CHEMICAL COMPOSITION OF *PORPHYRA* SPP. IN BRITISH COLUMBIA, CANADA

by

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CHEMICAL COMPOSITION OF PORPHYRA SPP. IN BRITISH COLUMBIA, CANADA

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ABSTRACT

Major chemical constituents and their seasonal changes were determined in several native Pacific Northwest species of *Porphyra*. The *Porphyra* samples collected at three different sites, Whiffin Spit (Sooke), Orlebar Pt. (Gabriola Island), and Wreck Beach (Vancouver), were *P. torta* Krishnamurthy, *P. perforata* J. Agardh, *P. pseudolanceolata* Krishnamurthy, *P. mumfordii* Lindstrom et Cole, and *P. fallax* Lindstrom et Cole.

Eicosapentaenoic acid, palmitic acid, floridosides, porphyran, ash content and dry weight showed significant but irregular fluctuations or seasonal changes over the growing period of *Porphyra*, which suggests the need for appropriate timing of harvest depending on the purpose of *Porphyra* utilization.

Total lipid content ranged from 0.10% to 1.70% (dw). The content of eicosapentaenoic acid, the major polyunsaturated fatty acid, varied from 0.01 mg $g.^{-1}$ to 1.69 mg $g.^{-1}$ (dw). The content of palmitic acid, the major polysaturated fatty acid, varied from 0.28 mg $g.^{-1}$ to 1.13 mg $g.^{-1}$.

Total amino acid content of *Porphyra* in this study was relatively high, and ranged from 16.65% to 43.79% (dw). The composition of total amino acids was dominated by methionine, alanine, and tyrosine, in 1991, and alanine, arginine and glutamic acid in 1992. Slightly different composition was observed in free amino acids, which were dominated by alanine, threonine, and glutamic acid in both collection years.

The content of porphyran ranged from 7.63% to 55.59% (dw), isofloridoside, from 0.37% to 8.26% (dw), and floridoside, from 0.13% to 4.91% (dw). Ash content ranged from 12.23% to 26.30% (dw).

Significant variations among species were observed in all chemical contents analyzed, except isofloridoside. Moreover, significant inter-site variations were recorded for total amino acids and eicosapentaenoic acid content in *P. perforata* collected at Whiffin Spit and Orlebar Point.

In conclusion, this study confirms the feasibility of *Porphyra* harvest for a highly valued product based on its chemical constituents.

DEDICATION

بِسْمِ اللهِ تَوَكَّلْتُ عَلَى اللهِ لَا حَوْلَ وَلَا قُوَّةَ إِلَّا بِاللهِ

I would like to dedicate this thesis
to my beloved wife and daughter,
Lucy and Putri Aulia,
for your patient, ever-lasting encouragement and love,
and also to my son, Buby Sultan,
who has been in God's hand,

Alkhamdulillah jaza kumullahu khoiron

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I. INTRODUCTION

BACKGROUND

Porphyra, also known as laver or nori, is a high value edible seaweed. The food value of *Porphyra* lies in its high nutritional content. It is rich in proteins, 25 - 50% of the dry weight (Nisizawa *et al.*, 1987), vitamins and mineral salts (Chapman, 1970) and for these reasons is considered to be a health food. Its vitamin C content is about 1 1/2 times that of oranges and it is also rich in B vitamins (Nisizawa, *et al.*, 1987). In addition, human beings digest 72-78% of the protein and carbohydrates of *Porphyra* (Fujiwara-Arasaki *et al.*, 1984). The quality of *Porphyra* also depends to a large extent on visual and organoleptic factors which are contributed by the chemical composition of *Porphyra* (Mumford and Miura, 1988).

Japan is considered as the most important producer as well as user of *Porphyra* (Chapman and Chapman, 1980). Recently, Japan produced approximately 10 billion sheets of nori worth nearly $2,000,000,000 annually (Anonymous, 1989). China and Korea are other major nori producers. In China, *Porphyra* has been utilized in soups and flavoring since 533 AD; in Korea, *Porphyra* was first cultivated in 1623 and its production has increased greatly during the last 10 years (Mumford and Miura, 1988).

In North America, most of the coastal Northwest American Indians from Washington to southern Alaska collect and eat

Porphyra. They dry and store it or sometimes chew and ferment it (Mumford and Miura, 1988). Commercial production of *Porphyra* began in Washington State, and it was hoped to begin in British Columbia in 1989 (Anonymous, 1989). Lindstrom (1989) has reported that there are 20 species of *Porphyra* in British Columbia, out of which 4-5 species could be considered for cultivation. These promising species are those proposed by Waaland, *et al.* (1986), namely *Porphyra fallax, P. abbottae, P. torta, P. pseudolanceolata, and P. nereocystis* (The samples of *P. fallax* were originally identified by Waaland *et al.*, 1986 as *P. perforata,* Lindstrom and Cole, 1990; and those of *P. pseudolanceolata* were *P. fallax* subsp. *conwayae*, Lindstrom, pers. commun.). In addition to developing *Porphyra* cultivation in North America, a research program to review the taxonomy (Conway *et al.* 1975; Lindstrom and Cole, 1990 and Lindstrom and Cole, 1992), and evaluate the biological and economic feasibility of *Porphyra* culture in the cool, nutrient-rich waters of the Northeast Pacific Ocean was conducted by the Washington Department of Natural Resources in 1980 and subsequent years (Mumford *et al.*, 1985; Mumford, 1988; Mumford, 1990), and Waaland and co workers (Waaland and Dickson, 1983, 1987; Waaland *et al.*, 1984, 1987, 1988, 1990; Waaland and Mumford, 1981).

The growth of *Porphyra*, like that of other seaweed species, depends on an interaction with its physicochemical environment (see Lobban *et al.*, 1985). Among the major environmental factors are light, temperature, salinity, water motion, and nutrient availability. Light, for example, affects metabolic rates, growth

rate as well as reproduction and life history of seaweeds (Dring, 1974). All of these environmental factors vary with season. Therefore, it seems reasonable to hypothesize that the biochemical composition of *Porphyra* also varies through the seasons.

A good deal of research has been done on seasonal variations in chemical and biochemical composition of seaweed species such as *Gracilaria tikvahiae* (Penniman and Mathieson, 1987), *Macrocystis integrifolia* and *Nereocystis luetkeana* (Rosell and Srivastava, 1984), *Sargassum muticum* (Gorham and Lewey, 1984), *Saccorhiza polychides* (Jensen *et al.*, 1985), *Eucheuma* spp. (Dawes *et al.*, 1974; Dawes *et al.*, 1977), *Cystoseira elegans* (Combaut *et al.*, 1981), *Pleurophycus gardneri* (Germann *et al.*, 1987), *Laminaria longicruris* (Chapman and Craigie, 1977; Chapman and Craigie, 1978), *Fucus* spp. and *Ascophyllum nodosum* (Macpherson and Young, 1952), *Ulva lactuca* (Medcalf *et al.*, 1975), and *Chondrus crispus* (Butler, 1936; Fuller and Mathieson, 1972). In contrast, in our knowledge only a few studies have been done on biochemical composition and its seasonal variation in species of *Porphyra* growing in North America (Wheeler and Björnsäter, 1992; Meng and Srivastava, 1993). This situation led me to investigate the changes in biochemical composition of native British Columbia *Porphyra* spp. during their growing season. This information may be useful in Canada in particular in the cultivation and production of *Porphyra* species that have an appropriate biochemical composition.

REVIEW OF LITERATURE

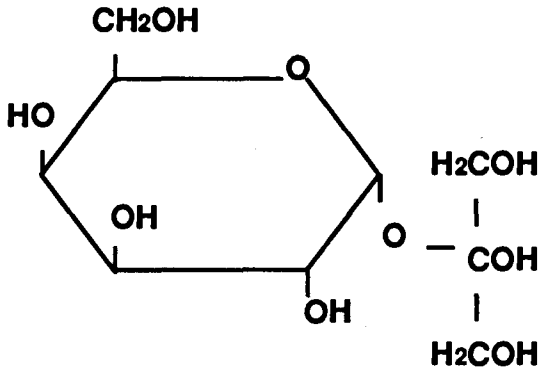
Carbohydrates

The constituents of major carbohydrates in red algae, in contrast to that in land plants, consist of floridean starch and floridosides as major storage products. Moreover, floridean starch is deposited free in the cytoplasm, rather than in chloroplasts, and generally is composed solely of amylopectin, rather than a mixture of amylose and amylopectin (Pueschel, 1990).

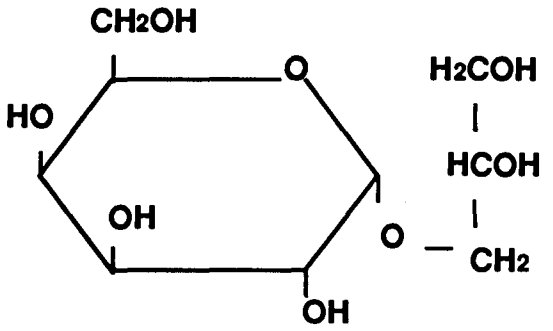
. The O-α-D-galactopyranosyl-(1->2)-glycerol, which is also called floridoside, was discovered in Rhodophyta by Colin and Guéguen in 1930. Later, Linberg (1955) and Wickberg (1958) described an isomeric form of floridoside, comprising the D- and L- glycerol derivates of O-α-D-galactopyranosyl-(1->1)-glycerol, which has been termed isofloridoside (Fig. 1A). Bean and Hassid (1955) suggested that floridoside was formed during photosynthesis by a condensation of UDP-galactose and α-glycerol-P to give galactosyl-glycerol-P which was subsequently hydrolyzed to galactosyl-glycerol. The biosynthesis of isofloridoside is unknown; according to Craigie (1974), it was consistently rather weakly ^{14}C-labelled during photosynthesis.

Floridoside and isofloridoside were shown to be the major alcohol-soluble, low molecular-weight carbohydrates in *Porphyra* (Craigie *et al.*, 1968; Reed *et al.*, 1980) and in all members of the Rhodophyta except Ceramiales (Majak *et al.*, 1966; Bidwell,

A.

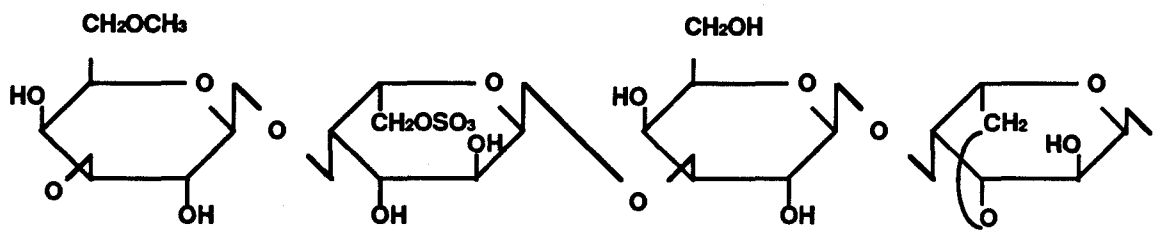


Floridoside



Isofloridoside

B.



Porphyran

Figure 1. Chemical structure of floridosides (A) and porphyran (B).

1958). In some *Porphyra* species, the amounts of floridoside and isofloridoside vary from 0.8 to 6.1% and 2.5 to 10.8%, respectively (McLachlan *et al.*, 1972). The absolute amounts of these carbohydrates were shown to vary with the level of salinity (for *P. purpurea*, see Reed and Collins 1980; for *P. umbilicalis*, see Wincke and Läuchli, 1981).

Porphyran is another carbohydrate found in red algae, particularly in *Porphyra*. Basically, porphyran is an agarose which is highly substituted by 6-O-sulfation of the L-galactose units and 6-O-methylation of the D-galactose units (Peat *et al.*, 1961). Its linear structure consists of 1,3-linked β-D-galactosyl and 1,4-linked α-L-galactosyl units in alternating sequence (Fig. 1B). The regularity of this repeating arrangement is masked by the partial occurrence of the D-galactosyl units as the 6-O methyl ether, and the occurrence of the L-galactosyl unit as both the 6-sulfate and the 3,6-anhydride. Thus, porphyran consists of four types of sugar units, namely D-galactose, L-galactose, 6-O-methyl-D-galactose, and 3,6-anhydro-L-galactose, together with ester sulfate. The proportions and linkage of the components are strictly regulated : 1. all the L-galactose units are sulfated at position 6, this accounts for nearly all the ester sulfate in the polysaccharide; 2. the sum of the proportion of L-galactose 6-sulfate and 3,6-anhydro-L-galactose is always equal to the sum of D-galactose and 6-O-methyl-D- galactose units; 3. the D-galactose and 6-O-methyl-D-galactose derivatives are each linked through position 3; and 4. the L-galactose 6-sulfate and 3,6-anhydro-L-

galactose are each linked through position 4 (Anderson and Rees, 1965). The existence of unsubstituted agarose has also been detected chemically (Turvey and Williams, 1964; Anderson and Rees, 1965) and enzymatically (Turvey and Christison, 1967; Duckworth and Turvey, 1969a; Duckworth and Turvey, 1969b).

Amino acids

The pattern of free and proteinaceous amino acids of *Porphyra* is roughly similar to that found in vegetables (that is, rich in alanine, aspartic acid, glutamic acid and glycine, see Noda, *et al.*, 1981). Amano and Noda (1990) reported that protein content of *Porphyra* was higher than that of soybean. The total protein content of commercially grown *Porphyra* spp. ranges from 30%-50% (Mumford and Miura, 1988). Generally, the higher the grade of *Porphyra* the greater its content of protein. Fujiwara-Arasaki *et al.* (1984) found that the value of essential amino acids, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (E:T value, mg of essential amino acid per g protein-N) in some *Porphyra* spp. ranged from 2,326 to 3,206. Similar results were also found by Arasaki and Arasaki (1983). These values are about the same as those for whole eggs (3040). The characteristic hoshi-nori taste is contributed by its amino acids (Nisizawa *et al.*, 1987). Moreover, the relatively high content of taurine, one of the unusual amino acids, is known to be effective for liver activity (prevents the

formation of gallstones) and for controlling blood cholesterol levels (Tsuji, *et al.*, 1981, 1983).

Lipids

Algae contain relatively small amounts of lipid, so that they are not utilized as a source of lipid or fatty acids. Instead they are used as a source of specific fatty acids (Wood, 1974). Fatty acids of particular interest in this study, eicosapentaenoic acid and palmitic acid, play an important role in biological membranes as major components of phospholipids and glycolipids. They also act as fuel molecules, which can be stored and/or mobilized as needed.

The fatty acid composition of Rhodophyta is distinctly different from that of higher plants in having polyunsaturated fatty acids of 20 carbon atoms and the saturated 16 carbon atoms as major components (Jamieson and Reid, 1972; Pohl and Zurheide, 1979; Khotimchenko and Svetashev, 1987; Khotimchenko and Vaskovsky, 1990). Several other studies, especially on *Porphyra* spp., support this finding (e.g., *Porphyra yezoensis*, Araki *et al.*, 1986, Kayama *et al.*, 1983; *P. tenera*, Sato, 1971; *Porphyra* sp., Johns *et al.*, 1979).

The importance of polyunsaturated C20 fatty acids in algae has been related to the fluidity change of biological membranes since this fatty acid is a major constituent of membrane lipids (Aro and Karunen, 1979), and also to antibacterial activity which is important in protection of algae from microbial attack,

especially after wave damage (Findlay and Patil, 1986). The utilization of the C20 fatty acid has been documented in the growth of oyster larvae (Langdon and Walcock, 1981; Chu and Webb, 1984; Wikfors *et al.*, 1984; Enright *et al.*, 1986); queen conch, *Strombus gigas*, larvae (Pillsbury, 1985); and baramundi, *Lates calcarifer*, fingerlings (Rimmer *et al.*, 1988). Moreover, Dyerberg *et al.* (1978) reported that C20 fatty acids have a therapeutic value for preventing atherosclerosis and serve as precursors for a powerful human hormone, prostaglandin (Bindra and Bindra, 1977).

Nutrients

The biochemical composition of marine algae is regulated to some extent by nutrient availability in the medium (DeBoer, 1981), primarily nitrogen content in the coastal waters in summer (Lobban *et al.*, 1985). Seawater nutrients in general are classified as essential or non-essential nutrients, and several criteria have been proposed to differentiate these two classes. Arnon (1953) suggested that an element was to be considered essential if algal growth ceased if the element was not provided and if optimal or near optimal growth was restored if the element was supplied. O'Kelley (1974) proposed that an element was essential if it could be demonstrated by *in vitro* experiments that the element had a non-replaceable role in a fundamental life process.

Among the essential nutrients, several studies have recognized the importance of nitrogen (Ryther and Dunstan, 1971; Topinka and Robbins, 1976; DeBoer and Ryther, 1977; Jackson, 1977;

Hanisak, 1979) and phosphorus as constituents of many biomolecules, in growth regulation, and in energy transfer (Kuhl, 1974; Lobban *et al.*, 1985). Nitrogen is available in seawater in forms of nitrate, nitrite, ammonium, or in the organic forms. In one study, *P. perforata* was shown to have preferential uptake of ammonium over other nitrogen ions (Thomas and Harrison, 1985), which might be because other ions have to be reduced by algae before further metabolism can take place (Kain and Norton, 1990).

Seasonal changes in the seawater nutrient availability have been found to affect the tissue nutrient level of red algae, *Chondrus crispus* (Asare and Harlin, 1983), *Gracilaria foliifera* (Rosenberg and Ramus, 1982), and brown alga, *Macrocystis integrifolia* (Wheeler and Srivastava, 1984). Some algae, *Valonia* and *Halicystis* (Jacques and Osterhout, 1938), *Laminaria longicruris* (Chapman and Craigie, 1977), and *L. saccharina* (Chapman *et al.*, 1978), were shown to accumulate nitrate in their tissue at much higher concentration than in seawater.

II. MATERIALS AND METHODS

SAMPLING

Porphyra

Samples of *Porphyra* spp. were collected monthly, January to May in 1991 and 1992. The species and locations where they were collected are shown in the following Table 1 and Figure 2.

Table 1: *Porphyra* Species and Collection Sites.

Species	Location
P. fallax	Wreck Beach ($123^{o}14'W$, $49^{o}17'N$)
P. pseudolanceolata	Whiffin Spit ($123^{o}49'W$, $48^{o}22'N$)
P. torta	Whiffin Spit
P. perforata	Whiffin Spit
P. mumfordii	Orlebar Point ($123^{o}49'W$, $49^{o}17'N$)
P. torta	Orlebar Point
P. perforata	Orlebar Point

Collections were scheduled for a time when the tide was at minimum level. The samples from Whiffin Spit were collected at about 11.00 to 12.00 AM, whereas those from Orlebar Pt. and Wreck Beach at 2.00 to 3.00 PM.

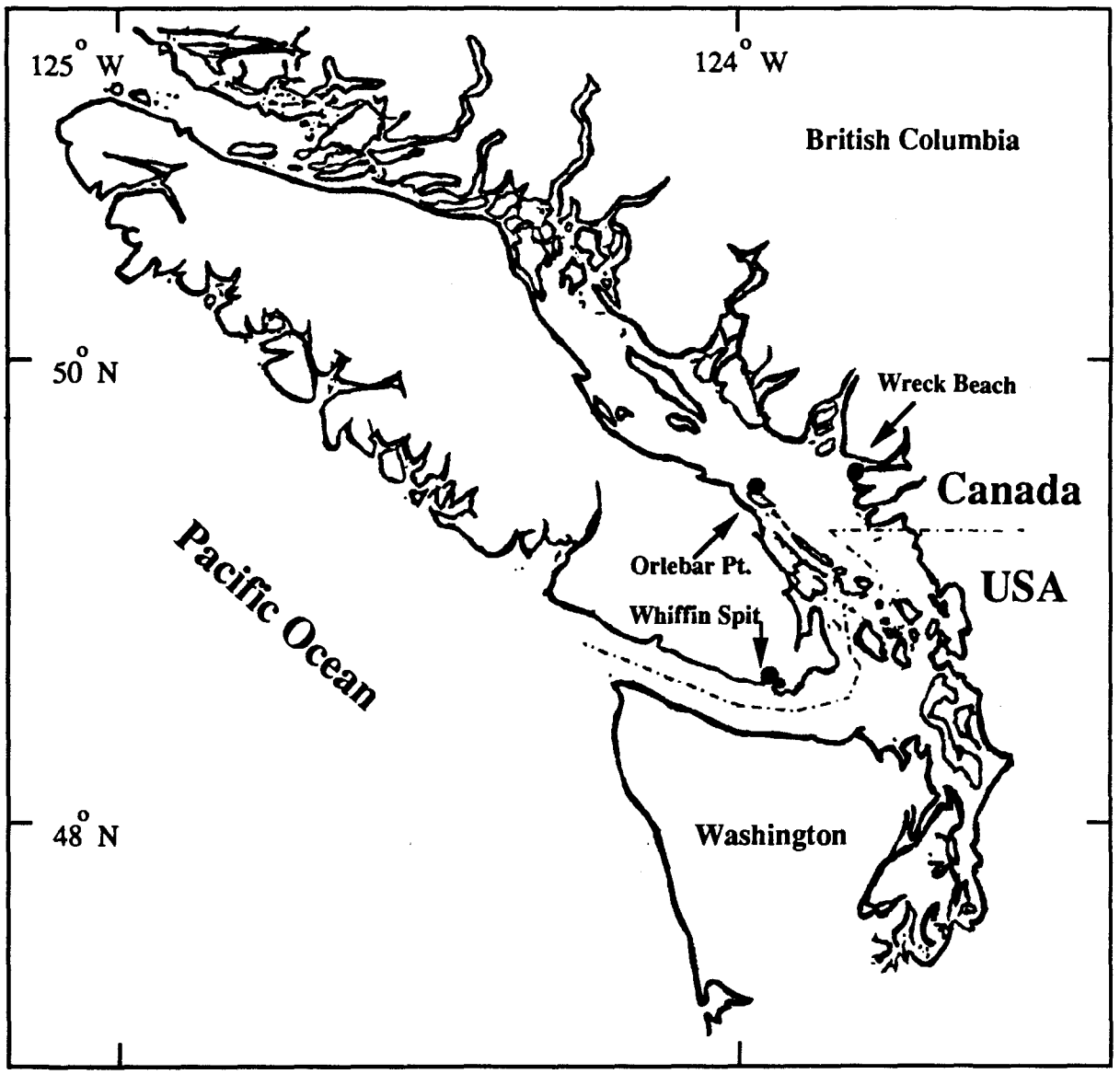


Figure 2. Map showing the collection sites of *Porphyra* spp.

Adhering materials were removed from samples by rinsing with filtered seawater and the samples were frozen in liquid nitrogen (LN_2). Fresh weight of tissue was determined after draining seawater from the samples using a fishnet. Then the samples were freeze-dried, ground to a powder, and stored in bottles covered by aluminium foil until analyzed.

Seawater

Seawater samples were collected from the three collection sites and stored in polyethylene bottles that had been rinsed twice with sample water before filling. The samples were filtered (0.22 µm, diameter) with suction and frozen until analyzed.

ANALYTICAL PROCEDURES

Seawater analysis

Determination of NO_3^-, NO_2^-, PO_4^{3-}, and NH_4^+ concentrations in seawater samples were carried out using a Technicon II auto analyzer at the University of British Columbia (courtesy Dr. P. J. Harrison).

The following chemical analyses were performed on the various samples of *Porphyra* spp. All analyses were done in duplicate.

Ash

Samples of freeze-dried powder were put in a porcelain crucible, weighed, and then burned at 400°C for 12 h in an

electric furnace. After cooling in a desiccator, the samples were reweighed (Larsen, 1978).

Total C and N analyses

Total carbon and nitrogen analyses in the tissue were done using Carlo Erba Strumentazione Elemental Analyzer-Model 1106.

Analyses of nutrients in plant tissue

Freeze-dried samples (0.5 g) were extracted with hot 80% EtOH for 1 h three times. The residue was rinsed with absolute EtOH. The combined extract was evaporated to dryness in a vacuum evaporator. Lipids in the extract were removed by partitioning against chloroform. The water phase was diluted to 250 mL as sample for analysis. Nutrient analyses were carried out as for seawater using the Technicon II auto analyzer.

Lipids

In this analysis, the concentrations of total lipids and of the major fatty acids in *Porphyra* spp., palmitic acid and eicosapentaenoic acids, were determined.

Extraction

Extraction of lipids was done following the methodology of Kates (1986). Methanol-chloroform (2:1, 6 mL) was added to 1 g of sample and the solution was homogenized. The homogenate was filtered with suction, and the residue was reblended with a

mixture of methanol-chloroform-water (2:1:0.8, 7.6 mL). The homogenate was filtered and the residue was washed with 3 mL of methanol-chloroform (2:1). To the first homogenate an internal standard, pentadecaenoic acid, was added.

