of 98.9% ^{12}C: 1.1% ^{13}C. For a more extensive treatment, see Smith and Epstein, 1971; Bender, 1971; and O'Leary, 1981.)

    Phyllospadix spp. can grow in exposed and high flow regimes and therefore might be expected to be isotopically light. Furthermore, **Amphibolis**, a seagrass genus that requires substantial water flow (den Hartog, 1970) was reported to have the second lightest mean $\delta^{13}C$ value (-14.2) among seagrass genera (McMillan, et al., 1980).

Phyllospadix spp. zonation observed by Phillips (1979) presents a possibility, however, of isolating isotopic effects due to water motion. Even submerged plants within an individual species are subjected to highly variable water motion, depending on whether they grow in tidal pools or surge zones. Thus it seemed useful to compare $\delta^{13}C$ values between *Phyllospadix* spp. growing in high flow regimes, for instance, surge zones, versus those plants growing in tidal pools that are very still at low tide.

Another possible explanation for more negative $\delta^{13}C$ values in *Phyllospadix* spp. is exposure to atmospheric carbon dioxide. Atmospheric carbon dioxide is 7°/oo to 10°/oo lighter isotopically than oceanic bicarbonate, depending upon temperature (Thode, et al, 1965; Deuser and Degins, 1967; Wendt, 1968; Mook, et al., 1974). Many aquatic plants can use bicarbonate (Raven, 1970; Steeman Nielsen, 1975; Beer, et al., 1977; Beer and Waisel, 1979; Millhouse and Strother, 1986). Most seagrasses are sublittoral (den Hartog, 1970), but at least one species of *Phyllospadix, P. serrulatus*, is well adapted to air exposure and grows at the highest levels in the intertidal (Phillips, 1979). This species showed the most negative mean $\delta^{13}C$ value (-15.4) of the three northeast Pacific species (McMillan, et al., 1980). Significantly, $\delta^{13}C$ values for several species of *Zostera* that can occur intertidally are also isotopically light. McMillan, et al. (1980) reported mean $\delta^{13}C$ values of -15.8 (North American *Z. japonicus* Makino), -16.8 (Asian *Z. japonicus*), -11.6 (*Z. noltii* Hornem.), and -15.8 (*Z. capensis* Setchell). This raises the possibility that *P. serrulatus* and other intertidal seagrasses may be isotopically light because they can assimilate bicarbonate when submerged and atmospheric carbon dioxide when emerged. The most obvious means of isolating this factor would be to analyze isotopic

variability over a gradient of tidal heights. An assumption has to be made that intertidal plants at different tidal heights, excluding those continually submerged in tidal pools, are subjected to the same flow velocities when submerged at high tide.

Raven's (1981) categorization of *Phyllospadix* spp. as an isotopically light haptophyte is thus complicated by potential factors of water motion and atmospheric exposure. Nevertheless these factors could be studied by testing for isotope variability with tidal elevation and with variable water motion. Examining isotope variability in marine algae over the same gradient allows testing of Raven's (1981) prediction that *Phyllospadix* spp. shows algal-like carbon isotope fractionation. Comparison of carbon isotope variability patterns between *Z. marina* and *Phyllospadix* spp. should reveal the extent that haptophytic *Phyllospadix* spp. differ from rhizophytic *Z. marina* in carbon isotope assimilation. These comparisons are covered in Chapter 3. This chapter is a study of carbon isotope variability in *P. serrulatus* and *P. torreyi* as well as two marine algae, *Egregia menziesii* (Turn.) Aresch. and *Halosaccion americanum* I.K. Lee. Also included is a study of isotopic variability in *Z. marina,* including the appraisal of an assumption that is implicit in Raven's treatment of submerged plant carbon isotope ratios.

Raven's comparison of rhizophyte versus haptophyte $\delta^{13}C$ values was based solely on leaves. Very little information is available on fractionation within plant organs other than leaves (O'Leary, 1981). Since seagrass rhizone biomass can be as great as twice leaf biomass (Sand-Jensen, 1975) differential fractionation in rhizome tissue could affect interpretations of how C is fractionated within the plant. *Z. marina* is better suited to examination of these two assumptions than *Phyllospadix* spp. because the congested

and invasive nature of *Phyllospadix* roots and rhizomes make separation of plant organs difficult.

Summary

The hypothesis that *Phyllospadix* spp. have developed specific adaptions to life in rocky shore habitats is examined by standpoints of anatomy and carbon isotope fractionation. Hypotheses involving the influence of isotopic discrimination factors external to Raven's suggestion of haptophytism are also tested to isolate those influences. These factors are leaf thickness, air exposure, and water motion. Comparative carbon isotope variability studies are also undertaken with marine algae and *Z. marina* to assess the extent to which carbon isotopic variability in *Phyllospadix* spp. is analogous or divergent to these other marine plants.

Chapter 2: Anatomical Adaptions to Rocky Substrates by *Phyllospadix* spp.

Introduction

Phyllospadix is the only flowering plant genus that grows predominately on hard substrates in the ocean (Chapter 1). This propensity to grow where there is no sediment, on exposed shoreline, (Ricketts, et al., 1985) and in competition with macroalgae (Turner, 1985) has led to a prediction (Phillips, 1979) concerning the mechanisms of adaption.

Phillips (1979) observed that the three northeastern Pacific species, *P. scouleri* Hook.; *P. serrulatus* Rupr. ex Aschers., and *P. torreyi* S. Watson, are distinctly zoned where they grow at the same location. He predicted that adaptive properties among the species would permit each to succeed in the zone that it dominates. Turner and Lucas (1985) tested for such differences in successional processes among the three species, but concluded that modes and final stages of succession were similar in all three species.

Based on anatomical and morphological evidence, Littler and Littler (1980) have predicted general relationships between form and function in marine algae. If this prediction can be extended to seagrasses growing at the same location, it follows that anatomical and morphological distinctions among co-occurring species should be related to the zones each dominates. Between *Phyllospadix* and the genus *Zostera*, which is rooted in soft sediments, differences could be related to the different substrates or degrees of exposure the plants are adapted to. Thus two separate, but related questions were addressed in this study: Are there anatomical differences between the genera

Phyllospadix and *Zostera* that can be related to the habitat of *Phyllospadix*? And if so, are there anatomical differences among species of *Phyllospadix* growing at the same location that can be related to the individual zones where a particular species is dominant? In addition to these comparisons among leaf, rhizome, and root anatomies, intra-specific changes in leaf anatomy over intertidal gradients were also assessed.

The anatomy of leaves, rhizomes, and roots of *Phyllospadix* spp. have been described on a number of occasions (Sauvageau, 1890; Chrysler, 1907; Miki, 1933; Tomlinson, 1980; 1982). In most cases, no attempt was made to relate descriptive observations to the ecologically distinct environments that the genus occupies. As with most aquatic vascular plants (Arber, 1920; Sculthorpe, 1967), *Phyllospadix* spp. are characterized by a reduction or elimination of lignified xylem elements, cuticle, stomata, and simplification of internal anatomy.

Materials and Methods

Leaves, roots, and rhizomes of *P. serrulatus* and *P. torreyi* were collected at North Cove, Cape Arago, Oregon (43°20'N; 124°22'W) on 28-29 August 1984 along an intertidal transect extending from -0.40 m to +0.94 m, mean lower low water (MLLW). Linear distance between samples was 4 m. *P. scouleri* were also collected from the South Cove of Cape Arago, where it grows in scattered tidal pools. *P. serrulatus* at Sandy Beach, Sitka, Alaska (57°03'N; 135°14'W) between approximately -0.88 m to +0.88 m MLLW were sampled on 11-12 August 1983. Tidal heights were estimated by interpolating tide tables (U.S. Department of Commerce, 1983; 1984) for several days of observations of water levels.

All plant samples were fixed in 5% glutaraldehyde, 0.1M cacodylate buffer with 3% sucrose for 24 hours, then rinsed three times over an hour and stored in a 0.1M cacodylate buffer with 22% sucrose at 2°C. Samples were sectioned and then dehydrated using a progressively stronger ethanol series follwed by processing in a Bomar critical point dryer. After mounting, samples were sputter coated with gold-palladium and examined using a JEOL Model JSM-35 Scanning Electron Microscope. This is the standard method used for marine specimens at the Electron Microscope Facility, University of Alaska - Fairbanks, M. Borchert, per. comm.).

In general, three leaves were collected at each sampling point along the intertidal gradients at Cape Arago and Sitka. The oldest leaf on a shoot at each sampled tidal height was sectioned 1 cm from the meristem to provide consistent samples. Leaf thicknesses were determined for all leaves collected and outer epidermal cell wall thicknesses were determined for most of these leaves.

Results

Morphometric determinations

P. torreyi and *P. serrulatus* leaf thicknesses tended to decrease as tidal height increased (Figures 1 and 2). Both parametric (Pearson product-moment coefficient) and non-parametric tests (Spearman's rho and Kendall's tau) indicated a significant relationship (Table 1). Epidermal cell wall thicknesses also showed a significant tendency to decline with increasing tidal height, but only at Cape Arago (Figure 3 and Table 1). At Sitka, no significant relationship was observed (Figure 4 and Table 1).

P. serrulatus mean leaf thicknesses showed a wider range at Sitka (0.0836 to
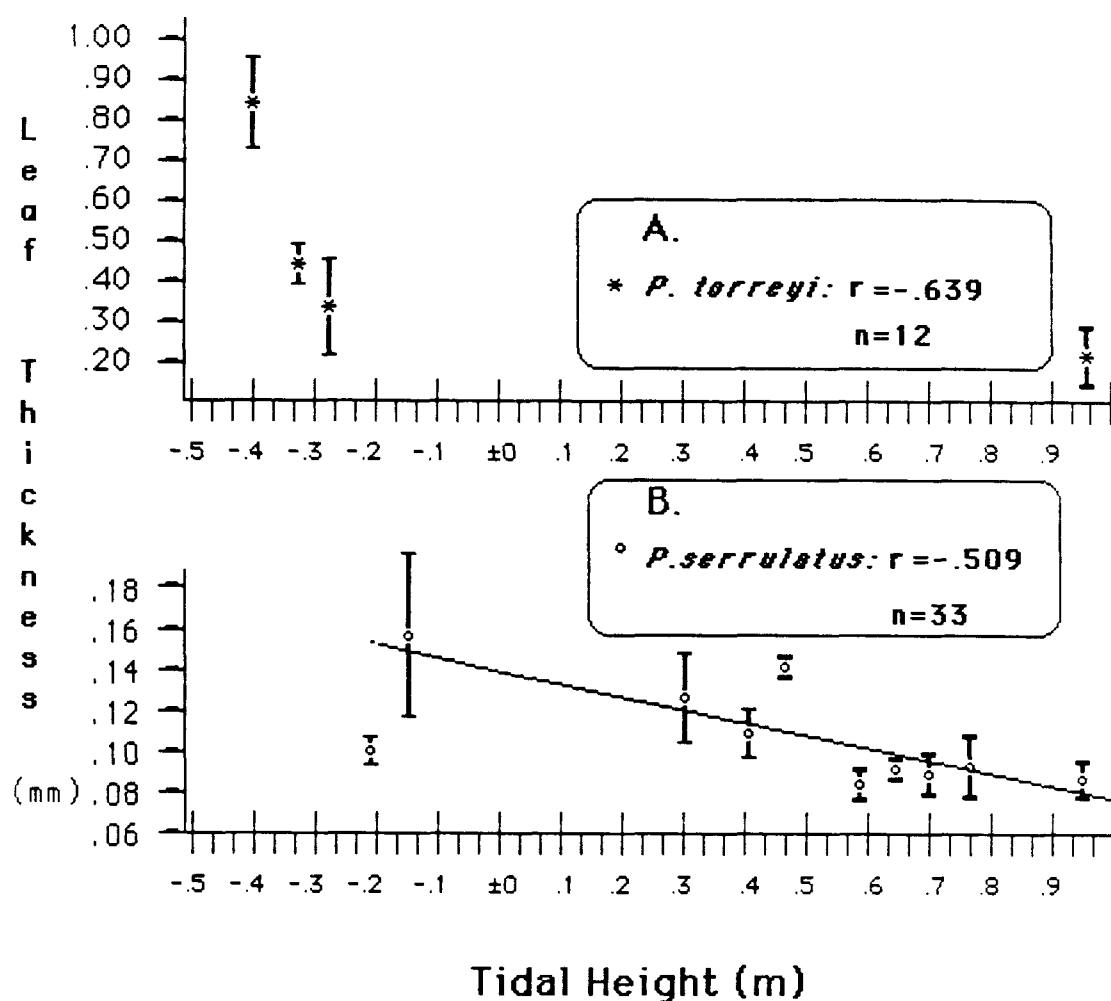
Figure 1.

Leaf thicknesses (mean ± SD) of *Phyllospadix torreyi* (A.) and *Phyllospadix serrulatus* (B.) in relation to tidal height at Cape Arago, Oregon (1984).
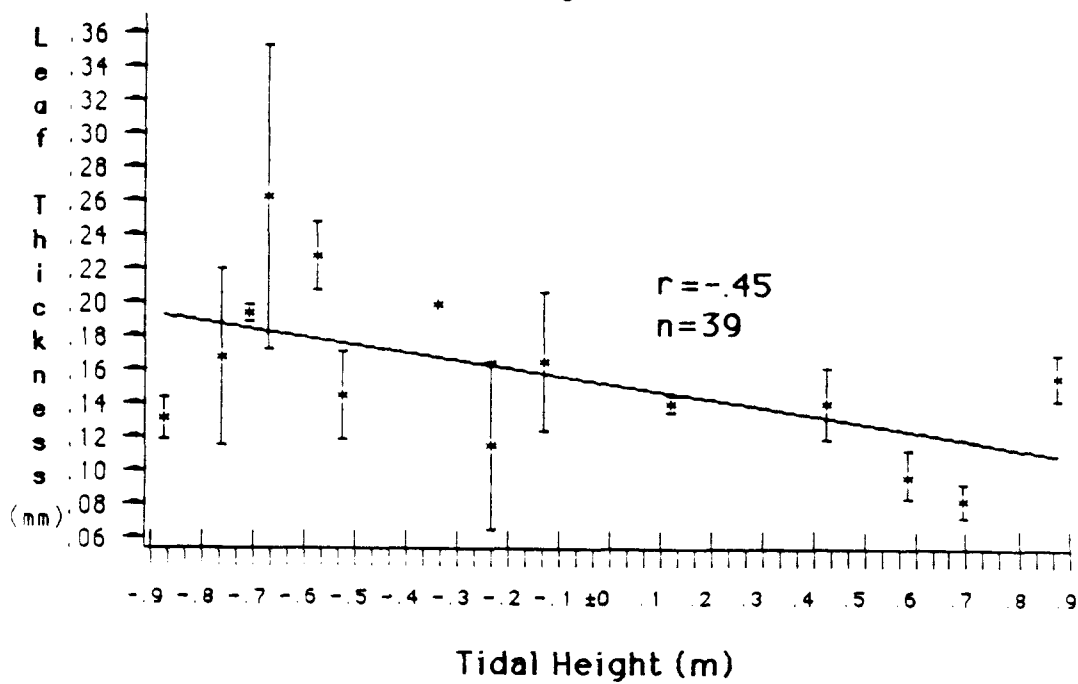
Figure 2.
Leaf thicknesses (mean ± SD) of *Phyllospadix serrulatus* in relation to tidal height at Sitka, Alaska (1983).
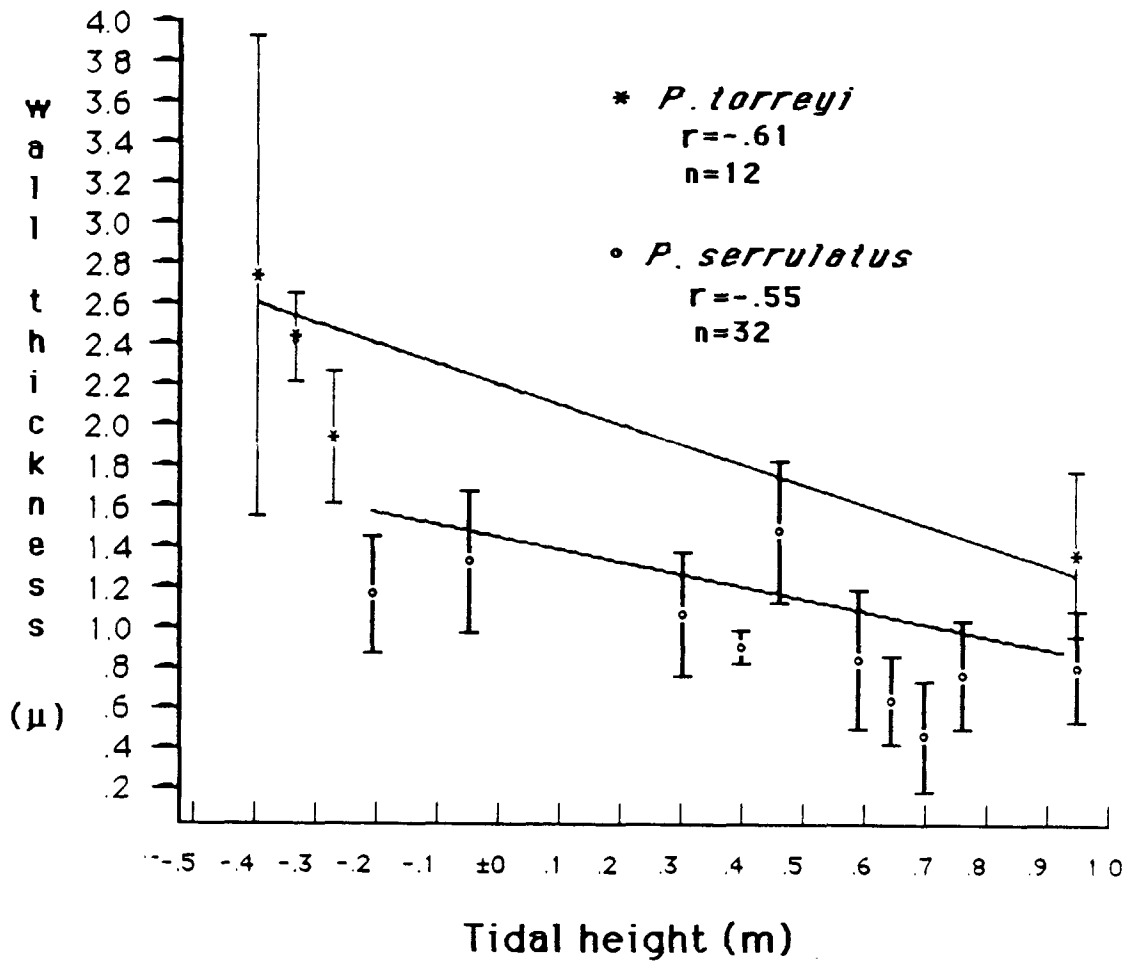
Figure 3.

Outer epidermal wall thicknesses (mean ± SD) of *Phyllospadix torreyi* and *Phyllospadix serrulatus* at Cape Arago, Oregon (1984).
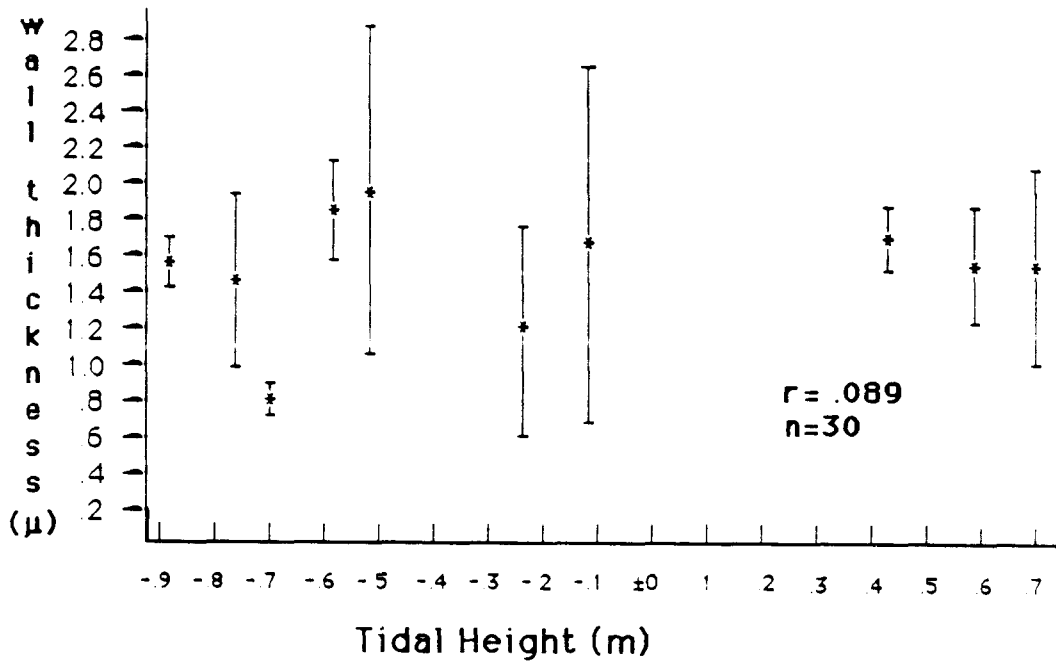
Figure 4.

