Short title:

Nitrogen and phosphorus for *Thalassia*

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Abstract

Yield-nutrient supply correlations indicate that significant amounts of phosphorus and virtually all nitrogen for growth of *Thalassia* are taken up from the sediments, and that growth of *Thalassia* is generally limited by the availability of nitrogen. Considerations of supply and demand suggest that the sediments could not be a primary source of phosphorus for *Thalassia*. However, it appears that the sediments may act as a sort of 'storage bank' for phosphate taken up from the sea water by *Thalassia*. Various evidence, including acetylene reduction assays, indicate that nitrogen for *Thalassia* is derived exclusively from N_2 fixation by anaerobic bacteria in the rhizosphere. Eh measurements and general observations indicate that a reduced root layer is essential for normal development of *Thalassia*; it is believed that this is associated with requirements of N_2-fixing bacteria, and with greater overall efficiency of the *Thalassia*-sediment N_2-fixing system under anaerobic conditions.

McGill University

THE ORIGIN OF NITROGEN AND PHOSPHORUS FOR GROWTH OF THE MARINE ANGIOSPERM THALASSIA TESTUDINUM KÖNIG

by

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CONTRIBUTION TO ORIGINAL KNOWLEDGE

Numerous studies have demonstrated high rates of primary production by the tropical marine angiosperm Thalassia testudinum König. The present study is a contribution to original knowledge in that it is an examination of some of the processes underlying, or necessary for, the high productivity of Thalassia. Evidence is presented that nitrogen for growth of Thalassia is derived exclusively from gaseous nitrogen fixed by anaerobic bacteria in the rhizosphere. It had previously been unsuspected, that such a phenomenon might be important in the ecology of aquatic plants.

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I. INTRODUCTION

Purpose

Rates of primary production by the tropical marine angiosperm Thalassia testudinum König rank amongst those of the most highly productive natural communities (Westlake, 1963). The question of basic concern to this study was: how are high rates of production maintained in the notably nutrient-poor (Smith, Williams and Davis, 1950; Williams, 1954; Beers, Steven and Lewis, 1968) tropical waters? Wood (1965) remarked that the marine angiosperm Zostera "seems to be confined to areas where sulphide is present, possibly because of the release of phosphate brought about by sulphate reduction." Marine angiosperms have extensive underground root-bearing stems, and it seems logical to suppose that they have direct access to nutrients in the sediments. However the question of whether roots of submerged vascular plants are functional in nutrient uptake has long been a subject of controversy (Scultborpe, 1967). The first conclusive experimental evidence of nutrient uptake by roots of a submerged angiosperm was reported in 1970 for Zostera (McRoy and Barsdate, 1970). The present study, which began in 1968 at the Bellairs Research Institute of McGill University in Barbados, is essentially an investigation of three questions: (1) does Thalassia obtain significant quantities of nitrogen and phosphorus from the sediments, (2) what is the origin of nitrogen and phosphorus in the

sediments, and (3) what is the influence of reducing conditions in the sediments on the availability of nitrogen and phosphorus?

Vegetative morphology and anatomy of Thalassia

The vegetative anatomy of Thalassia has been described in detail by Tomlinson and Vargo (1966), and a good description was given by Phillips (1960). Root anatomy was described by Tomlinson (1969a) and leaf anatomy, by Sauvageau (1890). The following is a brief description of the vegetative morphology and anatomy based on these studies. Descriptions of floral morphology and anatomy have been given by Tomlinson (1969b), and of floral morphology, fruit development and structure, seed anatomy and germination, by Orpurt and Boral (1964).

Thalassia is a monocotyledonous angiosperm belonging to the family Hydrocharitaceae. Essentially the Thalassia plant consists of a creeping rhizome, from which arise at more or less regular intervals, erect shoots bearing broad, flat, foliage leaves (Fig. 1, Plates I, II). Normally only the leaves are observed above the substrate. Non-assimilatory scale leaves occur on the rhizomes. The erect shoots are separated by a variable number of internodes, usually 9 to 13. Tomlinson and Vargo (1966) interpreted the erect shoots as lateral branches of the monopodial long shoot or rhizome. Foliage leaves are borne apically on the erect shoot; flowers arise laterally, and do not interrupt growth of the erect shoot. Thalassia produces no dormant buds, and growth of the rhizomes and erect shoots is apparently continuous. Tomlinson and Vargo (1966) observed, "Continuous growth of the organism is entirely dependent on activity of vigorous rhizome apices. No residual meristems are left behind so that adventitious growth, even of roots, is impossible." Tomlinson and Vargo (1966) described the rhizomes

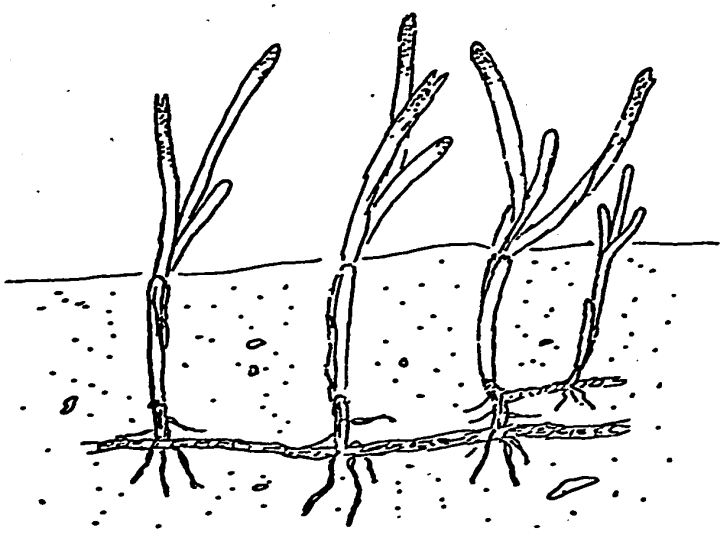

Fig. 1. Habit sketch of Thalassia. Old parts of leaves are encrusted with calcareous algae.

and erect shoots as "two seemingly unlike, but fundamentally similar, axes with different directions of growth producing entirely different kinds of leaf. The axes are autonomous. One kind cannot be converted directly into another, but only reciprocally by branching." Branching of the rhizome (to produce erect shoots) occurs regularly, but branching of the erect shoots (to produce rhizomes), only irregularly. With age, the attachment of the erect shoot to the rhizome weakens and the erect shoot breaks off. Tomlinson and Vargo (1966) remarked that Thalassia adjusts to an eroding substratum by downward growth of the rhizome, and adjusts to silting by upward growth of the rhizome, and by branching of the erect shoots.

Both the rhizomes and erect shoots bear roots. These are 5 to 10 cm in length and are densely covered with root hairs. Roots may be borne at every node on the rhizome, but they are usually most abundant (three to five) at the node bearing the erect shoot. They are borne irregularly on the erect shoot, commonly occurring at a frequency of about one per five nodes.

Foliage leaves are about 1 cm in width (range 0.3 to 1.8 cm). There are no stomata on the leaves, and only a thin cuticular layer. The mesophyll is homogeneous (no differentiation into palisade and spongy layers). Chloroplasts occur in both epidermal and mesophyll cells. Submerged angiosperms in general are characterized by very little lignin, even in vascular tissues. However the marine angiosperms are exceptional in having well lignified fibres. In Thalassia leaves these occur in longitudinally oriented bundles in a subepidermal position. These are believed to provide the strength necessary to withstand wave action (Sculthorpe, 1967).

Air filled lacunoate tissue occupies most of the leaf mesophyll and middle cortex of the shoots and roots. This is the 'spongy tissue' very characteristic of aquatic plants. The longitudinally oriented lacunae are

interrupted at intervals by perforated transverse diaphragms; these are believed to oppose strain exerted at right angles to the long axis, and to function in the prevention of waterlogging (Sculthorpe, 1967). The lacunae impart buoyancy to the various parts of the plant. The gases of the lacunae of several submerged angiosperms have been analyzed and found to contain N_2, O_2, and CO_2 (Sculthorpe, 1967). Studies by Laing (1940b) and Hartman and Brown (1967) have shown that the lacunal system functions as a reservoir for metabolic gases. Because of this, the latter authors questioned the reliability of estimating oxygen production of submerged angiosperms from changes in the dissolved oxygen content of the surrounding water.

Nine to 16 parallel veins are oriented lengthwise along the leaf. In the shoots and roots, vascular tissue is contained in a central stele. With the exception of the root, there are no detailed descriptions of vascular anatomy. In general the aquatic angiosperms are characterized by a reduced vascular system, particularly with respect to the xylem. The Hydrocharitaceae as a family is characterized by a complete lack of vessels (Cheadle, 1942), and a strong tendency towards reduction in the tracheal system (Arber, 1920). Tomlinson (1969a) observed that roots of Thalassia "have reached an extreme in xylem reduction because tracheids are absent from most of the root." Anatomy of the root is further discussed below under 'Nutrient uptake by submerged angiosperms'.

General ecology of Thalassia and other marine angiosperms

Thalassia testudinum is one of about 40 species of plants which constitute, in the terms of Arber (1920), the undemocratic, narrow and exclusive circle of the Marine Angiosperms. Although the fresh water aquatic angiosperms exhibit a great variety in habit and habitat, and it appears

"that the aquatic habit has been acquired by many unrelated angiosperms during the history of this group" (Sculthorpe, 1967), the marine angiosperms are all monocotyledons of the closely related families Hydrocharitaceae and Potamogetonaceae. They exhibit little variety in habit and habitat (Arber, 1920), and are believed to be of relatively ancient, Tethysian, origin (Setchell, 1935). Both of the families to which they belong are typically aquatic, and include a number of fresh water members. The marine angiosperms are all hydrophyllous and restricted to salt water (Sculthorpe, 1967). They are primarily tropical and subtropical in distribution, with only Zostera marina extending into cold boreal zones (Setchell, 1935).

Six species of marine angiosperms occur in the Caribbean; three species, Thalassia testudinum, Syringodium filiforme Kutzing, and Diplanthera wrightii (Ascherson) Ascherson are commonly encountered. Thalassia is readily distinguished by its broad, flat leaves and robust rhizome. Diplanthera has narrow (1 to 2 mm), flat leaves, and Syringodium, terete leaves. The rhizomes of the latter two species are thinner than that of Thalassia, and erect shoots are borne at each node. These three species are generally found in depths of less than 10 m, and only Diplanthera commonly occurs above the low water level (D.R. Moore, 1963). Thalassia appears to be restricted to areas where the salinity does not exceed 40 °/oo or go below 20 °/oo for long periods of time (D.R. Moore, 1963). Syringodium appears to have approximately the same salinity tolerances (Phillips, 1960). Diplanthera tolerates somewhat lower salinities (Phillips, 1960), and is the most tolerant of hypersaline conditions (McMillan and Mosely, 1967). Observations by Phillips (1960) and D.R. Moore (1963) suggest that Diplanthera survives mainly in very shallow water and hypersaline areas which are unfavorable for development of Thalassia and Syringodium. Syringodium is most commonly found

in mixed stands with Thalassia (Humm, 1956; Phillips, 1960). Welch (1965) observed that initial colonization of the substrate by the sea grasses varies with substrate stability. Diplanthera occurs on shifting sand, Syringodium, on sand of intermediate stabilities, and Thalassia, only on stable substrates; Thalassia tends to replace the other grasses, however, and thus to become "the dominant of the terminal submerged community." The studies of Tomlinson and Vargo (1966) indicate that "Thalassia is not well suited to asexual propagation by isolated fragments." Thus sexual reproduction must be the principal means by which Thalassia becomes established in areas not adjacent to previously existing Thalassia stands. Flowering of Thalassia, but not of other Caribbean marine angiosperms, has been commonly observed (Phillips, 1960). Open flowers have been observed from April to September (Phillips, 1960; Orpurt and Boral, 1964; Tomlinson, 1969b). The plants are dioecious and usually less than 10% of the plants are observed flowering at any one time (Phillips, 1960). Orpurt and Boral (1964) made observations on fruit development and seed germination. Fruits float, thus "affording an excellent means of dispersal." At maturity the fruits dehisce, and embryos, released from the seed coats, sink. Root hairs develop within 3 days from the lower side of the embryo, anchoring the plant to the substrate. Plumules may begin to emerge before opening of the fruit. Following anchorage to the substrate, a young rhizome emerges. The effectiveness (over short periods of time) of sexual reproduction in establishing Thalassia in new areas is unknown. The other three species of Caribbean marine angiosperms, Halophila baillonis Ascherson, Halophila engelmanii Ascherson and Halophila aschersonii Ostenfeld, are small, delicate forms with flat, oval leaves. These species are generally found in deeper water than the above species (Thorne, 1954).

In general, the marine angiosperms are confined to areas that are protected from violent wave action (Setchell, 1920), and they typically form extensive 'meadows' in fiords, bays, and lagoonal areas behind reefs and barrier beaches. The sea grasses are of major importance in these areas in three respects: (1) in modifying sedimentary processes, (2) as habitats of many invertebrates and fishes, and (3) as major primary producers. Ginsburg and Lowenstam (1958) noted that sea grasses stabilize sediments by growth of their rhizomes, and that the leaves slow down water motion just above the bottom. These conditions may lead to increased sedimentation in the sea grass beds, and sea grass beds are commonly observed to be elevated above the surrounding grass-free areas (Bernatowicz, 1952; Moulinier and Picard, 1952). Under certain conditions, increased sedimentation in the sea grass beds may lead to development of salt marshes (King, 1959) or mangrove communities (Welch, 1965). Thalassia bound sediments have been observed to be exceptionally resistant to hurricane damage (Thomas, Moore and Work, 1961; Ball, Shinn and Stockman, 1967). The grass blades themselves are habitats for many small invertebrates (Kikuchi, 1966; Nagle, 1968), and for large and small algal epiphytes (Humm, 1964). Numerous invertebrates and fish spend all or parts of their lives in sea grass beds (Petersen and Jensen, 1911; Petersen, 1918; Stephenson et al., 1931; Voss and Voss, 1955; MacNae and Kalk, 1962; Randall, 1963; Stephens, 1966; Kikuchi, 1966; O'Gower and Wacasey, 1967; Taylor and Lewis, 1970). Studies by Randall (1963) and Starck and Davis (1966) have shown that the Caribbean sea grass communities are the nocturnal feeding grounds of many reef dwelling fishes. High rates of primary production by sea grasses have been indicated by the classical study of Petersen (1914) on Zostera, and numerous studies on Thalassia (Odum, 1957; Odum and Hoskin, 1958; Odum, Burkholder and Rivero, 1959; Odum

and Wilson, 1962; Odum, 1963; Jones, MS, 1968; Zieman, MS, 1968). Odum (1957) estimated gross oxygen production of a Thalassia bed in the Florida Keys as 34 g O_2/m^2 per day, and Odum et al. (1959) estimated oxygen production of Thalassia beds in Puerto Rico as 8 to 15 g O_2/m^2 per day. Assuming, as in Westlake (1963), a photosynthetic quotient of 1.20 to 1.25 and a net-to-gross ratio of 0.45 to 0.50, these estimates are equivalent to net production of approximately 1 to 5 g C/m^2 per day. Zieman (MS, 1968), by direct measurement of the growth of leaves, estimated production of leaf tissue at two plots in Biscayne Bay, Florida, as 4.5 and 6 g leaf tissue/m^2 per day; these estimates are equivalent to production of 1.6 and 2.1 g C/m^2 per day, assuming 25% ash content (Burkholder, Burkholder and Rivero, 1959) and a carbon-to-organic matter ratio of 0.46 (from data of Brandt and Ruben, 1920, for Zostera). Except in areas near the geographical limits of Thalassia where there may be seasonal fluctuations in production (Phillips, 1960), high rates of production are maintained throughout the year. According to Odum (1959) and Westlake (1963), production rates of Thalassia rank amongst those of the most highly productive natural communities. Westlake's (1963) summary of primary production data of various natural and agricultural systems is given in Table I. These values are "the organic productivities that are likely to be obtained from vigorous communities under conditions close to the best attainable within the limits set by natural weather and economics." Few of the animals living in, or feeding in, sea grass beds feed directly on the sea grasses (Randall, 1965; Kikuchi, 1966). Even of those that do feed on sea grasses, there appear to be no studies made of the extent to which they utilize cellulose, the main constituent of the sea grasses. Burkholder et al. (1959) determined the proximate composition of Thalassia leaves and the amounts of different amino acids, and studied the

Table I. Probable annual average net primary productivity of fertile sites (from Westlake, 1963).

Approximate organic productivity (m.t./ha. year)	Range %	Climate	Type of ecosystem	Notes
1	50	arid	desert	much more if hot and irrigated
2	50	-	ocean phytoplankton	-
2	50	temperate	lake phytoplankton	little influenced by man
3	50	-	coastal phytoplankton	probably more in some polluted estuaries
6	50	temperate	polluted lake phytoplankton	agricultural or sewage drainage
6	20	temperate	freshwater submerged macrophytes	-
12	25	temperate	deciduous forest	-
17	25	tropical	freshwater submerged macrophytes	-
20	25	temperate	terrestrial herbs	possibly more if grazed
22	15	temperate	agriculture-annual plants	-
28	25	temperate	coniferous forest	-
29	15	temperate	marine submerged macrophytes	-
30	20	temperate	agriculture-perennial plants	-
30	20	tropical	agriculture-annual plants	including perennials in continental climates
30	20	-	salt marsh	-
35	15	tropical	marine submerged macrophytes	including coral reefs
38	20	temperate	reedswamp	-
40	15	sub-trop.	cultivated algae	more if carbon dioxide supplied
50	20	tropical	rain-forest	-
75	15	tropical	agriculture-perennial plants, reedswamp	-

growth of marine bacteria on leaf extracts. They observed that the most abundant amino acids are those best used by common marine bacteria, but considered that the relatively low content of tryptophan "may represent a weak nutritive element in the direct use of Thalassia proteins by fish life in tropical waters." Both Burkholder et al. (1959) and Kikuchi (1966) were of the opinion that invertebrates and fishes feeding on sea grasses may be utilizing the attached microorganisms rather than the grass itself. Detrital food chains are probably a major link between primary production of higher trophic levels (Fenchel, 1970).

Nitrogen, phosphorus and the redox potential of sediments

In initial considerations of the question of the origin of nitrogen and phosphorus for growth of Thalassia, it was thought that the sediments might contain relatively large amounts of these nutrients derived from (1) decomposition of organic matter deposited in the sediments, (2) nutrients taken up by biochemical or physical-chemical processes at the sediment surface, and (3) primary sources, such as phosphorus-containing minerals (of terrigenous origin or precipitated from sea water). Relatively little attention has been given to the nutrient content of marine sediments and the processes involved in exchange of nutrients between the sediments and sea water. However, studies on the sediments of estuaries (Moore, 1930, 1931; Stephensen, 1949; Rochford, 1951; Carritt and Goodgal, 1954; Jitts, 1959; Pomeroy, Smith and Grant, 1965), of lakes (for example Mortimer, 1941-1942; Hayes and Phillips, 1958; Harter, 1968), of shallow marine bays (Oppenheimer and Ward, 1963), and of marine basins (Miller, 1952; Rittenberg, Emery and Orr, 1955) have indicated relatively large quantities of nitrogen and phosphorus in the sediments derived from the above mentioned sources. A

recent study (Howard et al., 1970) has indicated significant rates of N_2 fixation in lake sediments. N_2-fixing bacteria have been isolated from marine sediments, but their significance in the nitrogen cycle is unknown (Wood, 1965).

Two of the most important parameters determining the forms and availabilities of nitrogen and phosphorus in soils and sediments are the pH and Eh. In waterlogged soils and stabilized sediments of aquatic systems, the latter is particularly subject to change from the norm of the biosphere because of the reduced availability of oxygen. Problems of measuring and interpreting Eh in natural environments have been discussed by Pearsall (1938), Pearsall and Mortimer (1939), ZoBell (1946a), Emery and Rittenberg (1952), Baas Becking, Kaplan and Moore (1960) and Garrels and Christ (1965). The reactions responsible for observed redox potentials in natural environments are not well understood, but they seem to involve both reversible and irreversible systems, and only those systems which react rapidly. Thus Eh readings of natural systems do not represent equilibrium conditions in the sense required by oxidation-reduction theory. Never the less, Eh Measurments are useful as empirical parameters of natural environments and calculations using observed Eh values and oxidation-reduction theory are useful as qualitative or semiquantitative information. Because of the unpredictable manner in which Eh varies with pH in complex systems, it is usual to express the pH at which Eh readings are made. Some workers, however, adjust the Eh values to a common pH by using an Eh/pH factor of -58 to -60 mv per pH unit. Studies on waterlogged soils and lake muds (Pearsall, 1938: Pearsall and Mortimer, 1939) rice paddy soils (Aomine, 1962) and experimental soil systems (Patrick, 1961) indicate that an Eh_5 (Eh at pH 5) of approximately 350 mv is critical for the existence of nitrate. Above this

value, nitrate accumulates, while below this value, existing nitrate disappears through denitrification (reduction to gaseous nitrogen compounds), and ammonium is not nitrified. Systems in which both oxidizing and reducing conditions exist are believed to be most susceptible to loss of nitrogen through denitrification (Aomine, 1962). Below Eh_5 320 to 350 mv, other products of reduction including manganous manganese, ferrous iron and sulfide also appear, and thus potentials below an Eh_5 of 320 mv are considered as "effectively reducing" and potentials above 350 mv, as "predominantly oxidizing" (Pearsall, 1938). Pearsall and Mortimer (1939) observed that relatively low oxygen concentrations (in the region of 8% saturation) are sufficient to maintain predominantly oxidizing conditions. Oxygen is believed to control the Eh of oxidizing environments, but the reactions involved are not understood, and "Variation in the molecular oxygen content of water appears to have no direct influence on the electrode potential, except at very low oxygen tensions" (Baas Becking et al., 1960). Although a number of reactions might determine the Eh of reducing environments, Eh values of less than -50 to -100 mv in estuarine and marine sediments appear to be determined largely by the presence of H_2S produced by sulfate-reducing bacteria (Baas Becking and Wood, 1955; Berner, 1962). The activities of other bacteria and oxidation states of inorganic compounds reflect rather than determine the redox potential in these conditions (Baas Becking and Wood, 1955; Baas Becking et al., 1960). For marine and estuarine sediments and sulfate reduction cultures of Eh less than about -50 to -100 mv, Berner (1962) found that pE-Eh measurements tended to fall along the curve representing the half cell

$$HS^- \rightleftharpoons S^o + H^+_{aq} + 2\,e$$

Sulfide in marine sediments is believed to be derived largely from sulfate reduction rather than from organic sulfur compounds (Kaplan, Emery and Rittenberg, 1963). From studies of enrichment cultures and field observations, Baas Becking and Wood (1955) reported growth of Desulfovibrio at redox potentials as high as Eh_7 +110 mv. Postgate (1959), on the other hand, claimed that for rapid growth in pure cultures, an initial Eh of -200 mv (pH 7 to 7.5) is required. Wood (1963) remarked, "Postgate's previous work on the synergic growth of aerobic and anaerobic contaminants suggests that the apparent discrepancy between these results may be due to the reducing capacity of these contaminants." Low pH of marine sediments under anaerobic conditions is often attributed to activities of sulfate-reducing bacteria. Berner (1966) attributed low pH of anaerobic sediments in Florida Bay containing "scattered grass remains" to production of H_2S and CO_2 by sulfate reducers. He estimated that partial pressures of CO_2 were up to 300 times the value for the overlying water, and high partial pressures of H_2S were also indicated.

Of particular interest to the present study was the remark of Wood (1965), based on the work of Wood (1953), Baas Becking and Wood (1955) and Baas Becking and MacKay (1956), that "Zostera seems to be confined to areas where sulphide is present possibly because of the release of phosphate brought about by sulphate reduction." Wood (1953) showed that the sea grasses Zostera and Posidonia excrete reducing substances from their leaves, including a sulfur compound and a compound with a nitrogenous base. Baas Becking and Wood (1955) noted that a number of other estuarine plants including the algae Ulva, Enteromorpha, Fucus and Polysiphonia had been found to excrete reducing substances (sulfonium salts and their derivatives such as dimethyl sulfide). These compounds stimulate the activity of sulfate

reducers by lowering the redox potential. Baas Becking and Wood (1955) observed, "Sea sand covered with a layer of these organisms will show sulphate-reduction within 12 hours at room temperature. It is no wonder then that both Zostera and Enteromorpha are constant companions of the sulphate reducers." Baas Becking and Mackay (1956) observed that treatment of suspensions of ferric phosphate, or of calcium orthophosphate and iron oxides, in sea water with H_2S results in an almost instantaneous release of phosphate, precipitation of ferrous sulfide, and increase in acidity. They believed that the concentration of inorganic phosphate in estuarine waters is closely related to the H_2S content of the water. It should be noted that a significant proportion of ferric iron may exist in ferrous form at redox potentials well above these required for sulfate reduction, and thus sulfate reduction per se should not be required for release of phosphate from ferric phosphate. However, in some systems, sulfide from sulfate reduction is believed to be the agent reducing ferric iron (Golterman, 1967). Also precipitation of ferrous iron as FeS effectively prevents diffusion of iron to oxidized zones where it might again precipitate phosphate as ferric phosphate. The influence of redox potential on the solubility of iron phosphates in lakes was recognized by Mortimer (1941-1942), and the phenomenon of increased availability of iron phosphates under reducing conditions in rice paddy soils is well known (Black, 1968). Production of bicarbonate by sulfate-reducing bacteria is another mechanism by which these bacteria may influence the phosphate concentration of marine sediments. The presence of excess bicarbonate would be expected to depress the concentration of calcium ion in solution in accordance with equilibrium in the reaction

$$CaCO_3(s) + H_2CO_3 \rightleftharpoons Ca^{++} + 2HCO_3^-$$

and thus allow a higher concentration of phosphate in solution in equilibrium with solid phase calcium phosphates (Kaplan and Rittenberg, 1963). Several studies have shown that the calcium ion concentration is depressed under reducing conditions in marine sediments (Berner, 1966; Presley and Kaplan, 1968; Brooks, Presley and Kaplan, 1968). Brooks et al. (1968) found a high degree of correlation ($r = -0.94$) between calcium and phosphate in interstitial waters of some marine sediments; concentrations of phosphates as high as 260 µg-at/l were observed. Studies by Rittenberg et al. (1955) also indicated high concentration of interstitial water phosphate or adsorbed phosphate in marine sediments under reducing conditions.

Nutrient uptake by submerged angiosperms

Marine angiosperms differ from other marine benthic plants in having extensive underground root-bearing stems. Thus it seemed logical to suppose that these plants would have direct access to nutrients in the sediments. However the question of whether any submerged hydrophyte takes up significant quantities of nutrients from the sediments has long been a subject of controversy (reviewed by Arber, 1920; Sculthorpe, 1967). Observations of reduction in the vascular system of hydrophytes, together with the demonstration of relatively free movement of ions between water and leaves, and the contradictory and equivocal results of physiological experiments designed to demonstrate absorption of nutrients by the roots and a flow of water in the vascular tissue, led many workers to conclude that roots of submerged hydrophytes serve mainly for anchorage. Tomlinson (1969a) observed that xylem is absent from most of the Thalassia root. However he also observed the existence of "a continuous pathway from the surface of the root to the stele, largely via cells with conspicuous nuclei, dense cytoplasm, and extensive pit connections"; he was prompted to remark, "The

possibility that this is the pathway for the selective absorption of ions or even large molecules has to be considered." Both Arber (1920) and Sculthorpe (1967) favored the point of view that roots may be important as organs of absorption. The latter author was particularly impressed with the presence of a well developed endodermis in aquatic plants. Sculthorpe (1967) remarked, "The frequent presence and fine development of the endodermis in aquatic roots in all manner of habitats contrast strongly with the trend towards structural reduction in the stele and cannot be dismissed as mere ancestral features. In view of the probable importance of the endodermis in regulating lateral movement of water and ions in terrestrial roots, it is difficult to reconcile the prominence of the endodermis in aquatic roots with the notion that absorption and transport do not occur."

With respect to the question of whether a sustained flow of water occurs in submerged plants, Sculthorpe (1967) remarked, "The only conclusion that can be safely drawn from the conflicting experimental data is that whilst a transpiration current of the type occurring in aerial organs clearly cannot exist in submerged plants, a transient flow of water and dissolved salts might occur, motivated either by exudation pressure in the root or shoot or by a gas-flow generated during periods of active photosynthesis." Odum (1967) noted that the sediment around roots of Thalassia "is strangely dry when brought to the surface, suggesting some water-using role by the plant roots." A serious objection to postulating a mass transport of water through plants such as Thalassia is the lack of structures, at least in intact young leaves, through which water could pass out of the plant (Sculthorpe, 1967). For halophytes, these questions are complicated further by the question of what happens to the dissolved salts. Scholander et al. (1962) found that the xylem sap of many mangroves has a salt content close

to that of fresh water. From experiments conducted with a variety of halophytes, including the marine angiosperm Enhalus acoroides, Scholander (1968) concluded, "xylem sap formation by roots in mangroves and other halophytes involves essentially an ultrafiltration of the sea water combined with an ion transport."

Sculthorpe (1967) saw particular promise in the techniques of Frank and Hodgson (1964). They devised a method of partitioning a container such that the medium surrounding roots and rhizomes could be separated from that surrounding the leaves, and were able to show uptake at the roots and some acropetal translocation of a ^{14}C labelled herbicide by Potamogeton pectinatus. The technique was applied by McRoy and Barsdate (1970) to a study of phosphate uptake by Zostera using ^{32}P. These workers observed that ^{32}P was taken up by the roots of Zostera, and thus provided the first direct evidence of nutrient uptake by roots of a submerged angiosperm. McRoy and Barsdate (1970) observed that phosphate was taken up by both roots and leaves, was rapidly translocated, and that uptake was greatest in the light. Some of the phosphate taken up by the roots was excreted by the leaves, and some of the phosphate taken up by the leaves was excreted by the roots. Field studies confirmed that phosphate is taken up by roots of Zostera in situ. Studies utilizing ^{32}P by Dr. Paul Burkholder of the Department of Marine Sciences, University of Puerto Rico, (personal communication, 1970) indicate that Thalassia is also able to take up phosphate by both roots and leaves. Parker (1966) observed that radioisotopes of the trace metals cobalt, manganese, iron, and zinc were rapidly taken up by leaves of Thalassia (or its epiphytes) in situ. Sodium-22, however, was not taken up by leaves.

When this work was initiated in 1968, it was unknown whether roots of marine angiosperms are functional in nutrient uptake. Thus to answer the

question, 'does Thalassia obtain significant quantities of nitrogen and phosphorus from the sediments?', it was of fundamental importance to determine whether roots of Thalassia are able to take up nutrients. Initially, an experimental investigation of this question was considered. It was reasoned that if it could be demonstrated that Thalassia roots are functional in nutrient uptake, then presumptive evidence of uptake of significant quantities of nutrients would be provided by demonstrating the existence of relatively large quantities of nutrients in the sediments. A different approach was adopted following recognition that marked differences in growth rate of Thalassia leaves occurred between Thalassia stands at the same depth, in the same water mass, and superficially at least, under the same sedimentary conditions (with respect to sediment grain size, mineralogy, and organic matter). These differences were suggested by pronounced differences in leaf length between adjacent stands. Large differences occurred in only a few areas, but these suggested that the lesser variations in leaf length between various stands might be associated with different growth rates and not with variation in wave action, epiphytism, or with normal genetic variation of the plant. Measurements of growth rates by Zieman's (MS, 1968) technique of stapling leaves confirmed that differences in leaf length reflected differences in growth rate, and provided empirical relationships for estimating growth rate and production from easily obtained leaf statistics. These studies are described in Appendix A of this thesis. Differences in growth rate of Thalassia under uniform conditions of turbidity and sea water nutrients suggested that one or more nutrient was obtained from the sediment, and that differences in the growth rate of Thalassia were due to differences in the supply of the nutrient or nutrients in the sediment. Investigation of nutrient uptake by Thalassia thus follows, in part, classical applications

of Liebig's 'Law of the Minimum' to agricultural problems (see Russell, 1950); it is assumed that if there exists a correlation between yield of Thalassia and some measure of nitrogen or phosphorus in the sediments, then this indicates that the nutrient with which yield is correlated is obtained from the sediments (via the roots), and is limiting.

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II. THE PRODUCTION-NUTRIENT CORRELATIONS AND THE NUTRIENT STATUS OF THE SEDIMENTS

MATERIALS AND METHODS

The sampling sites

Unless specified otherwise, results are based on samples and observations from Thalassia beds at Bath on the east coast, and St. Lawrence and Oistin Bay on the south coast, of Barbados. Location of each stand (B = Bath, S = St. Lawrence, O = Oistin Bay), approximate depth of the stand below mean low water, and the substrate type are given in the tables of results. Substrates are classified into 5 types: cobble framework (CF), mixed cobble and sand (CS), predominantly sand (PS), Porites rubble flats (PF), and an intermediate type between the CF and CS types, designated CCS. Descriptions of the various substrate types, and general characteristics of the Thalassia beds are given in Appendix B.

Estimation of leaf tissue production

At each stand for which leaf tissue production was to be estimated, a sample of leaves was cut at substrate level from a 3/16 m^2 area, and the following statistics taken: (1) L_{10}, the average length of the longest 10 leaves, (2) w, the average leaf width, estimated from a subsample of 30 or more leaves, and (3) the wet weight of the sample. The wet weight included epiphytes on Thalassia. If both Thalassia and Syringodium were present, they were separated and the wet weight determined for each. Production of leaf tissue (Thalassia only) per square meter, and production of leaf tissue per shoot were estimated by use of empirical relationships (Appendix A) between production and standing crop, and between growth rate and the maximum length

of leaves in a stand:

$$P_m = 0.0037\ SC$$

$$I_s = 0.0735\ L_{10} - 0.322$$

where P_m is the production per m^2 (g dry wt leaf tissue/m^2 per day), SC is the standing crop of Thalassia leaves and epiphytes (g wet wt/m^2), I_s is the average growth rate of the leaves expressed in terms of the total length of leaf tissue produced per shoot (cm/day), and L_{10} is in cm. P_s, the production of leaf tissue per shoot (mg/shoot per day) was estimated as:

$$P_s = I_s \times w \times c$$

where w is in cm and c is the dry weight of epiphyte-free leaf tissue (mg/cm^2). c varies with the average width of the leaves and is given by:

$$c = 3.38 + 1.43\ w$$

P_s and P_m estimates are believed to be accurate within limits of approximately $\pm 15\%$. A rough estimate of the number of shoots per m^2 may be obtained by dividing P_m by P_s. L_{10} values are reported for each stand. It should be noted that variations in P_s of the Barbados stands were determined mainly by variations in the growth rate (rate of elongation) of the leaves, rather than by variation in the leaf width. Dry weight of leaf tissue may be converted to organic matter by assuming an ash content of 20.1% (average of 9 samples from Barbados, range 19.7 to 22.0; analyzed by Mr. George Lilly of the Bellairs Research Institute, personal communication, 1970), and to organic carbon by assuming a carbon-to-organic matter ratio of 0.46 (from data given by Brandt and Ruben, 1920, for Zostera).

Sampling and analysis of plant tissues

Leaves and rhizomes from 13 stands were analyzed for nitrogen and phosphorus for the purpose of estimating requirements of Thalassia for nitrogen and phosphorus, and to determine if there were any correlations between yield and tissue nutrient levels.

Field sampling was done under conditions of maximum light intensity, that is between 1000 and 1400 hr when low tide occurred in this interval (turbidity was lowest at low tide), and only on days of little or no cloud cover. Relative growth rates of the stands could be estimated in the field by visual comparison of the maximum leaf lengths of the stands, and stands were selected such that a wide range of growth rates would be represented. At each stand leaves were cut at substrate level from a 3/16 m^2 area for estimation of P_s and P_m as described above. For tissue analyses, samples of leaves, shoots and rhizomes were removed from the substrate in as nearly intact condition as possible. Epiphyte-free, green (i.e. from above the sediment surface) portions of leaves were cut off and placed on absorbent paper to remove excess water. One portion of this material was retained for drying. A second portion was put in a 200 ml polyethylene bottle containing 100 ml frozen distilled water and subsequently frozen until analysis. Rhizomes were stripped of roots and similarly treated.

Samples of leaves and rhizomes dried at 70°C were analyzed for total nitrogen and total phosphorus. Total nitrogen was determined by a standard semimicro-Kjeldahl method (Bremner, 1965a) using a "Quickfit" steam distillation apparatus instead of the Hoskins apparatus. Samples for the determination of total phosphorus were combusted at 240°C to convert organic phosphorus to inorganic form, and the phosphate was extracted with hot HCl and measured by an absorptiometric technique (Olsen and Dean, 1965).

Duplicate analyses varied by less than 5%.

Each bottle containing a sample of frozen leaves or rhizomes was weighed to obtain the wet weight of the sample by difference from the predetermined weight of the bottle and contained water. The sample was thawed, macerated (in the water from the container) and filtered. The extract was analyzed for phosphate, nitrate, nitrite and ammonium by absorptiometric techniques described by Strickland and Parsons (1965). Applicability of these techniques to plant extracts was checked by adding 1 µg-at/l amounts of the nutrients to plant extracts on which the 'blanks' (equivalent to 1 µg-at/l or less) had been determined, and determining the recovery of the added nutrients. A recovery of 85% was considered acceptable. This method of determination of ammonium-N (oxidation of ammonium to nitrite by alkaline hypochlorite and determination of nitrite) determines also some of the nitrogen present in amino acids. To estimate what fraction of the nitrogen so determined originated from amino acids, a concentrated extract was steam distilled over magnesium oxide, a technique which does not release alkali-labile organic nitrogen (Bremner, 1965b). A direct method which does not determine amino acid-N (Solórzano, 1969) was unapplicable to these extracts.

Sampling and analysis of interstitial waters and sediments

Interstitial water samples were taken from approximately the middle of the root layer of 16 stands. The root layer includes that part of the sediment profile occupied by root-bearing tissues (shoots and rhizomes). At each of 6 stands, a series of interstitial water samples from the sediment surface to the bottom of, or below, the root layer was taken. Sediment samples were taken from selected stands. Leaves were removed from a 3/16 m^2 area for estimation of P_s and P_m. Sampling was done under conditions of

maximum light.

Interstitial water samples were taken with a 20 ml "B-D Luer-Lok" syringe fitted with a 16 gauge 4 inch cannula. The end of the cannula was plugged, and 8 small holes drilled within one centimeter of the tip as described by Johnson (1967). For samples from shallow depths in the sediment, the syringe was inserted vertically into the sediment to the desired depth. For deeper samples, a hole was dug with a shovel, about 8 cm of sediment were cleared away from the sides of the hole at the desired sampling level by hand, and the syringe was inserted horizontally to its maximum extent (11.5 cm). For each sample a total of 200 ml was taken, made up of ten 20 ml samples spaced at least 8 cm apart horizontally. Trials with the syringe sampler in an artificial sediment-sea water system indicated that negligible amounts of sea water would be taken in with the interstitial water. A cylinder was filled with sediment and sea water, sodium fluorescein dye was put into the supernatant sea water, and syringe samples were withdrawn from 2.5 and 11.5 cm depth in the sediment. The ratio of the concentration of dye in the test sample to the concentration in the supernatant water, determined by absorptiometry, was 0.025 for the 2.5 cm sample, and 0.0009 for the 11.5 cm sample.

Prior to analysis, each interstitial water sample was centrifuged to remove the small amount of sediment taken in with the interstitial water. The sample was then acidified to pH 3 with dilute HCl and air bubbled through for 5 to 10 minutes to remove sulfide. pH was readjusted. Samples for chlorinity determination were withdrawn prior to acidification. Nitrate, nitrite and phosphate were measured by the methods of Strickland and Parsons (1965). No nitrite was detected in initial samples, and other samples were only spot checked for presence of nitrite. Ammonium was measured in initial samples

by the method described by Strickland and Parsons (1965) which measures some of the amino acid-N also; other samples were analyzed by the method of Solórzano (1969) which is reported to measure only ammonium-N. Comparison of the two methods on interstitial waters of high and low quantities of ammonium (+amino acid)-N indicated little or no labile amino acid-N in these interstitial waters. The method used for ammonium is indicated in the results. Applicability of these methods to interstitial waters was checked as for plant extracts. Chlorinity was determined by the Mohr titration (Barnes, 1959) using ordinary burettes (results reproducible within $\pm$ 0.05 °/oo).

Sediment samples were taken in short lengths of 1½ inch O.D. cellulose acetate coring tubing, except in the CCS substrate at Oistin Bay in which it was necessary to use a 1 cm diameter tube because of the close packing of coarse material. The coring tube was inserted horizontally into the sediment at the desired sampling level after digging a hole. The ends were capped with Phleger core caps or rubber stoppers, and the cores frozen until analysis.