The combined filtrate was transferred to a separatory funnel, and chloroform (5 mL) and water (5.8 mL) were added. The phases were allowed to separate. The chloroform layer was withdrawn and concentrated in vacuo. For fatty acid analysis, the residual lipids were immediately methylated (Morrison and Smith, 1964) by adding 1 mL of 14% boron trifluoride in methanol, put in a capped bottle and heated in a boiling water bath for 30 min. The methylated sample was collected by partition in pentane:water (2:1). The pentane layer was dried by suction with nitrogen gas. The concentrate was diluted with ethyl acetate for injection in a gas liquid chromatograph (GLC).

Determination

Total lipids were determined by using the gravimetric method. The concentrated extract was weighed and calculated as a percentage of sample in dry weight. Fatty acid determination was carried out on a Hewlett Packard 5790A gas chromatograph, equipped with flame ionization detector and connected to a model 3390A electronic integrator. A fused silica capillary column (SE-30, 30m x 0.2mm) was employed, with helium as carrier gas.

Amino acids

Extraction of free amino acids

The freeze-dried powdered sample (1.0 g) was extracted with 80% aqueous ethanol (3x, 5 mL each) and the extract centrifuged. The supernatant was pooled, concentrated in vacuo, dissolved in 0.1 N HCl and filtered prior to determination using an HPLC (Rosell and Srivastava, 1985).

Extraction of total amino acids

The powdered sample was vacuum hydrolyzed with 6 N HCl in a hydrolysis tube at 110^{o}C for 23 h. After hydrolysis, the residue was rinsed with 80% aqueous ethanol, concentrated to dryness in a rotary evaporator, then dissolved in 0.1 N HCl, and filtered for determination.

Determination of amino acids

Free amino acids as well as total amino acids were analyzed with the PICO-TAG method on a WATERS high performance liquid chromatograph (HPLC) system.

Porphyran

Extraction of porphyran was carried out using the method of Nishide *et al.* (1988). The sample was treated with 3.7 % formaldehyde solution in a flask closed with a stopper at 30^{o}C for

24 h. Distilled water (2x volume) was added and the suspension was heated at 100°C with stirring for 12 h. The extract was cooled to room temperature and filtered. The clarified filtrate was dialyzed against running tap water for 24 h, and evaporated to 1/4 of its original volume under reduced pressure. Ethanol (4x volume) was added at room temperature. The gelatinous precipitate was collected by centrifugation, washed with ethanol and acetone, and dried at 30°C for 12 h. Then the dried porphyran extract was weighed, and the percentage of porphyran in the dry weight was calculated.

Floridosides

Extraction

Extraction of floridosides was performed following the methodology of Meng et al. (1987). Each sample (1 g) was extracted with 80% ethanol three times. Mannitol was added as an internal standard.

The extract was evaporated to dryness in a rotary evaporator. Pigments and lipids were removed by partition with chloroform and water. The water phase was desalted by passing through columns of cation/anion ion exchange resin and collected in a 250 mL evaporation flask. The neutral fraction was evaporated, then dissolved in water. The floridosides were methylated using TRI-SIL

"Z" (50 μL per sample) for 0.5 h at 70°C for determination using a gas liquid chromatograph (GLC).

Determination

Determination of floridosides was carried out on a Hewlett Packard 5790A gas chromatograph, as above. A fused silica capillary column (SE-30, 30m x 0.2mm) and isothermal program at 190°C were employed, with helium as carrier gas.

STATISTICAL ANALYSES

Analysis of variance was done for chemical constituents of *Porphyra* by using the general linear procedure model. As a model 1, interactions between factors was not included:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \chi_k + \varepsilon_{ijk}$$

where:

Y_{ijk}= the chemical concentration in year i, month j, for species k.

μ = the mean of chemical concentration in population.

α_i = the average effect of different conditions between 1991 and 1992.

β_j = the average effect of different conditions among collection months.

χ_k = the average effect of different characteristics among species.

ε_{ijk}= the residual effect of year, month, and species (level ijkl).

In addition to the above model, two other general linear models, one including factors for interaction of year and species (model 2), and the other including factors for the interaction of month and species (model 3), were also used to identify species that showed significant differences between years and among monthly collections, respectively.

A further analysis, i. e. least squares means, was applied to determine the difference of chemical contents between years, among monthly collections, and among species.

For determination of significance, a probability of 0.05 was used as a critical significant value in both, anova and least square means analyses.

III. RESULTS

COLLECTION SITES

The three collection sites, Orlebar Pt., Whiffin Spit, and Wreck Beach, are moderately exposed areas. Comparing their ecological conditions, the sites show some differences. As illustrated in Figure 3, Orlebar Pt., a rocky shore, is a substratum ideal for *Porphyra* spp. Whiffin Spit has a flat terrain with a few big rocks where *P. torta* and *P. pseudolanceolata* grow, and small rocks where *P. perforata* was found growing. Wreck Beach, a sandy shore, is slightly sloped with rocks where *P. fallax* attaches over a relatively wide range between mid and high levels (see Fig. 3).

On any particular date of collection, the air temperatures at the three sites were identical or very close, as shown in Table 2. However, generally lower temperatures were noted for 1991 than for 1992, although the pattern of temperature increase from January to May was similar in both years.

Table 2. Air Temperature at the Time of Collections for the Sites.

Collection Sites		Air Temperature (°C)				
		Jan	Feb	Mar	Apr	May
Orlebar Pt.	1991	4.0	6.0	9.0	12.0	14.0
	1992	8.0	8.0	12.0	13.0	17.0
Whiffin Spit	1991	4.5	6.0	9.0	12.0	12.0
	1992	8.0	8.5	12.0	13.0	19.0
Wreck Beach	1991	5.0	6.0	8.5	12.0	14.0
	1992	8.0	7.5	11.0	13.0	18.0

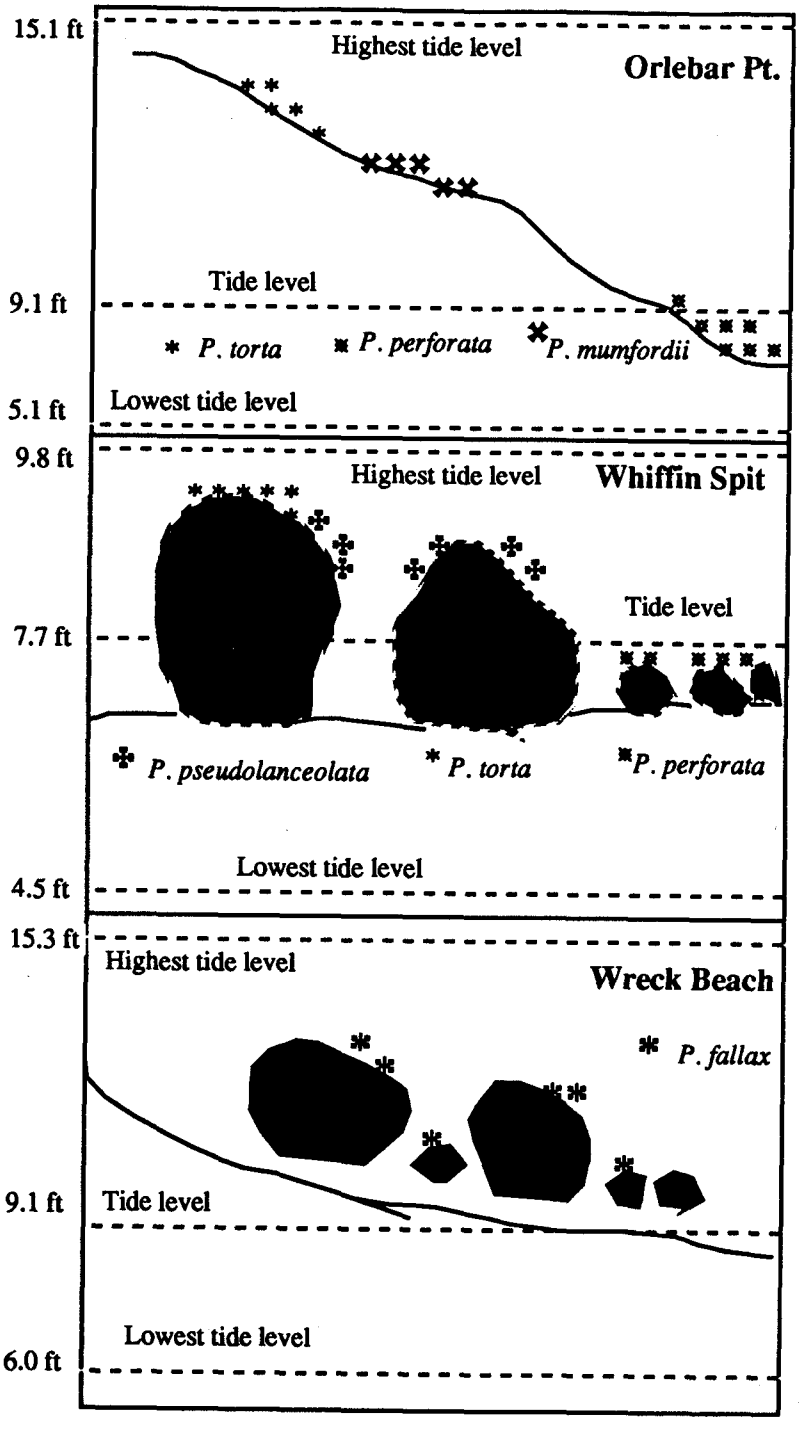


Figure 3. *Porphyra* spp. position relative to tide level at the time of January 1991 and 1992 collections.

Concerning their tide level, the three collection sites showed similar patterns (see Appendix I). Orlebar Pt. and Wreck Beach had a similar range of tide levels, whereas Whiffin Spit had a relatively smaller range than the other two sites. Despite the quantitative differences shown in Appendix I, the position of *Porphyra* growing at the sites relative to the tide level was similar for a particular species. For example, Figure 3 shows the tide levels at the collection sites when January samples were collected in both 1991 and 1992. As the season progressed, it was noted that there was a shift in the time of day when the minimum tide levels were reached. Consequently, *Porphyra* thalli experienced a longer periods of emergence during the day, while at the same time day-time air temperature also increased (Table 2).

OCCURRENCE OF *PORPHYRA*

In the present study, the samples of *Porphyra* were taken from the foliose haploid phase. All grew on rocky substrata. At Orlebar Pt., *P. torta* and *P. mumfordii* grew at almost the same intertidal level, so that at some spots they grew side by side, whereas *P. perforata* grew at a lower level together with the brown alga, *Fucus gardneri*. A similar pattern of distribution was also found at Whiffin Spit, in which *P. torta and P. pseudolanceolata* grew at almost the same intertidal level, or the latter a little bit lower, and *P. perforata* grew at a much lower level than the

previous two. At the third site, Wreck Beach, *P. fallax* grew over a relatively wide range in the upper-mid intertidal.

The collections showed that not all species of *Porphyra* had the same period of growth between the two collection years. As summarized in Figure 4, *P. perforata* from Orlebar Pt., at Whiffin Spit (1992 only), *P. fallax* at Wreck Beach, and *P. pseudolanceolata* from Whiffin Spit (1991 only) were found from January until May. However, *P. perforata* at Whiffin Spit 1991 appeared from April and *P. pseudolanceolata* at Whiffin Spit 1992 was found only until April. *P. torta* was found from January to April at both collection sites, Orlebar Pt. and Whiffin Spit, in both years. *P. mumfordii* showed the shortest period of growth. In 1991, *P. mumfordii* was found from January to March, but in 1992 it appeared only until February. *P. perforata* and *P. fallax*, which occurred until at least May, occurred somewhat lower on the shore than the other species, which were relatively more exposed to air, especially after March, and dry for prolonged periods.

SEAWATER NUTRIENTS

Nutrient status of the sites is presented in Figures 5 and 6. It was noted that there were some variations in individual nutrient concentrations among the sites, but all sites showed similar composition and seasonal changes.

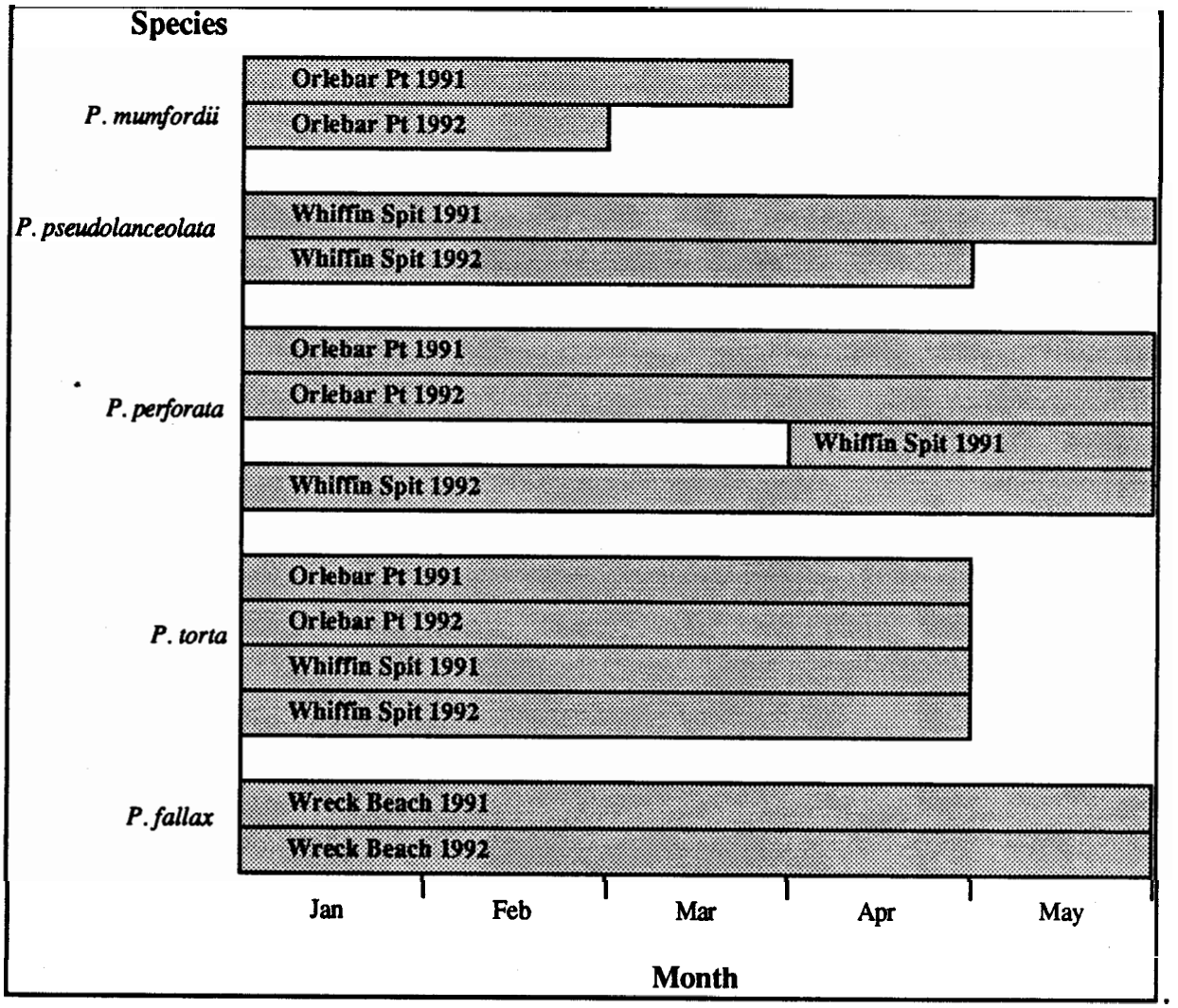


Figure 4. Seasonal occurrence of *Porphyra* spp. at the collection sites.

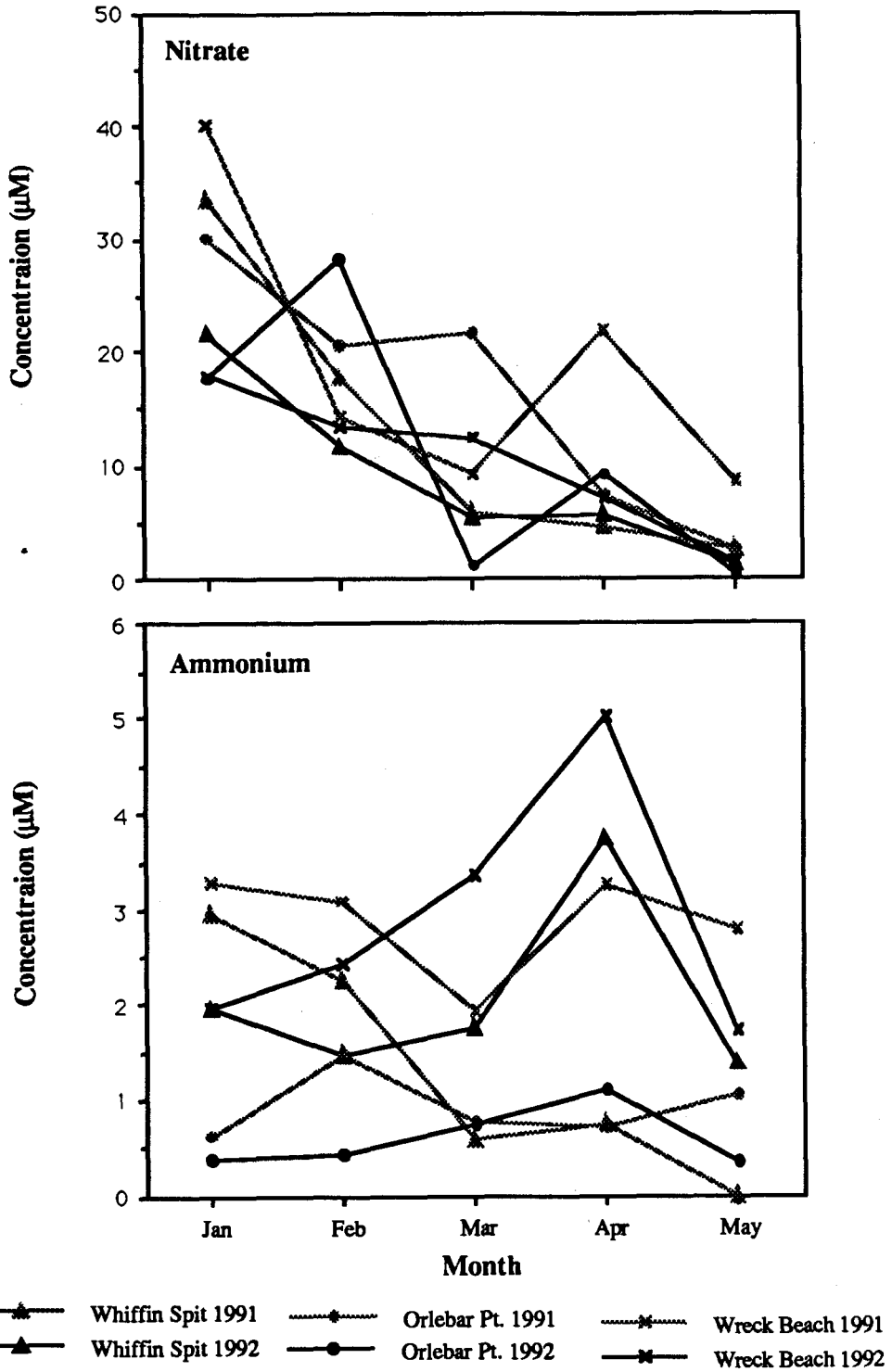


Figure 5. Changes in seawater nitrate and ammonium at the sites as observed at collection times.

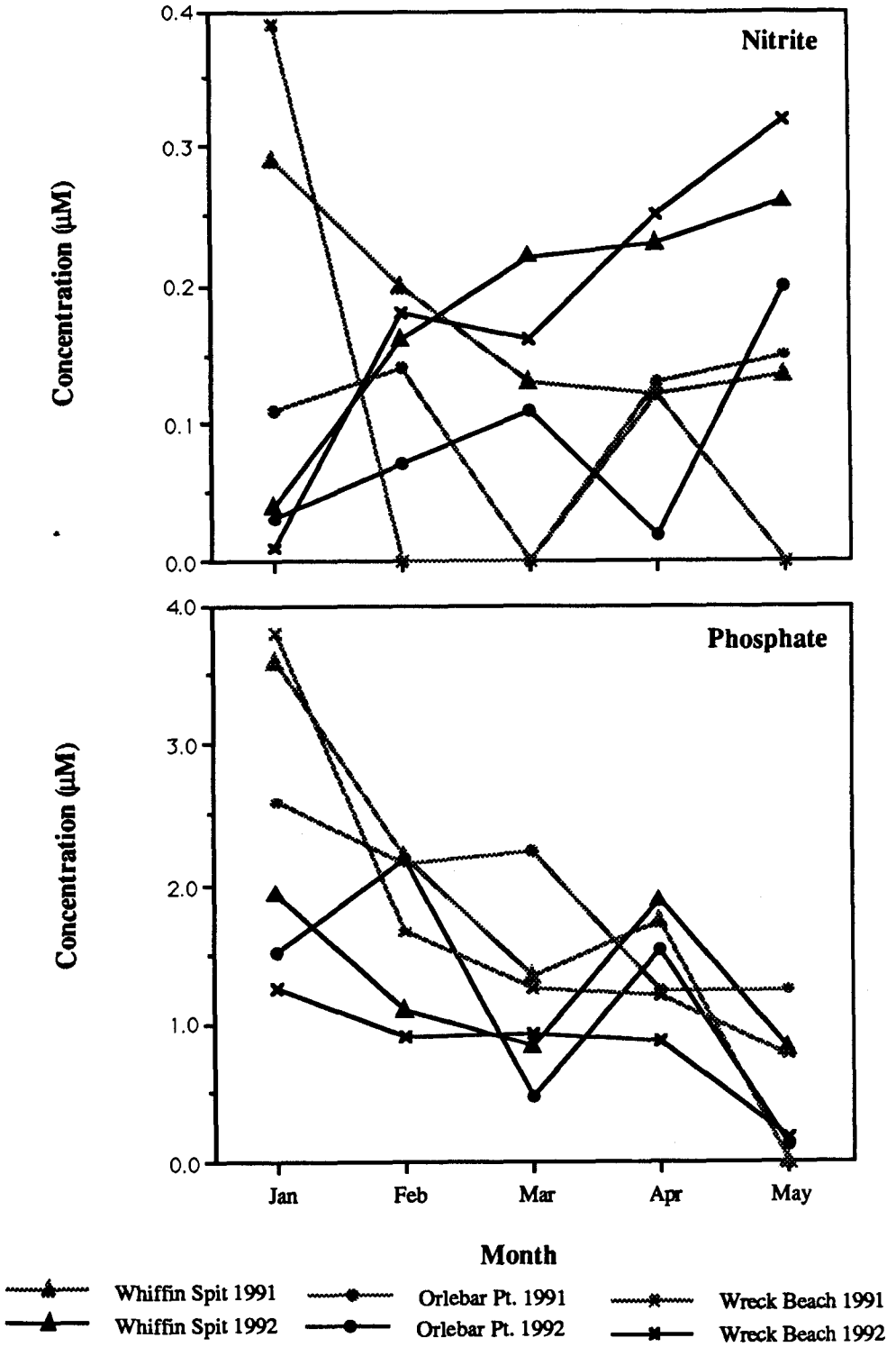


Figure 6. Changes in seawater nitrite and phosphate at the sites as observed at collection times.

Nitrate concentration in the seawater decreased markedly from January to May at all sites. In 1991, the nitrate concentration decreased from 29.9 to 2.6 μM, 33.3 to 2.5 μM and 40.0 to 8.7 μM at Orlebar Pt., Whiffin Spit and Wreck Beach, respectively (Fig. 5). In 1992, it decreased from 17.6 to 0.3 μM, 21.4 to 1.5 μM and 18.0 to 1.6 μM in Orlebar Pt., Whiffin Spit and Wreck Beach, respectively. In some cases, the decrease did not show a smooth pattern, e.g. Orlebar Pt. for both 1991 and 1992, and Wreck Beach, 1991, with slight increases in March or April over the previous month.

Ammonium did not show a clear seasonal pattern. In 1991, the ammonium concentration ranged between 0.6 to 1.5 μM, 0.0 (undetectable) to 2.9 μM and 1.9 to 3.3 μM at Orlebar Pt., Whiffin Spit and Wreck Beach, respectively (Fig. 5). In 1992, it ranged from 0.4 to 1.1 μM, 1.4 to 3.7 μM and 1.7 to 5.0 μM at Orlebar Pt., Whiffin Spit and Wreck Beach, respectively. In some instances, it showed an obvious increase pattern from January to April (1992 data for Whiffin Spit and Wreck Beach).