Outer epidermal cell wall thicknesses (mean ± SD)
of *Phyllospadix serrulatus* in relation to tidal
height at Sitka, Alaska (1983).

Table 1. Correlation analyses

Test	r	p	p (non-parametric)	n	site
P. torreyi leaf thickness vs. elevation	-0.639	0.0005< p<0.005	p<0.001	15	Cape Arago
P. serrulatus leaf thickness vs. elevation	-0.509	0.0005< p<0.005	p<0.001	30	Cape Arago
P. serrulatus leaf thickness vs. elevation	-0.45	0.0005< p<0.005	0.025<p <0.01	39	Sitka
P. torreyi cell wall thickness vs. elevation	-0.61	0.025<p <0.01	0.005<p <0.001	12	Cape Arago
P. serrulatus cell wall thickness vs. elev.	-0.55	0.0005< p<0.005	p<0.001	32	Cape Arago
P. serrulatus cell wall thickness vs. elev.	0.089	p>0.25	p>0.25	30	Sitka

0.2635 mm) than at Cape Arago (0.0896 to 0.1577 mm); (Figures 1B and 2). *P. torreyi*, which occupies the lower intertidal and subtidal zone at Cape Arago, but is not present at Sitka, showed a mean leaf thickness range of 0.2094 to 0.8467 mm, nearly an order of magnitude thicker than *P. serrulatus* plants growing immediately higher intertidally (Figure 1). The mean epidermal wall thickness of *P. torreyi* at Cape Arago ranged from 1.36 to 2.72 μ (Figure 4).

Leaf sections of *P. torreyi* were characterized by thicker epidermal walls than *P. serrulatus* (Figures 3, 4). (*P. scouleri* epidermal wall width was 4.55μ; n=1). *P. scouleri* and *P. torreyi* are distinguished by the presence of three vascular bundles in leaf cross-section (Figures 5, 6). Leaves of *P. torreyi* are considerably thicker and narrower than either of the other two species and the species is most readily recognized in the field. *P. serrulatus* has the widest leaves and is characterized by either 5 or 7 vascular bundles (Figure 7).

Other anatomical structures

P. serrulatus has a thin cuticle (Figure 8). Hypodermal fibers were present in all species, grouped below the leaf surface, around vascular bundles, and particularly concentrated on the lateral edges of the leaf (Figures 9, 10). Numbers of fibers per bundle and frequency of appearance appeared to be relatively constant in all three species at all tidal heights. Roots and rhizomes of all three *Phyllospadix* spp. showed extensive root-hair development (Figure 11). For comparison, root cross-sections of *Z. marina* did not show this extensive development (Figure 12). Outer epidermal walls of roots and rhizomes of the three *Phyllospadix* species were all thicker than *Z. marina*. Rhizomes and roots of *P. torreyi* and *P. scouleri* are more firmly attached to the substrate than *P. serrulatus* (per. obs.).