Trial extractions of sediments with sea water showed that nutrients were present in much larger quantities than could be accounted for by the nutrient concentrations in the interstitial waters. Most of the ammonium and nitrate could be extracted by mixing sea water and sediment in a 7.5:1 ratio (volume of sea water-to-wet wt of sediment) for one hour. 90% of the ammonium extracted in 7 successive one hour extractions was removed in the first extract, and no nitrate was detected after the first extract. Reducing mixing time, or use of distilled water instead of sea water, resulted in lower yields of ammonium. No nitrite, or only traces, was detected in these extracts. Only a small quantity of phosphate was removed from the

sediments by one sea water extraction, and significant quantities were removed even in the 28th successive extract of one sample. Extractable or 'available' nitrate and ammonium were thus estimated as the nitrate and ammonium that are extracted by mixing sea water and sediment in a 7.5:1 volume-to-wet wt ratio for one hour. Extracts were spot checked for nitrite. All extractions were done with nutrient-free sea water (collected from surface water at about 10 km offshore from Barbados). Extracted sediment samples were dried at 100°C to determine the dry weight and approximate interstitial water content.

Organic carbon and HCl soluble phosphate were determined on sediment samples dried at 70 to 80°C. Organic carbon was estimated by the Walkley-Black method as described by Allison (1965). For the determination of HCl soluble phosphate, samples were treated with 30% concentrated HCl and the dissolved phosphate was measured by an absorptiometric technique (Olsen and Dean, 1965). Duplicate determinations varied by less than 5%.

pH-Eh measurement and bacterial counts

Studies on these characteristics were undertaken as part of preliminary studies of Thalassia bed sediments prior to initiation of the nutrient studies described above.

Sediment samples were taken from a variety of conditions with respect to flora and fauna and aeration of the sediment. Samples for pH-Eh determinations were taken in 1½ inch coring tubes in which several holes had been cut and covered with masking tape. Determinations were made on shore with a portable pH meter using a platinum 6 x 6 mm plate type electrode and calomel reference electrode for Eh, and a combination glass-reference electrode for pH. Masking tape was removed from the holes in the coring tubes and the appropriate electrodes inserted. Performance of platinum electrodes was

tested by checking the potential difference between used and unused electrodes, and by reference to ZoBell's (1946a) ferric-ferrocyanide solution; the electrodes were washed in dilute chromic acid and cathodically cleaned when necessary. Samples of low Eh showed an initial rapid drift negatively, and then a gradual decrease in the rate of change; Eh readings were taken when the drift was less than 1 to 2 mv per minute, which was generally 15 to 30 minutes after insertion of the electrodes. Negative drift of this sort is typical of marine sediments of low Eh (ZoBell, 1946a). pH generally changed less than 0.1 pH unit after insertion of the electrode, but in some sediments of low pH, a rapid drift to higher pH occurred. Initial pH is reported. When possible, several readings of pH and Eh were taken from each core sample. Because of damage to electrodes in these coarse, highly compacted sediments, pH-Eh observations were necessarily limited in number. For the same reason, it was not always possible to obtain pH measurements for all samples for which measurements of Eh were obtained. However, the pH range of these sediments was not great, and the Eh measurements alone are considered reliable indicators of whether a sediment was strongly or mildly oxidized or reduced.

Samples for bacterial counts were taken in 1½ inch coring tubes which had been washed with detergent. Samples were held at ambient temperature, which was close to the temperature of the sea water (about 27°C), until subsequent treatment. The maximum time between taking of the samples and subsequent treatment was 2½ hours. 10 g wet wt were removed from the middle of each core sample, transferred to a 95 ml sea water dilution blank containing glass beads, and mechanically shaken for five minutes. A dilution series through 10^{-8} was prepared and transfers were made to culture media. For plate counts, 0.1 ml was spread over the surface of a medium of 1%

casein hydrolysate and 1½% agar in a basal medium prepared by boiling 500 g sediment in 1000 ml sea water followed by paper filtration. Burkholder et al. (1959) reported good growth of bacteria from Thalassia beds in a medium of this composition (without agar). Media described by ZoBell and Morita (1959) were used for estimation of the 'minimum numbers' (one tube per dilution) of aerobes, anaerobes, nitrate reducers, sulfate reducers and nitrifiers. To ensure strictly anaerobic conditions, the medium for anaerobes, which was dispensed hot into 15 ml screw-cap tubes, was covered by 2 cm mineral oil (also dispensed hot). A reducing agent (0.1 g ascorbic acid/ 1000 ml medium) was also added. Nitrate reducers were detected by the appearance of nitrite in the anaerobic cultures, sulfate reducers by the formation of ferrous sulfide in cultures for sulfate reducers, and nitrifiers by the appearance of nitrite in a medium of sea water enriched with 0.1% ammonium phosphate, dibasic. pH of the medium for nitrifiers was adjusted to 8.2, and grains of carbonate beach sand (in addition to the reagent grade $CaCO_3$ specified for the medium) were placed in the culture tubes. Nitrifying bacteria were not detected in 8 out of 18 samples. The cultures were kept for up to two months, and were checked for presence of nitrate also. For one of the samples, alternative media with lower concentrations of ammonium (30, 300 mg $(NH_4)_2SO_4$/l) were also inoculated, but without positive results. Cultures of anaerobes and nitrifiers in which nitrite was detected were kept for longer periods and examined for disappearance of the nitrite. Dr. E.J.F. Wood of the Institute of Marine and Atmospheric Sciences, University of Miami, suggested (personal communication, 1968) that media for the determination of sulfate reducers which contain peptone, as does the medium of ZoBell and Morita (1959), may give erroneously high results through splitting off of sulfhydryl groups from amino acids. Sulfate

reducers were cultured subsequent to this suggestion using a medium of sea water, ammonium chloride 0.1%, potassium dihydrogen phosphate 0.05%, calcium lactate 0.35%, ascorbic acid 0.01% and magnesium sulfate 0.1%, dispensed in screw-cap tubes containing, at Dr. Wood's suggestion, small amounts of steel wool.

RESULTS AND DISCUSSION

Nitrogen and phosphorus in plant tissues

Results of determinations of total nitrogen and phosphorus and water soluble ammonium (+amino acid)-N and phosphate in leaves and rhizomes, and estimates of production are given in Table II. Only four samples of leaves and rhizomes were examined for water soluble nitrate and nitrite. In two of the leaf extracts and two of the rhizome extracts, no nitrate was detected (sensitivity: 0.025, 0.01 μg-at NO_3-N/g wet wt leaves, rhizomes), and in the others only very small amounts were present (under 0.03 μg-at NO_3-N/g leaves and under 0.05 μg-at NO_3-N/g rhizomes). No nitrite was detected in any of these four samples (sensitivity: 0.005, 0.002 μg-at NO_2-N/g wet wt leaves, rhizomes).

Nutrient characteristics were tested for correlation with the production estimates, P_s and P_m, by means of Spearman's rank correlation coefficient. Significant correlations ($P < 0.05$) were found between P_s and rhizome water soluble ammonium (+amino acid)-N ($r_s = 0.96$, $P < 0.001$), P_s and rhizome water soluble phosphate ($r_s = 0.76$, $P < 0.01$), and P_m and rhizome water soluble ammonium (+amino acid)-N ($r_s = 0.80$, $P < 0.01$). P_s is plotted against rhizome water soluble ammonium (+amino acid)-N in Fig. 2 and against rhizome water soluble phosphate in Fig. 3. These correlations suggest that significant proportions of the nitrogen and phosphorus in the leaves are derived

Table II. N and P in leaves and rhizomes.

STAND	LOCATION SUBSTRATE DEPTH (m)	L_{10} (cm)	P_s (mg/sheet per day)	P_m (g/m^2 per day)	TOTAL N and P, % of dry wt				SOLUBLE N and P, μg-at/g wet wt			
					Leaf N	Leaf P	Rhiz N	Rhiz P	Leaf N	Leaf P	Rhiz N	Rhiz P
A-1	O-OS-1.9	46.0	18.2	7.6	3.05	0.108	0.97	0.050	21.8	3.35	39.2	1.49
A-2	B-OF-0.4	38.6	17.5	5.0	2.68	0.216			17.0	2.98	46.0	2.19
A-3	B-OF-0.4	38.0	16.3	4.8	2.28	0.121	2.04	0.175	18.7	4.50	27.6	2.87
A-4	B-OF-0.4	29.1	9.4	4.8	1.85	0.209	1.04	0.139	15.9	3.28	25.7	1.88
A-5	B-OS-0.3	26.4	9.0	6.7	2.27	0.174	0.92	0.081	15.5	3.10	20.9	1.49
A-6	S-PS-0.5	22.6	7.0	4.5	1.69	0.130	0.66	0.099	16.3	2.66	20.1	2.18
A-7	O-PS-0.7	22.3	6.2	5.9					17.4	3.22	29.1	1.11
A-8	S-PS-0.8	20.8	6.0	2.6					16.0	3.40	16.9	1.30
A-9*	B-PS-0.4	18.7	5.0	1.2	2.23	0.131	0.66	0.074	20.4	2.94	14.9	1.91
A-10*	S-PS-0.7	18.6	5.0	1.7					12.0	4.07	12.1	1.26
A-11	B-OS-0.4	17.0	4.1	2.0	2.14	0.229	1.54	0.228	21.2	3.63	11.7	1.91
A-12	B-OS-0.4	14.6	2.4	1.3					23.5	5.95	14.7	1.28
A-13	B-PP-0.2	11.1	1.1	1.5	2.38	0.093	0.64	0.079	22.1	2.59	8.7	1.13

*Mixed stand of Thalassia and Syringodium (513, 1190 g wet wt Syringodium /m^2 at A-9, A-10).

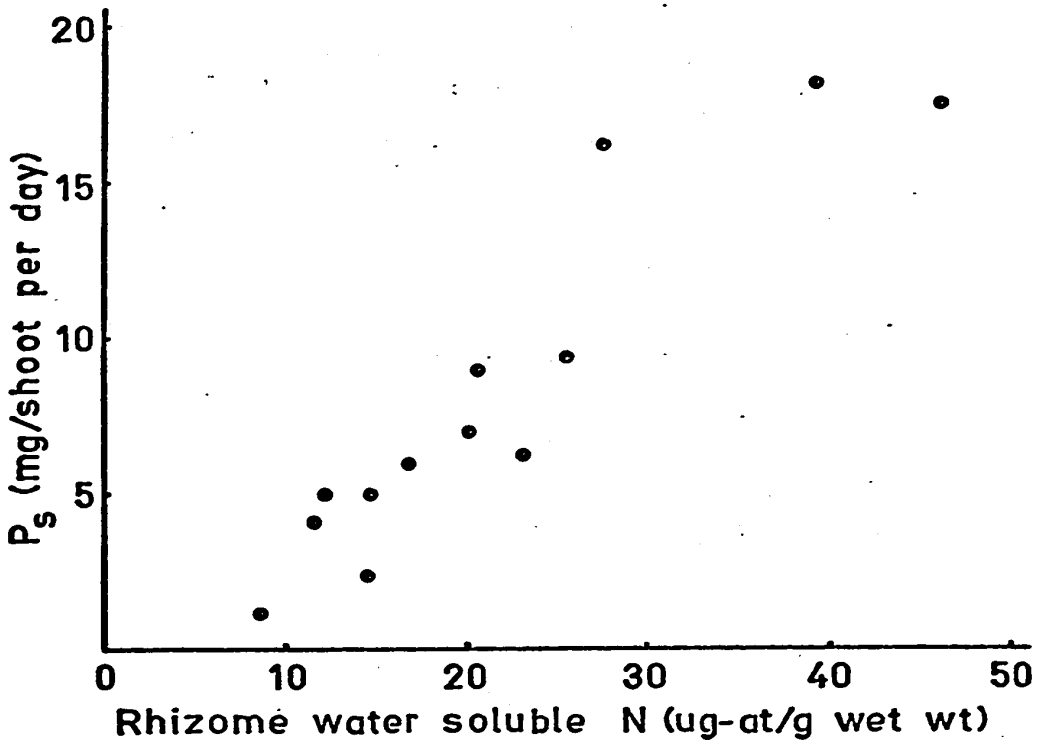


Fig. 2. Relation of production per shoot to rhizome water soluble ammonium(+amino acid)-N.

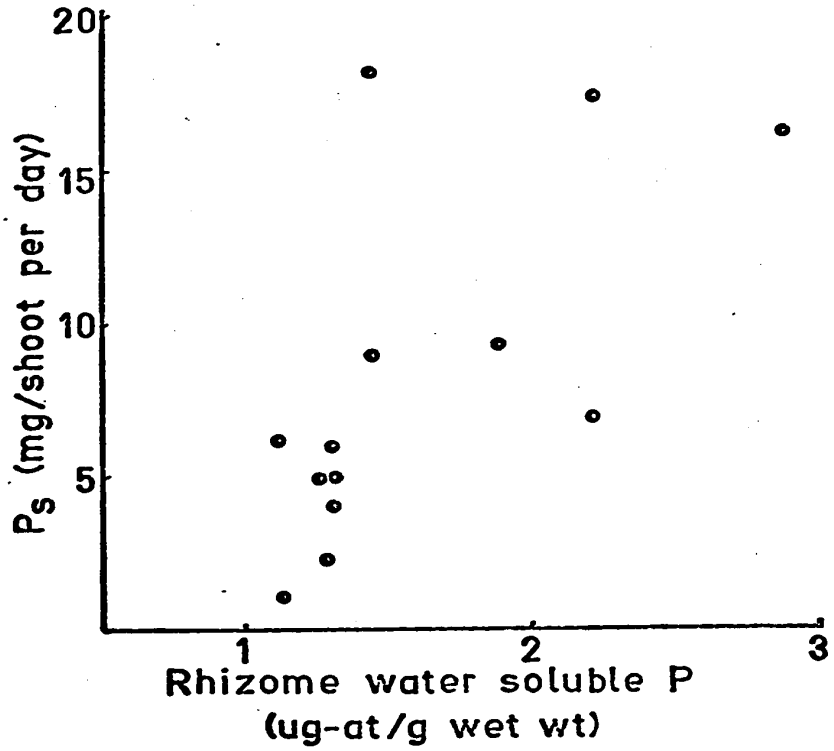


Fig. 3. Relation of production per shoot to rhizome water soluble phosphate.

from the rhizomes, and ultimately, from the sediments. For one sample it was estimated that 90% of the rhizome water soluble ammonium (+amino acid)-N was derived from amino acids. It has been demonstrated in certain terrestrial plants that amino acid synthesis occurs in the roots and that nitrogenous materials are translocated to the leaves mainly as amino acids and amides (Swanson, 1959; Kursanov, 1961, 1963). It appears that similar processes occur in Thalassia.

Requirements of a plant for nitrogen and phosphorus may be estimated by determining the concentrations of nitrogen and phosphorus in dead but undecomposed tissue, and multiplying these by the production of the plant. Because of the presence of calcareous epiphytes on old parts of Thalassia leaves, and breakage and initiation of decomposition of the leaf tissue associated with the epiphytes (Appendix A), it was not possible to determine nitrogen and phosphorus concentrations in senescent leaf tissue. Requirements for nitrogen and phosphorus estimated by using the concentrations of nitrogen and phosphorus in young leaf tissue may be high because of the possibility (Biddulph, 1959) of remobilization of nitrogen and phosphorus in scenescent tissue. The following data given by Jensen (1915) on the nitrogen content of Zostera leaves suggest that a maximum of 70% of the nitrogen in young leaves may be reutilized:

Living leaves, all green	2.97% N in dry matter
Mainly living, some tips brown	2.05
Mainly dead, some green leaves	1.10
Leaves dark brown, but still largely whole	0.88

The average of the values for total nitrogen content of the Thalassia leaves (Table II) is 2.3% N. Estimates of the nitrogen required for growth

of Thalassia leaves are thus given by multiplying leaf tissue production by 2.3% N (maximum estimate) and by 0.3 x 2.3% N (minimum estimate). Experiments utilizing ^{32}P with terrestrial plants (Biddulph, 1959) and Zostera (McRoy and Barsdate, 1970), show that a large proportion of the total phosphorus may be highly mobile, and thus would not be readily lost with death of leaf tissue. As a rough estimate, the non-mobile phosphorus fraction in Thalassia leaves is assumed to equal the difference between the water soluble phosphate and the total phosphorus. The water soluble phosphate values of the leaves were recalculated on a dry weight basis (dry wt/wet wt, average of 5 samples: 0.19) and subtracted from the total phosphorus values for the nine samples in which both fractions were determined. The estimated non-mobile fraction varies between 39 and 78% of the total phosphorus with a mean of 64%. The average of the total phosphorus values is 0.16%, and the average non-mobile phosphorus is thus estimated as 0.1% of the dry weight. Estimates of the phosphorus required for growth of Thalassia leaves are given by multiplying production by 0.1% P (maximum estimate), and by 0.3 x 0.1% P (minimum estimate, assuming 70% remobilization, as above).

Interstitial water nutrients

Ammonium, nitrate and phosphate concentrations in interstitial water from approximately the middle of the root layers, and estimates of P_s and P_m of 16 stands are given in Table III. There are no significant correlations between P_s or P_m and interstitial water nutrients.

P_s is plotted against interstitial ammonium in Fig. 4. The four points designated by 'X' in this figure were from stands (B-5, B-6, B-7, B-8 of Table III) in which the root layer extended vertically for more than 40 cm. Except for the root layer of stand B-15, which was 24 cm in vertical extent, the root layers of all other stands were less than 15 cm in vertical extent.

Table III. Mid root layer interstitial water nutrient concentrations.

STAND	LOCATION SUBSTRATE DEPTH (m)	L_{10} (cm)	P_s (mg/shoot per day)	P_a (g/m^2 per day)	INTERSTITIAL WATER NUTRIENTS (µg-at/l) NO_3-N	NH_4-N	PO_4-P
B-1 (A-1)	0-CCS-1.9	46.0	18.2	7.6	0.28	18.7	0.90
B-2	0-CCS-1.9	39.1	15.4	3.9	0.60	17.6*	0.90
B-3	S-CP-0.4	35.0	13.8	5.3	0.66	17.2	0.98
B-4	B-CP-0.4	34.1	13.6	3.6		15.7	1.00
B-5*	B-PS-0.4	30.3	10.0	1.1	2.60	3.7	0.61
B-6	S-PS-0.4	26.8	8.5 (7.2)**	6.0	1.30	2.0*	0.27
B-7*	S-PS-0.5	24.5	7.9	3.1	0.29	0.8*	0.30
B-8	B-CS-0.4	23.5	8.0	5.0	0.25	3.8	0.41
B-9	S-PS-0.9	22.8	6.7	3.1	0.34	7.0	0.92
B-10	S-PS-1.0	20.7	5.6	4.5	0.75	6.6	0.54
B-11 (A-10)*	S-PS-0.7	18.6	5.0	1.7	0.35	7.7	1.04
B-12	B-CS-0.5	16.2	3.9	1.8	0.45	5.5*	0.88
B-13	S-PS-0.6	14.9	3.6	1.8	0.60	3.6	0.51
B-14 (A-12)	B-CS-0.4	14.6	2.4	1.3		10.3	0.75
B-15	S-PS-1.1	12.8	2.8	2.1	0.98	7.0	0.79
B-16	B-PP-0.2	12.5	2.0	2.0	0.89	8.6	0.85

*Method of Solórzano (1969), others by method of Strickland and Parsons (1965).

**See Appendix A, p. 154.

*Mixed stand of Thalassia and Syringodium (633, 483, 1190 g wet wt Syringodium/m^2 at B-5, B-7, B-11).

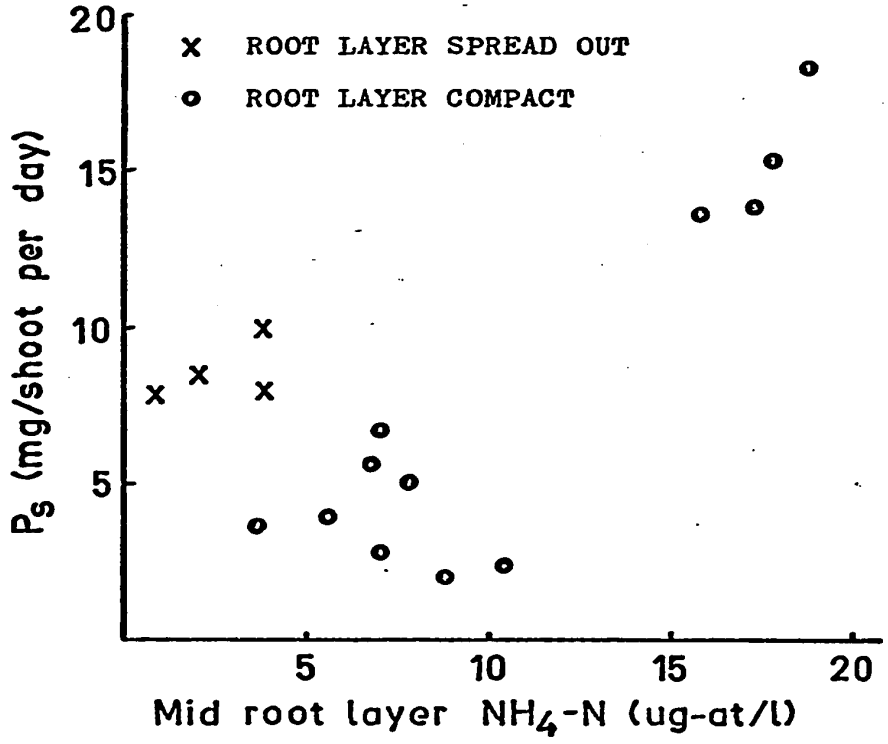
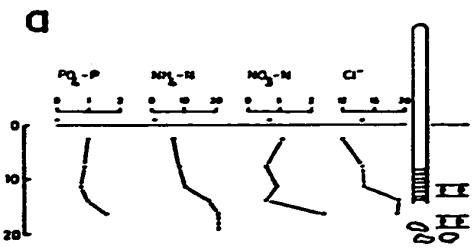


Fig. 4. Relation of production per shoot to mid root layer interstitial water ammonium concentration.

Certainly a simple relation between P_s and the mid root layer interstitial ammonium concentration is not evident in this figure. However, the relatively low concentrations of ammonium in the 'spread out' root layers compared to the other stands suggest that P_s might be proportional to the total ammonium in the root layer rather than the concentration per se. The relatively high concentrations of ammonium in the root layers of the stands of the three lowest P_s values, on the other hand, suggest that leaf growth of these stands was not limited by the supply of ammonium. For the remaining stands there appears to be a simple relation between P_s and the mid root layer interstitial water ammonium concentration.

Vertical profiles of nutrients obtained from six of these stands are given in Fig. 5. One of the profiles was from a stand (B-15) of very low P_s, two were from stands (B-6, B-7) with spread out root layers, and three were from stands (B-2, B-9, B-12) with root layers less than 15 cm in vertical extent. To estimate the (relative) total amounts of interstitial water ammonium in these stands, each ammonium profile was integrated over the interval within the root layer; a linear change in concentration between sampling points was assumed, and where the deepest part of the root layer could not be sampled because of the presence of rubble, the concentration in this area was assumed to be the same as that of the deepest sample. P_s is plotted against total ammonium in Fig. 6. Excluding the stand of lowest P_s, a simple linear relation between P_s and the total root layer interstitial ammonium is evident. The total root layer interstitial ammonium of the stand of lowest P_s was roughly 10 times what would be expected on the basis of the relation of P_s to root layer ammonium for the other stands. These data clearly demonstrate that ammonium is taken up from the sediments by Thalassia, and was the limiting factor for leaf growth for the majority

Fig. 5. Profiles of interstitial water nutrients and chlorinities at stands B-2, B-6, B-7, B-9, B-12, B-15 (Figs. a, b, c, d, e, f respectively). Nutrient concentrations are in µg-at/l, chlorinity in parts per thousand. Dashed lines at lower parts of ammonium profiles are assumed values. Vertical extent of rhizomes, positions of shallowest erect shoot apices, and top of rubble layer are shown diagramatically.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NH_4-N
NO_3-N
Cl⁻
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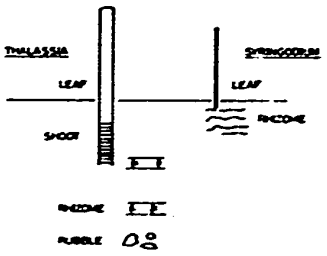
10
20


THALASSIA
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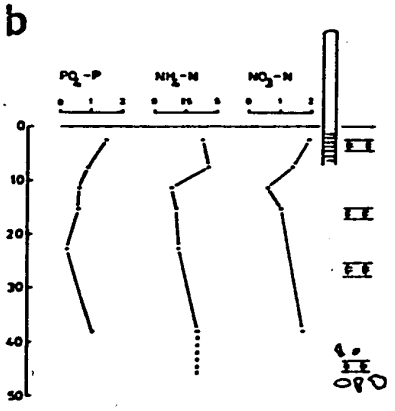
SHOOT
SYRINGODIUM
LEAF
RHIZOME
RHIZOME
RUBBLE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NO_3-N
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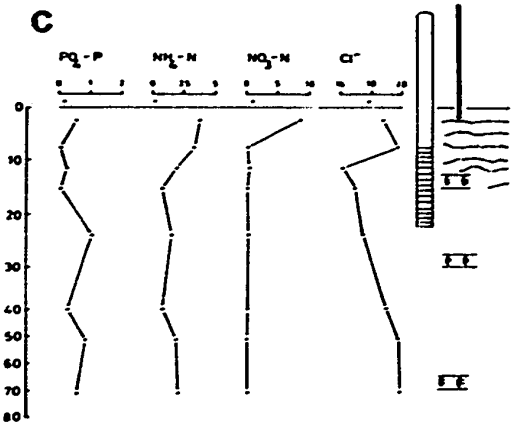
40
50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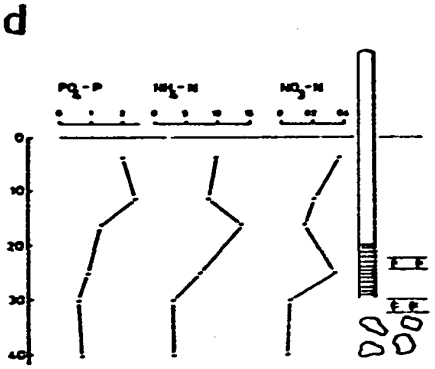
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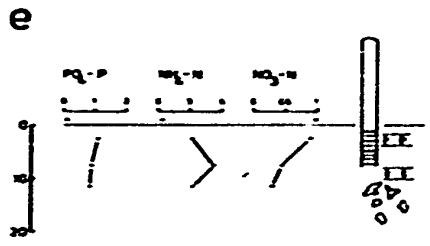
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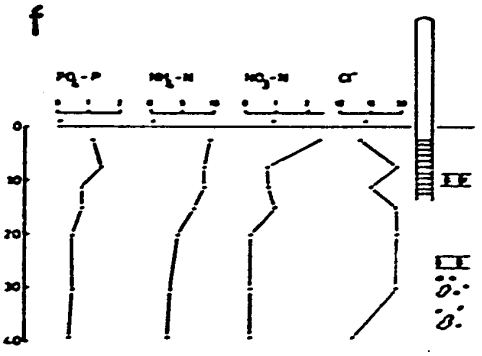
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NO_3-N
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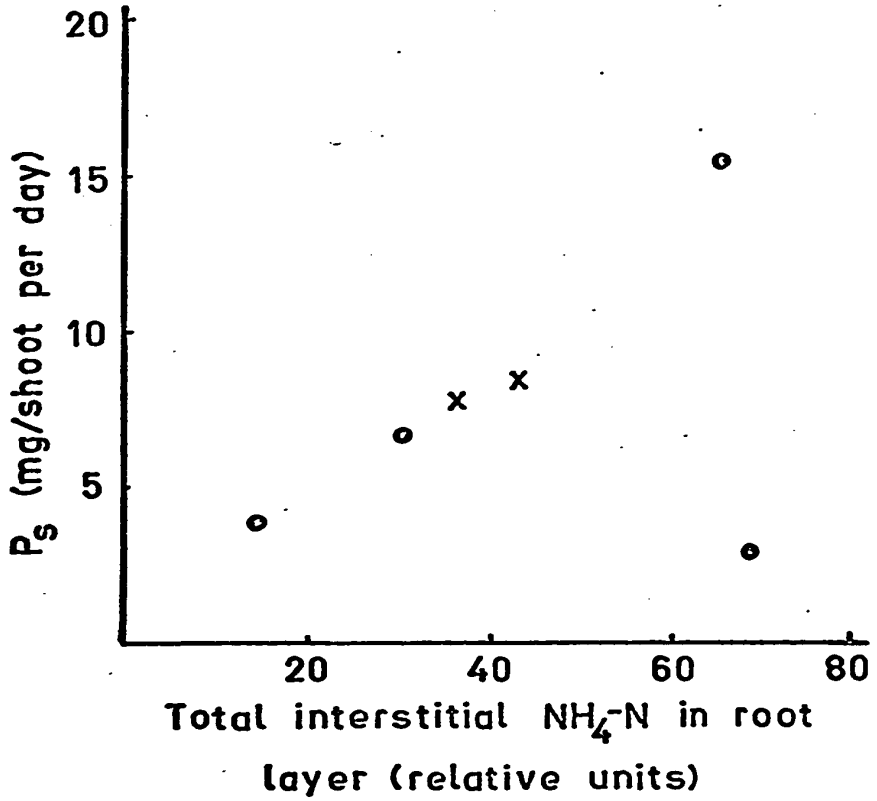

Fig. 6. Relation of production per shoot to total interstitial water ammonium in root layer.

of the stands studied.

The good correlation between P_s and total interstitial water ammonium in the root layer implies that the total ammonium in the root layer must be the same to maintain a given production per shoot regardless of whether there are few or many shoots per unit area. The density of shoots is determined in part by non-nutritional factors such as the stage of succession (Appendix B). Thus P_m, which is a function of both P_s and the shoot density, is only indirectly correlated with interstitial water ammonium.

Some factor other than nitrogen must have been limiting growth of leaves in the stands of very low P_s. Phosphate concentrations of the mid root layer interstitial water of these stands were not lower than those of the other stands, so it seems unlikely that phosphorus was limiting. One of the stands (B-16) was at a depth of about 15 cm below mean low water, and growth at that stand was probably limited simply by depth of the water. Low water of spring tide is approximately 0.2 m below mean low water at Barbados. In general it was observed that the length of leaves in depths less than 0.2 to 0.3 m below mean low water decreased with decreasing water depth (Appendix B), and similar observations have been made by other workers (Phillips, 1960; Strawn, 1961; D.R. Moore, 1963). The upper limit of Thalassia lies between mean low water and low water of spring tide (Phillips, 1960). Shortness of leaves in shallow water has been attributed at least partially to "leaf kill" (Phillips, 1960). However measurements of growth rates (Appendix A) indicates that the main cause of short leaf length in shallow water is simply low growth rate. Presumably leaf length is limited by depth of water and the growth rate is adapted to this situation through a sort of negative feedback. The two other stands of low P_s (and low growth rates) were at depths of greater than 0.3 m and lay adjacent to stands of longer

leaves. A pathological factor may have been limiting leaf growth at these stands. At one of the stands, B-15, the sediment surface was covered by a grey 'fuzz' which under the microscope was observed to consist of fungal mycelium. At the only other stand where such a fuzz was observed, the leaves were also very short. Whether or not the fungal mycelium was in any way related to the low growth rates of Thalassia can only be surmised, however.

The stands of highest P_s of Tables II and III occurred in CCS and CF substrates. Stands of moderately high P_s were associated with spread out root layers in PS and CS substrates. General survey studies (Appendix B) also showed that high P_s is associated with CF substrates and spread out root layers. In the stands of spread out root layers, erect shoots may be very long, with a maximum length equal to the vertical extent of the root layer (Plate IId). High P_s in such situations is probably associated with the large area of sediment in contact with each shoot or its roots. Stands B-12 and B-13, the stands of lowest P_s in Table III other than the stands in which nitrogen was probably not limiting, had 'shallow' root layers (Fig. 5e). Low P_s in these stands and high P_s in CF and CCS substrates may have been associated with 'high' and low redox potentials in these two situations respectively. This is further discussed below under 'General pH-Eh and bacterial characteristics of the sediments and observations on the occurrence of iron sulfide'.

The relation of P_s to interstitial water ammonium is an unusually good example of simple adherance to Liebig's Law of the Minimum. In many situations in agriculture, more than one nutrient is present in limiting or near limiting supply, and requirements for a nutrient may be affected by complex interactions between the nutrients (Russell, 1950; Reuther, 1961).

Liebig's Law implies a linear relation between yield and supply of the limiting nutrient. For agricultural systems in which only one nutrient is limiting, a sigmoid curve relating yield to nutrient supply is typically observed (Russell, 1950). As the maximum capacity of the plant for growth is approached the rate of change of yield with respect to nutrient supply decreases, and may approach zero. Similarly, calibration curves used for estimating the 'critical concentration' for the tissue analysis technique of evaluating nutrient supply are characterized by the presence of an 'adequate zone' in which increasing the supply of the limiting nutrient results in an increase in the concentration of the nutrient in the tissue, but no increase in yield (Ulrich and Hills, 1967). The critical concentration corresponds approximately to the lowest concentration of the limiting nutrient in the tissue associated with maximum yield. Gerloff and Krombholz (1966) successfully applied tissue analysis techniques to the study of nutrient availability for freshwater angiosperms. For several species the critical concentrations of total nitrogen and total phosphorus in the plants were 1.3% N and 0.13% P. Analyses of plants from nine lakes indicated that phosphorus rather than nitrogen was likely to be limiting growth of these plants. Even though very high rates of production by Thalassia were observed, there is no indication in the plots of P_s versus total interstitial ammonium (Fig. 6) and P_s versus rhizome water soluble ammonium (+amino acid)-N (Fig. 2) of a maximum yield of Thalassia being approached in situ. It is also apparent that the P_s-rhizome water soluble nutrient correlations do not indicate whether a particular nutrient is limiting growth of Thalassia. This is shown by the fact that there was a significant correlation between P_s and rhizome water soluble phosphate, even though phosphate was not limiting growth of Thalassia. Also, for stand A-13, at which growth of Thalassia

was probably limited by the depth of water rather than by nitrogen, the rhizome water soluble ammonium (+amino acid)-N concentration was what would be predicted from the values for the other stands, rather than anomalous. This type of relationship may be very useful in the study of aquatic angiosperms, however, as it does provide a simple means to determine whether significant quantities of nutrients are taken up from the substrate in situ. Some preliminary studies of Syringodium, for example, suggest uptake of nitrogen from the substrate by this plant:

Maximum leaf length	Rhizome water soluble ammonium (+amino acid)-N
28.0 cm	10.6 μg-at/g wet wt
23.9	3.16
23.7	3.48
19.4	1.12

Obviously, light must limit growth of Thalassia for a part of the day, and presumably at some depth, is continuously limiting. Jones (MS, 1968) found that oxygen production by Thalassia increased with irradiance up to an irradiance of 20 ly/hr. From his data he concluded that oxygen production is determined mainly by the standing crop and the length of day with irradiance greater than 20 ly/hr. The simple linear nature of the relation of P_g to total interstitial water ammonium suggests that at the depths sampled in Barbados (less than 2 m), most growth occurs during the period when nitrogen is limiting. The factor limiting oxygen production above an irradiance of 20 ly/hr in Jones's (MS, 1968) study was most probably nitrogen.

Interstitial water chlorinities

Interstitial water chlorinity profiles of three stands are given in

Fig. 5. A few sea water salinity samples taken from Thalassia beds during preliminary survey studies, and salinity data obtained by Mr. Finn Sanders of the Bellairs Research Institute (personal communication, 1969) had indicated little difference between salinities of inshore areas and oceanic salinities; thus the occurrence of low chlorinity water both over the Thalassia beds and in the sediments was unexpected. During the months in which the interstitial water samples were taken, December 1969 to February 1970, there were unusually heavy rains. Sampling was done at low tide when mixing of inshore water with oceanic water would have been least. Chlorinities of sea water taken at the three stands were 14.5, 17.8 and 15.4 °/oo (Figs. 5a, 5c, 5f, respectively). The minimum chlorinities of the interstitial waters of these stands were 12.0, 16.2 and 13.7 °/oo. The maximum chlorinities of the interstitial waters, 19.6, 19.8 and 19.6 °/oo, were close to oceanic chlorinities during this period (19.7 to 20 °/oo, calculated from salinity data obtained by Mr. Finn Sanders, personal communication, 1970).

Exchange between interstitial water and the overlying sea water is usually considered to be very slow. Emery, Stevenson and Hedgepeth (1957) remarked with respect to interstitial water salinities in estuaries, "the salinity of the interstitial water probably represents a rough median value of the salinity of the overlying water which flows past the point during the year." Thus the existence of apparently discrete layers of maximum salinity water next to layers of low chlorinity water is puzzling. Three phenomena which might account for the peculiar chlorinity distributions are (1) specific uptake of chloride by Thalassia, (2) some sort of mass transport of sea water through the sediments associated with uptake of sea water by Thalassia, and (3) upwelling of groundwater. Five interstitial water samples taken from positions between the sediment surface and bottom of the root layer

of a stand at Bath that was continually washed by offshore water had chlorinities of 19.1 to 19.7 °/oo. This suggests there is not a specific uptake of chloride by Thalassia. The demonstration of rapid rates of translocation of ^{32}P in the vascular system of Zostera (McRoy and Barsdate, 1970), and the evidence of this thesis for uptake of significant quantities of nutrients from the substrate by Thalassia, support arguments for the existence of a mass transport of some sort through the xylem of submerged plants. However, the whole subject of the physiology of water and solute movement in marine angiosperms is in need of careful experimental study. Whether movement of groundwater in the sediments would give rise to the type of chlorinity distributions observed is open to question. It might be useful to study the vertical chlorinity distributions of Thalassia sediments in areas definitely subject to groundwater movement, and in areas subject to varying chlorinity sea water, but not possibly subject to groundwater movement.

Inorganic nitrogen and phosphorus, organic matter in the sediments

Ten sediment samples, taken at levels at which interstitial water samples were also taken, were analyzed for extractable ammonium and nitrate, HCl soluble phosphate, and organic matter (Table IV). These values represent single samples of sediment, of approximately 120 ml volume, at the given depths. Interstitial water nutrient values, also given in Table IV, represent 10 individual 20 ml samples at the given depths.

Inorganic nitrogen

There is a significant correlation between interstitial water ammonium and extractable ammonium ($r_s = 0.87$, $P < 0.01$). Interstitial water ammonium is plotted against extractable ammonium in Fig. 7. It is apparent that most

Table IV. Analyses of sediments.

STAND	SAMPLE POSITION	INTERSTITIAL WATER NUTRIENTS (µg-at/l)			APPROX. INTERSTITIAL WATER CONTENT	EXTRACTABLE N (µg-at/kg dry wt)		HCl SOLUBLE P	ORGANIC C
		NH_4-N	NO_3-N	PO_4-P	(% of wet wt)	NH_4-N	NO_3-N	(% of dry wt)	(% of dry wt)
B-1	mid root layer	18.7*	0.28	0.90	35	869	3.1	0.0224	0.94
B-2 (Fig. 5a)	subsurface (2-3 cm)	6.9	1.06	0.98	28	196	8.7	0.0244	0.27
B-6 (Fig. 5b)	subsurface	3.8	1.94	1.49	29	129	3.8	0.0253	0.32
" "	13 cm in root layer**	1.5	0.8	0.7	33	40	4.3	0.0211	0.41
" "	37 cm in root layer	3.5	1.77	1.10	28	28	0.3	0.0213	0.29
B-9 (Fig. 5d)	subsurface	9.8*	0.38	2.00	25	189	87.7	0.0220	0.29
" "	25 cm in root layer	7.0*	0.34	0.92	35	294	4.2	0.0235	0.48
B-14	mid root layer	10.3*		0.75	44*	347	19.9	0.0247	1.19
B-15 (Fig. 5f)	subsurface	9.5*	2.39	1.15	31	250	49.1	0.0302	0.60
" "	15 cm in root layer	7.0*	0.98	0.79	32	178	30.5	0.0442	0.48

*Method of Strickland and Parsons (1965); others, including extractable NH_4-N, by method of Solórzano (1969).

**Interstitial values interpolated.

*Includes excess sea water taken in with sample.