Nitrite concentrations ranged between 0.0 (undetectable) to 0.2 μM, 0.1 to 0.3 μM and 0.0 to 0.4 μM at Orlebar Pt., Whiffin Spit and Wreck Beach, respectively, in 1991 (Fig. 6). In 1992, nitrite concentrations ranged from 0.0 to 0.1 μM, 0.0 to 0.3 μM and 0.0 to 0.3 μM at Orlebar Pt., Whiffin Spit and Wreck Beach, respectively. The phosphate concentrations ranged from 1.2 to 2.6 μM, 0.0 to 3.6 μM and 1.2 to 3.8 μM at Orlebar Pt., Whiffin Spit

and Wreck Beach, respectively, in 1991 (Fig. 6). In 1992, phosphate concentrations ranged from 0.1 to 2.2 µM, 0.8 to 1.9 µM and 0.2 to 1.3 µM at Orlebar Pt., Whiffin Spit and Wreck Beach, respectively. Nitrite concentrations did not show a clear seasonal patterns, whereas Phosphate showed a decreasing pattern from January through May (Fig. 6).

PORPHYRA SAMPLES AVAILABLE FOR CHEMICAL ANALYSES

Due to problems with the freeze drier, *Porphyra* collections in April 1991 for all sites and for January 1991 for Orlebar Pt. were lost and were not available for chemical analyses. For 1992, however, all monthly collections were available. For interpretation of seasonal trends, therefore, a greater reliance was placed on data from 1992.

STATISTICAL ANALYSES

Because of the nature of the data, in which species showed variations in availability among month of collections, comparison of the chemical contents among species was based on the analysis of variance model 1, i. e. without factor interactions, whereas those between year of collections was based on the model 2, i. e. with interaction of year-species to enable identifying species that showed significant differences between years.

Monthly variations among species could not be determined since analysis of variance model 3, i. e. with factor interaction

of month-species, failed to analyze the data which showed a high variations in availability among species. Therefore, in order to determine significant fluctuations of chemical content among months, analysis of variance model 1 was used (Appendix II).

DRY WEIGHT

The average dry weight of different species of *Porphyra* was about 20% of fresh weight. In 1991, it varied from 19.1% for *P. torta* at Whiffin Spit to 21.8% for *P. mumfordii*, whereas in 1992 it ranged from 20.1% for *P. pseudolanceolata* to 23.8% for *P. torta* at Orlebar Pt. (Fig. 7). *P. torta* at both Orlebar Pt. and Whiffin Spit showed significant differences in dry weight between years (Fig. 7). Among species (Fig. 8), *P. pseudolanceolata* was significantly lower in dry weight than all other species, except *P. fallax*. As shown in Figure 9, there was no clear pattern of changes in dry weight from month to month. The analysis of variance result, however, showed that there were significant differences between some of the months (Appendix II).

ASH

The average ash content of *Porphyra* spp., expressed as % dry weight (dw), ranged from 12.0% for *P. perforata* at Orlebar Pt. to 18.7% for *P. fallax* for the 1991 samples, and from 13.4% for *P. torta* at Whiffin Spit to 16.5% for *P. fallax* for the 1992 samples (Fig. 10). Figure 10 also shows that there is no significant

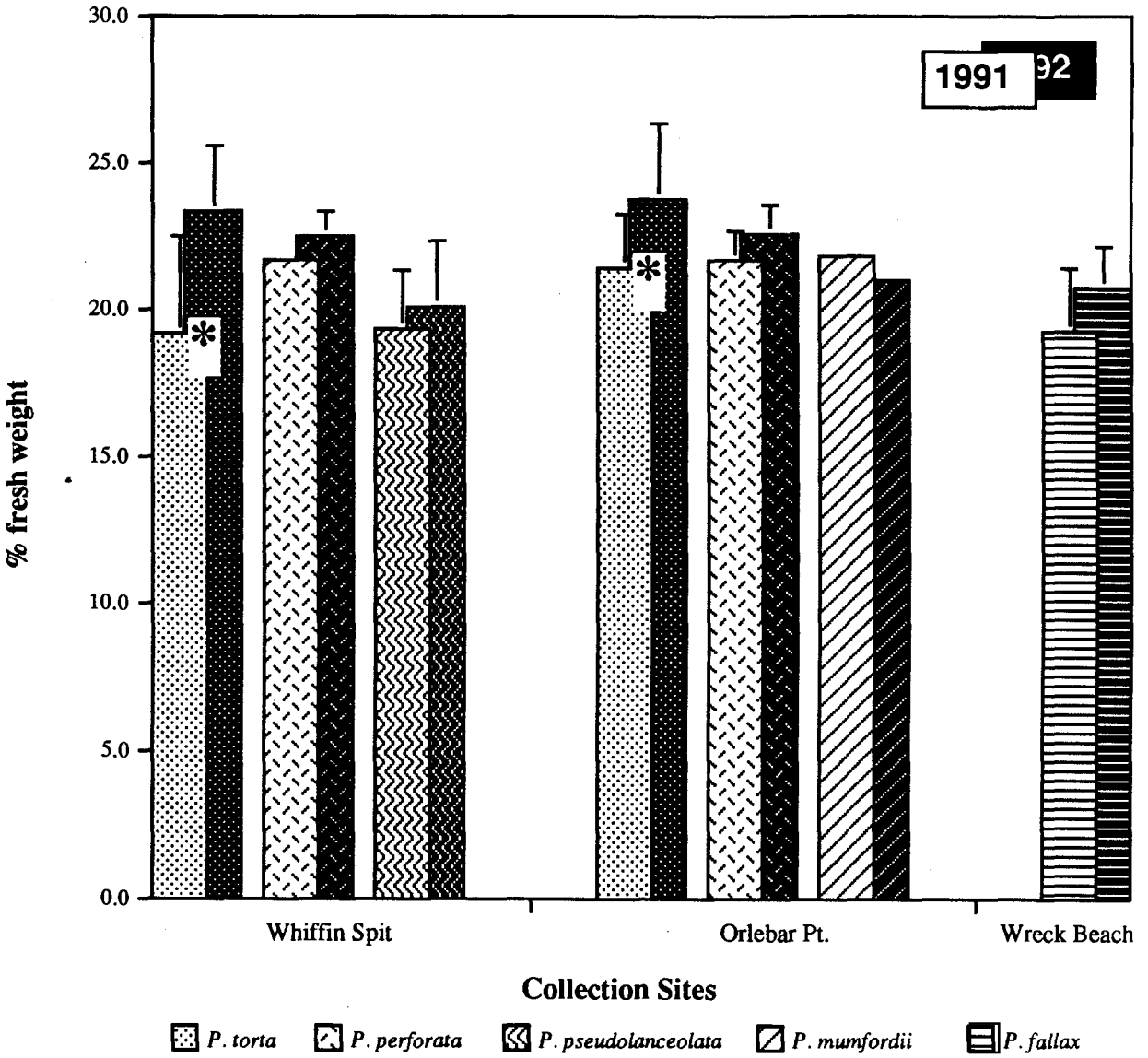


Figure 7. Dry weight of *Porphyra* spp. at the three sites. The values shown are averages of all collections in one year with their standard deviations (*P. perforata* from Whiffin Spit, 1991 has no standard deviation). Bars with asterisk (*) indicate significant differences between the two years ($p \leq 0.05$).

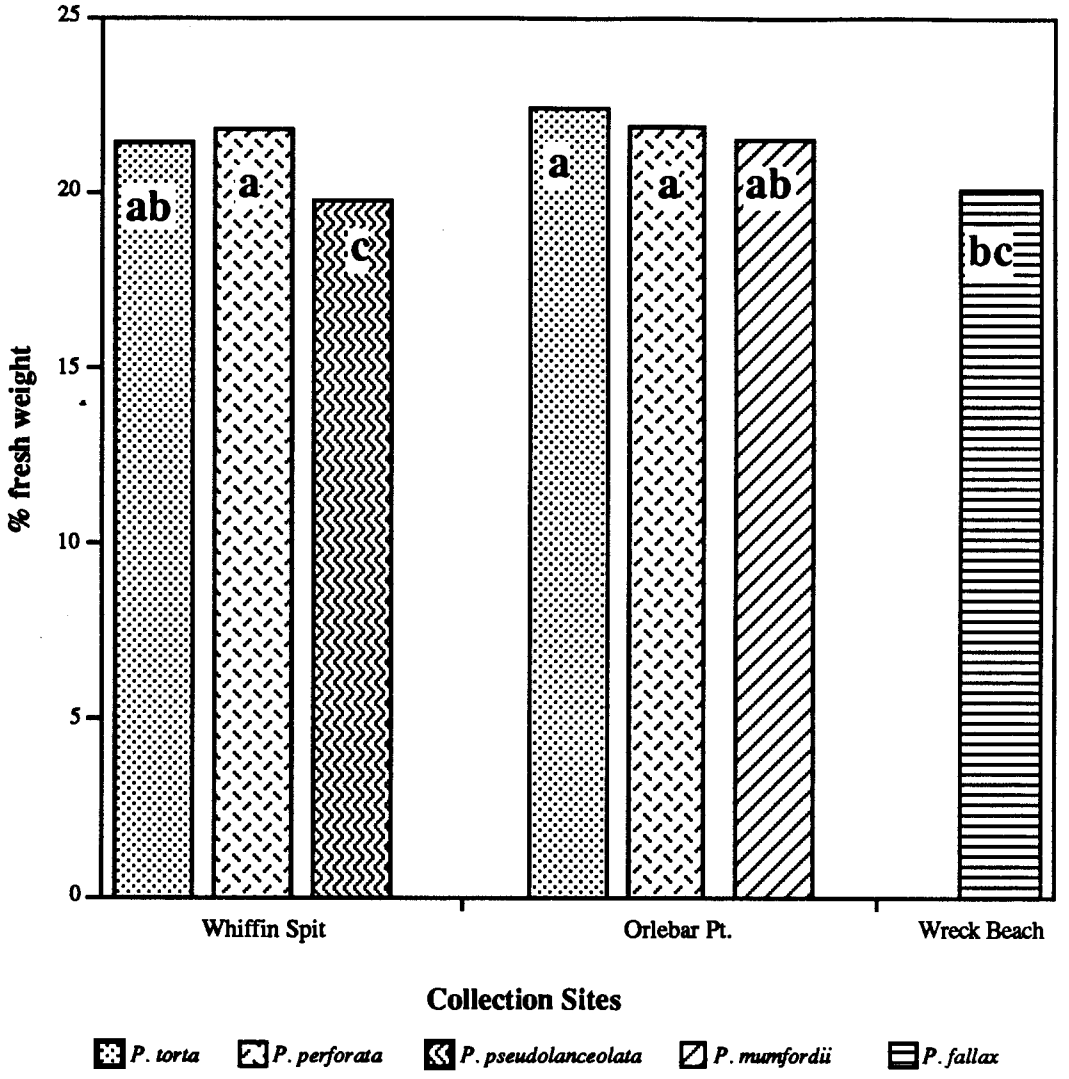


Figure 8. Average dry weight of *Porphyra* spp. for both 1991 and 1992 at the three sites. The bars shown are least square mean values of all collections. Bars with the same letter are not significantly different ($p > 0.05$).

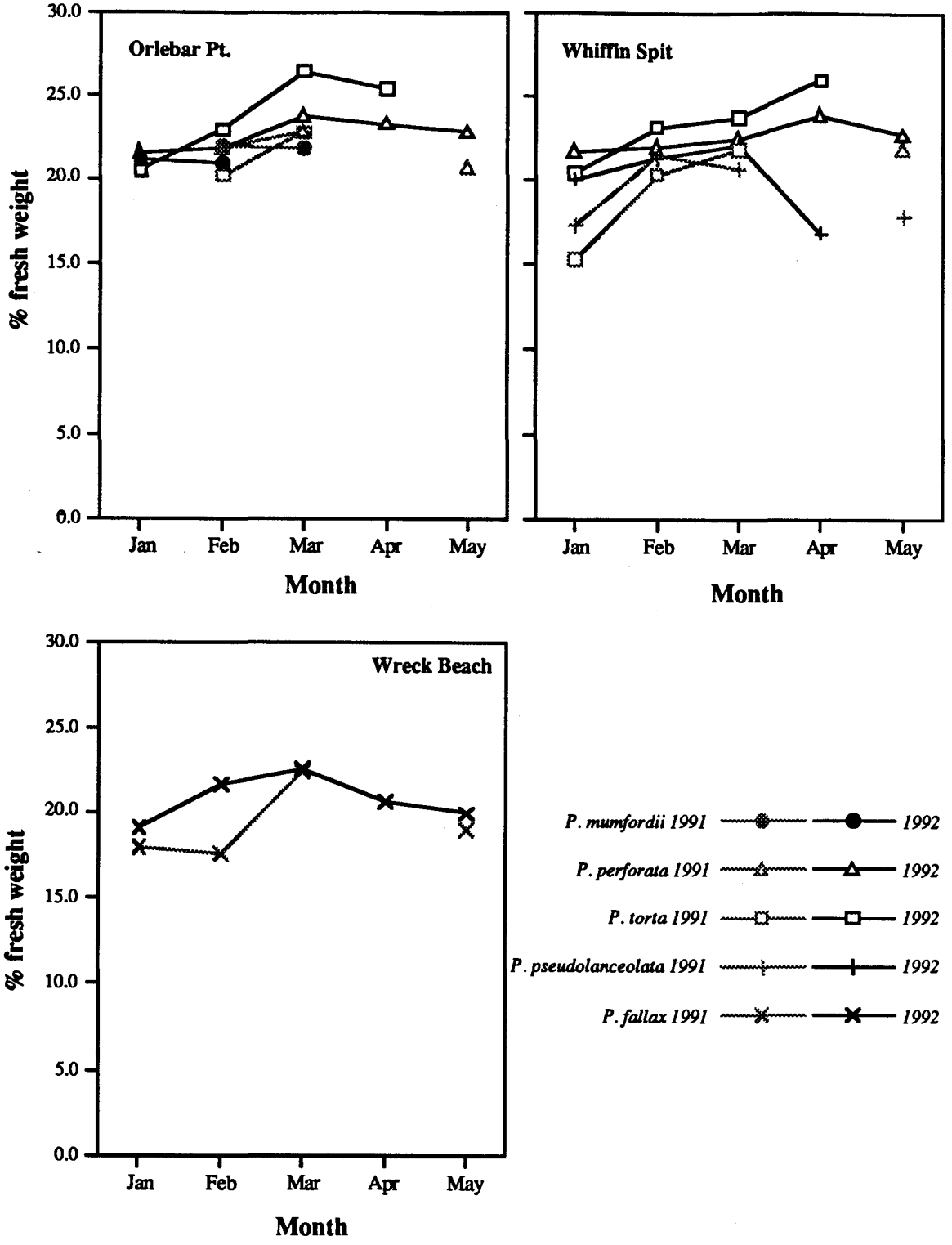


Figure 9. Changes in dry weight of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

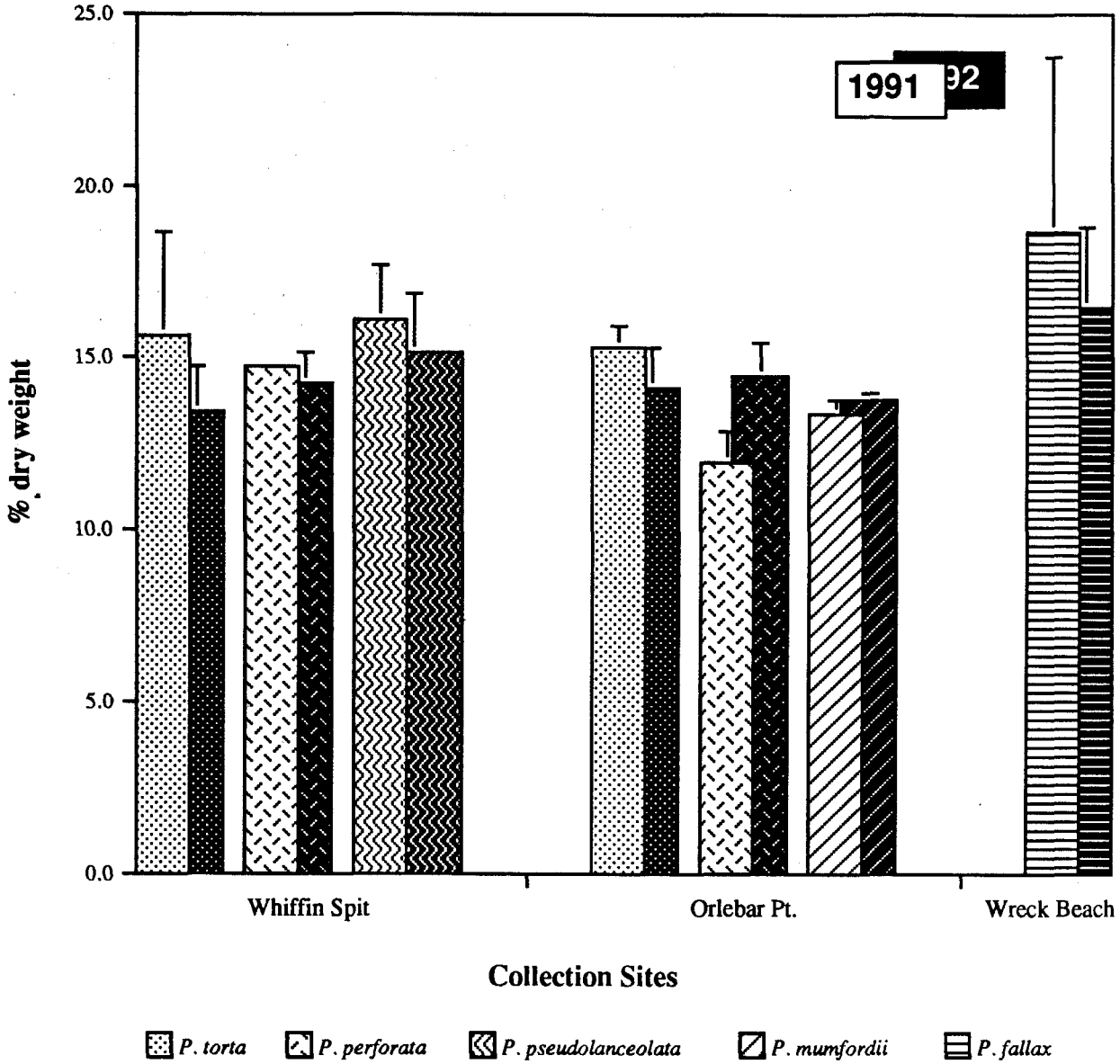


Figure 10. Ash content in *Porphyra* spp. at the three sites. The values shown are averages of all collections in one year with their standard deviations (*P. perforata* from Whiffin Spit, has no standard deviation). Bars with an asterisk (*) indicate significant differences between the two years ($p \leq 0.05$).

difference in ash content observed between year of collection in any species at a particular site. There were no significant differences among the species in ash content, except for *P. fallax*, which showed a significantly higher value (Fig. 11). Through the season, the values were significantly higher only in January (e. g. *P. fallax*); no differences were observed among the other months (Fig. 11, Appendix II).

TOTAL CARBON AND NITROGEN

Total tissue carbon, as percent of dry weight, varied little over the growing period. As presented in Figure 13, the value for total carbon ranged from 35.2% to 42.7% and 34.9% to 38.9% for *P. torta* at Orlebar Pt. and Whiffin Spit, 34.2% to 37.6% and 36.3% to 39.5% for *P. perforata* at Orlebar Pt. and Whiffin Spit, 34.7% to 38.0% for *P. pseudolanceolata*, 37.0% to 40.2% for *P. mumfordii*, 34.3% to 37.7% for *P. fallax*. There were no noticeable patterns of seasonal changes in total carbon, although the 1992 samples of *P. perforata* and *P. torta* from Orlebar Pt. showed some monthly fluctuations.

Total nitrogen, unlike total carbon, showed a distinct seasonal trend. The total nitrogen concentration varied from 3.6% to 5.3% and 3.9% to 4.4% (dw) for *P. torta* at Orlebar Pt. and Whiffin Spit, 3.1% to 4.5% and 4.1% to 5.4% (dw) for *P. perforata* at Orlebar Pt. and Whiffin Spit, 3.6% to 5.2% (dw) for *P. pseudolanceolata*, 4.6% to 5.5% (dw) for *P. mumfordii*, 3.1% to 5.7%

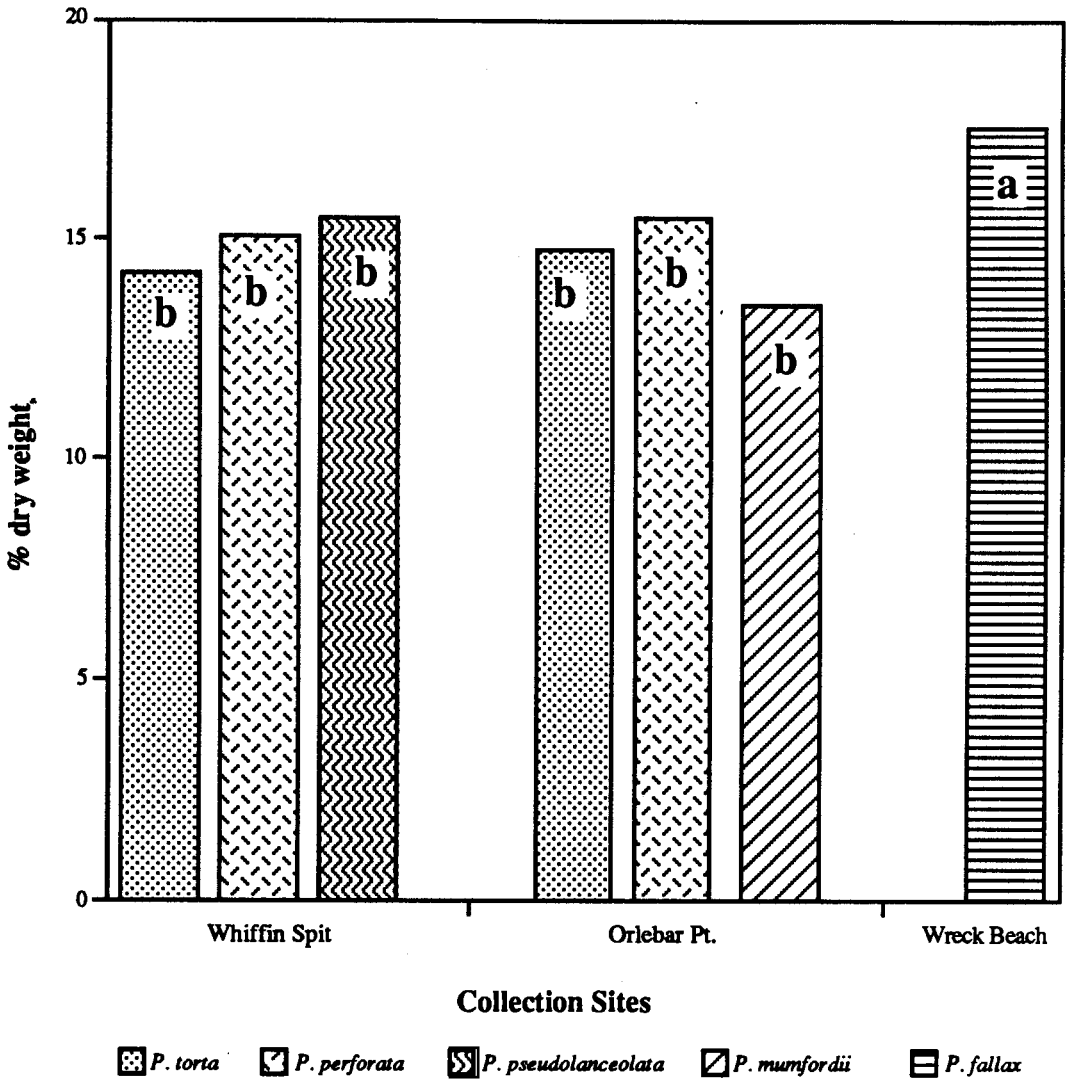


Figure 11. Average ash content in *Porphyra* spp. for both 1991 and 1992 at the three sites. The bars shown are least square mean values of all collections. Bars with the same letter are not significantly different ($p > 0.05$).