Perforated plates at the base of lacunae were observed in one cross-section of *P. serrulatus* (Figures 13 and 14). Lacunal air spaces in this genus are not as extensive as in *Z. marina.*
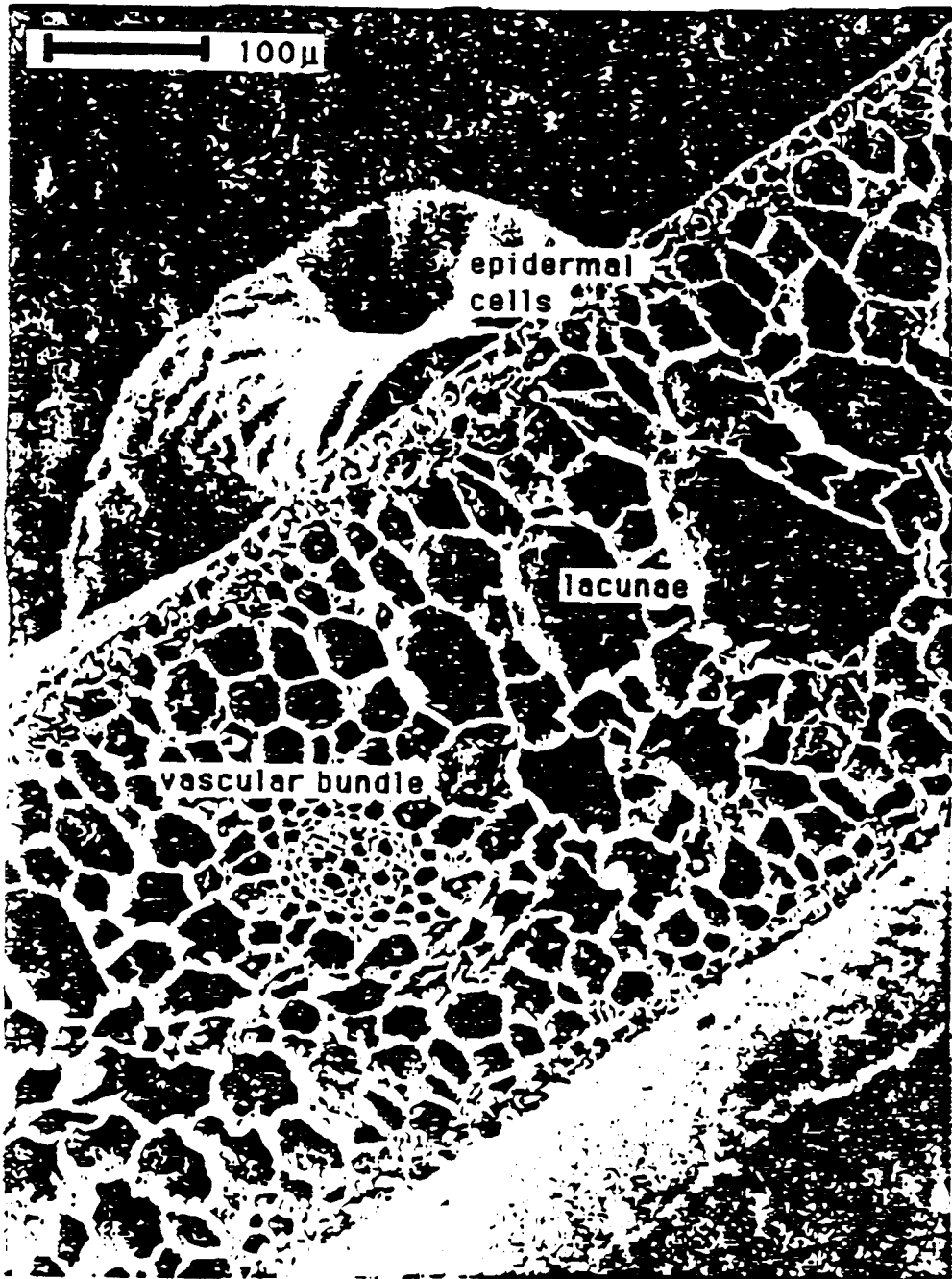
Figure 5. *P. scouleri*, leaf cross-section.
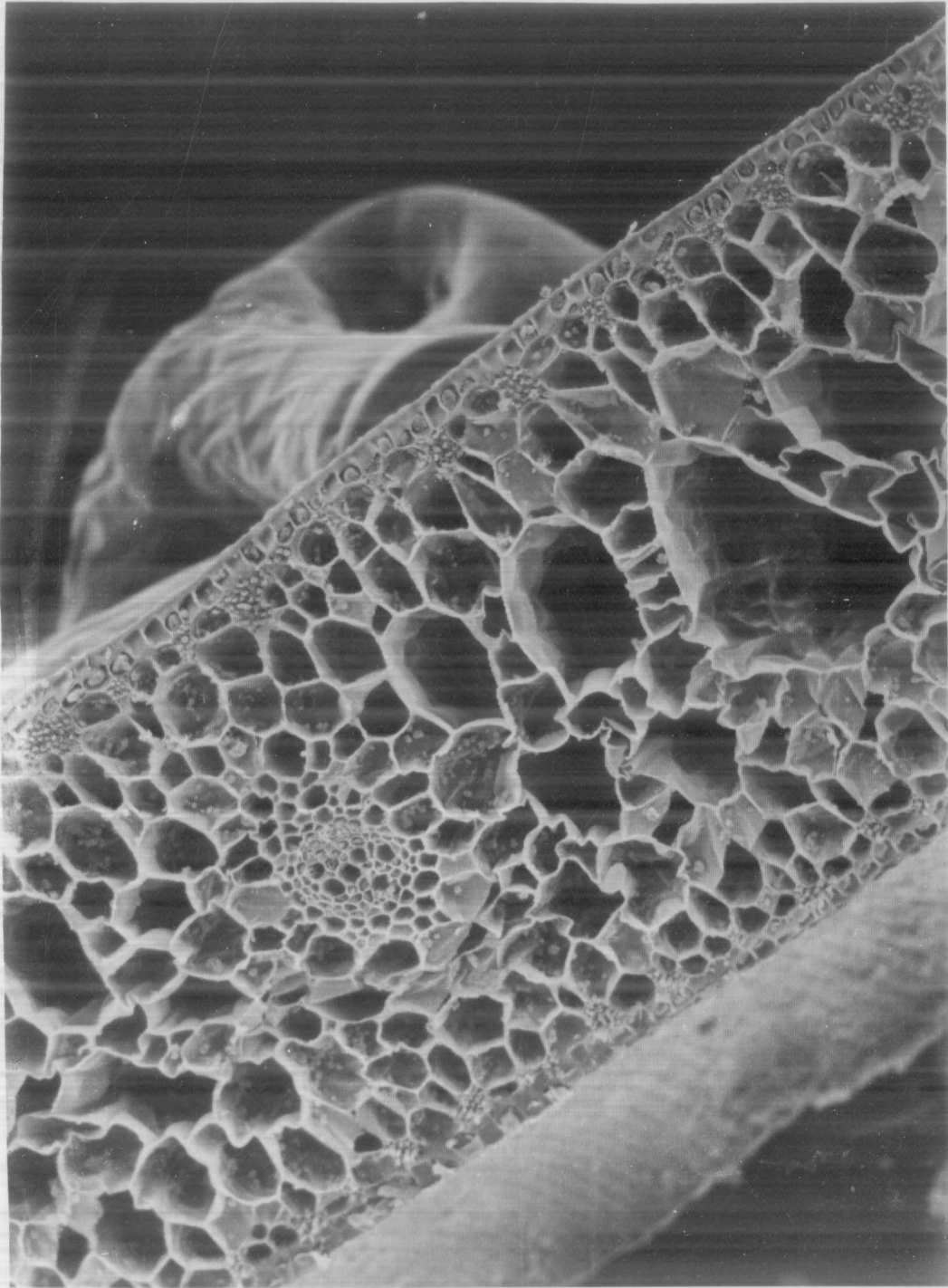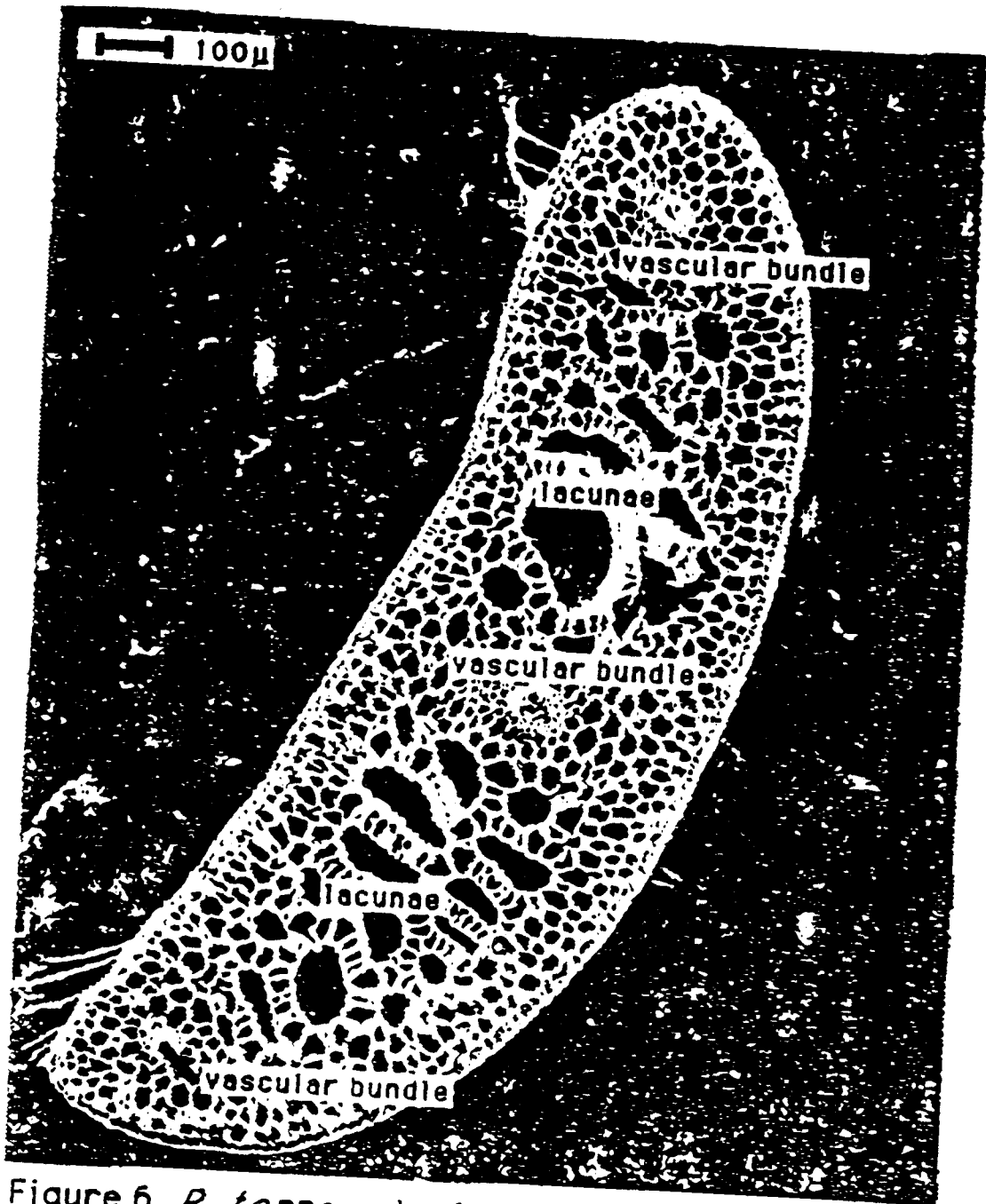
Figure 6. *P. torreyi* leaf cross-section.
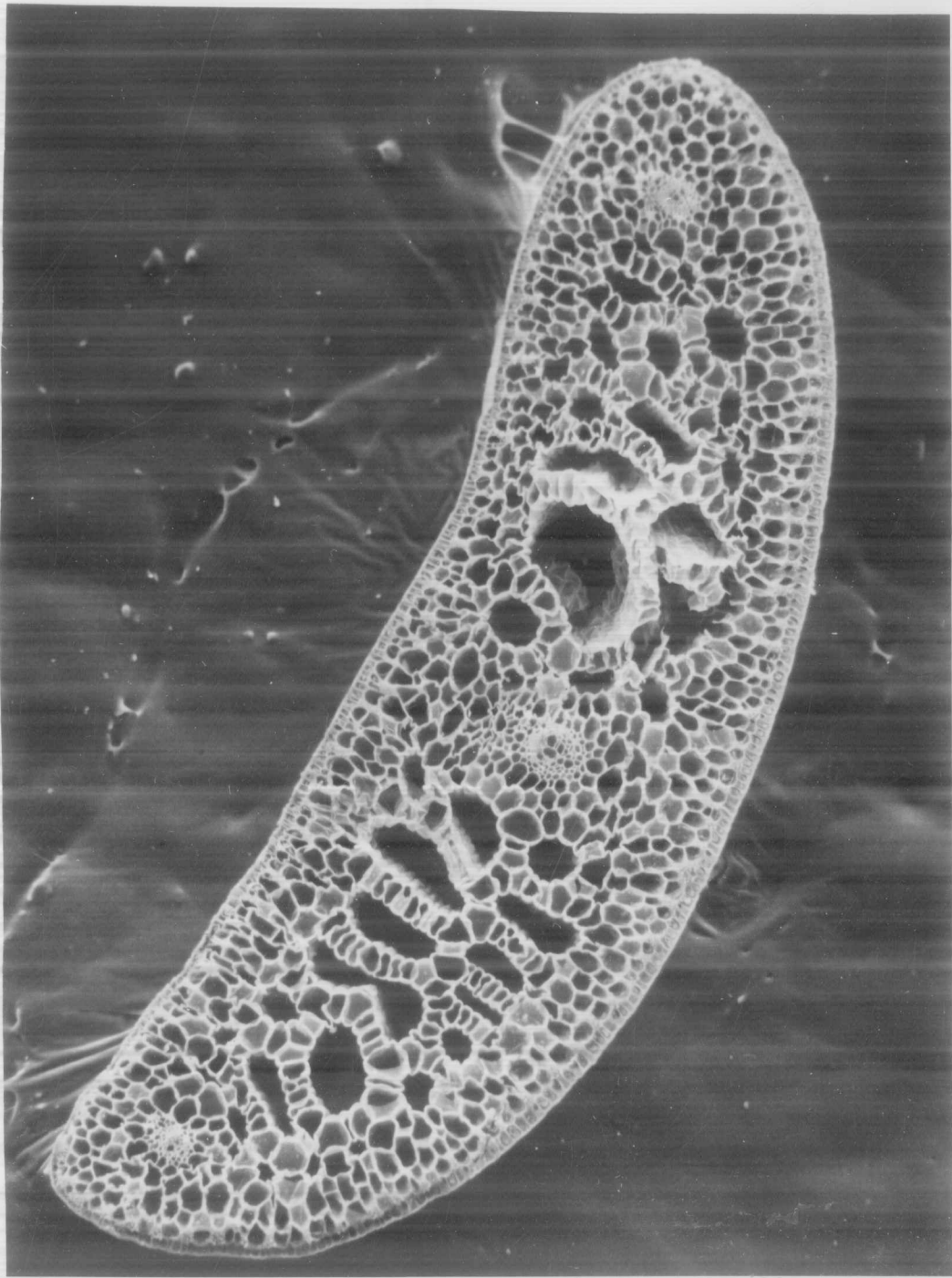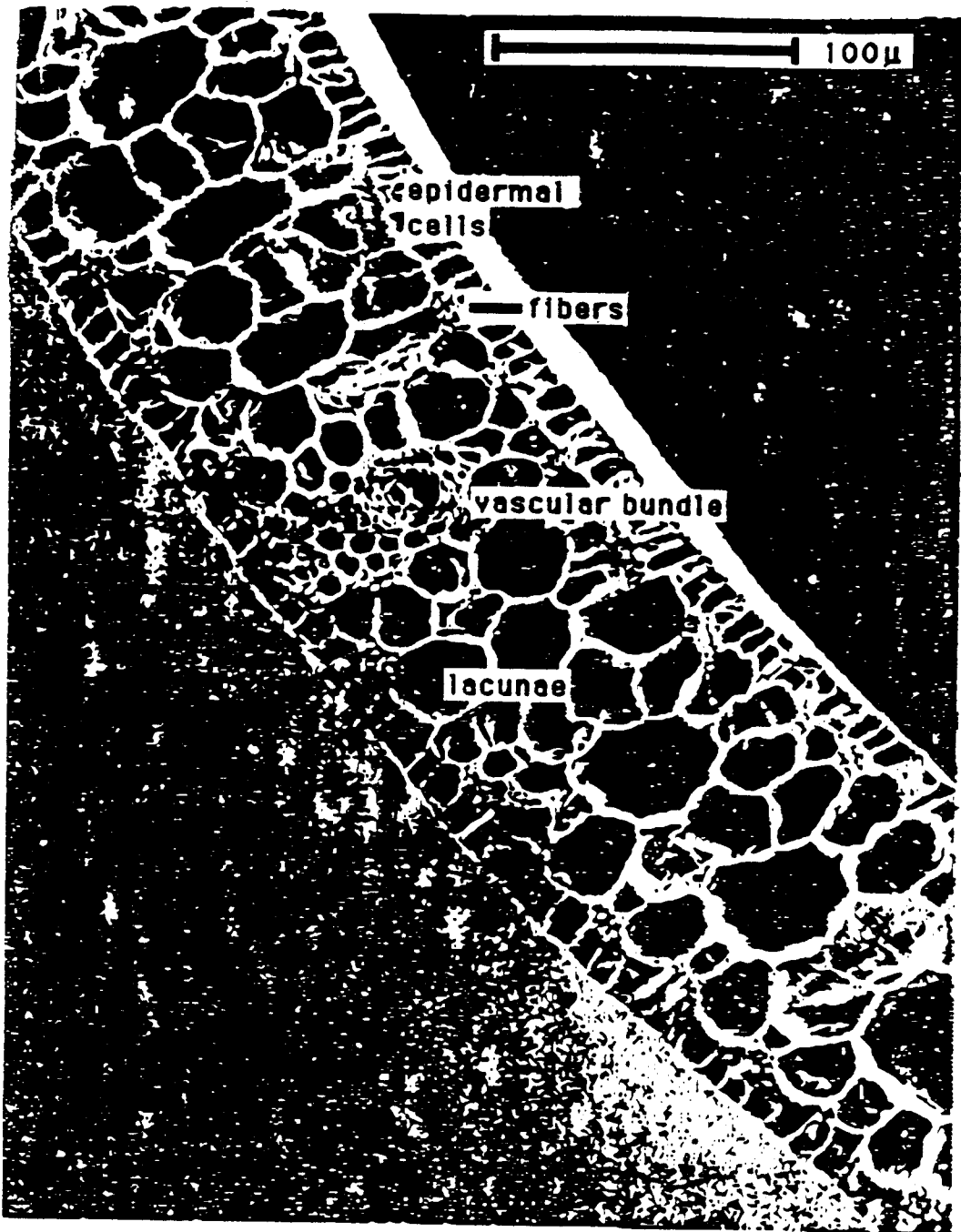
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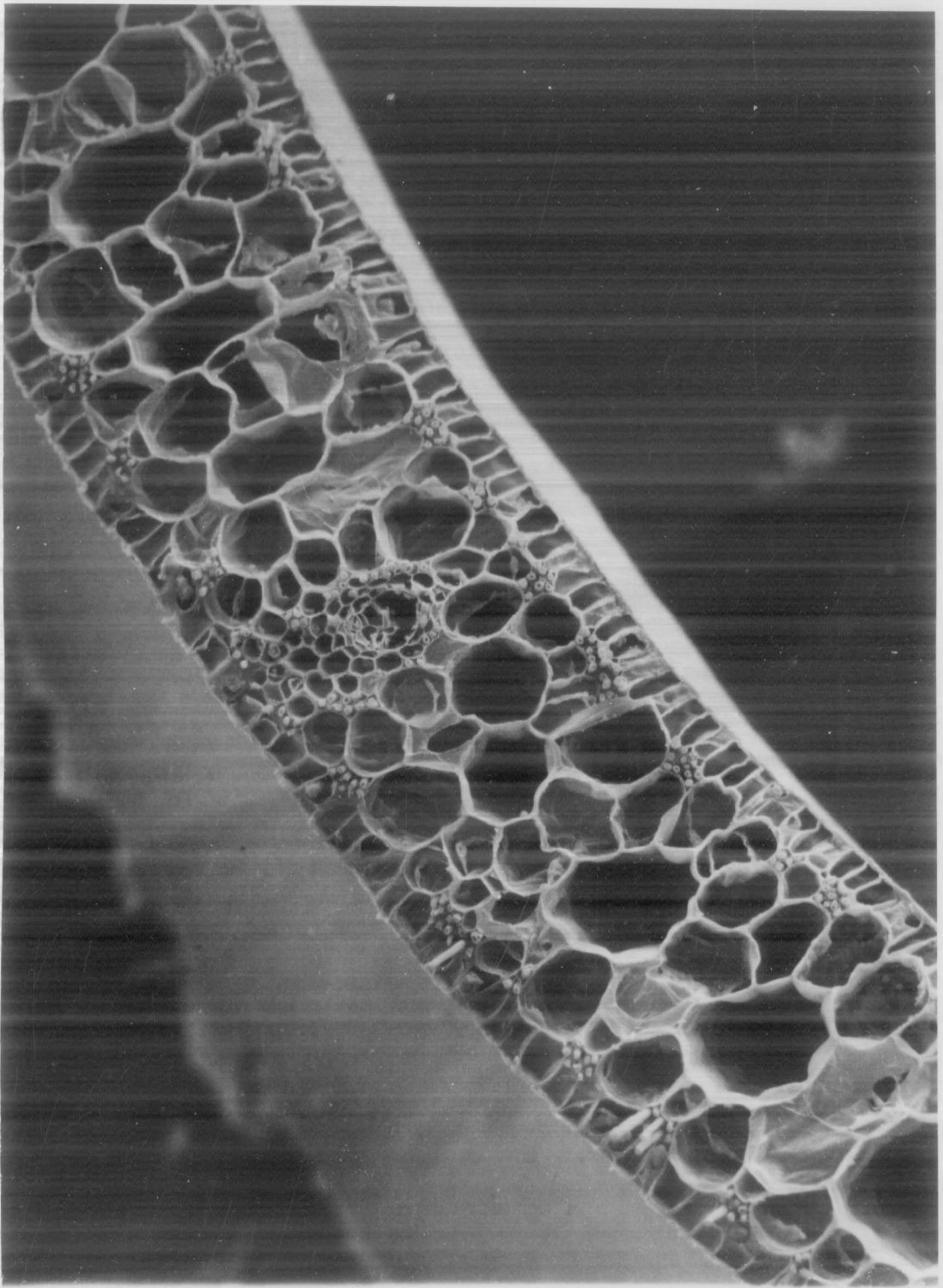
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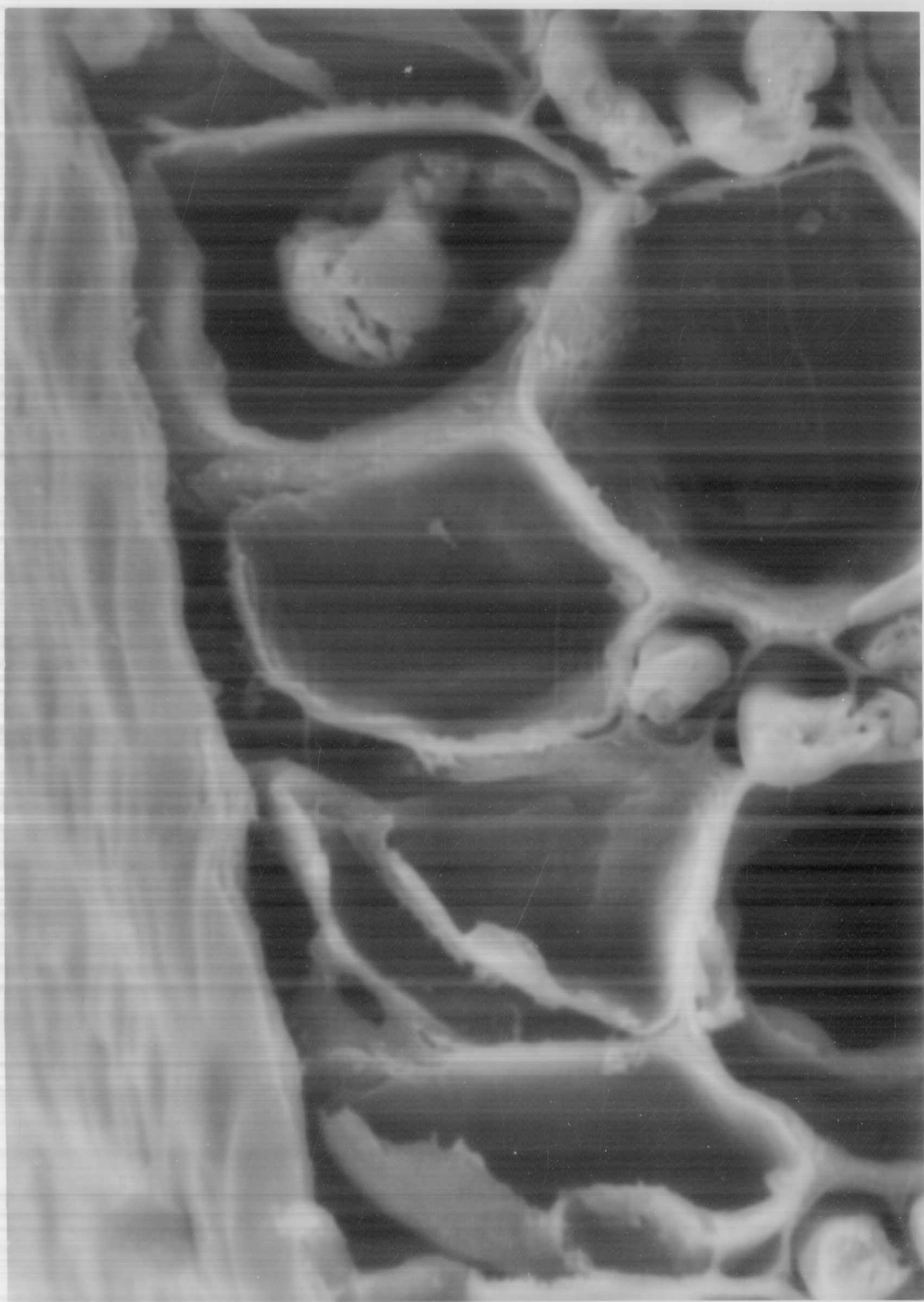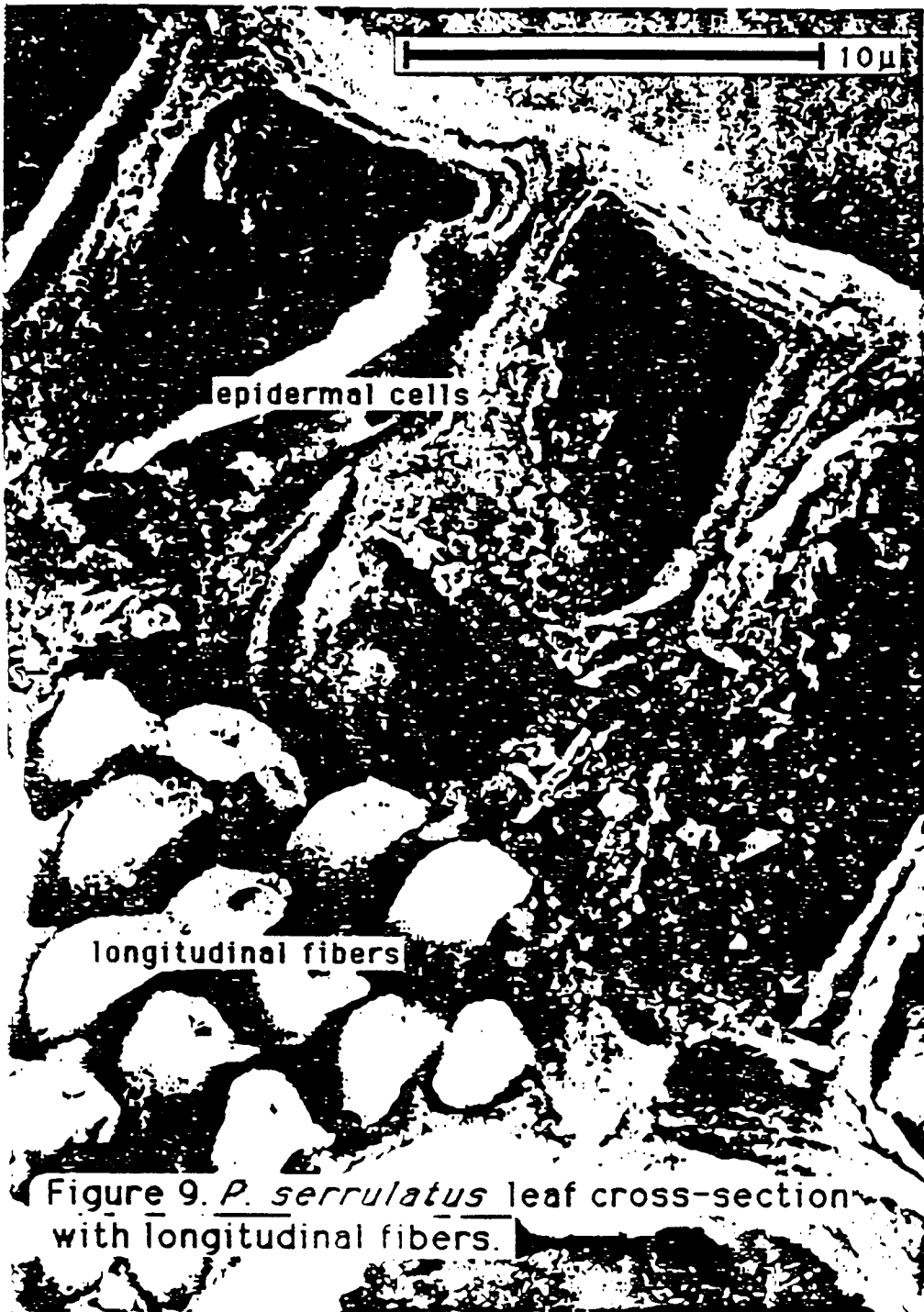
