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ECOLOGY OF CHEMICAL DEFENSES OF ALGAE AGAINST THE HERBIVOROUS SNAIL, LITTORINA LITTOREA, IN THE NEW ENGLAND ROCKY INTERTIDAL COMMUNITY

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ABSTRACT

In the New England rocky intertidal community, space is dominated by two perennial plant types, brown fucoid algae (Ascophyllum nodosum and several species of Fucus) in the mid zones and the red alga Chondrus crispus in the low zones. These algae are not grazed by the predominant herbivorous snail, Littorina littorea. Here I report the first direct evidence that these algae produce chemicals which inhibit feeding by the snails.

Polyphenols in Fucus vesiculosus and Ascophyllum nodosum were shown to be effective chemical defenses against the snails. Feeding experiments demonstrated that the presence in the diet of as little as 1% polyphenol (dry weight), extracted from these two algal species, caused a significant reduction in feeding by L. littorea; 10% polyphenol (dry wt.) in food media inhibited snail feeding nearly 100%. The phenol and polyphenol contents in different tissues of these two algal species and

in other New England rocky intertidal algal species were monitored monthly for one year. F. vesiculosus and A. nodosum showed highest polyphenol contents (1-17% dry wt.); these levels were sufficiently high in all tissues during all months to inhibit snail feeding. The mechanism of action of plant polyphenols against herbivores is through their binding to plant proteins and other nitrogenous compounds, rendering them indigestible. Polyphenol contents were therefore examined in relation to plant nitrogen contents (using polyphenol/nitrogen ratios) to estimate the unavailability of plant nitrogen to herbivores due to polyphenol binding.

Annual brown algal species such as Petalonia fascia and Scytosiphon lomentaria had significantly lower levels of phenols and polyphenols than the perennial F. vesiculosus and A. nodosum. These two species are highly preferred as food by L. littorea. C. crispus and the green alga Codium fragile also had low phenol and polyphenol levels, yet they are of low food preference to the snails. Methylene chloride extracts from C. crispus and volatile halocompounds from C. fragile inhibited snail feeding, hence these species have chemical defenses quite different from those of F. vesiculosus and A. nodosum. Factors such as physical defenses, nutritional content, and temporal and spatial escapes are also important in determining algal food preference to herbivores .

The release into seawater of volatile hydrocarbons and halomethanes from benthic algae and seagrass was measured to examine the possible role of these compounds as antiherbivore compounds. Bioassays indicated that CH_2I_2, a compound released into seawater from C. fragile, inhibited

feeding of L. littorea. $CHBr_3$, released into seawater by many algal species, appeared to have less activity against the herbivores.

This study represents one of the first examinations of plant chemical defenses against herbivores in the marine environment. The findings are discussed in relation to recent theories from terrestrial studies on the commitment of plants to chemical defense.

Name and Title of Thesis Supervisor: John M. Teal, Senior Scientist

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Mike Coen drafted all figures. Final manuscript preparation was facilitated by the cheerful and efficient help of the Biology Department secretaries (and the Wang Word Processor, which allowed me to type most of this thesis myself!).

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PREFACE

This thesis is composed of a general introduction and five chapters dealing with the ecology of algal chemical defenses against a marine herbivore. Chapters are written as discrete papers, each with introduction, methods, results, discussion and conclusion.

The general introduction to the thesis reviews the literature on plant chemical defenses in terrestrial and marine environments. It has been well documented in terrestrial environments that plants produce many diverse chemicals that deter feeding by invertebrate and vertebrate herbivores. Although many unusual natural products have been isolated from marine algae, and some have been shown toxic to bacteria, phytoplankton, fungi, fish, and insects, few have been tested for an antifeeding function against marine herbivores found in the environment of the alga from which they were extracted. The marine algae and periwinkles of the New England rocky intertidal community are suggested to comprise a suitable system in which to investigate the possible existence and importance of algal chemical defenses against herbivores.

Chapter 1 documents the first evidence for chemical defenses in several species of marine algae (Fucus vesiculosus, Ascophyllum nodosum, Chondrus crispus, and Codium fragile) against herbivores (Littorina littorea) in the New England rocky intertidal community. A bioassay was developed to determine the effect of algal compounds on the feeding of periwinkle snails.

In Chapter 2, the antiherbivore compounds in F. vesiculosus and A. nodosum are identified as polyphenols and their effective doses (ED_{50}) against L. littorea are determined. This work was done in cooperation with Dr. Oliver McConnell, Chemistry Department, Skidaway Institute of Oceanography (I carried out all bioassays of the algal extracts and compounds with L. littorea while Dr. McConnell performed all molecular weight determinations and spectroscopic analyses of the compounds). I concluded that polyphenols in F. vesiculosus and A. nodosum are functionally similar to terrestrial plant polyphenols (tannins) in their roles as chemical defenses against herbivores.

Chapter 3 reports the polyphenol contents in thirteen species of brown, red, and green algae in the New England rocky intertidal community. The seasonal and within-plant variations in polyphenol content in F. vesiculosus and A. nodosum are examined. Results are discussed in relation to the grazing patterns of L. littorea, the predominant macroalgal grazer in this New England rocky intertidal community.

In Chapter 4, the seasonal and within-plant variations in algal nitrogen content are examined in relation to the algal polyphenol contents reported in Chapter 3. The effect of algal polyphenols on nitrogen availability to herbivores is discussed and comparisons are drawn between brown algae and terrestrial plants in the nature and functioning of chemical defenses against herbivores.

Chapter 5 identifies volatile compounds produced by marine algae and seagrasses in New England intertidal and subtidal communitites and

examines their roles in algal chemical defense against herbivores. Part I is a paper (submitted to Journal of Phycology) that I coauthored with Philip Gschwend. It describes work we did in collaboration at Woods Hole Oceanographic Institution (I surveyed and identified all plant species in our study sites, assisted in the batch incubations of the samples, and interpreted the ecological roles of the algal compounds; Dr. Gschwend identified the algal compounds using gas chromatography/mass spectrometry and assessed the release rates of the compounds into seawater). We concluded that saturated and unsaturated hydrocarbons and di- and trihalomethanes were released by the algae at rates that may account for the concentrations of these volatile organic compounds in coastal seawater, and we hypothesized that some of these algal compounds may be important in allelochemic interactions. In Part II, I determined the effect of two of the volatile halomethanes (CH_2I_2 and $CHBr_3$) on feeding by L. littorea. CH_2I_2, a compound released by C. fragile and F. vesiculosus, was shown to inhibit feeding by L. littorea. In contrast, $CHBr_3$, a compound released by many algal species, did not significantly affect L. littorea feeding.

GENERAL INTRODUCTION

I. Statement of the Problem

Plant-herbivore interactions along with predation and competition provide most of the organization of community structure in both terrestrial and marine environments. Comparisons between communities are useful in determining whether similar mechanisms act to produce similar patterns. In terrestrial systems, it has been well documented that plants produce chemicals which act as defenses against herbivores and affect their feeding behavior (Fraenkel, 1959; Whittaker, 1970; Schoonhoven, 1972; Feeney, 1976). Indeed, a general theory of chemical coevolution between higher plants and their herbivores has been proposed by Ehrlich and Raven (1964) and Feeny (1975) which provides a partial explanation of the observed patterns of interaction. The evolution of defensive plant compounds and the stepwise evolutionary responses to these by phytophagous organisms are postulated to have been dominant factors in the evolution of both the herbivores and the plants and thus in the generation of terrestrial diversity.

In marine systems, direct evidence for algal chemical defenses has been lacking. Although many natural products have been isolated from marine algae (Baker and Murphy, 1976; Faulkner and Anderson, 1974; Fenical, 1975; Scheuer, 1973), very little is known about the biological activity of these compounds. A few of these compounds have been shown to have antibacterial or antialgal effects (Bhakuni and Silva, 1974; McLachlan and Craigie, 1966; Sieburth and Conover, 1965), but their ecological importance in plant-herbivore interactions has

largely been ignored. This research will investigate experimentally the importance of the organic compounds in algae as a means of defense against herbivores.

The intertidal algae and periwinkles (in particular, Littorina littorea) of the New England rocky intertidal community comprise a particularly conducive system in which to study this problem for the following reasons; 1) the snails and algae are readily accessible in the field and can be maintained under laboratory conditions; 2) Littorina littorea, the most abundant herbivore in the community, is a generalist which feeds on a wide variety of macroscopic and microscopic algae and exhibits a definite performance regime for particular algal species, avoiding some entirely; 3) a variety of compounds such as tannins and halogenated metabolites in these algae have demonstrated antibacterial and antialgal activity and hence are suspected as antiherbivore defenses; 4) the algae of this community include species in the Rhodophyta, Chlorophyta, and Phaeophyta; their life histories vary, including ephemerals, annuals, perennials, and species with alternating stages; 5) the importance of L. littorea in determining the distribution, abundance, and diversity of algae in the community has been established; feeding preferences of the snails and defenses of the algae together form the underlying cause of the patterns observed (Menge, 1975). The questions of what determines the feeding preferences of the snails and what constitutes the mechanism of defense of the algae remain unanswered. The role of algal

antiherbivore compounds is unexplored in this community as throughout most marine communities.

The questions which I will investigate in this thesis include:

1) Do the algae which are not eaten by L. littorea, in particular the dominant fucoids and perennial reds, possess compounds that act as deterrents to grazing? Is inhibition of feeding due to these antiherbivore chemicals rather than to size or toughness of the algae? To which classes of compounds do these compounds belong? What are their structures?

2) Do the algae vary in the production of antiherbivore chemicals during different stages of their life cycles, with age or size, or in different tissues? Are these seasonal variations? Do these variations result in differential palatability to herbivores?

3) What patterns emerge between the production of algal antiherbivore chemicals and the apparrency or successional status of the algae in the community? Within each algal taxonomic division, is commitment to chemical defense greater in perennials than in annuals and ephemerals as postulated by Cates and Orians (1975) for terrestrial plants?

This study combines the approaches of natural products chemistry and ecology to understand mechanisms of chemical ecology in the marine environment. Through comparisons to terrestrial studies this research will be useful in determining if similar mechanisms operate to produce similar patterns across marine and terrestrial communities.

II. Plant Chemical Defenses: Parallels Suggested Between Terrestrial and Marine Environments

A. Defenses of Plants Against Herbivores

Hairston, Smith and Slobodkin (1960) argued that because herbivores do not eat all plants and because populations of herbivores frequently increase greatly when predators are removed, herbivore populations must be limited by predators. This logic has serious flaws which were first pointed out by Murdoch (1966). The mere presence of plants in what appears to be unlimited supply does not necessarily mean that herbivores are not food-limited. Plants have evolved various defense systems which limit their availability to herbivores. Five types of plant antiherbivore strategies can be outlined as follows: 1) production of chemicals that are noxious or that decrease the quality of food, 2) mechanical and structural adaptations in the plant; 3) spatial escapes, 4) temporal escapes, and 5) size escapes. Because the production of defensive chemicals is so widespread and varied among plants, it has been considered the primary response to herbivore grazing pressure (Ehrlich and Raven, 1964). Stebbins (1950) labelled the other strategies as more specialized secondary responses evolving either in the absence or substantial failure of defensive compounds to discourage predation. A plant's best strategy against herbivores is to become inedible; this is well accomplished by the synthesis of metabolic compounds which are, for one reason or another, unacceptable to herbivores (Dethier, 1970).

B. Antiherbivore Chemicals in Terrestrial Plants

Allelochemics, as defined by Whittaker and Feeney (1971) are chemicals by which organisms of one species affect the growth, behavior, or population biology of another species (excluding substances used only as food by the second species). Within this group are 1) allomones, which give adaptive advantages to the producing organism, 2) kairomones, which give adaptive value to the receiving organism, and 3) depressants, which are wastes which harm the recipient but which have no adaptive value to the releaser. Antiherbivore chemicals are thus allomones in this scheme. Allomones may serve as defenses against herbivores as well as against parasites, bacteria, and competing plant species; one compound may have specific or multiple roles (Muller et al., 1964; Muller and Muller, 1964; Wells, 1964; Levin, 1971). Thus, adaptive advantage and selective preservation of certain plant chemicals cannot be evaluated solely in terms of herbivores. It is important to remember that the effects of herbivores on evolution of plant chemical defenses must be viewed against a broader coevolutionary background.

The chemicals involved in defending plants from herbivores generally belong among the secondary compounds, as do most plant allomones. They are not, in most cases, essential to the basic metabolism of the plant and hence are contrasted with primary substances such as proteins, carbohydrates, fats, and nucleic acids. Secondary compounds may be wastes or elaborated by-products of various synthetic pathways. The nutritive value of plants to herbivores is basically determined by

the primary metabolites; the secondary compounds are thus said to function as attractant or repellent triggers (Fraenkel, 1959). Dethier (1947) and Schoonhoven (1972) compiled extensive lists of plant secondary compounds known to act as feeding stimulants or deterrents to insects. They are of irregular or sporadic occurrence, reinforcing the view that they are not of primary metabolic concern to plants. The occurrence of the same or related secondary compounds in related plant species makes these compounds important concerns of chemical taxonomy as well as important in the evolutionary responses of groups of herbivores and their food-plants. However, some secondary compounds have appeared in plants of distant phylogenetic relation, implying independent evolution and convergence on chemical defenses (Whittaker, 1970).

The majority of the toxic or repellent compounds fall within the following chemical groups (which can overlap due to the complexity of the structures): alkaloids, glycosides, phenols and tannins, organic acids, saponins, terpenes and steroids, and other essential oils. These represent various offshoots from the major metabolic pathways but have broadly similar origins with acetic acid and amino acids as the major starting materials. Extensive discussion of secondary plant substances and their metabolic pathways may be found in texts and monographs (Bonner and Varner, 1965; Harborne, 1964; Haslam, 1966; Pridham, 1967; Manske and Holmes, 1950-1968).

1) Phenols and Tannins

Plant phenolics are second in abundance only to carbohydrates. Substances of this large grouping are aromatic in the sense of having one or more benzene rings in their structure. The compounds are structurally diverse and include coumarins, quinones, lignins, flavonoids, and tannins; many occur as glycosidic derivatives. Antibiotic properties of plant phenolics are well known (Levin, 1971). They become localized around points of infection where they increase in concentration and undergo oxidation or hydrolysis to form more toxic derivatives. This action is probably important when herbivores damage plants and may be considered as a defense (Miles, 1969).

Perhaps the most effective and the most common defensive phenols are the tannins. These polymeric phenols are subdivided into two groups: "hydrolyzable" tannins are readily hydrolyzed by acids or enzymes into a sugar or related alcohol and a phenolcarboxylic acid; "condensed" tannins do not readily break down with acid but, may undergo progressive polymerization. Tannins have long been used in the tanning (hence the name "tannin") of leather because of their affinities for proteins such as collagen. Because of their economic importance, much literature exists on the chemistry and distribution of tannins in higher plants (Nierenstein, 1935; Bate-Smith, 1957; Haslam, 1966).

Feeny (1968) suggested the ecological importance of tannins by hypothesizing that the presence of increasing amounts of tannins in oak leaves may be one factor influencing the selection for early lepi-

dopteran larval feeding periods on oak leaves. Just as tannins can combine with proteinaceous collagens, tannins can bind to enzymes and other proteins in animal digestive tracts. Goldstein and Swain (1965) demonstrated that tannins inhibit enzyme activity and suggested that condensed tannins are more effective than hydrolysable tannins because they form more stable cross-linkings with proteins. Feeny and Bostock (1968) discovered that moth larvae stopped feeding at the same time that condensed tannins appeared in oak leaves. Further experiments showed that oak leaf tannins formed complexes with leaf proteins and also bound to animal digestive enzymes such as trypsin at the physiological pH of the larval moth midgut; these effects were more marked with condensed than with hydrolysable oak leaf tannins (Feeny, 1969). Thus, tannins exhibit two mechanisms which lower the nutritional value of the plant proteins to herbivores: 1) tannins can bind to the herbivore's digestive enzymes and thus inhibit activity in digestive processes, and 2) tannins can combine with the proteins from the food plant and render them unavailable to the herbivore's digestive processes. The non-specific nature of the interaction between tannins and proteins probably accounts for their effectiveness against herbivores. The generalized nature of the tannin-protein complexes with extensive hydrogen and covalent bonds (Haslam, 1966) implies that it would be difficult for herbivores to develop detoxification pathways such as those that are known for alkaloids and other specific poisonous chemicals in plants (Hodgson, Self, and Guthrie, 1965). Experiments in which oak leaf tannins were radioactively labelled

showed that they were retained inside the peritrophic membrane of the winter moth midgut (Feeny, 1970). Plants, in order to prevent autotoxicity, sequester tannins into vacuoles or attach them to sugars, rendering them unable to bind to proteins. When cellular damage occurs by herbivores, parasites, bacteria, etc., the tannins are released and become active.

How do herbivores adapt to the presence of tannins in their food in view of the lack of specific detoxification mechanisms? Feeny (1968) hypothesized that alteration of life histories can take place in which feeding periods minimize the contact with tannins such as was proposed for larval moths and mature oak leaves. Herbivores such as leaf miners feed on spongy perenchyma of leaves rather than in the palisade layer, where tannins are believed to concentrate. Since tannin-protein complexes are known to dissociate at extremely high pH, it is possible that alkaline guts in herbivores may represent an adaptation to release a higher proportion of protein for digestion (Feeny, 1969).

2) Alkaloids

Alkaloids have been found primarily in higher plants. They are nitrogenous bases often with a heterocyclic ring structure. Like tannins, they are implicated as agents responsible for the bitter taste or unpalatability of many plants and tissues. Many are well known as toxins and highly effective poisons (Manske and Holmes, 1968). Alkaloids known for their effects on man include nicotine, caffeine, quinine, morphone, codeine, mescaline and lysergic acid.

These probably represent repellents and toxins which have evolved in response as defenses to plant enemies. Nicotiana alkaloids (e.g. nicotine) have been commonly used as insecticides and have been shown to be toxic to insect larvae in nature (Thorsteinson, 1960). The beetle Leptinotarsa is poisoned by fresh leaves of Nicotiana as well as by the addition of alkaloids from solanaceous plants into its diet (Fraenkel, 1969). Demissin and Tomatin, both alkaloids from Solanaceae, inhibit the feeding responses of larvae and hence cause death by starvation. Larval feeding of the diamond-back moth Plutella maculipennis is similarly inhibited by high concentrations of alkaloids in the leaves of Solanaceae such as Lycopersicum (Thorsteinson, 1960). The entire coffee family Rubiaceae is protected from feeding of lepidopteran larvae by the presence of quinine in leaves. Highly specific repellants to herbivores are also present within plant families. Ehrlich and Raven (1967) have shown that larvae of the moth Euchelia jacobaceae can be fed on most species in the compositae genus Senecio except S. visconcus. This species produces a gummy compound which renders its leaves inedible. When the alkaloid substance is removed by dissolution in alcohol, the leaves are eaten by the moth; application of the compound to leaves of normally edible species in the genus renders them unpalatable.

Alkaloid content of plants can vary with time as was demonstrated for tannins in oak leaves. Ehrlich and Raven (1964) found that insects feeding on leaves of alkaloid-rich species of Papaver prefer young leaves in which concentrations of the secondary substance have

not yet built up. Thus feeding strategies of the herbivores can adapt to the chemical defenses of the plants.

As with tannins, plants often sequester alkaloids in vacuoles or attach them to sugars to form glycosides. These detoxification mechanisms in plants appear to be general to prevent autotoxicity from defensive chemicals.

Herbivore detoxification of plant alkaloids appears to be more common than detoxification of tannins. This is presumably due to the nature and the mode of action of the chemicals. Microsomal mixed-function oxidase enzymes in herbivores are effective in the detoxification of some, but not all, plant secondary compounds. Animal microsomal systems have an overall similarity from species to species; their main functions are breakdown of internal steroidal hormones and the degradation of foreign chemicals by enzymatic oxidation, reduction, or hydrolysis often followed by conjugation with other molecules (Williams, 1959; Schuster, 1964; La Du, Mandel, and Way, 1972). Tannins apparently are resistant to detoxification by microsomal systems because of their large and complex structures and their abilities to combine with proteins and inhibit enzymes. In contrast, alkaloids and various other toxic compounds are more easily rendered inactive by microsomal enzymes; species-specificity can be quite high. Thus, herbivores have the potential to be released from toxic effects of certain plant secondary compounds.

Chrysolina beetles apparently have evolved a mechanism for detoxification of hypericin, an alkaloid present in plants of the genus

Hypericum which prevents feeding by nearly all other herbivores. The result is that Chrysolina has a food supply unlimited by other herbivores and, in fact, has cued in on hypericin as an attractant. In other evolutionary "arms races" between plants and animals (Whittaker and Feeny, 1971) using chemical defenses and detoxification counter-attacks, the plant instead has won. Chrysanthemum cinerariaefolium produces pyrethrin which inhibits herbivory. Certain animals have evolved oxidase systems which can detoxify the compound. The plant, in turn, has elaborated other chemicals which inhibit the animal oxidase systems, allowing pyrethrin to be effective as a defense. Kreiger, Feeny and Wilkinson (1971) studied the microsomal oxidase systems of ten lepidopteran families, using the rate of epoxidation of aldrin to dieldrin as an index of enzymatic activity. Polyphagous species were found to have higher levels of activity than do monophagous species. The implication followed that the polyphagous species are adapted to detoxify a greater range of secondary compounds than are oligophagous species, and monophagous species are most limited in their ability to detoxify a range of compounds.

3) Terpenes and Steroids

Several interesting lines of defense are found among the terpenes and their many derivatives, including the steroids. Ecdysones (molting and metamorphosis hormones) and their analogs occur in plants and can disturb the endocrine control of insect life cycles (Kaplanis et al., 1967). Precise timing and concentrations of these compounds are necessary for normal animal growth and development. Plants, by

means of hormones, can kill their herbivores by either accelerating or preventing their metamorphosis. In Cecropia moths, metamorphosis can be fatally accelerated by 1 ppb. ponasterone (Williams, 1970). Juvenile hormone (juvabione) present in balsam arrests the development of Pyrrhocoris at an immature stage (Slama and Williams, 1966). The importance of hormones as a means of herbivore control of plants is just beginning to be understood.

Sapogenins are plant steroids found in genera such as Yucca and Agave and are toxic to animals through hemolysis and other effects on membrane permeability. As with tannins and alkaloids, they occur as glycosides in plants to prevent autotoxicity. Cardiac glycosides consist of plant sterols linked to sugars; they are effective poisons which occur in the milkweed and dogbane families (Whittaker, 1970). Butterflies have evolved means to concentrate these antiherbivore compounds in their tissues and use them for defense against their predators (Brower and Brower, 1964).

Janzen, Juster, and Liener (1976) concluded that phytohemaglutinin in black beans (Phaseolus vulgaris) and other legumes is of adaptive significance in preventing attack by insects. Several hundred species of neotropical legumes are eaten by larvae of bruchid beetles. However, the bruchids are extremely species-specific; nearly all the species of bruchids in a given habitat cannot feed on any given species of potential prey. Callosobruchus maculatus feeds on cow peas (Vigna unguiculata) but not on nearby black beans. Black bean phytohemagglutinin added to the normal diet of C. maculatus in the labora-

tory killed them. Phytohemagglutinin is lethal apparently because the bruchid has not evolved a detoxification mechanism.

4) Cyanogenic Glucosides

Lotus corniculata (bird's-foot trefoil) has been shown to be polymorphic for the presence or absence of cyanogenic glucosides (Jones, 1962). When the stem or leaves are damaged, as when herbivores graze, the glucosides are hydrolyzed and hydrocyanic acid is evolved. Cyanogenesis involved complementary gene interaction; cyanide production requires the presence of the glucoside plus enzyme, each produced by dominant alleles at unlinked loci. Jones (1972) hypothesized that the continuous and gradual increase in geographic frequency of cyanogenic L. corniculata in the Netherlands from 3% to 96% is a cline that was originated by and is maintained by grazing pressure of rabbits. Rabbits and slugs prefer acyanogenic strains of the plant species, conveying selective advantage to cyanogenic strains in habitats where the herbivores are abundant. Some herbivore species have, in turn, evolved detoxification mechanisms for cyanide and are not selective between the strains (Jones, 1966).

5) Costs and Benefits of Antiherbivore Chemicals to Plants

From the discussion of plant chemicals thus far, a few patterns begin to emerge. Plant secondary compounds are diverse in their structures and modes of action against herbivores, and animals are affected by them in various ways and degrees. The effects of compounds such as tannins, which are not easily detoxified by microsomal enzymes, can be circumvented by herbivores that avoid their presence

in time or space, seasonally and within or between plant individuals or species. Other toxic substances, such as some alkaloids, steroids, and cyanogenic glucosides, can be detoxified by animals with the proper specific enzymes. Further, animals can cue in on these detoxified plant substances to use them as attractants or to concentrate them for defense against their own predators. Plants, in turn, can add to their protective systems to counteract animal detoxification mechanisms. The result is the creation of numerous interaction pairs that illustrate the reciprocal adaptations of plants and herbivores. The recognition of the antiherbivore nature of plant secondary compounds is the first step in analysis of the patterns and ways in which plants and herbivores have been of mutual evolutionary influence.

Considering plant chemicals as a means of defense against herbivores implies that they are of advantage to a plant. Jones (1971) points out that it is essential to show that chemical differences between individuals in a polymorphic or continuously varying population convey differential advantages as far as protection against herbivores is concerned. That is, selection for the production of chemical defenses occurs on an intraspecific level. For example, cyanogenic strains of L. corniculatus are favored over acyanogenic strains when slug or rabbit densities are high because these herbivores preferentially feed on acyanogenic strains (Jones, 1962, 1966, 1972). However, in the absence of herbivores, the acyanogenic strains are favored because they grow and clone faster and produce more seeds than do cyanogenic strains. This suggests that there is some cost to the

plant in the production or maintenance of the chemicals which act as antiherbivore defenses. Costs to the plant are dependent upon the amount of energy, time, and nutrient resources which must be 1) incorporated into the compound and spent in its biosynthesis, and 2) expanded in the elaboration of means to concentrate and store the compounds and to prevent autotoxicity, e.g. formation of vacuoles, ducts, storage cells or organs; synthesis of enzymes for glycoside production. The time, energy, and nutrients expended on chemical defense could instead be allocated to basic metabolic processes which could result in increased growth and reproduction rates. A trade-off in resource allocation is implicit: with no grazing pressure, plants that allot the majority of their time, energy and nutrients to growth and reproduction have a competitive advantage; with grazing pressure, those plants posessing chemical defenses have a selective advantage in survival and reproduction. A plant's production of chemical defenses can be interpreted in terms of a compromise between minimizing risk of mortality and maximizing growth and reproduction rates. This view is supported by data collected on populations of wild ginger which are polymorphic for growth rate, seed production, and palatability to slugs (Cates, 1975). In habitats where slugs are not abundant, populations of wild ginger are dominated by individuals which grow rapidly, produce large seed sets, and are palatable to slugs. In habitats where slugs are abundant, populations are dominated by individuals which are highly unpalatable to slugs but which grow slowly and produce small seed sets. Though no direct evidence

is yet available, palatability of the ginger to slugs is postulated to be determined by secondary compounds.

The content of secondary compounds in different parts of a plant may vary seasonally or as a function of age. In many young, actively growing plant shoots and foliage, low frequency and low concentrations are often found, presumably because of the high costs of the chemicals in terms of energy and resource commitment and their autotoxity. For example, young oak leaves do not produce tannins (Feeny, 1970) and young Papaver leaves do not produce alkaloids (Ehrlich and Raven, 1964). It appears that most seedlings and young tissues are indeed tasty to herbivores (Orians and Janzen, 1974). There are exceptions to this generalization; some plant species are able to concentrate toxic substances in actively growing shoot tips, perhaps in response to strong grazing pressure (McKey, 1974).

6) Plant Apparency and Successional Status: Commitment to Chemical Defense

Cates and Orians (1975) hypothesized that annuals or early successional plant species should make, proportionately, a lesser commitment to defense against herbivores than perennials or later successional or climax species. Measuring palatability of 100 plant species to slugs, they found that early successional perennials were significantly more palatable than later successional species (palatability was assumed to be of chemical determination). These results agree with the suggestion of Odum (1969) that the development of chemical defenses against herbivores is directly correlated with the

time involved in uninterrupted succession. Early successional plants and annuals tend to mature and reproduce rapidly and have abilities to disperse and colonize, hence they are subject to herbivore grazing pressure during a short time span or may not be discovered at all (thus posessing temporal or spatial escapes). Because of their "unapparency" (Feeny, 1976) to herbivores, i.e. low vulnerability to discovery, the price of chemical defenses may be be worth their investment. In contrast, perennial, late successional, and climax plants tend to grow more slowly and are more highly predictable in space and time. They are highly "apparent" (Feeny, 1976), i.e. easy to find by herbivores, and will not survive unless protected, hence chemical defenses are advantageous and worth the cost to the plants. Besides persistence and predictability in the community, other factors which determine plant "apparency" include size, growth form, and relative abundance of its species. The more apparent the plant, the greater is the advantage from, and hence more likely commitment to, chemical defense.

C. Chemicals in Marine Algae: Potential Antiherbivore Defenses

Marine algae produce a variety of compounds which resemble the antiherbivore chemicals of higher plants and also a variety of compounds which are unique to the marine environment (Faulkner and Anderson, 1975; Fenical, 1975; Scheuer, 1973). Few of these compounds have been investigated for their roles in marine plant-herbivore interactions. Their potential importance is suggested by the defense/response parallels with terrestrial higher plants and animals

and by the activities that they have exhibited against certain pathogens and pests.

1) Phenols and Tannins

Among the phenols, the tannins are the most abundant compounds that are found in both marine and terrestrial plants and that most likely function as feeding deterrents. Tannins have been found in many brown algae (Phaeophyta) and in a few green algae (Chlorophyta) (Ogino, 1962; Glombitza, 1977). As in terrestrial plants, the tannins in marine algae are localized in vacuoles or in special vesicles called physodes, presumably to prevent autotoxicity. Their exact function is unknown, although they are believed to be metabolic wastes or by-products (Craigie and McLachlan, 1964). Conover and Sieburth (1964) demonstrated that tannic compounds from Sargassum inhibited bacterial growth and correlated the presence or absence of epiphytes and eipfauna on the alga with the antibacterial activity of its tannic extracts. Branch tips that were fouled had cells with 2-12 physodes, while unfouled branches had cells with 40-68 physodes (Sieburth and Conover, 1965). Ragan and Craigie (1975) studied the chemical composition of physodes of Fucus vesiculosus. A series of vanillin-reactive compounds isolated from the extracts of this brown alga were shown to be composed of phloroglucinol and its phenyl-linked dimers and trimers.

The complexing action of tannins on proteins and enzymes would presumably affect the digestive abilities of marine herbivores just as they do of terrestrial animals. That is, tannins in marine algae may

be quantitative, dosage-dependent barriers whose mode of action is to reduce growth rate and fitness of all herbivores by reducing availability of plant proteins and nutrients and whose potential for biochemical detoxification and counteradaptation by animals is limited. As in terrestrial plants, they may require a relatively large investment by marine algae in terms of energy and time and may be of advantage primarily in "apparent" (Feeny, 1976) or late successional (Cates and Orians, 1975) species. Parallels are suggested between tannin-containing terrestrial plants and tannin-containing marine algae with respect to herbivores. Menge (1975) showed that the phaeophytes Agarum, Ascophyllum, Chorda, Chordaria, Fucus, Laminaria, and Ralfsia are of very low food preference to the generalist herbivore Littorina littorea. All of these brown algae have tannin-filled physodes (Craigie and McLachlan, 1964) and are "apparent" or are late successionals (though not in obligate sense, J. Menge, unpub. data). That herbivores do graze young sporeling stages of brown algae suggests that the sporelings may not have high concentrations of tannins (cf. Orians and Jansen, 1974); likewise, the ephemeral or "unapparent" brown algae may not be protected chemically by high concentrations of tannins. Alternative, but not mutually exclusive, hypotheses which do not include the role of tannins may explain these herbivore feeding preferences, e.g. toughness of the algae may be a factor (Menge, 1975). However, Feeny (1970) postulated that increasing leaf toughness is a proximate, though not ultimate, factor in preventing feeding by the winter moth on mature oak leaves.

He reasoned that a factor other than toughness must make oak leaves less suitable as food as the leaves mature because selection in the herbivore has not favored the development of stronger mandibular muscles. Experiments to demonstrate the physiological effects of tannins on marine herbivores and the role of tannins in their food selection are needed, along with studies of the distribution and abundance of tannins in marine algae in relation to algal "apparency" or successional status in the community.

Other phenolic compounds in marine algae besides tannins are potential defenses against herbivores. Many have been shown to be antibacterial and antifungal (Bhakuni and Silva, 1974). McLachlan and Craigie (1966). demonstrated that a number of algal phenols inhibited the growth of other algal species. Phenols may play an important role in regulating the abundance and occurrence of algal endophytes, epiphytes, and parasites as well as in deterring herbivores.

2) Halogenated Compounds

Halogenated compounds are extremely abundant in marine algae. A variety of metabolites containing chlorine, bromine, and iodine have been discovered, with structures ranging from simple aliphatic haloketones and brominated phenols to more complex halogenated mono-, sesqui-, and diterpenes. The structural formulas of these compounds and their distributions in organisms have been recently examined and summarized (Baker and Murphy, 1976; Bhakuni and Silva, 1974; Faulkner and Anderson, 1974; Faulkner and Fenical, 1977; Fenical, 1975; Scheuer, 1973). Two of the halogens, iodine and chlorine, are

reported in metabolic processes of some terrestrial plants and animals, but bromine metabolism is generally not recognized in terrestrial organisms. Because of the high concentrations of halide ions in seawater, it is not surprising that marine organisms have developed pathways that utilize halogens in metabolic compounds. However, the biological functions of halogenated compounds in marine algae have been little studied. These compounds do not appear to be involved in primary metabolic processes; Fenical (1975) postulated that they are probably synthesized as chemical defenses against epiphytes, parasites, herbivores, and bacteria.

Although brown algae are known to contain iodine, the red algae have been more intensively studied by marine natural products chemists and have been shown to utilize all three halogens (bromine, chlorine, and iodine) in metabolic products (Fenical, 1975). Less information exists on the presence or absence of halogenated compounds in the green algae. In the red algae halogenated compounds are often localized in specialized vesicular cells of the thallus (Fritsch, 1945; Chan and McManus, 1969; Pedersen et al., 1974; Wolk, 1968). This may be a means of preventing autotoxicity, similar to the sequestering vacuoles of higher plants.

Studies of the "biological activity" of halogenated compounds in marine algae have been limited primarily to the effect of red algal extracts on bacterial or fungal growth. Mautner et al. (1953) conducted one of the first studies of the antibiotic activity of Rhodophyte extracts. Since then, many bromophenols, haloterpenes, and

haloketones have been shown to have antibacterial and antifungal effects (Peguy and Heim, 1961; Glombitza and Stoffelen, 1972; Glombitza et al., 1974; Fenical, 1974). Bromophenols from Polysiphonia lanosa were demonstrated to be toxic to single-cell algal cultures (McLachlan and Craigie, 1966), but their function in nature as antiepiphyte agents has only been hypothesized and not tested. Chlorinated terpenes from Plocamium cartilagineum have been tested for toxic effects on goldfish and insects (Crews and Kho, 1974, 1975), but their function against herbivores, algae, or bacteria found in the natural environment of the alga has been ignored, perhaps for lack of appropriate experimental designs. This thesis research will examine the effect of halogenated compounds in algae against herbivores found in their community and hence will contribute to the understanding of plant-animal chemical interactions in the marine environment.

It has been shown that herbivores can concentrate halogenated compounds from their algal food sources. Stallard and Faulkner (1974) found large quantities of halogenated metabolites from Laurencia and Plocamium in the sea-hare Aplysia, but no enzymatic degradation products of these compounds were found. It appears that Aplysia, which is a specialist herbivore preferring these two species of red algae, is adapted to store these potentially toxic substances in its body tissues, resulting in a bad taste and hence chemical protection from its predators. Similarly, in terrestrial systems, monarch butterflies have become adapted to plant poisons in their tissues which serve as protection against bird predators (Brower and Brower, 1964).

Despite their abundance in the Rhodophyta, halogenated compounds are not ubiquitous in the division. Fenical (1975) reported that they have been found in seventeen genera from five orders including Laurencia, Plocamium, Ceramium, Odonthalia, Polysiphonia and Rhodymenia; no significant concentrations have been found in Porphyra, Pterocladia, Halosaccion, and Spyridia. Synthesis of halogenated compounds appears to be similar within families, and halogenated metabolites have been used as taxonomic markers to study polymorphism (Fenical and Norris, 1975; Mynderse and Faulkner, 1974).

Halogenated compounds thus compare in several ways to chemical defenses in terrestrial plants such as alkaloids and glucosinolates. They are probably effective in relatively small concentrations as toxins or feeding deterrents to non-adapted herbivores, but may be susceptible to counteradaptation and may have little inhibitory effect on the growth and fitness of adapted herbivores, which may exploit them as attractants or use them for their own chemical defense against predators. Furthermore, potentially toxic halogenated compounds may occur along algal family lines and may vary in production between and within species and among habitats, perhaps in relation to herbivore grazing pressure, thus suggesting parallels to the alkaloids and cyanogens in the terrestrial plant-herbivore systems. Halogenated metabolites are probably relatively inexpensive energetically for marine algae to synthesize as chemical defenses, partly because of the high concentrations of halide ions in seawater and partly because many of the structures are relatively simple.