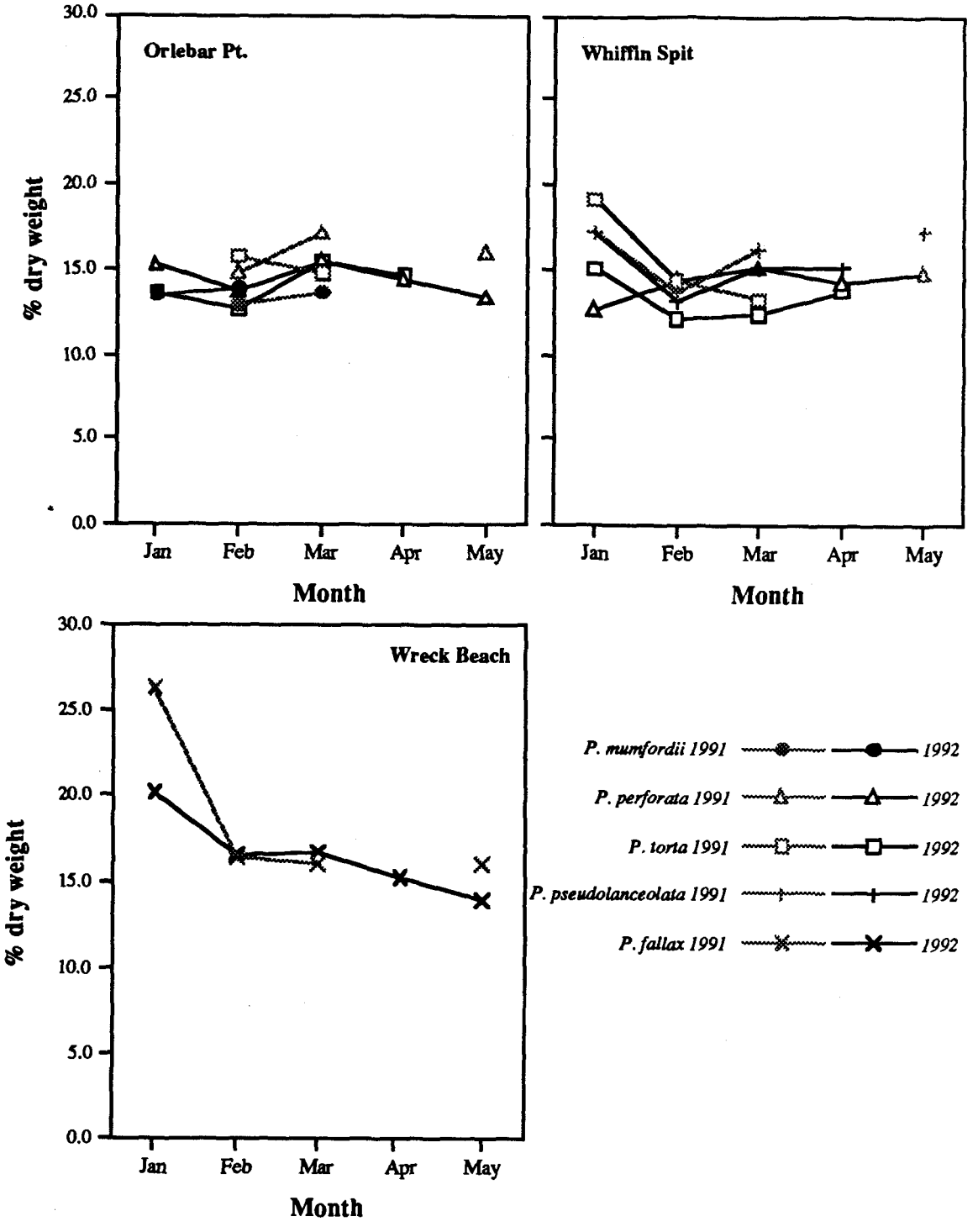


Figure 12. Changes in ash content of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

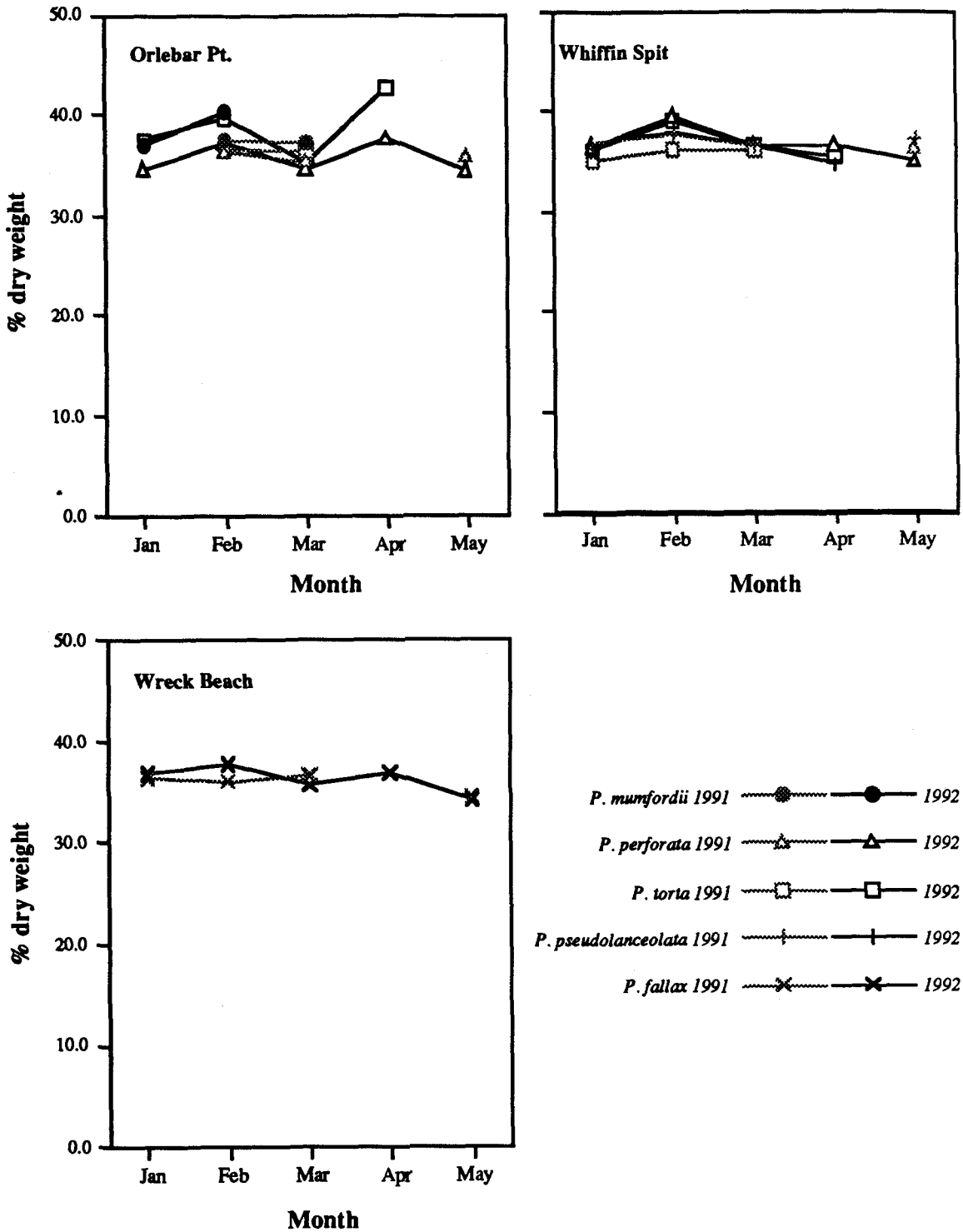


Figure 13. Changes in total carbon of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

(dw) for *P. fallax* (Fig. 14). In most instances there was a general decline in total nitrogen value as the season progressed from January through May.

As expected from the above data, the C/N ratio showed a gradual increase from January to May (as an example, the ratio for 1992 is plotted in Fig. 15).

TISSUE INORGANIC NUTRIENTS

Nitrate concentration of tissue samples showed the highest values in January or a gradual increase and then decrease through the season (Fig. 16). The magnitude of change in nitrate concentration in some cases was quite high. For example, nitrate concentration in *P. torta* at Orlebar Pt. 1991, *P. perforata* at Whiffin Spit 1992 and *P. fallax* 1992 showed changes from 15.31 to 4.56 µmol. g^{-1} dw, 14.98 to 0.95 µmol. g^{-1} dw, and 10.69 to 0.56 µmol. g^{-1} dw, respectively.

The seasonal variations in tissue nitrite content showed different trends between the two years. The available 1991 samples (Fig. 17) showed a sharp decreasing pattern, except for *P. mumfordii*, which showed an increase. The 1992 samples, on the other hand, showed no significant pattern of change from January through May.

Tissue ammonium showed an increase in the early season, followed by a decrease in 1991 (Fig. 18). In the 1992 samples,

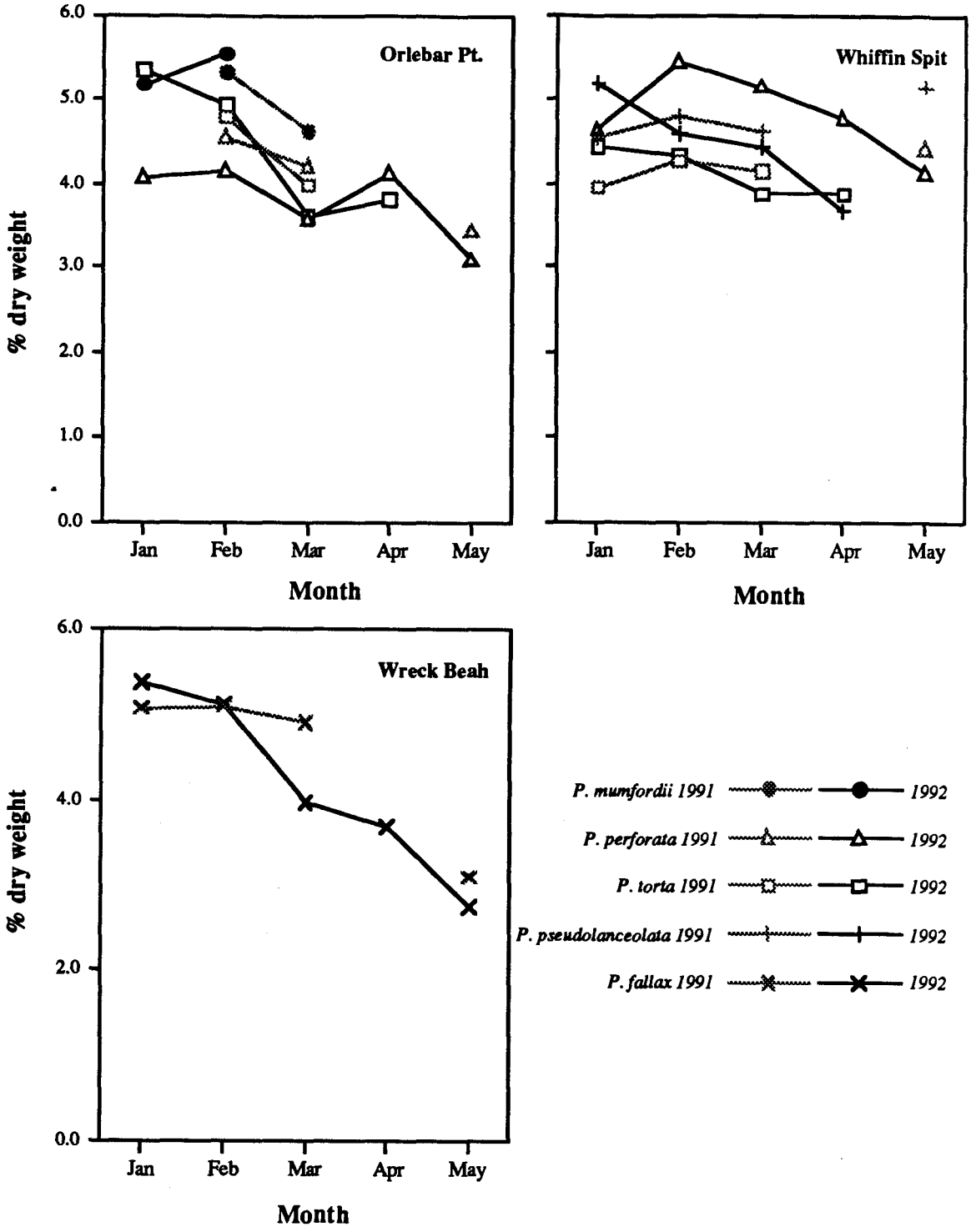


Figure 14. Changes in total nitrogen of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

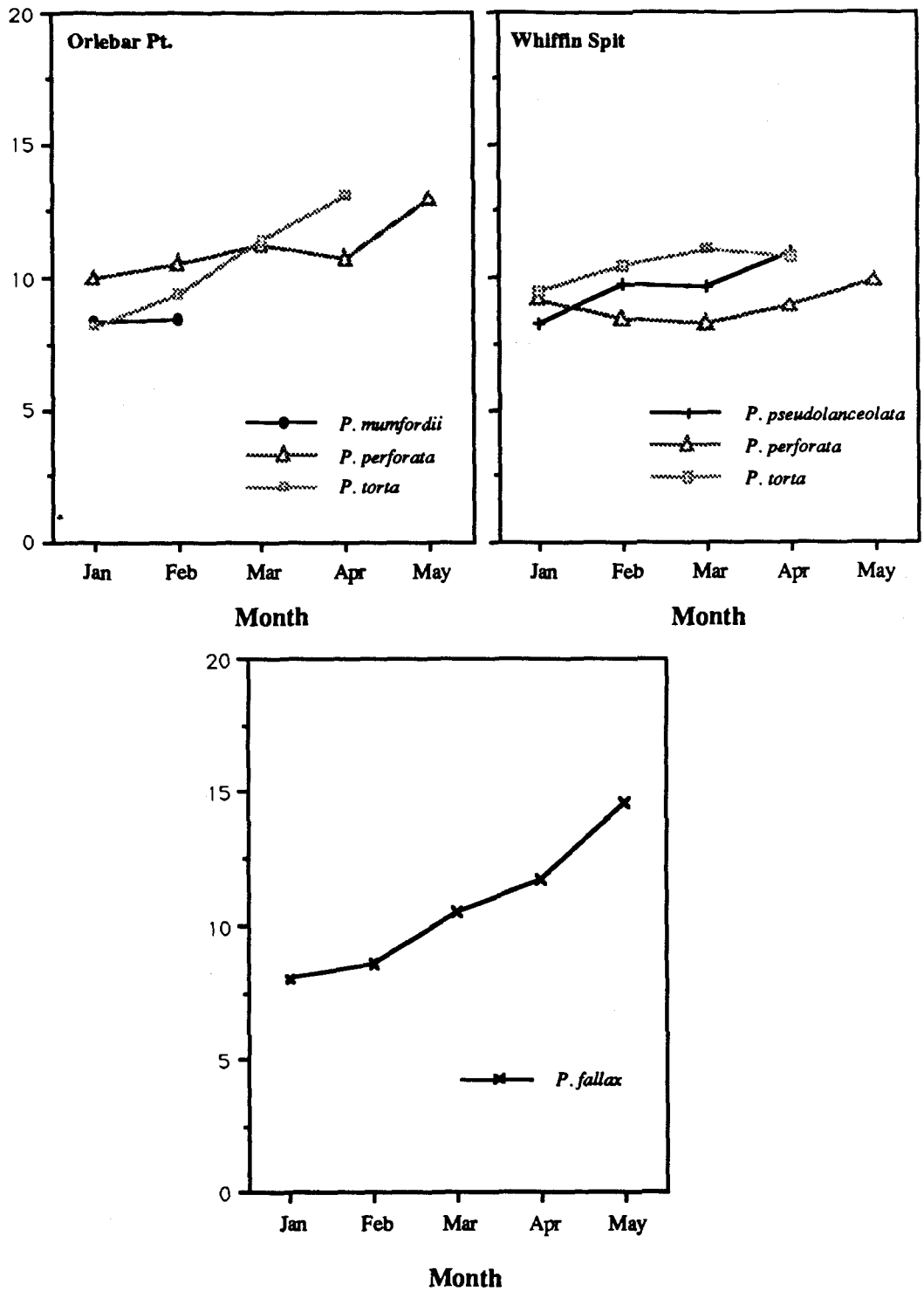


Figure 15. Changes in C/N ratio (by atom) in *Porphyra* spp. collected January to May 1992 at the three collection sites.

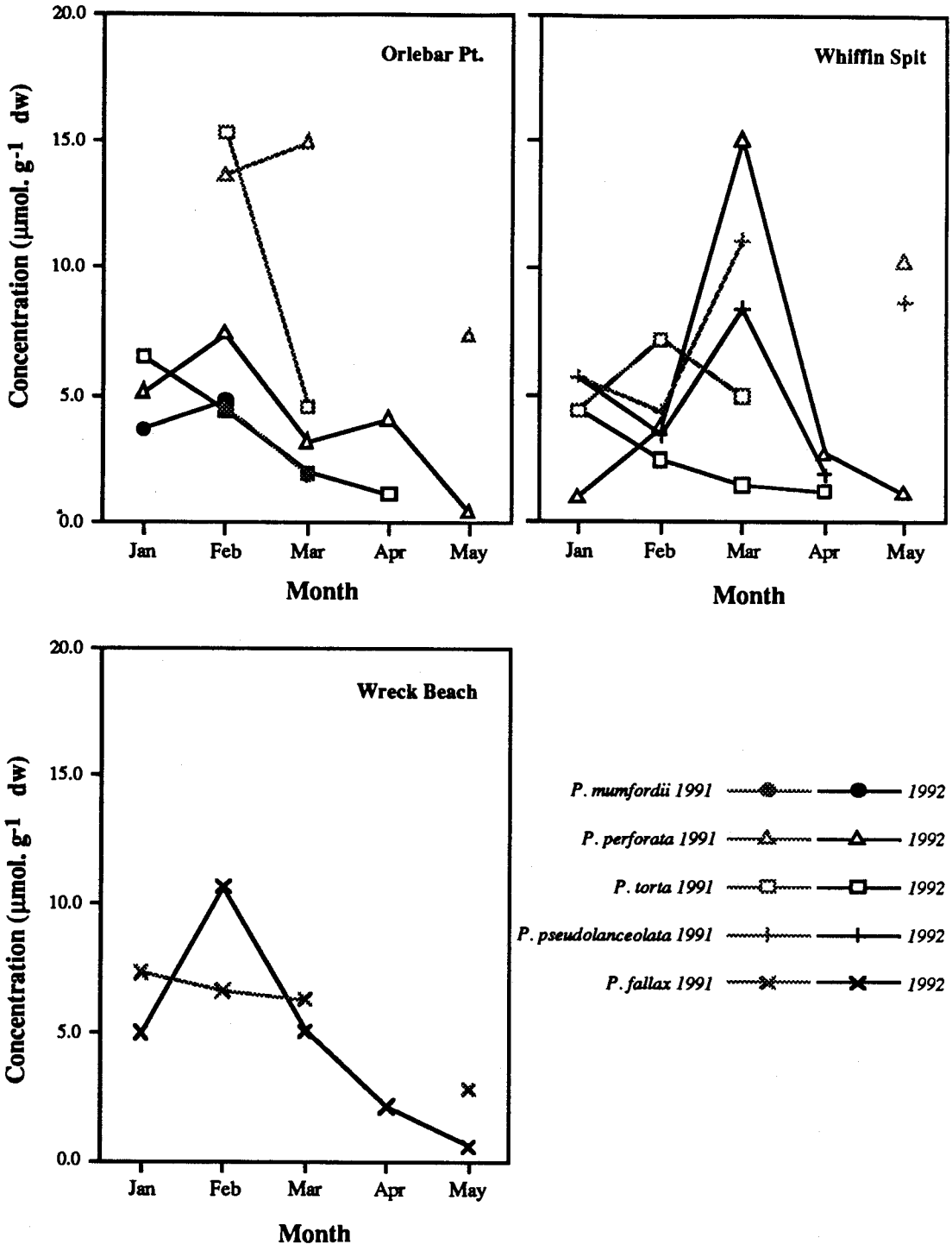


Figure 16. Changes in tissue nitrate of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

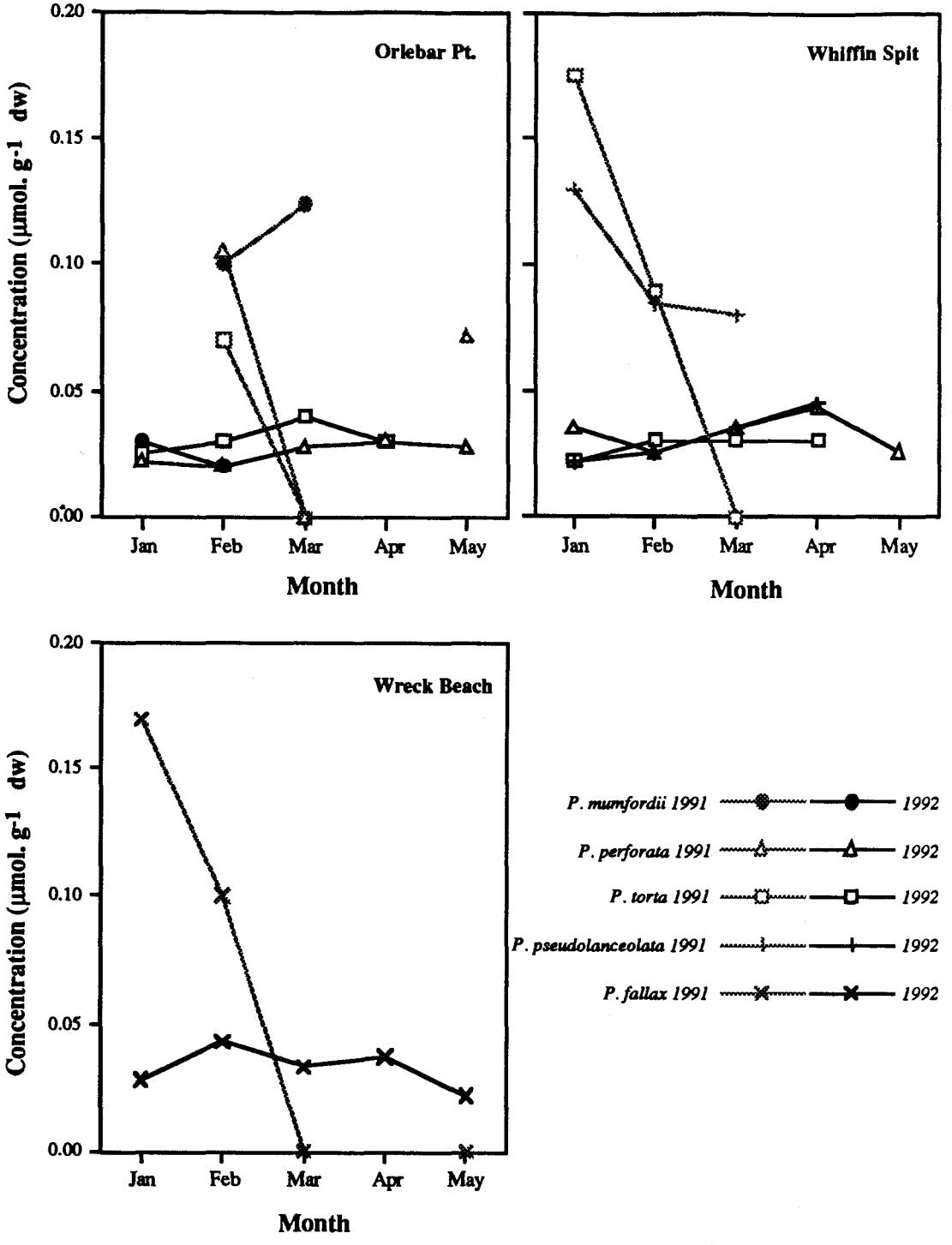


Figure 17. Changes in tissue nitrite of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

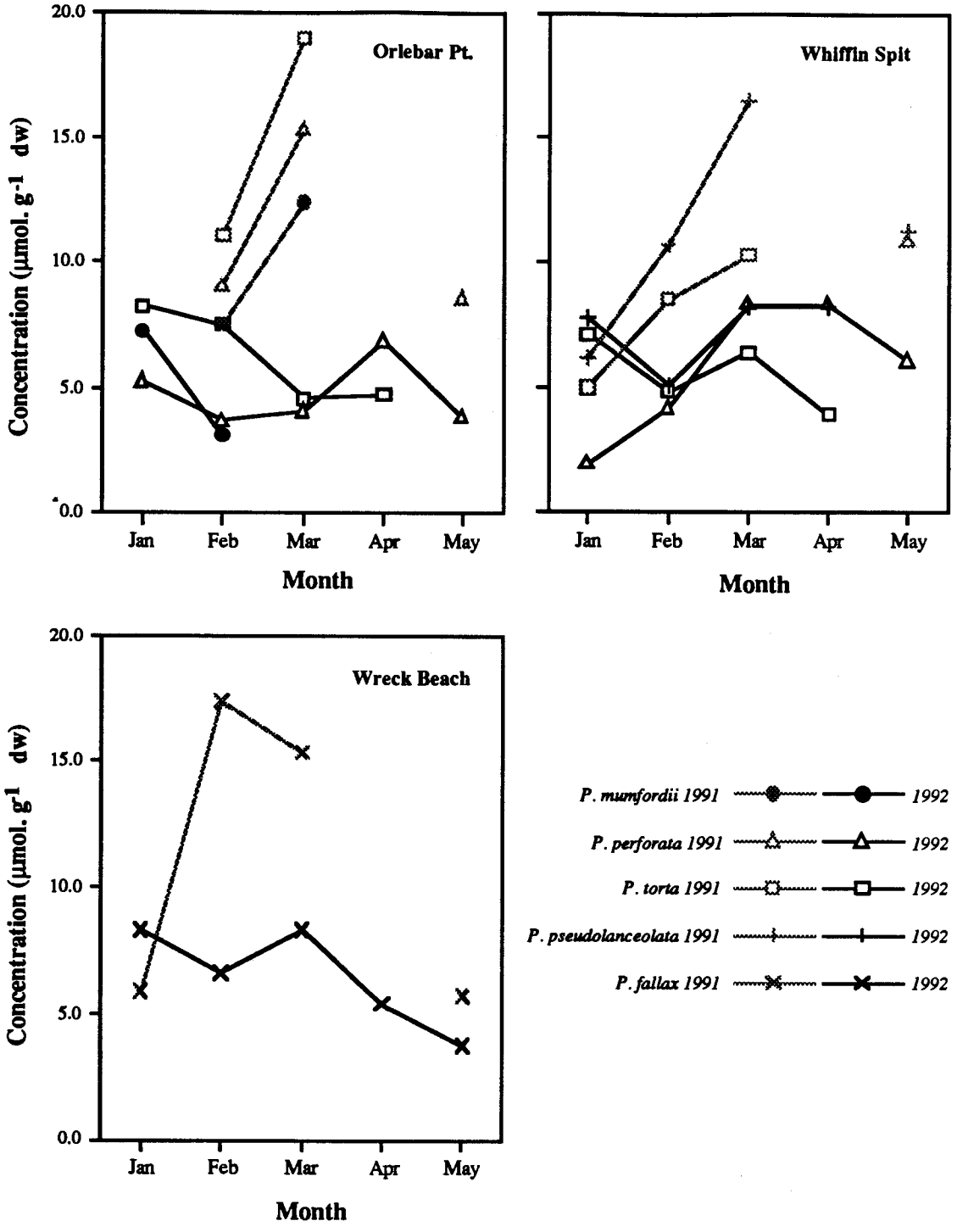


Figure 18. Changes in tissue ammonium of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

however, there did not seem to be any specific pattern of increase or decrease.