Figure 9. *P. serrulatus* leaf cross-section with longitudinal fibers.
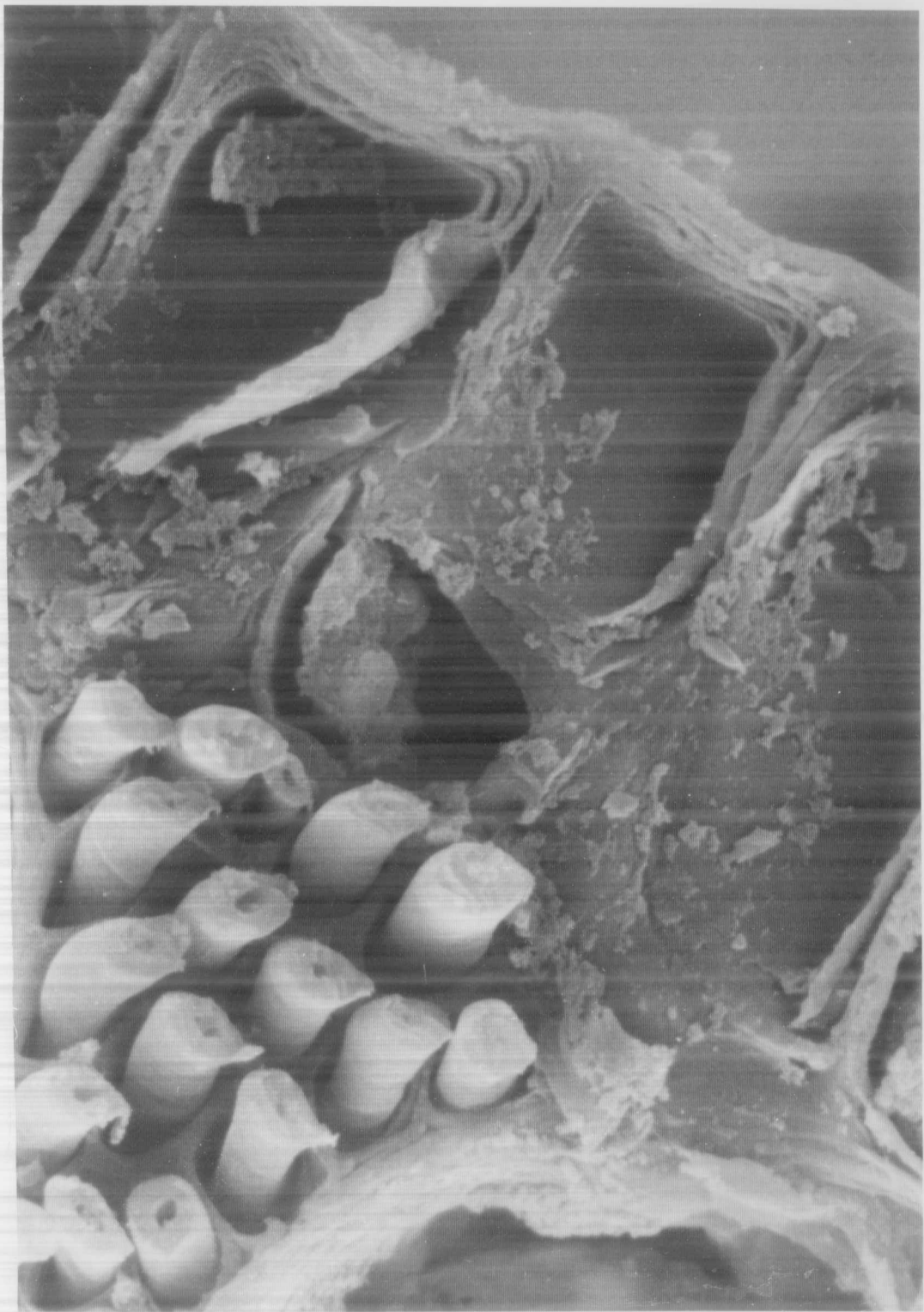
Figure 10. *P. serrulatus*, hypodermal fibers, longitudinal view
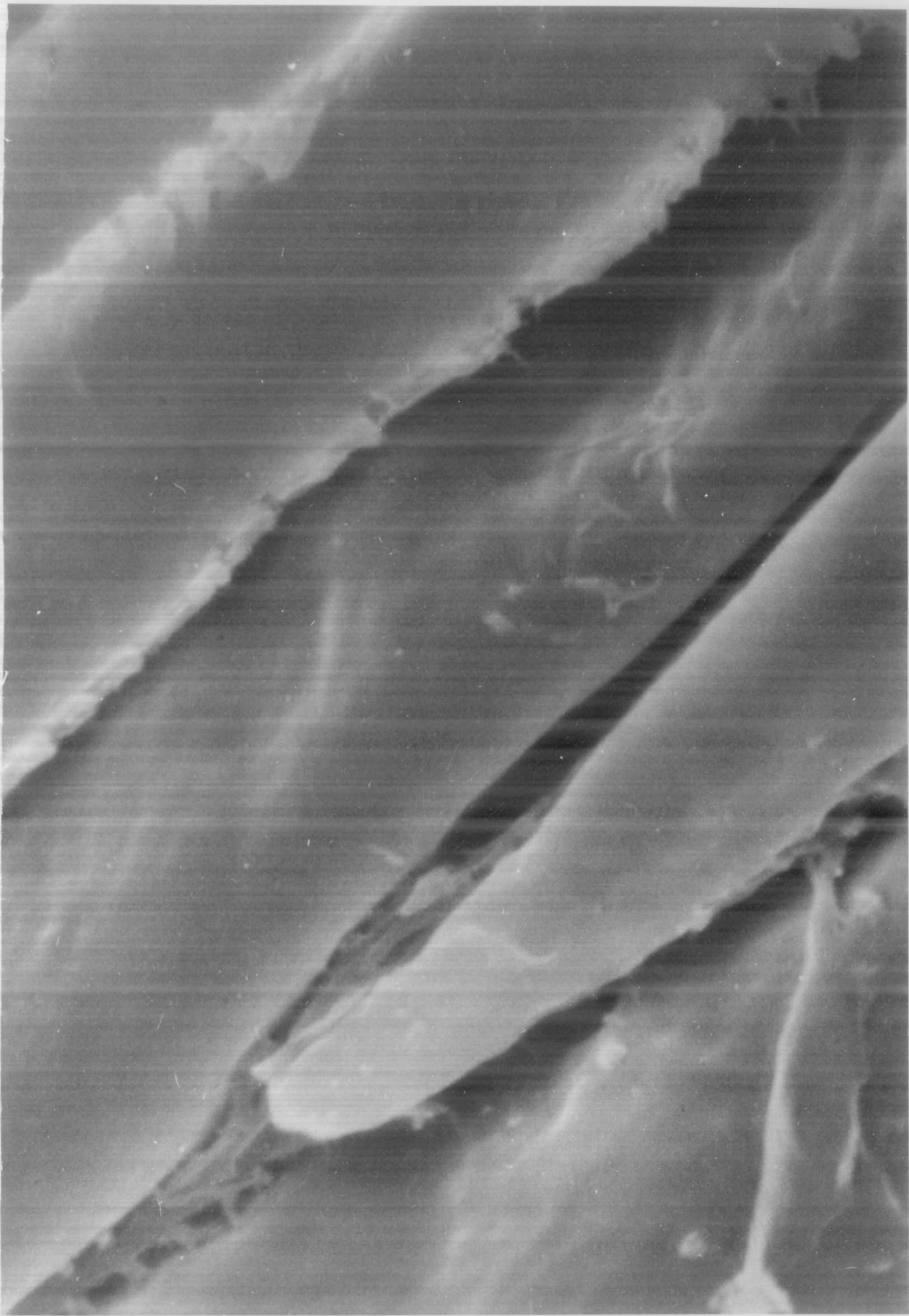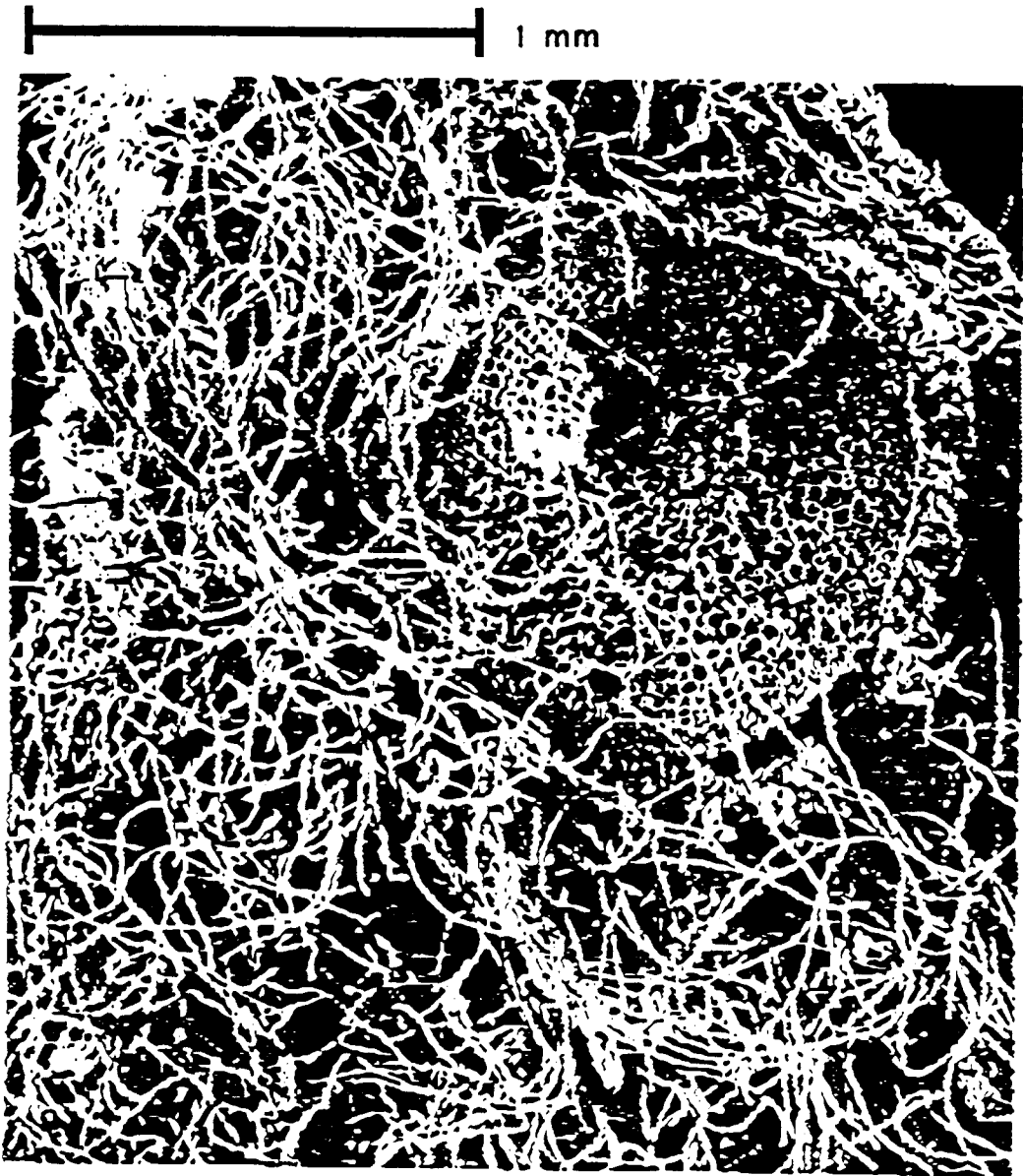
Figure 11. *P. serrulatus* root and roothairs
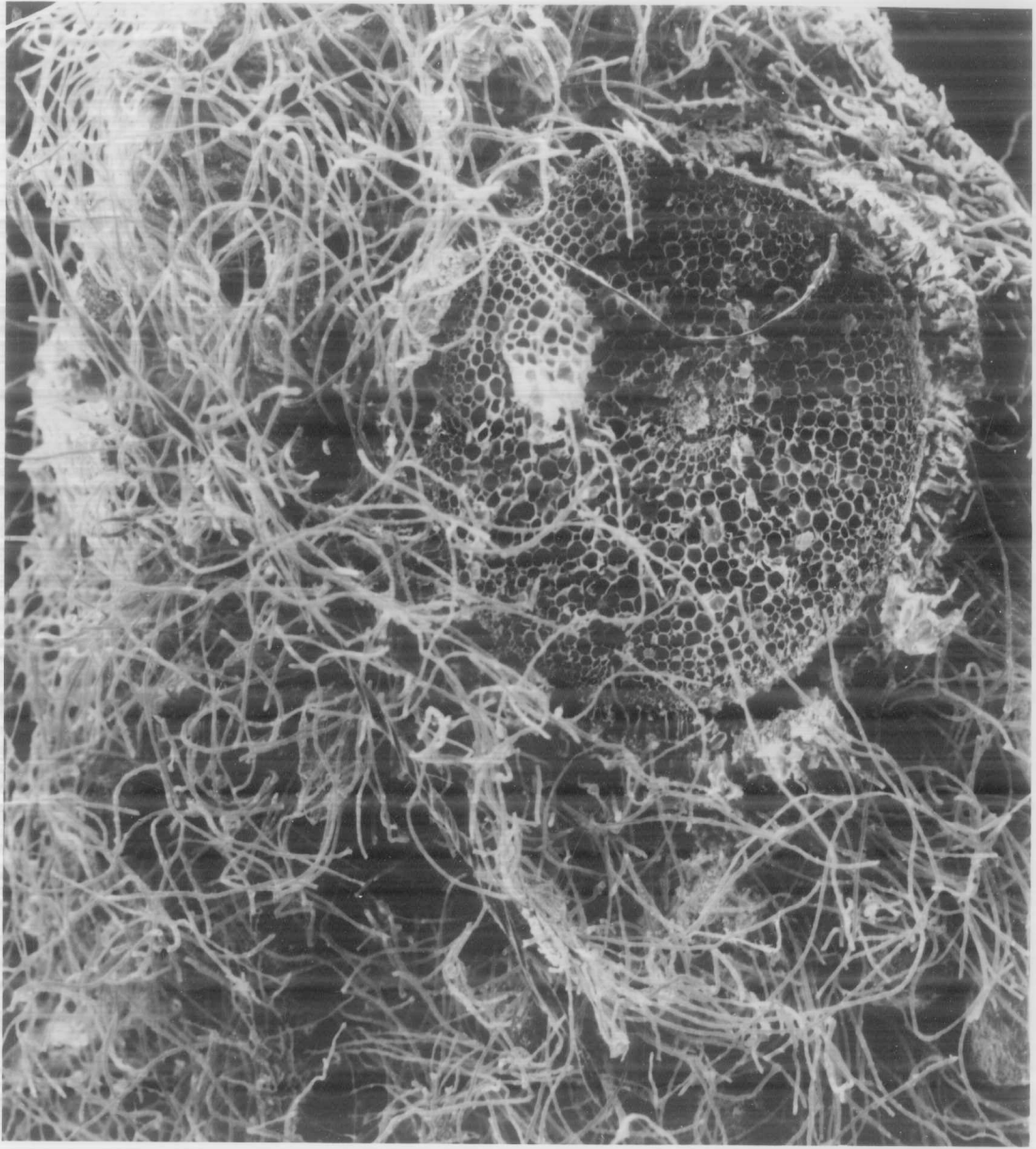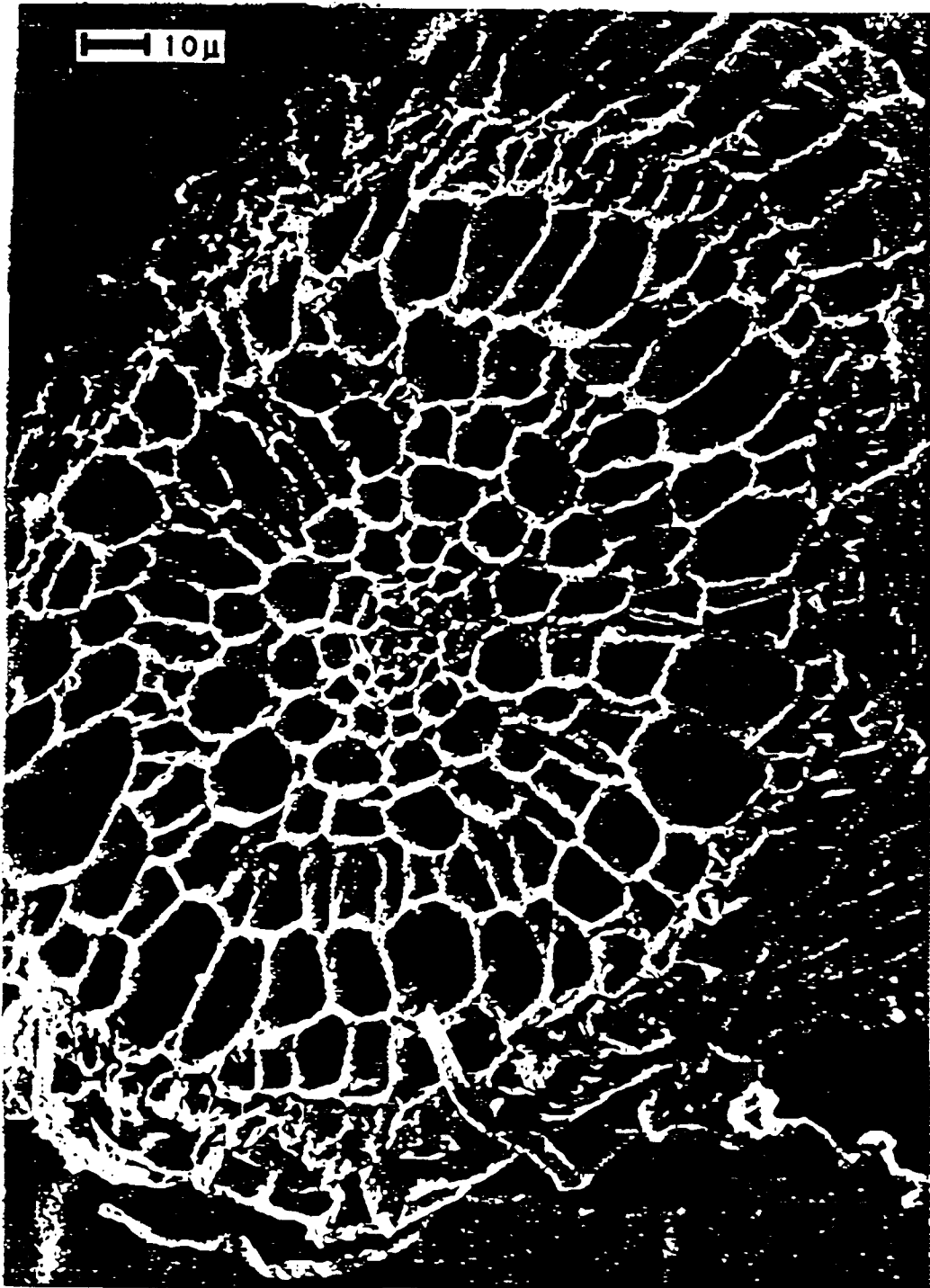
Figure 12. *Z. marina* root and roothairs.
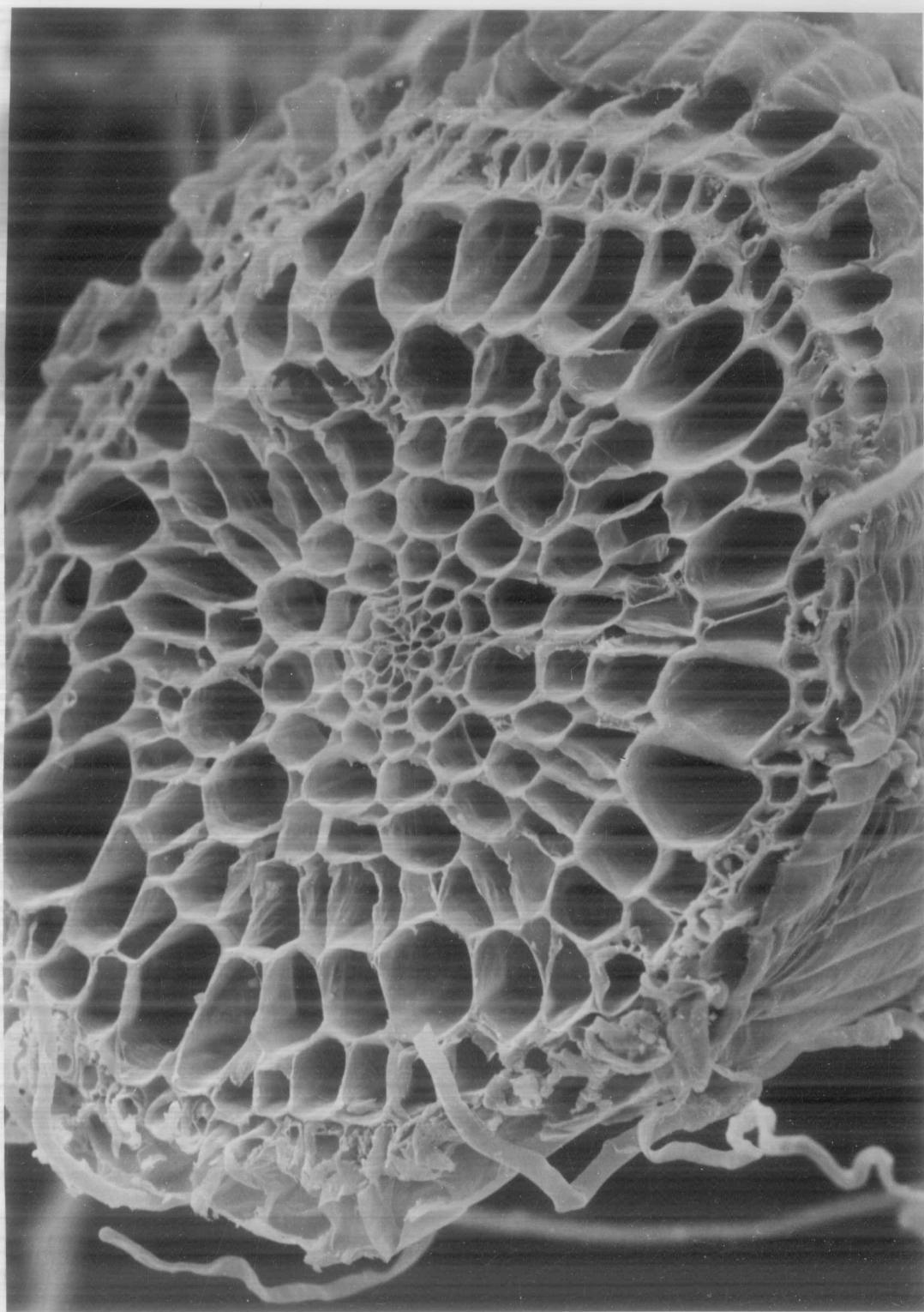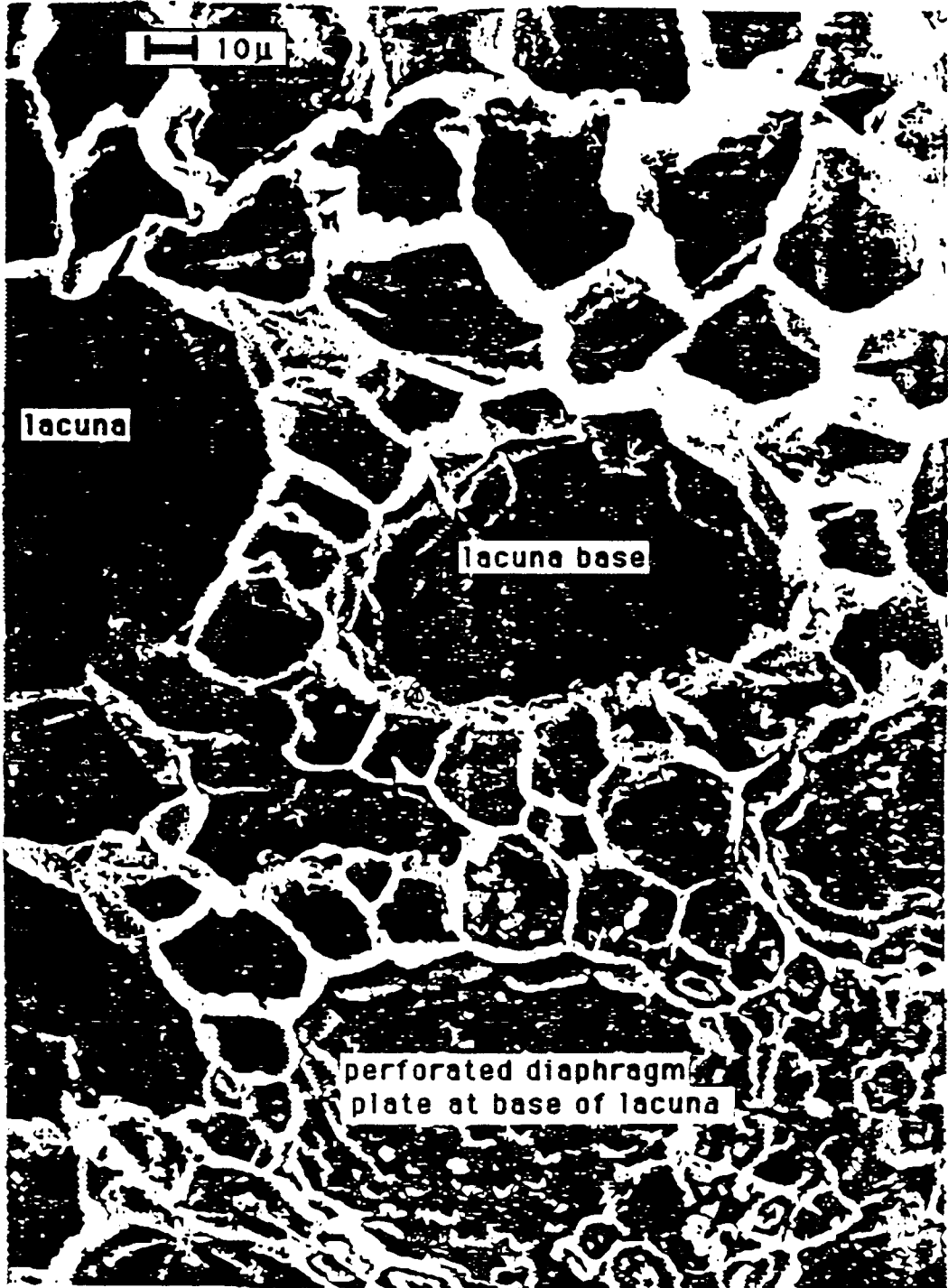
Figure 13. *P. serrulatus*, perforated lacunal diaphragms.
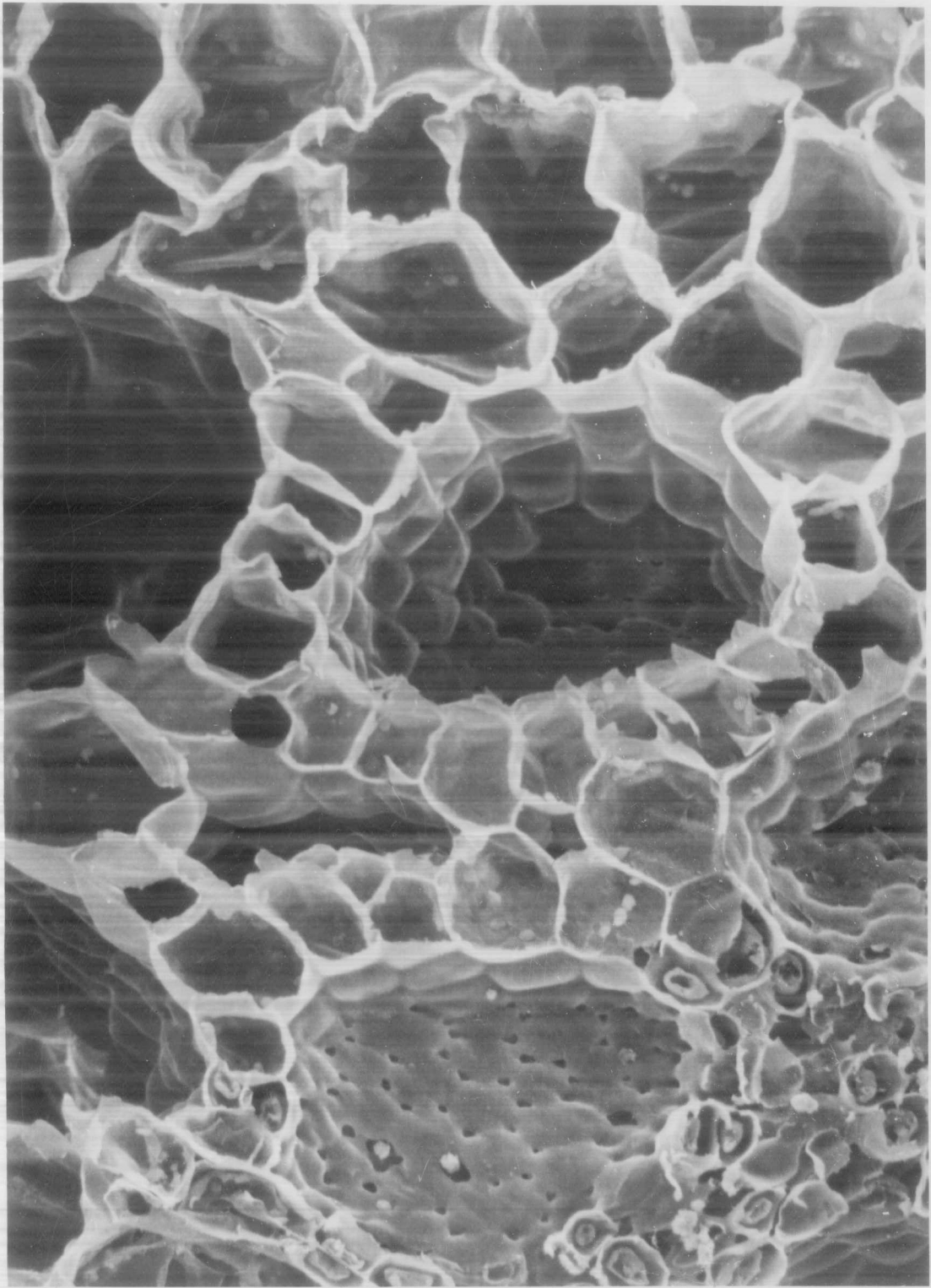
Figure 14. *P. serrulatus*, perforated lacunal diaphragms.
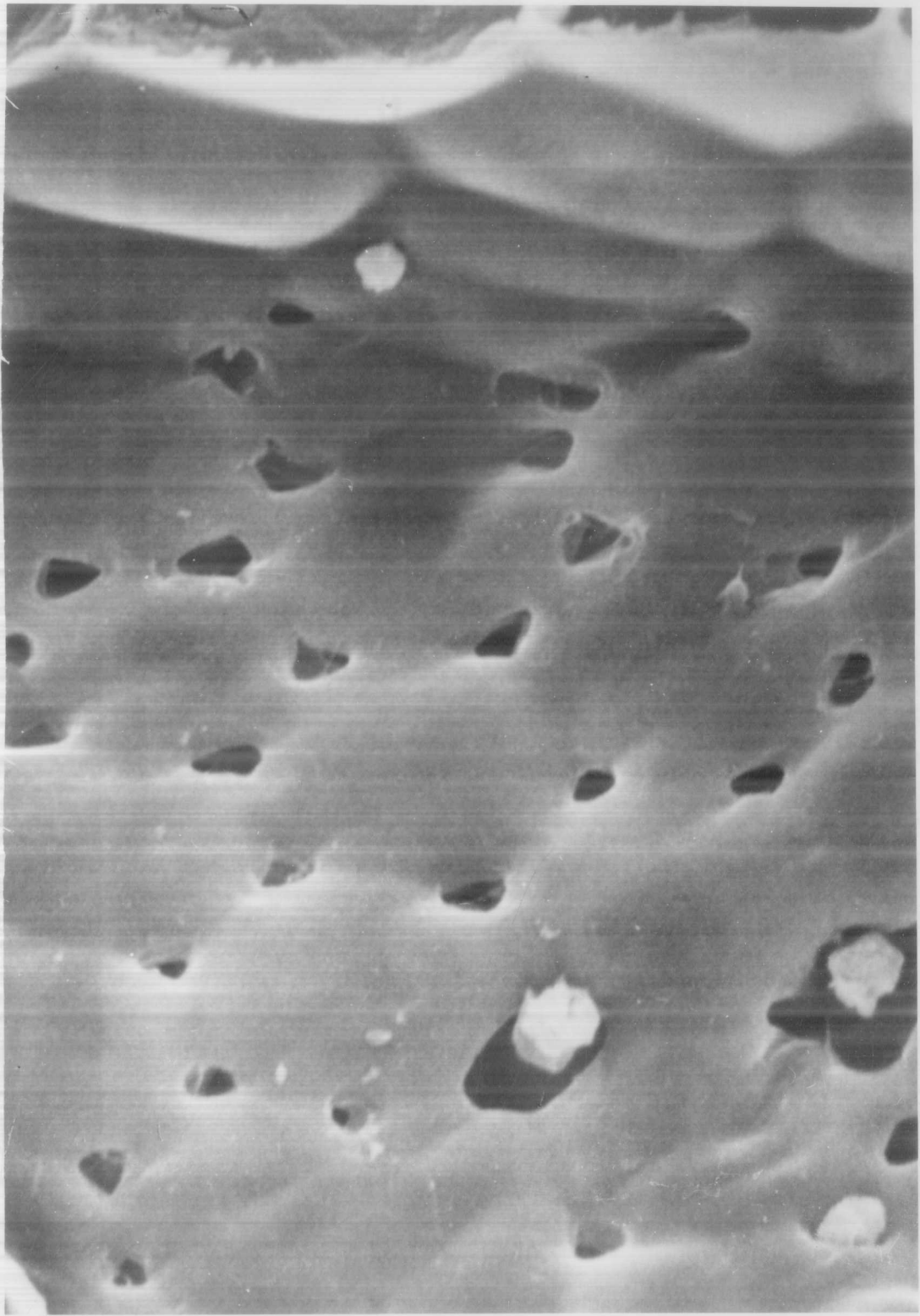
Discussion