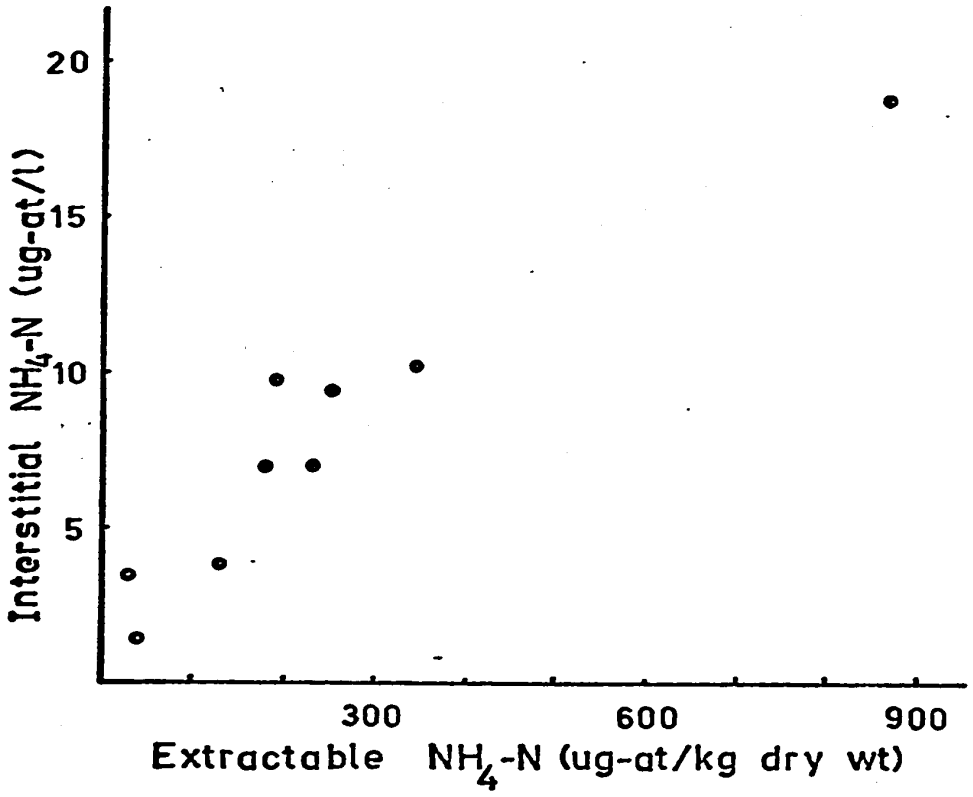

Fig. 7. Relation of interstitial water ammonium to ammonium in sea water extracts of the sediments.

of the ammonium in the sediments is adsorbed in some manner, and that this adsorbed ammonium is readily exchangeable with other cations in sea water. The correlation between interstitial water ammonium and extractable ammonium indicates that the two phases are in equilibrium, and that the various sediments possessed similar ammonium adsorption characteristics. The Thalassia sediments consisted predominantly of sand sized skeletal carbonates (Appendix B). There were only small amounts of organic matter and negligible amounts of clay minerals, the two characteristics which account for most of the cation-exchange capacity of soils (Black, 1968), in these sediments. Adsorption of ammonium in these sediments is probably a coulombic type of adsorption associated with the negative charge (Pravdić, 1970) of the sediments. Pravdić (1970) found that all sediments he examined were negatively charged in sea water and positively charged in fresh water with the reversal occurring between 2 and 6 °/oo salinity. The particle surfaces are apparently charged by surface interactions, both coulombic and specific in nature, with the more abundant ions in sea water. Pravdić (1970) remarked, "Once charged, the mineral surface tends to adsorb ions of opposite charge to maintain the electrical neutrality of the system. These ions are weakly held and readily exchange if their concentration in the solution varies or if the charge of the surface is reversed by some chemical interaction."

There is no correlation between interstitial water nitrate and extractable nitrate. Relatively large amounts of nitrate were present in some of the surface samples. Only traces of nitrite, or none at all, were detected in the sea water extracts.

Inorganic phosphorus

The HCl soluble phosphate values are considered to be good estimates of the total inorganic phosphorus in the sediments as most of the inorganic

phosphorus was probably present in the form of (acid soluble) calcium phosphates. Phosphate is precipitated in the marine environment as calcium phosphates (Nelson, 1967). Under alkaline conditions, as in sea water or in limed soils, phosphate is released from iron and aluminum phosphates, the iron and aluminum remaining in insoluble form as hydroxides (Black, 1968). The Thalassia sediments consisted predominantly of skeletal carbonates, and the south coast sediments were notably low in iron (see p. 69), so it is unlikely that there were significant amounts of iron and aluminum phosphates in these sediments even in metastable form. Sea water is believed to be approximately saturated with respect to calcium carbonate and apatite (Sillén, 1961; Kramer, 1964; Roberson, 1966). Small, discrete particles of apatite would not be deposited in the relatively high energy environments of the Barbados Thalassia beds unless formed after stabilization of the sediments. Gulbrandsen (1969) noted that the ratio of carbonate to phosphate in limestone (which averages about 0.04% P) is of the same order as the ratio of carbonate to phosphate in sea water. He suggested that coprecipitation of carbonate and phosphate "may be a significant feature of carbonate deposition." The HCl soluble phosphate values of the Thalassia sediments are of the same order as the total phosphorus values given by Vinogradov (1953) for mollusc skeletal parts (trace to 0.61% P, most values < 0.01% P), corals (trace amounts), Halimeda (trace amounts), coralline algae (trace to 0.20% P, most values < 0.070% P) and by Clarke and Wheeler (1922) for foraminifera (trace amounts). Debris of these organisms made up the main part of the Thalassia bed sediments (Appendix B). It seems likely that most of the inorganic phosphorus in the Thalassia sediments is in the form of calcium phosphates occluded within the skeletal carbonates. Occluded phosphate would be unable to go into solution to replace phosphate removed from the

interstitial waters by Thalassia. Thus while the HCl soluble phosphate values indicate relatively large amounts of phosphate in these sediments, most of it may not be available to Thalassia. Some indication of the amount of phosphate that is readily available is given by the amount of phosphate extracted in 28 successive washings of a Thalassia root layer sediment with sea water. The HCl soluble phosphate of this sediment was 0.024% of the dry weight, and the total phosphate extracted with sea water was 6.6 mg/kg dry wt, or 2.8% of the HCl soluble phosphate. This is of course a minimum estimate of the available phosphate as considerably more might have been extracted with continued washings (see Fig. 8).

The decrease in the concentration of phosphate in 28 successive extracts of a root layer sediment, and in 7 successive extracts of two other root layer sediments (Fig. 8), is suggestive of desorption of phosphate as observed in soils. Adsorption processes are known to be of major importance in regulating availability of phosphate for plant growth in soils. It is pertinent to briefly review work of the soil sciences concerned with phosphorus solubility, particularly that concerned with limed and calcareous soils, as similar processes probably control the availability of phosphate in the Thalassia sediments. The availability of phosphate for plant growth in soils is a function of both the concentration of phosphate in the solution phase, and the capacity of the soil to renew the solution phase phosphate (Fried and Shapiro, 1962). The soil-plant relationship may be represented as (Fried and Shapiro, 1962)

$$\text{Soil P} \rightleftharpoons \text{Solution P} \longrightarrow \text{Plant}$$

Apatite, in particular fluorapatite, is considered to be the only stable crystalline phosphate in alkaline soils in the presence of calcium (Murrmann

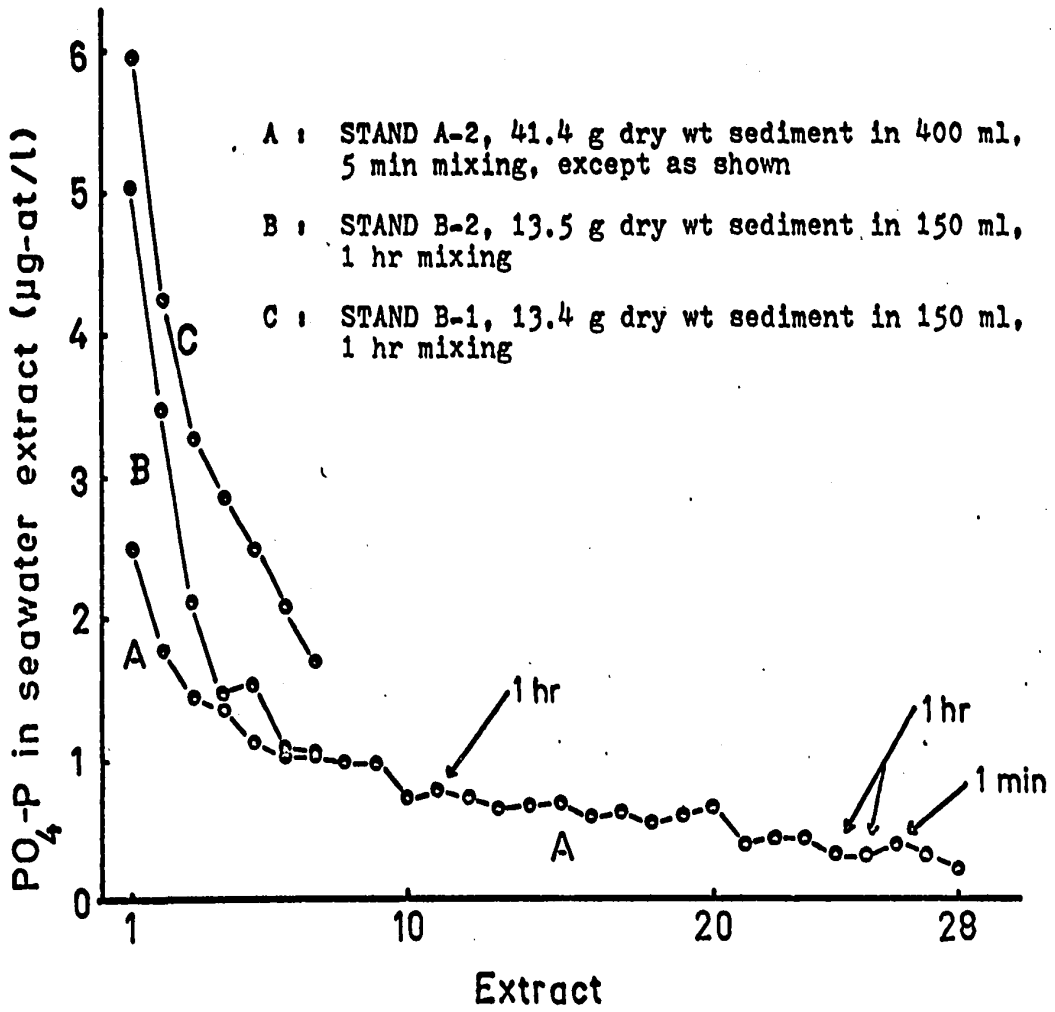

Fig. 8. Phosphate concentrations in successive sea water extracts of root layer sediments.

and Peech, 1968). Other metastable calcium phosphates such as dicalcium phosphate and octocalcium phosphate may occur as transitional forms when phosphate fertilizer is added to soils (Fried and Shapiro, 1962). In addition to solid phase crystalline phosphate, phosphate is adsorbed on the surface of soil particles. Some characteristics of the adsorbed phosphate are as follows. (1) Adsorption and desorption of phosphate on calcium carbonate (Cole, Olsen and Scott, 1953; Cole and Olsen, 1959) and other minerals (Fried and Shapiro, 1956; Olsen and Watanbe, 1957; Cole and Olsen, 1959; Hsu, 1964) are accurately described by the Langmuir equation. (2) Equilibrium between adsorbed phosphate and solution phosphate is attained rapidly (one minute or less in well mixed experimental systems - Fried and Shapiro, 1956). (3) Essentially all of the adsorbed phosphate is in equilibrium with the solution phosphate (i.e. exchangeable with ^{32}P), at least within the first 24 hours after adsorption (Cole et al., 1953; Olsen and Watanbe, 1957). Cole et al. (1953) considered the slight decrease in the amount of exchangeable phosphate with aging to be due to "gradual penetration of phosphate ions into cracks and crevices inaccessible to the solutions or of solid diffusion of the ions into the crystal lattice." By comparison, only a small fraction of crystalline phosphate is exchangeable with ^{32}P (Black, 1968). (4) the adsorption maximum, as estimated from the Langmuir equation, is closely correlated with soil particle surface area (Olsen and Watanbe, 1957). These four characteristics are taken as evidence that (i) phosphate is adsorbed as a monolayer, and (ii) adsorption processes are of fundamental importance in determining the solubility of phosphate in dilute solutions. The exact nature of the adsorption process is unknown, but it seems to be generally agreed that it is an exchange process involving primary valence bonds. There has been considerable confusion over the question of whether the

adsorbed phosphate or the crystalline phosphate determines phosphate solubility. Consistent with what would be predicted from the Langmuir equation, Cole and Olsen (1959) found that phosphate solubility in various calcareous soils was directly related to the amounts of surface area and the percentage phosphate saturation of these surfaces. Murrmann and Peech (1968), on the other hand, found that the solubility of phosphate in various calcareous soils corresponded closely with that predicted by the solubility of fluorapatite. However Murrmann and Peech (1969a) subsequently found that when the pH of these soils was varied, the phosphate solubility was determined by the amount of labile (surface) phosphate rather than by the solubility of fluorapatite. These workers proposed (Murrmann and Peech, 1969b) that labile phosphate and fluorapatite should coexist according to the equilibrium

$$\text{Labile P} \rightleftharpoons \text{Solution P} \rightleftharpoons \text{Crystalline P}$$

They noted that apatite dissolves extremely slowly, and suggested that when the equilibrium between crystalline phosphate and labile phosphate is shifted by altering pH, or by plant uptake of phosphate, it is the labile phosphate rather than crystalline phosphate that determines the concentration of phosphate in solution. They remarked, "It should be obvious that, regardless of whether or not any highly insoluble crystalline phosphate like apatite or variscite is in equilibrium with the labile phosphate of the soil, it is the labile phosphate that determines the concentration of phosphate in solution and the rate at which this equilibrium is replenished upon leaching or removal of phosphate by plant roots."

Assuming that the phosphate concentrations in the sea water extracts of the root layer sediments (Fig. 8) were equilibrium values, then concentrations

of the order of 5 µg-at PO_4-P/l would be expected in the interstitial waters of the Thalassia root layer. Values of 0.1 to 1.5 µg-at PO_4-P/l were observed (Table III and Fig. 5). The difference might in part be accounted for by bacterial activities in situ. Studies by Hayes and associates (Hayes and Phillips, 1958; Watt and Hayes, 1963) have shown that bacteria are important in the regulation of phosphate concentration in aquatic systems. However rates of exchange of phosphate between bacteria and the water phase vary by several orders of magnitude for different systems, and it is not yet possible to predict what the effect of bacteria will be in a given system. The low phosphate concentrations in situ might also be a result of sulfate reduction. With respect to adsorption characteristics, sulfate behaves similarly to phosphate in soils (Barrow, 1967). Sulfate is probably the main anion in the sea water extracting solutions which exchanged with adsorbed phosphate. Sulfate initially adsorbed on calcium carbonate and in interstitial water of the Thalassia root layer must be at least partially removed by sulfate reduction. This would be expected to result in more adsorption of phosphate, and correspondingly lower interstitial water phosphate concentrations in the Thalassia root layer sediments than would occur in systems in equilibrium with sea water sulfate.

Because of the complex nature of apatites, and uncertainty regarding activity coefficients in sea water, it is difficult to make reliable estimates of the solubility of apatites in sea water. Roberson's (1966) experimental data on the solubility of apatites in sea water (calcium ion concentration 1.02×10^{-2}M) indicate equilibrium concentrations of phosphate of approximately 0.2 µg-at/l at pH 8.2, 1 µg-at/l at pH 7.5, and 1.8 µg-at/l at pH 7.0. Gulbrandsen (1969) considered that these are probably slight underestimates of the solubility of phosphate. Under conditions of reduced

calcium ion concentration, the equilibrium phosphate concentrations would be higher. The phosphate concentrations in interstitial waters of the Thalassia sediments were of this order. This does not necessarily imply that solid phase apatite was present and in equilibrium with the interstitial water phosphate, but it may safely be concluded that none of the more soluble non-apatitic calcium phosphates were present.

Organic matter

The organic carbon values are intended to be estimates of the non-living organic matter (except for bacteria). Large pieces of obviously fresh rhizome tissue were removed from the samples prior to drying, but portions of living roots may have been taken in the samples. On the other hand, much of the dead Thalassia tissue remains closely attached to the plant until it is decomposed, and this material may have been undersampled. In any case, the estimates indicate that only small amounts of organic matter were present in the sediments. Much larger organic carbon values (6 to 8%) characterize muds of the 'tidal zone', the region of Zostera occurrence, of Australian estuaries (Rochford, 1951). Most of the organic matter in the Thalassia sediments was probably derived from dead underground parts of Thalassia. Infaunal organisms were sparsely distributed in these sediments, particularly in the Thalassia root layer (Appendix B). Significant amounts of externally produced organic matter would not be deposited in these shallow, relatively high wave energy environments. The organic carbon values of the surface samples can be considered maximum estimates of the amounts of organic matter present in the sediments when they are first colonized by Thalassia. It is somewhat surprising that there were not in the sediments larger amounts of organic matter derived from dead underground parts of Thalassia. In some Thalassia beds elsewhere, I observed large amounts

of dead rhizome tissue in the sediments. In a lagoonal Thalassia bed at Grenada, dead rhizome tissue, consisting largely of vascular bundles, occupied an estimated 80% of the sediment in a layer about 15 cm thick lying below the layer of living rhizomes. At a Bermuda Thalassia stand (Section III), dead, largely intact rhizomes were observed in a layer below the layer of living rhizomes. The substrates at these stands were highly sulfidic and decomposition rates may have been unusually low compared to those in the Barbados Thalassia sediments. Distribution of cellulose digesters may also be somewhat sporadic. Wood (1959a) failed to isolate either aerobic or anaerobic cellulose digesters from Zostera beds. He remarked, "This is surprising in the light of the quantity of cellulose-containing plants occurring in Lake Macquarie and in Australian estuaries. However, there are deep beds of the cellulosic parts of Zostera above the water level round the lake, giving field support to the laboratory findings regarding the absence of cellulose digesters. It is difficult, however, to believe that cellulose-digesting bacteria or fungi were entirely absent." Bavendamm (1932; reviewed by ZoBell, 1946b) isolated aerobic cellulose digesters from all samples of calcareous mud he examined, and anaerobic cellulose digesters from most samples. Other workers have reported difficulty in isolating cellulose digesters from marine sources (Oppenheimer, 1968, p. 221 to 222). The Barbados Thalassia stands are subject to recurrent erosion-succession processes (Appendix B), and, in part, the low amounts of organic matter in the sediments may reflect the relative youth of the Barbados stands.

General pH-Eh and bacterial characteristics of the sediments and observations on the occurrence of iron sulfide

The results of pH-Eh determinations, and estimates of bacterial numbers are given in Table V. All samples were from PS or CS substrates. It was not possible to get large, intact sediment samples from CF and CCS substrates. The purposes of these observations, which were carried out as part of preliminary studies on Thalassia beds, were primarily to determine (1) whether Thalassia root layer sediments, Syringodium root layer sediments and non-root layer sediments are characterized by differences in pH-Eh, and by pronounced differences in numbers of various physiological types of bacteria, particularly of sulfate reducers, and (2) whether the occurrence of ferrous sulfide at Bath and its absence at St. Lawrence reflect differences in bacterial activities in these two areas.

pH of all samples was less than 8.1 to 8.3, the pH of sea water in equilibrium with atmospheric CO_2. Lowest pH was observed in sediments covered by blue-green algal mats. These sediments were strongly reduced and smelled of sulfide. pH of samples from the Syringodium root layer varied from 6.8 to 7.4. pH of Thalassia root layer samples was, on the whole, somewhat higher, varying from 7.0 to 7.9. There may be some dissolution of skeletal carbonates in Thalassia sediments associated with low pH in microenvironments. Taylor and Lewis (1970) observed that some of the coarse particles in Thalassia sediments at Mahé, Seychelles, have a "corroded" appearance, and this was noted for the Barbados Thalassia sediments also. Wave disturbed (rippled) subsurface sediments from above the Thalassia and Syringodium root layers had, as would be expected, high redox potentials. Undisturbed subsurface sediments and sediments of the Syringodium root

Table V. pH-Eh and bacterial characteristics of sediments. U = undetected.

SEDIMENT TYPE	LOCATION & NO.	pH	Eh (mv)	BACTERIA (log of minimum no./g wet wt)					PLATE COUNT
				AEROBE	ANAEROBE	SO_4-REDUCING	NITRIFYING	NO_3-REDUCING	
SUBSURFACE (2 - 3 cm), WAVE DISTURBED	S-S1		+246, +337	6	4	4	U	4	
	S-S2			4	3	3*	U	3	1.2×10^4
	S-S3	7.7, 7.7	+252, +302						
SUBSURFACE, NOT WAVE DISTURBED	S-S4		+212, +382	5	5	3	U	5	
	S-S5	7.3, 7.4	0, +133	4	4	2 (2*)	4	4	2.7×10^4
	B-S6	7.6, 7.7, 7.7	-188, +14, +272	5	5	4	3	4	3.1×10^5
	B-S7	7.5, 7.7							
	B-S8	7.6, 7.6	+350, +394						
	B-S9	7.4, 7.4, 7.5, 7.7	-188, -119, -110, -98, +232						
SUBSURFACE, COVERED BY BLUE-GREEN ALGAL MAT	S-S10	6.5, 6.9	-189, -107	4	4	1 (2*)	3	3	6.5×10^4
	B-S11	7.3, 7.4, 7.5	-223, -188, -188			3			
	B-S12	6.4	-74						
	B-S13	6.6							
	B-S14		-138						

Table V -- Continued

SEDIMENT TYPE	LOCATION & NO.	pH	Eh (mv)	BACTERIA (log of minimum no./g wet wt) AEROBE	ANAEROBE	SO_4-REDUCING	NITRIFYING	NO_3-REDUCING	PLATE COUNT
STRINGODIUM									
ROOT LAYER	S-S15		-138	5	5	4	3	5	
	B-S16			5	4	3*	3	4	9.1×10^4
	B-S17	7.1, 7.1, 7.4	-229, +152, +182 +198	5	6	3	2	6	5.4×10^5
	B-S18	6.9, 7.1, 7.1	+105, +150	4	6	4	U	6	4.6×10^4
	B-S19	6.9, 7.3, 7.4	-190, -168, -108, +287	5	5	3	2	3	2.7×10^5
	B-S20	7.1	-242						
	B-S21	6.8							
THALASSIA									
ROOT LAYER	S-S22		-178, -172	5	5	3	1	5	
	S-S23		-170, -158	6	6	5	U	4	1.6×10^6
	S-S24			6	5	5*	3	5	3.9×10^6
	S-S25		0, +22, +46, +74	5	6	4	U	6	

Table V. Concluded.

SEDIMENT TYPE	LOCATION & NO.	pH	Eh (mv)	BACTERIA (log of minimum no./g wet wt)					PLATE COUNT
				AEROBE	ANAEROBE	SO_4-REDUCING	NITRIFYING	NO_3-REDUCING	
THALASSIA ROOT LAYER	B-826	7.4, 7.5, 7.6	-157, -8	6	5	4	2	4	2.5×10^5
	B-827	7.0, 7.5	-38	6	7	3 (2*)	U	7	2.4×10^6
	B-828			4	5	4*	U	5	
	B-829	7.5, 7.6							
	B-830	7.9							
	B-831	7.6							

*Medium containing peptone; others on medium with calcium lactate as only hydrogen donor.

layer were highly variable with respect to Eh, even within single sediment samples. Redox potentials of four of the five Thalassia root layer sediments examined were negative. The Eh data and general observations suggest that a reduced root layer is essential for normal development or good growth of Thalassia. The one sample with positive Eh was from a stand of notably poor growth (short leaves) of Thalassia. In areas where the surface sediments were well aerated, the erect shoots and rhizomes were found well below the sediment surface. At one area at Bath where wave action and currents were very strong and Thalassia was growing in a coarse sand substrate, I dug down approximately 30 cm without encountering the erect shoot apices. Where the sediment was uniformly reduced close to the sediment surface (indicated by iron sulfide formation at Bath), the top of the root layer was also close to the sediment surface. In situations where Thalassia was observed growing in a thin sediment layer over the rubble layer (such as stands B-12 and B-13 of Table III) or in sand filled crevices in rocks, both situations in which highly reduced conditions could not be maintained, the leaves were invariably short, indicating low growth rates. Similarly it was observed that where the underground parts of Thalassia had been partially or wholly exposed by erosion of the substrate, the leaves were very short, while in the adjacent uneroded areas, the leaves were of normal (longer) length. The occurrence of high P_g in CF and CCS substrates might be associated in part with strongly reducing conditions in these substrates. Eh observations were not obtained because of sampling difficulties. However, strong odor of sulfide and the uniformly blackened and tacky nature of sediments in CF substrate areas at Bath suggest that Eh is low. Thalassia and Syringodium appear to differ in their tolerances of oxidizing conditions about their erect shoots and rhizomes. Thalassia rhizomes were never

observed on the sediment surface except where they had been exposed by erosion. In contrast to this, the apical regions of Syringodium rhizomes were commonly observed 'creeping' over the substrate surface into grass-free areas. Eh data indicate that Syringodium rhizomes may occur in both strongly reduced and well aerated sediments. Erosional scarps in the Thalassia beds provided excellent exposures of the underground parts of Thalassia and Syringodium. At all scarps observed in areas of mixed Thalassia and Syringodium stands, the Syringodium rhizomes and erect shoots occupied a layer of 10 to 20 cm thickness next to the sediment surface, while the erect shoots and rhizomes of Thalassia occurred in a layer extending from just below or within the Syringodium root layer down to the base of the scarp (which varied in relief from about 15 to 60 cm). Replacement of a Syringodium stand by a Thalassia stand appears to involve initial growth of Thalassia rhizomes below those of Syringodium, and subsequent growth of Thalassia rhizomes into the Syringodium layer. Welsh (1965) related the Syringodium-Thalassia successional sequence to substrate stability. The requirement of Thalassia for a stable substrate may in fact be a requirement for a reduced substrate. Possibly Syringodium prepares the substrate for Thalassia colonization by stimulating development of reducing conditions.

Differences between the sediment samples in the numbers and proportions of various physiological types of bacteria were not pronounced, but there appear to have been generally higher numbers of bacteria in the Thalassia root layer sediments than in other sediments. The numbers of bacteria in the Thalassia root layer sediments are of the same order as the number reported by Burkholder et al. (1959), 3.7×10^6, in a Thalassia sediment at Puerto Rico, and numbers reported by Wood (1959a), 6.8×10^5 to 6.5×10^6, in muds of Zostera beds. Much larger numbers of bacteria are found

associated with leaves and detritus of Thalassia above and on the sediment surface. Burkholder et al. (1959) reported 15 x 10^6 bacteria per gram of leaf tissue, and Fenchel (1970) reported numbers of 1 x 10^6 to 9 x 10^6 per cm^2 of decaying Thalassia leaf tissue. For one sample of leaves (including young and old parts) I estimated a number of 2.5 x 10^6 per cm^2 by plate count. Minimum number estimates of aerobes using the medium of ZoBell and Morita (1959) agreed with estimates by plate counts using the medium of Burkholder et al. (1959). Minimum number estimates of aerobes were generally the same as the minimum number estimates of anaerobes, suggesting that a large proportion of the anaerobes were facultative anaerobes. ZoBell (1946b) found that over 90% of the marine bacteria isolated from either aerobic or anaerobic plates were facultative anaerobes. Ability to respire using nitrate as a hydrogen acceptor is characteristic of about 50% of bacteria isolated from the sea (ZoBell, 1946b). Fewer bacteria reduce nitrite (Liston, p. 301 in Oppenheimer, 1968). In the anaerobic cultures, disappearance of the nitrite produced by reduction of nitrate occurred in only the lowest dilutions (10^{-1}, 10^{-2}) except for sample B-S18 in which disappearance of nitrate occurred up to and including the 10^{-5} dilution. This might be due to assimilation of nitrite (reduction to ammonium) or to denitrification (reduction to gaseous nitrogen compounds). Nitrifying bacteria were either present in numbers less than 10/g or were not detected in 8 out of 18 samples including two samples from well oxygenated subsurface sediments. In most of the cultures in which nitrite did appear, only small amounts were evident (at about four to seven days after inoculation); subsequent disappearance of the nitrite indicated the presence of nitrite oxidizers in most of the cultures. Relatively large numbers of sulfate reducers were detected in all samples, including samples from well aerated subsurface sediments.

It is not unusual to isolate nitrifying bacteria from predominantly reducing sediments or soils (see for example, Kadota, p. 244 in Oppenheimer, 1968; Rinaudo, 1970). It is not understood, however, why nitrifiers were not isolated from so many of the samples, particularly those from well aerated sediments. The weak nature of the positive tests for nitrite in cultures from the other sediments suggests that technical difficulties were involved. However, it is also considered possible that production of sulfur compounds by Thalassia and sulfate-reducing bacteria could lead to a sporadic distribution of nitrifiers in the Thalassia sediments. Certain sulfur compounds inhibit nitrification when added to soils. Frederick, Starkey and Segal (1957) believed that the toxicity of methionine to nitrifiers is associated with the end products of sulfur decomposition of this compound, which are methylthiol and dimethyl sulfide rather than sulfate (or H_2S under anaerobic conditions) as for most other sulfur compounds. Wood (1965) was of the opinion that the reducing substances excreted by Zostera include dimethyl sulfide, or a similar compound.

Quite obviously sulfate reducers were not active at the Eh, +246 and +337 mv, of sample S-S1. Two phenomena which might account for the occurrence of large numbers of sulfate-reducing bacteria in this sample and sample S-S2 are (1) stirring up of sediments from deeper anaerobic layers, and (2) existence of reduced microenvironments. Wood (1959a) isolated sulfate reducers from muds containing decaying Zostera (Eh -130 mv at pH 5.9) but did not isolate sulfate reducers from surface muds of positive Eh in regions of growing Zostera. When winds caused stirring up of decaying Zostera, sulfate reducers were isolated from surface water, even though oxygen was not depleted. Wood (1959b) attributed occurrence of sulfate reducers and other strict anaerobes in surface water of Eh +430 mv to stirring up of bottom muds.

Evidence for the existence of reduced microenvironments within well aerated sediments is presented below.

The generally high numbers of sulfate reducers in the Thalassia sediments suggest that these bacteria are very active in these areas. The sulfate reducers undoubtedly play a major role in determining the pH-Eh characteristics of the Thalassia sediments. Presence of sulfide in Thalassia root layer sediments was indicated by odor of sulfide and blackening of the sediment associated with ferrous sulfide formation. Ferrous sulfide was observed in the Bath Thalassia sediments, but not in sediments of south coast Thalassia beds; lack of ferrous sulfide in the latter is attributed to a lack of iron rather than lack of sulfide (see below). Sulfide may be of more importance in the control of Eh in sediments in which iron is reasonably abundant than in iron-poor sediments because dissociation of iron sulfide would tend to buffer the sulfide concentration in the presence of oxidizing agents. All Thalassia root layer sediment samples from Bath smelled of sulfide. St. Lawrence sediment samples S-S10, S-S15 and S-S23 smelled strongly of sulfide and had correspondingly low Eh values (Table V). However sample S-S22, from the Thalassia root layer, had a low Eh but did not smell of sulfide. Activities of sulfate-reducing bacteria are probably at least partially responsible for low pH under anaerobic conditions in Thalassia bed sediments. Production of organic acids from the incomplete decomposition of carbohydrates by bacteria other than sulfate reducers may also contribute to lowering of the pH under anaerobic conditions.

Under oxidizing conditions, iron exists mainly as highly insoluble colloidal or particulate ferric hydroxide (or hydrous ferric oxide) which is uniformly dispersed through the sediments. Under reducing conditions, iron may become concentrated through diffusion of ferrous iron to regions of high

sulfide concentration where it is precipitated as ferrous sulfide. Manganese may become concentrated through similar processes. Subsequent exposure of the ferrous sulfide, which is black in color, to oxidizing conditions may result in an orange coloration due to the presence of concentrated ferric hydroxide (Oppenheimer, 1960, 1966). Under certain conditions pyrite (FeS_2) is formed, but the mechanisms are controversial (see for example, Berner, 1962). Blackening of sediments at Bath associated with iron sulfide formation corresponded with what would be expected on the basis of the occurrence of reducing conditions as indicated by Eh measurements. Subsurface sediments and sediments of the Syringodium root layer were mottled in appearance indicating non-uniform reduction of the sediments. Sediments under blue-green algal mats were uniformly blackened, as were sediments of the Thalassia root layer. Beach sediments and wave disturbed sediments were not discolored, with the exception of certain coarse grains. Surfaces of coral-coralline algal cobble sized debris buried in the sediment were often blackened, and surfaces of some unburied cobble sized debris were stained orange, suggesting previous burial in reducing sediments. Coarse (over 0.3 mm diameter) sediment grains derived from the encrusting foraminiferan Homotrema rubrum were peculiar in that, unlike smaller sized grains and most other grains in the coarse fractions of the sediments, the black discoloration was not lost when the grains were exposed to air or bleach. About 80% of the Homotrema grains remained so darkly stained that they could not be recognized as skeletal carbonates without sectioning. In thin section (see Appendix B for methods of examination of sediments) the chambers of the Homotrema debris were observed to be packed with a black substance (Plate III). The solubility of this substance in acid and the strong positive result of a test for ferrous iron (red color of a slightly acidic test solution with 2,2'-bipyridine;

Feigl, 1958) indicate the black substance was ferrous sulfide. Partial black discoloration of bleached sediment grains derived from corals, red algae and Halimeda was also observed, but few grains derived from molluscs, echinoderms or other foraminifera--in addition to Homotrema, Rotorbinella rosea, Amphistegina gibbosa and Puteolina angulata were abundant in these sediments--retained any black discoloration. Patches on the surfaces of some Halimeda grains were stained orange.

A number of workers have observed discoloration of skeletal carbonates associated with iron sulfide formation (see for example, Maiklem, 1967; Macintyre, 1970). Emery and Rittenberg (1952) and Kaplan, Emery and Rittenberg (1963) observed pyrite particles having the shapes of radiolaria and foraminifera. To account for the occurrence of large amounts of authigenic pyrite in oxidizing surface sediments (Emery and Rittenberg, 1952) and for sulfur isotope data which indicated the pyrite was formed from a large pool of sulfate (Kaplan et al., 1963), these workers postulated that iron sulfide formation takes place mainly at the sediment-water interface within reduced microenvironments. Berner's (1969) diagenetic models provide support for this hypothesis by showing that in sediments of 'high' reactive iron content and low organic content, sulfate reduction within an organic rich layer or microenvironment would lead to enrichment of the boundary of the layer with iron sulfide. An argument for the initiation of sulfate reduction within the Homotrema grains while the grains were still in externally oxidizing environments is simply that it is difficult to understand otherwise how sufficient organic matter would be preserved for the activities of sulfate reducers. The occurrence of large amounts of iron sulfide in the Homotrema grains seems to imply efficient use of the organic matter originally present by a bacterial population composed mainly or entirely of sulfate reducers.

At Bath, Homotrema grows mainly on the undersides and in cavities of rocks in very turbulent areas seaward of the Thalassia beds. When the tests are broken off from their substrates, they must remain in an oxidizing environment for a relatively long period. Unless sulfate reduction were initiated soon after death of the organism, it seems likely that other bacteria would consume most of the organic matter present. These considerations suggest that the occurrence of large numbers of sulfate reducers in well aerated surface sediments of the Thalassia beds may have been associated with activity of these bacteria in reduced microenvironments in skeletal carbonates.

The lack of iron sulfide in Thalassia sediments at St. Lawrence and in other Thalassia beds on the south coast of Barbados must be attributed to a lack of iron rather than a lack of sulfide. The low Eh, odor of sulfide, and large numbers of sulfate reducers in some of the St. Lawrence sediments indicate sulfate reducers were active in these sediments. There is no reason to believe that bacterial processes in the south coast Thalassia beds are fundamentally different from those in the Bath Thalassia beds. Kaplan et al. (1963) considered that of the three possible sources of iron, sea water, marine organic matter and detrital minerals, only the last could be a major source of iron for iron sulfide formation. Kaplan and Rittenberg (1963) remarked that the total amount of iron available may be important in determining the color of marine sediments, and suggested that high iron content would be expected in areas subject to runoff and, perhaps, in areas with a high content of volcanic rocks. High iron content of the Bath sediments may be associated with the occurrence of volcanic ash deposits in this area. At Carriacou, an island of volcanic origin, blackening of sediments was observed in Thalassia beds in near-shore areas, where there were large amounts of detrital minerals in the sediments; at 200 to 300 m offshore, where the

sediments consisted predominantly of skeletal carbonates (Appendix B), blackening of sediments in Thalassia beds was not observed. Thus, while the presence of ferrous sulfide in Thalassia bed sediments may indicate reducing conditions, its absence in Thalassia bed sediments appears to indicate a sparcity of iron, rather than oxidizing conditions. This is an important point because the occurrence of reducing conditions in near shore areas is frequently inferred from casual observations on the occurrence of ferrous sulfide.

Considerations of supply and demand

Estimates of the nitrogen and phosphorus required for leaf growth (above ground parts only) and the amounts of nitrogen and phosphorus available in the root layer were made for stand B-9 (Fig. 5d). In terms of P_g and P_n, this stand was more or less typical of stands observed at Barbados and Carriacou (Appendix B).

The amounts of nitrogen and phosphorus required for leaf growth were estimated by multiplying the estimate of P_n, 3.1 g/m^2 per day, by the values of nitrogen and phosphorus given for this purpose previously, i.e. by 2.3% N and 0.3 x 2.3% N for maximum and minimum estimates of nitrogen requirements, and by 0.1% P and 0.3 x 0.1% P for maximum and minimum estimates of phosphorus requirements. The volume of the root layer was estimated as 127 l/m^2. By drying of sediment samples of known volume and wet weight, one liter of the root layer was estimated to contain 490 ml interstitial water and 1140 g dry wt sediment; these estimates are uncorrected for the volume occupied by plant tissues, but a rough estimate indicates this was less than 2% of the root layer volume. Average concentrations of phosphate and ammonium in the interstitial water were estimated by integration of the profiles in Fig. 5d. The average concentration of NH_4-N in the interstitial water of the root layer was estimated as 6.1 µg-at/l; this implies (Fig. 7) an average of

approximately 170 µg-at extractable NH_4-N/kg dry wt sediment. Available phosphate was assumed to be 213 µg-at/kg dry wt sediment (the total of 28 extractions of sediment A, Fig. 8). These values were converted to total amounts in the root layer. Average quantities of nutrients in the sea water are based on 14 observations in the Thalassia beds (Table VI). The estimates are thus:

N required for leaf growth:	21 to 71 mg/m^2 per day
P required for leaf growth:	0.9 to 3.1 mg/m^2 per day
Total interstitial water NH_4-N in root layer:	5.3 mg/m^2
Total interstitial water PO_4-P in root layer:	1.7 mg/m^2
Total extractable NH_4-N in root layer:	340 mg/m^2
Total available PO_4-P in root layer:	960 mg/m^2
Average NO_3-N + NO_2-N + NH_4-N in sea water	14 mg/m^3
Average PO_4-P in sea water:	6.1 mg/m^3

Thus it is estimated that in the root layer of this stand there was sufficient phosphate to satisfy requirements of leaf growth for 300 to 1000 days, while the total ammonium was sufficient to satisfy requirements of leaf growth for only 5 to 15 days. Nitrate was present in only small quantities in the root layer of this stand (Fig. 5d and Table IV), hence inclusion of nitrate as a source of nitrogen would not significantly alter these estimates. At the average concentrations of nutrients in the sea water, the inorganic nitrogen would have to be completely removed from 5 m^3 of sea water to satisfy one day's requirements of Thalassia leaves for nitrogen but the

Table VI. Nutrients in surface sea water at Barbados.
U = undetected.

LOCATION	DATE	NO_3+NO_2-N	NH_4-N (µg-at/l)	PO_4-P	REFERENCE
1. WATER IN THALASSIA BEDS					
Bath	May 7 '68	0.08	0.60	0.18	
Bath, inshore amongst decaying seaweed	May 7 '68	0.21	0.47	0.08	
Bath	Nov 7 '68	U	0.10	1.70	
Bath	Nov 21 '68				
	0630 hr	U	U	U	
	0800	U	0.46	0.21	
	1000	U	0.62	U	
	1200	5.00	U	0.11	
	1430	U	U	U	
	1730	U	U	U	
St. Lawrence	Dec 23 '69	0.92	0.28	0.10	
St. Lawrence	Jan 13 '70	0.88	U	0.17	
Oistin Bay	Jan 21 '70	0.70	0.81	U	
Bath	Jan 28 '70	1.00	0.57	0.11	
St. Lawrence	Feb 26 '70	0.98	0.33	0.18	
	Sensitivity:	0.06-NO_3 0.02-NO_2	0.15	0.05	
	Average:	0.70	0.30	0.20	
2. WEST COAST OF BARBADOS, INSHORE	38 samples taken July '68 to June '70, Means and ranges:	0.80 (0.08 -3.16)		0.06 (U -0.12)	F. Sanders (personal communication, 1971)
3. WEST COAST OF BARBADOS, 10 to 15 km OFFSHORE	30 samples taken Dec '61 to Nov '64, Means and ranges:	0.25 (U -1.42)		0.12 (U -0.68)	Beers, Steven and Lewis (1968)
	42 samples taken Aug '67 to May '69, Means and ranges	0.44 (0.05 -2.40)	1.30 (U -3.27)	0.06 (U -0.39)	Steven, Brooks and Moore (MS, 1970)

inorganic phosphorus would have to be removed from only 0.5 m^3 to supply the phosphorus required for one day's growth (or from 1.5 m^3 for nitrogen and from 0.15 m^3 for phosphorus assuming the minimum estimates of requirements). These supply-demand comparisons illustrate why nitrogen rather than phosphorus is limiting growth of Thalassia. In making these comparisons, it is assumed that a closed system exists with respect to the nitrogen and phosphorus of underground parts, i.e. that uptake of nitrogen and phosphorus for synthesis of underground parts is balanced by regeneration of nitrogen and phosphorus accompanying decomposition of underground parts.