Phosphate concentration in the tissue was found to be lower in 1992 than in 1991 (Fig. 19). Figure 19 also shows that tissue phosphate did not follow a clear pattern of seasonal changes.

TOTAL LIPIDS AND FATTY ACIDS

The yearly average concentration of total lipids (some scientists use the term crude lipids) was marked by a considerable seasonal fluctuation in values as shown by the high standard deviations (Fig. 20). *P. torta* showed high values, more than 0.8% dry weight at Orlebar Pt. both in 1991 and 1992, and at Whiffin Spit in 1991. However, at Whiffin Spit in 1992, the total lipids in this species amounted to an average of only 0.4% dry weight. The difference between the two collection years, however, was not significant (Appendix II).

There were no significant differences in total lipid content of *Porphyra* under study, except for *P. torta* at Orlebar Pt., which had a significantly higher value than *P. perforata* at the same site (Fig. 21).

Eicosapentaenoic acid, a polyunsaturated fatty acid, showed relatively high variations in monthly concentrations (as evidenced by the magnitude of the standard deviations in Fig. 22). Only *P. pseudolanceolata* showed a significant difference in eicosapentaenoic acid content between the two collection years

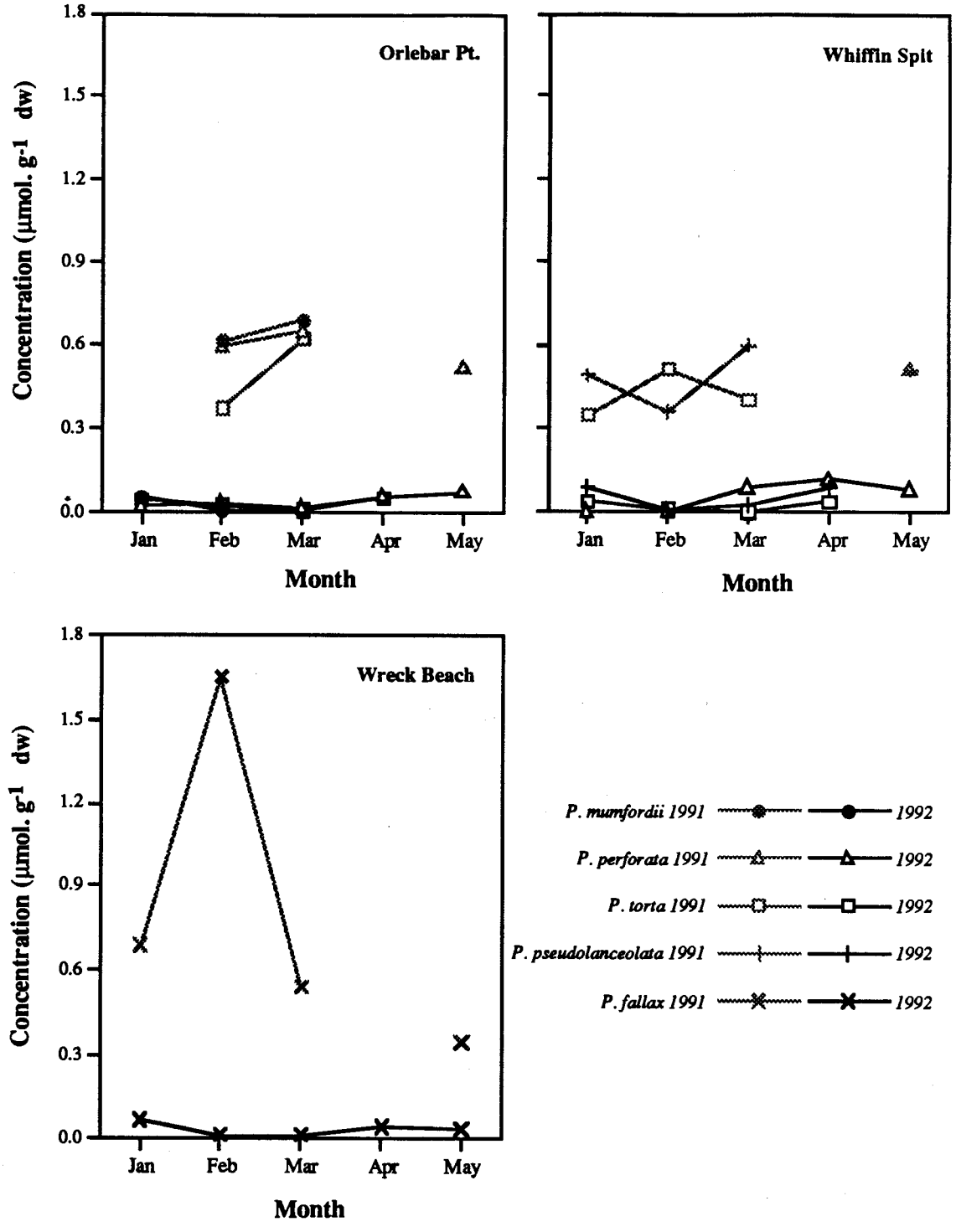


Figure 19. Changes in tissue phosphate of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

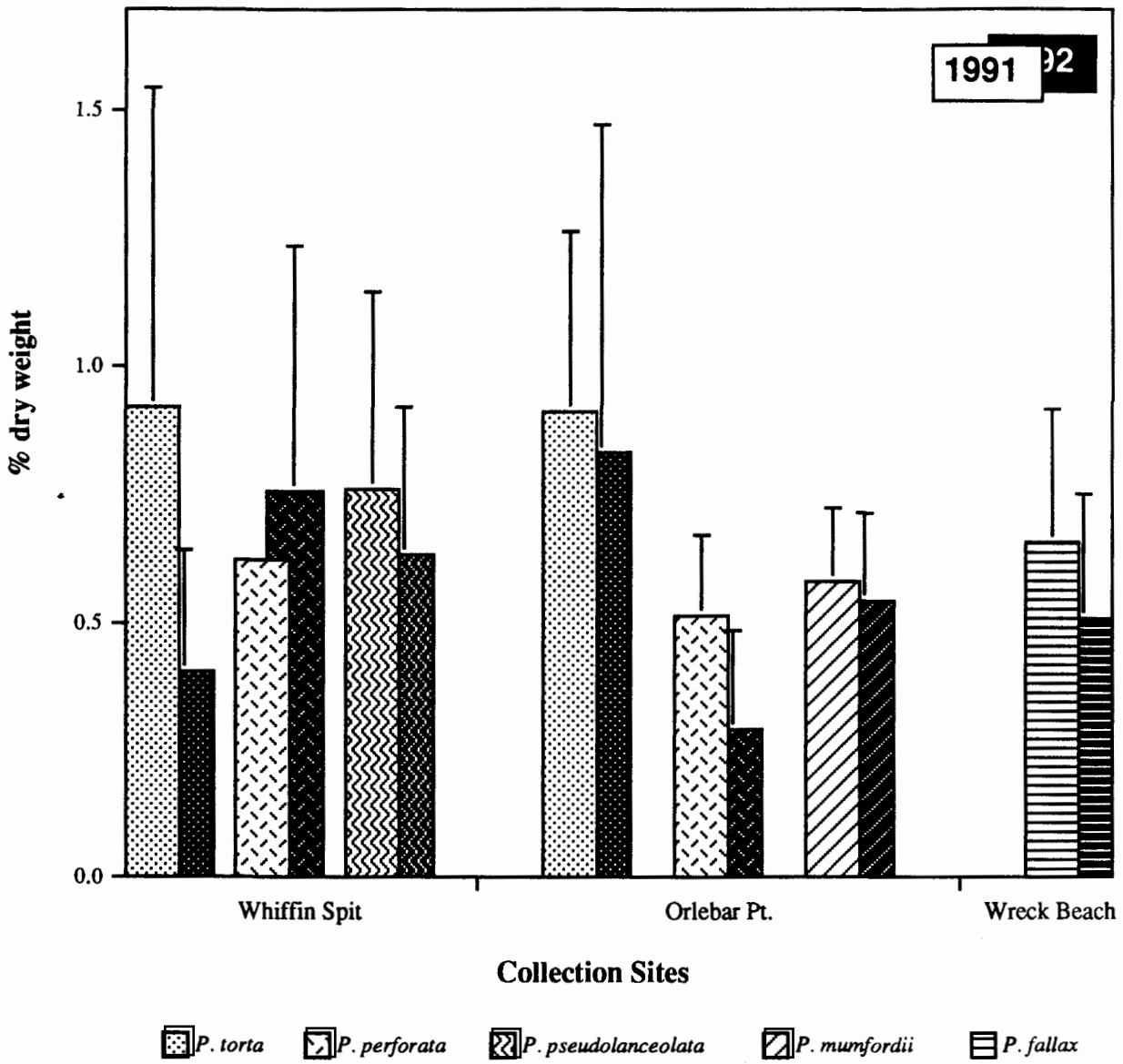


Figure 20. Total lipid content in *Porphyra* spp. at the three sites. The values shown are averages of all collections in one year with their standard deviations (*P. perforata* from Whiffin Spit, 1991 has no standard deviation). Bars with an asterisk (*) indicate significant differences between the two years ($p \leq 0.05$).

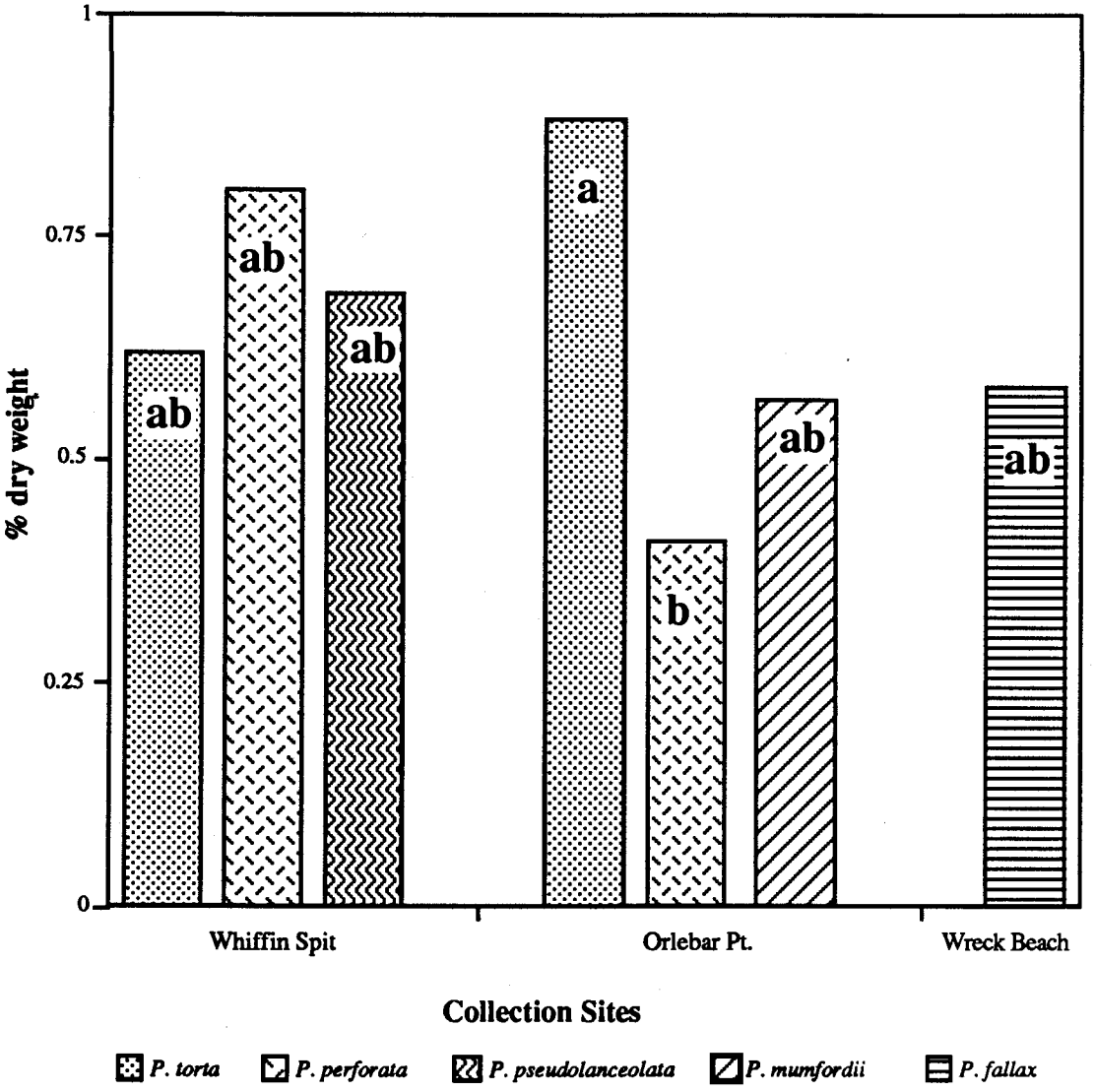


Figure 21. Average total lipid content in *Porphyra* spp. both 1991 and 1992 at the three sites. The bars shown are least square mean values of all collections. Bars with the same letter are not significantly different ($p > 0.05$).

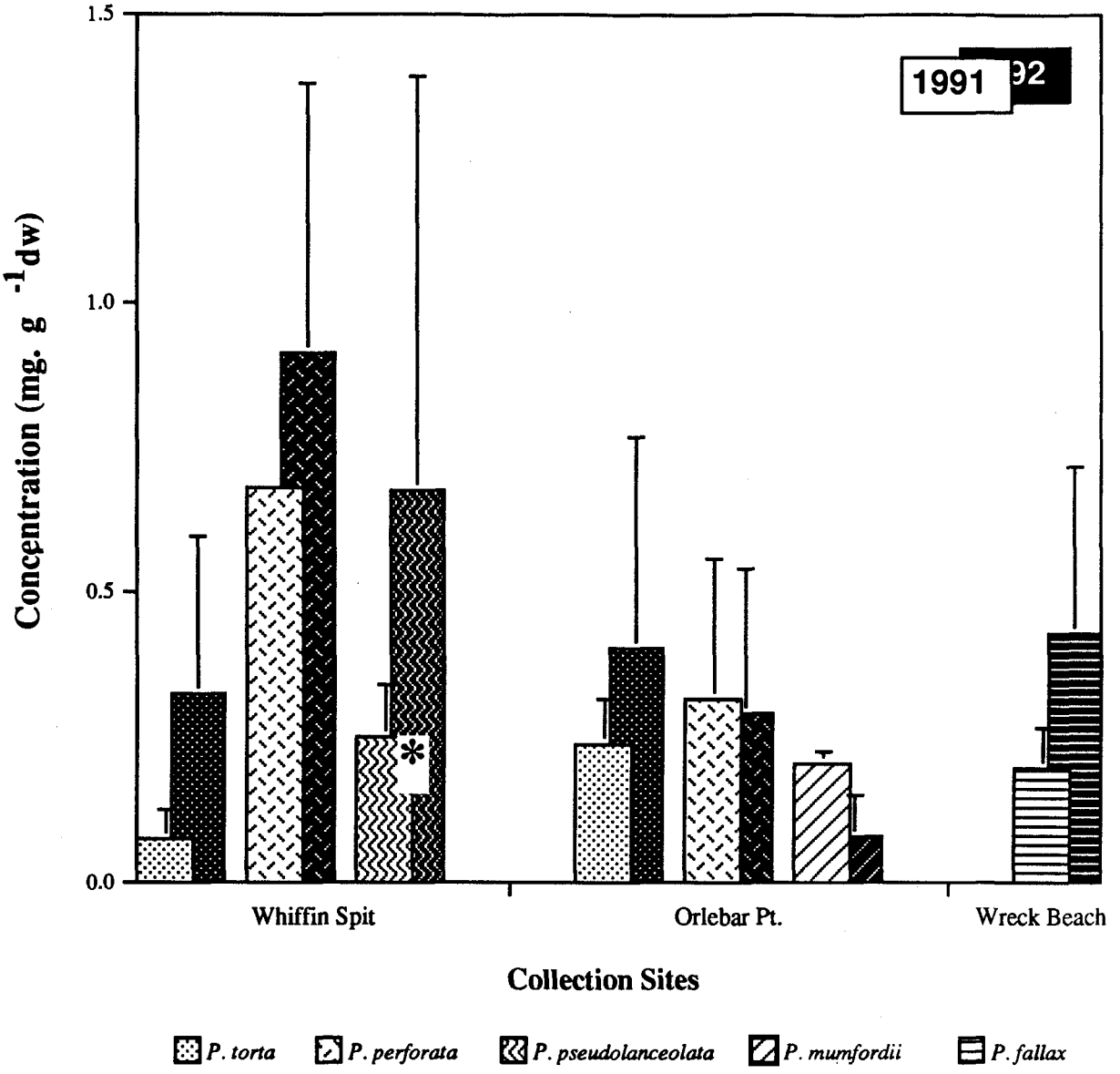


Figure 22. Eicosapentaenoic acid content in *Porphyra* spp. at the three sites. The values shown are averages of all collections in one year with their standard deviations. (*P. perforata* from Whiffin Spit, 1991 has no standard deviation). Bars with an asterisk (*) indicate significant differences between the two years ($p \leq 0.05$).

(Fig. 22). Among species, *P. perforata* at Whiffin Spit showed a significantly higher concentration of this fatty acid than any of the other species (Fig. 23).

The average concentrations of palmitic acid ranged from 0.43 mg. g^{-1} (dw) for *P. torta* at Whiffin Spit in 1992 to 0.92 mg. g^{-1} (dw) for *P. mumfordii* in 1991 (Fig. 24). The 1991 samples of *P. pseudolanceolata*, and *P. perforata* and *P. mumfordii* at Orlebar Pt. showed a lower content of palmitic acid than the 1992 samples (Fig. 24). Palmitic acid content showed some variations among the species. *P. torta* at Whiffin Spit showed a significantly lower palmitic acid content than all other species (Fig. 25).

Considering the pattern of seasonal changes, total lipids showed irregular fluctuations and varied among species (Fig. 26). Some species showed an increase, followed by a decrease (e. g. *P. perforata* at Whiffin Spit and *P. fallax*); others showed a sharp decrease preceding the previous pattern (e. g., *P. torta* and *P. pseudolanceolata* at Whiffin Spit, and *P. torta* and *P. perforata* at Orlebar Pt.). The monthly changes in eicosapentaenoic acid and palmitic acid contents showed similar patterns (Figs. 27 and 28). As shown in appendix II, Eicosapentaenoic acid showed a gradual increase from January to March, which was statistically significant, and then a significant decrease afterward. Similarly, palmitic acid showed a significantly higher values in February and March than the other months.

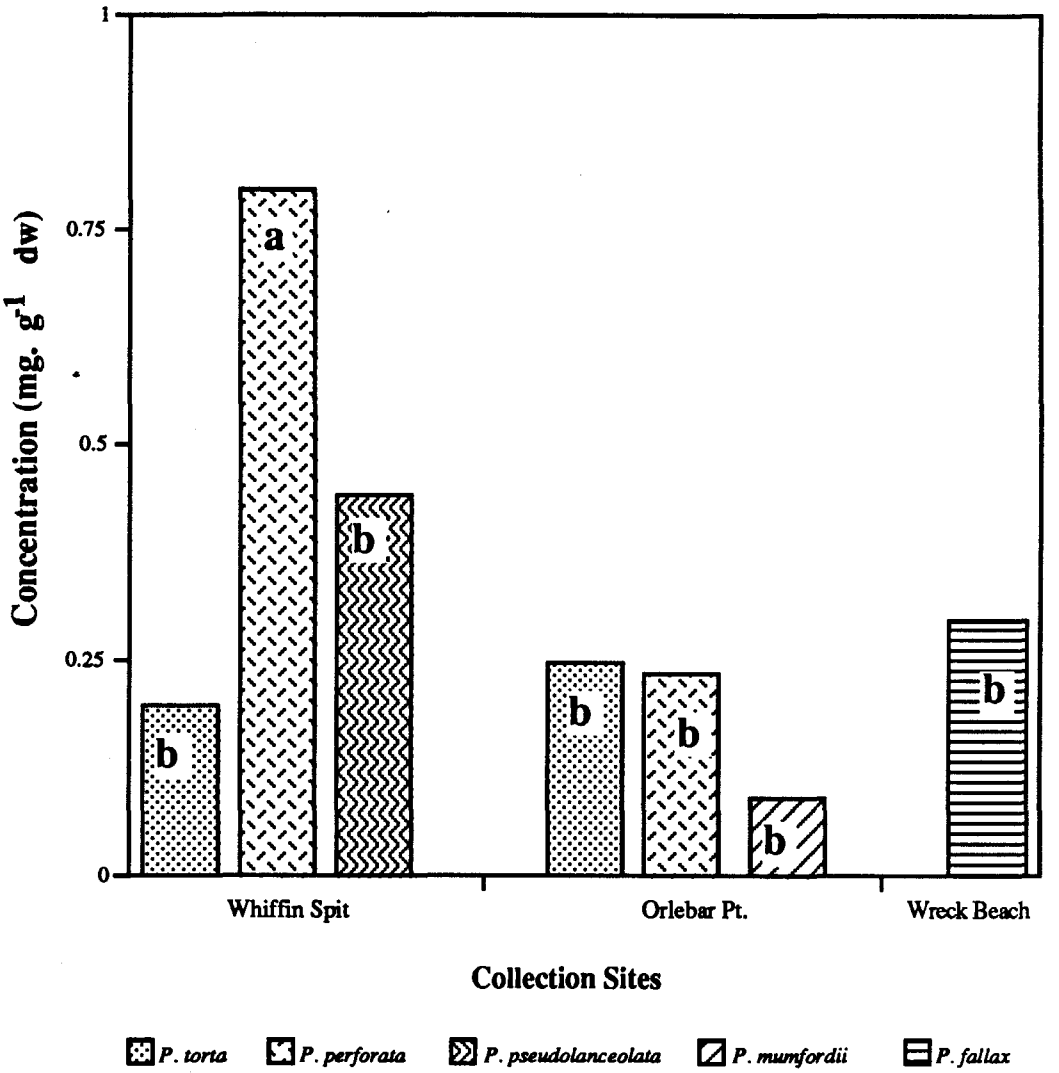


Figure 23. Average eicosapentaenoic acid content in *Porphyra* spp. both 1991 and 1992 at the three sites. The bars shown are least square mean values of all collections. Bars with the same letter are not significantly different ($p > 0.05$).