In the New England intertidal community, Menge (1975) found that herbivorous snails preferentially feed on ephemeral red algae such as Ceramium sp. and Porphyra umbilicalis and avoid perennial reds such as Chondrus crispus and Polysiphonia lanosa. Certain halogenated compounds in the non-preferred algae may be acting as chemical defenses. For example, the diverse bromophenolic compounds in Polysiphonia lanosa that have demonstrated antibacterial and anti-algal effects (Augier and Hofmann, 1952; Glombitza and Stoffelen, 1972; Glombitza et al., 1974; Stoffelen et al., 1972) may be antiherbivore in nature. In contrast, no halogenated compounds have been found in Porphyra. Likewise, the ephemeral algae which are most highly preferred as food by the snails are dominated by green algae, few of which have been shown to contain halogenated metabolites. Studies of the effect of halogenated compounds on the feeding preferences of these snails could elucidate the function of the algal chemicals in herbivore relationships.

3) Terpenes, Steroids, and Other Chemicals

Many other chemicals in marine algae may be involved in plant-herbivore feeding relationships. Caulerpa spp. are well known for the toxins caulerpicin and caulerpin, which are complex dimeric structures and which have been shown to concentrate in herbivore tissues (Doty and Aguilar-Santos, 1974). Recently, Sun and Fenical (1979) showed that rhipocephalin and rhipocephenal, compounds from the tropical marine alga Rhipocephalus phoenix, were toxic to fish. A variety of substances from algae have been shown to be toxic to man

(Hashimoto and Fusetani, 1971). Marine unicellular algae, especially dinoflagellates, produce toxins which are responsible for shellfish poisoning.

Sterols and steroids are common in marine algae (Patterson, 1971). One of the most clever defenses of terrestrial plants against herbivores is the production of steroidal molting hormones and their analogs that disrupt normal development of the animals. Closely related ecdysones and crustecdysones have been isolated from both insects and marine crustaceans (Gagosian and Bourbonniere, 1976). A synthetic juvenile hormone mimic was shown to cause premature metamorphosis of marine crustaceans (Gomez, Faulkner, Newman, and Ireland, 1973). Further research may show that marine plants can produce such hormones and analogs that lethally accelerate or inhibit development of their herbivores, just as Williams (1970) demonstrated for terrestrial plants and their insect herbivores.

D. Conclusions

Examination of the chemical defenses and responses of higher plants and their herbivores in terrestrial communities suggests patterns which may have parallels in marine communities. Marine algae produce a variety of chemicals which resemble antiherbivore chemicals of higher plants and also a variety of chemicals which are unique to the marine environment. Tannins and many diverse halogenated compounds in marine algae have demonstrated antibacterial, antifungal, and antialgal activity, but their function in plant-herbivore relationships has not been studied. The following research will examine

experimentally the importance of chemicals in algae as a means of defense against marine herbivores.

III. Plant-Herbivore Interactions in the New England Rocky Intertidal Community

A. Importance of Herbivores in Structure of Plant Community

The rocky intertidal shores of protected to moderately exposed coasts of New England are characterized by a large biomass of macroscopic algae. Space is dominated by two perennial plant types, fucoids in the mid-zones and Irish moss (Chondrus crispus) in the low zones. Periwinkle snails of the genus Littorina, in particular L. littorea, are the predominant herbivores of the New England rocky intertidal community. The fucoid algae and Irish moss have effective escapes from these herbivores. Chondrus crispus is apparently unpalatable to periwinkles at all stages of its life cycle. Fucoids are grazed by periwinkles during sporeling stages but are not eaten once they attain a size of 3-5 cm. (Menge, 1975). Thus, these herbivores are ineffective at preventing colonization and monopolization of space by fucoids and Irish moss.

Ephemeral (short-lived) algae occur seasonally in varying degrees of abundance in the intertidal zone on rocks and as epiphytes and constitute the preferred food of the snails. Experimental removal of the herbivores and Fucus spp. allowed continual growth of a high abundance of ephemerals throughout the year, thus demonstrating that the periwinkles are directly responsible for much of the disappearance of ephemeral algae (Menge, 1975).

In high rocky intertidal pools, field experiments demonstrated that L. littorea controls the abundance and type of algae (Menge, 1975). Highest species diversity of algae occurs at intermediate periwinkle densities because the herbivore preferentially feeds on the competitively dominant algae, Enteromorpha spp. Absence of herbivore grazing results in a pure stand of Enteromorpha; moderate grazing creates space which allows competitively inferior algal species to persist; intense grazing eliminates most algae in numbers of both individuals and species. This effect of L. littorea in high tide pools is contrasted on mid and low rocky emergent substrata where the herbivore's preferred food is competitively inferior, thereby decreasing algal diversity at all intensities of grazing.

In summary, plant-herbivore interactions are important in the community structure and organization of the New England rocky intertidal region. The most abundant herbivores, in particular the periwinkle snails L. littorea, because of their grazing preference regimes, affect the distribution, abundance, and diversity of algae in the mid, low and high intertidal zones.

B. Factors Determining Algal Preferences of Herbivores

To date, little is known of factors determining the algal preferences of marine herbivores. Evidence of the protective function of certain chemicals in terrestrial plants and their role in determining herbivore feeding patterns has accumulated, but counterpart studies in marine environments have done little more than to implicate the possible importance of algal chemical defenses against grazers.

Vadas (1968) hypothesized that chemical defenses of the phaeophyte Agarum reduces grazing and adversely affects the growth the reproduction of herbivores, leading to the evolution of preferential herbivory. Field experiments combined with laboratory food preference tests by Ogden (1976) showed that some algae or algal tissues are specifically avoided during grazing; chemical and structural features were merely suggested, without experimentation, to be the responsible factors. Some hypotheses attempt to explain herbivore feeding preferences on the bases of algal caloric content, nitrogen content, storage products, cell-wall composition, or other various factors. All of these possibilities are, of course, not mutually exclusive, and certain algal species due to particular combinations of these factors may be highly preferred or non-preferred as food, depending upon the herbivore involved. Many studies have attempted to correlate marine herbivore food preference with these various factors, but no clearly defined trends have emerged (Carefoot, 1967, 1973; Paine and Vadas, 1969; Kristenson, 1972; Nicotri, 1978). Correlations are merely suggestive and do not demonstrate causative factors and effects. The importance of controlled, manipulative experiments in ecological studies has recently been stressed (Connell, 1975). This study will attempt to investigate experimentally the importance of chemicals in algae as means of defense against their herbivores.

CHAPTER 1

EVIDENCE FOR ALGAL CHEMICAL DEFENSE AGAINST A MARINE HERBIVORE

ABSTRACT

A method was developed to test compounds extracted from algae for their effect on the feeding of snails. Using this method it was shown that in three of the dominant perennial algae of the New England rocky intertidal community (Fucus vesiculosus, Ascophyllum nodosum, and Chondrus crispus), a chemical factor rather than size or toughness is responsible as a feeding deterrent against the most abundant herbivore, Littorina littorea. The antiherbivore factor in Codium fragile, an annual green alga which is of low food preference to L. littorea, appeared to be partially lost during extraction procedures and feeding experiments. In the two brown algal species, the antiherbivore compounds are methanol-extractable, probably polyphenols, while in the red alga the active component is methylene chloride-extractable.

INTRODUCTION

In the New England rocky intertidal community, space is dominated by two perennial plant types, fucoids (Ascophyllum nodosum and several species of Fucus) in the high and mid zones and Irish moss (Chondrus crispus) in the low zones. These algae have effective escapes from the predominant herbivores, periwinkle snails of the genus Littorina (Lubchenco, 1978). Here I report the first direct evidence that these algae produce chemical compounds which inhibit feeding by the snails and describe the method used. Although many unusual natural products isolated from marine algae have toxic effects on bacteria, single-celled algae, fungi, goldfish, and insects (Conover and Sieburth, 1964; McLachlan and Craigie, 1966; Crews and Kho, 1974 and 1975; Fenical, 1975), few have been tested for an antifeeding function against marine herbivores found in the environment of the alga from which the compounds were extracted (only recently, Sun and Fenical, 1979, reported the activity of two sesquiterpenoids from a Caribbean alga against reef fish). In contrast, it has been well documented in terrestrial environments that plants produce many diverse chemicals that deter feeding by invertebrate and vertebrate herbivores (Feeny, 1968, 1970, 1976; Gilbert et al., 1967; Janzen et al., 1976; Meinwald et al., 1978).

EXPERIMENTAL PROCEDURES AND RESULTS

Periwinkles and algae were collected from rocky outcrops along the coast of Massachusetts. Food preference studies conducted by Lubchenco

(1978) and by me indicate that some macroalgae are highly preferred by periwinkles while others are rarely eaten. Lubchenco (personal communication) found a correlation between degree of preference and toughness of the algae. To test the hypothesis that certain algae are not eaten because of their toughness, nine species of algae, ranging from preferred to non-preferred as food in the Littorina littorea's preference regime, were homogenized with a Brinkman Polytron and solidified with 1.8% agar-seawater in petri plates. Two sets of feeding experiments were run. In the first, the snails were presented with a single choice of algal-agar media and tested for a feeding response. In the second, the snails were presented with a choice of four types of media and tested for a preferential feeding response. These experiments were conducted under running seawater in "test arenas" consisting of screened containers (8 cm. high x 11 cm. diam.) with four-way divided petri plates offering the media to four L. littorea which were allowed to graze for four days (Figure 1). Amounts of media consumed were measured using a 0.5 cm. gridded plate over the petri plate. The results are summarized in Tables 1 and 2. Snails consumed media prepared with their normally preferred algal species such as Ulva lactuca, Enteromorpha intestinalis, Porphyra umbilicalis, and Ceramium rubrum and did not eat agar controls (without algal homogenates added), indicating that agar itself was not acting as an attractant. Media prepared with Fucus vesiculosus, Ascophyllum nodosum, and Chondrus crispus (normally non-preferred algal species) were avoided. These results indicate that there is a factor other than toughness or size that is acting as a feeding deterrent and suggest that

Figure 1. "Test arena" with divided petri plate offering media to snails.

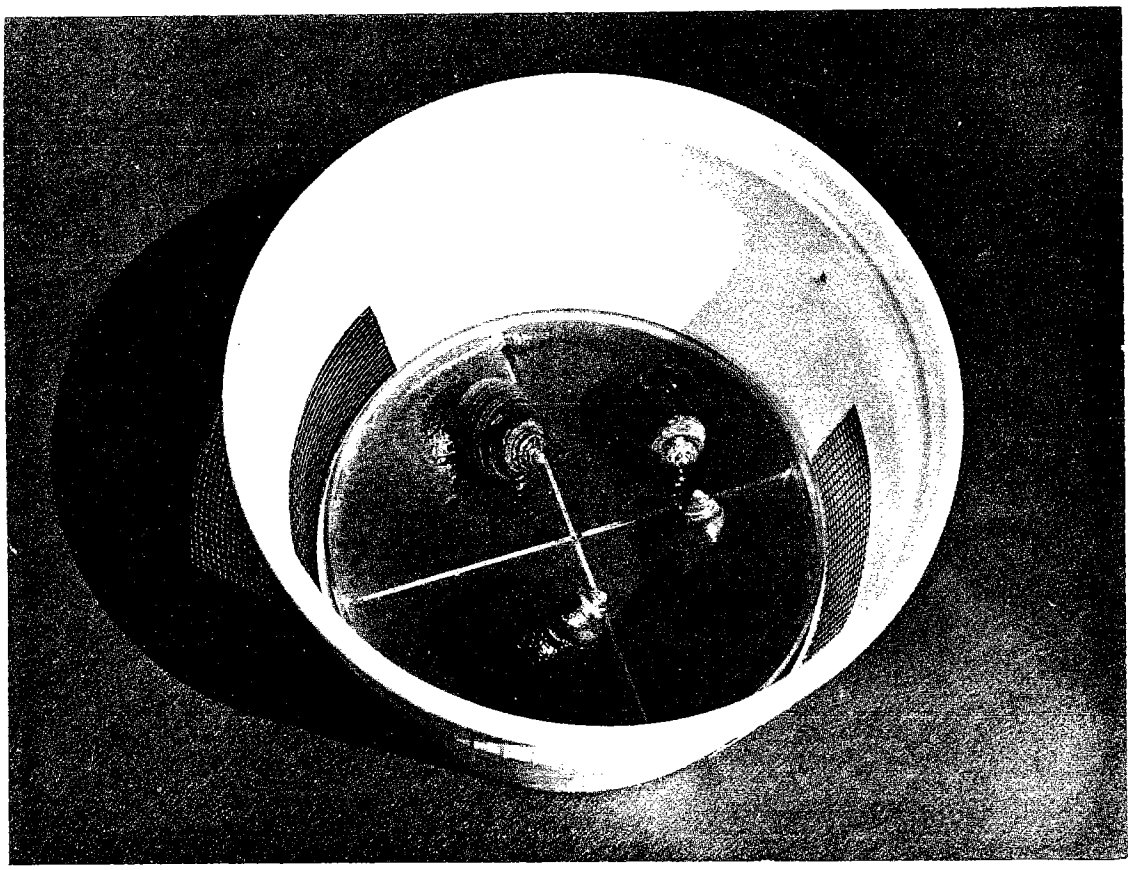


TABLE 1

Feeding Preferences of *Littorina littorea* for Algal Species in Agar Medium

1. Single Choice Experiments

Alga used in Agar Medium	Preference of Alga to *L. littorea**	% Medium consumed by four snails after four days (mean ± s.e., n=4)
CHLOROPHYTA		
Ulva lactuca	high	90 ± 5
Enteromorpha intestinalis	high	90 ± 5
Codium fragile	low	90 ± 10
RHODOPHYTA		
Porphyra umbilicalis	high	90 ± 5
Ceramium rubrum	high	65 ± 10
Polysiphonia flexicaulis	medium	40 ± 5
Chondrus crispus	low	10 ± 2
PHAEOPHYTA		
Ectocarpus siliculosus	high	70 ± 2
Sphaerotrichia divaricata	medium	40 ± 5
Fucus vesiculosus	low	10 ± 0
Ascophyllum nodosum	low	5 ± 2
Agar control		10 ± 2

*Data from field and laboratory experiments of Menge (1975), Lubchenco (1978), and Geiselman (unpublished data).

TABLE 2

Feeding Preferences of *Littorina littorea* for Algal Species in Agar Medium

2. Four-way Choice Experiments

Alga Used in Agar Medium	Preference of Alga to *L. littorea**	% Medium consumed after four days (mean ± s.e., n=4)
1. *Fucus vesiculosus*	low	0 ± 0
2. *Codium fragile*	low	30 ± 10
3. *Enteromorpha intestinalis*	high	95 ± 5
4. Agar control		0 ± 0
1. *Chondrus crispus*	low	10 ± 5
2. *Ceramium rubrum*	high	65 ± 5
3. *Porphyra umbilicalis*	high	98 ± 2
4. Agar control		0 ± 0
1. *Ascophyllum nodosum*	low	0 ± 0
2. *Sphaerotrichia divaricata*	medium	40 ± 2
3. *Ulva lactuca*	high	95 ± 5
4. Agar control		0 ± 0

*Data from field and laboratory experiments of Menge (1975), Lubchenco (1978), and J. Geiselman (unpublished data).

a chemical factor is involved. That is, those species of algae that are not eaten in the field or laboratory (Menge, 1975; Geiselman, unpubl. data) were still not acceptable as food when homogenized and incorporated into agar media. Those species of algae normally eaten in the field and laboratory were the preferred food when homogenized into agar media. One exception was found. Codium fragile, which normally is of low food preference to the snails, was acceptable as food when homogenized and incorporated into agar. This suggests that its low preference is due to some factor, perhaps a volatile chemical (Chapter 5) or the tough coenocytic cell wall, which is lost or destroyed during the homogenization and incorporation into warm agar media. However, when offered a choice between media, snails selectively consumed media prepared with preferred food species over media prepared with C. fragile. This suggests that the antiherbivore factor may be partially destroyed but not entirely lost during the experimental procedures.

The experiments described above led to the development of a method to test the effect of algal chemicals on the feeding preferences of the herbivores. Suspected defensive chemicals extracted from the algae were added to an agar medium containing homogenized algae preferred by the snails. The effect of the chemicals on the feeding of the snails was then observed and quantified by comparing the amount of the experimental media consumed to the amount of control media consumed, using the divided petri plates and test arenas described above.

Chemical investigation of the brown algae F. vesiculosus and A. nodosum was begun by comparing the effect on grazing of compounds

extracted with methanol versus those extracted with methylene chloride. For each alga, a 50 g. sample (wet weight) in 300 ml. of CH_3OH and another in 300 ml. of CH_2Cl_2 were allowed to stand for 24 hours and then homogenized and filtered. The extracts were evaporated to near dryness using a Buchi Rotavapor at 35°C. Residues were mixed into 50 g. of food media consisting of a mixture of Ulva lactuca and Enteromorpha intestinalis homogenized in 1.8% agar-seawater. Methanol and methylene chloride extracts of a mixture of U. lactuca and E. intestinalis were similarly tested in the food media. The amounts consumed of these media with added extracts were compared to the amount consumed of the control food media with no added extracts (control media did have an equal volume of CH_3OH or CH_2I_2 added). Results indicated that compounds extractable with methanol from both F. vesiculosus and A. nodosum cause inhibition of feeding by L. littorea (Figure 2).

The next set of experiments was designed to partition the components in the active methanol extracts and to bioassay each fraction. For each alga, a 50 g. sample was extracted for 24 hr. in 300 ml. of methanol and the extract partitioned between methylene chloride and methanol-water (3:1). Both fractions were concentrated and bioassayed in the food medium with L. littorea. Figure 3 shows that the active compounds in F. vesiculosus and A. nodosum are concentrated in the methanol-water fractions. Thin layer chromatography of these active fractions on silica gel and polyamide revealed spots which were UV-absorbent and which stained reddish-brown with the Lindt reagent (vanillin-HCl). This

Figure 2. Effect of algal methanol and methylene chloride extracts on feeding of L. littorea.

$$\% \text{ media consumed} = \frac{\text{experimental media consumed}}{\text{control media consumed}} \times 100\%,$$

(mean $\pm$ s.e., n = 4).

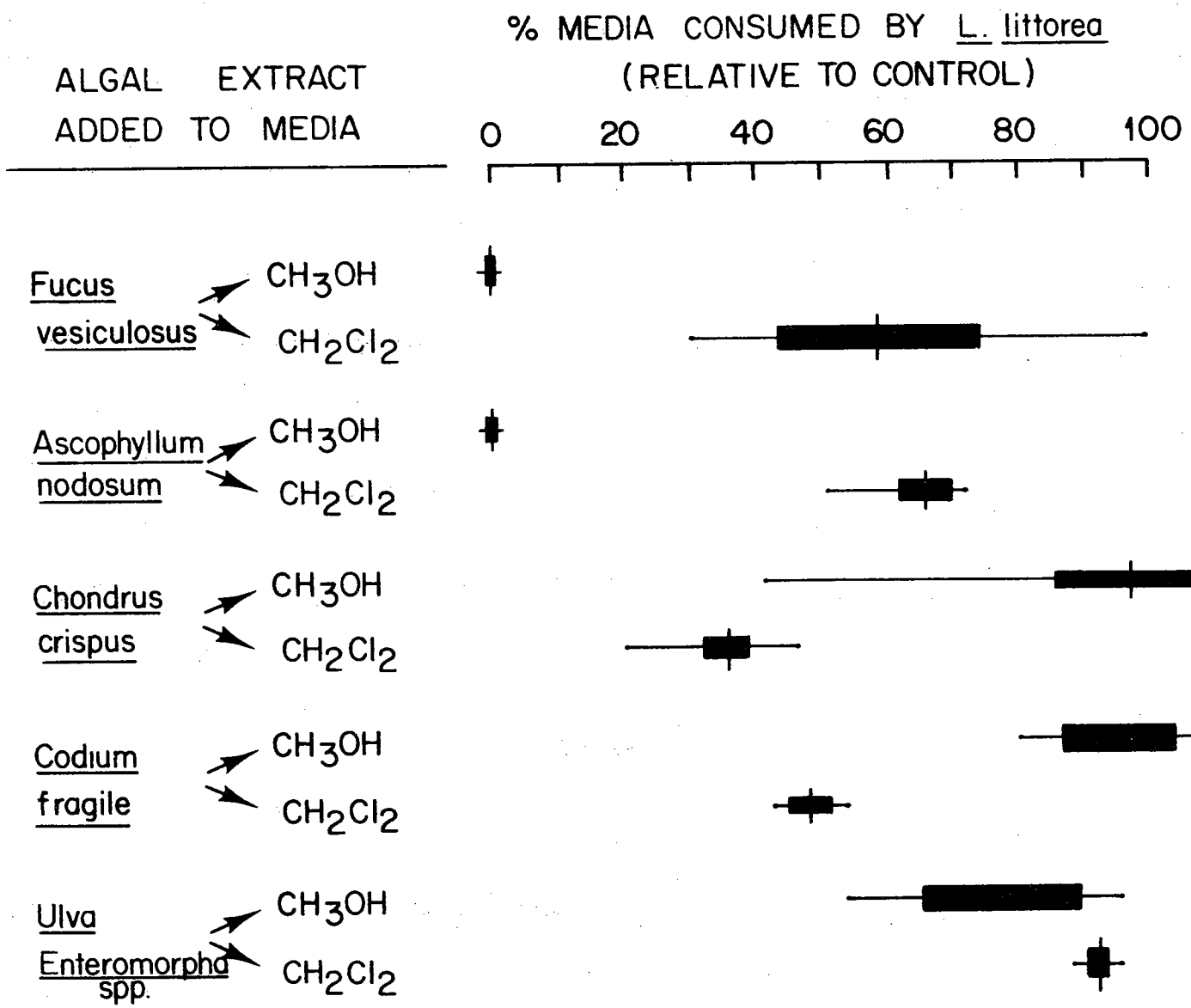
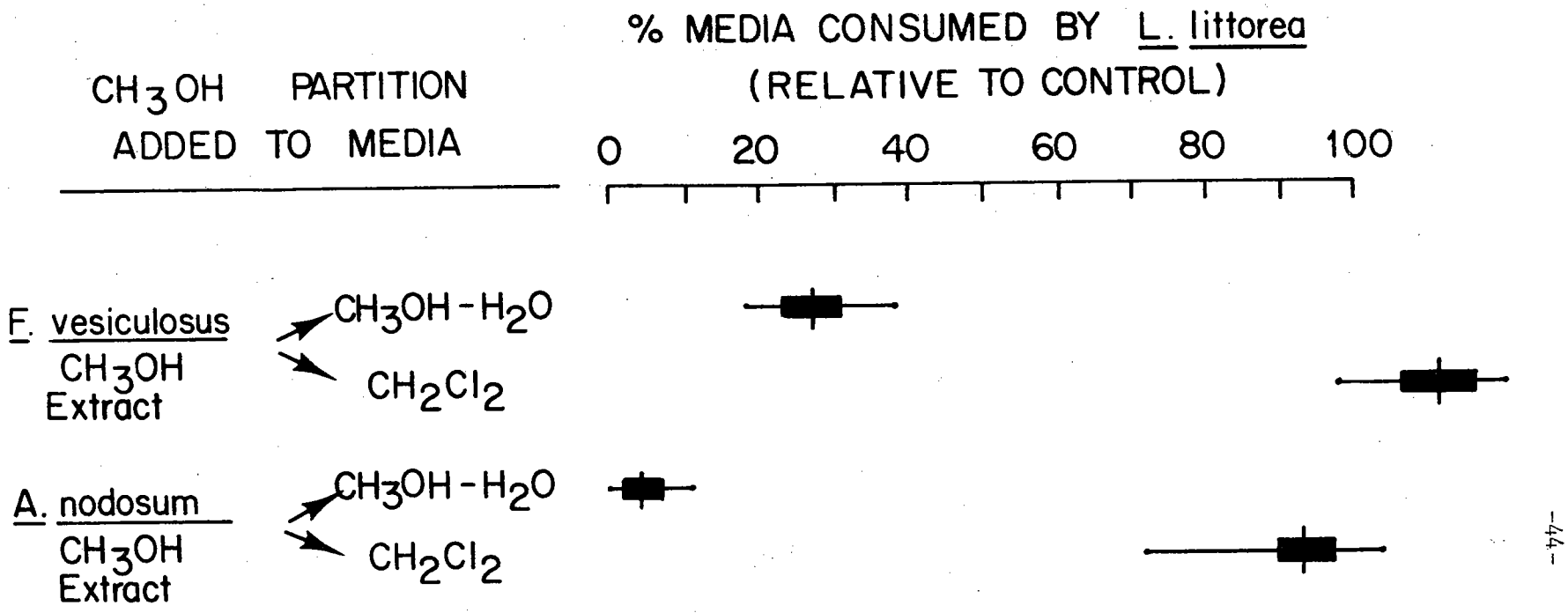

% MEDIA CONSUMED BY L. littorea
(RELATIVE TO CONTROL)
ALGAL EXTRACT
ADDED TO MEDIA
0
20
40
60
80
100
Fucus vesiculosus
CH3OH
CH2Cl2
Ascophyllum nodosum
CH3OH
CH2Cl2
Chondrus crispus
CH3OH
CH2Cl2
Codium fragile
CH3OH
CH2Cl2
Ulva Enteromorpha spp.
CH3OH
CH2Cl2

Figure 3. Effect of methanol-water and methylene chloride partitions of *F. vesiculosus* and *A. nodosum* methanol extracts on feeding of *L. littorea*.

$$\% \text{ media consumed} = \frac{\text{experimental media consumed}}{\text{control media consumed}} \times 100\%,$$

(mean $\pm$ s.e., n = 4).


% MEDIA CONSUMED BY L. littorea
(RELATIVE TO CONTROL)
CH3OH PARTITION
ADDED TO MEDIA
0
20
40
60
80
100
F. vesiculosus
CH3OH
Extract
CH3OH - H2O
CH2Cl2
A. nodosum
CH3OH
Extract
CH3OH - H2O
CH2Cl2

suggests that the active fractions contain phenolic or tannic compounds, perhaps similar to those identified in brown algae by Glombitza (1977) and Ragan and Craigie (1976) or to those found in terrestrial plants which have been shown to be effective antiherbivore compounds (Feeny, 1970; Dement and Mooney, 1974).

Chondrus crispus and Codium fragile samples were similarly extracted with methanol and methylene chloride. The methanol extracts of these algae were ineffective in deterring L. littorea feeding in contrast to those of the brown algae. The methylene chloride extracts of both algae reduced feeding but were not as effective as brown algal methanol extracts. C. crispus extract was more effective than that of C. fragile, agreeing with the preliminary experiments which indicated that some compounds in C. fragile may be active against feeding but are, perhaps, volatile and partially lost or transformed to other products during the extraction procedures or feeding experiments (in spite of precautions taken such as using low temperatures for rotoevaporation of the extracts and incorporation of extracts into cooled agar media).

CONCLUSION

I have developed a method to test compounds extracted from marine algae for their effect on the feeding of herbivorous snails. Analyses of the active extracts from algae not preferred as food by L. littorea are underway to identify the antiherbivore compounds and determine if the production of these defenses varies seasonally, during different stages of life cycles, with age or size, or in different parts of the plants; results will be reported in subsequent papers. At this point I conclude

that three dominant, perennial algae of the New England rocky intertidal community (F. vesiculosus, A. nodosum, and C. crispus) have chemical defenses against the major herbivore in the community, L. littorea. The low food preference to L. littorea of C. fragile, an annual alga recently introduced to this community, appears to be due to factors partially lost during experimental procedures, perhaps volatile chemicals or structural toughness.

ACKNOWLEDGMENTS

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CHAPTER 2

Polyphenols in *Fucus vesiculosus* and *Ascophyllum nodosum*: Chemical Defenses against the Herbivorous Snail, *Littorina littorea*

ABSTRACT

Polyphenols from two brown algae, Fucus vesiculosus and Ascophyllum nodosum, were shown to inhibit feeding by the herbivorous snail, Littorina littorea. This research is the first demonstration that chemical compounds defend these two dominant, perennial marine algae from the major herbivore found in their community. The active compounds from the algae were characterized as high molecular weight polymers of phloroglucinol (polyphenols) by means of thin layer chromatography, infra-red spectroscopy, ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy, and membrane ultrafiltration. These compounds were previously shown to be localized intracellularly in brown algal physodes. As little as 1% (dry wt.) polyphenol in food media reduced feeding by more than 50%. Certain polyphenolic extracts inhibited feeding entirely when present in concentrations of 2 - 5% (dry wt.) in food media. Phloroglucinol dihydrate and gallotannin, which are commercially available phenols known to be herbivore deterrents in terrestrial plants, inhibited L. littorea feeding when added to food media in concentrations similar to those above. I conclude that polyphenols in F. vesiculosus and A. nodosum are functionally similar to terrestrial plant polyphenols (tannins) in their roles as chemical defenses against herbivores.

INTRODUCTION

Fucus vesiculosus and Ascophyllum nodosum are dominant, perennial plants in high and mid rocky intertidal zones of New England. The major herbivore on macroscopic algae in this intertidal community is the periwinkle snail, Littorina littorea. Although in abundant supply, neither of these fucoid algae is utilized for food by this herbivore. The only exceptions to this observation appear to be certain instances of littorine grazing on sporeling stages or on mature fucoids only in winter when all other food species are unavailable (Menge, 1975). Lubchenco (1978) hypothesized that there is some chemical or mechanical change in fucoids correlated with larger size that makes F. vesiculosus and A. nodosum less attractive as food for L. littorea. It has since been demonstrated that methanol extracts of F. vesiculosus and A. nodosum inhibit feeding by L. littorea when added to preferred food sources (Chapter 1). The purpose of this investigation was to isolate and identify the compounds in these extracts which are active as chemical defenses and to determine their effective concentrations ("ED_{50}" = dose required to inhibit feeding by 50%; e.g., see Russell et al.,1978) against L. littorea.

METHODS AND RESULTS

Algal Collection, Extraction, and Bioassay

F. vesiculosus and A. nodosum were collected during low tides along the coast of Vineyard Sound, Massachusetts. After removal of epiphytes and animals from their surfaces, the algae were extracted either immediately or after drying. Preliminary experiments showed that methanol extracts from dried fucoids showed similar levels of antiherbivore activity as extracts from fresh plants. Hence, analyses were initially carried out on dried plants, and, as a precaution against structural changes on drying, were thereafter performed on fresh plants; results are compared below.

The bioassay used to determine the effects of compounds on feeding by L. littorea was similar to that described previously (Chapter 1). Food media consisted of Ulva and Enteromorpha spp. homogenized in seawater (1:1 wet wt.) and solidified with agar. Extracted compounds were dissolved in a small volume of ethanol/water (depending on solubility), and an equal volume of ethanol/water was added to the control food media. Extracts were incorporated into the food media in concentrations equivalent to those in the fresh plant unless otherwise noted (e.g., in dose-response experiments). The experimental and control media were offered in pairs in divided petri plates which were placed in running seawater tanks (18^{o}C). Rather than being confined to individual plates in screened containers (as described in Chapter 1), the snails were allowed to roam the tank, feeding freely on the plates and media. This

procedure change was initiated because snails tended to climb the walls of the containers when enclosed, causing time delays in the feeding experiments. A grid was used to determine the area (%) of experimental and control media consumed. Results for each experimental medium were expressed as % of control medium consumed.

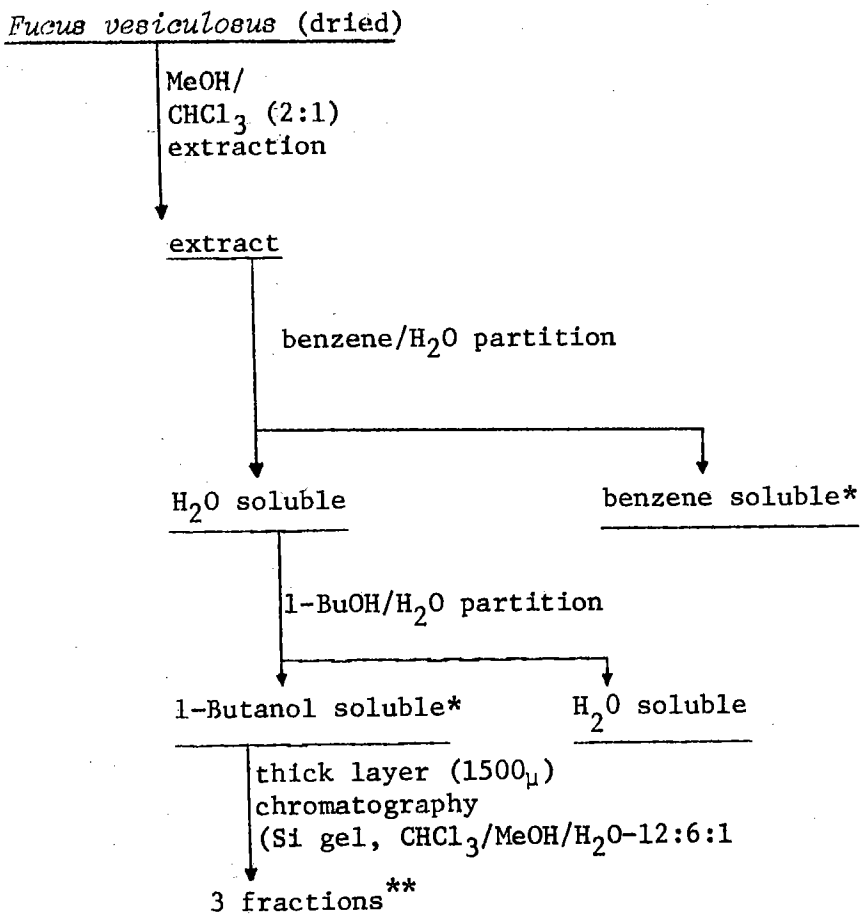
Isolation of Activity in Crude Fractions of Fucus vesiculosus(dried)

F. vesiculosus was extracted according to the scheme depicted in Figure 1. Bioassay of the benzene and 1-butanol partitions indicated that the 1-butanol partition totally inhibited feeding by L. littorea (Figure 1). The benzene partition also exhibited antiherbivore activity, but it was less than that of the 1-butanol partition. Therefore, further analyses proceeded with the more active 1-butanol partition. Thin layer chromatography (silica gel, ethyl ether/benzene - 1:1) of the benzene partition revealed expected lipid components, and the NMR spectral data indicated an abundance of triglyceride absorptions where linolenic or linoleic was the predominant type of fatty acid . Thin layer chromatography (silica gel, $CHCl_3$/MeOH/H_2O - 12:6:1) (Table 1) and NMR spectral data of the 1-butanol partition indicated that polyaromatic or tannin-like substances were present because of the low mobility and UV fluorescence on the TLC plates and the NMR proton absorbtions near 6d .

The 1-butanol partition of F. vesiculosus was next separated into three fractions by preparative thick layer (1500 microns) silica gel chromatography ($CHCl_3$/MeOH/H_2O - 12:6:1). The most polar section (R_f = 0.0 - 0.3) contained most of the mass of the extract, exhibited

Figure 1. First extraction scheme for Fucus vesiculosus (dried) and results from bioassays.

EXTRACTION SCHEME:



BIOASSAY RESULTS:

	Addition to media	% media consumed (relative to control) mean ± S. E., n = 3
*I.	Benzene partition	23.3 ± 8.8
	1-Butanol partition	0 ± 0
**II.	Fraction 1 (R_f 0.6-0.8)	100
	Fraction 2 (R_f 0.3-0.6)	100
	Fraction 3 (R_f 0.0-0.3)	0

TABLE 1. Thin layer chromatography of 1-butanol partition of *F. vesiculosus* MeOH/$CHCl_3$ extract. Solvent system = $CHCl_3$/MeOH/H_2O) (12:6:1).

R_f	Color from 50% H_2SO_4 (aqueous) char	Fluorescence from UV	Fluorescence from VIS
0.83	green and brown	-	-
0.78	blue	-	✓
0.73	purple	-	✓
0.64	light green	-	✓
0.55	brown/purple	-	✓
0.45	purple/pink	-	✓
0.35	light tan/orange	-	✓
0.11-0.26	light tan/green	✓	-
0.0	brown/black (origin)	✓	-

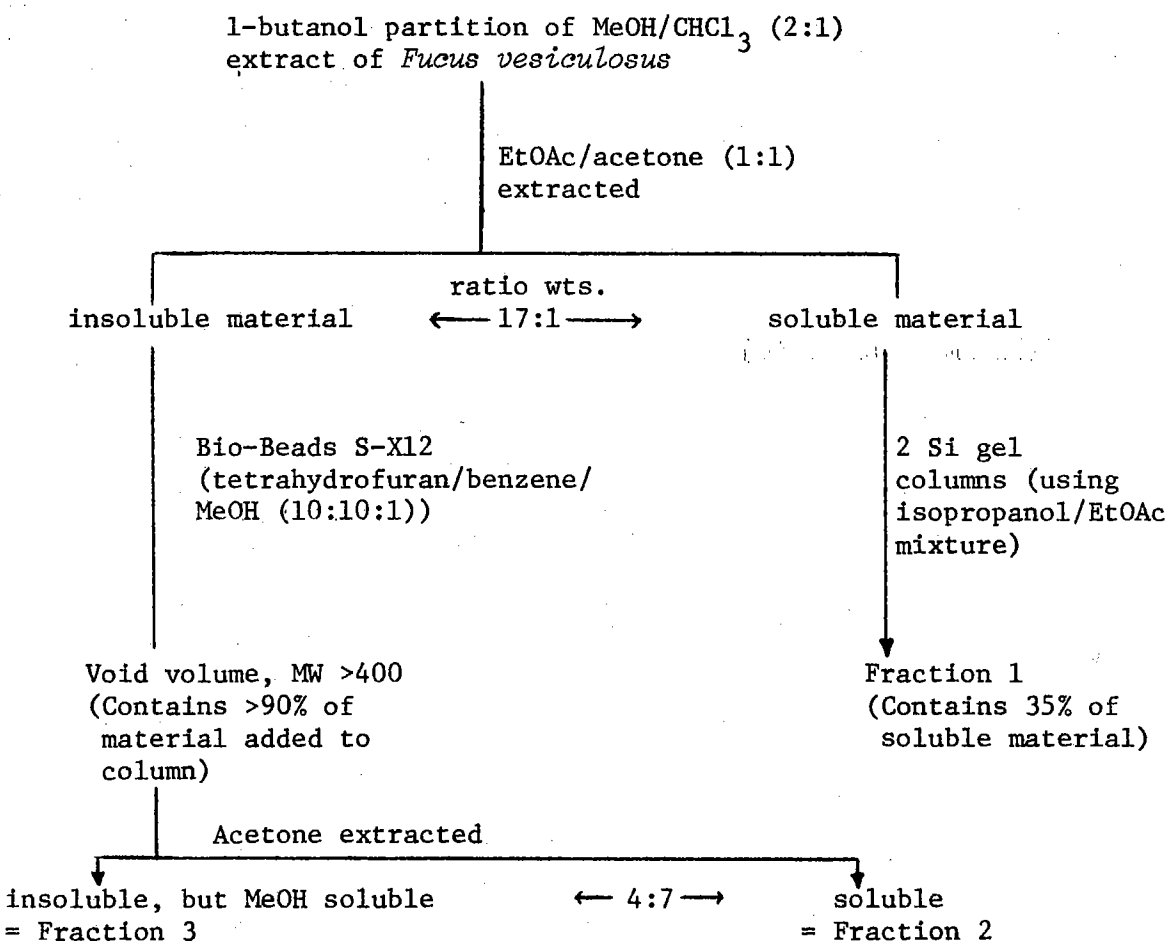
absorptions at δ5.9 - 6.2 in the ^{1}H NMR, and showed a positive reaction with vanillin. The less polar (top, middle) layers exhibited aromatic absorptions in the NMR at δ7.5 but did not react as strongly with vanillin. The bioassay of the three fractions showed that feeding deterrent activity was isolated in the most polar fraction (Figure 1).