The good correlation of P_s with interstitial ammonium indicates that virtually all nitrogen for growth comes from the sediments, and also that a steady state must exist with respect to the nitrogen status of the sediments. The correlation of P_s with rhizome water soluble phosphate indicates that significant amounts of phosphate are taken up from the sediments for leaf growth. It is apparent from the above considerations, however, that day to day replenishment of the phosphorus removed from the sediments is far less critical than for nitrogen. How is the nitrogen status of the sediments maintained? Is the phosphorus status of the sediments maintained, or is there a steady decline in the reserves of phosphorus in the sediments? There is no possibility of an efficient recirculation of nutrients. Growth of calcareous epiphytes on the leaves results in continual fragmentation of old parts of the leaves into small pieces (Appendix A), most of which are immediately carried away in the water. The environments in which Thalassia grows in Barbados are shallow, relatively high wave energy environments not favorable to accumulation of organic matter on the sediment surface or in the sediments. The root layer may lie well below the sediment surface; even assuming that nutrients were taken up at the sediment surface by biochemical

or physical-chemical processes, the nutrient profiles (Fig. 5) are largely inconsistent, even qualitatively, with a diffusion process from the sediment surface into the root layer. Assuming a diffusion coefficient of 2×10^{-5} $cm^2 sec^{-1}$ (an approximate value for sea water; Sverdrup, Johnson and Fleming, 1942), then gradients of the order of 300 µg-at N/l per cm and 6 µg-at P/l per cm are predicted by Fick's Law to account for the flux of nitrogen and phosphorus by simple diffusion. Obviously there cannot be significant movement of nitrogen and phosphorus into the root layer by simple diffusion.

The following two considerations suggest that crystalline phosphate in the sediments could not be a major source of phosphorus for Thalassia: (1) it appears likely that most of the crystalline phosphate in the sediments is occluded within skeletal carbonates, and thus is not available to go into solution; (2) even if there were crystalline phosphate (apatite) in contact with the interstitial water, it is unlikely that dissolution of this could occur at a rate approaching the rate at which phosphate is taken up by Thalassia. Occluded phosphate could become available only through dissolution of the skeletal carbonates. Calcium carbonate is unaffected by Eh. Decreased pH of the interstitial waters or within microenvironments might cause some dissolution of calcium carbonate, but it is obvious that large scale dissolution of the sediments could not occur. Further, it should be noted that the effect of production of bicarbonate by sulfate-reducing bacteria is to oppose dissolution of calcium carbonate associated with lowered pH. Crystalline phosphate in contact with the interstitial water would tend to go into solution as phosphate was removed from the interstitial water by Thalassia in accordance with the equilibrium

$$\text{Crystalline P} \rightleftharpoons \text{Interstitial P} \rightleftharpoons \text{Adsorbed P}$$

However, because of the slow rate at which apatite dissolves, it seems unlikely that the observed levels of interstitial water phosphate could be maintained in the sediments by this mechanism, given rates of removal equal to the requirements of Thalassia. The above supply-demand estimates indicate that for stand B-9 interstitial water phosphate would have to be renewed every 13 to 45 hours to satisfy the requirements of Thalassia. According to Roberson's (1966) data on the solubility of apatites in sea water, at pH 7.5 and calcium ion concentration 1.2×10^{-2}M, the equilibrium concentration of phosphate is approximately 1 µg-at/l. This concentration is of the same order as the concentrations of phosphate in the root layer interstitial water of stand B-9 (Fig. 5d). Thus if crystalline phosphate were to go into solution at a rate equal to uptake of phosphate by Thalassia, this implies an equilibration period of the order of 13 to 45 hours. If the calcium ion concentration is depressed in the Thalassia bed interstitial waters, then the equilibrium concentration of phosphate might be much higher. However, even in systems greatly undersaturated with respect to apatite, dissolution of apatite is extremely slow. In experimental soil systems at pH 4.4 to 5, Murrmann and Peech (1969b) found no significant increase in labile and soluble phosphate originating from added hydroxyapatite after a two month period (30 minutes stirring per day). Roberson (1966) used a two week equilibration period for studying solubility of apatites in sea water at initial pH 2.8 and 3.5 (continual mixing). In terrestrial systems, mobilization of non-mobile phosphate is not considered to constitute a significant source of phosphate for the current growing season (Sutton and Gunary, 1969). The significance of phosphate released from the crystalline phase depends on the net rate of removal of phosphorus from the system. In nearly closed systems, even a very slow rate of release of phosphate from the crystalline phase may

be critical to maintaining the phosphorus status of the soils over long periods of time. Cropping of Thalassia leaves may be considered 100% efficient. Thus for dissolution of crystalline phosphates to contribute significantly to the supply of phosphate for Thalassia, even over long periods of time, the rate of formation of interstitial water phosphate would have to approach the rate of use by Thalassia. It is concluded that this does not occur, even under conditions of sulfate reduction.

That there was not large scale net removal of phosphate from these sediments is suggested by the fact that the HCl soluble phosphate values of the root layer sediments were not lower, or were only slightly lower, than the HCl soluble phosphate values of the surface sediments of the same stands (Table IV; the surface sediment of stand B-2 and the root layer sediment of stand B-1 may be compared in this respect as these were adjacent stands). In fact the anomalously high value of the sample from the root layer of stand B-15 suggests that there may have been a net deposition of phosphate in the root layer of this stand.

These conclusions are in apparent contradiction to the evidence of the P_s-rhizome water soluble phosphate correlation that significant amounts of phosphate are taken up from the sediments by Thalassia. There is no reason to suppose that Thalassia would not take up significant amounts of phosphate from the interstitial water, given the ability of the plant to do so, the frequently negligible quantities of phosphate in the sea water (Table VI) and quantities of phosphate in the interstitial water averaging approximately 0.7 µg-at/l. The absence of equivalent rates of renewal of the interstitial water phosphate and adsorbed phosphate would be expected to result in depletion of phosphate in the sediments, or at least in interstitial water phosphate concentrations not appreciably above the concentrations in sea water.

This was not the case. Accomodation of these apparently contradictory or incompatible conclusions may lie in the suggestion of McRoy and Barsdate (1970) that "seagrass may...act as a source or sink of dissolved phosphate in estuarine waters." This suggestion was based on the observation that some of the ^{32}P taken up by roots of Zostera was excreted by the leaves, and vice versa, that some of the ^{32}P taken up by the leaves was excreted by the roots. McRoy and Barsdate (1970) believed that the site of phosphate uptake in situ would be determined by the relative concentrations of phosphate in the sea water and interstitial water. For the Barbados Thalassia beds, the mid root layer interstitial water phosphate concentrations averaged 0.73 µg-at/l; the average concentration in the sea water was 0.20 µg-at/l. The minimum value of the mid root layer interstitial water was 0.27 µg-at/l, and the maximum value was 1.04 µg-at/l, while the maximum value observed in the sea water was 1.70 µg-at/l. Thus, at certain times, it may require less expenditure of energy to take up phosphate from the sea water than from the interstitial water. The P_s-rhizome water soluble phosphate correlation indicates that most plants were taking up significant amounts of phosphate from the interstitial water during the period of most active photosynthesis. Perhaps phosphate is returned to the sediments during darkness, or at any time that phosphate concentrations in sea water are high and phosphate is taken up in excess of demand. The Thalassia-sediment phosphate system might thus be represented as

$$\text{Apatitic P} \rightleftharpoons \text{Interstitial P} \rightleftharpoons \text{Adsorbed P}$$
$$\Updownarrow$$
$$\text{Thalassia} \rightleftharpoons \text{Sea water P}$$

A system in which phosphate is both taken up from and returned to the sediments by Thalassia, coupled with interstitial phosphate-adsorbed phosphate

reactions in the sediments, may ensure that there is always an adequate supply of phosphate for Thalassia when it is in greatest demand. Thus although the sediments may not be a primary source of phosphate for Thalassia, they may function as a sort of storage bank for phosphate.

There seems to be no way to account for maintenance of the nitrogen status of the sediments other than by postulating high rates of N_2 fixation. Various considerations suggest that N_2 fixation by heterotrophic anaerobic bacteria in the sediments is in fact the source of nitrogen for Thalassia. Large amounts of nitrogen are present in sea water in dissolved gaseous form. At 25°C and 18 °/oo chlorinity, N_2 saturation is approximately 11 mg/l (Sverdrup et al., 1942). Dissolved N_2 undoubtedly enters the sediment both through simple diffusion and via the lacunal system of Thalassia. It is pertinent that a reduced root layer appears to be essential for normal development of Thalassia and that the maximum concentration of interstitial water ammonium occurred well below the sediment surface in those stands where the top of the root layer was well below the sediment surface (Fig. 5a, d). Often Thalassia sediments are reduced to within a few millimeters of the sediment surface. Thus if N_2 fixation is the source of nitrogen in the sediments, then it seems likely that a heterotrophic anaerobe is involved. A number of workers have isolated N_2-fixing Azotobacter and Clostridium from marine sources (reviewed by Waksman, Hotchkiss and Carey, 1933; ZoBell, 1946b). Sisler and ZoBell's (1951) claim that some strains of marine Desulfovibrio fix N_2 has been verified by several studies (LeGall, Sēnez and Pichnoty, 1959; Riederer-Henderson and Wilson, 1970; Postgate, 1970). In addition to these genera, there is evidence for N_2 fixation by members of at least 12 other bacterial genera, and most of these fix N_2 under anaerobic conditions (Stewart, 1969). There is then, no reason to believe that N_2 fixation in

Thalassia sediments would be limited by a lack of suitable organisms. Lack of organic matter is believed to be the main factor limiting N_2 fixation by heterotrophic bacteria in terrestrial systems (Stewart, 1969). Reported 'efficiencies of fixation' by Clostridium, a strict anaerobe, vary from 2 to 27 mg N_2 fixed per gram carbohydrate utilized. Assuming an efficiency of 27 mg N_2/g carbohydrate, and requirements of Thalassia for nitrogen equal to 2.3% N times production, then in order for heterotrophic N_2 fixing bacteria to supply sufficient nitrogen to satisfy demands of Thalassia, carbohydrate would have to be supplied to these bacteria at a rate approximately equal to the rate of production of leaf tissue organic matter. Two possible sources of this material are (1) organic matter of underground parts, and (2) organic matter excreted by Thalassia. Production of underground parts of Thalassia is estimated to occur at rates equal to 1/3 to 1.5 times the rates of production of leaf tissue above ground (Appendix A). Studies by Wetzel (1969) indicate that the fresh water angiosperm Najas may excrete organic carbon at rates equal to those of net fixation of carbon by this plant.

Until recently it was generally assumed that there could not be significant N_2 fixation under anaerobic conditions because of the inefficient use of energy substrates under anaerobic conditions. However studies by Chang and Knowles (1965) and Brouzes, Lasik and Knowles (1969) on the incorporation of $^{15}N_2$ in soil samples under both aerobic and anaerobic conditions point to significantly greater N_2 fixation under anaerobic conditions than under aerobic conditions. Brouzes et al. (1969) suggested that the reason for this may be that there is less competition for energy substrates under anaerobic conditions. Evidence presented in this thesis indicates that a reduced root layer is essential for normal development or good growth of Thalassia; this may represent a requirement of the N_2-fixing bacteria.

III. ASSAY OF N_2 FIXATION IN A THALASSIA SEDIMENT

INTRODUCTION

Very little is known concerning the quantitative significance of N_2 fixation by free-living organisms. This is largely attributable to lack of convenient methods for measuring N_2 fixation in field systems. Measurement of N_2 fixation in field systems in the past has been based mainly on two methods: (1) determination of nitrogen gains in a system by Kjeldahl analysis, and (2) measurement of incorporation of $^{15}N_2$ into samples taken from the field. The first method suffers from a lack of sensitivity, measures gains of nitrogen from all sources, and measures only net gains of nitrogen. The second method has had only limited application because of the complexity and expense involved. The recent development of the acetylene-ethylene assay for N_2 fixation has made the measurement of N_2 fixation in field and artificial systems relatively simple and inexpensive. The methodology, characteristics and applications of this technique are given in detail by Hardy et al. (1968). The assay is based on the nitrogenase catalysed reduction of acetylene to ethylene. An opportunity for assaying N_2 fixation in Thalassia sediments by this technique was kindly provided by Dr. Roger Knowles of the Department of Microbiology, Macdonald College of McGill University. Dr. Knowles has used this technique in the assay of N_2 fixation in agricultural and forest soils.

MATERIALS AND METHODS

Field

Samples of Thalassia root layer sediments were obtained from a Thalassia bed at Fort St. Catherine, Bermuda, on August 30, 1970, and taken to

Macdonald College on the same day. The Thalassia bed in which sampling was carried out was selected because dense growth and long length of the Thalassia leaves indicated high rates of production. This bed was shallow (about 1 m below low water) and was subject to gentle wave action at the time of sampling. The sediments were uniformly reduced within a few millimeters of the sediment surface (indicated by FeS formation), and consisted predominantly of sand sized skeletal carbonates. The substrate is classified as a PS type. The top of the root layer was approximately 4 cm below the sediment surface, and extended 22 to 30 cm below the sediment surface. The root layer overlay layers of sediment in which there occurred large amounts of dead, largely undecomposed, rhizome tissue. There were few infaunal organisms, and except for the occasional sponge, the sediment surface was bare of epifauna. Production of the stand was estimated from data obtained by direct measurement of the growth of leaves. Details of these measurements, and estimates of the underground production are included in Appendix A.

Sediment samples from the Thalassia root layer were taken between 0600 and 0900 hours. These were taken in a short length of $1\frac{1}{2}$ inch O.D. cellulose acetate tubing, as at Barbados, and immediately transferred to the assay tubes. The assay tubes were made of 8 inch lengths of $1\frac{1}{2}$ inch I.D. polyvinylchloride tubing, the ends of which were plugged with rubber stoppers. The assay tubes were filled with sediment, and the stoppers taped on to ensure they remained in place during transit. Initial processing of the samples at Macdonald College was begun at 1600 hours, and completed by 1800 hours.

Laboratory

The general procedure used in the acetylene reduction assay of N_2 fixation has been given by Hardy et al (1968). As applied in this study, the following steps were involved. (1) The top 3 to 4 cm of sediment in each

assay tube were scooped out to create a gas phase above the sediment. The samples were amended with glucose and sulfate where appropriate, and the assay tubes serum capped. (2) Samples were evacuated to 1 atm and back-filled with 1 atm Ar for samples to be incubated anaerobically, or 1 atm Ar and O_2 ($Ar:O_2$, 4:1) for samples to be incubated aerobically. This procedure was repeated three times. (3) The samples were evacuated to 1 atm, and back-filled with 0.9 atm Ar or $Ar-O_2$, and 0.1 atm C_2H_2. (4) The samples were incubated at 30°C. (5) At intervals of 1,2,3,4 days, 0.7 ml gas phase was removed from each sample. This was analyzed for acetylene and ethylene by use of a Hewlett-Packard Model 5750 gas chromatograph with an activated alumina column (183 x 6 mm) at 200°C, a flame ionization detector, and with He carrier gas at a flow rate of 50 ml per min. (6) Following completion of the final assays on the fourth day, the gas phase volumes were determined.

High pressures built up in most of the amended samples after the first day. Gas samples were equilibrated to atmospheric pressure and hence corrections for estimating true C_2H_4 production were applied by calculating the changes in the total amounts of $C_2H_2+C_2H_4$ in the samples.

Six samples were used as controls to determine background levels of acetylene and ethylene, and to determine whether significant adsorption or metabolism of ethylene occurred in these sediments. The 16 test samples were divided into two groups, one of which was incubated aerobically, and the other, anaerobically. Two samples in each group were amended with 10 ml of a basal solution (NaCl, 0.3M; KCl, 0.01M; $MgSO_4$, 0.05M) containing 2 g glucose; each sample contained approximately 200 g dry wt sediment, so this was an

approximately 1% glucose amendment. One sample in each group was amended with glucose solution plus 5 ml saturated sodium sulfate (400 g/l) solution. The other five samples in each group were unamended. Sulfate solution was added with the intention of stimulating sulfate-reducing bacteria. Following final assays, most-probable-number (MPN) counts (five tubes per dilution) were made of sulfate reducers in selected samples. The calcium lactate medium for sulfate reducers given in Section II of this thesis was used, with the exception that sea water was replaced by a solution 0.3M in NaCl, 0.05M in $MgSO_4$, 0.01M in KCl, 0.01M in $CaCl_2$ and 0.025M in $NaHCO_3$, adjusted to pH 7.5. Results of the acetylene reduction assays are given as mg N_2 fixed/m^2 per day, and were calculated as:

$$\text{mg } N_2 \text{ fixed/m}^2 \text{ per day} = (C_2H_4) \times 28/3 \times 220000/SV$$

where

(C_2H_4) is the ethylene produced in a sample over a 24 hour period, in millimoles

28/3 is a conversion factor for converting millimoles of ethylene to the equivalent in N_2 fixed

220000 is the estimated volume of the sediment in the root layer of the Thalassia stand, in ml/m^2

SV is the sample volume, in ml

For converting the results to a dry wt sediment basis, a bulk density of 1.13 g/ml may be assumed (average of the dry wt-to-volume ratios of 10 unamended samples). There was little or no adsorption or metabolism of ethylene in two control samples to which ethylene had been added. Small amounts of ethylene were detected in three of four control samples to which no acetylene

or ethylene had been added. The largest amount was equivalent to approximately 1.6 mg N_2 fixed/m^2 per day, and thus only figures above this value are considered significant.

Since six electrons are required for reduction of N_2 to NH_3 and 2 electrons for reduction of C_2H_2 to C_2H_4, reduction of three moles of acetylene should be equivalent to reduction of one mole of N_2. Comparisons by Hardy et al. (1968) of acetylene reduction and N_2 fixation (Kjeldahl measurement) by Azotobacter cultures gave empirical ratios varying from 3 to 4.5. Comparisons by Rinaudo (1970) of C_2H_2 reduction and N_2 fixation (Kjeldahl measurement) in the rhizosphere of rice also validated the 3:1 ratio for that system. However, work in Dr. Knowles laboratory on acetylene reduction and $^{15}N_2$ incorporation in soils has given empirical ratios varying from 10 to 22 (Brouzes, Mayfield and Knowles, 1971). A ratio of 3 was assumed in calculation of N_2 fixation in the Thalassia bed sediments. It is not known whether there was complete exchange between the gas phase and the sediment in these samples. For these reasons, the results should probably be considered semi-quantitative.

It is unlikely that any of the 'aerobic' samples were in fact aerobic or even partially aerobic for more than part of the incubation period. Ferrous sulfide would have acted as a poising agent; H_2S odor was evident in all samples at the end of the incubation period, and was particularly strong in the amended samples.

RESULTS AND DISCUSSION

The Bermuda Thalassia stand had the highest P_m, 14.1 g/m^2 per day, of any Thalassia stand examined in this study. P_s, 8.75 mg/shoot per day, was not higher than values observed for Barbados stands but the shoot density, 1611 shoots/m^2, was unusually high (see Appendix B for shoot density values).

The maximum and minimum estimates of nitrogen requirements of this stand (estimated as in Section II) are 97 and 324 mg N/m^2 per day. The estimates of N_2 fixation in the root layer sediments are given in Table VII. Grouping the samples into aerobic and anaerobic, amended and unamended samples, the mean values and standard deviations are:

	$\bar{x}$ (mg N_2 fixed/m^2 per day)		SD
unamended, aerobic	3.8	(0.76)	3.9
unamended, anaerobic	1.7	(0.34)	2.7
amended, aerobic	339	(68)	146
amended, anaerobic	581	(116)	230

Values in brackets are the N_2 fixation rates assuming a 15:1 ratio in estimating N_2 fixation from acetylene reduction. In the unamended samples significant reduction occurred only sporadically in the anaerobic samples; in the aerobic samples the reduction values were significantly higher. However, it is apparent from comparison of these estimates with the estimates of the nitrogen requirements of the Thalassia stand that the rates of N_2 fixation in these sediments as indicated by the assays of the unamended samples would be insufficient to renew nitrogen removed from the sediment by Thalassia. Rates of acetylene reduction were of the order of 100 times greater in the glucose and glucose+sulfate amended samples than in the unamended samples, and rates in anaerobic amended samples were almost twice as high as the rates in the aerobic amended samples. Rates of acetylene reduction were high in the amended samples from the first day of incubation, indicating the presence of a large population of N_2 fixing bacteria in situ. It is evident that N_2 fixation could account for renewal of nitrogen removed from the sediments by Thalassia provided there were readily assimilable energy substrates available to the N_2 fixing bacteria.

Table VII. N_2 fixation in Bermuda Thalassia sediments estimated from acetylene reduction assays.
G = glucose, S = sulfate, O = aerobic, ø = anaerobic

Sample	Amendment	Incubation	Calculated N_2 fixation mg N_2/m^2 per day			
			Day 1	Day 2	Day 3	Day 4
7		O	3.4	0.7*	11.5	0
8		O	13.6	5.7	8.7	0
9		O	6.5	3.2	0	0
10		O	3.3	2.8	5.2	0
11		O	2.8	0.5*	4.9	3.8
12	G	O	250	234		
13	G	O	259	351	649	388
14	G + S	O	335	402	422	99
15		ø	1.4*	0.9*	4.4	0
16		ø	0.7*	0.8*	10.8	0
17		ø	0.5*	0.4*	6.6	2.4
18		ø	0.6*	0.4*	0.3*	0.3*
19		ø	1.0*	0.8*	0.7*	0
20	G	ø	454	540	521	418
21	G	ø	265	719	923	972
22	G + S	ø	455	665	757	278

* Not significant.

Addition of glucose to the samples represented about a 50% increase in the total amount of organic matter in the samples, assuming that the sediments contained approximately 2 g organic matter/100 g dry wt sediment (Table IV, Section II). It may be concluded that although organic matter was present in the unamended samples, it was largely unavailable to (unassimilable by) the N_2 fixing bacteria. Since this organic matter probably consisted largely of cellulose derived from underground parts of Thalassia and the common N_2-fixing bacteria are not cellulose decomposers, this conclusion is not surprising. High levels of N_2 fixation in straw amended soils have been attributed to a two phase system in which plant residues are broken down under aerobic conditions, and N_2 fixation occurs under anaerobic conditions (Rice, Paul and Wetter, 1967). The somewhat greater rates of acetylene reduction in the 'aerobic' unamended samples compared to the anaerobic unamended samples may have been due to increased breakdown of cellulose in the former. However it is apparent that even if microenvironments of oxidizing conditions exist in the root layer sediments, there still would not be sufficient organic matter available to the N_2 fixing bacteria to allow N_2 fixation to the degree required to satisfy the demands of Thalassia. It is concluded that the organic matter of underground parts of Thalassia is not a significant source of energy for N_2-fixing bacteria in situ. If N_2 fixation is in fact the source of nitrogen for growth of Thalassia, then there must be a source of energy available to the N_2 fixers in situ that was not available in these sediment samples.

Synthesis of amino acids appears to occur in the roots of Thalassia as in certain terrestrial plants; this implies a regular transport of carbon skeletons to the roots via the phloem. In terrestrial plants, phloem transport of carbon skeletons to the roots is stimulated by and regulated by the

demand of the root for the carbon skeletons (Kursanov, 1961, 1963). Given (1) the high potential of the sediments for fixing N_2, (2) the evidence that nitrogen limits growth of Thalassia, (3) the limited standing supply of nitrogen in the sediments, (4) the evidence that all nitrogen for growth of Thalassia comes from the sediments, and (5) the lack of possible alternative sources of nitrogen in the sediments, there can be little doubt that organic matter is excreted into the sediments by Thalassia and serves as a source of energy for the N_2 fixing bacteria. It is probably not necessary for cell autolysis to occur for reduced N_2 to be available to Thalassia; N_2-fixing bacteria excrete reduced N_2, predominantly in the form of ammonium, during healthy growth (Stewart, 1966). A finely balanced relationship between Thalassia and the nitrogen status of the sediments is implicit in the simple nature of the relation of P_s to interstitial water ammonium. The available evidence points to a sensitively regulated, symbiotic type of relationship between Thalassia and the N_2-fixing bacteria.

If organic matter and ammonium substrates move between Thalassia roots and N_2-fixing bacteria mainly by diffusion, then most N_2 fixation must take place within a fairly small distance from the root. At the Bermuda stand there were an estimated 1611 erect shoots per square meter. The maximum volume of sediment per shoot was thus 220 l ÷ 1611 = 136 cm^3. As a conservative estimate, there would be approximately 40 cm root per shoot, and thus the maximum volume of sediment per shoot is equivalent to a band of approximately 1 cm radius around the root. Assuming that this stand was overcrowded and that P_s could be increased simply by removing some of the shoots (i.e. allowing a greater volume of sediment per shoot), then an increase in P_s by a factor of three to 26.1 mg leaf tissue/shoot per day, a larger value than was observed for any Thalassia stand in this study, represents a cylinder

of only 1.8 cm radius. This is thus a maximum estimate of the extent of the rhizosphere of Thalassia. This estimate is similar to estimates, which are few in number, of the extent of the rhizosphere of terrestrial plants. Papavizas and Davey (1961), for example, concluded from studies on the distribution of microflora around roots of the blue lupine that the rhizosphere effect was "very pronounced to 3 mm, but was still evident at 18 mm." Thalassia roots are covered with a layer of mucilaginous material which binds sand grains, and much of the N_2-fixing activity may take place in this region or 'rhizoplane'.

Rates of acetylene reduction in the glucose+sulfate amended samples were not significantly higher than those in the glucose amended samples. However it was apparent from the strong odor of sulfide in the glucose amended samples that activity of sulfate reducers was not limited in these samples by a lack of sulfate. Thus there is no indication given by these data of whether the sulfate-reducing bacteria are important N_2 fixers in the Thalassia sediments. The numbers of sulfate reducers in both amended and unamended samples, 7800 to 330,000 (Table VIII), are of the same order as the numbers found in the Barbados root layer sediments (10^3 to 10^5, Table V). It had been expected that much higher numbers would occur in the amended samples. The results indicate that factors other than the availability of organic matter or sulfate were limiting increase in numbers, but not necessarily biochemical activity, of sulfate reducers in these sediments.

Table VIII. Sulfate-reducing bacteria in acetylene reduction assay samples.

Sample	Amendment	Incubation	Sulfate reducing bacteria M.P.N. (per g wet wt)	95% confidence intervals
2	G + S	ø	100000*	(24000-1100000)
3		0	330000	(100000-1100000)
5		ø	17000	(5200-56000)
6	G + S	ø	330000	(100000-1100000)
11		0	24000	(7300-80000)
17		ø	7900	(2400-26000)
20	G	ø	7800	(2400-26000)
22	G + S	ø	100000*	(24000-1100000)

* 1/1 tubes positive at 10^{-5} dilution,
5/5 tubes negative at 10^{-6} dilution.

IV. GENERAL DISCUSSION

Speculations regarding the nature of the *Thalassia*-sediment N_2-fixing system

The question, 'what limits N_2 fixation in situ?', leads directly to a hypothesis concerning the nature of the *Thalassia*-sediment N_2-fixing system. For the purpose of presenting this hypothesis, the following abbreviations are used.

EOM	excreted organic matter
P_{eom}	the rate of excretion of organic matter into the rhizosphere by *Thalassia*
N_a	the rate at which combined nitrogen from N_2 fixation is made available to *Thalassia* or is taken up by *Thalassia*
E_s	the efficiency of the *Thalassia*-sediment N_2-fixing system, defined as the ratio of N_a-to-P_{eom}

Evidence presented in this thesis points to a sensitively regulated, symbiotic type of relationship between *Thalassia* and the N_2-fixing bacteria in which there is reciprocal exchange of organic matter and nitrogen substrates, the amount of inorganic combined nitrogen transferred to *Thalassia* being in proportion to the amount of EOM transferred to the bacteria, and vice versa. It is reasonable to assume that there is a limit to the proportion of gross photosynthesis that can be used for obtaining nitrogen, i.e. to the proportion used for synthesis of EOM. There must also be a limit to the extent of the *Thalassia*-sediment N_2-fixing system, i.e. limits to factors such as the number and length of *Thalassia* roots and the extent of the rhizosphere. Given these conditions, then to account for the fact that nitrogen limits growth in any given *Thalassia* stand, it is necessary to postulate that increasing P_{eom} above the level actually occurring in that system results in a

proportionally small increase in N_a in comparison to increments in N_a associated with similar increases of P_{eom} from initially lower levels of P_{eom}. In other words, E_s decreases with increasing P_{eom}. Without this type of relation, production of Thalassia could be increased simply by excreting more organic matter, and production would go on increasing until some factor other than nitrogen was limiting, which was clearly not the case for the Thalassia stands studied. According to this hypothesis, the nature of the N_a-P_{eom} relationship, and the maximum limit of the proportion of gross photosynthesis which can be used for supplying organic matter to the N_2-fixing bacteria, would determine the actual P_s in any given Thalassia system.

Assuming that the nitrogen requirements of Thalassia are given by multiplying production by $\frac{1}{2}$ x 2.3% N, then the relation between P_{eom}, P_s and E_s ($= N_a/P_{eom}$) is given by

$$\frac{P_{eom}}{P_s} = \frac{1.15}{E_s}$$

It can be seen that a maximum value of the ratio P_{eom}-to-P_s (which can be considered directly proportional to a maximum value of the proportion of gross photosynthesis which can be used for synthesis of EOM) corresponds to a minimum value of E_s. Hypthetical examples of the $P_s(N_a)$-P_{eom} relationships of five different Thalassia-sediment systems are given in Fig. 9. The five different systems may be regarded as five Thalassia stands with different P_s values, or as a single stand in different states brought about, perhaps, by changes in substrate Eh.

Synthesis of organic matter for the N_2 fixing bacteria is equivalent to expenditure of energy for taking up nutrients from dilute solutions, and thus should not be considered part of the net photosynthesis of Thalassia. Net-

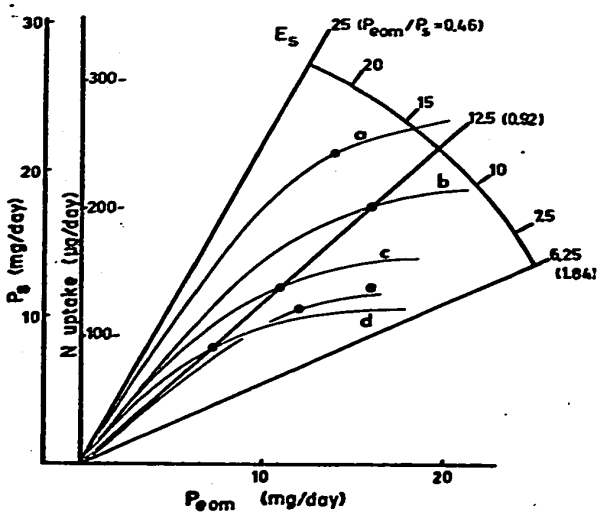

Fig. 9. Relation of nitrogen uptake to organic matter excretion in hypothetical Thalassia-sediment N_2-fixing systems. See text for hypothesis on which this figure is based. In systems b, c, and d it is assumed that the maximum value of P_{eom}/P_s tolerated by Thalassia is 0.92 (equivalent to a minimum tolerable E_s of 12.5 ug N/mg EOM). The actual P_s of these three systems is thus given by the points on the P_s-P_{eom} curves at which P_{eom}/P_s = 0.92. P_s cannot be increased above these values because this would require higher values of P_{eom}/P_s; while the system efficiency is higher at lower levels of P_{eom}, the absolute amounts of nitrogen available to Thalassia are less. In systems a and e, it is assumed that the maximum value of P_{eom}/P_s is determined by the nature of the P_s-P_{eom} relationship, and the actual P_s is an optimal value representing the best combination of efficiency and production.

to-gross photosynthesis ratios of plants are typically in the range 0.4 to 0.7 (Westlake, 1963). Teal (1962) reported a ratio of 0.19 for the salt marsh grass, Spartina. It seems unlikely that the amount of organic matter excreted by Thalassia could be greater than 2 x the net production of organic matter. Figures given by Wetzel (1969) for the ratio organic carbon excreted-to-net fixation of carbon for Najas under a variety of experimental conditions, vary between 0.005 to 1.79, with most values between 0.2 and 1. Assuming a ratio of P_{eom}/P_s for Thalassia of 1 (remembering that leaf tissue includes approximately 20% ash), and that nitrogen requirements are given by multiplying production by $\frac{1}{2}$ x 2.3% N, then this implies a system efficiency of 11.5 mg N/g EOM. The real system efficiency might be considerably higher than this, but could not be much less than about one half this value. This figure, 11.5 mg N/g EOM is of the same order as reported efficiencies of fixation of N_2-fixing bacteria. It is obvious that the system efficiency must be less than the efficiency of fixation.

The essential feature of the hypothesis regarding the nature of the Thalassia-sediment N_2-fixing system is that under any given set of conditions, the system efficiency decreases with increasing amounts of organic matter excreted by Thalassia. This might be associated with less efficient use of EOM by the N_2-fixing bacteria, and with greater loss of EOM to competing organisms as P_{eom} is increased. One of the reasons it is generally assumed that the free living heterotrophic N_2 fixers do not, in most circumstances, fix large amounts of N_2 is that reported efficiency of fixation values indicate "nitrogen-fixing heterotrophs are inefficient users of carbohydrate and there is intense competition for that which is available not only between nitrogen-fixing forms, but between nitrogen-fixing and non-nitrogen-fixing forms" (Stewart, 1969). Reported efficiencies of fixation are based

on cultures of N_2 fixers in solutions of initial concentrations of sugar of 0.5% to 2% (see for example, Parker, 1954; Proctor and Wilson, 1958). The above considerations suggest that these efficiency of fixation values may give a very misleading impression of the use of energy substrates by N_2 fixing bacteria in situ where concentrations of readily available organic matter are likely to be several orders of magnitude less than in the culture solutions. It is to be expected that bacteria are most efficient in the use of substrates when they are supplied at levels likely to be found in nature. The bacteria may be more productive even in absolute terms when substrates are not supplied in great excess. For example Kadota (p. 171 in Oppenheimer, 1968) reported that the number of viable cells of nitrifying bacteria and the rate of ammonium oxidation by resting cells were both maximal at a concentration of $(NH_4)_2SO_4$ of 30 mg/1000 ml sea water, and decreased at lower and higher concentrations of ammonium sulfate. This concentration is about 1/100 the concentration normally used for soil and marine nitrifiers. Increasing P_{eom} might also result in greater loss to non-nitrogen fixers because those bacteria capable of rapid uptake from more concentrated solutions without regard to efficiency would be favored. Lowering the concentration would favor bacteria which make most efficient use of energy substrates. Evidence of a crude sort that E_s decreases with increasing supply of organic matter is provided by comparison of the estimated nitrogen requirements of the Bermuda Thalassia stand with the results of the assays of N_2 fixation in the amended, anaerobic samples. The assay samples were amended with approximately 2 g glucose/200 g sediment. Assuming that in situ Thalassia supplied all of the carbohydrate for four days' requirements of the N_2 fixers at once, this would be equivalent (assuming as above, $P_{eom}/P_s = 1$) to an amendment of approximately 50 mg carbohydrate/200 g sediment, i.e. 1/40 of the above. The

estimated N_2 fixation by the acetylene reduction assay was 581 mg N_2/m^2 per day, and the minimum estimate of the nitrogen requirements was 97 mg N/m^2 per day. In other words, a more than 40 fold increase in readily available carbohydrate resulted in a less than 6 fold increase in N_2 fixation.

The exact nature of the N_a-P_{eom} relationship might be determined by a large number of factors; most important would be those factors which influence the following characteristics: (1) the total volume of sediment available to a Thalassia plant for N_2 fixation, (2) rates of diffusion in the sediment, (3) the size, composition and relative activities of the various components of the bacterial population, (4) the proportion of organic matter excreted which is available to the N_2-fixing bacteria, and (5) the efficiency of fixation. These characteristics are interrelated. In connection with (1), it was noted that high P_s is associated with long erect shoots. This is consistent with the above hypothesis. Variations in grain size characteristics, particularly with respect to the amount of clay in the sediments, would be expected to influence some of the above characteristics. All Thalassia beds examined at Barbados and Carriacou occurred in sediments with small amounts of silt and clay. On the whole, similar P_s values characterized Thalassia growing on predominantly sand and cobble-sand substrates at Bath, St. Lawrence and Carriacou (Appendix B). Generally higher P_s values may characterize Thalassia growing in predominantly silt and clay substrates. During very cursory observations of Thalassia at La Parguera, Puerto Rico, Thalassia with notably long and wide leaves (approximately 40 to 50 cm length and 12 mm width) was observed growing in what appeared to be predominantly silt and clay substrates. Ginsburg (1956) observed "luxuriant" growths of Thalassia on Florida Bay mud banks which consist predominantly of silt and clay. Evidence that reducing conditions in the substrate are essential to

the normal development of Thalassia has been presented. Redox potential may affect both the efficiency of fixation and the proportion of organic matter excreted which is used by the N_2-fixing bacteria (this may be referred to as the 'efficiency of transfer'). Cell-free extracts fix N_2 only under anaerobic conditions, and most facultative anaerobes fix N_2 only under anaerobic conditions (Stewart, 1969). Even the classical aerobic N_2-fixing bacteria, Azotobacter, fix N_2 most efficienctly under low levels of oxygen (Dalton and Postgate, 1969). Redox potential may have a pronounced effect on the efficiency of transfer. The remarks of Brouzes et al. (1969) are considered pertinent: "It is often argued that under anaerobic conditions both the rate of liberation of low molecular weight energy substrates and the efficiency of their use by nitrogen fixers must be low (Barrow and Jenkinson, 1962) so that anaerobic fixation could not be great. However, it may be that a much more intense competition for energy substrates exists in aerobic conditions (Nykvist, 1963) and that this becomes a factor of overriding importance." Another factor which may lead to decreased system efficiency at high Eh or in association with oxidizing microenvironments is a nitrification-denitrification process. At the redox potentials observed in the Thalassia sediments there would appear to be little possibility of nitrification occurring in the root layer. However, small amounts of nitrate were present in the root layer, the largest amounts of extractable nitrate occurring in the stands of lowest P_s. It seems likely that this nitrate originated from oxidation of reduced N_2. Oxidized microenvironments may exist within the sediments, or perhaps periodically in the rhizosphere. Once nitrate is formed, then the possibility exists for loss of nitrogen from the system to occur by denitrification.

A number of processes probably contribute to lowering of the redox potential in the Thalassia sediments. Growths of Thalassia and associated

sessile organisms stabilize the sediments and thus reduce exchange of gases with the overlying water. Organic matter is continually produced by underground parts and is eventually available for anaerobic or aerobic decomposition. Low redox potentials may be brought about by reducing substances excreted by Thalassia, by H_2S produced by sulfate reduction, and by reducing substances excreted by anaerobes. The extent to which sulfate reducers control the Eh in Thalassia sediments is not clear. pS-Eh measurements might be useful in investigating this problem. There may be somewhat different controls over Eh in rhizosphere and non-rhizosphere sediments. Sulfate reduction alone would probably be sufficient to maintain low redox potentials in stabilized sediments where penetration of O_2 occurs mainly by simple diffusion. The region of the rhizosphere, however, may be subject to periodic flushes of oxygen emanating from the roots of Thalassia. If so, then the nature of the poising system in that region would determine whether low redox potentials could be maintained over short periods of time, and maintenance of a low Eh over long periods of time would be dependent on the continued activity of microorganisms. In such conditions, activity of sulfate reducers might be limited by the availability of sulfate. Low redox potentials are generated in cultures of anaerobes as a result of production of substances such as dioxyacetone (Rabotnova et al., 1963). Such substances are probably produced by anaerobic N_2 fixers in the rhizosphere, and may play an important role in the regulation of Eh. Thalassia may have a definite physiological requirment for reducing conditions in the substrate. Aquatic angiosperms typically occur in substrates with little or no oxygen, and the well developed lacunar system ensures that an adequate supply of oxygen reaches at least most of the underground parts for most of the day; however, oxygen may be deficient at the extremities of the underground parts, or more

generally during the night (Sculthorpe, 1967). Laing (1940a, 1941) found that rhizomes of 10 species of aquatic angiosperms he studied are capable of anaerobic respiration with production of alcohol. The plants varied, however, in the oxygen required to maintain the plants in an active (versus dormant) state, and habits of the plants could be accounted for by their physiological requirements for oxygen. Growth of rhizomes of Nuphar advenum was found to be uninfluenced by the presence or absence of oxygen in the lacunae, but the presence of oxygen around the rhizome apex actually inhibited growth of the rhizome. Such may also be the case for Thalassia. It is also conceivable that the energy substrates for the N_2-fixing bacteria are the products of anaerobic respiration by underground parts.