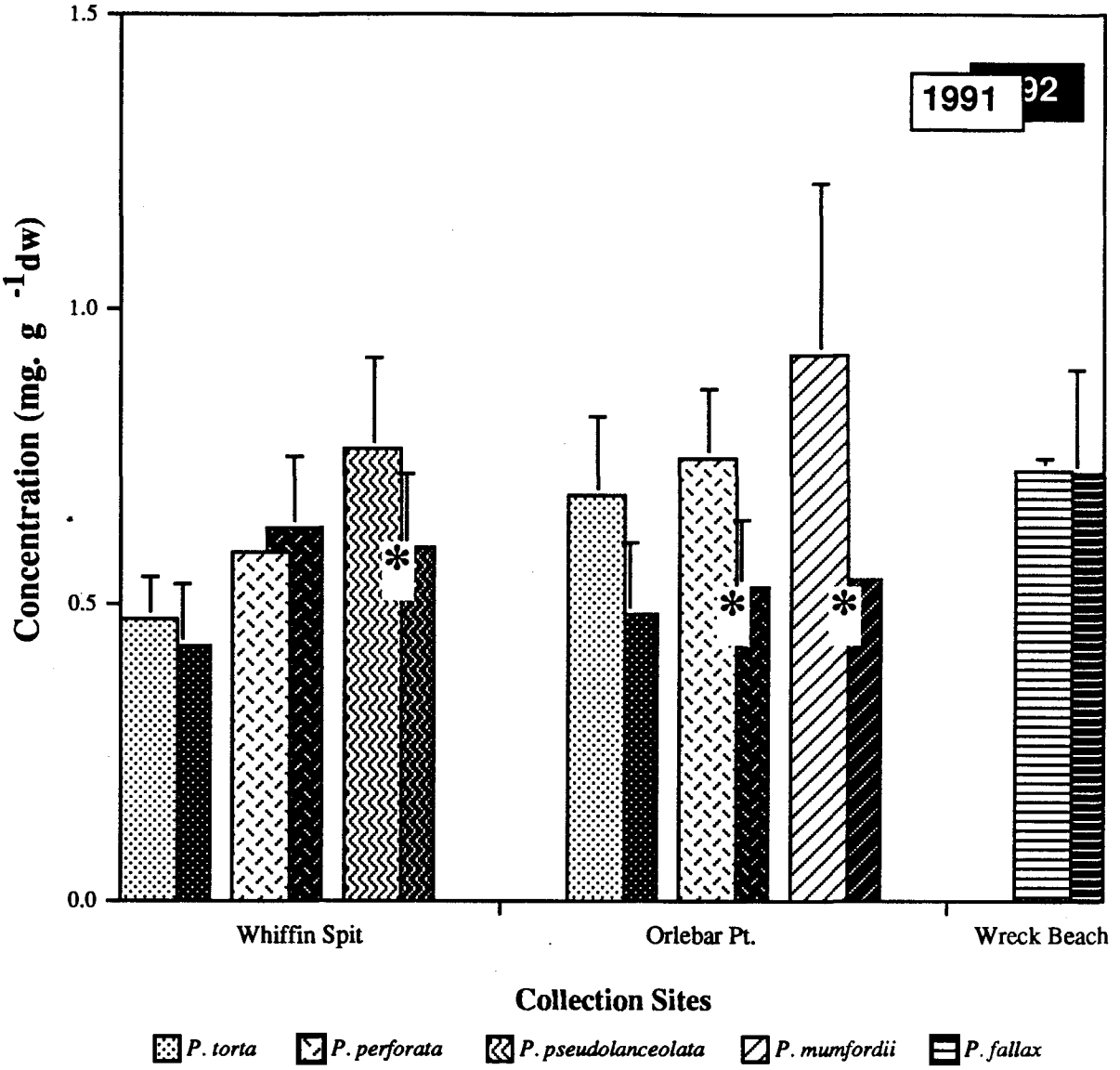


Figure 24. Palmitic acid content in *Porphyra* spp. at the three sites. The values shown are averages of all collections in one year with their standard deviations (*P. perforata* from Whiffin Spit, 1991 has no standard deviation). Bars with an asterisk (*) indicate significant differences between the two years ($p \leq 0.05$).

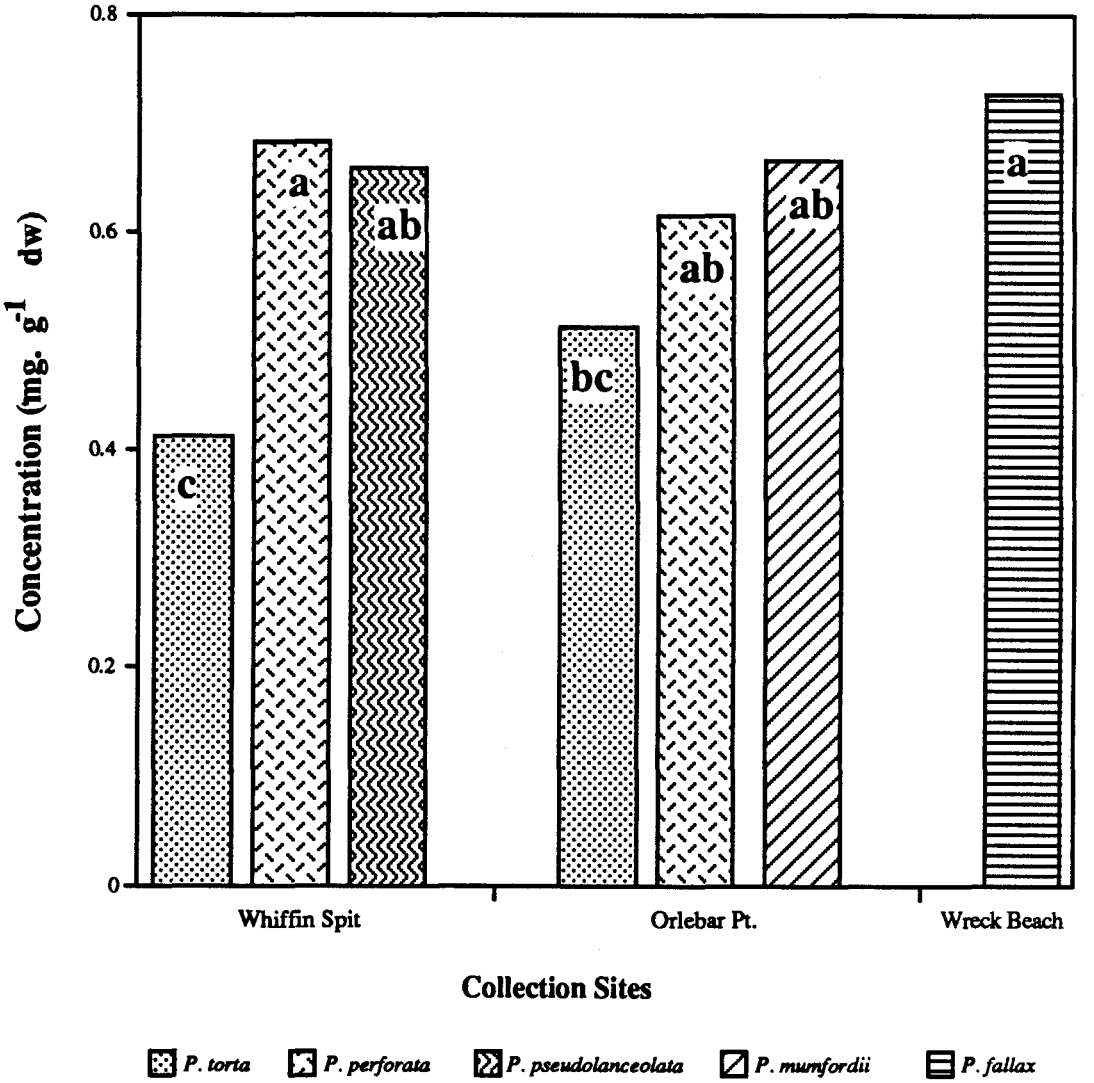


Figure 25. Average palmitic acid content in *Porphyra* spp. both 1991 and 1992 at the three sites. The bars shown are least square mean values of all collections. Bars with the same letter are not significantly different ($p > 0.05$).

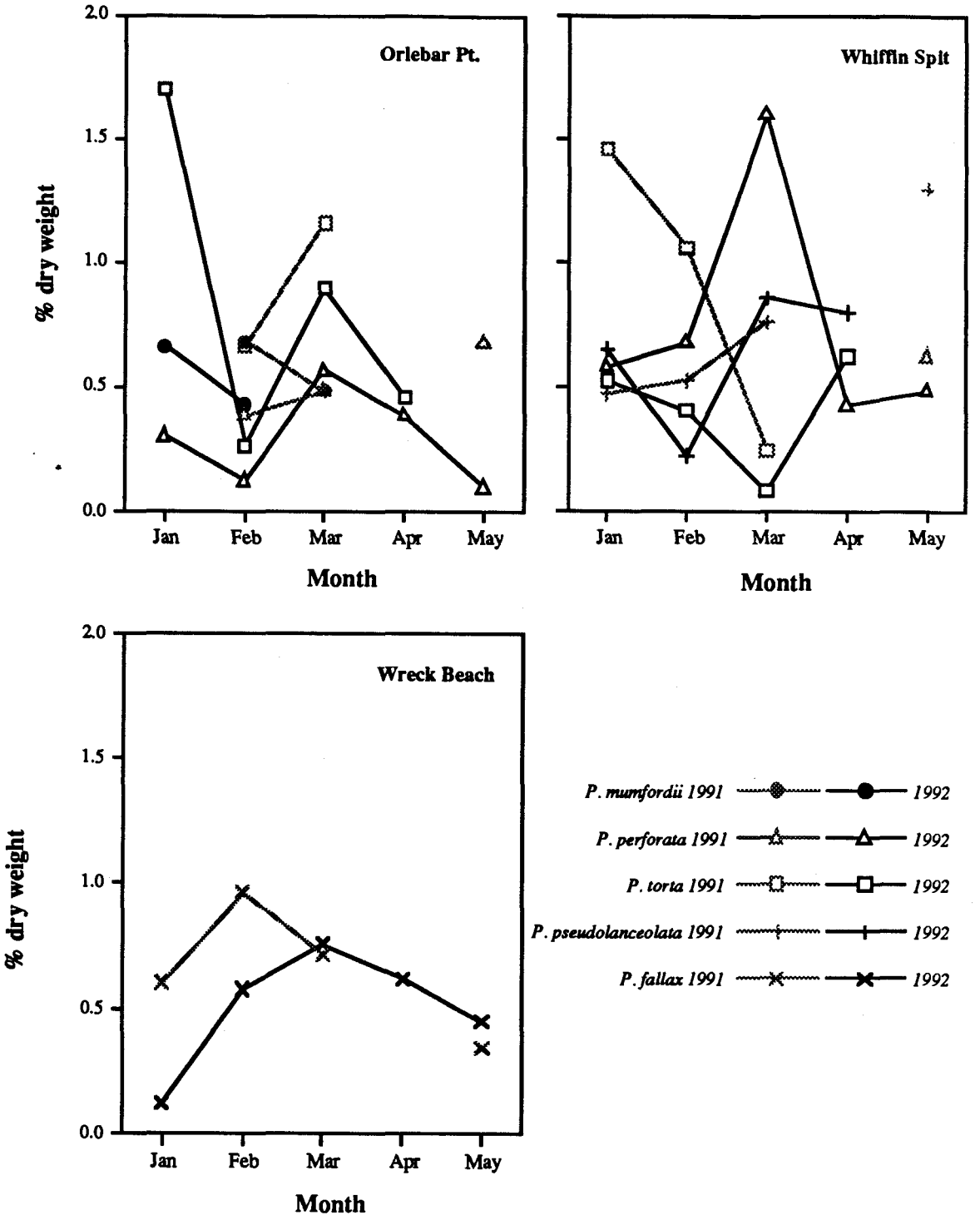


Figure 26. Changes in total lipids of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

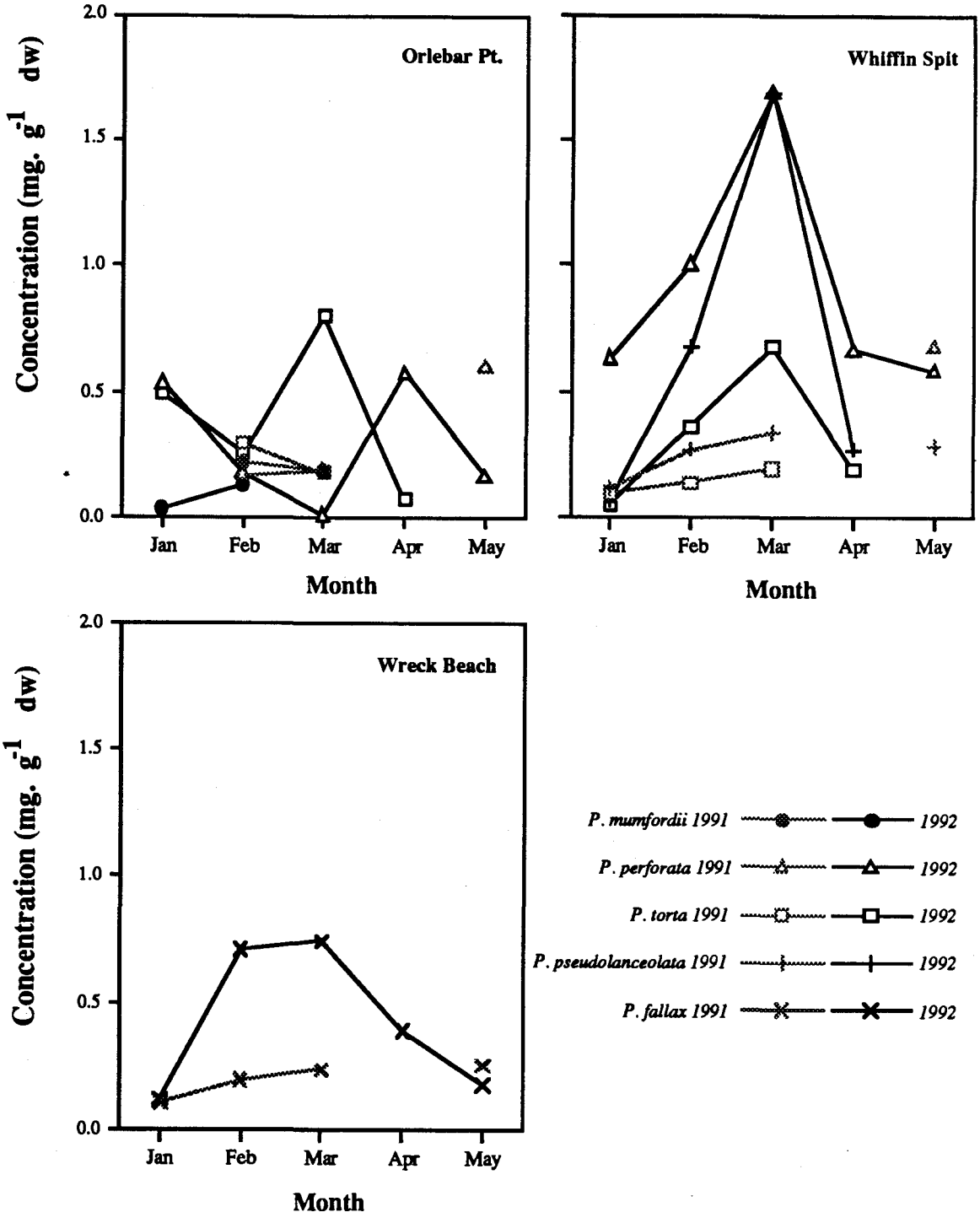


Figure 27. Changes in eicosapentaenoic acid of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

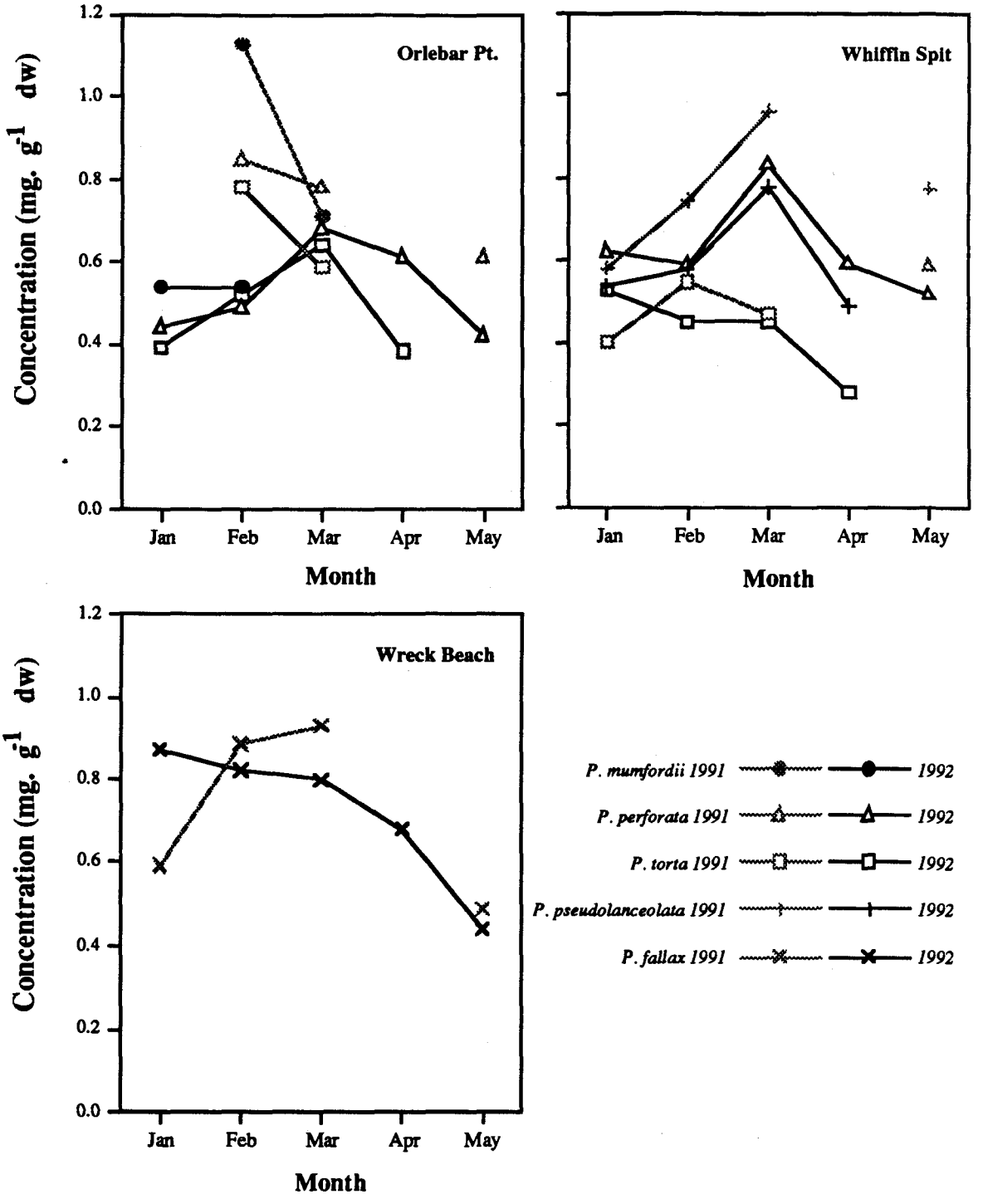


Figure 28. Changes in palmitic acid content of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

AMINO ACIDS

The yearly average of total amino acid content of *P perforata* at Whiffin Spit , *P. mumfordii*, and *P. fallax* showed significantly higher concentrations in 1992 than 1991 (Fig. 29). Among the species, total amino acid content of *P. perforata* at Whiffin Spit was significantly higher than that of the same species at Orlebar Pt. and of *P. fallax* (Fig. 30).

The free amino acids in *Porphyra* accounted for about 5% to 10% of the total amino acids. Their average values ranged from 0.5% dw (*P. perforata*, Orlebar Pt., 1991) to 4.1% dw (*P. fallax*, Wreck Beach, 1992, Fig. 31). It was also noted that the free amino acid content of the 1992 samples of *P. pseudolanceolata* and *P. fallax* were higher than those for 1991. Among species, only *P. perforata* at Orlebar Pt. showed significantly lower free amino acid content than that of *P. fallax* (Fig. 32).

Seasonal variations in total amino acids showed a gradual decrease from January to May or an increase followed by a decrease (Fig. 33). The only exceptions to these patterns were the 1991 collections of *P. perforata* at Orlebar Pt. and *P. pseudolanceolata* at Whiffin Spit, which showed high values in May. Statistically, however, the monthly fluctuations were not significant (Appendix II).

The seasonal changes in free amino acids showed a pattern similar to that of total amino acids, either a gradual decrease or

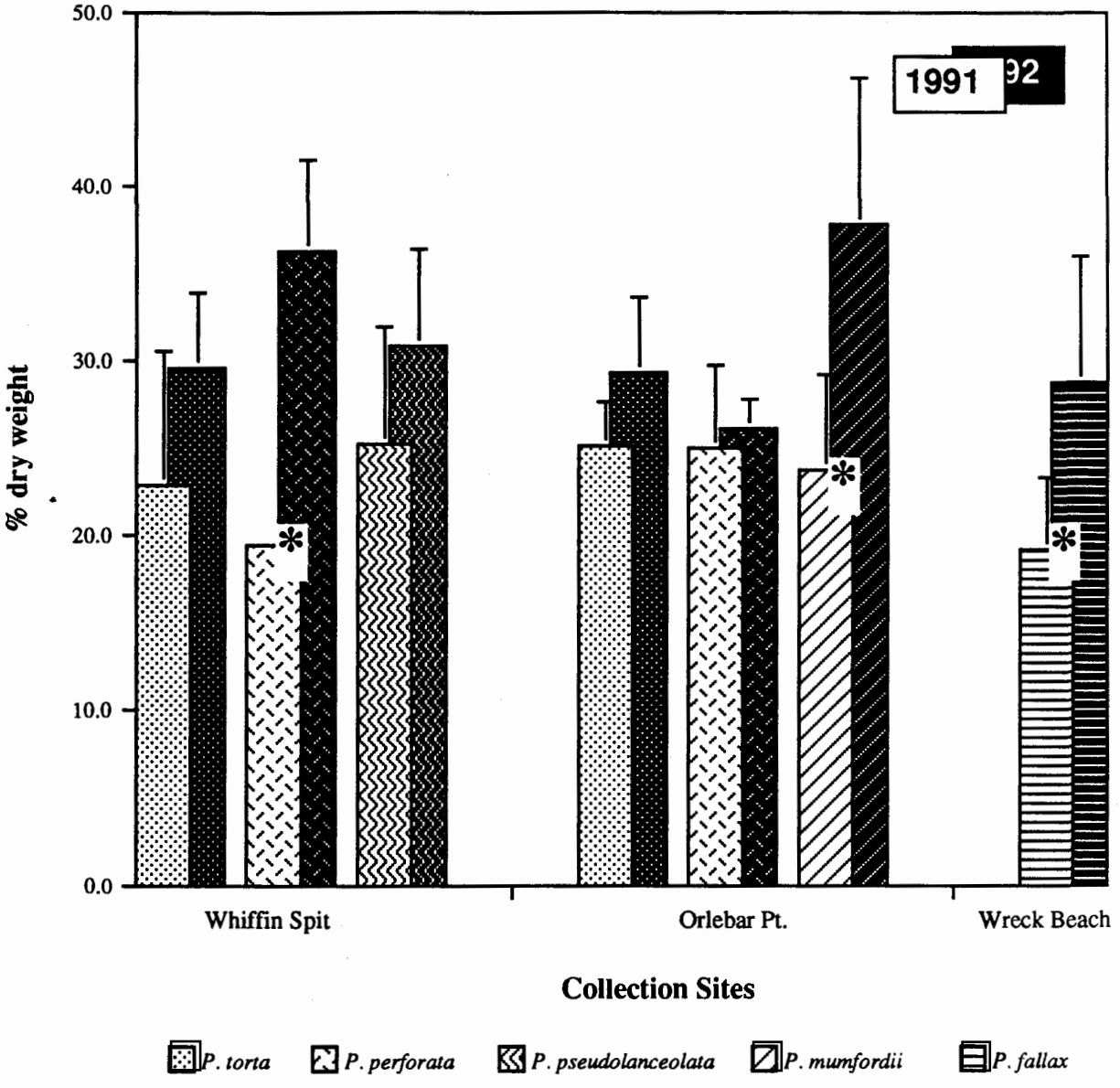


Figure 29. Total amino acid content in *Porphyra* spp. at the three sites. The values shown are averages of all collections in one year with their standard deviations (*P. perforata* from Whiffin Spit, 1991 has no standard deviation). Bars with an asterisk (*) indicate significant differences between the two years ($p \leq 0.05$).