Comparison with the closely related species *Z. marina* was most useful in defining those characteristics that give *Phyllospadix* spp. the ability to live in rocky, exposed environments. These characteristics include thickened leaf and rhizome epidermal tissue, reduction in lacunae, greater roothair development and more extensive development of hypodermal fibers. Leaf thicknesses vary according to tidal elevation, although epidermal wall thicknesses only varied in this way at one of two locations.

Examination of roots of *P. serrulatus* (Figure 11) indicated that compared with *Z. marina* (Figure 12), the plant shows differences which may be related to life in exposed rocky environments. Rhizome epidermal tissue is thickened and this may mechanically strengthen the plant. The plant also typically shows greater roothair development than in *Z. marina*. This could be the physical mechanism by which sediment is collected about the roots of the plant (Gibbs, 1902; Phillips, 1979).

The observation of extensive roothair development suggests that the plant may be able to obtain a significant portion of inorganic nutrients through its roots. If this is the case, it would contradict an assertion that the genus obtains inorganic nutrients from the bulk water phase (Raven, 1981). The observation of small amounts of sediment trapped within the roothairs also suggests that this nutrient assimilation categorization (Raven, 1981) is inappropriate.

One anatomical feature of *Phyllospadix* spp. that particularly appears related to water motion in the rocky intertidal is the presence of numerous non-lignified hypodermal

leaf fibers (Figures 9 and 10). *Phyllospadix* spp. share these fibers with *Posidonia oceanica* (L.) Delile (Sauvageau, 1889), a seagrass with an apparently obligate need to live in areas of high relative water motion (Molinier and Picard, 1952). The species of *Zostera* are regarded as the closest relatives phylogenetically to *Phyllospadix* species (Tomlinson, 1982). These species are also characterized by these hypodermal fibers (Tomlinson, 1982), although in reduced numbers compared to *Phyllospadix* (Miki, 1933; Tomlinson, 1982). Sauvageau (1890) considered these numerous hypodermal fibers to one of the major distinguishing features of the genus *Phyllospadix*. Colombo, et al. (1983) observed fewer hypodermal fibers in *Posidonia oceanica* leaves found at depth of up to 30 m as opposed to shoots growing near the surface and attributed this to greater hydrodynamic forces nearer the surface. In this study of *Phyllospadix* spp., the number of bundles and the spacing of bundles within the leaf did not vary irrespective of species or tidal height.

Miki (1933), in a size comparison of lacunae between the two genera, characterized *Phyllospadix* spp. lacunae as being smaller than *Zostera* spp. lacunae. Sauvageau (1891) also observed extensive lacunae in *Z. marina* (Figure 15). Since lacunae in aquatic plants have been implicated in gas exchange during photosynthesis (Hartman and Brown, 1967), the smaller lacunae in Phyllospadix are consistent with a plant living in a more oxic environment. The freshwater Podostemaceae, which lack lacunae entirely, also are restricted to high flow regimes (Sculthorpe, 1967).

The character of the perforated lacunal plates (Figures 13 and 14) may be influenced by the environment in which *Phyllospadix* spp. grow because there have been few reports of perforation plates within submerged plant leaves (Tomlinson, 1980;
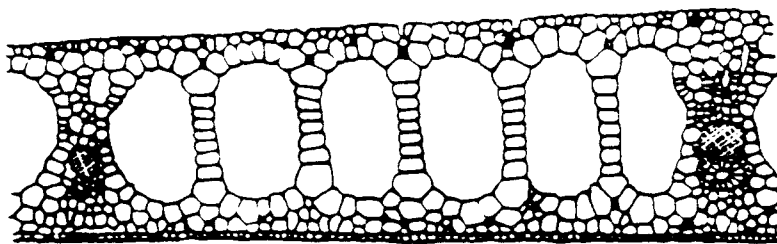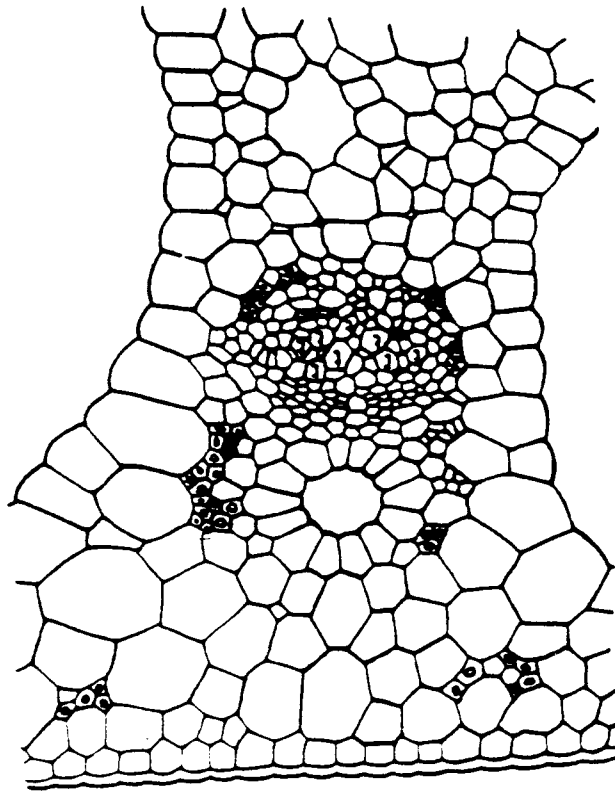
Figure 15. Leaf cross-section of *Z. marina*, showing enlarged lacunal spaces. (from Sauvageau, 1891)

1982). These few records of perforated plates within submerged plant protoxylem or lacunae are limited to the nodal vascular complex in the stem, close to where all the leaves in this study were sectioned. The perforation plates in this study were only observed in one cross-section.

The presence of a thin cuticle in *P. scouleri* has been reported by Tomlinson (1982), who also observed that the outer epidermal wall was "quite thick." *P. scouleri* showed the greatest epidermal wall thickness of the three species examined in this study. At Cape Arago, *P. scouleri* grows submerged in tidal pools in the middle intertidal zone, but it is not particularly common. The discovery of a cuticle on leaves of *P. serrulatus* is not particularly suprising in light of the presence of a cuticle on *P. scouleri*. Tomlinson (1982) attributed the presence of aquatic plant cuticles to the "specialized exchange function of submerged photosynthetic epidermal cells, especially in salt water."

Morphometric variation in epidermal wall thickness showed a consistent pattern at Cape Arago, becoming increasingly thin at the higher tidal elevations, suggesting a physiological relationship to wall thickness. This pattern was not observed at Sitka, however. Inasmuch as thinner epidermal walls could be expected on thinner leaves, some counteracting factor may be present at Sitka that causes thicker epidermal walls on upper intertidal plants. Differences between the two sites include colder water and air temperatures at Sitka, as well as greater precipitation. These factors might cause thickening of epidermal walls on plants growing at the higher intertidal levels.

The Sitka and Cape Arago sites also differ fundamentally in that there is no intra-genus competition at Sitka as there is at Cape Arago. *P. serrulatus* at Sitka grows over a wider

tidal elevation range than at Cape Arago, replacing *P. torreyi* in the lower intertidal and subtidal (Figures 1, 2). The range of *P. torreyi* extends as far north as Vancouver Island (Phillips, 1979), and it may be limited at that point by colder water. By contrast, *P. serrulatus* is the most northern species in the genus, ranging from Unimak Island, Alaska (54°41'N, 163°11'W) north and east to Prince William Sound (60°N, 147°W) and then south to southern Oregon (Appendix 8). This suggests that where both species occur, *P. torreyi* may out-compete *P. serrulatus* in the subtidal and lower intertidal. *P. serrulatus* nevertheless grows higher intertidally and dominates upper zones at Cape Arago (Phillips, 1979; Turner and Lucas, 1985; pers. obs.). Rhizomes of *P. serrulatus* have only 2 roots per node versus 6 to 10 per node in *P. torreyi* and *P. scouleri* (den Hartog, 1970; Phillips, 1979). One consequence of this is that *P. serrulatus* is not as firmly attached to the substrate as the other two species (pers. obs.). Thus while *P. serrulatus* can live at lower tidal levels (e.g. Sitka), it may be out-competed when *P. torreyi* co-occurs if it is more vulnerable to disturbance. The degree of rhizome attachment suggests that *P. serrulatus* cannot live competitively in as exposed an environment as *P. torreyi* and *P. scouleri*. While not contradicting the findings of Turner and Lucas (1985) for the Cape Arago site they used, this distributional pattern suggests that competition during succession may be different for the three species in places of greater exposure.

P. serrulatus leaf thicknesses at Cape Arago were less than those at Sitka (Figures 1B and 2). Sitka *P. serrulatus* leaves at the same tidal heights where *P. torreyi* grows at Cape Arago (below -0.2 m) approached the robustness of *P. torreyi* at Cape Arago (Figures 1A and 2). This supports the suggestion that *P. serrulatus* can grow well at the

lower tidal elevations except when in competition with *P. torreyi.*

Leaf thicknesses of *P. serrulatus* at both locations varied consistently, becoming thinner with increasing tidal height. This probably represents an adaptation in higher intertidal plants to stresses of exposure and greater temperature variation. Robustness of *P. serrulatus* at the upper tidal levels, particularly at Cape Arago, also could be affected by air temperatures. Cape Arago was reported to be the southern limit for the species (Phillips, 1979), although it was observed in this study growing mixed with *P. torreyi* as far south as Cape Blanco, Oregon (42°50'N, 124°33'W, Appendix 8).

The leaf thickness pattern observed here differs from that found in a *Posidonia oceanica* community in the Mediterranean (Colombo, et al., 1983). In that study, leaves were thickest 1 m below the sea surface and became increasingly thinner with greater depth. One difference between *Posidonia oceanica* and *P. serrulatus* is the former cannot withstand emersion (Molinier and Picard, 1952). Among the northeast Pacific species of *Phyllospadix*, *P. serrulatus* appears best adapted to life in the intertidal (Phillips, 1979; Turner and Lucas, 1985). *Posidonia oceanica* was observed to have thinner leaves in increasingly light limited waters, with the thinnest leaves observed at 30 m depth. Thinnest leaves observed in this study were at the highest intertidal levels, indicating that exposure stresses rather than light limitations were the predominant influence.

Although Phillips (1979) articulated a need for investigation of adaptive properties of niche differentiation among the three North American *Phyllospadix* species, this interspecific anatomical comparison has not revealed features unique to one or another species. Turner and Lucas (1985) came to an analogous conclusion in analyzing

succession processes that the three species dominate: "these seagrasses appear to play similar roles in their communities." Nevertheless, the scarcity of *P. scouleri* at Cape Arago, where all three species co-occur, suggest that it may be out-competed by the other two. Furthermore, the distributional patterns of *P. serrulatus* and *P. torreyi* suggest that *P. torreyi* may out-compete *P. serrulatus* in the lower intertidal and subtidal when both occur. Previous observations that *P. serrulatus* is well adapted to air exposure (Phillips, 1979; Turner and Lucas, 1985) are corroborated here and in Chapter 3.

Chapter 3: Stable Carbon Isotope Ratio Variations in Marine Macrophytes Along Intertidal Gradients

Introduction

For thirty years it has been known that submerged aquatic plants, both freshwater and marine, are enriched in the heavy carbon isotope ^{13}C when compared with most other plants (Wickman, 1952; Craig, 1953; Smith and Epstein, 1971; McMillan, et al., 1980; DeNiro and Epstein, 1981). Relatively high ^{13}C : ^{12}C ratios (or less negative $\delta^{13}C$ values) have been explained in terrestrial plants through discovery of alternate photosynthetic mechanisms such as C-4 (Hatch-Slack) metabolism or crassulacean acid metabolism (Bender, 1971; Smith and Epstein, 1971), but there is evidence for these alternate carbon metabolism sequences in only a few aquatic plants (Beer, et al., 1980; Holaday and Bowes, 1980; Keeley, 1981). As a result, the explanation for comparatively high ^{13}C : ^{12}C ratios in submerged aquatic plants is commonly attributed to diffusion limitations in plants taking up carbon dioxide or bicarbonate in water (Raven, 1970; 1981; Andrews and Abel, 1979; Smith and Walker, 1980; O'Leary, 1981; Edwards and Walker, 1983).

Osmond, et al. (1981) and Raven, et al. (1982) tested this attribution of diffusion limitation by examining stable carbon isotope ratios of plants growing in different water flow regimes, while monitoring dissolved inorganic $\delta^{13}C$ source values. The hypothesis was that plants growing in fast-flowing streams are associated with smaller boundary diffusion barriers. As a result, these plants should be isotopically lighter (exhibit more negative $\delta^{13}C$

values) than plants growing in still water. The more negative $\delta^{13}C$ values expected would be consistent with the known isotopic discriminating behavior of ribulose-1,5 bisphosphate carboxylase (Smith and Epstein, 1971; Bender, 1971; O'Leary, 1981), the first carboxylating enzyme in Calvin cycle photosynthesis. This discrimination was hypothesized to be masked in aquatic plants because of diffusion boundary layers affecting the transport of inorganic carbon to the plant (Raven, 1981; Chapter 1). The findings of Osmond, et al. (1981) and Raven et al. (1982) tended to validate this hypothesis, although both studies acknowledged the problem of variability in dissolved inorganic $\delta^{13}C$ values present in different freshwater bodies sampled. Plants sampled were also members of diverse taxonomic groups, including red algae (*Lemanea mamillosa*), green algae (*Cladaphora glomerata*), mosses (*Fontinalis antipyretica*), and several vascular plants (*Potamogeton* spp., *Ranunculus* spp., among others). This taxonomic diversity complicates interspecific comparisons because physiological factors unique to an individual species could also affect isotopic fractionation.

In this study, stable carbon isotope ratio variations were examined in two species of seagrasses within a single genus growing along intertidal elevation gradients. Variability in isotope ratios was also examined in two macroalgae growing along the same intertidal gradients to examine fractionation effects due to individual species differences rather than water motion.