The active N_2 fixer in the Thalassia sediments might be any one of a number of species. There can be little doubt that an obligate or facultative anaerobe is involved. Wood (1959a) was unable to isolate Azotobacter from Zostera sediments. Other workers have had inconsistent results in attempting to isolate Azotobacter from marine sediments (reviewed by ZoBell, 1946b). Clostridium, on the other hand, has been consistently isolated from marine sediments. Waksman et al. (1933) concluded that "anaerobic nitrogen-fixing bacteria of the Clostridium pastorianum type are universally distributed in the sea bottom." Recent studies have shown nitrogen fixation to be "far more widespread among the sulphate-reducing bacteria of the genus Desulfovibrio than was earlier thought" (Postgate, 1970). Conclusive evidence that sulfate reducers are the active N_2 fixers in the Thalassia rhizosphere would have to include a demonstration that sulfate is not limiting in situ. In view of the rather remarkable physiology of sea grasses in other respects, the possibility that Thalassia mediates a flow of sulfate into the sediments should not be excluded. Members of the genera Pseudomonas

and Vibrio have been found to fix N_2 (Stewart, 1966). According to ZoBell (1946b), species of these genera rank first and second in predominance in the sea, and the active N_2 fixer in the Thalassia beds might be a member of one of these genera.

Nitrogen and phosphorus nutrition of other sea grasses

The Thalassia-N_2 fixing bacteria type of relationship may be characteristic of many, if not most, of the marine angiosperms. Preliminary studies indicate that Syringodium obtains nitrogen from the sediments. A comparative study of the nitrogen nutrition of the three marine angiosperms Thalassia, Syringodium and Diplanthera would be interesting because of the successional relations between these sea grasses, and the variation in the redox potential of the substrates in which they occur. Thalassia is restricted to reduced substrates, while Syringodium and Diplanthera occur on both well aerated and strongly reducing substrates. Requirements of Zostera for nitrogen and phosphorus are probably similar to those of Thalassia. Reported values of the nitrogen content of healthy green leaves of Zostera (Jensen, 1915; Brandt and Ruben, 1920; Udell et al., 1969) have a mean of 2.2% N. Jensen (1915) observed lower values in January than in September. Brandt and Ruben (1920) reported the phosphorus content of three samples of Zostera leaves as 0.65, 0.35 and 0.20% P. Productivity of Zostera is of the same order as that of Thalassia. According to Jensen (1915), productivity at a good locale is 1920 g dry matter/m^2 per year, and productivity at a moderate locale is 1120 g dry matter/m^2 per year. The habits of Zostera are similar to those of Thalassia with respect to the redox potential of the sediments. Wood's (1965) remarks on this subject have been discussed. The observations of Ostenfeld (1908) are particularly interesting. He observed that leaves

of Zostera are long and broad on soft (muddy) bottoms, and short and narrow on hard (predominantly sand) bottoms, and suggested that the difference is due to a lack of soil development on hard bottoms. He noted a darkening of the sediment in areas of good growth, and cited this as evidence of "fertilization" of the sediments. The similarity in requirements and habits of Zostera and Thalassia, as well as the fact that the marine angiosperms are all closely related, suggest Zostera obtains nitrogen from the sediments by the same mechanisms as Thalassia. Jensen (1915) in fact suggested that N_2 fixation by Clostridium and Azotobacter might be responsible for nitrogen enenrichment of Zostera detritus in the sediments. Inorganic nitrogen concentrations in temperate coastal and estuarine waters may undergo large seasonal fluctuations, and may be completely depleted during phytoplankton blooms. At other times, concentrations of NO_3-N considerably higher than the highest NH_4-N concentration in the Thalassia interstitial waters may be observed (Rochford, 1951; Barnes, 1957). In view of the evidence that amino acid synthesis occurs in the root of Thalassia, it would be interesting to know whether Zostera takes up nitrates from the water when concentrations are high.

It seems likely that if there is an obligatory association between Zostera and reducing conditions, as Wood (1965) suggested, that it is associated with requirements for nitrogen rather than phosphorus. Does the existence of reducing conditions in fact have any influence on the availability of phosphorus to Zostera? The reasons for believing that reducing conditions do not have a significant influence on the availability of phosphorus to Thalassia have already been stated. The basic argument was that even under reducing conditions (implying also production of bicarbonate by sulfate-reducing bacteria) the dissolution of apatite would be too slow to supply

phosphorus at rates approaching the uptake of phosphorus by Thalassia. The situation with respect to Zostera may be somewhat different. Zostera is typically an estuarine plant. Ostenfeld (1908) found Zostera growing in regions of salinity of 10 to 30 °/oo. A large proportion of the total inorganic phosphorus in estuarine sediments may occur as iron phosphates. Nelson (1967) used a soil phosphorus fractionation procedure to study the distribution of phosphate in the sediments of an estuary. He observed that iron and calcium phosphates varied systematically along the estuary. For regions of the estuary of maximum bottom salinities of 10 to 30 °/oo, the ratio iron phosphate-to-iron phosphate+calcium phosphate varied between 0.67 and 0.18. For 16 samples of marine origin, the average was 0.04. Iron phosphates and hydrous ferric oxide (on which phosphate may be adsorbed) are directly affected by Eh because of the reduction of ferric iron to ferrous iron under reducing conditions. Ferrous phosphate, unlike ferric phosphate, is very soluble. In the presence of sulfide, the ferrous iron is precipitated as ferrous sulfide, as previously discussed. These reactions occur very rapidly (Baas Becking and MacKay, 1956; Sperber, 1958). Sperber (1958) observed that the presence of iron oxides suppressed release of phosphate from iron phosphate associated with sulfide treatment through competition for the sulfide; he also observed, "The level of phosphate in solution reached a maximum after several hours and thereafter fell steadily, suggesting refixing of phosphate on the surface of sesquioxide particles." Increased availability of phosphate in rice paddy soils following flooding occurs rapidly (current growing season), and is correlated with the amount of phosphate initially present as iron phosphate (Black, 1968). Thus it is likely as Wood (1965) suggested, that the development of reducing conditions in Zostera sediments is accompanied by a release of phosphate from iron

phosphates. The released phosphate is probably adsorbed on the surface of the various mineral particles in the sediments, and the interstitial water phosphate concentration increased in accordance with the adsorbed phosphate-solution phosphate equilibrium of the particular system. Thus phosphate released from minerals might constitute a significant source of phosphate for Zostera, at least following the initial colonization of the substrate by Zostera. However the work of McRoy and Barsdate (1970) suggests that the effect of Zostera growing in the sediments is to bring the internal parts of the sediments into, or closer to, a state of dynamic equilibrium with the water phosphate or phosphate "pool" (Hayes, 1963) of the estuarine ecosystem. It seems likely that this, together with the increased solubility of iron phosphates under reducing conditions, would lead to fairly rapid depletion of the available phosphate in the sediments. Rochford (1951) observed for sediments of the 'tidal zone', the region of Zostera occurrence in Australian estuaries, low total phosphorus concentrations, maximum amounts of adsorbed phosphate, and intermediate amounts of interstitial water phosphate in comparison to sediments of the other estuarine zones (marine, gradient and freshwater)[1]. These observations are consistent with the above considerations concerning the effect of Zostera on the sedimentary phosphate. It seems then, that as for Thalassia, the sediments could not be a primary source of phosphate for Zostera over long periods of time. The sediments may have an important function in the phosphorus nutrition of Zostera and other sea grasses, however, by providing a sort of buffer mechanism for the

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[1]McRoy and Barsdate (1970) stated, "Rochford (1951) determined that high concentrations of phosphate in interstitial waters resulted in high standing stocks of eelgrass." In a careful reading of Rochford's (1951) paper, I could find no basis for this statement. Presumably McRoy and Barsdate (1970) were referring to adsorbed phosphate, not interstitial water phosphate.

phosphate supply, as was suggested for Thalassia.

Studies by Hayes and associates (Hayes and Phillips, 1958; Watt and Hayes, 1963) have shown that in aquatic systems a state of dynamic equilibrium exists between phosphate in various phases, i.e. between dissolved inorganic phosphate, dissolved organic phosphate, particulate phosphate (includes living organisms) and the sediments (surface layers). The rates of exchange between these phases vary by several orders of magnitude, both for the different phases in a given system, and between similar phases in different systems. The causes for these variations are not well understood, but it seems that traditional concepts or measures of the supply of phosphate in aquatic systems are not valid. In the context of the work of Hayes and associates (op. cit.), the internal parts of the sea grass sediments might be regarded simply as an anion exchange resin which is in equilibrium or quasi-equilibrium with the other phases of the marine or estuarine ecosystem with respect to phosphate, but to which the sea grasses have more or less exclusive access. In the same context, it would be interesting to know what role the large bacterial populations on the leaves play in phosphate uptake.

The significance of N_2 fixation by free-living heterotrophic bacteria in some aquatic and terrestrial systems

Assuming that a reasonable estimate of N_2 fixation in the Thalassia sediments is given by multiplying production by $\frac{1}{2}$ x 2.3% N, then N_2 fixation in a typical Thalassia stand (P_n = 4 g/m^2 per day) is estimated as 170 kg N_2 fixed/ha per year, and maximum fixation (the Bermuda stand) is estimated as 590 kg N_2/ha per year. Some comparative estimates for other systems are given in Table IX. It is evident that N_2 fixation in the

Table IX. Estimates of N_2 fixation in various systems.

NO.	SYSTEM	N_2 FIXATION (kg/ ha per year)		METHOD	REFERENCE
1	Legumes	avg max	110 - 220 820	N gains in plant and soil	Stewart (1966), review of reported values
2	Nodulated non-legumes	range	9 - 220	"	"
3	Paddy soils (blue-green algae)		10, 19, 55	$^{15}N_2$ incorporation	MacRae and Castro (1967)
4	Lake Windermere (mainly algal)		7	$^{15}N_2$ incorporation	Fogg and Horne (1967)
NON-SYMBIOTIC BACTERIA					
5	Quebec forest and agricultural soils	commonly max	10 - 50 226	$^{15}N_2$ incorporation; potential values - include amended samples	Brouzes, Lasik and Knowles (1969)
6	Quebec forest soils agricultural soils	avg avg	1 (for 100 day 0 growing season)	C_2H_2 reduction; replicate sampling through growing season	R. Knowles (personal communication, 1971)
7	Grassland soil, N poor -soil alone -inverted lawn (decaying grass) -growing grass -10 g soil + 780 mg sucrose		 2.9 158 0.14 4500	$^{15}N_2$ incorporation	Delwiche and Wijler (1956)
8	Latosolic soil under regenerated rain forest		720	N gain in soil	Jaibeyo and Moore (1963)
9	Anoxic zone of Lake Mary, Wisconsin		3.8	C_2H_2 reduction	Brezonik and Harper (1969)
10	Sediments of Lake Erie		44*	C_2H_2 reduction	Howard et al. (1970)
11	Thalassia	typical max	170 590		

*Calculated for 6 cm depth of sediment from data given by Howard et al. (1970) by assuming 50% interstitial water, sediment particle specific gravity of 2.6, and 3:1 ratio in converting moles C_2H_4 produced to moles N_2 fixed.

Thalassia-sediment system is comparable to N_2 fixation associated with legumes.

Very little is known concerning the quantitative significance of N_2 fixation in aquatic systems, particularly in the oceans. There is evidence of significant N_2 fixation by blue-green algae in lakes, and either by or associated with blue-green algae in the sea (Stewart, 1969). Two studies utilizing the acetylene reduction assay have indicated appreciable rates of N_2 fixation by bacteria (probably heterotrophic anaerobes) in sediments and anoxic waters of lakes (entries nos. 9 and 10, Table IX). Other than those of the present study, no assays have been made of N_2 fixation in marine sediments.

Because the input of nitrogen into the oceans from rivers and rain water is much greater than the nitrogen removed from the oceans by sedimentation, it appears that denitrification is more important than N_2 fixation in regulating the nitrogen content of the oceans as a whole (Rittenberg, 1963). Denitrification in the oceans is also believed to be the major route by which N_2 fixed in terrestrial systems is returned to the atmosphere (Hutchinson, 1944). Thus it is apparent that for the oceans as a whole, N_2 fixation cannot assume the significance it has in terrestrial systems in maintaining the nitrogen status, unless denitrification is actually occurring at a much greater rate than that required to balance the excess of input of nitrogen into the sea. In spite of the large input of nitrogen into the sea, however, nitrogen is probably the nutrient most frequently limiting production in the euphotic zone (Ryther and Dunstan, 1971). Thus N_2 fixation could be of significance in the euphotic zone.

There is good reason to believe that N_2 fixation is of general significance in the euphotic zone of tropical waters. It is only in tropical

waters that significant N_2 fixation has in fact been demonstrated; rates as high as 2 ug N_2 fixed per litre per hour have been demonstrated in tropical waters during heavy blooms of the blue-green alga Trichodesmium (Dugdale, Goering and Ryther, 1964; Dugdale and Goering, 1967). Trichodesmium is often the dominant member of tropical phytoplankton (Wood, 1965). Correlation between $^{14}CO_2$ uptake and N_2 fixation suggests that Trichodesmium is the active N_2 fixer. There is strong presumptive evidence for N_2 fixation by the pink yeast Rhodotorula, an organism which has been observed in high numbers in tropical waters (Allen, 1963). The present study has demonstrated the importance of N_2 fixation to Thalassia. Thalassia and coral reef communities are the most highly productive communities in tropical waters. Odum and Odum (1955) and Wood (1965) expressed the opinion that N_2 fixation by Nostocaceae may be of importance to the coral reef ecosystem.

Several workers in the early 1900's demonstrated large numbers of Azotobacter in association with the slime of seaweeds, and also in association with phytoplankton, and it was suggested that there may be a symbiotic type of relationship between algae and N_2-fixing bacteria (reviewed by Waksman et al., 1933; ZoBell, 1946b). This suggestion deserves reinvestigation. Algae are known to excrete large amounts of organic matter, (Fogg, 1962). Given an association of N_2-fixing bacteria with algae, there seems to be no reason to suppose that these bacteria would not fix significant amounts of nitrogen, particularly if nitrogen were limiting. It should also be noted that it has not been demonstrated conclusively that N_2 fixation associated with Trichodesmium--unlike other N_2-fixing Cyanophyceae except possibly Gleocapsa (Wyatt and Silvey, 1969), Trichodesmium does not possess heterocysts--is not bacterial in origin. A possibly analagous situation occurs in the phyllosphere of tropical rain forests where Azotobacter and

Beijerinckia are believed to fix significant amounts of N_2 using leaf exudates as energy substrates (Ruinen, 1961, 1965).

The significance of N_2 fixation by free-living bacteria in terrestrial systems is not well understood. Prior to 1949, Azotobacter and Clostridium were the only N_2 fixers known. In general Azotobacter was considered unimportant in N_2 fixation because of the low numbers in which it occurs. Clostridium was rarely considered at all, presumably because it was assumed that there could not be significant N_2 fixation under anaerobic conditions, or that anaerobic conditions did not exist in the soils. Although members of many genera have since been found to fix N_2, little is known of the distribution and activities of N_2-fixing bacteria other than Clostridium, Azotobacter and Beijerinckia. It is believed however that N_2 fixation is not limited by a lack of N_2-fixing bacteria in the soil. The main factor believed to be limiting N_2 fixation by heterotrophic bacteria is a shortage of readily available organic matter. Experiments such as those of Delwiche and Wijler (1956; entry no. 7, Table IX of this thesis), which show a marked increase in N_2 fixation following organic matter amendment, are cited as evidence that organic matter is normally limiting. Stewart (1969), in a review of the ecological significance of N_2 fixation by free-living bacteria, concluded, "On the whole it appears that heterotrophs play their main role by being widely distributed, and fixing small quantities of nitrogen over prolonged periods." The significance of "fixing small quantities of nitrogen over prolonged periods" would of course depend on the rate at which nitrogen was being removed from the system. Moore (1966) remarked, "In natural systems which are virtually closed, e.g. some forest and grassland communities, low rates of fixation (say up to 10 lb/ac./an.) would be adequate to maintain the nitrogen status, whereas under intensive farming conditions, where

large quantities of nitrogen are removed in crops and by leaching and volatilization, even 50 or more lb/ac./an. may be inadequate." Assays of N_2 fixation by the ^{15}N technique in temperate forest and agricultural soils pointed to high potentials for N_2 fixation by heterotrophic bacteria (entry no. 5, Table IX). However, more extensive sampling of these soils through the 1970 growing season using the acetylene reduction assay has indicated "low and irregular activities or no activity" (Dr. R. Knowles, personal communication, 1971) and low average fixation (entry no. 6, Table IX). There is good evidence of high rates of N_2 fixation by free-living heterotrophs in organic-rich surface layers of some tropical soils (entry no. 8, Table IX).

Little is known concerning N_2 fixation by 'free-living' heterotrophs in the rhizosphere of plants. Both sloughed off root tissue and root excretions are considered possible sources of energy for N_2 fixers in the rhizosphere (Moore, 1966). The composition of root excretions has been studied in detail for a number of plants (for example, Rovira, 1956; Vancura, 1964), but less is known of the quantities of organic matter excreted. Harmsen and Jager (1962) detected excretion of up to 1.7 mg organic carbon per gram rhizosphere soil of wheat over a two month period: this does not include the organic matter respired by bacteria. There is evidence of both stimulation and inhibition of N_2-fixing bacteria in the rhizosphere (Jurgensen and Davey, 1970). The only ^{15}N study of an intact plant-soil system is that of Delwiche and Wijler (1956). They observed very little N_2 fixation in a nitrogen-poor soil with growing grass (entry no. 7, Table IX). A.W. Moore (1963) reported nitrogen gains in a tropical soil-plant system over a four month period equivalent to N_2 fixation of 330 to 440 kg N_2 fixed/ha per year, while there was no change in the nitrogen content of an unplanted soil. It seems likely

that short term gains of this order are associated largely with use of plant root excretions. Hassouana and Wareing (1964) attributed nitrogen gains in sand dune soils to fixation by heterotrophs in the rhizosphere of the pioneer plant Ammonophila arenaria. Studies by Rinaudo (1970) have provided the first conclusive evidence of a significant rhizosphere effect on N_2 fixation. He carried out studies of acetylene reduction and N_2 fixation (Kjeldahl technique) on an intact rice-plant soil system. The rates of acetylene reduction observed in the rhizosphere soil, 8.2 to 22.8 n moles C_2H_4 produced/g soil per hour for the 24th hour, are of the same order as the rates observed for the glucose amended Thalassia sediments (10.3 n moles C_2H_4 produced/g sediment per hour, average for the anaerobic amended samples). Cutting of the rice plants resulted in an 80% decrease in the rates of acetylene reduction within 24 hours, and in negligible rates after five days. Comparison of acetylene reduction with N_2 fixation validated the 3:1 ratio for estimating N_2 fixation from acetylene reduction. It is well known that growth of rice is best under conditions of flooding, and that such conditions favor the maintenance of soil fertility. Prior to Rinaudo's (1970) study, this effect was attributed largely to N_2 fixation by blue-green algae. Rinaudo's (1970) work included measurements of a number of microbial processes in situ, and experimental studies of the effects on N_2 fixation of submergence, illumination, organic amendment, nitrogen amendment, oxygen and (as discussed above) rhizosphere stimulation. In situ studies indicated that submersion creates conditions favorable for N_2 fixation by raising the pH and inducing establishment of semianaerobic conditions. From counts of N_2-fixing organisms he concluded that N_2 fixation in rice soils is essentially the work of blue-green algae and Clostridium; densities of these organisms in situ of the order of 10^5/g were consistently observed. Experimental studies

provided evidence for the following points as well as the above mentioned rhizosphere effect.

(1) Submersion favors N_2 fixing activity of rice soils.

(2) Periodic submersion has on the whole greater effect than constant submersion.

(3) Oxygen has an inhibitory effect on N_2 fixation which is appreciable at a P_{O_2} of 0.01 atm.

(4) Addition of NH_4-N to soils slows down or stops N_2 fixation; however, there appears to be a fairly high threshold value for native (versus added) NH_4-N. Beyond this threshold, N_2 fixing activity decreases rapidly, and a loss from denitrification becomes apparent.

(5) Vegetative residues (straw or roots) give rise to a fixing activity less important than one might think.

(6) Light energy permits an intense N_2 fixation by photosynthetic microorganisms, in the first rank of which are blue-green algae. However, the light effect is appreciable only at the level of the soil surface.

With respect to rhizosphere N_2 fixation he concluded, "La fixation d'azote au niveau de la rhizosphère est d'un niveau remarquablement élevé ... En raison de l'épaisseur de sol prospectée par les racines, et de lar densité de l'enracinement, 'l'effet rhizosphère' paraît etre le facteur majeur de l'enrichissement en azote des sols de rizières." The similarity of the rice-soil N_2 fixing system to the Thalassia-sediment N_2-fixing system in many respects cannot be considered coincidental. It is noteworthy that several of the most highly productive communities listed in Table I are systems in which the soils are submerged and probably strongly reducing. It does not

seem unwarranted to suggest that rhizosphere N_2 fixation may be important in the maintenance of the nitrogen status of communities such as salt marshes, mangrove swamps and reed swamps, as well as of sea grass beds and rice paddies. Because of the simplicity of the Thalassia sediment N_2 fixing system, studies on this system may be of value in understanding more general aspects of plant-N_2-fixing bacteria relationships.

V. SUMMARY

1. The question of basic concern to this study was: how are high rates of production by the tropical marine angiosperm Thalassia testudinum König maintained in the notably nutrient-poor tropical waters?
2. Correlations between production of leaf tissue per erect shoot (P_s) and (i) rhizome water soluble ammonium (+amino acid)-N, and (ii) rhizome water soluble phosphate, indicate that significant proportions of the nitrogen and phosphorus for leaf growth are taken up from the substrate.
3. For one sample it was estimated that 90% of the rhizome water soluble ammonium (+amino acid)-N was derived from amino acids.
4. A simple linear relation between P_s and total interstitial water ammonium in the root layer indicates that (i) leaf growth at the majority of stands studied was limited by availability of nitrogen, (ii) virtually all nitrogen for leaf growth comes from the substrate, and (iii) a steady state with respect to the nitrogen status of the sediments is maintained over relatively short periods.
5. During periods of heavy rains at Barbados, chlorinity of interstitial water in the Thalassia sediments varied irregularly with depth in the sediment.
6. There is a correlation between interstitial water ammonium and the amount of ammonium in sea water extracts of the sediments. Per unit volume of sediment, interstitial water ammonium amounts to only about 2% of the ammonium in sea water extracts of the sediments. It appears that most of the ammonium in the sediments is weakly adsorbed on sedimentary particles (sand sized skeletal carbonates predominate) as a result of the negative charge of sedimentary particles in sea water.
7. Most of the sediments contained approximately 0.02+% HCl soluble

phosphate phosphorus. Only a small proportion of this phosphate is readily available in sea water; most is probably occluded within skeletal carbonates, and is thus not available to Thalassia.

8. The decrease of phosphate in successive sea water extracts of Thalassia sediments suggests that the readily available phosphate is adsorbed on the surfaces of sedimentary particles, as has been observed for soils. This is a chemical type of adsorption, as opposed to the coulombic type of adsorption of ammonium.

9. The phosphate concentrations in sea water extracts of the Thalassia sediments were greater than the concentrations of phosphate in the interstitial water in situ. It is believed that the difference is associated with (i) bacterial retention of phosphate in situ, and (ii) reduced amounts of interstitial water and adsorbed sulfate in situ as a result of sulfate reduction, and consequent greater adsorption of phosphate.

10. Because (i) the Thalassia sediments are predominantly of marine origin, and (ii) interstitial water phosphate concentrations are low, it is believed apatite is the only crystalline phosphate that could be in contact with the interstitial water.

11. pH of sediment samples from the Thalassia root layer varied from 7.0 to 7.9, and Eh, from -178 to +74 mv. Eh data and general observations indicate that a reduced root layer is essential for normal development or good growth of Thalassia. Occurrence of ferrous sulfide is not a reliable indicator of the occurrence of reducing conditions in Thalassia sediments because availability of iron rather than sulfide may limit ferrous sulfide formation.

12. Total numbers of bacteria in Thalassia root layer sediments estimated by plate counts and dilution-extinction counts (aerobic and anaerobic

incubation) were of the order of 10^5 to 10^6 per gram wet weight sediment. Numbers of sulfate reducers were of the order 10^3 to 10^5.

13. High numbers of sulfate reducers were detected in two samples from well aerated surface sediments. Circumstantial evidence indicates these were active within reduced microenvironments in skeletal carbonate grains.

14. For a typical Thalassia stand it was estimated there was sufficient available phosphate in the sediment to satisfy phosphorus requirements of Thalassia for 300 to 1000 days, and sufficient ammonium-N to satisfy nitrogen requirements for 5 to 15 days.

15. Renewal of nitrogen and phosphorus removed from the sediments by Thalassia can not be accounted for by decomposition of organic matter, or by diffusion of nutrients from the sediment-water interface.

16. It is believed that apatitic phosphate in the sediments could not be a significant source of phosphate for Thalassia because of the slow rate at which apatite dissolves.

17. It is concluded that the sediments are not a primary source of phosphate for Thalassia. It is believed, however, that the sediments act as a sort of storage bank for phosphate taken up from the sea water by Thalassia.

18. The only way to account for the maintenance of the nitrogen status of the sediments is by postulating high rates of N_2 fixation, presumably by heterotrophic anaerobes.

19. Acetylene reduction assays of N_2 fixation in sediment samples from a Thalassia stand were carried out with the cooperation of Dr. R. Knowles of Macdonald College, McGill University. Negligible rates were observed in unamended samples, but for samples to which glucose had been added,

the rates of N_2 fixation corresponded to estimates of the rates required to satisfy demands of Thalassia for nitrogen.

20. It is concluded that rhizosphere N_2 fixation by heterotrophic anaerobes is the source of nitrogen for Thalassia. Organic matter excreted by Thalassia serves as energy substrates for the N_2-fixing bacteria.

21. Although large amounts of organic tissue are produced underground, it is apparent that this material does not serve as a significant source of energy for N_2-fixing bacteria.

22. Considerations based on shoot densities indicate that the rhizosphere has a maximum extent of 1.8 cm from the root (with respect to N_2 fixation).

23. It is believed that the requirement of Thalassia for a reduced substrate is associated with requirements of the N_2-fixing bacteria, and with greater overall efficiency of the Thalassia-sediment N_2-fixing system under anaerobic conditions.

VI. LITERATURE CITED

Allen, M.B. 1963. Nitrogen-fixing micro-organisms in the sea. In: Symposium on marine microbiology, p. 85 to 92. Ed. by C.H. Oppenheimer. Springfield, Illinois: C.C. Thomas.

Allison, L.E. 1965. Organic carbon. In: Methods of soil analysis, p. 1367 to 1378. Ed. by C.A. Black et al. Madison, Wisconsin: American Society of Agronomy.

Aomine, S. 1962. A review of research on redox potentials of paddy soils in Japan. Soil Sci. 94: 6-13.

Arber, A. 1920. Water plants, 436 p. London: Cambridge University Press.

Baas Becking, L.G.M. and E.J.F. Wood. 1955. Biological processes in the estuarine environment. I, II. Ecology of the sulphur cycle. Kon. Ned. Akad. Weten. Proc. B58: 160-181.

Baas Becking, L.G.M. and M. Mackay. 1956. Biological processes in the estuarine environment. V. The influence of Enteromorpha on its environment. Kon. Ned. Akad. Weten. Proc. B59: 109-123.

Baas Becking, L.G.M., I.R. Kaplan and D. Moore. 1960. Limits of the natural environment in terms of pH and oxidation-reduction potentials. J. Geol. 68: 243-284.

Ball, M., E.A. Shinn and K. Stockman. 1967. The geologic effects of Hurricane Donna in south Florida. J. Geol. 75: 583-597.

Barnes, H. 1957. Nutrient elements. In: Treatise on marine ecology and paleoecology. I. Ecology, p. 297-343. Ed. by J.W. Hedgepeth. Geol. Soc. Amer. Mem 67.

_____. 1959. Apparatus and methods of oceanography, 341 p. London: Allan and Unwin.

Barrow, N.J. 1967. Studies on the adsorption of sulfate by soils. Soil Sci. 104: 342-349.

Barrow, N.J. and D.S. Jenkinson. 1962. The effect of water-logging on fixation of nitrogen leached by soil incubated with straw. Plant Soil 16: 258-262.

Bavendamm, W. 1932. Die mikrobiologische Kalkfallung in der tropischen sea. Arch. F. Mikrobiol. 3: 205-276.

Beers, J.R., D.M. Steven and J.B. Lewis. 1968. Primary productivity in the Caribbean Sea off Jamaica and the tropical north Atlantic off Barbados. Bull. Mar. Sci. Gulf and Carib. 18: 86-104.

Bernatowicz, A.J. 1952. Marine monocotyledonous plants of Bermuda. Bull. Mar. Sci. Gulf and Carib. 2: 338-345.

Berner, R.A. 1962. Experimental studies of the formation of sedimentary iron sulfides. In: Biogeochemistry of sulfur isotopes, p. 107 to 120. Ed. by M.L. Jensen. New Haven: Yale University Press.

_____. 1966. Chemical diagenesis of some modern carbonate sediments. Amer. J. Sci. 264: 1-36.

_____. 1969. Migration of iron and sulfur within anaerobic sediments during early diagenesis. Amer. J. Sci. 267: 19-42.

Biddulph, O. 1959. Translocation of inorganic solutes. In Plant Physiology 2, p. 553 to 603. Ed. by F.C. Steward. New York: Academic Press.

Black, C.A. 1968. Soil-plant relationships, 792 p. New York: John Wiley and Sons.

Brandt, K. and E. Ruben. 1920. Zur Kenntnis der chemischen Zusammensetzung des planktons und eineger Bodenorganismen. Wiss. Meeres. Untersuchungen, N.F. 19: 175-210.

Bremner, J.M. 1965a. Total nitrogen. In: Methods of soil analysis, p. 1149 to 1178. Ed. by C.A. Black et al. Madison, Wisconsin: American Society of Agronomy.

_____. 1965b. Inorganic forms of nitrogen. In: Methods of soil analysis, p. 1179 to 1237. Ed. by C.A. Black et al. Madison, Wisconsin: American Society of Agronomy.

Brezonik, P. and C. Harper. 1969. Nitrogen fixation in some anoxic lacustrine environments. Science 164: 1277-1279.

Brooks, R.R., B.J. Presley and I.R. Kaplan. 1968. Trace elements in the interstitial waters of marine sediments. Geochim. Cosmochim. Acta 32: 397-414.

Brouzes, R., J. Lasik and R. Knowles. 1969. The effect of organic amendment, water content, and oxygen on the incorporation of $^{15}N_2$ by some agricultural and forest soils. Can. J. Microbiol, 15: 899-905.

Brouzes, R., C.I. Mayfield and R. Knowles. 1971. Effect of oxygen partial pressure on nitrogen fixation and acetylene reduction in a sandy loam soil amended with glucose. Plant Soil (in press).

Burkholder, P.R., L.M. Burkholder and J.A. Rivero. 1959. Some chemical constituents of turtle grass, Thalassia testudinum. Bull. Torrey Bot. Club 86: 88-93.

Carritt, D.E. and S. Goddgal. 1954. Sorption reactions and some ecological implications. Deep Sea Res. 1: 224-243.

Chang, P.C. and R. Knowles. 1965. Non-symbiotic nitrogen fixation in some Quebec soils. Can. J. Microbiol. 11: 29-38.

Cheadle, V.I. 1942. The occurrence and types of vessels in the various organs of the plant in the Monocotyledonaceae. Amer. J. Bot. 29: 441-450.

Clarke, F.W. and W.C. Wheeler. 1922. The inorganic constituents of some marine invertebrates. U.S. Geol. Surv. Prof. Paper 124, 62 p.

Cole, C.V., S.R. Olsen and C.O. Scott. 1953. The nature of phosphate sorption by calcium carbonate. Soil Sci. Soc. Amer. Proc. 17: 352-356.

Cole, C.V. and S.R. Olsen. 1959. Phosphorus solubility in calcareous soils: II. Effects of exchangeable phosphorus and soil texture on phosphorus solubility. Soil. Soc. Amer. Proc. 23: 119-121.

Dalton, H. and J.R. Postgate. 1969. Effect of oxygen on growth of Azotobacter chroococcum in batch and continuous cultures. J. Gen. Microbiol. 54: 463-473.

Day, P.R. 1965. Particle fractionation and particle size analysis. In: Methods of soil analysis, p. 545 to 567. Ed. by C.A. Black et al. Madison, Wisconsin: American Society of Agronomy.

Delwiche, C.C. and J. Wijler. 1956. Non-symbiotic nitrogen fixation in soil. Plant Soil 7: 113-129.

Dugdale, R.C., Goering, J.J. and J.H. Ryther. 1964. High nitrogen fixation rates in the Sargasso Sea and Arabian Sea. Limnol. Oceanogr. 9: 507-510.

Dugdale, R.C. and J.J. Goering. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr. 12: 196-206.

Emery, K.O. and S.C. Rittenberg. 1952. Early diagenesis of California basin sediments in relation to the origin of oil. Bull. Amer. Soc. Petrol. Geol. 36: 735-806.

Emery, K.O., R.E. Stevenson and J.W. Hedgepeth. 1957. Estuaries and lagoons. In: Treatise on marine ecology and paleoecology. I. Ecology, p. 673 to 750. Ed. by J.W. Hedgepeth. Geol. Soc. Amer. Mem. 67.

Feigl, F. 1958. Spot tests in inorganic analysis, 600 p. Amsterdam: Elsevier.

Fenchel, T. 1970. Studies on the decomposition of organic detritus derived from the turtle grass Thalassia testudinum. Limnol. Oceanogr. 15: 14-20.

Fogg, G.E. 1962. Extracellular products. In: Physiology and biochemistry of algae, p. 475 to 489. Ed. by R.A. Lewin. New York: Academic Press.

Fogg, G.E. and A.J. Horne. 1967. The determination of nitrogen fixation in aquatic environments. In: Chemical environment in the aquatic habitat, p. 115 to 120. Ed. by H.L. Golterman and R.S. Clymo. Amsterdam: N.V. Noord-Hollandsche Uitgerers Maatschappij.

Folk, R.L. 1965. Petrology of sedimentary rocks, 159 p. Austin, Texas: Hemphill's.

Frank, P.A. and R.H. Hodgson. 1964. A technique for studying absorption and translocation in submerged plants. Weeds 12: 546-555.

Frederick, L.R., R.L. Starkey and W. Segal. 1957. Decomposability of some organic sulfur compounds in soil. Soil Sci. Amer. Proc. 21: 287-292.

Fried, M. and R.E. Shapiro. 1956. Phosphate supply pattern of various soils. Soil Sci. Soc. Amer. Proc. 20: 471-475.

Fried, M. and R.E. Shapiro. 1962. Soil-plant relationships in ion uptake. Ann. Rev. Plant Physiol. 12: 91-112.

Garrels, R.M. and C. Christ. 1965. Minerals, solutions and equilibria, 450 p. New York: Harper and Row.

Gerloff, G.C. and P.H. Krombholz. 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. Limnol. Oceanogr. 11: 529-537.

Ginsburg, R.N. 1956. Environmental relationships of grain size and constituent particles in some south Florida carbonate sediments. Bull. Amer. Assoc. Petrol. Geol. 40: 2384-2427.

Ginsburg, R.N. and H.A. Lowenstam. 1958. The influence of marine bottom communities on the depositional environment of sediments. J. Geology 66: 310-318.

Golterman, H.L. 1967. Influence of the mud on the chemistry of water in relation to productivity. In: Chemical environment in the aquatic habitat, p. 297-313. Ed. by H.L. Golterman and R.S. Clymo. Amsterdam: N.V. Noord-Hollandsche Uitgerers Maatschappij.

Gulbrandsen, R.A. 1969. Physical and chemical factors in the formation of marine apatite. Econ. Geology 64: 365-382.

Hardy, R.W.F., R.D. Holsten, E.K. Jackson and R.C. Burns. 1968. The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol. 43: 1185-1207.

Harmsen, G.W. and G. Jager. 1962. Determination of the quantity of carbon and nitrogen in the rhizosphere of young plants. Nature 195: 1119-1120.

Harter, R.D. 1968. Adsorption of phosphorus by lake sediment. Soil Sci. Soc. Amer. Proc. 32: 514-517.

Hartman, R.T. and D.L. Brown. 1967. Changes in internal atmosphere of submerged vascular hydrophytes in relation to photosynthesis. Ecology 48: 252-258.

Hassouna, M.G. and P.F. Wareing. 1964. Possible role of rhizosphere bacteria in the nitrogen nutrition of Ammophila arenaria. Nature 202: 467-469.

Hayes, F.R. 1963. The role of bacteria in the mineralization of phosphorus in lakes. In: Symposium on marine microbiology, p. 654 to 663. Ed. by C.H. Oppenheimer. Springfield, Illinois: C.C. Thomas.

Hayes, F.R. and J.E. Phillips. 1958. Lake water and sediment. IV. Radiophosphorus equilibrium with mud, plants, and bacteria under oxidized and reduced conditions. Limnol. Oceanogr. 3: 459-475.

Hoskin, C.M. 1963. Recent carbonate sedimentation on Alacran Reef, Yucatan, Mexico, 160 p. Pub. 1089, National Academy of Sciences, Washington.

Howard, D., J. Frea, R. Pfister and P. Dugan. 1970. Biological nitrogen fixation in Lake Erie. Science 169: 61-62.

Hsu, P.H. 1964. Adsorption of phosphate by aluminum and iron in soils. Soil Sci. Soc. Amer. Proc. 28: 474-478.

Humm, H.J. 1956. Sea grasses of the northern Gulf coast. Bull. Mar. Sci. Gulf and Carib. 6: 305-308.

_____. 1964. Epiphytes of the sea grass, Thalassia testudinum in Florida. Bull. Mar. Sci. Gulf and Carib. 14: 306-341.

Hutchinson, G.E. 1944. Nitrogen in the biogeochemistry of the atmosphere. Amer. Sci. 32: 178-195.

Inman, D.L. 1949. Sorting of sediments in the light of fluid mechanics. J. Sediment. Petrol. 19: 51-70.

Jaibeyo, E.O. and A.W. Moore. 1963. Soil nitrogen accretion under different covers in a tropical rain forest environment. Nature: 317-318.

Jensen, P.B. 1915. Studies concerning the organic matter of the sea bottom. Rept. Danish Biol. Sta. 22: 1-39.

Jitts, H.R. 1959. The adsorption of phosphate by estuarine bottom deposits. Aust. J. Mar. Freshw. Res. 10: 7-21.

Johnson, R.G. 1967. Salinity of interstitial water in a sandy beach. Limnol. Oceanogr. 12: 1-7.

Jones, J.A. MS, 1968. Primary productivity by the tropical marine turtle grass, Thalassia testudinum König, and its epiphytes, 196 p. PhD thesis. Univ. Miami, Miami.

Jurgensen, M.F. and C.B. Davey. 1970. Nonsymbiotic nitrogen-fixing microorganisms in acid soils and the rhizosphere. Soils Fertil. 33: 435-446.

Kaplan, I.R. and S.C. Rittenberg. 1963. Basin sedimentation and diagenesis. In: The sea, Volume III, p. 583 to 619. Ed. by M.N. Hill. New York: Interscience.

Kaplan, I.R., K.O. Emery and S.C. Rittenberg. 1963. The distribution and isotropic abundance of sulfur in recent marine sediments off southern California. Geochim. Cosmochim. Acta 27: 297-331.

Kikuchi, T. 1966. An ecological study on animal communities of the Zostera marina belt in Tomioka Bay, Amakusa, Kyushu. Publ. Amakusa Mar. Biol. Lab. 1: 1-106.

King, C.A.M. 1959. Beaches and coasts, 403 p. London: Edward Arnold.

Kramer, J.R. 1964. Sea water: saturation with apatites and carbonates. Science 146: 637-638.

Kursanov, A.L. 1961. The transport of organic substances in plants. Endeavor 20: 19-25.

_____. 1963. Metabolism and the transport of organic substances in the phloem. Adv. in Bot. Res. 1: 209-278.

Laing, H.E. 1940a. Respiration of the rhizomes of Nuphar advenum and other water plants. Amer. J. Bot. 27: 574-581.

_____. 1940b. The composition of the internal atmosphere of Nuphar advenum and other water plants. Amer. J. Bot. 27: 861-868.

_____. 1941. Effect of concentration of oxygen and pressure of water upon growth of rhizomes of semi-submerged water plants. Bot. Gazette 102: 712-724.

Land, L.S. 1970. Carbonate mud production by epibiont growth on Thalassia testudinum. J. Sediment. Petrol. 40, 1361-1363.

LeGall, J., J.C. Senez and F. Pichnoty. 1959. Fixation de l'azote par les bactéries sulfato-réductrices. Ann. Inst. Pasteur 96: 223-230.

Lewis, J.B. 1960a. The fauna of rocky shores of Barbados, West Indies. Can. J. Zool. 38: 391-435.

_____. 1960b. The coral reefs and coral communities of Barbados, W.I. Can. J. Zool. 38: 1133-1145.

Macintyre, I.G. 1970. Sediments off the west coast of Barbados: diversity of origins. Mar. Geol. 9: 5-23.

Macnae, W. and M. Kalk. 1962. The fauna and flora of sand flats at Inhaca Island, Moçambique. J. Anim. Ecol. 31: 93-128.

MacRae, I.C. and T.F. Castro. 1967. Nitrogen fixation in some tropical rice soils. Soil Sci. 103: 277-280.

Maiklem, W.R. 1967. Black and brown speckled foraminiferal sand from the southern part of the Great Barrier Reef. J. Sed. Petrol. 37: 1023-1030.