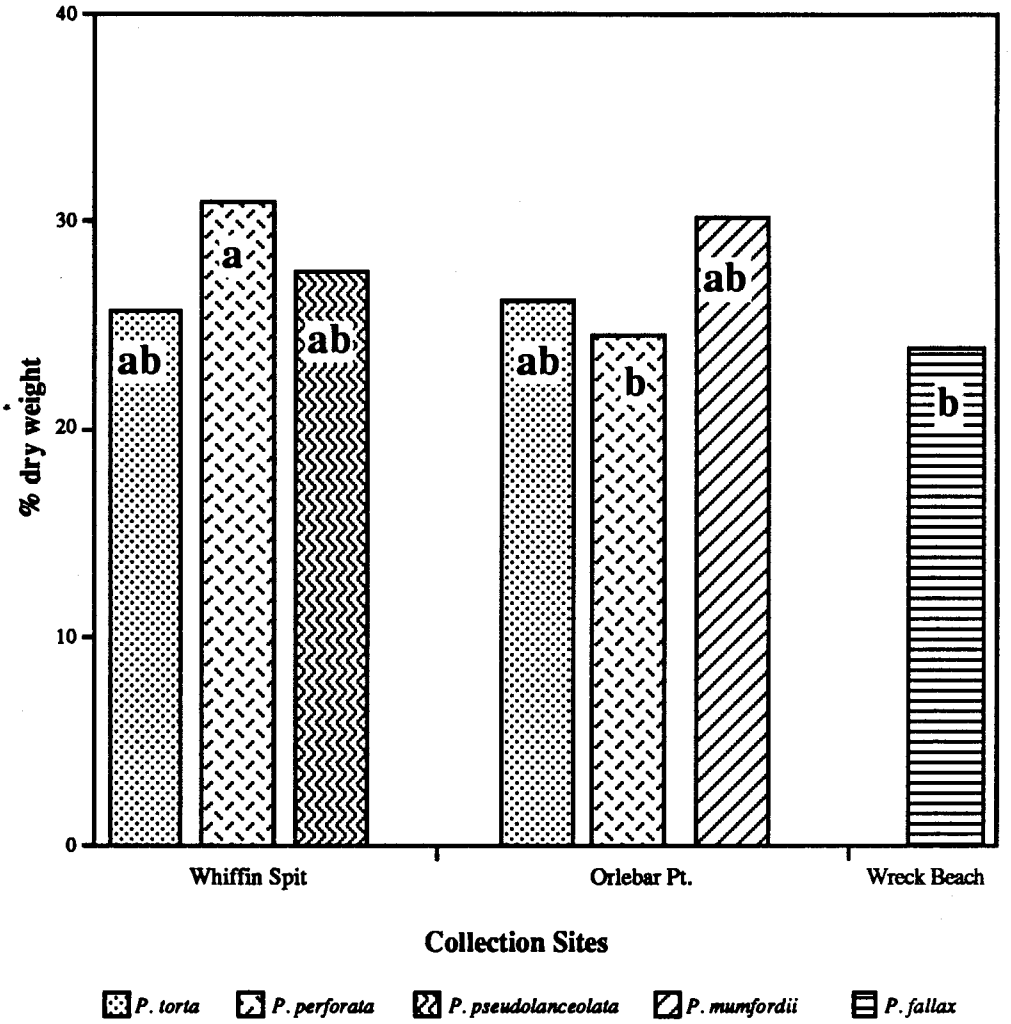


Figure 30. Average total amino acid content in *Porphyra* spp. both 1991 and 1992 at the three sites. The bars shown are least square mean values of all collections. Bars with the same letter are not significanty different ($p > 0.05$).

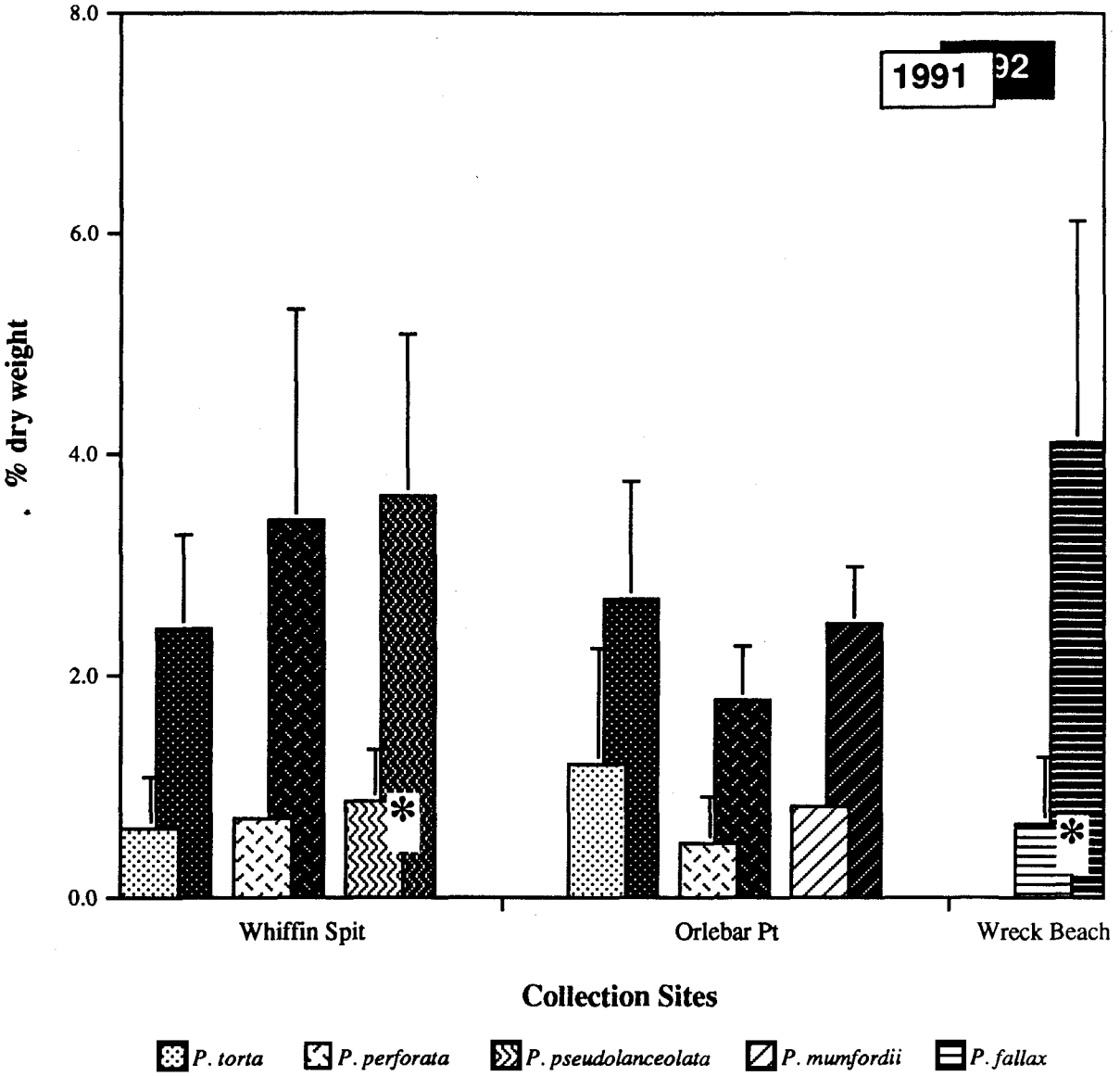


Figure 31. Free amino acid content in *Porphyra* spp. at the three sites. The values shown are averages of all collections in one year with their standard deviations (*P. perforata* from Whiffin Spit, 1991 has no standard deviation). Bars with an asterisk (*) indicate significant differences between the two years ($p \leq 0.05$).

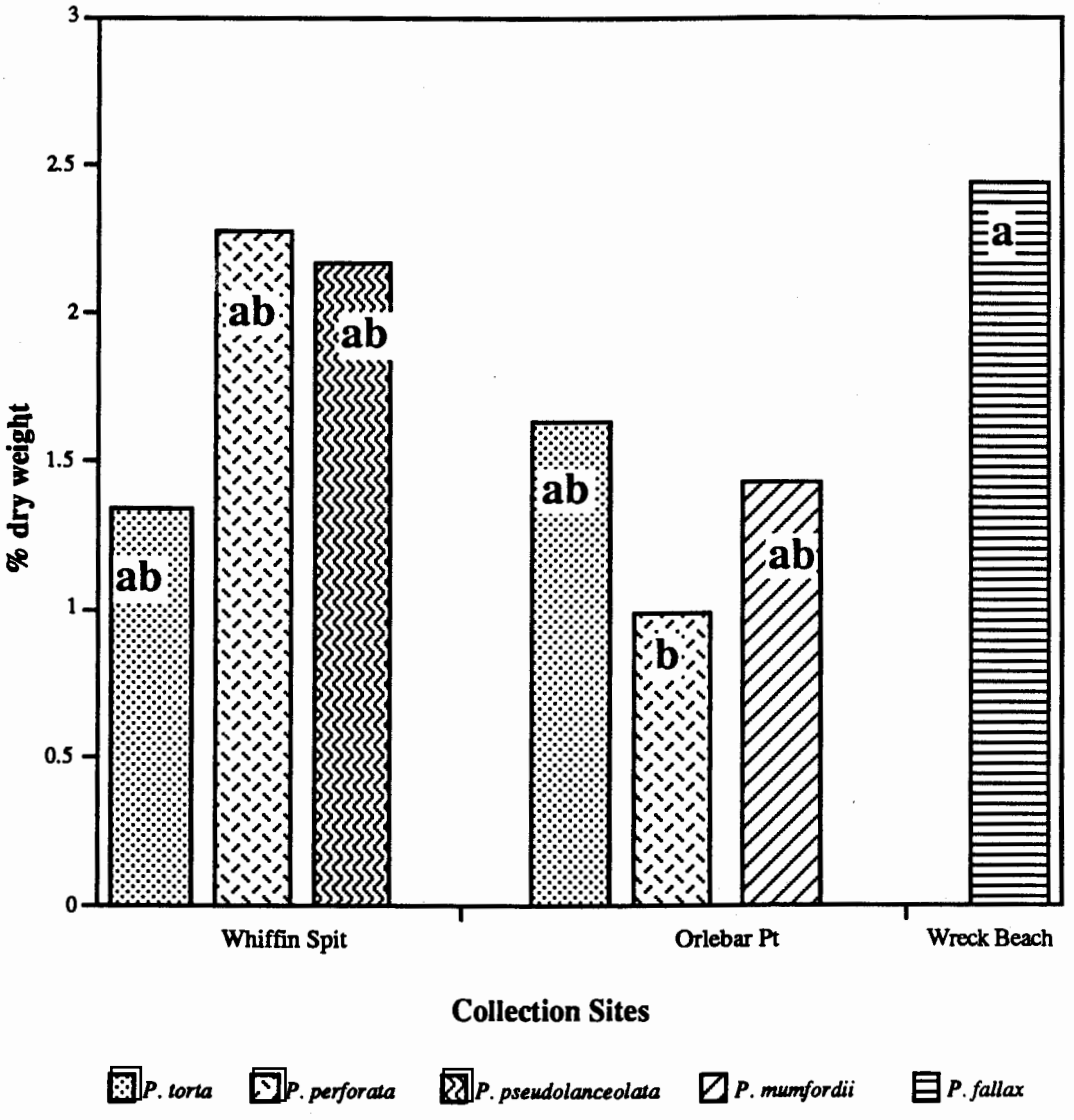


Figure 32. Average free amino acid content in *Porphyra* spp. both 1991 and 1992 at the three sites. The bars shown are least square mean values of all collections. Bars with the same letter are not significantly different ($p > 0.05$).

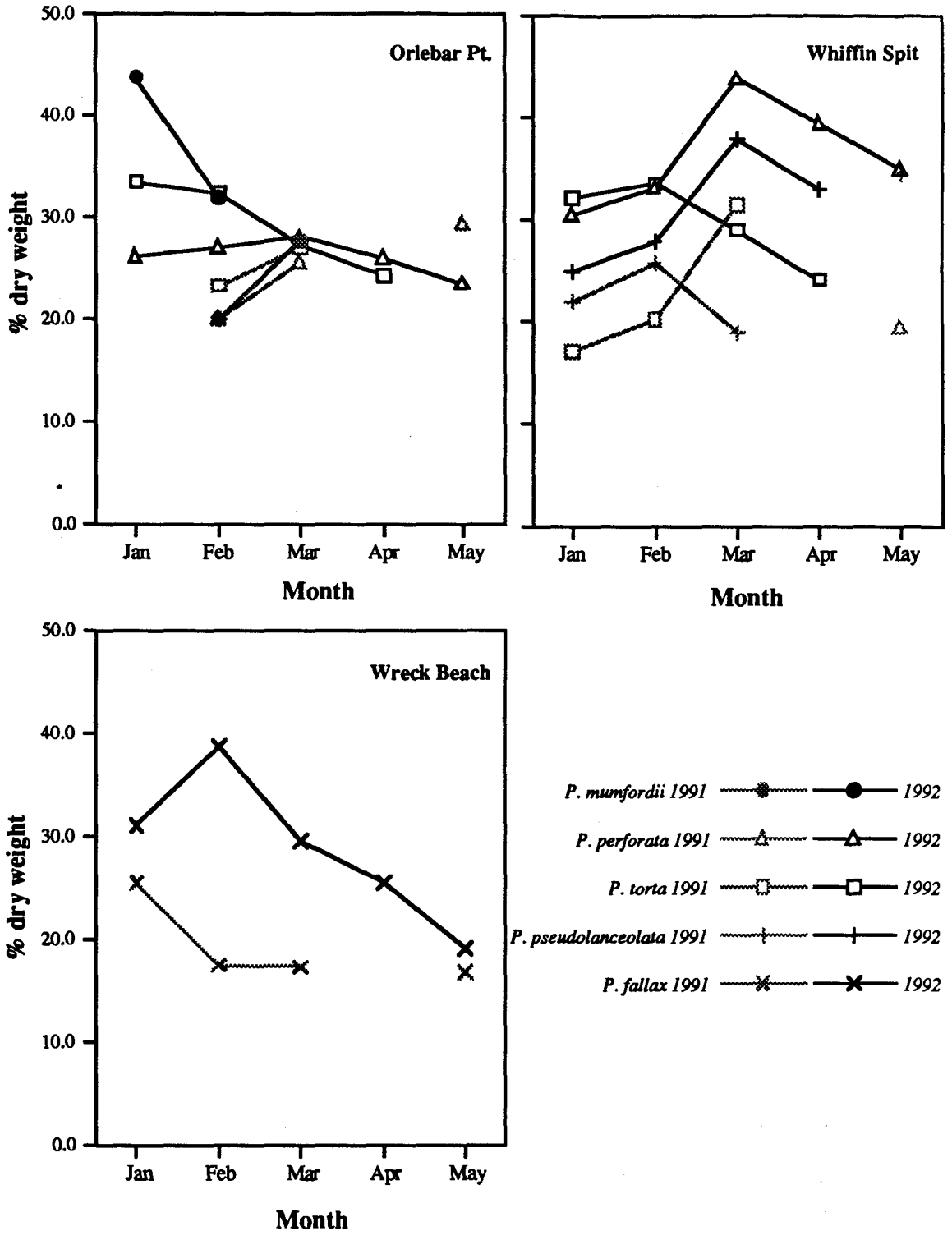


Figure 33. Changes in total amino acids of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

a slight increase followed by a gradual decrease and statistically were not significant (Fig. 34, Appendix II).

The total amino acid composition of *Porphyra* spp. was dominated by methionine, alanine, and tyrosine in 1991 samples; however, in 1992 samples, alanine, arginine, and glutamic acid were the dominant amino acids (Figs. 35 and 36). Slightly different from the composition of total amino acids, the free amino acid composition in both years was dominated by alanine, threonine, glutamic acid (Figs. 37 and 38).

PORPHYRAN

The average porphyran concentration ranged from 15.3% (*P. pseudolanceolata*, 1991) to 45.8%, dw (*P. torta*, Orlebar Pt., 1992, Fig. 39). The 1992 samples of *P. pseudolanceolata*, and of *P. torta and P. mumfordii* at Orlebar Pt. had significantly higher porphyran contents than those of 1991 (Fig. 39). *P. perforata* and *P. torta* at Orlebar Pt. appeared to have a higher content of porphyran than the same species at Whiffin Spit, relative to other samples (Fig. 40), but they were not significantly different.

Porphyran content did not show any clear pattern of seasonal change (Figs. 41). In general, the porphyran content showed irregular fluctuations, decreasing and increasing with no defined pattern. The only significant increase in porphyran content was observed from January to February (Appendix II).

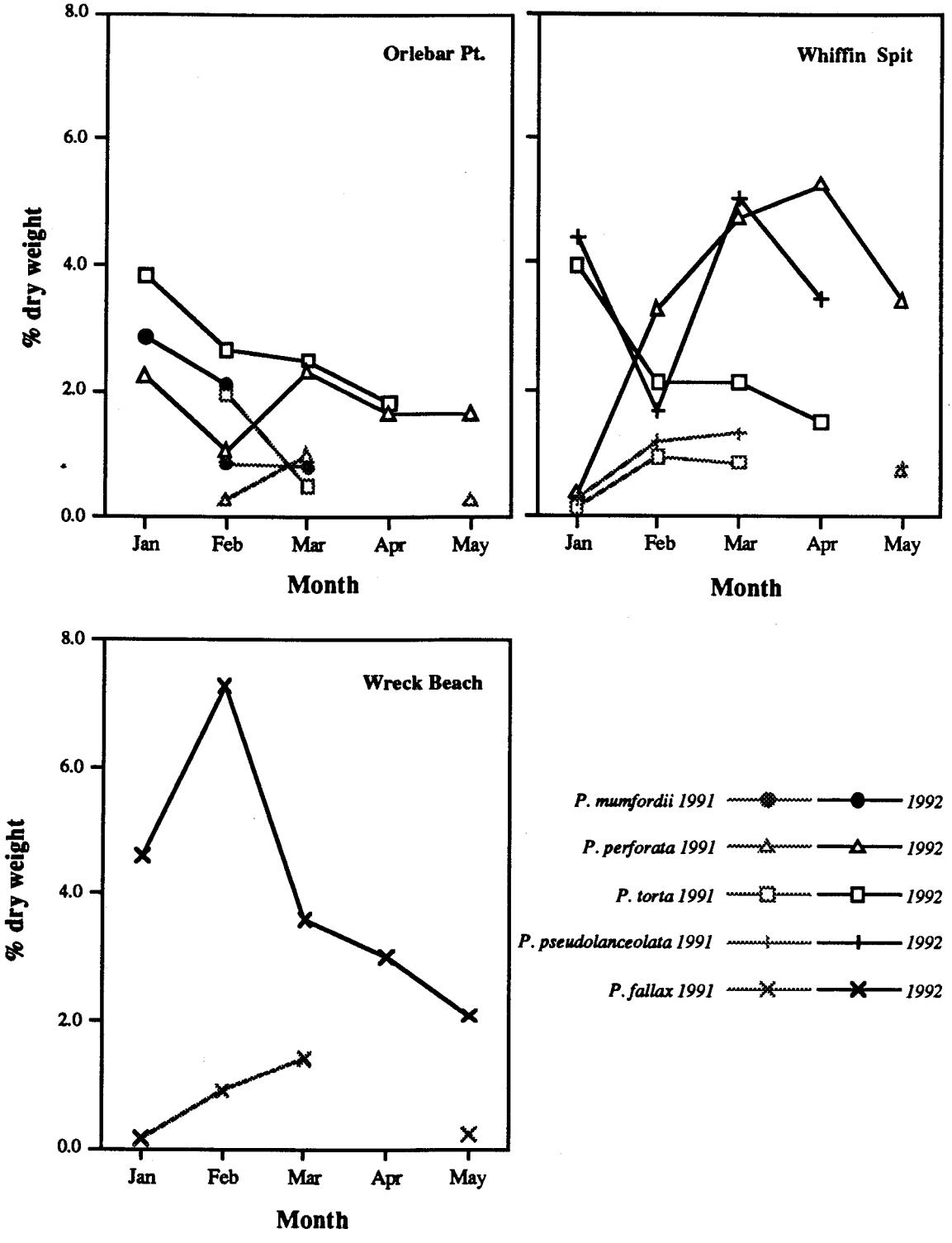


Figure 34. Changes in free amino acids of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

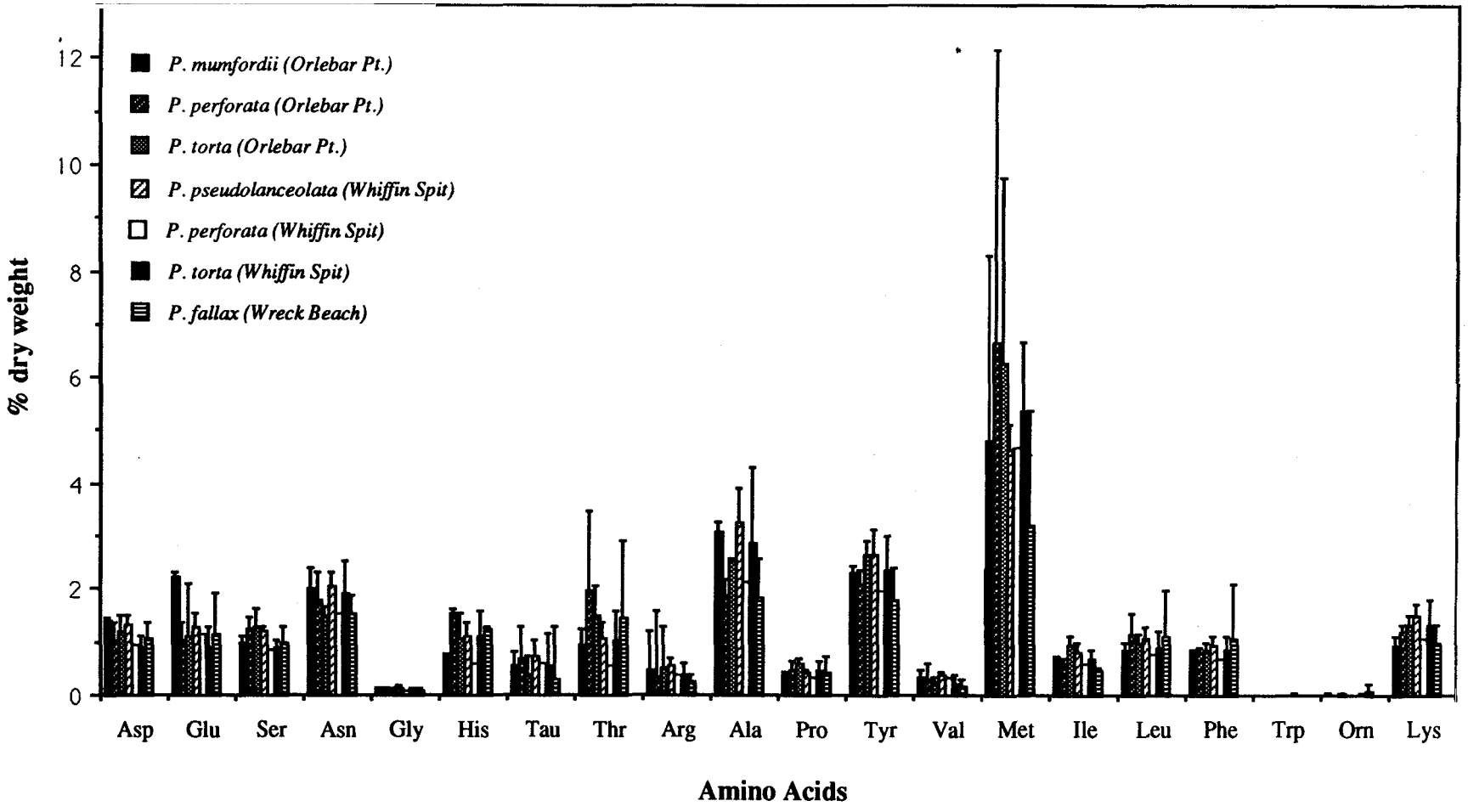


Figure 35. Total amino acids composition of *Porphyra* spp. collected 1991. The values shown are an average of all collections in one year with their standard deviations.