Activity of Polyphenolic Fractions from F. vesiculosus

The results above showed that feeding deterrent activity against L. littorea was isolated in polar fractions of 1-butanol partitions of F. vesiculosus MeOH/$CHCl_3$ extracts. Because the thick-layer chromatographic separations did not yield large enough quantities for several bioassays, the 1-butanol partition was next separated into three polyphenolic fractions using solvent partitioning and gel and adsorption chromatography (Figure 2). This separation yielded a large enough quantity of each fraction to bioassay in a dose-response experiment. Fraction 3 was most active, reducing feeding by 50% (ED_{50}) at a concentration of 0.2 mg./g. media (=0.2% dry wt.) (Figure 3). Fractions 1 and 2 reduced feeding by 50% at the higher concentrations of 4.0 - 4.6 mg./g media (4.0 - 4.6% dry wt.). Since fraction 1 was present in the F. vesiculosus extracts in a much lower amount than fractions 2 and 3 (ratio of weights of fraction 1 to weight of fractions 2 and 3 combined = 1:17), it appears that the compounds in fractions 2 and 3 account for most of the chemical resistance of F. vesiculosus to L. littorea.

Fractions 2 and 3 were then subjected to molecular weight fractionation by ultrafiltration using an Amicon membrane (XM100A) with

Figure 2. Second extraction scheme for Fucus vesiculosus (dried).



Fraction 1 = relatively nonpolar, low molecular weight polyphenolic

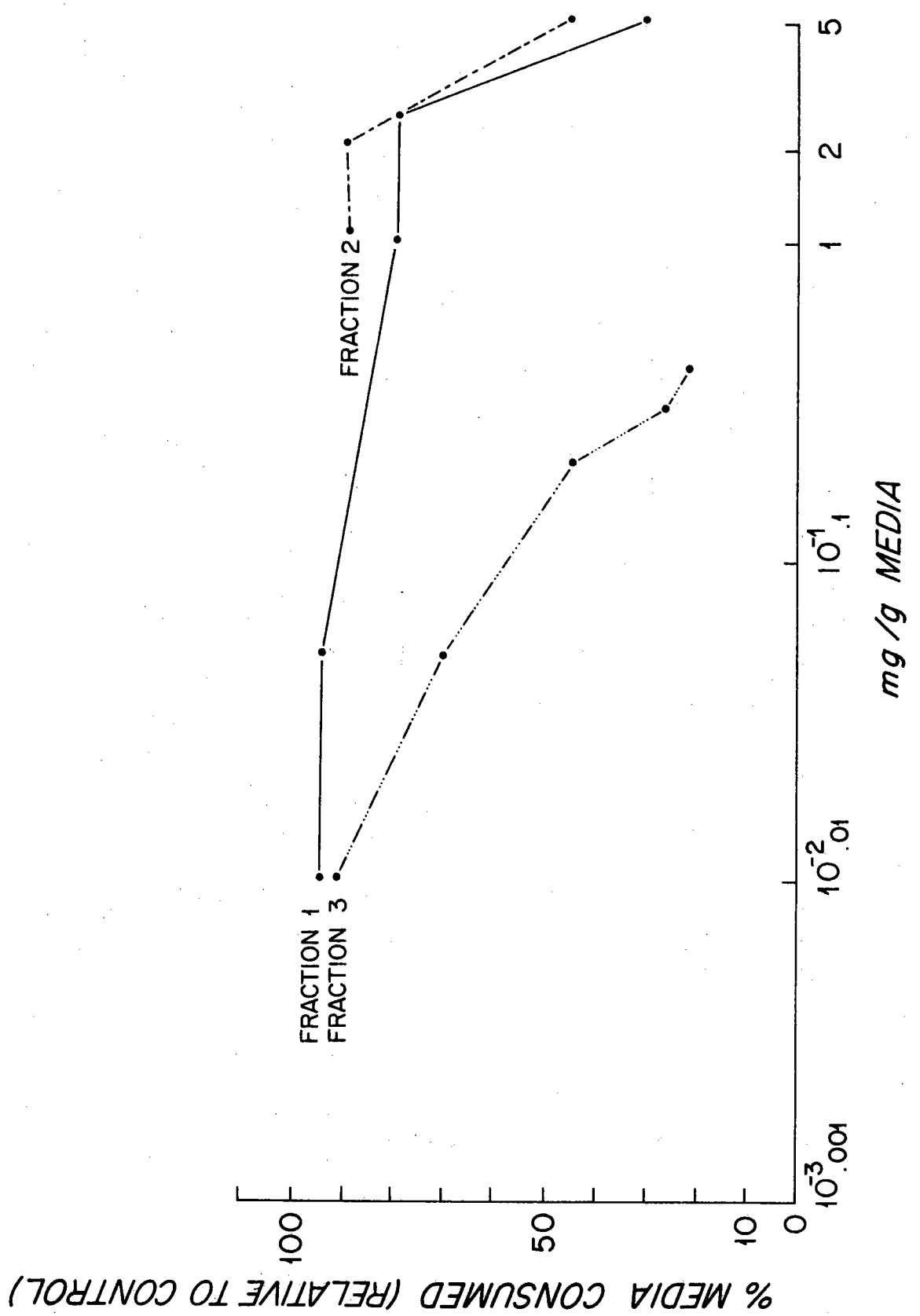
Fraction 2 = high molecular weight, acetone soluble mixture of polyphenolics [including 1) and 3)]

Fraction 3 = high molecular weight, acetone insoluble but methanol soluble mixture of polyphenolics

Figure 3. Dose-response curve for the effect of fractions 1, 2, and 3 from Fucus vesiculosus (second extraction scheme, Figure 2) on feeding by L. littorea.

$$\% \text{ media consumed} = \frac{\text{experimental media consumed}}{\text{control media consumed}} \times 100\%,$$

(n = 1 because of the small quantities of fractions obtained).


% MEDIA CONSUMED (RELATIVE TO CONTROL)
100
50
10
0
FRACTION 1
FRACTION 3
FRACTION 2
10^{-3} .001
10^{-2} .01
10^{-1} .1
1
2
5
mg/g MEDIA

molecular weight cut-off at 100,000 (note: gel chromatography with Sephadex and BioBeads gels had been ineffective in separating higher molecular weight polyphenols). Results are shown in Table 2. Recovery for each fraction was 98% and 85%, respectively. Fraction 2 had a higher percentage of molecular weights over 100,000 (58.2%) than did fraction 3 (37.6%). Each of these m.w. fractionations were than bioassayed. Each reduced feeding (compared to control) by a significant amount (greater than 50%), and the most active were greater than m.w. 100,000 in the acetone-soluble fraction Table 3.

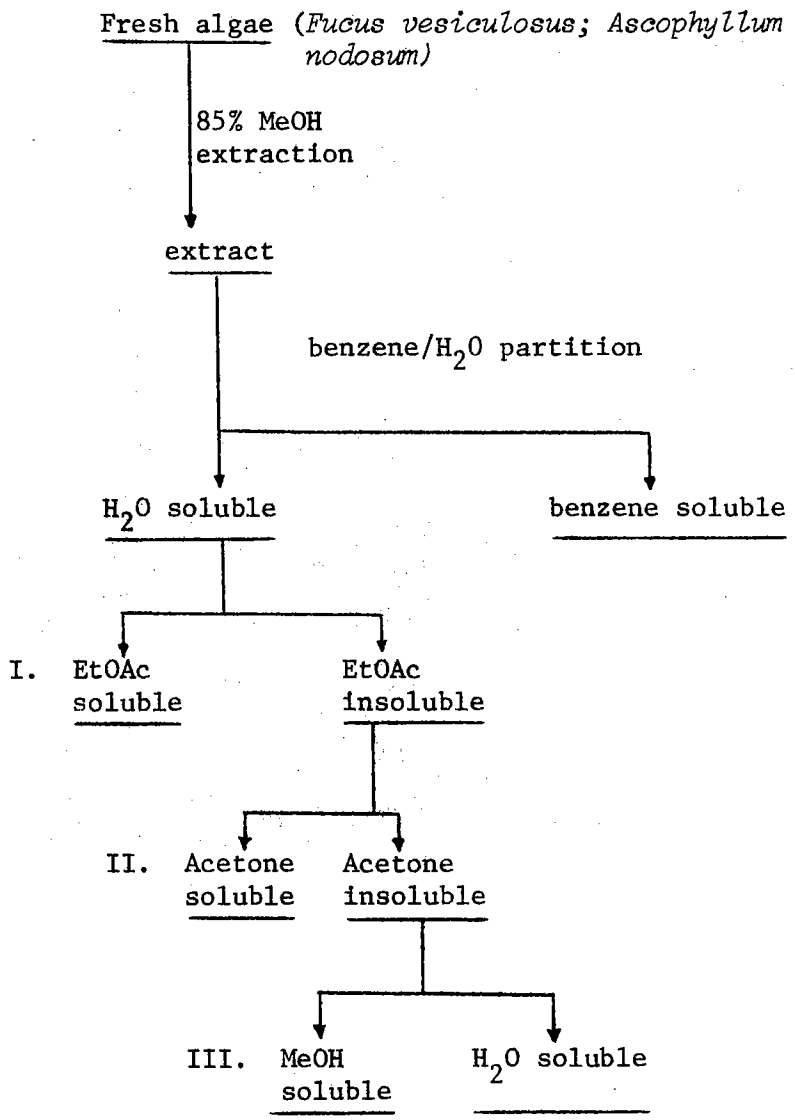
After these results from extracts of dried F. vesiculosus, work was begun on extracts of fresh F. vesiculosus and A. nodosum using similar extraction schemes and molecular weight fractionations. Each fraction was bioassayed with L. littorea in concentrations of 0.1%, 1.0%, and 2.0% (dry wt. of media). These concentrations are similar to polyphenol contents determined in F. vesiculosus and A. nodosum during a year-long study (Chapter 3) and hence are realistic in terms of what the snails encounter in these two algal species.

Freshly collected F. vesiculosus (1595 g.) and A. nodosum (1405 g.) were each homogenized and extracted with 7 1. 85% aqueous methanol (Figure 4). All organic solvents were distilled in glass before use. Water was deionized and distilled twice. Solvent and algal grounds were placed in 3 to 4 1. Erlenmeyer flasks, flushed with nitrogen, stoppered, stored at 10°C in the dark, and swirled periodically. After two days, the extraction solvent was filtered, and the methanol removed in vacuo and recycled. The algae were extracted two additional times under the

TABLE 2. Molecular weight fractionation of *F. vesiculosus* acetone soluble and methanol soluble fractions.

	Recovery	MW < 100,000	MW > 100,000
Fraction 2 - Acetone soluble	98	58.2	41.8
Fraction 3 - Methanol soluble	85	37.6	62.4

Figure 4. Extraction scheme for Fucus vesiculosus and Ascophyllum nodosum (fresh).



I. Phloroglucinol + Low MW phloroglucinol polymers

II. High MW phloroglucinol polymers

III. High MW phloroglucinol polymers

TABLE 3. Bioassay of *F. vesiculosus* acetone soluble and methanol soluble fractions with MW cut-offs at 100,000).

	% media consumed, relative to control (mean ± $s_{\bar{x}}$, n = 2)
Fraction 2 - Acetone soluble	
<100,000	7.5 ± 0
>100,000	2 ± 0
Fraction 3 - Methanol soluble	
<100,000	31 ± 7
>100,000	25 ± 7

same conditions for one day periods. The residual aqueous layer (1 - 1.2 1.) from the first extraction was shaken twice with 0.5 1. benzene and the benzene layers combined. Water was removed from the aqueous phase in vacuo at approximately 10 mm. Hg and 40°C. The residue was successively extracted with 1 1. ethyl acetate (EtOAc), 1 1. acetone, and 1 1. MeOH. The second and third extractions were handled similarly, and the solvent partitions combined. NMR and IR data for the F. vesiculosus and A. nodosum solvent partitions are given in Tables 4 - 8.

Molecular weight fractions of these solvent partitions were subsequently obtained by ultrafiltration (gel chromatography with Sephadex and Bio-Beads gels was ineffective in separating higher molecular weight phloroglucinol polymers in F. vesiculosus and A. nodosum). A 400 ml. Amicon stirred-cell with 76 mm. membranes was used for all molecular weight separations. Approximately 100 ml. of deionized and twice-distilled water was used for per 100 mg. of sample. The membranes were prepared by soaking in distilled water at least one hour prior to use followed by washing in the cell under pressure with approximately 150 - 250 ml. water. During molecular weight separation, the membrane integrity was monitored by observing the filtrate flow rate. When the flow rate decreased for a given nitrogen pressure, the membrane was changed. The filtrate was subsequently passed through a new membrane prepared as discussed above. The higher molecular weight or retained fractions were combined. Water was removed by freeze-drying. Adsorption on the the Amicon membranes was significant but tolerable

TABLE 4. ^{1}HNMR data from *F. vesiculosus* (fresh) EtOAc, Acetone, and MeOH solvent partitions.

I. EtOAc soluble (d_6-acetone/TMS, 60 MHZ)

δ	rel. #H	
5.83	1	
6.00-6.03	7	
6.13	5	
6.33	4	
6.97	-	D_2O exchangeable
7.27	-	"
7.73	-	"
8.00	-	"

II. Acetone soluble (d_6-acetone + d_4-MeOH [trace]/TMS; 60 MHZ

δ	rel. #H	
5.80	2	
5.97-6.0	12	
6.07-6.10	7	
6.67	5	
7.43	-	D_2 exchangeable
7.83	-	"
8.1	-	"

III. MeOH soluble (d_6-acetone + D_2O [1:1]/TMS + DSS [sodium 2, 2-dimethyl-2-silapentane-5-sulfonate], 60 MHZ)

δ	rel. #H
5.85	1
5.97-6.02	6
6.13-6.20	5
6.30	3

TABLE 5. 'HNMR data from *A. nodosum* (fresh) EtOAc, Acetone, and MeOH solvent partitions.

I. EtOAc soluble (d_6-acetone/TMS, 60 MHZ)

δ	rel. #H	
5.88	3	
5.98-6.02	11	
6.17	8	
6.32	5	
7.87-8.03	-	D_2O exchangeable

II. Acetone soluble (d_6-acetone + d_4-MeOH [trace]/TMS, 60 MHZ)

δ	rel. #H	
5.87	4	
5.98	17	
6.13	12	
6.3	7	
7.97-8.2	-	D_2O exchangeable

III. MeOH soluble (d_6-acetone + D_2O [1:1]/TMS + DSS, 60 MHZ)

δ	rel. #H
5.93	4
6.10	11
6.23	7
6.33	5

TABLE 6. ^{13}C NMR data from *F. vesiculosus* (fresh) acetone partition.

II. Acetone soluble, MW >100,000
(d_6-acetone/TMS, 50 MHZ)

δ 94-100, 123-125, and 149-161

δ	rel. int.	δ	rel. int.	δ	rel. int.
94.2	1.9	96.3	1.8	123.8	1.9
94.4	4.0	96.5	1.4	124.0	1.0
94.5	4.0	96.6	1.8	124.2	1.7
94.7	3.2	96.7	3.7	124.2	1.5
94.8	3.2	96.9	2.4	124.3	1.1
94.9	3.3	97.2	1.2	124.7	1.7
95.0	3.4	97.5	3.3	125.0	1.7
95.2	7.3	97.6	1.6	149.5	1.0
95.7	19.4	100.1	1.2	149.9	4.0
96.1	1.6	123.5	1.1	150.4	1.3
96.2	1.6	123.7	2.0	150.5	1.7
150.7	2.9	153.5	1.4	155.5	5.9
151.1	12.0	153.6	1.7	155.6	6.2
151.4	2.5	153.8	1.7	157.0	1.1
151.7	1.7	153.9	1.7	157.2	1.9
151.9	1.1	154.0	1.8	157.6	6.0
152.5	1.2	154.1	1.3	157.9	5.2
152.7	3.4	155.0	1.5	158.6	1.1
152.8	3.7	155.2	3.4	158.7	1.1
152.8	3.7	155.4	5.2	159.1	2.1
153.1	3.3			161.2	1.4
153.3	1.2				
153.4	1.1				

TABLE 7. IR data from *F. vesiculosus* and *A. nodosum* (fresh) EtOAc, acetone, and MeOH solvent partitions. (The IR's of solvent partitions I, II, and III of *F. vesiculosus* and *A. nodosum* were identical.)

I. EtOAc soluble (Tetrahydrofuran solution, NACl plates)

ν (cm^{-1})	Shape
3280	Strong, Broad
3480	Strong, Broad
1610	Med., Broad

II. Acetone soluble (Nujol mull, MaCl plate)

ν (cm^{-1})	Shape
3260-3500	Very Broad
1600	Med., Broad

III. MeOH soluble (Nujol mull, NaCl plate)

ν (cm^{-1})	Shape
3300-3500	Very Broad
1600	Med., Broad

TABLE 8. Percent recovery during molecular weight separations of *F. vesiculosus* and *A. nodosum* solvent partitions.

Amicon membrane (MW cut-off)	% Recovery - Avg. (range)	
	II. Acetone sol.	III. MeOH sol.
XM 300 (300,000)	75	-
XM 100 (100,000)	96 (86-100)	85
XM 50 (50,000)	61	76
YM 30 (30,000)	-	93 (85-100)

(Table 8). The molecular weight data from ultrafiltration of F. vesiculosus and A. nodosum solvent partitions are summarized in Table 9.

The chemical data for the extracts from dried and fresh F. vesiculosus and A. nodosum were very similar; only two differences were noted: 1) there were greater relative amounts of EtOAc soluble phloroglucinol polymers in fresh than in dry algae, and 2) there were greater relative amounts of low molecular weight (less than 100,000) phloroglucinol polymers in acetone soluble fractions in fresh algae than in dry algae.

The ethyl acetate, acetone, and MeOH partitions from fresh F. vesiculosus and A. nodosum were bioassayed at concentrations of 0.1%, 1.0%, and 5.0% (dry wt. media). The results were similar to those of extracts from the dried algae. All the partitions from both algal species caused significant reduction of feeding (less than 50% experimental media consumption relative to control) at 5.0% concentration; the acetone and MeOH partitions were also active at the lower concentrations of 0.1% and 1.0% (Table 10). These more active acetone and MeOH partitions from F. vesiculosus and A. nodosum were then divided into molecular weight fractions by ultrafiltration, and each fraction was bioassayed at concentrations of 0.1%, 1.0%, and 2.0%. In addition, F. vesiculosus EtOAc, acetone, and MeOH partitions were combined, divided by molecular weights, and bioassayed to determine if molecular weight alone was important in determining activity of compounds. Results indicated that all molecular weight fractions of acetone and MeOH partitions from both F. vesiculosus and A. nodosum

TABLE 9. Solvent partition and molecular weight data of *F. vesiculosus* and *A. nodosum* (fresh) samples.

1. Solvent partitions

	% wet wt.	wt. (gm.)
F. vesiculosus:		
I. EtOAc	0.2	3.6
II. Acetone	0.5	7.7
III. MeOH	0.9	14.3
A. nodosum:		
I. EtOAc	0.6	8.1
II. Acetone	0.5	6.4
III. MeOH	1.2	17.8

2. Molecular weight data from ultrafiltration

Combined EtOAc, acetone and MeOH solvent partitions	MW	Avg.-%	Range - %
F. vesiculosus:			
	>100,000	19	17-21
	30,000-100,000	41	38-44
	< 30,000	40	36-44
II. Acetone soluble	>100,000	29	23-33
	<100,000	71	67-76
III. MeOH soluble	> 30,000	55	48-65
	< 30,000	45	35-52
A. nodosum:			
II. Acetone soluble	>100,000	32	32-33
	<100,000	68	67-68
III. MeOH soluble	> 30,000	35	35
	< 30,000	65	65

TABLE 10. Consumption by *L. littorea* of media treated with various solvent partitions of *F. vesiculosus* and *A. nodosum* (mean ± S. E.; n=2).

Solvent Partition		% concentration of partition in media		
		0.1	1.0	5.0*
		% of media consumed (relative to control)		
F. vesiculosus				
I.	EtOAc	50	55	45
II.	Acetone	17±6	16±5	13%
III.	MeOH	12±1	12±0	0
A. nodosum				
I.	EtOAc	59±13	41±3	40
II.	Acetone	33±3	38±5	13%
III.	MeOH	20±6	20±8	10

*n=1 for all bioassays of this highest concentration because of limited amounts of material obtained from extraction scheme.

reduced feeding significantly (less than 50% experimental media consumed relative to control) at 1.0% and 2.0% (dry wt. media) (Table 11). At the lower concentration of 0.1%, both molecular weight fractions (one with MW's less than 100,000, the other with MW's greater than 100,000) of the F. vesiculosus acetone partition appeared more active than the methanol partition molecular weight fractions. The A. nodosum acetone fraction with molecular weight greater than 100,000 was more active than that with molecular weight less than 100,000 and both molecular weight fractions of the methanol partition. Bioassays of the combined partitions which were divided by molecular weight showed that all fractions inhibited feeding at 1.0% and 2.0% without any apparent relation to molecular weight; at 0.1% the fractions with molecular weight less than 100,000 appeared more active than those with molecular weight greater than 100,000.

Because high molecular weight polyphenols in F. vesiculosus and A. nodosum were previously shown to be polymers of phloroglucinol (Ragan and Craigie, 1976; Glombitza, 1977), it was decided to bioassay this compound (Aldrich, C_6H_3-1,3,5-$(OH)_3 \cdot 2H_2O$, m.w. 162.14) to see if this low molecular weight precursor to the polymers inhibits feeding of snails. Phloroglucinol is reported to be a constituent of brown algal physodes and is found in terrestrial plants as well. In addition, gallotannin (Pfaltz and Bauer, $C_{76}H_{52}O_{46}$, m.w. 1700) was bioassayed. This higher molecular weight polyphenol is common in terrestrial plants and has demonstrated antiherbivore activity against insects (Todd et al., 1971). Results are summarized in the dose-response curves of Figure 5. Phloroglucinol inhibited snail feeding by at least

TABLE 11. Consumption by *L. littorea* of media treated with various molecular weight fractions of *F. vesiculosus* and *A. nodosum* solvent partitions (mean ± S. E., n = 2).

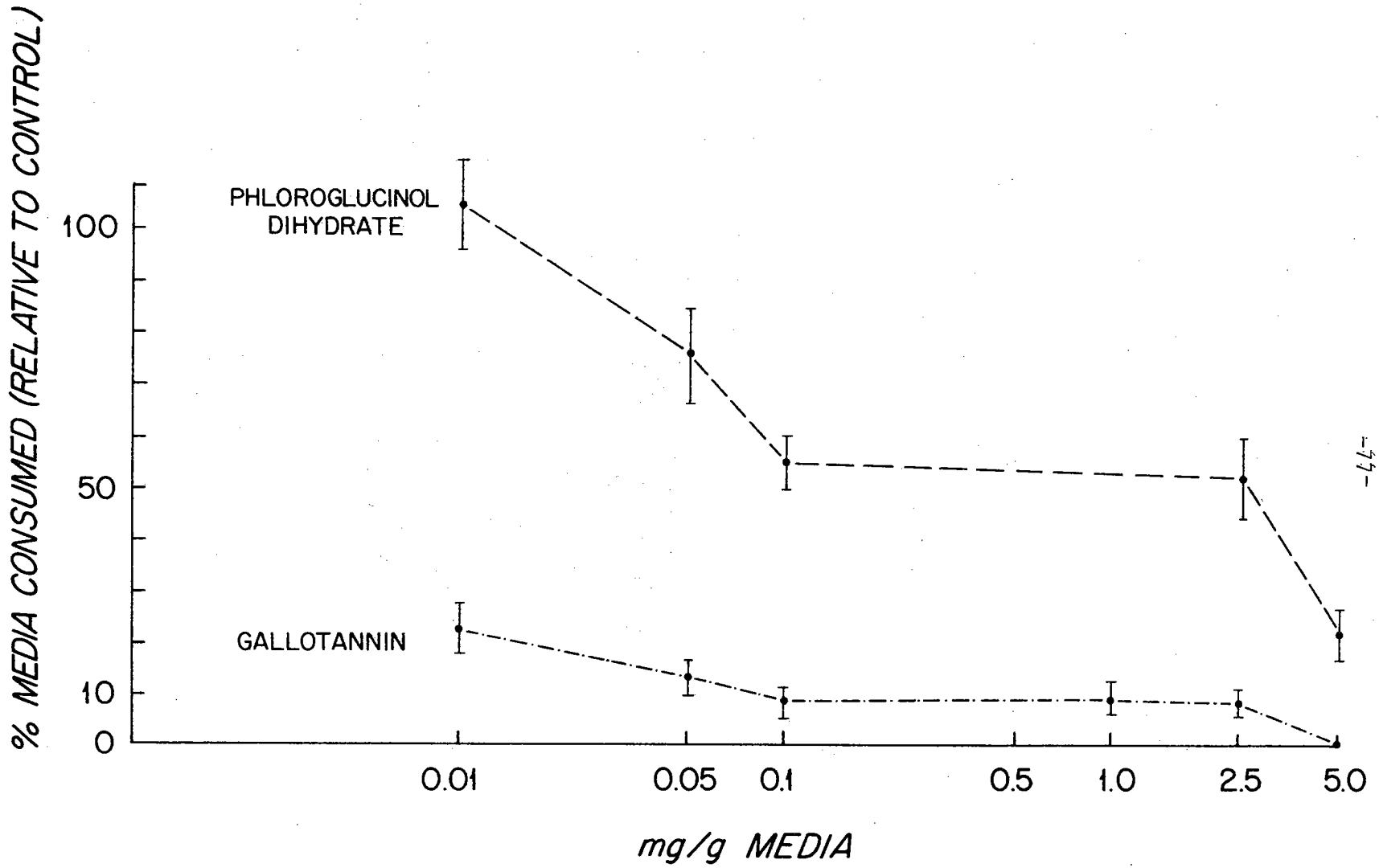
	% concentration of MW fraction in media		
	0.1	1.0	2.0*
	% of media consumed (relative to control)		
F. vesiculosus			
II. Acetone:			
MW <100,000	7± 2	8± 7	-
MW >100,000	7± 1	4± 1	-
III. MeOH:			
MW < 30,000	69± 0	30± 0	0
MW > 30,000	75± 0	14± 5	9
EtOAc(I), Acetone(II), and MeOH(III) partitions combined:			
MW < 30,000	27± 5	34± 9	3
MW >100,000	28± 8	32± 7	11
30,000 <MW <100,000	73±13	20± 8	15
MW >100,000	66±13	14±10	6
A. nodosum			
II. Acetone:			
MW <100,000	28± 8	15± 5	-
MW >100,000	8± 1	3± 1	-
III. MeOH:			
MW < 30,000	23±10	33± 8	17
MW > 30,000	60± 7	38± 8	33

* n = 1 for all bioassays of this highest concentration because of limited amounts of material obtained from extraction/molecular wt. separation.

Figure 5. Dose-response curve for the effect of phloroglucinol dihydrate and gallotannin on feeding by L. littorea.

$$\% \text{ media consumed} = \frac{\text{experimental media consumed}}{\text{control media consumed}} \times 100\%,$$

(mean $\pm$ s.e., n = 4).


% MEDIA CONSUMED (RELATIVE TO CONTROL)
100
50
10
0
PHLOROGLUCINOL DIHYDRATE
GALLOTANNIN
0.01
0.05
0.1
0.5
1.0
2.5
5.0
mg/g MEDIA

50% when present in concentrations of 0.2 - 0.5% fresh wt. of media (2 - 5% dry wt.). Gallotannin inhibited snail feeding by at least 50% when present in concentrations as low as 0.001% fresh wt. (0.01% dry wt.). The effective concentration (ED_{50}) of phloroglucinol is similar to the effective concentrations of F. vesiculosus polyphenolic fractions shown in Figure 3. Gallotannin is apparently more active than phloroglucinol and the F. vesiculosus polyphenolic fractions.

DISCUSSION

Stahl (1888) long ago suggested that plants produce compounds that deter animals and showed that terrestrial snails would eat certain leaves only after removal of tannic substances by alcoholic extraction. Hunger (1902) was the first to suggest that marine algae contained compounds that could provide protection against herbivores because he found that the sea hare Aplysia would eat the brown alga Dictyota dichotoma only after it had been subjected to alcoholic extraction. Since these initial studies, most investigations of plant chemical defenses against herbivores have been directed in terrestrial environments. However, evidence has accumulated that physodes and their phenolic contents in brown algae are important as antifouling, antialgal, and antibacterial agents (Conover and Sieburth, 1964; McLachlan and Craigie, 1964; Al-Ogily and Knight-Jones, 1977). Our investigations have now provided evidence that two dominant, perennial brown algae, F. vesiculosus and A. nodosum,

contain compounds which inhibit feeding by the major herbivore found in their community, L. littorea.

As shown by the ^{1}H NMR, ^{13}C NMR, UV, IR, and TLC data, the compounds in F. vesiculosus and A. nodosum that inhibited feeding of snails are clearly polymeric phenols (polyphenols). The most diagnostic structural information came from the ^{13}C NMR absorption values. These compared to the calculated values of monomers, dimers, trimers, tetramers, etc. identified in brown algae by Glombitza et al. (1973, 1975, 1977), thus indicating the presence of phloroglucinol polymers which contain primarily carbon-carbon linkages rather than ether linkages. Ragan and Craigie (1976) isolated from F. vesiculosus a series of phloroglucinol polymers with molecular weights ranging as high as 650,000 daltons. Because the linkages are chemically stable, Ragan and Craigie hypothesized that polymerization in vivo could be irreversible and could give rise to physodes increasingly rich in polymeric phenols ("aging" of physodes).

Our bioassays of polyphenols from F. vesiculosus and A. nodosum showed that a series of polymers with a wide range of molecular weights are all active in inhibiting feeding by snails as is phloroglucinol itself. The presence of these compounds in concentrations as low as 1% dry weight of media reduced feeding by more than 50%. Therefore it appears that chemical protection of these algae from herbivores is afforded by a rather heterogeneous mixture of polymeric phenols rather than a single compound. This has been found true for many terrestrial plants (Rhoades and Cates, 1976). There are many other similarities

between polyphenols in brown algae and those in terrestrial plants. First, the structures and activities of the compounds, including the effective concentrations against herbivores, are much the same. Second, the plants tend to sequester the compounds in special vacuoles so as to prevent autotoxicity. Third, there are seasonal and within-plant variations in tissue polyphenol contents (Fritsch, 1945; Levin, 1976; Feeny, 1976; Chapter 3). It has been suggested for terrestrial plants that attacks by herbivores and pathogens stimulates a defense mechanism based upon the release of and immediate oxidation and/or polymerization of phenols and polyphenols (Miles, 1969). These compounds then bind to amino acids, proteins, carbohydrates, vitamins, and other plant nutrients (e.g., iron), rendering them unavailable to herbivore digestive processes (Van Sumere et al, 1975; DeAlarcon et al., 1979). In addition, polyphenols complex with herbivores' enzymes, inactivating them, and herbivores' salivary proteins, causing astringent, bitter sensations which are presumably unpleasant and repellent. We hypothesize that the polyphenols in F. vesiculosus and A. nodosum are functionally similar to terrestrial plant polyphenols in their roles as chemical defenses against herbivores.

CHAPTER 3

SEASONAL VARIATION IN THE PHENOL AND POLYPHENOL CONTENTS IN NEW ENGLAND ROCKY INTERTIDAL ALGAE

ABSTRACT

Total phenol and polyphenol contents were determined seasonally in ten species of New England rocky intertidal algae, ranging from those preferred to those avoided as food for Littorina littorea, the predominant herbivore in this algal community. Fucus vesiculosus and Ascophyllum nodosum, the perennial algae that dominate space and that are not grazed by L. littorea, had highest phenol and polyphenol contents (1-17% dry wt.). Red, green, and ephemeral brown algae had much lower levels of phenols and polyphenol or none.

Within all F. vesiculosus tissues (apices, receptacles, midsections, and stipes), phenol and polyphenol levels were lowest during winter months, when the snails are inactive and not grazing. Phenol and polyphenol levels in all tissues increase rapidly to high levels (up to 17%) during spring months, at which time snails are actively grazing in this algal community, crawling over the surfaces of the fucoids but not consuming them. Apices, midsections, and stipes were similar in the seasonal variation of phenol and polyphenol contents; receptacles had lower contents

A. nodosum had a range of phenol and polyphenol contents during the year similar to that of F. vesiculosus, but two differences between these species were noted. First, A. nodosum phenol and polyphenol levels did not show as pronounced seasonal lows and highs. Second, there were greater differences between different plant tissues; stipes had higher levels than apices and receptacles.

It is concluded that the levels of phenols and polyphenols in F. vesiculosus and A. nodosum are sufficient to deter L. littorea feeding during the season when they are active.

INTRODUCTION

Phenols and polyphenols in terrestrial plants have been shown to act as antiherbivore defenses (Feeny, 1968; Todd et al.,1971; Beck and Reese, 1976; Rhoades and Cates, 1976). Generally, polymeric phenols of high molecular weight (greater than 500), loosely termed tannins, show greater biological activity than simple phenols (Levin, 1976). Feeny (1970) and Dement and Mooney (1974) showed that the seasonal variation of tannins in terrestrial plants accounts for differences in the grazing rates and patterns of herbivores. Similarly, variations in tannin content in different tissues of a plant can result in differential predation by animals (Rhoades and Cates, 1976).

Marine algae produce a variety of phenols and polyphenols, some of which closely resemble the tannins of terrestrial plants (Glombitza, 1977; Ragan and Craigie, 1978). While these compounds have demonstrated antibacterial, antialgal, and antifouling effects (McLachlan and Craigie, 1964; Conover and Sieburth, 1964; Sieburth and Conover, 1965), their role against herbivores has not previously been shown. The work described here is part of a project studying the ecology of algal chemical defenses against the herbivorous snail, Littorina littorea, in the New England rocky intertidal community.

Fucus vesiculosus and Ascophyllum nodosum dominate space in the mid- and high intertidal zones of this community. These fucoids escape predation by the predominant herbivore, L. littorea, once they attain a

germling size of 3 - 5 cm. (Menge, 1975). In previous experiments I showed that polyphenols in these two fucoids inhibit grazing and serve as chemical defenses against L. littorea (Chapters 1 and 2). The purpose of this study was to determine the phenol and polyphenol content seasonally in different tissues of F. vesiculosus and A. nodosum, and to survey other species of brown, red, and green algae (ranging from those preferred to those avoided as food for L. littorea) for phenol and polyphenol content.

MATERIALS AND METHODS

Collection and extraction

Algae were collected at low tide from rocky outcrops along the coast of Massachusetts (F. vesiculosus and A. nodosum samples for seasonal analysis were collected at Woods Hole, Ma. unless otherwise noted; other specimens were collected at Falmouth and Manomet Point, Ma.). Care was taken to shield the plants from sunlight and heat during transportation to the laboratory, where they were then placed in running seawater tanks. The algae were immediately prepared for extraction of phenols and polyphenols using 85% aqueous methanol (Goldstein and Swain, 1963; Ragan and Craigie, 1978). First, the plants were quickly rinsed in distilled water and blotted dry; all visible epiphytes were removed. For F. vesiculosus and A. nodosum, 8 - 10 full-size healthy plants were selected and the following parts were excised using a razor blade: apices, midsections, receptacles, and stipes. Similarly, Laminaria agardhii

thalli were sectioned into frond edge, meristem, and stipe samples. For all other species, samples consisted of whole thalli chopped with a razor blade.

For each sample of plant parts or thalli, duplicate 1 g. (wet weight) subsamples were taken for dry weight determination (48 hr., 65°C.), and duplicate 1 g. (wet wt.) subsamples were extracted in 20 ml. 85% methanol. After 48 hr. extraction in the dark, the methanol subsamples were finely ground with a Polytron homogenizer and centrifuged. The methanol extracts were decanted, and the residues were re-extracted twice, each time with 20 ml. 85% methanol (previous assays had shown that further washings with 85% methanol, 100% methanol, and distilled water did not yield appreciable phenol levels). Volumes were measured, and the extracts were then stored in darkness at 5°C. for no longer than 2 days until assayed for phenols and polyphenols.