McMillan, C. and F.N. Mosely. 1967. Salinity tolerances of five marine spermatophytes of Redfish Bay, Texas. Ecology 6: 503-506.

McRoy, C.P. and R.J. Barsdate. 1970. Phosphate absorption in eelgrass. Limnol. Oceanogr. 15: 6-13.

Miller, S.M. 1952. Phosphorus exchange in a sub-tropical marine basin. Bull. Mar. Sci. Gulf and Carib. 1: 257-265.

Moore, A.W. 1963. Nitrogen fixation in latosolic soil under grass. Plant Soil 19: 127-138.

_____. 1966. Non-symbiotic nitrogen fixation in soil and soil-plant systems. Soils Fertil. 29: 113-128.

Moore, D.R. 1963. Distribution of the sea grass Thalassia in the United States. Bull. Mar. Sci. Gulf and Carib. 13: 329-342.

Moore, H.B. 1930. Muds of the Clyde Sea area. I. Phosphate and nitrogen contents. J. Mar. Biol. Ass. U.K. 16: 595-607.

_____. 1931. Muds of the Clyde Sea area. III. Chemical and physical conditions; rate and nature of sedimentation; and fauna. J. Mar. Biol. Ass. U.K. 17: 325-358.

Mortimer, C.H. 1941-1942. The exchange of dissolved substances between mud and water in lakes. J. Ecol 29: 280-329; 30: 147-201.

Moulinier, R.M. and J. Picard. 1952. Recherches sur les herbiers de phanérogames marine du littoral Mediterranéen Francais. Annales Inst. Oceanogr. Paris 27: 157-234.

Murrmann, R.P. and M. Peech. 1968. Reaction products of applied phosphate in limed soils. Soil Sci. Soc. Amer. Proc. 32: 493-496.

Murrmann, R.P. and M. Peech. 1969a. Effect of pH on labile and soluble phosphate in soils. Soil Sci. Soc. Amer. Proc. 33: 205-210.

Murrmann, R.P. and M. Peech. 1969b. Relative significance of labile and crystalline phosphates in soil. Soil Sci. 107: 249-255.

Nagle, J.S. 1968. Distribution of the epibiota of macroepibenthic plants. Pub. Inst. Mar. Sci. Univ. Texas 13: 105-144.

Nelson, B.W. 1967. Sedimentary phosphate method for estimating paleosalinities. Science 158: 917-920.

Nykvist, N. 1963. Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter. Stud. Forest. Suecica, No. 3.

Odum, E.P. 1959. Fundamentals of ecology, 546 p. Philadelphia: W.B. Saunders.

Odum, H.T. 1957. Primary production measurements in eleven Florida springs and a marine turtle grass community. Limnol. Oceanogr. 2: 85-97.

_____. 1963. Productivity measurements in Texas turtle grass and the effects of dredging an intracoastal channel. Pub. Inst. Mar. Sci. Univ. Texas 9: 48-58.

_____. 1967. Biological circuits and the marine systems of Texas. In: Pollution and marine ecology, p. 99 to 157. Ed. by T.A. Olson and F.J. Burgess. New York: John Wiley and Sons.

Odum, H.T., P.R. Burkholder and J. Rivero. 1959. Measurements of productivity of turtle grass flats, reefs, and the Bahia Fosforente of southern Puerto Rico. Publ. Inst. Mar. Sci. Univ. Texas 6: 159-170.

Odum, H.T. and C.M. Hoskin. 1958. Comparative studies on the metabolism of marine waters. Pub. Inst. Mar. Sci. Univ. Texas 5: 65-96.

Odum, H.T. and E.P. Odum. 1955. Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. Ecol. Mono. 25: 291-320.

Odum, H.T. and R.F. Wilson. 1962. Further studies on reaeration and metabolism of Texas bays. Pub. Inst. Mar. Sci. Univ. Texas 8: 23-55.

O'Gower, A.K. and J.W. Wacasey. 1967. Animal communities associated with Thalassia, Diplanthera and sand beds in Biscayne Bay I. Analysis of communities in relation to water movements. Bull Mar. Sci. Gulf and Carib. 17: 175-210.

Olsen, S.R. and L.A. Dean. 1965. Phosphorus. In: Methods of soil analysis, p. 1035 to 1049. Ed. by C.A. Black et al. Madison, Wisconsin: American Society of Agronomy.

Olsen, S.R. and F.S. Watanbe. 1957. A method to determine a phosphorus adsorption maximum of soils as measured by the Langmuir isotherm. Soil Sci. Soc. Amer. Proc. 21: 144-148.

Oppenheimer, C.H. 1960. Bacterial activity in sediments of shallow marine bays. Geochim. Cosmochim. Acta 19: 244-260.

_____. 1966. pH and Eh of marine sediments. In: The encyclopedia of oceanography, p. 698 to 705. Ed. by R.W. Fairbridge. New York: Reinhold.

_____. 1968. Ed. Marine Biology IV. Unresolved problems in marine microbiology, 485 p. New York: New York Academy of Sciences.

Oppenheimer, C.H. and R.A. Ward. 1963. Release and capillary movement of phosphorus in exposed tidal sediments. In: Symposium on marine microbiology, p. 664 to 673. Ed. by C.H. Oppenheimer. Springfield, Illinois: C.C. Thomas.

Orpurt, P. and L. Boral. 1964. The flowers, fruits and seeds of Thalassia testudinum König. Bull. Mar. Sci. Gulf and Carib. 14: 296-302.

Ostenfeld, C.H. 1908. On the ecology and distribution of Grass-Wrack (Zostera marina) in Danish waters. Rept. Danish Biol. Sta. 17: 1-62.

Papavizas, G.C. and C.B. Davey. 1961. Extent and nature of the rhizosphere of Lupinus. Plant Soil 14: 215-236.

Parker, C.A. 1954. Non-symbiotic nitrogen-fixing bacteria in soil. I. Studies on Clostridium butyricum. Aust. J. Agric. Res. 5: 90-97.

Parker, P.L. 1966. Movement of radioisotopes in a marine bay: cobalt-60, iron-59, manganese-54, zinc-65, sodium-22, Pub. Inst. Mar. Sci. Univ. Texas 11: 102-107.

Patrick, W.H. 1961. Nitrate reduction ratio in a submerged soil as affected by redox potential. Trans. 7th Intern. Congr. Soil Sci. 2: 494-500.

Pearsall, W.H. 1938. The soil complex in relation to plant communities. I. Oxidation-reduction potentials in soils. J. Ecol. 26: 180-193.

Pearsall, W.H. and C.H. Mortimer. 1939. Oxidation-reduction potentials in waterlogged soils, natural waters and muds. J. Ecol. 27: 483-501.

Petersen, C.J.G. 1914. Om Baendeltangens (Zostera marina) Aars-Produktion i de danske farvande. Mindeskr. Steenstr. Fds. Kbh. 9: 1-20.

_____. 1918. The sea bottom and its production of fish food. Rept. Danish Biol. Sta. 25 No. 7, 62 p.

Petersen, C.J.G. and P.B. Jensen. 1911. Valuation of the sea. I. Animal life of the sea bottom, its food and quantity. Ber. f. Dansk. Biol. Sta. 20, 76 p.

Phillips, R.C. 1960. Observations on the ecology and distribution of the Florida sea grasses. Florida Bd. Conserv. Prof. Paper Ser. 2, 72 p.

Pomeroy, L.R., E.E. Smith and C.M. Grant. 1965. The exchange of phosphate between estuarine water and sediments. Limnol. Oceanogr. 10: 167-172.

Postgate, J.R. 1959. Sulphate reduction by bacteria. Ann. Rev. Microbiol. 13: 505-520.

_____. 1970. Nitrogen fixation by sporulating sulphate-reducing bacteria including rumen strains. J. Gen. Microbiol. 63: 137-139.

Pravdić, V. 1970. Surface charge characterization of sea sediments. Limnol. Oceanogr. 15: 230-233.

Presley, B.J. and I.R. Kaplan. 1968. Changes in dissolved sulfate, calcium and carbonate from interstitial water of near-shore sediments. Geochim. Cosmochim. Acta 32: 1037-1048.

Proctor, M.H. and P.W. Wilson. 1958. Nitrogen fixation by gram-negative bacteria. Nature 182: 891.

Rabotnova, I.L., V.G. Plakunova, M.A. Paleeva and L.V. Shenderova. 1963. On the causes of a decrease in the redox potential in cultures of microorganisms [in Russian, English summary]. Mikrobiologiia 32: 954-960.

Randall, J.E. 1963. An analysis of the fish populations of artificial and natural reefs in the Virgin Islands. Carib. J. Sci. 3: 31-47.

_____. 1965. Grazing effect on sea grasses by herbivorous reef fishes in the West Indies. Ecology 46: 255-260.

Reuther, W. 1961. Ed. Plant analysis and fertilizer problems, 454 p. Washington: American Institute of Biological Sciences.

Rice, W.A., E.A. Paul and L.R. Wetter. 1967. The role of anaerobosis in asymbiotic nitrogen fixation. Can. J. Microbiol. 13: 829-836.

Riederer-Henderson, M.A. and P.W. Wilson. 1970. Nitrogen fixation by sulphate-reducing bacteria. J. Gen. Microbiol. 65: 27-31.

Rinaudo, G. 1970. Fixation biologique de l'azote dans trois types de sols de rizières de Cote d'Ivoire, 121 p. Paris: Office de la recherche scientifique et technique outre-mer.

Rittenberg, S.C. 1963. Marine bacteriology and the problem of mineralization. In: Symposium on marine microbiology, p. 48-60. Ed. by C.H. Oppenheimer. Springfield, Illinois: C.C. Thomas.

Rittenberg, S.C., K.O. Emery and W.L. Orr. 1955. Regeneration of nutrients in sediments of marine basins. Deep Sea Res. 3: 23-45.

Roberson, C.E. 1966. Solubility implications of apatite in sea water. U.S. Geol. Surv. Prof. Paper 550-D: D178-D185.

Rochford, D.J. 1951. Studies in Australian estuarine hydrology. I. Introductory and comparative features. Aust. J. Mar. Freshw. Res. 2: 1-116.

Rouse, W.R. 1966. The moisture balance of Barbados and its influence on sugar cane yield. McGill Climatological Res. Ser. 1: 1-54.

Rovira, A.D. 1956. Plant root excretions in relation to the rhizosphere effect. I, II, III. Plant Soil 7: 178-217.

Ruinen, J. 1961. The phyllosphere. I. An ecologically neglected milieu. Plant Soil 15: 81-109.

_____. 1965. The phyllosphere. III. Nitrogen fixation in the phyllosphere. Plant Soil: 22: 375-394.

Russell, E.J. 1950. Soil conditions and plant growth, 635 p. London: Longmans, Green and Co.

Ryther, J.H. and W.M. Dunstan. 1971. Nitrogen, phosphorus and eutrophication in the coastal marine environment. Science 171: 1008-1013.

Sauvageau, M.C. 1890. Sur la feuille des Hydrocharidées marines. II. Thalassia. J. de Bot. 4: 289-293.

Scholander, P.F., H.T. Hammel, E. Hemmingsen and W. Garey. 1962. Salt balance in mangroves. Plant Physiol. 37: 722-729.

Scholander, P.F. 1968. How mangroves desalinate seawater. Physiologia Plantarum 21: 251-261.

Sculthorpe, C.D. 1967. The biology of aquatic plants, 610 p. London: Edward Arnold.

Setchell, W.A. 1920. Geographical distribution of the marine spermatophytes. Bull. Torrey Bot. Club 47: 563-579.

_____. 1935. Geographic elements of the marine flora of the north Pacific ocean. Amer. Naturalist 69: 560-577.

Sharp, J.H. 1969. Blue-green algae and carbonates: Schizothrix calicola and algal stromatolites from Bermuda. Limnol. Oceanogr. 14: 568-578.

Sillén, L.G. 1961. The physical chemistry of sea water. In: Oceanography, p. 549 to 582. Ed. by M. Sears. Washington: American Association for the Advancement of Science.

Sisler, F.D. and C.E. ZoBell. 1951. Nitrogen fixation by sulfate-reducing bacteria as indicated by nitrogen/argon ratios. Science 113: 511.

Smith, F.G.W., R.H. Williams and C.C. Davis. 1950. An ecological survey of the subtropical inshore waters adjacent to Miami. Ecology 31: 119-146.

Solórzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14: 799-801.

Sperber, J.I. 1958. Release of phosphate from soil minerals by hydrogen sulphide. Nature 181: 934.

Starck, W.A. and W.P. Davis. 1966. Night habits of fishes of Alligator Reef, Florida. Icthylogica 38: 313-356.

Stephens, W.M. 1966. Life in the turtle grass. Sea Frontiers 12: 264-275.

Stephensen, W. 1949. Certain effects of agitation upon release of phosphate mud. J. Mar. Biol. Assoc. U.K. 28: 371-380.

Stephenson, T.A., Anne Stephenson, G. Tandy and M. Spender. 1931. The structure and ecology of Low Isles and other reefs. Brit. Mus. Nat. Hist. Sci. Rep. Great Barrier Reef Exped. 3: 17-112.

Steven, D.M., A.L. Brooks and E.A. Moore. MS, 1970. Primary and secondary production in the tropical Atlantic, 124 p. Final report submitted to Biology Branch, Office of Naval Research. Bermuda Biological Station, Bermuda.

Stewart, W.D. 1966. Nitrogen fixation in plants, 168 p. London: Athlone Press.

_____. 1969. Biological and ecological aspects of nitrogen fixation by free-living micro-organisms. Proc. Roy. Soc. B. 172: 367-388.

Strawn, K. 1961. Factors influencing the zonation of submerged monocotyledons at Cedar Key, Florida. J. Wildlife Manage. 25: 178-189.

Strickland, J.D.H. and T.R. Parsons. 1965. A manual of sea water analysis, 203 p. Bull. Fish. Res. Bd. Canada No. 125.

Sutton, C.D. and D. Gunary. 1969. Phosphate equilibria in soil. In: Ecological aspects of the mineral nutrition of plants, p. 127 to 134. Ed. by I.H. Rorison. London: Blackwell.

Sverdrup, H.U., M.W. Johnson and R.H. Fleming. 1942. The oceans, 1087 p. New York: Prentice-Hall.

Swanson, C.A. 1959. Translocation of organic solutes. In: Plant physiology 2, p. 481 to 551. Ed. by F.C. Steward. New York: Academic Press.

Taylor, W.R. 1960. Marine algae of the eastern tropical and subtropical coasts of the Americas, 870 p.; 80 pl. Ann Arbor, Michigan: University of Michigan Press.

Taylor, J.D. and M.S. Lewis. 1970. The flora, fauna and sediments of the marine grass beds of Mahé, Seychelles. J. Natur. Hist. 4: 199-220.

Teal, J.M. 1962. Energy flow in the salt marsh ecosystem of Georgia. Ecology 43: 614-624.

Thomas, L.P., D.R. Moore and R.C. Work. 1961. Effects of Hurricane Donna on the turtle grass beds of Biscayne Bay, Florida. Bull. Mar. Sci. Gulf and Carib. 11: 191-197.

Thorne, R.F. 1954. Flowering plants of the waters and shores of the Gulf of Mexico. Fish. Bull. U.S. 89: 193-202.

Tomlinson, P.B. 1969a. On the morphology and anatomy of turtle grass, Thalassia testudinum (Hydrocharitaceae) II. Anatomy and development of the root in relation to function. Bull. Mar. Sci. Gulf and Carib. 19: 57-91.

_____. 1969b. On the morphology and anatomy of turtle grass, Thalassia testudinum (Hydrocharitaceae) III. Floral morphology and anatomy. Bull. Mar. Sci. Gulf and Carib. 19: 286-305.

Tomlinson, P.B. and G.A. Vargo. 1966. On the morphology and anatomy of turtle grass, Thalassia testudinum (Hydrocharitaceae) I. Vegetative morphology. Bull. Mar. Sci. Gulf and Carib. 16: 748-761.

Udell, H.F., J. Zarudsky, R.E. Doheny and P.R. Burkholder. 1969. Productivity and nutrient values of plants growing in the salt marshes of the Town of Hempstead, Long Island. Bull. Torrey Bot. Club 96: 42-51.

Ulrich, A. and F.S. Hills. 1967. Principles and practices of plant analysis. In: Soil testing and plant analysis. II. Plant analysis, p. 11 to 24. Ed. by G.W. Hardy. Madison, Wisconsin: Soil Science Society of America.

Vancura, V. 1964. Root exudates of plants. I. Analysis of barley and wheat in their initial phases of growth. Plant Soil 21: 231-247.

Vinogradov, A.P. 1953. The elementary composition of marine organisms, 647 p. Translated from Russian by J. Efron and J.K. Setlow, with biblio. by V.W. Odum. Sears Found. Mar. Res. Mem. 2. New Haven: Yale University Press.

Voss, G., and N. Voss. 1955. An ecological survey of Soldier Key, Biscayne Bay, Florida. Bull. Mar. Sci. Gulf and Carib. 5: 203-229.

Waksman, S.A., M. Hotchkiss and C.L. Carey. 1933. Marine bacteria and their role in the cycle of life in the sea. II. Bacteria concerned in the nitrogen cycle in the sea. Biol. Bull. 65: 137-166.

Watt, W.D. and F.R. Hayes. 1963. Tracer study of the phosphorus cycle in sea water. Limnol. Oceanogr. 8: 276-285.

Welch, B.L. 1965. Succession in the Caribbean Thalassia community. Trans. Joint Conf. Mar. Tech. Soc. and Amer. Soc. Limnol. Oceanogr. June 1965 1: 297 (abstr).

Westlake, D.F. 1963. Comparisons of plant productivity. Biol. Rev. 38: 385-425.

_____. 1965. Some basic data for investigations of the productivity of aquatic macrophytes. Mem. 1st Ital. Idrobiol. Suppl. 18: 229-48.

Wetzel, R.G. 1964. A comparative study of the primary productivity of higher aquatic plants, periphyton, and phytoplankton in a large shallow lake. Int. Rev. Ges. Hydrobiol. 49: 1-61.

_____. 1969. Factors influencing photosynthesis and excretion of dissolved organic matter by aquatic macrophytes in hard-water lakes. Verh. Intern. Verein. Limnol. 17: 72-85.

Williams, R.H. 1954. Distribution of chemical constituents of sea water in the Gulf of Mexico. Fish. Bull. U.S. 89: 143-151.

Wood, E.J.F. 1953. Reducing substances in Zostera. Nature 172: 916.

_____. 1959a. Some aspects of the ecology of Lake Macquarie, N.S.W. with regard to an alleged depletion of fish. IV. Bacterial and fungal studies. Aust. J. Mar. Freshw. Res. 10: 304-315.

_____. 1959b. Some aspects of marine microbiology. J. Mar. Biol. Assoc. India 1: 26-32.

_____. 1963. Heterotrophic micro-organisms in the oceans. Oceanogr. Mar. Biol. Ann. Rev. 1: 197-222.

_____. 1965. Marine microbial ecology, 243 p. London: Chapman and Hall.

Wyatt, J.T. and J.K.G. Silvey. 1969. Nitrogen fixation by Gleocapsa. Science 165: 908-909.

Zieman, J.C. MS, 1968. A study of the growth and decomposition of the sea-grass, Thalassia testudinum, 50 p. MSc thesis. Univ. of Miami, Miami.

ZoBell, C.E. 1946a. Studies on redox potential of marine sediments. Bull. Amer. Assoc. Petrol. Geol. 30: 477-513.

_____. 1946b. Marine microbiology, 240 p. Waltham, Mass.: Chronica Botanica Co.

ZoBell, C.E. and R.Y. Morita. 1959. Deep sea bacteria. Galathea Rep. 1: 139-154.

PLATE I

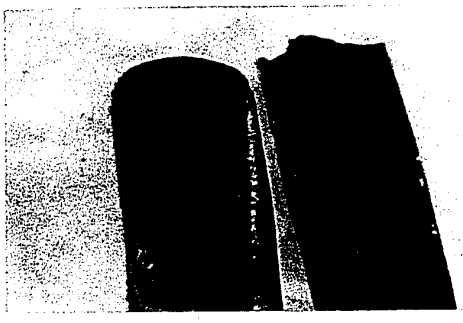
PORTIONS OF THE THALASSIA PLANT

a. (left) Distal end of young leaf, tip intact; (right) distal end of older leaf, encrusted with calcareous algae, and tip broken off. X2.

b. A portion of an erect shoot with dead leaf tissue removed to show leaf scars.

c. Rhizome growing tip and young erect shoot. X 1.

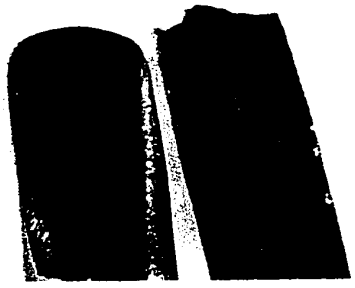
a

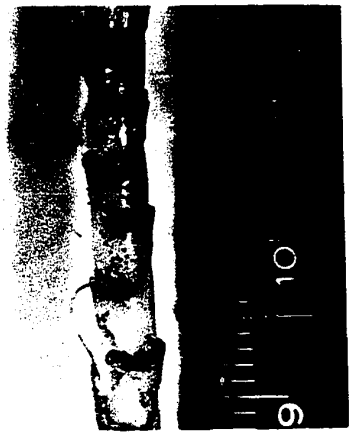


b

c

a





b

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PLATE II

UNDERWATER PHOTOGRAPHS OF THALASSIA BEDS

a. Typical *Thalassia* stand. Leaves approximately 25 cm height.

b. *Thalassia* stand overgrown by *Avrainvillea rawsonii* and *Avrainvillea* nigricans. Flabellate alga (*A. nigricans*) approximately 15 cm height.

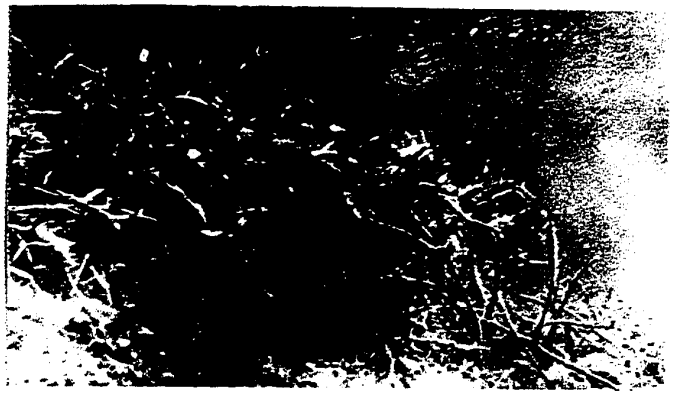
c. Erosional scarp in cobble framework substrate area. Approximately 0.5 m relief.

d. Erosional scarp in predominantly sand substrate area. Long, unbranched erect shoots of *Thalassia* are approximately 25 cm length.

a

b

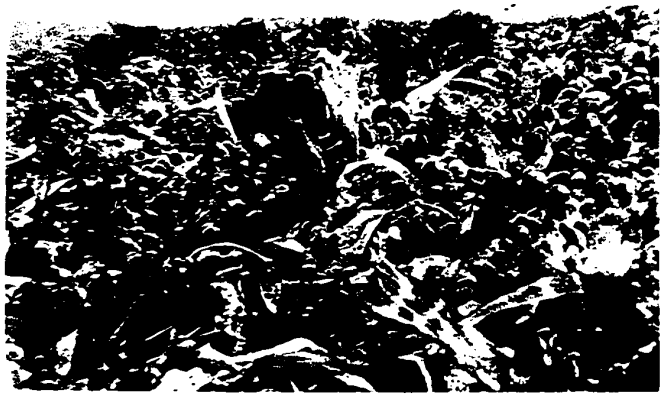
c



d

a

b



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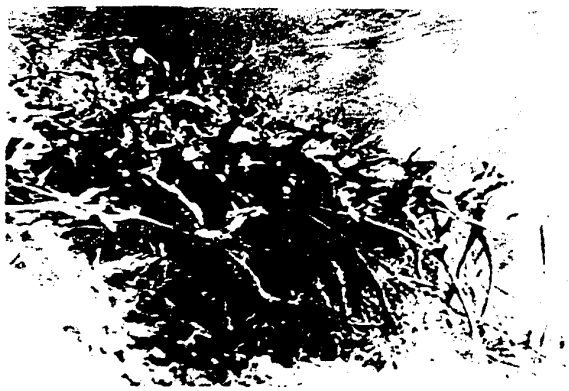

d

PLATE III

PHOTOMICROGRAPHS OF THIN SECTIONS OF HOMOTREMA DEBRIS IN THE SEDIMENT

a. All chambers infilled with iron sulfide. X 28.

b. As for (a). X 135.

c. Iron sulfide infilling restricted to few chambers. X 28.

d. No iron sulfide infilling. X 135.

a

b

c

d

a

b

c

d

APPENDIX A. ESTIMATION OF GROWTH RATE, PRODUCTION AND AGE OF THALASSIA

INTRODUCTION

Methods of estimating production based on seasonal increases in biomass are not applicable to plants such as Thalassia because rates of production and losses of leaf tissue are approximately constant throughout the year. The validity of applying techniques of estimating production based on measurements of oxygen production to aquatic plants has been questioned because of the retention of metabolic gases within the lacunal system (Hartman and Brown, 1967). The ^{14}C technique as applied to aquatic plants (Wetzel, 1964), while useful in experimental studies, is obviously limited in application to routine studies of production in the field. Zieman (MS, 1968) measured growth of Thalassia leaves by the elegantly simple technique of marking the leaves with metal staples. Very little is known of the age, growth rate and production of underground parts of aquatic angiosperms (Westlake, 1965).

Observations of Thalassia at Barbados indicated that there might be a simple relation between the maximum length of leaves in a stand and the average growth rate of the leaves. Variation in length of Thalassia leaves has been observed by several workers (Phillips, 1960; Strawn, 1961; D.R. Moore, 1963) and has been associated with variations in depth of water, tides, tidal zone, air and water temperatures, light and turbidity, and growth of epiphytes. However, no clear distinction has been made between variation in leaf length due to actual breaking off of the leaves, and variation due to differences in growth rates of the leaves. Such a distinction is not easily made because the actual leaf length in a given situation appears to be influenced by a number of factors. One of the most important factors determining the maximum length of leaves is the degree or rate of encrustation of the leaves by

calcareous epiphytes (Melobesia and Fosliella spp). Dense growth of these epiphytes on Thalassia leaves is a common phenomenon (Phillips, 1960; Taylor, 1960; Humm, 1964; Land, 1970). Growth of Thalassia leaves takes place largely or entirely at the basal meristematic region of the leaves. New leaves arise at the apex of the shoot; successively older leaves are progressively further removed both laterally and vertically from the shoot apex, and are alternately arranged. Thus the relative ages of leaves on a shoot may be determined by their positions relative to the shoot apex or youngest, innermost leaf (Phillips, 1960). At Barbados, dense growths of calcareous epiphytes occurred on the distal (older) parts of all leaves except for the youngest leaf on a shoot on which there were no epiphytes or they were just beginning to develop. The original, rounded leaf tip was observed only on the youngest one or two leaves on a shoot; the original tips of older leaves had been broken off and the distal parts were heavily encrusted by calcareous algae (Plate Ia). Infestation by calcareous epiphytes apparently weakens the blade, probably both through stimulating decay and through the strain imposed by the weight of the calcareous material, and eventually results in breaking off of the infested parts of the leaves. It was postulated that in the absence of heavy grazing, the maximum length reached by a leaf is determined mainly by (1) the growth rate of the leaf, (2) the rate of infestation by calcareous epiphytes, and (3) factors such as wave action which mechanically aid the actual breaking off process. Examination of leaves indicated little variation in the degree of encrustation by calcareous epiphytes between various stands at Barbados. This, and observations of marked differences in maximum leaf length between adjacent stands (i.e. stands subject to the same level of wave action), suggested that variations in leaf length are due mainly to variations in growth rate. Observations on growth rates utilizing

Zieman's (MS, 1968) technique of stapling the leaves were made at a series of stands of differing leaf lengths to determine if there was a simple relation between the maximum leaf length in a stand and the average growth rate. At the same time, data were obtained for the purpose of examining the relation between production and standing crop (wet wt including epiphytes), and examination of certain characteristics of underground parts suggested a means of estimating age, growth rate and production of the underground parts. In previous studies of Thalassia beds (Appendix B), Thalassia stands had been described in terms of the standing crop (wet wt including epiphytes), average leaf width, and the maximum leaf length (given as the average length of the longest 10 leaves in a sample of leaves from a 3/16 m^2 area); thus it was desired to determine the relation of growth rate and production to these statistics.

MATERIALS AND METHODS

Initially 13 stands of differing maximum leaf lengths were selected for study (Series 1). Nine of these stands were at Bath and four at St. Lawrence (Barbados). The Bath Thalassia bed is only partially protected from wave action; stands in the seaward area of this bed were subject to strong wave action, and stands in the inshore area, to moderate wave action. The St. Lawrence Thalassia bed lies in the lee of a rubble reef, and was subject to moderate wave action. Growth rates were determined by placing metal staples at a fixed height above the substrate on 50 leaves within a 1 m^2 area of uniform growth of Thalassia and determining the height of the staples after 5 days. Staples, oriented vertically, were placed at 3 cm height in stands of generally short leaves, and at 5 cm height in stands of longer leaves. Posts were inserted in the substrate to check for changes in the substrate (reference) level. On each of 10 leaves in a stand of long leaves (Series 1,

no. 13), a second staple was placed 3 cm above the first staple and the interval measured after 5 days; the average interval for the 8 recovered leaves was 3.2 cm, indicating little or no elongation above the level of the first staple (Zieman, MS 1968, reported that 18% of the total elongation of the leaves he studied took place above staples placed at 3 cm height above the substrate). Average growth rates were calculated by dividing the sum of all measured growth increments by the number of leaves observed. A sample of leaves at each of the stands was taken from a 3/16 m^2 area (three separate 1/16 m^2 areas combined) by cutting leaves at substrate level. For each sample, the leaves were counted, lengths of 20 to 30 of the longest leaves and widths of 50 leaves measured, and the wet weight of the sample was determined.

The observations of the Series 1 stands demonstrated surprisingly large differences between the growth rates of leaves within a stand; for example, in the stand of highest average growth rate, the individual growth rates varied over the entire range 0.to 17.9 mm/day. Because of this, it was thought that the average growth rate estimates might be highly biased because the youngest leaves (less than 5 or 3 cm at the time of marking) were not included in the measurements. Two other stands (Series 2) in which the individual shoots were widely separated and thus easily distinguished, were selected for growth rate observations. One stand was in the seaward area of the Bath Thalassia bed (strong wave action), and one was at St. Lawrence at 1.8 m depth (moderate wave action). All leaves on 25 shoots at Bath and on 12 shoots at St. Lawrence were marked. Those too short to be marked at the 5 cm level were marked by a horizontally oriented staple, and the length of the leaf and its particular shoot noted. After 5 days, the position of the staple, the total length of the leaf, the leaf width, and the presence or absence of the original leaf tip were determined for each leaf. Wet weight of

all leaves combined was determined. Average growth rates of these stands were calculated by adding all increments of growth, including the total lengths of leaves which emerged after marking.

The dry wt/wet wt ratio of Thalassia+epiphytes was determined (as part of previous studies) on 14 samples varying in wet weight between 40 and 285 g; the dry wt/wet wt ratio of Syringodium+epiphytes was determined on 11 samples varying in wet weight between 59 and 269 g. The proportion of the dry weight of Thalassia+epiphytes due to calcareous material was estimated for one sample of Thalassia as the loss of dry wt following treatment of 23 g dry wt Thalassia+epiphytes with dilute HCl to dissolve the calcareous material.

Epiphyte-free portions of leaves were cut from 8 samples of Thalassia of differing average widths and dried to constant weight to determine the dry wt/cm^2 of epiphyte-free leaf tissue.

Leaf growth rate and production data obtained in a subsequent study of a Bermuda Thalassia stand (Section III) are included here for comparison with the Barbados data. Wave action and the degree of encrustation by epiphytes were noticeably less than at Barbados. All leaves on 13 shoots were marked in the same way as were the Series 2 Barbados stands, and the positions of the staples were determined after 43 hours (1400 hr Aug. 28 to 0900 hr Aug. 30). To estimate daily rates, results were multiplied by 48/43x2. Leaf statistics (average width, maximum leaf length, wet wt, dry wt/wet wt ratio, dry wt/cm^2 of epiphyte-free leaf tissue) are based on a sample of leaves from 20 shoots in the same stand.

For examination of underground parts, sections of rhizomes and associated shoots and foliage leaves were obtained at erosional scarps where the underground parts had been exposed by erosion and thus were easily removed in

intact condition.

RESULTS AND DISCUSSION

Average growth rate and the maximum length of leaves in a stand

Two measures of the 'average maximum leaf length' of a stand were calculated from the data of the 3/16 m^2 samples of the Series 1 stands: (1) $L_{5\%}$, the average length of the longest 5% of the leaves, and (2) L_{10}, the average length of the longest 10 leaves in the sample. The locations, approximate depths below mean low water (the mean tidal range is approximately 0.7 m, the diurnal range 1.1 m; Lewis 1960b), the number of leaves in the 3/16 m^2 samples, the length of the longest leaf, the L_{10} and $L_{5\%}$ measures of the average maximum leaf length, and the observed average growth rates of the Series 1 stands are given in Table X. Average growth rate is plotted against $L_{5\%}$ in Fig. 10. It is evident that the differences in maximum leaf length between the various stands were associated mainly with differences in the growth rates. The least-squares regression of average growth rate (G) on $L_{5\%}$ is

$$G = 0.318\ L_{5\%} - 1.40$$

where G is in mm/day and $L_{5\%}$ is in cm.

The estimates of average growth rates of the Series 1 stands may have been biased because (1) leaves less than 3 or 5 cm at the time of marking were not marked, and (2) old or young leaves may not have been marked in proportion to their occurrence. The growth rate estimates of the Series 2 stands (Table XI) were not subject to these sources of bias, and thus can be expected to be more accurate. Comparison of the observed average growth

Table X. Leaf length and growth rate observations of Series 1 stands.

BI = Bath inshore, BS = Bath seaward, SL = St. Lawrence.

STAND	LOCATION & DEPTH (m)	NO. LEAVES per 3/16 m^2	LENGTH OF LONGEST LEAF (cm)	L_{10} (cm)	$L_{5\%}$	$L_{10}/L_{5\%}$	G (mm/day)	NO. GROWTH MEASUREMENTS
1	BI 0.2	271	19	11.0	10.5	1.05	1.9	25
2	SL 1.0	251	17	14.6	14.2	1.03	3.9	33
3	BS 1.0	264	21	16.5	15.8	1.04	2.9	28
4	BS 0.6	200	22	18.1	18.1	1.00	4.5	29
5	SL 1.2	92	21	19.6	20.2	0.97	4.6	32
6	SL 1.0	212	22	20.5	20.4	1.00	5.5	31
7	BI 0.6	129	24	22.3	23.0	0.97	4.5	38
8	BS 0.5	419	26	23.3	22.0	1.06	7.0	37
9	BS 1.0	206	26	23.9	23.9	1.00	4.8	21
10	SL 1.8	73	30	25.8	27.5	0.94	9.1	33
11	BS 0.6	264	29	26.0	25.3	1.03	6.2	17
12	BS 0.6	116	29	27.0	27.3	0.99	7.3	27
13	BS 0.4	374	41	37.4	36.3	1.03	10.0	42

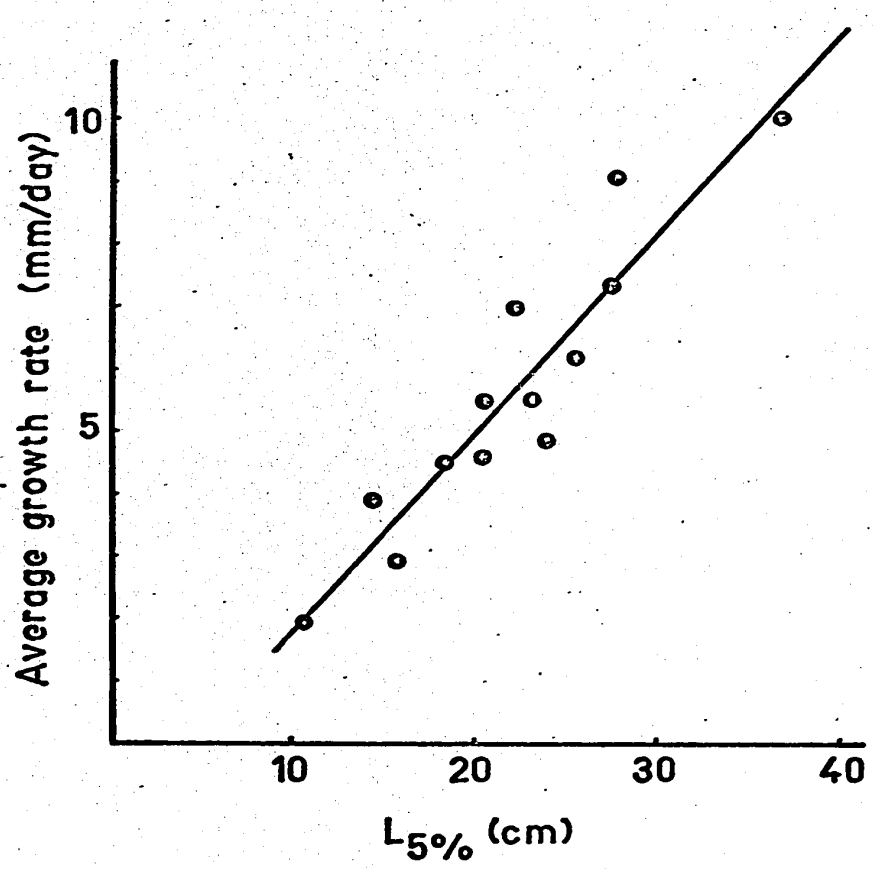

Fig. 10. Relation of leaf growth rate to the maximum length of leaves ($L_{5\%}$) in Thalassia stands.

Table XI. Production data of Barbados Series 2 stands and a Bermuda stand.

	SERIES 2		BERMUDA
	BATH	ST. LAWRENCE	
No. shoots in sample	25	12	13
No. leaves in sample	80	46	54
Period of growth observations	5 days	5 days	49 hr
No. new leaves emerged during period of observation	8	5	2
Estimate of T (days)	15.6	12.0	11.6
Non-growing leaves, % of total	12.5	19.6	29.6
Avg no. leaves per shoot	3.20	3.83	4.15
Avg width (mm)	14.0	15.1	7.28
e (mg/cm^2)	5.38*	5.54*	4.28+
Avg growth rate (mm/day)	7.12	5.31	6.76
I_s (cm/shoot per day)	2.28	2.04	2.81
P_s (mg/shoot per day)	17.2	17.0	8.75
P/SC	0.00375	0.00360	0.00343
$L_{5\%}$	36.0	31.5	41.0

*Estimated, +Observed

rates of the Series 2 stands with their growth rates predicted by their $L_{5\%}$ measures provides a check on the accuracy of growth rates estimated from the above regression. The observed average growth rates were 7.1 and 5.3 mm/day for the Bath and St. Lawrence stands respectively, and the growth rates predicted from their $L_{5\%}$ measures were 10.0 and 8.6 mm/day. Examination of the original data indicates that the older, slower growing leaves were probably undersampled in the Series 1 stands; 15% of the leaves marked in the Series 2 stands exhibited no growth over the 5 day period, whereas only 7% of all leaves in the Series 1 stands exhibited no growth. A correction is applied to the above regression by multiplying the intercept and slope by 0.66, the average of the observed growth rate-to-estimated growth rate ratios of the Series 2 stands. The corrected regression is

$$G = 0.210\ L_{5\%} - 0.92$$

The ratios $L_{10}/L_{5\%}$ of the Series 1 samples are given in Table X. L_{10} is equal to $L_{5\%}$ when the number of leaves in the 3/16 m^2 sample is 200. At very low leaf densities, L_{10} would tend towards being a measure of the average length rather than the maximum length, and it is obviously necessary to use a weighted (by the number of leaves) measure of the average maximum leaf length at very low leaf densities. At densities above about 75 leaves per 3/16 m^2 area there is little difference between L_{10} and $L_{5\%}$, even at very high leaf densities. For one sample of 807 leaves, the $L_{10}/L_{5\%}$ ratio was 1.06. For most purposes it is probably satisfactory to use an unweighted measure of the average maximum leaf length, or the weighting may be somewhat arbitrary, perhaps 3 leaves in samples of low leaf density, 10 leaves in samples of intermediate leaf density, and 30 leaves in samples of high leaf density.