The algal - seagrass isotope comparison also permitted evaluation of a general model discussed by Raven (1981). The seagrass genus sampled, *Phyllospadix*, is

unusual among seagrasses in that it grows upon rocks and lives in an environment relatively exposed to waves and swells. Raven categorized the genus as "haptophytic" together with most benthic algae. Raven suggested that carbon transport resistances would be less apparent in haptophytes and as a consequence they would be in general isotopically lighter. Included in the categorization of *Phyllospadix* as a haptophyte was a suggestion that its carbon fractionation should bear a stronger resemblance to haptophytic algal patterns than rhizophytic patterns predicted for other seagrasses. To provide a basis for comparing carbon isotope variability between haptophytic and rhizophytic seagrasses, a separate study was undertaken of the sediment-rooted seagrass *Zostera marina* L.

Isotopic comparisons between marine plants are not complicated by spatial and temporal variations in dissolved inorganic carbon isotope variability because ratios are relatively constant throughout the marine environment (Broecker, 1982; Galimov, 1985). Also, while differences in water motion often bear a non-causative relationship to pH in freshwater, this is not the case in the ocean where water brought on shore by waves is drawn from a large homogenous source and this water is replaced during each high tide. Intertidal gradients allow for comparison between plants growing in still water versus fast flowing water because of the quiet conditions present in upper intertidal pools during each low tide.

Additional potential sources of isotopic variability were physical factors and physiological or anatomical changes in the plants over these intertidal gradients. It was expected that submerged tidal pool plants would be somewhat different in physiology and robustness than plants of the same species growing in the lower intertidal. Two additional isotopic variability comparisons were made to help isolate these potential sources.

Representative leaf thicknesses were measured in *Phyllospadix torreyi* S. Watson at one elevation to see if there was any relationship between individual leaf thickness and individual $\delta^{13}C$ value. Leaf, cell, or thallus size has been predicted to affect the size of diffusional boundary layers (Smith and Walker, 1980; Raven, 1981; MacFarlane and Raven, 1985). Representative leaf and epidermal cell wall thicknesses of both *P. torreyi* and *P. serrulatus* Rupr. ex Aschers. were measured along intertidal gradients (Chapter 2) to provide a basis for interpreting any leaf thickness fractionation effect observed.

Plants emersed, rather than submerged in intertidal pools, also were compared isotopically. The isotopic consequence of emersion was examined because atmospheric carbon dioxide is 7°/oo to 10°/oo lighter isotopically than oceanic bicarbonate (Thode, et al., 1965; Deuser and Degens, 1967; Wendt, 1968; Mook, et al., 1974). Some aquatic plants, including seagrasses, can utilize bicarbonate (Raven, 1970; Steeman-Nielsen, 1975; Beer et al., 1977; Beer and Waisel, 1979; Millhouse and Strother, 1986). Marine plants utilizing dissolved or atmospheric carbon dioxide could be isotopically lighter solely for that reason. This effect was separated from water flow effects by comparing plants submerged in upper intertidal pools with adjacent emerged plants growing at the same tidal elevations.

Another form of isotopic variation that has been recently observed in marine macrophytes is seasonal in nature (Stephenson, et al., 1984; Fry and Sherr, 1984; Simenstad and Wissmar, 1985). It was therefore necessary to compare plants collected at different times of the year to insure that any isotopic distinctiveness was not due to seasonal variability.

Three hypotheses were examined to account for observed variability of carbon isotope ratios: 1) Variations observed were related to relative water motion around the plants; 2) Variations observed were related to tidal elevation and consequently proportions of exposure to atmospheric carbon dioxide; 3) Variation in leaf ratios was related to the thickness of those leaves. Raven's (1981) suggestion that *Phyllospadix* is haptophytic was evaluated by comparing carbon isotopic variability among two species in the genus with that of a rhizophytic seagrass and marine algae co-occuring with *Phyllospadix* spp.

Materials and Methods

Leaves of *P. serrulatus* were collected at Sandy Beach, Sitka, Alaska (57°03'N, 135°14'W) on 9 and 10 August 1983 along an intertidal transect extending from -0.88 m to +0.88 m, mean lower low water (MLLW). Leaves of the seagrasses *P. serrulatus* and *P. torreyi* and thallus tissue of the algae *Egregia menziesii* (Turn.) Aresch. and *Halosaccion americanum* I.K. Lee were collected at North Cove, Cape Arago, Oregon (43°20'N, 124°22'W) on 25 to 27 August 1984. This intertidal transect extended from -0.40 m to +0.94 m (MLLW). Algal and seagrass samples were collected along this same Cape Arago transect on 1 to 3 June 1985. The seagrass sampling protocol used on this occasion was slightly different. Instead of using unsorted leaves of all ages, at each sampled elevation, 3 of the youngest leaves from 3 separate shoots were collected. Additionally 3 of the oldest leaves from these same 3 shoots were collected and analyzed separately. Two additional transects parallel to this first transect were also sampled at that time, but leaves of all ages were sampled. Tidal ranges for all transects at Cape Arago were essentially identical. Tidal elevations were estimated at Sitka and Cape Arago from tide

table interpolations using base data for Sitka and Humboldt Bay, California (U.S. Department of Commerce, 1983; 1984; 1985). Relative elevations within the transects are precise to .01 m, although absolute elevations relative to base stations are less accurate.

Eelgrass (*Z. marina*) shoots and adhering sediments were collected along a transect in Izembek Lagoon, Alaska (55°16'N; 163°07'W) that was approximately the same transect described by Short (1983). Samples were collected 21 June to 28 July 1982. The transect traversed an ecological cross-section of eelgrass beds, ranging from plants perceived to be early successional and limited by sediment nutrients in the upper end to late successional plants limited by light in the lower end (Dennison, 1979; Short, 1983; Roth, 1986). Of the 36 stations, 35 were subtidal, although water depths at low tide were typically less than 1 m. Each sample, consisting of numerous shoots, was divided into leaves, roots, and rhizomes. Plant parts were washed thoroughly in seawater and freeze-dried. All of the Sitka and Cape Arago seagrass and marine algal samples were air dried in the field and within a week were further dried at 60°C for 24 hours.

Sub-samples of 5 mg of well homogenized plant tissue for each sample were combined with 700 mg Cuprox™ copper wire and isolated in a vacuum within 6 mm Pyrex™ glass tubing. Samples were combusted at 585°C for 2 hours. The liberated carbon dioxide was isolated using liquid nitrogen and cryrogenically cooled alcohol, then transferred via a new sealed glass tube to the mass spectrometer. Mass spectrometry was performed using a VG Instruments Sira-9™ for the Cape Arago and Izembek Lagoon samples and AEI MS 20™ for Sitka samples at the Institute of Marine Science, University of Alaska, Fairbanks. This method is a modification of that used by Dunton (1985).

Samples collected were free of epiphytes. For *E. menziesii*, samples constituted

roughly equal proportions of blades, collapsed pneumatocysts, and stipe. For *H. americanum*, samples consisted of collapsed, shredded sacs.

On 4 June 1985, 9 leaves of *P. torreyi* from -1.6 m at Cape Arago were collected for subsequent isotopic analysis. Each leaf was later examined individually using scanning electron microscopy (SEM) for leaf and epidermal cell wall layer thicknesses. Preservation and SEM procedures are described in Chapter 2. Leaf thicknesses measured were averages taken from at least 2 photographs of each leaf. Leaves were sectioned within 1 cm of the point of initiation and thicknesses measured were at this point. Isotopic values were measured on a homogenate of the rest of the leaf. One of the 9 leaves was not homogenated, but rather divided into 5 additional sub-samples, providing isotopic ratios on 5 segments within the leaf.

The $\delta^{13}C$ values reported are relative to the Peedee Belemnite standard (PDB; Craig, 1953) although 2 internal standards were employed to calibrate the mass spectrometers to PDB. A correction was made for the contribution of ^{17}O to the mass 45 beam as given by Craig (1957). Instrumental error determined in consecutive analyses of a single CO_2 sample averaged less than $\pm0.05°/oo$. Average replicate sample standard deviation for seagrass samples from Sitka on the AEI Mass Spectrometer, including all instrumental errors and errors of preparation, was $\pm0.15°/oo$ (n=52). Average replicate sample standard deviations for seagrass and marine algal samples from Cape Arago on the VG Mass Spectrometer, including all instrumental errors and errors of preparation were $\pm0.21°/oo$ (n=45) and $\pm0.24°/oo$ (n=42), respectively. Spot checks of eelgrass samples from Izembek Lagoon indicated a similar range of error.

Several statistical procedures were used to analyze isotopic patterns observed. A microcomputer statistical package (Statview, Brainpower, Inc., Calabasas, Calif.) was used for each statistical procedure. Data from samples collected at Cape Arago at 2 different times of year using slightly different sampling protocols were analyzed to see if sampling variability could explain any of the isotopic variability observed. A Friedman Test, which tests for differences in 3 or more linked sample sets, was used to test the null hypothesis that samples collected at different times of the year and/or using different sampling protocols were drawn from the same population. A Wilcoxon signed-rank test, which tests for differences between 2 linked samples, was used to test the null hypothesis that isotopic values observed in young leaves were not significantly different from older leaves from the same plants.

A Mann-Whitney test, which tests for differences between independent samples, was used to test the null hypothesis that $\delta^{13}C$ values of marginal subtidal seagrasses were not significantly different from tidal pool plants. A Mann-Whitney test was also used to test if $\delta^{13}C$ values of all submerged plants were significantly different than values of all plants that were emersed during low tides. Pearson product-moment correlation analyses, as well as Spearman's rho, were used to assess the influence of atmospheric exposure and leaf thickness upon isotopic ratios.

Results

Sample Treatment

Isotopic values observed at Sitka (Figure 1) are based on a single sampling period using unsorted leaves of all ages, so there is no seasonal variability in the data.
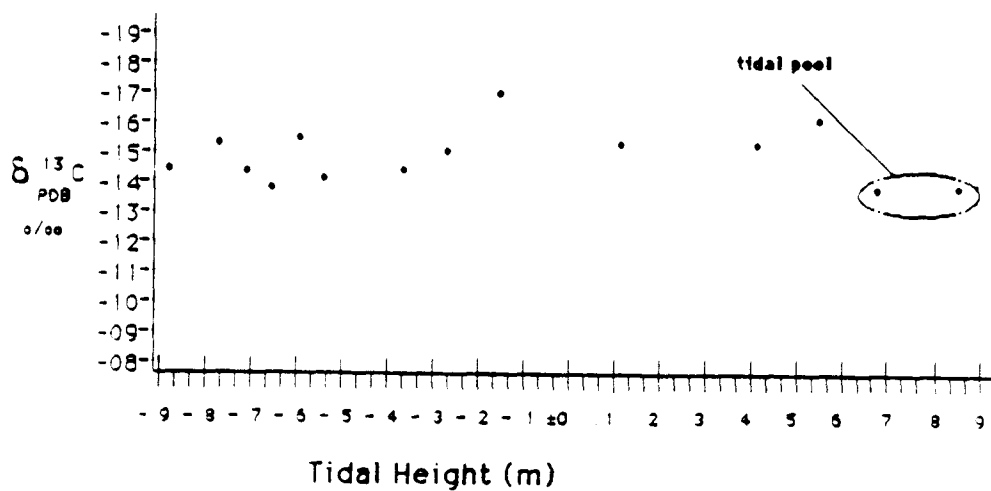
Figure 1.

Stable carbon isotope ratios of *Phyllospadix serrulatus* (single observations) in relation to tidal height at Sitka, Alaska.

A Friedman Test indicated that samples collected along 1 of the transects at Cape Arago at 2 different times of year (June, 1985 and August, 1984) and using 2 different sampling protocols (old and young leaves versus unsorted) were drawn from the same population ($0.3 < p < 0.5$). A Wilcoxon signed-rank test showed further that there was no significant difference between isotopic values of 3 older leaves and 3 younger leaves from the same plants ($p = 0.2$; $n = 12$). As a result, seagrass isotopic data collected at Cape Arago were grouped together for age of leaf and sampling times (Figure 2).

A Wilcoxon signed-rank test indicated that there was no significant difference between isotopic values in marine algae collected in August, 1984 and June, 1985 ($p > 0.5$; $n = 14$). As a result, data for marine algae collected at Cape Arago for both times were also grouped together (Figure 3).

Isotopic Variability - General

$\delta^{13}C$ variability for *P. serrulatus* at Cape Arago was as great as 7°/oo for homogenized leaves collected less than 20 m apart. $\delta^{13}C$ variability extremes for *P. torreyi* collected 8 m apart were 3°/oo. Ranges for *E. menziesii* and *H. americanum* were 6°/oo collected 16 m apart and 3°/oo collected 1 m apart, respectively.

Variability due to Water Motion

At the Cape Arago site, a Mann-Whitney test indicated no significant differences between *P. torreyi* growing in marginal subtidal surge zones (below -0.2 m) and *P. torreyi* growing submerged in upper tidal pools, where average water motion was lower ($p > 0.10$; $n = 19$; Figure 2). *P. serrulatus* growing in tidal pools at Sitka also were not significantly different from plants growing at the lowest tidal elevations (Mann-Whitney
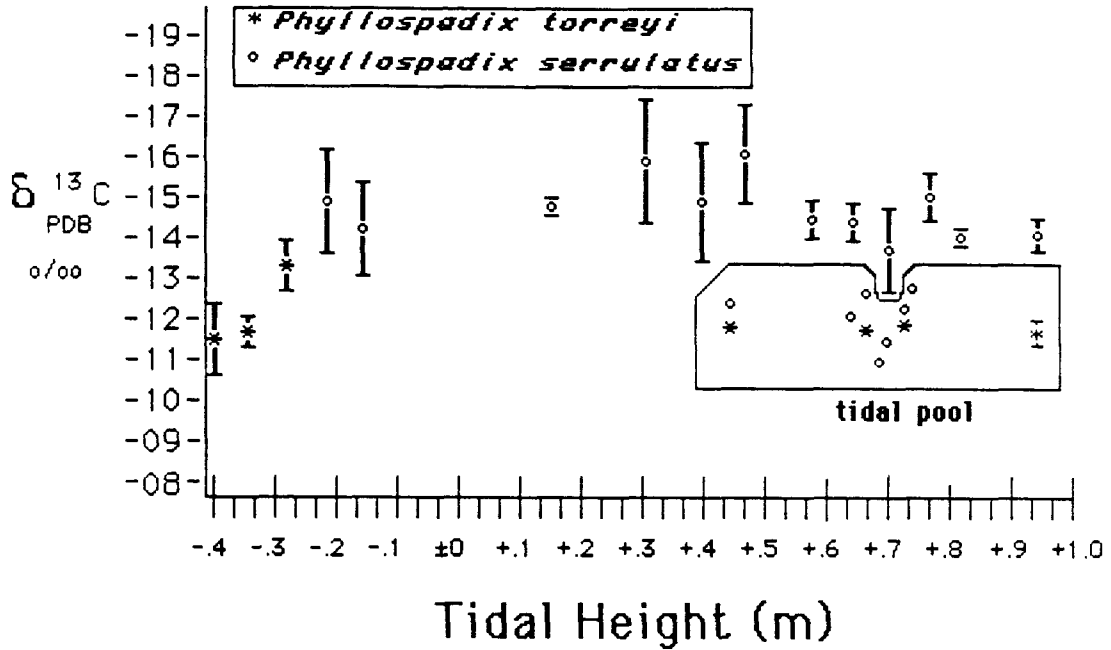
Figure 2.

Stable carbon isotope ratios (mean ± SD) of seagrass leaves in relation to tidal height at Cape Arago, Oregon (1984 and 1985 data, n=79).
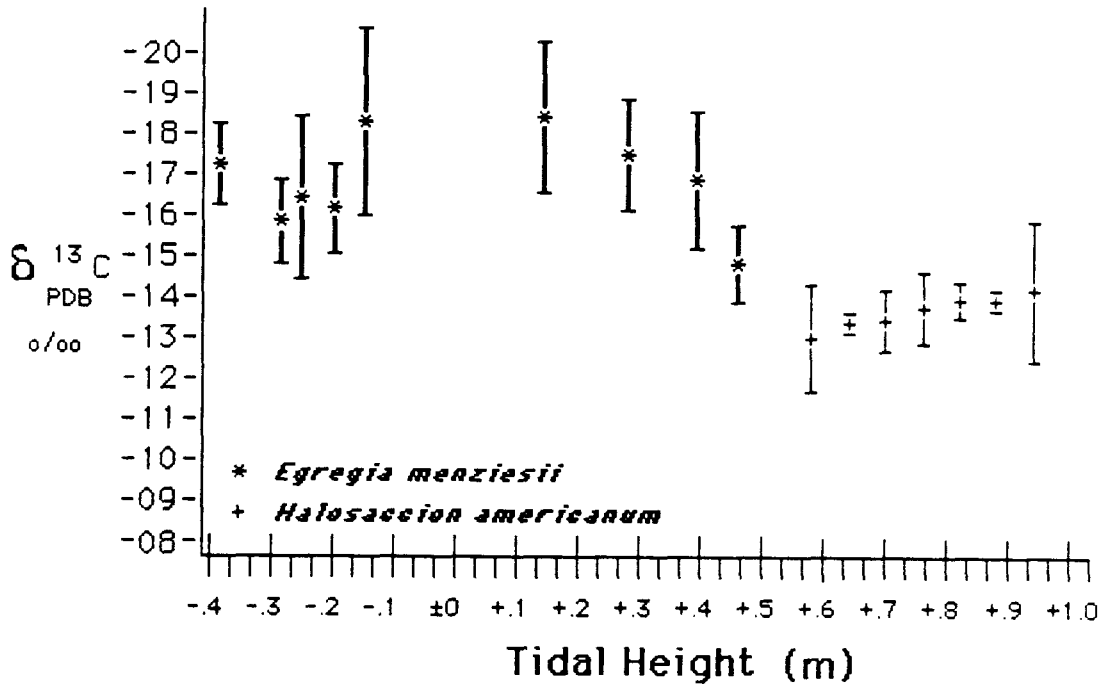
Figure 3.

Stable carbon isotope ratios (mean ± SD) for two algae in relation to tidal height at Cape Arago, Oregon (1984 and 1985 data, n=60).

p=0.27; n=6; Figure 1).

Variability due to Atmospheric Exposure (Tidal Height)

P. serrulatus growing in submerged tidal pools at Cape Arago were significantly heavier than other _P. serrulatus_ plants (Mann-Whitney, p<0.001; n=57). It appeared that this was related to atmospheric exposure rather than water motion because almost all _P. serrulatus_ exposed to air, even for short periods, were isotopically lighter than any growing submerged in tidal pools, even at the same elevations (Figure 2). A similar effect was observed at Sitka, where only _P. serrulatus_ is present (Figure 1). For _P. torreyi_ growing at Cape Arago, and for _P. serrulatus_ growing at Sitka, there were signficant correlations between tidal elevation and $\delta^{13}C$ values, with both species becoming isotopically lighter with increasing elevation (Table 1). At Cape Arago, for _P. serrulatus_, no relationship was observed between $\delta^{13}C$ and tidal elevation (Figure 2; Appendix 2) although the species does not grow subtidally at Cape Arago as it does at Sitka.

For _E. menziesii_ at Cape Arago, no significant relationship (p>0.05) was observed between $\delta^{13}C$ and tidal elevation (Figure 3; Appendix 5). Variances among samples, even at the same elevations, were the largest of any of the species sampled. _H. americanum_ $\delta^{13}C$ became lighter with increasing elevation (Figure 3; Table 1) although the average change was small (about 1°/oo) and the standard deviations about the mean were relatively large. When only data from June, 1985 was used, correlations were slightly more significant (Table 1).

Table 1. Correlation Analyses (0.05›p) of Isotopic Values of Seagrass and Algae in Relation to Tidal Height

Test	r	p	Spear-man's rho	p	n	Site
P. torreyi $\delta^{13}C$ vs. elevation	-0.714	0.01›p›0.001	-0.700	0.01›p›0.005	15	Cape Arago
P. serrulatus $\delta^{13}C$ vs. elevation	-0.573	0.025›p›0.01	-0.615	0.025›p›0.01	12	Sitka
H. americanum $\delta^{13}C$ vs. elevation	-0.420	0.05›p›0.01	-0.337	0.05›p›0.025	25	Cape Arago
H. americanum $\delta^{13}C$ vs. elevation, 6-85	-0.638	0.01›p›0.001	-0.589	0.005›p›0.001	20	Cape Arago

Variability due to Leaf Thickness

In a direct comparison at one intertidal height of leaf thicknesses versus $\delta^{13}C$ values for those individual leaves, a correlation was found, with thicker leaves of *P. torreyi* tending to be isotopically heavier (Figure 4; Table 2). Epidermal cell wall thicknesses were not well correlated with $\delta^{13}C$ values (p>0.05; Appendix 4).