Assay for Total Phenol Content

Total phenol levels were measured using an adaptation of the Folin-Denis colorimetric method (Folin and Denis, 1915; Swain and Hillis, 1959; Goldstein and Swain, 1963; Assoc. of Official Anal. Chemists, 1970). This assay is based on the oxidation of phenols by addition of a phosphomolybdo-tungstic acid complex resulting in the formation of tungsten and molybdenum blues with broad λ max 725-750 mμ. Other reducing agents such as ascorbic acid, but not glucose, are Folin-Denis reactive, but it is held that phenols constitute the large bulk of Folin-Denis reactive compounds found in most terrestrial and marine

plants (D. F. Rhoades, pers. comm.; Fox and Macauley, 1977; Ragan and Craigie, 1978; Van Sumere et al., 1975).

Results are expressed as % dry weight of phloroglucinol dihydrate, a commercially available phenol. The majority of phenols in the brown algae consist of phloroglucinol units (Ragan and Craigie, 1978).

Reagents:

1) Folin-Denis reagent, 0.25 N in Na_2Wo_4

41.25 g. sodium tungstate (Na_2Wo_4 $2H_2O$), 8.25 g. phosphomolybdic acid ($20MoO_3 \cdot 2H_3PO_4 \cdot 48H_2O$), and 20 ml. phosphoric acid were added to 300 ml. distilled H_2O. The solution was refluxed 2 hr.,cooled, then diluted to 1 l. with distilled H_2O.

2) Sodium Carbonate, 2 N

Method:

1) Preparation of phloroglucinol dihydrate dilutions for standard curve

0.01 g. phloroglucinol dihydrate was dissolved in distilled H_2O to a volume of 100 ml. 0 - 10 ml. of this solution were pipetted into 100 ml. volumetric flasks and brought to volume with distilled water.

2) Preparation of algal methanol extracts for assay

1 - 3 ml. of each algal methanol extract were pipetted into 100 ml. volumetric flasks and brought to volume with distilled water.

3) Assay

1 ml. of diluted sample was added to duplicate 10 ml. test tubes, followed by 1 ml. of Folin-Denis reagent with thorough mixing. After 3 min., 1 ml. of sodium carbonate was added and thoroughly mixed. After 1 hr. the test tubes were centrifuged (5 min., 3000 rpm) to remove any

precipitates, and the absorbance of each solution was read against a suitable blank at 725 mμ on a Gilford Spectrophotometer (model 252). If the absorbancy of any sample fell outside the range of the standard curve, the methanol extract was rediluted accordingly.

Assay for Polyphenol Content

Polyphenol levels were measured by an adaptation of the Folin-Denis colorimetric method using polyamide binding (Sieburth and Jensen, 1968; Ragan and Craigie, 1978). Excess polyamide powder quantitatively adsorbs polyphenols from solutions, whereas most simple phenols such as phloroglucinol, and other Folin-Denis interfering compounds such as ascorbic acid, remain primarily in solution. Measuring phenol levels by the Folin-Denis method before and after polyamide adsorption treatment therefore gives an estimation of total polyphenol content.

Method: 0.25 g ?

1) 2.5 g. of polyamide powder (Woelm) was added to 10 ml. of diluted algal sample (#2 in above Method). After 24 hr. of rotary shaking the solution was centrifuged to remove the polyamide.

2) The solution was then assayed as in 3) above.

RESULTS

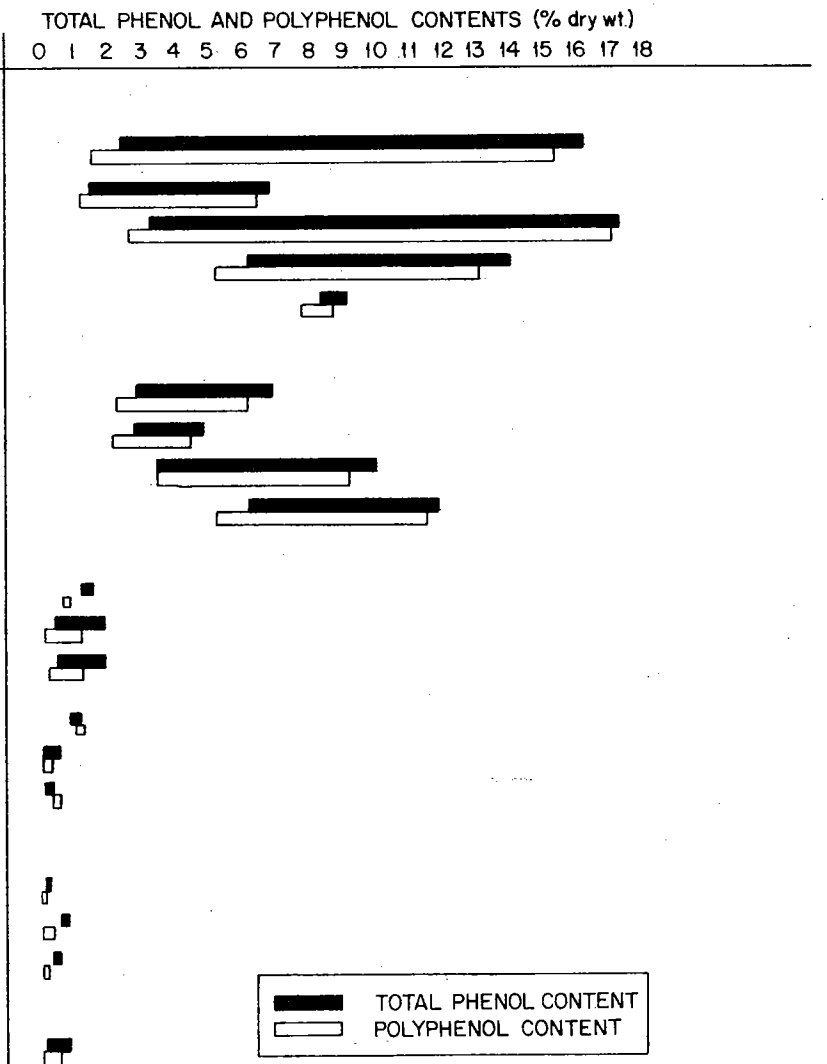
Survey of Total Phenol and Polyphenol Content in Algal Species of the New England Rocky Intertidal Community

Total phenol and polyphenol contents (% dry weight) were measured in eleven species in the Phaeophyta, Rhodophyta, and Chlorophyta (Figure 1).

Figure 1. Range of total phenol and polyphenol contents (% dry wt.) in New England rocky intertidal algal species.

Table I. Seasonal ranges of total phenol and polyphenol contents in New England intertidal algae

ALGAE	MONTHS COLLECTED	NUMBER OF SAMPLES
PHAEOPHYTA:		
Fucus vesiculosus		
Apices	1-12/79	34
Receptacles	1-12/79	32
Midsections	1-12/79	34
Stipes	2-12/79	34
Germling	6/79	2
Ascophyllum nodosum		
Apices	2-12/79	28
Receptacles	2-4,10-12/79	12
Midsections	2-12/79	28
Stipes	2-12/79	28
Laminaria agardhii		
Fronds	5/79	2
Meristems	5/79	2
Stipes	5/79	2
Petalonia fascia	3/79	2
Scytosiphon lomentaria	1-3/79	4
Ralfsiod crust	7/79	2
RHODOPHYTA:		
Chondrus crispus	6/79	2
Polysiphonia flexicaulis	9/79	2
Polysiphonia lanosa	6/79	2
CHLOROPHYTA:		
Ulva - Enteromorpha spp.	1,3,5,6/79	8
Codium fragile	7/79	2



Species of brown algae showed higher contents of both phenols and polyphenols than species of red and green algae. Within the brown algae, F. vesiculosus and A. nodosum had the highest phenol and polyphenol contents. Their levels were 2 - 20 times greater than the levels in all other species examined.

Seasonal Variation in the Total Phenol and Polyphenol Content in Different Tissues of F. vesiculosus and A. nodosum

Total phenol and polyphenol contents (% dry weight) were measured at monthly intervals for one year in the apices, midsections, receptacles, and stipes of F. vesiculosus (Figures. 2 and 3) and A. nodosum (Figures. 4 and 5). Figures 6 and 7 show the seasonal variation in the percentage of phenols that were polymeric in the different plant parts of the two species

In all parts of F. vesiculosus, total phenol and polyphenol contents were lowest during the winter months of December and January (1-3.3% dry wt.), after which they increased rapidly to high levels (8.6-13% dry wt.) during the spring months of March through May. Levels slowly decreased during the summer (June through August), with levels in the receptacles showing a marked decrease in June after the peak reproduction period. Gradual decreases continued during the fall except in the midsections, where total phenol and polyphenol contents increased in September. From January through June, there was no significant difference between apices, midsections, and stipes in the monthly variation of total phenol and polyphenol levels. Beginning in July, the apices had lower levels of these compounds than the midsections and stipes. Receptacles had

Figure 2. Seasonal variation in phenol content of Fucus vesiculosus apices, receptacles, midsections, and stipes. (% dry wt., mean ± s.e., n = 2).

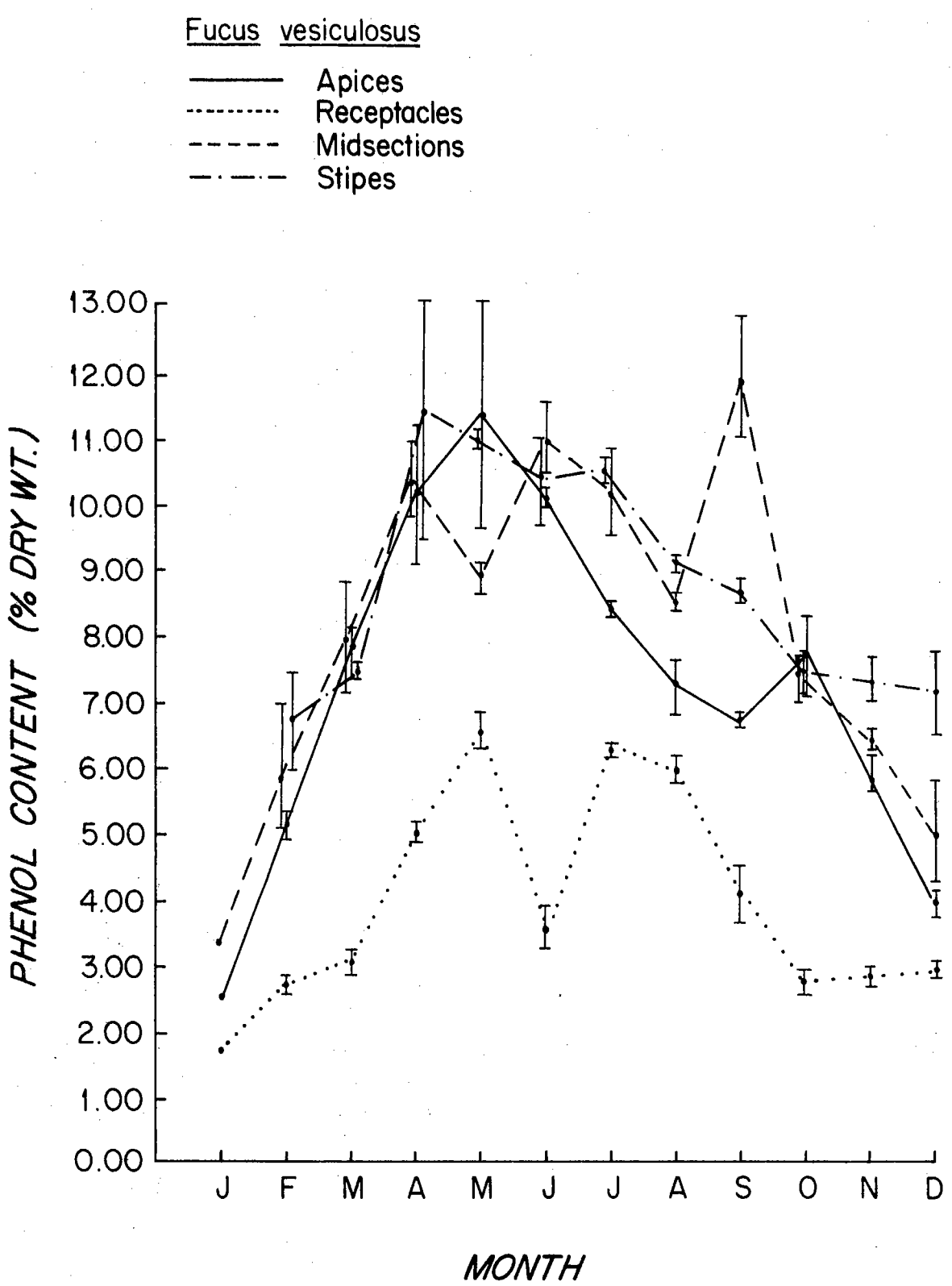

Fucus vesiculosus
Apices
Receptacles
Midsections
Stipes
PHENOL CONTENT (% DRY WT.)
13.00
12.00
11.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
J F M A M J J A S O N D
MONTH

Figure 3. Seasonal variation in polyphenol content of Fucus vesiculosus apices, receptacles, midsections, and stipes. (% dry wt., mean ± s.e., n = 2).

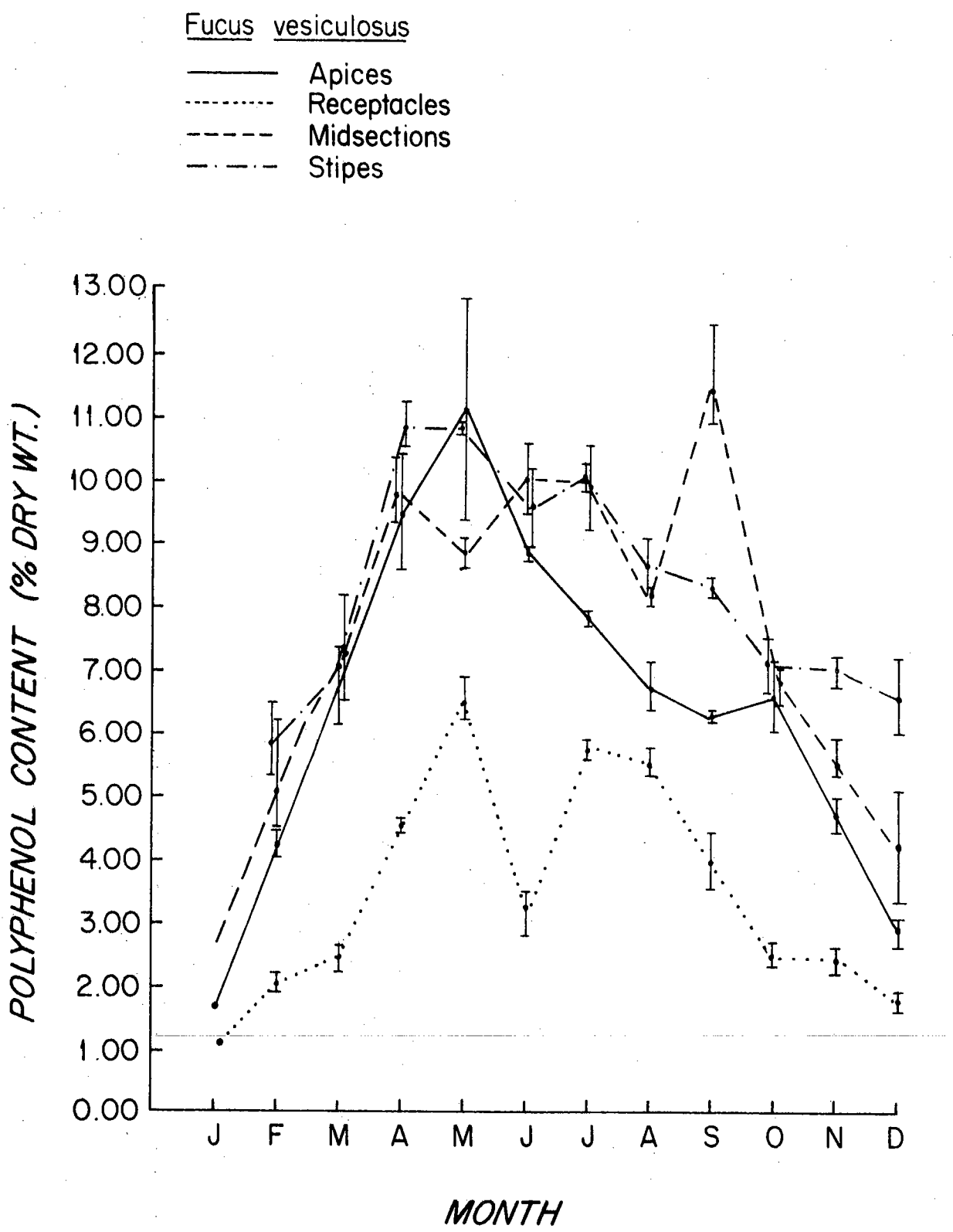

Fucus vesiculosus
Apices
Receptacles
Midsections
Stipes
POLYPHENOL CONTENT (% DRY WT.)
13.00
12.00
11.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
J F M A M J J A S O N D
MONTH

Figure 4. Seasonal variation in phenol content of Ascophyllum nodosum apices, receptacles, midsections, and stipes.
(% dry wt., mean ± s.e., n = 2).

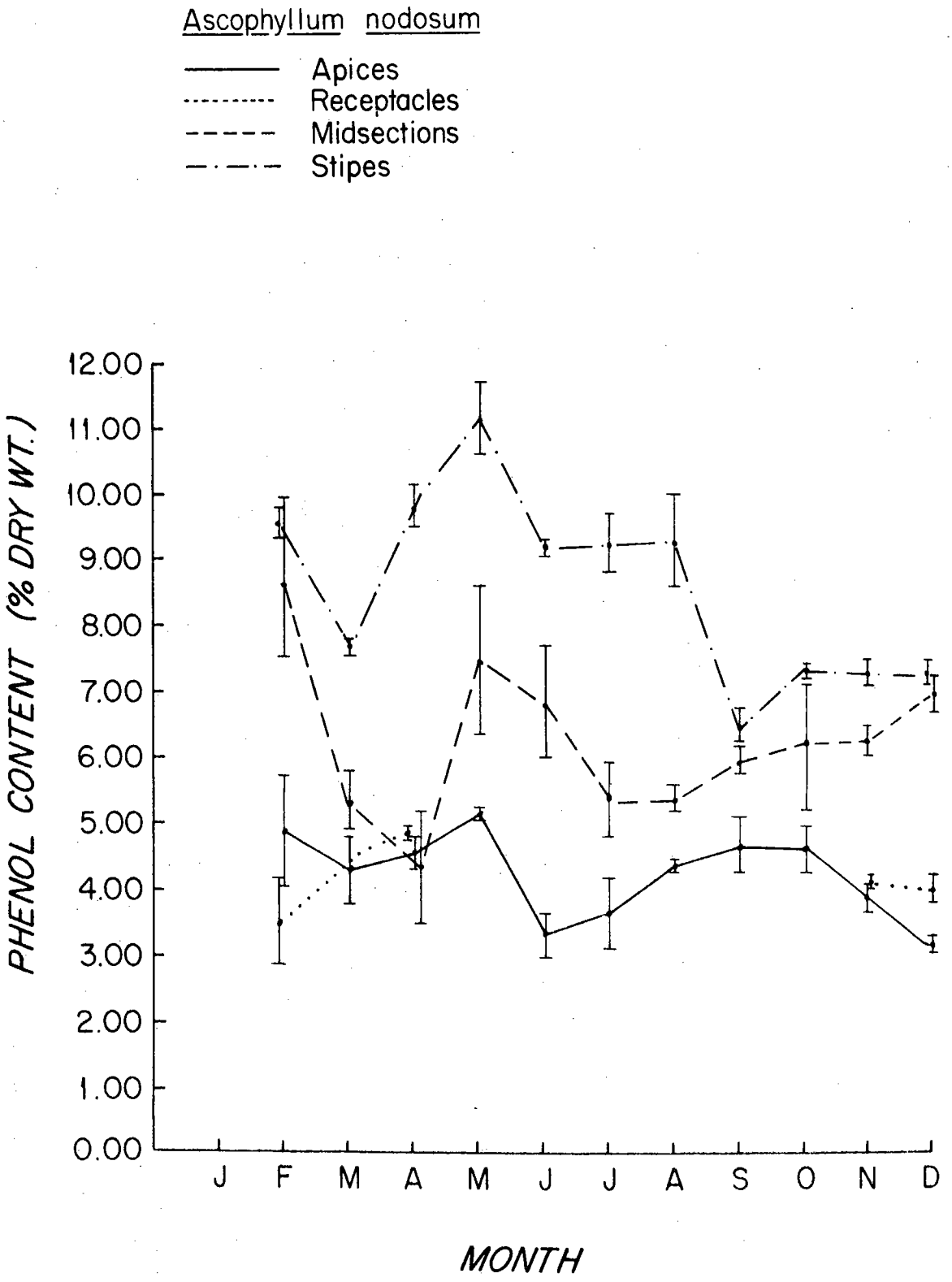

Ascophyllum nodosum
Apices
Receptacles
Midsections
Stipes
PHENOL CONTENT (% DRY WT.)
12.00
11.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
J F M A M J J A S O N D
MONTH

Figure 5. Seasonal variation in polyphenol content of Ascophyllum nodosum apices, receptacles, midsections, and stipes.
(% dry wt., mean ± s.e., n = 2).

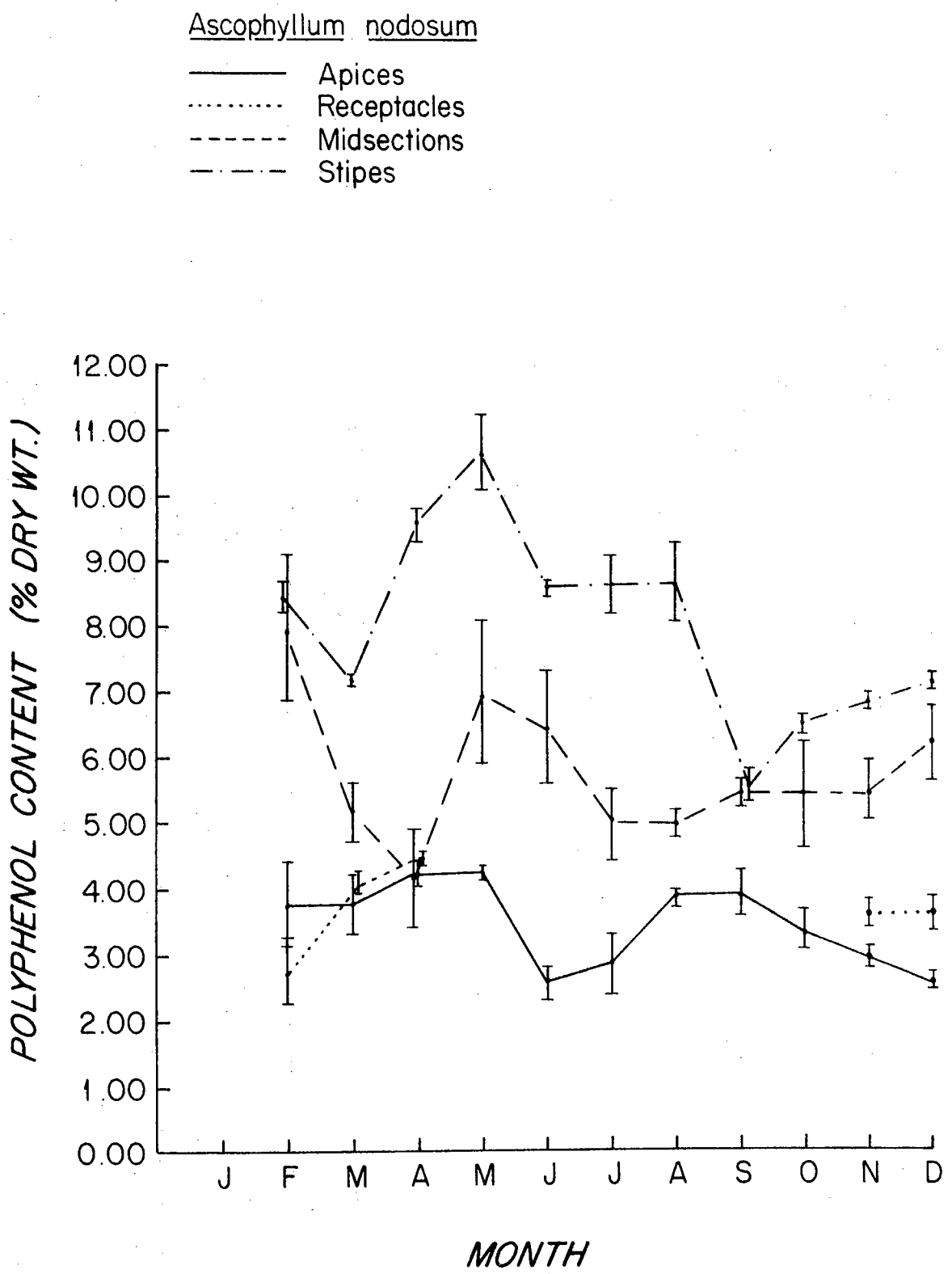

Ascophyllum nodosum
Apices
Receptacles
Midsections
Stipes
POLYPHENOL CONTENT (% DRY WT.)
12.00
11.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
J F M A M J J A S O N D
MONTH

Figure 6. Percent of phenols which are polyphenolic in Fucus vesiculosus apices, receptacles, midsections, and stipes. (mean ± s.e., n = 2).

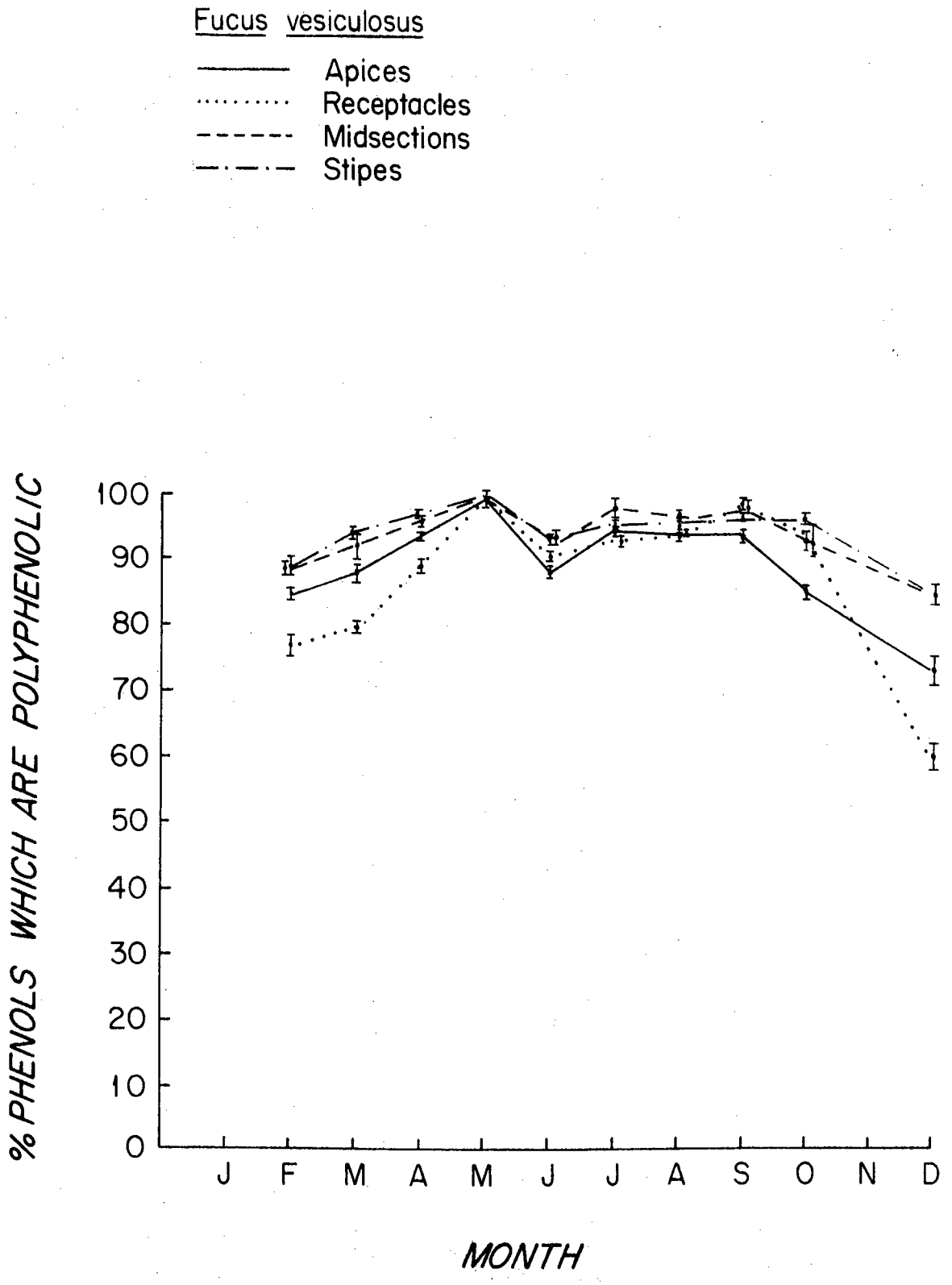
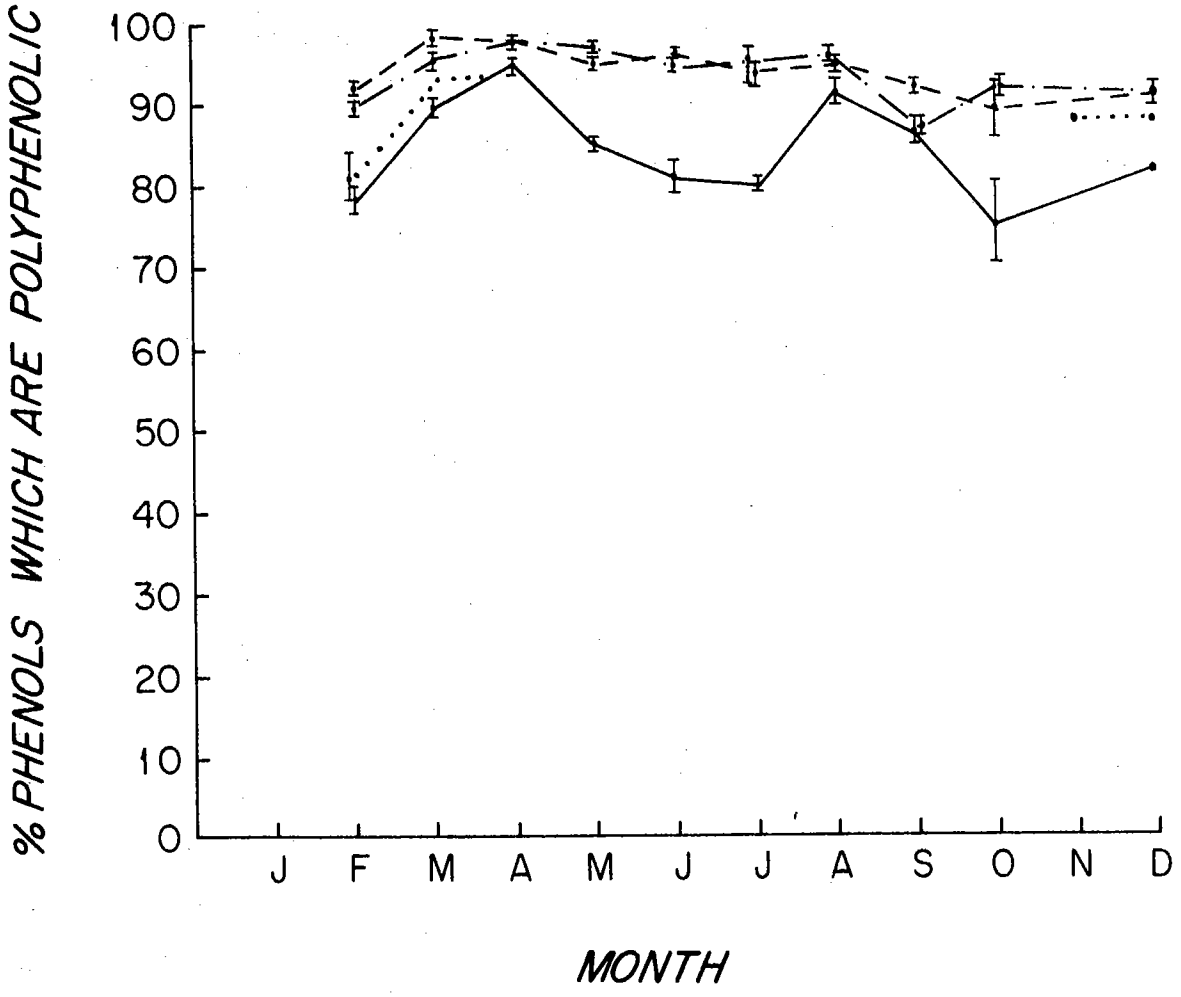

Fucus vesiculosus
Apices
Receptacles
Midsections
Stipes
% PHENOLS WHICH ARE POLYPHENOLIC
100
90
80
70
60
50
40
30
20
10
0
J
F
M
A
M
J
J
A
S
O
N
D
MONTH

Figure 7. Percent of phenols which are polyphenolic in Ascophyllum nodosum apices, receptacles, midsections, and stipes. (mean ± s.e., n = 2).

Ascophyllum nodosum

——— Apices
····· Receptacles
- - - - - Midsections
—·—·— Stipes



significantly lower levels of total phenols and polyphenols than apices, midsections, and stipes during all months. Percentage of phenols that were polymeric varied most throughout the year in receptacles (from 75% in February to 99% in May and September); these values were significantly lower than those in apices, midsections, and stipes during February through April. For all parts, polyphenols constituted the highest percentage of the total phenols (98-100%) during May .

The total phenol and polyphenol levels in A. nodosum did not show marked seasonal variations as did those in F. vesiculosus, but they did indicate that levels varied within the plants throughout the year. Stipes consistently contained higher levels of phenols and polyphenols than apices, midsections, and receptacles. Except during March through April, midsections had higher levels of phenols and polyphenols than apices and receptacles. Percentage of phenols that were polymeric varied most throughout the year in the apices and receptacles, with lows of 76-80% in February and July and a high of 95% in April. These values were significantly lower throughout the year than those in midsections and stipes, which gradually changed from highs of 95-99% in March through June to lows of 86-93% in September.

The total phenol and polyphenol contents throughout the year for Fucus vesiculosus and A. nodosum plant parts exhibited similar ranges. Total phenol levels ranged from 1.6-17.4% in F. vesiculosus and from 2.9-11.6% in A. nodosum. Polyphenol levels ranged from 1.2-17.2% in F. vesiculosus and 2.2-11.3% in A. nodosum. There is some evidence that the levels and patterns of seasonal variation of total phenols and

polyphenols vary in plants collected from different localities for both F. vesiculosus and A. nodosum (Figures 8 and 9).

DISCUSSION

Total phenol and polyphenol contents measured in marine algae of the New England rocky intertidal community were highest in F. vesiculosus and A. nodosum, the perennial brown algae that dominate mid and high intertidal space and that are not grazed by the major herbivore, L. littorea. Previous experiments demonstrated that polyphenols in these two species inhibit feeding of the periwinkles and hence act as chemical defenses against herbivores (Chapter 1). Similar studies of terrestrial plants indicated that their chemical defenses may vary in concentration within a plant and seasonally and that these changes may relate to changes in herbivore feeding patterns (Feeny, 1976; Rhoades and Cates, 1976).

This study has shown that polyphenol levels in F. vesiculosus plant parts are lowest during cold winter months, at which time snails are inactive and do not feed. Phenol and polyphenol levels in all F. vesiculosus tissues increase rapidly to high levels during spring months (March - May) and remain high until a sudden decrease in late fall. This period corresponds to the time when snails are actively grazing in this intertidal community, crawling over the surfaces of the fucoids yet not consuming them. Apices, midsections, and stipes of F. vesiculosus are protected with similar levels of phenols and polyphenols throughout most

Figure 8. Seasonal variation in tissue polyphenol contents of *Fucus vesiculosus* plants collected at different locations. (% dry wt., mean ± s.e., n = 2).