Production and standing crop

Leaf tissue production of the Series 1 stands was estimated as

$$P_m = n \times G_c \times w \times c$$

where P_m is production (g dry wt leaf tissue/m^2 per day), n is the number of leaves per m^2, G_c is the observed average growth rate (Table X) multiplied by 0.66 (cm/day), w is the average leaf width (cm), and c is the dry wt/cm^2 of unepiphytized leaf tissue (g/cm^2). c varies with the average width of the leaves (Fig. 11)

$$c = 3.38 + 1.43\ w$$

where c is in mg/cm^2, and w is in cm. Standing crop (wet wt including epiphytes), data for the estimation of production and production-to-standing crop (P/SC) ratios of the Series 1 samples are given in Table XII. The mean of P/SC ratios is 0.0037, and the standard deviation is 0.00057. The P/SC ratios of the Series 2 samples were calculated by adding all increments of growth (in cm^2) over the 5 day period, multiplying by c and dividing by 5 times the weight of all leaves. The ratios, 0.00375 and 0.00360 for the Bath and St. Lawrence samples respectively, are in close agreement. The agreement of these ratios, which were subject to fewer errors than were the ratios of the Series 1 samples, and the agreement of the Series 2 ratios with the mean of the Series 1 ratios, suggest that the true P/SC ratio does not vary as greatly as suggested by the variation of the Series 1 ratios. At least as a first approximation, production may be estimated by multiplying standing crop (wet wt including epiphytes) by 0.0037.

Removal of calcareous material from large samples of Thalassia is a tedious procedure. Unless epiphytes are removed, there is little advantage in

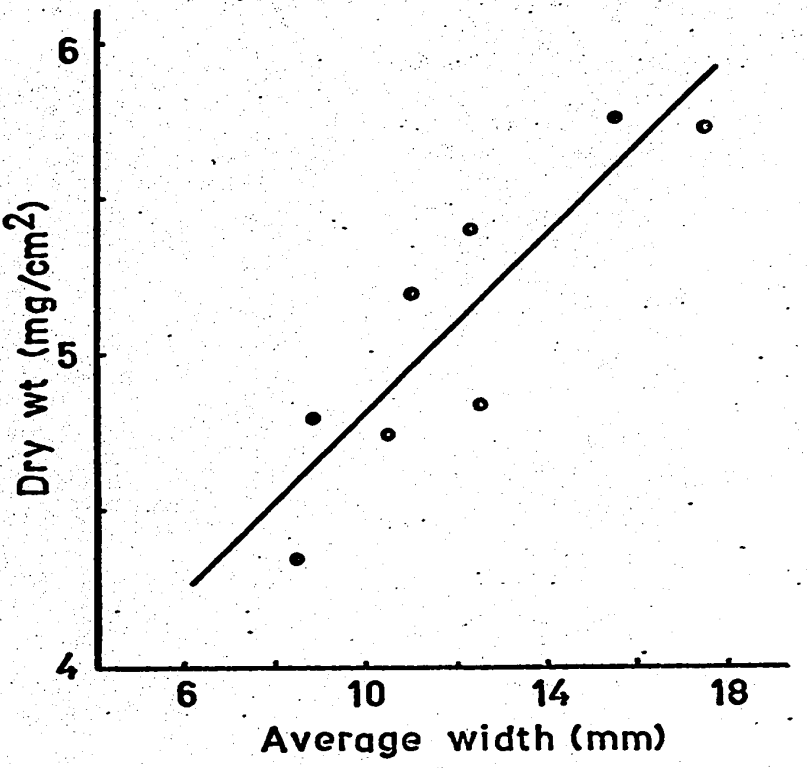

Fig. 11. Dry weight of epiphyte-free leaf tissue as a function of average leaf width.

Table XII. Production estimates of Series 1 stands.

STAND	n (leaves/m^2)	G_e (mm/day)	w (mm)	e (mg/cm^2)	P_m (g/m^2 per day)	SC (g/m^2)	P/SC
1	1445	1.3	7.6	4.5	0.64	197	0.0033
2	1338	2.6	9.9	4.8	1.65	427	0.0040
3	1408	1.9	8.5	4.6	1.05	411	0.0026
4	1066	3.0	8.7	4.6	1.28	301	0.0043
5	491	3.0	9.8	4.8	0.69	197	0.0035
6	1131	3.6	10.1	4.8	1.97	603	0.0033
7	688	3.0	8.6	4.6	0.82	224	0.0037
8	2202	4.6	11.0	5.0	5.57	1760	0.0032
9	1099	3.2	9.8	4.8	1.65	432	0.0038
10	389	6.0	15.8	5.6	2.07	545	0.0038
11	1408	4.1	9.4	4.7	2.55	587	0.0043
12	618	4.8	9.3	4.7	1.30	272	0.0048
13	1993	6.6	11.0	5.0	7.23	1810	0.0040

using dry weight over using wet weight as a measure of the standing crop. In fact, because wet weight is proportionally less influenced by variations in the amount of calcareous material than is dry weight, variations in the wet weight probably more closely reflect variations in the amount of Thalassia tissue than do variations in the dry weight. For one sample of Barbados Thalassia leaves, the amount of calcareous material was estimated as 45% of the dry weight of Thalassia+epiphytes. The dry wt/wet wt ratio of Barbados Thalassia+epiphytes averaged 0.190 (SD = 0.020); thus the ratio of dry wt Thalassia tissue only-to-wet wt Thalassia+epiphytes was approximately 0.105. The dry wt/wet wt ratio of Barbados Syringodium, a marine angiosperm with terete leaves on which there was almost no encrustation by calcareous epiphytes, was 0.128 (SD = 0.010). The dry wt/wet wt ratio of a Bermuda Thalassia+epiphytes sample was 0.144; there was significant growth of calcareous epiphytes on the Bermuda Thalassia, but it was conspicuously less than at Barbados. The differences in the dry wt/wet wt ratios are obviously due mainly to variations in the amount of calcareous material.

Growth of individual leaves

In studies of growth and production of Thalassia in Florida, Zieman (MS, 1968) observed the appearance of 6 new leaves on 6 shoots over a 14 day period, 11 new leaves on 10 shoots over a 16 day period and 8 new leaves on 4 shoots over a 32 day period, and concluded that "under normal conditions a branch produces a new blade every 14 to 16 days." In the observations of the Series 2 stands, 8 new leaves were observed on the 25 shoots at Bath over a 5 day period, and 5 new leaves on the 12 shoots at St. Lawrence. The frequency of new leaf production estimated from these observations is 15.6 days for the Bath sample and 12 days for the St. Lawrence sample. 95% confidence intervals for these estimates (from the binomial distribution

applied to the ratio no. of new leaves-to-shoots) are 9.4 to 33 days, and 7.4 to 32 days. These observations are therefore considered consistent with the observations of Zieman (MS, 1969).

Another estimate of the periodicity in leaf production was obtained from data of the Series 2 observations as follows. It is assumed: (1) the growth of all leaves on a given shoot follow a similar pattern of growth from time of initial emergence from the substrate to maturity and cessation of growth; thus if two leaves with their original tips present occur on the same shoot, the length of the oldest of the two is the length the youngest would reach after growing for an interval (T) equal to the difference in their ages; (2) the growth rate decreases linearly with age of a leaf; (3) the observed growth rates (of individual leaves) were the instantaneous growth rates at time 2.5 days after they were marked (i.e. in the middle of the 5 day period). Based on these assumptions, the periodicity in leaf production was estimated from data of the Series 2 stands as

$$T = (A + 2.5\ D) \div (G_y - 0.5\ D)$$

where A is the difference in length of two leaves with original tips on a shoot (mm), D is the difference in their average growth rates over the 5 day period (mm/day), and G_y is the average growth rate of the youngest of the two leaves over the 5 day period. Leaf data from 6 shoots were suitable for making this estimate (Table XIII). The estimates of T range from 11.3 to 19.7 days, with a mean of 15.1 days.

The longest unmarked leaf (leaf which emerged after marking) of the Series 2 stands was on a shoot at the St. Lawrence stand and was 11.0 cm in length. Assuming that this leaf emerged immediately after marking, then its average growth rate over the 5 day period was 22 mm/day. The growth rates

Table XIII. Growth rate-leaf length data for estimation of T.

SHOOT	YOUNGEST LEAF WITH TIP		NEXT YOUNGEST LEAF WITH TIP		OTHER LEAVES		T
	Length (cm)	G (mm/day)	Length	G	Length	G	(days)
Bath 1	11	15.0	27	13.8	15	2.0	11.3
Bath 2	8.5	10.4	23	8.6	8.5	0.6	15.7
Bath 3	18.5	11.4	33.5	7.6	24	0	16.8
Bath 4	19	9.0	36	8.4	26.5	0	19.7
SL 5	17	12.0	30	8.0	1.6*	-	14.0
					11	0	
SL 6	22.5	12.0	33	6.0	5*	-	13.3
					9	0.4	

*Leaf emerged after marking.

of the other leaves on this shoot were 10.4, 1.4, and 0 mm/day. Assuming, as above, that all leaves on a shoot have a similar growth rate pattern, and that these values represent the instantaneous growth rates at time 2.5, 17.5, 32.5, and 47.5 days, then the growth of an individual leaf on this shoot may be represented as in Fig. 12. This figure suggests that the growth rate of a leaf decreases more or less regularly with age, and cessation of growth occurs sometime between 35 and 45 days.

Average growth rate, length of leaf tissue produced per shoot, production per shoot

It is evident that the concept of an 'average growth rate' may be somewhat misleading when used in the sense of 'the average growth rate of all leaves in a stand'; this is so for two reasons: (1) it includes leaves at all stages of maturity growing at very different rates, and (2) its magnitude is in part determined by the length of time dead leaves remain attached to the shoot. The length of time dead leaves remain attached to a shoot is influenced by wave action as is shown by comparison of data on the number of dead leaves and the average number of leaves per shoot at the Series 2 Bath stand (strong wave action), Series 2 St. Lawrence stand (moderate wave action), and the Bermuda stand (gentle wave action) in Table XI. The high number of dead leaves at the Bermuda stand may also have been associated with a lower degree of epiphyte infestation at this stand. The average growth rate of the leaves in a stand is thus meaningful only as a parameter relating production per unit area to the number of leaves. A suggested statistic for the purpose of comparing growth rates, in the physiological sense, between different stands is I_s, the 'average length of leaf tissue produced per shoot per day'. Variation in wave action apparently has much less effect on the 'average maximum leaf length' than on the number of leaves per shoot; this

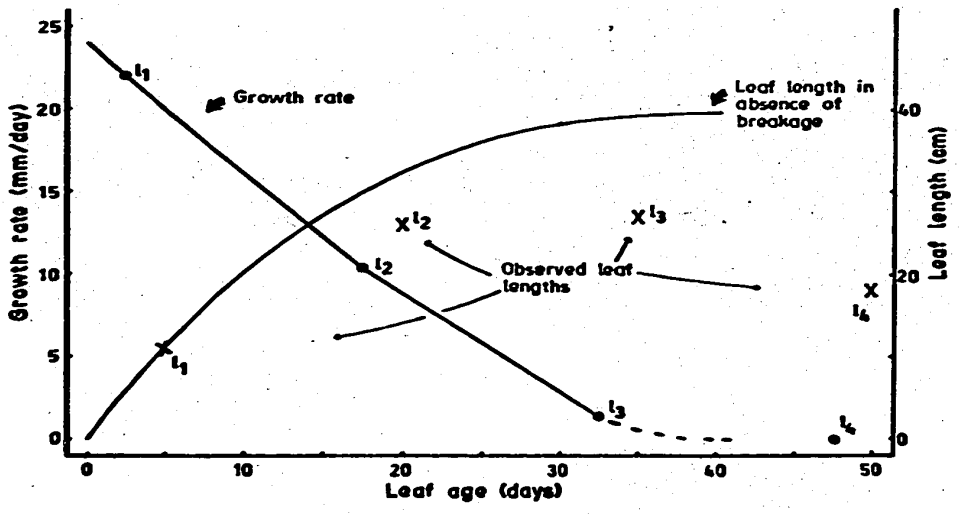

Fig. 12. Growth of an individual leaf. Projected from observed growth rates of four leaves (l_1, l_2, l_3, l_4) on a shoot.

is shown by the data in Fig. 10, and by comparison of the ratios given below (G is the observed average growth rate)

	I_s/I_{sBath}	$L_{5\%}/L_{5\%\ Bath}$	G/G_{Bath}
Bermuda	1.23	1.30	0.95
Series 2 St. Lawrence	0.89	0.87	0.74
Series 2 Bath	1.00	1.00	1.00

Thus 'average growth rates' estimated from the leaf length-growth relationship are comparable in the physiological sense. This is shown also by the studies of Section II. I_s may be estimated from the G-$L_{5\%}$ relation by multiplying the estimate of G by 3.5 (the Series 2 Bath stand has 3.20 leaves per shoot, and the Series 2 St. Lawrence stand, 3.83); thus the relation between I_s and $L_{5\%}$ is given by

$$I_s = 0.735\ L_{5\%} - 3.22$$

where I_s is in mm/day and $L_{5\%}$ is in cm.

For some purposes it may be wished to know the production of leaf tissue per shoot; this is given by

$$P_s = I_s \times w \times c$$

where P_s is the production in mg leaf tissue/shoot per day, w is the average leaf width in cm and c is dry weight of epiphyte-free leaf tissue in mg/cm^2. c varies with the average leaf width, as given above. In the Barbados-Grenadine Islands region, narrow leaves were generally associated with low growth rates, and wide leaves with high growth rates (Fig. 13), so that in general, increasing leaf length indicated increasing production per shoot.

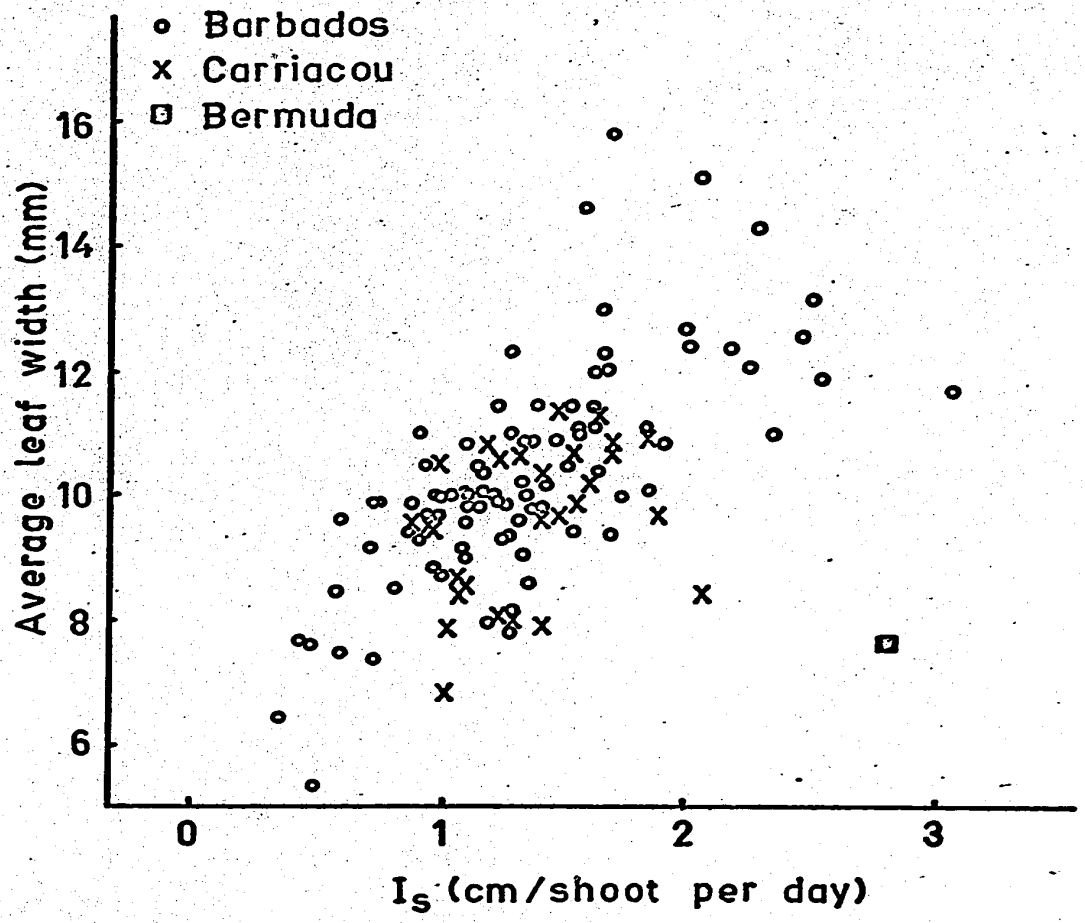

Fig. 13. Relation of average leaf width to observed or estimated leaf growth rate (I_s).

However, growth-width relations may be quite different elsewhere, as is shown by the position of the Bermuda sample in Fig. 13, and it is important to distinguish between variation in growth rate (I_s) and variation in P_s when factors limiting growth are being considered. Divisation of P_m by P_s gives a rough estimate of the number of shoots per square meter. For stand B-9, the number of shoots per square meter estimated by a count of shoots in a 1/8 m^2 area was 488, while the number estimated by dividing P_m by P_s was 463. It is generally difficult to count the shoots per unit area in the field, so this is a convenient way of obtaining this estimate.

Note concerning calculation of P_s for stand B-6

Zieman (MS, 1968) observed that leaves of young erect shoots near the rhizome apex are narrower and have somewhat lower growth rates than do leaves further removed from the rhizome apex. It was also noted in the present study that the narrowest leaves were never as long as the other leaves in a stand. Thus it appears that P_s of the youngest shoots is limited by physiological processes rather than by the supply of nutrients. When P_s estimates are used for purposes such as the yield-nutrient correlations of Section II, the P_s should refer only to nutrient limited production. In such instances it might be better to use the average width of the longest leaves rather than the average width of all leaves in calculating P_s. In most instances, the young shoots constitute a small and relatively constant proportion of the shoots in a stand, and thus this distinction would not be important. However, stand B-6 was unusual in that there was an unusually large number of shallow rooted young shoots, and this was reflected in a bimodal leaf width distribution (Fig. 14). Thus in calculating P_s of this stand, an average width of 10.5 mm, corresponding to the modal width of the longer leaves, was used in place of the true average width, 9.3 mm. The P_s given in brackets for stand

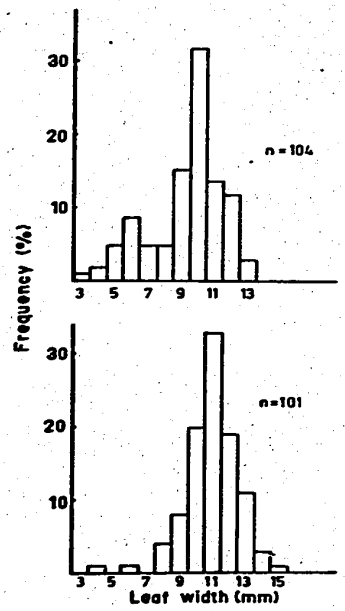

Fig. 14. Frequency distribution of leaf widths for stand B-6 (above). Frequency distribution of leaf widths for typical Thalassia stand is included for comparison.

B-6 in Table III (Section II), is the P_s based on the true average width.

General application of the Barbados leaf growth-leaf statistics relationships

According to the hypothesis stated in the Introduction to Appendix B, maximum leaf length is determined in part by the rate of infestation of leaves by calcareous epiphytes. Thus one might expect a significant difference in the growth rate-maximum leaf length relation between areas in which the rate of infestation by calcareous epiphytes varies significantly. The degree of infestation by calcareous epiphytes was significantly less at Bermuda than at Barbados (see comparison of dry wt/wet wt ratios above). However, the I_s of the Bermuda stand predicted from the Barbados I_s-$L_{5\%}$ relation, 2.7 mm/day, is in good agreement with the observed I_s, 2.8 cm/day. There did appear to be somewhat more "scollaping" of the leaves--Thomas et al. (1961) believe this to be due to grazing--than at Barbados. However the agreement may not be entirely coincidental. There must be some negative feedback between leaf length and growth rate; leaf length per se influences growth rate through photosynthesis, and growth rate influences leaf length. The same considerations apply to the limitation of leaf length in very shallow water. It is apparent that in depths of less than 0.2 to 0.3 m at Barbados leaf length per se is limited by depth of water (see p. 41 Section II, and p.188 Appendix B). The growth rate measurements for stand 1, Series 1 (Table X) indicate that the growth rate of these stands is correspondingly low. Differences in the lengths of other sea grasses, for example as reported for Zostera (Ostenfeld, 1908), Syringodium and Diplanthera (Phillips, 1960; Strawn, 1961) are probably also related to variations in growth rate. Even without knowledge of the quantitative relation of growth

rate to maximum leaf length, the maximum length is a useful and easily obtained statistic.

The P/SC ratio of the Bermuda stand, 0.0034, is close to the P/SC ratios of the Series 2 Barbados stands. In general I have observed that there tends to be poorer growth of calcareous epiphytes in calm water in comparison to turbulent water, and Land (1970) has observed this also. This would tend to result in a higher P/SC ratio in calm water conditions. On the other hand, leaves tend to remain attached longer under calm conditions, and this would tend to result in smaller P/SC ratios. While the relation between production and standing crop (wet wt including epiphytes) is theoretically complicated, it may be reasonably constant.

All observations at Barbados were made on Thalassia stands in depths of less than 2 m, and there was probably little variation in the amount of light reaching these stands. It might not be reliable to use the relations based on observations in shallow water for estimating production parameters of deeper water stands where the amount of light reaching the stands differs significantly. Jones (MS, 1968) found that oxygen production by Thalassia is influenced mainly by standing crop and the length of day with illumination greater than 20 ly/hr. Again, however, there must be some adaptation of standing crop to ambient light conditions, at least on a long term basis.

It may be possible to correlate growth of Thalassia with a leaf statistic that is uninfluenced by variation in environmental parameters, at least not on a proximate basis. A suggested statistic is the average length of the youngest leaves on a number of shoots; the original tips are almost invariably present on these leaves. If the growth rate patterns of all leaves on a shoot are similar, which seems likely, then there should be a high degree of correlation between growth rate and this statistic. If this

is so, then it would be possible to work out other production-leaf statistic relations in any area without having to make any growth rate measurements.

Age, growth rates and production of underground parts

The erect shoots of Thalassia have very distinct leaf scars (Plate Ib). Assuming a 15 day periodicity in leaf production, the age of an erect shoot can be estimated by counting the leaf scars and leaves on a shoot, and multiplying this number by 15 days. Tomlinson and Vargo (1966) observed that meristematic tissue is restricted to the rhizome apex, and concluded that growth of the rhizome and production of new erect shoots are restricted to the apical region of the rhizome. Hence an estimate of the growth rate of the rhizome may be obtained by dividing the length of the rhizome between two erect shoots by the difference in their ages.

Observations of the difference in ages between erect shoots on 10 rhizome fragments suggest that there is a periodicity in production of erect shoots. Of 21 pairs of adjacent erect shoots examined, 10 pairs differed in the number of leaf scars+leaves by 1, and 11 pairs differed by 2. Since leaf scars and leaves can be counted in only whole numbers, these observations suggest that the difference in age between adjacent erect shoots may be approximately constant and is some figure between 15 and 30 days. The average difference in number of leaf scars+leaves between adjacent erect shoots was 1.52, which is equivalent to a difference in age of 22.8 days. Estimates of the average difference in age of adjacent erect shoots (the frequency of production of new erect shoots) of 5 rhizome fragments with 5 or more erect shoots are given in Table XIV, together with the estimated average growth rates of the rhizomes. The estimates of the periodicity in production of erect shoots vary from 22.5 to 26.2 days with a mean of 24.7 days. The

Table XIV. Estimation of frequency of new shoot production and rhizome growth rate.

NO. ERECT SHOOTS ON RHIZOME FRAGMENT	LEAVES + LEAF SCARS ON YOUNGEST SHOOT	LEAVES + LEAF SCARS ON OLDEST SHOOT	DISTANCE BETWEEN YOUNGEST AND OLDEST SHOOT (cm)	ESTIMATED DIFFERENCE IN AGE BETWEEN SUCCESSIVE SHOOTS (days)	ESTIMATED AVG GROWTH RATE OF RHIZOME (mm/day)
8	15	26	45	29.6	2.7
7	11	21	36	25.0	2.4
5	7	13	28	22.5	3.1
5	7	14	26	26.3	2.5
5	19	26	31	26.3	3.0

estimated average growth rates vary between 2.4 and 3.1 mm/day. Intershoot distance does not vary much between plants, and rhizomes produce no dormant buds (Tomlinson and Vargo, 1966); if new erect shoots are produced at regular intervals, this implies that growth rates of rhizomes do not vary greatly. Several of the rhizome fragments and associated erect shoots examined included growing tips of the rhizome. The erect shoot next to the apex on these fragments (Plate Ic) had 6 or 7 leaves including scale leaves (similar to those on the rhizome) and intermediates between scale leaves and foliage leaves (these are described by Tomlinson and Vargo, 1966). The erect shoot next to the youngest differed in the number of leaf scars+leaves from the youngest by 1 or 2, as described above. Since the interval between production of new erect shoots is estimated as approximately 24 days, the first 6 or 7 leaves must be produced within this interval, and the 15 day periodicity is probably initiated subsequent to development of the 6th or 7th leaf. In aging an erect shoot, the first 7 leaf scars should thus be counted as 24 days. The age of the rhizome is the same as that of the attached erect shoot. Preliminary studies, made at 17 different Thalassia stands, indicate that except in very young stands it is common to find shoots at least $4\frac{1}{2}$ years of age, and not uncommon to find shoots 8 to 10 years of age. The oldest erect shoot observed was 10.5 years of age. There are large differences in the spacing of leaf scars (shoot growth rates), and these may reflect differences in sedimentary conditions. In an area where it was evident that accretion of sediment was occurring at a relatively rapid rate, the average distance between leaf scars was 3.2 mm, while in an adjacent area which appeared to be quite stable (well developed epifauna), the average distance between leaf scars was 0.30 mm.

Production of erect shoot and rhizome tissue may be estimated as

$$P_m = n \times G \times c$$

where P_m is the production (g dry wt shoot or rhizome tissue/m^2 per day), n is the number of erect shoots or rhizome growing tips per m^2, G is the average growth rate of the shoots or rhizomes (cm/day), and c is the dry wt (g/cm) of shoots or rhizomes. To determine c it may be necessary to remove the roots from the erect shoots and rhizomes because of the adherent sediment, and thus production of root tissue would not be included; this is probably small in comparison to production of erect shoot and rhizome tissue in any case. Dead leaves should be removed from the erect shoots for determination of c, and the length of both erect shoots and rhizomes should be determined in fresh condition. It is generally difficult to remove the underground parts from the substrate in intact condition. Average growth rates of the erect shoots and rhizomes may be estimated by determining the average internodal distance on fragments of erect shoots and the average intershoot distance on a number of rhizome fragments, and dividing by 15, 24 days. The number of rhizome growing tips may be estimated by digging up the substrate under a given surface area and counting the growing tips exposed and which float to the surface.

Because the erect shoot apices may lie well under the substrate surface, a significant amount of leaf tissue may be produced underground that is not included in the estimate of leaf tissue production above the substrate. This material is decomposed under the sediment surface, and thus should be included in estimates of underground production. Assuming one new leaf is produced on each erect shoot every 15 days, production of leaf tissue underground

may be estimated as

$$P_m = n \times w \times l \times c \quad - \quad 15 \text{ days}$$

where P_m is production (g dry wt leaf tissue/m^2 per day), n is the number of shoots per m^2, w is the average leaf width (cm), l is the average depth of the shoot apices below the substrate surface (cm) and c is the dry wt (g/cm^2) of epiphyte-free leaf tissue (Fig. 11).

Estimates of the underground production at a Barbados Thalassia stand and a Bermuda Thalassia stand are given along with the data for these estimates, and the estimates of the above ground production in Table XV. It can be seen that there may be significant production of leaf tissue underground; production of shoot and rhizome tissue was less than 1/7 of the total production in these stands.

Table XV. Underground production at two Thalassia stands.

	BARBADOS	BERMUDA
Erect shoots/m^2	488	1611
Avg distance between leaf scars	2.1 mm	1.5 mm
Avg growth rate of erect shoots	0.14 mm/day	0.10 mm/day
Dry wt of shoots	0.041 g/cm	0.032 g/cm
PRODUCTION OF ERECT SHOOT TISSUE	0.28 g/m^2 per day	0.52 g/m^2 per day
Rhizome growing tips/m^2	73	196
Avg intershoot distance	6.8 cm	5.2 cm
Avg growth rate of rhizomes	2.8 mm/day	2.2 mm/day
Dry wt of rhizomes	0.037 g/cm	0.035 g/cm
PRODUCTION OF RHIZOME TISSUE	0.76 g/m^2 per day	1.5 g/m^2 per day
Depth of shoot apices (range)	18 to 25 cm	4 to 10 cm
Assumed avg depth of shoot apices	21.5 cm	7 cm
Avg width of leaves	10.2 mm	7.3 mm
Dry wt of leaves	4.8 mg/cm^2	4.3 mg/cm^2
PRODUCTION OF LEAF TISSUE UNDERGROUND	3.4 g/m^2 per day	2.4 g/m^2 per day
TOTAL UNDERGROUND PRODUCTION	4.4 g/m^2 per day	4.4 g/m^2 per day
LEAF TISSUE PRODUCTION (above ground)	3.1 g/m^2 per day	14.1 g/m^2 per day

APPENDIX B. A GENERAL DESCRIPTION OF THE *THALASSIA* BEDS AND ADDITIONAL DATA ON PRODUCTION

REGIONAL SETTING

Barbados (Fig. 15) is a small island of non-volcanic origin lying in the trade wind belt and region of equatorial currents at 13°10' N, 59°30' W. Most of the island is covered by Pleistocene coral limestone; outcroppings of poorly indurated Tertiary sediments occur in the Scotland district in the NE section of the island. The coastline is regular, there are no offshore islands, and living reefs are limited to small fringing reefs on the west coast (Lewis, 1960a). Rubble banks, supporting only sparse coral growth, occur close to shore on the south coast and at a distance of approximately 0.7 km off the SE coast. Rainfall averages about 50 cm/yr, winds are predominantly from the eastern sector and mean annual wind speed is 11.0 m.p.h. (Rouse, 1966). Lewis (1960b) reported observations on tidal, water temperature and wave conditions at Barbados. The mean tidal range is approximately 0.7 m, and the diurnal range, 1.1 m. Surface temperatures of coastal water varied between 25.2 and 28.5°C over a one year period. Wave amplitudes are four to eight times greater on the east coast than on the west coast. Because Barbados lacks large lagoons and semiprotected bays, *Thalassia* beds there are not extensive. The largest beds occur at Bath and at St. Lawrence, where most of the nutrient studies were carried out. A general survey of the substrate types, sea grasses and associated flora and fauna in these beds was carried out prior to initiation of the nutrient studies.

Data on production and some sedimentary characteristics were also obtained from *Thalassia* beds at Carriacou. This is a small island of volcanic origin, lying approximately 200 km SW of Barbados (Fig. 15). *Thalassia* beds are more extensive at Carriacou than at Barbados, and can probably be

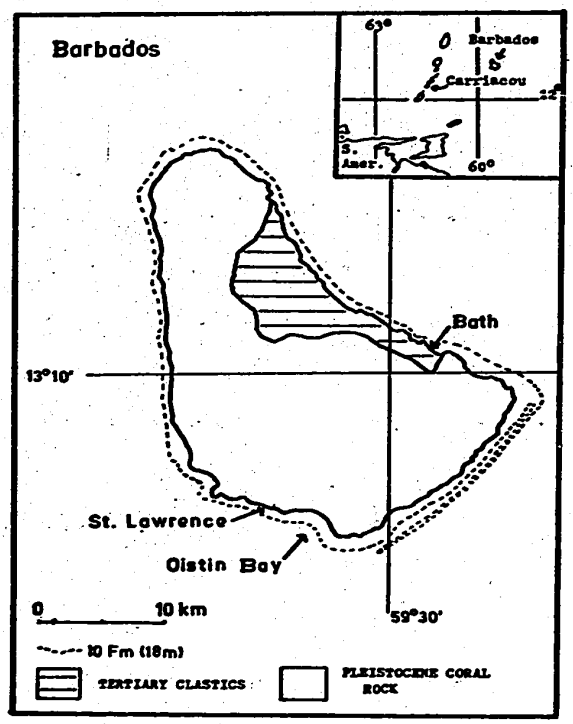

Fig. 15. Index map of Barbados.

considered representative of typical Thalassia growth in the southern Caribbean.

MATERIALS AND METHODS

For the purpose of mapping the Bath and St. Lawrence Thalassia beds, base maps were prepared from aerial photographs (Hunting Surveys Ltd., taken in 1964). A series of transects were swum out from positions on shore located on the base maps; orientation was maintained by lining up two targets on shore. Distances and depths along the transects were measured with a 2 m pole, and these were recorded on an underwater slate together with observations on the substrate type and flora and fauna. Observations of sea level were made at ½ hour intervals at fixed reference points during the periods of transect observations, and observations were adjusted to a common tidal level which is approximately mean low water (accurate tidal data for Barbados are lacking). Maps of the hydrography, distribution of sea grasses and bottom types were prepared by making use of details which could be distinguished in the aerial photographs together with data of the transect observations. Surveys of the macro-infauna, sea grasses and sediments were carried out by sampling 20 randomly located positions at Bath, and 27 positions at St. Lawrence. At each position, a 3/16 m^2 sample of the sea grasses was obtained. This was made up of cuttings from three separate 1/16 m^2 areas, positions of which were selected by throwing a quadrat within a total area of approximately 100 m^2. If the Thalassia stand was not reasonably uniform with respect to length of the leaves--this was the case for only one stand, at Bath--then the area was subjectively subdivided into uniform stands, and the subdivisions were sampled separately. To sample the macro-infauna, the substrate was dug up under a surface area of approximately 0.15 m^2, and immediately washed through a 2 mm mesh sieve. Sediment samples were taken

from all positions but only selected samples were analyzed. The proportions of coarse material (> 5.2 mm) in substrates which had been subjectively classified as predominantly sand, cobble-sand and Porites rubble flats were estimated by digging up a measured volume of substrate, sieving the sample through a 5.2 mm mesh sieve, and determining the volume of water displaced by the coarse material. This was done for several positions in each of the above substrates. The proportion of coarse material in substrates which had been subjectively classified as cobble framework or cobble-cobble-sand was estimated from visual observations at erosional scarps. Observations of Thalassia beds in Oistin Bay were made during the course of nutrient investigations.

A map of sea grass distribution at Carriacou was prepared by examination of aerial photographs (United Kingdom, Directorate of Overseas Surveys, Contract 85, taken in 1966). Approximate depths were taken from Admiralty Chart 2872, and 27 positions were selected for sampling such that Thalassia beds from various depths and degrees of exposure to wave action were represented. At each position, observations were made on flora and fauna, substrate type, and samples of sea grass and sediment were taken as at Barbados. Depths were measured by use of a 'venturi' type depth gauge.

For each of the 3/16 m^2 samples, the Thalassia and Syringodium leaves were separated, and then the wet weight of each determined. For the Thalassia leaves, the lengths of the 10 longest leaves and widths of 30+ leaves were measured. L_{10} was used as a measure of the maximum length (L_m), except in samples of few leaves in which the average length of the three longest leaves was used as a measure of maximum leaf length. P_s and P_m, and the number of erect shoots per m^2 were estimated from these data by the relations given in Appendix A.

Particle size analyses were made on 50 to 100 g splits of the sediment samples. The samples (or splits of) were oxidized in commercial bleach and washed several times with distilled water using centrifugation to remove water after each wash. Each sample was then shaken in distilled water and wet sieved through a 300 mesh (47 μ) sieve. Material passing through the sieve was flocculated by adding 5 ml saturated potassium alum solution, centrifuged to remove excess water, and then dried in a tarred beaker. The clay content of two samples was determined by pipette analysis (Day, 1965). Coarse material was dry sieved by means of a mechanical shaker through sieves of the following mesh sizes: 5.16 mm, 3.35 mm, 2.46 mm, 1.52 mm, 0.98 mm, 0.52 mm, 0.28 mm, 0.14 mm, 0.074 mm and 0.047 mm. Results were plotted as cumulative curves on probability paper using 'phi' units of grain size. From these curves the graphic mean (M_z) and inclusive graphic standard deviation (σ_I), measures of average size and sorting, were determined (Folk, 1965). M_z is reported in units of millimeters. σ_I values are reported according to the verbal classification scale of Folk (1965) which is as follows.

σ_I	
	under .35ϕ, very well sorted
	.35-.50ϕ, well sorted
	.50-.71ϕ, moderately well sorted
	.71-1.0ϕ, moderately sorted
	1.0-2.0ϕ, poorly sorted
	2.0-4.0ϕ, very poorly sorted
	over 4.0ϕ, extremely poorly sorted

The percentage of each sediment sample in standard (U.S. Dept. of Agriculture) size intervals was interpolated from the cumulative frequency-grain size plot. For selected samples, the percentage of acid insoluble material in

each of the coarse (> 47u) sieve separates was estimated as the percentage of grains in a sample of 300 to 500 grains which did not dissolve in hydrochloric acid (15% concentrated). The percentage of acid insoluble material in the silt and clay fraction (< 47u) was estimated from the loss of weight following treatment with hydrochloric acid. Values for the standard size intervals were interpolated from plots of percentage of size class acid insoluble versus size class median diameter (phi units). Observations were made on the constituent nature of both the acid soluble and acid insoluble fractions. For this purpose, and also for the purpose of examining ferrous sulfide formation in skeletal carbonate grains (Section II), selected size fractions were embedded in polyester resin, thin sectioned, and examined under a petrographic microscope. Mr. Noel James of the Geology Department, McGill University, prepared the thin sections, and aided in identification of sediment constituents.

RESULTS AND DISCUSSION

Substrate classification

On the basis of the proportion of coarse material in the substrates, Thalassia substrates at Barbados and Carriacou were classified into four types. A fifth substrate type was distinguished from the others because of its unique vertical position. The under 5.2 mm fraction of almost all sediments examined at Barbados and Carriacou contained less than 10% silt and clay (Table XVI). In the following descriptions, 'sandy sediment' refers to sediment of particle size diameters less than 5.2 mm, while 'cobble sized' refers to material coarser than 5.2 mm. The substrate types are characterized as follows.

Table XVI. Grain size characteristics of sediments.

NO.	SAMPLE SITE CHARACTERISTICS	% OF SAMPLE IN SIZE SEPARATES % OF SIZE SEPARATE ACID INSOLUBLE (in brackets) (GRAVEL) 5.2 -2.0 mm	VERY COARSE SAND 2.0 -1.0	COARSE SAND 1.0 -0.5	MEDIUM SAND 0.5 -0.25	FINE SAND 0.25 -0.10	VERY FINE SAND 0.10 -0.05	SILT & CLAY < 0.05	CLAY < 0.002	M_z (mm)	SORTING
	BATH										
1	PS substrate, 0.5m	0	0.9 (0)	1.1 (1)	3.2 (9)	78.8 (20)	13.7 (26)	2.3 (50)		0.13	well sorted
2	PS substrate, 0.3m	2.0	1.0	1.3	1.7	74.0	18.2	1.8		0.12	well sorted
3	PS substrate area, under blue-green mat, 0.5m	0	0	0.2	0.8	79.0	17.2	2.8		0.12	very well sorted
4	OS substrate, 0.7m	6.3	33.7	27.0	3.5	12.5	12.5	4.5		0.50	poorly sorted
5	OS substrate, 0.5m	5.0	6.0	7.0	6.0 (0)	49.0 (11)	25.0 (24)	8.0 (43)	3.2	0.18	poorly sorted
6	Adjacent to No. 5, but in grass-free area, 0.8m	10.0	37.0	33.0	6.2	12.0	1.8	0.01		0.78	poorly sorted
7	PF substrate, 0.2m	2.3	6.9	7.8	11.3	54.8	12.4	4.5		0.21	poorly sorted
8	OF substrate, 0.3m	5.5	11.0	14.0	9.5 (1)	26.0 (2)	19.0 (7)	15.0 (86)		0.20	very poorly sorted
9	OF substrate, 0.4m	0.9	2.5	12.6	22.0	46.0	12.9	3.1		0.21	poorly sorted
10	Adjacent to No. 9, but in grass-free area, 0.5m	28.5	37.5	25.6	3.1	3.9	1.3	0.1		1.45	poorly sorted

Table XVI -- Continued

NO.	SAMPLE SITE CHARACTERISTICS	% OF SAMPLE IN SIZE SEPARATES % OF SIZE SEPARATE ACID INSOLUBLE (in brackets) (GRAVEL) 5.2 -2.0	VERY COARSE SAND 2.0 -1.0	COARSE SAND 1.0 -0.5	MEDIUM SAND 0.5 -0.25	FINE SAND 0.25 -0.10	VERY FINE SAND 0.10 -0.05	SILT & CLAY 0.05	CLAY 0.002	M_z (mm)	SORTING
	BATH (continued)										
11	Transient sand on coral-coralline algal bottom seaward of *Thalassia* bed	2.9	29.6	73.3	0.2	0	0	0		0.91	well sorted
	SOUTH COAST - ST. LAWRENCE										
12	Upstream of *Thalassia* bed, grass-free area, 0.5m	0	0.4	4.5	6.4	82.7	6.0	0		0.16	moderately well sorted
13	Upstream part of *Thalassia* bed, in mixed *Thalassia-Syringodium* stand, 0.5m	0	0	0	0.4	77.6	20.8	1.2		0.12	very well sorted
14	As for No. 13, but at 0.9m	0	0	0	0.5	51.5	45.0	3.0		0.10	well sorted
15	Central part of bed, pure *Thalassia*, rich fauna, 0.6m	3.7	6.3	10.4	14.6 (0)	49.5 (1)	11.8 (2)	3.7 (24)		0.23	poorly sorted
16	As for No. 15, but at 0.9m	3.7	4.8	9.5	16.0	49.8	12.7	3.5		0.21	poorly sorted
17	Downstream part of bed, 1.6m, mixed *Thalassia-Syringodium*	1.6	1.8	3.9	9.7	56.0	23.7	3.3		0.14	moderately sorted
18	In *Diplanthera* growth inshore of *Thalassia* bed	0	1.4	3.2	6.6	70.3	18.5	0		0.14	moderately well sorted

Table XVI. Concluded.