Table 2. Correlation Analysis of Leaf Thickness in Relation to Isotopic Value

Test	r	p	Spear-man's rho	p	n	Site
P. torreyi $\delta^{13}C$ vs. leaf thickness	0.639	0.05›p›0.01	0.667	0.05›p›0.01	9	Cape Arago
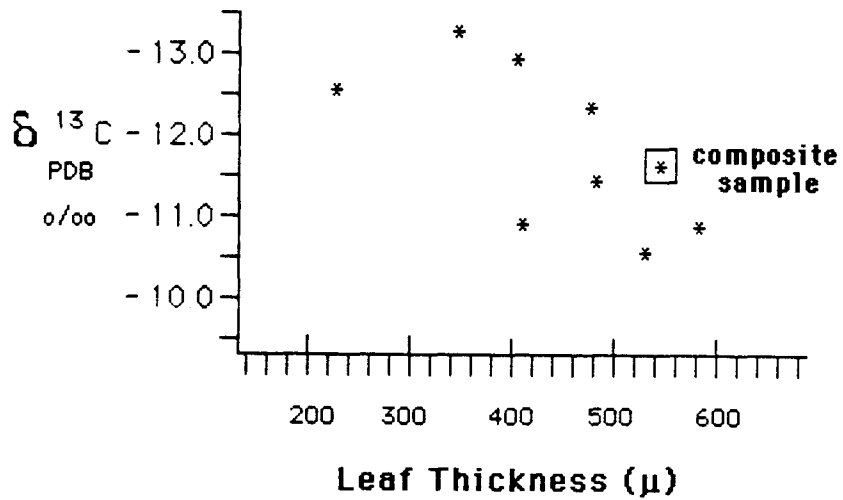
Figure 4.

Stable carbon isotope ratios of leaves of *Phyllospadix torreyi* in relation to leaf thickness (single observations).

One isotopic value reported in Figure 4 (-11.4°/oo) is an integrated, weight averaged composite or the isotopic values observed for each of 5 leaf segments. A pattern of isotopic lightening from base to tip was observed in this leaf ranging from -10.1°/oo to -12.8°/oo (Figure 5). Because of the preliminary nature of this one leaf segmentation, no statistical analyses were performed.

Variability of Isotopic Ratios in Z. marina

Leaf $\delta^{13}C$ values of *Z. marina* from Izembek Lagoon ranged from -7.0°/oo to -9.5°/oo (Figure 6). Rhizome $\delta^{13}C$ values ranged from -7.5°/oo to -10.1°/oo (Figure 7). Root $\delta^{13}C$ values ranged from -7.4°/oo to -10.9°/oo (Figure 8). In all 3 plant organs, the most negative $\delta^{13}C$ values were from the intertidal station (Table 3).

Table 3.

$\delta^{13}C$ Values for Eelgrass from Izembek Lagoon, Alaska.

Leaf	Root	Rhizome	
-9.54 (n=1)	-10.88 (n=1)	-10.12 (n=1)	intertidal
-8.18 (n=31)	-8.59 (n=33)	-8.90 (n=32)	mean subtidal
±0.48	±0.66	±0.62	± SD

A one-way analysis of variance indicated that subtidal leaves, roots, and rhizomes were significantly different isotopically. (Table 4).

meristem $\delta^{13}C$ tip

-10.1   -10.4   -11.5   -12.2   -12.8

23.2 mg   83.6 mg   62.3 mg   46.1 mg   60.4 mg

Weight of segment
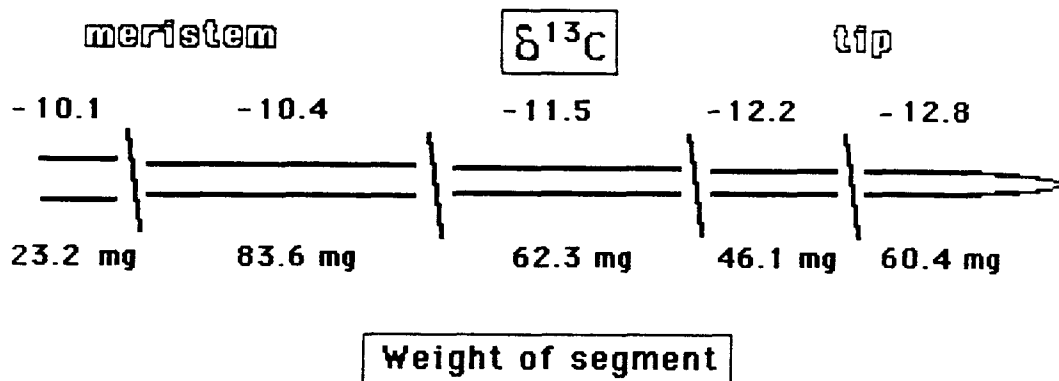
Figure 5.

Stable carbon isotope ratios of leaf segments
from *Phyllospadix torreyi*
(Cape Arago, Oregon, 4 June 1985).
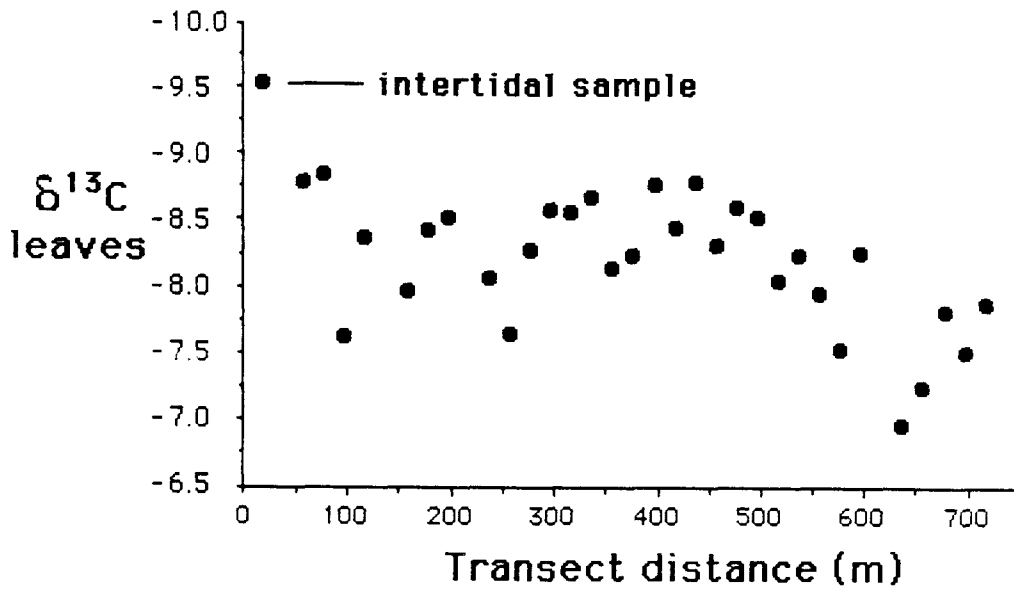
Figure 6.
Stable carbon isotope ratios of *Zostera marina* leaves (single observations) from Izembek Lagoon, Alaska.
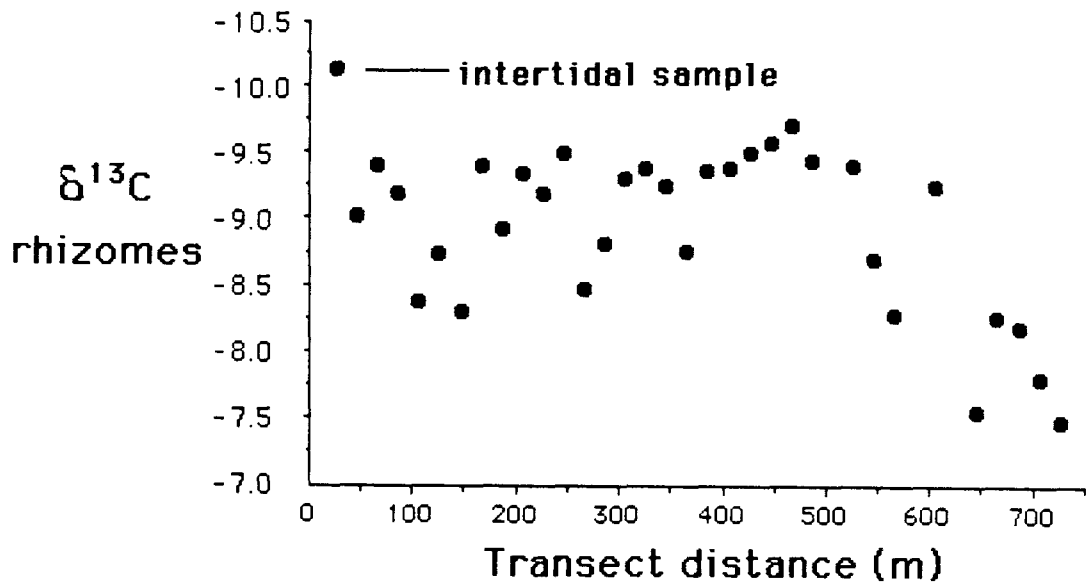
Figure 7.
Stable carbon isotope ratios of *Zostera marina* rhizomes (single observations) from Izembek Lagoon, Alaska.
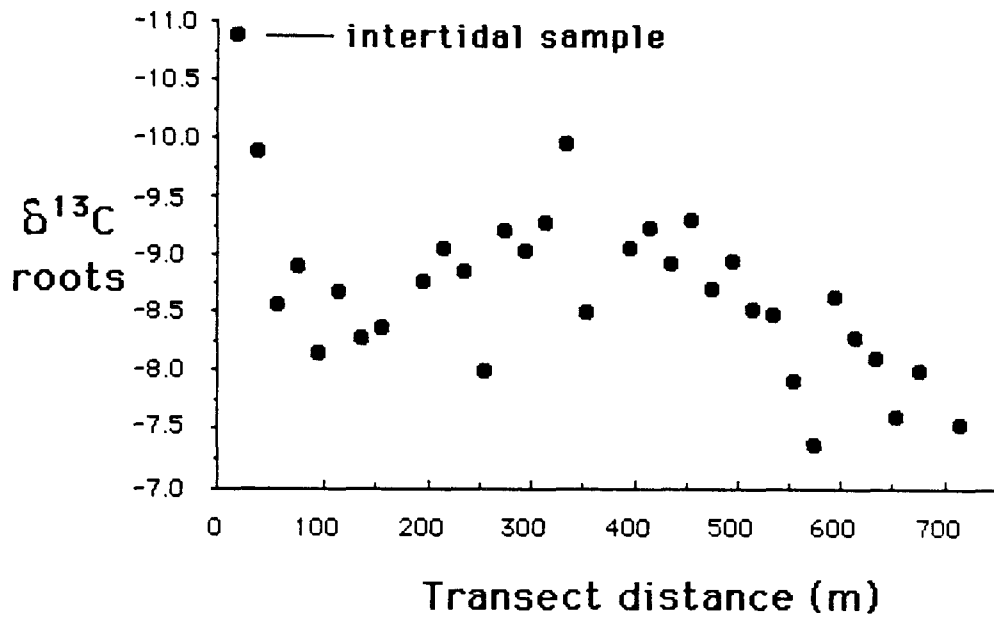
Figure 8.
Stable carbon isotope ratios of *Zostera marina* roots (single observations) from Izembek Lagoon, Alaska.

Table 4.

One Way ANOVA for 3 Groups: Leaves, Roots, and Rhizomes from Izembek Lagoon, Alaska.

Analysis of variance

Source	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	2	8.11	4.055	11.489
Wihin groups	93	32.823	.353	p ≤ .0001
Total	95	40.933		

Model II estimate of between component variance = .116

For rhizomes and leaves, the null hypothesis that there was no signficant difference between leaves and rhizomes was rejected using a Wilcoxon signed-rank test (p<0.005. n=31). A null hypothesis relating to the difference between roots and rhizomes was also rejected using a Wilcoxon signed-rank test (0.005<p<0.01; n=31). As could be expected from these results, leaves and roots were also found to be significantly different (p<0.005; n=30). Examination of leaf - root, rhizome - root, and leaf - root correlations consistently showed strong, direct relationships (p<0.005) indicating that where one plant component varied, other plant components varied in similar magnitudes and directions (Figure 9).

Discussion

Variability of $\delta^{13}C$ values within the seagrasses studied appear to be most heavily influenced by exposure to atmospheric carbon dioxide. Continually submerged plants were consistently heavier than those exposed to air, even if exposed for relatively short times. Above about -0.2 m (MLLW), at both Sitka and Cape Arago, emersed *P. serrulatus* plants did not continue to become significantly lighter, suggesting the influence of isotopically light atmospheric carbon dioxide on these intertidal seagrasses is

Figure 9.

Correlation coefficients among $\delta^{13}C$ values for *Zostera marina* plant organs from Izembek Lagoon, Alaska.

a threshold phenomenon. Additional exposure to air does not lead to increasingly lighter plants. It is possible that this threshold effect takes the form of a reduction in photosynthesis or respiration in plants growing at the highest tidal levels; perhaps all of the plants growing above the -0.2 m level incorporate atmospheric carbon dioxide in roughly the same proportion of total photosynthetic activity, but photosynthesis is increasingly limited at higher tidal elevations because of desiccation. These data do not address directly whether those submerged plants are using dissolved carbon dioxide or bicarbonate as the initial substrate, but it does provide isotopic evidence that emerged plants are actively incorporating atmospheric carbon dioxide. This indicates that differential use of isotopically distinct bicarbonate by submerged plants and carbon dioxide by emerged plants may be the basis for the observed fractionation. This finding is somewhat unexpected because Barbour and Radosevich (1979) concluded that *P. scouleri* Hook. is photosynthetically inactive at low tide, based on ^{14}C tracer studies. Nevertheless *P. serrulatus* grows higher intertidally than does *P. scouleri* (Phillips, 1979) and may be better adapted to photosynthesizing when emersed.

Leaves, roots, and rhizomes of eelgrass, *Zostera marina*, from Izembek Lagoon, also showed this same pattern of more negative $\delta^{13}C$ values at the one intertidal station (Figures 6, 7, 8), indicating that atmospheric carbon dioxide was the one most important factor causing variability in $\delta^{13}C$ values in the seagrasses studied.

By contrast, algal isotopic variability, while showing the same general range as in the seagrasses, could not be tied to atmospheric carbon dioxide exposure. For *E.*

menziesii. $\delta^{13}C$ values ranged from -14.3 to -20.0 for all elevations and showed a maximum $\delta^{13}C$ variability range at one elevation of 4.9 at -0.15 m. This range of variation is similar to what was observed for individual specimens of the brown alga *Laminaria longicuris*, in Nova Scotia (Stephenson, et al., 1984). Variability for *H. americanum* was smaller ($\delta^{13}C$ range was -11.6 to -16.0 for all elevations and a maximum change in $\delta^{13}C$ of 3.4 at +0.94 m), but this may simply be a scale related problem since it is more difficult to obtain representative samples in a larger species. There was an indication that *H. americanum* became isotopically lighter with increasing elevation (Table 1), but the change was small (1°/∘∘) and variability at individual locations was too high to resolve any trends with certainty. The demonstration that this alga uses dissolved inorganic carbon stored within its saccate, water filled thallus (Oates, 1986) indicates that isotopic lightening due to atmospheric exposure would be difficult to detect.

$\delta^{13}C$ values of submerged *Phyllospadix* spp. were not significantly different in surge zones and still water pools, indicating that boundary layers around these plants did not influence isotopic fractionation. It is possible, however, that isotopic effects due to boundary layers are present and could be obscured under the following scenario: if pH in upper tidal pools decreases in response to invertebrate respiration, isotopically light dissolved CO_2 would be more available to submerged plants. Uptake of this CO_2 would mask the heavier $\delta^{13}C$ expected because of larger boundary layers. In this scenario, submerged lower intertidal and subtidal plants primarily use bicarbonate and upper intertidal plants primarily use atmospheric or dissolved CO_2. The maximum size of the

hypothesized boundary layer effect is equal to the $\delta^{13}C$ difference between oceanic bicarbonate and dissolved CO_2, from 7°/oo to 10°/oo, depending upon temperature (Galimov, 1985). Submerged tidal pool plants are not in still water at high tide, so the $\delta^{13}C$ difference due to boundary layer effects, if it is present at all, is no doubt more modest.

The leaf thickness data provides evidence that leaf anatomy may be related to inorganic carbon diffusion and as a consequence, stable carbon isotope ratios. Preliminary evidence from the segmentation of an individual *P. torreyi* leaf (Figure 5) indicates that within-leaf variability can be 3°/oo, and that there may be an ontogenetic pattern to the fractionation. The later formed (and thicker) portions of the leaf are isotopically heavier with a progressive lightening in the direction of the thinner tip. Lowdon and Dyck (1974) observed a similar pattern in maple leaves and grass on a seasonal basis, with leaves becoming isotopically lighter as autumn approached, and suggested increased photorespiration with age as a possible cause. Photorespiration has been observed in some seagrasses (Hough, 1976). Except for Lowdon and Dyck's observations, and the possibility of ontogenetic effects observed in this study, age related isotope effects in plants have not been documented (Wickman, 1952; Craig, 1954; Jansen, 1962).

The correlation between leaf thickness and isotopic fractionation in *P. torreyi* at a single elevation is based on a small sample (n=9) and the effect is relatively small, about 2°/oo. The observed effect, of thicker-leaved plants tending to be isotopically heavier, may be only indirectly related to anatomy and could also be a consequence of recirculation of photorespired CO_2 within larger lacunae.

Stephenson, et al. (1984) in studying isotopic fractionation in two eelgrass leaves, observed variation between younger and older leaves from the same plant of up to 2 °/oo, but over the course of a year, different aged leaves were not consistently heavier or lighter. Simenstad and Wissmar (1985) also found seasonal variations in *Z. marina* $\delta^{13}C$ values of more than 3°/oo. In this study, the effects of individual leaf variation were minimized by performing mass spectrometric analyses on homogenized leaves of a number of plants growing at any particular tidal elevation.

The one exception to the use of unsorted homogenized leaves was the collection of 3 older leaves from 3 separate *P. serrulatus* plants for isotopic comparison with 3 younger leaves from the same plants at each of 12 different elevations (Appendix 2). $\delta^{13}C$ values varied by up to 1°/oo, but no consistent pattern of age related fractionation was observed. It is possible that the lack of a pattern between the old and young leaves of the same *P. serrulatus* plants was due to orientation of the leaves in reference to air exposure.

At Cape Arago, *P. torreyi* leaves were roughly an order of magnitude thicker than typical *P. serrulatus* leaves (see Chapter 2). Thus the lack of a similar leaf age (or thickness) pattern for *P. serrulatus* could be due to species differences or it could be due to sampling protocol. Only in the case of *P. torreyi* were individual leaves measured both morphometrically and isotopically.

With respect to Raven's (1981) suggestion that *Phyllospadix* shows haptophytic carbon isotope patterns, these data are equivocal. $\delta^{13}C$ values observed were similar to

δ^{13}C values of 2 co-occuring marine algae and heavier than most other seagrasses (McMillan, et al., 1980). δ^{13}C values for **Z. marina** did not overlap any of the other seagrass or marine algae species. Eelgrass was consistently heavier, supporting Raven's prediction that rhizophytes would exhibit less negative δ^{13}C values. Nevertheless, δ^{13}C values for **E. menziesii** and **H. americanum** were slightly heavier than has been reported for many other species of marine algae (Craig, 1953; Smith and Epstein, 1971; DeNiro and Epstein, 1981), so the δ^{13}C values observed for **P. torreyi** and **P. serrulatus** may actually be intermediate between most seagrass values and those for most algae. All of the seagrass species studied were isotopically lighter if emersed and it was concluded that this was due to uptake of isotopically lighter atmospheric carbon dioxide. Neither of the marine algae clearly showed this pattern, suggesting that differential use of bicarbonate and atmospheric carbon dioxide does not occur in an isotopically detectable manner. This conflicts with the conclusion of a recent study (Faganeli, et al., 1986) which suggested that carbon isotopic variability in the green alga **Ulva rigida** C. Ag. and the brown alga **Fucus virsoides** J. Ag. was due to differential use of bicarbonate and atmospheric carbon dioxide. Faganeli et al. (1986) did not attempt to correlate tidal exposure with isotopic ratios, but simply associated the presence of variability with differential use of inorganic carbon sources. Similar ranges of isotopic variability in **E. menziesii** and **H. americanum** were not associated with intertidal exposure, so this explanation of algal carbon isotope variability is not satisfactory.

Evidence that **Phyllospadix** spp. trap small grains of sediment through extensive roothair development (Gibbs, 1902; den Hartog, 1970; Phillips, 1979; Chapter 2) also

indicates that the categorization of **Phyllospadix** as haptophytic is too simplistic. Small sediment accumulations within the roothair network may create an environment for organic matter accumulation and hence a rhizosphere with higher concentrations of inorganic nutrients. It is possible that this network permits the plant to absorb inorganic nutrients through its roots.