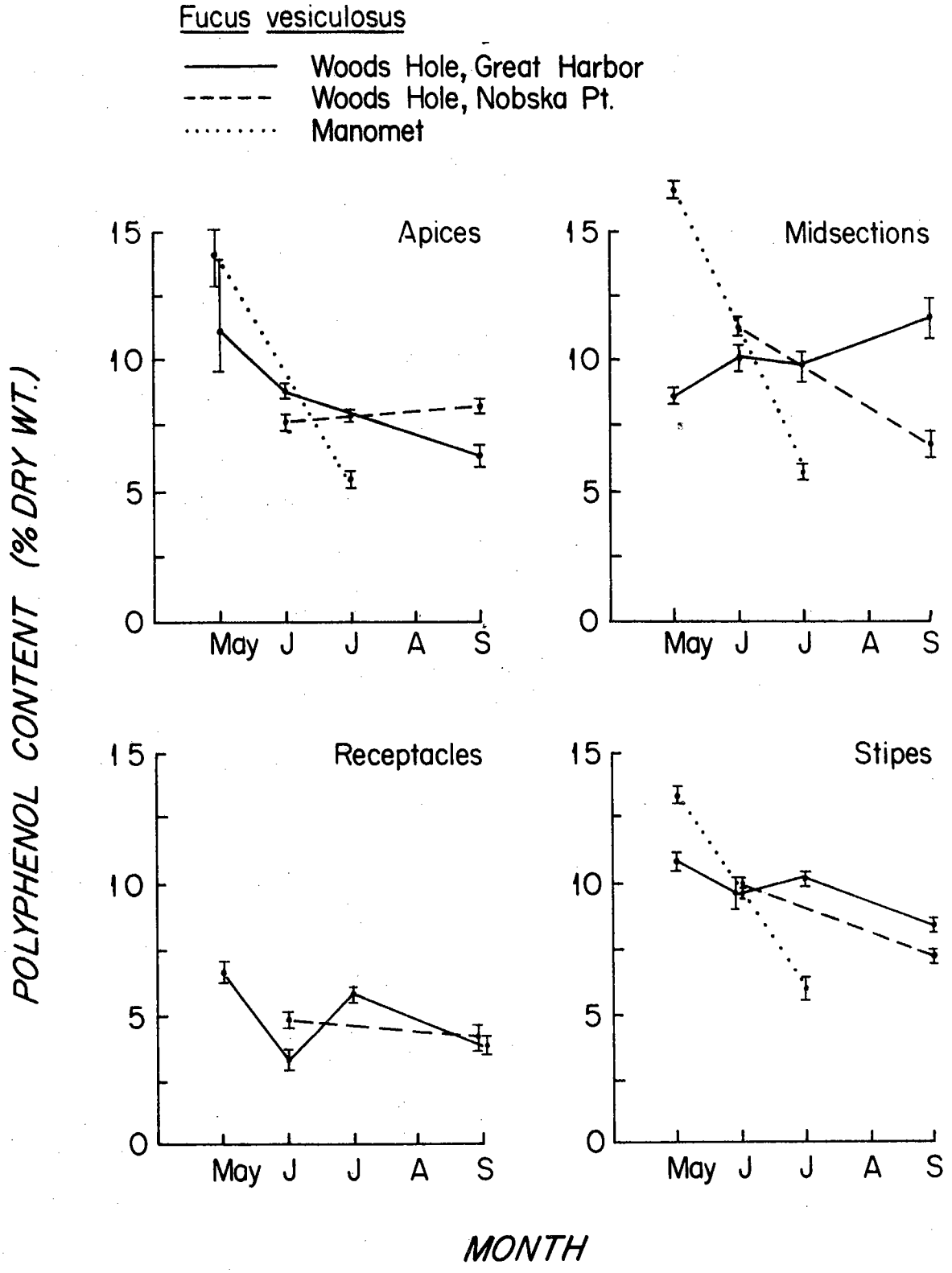
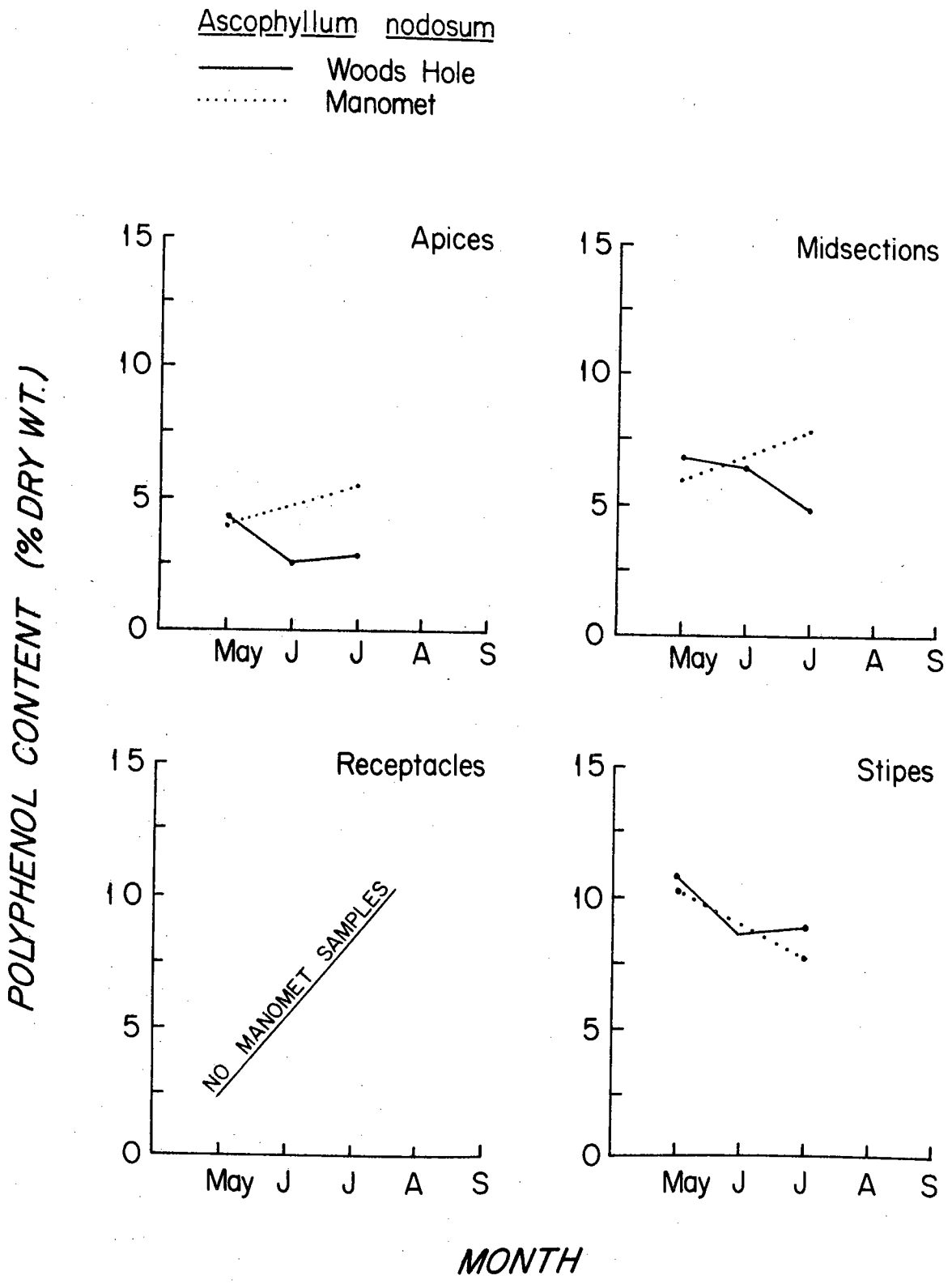

Fucus vesiculosus
Woods Hole, Great Harbor
Woods Hole, Nobska Pt.
Manomet
Apices
Midsections
Receptacles
Stipes
15
10
5
0
May
J
J
A
S
POLYPHENOL CONTENT (% DRY WT.)
MONTH

Figure 9. Seasonal variation in tissue polyphenol contents of Ascophyllum nodosum plants collected at different locations. (% dry wt., mean ± s.e., n = 2).


Ascophyllum nodosum
Woods Hole
Manomet
Apices
Midsections
Receptacles
Stipes
NO MANOMET SAMPLES
15
10
5
0
May J J A S
POLYPHENOL CONTENT (% DRY WT.)
MONTH

of the year. Receptacles, however, appear to be less protected with these compounds than the other plant parts during all months.

A. nodosum showed a similar range of phenol and polyphenol levels during the year as F. vesiculosus (1-13% dry weight), but two differences between the species were apparent. First, A. nodosum total phenol and polyphenol levels did not show pronounced seasonal lows in the winter or rapid increases in the spring but instead showed more minor fluctuations monthly. Second, total phenol and polyphenol levels in A. nodosum varied more between different plant tissues than did levels within F. vesiculosus. During most months the stipes were protected with highest levels of total phenols and polyphenols; midsections had intermediate values, and receptacles and apices had lowest total phenol and polyphenol contents.

The levels of phenols and polyphenols in F. vesiculosus and A. nodosum reported here show approximately the same range as levels reported by Ragan and Jensen (1977, 1978) for these two species in Norway. They analyzed the plants as a whole and not as individual parts so comparisons can only be general. Levels of polyphenols in the Norwegian plants varied from 7-14% in F. vesiculosus and from 8-13% in A. nodosum. Differences in the patterns of variation of polyphenol levels in plants collected from two different coasts are not surprising. In New England, these two species of algae collected from different locations showed similar ranges but somewhat different monthly patterns of phenol and polyphenol levels.

Feeding experiments demonstrated that the presence in the diet of as little as 1% polyphenol (dry wt.), extracted from F. vesiculosus and A. nodosum, caused a significant reduction in consumption by L. littorea. Further, 10% polyphenol (dry wt.) in food media inhibited snail feeding nearly 100% (Chapter 2). Thus, the effective doses ranged from 1-10% polyphenol in the diet. The results of this seasonal survey of polyphenol content in F. vesiculosus and A. nodosum indicate that these two species have polyphenol contents in excess of 1% (dry wt.) during all months of the year, in all tissues examined. During months when snails are actively grazing in the intertidal, polyphenol contents in these two fucoids reach as high as 17.5% (dry wt.). It therefore appears that F. vesiculosus and A. nodosum are well protected with chemical defenses against L. littorea.

Fucoid germlings (3-5 cm.) had polyphenol levels of 8-9% (dry wt.). Sporelings of this size range have usually become resistent to grazing by L. littorea (Menge, 1975; Cheney and Sideman, pers. comm.). Difficulties in obtaining a large enough sample of smaller germlings prevented analysis by the Folin-Denis method, but McConnell (pers. comm.) reported that analysis by thin layer chromatography indicated very low levels of vanillin-reactive phenols. Factors such as spatial and temporal refugia probably play important roles in the escapes of very small fucoid germlings from herbivores.

Comparisons of the levels of phenols and polyphenols in F. vesiculosus and A. nodosum to levels reported in terrestrial plants show similarities in the range of concentrations (% dry weight) and in the

effective doses for deterring herbivores. Feeny and Bostock (1968) reported that polyphenols in oak leaves increased from 0.5% of leaf dry weight in April to 5.5% of leaf dry weight in September. Experiments showed that the presence in the diet of as little as 1% tannin (wet wt.), extracted from September oak leaves, caused a significant reduction in larval growth rate and pupal weight of winter moth, Operophtera brumata (Feeny, 1968). Feeny (1970) concluded that oak leaf tannins serve as chemical defenses against these herbivores by reducing the availability of nitrogen and influencing leaf palatability. Other studies of diverse terrestrial plants have since shown similar ranges of tannins and have implicated their importance in defending plants against herbivores. For example, leaves of the chaparral shrub Heteromeles arbutifolia contain 4-12% tannin (dry wt.)(Dement and Mooney, 1974), and leaves of African rain forest trees that are avoided by herbivorous monkeys contain an average of 7.8% phenols (dry wt.)(McKey et al., 1979). Between-plant comparisons of phenol and polyphenol levels must be viewed cautiously because of the differences used in extraction methods and the variations in reactivity for individual phenolic compounds. The dry weight figures for these compounds represent a quantification that is more relative than absolute for each plant studied. With these limitations in mind, it is, however, interesting that the range of polyphenol contents in F. vesiculosus and A. nodosum (1-17% dry wt.) and their effective concentrations against herbivores (1-10% dry wt.) are similar to the tannin contents of terrestrial plants and their effective doses against herbivores (0.5-12% dry leaf content).

Based on many terrestrial studies, Feeny (1976) and Rhoades and Cates (1976) recently hypothesized that commitment to chemical defense is related to a plant's "apparency" or predictability and successional status in a community. Within this framework, tannins (polyphenols) are identified as "quantitative defenses", which reduce availability of nutrients and lower palatability and are found primarily in perennial, late successional plants, which tend to grow more slowly and hence are more highly predictable in space and time. I propose that the polyphenols in F. vesiculosus and A. nodosum , two species which are perennial and highly "apparent" in the New England rocky intertidal community, act similarly as "quantitative" chemical defenses against L. littorea. This further substantiates the "broad spectrum" action of polyphenols against herbivores and their widespread occurrence in plants, including species in marine as well as terrestrial environments.

Annual brown algal species such as Petalonia fascia and Scytosiphon lomentaria are shorter-lived and less predictable than F. vesiculosus and A. nodosum. As predicted by the above hypotheses, these algae have very low levels of antiherbivore phenols and polyphenols, and they are highly preferred as food for herbivores when they are available. Both of these species have an alternation of dimorphic stages in their life cycles (Wynne, 1969). Upright blades and thalli are present and fruiting only in the winter, followed by Ralfsioid crusts which are present and fruiting only in the summer. Hence it appears that the upright thalli of P. fascia and S. lomentaria escape heavy grazing pressure by timing of

their growth and reproduction rather than by chemical defenses. L. littorea does consume both of these species during early spring when both are still present and the snails are just beginning to feed actively (Menge, 1975). The Ralsioid crustose stages, which are not heavily grazed by the snails, likewise do not have high phenol or polyphenol levels but may be defended against these herbivores in the summer by means of a physical factor such as toughness (Chapter 1 and Lubchenco, pers. comm.). Similarly, Laminaria agardhii, of medium food preference to the snails, has a lower polyphenol content than F. vesiculosus and A. nodosum and may be defended by a combination of physical and chemical factors.

Phenol and polyphenol contents in F. vesiculosus and A. nodosum may not be the only factors important in deterring herbivores; they may act in conjunction with others such as toughness. Lubchenco (pers. comm.) found a correlation between degree of algal toughness and their preference as food to snails. That polyphenols can form complexes with proteins and result in toughness of tissues is exemplified by the tanning process of leather. It is possible that polyphenols in plants cause toughness of tissues. Nutritional value of algae may be important in herbivore preference. Since polyphenols can bind to proteins and reduce availability of nitrogen (Goldstein and Swain, 1964; Feeny, 1970), they, too, are involved as nutritional factors (Chapter 4).

The physiological and ecological roles of phenols and polyphenols in terrestrial and marine plants have long been subjects of controversy. Proposed roles include storage of metabolic reserves, protection against

desiccation, synthesis of mucilage, or merely waste accumulation (Fritsch, 1945; Esau, 1965). None of these have been proven. Biosynthesis of phenols and polyphenols from acetate via the acetate and shikimic acid pathways involve considerable quantities of energy (Haslam, 1966), hence it is believed that there must be some compensating adaptive advantage conferred to the plants that produce them. That these compounds might serve protection against animals was suggested long ago by Stahl (1888) and Hunger (1902), but experimental evidence was lacking. There now are considerable data demonstrating the widespread occurrence and function of phenols and polyphenols as chemical defenses against herbivores and other organisms in marine and terrestrial environments.

CHAPTER 4

SEASONAL VARIATION IN THE NITROGEN CONTENT AND POLYPHENOL/NITROGEN RATIO IN NEW ENGLAND ROCKY INTERTIDAL ALGAE

ABSTRACT

Nitrogen contents and polyphenol/nitrogen ratios in Fucus vesiculosus and Ascophyllum nodosum tissues (apices, receptacles, midsections, and stipes) and in ten other red, green, and ephemeral brown algal species were examined seasonally in relation to algal feeding preferences of the periwinkle snail, Littorina littorea. The results showed that nitrogen contents of low preference species such as F. vesiculosus and A. nodosum were as high as or higher than those of highly preferred species such as Scytosiphon lomentaria, Ceramium spp., and Ulva-Enteromorpha spp. However, polyphenol/nitrogen ratios of F. vesiculosus and A. nodosum were very high, in contrast to those of the other species. Hence, it is likely that the nitrogen of F. vesiculosus and A. nodosum is less available nutritionally to herbivores because of polyphenols. I conclude that F. vesiculosus and A. nodosum are similar to terrestrial plants such as oaks and chaparral in their patterns of "quantitative" chemical defense.

INTRODUCTION

In the New England rocky intertidal community, space is dominated by brown fucoid algae (Ascophyllum nodosum, Fucus vesiculosus, and other Fucus spp.) in the high to mid zones, and a red alga, Chondrus crispus, in the low zone. Lubchenco (1978) showed in field and laboratory experiments that these large, perennial algae are not eaten by the predominant macroalgal herbivores, periwinkle snails (Littorina littorea). She concluded that Fucus spp. are vulnerable to these generalist herbivores only when in young sporeling stages; after these stages become established by means of temporal or spatial escapes, Fucus spp. are either never eaten by L. littorea or are eaten only if no other food has been available for a considerable length of time (e.g., during winter months). A. nodosum and C. crispus are seldom eaten, even if no other food is available.

Recent experiments demonstrated that compounds extracted from F. vesiculosus, A. nodosum, and C. crispus inhibit feeding of L. littorea when added to preferred food sources (Chapter 1). The active antiherbivore compounds in the brown algae were shown to be polyphenols (Chapter 2), similar to terrestrial plant polyphenols ("tannins"), which are well documented herbivore defenses (Feeny, 1968 and 1970; Dement and Mooney, 1974; Levin, 1976).

The term "tannin" is derived from the fact that polyphenols "tan" proteins; that is, they can indirectly or directly bind to polypeptides by means of hydrogen bonds or ester and amide linkages (Van Sumere et al., 1975). This ability of plant polyphenols has several repercussions to herbivores: 1) plant polyphenols may bind to plant proteins, forming complexes refractory to herbivore digestive processes; 2) plant polyphenols may bind to herbivore digestive enzymes in the gut and reduce digestive activities; and 3) plant polyphenols may bind to proteins in herbivore mouths, causing unpleasant, astringent sensations and the reduction of lubricant action of glycoproteins in saliva. These effects may reduce food attractiveness of the plant to herbivores (Goldstein and Swain, 1965; Feeny, 1968; Bate-Smith, 1973).

Polyphenols are characteristic of terrestrial plants or plant tissues that are highly "apparent" in terms of size, growth form, persistence, and predictability (Feeny, 1976). They are present in relatively large amounts (up to 12% dry wt.) in certain plant tissues (Dement and Mooney, 1974). Rhoades and Cates (1976) and Feeny (1976) postulated that polyphenols in terrestrial plants act as "quantitative" defenses, which are dosage-dependent and whose mode of action against herbivores is in reducing availability of nutrients, particularly nitrogen in the form of protein. Nitrogen has been shown to be a limiting factor in the growth and reproduction of many herbivores and hence critical in the selection of their food (Cannon and Connell, 1965; Van Emden, 1966; Soo Hoo and Fraenkel, 1966; Onuf, 1977; Slansky and Feeny, 1979; Vince, 1979). One

can predict that plants in their evolutionary "arms race" with their herbivores use non-availability of the nitrogen of proteins as a defense mechanism.

Because polyphenols bind proteins, total nitrogen or protein content measured in a plant may not reflect the amount of nitrogen or protein actually available to an herbivore. In terrestrial plants containing polyphenols, protein digestibilities (nitrogen availabilities) for different tissues have been compared as polyphenol/protein ratios (Feeny, 1970; Rhoades and Cates, 1976). Generally, polyphenol content increases and protein content (usually measured as nitrogen) decreases with tissue age. Thus, polyphenol/protein (nitrogen) ratios increase greatly as plant tissues mature, implying that plant proteins (nitrogen) become less available to herbivores by digestibility-reducing polyphenols.

Previously I showed that F. vesiculosus and A. nodosum, the large, dominant perennial algae of the high and mid intertidal zones in New England, are similar to "apparent" terrestrial plants because of their high content of polyphenols (up to 17% dry wt.) (Chapter 3) and the inhibitory effect of these polyphenols on feeding by the generalist herbivore, L. littorea. In this paper I will examine the seasonal nitrogen contents and polyphenol/nitrogen ratios of F. vesiculosus and A. nodosum tissues (apices, receptacles, midsections, and stipes) and other perennial, annual, and ephemeral algae (Phaeophyta, Rhodophyta, and Chlorophyta) to determine if mature plant tissues and perennial species tend to have lower nitrogen availabilities (higher polyphenol/nitrogen

ratios) than young, growing tissues and annual and ephemeral species, as hypothesized for terrestrial plants by Feeny (1976) and Rhoades and Cates (1976).

MATERIALS AND METHODS

Algae were collected and prepared for analysis as previously described in Chapter 3. Procedures were reported there for the determination of polyphenol content, dry weight and water content. Carbon, hydrogen, and nitrogen contents were determined on a Perkin-Elmer Model 240 CHN Analyzer after samples had been oven-dried (65°C, 48 hr.) to constant weight and ground in a Wiley Mill.

RESULTS

The range of values for seasonal nitrogen contents, water contents, carbon/nitrogen ratios, and polyphenol/nitrogen ratios are summarized in Table 1 for thirteen species of brown, red, and green algae. Information on algal life history patterns and preference as food to the herbivore L. littorea is included. F. vesiculosus and A. nodosum were examined in detail since previous studies had shown that their polyphenols cause inhibition of feeding by L. littorea (Chapters 1, 2). Each of these species were sampled monthly for one year, and different tissues (apices, receptacles, midsections, and stipes) were analyzed separately to determine the seasonal within-plant variations in nitrogen content, water

Table 1. Seasonal ranges of nitrogen content, water content, carbon/nitrogen ratio, and polyphenol/nitrogen ratio in New England intertidal algae

ALGAE	LIFE HISTORY	PREFERENCE TO *L. littorea*	MONTHS COLLECTED	NUMBER OF SAMPLES	H_2O (% FRESH WT.) $\bar{X} \pm$ S.E.	NITROGEN (% DRY WT. RANGE)	CARBON/NITROGEN RATIO (RANGE)	POLYPHENOL/NITROGEN RATIO (RANGE)
PHAEOPHYTA:								
Fucus vesiculosus	perennial	low						
Apices		low	1-12/79	34	83.1 ± 1.0	1.03 - 4.49	8.30 - 31.62	0.413 - 8.450
Receptacles		low	1-12/79	32	86.1 ± 1.0	0.56 - 4.46	8.00 - 40.97	0.275 - 9.056
Midsections		low	1-12/79	34	76.0 ± 1.3	0.70 - 3.97	10.30 - 49.30	1.350 - 14.163
Stipes		low	2-12/79	34	67.2 ± 0.9	0.72 - 4.34	9.04 - 47.95	1.631 - 14.044
Germling		medium	6/79	2	83.0 ± 0	0.66 - 0.70	48.53	12.044
Ascophyllum nodosum	perennial	low						
Apices		low	2-12/79	28	78.1 ± 0.8	0.74 - 3.07	12.40 - 43.11	1.225 - 4.375
Receptacles		low	2-4,10-12/79	12	86.7 ± 3.9	0.88 - 2.26	14.40 - 32.81	1.231 - 5.000
Midsections		low	2-12/79	28	71.2 ± 0.8	0.76 - 1.78	21.70 - 48.23	4.525 - 8.781
Stipes		low	2-12/79	28	68.1 ± 0.9	0.83 - 1.81	21.71 - 42.14	3.706 - 10.375
Laminaria agardhii	annual	medium						
Fronds		medium	5,10/79	4	87.0 ± 1.0	0.83 - 2.17	15.14 - 22.65	0.413
Meristems		medium	5,10/79	4	88.0 ± 1.0	1.43 - 2.90	14.98 - 16.42	0.463
Stipes		medium	5,10/79	4	87.0 ± 1.0	1.30 - 1.68	19.17 - 19.18	0.550
Petalonia fascia	ephemeral	high	3/79	2	91.0 ± 1.0	2.66 - 2.78	12.7	0.369
Scytosiphon lomentaria	ephemeral	high	1-3,10/79	4	91.8 ± 3.2	1.01 - 2.08	9.3 - 10.4	0 - 0.144
Ralfsiod crust	perennial	low	7/79	2	71.0 ± 1.0	1.66 - 1.68	23.4	0.813
RHODOPHYTA:								
Chondrus crispus	perennial	low	6,10/79	4	81.0 ± 1.0	1.79 - 1.85	15.17	0
Polysiphonia flexicaulis	annual	medium	9/79	2	90.0 ± 1.0	2.81 - 2.83	10.25	0.194
Polysiphonia lanosa	annual	medium	6/79	2	87.0 ± 1.0	2.57 - 2.93	10.47	0
Porphyra umbilicalis	ephemeral	high	10/79	2	90.0 ± 1.0	3.04 - 3.09	8.83	
Ceramium	annual	high	10/79	2	87.0 ± 1.0	2.36 - 2.44	8.17	
CHLOROPHYTA:								
Ulva - Enteromorpha spp.	annual	high	1,3,5,6,10/79	8	91.1 ± 1.1	1.90 - 4.41	7.99 - 15.74	0 - 0.244
Codium fragile	annual	low	7,10/79	4	94.1 ± 1.0	1.07 - 2.22	8.43 - 14.55	0.206

content, nitrogen/carbon ratio, and polyphenol/nitrogen ratio; results are shown in Figures 1 - 8. Other annual and ephemeral algal species were collected and analyzed at their seasonal peaks (Table 1); Ulva-Enteromorpha spp., which are annuals but present throughout the year and of high food preference to L. littorea, were sampled once in the winter, spring, summer, and fall (Figures 9 and 10).

Nitrogen

Nitrogen contents (% dry weight) varied from 0.56 - 4.49% for the brown algal species, 1.79 - 3.09% for red algal species, and 1.07 - 4.41% for green algal species. Species highly preferred as food by L. littorea had a range of nitrogen contents similar to those of species of low food preference. Ephemeral and annual species had ranges similar to those of perennials.

F. vesiculosus and A. nodosum showed a seasonal pattern of high nitrogen contents during January - February, dropping to low values during April through June (Figures 1 and 2). Between-plant and within-plant differences in this seasonal pattern were slight. During the winter, F. vesiculosus tissues were generally slightly higher in nitrogen than were A. nodosum tissues. During the remainder of the year, the two species had similar tissue nitrogen contents. Apices and receptacles of both species had somewhat higher nitrogen contents than stipes and midsections during January - February, but during the rest of the year the within-plant nitrogen contents of the different tissues generally did not vary significantly and showed similar trends.

Figure 1. Seasonal variation in nitrogen content of Fucus vesiculosus apices, receptacles, midsections, and stipes. (% dry wt., mean ± s.e., n = 2).

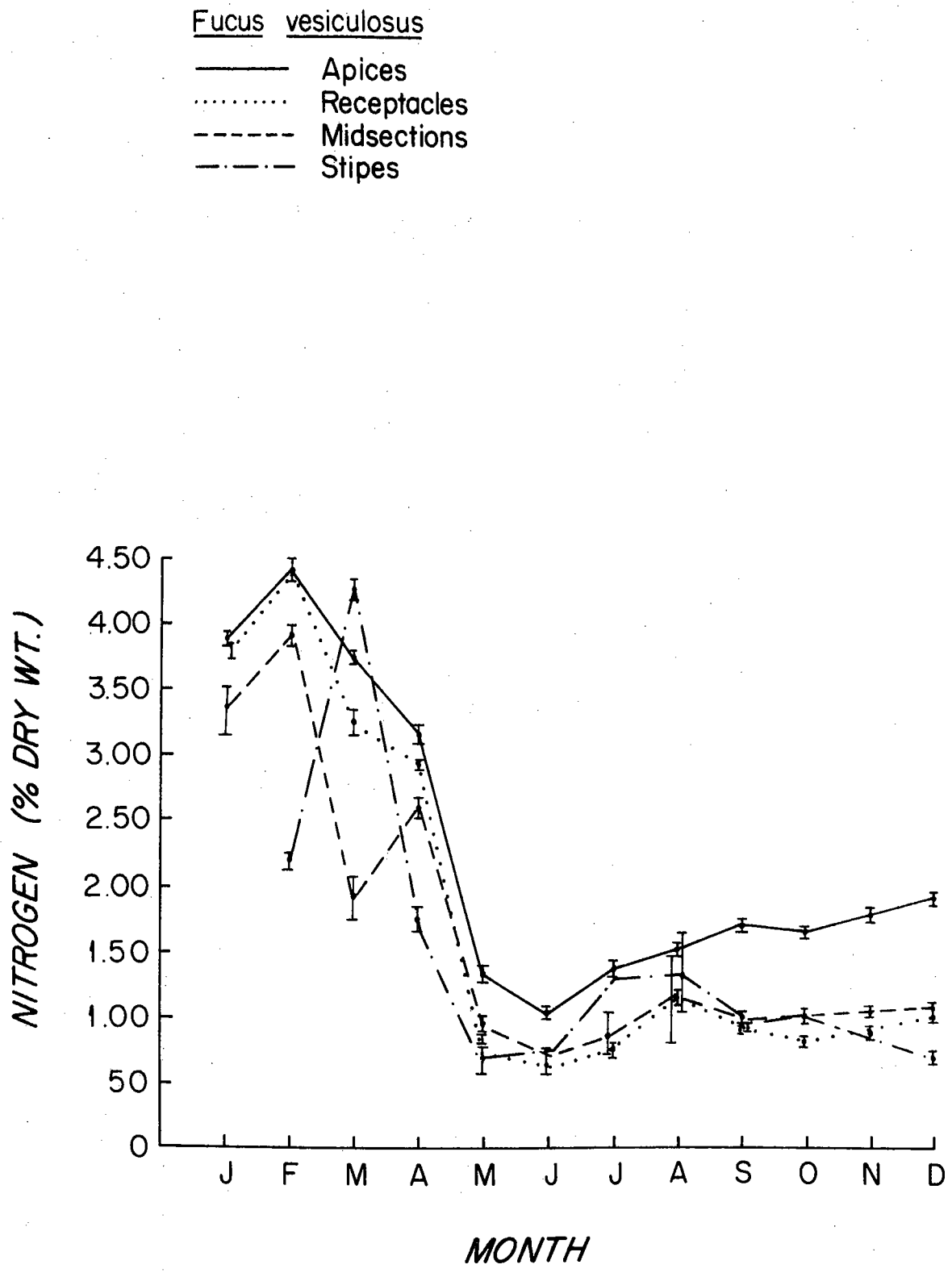

Fucus vesiculosus
Apices
Receptacles
Midsections
Stipes
NITROGEN (% DRY WT.)
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
50
0
J F M A M J J A S O N D
MONTH

Figure 2. Seasonal variation in nitrogen content of Ascophyllum nodosum apices, receptacles, midsections, and stipes. (% dry wt., mean ± s.e., n = 2).

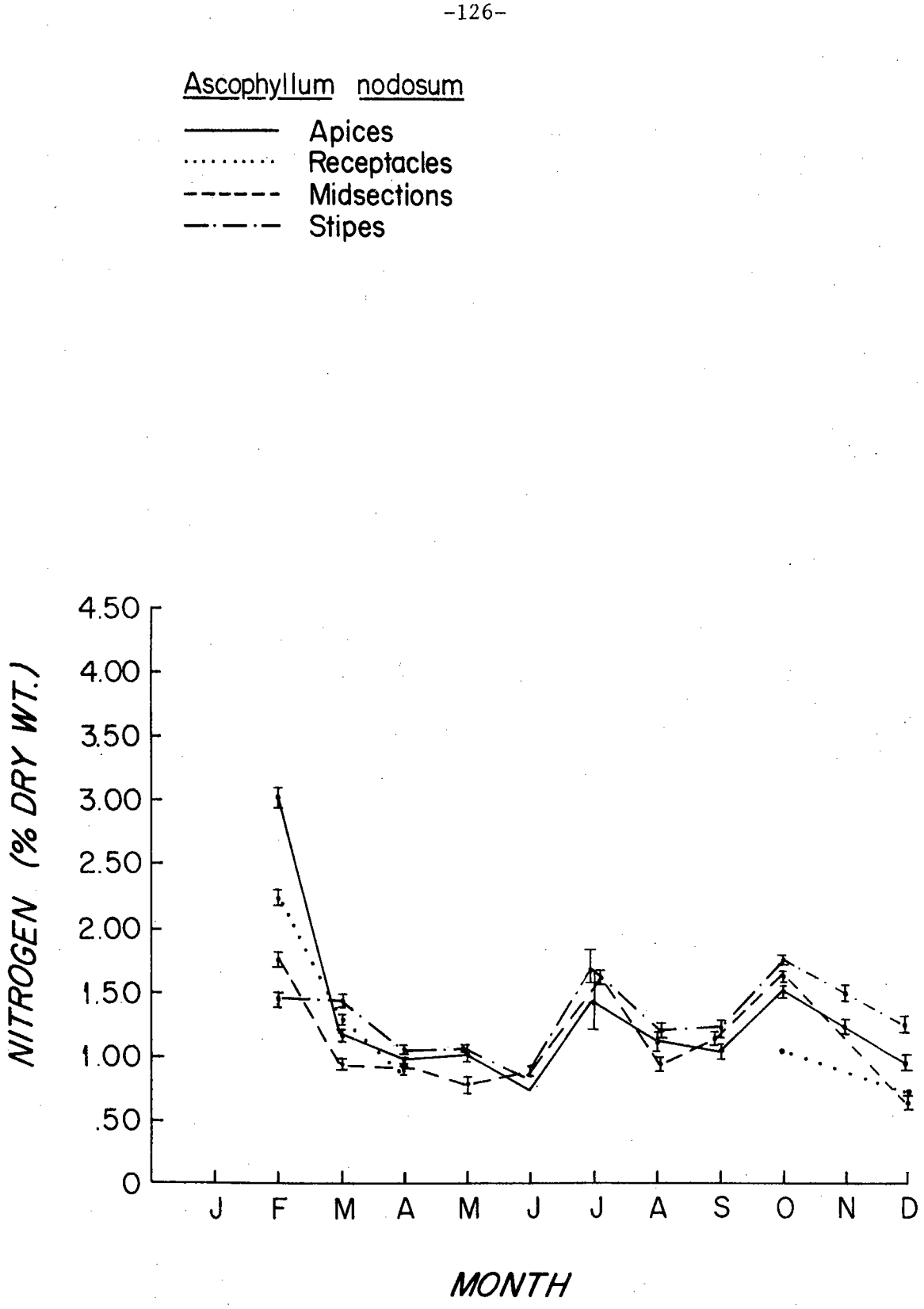

Ascophyllum nodosum
Apices
Receptacles
Midsections
Stipes
NITROGEN (% DRY WT.)
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
.50
0
J F M A M J J A S O N D
MONTH

Figure 3. Seasonal variation in the carbon/nitrogen ratio in *Fucus vesiculosus* apices, receptacles, midsections, and stipes. (mean ± s.e., n = 2; ratios were computed using % dry wt. carbon and % dry wt.nitrogen; ratio standard errors were computed using first order perturbation theory of Deming, 1944).

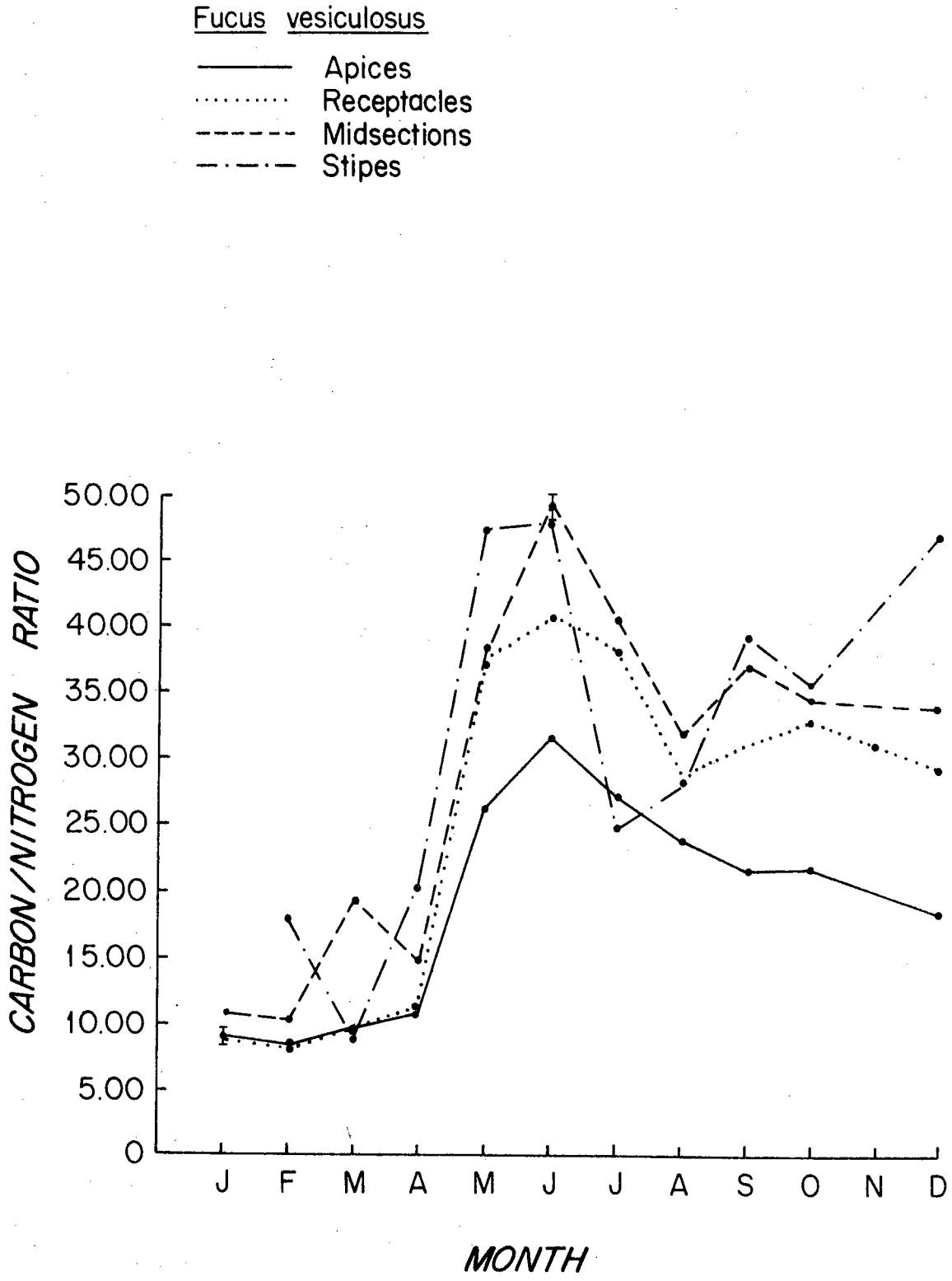

Fucus vesiculosus
Apices
Receptacles
Midsections
Stipes
CARBON/NITROGEN RATIO
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0
J F M A M J J A S O N D
MONTH

Figure 4. Seasonal variation in the carbon/nitrogen ratio in Ascophyllum nodosum apices, receptacles, midsections, and stipes.
(mean ± s.e., n = 2; ratios were computed using % dry wt. carbon and % dry wt. nitrogen; ratio standard errors were computed using first order perturbation theory of Deming, 1944).