NO.	SAMPLE SITE CHARACTERISTICS	% OF SAMPLE IN SIZE SEPARATES % OF SIZE SEPARATES ACID INSOLUBLE (in brackets) (GRAVEL) 5.2 -2.0 mm	VERY COARSE SAND 2.0 -1.0	COARSE SAND 1.0 -0.5	MEDIUM SAND 0.5 -0.25	FINE SAND 0.25 -0.10	VERY FINE SAND 0.10 -0.05	SILT & CLAY 0.05	CLAY 0.002	M_z (mm)	SORTING
	SOUTH COAST - OISTIN BAY										
19	Oistin Bay west (stand A-1), 1.9 m	1.9	2.6	3.3	10.2 (0)	72.0 (1)	9.3 (6)	0.7		0.17	moderately sorted
20	Oistin B. south (A-7), 0.7m	1.6	3.9	7.0	20.0	66.9	0.5	0.1		0.23	moderately sorted
	GARRIACOU (all in Thal. beds)										
21	Watering B., 3m, strong current	0.3	3.4	12.8	29.5	52.0	5.8	2.2		0.25	moderately sorted
22	Watering Bay, 1.2m, in lee of patch reef	0.7	4.4	12.2	14.7	22.5	26.5	19.0	3.2	0.19	poorly sorted
23	Jew Bay, 66m from shore, 3m	1.2 (10)	6.8 (30)	20.9 (24)	32.7 (27)	22.1 (49)	9.0 (60)	7.9 (56)		0.28	poorly sorted
24	Jew Bay, 330m offshore, 4m, strong current	0.5	2.7	6.8	14.0	64.7 (0)	5.6 (8)	5.7 (26)		0.20	moderately sorted
25	Jew Bay, 8m, strong current	9.3	21.2	30.5	24.0	11.7	1.5	1.8		0.66	poorly sorted
26	Grand Bay, 5m, weak current	2.5	12.5	21.0	22.5	27.5	9.7	4.3		0.33	poorly sorted
27	Ot. Bretache Bay, 4.5m	0.3	2.3	15.4	38.0	42.8	0.4	0.8		0.30	moderately sorted
28	L'Esterre B., OF substrate, 1.2m	5.6	10.7	1.7	42.0	30.1	1.7	8.2		0.36	poorly sorted
29	Hillsborough Bay, 4m	0.4	8.1	21.5	22.0	41.0	4.6	2.4		0.30	poorly sorted

Cobble framework (CF) substrates

These are substrates in which cobble sized material forms a 'structural framework' and sandy sediment fills in the spaces (Plate IIc). The cobble sized material occupies approximately 70% and greater of the sediment volume. Exposures at erosional scarps indicate that the Thalassia root layer is usually restricted to the upper 15 to 20 cm of these substrates. These substrates are very strongly bound together, and are sampled only with considerable difficulty.

Predominantly sand (PS) substrates

These are substrates in which cobble sized material occupies less than about 5% of the substrate volume. At Barbados, the PS substrate overlies a coral rock basement or a layer of densely packed cobble sized coral rubble referred to here as the 'rubble layer'. The transition between the PS substrate and the rubble layer is generally abrupt, and the Thalassia rhizomes do not penetrate the rubble layer. The root layer in PS substrates at Barbados generally extends from the bottom of the PS substrate layer, at about 10 cm to 1 m below the sediment surface, to within 30 to 2 cm of the substrate surface. Where the root layer is spread out, erect shoots of Thalassia may be very long and largely unbranched (Plate IId). At most areas in Carriacou, the PS substrate is of undetermined thickness (but over 1 m), and the Thalassia root layer occurs within the top 75 cm of the PS substrate layer, and commonly within the top 20 cm.

Cobble-sand (CS) substrates

In these substrates, cobble sized material occupies approximately 5 to 45% of the substrate volume. At Barbados, CS substrates overlie a coral rock basement or rubble layer, as described for the PS substrates.

Cobble-Cobble-sand (CCS) substrates

This substrate type is intermediate between the CF and CS substrate types, with cobble sized material occupying approximately 50 to 70% of the substrate volume. This substrate type was encountered at only one position, in Oistin Bay. Here, a layer of sand overlies the CCS substrate, and a rubble layer occurs below the CCS substrate layer. Thalassia rhizomes are restricted to the CCS substrate layer.

Porites rubble flats (PF)

This substrate type occurs at Bath. In several near-shore areas at Bath, converging waves have caused piling up of skeletons of the coral Porites furcata to approximately 10 cm below mean low water. These areas are exposed at low water of spring tides. The skeletons of Porites furcata are irregularly branched cylindrical structures about 1 cm in diameter. The skeletons form a structural framework, and sandy sediment fills in the spaces. An estimated 50% of the substrate volume is occupied by Porites skeletons. Thalassia rhizomes are restricted to the upper 15 cm of these substrates.

The Bath Thalassia bed

General hydrography

The generalized bathymetry and distribution of sea grasses are shown in Fig. 16. The Thalassia bed lies partially in the lee of large rocks, the 'breaker zone rocks', which rise close to and above mean low water. Shallow areas bound the NW and SE regions of the bed. Because of the spaces between shallow areas seaward of the Thalassia bed, the shallowness of the Thalassia bed, and the generally high level of wave action of the east coast of Barbados, conditions in the Thalassia bed are generally turbulent and the

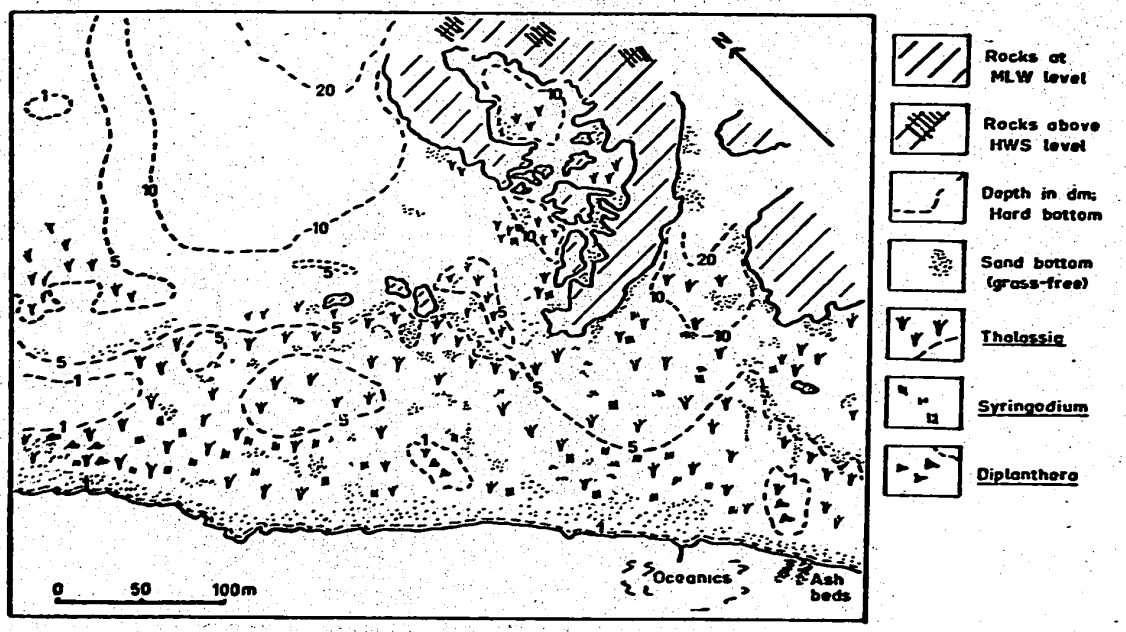

Fig. 16. Generalized bathymetry, and distribution of sea grasses at Bath.

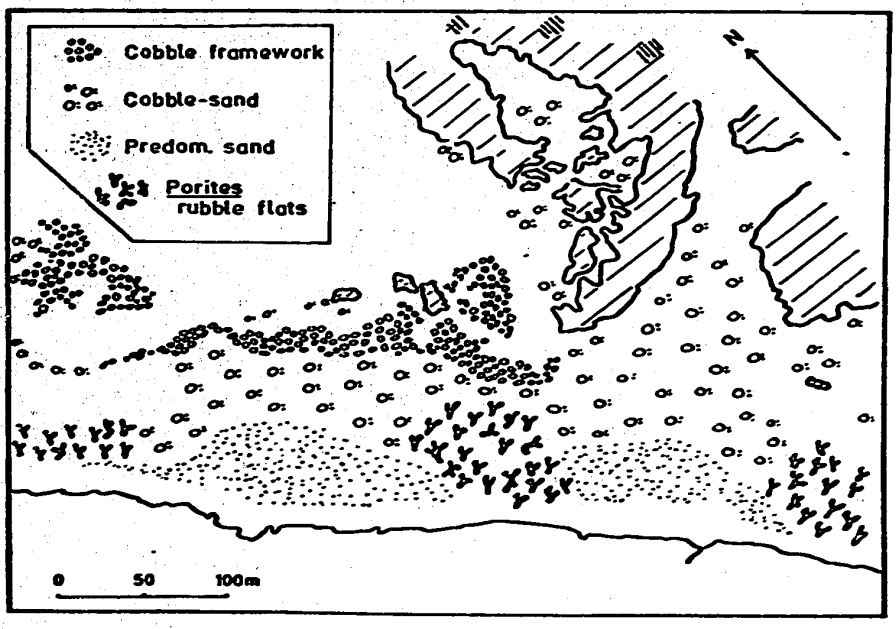

Fig. 17. Distribution of substrate types in sea grass beds at Bath.

water turbid from stirred up bottom sediment, except for a few hours at low water. Currents over the Thalassia bed are generally weak and irregular, but there is an overall flow of water towards the NW and currents are strong in channels cutting through the shallow areas at the NW and SE boundaries of the bed. Seaward of the Thalassia bed is a coral-coralline algal bottom with only transient sand cover.

Substrate types

The distribution of substrate types in the Thalassia bed is shown in Fig. 17. A CF substrate occurs at the seaward face of the Thalassia bed where wave action has caused piling up of coarse debris originating from the coral-coralline algal bottom. This material is piled up to about 25 cm below mean low water. The coarse debris consists largely of 'algal balls' 5 to 15 cm in diameter which were formed by growth of encrusting coralline algae around loose coral fragments; these algal balls were observed rolling about in pockets in the coral-coralline algal bottom. Plate IIc is a photograph of an erosional scarp in the CF substrate area. Skeletons of Porites furcata are piled up at several nearshore areas (Fig. 17) as described above. The upper limit of accumulation of the Porites skeletons is probably determined by factors limiting the growth of Thalassia, i.e. tidal level. In the shallowest areas of these flats, growths of Diplanthera occur, and associated with the Diplanthera is an accumulation of sandy sediment to about MLW level. PS substrates occur in inshore areas between the Porites rubble flats. CS substrates occur over most of the Thalassia bed area.

Sediment size and constituent characteristics

Grain size characteristics and the proportion of acid insoluble material of samples from the different substrates are given in Table XVI. There is

some variation in the relative amounts of coarse, medium and fine sand, but all samples are characterized by small amounts of silt and clay. Samples from grass-free areas differ from samples from the Thalassia stabilized sediments in having much smaller proportions of grains smaller than 0.5 mm; this illustrates the effect of Thalassia in modifying sorting of sediments by waves. The good sorting and coarse grain size of sample 11 (Table XVI) from the coral-coralline algal bottom is indicative of the strong wave action in that area.

Sediments at Bath are derived from several sources, and this is reflected in the constituent composition. Skeletal carbonates constitute predominant class of sediment constituents, making up approximately 75 to 92% (equivalent to the acid soluble fraction) of the sediments. These are derived largely from molluscs, corals, Halimeda (green alga), foraminifera and red algae growing in the Thalassia beds and on the hard coral-coralline algal bottom. Minor amounts of debris derived from echinoderms, alcyonarians, crustacea and ostracods were recognized in examination of the sediments. A few composite grains, probably derived from the Pleistocene coral cap or rocks of the breaker zone, were also observed. The predominant minerals of the acid insoluble fractions of these samples are quartz, feldspar and hornblende, in that order. These may have been derived in part from outcroppings of volcanic ash beds in this area (Fig. 16). Radiolarian tests, derived from Tertiary Oceanic deposits on shore (Fig. 16) were also observed in the acid insoluble fraction. Soil erosion, which is severe in this part of the island, probably contributes silt and clay sized material to the Bath sediments.

Substrate stability

Sediments are stabilized by growth of Thalassia, as was pointed out by Ginsburg and Lowenstam (1958), both through the binding effect of rhizomes,

and through the slowing down of water motions at the sediment surface associated with the presence of leaves. In addition, growths of sessile organisms of all sorts (see 'Epifauna and flora' below) help stabilize the sediment surface, and in a Thalassia stand with a well developed epifauna and flora, there is very little disturbance of the sediment surface even under conditions of strong wave action. However, once rhizomes are exposed, then erosion, directed horizontally from the place of exposure, may take place fairly rapidly and under conditions of only moderate wave action. Grass-free, depressed areas or 'blowouts' similar to those described by Hoskin (1963) occur throughout the PS and CS substrate areas at Bath. Hoskin (1963) noted that the steep, seaward edges of these depressed areas expose a well developed root system of Thalassia (see Plate IId, this thesis), and he believed that the blowouts are produced by wave erosion during storms. This may be so, but it is also apparent that once formed, erosion at the seaward face (erosional scarp) of the blowout may continue for some time. Measurements of erosion at two such areas at Bath were carried out over a one year period; the seaward faces of the grass-free areas were eroded 1.2 and 1.6 m during this period and rates of erosion did not vary much from month to month. At the same time as erosion took place at the seaward face of the grass-free areas, Syringodium advanced into the leeward regions, restabilizing the sediments. Irregularly oriented erosional scarps and depressed grass-free areas also occur in the CF and PF substrate areas. Erosion in these areas is probably slower. The Bath Thalassia bed thus appears to be subject to continuous erosion-succession processes, erosion occurring in some areas, and growth of Syringodium and subsequent development of Thalassia and associated epifauna and flora in other areas. Emery et al. (1957) remarked that graded bedding would be expected in shallow lagoon areas subject to

continuous erosion and deposition of sediments by tidal currents. It is probable that the occurrence of a rubble layer below the CS and PS substrates at Bath is a result of recurrent erosion-succession processes.

Marine angiosperms

Diplanthera is restricted in occurrence to the shallowest parts of the Porites rubble flats, and occasional narrow bands at the landward border of Thalassia and Syringodium growth. Syringodium occurs in the PS and CS substrates, mainly in mixed stands with Thalassia; pure stands occur bordering the grass-free depressed areas, and Syringodium rhizomes can be observed growing into these areas. Thalassia occurs in pure stands in the CF substrates, mainly in pure stands in the Porites rubble flats (mixed with Diplanthera in some of the shallower areas), in pure and mixed (with Syringodium) stands in the CS substrates, and in mixed stands only in the PS substrates. In the absence of recurrent erosion processes at Bath, Syringodium would probably be completely replaced by Thalassia.

Epifauna and flora

A rich epifauna and flora occurs in the Bath Thalassia bed. The most abundant and conspicuous organisms are (substrate types in brackets): the corals Porites furcata and Siderastrea radians (CS); a sponge Anthosigmella varians ? (PF,CF,CS); the anemone Homostichanthus duerdeni (PS,CS); the sabellid polychaete Branchiomma nigromaculata (PF,PS,CS); the Queen conch Strombus gigas (PS,CS) and a number of small gastropods such as Columbella mercatoria and Smaragdia viridis viridemaris which feed on Thalassia or its epiphytes; hermit crabs Calcinus tubicen (all substrates) and Clibanarius tricolor (PF), a number of spider crabs including Pitho aculeata and Microphyrs bicornutus (all substrates); the spiny lobster Panulirus argus

(PF,CS,PS); the sea urchin Tripneustes esculentus (PS,CS); the large ophiuran Ophiocoma echinata; the green algae Caulerpa spp. (CS,CF,PS), Avrainvillea nigricans (CS,CF), Avrainvillea rawsonii (CF,CS), Udotea spp. (CS,PS), and Halimeda opuntia (CF,CS); red algae Amphiroa spp.; and a number of blue-green algae. Blue-green algae (Microcoleus sp?) form extensive mats in some of the PS and CS substrate areas, and appear to trap fine sand (sample no. 3, Table XVI) as described by Sharp (1969). Blue-green algal growths were examined for the presence of heterocystous (N_2-fixing; Stewart, 1966) forms, but none were observed. In some CS substrate areas, Halimeda opuntia, Porites furcata and Avrainvillea rawsonii have completely overgrown the substrate, causing a reduction in the number of Thalassia shoots. Growths of Avrainvillea rawsonii in the CF substrate areas have the same effect (Plate IIb and Table XVII below).

Infauna

With the exception of a maldanid polychaete and a jawfish, infaunal organisms are very sparsely distributed at Bath. Tubes of about 5 cm length of a small maldanid polychaete (Clymenella torquata ?) number in the thousands per square meter in some PS and CS substrate areas. They are most common in areas of Syringodium growth where the sediment consists predominantly of fine sand (such as for sample 1, Table XVI). Burrows of a jawfish (probably Opistognathus aurifrons) number several per square meter in a few restricted CS substrate areas of rather sparse Thalassia growth. With the exception of these organisms, which may cause some overturning of the top 10 cm of sediment, there was no evidence of significant overturning of the Bath sediments by infaunal organisms. No bivalves other than the occasional Atrina seminuda were observed. A terrebellid was common on the undersides of algal balls in the surface layer of CF substrates. Other than these

organisms, in twenty-one 0.15 m^2 infaunal samples, only 4 polychaetes, 3 sipunculoids, 5 enteropneusts (Ptychodera bahamensis), and 1 callianasid were collected.

The St. Lawrence Thalassia bed

General hydrography

The general hydrography and distribution of sea grasses at St. Lawrence is shown in Fig. 18. A rubble reef borders the shore at distances of 70 to 170 m from shore. Sand covers the bottom in the lee of the reef, and the sea grasses Thalassia, Syringodium and Diplanthera are all common in the leeward area. The 'Thalassia bed' refers to the largest, central sea grass bed in Fig. 18. Wave action over the leeward area is generally gentle, but at high tide is usually sufficient to cause stirring up of the sediment surface. A continual current flows westward over the leeward area, presumably resulting from the easterly component in wave approach on this coast; velocities of 4.7 and 10.7 cm/sec were observed at low and high tide on a day of moderate sea conditions.

Substrate types

Thalassia grows in a CF substrate at the inner borders of the rubble reef. The cobble framework is made up of flattened and rounded coral debris. PS substrates occur over the entire leeward area. A rubble layer occurs at a depth of 10 cm to 1 m or more below the substrate surface.

Sediment size and constituent characteristics

Grain size characteristics of sediment samples from St. Lawrence are given in Table XVI. All samples are characterized by a predominance of fine sand sized sediment, and less than 5% silt and clay. South coast sediments

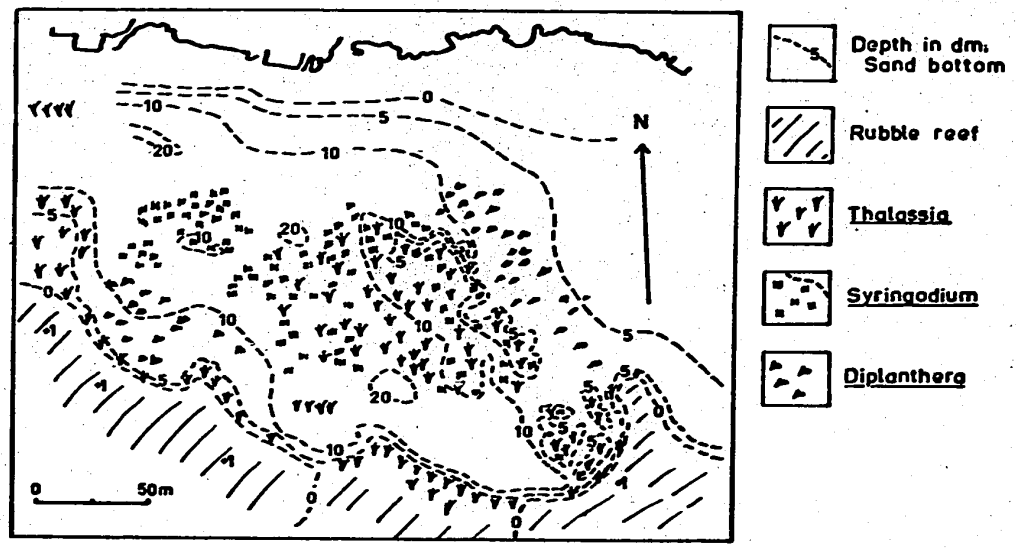

Fig. 18. Generalized bathymetry and distribution of sea grasses at St. Lawrence.

contain very little (less than 2%) non-carbonate material. The carbonate fraction of St. Lawrence sediments is similar in constituent composition to that described for Bath.

Substrate stability

Noticeable changes in substrate level in the grass-free sandy areas over periods of several weeks indicate significant motion of the sediment in these areas. Considerations based on settling velocity, threshold velocity and roughness velocity indicate that particles of about 0.18 mm in diameter require the least disturbance to be moved in comparison to both larger and smaller particles (Inman, 1949). The St. Lawrence sediments are in general well sorted and have median diameters close to this value. Thus even though wave action is not particularly strong in this area, the sediments are easily moved. There was little change in substrate level noted within the Thalassia bed, but, at high tide, there was usually noticeable disturbance of the surface sediments in most areas where the surface was not stabilized by blue-green algae or other organisms. Erosional scarps border much of the leeward margin of the bed, and large changes in the limits of the bed occurred subsequent to mapping of the Thalassia bed in July, 1968, erosion occuring in some areas, and extension of Syringodium into grass-free areas elsewhere.

Marine angiosperms

Thalassia occurs in pure stands at the leeward edge of the rubble reef, and in pure stands and mixed Thalassia-Syringodium stands in PS substrates. Syringodium occurs in pure stands and mixed stands. Diplanthera is restricted to small pure stands outside the main Thalassia bed. The substrate in areas where Diplanthera grows is noticeably unstable, and Diplanthera is

alternately covered by sand (including leaves) and then exposed (including rhizomes) with no apparent ill effect. Recurrent erosion of Thalassia-Syringodium stands apparently favors maintenance of Syringodium at St. Lawrence, and shallower areas, because of the instability of the substrates, are suitable only for Diplanthera.

Epifauna and flora

The epifauna and flora are best developed in the pure Thalassia stands in the central part of the Thalassia bed. In other areas the sediment surface is largely bare. The most abundant and conspicuous elements of the epifauna and flora are the corals Porites furcata and Siderastrea radians; sponges (Haliclona spp. and others); the anemone Homostichanthus duerdeni, the sea urchin Tripneustes esculentus, the ophiuran Ophiothrix orstedii, the queen conch Strombus gigas and small gastropods as at Bath; spider crabs as at Bath; the bivalve Atrina seminuda and the ascidian Microsomus helleri are are partially buried in the sediment; blue-green algal mats as at Bath; and several species of Caulerpa are common. In some areas, sponges have overgrown the substrate, apparently 'choking out' Thalassia (Table XVII, below).

Infauna

Macro-infauna are abundant in comparison with the infauna at Bath, but not abundant in comparison with temperate water infauna. Infaunal organisms are more or less uniformly distributed in the Thalassia bed, and are limited largely to the top 10 cm of substrate. The more abundant organisms, and the approximate numbers per square meter are: bivalves Codakia orbiculata (4.4), C. pectinella (26), C. orbicularis (15), Chione pygmaea (8.5); gastropods Bulla striata (1.5), Olivella nivea (2.3) and Jaspidella jaspidea (3.6); the the sipunculoid Siphonosoma cumanense (5.1); the holothurian

Thyoneria cognata (3.9); the enteropneust Ptychodera bahamensis (4.4); polychaetes all species (9.1) of which the most abundant are Glycera sp. (1.5) and two Capitellid spp. (2.4).

The Oistin Bay Thalassia beds

Two Thalassia beds at Oistin Bay were sampled in the nutrient studies. There are no offshore reefs in Oistin Bay, and the area is subject to strong wave action. Thalassia is the only sea grass occurring in Oistin Bay. Stands A-1 and B-2 are adjacent stands in a small patch of Thalassia close to shore at the western extremity of Oistin Bay. Stand A-7 is in a small, near-shore patch of Thalassia at the southern extremity of Oistin Bay. The CCS substrate at the former position is described above under 'Substrate classification'. The substrate at the Oistin Bay S position is a PS substrate overlying a coral rock basement. At both areas the substrate surface is disturbed by wave action, and is devoid of attached epifauna and flora. The infaunal populations are similar to that described for St. Lawrence. Sediments (samples 19, 20, Table XVI) consist predominantly of fine sand sized skeletal carbonates.

The Carriacou Thalassia beds

The distribution of sea grasses at Carriacou is shown in Fig. 19. On the east, windward coast of Carriacou, a N-S oriented reef lies at distances of 750 to 1850 m offshore. The lagoonal areas behind the reef have a maximum depth of about 14 m. An almost continuous Thalassia (Thalassia-Syringodium) bed fringes the shore from the northern part of Watering Bay to the southern edge of Grand Bay, extending seaward 200 to 300 m. Thalassia beds are irregularly distributed through the remainder of the lagoonal area. Some of these areas are subject to strong tidal currents. Thalassia beds on

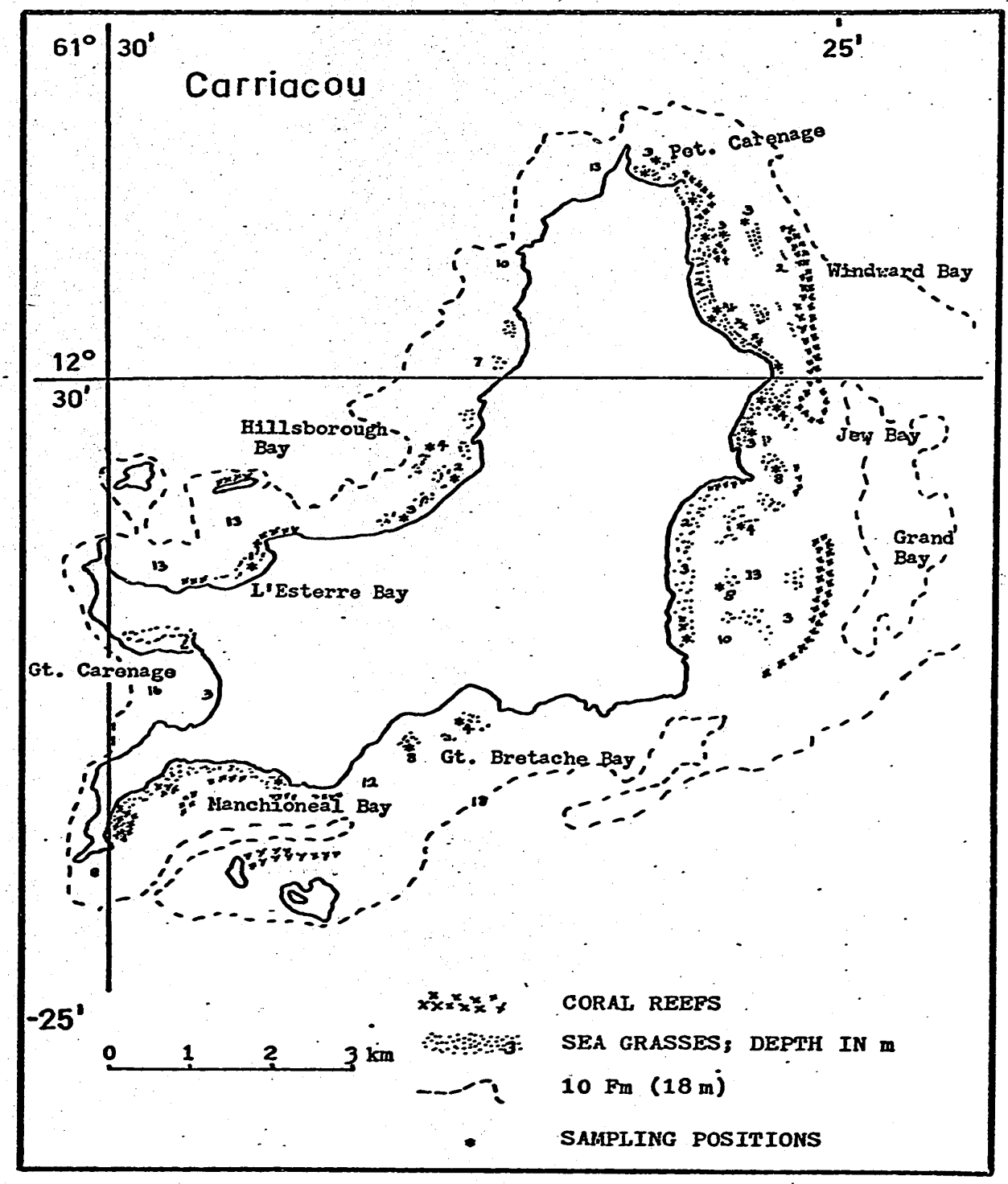

Fig. 19. Distribution of sea grasses at Carriacou.

other coasts are less extensive than on the east coast. Diplanthera is not common at Carriacou, occurring in a few shallow near-shore areas, and in some areas of Grand Bay at about 6 m depth. Halophila baillonis occurs in Thalassia-Syringodium stands in some of the deeper beds. Almost all beds at depths greater than 6 m are mixed Thalassia-Syringodium beds; Moore (1963) also noted that deep beds are usually mixed Thalassia-Syringodium. Substrates are most commonly the PS type, with less than 1% cobble sized material. CS substrates occur in some patch reef-Thalassia complexes. CF substrates occur in L'Esterre Bay, an area generally subject to turbulent conditions, and a CF substrate also occurs at a near-shore position in Hillsboro Bay. Sediment size characteristics (Table XVI) are similar to those for Barbados Thalassia sediments, with a predominance of sand sized material, and generally small amounts of silt and clay. Except in immediate near-shore areas where a large proportion of the sediments consists of non-carbonate material (Sample no. 23, Table XVI), the sediments consist predominantly of skeletal carbonates (sample no. 24, Table XVI). The epifauna and flora include many of the organisms observed at Barbados, such as Porites furcata, Siderastrea radians, Haliclona spp., Strombus gigas, Atrina seminuda, Tripneustes esculentus, various spider crabs and small gastropods, Caulerpa spp., Avrainvillea nigricans, Halimeda opuntia, Udotea spp., Amphiroa spp. and blue-green algae. In addition, organisms characteristic of Thalassia beds elsewhere in the Caribbean but not observed in Barbados Thalassia beds, occur in the Carriacou Thalassia beds. These include the coral Manicina areolata, the loggerhead sponge Spheciospongia vesparia, the basket star Oreaster reticulatus, many sponges and alcyonarians, and the green algae Halimeda simulans, Halimeda incrassata, and Penicillus dumetosus. Infaunal organisms were in general surprisingly sparse, with only the tube-dwelling

polychaete Onuphis erimata being commonly observed. In a few shallow water areas there was some evidence of overturning of Thalassia sediments by Callianassa, but there was little evidence of biogenic overturning of Thalassia sediments elsewhere.

Production data

Production data from surveys of Thalassia beds at Bath, St. Lawrence and Carriacou are given in summarized form in Table XVII. Except where stated, the following remarks are based on these data.

1. At depths less than 0.2 to 0.3 m below mean low water at Barbados, leaf length, and thus growth rate and P_s are limited by depth of water (see remarks, p. 41, 156).
2. The mean values of L_m, P_s, P_m and the no. shoots/m^2 of PS and CS substrate stands at Bath, St. Lawrence and Carriacou are remarkably similar.
3. Examination of the original data for individual Thalassia stands at Carriacou revealed no significant trends of change in production parameters with depth. However, data were obtained for only 7 stands in the depth range 4 to 9 m. Further, it may not be reliable to use the relations of Appendix A to estimate production of stands at depths much greater than 2 m.
4. P_s of CF substrate stands is, on the whole, significantly higher than P_s of CS and PS substrate stands. However, the maximum values on each substrate type are approximately the same. The maximum P_s observed for a CF stand was 17.5 mg/shoot per day (Stand A-2, Table II), while the maximum P_s for a PS substrate was 17.0 mg/shoot per day (Series 2 St. Lawrence stand, Table XI). These values are similar to those observed for the CCS substrate in Oistin Bay (Table III),

Table XVII. Production data of Barbados and Carriacou *Thalassia* beds.

SAMPLE GROUP CHARACTERISTICS	ALL STANDS							MAXIMUM STANDING CROP			THALASSIA STANDS ONLY				
		L_m			P_g			*Thalassia*	*Syring.*	*Thalassia* + *Syring.*		P_m		SHOOTS	
	n	$\bar{x}$	SD	RANGE	$\bar{x}$	SD	RANGE				n	$\bar{x}$	SD	RANGE	$\bar{x}$	SD	RANGE
		(cm)			(mg/shoot per day)			(g wet wt/m^2)				(g/m^2 per day)			(no./m^2)		
1. St. Lawrence																	
PS substrate	25	21.9	3.2	17.0-27.0	6.8	1.9	4.2-12.6	1510[P]	1570[P]	1822	5	4.1		1.7-5.6	650		540-800
Overgrown by sponges	1	26.3			9.2			407[P]			1	1.5			160		
2. Bath																	
CS substrate 0.5m+	10	21.3	4.6	14.3-29.3	6.5	2.7	3.2-11.4	1360[P]	815[M]	1600	5	3.2		2.6-5.0	550		470-680
CS substrate 0.25m	2	21.6		21.0-22.1	4.5		4.4-4.6	468[M]	1358[M]	1660	0						
CS substrate 0.25m stunted growth	1	10.2			1.5			272[M]	60[M]	332							
PS substrate 0.25m	1	18.4			3.6			330[M]	468[M]	800							
PP substrate 0.2m+	2	10.8		9.3-12.3	1.7		1.0-2.3	540[P]			2	1.0		0.7-1.3	800		300-1300

Table XVII. Concluded.

SAMPLE GROUP CHARACTERISTICS	ALL STANDS n	L_m $\bar{x}$ (cm)	L_m SD	L_m RANGE	P_s $\bar{x}$ (mg/shoot per day)	P_s SD	P_s RANGE	MAXIMUM STANDING CROP Thalassia (g wet wt/m²)	Syring. (g wet wt/m²)	Thalassia + Syring. (g wet wt/m²)	THALASSIA STANDS ONLY n	P_m $\bar{x}$ (g/m² per day)	P_m SD	P_m RANGE	SHOOTS $\bar{x}$ (no./m²)	SHOOTS SD	SHOOTS RANGE
2. Bath (continued)																	
CF substrate 0.4m	3	27.9		25.0-31.7	10.5		8.8-12.5	1207[P]			3	3.6		3.0-4.5	340		290-360
CF substrate 0.3m	2	21.2		19.7-22.6	5.4		5.3-5.4	1177[P]			2	2.8		1.1-4.4	520		210-820
CF substrate 0.25m (overgrown by Avrainvillea)	1	16.7			4.3						1	0.16			97		
3. Carriacou																	
PS substrate	22	22.5	4.1	16.5-32.3	5.9	1.9	3.0-8.9	1615[P]	1066[M]	1700	10	3.8	1.6	2.0-6.0	650	245	310-930
OS substrate	4	20.4		18.0-23.7	5.9		4.7-7.2	1238[P]			4	2.3		0.8-4.6	450		170-640
CF substrate	2	29.3		29.1-29.5	10.6		9.9-11.3	1811[P]			2	5.6		4.4-6.7	540		390-680

[P]Pure stand, [M]Mixed stand

18.2 and 15.4 mg/shoot per day.

5. Routine observations were not made of the thickness of the root layer in the surveys represented in Table XVII. However, examination of a number of areas in which long erect shoots occurred, and of a number of CS and PS substrate areas in which long leaves occurred, indicate that high P_s in PS and CS substrates is invariably associated with long erect shoots, and vice versa, that long erect shoots invariably have high P_s. Similar observations indicate that P_s in the range 3 to 4 mg/shoot per day in PS and CS substrate stands is associated with shallow, presumably partially aerated (see p. 62) root layers. 'Stunted' Thalassia growths, i.e. stands with very short leaves and low P_s (less than about 3 mg/shoot per day) not in depths less than 0.3 m and for which the nutrient studies indicate growth is not limited by availability of nitrogen, are unusual at Barbados, and were not observed at Carriacou. The question of what limits growth at these particular stands is the question which led to the approach used in this study for investigating the origin of nitrogen and phosphorus for growth of Thalassia. It remains unanswered.

6. There is a roughly inverse relation between standing crop of Thalassia and standing crop of Syringodium. This is shown by the plot of the standing crop of Thalassia versus standing crop of Syringodium at St. Lawrence (Fig. 20), and is also suggested by the similar maximum standing crops of Thalassia and Syringodium on PS and CS substrates (Table XVII). It is tempting to suggest that this indicates similar P/SC ratios for Thalassia and Syringodium. However, it is not clear why the maximum P_m observed for the Barbados-Carriacou Thalassia stands, 7.6 g/m^2 per day (Stand A-1, Table II), should be

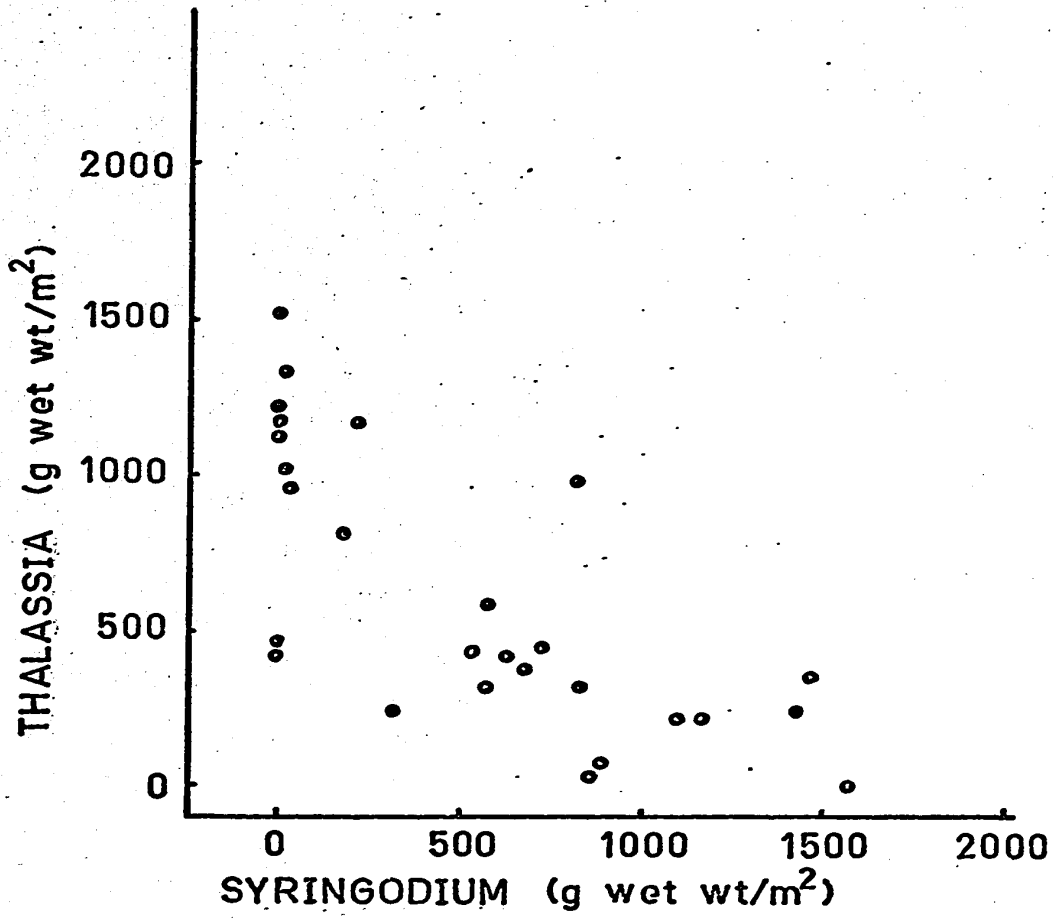

Fig. 20. Relation of standing crop of Thalassia to standing crop of Syringodium at St. Lawrence.

only one-half the P_m, 14.1 g/m^2 per day, of the Bermuda stand.