Mean rhizome $\delta^{13}C$ values of **Z. marina** were 0.7°/∘∘ lighter than mean leaf values. The analysis of variance and the Wilcoxon signed-rank test both indicated that this difference was signficant, so strictly speaking, eelgrass leaf $\delta^{13}C$ values are not representative of the plant as a whole. Such a small difference should have little practical effect on applied uses of carbon isotopes, such as in food web tracer studies. However, it does indicate the presence of isotope fractionation during carbon metabolism and transport within the plant. This might be biochemically driven by enzymatic discrimination or it could be a physical effect caused by diffusion limitations as photosynthate is transported from sources to sinks within the plant. Subtidal $\delta^{13}C$ values of eelgrass rhizomes, roots and leaves varied consistently with each other (Figure 9), confirming that individual samples were isotopically distinct. The reasons for this isotopic variability in subtidal **Z. marina** (Figures 6, 7, 8) are not clear. This portion of Izembek Lagoon has little freshwater input (per. comm., C. Peter McRoy) and the homogenization of plant samples presumably would obscure any plant organ thickness or age effects. It is possible that differential storage of isotopically distinct chemical fractions by plants in different parts of the lagoon is responsible for the patterns shown on Figures 6, 7, and 8. Examination of isotopic variability using a single organic fraction such as cellulose would test this suggestion.

Chapter 4: Conclusions

The inclusive hypothesis upon which this work is based is that the genus *Phyllospadix* shows adaptations to life in rocky marine habitats that distinguish it from other seagrasses. The working hypotheses tested were based on a niche differentiation prediction of Phillips (1979) and a carbon assimilation model of Raven (1981).

The niche differentiation hypothesis was based on an observation of Phillips that three species in the genus, when they occur together, are distinctly zoned in the littoral zone. It was predicted that each species of *Phyllospadix* dominates its zone through specific adaptations. This prediction was approached anatomically (Chapter 2). The three North American species of *Phyllospadix* were examined for differences that might be related to different rocky microhabitats in the littoral. Although each species was found to be anatomically distinct, no distinguishing features were found that could be related to specific littoral zones. These conclusions are analogous to an ecological study of the same three species which found that the three species followed similar successional sequences (Turner and Lucas, 1985).

Anatomical characteristics for the genus as a whole, particularly numerous hypodermal fibers, are adaptations to exposed conditions. Comparison with the closely related genus *Zostera* also showed less extensive lacunal development, which would be consistent with plants living in turbulent, more oxic environments. *Phyllospadix* spp. show greater roothair development than *Z. marina* and these roothairs trap sediment. This suggests modifications to the categorization of the plant as a strict haptophyte,

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dependent on the water column for inorganic nutrients (Raven, 1981).

The suggestion of haptophytic nutrient acquisition in the genus (Raven, 1981) was the initial point for testing a carbon assimilation model. Raven predicted that rhizophytic (substrate penetrating) aquatic plants would exhibit less enzymatic discrimination against ^{13}C because of greater diffusion resistances to inorganic carbon uptake in plants that obtain scarce nutrients from the substrate. He also predicted that submerged aquatic plants growing in still water conditions would tend to be isotopically heavier than those in fast flowing water because of diffusion limitations associated with boundary layers around leaves. This latter prediction was tested in a comparison of submerged surge zone *P. serrulatus* and *P. torreyi* with plants of those same species growing submerged in upper tidal pools. In this case, no evidence was found that tidal pool plants were isotopically heavier (Chapter 3).

With respect to the general carbon assimilation model of Raven, *Phyllospadix* spp. were compared to 2 co-occurring haptophytic marine algae to see if the seagrass genus showed a similar pattern of carbon isotope variation (Chapter 3). A study also was made of isotopic variability in *Z. marina* to provide a basis of comparison between rhizophytic and haptophytic seagrassses (Chapter 3). *Phyllospadix* spp. had $^{13}C : ^{12}C$ similar to the algae, *Egregia menziesii* and *Halosacccion americanum*, but the seagrasses alone varied consistently in response to intertidal exposure. Seagrasses growing emersed intertidally tended to become isotopically lighter and it was concluded that this was due to uptake of isotopically lighter atmospheric carbon dioxide. The marine

algae, by contrast, did not vary consistently with additional atmospheric exposure indicating that differential uptake of isotopically distinct inorganic carbon sources (i.e. bicarbonate and atmospheric carbon dioxide) was not present to an extent that could be isotopically detected.

Phyllospadix spp. nevertheless were consistently isotopically heavier than *Z. marina*, a closely related rhizophytic seagrass. Isotopic variability patterns bore similarity to that observed in *Phyllospadix* spp. because *Z. marina* also became lighter when exposed to air.

These patterns suggest, at least in isotopic variability, that *Phyllospadix* spp. exhibit intermediate characteristics between classically defined haptophytes and rhizophytes (Raven, 1981). Although the genus clearly has characteristics adapting it to growth on rocky marine shores (Chapter 2), and exhibits the lightest mean carbon isotope ratios of any seagrass genus (McMillan, et al., 1980), the isotopic lightening observed in upper tidal plants was identical to that observed in *Z. marina* (Chapter 3).

An anatomical relationship with carbon isotopic ratios was also observed in *P. torreyi*. Thicker leaves were correlated with heavier isotopic ratios (Chapter 3). It was concluded that this correlation may not be necessarily related to anatomy. Such a relationship could have an ontogenetic or other origin and the relationship merits further investigation.

The study of isotopic variability in *Z. marina* indicated that leaves are isotopically heavier than rhizomes, which are in turn lighter than roots (Chapter 3). It was concluded that this small effect, less than 1°/oo at each step, is probably due to fractionation during

photosynthate transport, differential storage, or enzymatically driven metabolic processes. Carbon isotope ratios of roots, rhizomes, and leaves were well correlated with each other, indicating that where individual plant organs varied, other components of those same plants varied in the same manner.

Clearly one of the larger questions raised by this work remains the usefulness of Raven's aquatic plant model of rhizophytes and haptophytes. Raven (1981) specifically predicted similar isotopic fractionation patterns for all haptophytes regardless of taxon. Nevertheless the single most important factor in determining carbon isotopic variability in both a haptophytic and rhizophytic seagrass, air exposure, does not apparently affect the two haptophytic marine algae studied. Although Raven's dual model specifically concerns submerged aquatic plants, the differences in response to air exposure suggest that similar and dissimilar physiologies in taxonomically related and unrelated plants must play a more important role in determining carbon isotopic variability than has been acknowledged. The division of submerged aquatic plants into two categories was useful in posing predictions of two nutrient assimilation strategies, but the results of this study also indicate that a gradient exists between the two extremes.

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Appendix 1. Sample data for *Phyllospadix serrulatus* collected in Sitka, Alaska, 7-12 August 1983.

δ¹³C Leaves *	Number of Instrumental Replicates	Tidal Height (m; MLLW)	Mean Leaf Thickness (mm)	SD (±)	Number of Leaf Thicknesses Measured	Mean Epidermal Cell Wall Thickness (μ)	SD (±)	Number of Epidermal Cell Walls Measured
-14.40	4	-.88	.1308	.0131	3	1.56	.14	4
-15.24	3	-.76	.1641	.0525	3	1.46	.48	3
-14.28	3	-.70	.1942	.0016	3	.81	.09	3
-13.81	3	-.64	.2635	.0898	2	●	●	●
-15.49	5	-.58	.2306	.0202	3	1.85	.28	3
-14.02	4	-.52	.1461	.0273	3	1.95	.91	3
-14.54	5	-.34	.2000	●	1	●	●	●
-15.14	4	-.24	.1178	.0495	3	1.21	.58	3
-17.18	4	-.12	.1697	.0417	3	1.66	1.01	3
-15.33	3	+.12	.1481	.0028	2	●	●	●
-15.46	3	+.43	.1489	.0218	3	1.7	.18	3
-16.05	3	+.58	.1034	.0126	3	1.54	.32	3
-13.95	3	+.70	.0836	.0104	3	●	.54	2
-14.05	5	+.88	.1600	.0139	3	●	●	●

*sample values reported are a mean of at least three instrumental replications prepared and analyzed separately (N=52; avg. SD =±0.15).

Appendix 2. $\delta^{13}C$ data for *Phyllospadix* spp. from Cape Arago, Oregon

					Tidal Height (m; MLLW)	$\delta^{13}C$ mean for all transects	$\delta^{13}C$ SD (±)	
Transect	A	B ‡	B old leaves	B young leaves	C			Species
Date	6/85	8/84	6/85	6/85	6/85			
	-10.91	-11.15	-12.74	-11.99	-10.68	-.40	-11.49	.85	*P. torreyi*
	-11.71	-11.06	-11.92	-11.65	-11.91	-.33	-11.65	.35	*P. torreyi*
	-13.10	-14.17	-13.31	-13.10	-12.47	-.27	-13.23	.61	*P. torreyi*
	-14.70	-17.20	-14.03	-14.50	-14.14	-.21	-14.43	1.30	*P. serrulatus*
	-15.09	-12.40	-14.67	-13.73	-15.18	-.15	-14.21	1.16	*P. serrulatus*
	●	●	-14.73	-14.68	●	+.15	-14.70	.03	*P. serrulatus*
	-14.80	-16.04	●	-17.96	-14.61	+.30	-15.85	1.54	*P. serrulatus*
	-15.50	-13.54	-15.69	-15.45	-13.35	+.40	-14.95	1.51	*P. serrulatus*
	-17.23	-16.76	-16.03	●	-14.38	+.46	-16.10	1.25	*P. serrulatus*
	-14.01	-14.22	-14.63	-14.28	-15.32	+.58	-14.49	.51	*P. serrulatus*
	-14.65	-14.80	-14.33	-14.66	-13.53	+.64	-14.49	.51	*P. serrulatus*
	●	-14.06	●	-12.48	-14.44	+.70	-13.66	1.04	*P. serrulatus*
	-14.76	-14.13	-15.09	-15.81	-15.13	+.76	-14.98	.61	*P. serrulatus*
	●	●	-14.06	-13.98	●	+.82	-14.04	.06	*P. serrulatus*
	-14.39	-14.32	-13.49*		-14.34	+.94	-14.13	.43	*P. serrulatus*
	-12.23	-11.75	-11.76	-11.19	-11.70	+.94	-11.51	.33	*P. torreyi* ‡

* sample was not divided into older and younger leaf samples

✝ submerged plants. Other tide pool values reported in Appendix 3.

‡ sample values are means of at least duplicates prepared and analyzed separately (N=45; avg. SD=±0.21)

Appendix 3. δ^{13}C values of tidal pool plants.

Tidal Height
(m; MLLW) δ^{13}C

.43	-12.51
.63	-12.05
.66	-12.66
.68	-10.99
.70	-11.68
.73	-12.25
.74	-12.76

Phyllospadix serrulatus
Cape Arago,
1-3 June 1985

.70	-14.18
.88	-14.28

Phyllospadix serrulatus
Sitka, 9-10 August 1983

.45	-11.84
.66	-11.74
.73	-11.89
.94	-12.23
.94	-11.76
.94	-11.19
.94	-11.75
.94	-11.70
.94	-11.84

Phyllospadix torreyi
Cape Arago, 25-27 August
1984 and 1-3 June 1985

Appendix 4. $\delta^{13}C$ values and individual leaf thickness data for *Phyllospadix torreyi* collected 4 June 1985.

Leaf $\delta^{13}C$	Leaf Thickness (μ)	Range (μ)	Cell Wall Thickness (μ)	Range (μ)
-10.92*	410	3	1.66	.32
-11.41	482	75	1.83	.33
-13.28	348	34	2.16	.72
-12.93*	403	6	.69	.28
-12.36	478	44	1.59	.82
-11.69†	545	34	1.39	.29
-10.79	531	29	2.26	.11
-12.57	228	19	1.83	.33
-11.09*	562	61	1.56	.63

* mean of three instrumental samples; avg. s. d. =±0.22

† sample was a weight averaged composite of five sub-samples of an individual leaf

Appendix 5. $\delta^{13}C$ data for the macroalgae *Halosaccion americanum* and *Egregia menziesii*, Cape Arago, Oregon, 28-29 August 1984.

Tidal Height (m; MLLW)							
$\delta^{13}C$ SD (±)							
$\delta^{13}C$ mean for all transects							
Transect A	B	C	B*				
Date 6/85	6/85	6/85	8/84				Species
-17.77	-17.45	-17.99	-15.72	-17.23	1.03	-40	*E. menziesii*
-15.88	-15.10	-15.05	-17.24	-15.82	1.02	-33	*E. menziesii*
-14.27	-15.64	-16.73	-18.99	-16.41	1.99	-27	*E. menziesii*
-17.28	●	-15.41	-17.79	-16.83	1.25	-24	*E. menziesii*
-16.82	-16.69	-16.70	-14.55	-16.19	1.09	-21	*E. menziesii*
-19.86	-18.05	-19.99	-15.06	-18.25	2.29	-15	*E. menziesii*
-19.60	-19.70	-19.15	-16.02	-18.43	1.90	+15	*E. menziesii*
-18.92	-17.69	-17.71	-15.66	-17.49	1.35	+30	*E. menziesii*
●	-18.20	-14.32	-15.40	-16.84	1.71	+40	*E. menziesii*
-14.51	-14.06	●	-15.88	-14.82	94	+46	*H. americanum*
-13.58	-13.62	-11.63'	-14.49	-12.99	1.29	+58	*H. americanum*
-13.63	-13.13	-13.28	-13.51	-13.38	22	+64	*H. americanum*
●	-12.88	●	-13.90	-13.39	72	+70	*H. americanum*
-14.30	-14.53	-12.68	-13.31	-13.71	86	+76	*H. americanum*
-13.73	-13.57	-14.40	●	-14.00	44	+82	*H. americanum*
-13.67	-14.08	-14.01	●	-13.92	22	+88	*H. americanum*
-15.97	-14.15	●	-12.53	-14.22	1.72	+94	*H. americanum*

* sample values are means of at least two duplicates
prepared and analyzed separately (N=42; avg. SD=±0.24)

' sample is mean of two instrumental replicates
(-11.72 and -11.54) prepared and analyzed separately.

Appendix 6. Leaf thickness data for *Phyllospadix torreyi* and *Phyllospadix serrulatus*, Cape Arago, Oregon, 28-29 August 1984

	Tidal Height (m)	Mean Leaf Thickness (mm)	SD (±)	Number of Leaves Measured	Mean Epidermal Wall Thickness (µ)	SD (±)	Number of Leaves Measured
P. *torreyi*	-.40	.8467	.1108	3	2.72	1.19	3
P. *torreyi*	-.33	.4474	.0484	3	2.43	.21	3
P. *torreyi*	-.27	.3426	.1174	3	1.92	.32	3
P. *serrulatus*	-.21	.1096	.0078	3	1.19	.30	3
P. *serrulatus*	-.15	.1577	.0410	3	1.34	.36	4
P. *serrulatus*	+.30	.1339	.0222	3	1.14	.32	3
P. *serrulatus*	+.40	.1147	.0125	4	.91	0	3
P. *serrulatus*	+.46	.1488	.0046	3	1.49	.09	3
P. *serrulatus*	+.58	.0896	.0071	5	.83	.36	3
P. *serrulatus*	+.64	.0975	.0025	4	.65	.22	3
P. *serrulatus*	+.70	.0942	.0096	3	.48	.29	3
P. *serrulatus*	+.76	.0979	.0155	2	.76	.34	3
P. *torreyi*	+.94	.2094	.0087	3	1.36	.16	4
P. *serrulatus*	+.94	.0939	.0683	3	.80	.40	3

Appendix 7. Eelgrass data from Izembek Lagoon, Alaska, 21 June – 28 July 1982.

transect distance (m)	δ¹³C root	rhizome δ¹³C	leaf δ¹³C
20	-10.88	-10.12	-9.54
40	-9.89	-9.01	●
60	-8.58	-9.39	-8.79
80	-8.90	-9.18	-8.86
100	-8.16	-8.36	-7.63
120	-8.69	-8.73	-8.38
140	-8.29	-8.28	●
160	-8.38	-9.39	-7.97
180	●	-8.92	-8.43
200	-8.77	-9.34	-8.52
220	-9.05	-9.18	●
240	-8.86	-9.49	-8.06
260	-8.01	-8.46	-7.65
280	-9.21	-8.80	-8.27
300	-9.04	-9.30	-8.58
320	-9.28	-9.37	-8.56
340	-9.96	-9.24	-8.68
360	-8.50	-8.74	-8.15
380	●	-9.36	-8.25
400	-9.05	-9.38	-8.77
420	-9.24	-9.48	-8.46
440	-8.93	-9.57	-8.80
460	-9.30	-9.70	-8.31
480	-8.71	-9.43	-8.61
500	-8.95	●	-8.53
520	-8.52	-9.39	-8.05
540	-8.49	-8.68	-8.24
560	-7.91	-8.26	-7.95
580	-7.38	●	-7.53
600	-8.65	-9.24	-8.26
620	-8.28	●	●
640	-8.12	-7.53	-6.96
660	-7.61	-8.24	-7.25
680	-8.01	-8.17	-7.82
700	-7.05	-7.79	-7.51
720	-7.55	-7.46	-7.87

intertidal samples → (20)
subtidal samples ↓ (40 onward)

Appendix 8. Seagrass survey sites.

Location	Seagrasses observed	Date
Northeast Harbor, Sanak Island, 54°26'30"N, 162°35'00"W	NONE	JULY 1980 *
Caton Harbor, Sanak Island, 54°24'N, 162°32'W	NONE	JULY 1980
Pauloff Harbor, Sanak Island, 54°27'45"N, 162°41'30"W	*Zostera marina*	JULY 1980
Salmon Bay, Sanak Island, 54°23'25"N, 162°36'00"W	NONE	JULY 1980
Shoal between Sanak Island, Long Island, 54°24'N, 162°44'W	*Zostera marina*	JULY 1980
West Anchor Cove, Unimak Island, 54°41'N, 163°11'W	*Phyllospadix serrulatus*	JULY 1980 †
Tigalda Bay, Tigalda Island, 54°07'45"N, 165°00'00"W	NONE	JULY 1980
Nazan Bay, Atka Island, 52°12'N, 174°06'W	NONE	JULY 1980
Korovin Bay, Atka Island, 52°15 N, 174°27'W	NONE	JULY 1980
Bechevin Bay, Atka Island, 52°03'N, 175°06'W	NONE	JULY 1980
Portage Lagoon, Atka Island, 52°03'10"N, 175°02'30"W	*Zostera marina*	JULY 1980
Deep Bay, Atka Island, 52°06'50"N, 174°37'00"W	NONE	JULY 1980
Explorer Bay, Atka Island, 52°03'15"N, 174°32'00"W	NONE	JULY 1980
Clam Lagoon, Adak Island, 51°56 00"N, 176°34'30"W	*Zostera marina*	JULY 1980
Bogoslov Island, 53°56'N, 168°02'W	NONE	JULY 1980
Grosvold Bay, Korovin Island, 55°27'N, 160°12'W	*Phyllospadix serrulatus*	AUG. 1979 *
Voidipoini Point, Alaska Peninsula, 55°02'10"N, 162°24'30"W	*P. serrulatus, Z. marina*	JULY 1982
between Cold and Morzhovoi Bays, Alaska Peninsula, 54°56'N, 162°54'W	*P. serrulatus, Z. marina*	JULY 1982
Fossil Beach, Kodiak Island, 57°25'N, 152°20'W	*Phyllospadix serrulatus*	JUNE 1982
Kachemak Bay, between Kasitsna and Seldovia Bays, 59°28'N 151°35'W	NONE	MAR. 1982
Hanning Bay, Montague Island, 59°58'N, 147°43'W	*Phyllospadix serrulatus*	AUG. 1980 *
Zaikov Point, Montague Island, 60°19'N 146°55'W	*Phyllospadix serrulatus*	JUNE 1972 *
Kirushkin Island, Sitka Sound, 57°02'40"N, 135°23'00"W	*Phyllospadix scouleri*	AUG. 1983
Watson Point, Sitka Sound, 57°04'00"N, 135°21'50"W	*Phyllospadix serrulatus*	AUG. 1983
Crane Cove, Baranof Island, 56°50'55"N, 135°22'30"W	*P. serrulatus, Z. marina*	AUG. 1979 *
White Cliff Island, Davidson Inlet, 55°59'30"N, 133°29'00"W	*Phyllospadix serrulatus*	AUG. 1975 *
Cape Blanco, Oregon, 42°50'N, 124°33'W	*P. serrulatus, P. torreyi*	JUNE 1985 †

* observations made by C.P. McRoy and others.
† western range extension ‖ *P. serrulatus*
‡ southern range extension ‖ *P. serrulatus*