Ascophyllum nodosum

——— Apices
..... Receptacles
- - - - - - Midsections
—·—·— Stipes

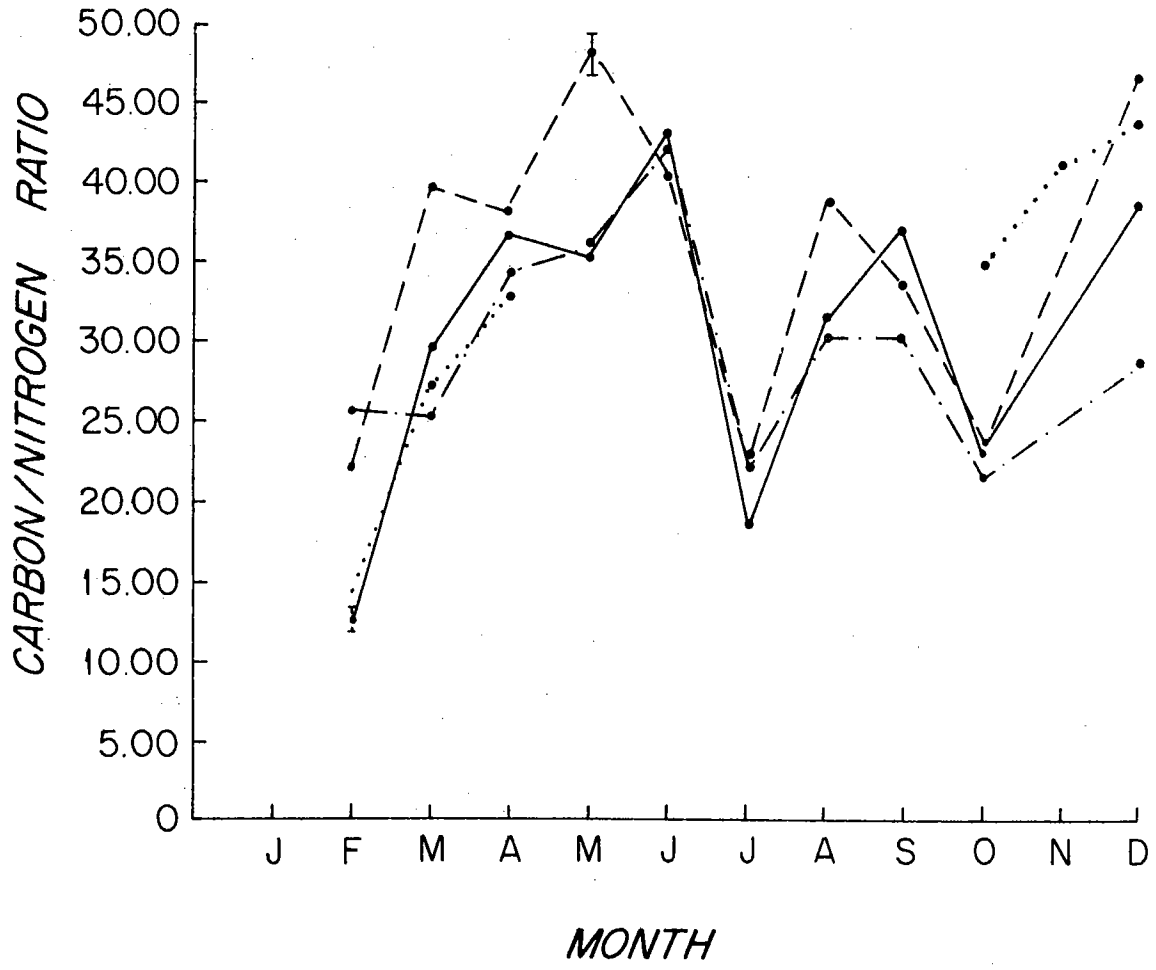


Figure 5. Seasonal variation in the polyphenol/nitrogen ratio in Fucus vesiculosus apices, receptacles, midsections, and stipes. (mean ± s.e., n = 2; ratios were computed using % dry wt. polyphenol and % dry wt. nitrogen; ratio standard errors were computed using first order perturbation theory of Deming, 1944).

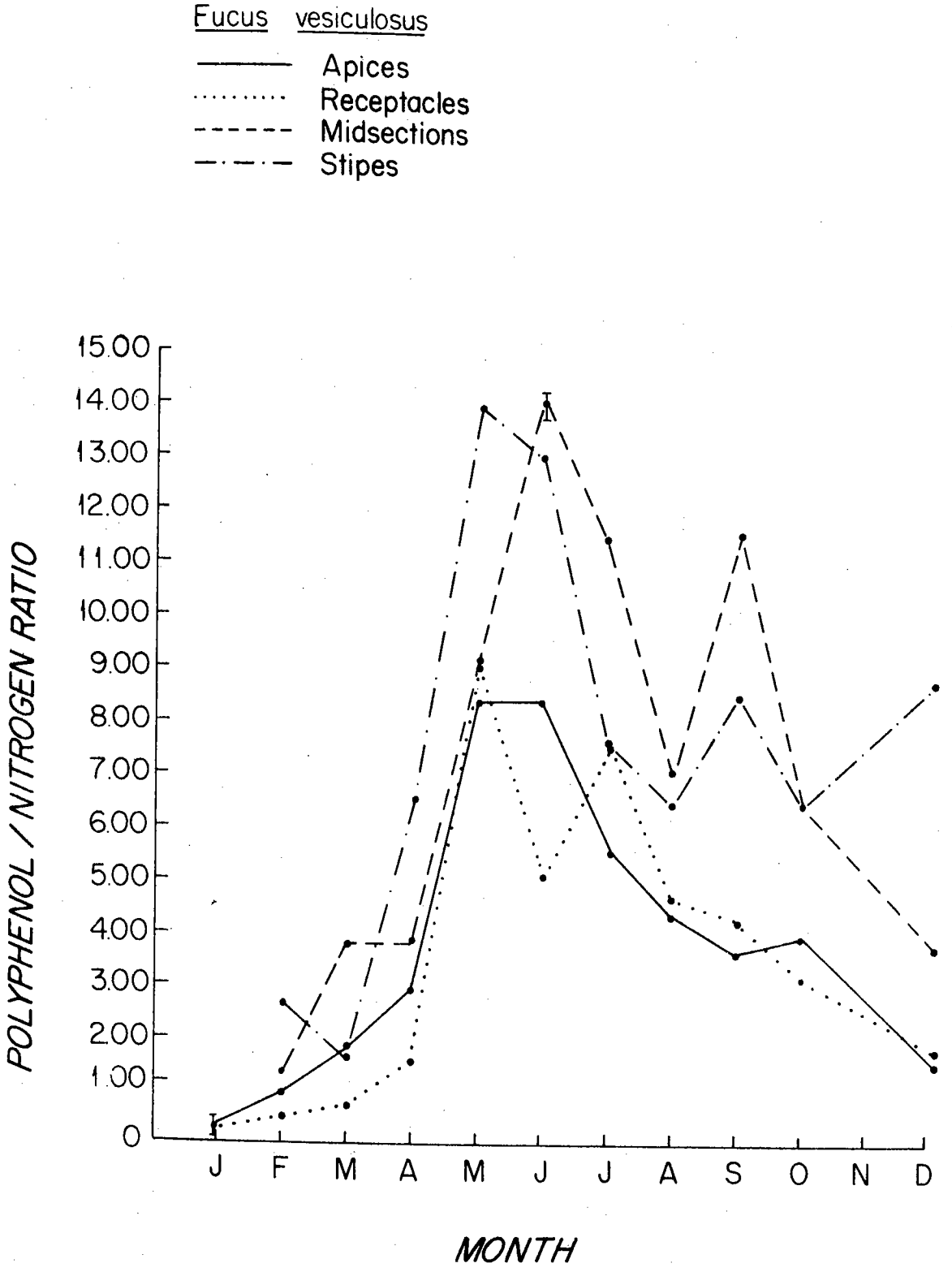

Fucus vesiculosus
Apices
Receptacles
Midsections
Stipes
POLYPHENOL / NITROGEN RATIO
15.00
14.00
13.00
12.00
11.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0
J F M A M J J A S O N D
MONTH

Figure 6. Seasonal variation in the polyphenol/nitrogen ratio in Ascophyllum nodosum apices, receptacles, midsections, and stipes.
(mean ± s.e., n = 2; ratios were computed using % dry wt. polyphenol and % dry wt. nitrogen; ratio standard errors were computed using first order perturbation theory of Deming, 1944).

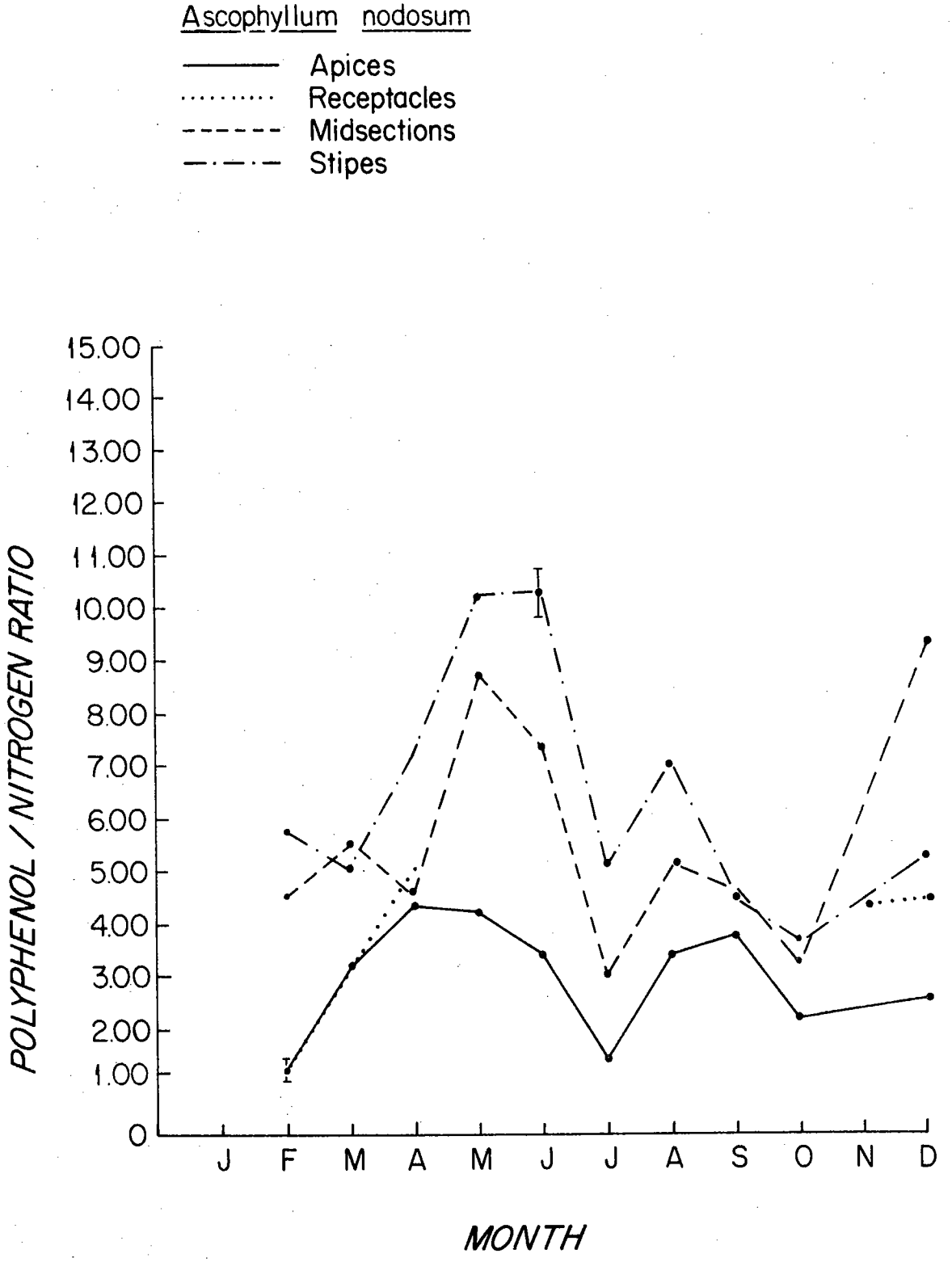

Ascophyllum nodosum
Apices
Receptacles
Midsections
Stipes
15.00
14.00
13.00
12.00
11.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0
POLYPHENOL / NITROGEN RATIO
J F M A M J J A S O N D
MONTH

Figure 7. Seasonal variation in water content of Fucus vesiculosus apices, receptacles, midsections, and stipes. (% fresh wt., mean ± s.e., n = 2).

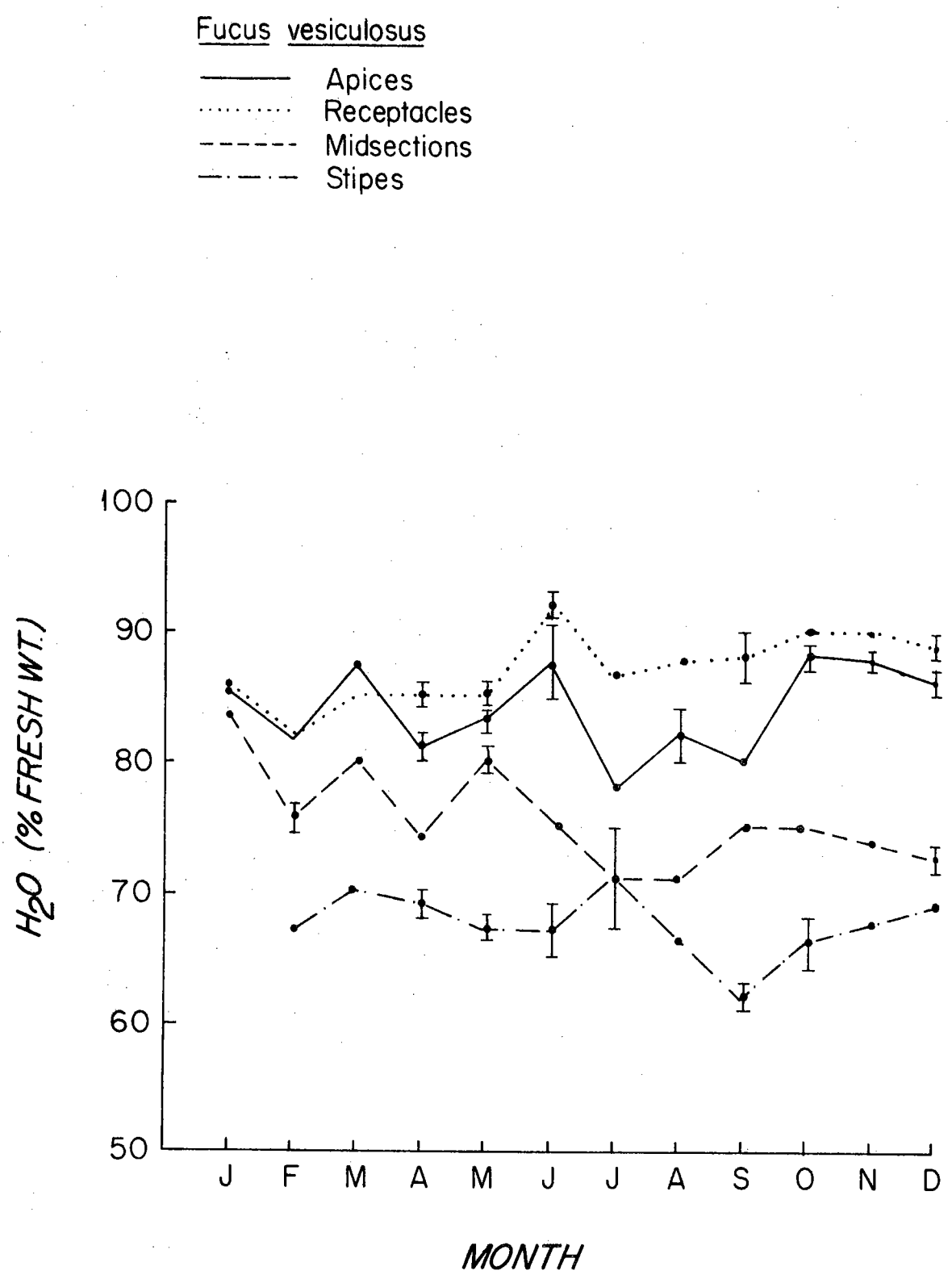
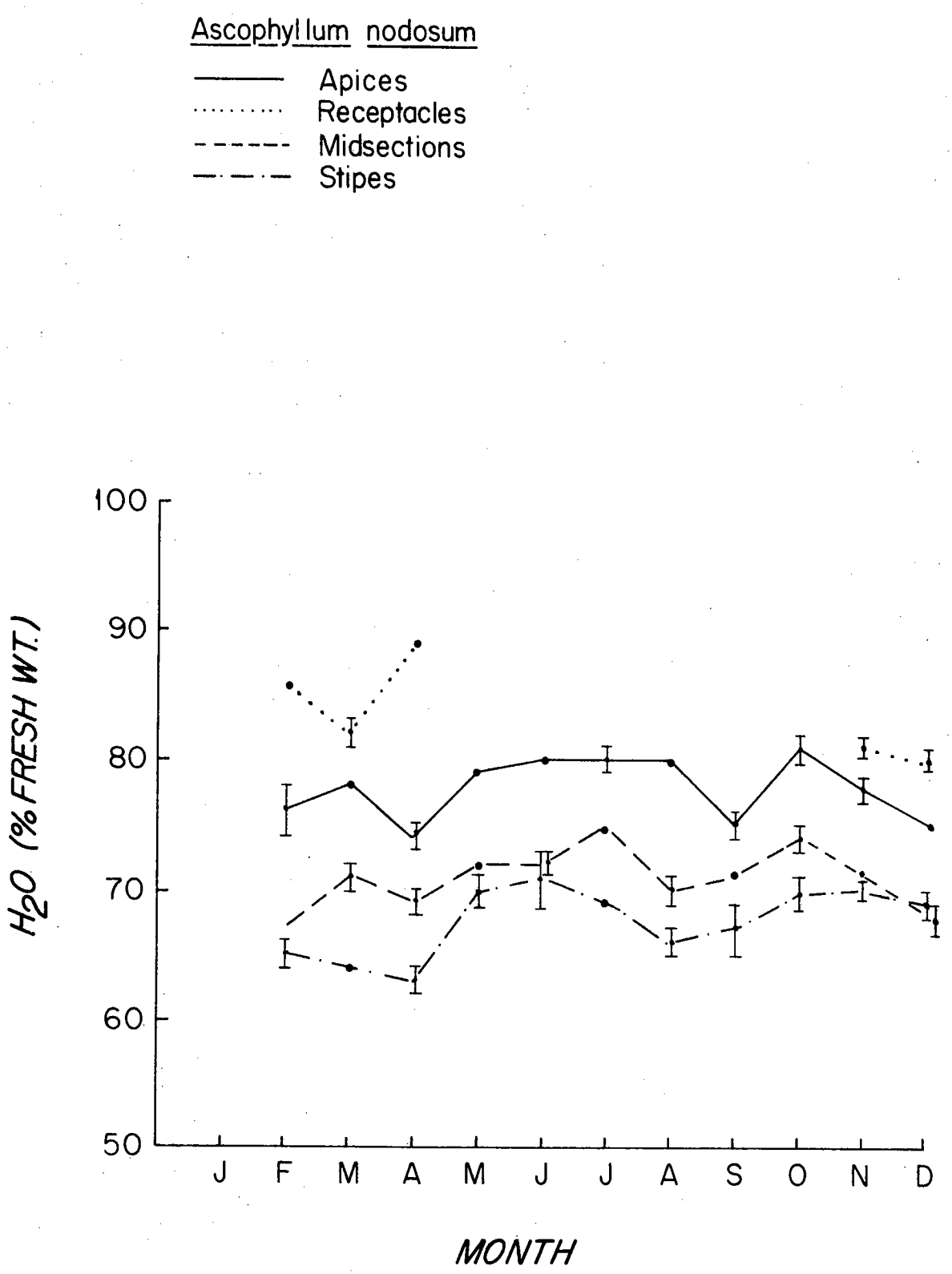

Fucus vesiculosus
Apices
Receptacles
Midsections
Stipes
100
90
80
70
60
50
H2O (% FRESH WT.)
J F M A M J J A S O N D
MONTH

Figure 8. Seasonal variation in water content of Ascophyllum nodosum apices, receptacles, midsections, and stipes. (% fresh wt., mean ± s.e., n = 2).


Ascophyllum nodosum
Apices
Receptacles
Midsections
Stipes
100
90
80
70
60
50
H2O (% FRESH WT.)
J
F
M
A
M
J
J
A
S
O
N
D
MONTH

Ulva-Enteromorpha spp. similarly showed highest nitrogen contents in winter/early spring, with values dropping in the summer. The values were similar to those of F. vesiculosus and A. nodosum during the winter but were higher during the summer (Figure 9).

Carbon/nitrogen ratio

Carbon/nitrogen (C/N) ratios varied greatly, from 8.30 - 49.30 in the brown algae, 8.17 - 15.17 in the red algae, and 7.99 - 15.74 in the green algae (Table 1). In all tissues of F. vesiculosus and A. nodosum and in Ulva-Enteromorpha spp., C/N showed a seasonal pattern inverse to that of nitrogen content: low values in winter/spring increased to highs in the summer which then decreased in the fall (Figures 3, 4, and 10).

Polyphenol/nitrogen ratio

Brown algae had the greatest range of polyphenol/nitrogen ratios (0.144 - 14.163), with highest values in the perennial and low food preference species (F. vesiculosus and A. nodosum), and lowest values in the ephemeral and high food preference species (Petalonia fascia and Scytosiphon lomentaria) (Table 1). All red and green algae had low polyphenol/nitrogen ratios (0 - 0.244).

The polyphenol/nitrogen ratios in F. vesiculosus and A. nodosum showed seasonal variations similar to those of their C/N ratios, with low values in the winter and high values in the summer (as carbon and polyphenol contents increased, nitrogen contents decreased) (Figures 5 and 6). The seasonal differences in these values were greater for F. vesiculosus tissues than for A. nodosum tissues. There were within-plant variations in polyphenol/nitrogen ratios in both species during certain

Figure 9. Seasonal variation in the nitrogen content (% dry wt.) and water content (% fresh wt.) of Ulva and Enteromorpha spp. (mean ± s.e., n = 2).

Ulva - Enteromorpha spp.

——— H_2O
------ Nitrogen

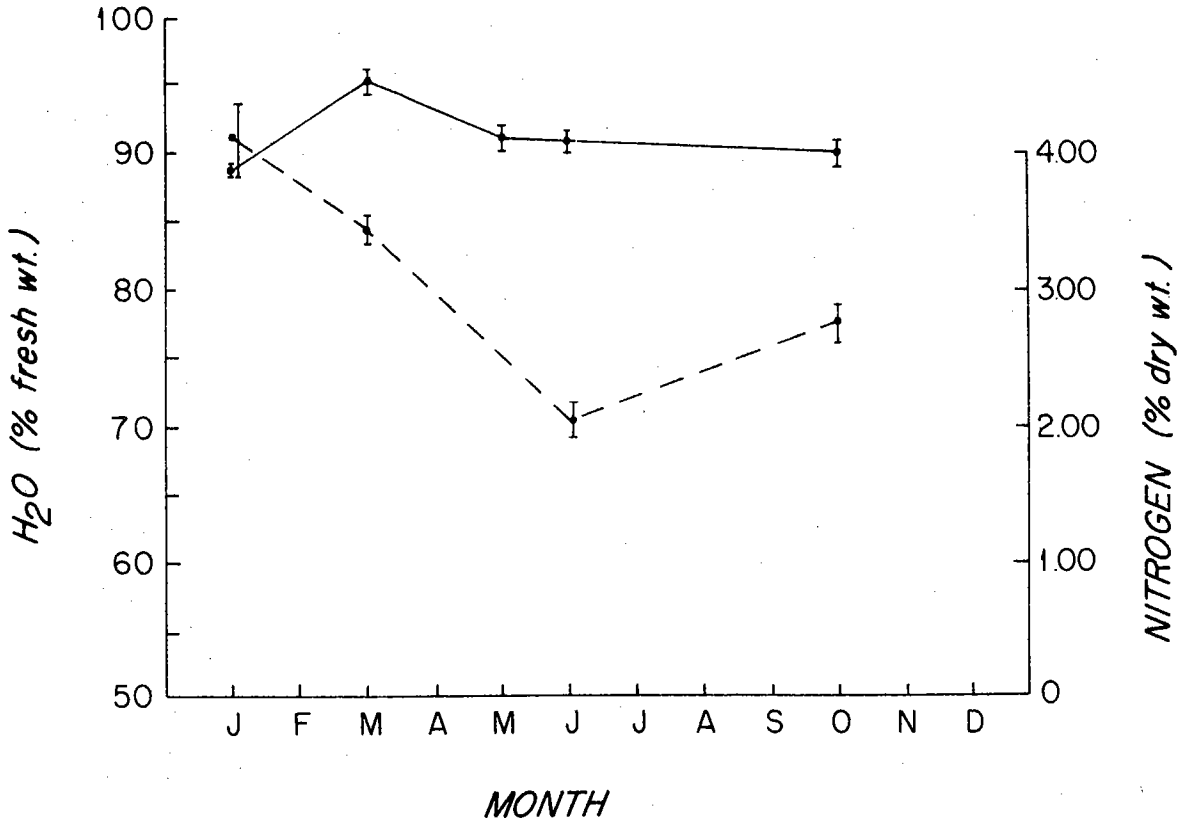
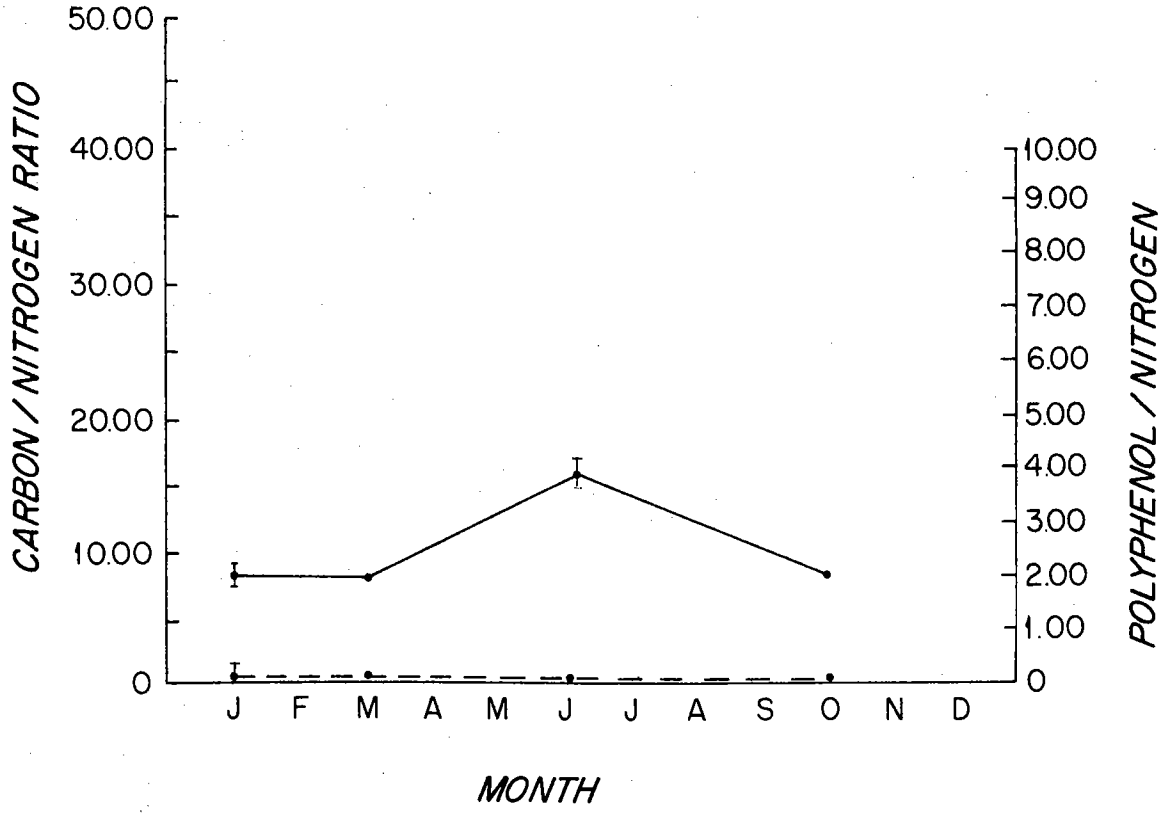


Figure 10. Seasonal variation in the carbon/nitrogen and polyphenol/nitrogen ratios of Ulva and Enteromorpha spp.
(mean ± s.e., n = 2; ratios were computed using dry wt. values for carbon, nitrogen, and polyphenol contents; ratio standard errors were computed using perturbation theory of Deming, 1944).

Ulva - Enteromorpha spp.

——— Carbon / Nitrogen

------ Polyphenol / Nitrogen



or all months. The stipes of A. nodosum had higher ratios than the apices throughout all months; midsections had ratios intermediate between or similar to these. A. nodosum receptacles are formed during the fall and are shed during the spring after releasing gametes; their polyphenol/nitrogen ratios were similar to those of older tissues during the fall and dropped during the winter to levels of apices until they dehisced. In contrast, F. vesiculosus receptacles were present throughout the year. Their polyphenol/nitrogen ratios were significantly lower than those of stipes and midsections from May-June and August-December, as were those of apices. During the other months, the ratios for apices, midsections, receptacles, and stipes were not significantly different. Because of very low polyphenol contents, Ulva-Enteromorpha spp. had polyphenol/nitrogen ratios near zero during all months (Figure 10).

H_2O

Water contents ranged from 67.2 - 91.8% in brown algae, 81.0 - 90.0% in red algae, and 91.1 - 94.1% in green algae (Table 1). Apices, receptacles, and germlings of F. vesiculosus and A. nodosum had higher water contents than midsections and stipes throughout all months; there did not appear to any significant seasonal variations in these values (Figures 7 and 8). Ulva-Enteromorpha spp. had high water content throughout all months with little seasonal variation.

Determining water contents accurately for intertidal algae is difficult because of the problems inherent in taking the fresh weight of plants that grow in seawater and which are subjected to varying degrees

of desiccation during low tides (dependent on temperature, sunlight, length of exposure). The algae must be rinsed quickly in distilled water to remove salt or saltwater from their surfaces, then blotted dry to remove the excess water before weighing. Thus, factors that would introduce errors in taking fresh weights include: degree of algal desiccation at low tide prior to collection; imprecision in blotting dry algal surfaces, with variable amounts of excess water remaining; and, seasonal or species differences in algal morphology which would cause variations in degree of desiccation or amount of excess water remaining after blotting. Water contents of these algae should therefore be considered with these limitations in mind. For most determinations (e.g., nitrogen, polyphenol contents), results are expressed as dry weight percentages since dry weights can be taken more accurately; using ratios to compare these contents eliminates fresh vs. dry weight considerations. Nevertheless, water contents were measured so that comparisons could be made to studies of terrestrial plants which have indicated the importance of water content as a factor in food plant selection by herbivores (Scriber and Feeny, 1979).

DISCUSSION

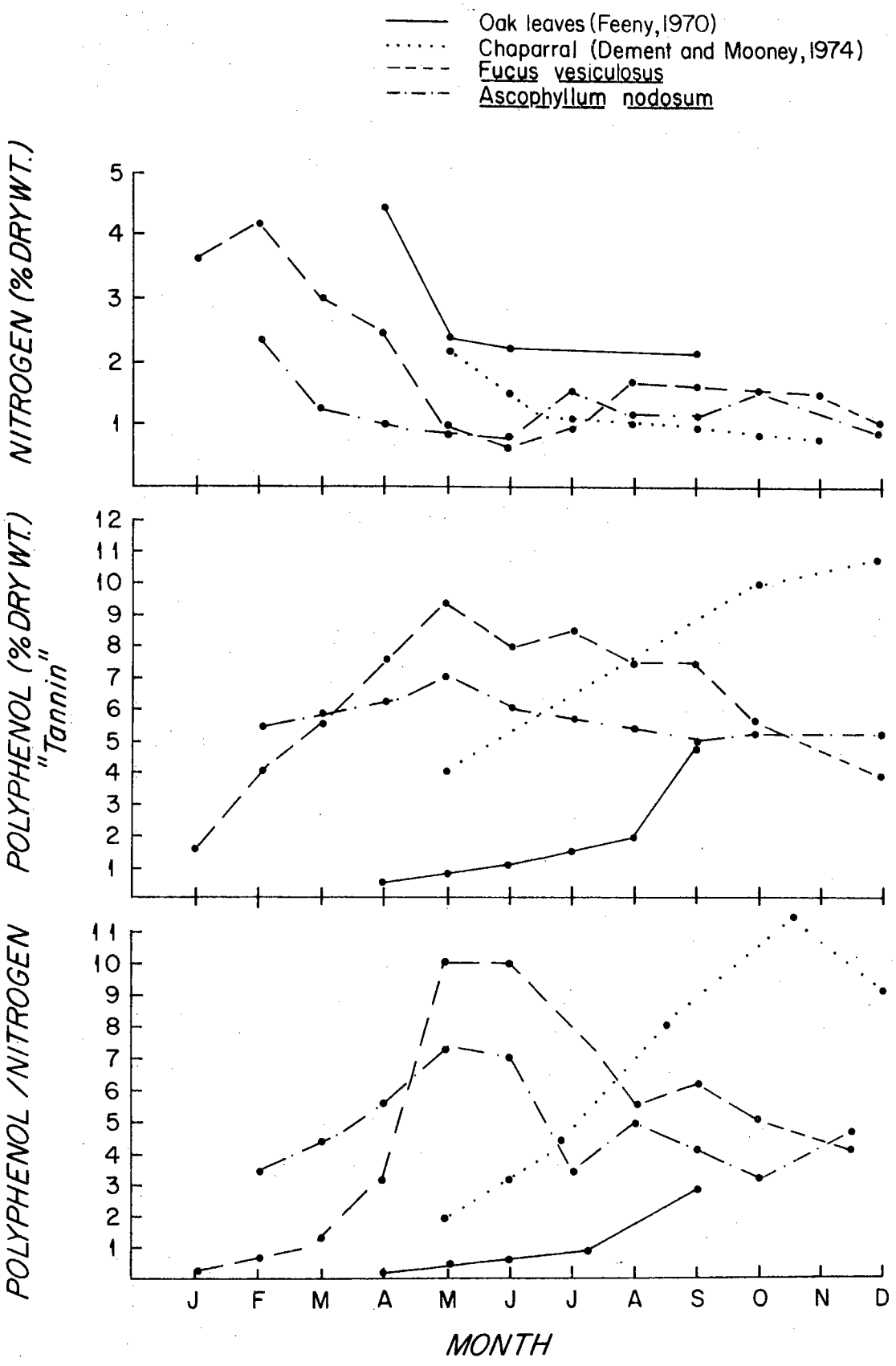
Nitrogen contents of these algae followed the general patterns observed for most terrestrial and marine plants (Feeny, 1970; Dement and Mooney, 1974; Mann, 1972): they were highest early in the growing season during periods of rapid growth, and they dropped as the season progressed

and as growth slowed or ceased. This pattern is most obvious in perennial algae such as F. vesiculosus and A. nodosum. Seasonal variations in their nitrogen contents are very similar to those of perennial terrestrial plants such as oaks and chaparral (Figure 11). Shorter-lived annual and ephemeral species (e.g., Ulva-Enteromorpha spp.) may complete several cycles during a growing season, growing rapidly and maintaining high nitrogen contents throughout the year (Fig. 9).

Plant nitrogen contents are important in determining plant nutritional values to herbivores. Studies have shown that many herbivores are limited by the availability of nitrogen in their food plants, and herbivores' feeding rates and preferences are often correlated with plant nitrogen contents (Van Emden, 1966 and 1972; Slansky and Feeny, 1977; McNeil and Southwood, 1978; Vince, 1979). However, plant nitrogen content does not necessarily reflect plant nitrogen availability to herbivores. Plant nitrogen may be bound in complexes refractory to herbivores' digestive processes. Polyphenols in plants are especially important in this consideration because of their known abilities to bind to proteins (Van Sumere et al., 1975). Therefore, it is important to consider the interaction between polyphenol and nitrogen contents in relation to herbivore food plant preferences.

An estimate of the unavailability of plant nitrogen to herbivores due to polyphenols may be obtained from plant polyphenol/nitrogen ratios: the higher the ratio, the more likely that nitrogen in the form of protein is bound to polyphenols in refractory complexes (Feeny, 1970; Rhoades and Cates, 1976). In these previous studies, "protein" was determined by

Figure 11. Comparison of Fucus vesiculosus and Ascophyllum nodosum with oak leaves and chaparral shrub in their seasonal variations of nitrogen content, polyphenol content, and polyphenol/nitrogen ratio (data from Figures 1, 2, 5, and 6 in this chapter; Figures 3 and 5 in Chapter 3; Feeny, 1970; and Dement and Mooney, 1974).


Oak leaves (Feeny, 1970)
Chaparral (Dement and Mooney, 1974)
Fucus vesiculosus
Ascophyllum nodosum
NITROGEN (% DRY WT.)
POLYPHENOL (% DRY WT.) "Tannin"
POLYPHENOL /NITROGEN
MONTH
J F M A M J J A S O N D

measuring plant nitrogen content and multiplying by a predetermined factor of 6.25 (Long, 1961); plant polyphenol/protein ratios were then computed and compared. But since this factor does always accurately convert nitrogen to protein content (Staresinic, 1978), I have used what was actually measured (nitrogen, not protein) in the ratios.

In the New England rocky intertidal algae, high levels of polyphenols are characteristic of the dominant perennial browns, F. vesiculosus and A. nodosum; ephemeral browns have much lower levels. Red and green algae, including perennials, annuals, and ephemerals, contain zero or very low levels of polyphenols (Chapter 3). Algal polyphenols were shown to be dosage-dependent feeding inhibitors to L. littorea. Therefore, I suggest that polyphenols in the brown algae act as "quantitative" defenses. This supports the hypotheses of Feeny (1976) and Rhoades and Cates (1976), based on studies of terrestrial plants, that polyphenols are general and nonspecific defenses which tend to be characteristic of highly predictable "apparent" plants or plant tissues. The results reported here and in Chapters 1, 2, and 3 provide the first evidence that two marine algae, F. vesiculosus and A. nodosum, are similar to terrestrial plants in the nature and functioning of their chemical defenses.

Red and green algae appear to differ from the browns because of their lack of high polyphenol levels. Species that are not eaten by L. littorea such as C. crispus and Codium fragile must have other chemical or physical adaptations that deter feeding by this herbivore (Chapters 1, 3, 5).

Because low nitrogen availability has been shown to be important in determining food preferences of plants to herbivores, and because certain species in this algal community contain high levels of polyphenols which can render plant nitrogen unavailable to herbivores, nitrogen content and polyphenol/nitrogen ratios of these algae were examined in relation to algal feeding preferences of L. littorea. The results showed that nitrogen contents of low preference species such as F. vesiculosus and A. nodosum were as high or higher than those of highly preferred species such as Scytosiphon lomentaria, Ceramium spp., and Ulva-Enteromorpha spp. However, polyphenol/nitrogen ratios of F. vesiculosus and A. nodosum were very high in comparison to these other species, so it is likely that the nitrogen of F. vesiculosus and A. nodosum is less available nutritionally to herbivores. I suggest that F. vesiculosus and A. nodosum are similar to terrestrial plants such as oaks and chaparral in their patterns of chemical defense (Feeny, 1970 and 1976; Dement and Mooney, 1974; Rhoades and Cates, 1976). Figure 11 summarizes the comparisons. Early in the growing season, polyphenol levels are low and nitrogen levels are high. As the season progresses and rapid growth ceases and tissues mature, polyphenol levels increase and nitrogen levels decrease. Thus, polyphenol/nitrogen ratios are quite low during the early growing season and become quite high as the season progresses. This means that plant nitrogen becomes increasingly susceptible to binding by polyphenols and hence unavailable to herbivores. F. vesiculosus and A. nodosum are best protected with these digestibility-reducing systems during the period of greatest grazing

pressure by L. littorea (late spring through late summer, because the herbivores become less active when air and water temperatures are low), just as was shown in the case of oaks and chaparral and their herbivores.

F. vesiculosus showed greater seasonal changes in polyphenol/nitrogen ratios than A. nodosum, with lower values in the winter and higher values in the summer than the latter. The winter polyphenol/nitrogen ratios in F. vesiculosus were so low that they approached the levels of ephemeral browns preferred by L. littorea such as P. fascia. Menge (1975) found that L. littorea does not eat Fucus plants (greater than 3-5 cm.) during the summer, but will eat it, but not A. nodosum, during the late fall-early winter when other food is scarce. Thus, it appears that F. vesiculosus may become more edible during the winter due to lower polyphenol content and higher nitrogen availability. That is, F. vesiculosus is less defended by digestibility-reducing systems from the herbivores during the winter.

Previous studies have shown that fucoid algae exude polyphenols, especially under stress conditions (Craigie and McLachlan, 1964; Sieburth and Jensen, 1968, 1969). It is possible that exudation of these compounds because of low air and water temperatures and freezing of algal tissues during the winter, combined with lowered levels of polyphenol synthesis during the latter part of the growing season, result in levels too low to afford plant protection from herbivores during the winter. With increased growth and production in the spring, the plants again attain levels of these compounds sufficient to deter herbivores. Similarly, certain freshwater plants are not eaten until tannins are

leached and their physical structures altered by rainfall and microbial action (Cameron and LaPoint, 1978).

Polyphenol/nitrogen ratios are somewhat lower in apices and receptacles than in older portions (stipes and midsections) of these plants (Figures 5 and 6). Thus, it appears that older tissues of these fucoids may be better defended than young growing tips and reproductive bodies (which are ephemeral in A. nodosum). However, polyphenol levels relative to nitrogen contents may be high enough to inhibit feeding of all fucoid tissues during the spring, summer, and fall but not during the winter.

Very small Fucus germlings have been reported to be susceptible to L. littorea grazing (Menge, 1975; D. Cheney, pers. comm.). I found in laboratory feeding experiments and in field observations that these herbivores may take bites from the germlings but feed preferentially on other ephemeral brown, red, and green algae when the choice is available. However, while foraging, the snails "bulldoze" down the small germlings, often resulting in damage to them; this activity could mistakenly be interpreted as grazing. Although the ramifications for the plants are the same, it is important to distinguish between loss due to grazing and loss due to disruption, lest the "right prediction for the wrong reason" (Dayton, 1973) be made in these plant-herbivore interactions. Fucus germlings (1-3 cm., collected in summer) had polyphenol contents and polyphenol/nitrogen ratios nearly as high as those of mature plants (Table 1), so it appears that the plants become chemically defended while relatively small and young. Minute germlings

(approximately 0.5 cm.) could not be collected in large enough quantities to allow for polyphenol analysis by the Folin-Denis method; when examined by thin layer chromatography, they showed vanillin-reactive compounds similar to, but perhaps in lower concentrations than, those of adult plants (O. McConnell, pers. comm.). Thus, chemical defenses may be present in germling stages but not effective in protecting them from L. littorea because of the susceptibility of germlings to the "bulldozing" activities of the herbivores. These observations on chemical defense for fucoid germlings differ from the patterns observed for most terrestrial "apparent" plants (such as oaks and chaparral), whose young tissues have less commitment to chemical defense than mature plants (Feeny, 1976; Rhoades and Cates, 1976).

The algae in this rocky intertidal community having very low polyphenol contents or none (and hence very low polyphenol/nitrogen ratios) are either 1) of low food preference because of other chemical or physical factors (e.g., C. crispus, C. fragile, Ralfsia crusts; see Chapters 1, 3, 5 for discussion), or 2) of high food preference, relying on temporal or spatial escapes from herbivores for survival (e.g., P. Fascia, P. umbilicalis, Ulva-Enteromorpha spp.; see Menge, 1975 and Lubchenco, 1978). Factors determining high food preference of algae to L. littorea probably include high nitrogen availability (high nitrogen content and absence of polyphenols) and high water content, both of which have been shown to be important in correlating terrestrial plant preferences to herbivores (Slansky and Feeny, 1977; Scriber and Feeny, 1979).

It can be concluded that low preference of F. vesiculosus and A. nodosum is due to high contents of polyphenols and the presumed consequent decrease in nitrogen availability. The observations on polyphenol and nitrogen contents in these two fucoids and other algae in the New England rocky intertidal community suggest many similarities to terrestrial plants and their patterns of chemical defense against herbivores.

CHAPTER 5

Volatile Compounds from Algae: Effect of Halomethanes on *Littorina littorea* Feeding Activity

Part I. Volatile Hydrocarbons and Halomethanes Released from Benthic Algae and Seagrass. P. Gschwend and J. A. Geiselman.

Part II. Effect of CH_2I_2 and $CHBr_3$ on Feeding Activity of *Littorina littorea*.

CHAPTER 5, PART I

Volatile Hydrocarbons and Halomethanes Released from Benthic Algae and Seagrass[1]

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ABSTRACT

Several species of benthic algae and a seagrass were batch incubated to estimate their release rates of volatile hydrocarbons and halomethanes into seawater. Saturated and unsaturated hydrocarbons and di- and trihalomethanes were released at rates which may account for the concentrations of these volatile organic compounds in coastal seawater. Certain of these compounds may be involved in allelochemic interactions between species.

Key words: benthic algae, halomethanes, hydrocarbons, volatile

INTRODUCTION

In a seasonal study of the volatile organic compounds occurring in coastal seawater, the temporal variations of the concentrations of several compounds suggested benthic algal sources (Schwarzenbach et al., 1978; Gschwend, 1979). These volatiles included hydrocarbons such as pentadecane and halogenated compounds such as bromoform. Some species of benthic algae are known to contain these and similar compounds (Youngblood et al., 1971; Youngblood and Blumer, 1973; Mynderse and Faulkner, 1975; Burreson et al., 1975 and 1976; Fenical, 1975; Crews, 1977; Moore, 1977). However, evidence for release of these compounds into the seawater environment is lacking, and their ecological roles are unknown. In contrast, the release of volatile compounds from terrestrial plants and their importance in allelochemic interactions between species has been well documented (Asplund, 1968; Muller and Chou, 1972; Halligan, 1975; Scholl et al., 1977; Vernon et al., 1977; Bergstrom, 1978; Sturgeon, 1979). A current study of chemical aspects of plant-herbivore interactions in the New England rocky intertidal community has suggested the involvement of volatile compounds in certain algal species as defenses against herbivores (Geiselman, in prep.). The objectives of this investigation were: 1) to assess the rate of release into seawater of volatile compounds from benthic algae and seagrass to determine if these plants could be the source of volatile hydrocarbons and halomethanes found in Vineyard Sound, Massachusetts, and 2) to examine

plant species ranging from those preferred to those avoided as food sources for the herbivorous snail, Littorina littorea, for their production and release of volatile compounds, particularly the halomethanes, as possible antiherbivore agents.

METHODS

Algae and seagrass were collected in Vineyard Sound from Nobska Point to Chemotaxis Dock (CD), Falmouth, Ma. (Table 1). Efforts were made to avoid plants with macroscopic epiphytes; microscopic epiphytes could not be excluded. The plants were placed in 2 liter reagent flasks filled with seawater. The flasks were stoppered and then anchored at a depth of approximately 1.5 m. at CD or in outdoor running-seawater tanks at a nearby laboratory for incubation at ambient sunlight and temperature conditions. After 24 hr., the flasks were brought to the lab and purged for one hour (with the plants still in place) with recycled air at 20°C (except the May samples at 35°). The effluent volatile organic compounds were trapped on a charcoal trap which was subsequently spiked with 20 ng. 1-chloro-n-octane in 2 µl. CS_2 and extracted with 15 µl. of CS_2 or CH_2Cl_2 and then extracted with 15 ml CS_2 or CH_2Cl_2 (Grob, 1973; Grob and Zurcher, 1976).

Gas chromatographic analyses were made using a Carlo Erba model 2101 gas chromatograph equipped with a flame ionization detector (FID) and splitless injector. Samples were chromatographed on an SE 54 glass capillary column (0.32 mm. i.d. x 20 m. long) purchased from Jaeggi

TABLE 1: Date of collection, seawater temperature, and dry weight of benthic algal samples collected near CD.

Sample	Date (1978)	Dry Wt. (g)	Seawater Temp.(°C)
PHAEOPHYTA			
Fucus vesiculosus Linnaeus	May 11	10	12
Fucus vesiculosus	Aug.22	5	22
Fucus vesiculosus	Oct. 2	9.6	17
Fucus vesiculosus, (germlings)	Oct.18	10.0	14
Petalonia fascia (Muller)Kuntze/ Scytosiphon lomentaria (Lyngbye)C. Agardh	May 11	5	12
Sargassum filipendula C. Agardh	Oct. 2	6.6	17
Ascophyllum nodosum (Linnaeus)Le Jolis	Oct.18	21.8	14
CHLOROPHYTA			
Codium fragile (Suringar)Hariot	Oct. 2	5.5	17
Enteromorpha spp.	Oct. 2	3.4	17
RHODOPHYTA			
Polysiphonia spp.	Oct. 2	3.8	17
Porphyra umbilicalis (Linnaeus)J. Agardh	Oct. 2	4.1	17
Chondrus crispus Stackhouse	Oct.18	8.3	14
Ceramium spp.	Oct.18	1.3	14
SEAGRASS			
Zostera marina Linnaeus	Aug.22	2	22

(9043, Trogen, Switzerland) and on a UCON HB 5100 glass capillary column (0.3 mm. i.d. x 20 m. long) supplied by Dr. K. Grob (EAWAG, Dubendorf, Switzerland). The columns were held at room temperature for 8 min. then programmed from 20 - 200°C at 3°C/min. for the SE54 or 20 - 180°C at 3°C/min. for the UCON. Helium was the carrier gas (ca. 1 ml./min.). Compound concentrations were calculated based on peak height relative to the 1-chloro-n-octane standard and were not corrected for inefficient stripping or differential FID response.

Gas chromatography-mass spectrometry was performed on a Finnegan 3200 GC-MS system using an SE52 glass capillary column (0.3 mm. i.d. x 20 m. long). Electron impact (EI) spectra were acquired with an electron potential of 70 eV. Chemical ionization (CH_4-CI) spectra were obtained with methane as the reagent gas at 950 microns ion source pressure and with electron energy of 130 eV. Compound identifications were based on retention times from two columns and both EI and CH_4-CI spectral comparisons with standards.

RESULTS

Release of individual volatile organic compounds from the plant samples was estimated by subtracting the seawater control concentration from the seawater plus plant concentration, then dividing by the dry weight of the plant sample. Table 2 summarizes the results for the hydrocarbons and halogenated methanes. Most of the discussion will be restricted to samples which showed more than 5 times the concentration of

TABLE 2: Hydrocarbon and halomethane release rates (ng/g dry wt/day) from algae into seawater. Values in parentheses indicate that seawater concentrations of volatiles after algal incubation were two to five times greater than concentrations found in the control seawater. Other values represent increases in seawater concentrations of hydrocarbons of more than five times over the concentrations in the control seawater.

	Release Rates (ng/g dry wt./day)			
	nC_{15}	nC_{17}	$nC_{17:1}$	$nC_{17:1}$
PHAEOPHYTA				
Fucus vesiculosus, May	20			
Fucus vesiculosus, Aug.		(2)		
Fucus vesiculosus, Oct.	(3)			
Fucus vesiculosus, Oct. (germlings)	(0.4)			
Petalonia fascia/ *Scytosiphon lomentaria*	40			
Sargassum filipendula	(0.7)			
Ascophyllum nodosum	(0.9)			
CHLOROPHYTA				
Codium fragile	(1)	(2)		30
Enteromorpha spp.	60		200	
RHODOPHYTA				
Polysiphonia spp.				
Porphyra umbilicalis	(4)			
Chondrus crispus				
Ceramium spp.				
SEAGRASS				
Zostera marina		(2)		

TABLE 2: (Continued)

	Release Rates (ng/g dry wt./day)			
	$CHBr_2Cl$	CH_2BrI	$CHBr_3$	CH_2I_2
PHAEOPHYTA				
Fucus vesiculosus, May			10	1
Fucus vesiculosus, Aug.	10	(0.2)	20	1
Fucus vesiculosus, Oct.	(0.8)	2	20	
Fucus vesiculosus, Oct. (germlings)	(0.1)		4	(0.3)
Petalonia fascia/ *Scytosiphon lomentaria*	1	(0.2)	30	
Sargassum filipendula			(1)	
Ascophyllum nodosum	(0.5)		8	
CHLOROPHYTA				
Codium fragile			(1)	9
Enteromorpha spp.	(0.6)		20	
RHODOPHYTA				
Polysiphonia spp.				
Porphyra umbilicalis				
Chondrus crispus	(0.2)		4	
Ceramium spp.				
SEAGRASS				
Zostera marina			2	(0.1)

a given compound relative to the control seawater. Increases of only 2 - 5 times over control levels are shown in parentheses in the tables.

DISCUSSION

Pentadecane was released in large quantities in three samples: 1) Fucus vesiculosus, collected May 11; 2) Petalonia/Scytosiphon, collected May 11; 3) Enteromorpha spp., collected Oct. 2. All other brown algal samples showed small releases (less than 5 times that of control seawater) of this compound. Pentadecane release by F. vesiculosus samples collected in August and October may be lower than that of May samples for reasons of method or plant physiological differences. May samples were purged at 35°C; all following samples were purged at 20°C and hence may have shown lower pentadecane release due to lower temperature of stripping. However, this interpretation was not supported when we found that an October F. vesiculosus sample stripped at 50°C released 6 ng./g., while the May sample stripped at 35°C released 20 ng./g. Thus, while there may be some effect of temperature of stripping on pentadecane release it is likely that other factors are important.

Seasonal physiological differences affect algal chemical content and release. For Norwegian F. vesiculosus and Ascophyllum nodosum, accumulation and exudation of various plant products have been shown to vary between summer and winter, particularly with respect to fructification periods (Haug and Larsen, 1958; Jensen, 1969; Baardseth, 1970; Ragan and Jensen, 1978). Conover (1958) found that F. vesiculosus

from Vineyard Sound was "dormant" during summer months of July and August and that maximum growth took place in months before and after this time. Mathieson et al. (1976) showed that maximum growth and reproduction of F. vesiculosus in a New Hampshire estuary occurred during spring and early summer. Thus, the variations in the release of pentadecane between May and October F. vesiculosus samples may be related to the seasonal periodicity of growth and reproduction.

Blumer and his coworkers (Clark and Blumer, 1967; Youngblood et al., 1971; Youngblood and Blumer, 1973) reported the presence of pentadecane in brown algae, with values of 10 - 100 μg./g. dry wt. algae. Even the largest release rates, calculated for the brown algal samples from the present work, would take many months to empty such a reservoir of pentadecane from these algae. Therefore, the release rates appear reasonable. These investigators also reported somewhat lower levels of pentadecane (1 μg./g.) in the green alga, Enteromorpha sp. The higher release rates of pentadecane from our sample of Enteromorpha may indicate greater cell excretion by this plant compared to the others examined. Enteromorpha spp. have a high surface-to-volume ratio compared to species such as F. vesiculosus and A. nodosum, yet it is similar to that of Porphyra umbilicalis, which released very low levels of pentadecane. Damage from handling this seemingly fragile form during the experiment is unlikely to have caused greater rates of release than those which occur in its environment, where it is exposed to breaking waves and extremes of temperature and desiccation during low tides.

Simple calculations indicate that the release rates found in these experiments can account for the observed levels of pentadecane in Vineyard Sound seawater. A typical benthic algal biomass for this region is between 1.5 and 4 kg. wet wt./$m.^2$ (Conover. 1958). Assuming these plants are 80% water, this corresponds to 300 - 800 g. dry wt. algae/m^2. At a production and release rate of 30 ng. pentadecane/g. dry wt./day and assuming the average residence time of water at CD is 2 days (1.5 - 2.5 m. depth, 0.6 m. tidal amplitude), a standing stock of pentadecane from 18 - 48 µg./$m.^2$ may be expected. For a 2 m. water column, this corresponds to 10 - 20 ng. pentadecane/l. seawater. Nearly all of the seawater samples assessed in a year-round study at CD were of similar concentration (Gschwend, 1979). On three occasions during that study (June and September, 1977 and May - June, 1978), pentadecane levels in the coastal seawater rose dramatically . These incidents may reflect particularly strong storm activity breaking up macroalgae and releasing pentadecane to the seawater, or they may relate to seasonal physiological changes in algal growth and reproduction.

Heptadecane was not produced and released at rates similar to those of pentadecane in these incubation experiments. This observation is surprising since previous workers reported the importance of this hydrocarbon, particularly in red algae (Youngblood et al., 1971; Youngblood and Blumer, 1973). They found between 100 and 1000 µg. heptadecane/g. dry wt. red algae. At most, only a few ng. heptadecane/g. dry wt. algae/day were released in our experiments. A standing crop calculation similar to that performed for pentadecane indicates that this

rate would support about 0.5 - 2 ng. heptadecane/1. seawater. Heptadecane concentrations reported throughout the year at CD equal or exceed this concentration range. Peak heptadecane concentrations at CD may be derived from algal species or physiological stages of algae not included in these studies.

The two green algae studied revealed very high production rates for two unsaturated 17-carbon compounds. The retention time and GCMS data, along with the work of Youngblood et al. (12), suggest that the compound from Enteromorpha spp. was cis-3-heptadecene. Codium fragile demonstrated production and release of a different heptadecene. Since these compounds showed such high release rates, one might expect to observe them along with pentadecane in coastal seawater. However, neither unsaturated compound was seen in CD seawater. This reaffirms the suggestion made by Schwarzenbach et al. (1978) that brown benthic algae are the major source of pentadecane in this coastal region and suggests that the green algae are less important as a source of hydrocarbons to this coastal seawater, perhaps due to smaller production rates and standing crops compared to the brown algae and perhaps because their olefins are degraded rapidly. The biochemical and ecological roles of these natural hydrocarbons remain unknown.

Halogenated methanes were released by plants in our incubation experiments. All of these compounds were previously reported from a red alga, Asparagopsis taxiformis (Burreson et al., 1975 and 1976). Halogenation in the Rhodophyta has been extensively documented (Fenical, 1975), but little work has been done on other algal divisions.

In our experiments, bromoform (tribromomethane) was released by species in the Rhodophyta, Phaeophyta, and Chlorophyta, as well as by the marine vascular plant, Zostera marina. Epiphytic algae or microbes on the seagrass may have been responsible for production of this compound, or, the seagrass may have accumulated it from the surrounding seawater, only to release it upon purging. Production artifacts such as these may also have been operating for some of the algae examined and may explain the prevalence of bromoform release.

An examination of the F. vesiculosus data suggests that maximum release of bromoform occurs in the middle of the summer, when the algae are "dormant" (Conover, 1958) but are subjected to pest pressures such as settling of epiphytes and grazing by herbivores.

Lesser amounts of chlorodibromomethane were frequently produced with bromoform. High release rates of this compound were confined to the brown algal samples.

Release rates of 20 - 40 ng. haloform/g. dry wt./day suggest standing stocks of 1 - 2 ng. haloform/l. seawater in coastal seawater (these weight values, however, underestimate the true haloform levels due to insensitivity of an FID relative to the 1-chloro-n-octane internal standard). Such levels of bromoform were observed in some samples of Vineyard Sound seawater during summer months (Gschwend, 1979; Schwarzenbach et al., 1978) and in the August and October control seawater samples of this study.

Two dihalomethanes were also found. These were produced at about one-tenth the rate of the trihalomethanes. Since a 1, 1, 1-tribromo-2-

keto-compound, released into seawater, will react to produce bromoform (Burreson et al., 1975, 1976), the observed lower rate of production ofdihalomethanes may reflect the greater difficulty of cleavage of the dihalo-2-keto-compounds in seawater relative to the trihalo analogues. Although most dihalo-2- keto-compounds would not be expected to be cleaved in weakly basic seawater, the presence of iodine in both of these dihalocompounds would serve to enhance and possibly permit this cleavage.

$$XBr_2C\text{-}C(=O)\text{-}R \xrightarrow{K} XBr_2CH + HOC(=O)R \qquad X=Cl$$

$$HXIC\text{-}C(=O)\text{-}R \xrightarrow{K/10\,?} XICH_2 + HOC(=O)R \qquad X=Br$$

Thus, the keto-compounds may be the chemical agents actually produced and released by the algae.

Release rates on the order of several ng. dihalomethane/g. dry weight/day suggest that standing stocks of less than 1 ng. dihalomethane/l. seawater would result. Thus, our detection of these compounds would not be expected and, indeed, the CD year-round seawater samples did not reveal the presence of these compounds. Future work utilizing an electron capture detector may yield more information on the levels of these compounds in seawater because this detector is particularly sensitive to halogenated compounds.

Other compounds with other combinations of halogen substitution were likely present in the plants we examined but were not detected due to limitations of our methods (e.g., iodoform may be too soluble and have too high a boiling point to be purged from seawater).

Surprisingly, our red algal samples showed less inclination to release halogenated compounds than brown and green algal samples. Red algae are well known to contain halogenated terpenes and other metabolites (Fenical, 1975; Crews, 1977). Previous analyses may have used extraction techniques which disrupted plant cells and organelles and caused release of compounds not normally released into seawater. Alternatively, halocompounds may not have been released from our red algal samples due to their seasonal physiological status.

The physiological and ecological roles of the halocompounds in these algae are unknown. It has been hypothesized that these compounds serve as algal defenses against epiphytes, parasites, and herbivores (Fenical, 1975). It may be advantageous for the plant to produce or store these compounds near the surfaces subject to attack, or to release the compounds into seawater to deter pests from approaching. Many halocompounds are synthesized or stored in specialized vesicular cells or in large refractile bodies in vegetative cells (termed "gland cells"). Similarly, chemicals used for defense in terrestrial plants are often sequestered in specialized cells or organelles so as to protect these plants from autotoxicity (Levin, 1976). Volatile and non-volatile compounds in glandular trichomes of terrestrial plants are either continuously secreted at low levels to deter pathogens and herbivores or are discharged in high concentrations when cell walls are ruptured by an attack (Levin, 1973). Release of compounds to seawater would be an especially effective mode of defense since pests would be repelled prior to inflicting damage to the plant. Plants may maximize production of

these defensive compounds during periods of intense grazing pressure or epiphyte settlement, such as spring and early summer for the species we examined. Furthermore, plants unable to synthesize these protective compounds may accumulate them from seawater during this time and derive survival advantage.

One objective of this investigation was to look for production of compounds that may deter grazing by the most abundant herbivore in this marine community, Littorina littorea. Bromoform, since it was released by preferred food sources such as Enteromorpha and Petalonia/Scytosiphon spp. as well as by algal species not eaten by this snail, appears not to be a feeding deterrent for L. littorea. The halomethanes CH_2BrI and CH_2I_2 are suspected as defenses since they were produced by F. vesiculosus and Codium fragile, algae not grazed by this snail. These two compounds were not released by algal species that the snail prefers as food. Furthermore, field observations and laboratory experiments indicated that C. fragile becomes edible to the snails upon aging and decomposition or grinding and heating. This suggests that with these processes, C. fragile loses its defense, possibly these protective compounds. Results of L. littorea feeding experiments bioassaying CH_2I_2 and $CHBr_3$ will be reported elsewhere in the next section.

In summary, certain marine algal species released hydrocarbons and halocarbons into seawater at significant rates. The observed rates of release were consistent with the levels of volatiles reported in seawater samples in a year-round study of the region. Seasonal physiological changes of the algae may be the most important factors controlling large

inputs of volatile organic compounds to seawater. Certain of these compounds may have ecological roles in plant defense against pests.

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CHAPTER 5, PART II

EFFECT OF CH_2I_2 AND $CHBr_3$ ON FEEDING ACTIVITY OF *LITTORINA LITTOREA*

INTRODUCTION

In the preceding section we reported that diiodomethane (CH_2I_2) is released into seawater by Codium fragile and Fucus vesiculosus. Because these algal species are not grazed by Littorina littorea and because halocompounds have been hypothesized, but not proven, as chemical defenses against herbivores, I chose to test the effect of CH_2I_2 on the feeding activity of L. littorea. In addition, bromoform ($CHBr_3$), a halocompound we found to be released by nearly all algal species, was bioassayed for its effect on snail feeding.

METHODS

Bioassays were conducted using L. littorea and the algal-agar feeding media as described in Chapter 1. CH_2I_2 (Pfaltz and Bauer) and $CHBr_3$ (Eastman) were diluted in 100% ethanol and added using a syringe to the media as it cooled (final concentration range: 10^{-7} - 10^{-1} mg./ml. Two sets of experiments were run. The first consisted of 2-way choice experiments using divided petri plates to offer the snails the control media and the experimental media to which the chemicals had been added. Results for each compound concentration were expressed as % experimental media consumed relative to the control. The second set of experiments offered a single choice of media in each plate to the snails; the plates were in isolated seawater tanks to prevent possible cross-contamination

of media with the added chemicals. Results are expressed as % media consumed, both independent and relative to the control.

RESULTS

The dose-response curve for the effect of CH_2I_2 on feeding by L. littorea in 2-way choice experiments is given in Figure 1. CH_2I_2 reduced feeding by at least 50% at concentrations between 0.0035 - 0.1 mg./ml. when the snails were offered a choice between experimental and control media. When a single medium was offered, CH_2I_2 at a concentration of 0.1 mg./ml. reduced feeding to 25% compared to the control during the first two days; after 5 days, the media to which CH_2I_2 had been added were consumed at levels greater than the control (Table 3).

Figure 2 shows the dose-response curve for the effect of $CHBr_3$ on feeding by L. littorea in 2-way choice experiments. Results of single medium experiments are summarized in Table 4. The snails exhibited large ranges of feeding responses to media with added $CHBr_3$, with consumption levels from 25 - 200% those of controls. For each concentration of $CHBr_3$, the range included 100% consumption; thus, the addition of $CHBr_3$ did not significantly reduce L. littorea feeding.

Figure 1. Dose-response curve for the effect of CH_2I_2 on feeding by *L. littorea*.

$$\% \text{ media consumed} = \frac{\text{experimental media consumed}}{\text{control media consumed}} \times 100\%,$$

(mean $\pm$ s.e., n = 4).

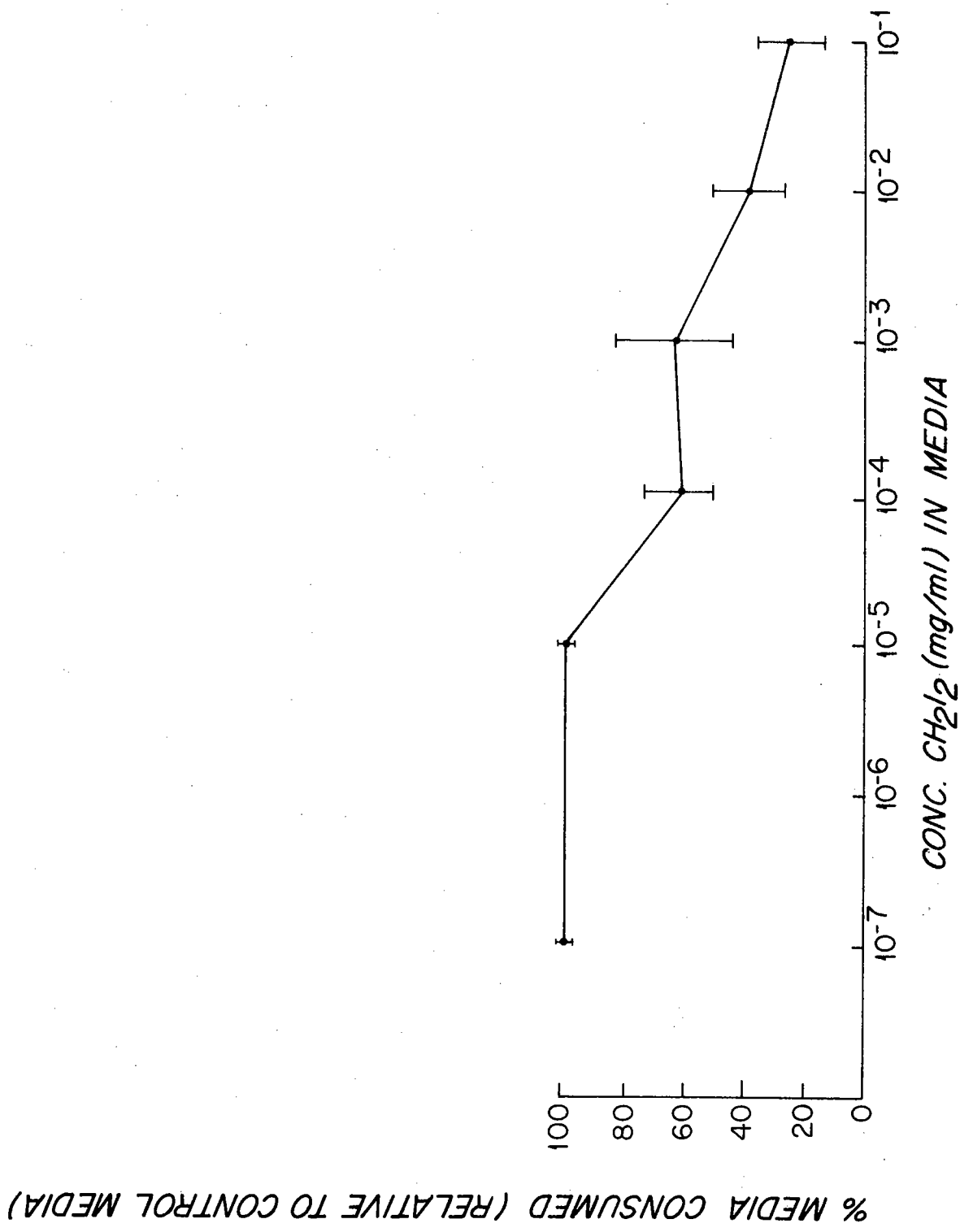

% MEDIA CONSUMED (RELATIVE TO CONTROL MEDIA)
100
80
60
40
20
0
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
CONC. CH_2I_2 (mg/ml) IN MEDIA

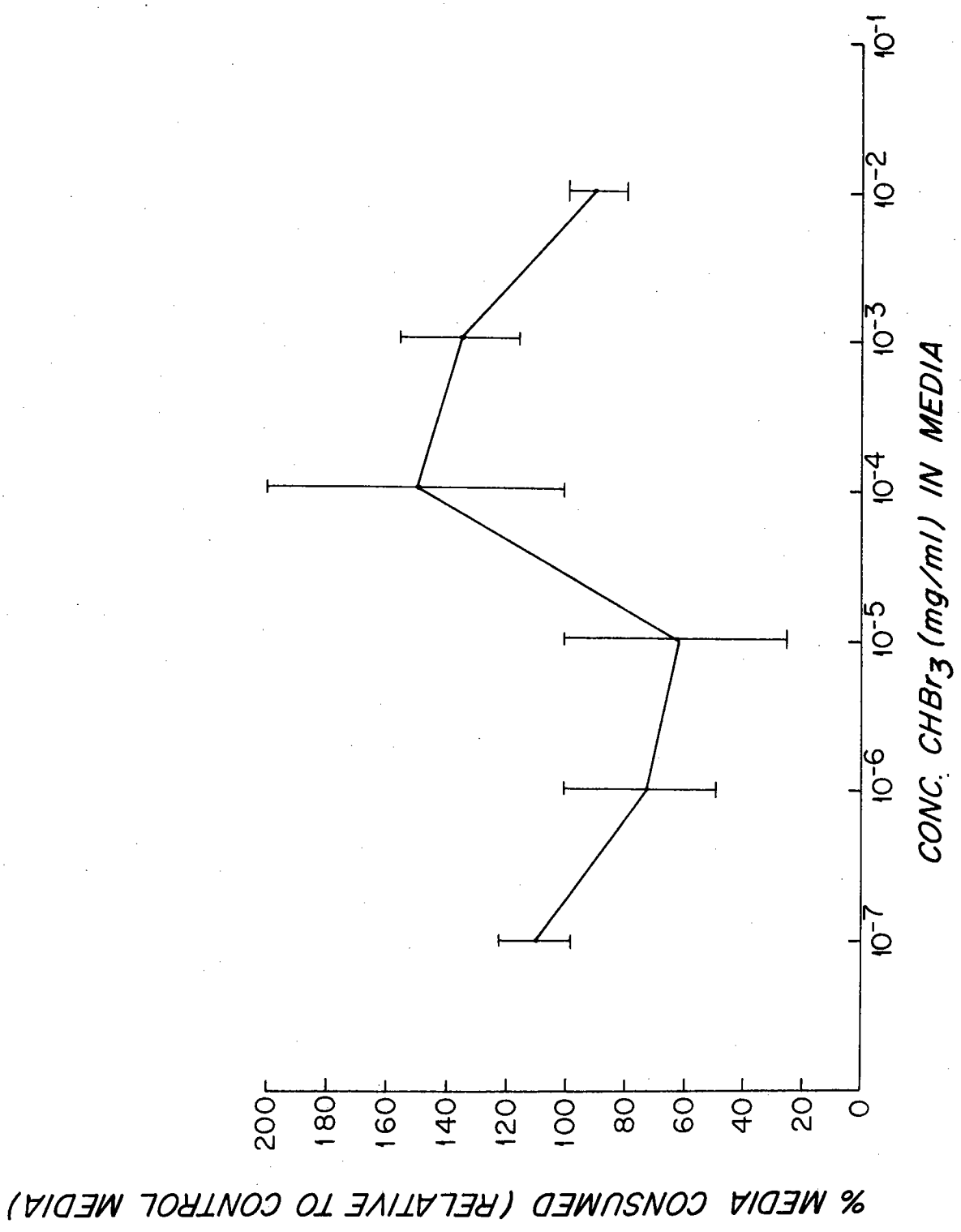
TABLE 3: Effect of CH_2I_2 and $CHBr_3$ on feeding by L. littorea (Mean std. error = ± 5% media consumed).

Halomethane In Media (mg/ml)	Media Consumed After 2 days,	After 5 days	Media Consumed, Rel. to Control After 2 days,	After 5 days
CH_2I_2, 10^{-2}	25%	50%	63%	126%
CH_2I_2, 10^{-1}	10%	55%	25%	126%
$CHBr_3$, 10^{-2}	35%	100%	88%	250%
$CHBr_3$, 10^{-1}	40%	45%	100%	113%
Control	40%	40%	(100%)	(100%)

Figure 2. Dose-response curve for the effect of $CHBr_3$ on feeding by L. littorea.

$$\% \text{ media consumed} = \frac{\text{experimental media consumed}}{\text{control media consumed}} \times 100\%,$$

(mean $\pm$ s.e., n = 4).


% MEDIA CONSUMED (RELATIVE TO CONTROL MEDIA)
200
180
160
140
120
100
80
60
40
20
0
10-7
10-6
10-5
10-4
10-3
10-2
10-1
CONC. CHBr3 (mg/ml) IN MEDIA

DISCUSSION

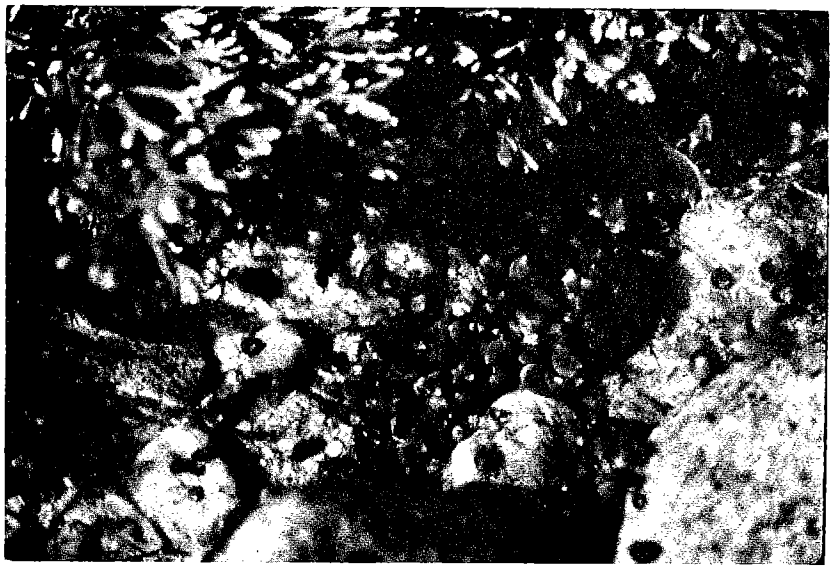
Diiodomethane deterred feeding by L. littorea and hence may account for a significant part of the resistance of certain algal species to this herbivore. In our experiments, this compound was released at a rate of 9 ng./g. dry wt. alga/day by C. fragile and 1 ng./g. dry wt. alga/day by F. vesiculosus. Both of these algae (mature plants) are of low food preference to L. littorea (it should be noted here that germlings of F. vesiculosus, which are of medium preference, released lesser amounts of CH_2I_2 than mature plants). Furthermore, extracts from these two species inhibit grazing by L. littorea when added to a preferred food source (Chapter 1). F. vesiculosus has high levels of phenols and polyphenols which deter herbivores, hence this plant species may have several classes of compounds which are antiherbivore in nature. C. fragile does not have high levels of phenols or polyphenols (Chapter III); whether CH_2I_2 is the primary feeding deterrent in its active extracts remains to be determined.

Several lines of evidence suggest that the resistance of C. fragile to L. littorea is due to a labile factor such as a volatile compound. For example, in the field, C. fragile becomes edible to L. littorea when it is old and beginning to decompose (Figures 3a, 3b). In laboratory feeding experiments, C. fragile is eaten when it is macerated and heated (Chapter 1). In these cases, it could be that the active deterrents are volatile compounds that are lost upon decomposition of the alga.

Figures 3a and 3b. Codium fragile (last year's thalli which had broken down during the winter) being eaten by L. littorea during early spring (March, 1978; Falmouth, Mass.).

a.

b.



However, other chemicals and factors such as toughness and nutritional content may be important in addition to the volatile defense.

In the bioassays, CH_2I_2 caused reduced levels of grazing by L. littorea during the first few days of the experiments, but activity appeared to be lost after 2 - 5 days. This suggests that the compound was either lost from the media to the seawater or transformed to less active compounds (e.g., Moore, 1977, reported that CH_2I_2 liberates free iodine upon exposure to air and light). Further experiments are necessary to determine the levels of CH_2I_2 in the media and in the seawater with time.

Volatile compounds from terrestrial plants have been shown to repel or attract herbivores in feeding and oviposition experiments (Vernon et al., 1977; Bergstrom, 1978; Brattsen et al., 1977). In some plants, the active compounds are emitted continuously at low concentrations, while in other plants the compounds are sequestered in special structures and are released in high concentrations upon damage to plant tissues (Levin, 1973). We found that C. fragile released CH_2I_2 to the surrounding seawater when incubated in flasks; whether this release is naturally continuous or due to stress remains to be determined. At concentrations of 0.0035 - 0.1 mg./ml. of food media, CH_2I_2 inhibited L. littorea feeding. Experiments to determine the concentrations of CH_2I_2 within C. fragile tissues and released by the tissues when damaged by an herbivore are needed.

Moore (1977) suggested that CH_2I_2 from the red alga Asparagopsis taxiformis may result from photooxidation of iodoform,

bromodiiodomethane, and dibromoiodomethane. This may also hold for C. fragile. Regardless of origin, the compound's activity against herbivores remains; in fact, studies of terrestrial plants have shown that herbivore damage often induces formation of toxic compounds from non-toxic precursors (Berenbaum, 1978; Miles, 1969).

Bromoform did not deter L. littorea feeding significantly when added to a preferred food medium. It sometimes appeared to enhance feeding while it at other times slightly reduced feeding. It could be that bromoform was lost from the media or degraded more rapidly than was CH_2I_2. Or, bromoform may indeed have little or no activity against L. littorea, as suggested by its release into Vineyard Sound by many algal species , including those highly preferred as food to L. littorea, such as Enteromorpha spp. Bromoform has also been reported in algae from other marine communities and in fish, shrimp, and mussels (Lunde, 1973; Moore, 1977), suggesting that the compound may be ubiquitous and not highly toxic. The effect of plant chemicals on herbivores invariably depends on concentration, and in some cases, low concentrations are phagostimulatory while high concentrations are inhibitory (Chapman, 1974); bromoform may act in this manner. However, even at high concentrations, few plant chemicals have inhibitory effects to all herbivores. These suggestions could be affirmed or disproved by further experiments monitoring bromoform's release and effect on other organisms.

Other halogenated compounds such as brominated phenols and ketones have shown antialgal, antifungal, and antibacterial effects (see Fenical, 1975 for review). Hence, algal-produced CH_2I_2 and $CHBr_3$ may affect

other organisms in the plant community in addition to herbivores such as L. littorea , conveying selective advantages to those algae producing them. Neither CH_2I_2 or $CHBr_3$ had previously been studied for effects on herbivores; indeed, I know of no studies demonstrating antiherbivore activity of any halogenated compounds from marine algae.

In summary, CH_2I_2, a compound released by C. fragile and F. vesiculosus, was shown to inhibit feeding by L. littorea when added to its preferred food source. Bromoform, in contrast, did not consistently or significantly affect L. littorea feeding, perhaps because of its rapid loss or degradation.

REFERENCES

Al-Ogily, S. M. and E. W. Knight-Jones. 1977. Antifouling role of antibiotics produced by marine algae and bryozoans. Nature 265: 728-729.

Asplund, R. O. 1968. Monoterpenes: relationship between structure and inhibition of germination. Phytochemistry 7: 1995-1997.

Association of Official Analytical Chemists. 1970. Official Methods of Analysis. 11th ed. Washington, D.C, 154 p.

Augier, J. and G. Hoffman. 1952. Sucre et brome dans le Polysiphonia fastigiata. Bull. Soc. Fr. 99: 80-82.

Baardseth, E. 1970. Seasonal variation in Ascophyllum nodosum (L.) Le Jol. in the Trondheimsfjord with respect to the absolute live and dry weight and the relative contents of dry matter, ash, and fruit bodies. Botanica Marina 13: 13-22.

Baker, J. T. and V. Murphy. 1976. Handbook of Marine Science. Compounds from Marine Organisms, Volume 1. CRC Press, Cleveland, 226 p.

Bate-Smith, E. C. 1957. The distribution of tannins in dicotyledonous plants. Journal of the Linnean Society of London-Botony 55: 669-705.

Bate-Smith, E. C. 1973. Haemoanalysis of tannins: the concept of relative astringency. Phytochemistry 12: 907-912.

Beck, S. D. and J. C. Reese. 1976. Insect-plant interactions: nutrition and metabolism. pp. 41-92. In: J. Wallace and R. Mansell (eds.), Recent Advances in Phytochemistry, Vol. 10. Biochemical interaction between plants and insects. Plenum Press, New York, 425 p.

Berenbaum, M. 1978. Toxicity of a furanocoumarin to armyworms: a case of biosynthetic escape from insect herbivores. Science 201: 532-534.

Bergstrom, G. 1978. Role of volatile chemicals in Ophrys - pollinator interactions. pp. 207-232. In: J. B. Harborne (ed.) Biochemical Aspects of Plant and Animal Coevolution. Academic Press, New York, 435 p.

Bhakuni, D. S. and M. Silva. 1974. Biodynamic substances from marine flora. Botanica Marina 17: 40-51.

Bonner, J. and J. E. Varner (eds.). 1965. Plant Biochemistry. Academic Press, New York, 1054 p.

Brattsten, L. B., C. F. Wilkinson, and T. Eisner. 1977. Herbivore-plant interactions: mixed function oxidases and secondary plant substances. Science 196: 1349-1352.

Brower, L. P. and J. V. Z. Brower. 1964. Birds, butterflies and plant poisons: A study in ecological chemistry. Zoologica 49: 137-159.

Burreson, B. J., R. E. Moore, and P. P. Roller. 1975. Haloforms in the essential oil of the alga Asparagopsis taxiformis (Rhodophyta). Tetrahedron Letters 7: 473-476.

Burreson, B. J., R. E. Moore, and P. P. Roller. 1976. Volatile halogen compounds in the alga Asparagopsis taxiformis (Rhodophyta). J. Agric. Food Chem. 24: 856-861.

Cameron, G. N. and T. W. LaPoint. 1978. Effects of tannins on the decomposition of chinese tallow leaves by terrestrial and aquatic invertebrates. Oecologia 32: 349-366.

Cannon, W. N., Jr. and W. A. Connell. 1965. Populations of Tetranychus atlanticus M.G. (Acarina: Tetranychidae) on soybean supplied with various levels of nitrogen , phosphorous, and potassium. Entol. Exp. Appl. 8: 153-161.

Carefoot, T. H. 1967. Growth and nutrition of Aplysia punctata feeding on a variety of marine algae. Jour. Mar. Biol. Assoc. U.K. 47: 565-589.

Carefoot, T. H. 1973. Feeding, food preferences, and the uptake of food energy by the supra-littoral isopod Ligia pallasii. Mar. Biol. 18: 228-B6.

Cates, R. G. 1975. The interface between slugs and wild ginger: some evolutionary aspects. Ecology 56: 391-400.

Cates, R. G. and G. H. Orians. 1975. Successional status and the palatability of plants to generalized herbivores. Ecology 56: 410-418.

Chan, E. C. S. and E. A. McManus. 1969. Distribution, characterization and nutrition of marine microorganisms from the algae Polysiphonia lanosa and Ascophyllum nodosum. Can. J. Microbiol. 15: 409-420.

Chapman, R. F. 1974. The chemical inhibition of feeding by phytophagous insects: A review. Bull. Ent. Res. 64: 339-363.

Clark, R. C., Jr., and M. Blumer. 1967. Distribution of n-paraffins in marine organisms and sediment. Limnol. Oceanogr. 12: 79-87.

Connell, J. 1975. Some mechanisms producing structure in natural communities. pp. 460-490. In: M.L. Cody and J. Diamond (eds.), Ecology and Evolution of Communities. Belknap Press, Cambridge, Massachusetts.

Conover, J. T. 1958. Seasonal growth of benthic marine plants as related to environmental factors in an estuary. Publ. Inst. Mar. Sci. 5: 97-147.

Conover, J. T. and J. McN. Sieburth. 1964. Effect of Sargassum distribution on its epibiota and antibacterial activity. Botanica Marina 6: 147-157.

Craigie, J. S. and J. McLachlan. 1964. Excretion of colored ultraviolet-absorbing substances by marine algae. Can. J. Bot. 42: 23-33.

Crews, P. 1977. The breadth of monoterpene synthesis by marine red algae: Potential difficulties in their application as taxonomic markers. pp. 211-223. In: Faulkner, D. J. and W. H. Fenical (eds.), Marine Natural Products Chemistry, Plenum Press, New York.

Crews, P. and E. Kho. 1974. Cartilagineal. An unusual monoterpene aldehyde from marine alga. J. Org. Chem. 39: 3303-3304.

Crews, P. and E. Kho. 1975. Plocamene B, a new cyclic monoterpene skeleton from a red marine alga. J. Org. Chem. 40: 2568-2570.

Dayton, P. K. 1973. Two cases of resource partitioning in an intertidal community: making the right prediction for the wrong reason. Amer. Natur. 107: 662-670.

DeAlarcon, P. A., M. E. Donovan, G. B. Forbes, S. A. Landau, and J. A. Stockman. 1979. Iron adsorption in the Thalasemia syndromes and its inhibition by tea. New England J. Med. 300: 5-8.

Dement, W. A. and H. A. Mooney. 1974. Seasonal variation in the production of tannins and cyanogenic glucodes in the chapparal shrub, Heteromeles arbutifolia. Oecologia 15: 65-76

Deming, W. E. 1944. Statistical Adjustment of Data. John Wiley and Sons, Inc., New York, 262 p.

Dethier, V. G. 1947. Chemical Insect Attractants and Repellents. Blakiston Co., Philadelphia, 289 p.

Dethier, V. G., 1970. Chemical interactions between plants and insects. pp. 83-99. In: E. Sonderheimer and J.B. Simeone (eds.), Chemical Ecology. Academic Press, New York, 336 p.

Doty, M. S. and G. Aguilar-Santos. 1970. Transfer of toxic algal substances in marine food chains. Pacific Science 24: 351-355.

Ehrlich, P. R. and P. H. Raven. 1964. Butterflies and plants: A study in coevolution. Evolution 18: 586-608.

Ehrlich, P. R. and P. H. Raven. 1967. Butterflies and plants. Scientific American 216: 104-113.

Esau, K. 1965. Plant Anatomy. 2nd ed. John Wiley and Sons, Inc., New York, 767 p.

Faulkner, D. J. and R. J. Anderson, 1974. Natural products chemistry of the marine environment. pp. 679-714. In: E. Goldberg (ed.), The Sea, Vol. 5, Marine Chemistry. Wiley and Sons, New York.

Feeny, P. P. 1968. Effect of oak leaf tannins on larval growth of the winter moth, Operophtera brumata. J. Insect Physiol. 14: 805-817.

Feeny, P. P. 1969. Inhibitory effect of oak leaf tannins on the hydrolysis of proteins by trypsin. Phytochemistry 8: 2119-2126.

Feeny, P. P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51: 565-581.

Feeny, P. P. 1975. Biochemical coevolution between and their insect herbivores. pp. 3-19. In: L.E. Gilbert and P.H. Raven (eds.), Coevolution of Animals and Plants. U. Texas Press, Austin.

Feeny, P. P. 1976. Plant apparency and chemical defense. pp. 1-40. In: J. Wallace and R. Mansell (eds.), Recent Advances in Phytochemistry, Vol. 10. Biochemical interaction between plants and insects. Plenum Press, New York, 425 p.

Feeny, P. P. and H. Bostock. 1968. Seasonal changes in the tannin content of oak leaves. Phytochemistry 7: 871-880.

Fenical, W. 1974. Polyhaloketones from the red seaweed, Asparagopsis taxiformis. Tetrahedron Lett. 1974: 4463-4464.

Fenical, W. 1975. Halogenation in the Rhodophyta. A review. J. Phycol. 11: 245-259.

Fenical, W. and J. N. Norris. 1975. Chemotaxonomy in marine algae: chemical separation of some Laurencia species (Rhodophyta) from the Gulf of California. J. Phycol. 11: 104-108.

Folin, O. and W. Denis. 1915. A colorimetric method for the determination of phenols (and phenol derivatives) in urine. J. Biol. Chem. 22: 305-308.

Fox, L. R. and B. J. Macauley. 1977. Insect grazing on Eucalyptus in response to variation in leaf tannins and nitrogen. Oecologica 29: 145-162.

Fraenkel, G. S. 1959. The raison d'etre of secondary plant substances. Science 129: 1466-1470.

Fraenkel, G. S. 1969. Evaluation of our thoughts on secondary plant substances. Enotomologia Experimentalis et Applica 12: 473-486.

Fritsch, F. E. 1945. The Structure and Reproduction of the Algae, Vol. II. Cambridge University Press, London, 939 p.

Gagosian, R. B. and R. A. Bourbonniere. 1976. Isolation and purification of the molting hormones from the American lobster (Homarus americanus). Comp. Biochem. Physiol. 53B: 155-161.

Gagosian, R. B., R. A. Bourbonniere, W. B. Smith, E. F. Couch, C. Blanton and W. Novak, 1974. Lobster Molting hormones: Isolation and biosynthesis of ecdysterone. Experientia 30: 723-724.

Gilbert, B. L., J. E. Baker, and D. M. Norris. 1967. Juglone (5-hydroxy-1, 4-naphthoquinone) from Carya ovata, a deterrent to feeding by Scolytus multistriatus. J. Insect. Physiol. 13: 1453-1459.

Glombitza, K. W. 1977. Highly hydroxylated phenols of the Phaeophyceae. pp. 191-204. In: Faulkner, D. J. and W. H. Fenical (eds.), Marine Natural Products Chemistry. Plenum Press, New York.

Glombitza, K. W., U. Murawski, J. Bielaczek and H. Egge. 1974. Bromophenole aus Rhodomelaceen (antibiotica aus algen 9. mitt.). Planta Med. 25: 105-114.

Glombitza, K. W. and H. Stoffelen. 1972. 2,3-Dibromo-5-hydroxylbenzyl-1,4-disulfat (dikalium salz) aus Rhodomelaceen (antibiotica aus algen 6. mitt.). Planta Med. 22: 391-395.

Glombitza, K. W., H. W. Ranwald, and G. Eckhardt. 1975. Fucole, polyhydroxyligophenyle, and Fucus vesiculous. Phytochemistry 14: 1403-1405.

Glombitza, K. W., H. U. Rosener, H. Vilter, and W. Ranwald. 1973. Antibiotica aus algen 8. mitt. phloroplucin aus Braunalgen. Planta Medica 24: 301-303.

Glombitza, K. W. and H. Stoffelen. 1972. 2,3-Dibromo-5-hydroxylbenzyl- 1,4-disulfat (dikalium salz) aus Rhodomelaceen (antibiotica aus algen 6. mitt.). Planta Med. 22: 391-395.

Goldstein, J. L. and T. Swain. 1963. Changes in tannins in ripening fruits. Phytochemistry 2: 371-383.

Goldstein, J. R. and T. Swain. 1965. The inhibition of enzymes by tannins. Phytochemistry 4: 185-192.

Gomez, E. D., D. J. Faulkner, W. A. Newman and C. Ireland. 1973. Juvenile hormone mimics: Effect on cirriped crustacean metamorphosis. Science 179: 1813-1814.

Grob, K. 1973. Organic substances in potable water and in its precursor. Part I. Methods for the determination by gas-liquid chromatogrphy. J. Chromatogr. 84: 255-273.

Grob, K. and F. Zurcher. 1976. Stripping of trace organic substances from water. Equipment and procedure. J. Chromatogr. 117: 285-294.

Gschwend, P. M. 1979. Volatile organic compounds in seawater. Ph.D. thesis, Woods Hole Oceanographic Institution, 271 p.

Hairston, N. G., F. F. Smith and L. B. Slobodkin. 1960. Community structure, population control, and competition. Amer. Natur. 94: 421-425.

Halligan, J. P. 1975. Toxic terpenes from Artemisia californica. Ecology 56: 999-1003.

Harborne, J. B. (ed.). 1964. Biochemistry of Phenolic Compounds. Academic Press, New York, 618 p.

Hashimoto, Y. and N. Fusetani. 1974. Screening of the toxic algae on coral reefs. In: Kazutosi Nizizawa (ed.). VIIth Int. Seaweed Symposium. Wiley and Sons.

Haslam, E. 1966. Chemistry of Vegetable Tannins. Academic Press, London and New York, 179 p.

Haug, A. and B. Larsen. 1958. Chemical composition of the brown alga Ascophyllum nodusum (L.) Le Jol.: influence of habitat on the chemical composition of Ascophyllum nodosum. Nature 181: 1224-1225.

Hodgson, E., L. S. Self and F. E. Guthrie. 1965. Adaptation of insects to toxic plants. Proc. 12th Int. Congr. Entomol. London (1964), 210 p.

Hunger, F. W. T. 1902. Uber das assimilations-product der dictyotaceen. Jb. Wiss. Bot. 38: 70-82.

Irie, T., M. Suzuki, E. Kurosawa and T. Masamune. 1970. Laurinterol, debromolaurinterol and isolaurinterol, constituents of Laurencia intermedia Yamada. Tetrahedron 26: 3271-3277.

Janzen, D. H., H. B. Juster, and I. E. Liener. 1976. Insecticidal action of the phytohemagglutin in black beans on a bruchid beetle. Science 192: 795-796.

Jensen, A. 1969. Tocopherol content of seaweed and seaweed meal. II. Individual, diurnal and seasonal variations in some Fucaceae. J. Sci. Food Agric. 20: 454-458.

Jones, D. A. 1962. Selective eating of the acyanogenic form of the plant Lotus corniculatus L. by various animals. Nature 193: 1109-1110.

Jones, D. A. 1966. On the polymorphism of cyanogenesis in Lotus corniculatus L. selection by animals. Can. J. Genet. Cytol. 8: 556-567.

Jones, D. A. 1971. Chemical defense mechanisms and genetic polymorphisms. Science 173: 945.

Jones, D. A. 1972. On the polymorphism of cyanogenesis in Lotus corniculatus L. IV. The Netherlands. Genetica 43: 394-406.

Jones, W. T., R. B. Broadhurst and J. W. Lyttleton. 1976. The condensed tannins of pasture legume species. Phytochemistry 15: 1407-1409.

Kaplanis, J. N., M. J. Thompson, W. E. Robbins and B. M. Bryce. 1967. Insect hormones: Alpha ecdysone and 20-hydrosyecdysone in bracken fern. Science 157: 1436-1438.

Kristensen, J. H. 1972. Carbohydrases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. Marine Biology 14: 130-142.

Krieger, R. I., P. P. Feeny and C. F. Wilkinson. 1971. Detoxification in the guts of caterpillars: An evolutionary answer to plant defense? Science 172: 579-580.

LaDu, B. N., H. G. Mandel and E. L. Way (eds.). 1972. Fundamentals of Drug Metabolism and Drug Disposition. Williams and Wilkins, Baltimore, 615 p.

Levin, D. A. 1971. Plant phenolics: An ecological perspective. Amer. Natur. 105: 157-181.

Levin, D. A. 1973. The role of trichomes in plant defense. Quart. Rev. Biol. 48: 3-15.

Levin, D. A. 1976. The chemical defenses of plants to pathogens and herbivores. Annual. Rev. Ecol. Syst. 7: 121-159.

Long, C. (ed.). 1961. Biochemist's Handbook. Van Nostrand, Princeton, New Jersey, 1192 p.

Lubchenco, J. 1978. Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. Amer. Natur. 112: 23-39.

Lunde, G. 1973. The presence of volatile, nonpolar bromo-organic compounds synthesized by marine organisms. J. Amer. Oil Chem. Soc. 50: 24-25.

MacArthur, R. H. and E. O. Wilson. 1967. The Theory of Island Biogeography. Princeton University Press, Princeton, 203 p.

Mann, K. H. 1972. Ecological Energetics of the seaweed zone in a marine bay on the Atlantic coast of Canada. I. Zonation and biomass of seawaeeds. Mar. Biol. 12: 1-10.

Manske, R. H. F. and H. L. Holmes (eds.). 1950-1968. The Alkaloids: Chemistry and Physiology, 11 Volumes. Academic Press, New York.

Mathieson, A. C., J. W. Shipman, J. R. O'Shea, and R. C. Hasenlat. 1976. Seasonal growth and reproduction of estuarine Fucoid algae in New England. J. Exp. Mar. Biol. Ecol. 25: 273-284.

Mautner, H. G., G. M. Gardner and R. Pratt. 1953. Antibiotic activity of seaweed extracts. II. Rhodomela larix. J. Am. Pharm. Assoc. Sci. Ed. 42: 294-296.

McKey, D. 1974. Adaptive patterns in alkaloid physiology. Amer. Natur. 108:305-320.

McKey, D., P. G. Waterman, C. N. Mbi, J. S. Gartlan, and T. T. Struhsaker. Phenolic content of vegetation in two African rain forests: ecological implications. Science 202: 61-63.

McLachlan, J. and J. S. Craigie. 1966. Antialgal activity of some simple phenols. J. Phycol. 2: 133-135.

McNeill, S. and T. R. E. Southwood. 1978. The role of nitrogen in the development of insect/plant relationships. pp. 78-98. In: J. B. Harborne (ed.). Biochemical Aspects of Plant and Animal Coevolution. Academic Press, New York, 435 p.

Meinwald, J., G. D. Prestwich, K. Nakanishi, and I. Kubo. 1978. Chemical ecology: studies from East Africa. Science 199: 1167-1173.

Menge, J. L. 1975. Effect of herbivores on community structure of the New England rocky intertidal region: distribution, abundance, and diversity of algae. Ph.D. thesis, Harvard University, Cambridge, Mass., 164 p.

Miles, P. W. 1969. Interaction of plant phenols and salivary phenolases in the relationship between plants and Hemiptera. Entomol. Exp. Appl. 12: 736-744.

Moore, R. E. 1977. Volatile compounds from marine algae. Acc. Chem. Res. 10: 40-47.

Muller, C. H. and C-H. Chou. 1972. Phytotoxins: an ecological phase of phytochemistry. pp. 201-216, In: J. B. Harborne (ed.), Phytochemical Ecology. Academic Press, New York, 272 p.

Muller, C. H. and W. H. Muller. 1964. Antibiosis as a factor in vegetation patterns. Science, 144: 889-890.

Muller, C. H., W. H. Muller and B. L. Haines. 1964. Antibiosis as a factor in vegetation patterns. Science 143: 471.

Murdoch, W. H. 1966. Community structure, population control and competition - a critique. Amer. Natur. 100: 219-226.

Mynderse, J. S. and D. J. Faulkner. 1974. Violacene, a halogenated monocyclic monoterpene from the red alga Plocamium violaceum. J. Am. Chem. Soc. 96: 6771-6772.

Nicotri, M. E. 1978. Impact of crustacean herbivores on cultured seaweed populations. Aquaculture 12: 127-136.

Nierenstein, M. 1935. The Natural Organic Tannins. History: chemistry:distribution. Sherwood Press, Cleveland, Ohio, 313 p.

Odum, E. P. 1969. The strategy of ecosystem development. Science 164: 252-270.

Ogden, J. C. 1976. Some aspects of herbivore-plant relationships on Caribbean reefs and sea grass beds. Aquatic Botany 2: 103-116.

Ogino, C. 1962. Tannins and vacuolar pigments. pp.437-455. In: R.A. Lewin (ed.), Physiology and Biochemistry of Algae. Academic Press, New York and London, 929 p.

Onuf, C. P., J. M. Teal, and I. Valiela. 1977. Interactions of nutrients, plant growth, and hebivory in a mangrove ecosystem. Ecology 58: 514-526.

Orians, G. H. and D. H. Janzen. 1974. Why are embroyos so tasty? Amer. Natur., 108: 581-592.

Paine, R. T. and R. L. Vadas. 1969. Calorific values of benthic marine algae and their postulated relation to invertebrate food preference. Mar. Biol. 4: 79-86.

Pedersen, M., P. Saenger and L. Fries. 1974. Simple bromophenols in red algae. Phytochemistry 13: 2273-2279.

Peguy, M. and R. Heim. 1961. A propos de l'activite antiobiotique presumee d'un solution aqueuse du corps phenolique brome et sulphone extrait du Polysiphonia lanosa (Linne) Tandy (Floridee, Rhodomelacee). Comp. Rend. Acad. Sci. Paris, Ser. D. 252: 2134-2135.

Pridham, J. B. (ed.). 1967. Terpenoids in Plants. Academic Press, New York, 257 p.

Ragan, M. A. 1976. Physodes and the phenolic compounds of brown algae. Composition and significance of physodes in vivo. Botanica Marina 19: 145-154.

Ragan, M. A. and J. S. Craigie. 1978. Phenolic compounds in brown and red algae. pp. 157-179. In: Hellebust, J. A. and J. S. Craigie (eds.). Handbook of Phycological Methods, Vol II. Cambridge University Press, Cambridge, 512 p.

Ragan, M. A. and J. S. Craigie. 1976. Physodes and the phenolic compounds of brown algae. Isolation and characterization of phloroglucinol polymers from Fucus vesiculosus (L.). Can. J. Biochem. 54: 66-73.

Ragan, M. A. and A. Jensen. 1978. Quantitative studies on brown algal phenols. II. Seasonal variation in polyphenol content of Ascophyllum nodosum (L.) Le Jol. and Fucus vesiculosus (L.). J. Exp. Mar. Biol. Ecol. 34: 245-258.

Ragan, M. A. and A. Jensen. 1977. Quantitative studies on brown algal phenols. I. Estimation of absolute polyphenol content of Ascophyllum nodosum (L.) Le Jol. and Fucus vesiculosus (L.). J. Exp. Mar. Biol. Ecol. 30: 209-221.

Rhoades, D. F. and R. G. Cates. 1976. Toward a general theory of plant antiherbivore chemistry. pp. 168-213. In: J. Wallace and R. Mansell (eds.). Recent Advances in Phytochemistry, Vol. 10. Biochemical interaction between plants and insects. Plenum Press, New York, 425 p.

Robson, E. M. and L. C. Williams. 1971. Relationships of some species of digenea with the kmarine prosobranch Littorina littorea (L.). II. The effect of larval diagenea on the reproductive biology of L. littorea. J. Helminth. 45: 145-159.

Russell, G. B., O. R. W. Sutherland, R. F. N. Hutchins, and P. E. Christmas. 1978. Vesitol: a phytoalexin with insect feeding-deterrent activity. J. Chem. Ecol. 4: 571-579.

Scheuer, P. J. 1973. Chemistry of Maine Natural Products. Academic Press, New York, 201 p.

Scholl, J. P., R. G. Kelsey, and F. Shafizadeh. 1977. Involvement of volatile compounds of Artemisia in browse preference by mule deer. Biochem. Syst. and Ecol. 5: 291-295.

Schoonhoven, L. M. 1972. Secondary plant substances and insects. pp. 197-224. In: V.C. Runeckles and T.C. Tso (eds.), Recent Advances of Phytochemistry. Academic Press, New York.

Schuster, L. 1964. Metabolism of drugs and toxic substances. Ann. Rev. Biochem. 33: 571-596.

Schwarzenbach, R. P., R. H. Bromund, P. M. Gschwend, and O. C. Zafiriou. 1978. Volatile organic compounds in coastal seawater. Org. Geochem. 1: 93-107.

Scriber, J. M. and P. Feeney. 1979. Growth of herbivorous caterpillars in relation to feeding specialization and to the growth form of their food plants. Ecology 60: 829-850.

Sieburth, J. McN. and J.T. Conover. 1965. Sargassum tannin, an antibiotic which retards fouling. Nature 208: 52-53.

Sieburth, J. McN. and A. Jensen. 1969. Studies on algal substances in the sea. II. The formation of Gelbstoff (humic material) by exudates of Phaeophyta. J. Exp. Mar. Biol. Ecol. 3: 275-289.

Sieburth, J. McN. and A. Jensen. 1968. Studies on algal substances in the sea. I. Gelbstoff (humic material) in terrestrial and marine waters. J. Exp. Mar. Biol. Ecol. 2: 174-189.

Slama, K. and C. M. Williams. 1966. "Paper factor" as an inhibitor of the embryonic development of the European bug, Pyrrhocoris apterus. Nature 210: 329-330.

Slansky, F. Jr. and P. Feeney. 1979. Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecological Monographs 47: 209-228.

Soo Hoo, C. F. and G. Fraenkel. 1966. The selection of food plants in a polyphagous insect, Prodemia eridania (Cramer). J. Insect. Physiol. 12: 693-709.

Stahl, E. 1888. Pflanzen and schnecken: ein biologische studie iber die schutzmittel der pflanzen gegen schnecken frass. Jena. Z. Naturwiss u. Medezin 15: 557-684.

Stallard, M. O. and D. J. Faulkner. 1974. Chemical constituents of the digestive gland of the sea hare Aplysia californica. I. Importance of diet. Comp. Biochem. Physiol. 49B: 25-36.

Staresinic, N. 1978. The vertical flux of particulate matter in the Peru coastal upwelling as measured with free- drifting sediment traps. Ph.D. thesis, Massachusetts Institute of Technology/ Woods Hole Oceanographic Institution, 255 p.

Stebbins, G. L., Jr. 1950. Variation and Evolution in Plants. Columbia University Press, New York.

Stoffelen, H., K. W. Glombitza, U. Murawski, J. Bielaczek and H. Egge. 1972. Bromophenole aus Polysiphonia lanosa (L.) Tandy. (antibiotica aux algen 7. mitt.). Planta Med. 22: 396-401.

Sturgeon, K. B. 1979. Monoterpene variation in Pondersosa Pine xylem resin related to western pine beetle predation. Evolution 33: 803-814.

Sun, H. H. and W. Fenical. 1979. Rhipocephalin and rhipocephenal; toxic feeding deterrents from the tropical marine alga Rhipocephalus phoenix. Tetrahedron Letters 8: 685-688.

Swain, T. S. and J. L. Goldstein. 1964. The quantitative analysis of phenolic compounds. pp. 131-145. In: J.B. Pridham (ed.). Methods in Polyphenol Chemistry. Macmillan, New York, 164 p.

Swain, T. and W. E. Hillis. 1959. The phenolic constituents of Prunus domestica. I. The quantitative analysis of phenolic constitutents. J. Sci. Food Agr. 10: 63-68.

Todd, G. W., A. Getahun, and D. C. Cress. 1971. Resistance in barley to the greenbug, Schizaphis graminum. I. Toxicity of phenolic and flavonoid compounds and related substance. Ann. Ent. Soc. Amer. 64: 718-722.

Thorsteinson, A. J. 1960. Host selection in phytophagous insects. Ann. Rev. Ent. 5: 193-218.

Vadas, R. L. 1968. The ecology of Agarum and the kelp bed community. Ph.D. thesis, University of Washington, Seattle, Washington, 280 p.

Van Emden, H. F. 1966. Studies on the relations of insect and host plant. III. A comparison of the reproduction of Brevicoryne brassicae and Myzus persicae (Hemiptera: Aphididae) on brussels sprout plants supplied with different rates of nitrogen and potassium. Entomol. Exp. Appl. 9:444-460.

Van Emden, H. F. 1972. Aphids as phytochemists. pp. 25-43. In: J. B. Harborne (ed.). Phytochemical Ecology. Academic Press, New York, 273 p.

Van Sumere, C. F., J. Albrecht, A. Dedonder, and H. De Pooter, and I. Pe. 1975. Plant proteins and phenolics. pp. 211-256. In: J. B. Harborne and C. F. Van Sumere (eds.). The Chemistry and Biochemistry of Plant Proteins. Academic Press, New York, 326 p.

Vernon, R. S., J. H. Borden, H. O. Pierce, Jr., and A. C. Oehlschlager. 1977. Host selection by Hylemya antiqua. Laboratory bioassay and methods of obtaining host volatiles. J. Chem. Ecol. 3: 359-368.

Vince, S. W. 1979. Response of herbivores to salt marsh fertilzation. Ph.D. thesis. Boston University, 86 p.

Wells, P. V. 1964. Antibiosis as a factor in vegetation patterns. Science 144: 889.

Whittaker, R. H. 1970. The biochemical ecology of some higher plants. pp. 43-70. In: E. Sonderheimer and J.B. Simone (eds.), Chemical Ecology. Academic Press, New York, 336 p.

Whittaker, R. H. and P. P. Feeny. 1971. Allelochemics: Chemical interactions between species. Science 171: 757-770.

Williams, C. M. 1970. Hormonal interactions between plants and insects. pp. 103-129. In: E. Sonderheimer and J.B. Simeone (eds.), Chemical Ecology. Academic Press, New York, 336 p.

Williams, E. E. 1964. The growth and distribution of Littorina littorea (L.) on a rocky shore in Wales. J. Anim. Ecol. 33: 413-432.

Williams, R. T. 1959. Detoxification Mechanisms. Wiley and Sons, New York, 769 p.

Wolk, C. P. 1968. Role of bromine in the formtion of the refractile inclusions of the vesicle cells of the Bonnemaisoniaceae (Rhodophyta). Planta 78: 371-378.

Wynne, M. J. 1969. Life History and Systematic Studies of Some Pacific North American Phaeophyceae (brown algae). Univ. Calif. Press, Berkeley, 88 p.

Youngblood, W. W., and M. Blumer. 1973. Alkanes and alkenes in marine benthic algae. Mar. Biol. 21: 163-172.

Youngblood, W. W., M. Blumer, R. L. Guillard, and F. Fiore. 1971. Saturated and unsaturated hydrocarbons in marine benthic algae. Mar. Biol. 8: 190-201.

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Geiselman, J. A. 1980. Ecology of chemical defenses of algae against the herbivorous snail, Littorina littorea, in the New England rocky intertidal community. Ph.D. thesis, Massachusetts Institute of Technology/Woods Hole Oceanographic Institution, 209 p.

Morris, B. F., J. Cadwallader, J. Geiselman, and J. N. Butler. 1976. Transfer of petroleum and biogenic hydrocarbons in the Sargassum community. pp. 235-259 In H. E. Windom and R. A. Duce (eds.). Marine Pollutant Transfer. D. C. Heath and Co. Lexington, Ma. 391 p.

Presented Papers

Geiselman, J. A. 1978. Evidence for algal chemical defense against a marine herbivore. American Society of Limnology and Oceanography Annual Meeting, June, 1978, Victoria, British Columbia.

Geiselman, J. A. and O. J. McConnell. 1979. Chemical defenses of brown algae against the periwinkle, Littorina littorea, in the New England rocky intertidal community. Gordon Research Conference on Marine Natural Products, June, 1979, Santa Barbara, California.

Papers in Preparation for Publication

Geiselman, J. A. Evidence for algal chemical defense against a marine herbivore.

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