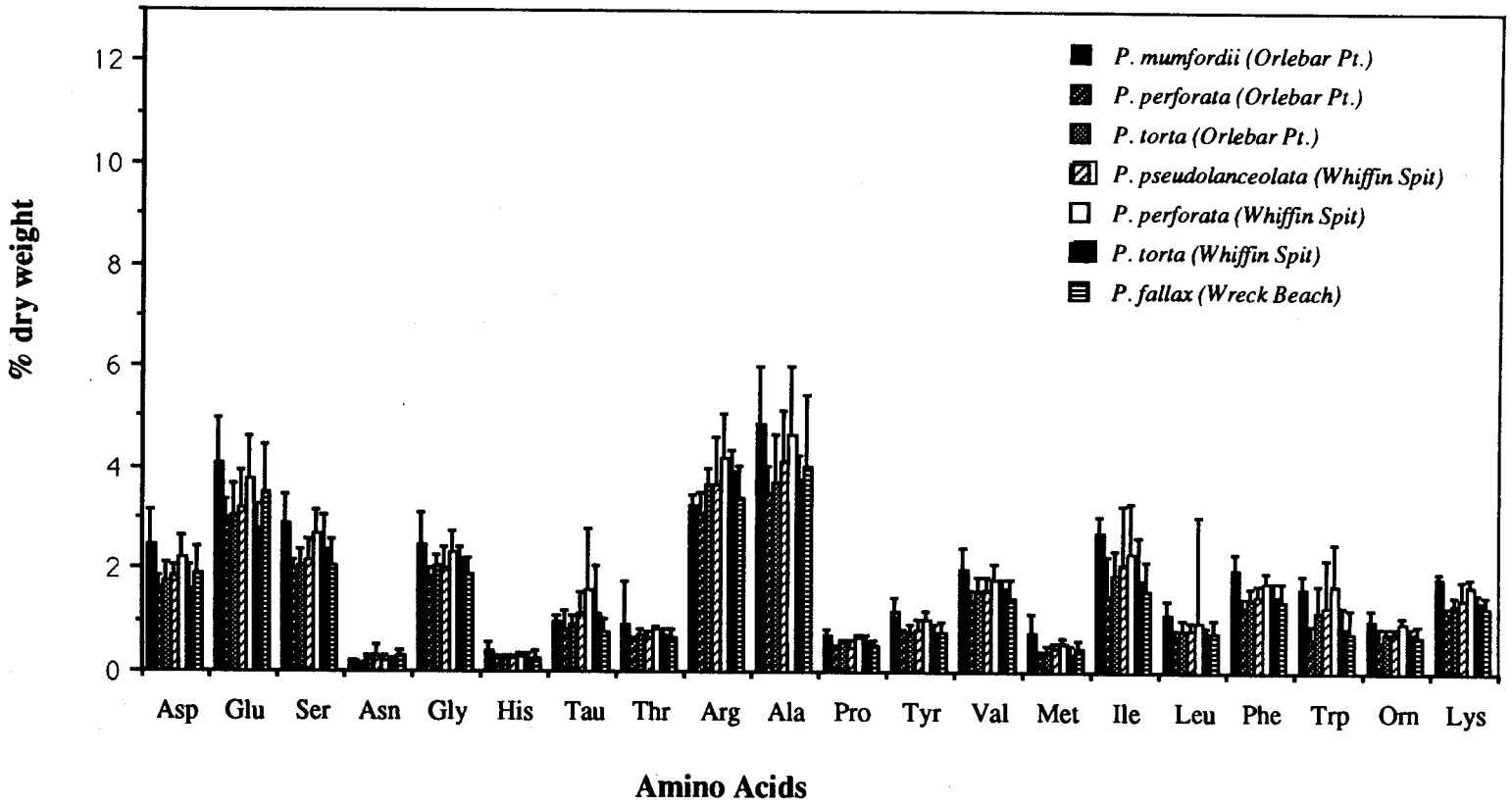


Figure 36. Total amino acids composition of *Porphyra* spp. collected 1992. The values shown are an average of all collections in one year with their standard deviations.

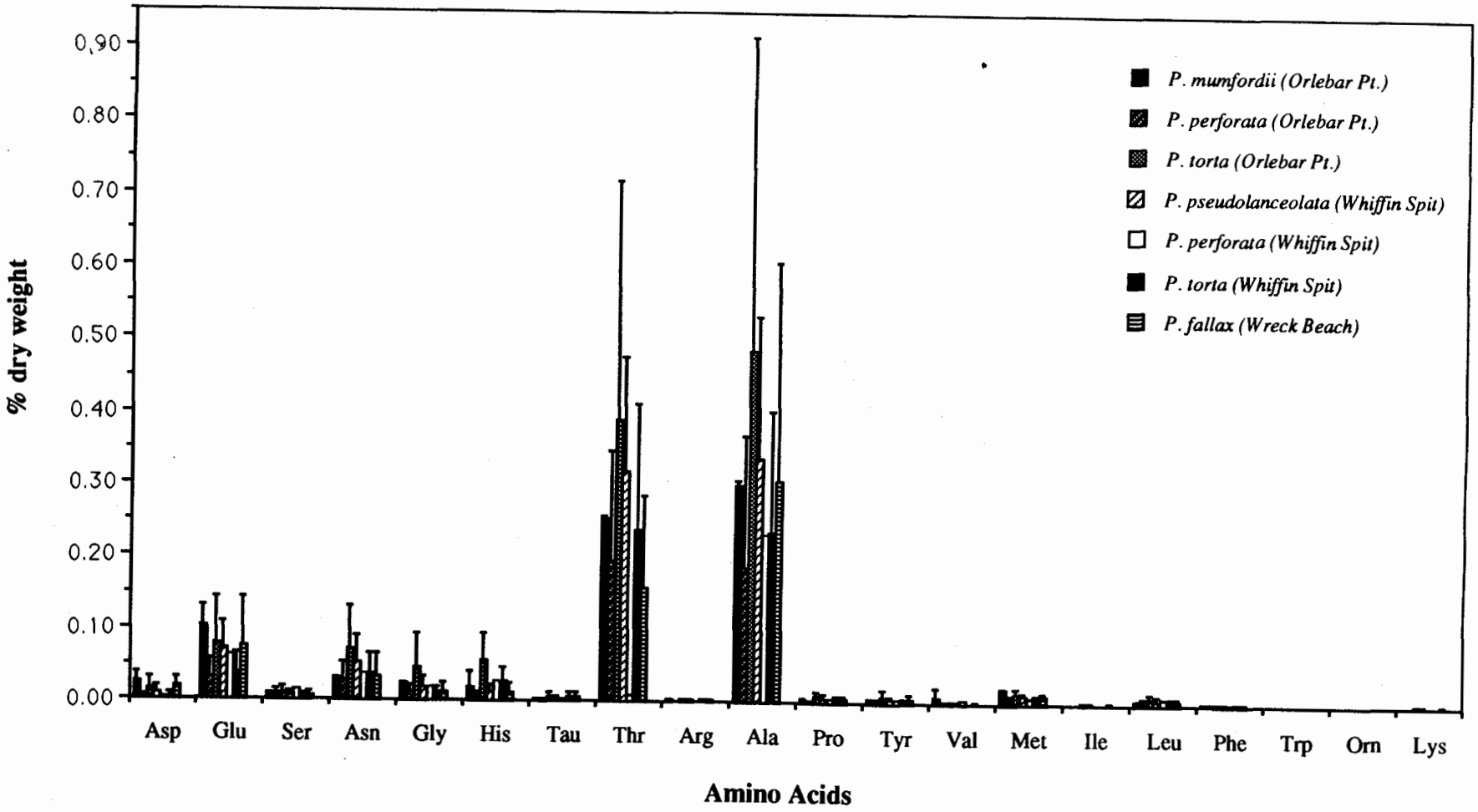


Figure 37. Free amino acids composition of *Porphyra* spp. collected 1991. The values shown are an average of all collections in one year with their standard deviations.

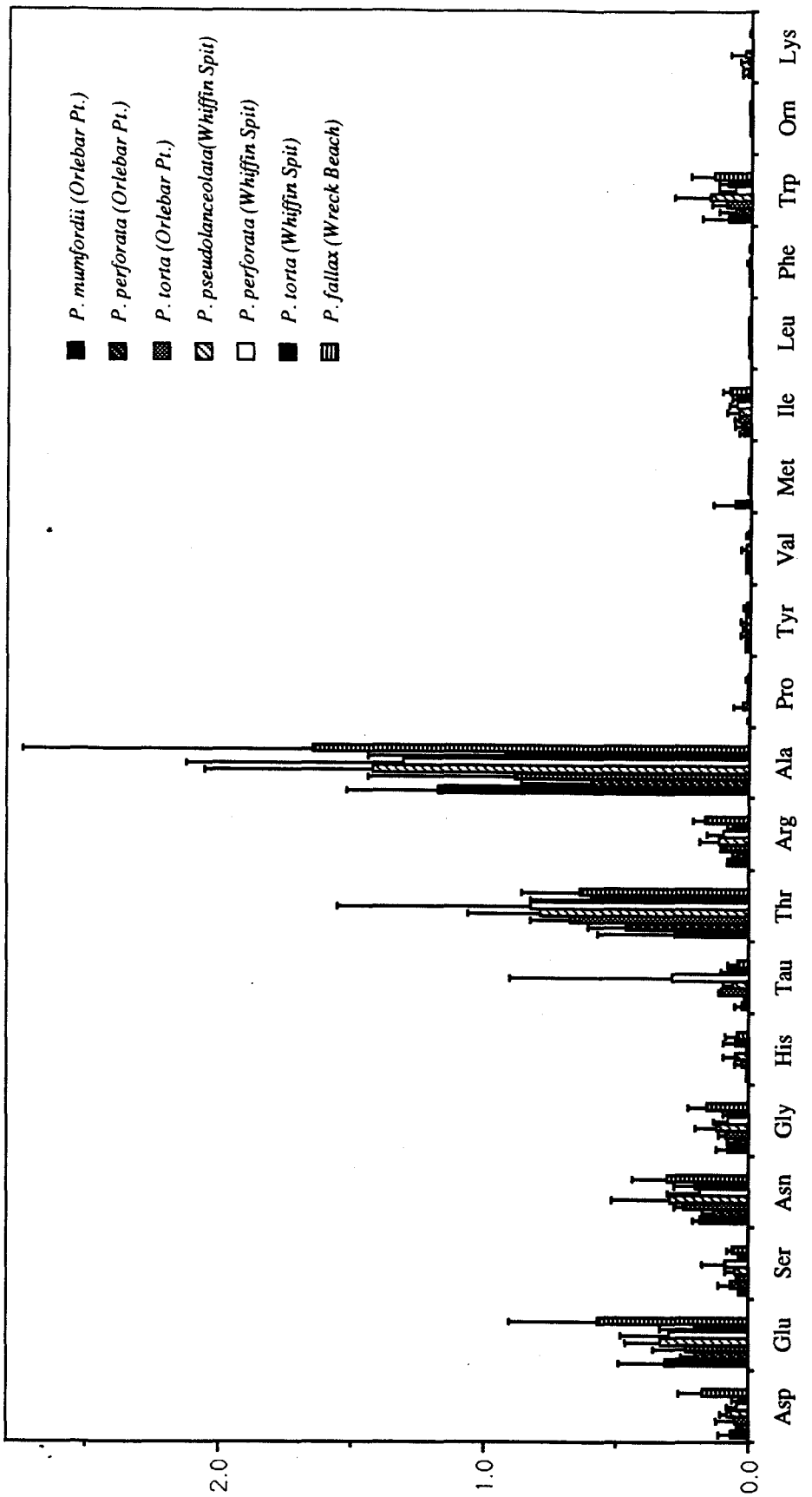


Figure 38. Free amino acids composition of *Porphyra* spp. collected 1992. The values shown are an average of all collections in one year with their standard deviations.

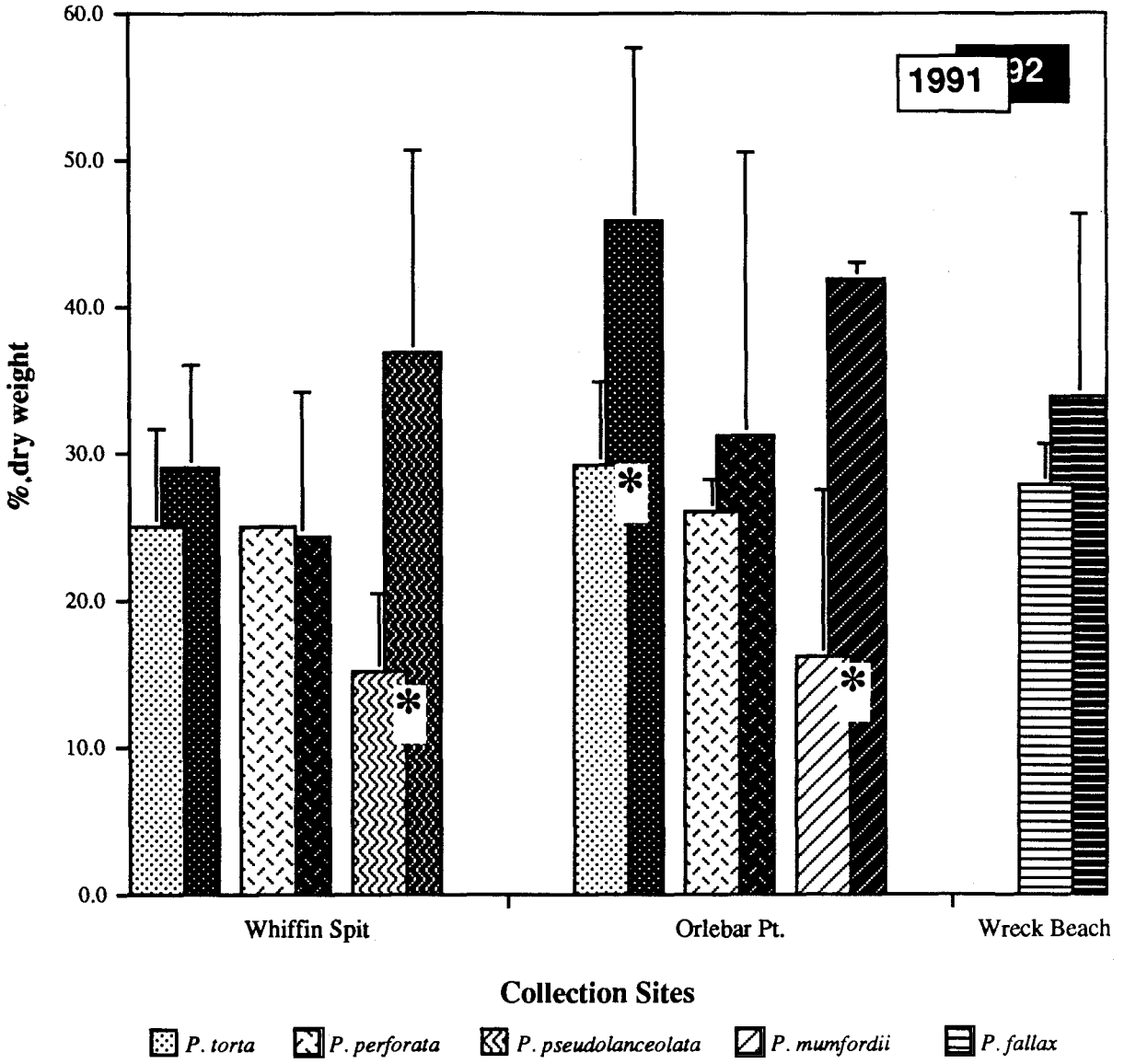


Figure 39. Porphyran content in *Porphyra* spp. at the three sites. The values shown are averages of all collections in one year with their standard deviations (*P. perforata* from Whiffin Spit, 1991 has no standard deviation). Bars with an asterisk (*) indicate significant differences between the two years ($p \leq 0.05$).

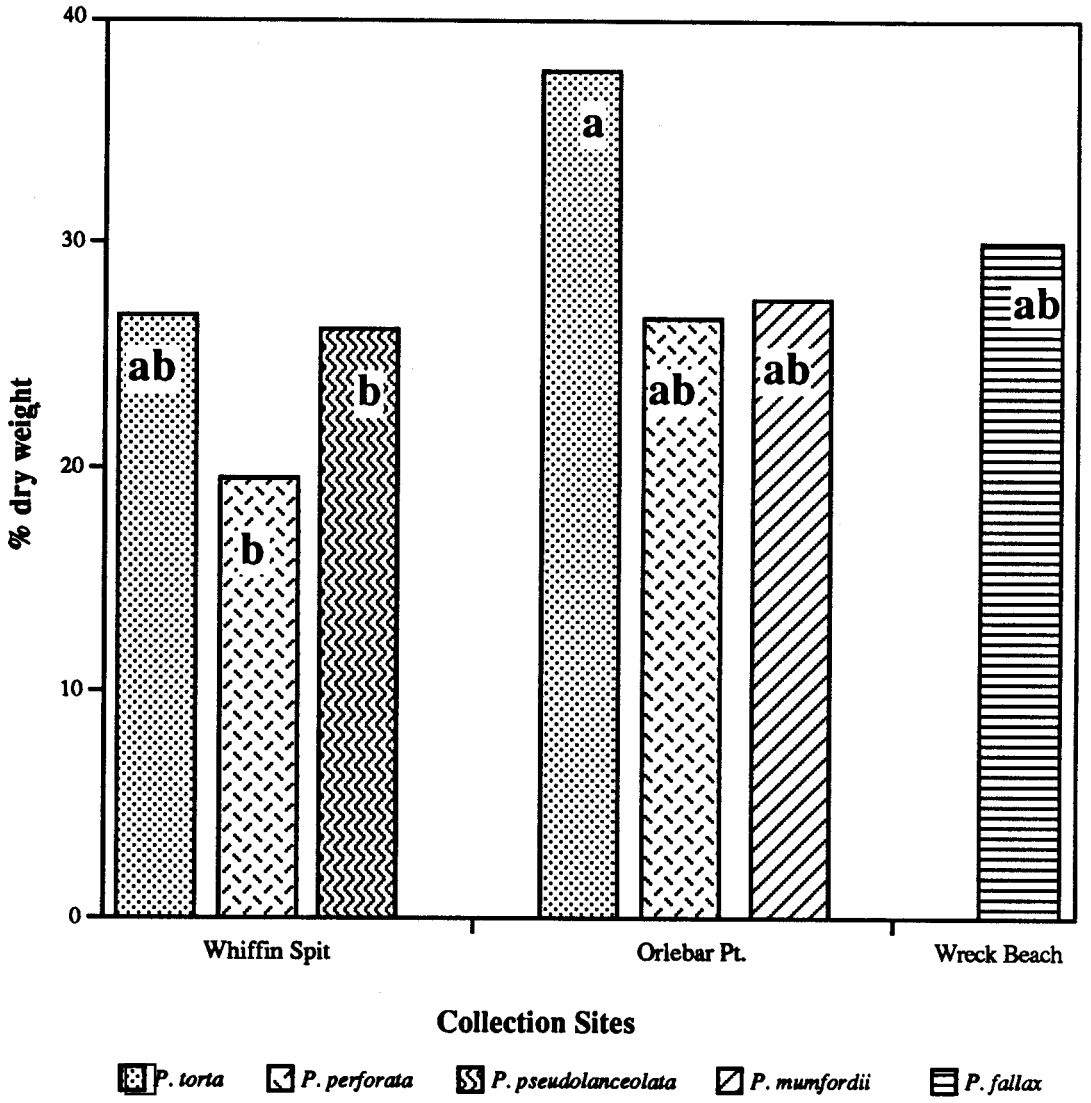


Figure 40. Average porphyran content in *Porphyra* spp. both 1991 and 1992 at the three sites. The bars shown are least square mean values of all collections. Bars with the same letter are not significantly different ($p > 0.05$).

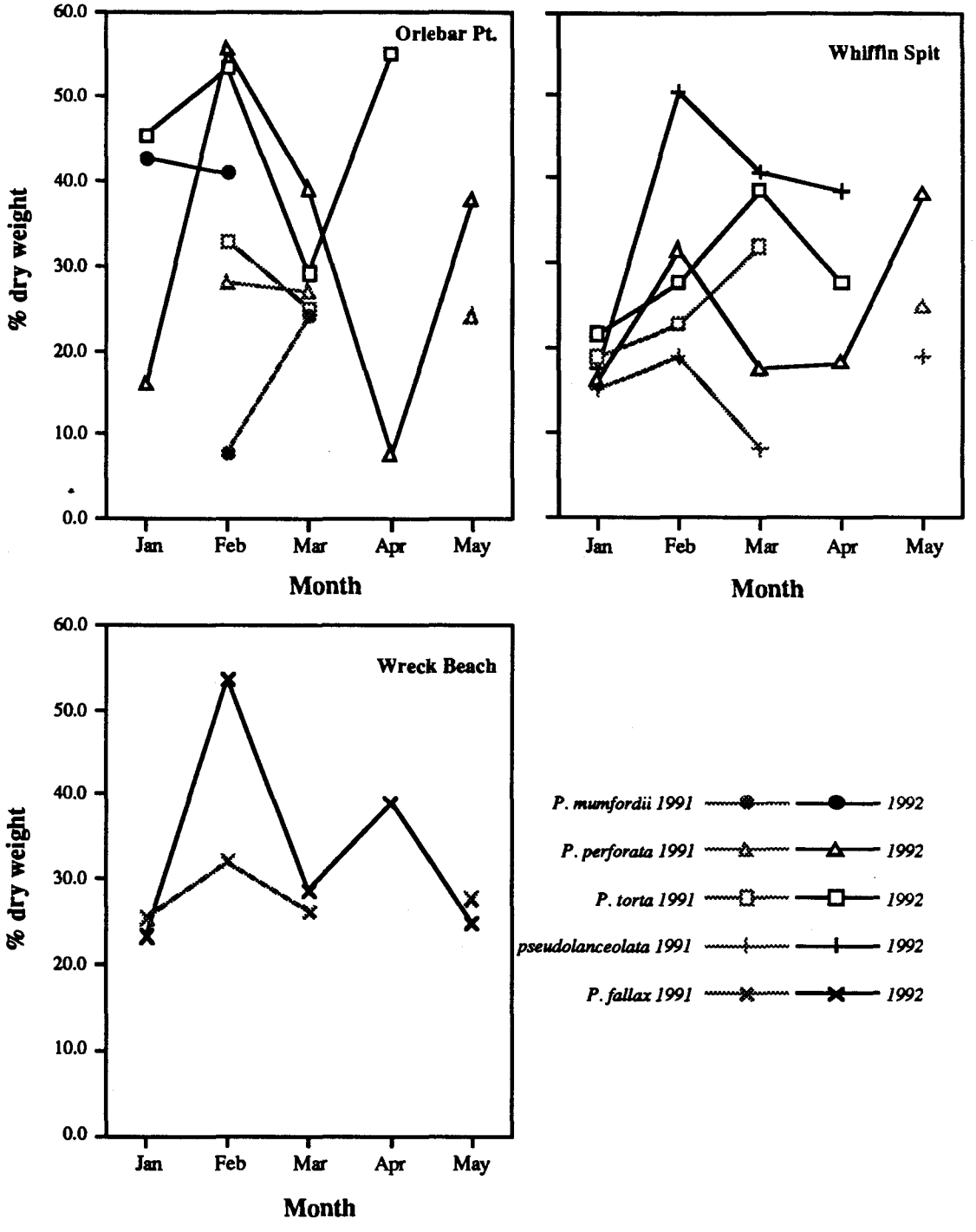


Figure 41. Changes in porphyran content of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

FLORIDOSIDES

All samples had a higher content of isofloridoside than floridoside (Figs. 42 and 44). The average concentration of isofloridoside varied between 1.6% (dw) for *P. torta* at Whiffin Spit (1991) to 5.0% (dw) for *P. perforata* at Orlebar Pt. (1992), and the isofloridoside content of *P. perforata* at Orlebar Pt. was significantly higher than in 1991 (Fig. 42). In contrast, average concentration of floridoside varied from 0.83% (dw) for *P. torta* at Whiffin Spit (1991) to 3.05% (dw) for *P. fallax* (1992), and no significant differences were observed in floridoside content between collection years (Fig. 44).

Isofloridoside content did not show any significant differences among species (Fig. 43). In contrast, floridoside of *P. fallax* was significantly higher than that of all but *P. perforata* and *P. pseudolanceolata* at Whiffin Spit (Fig. 45).

Among the months of collection, slightly different fluctuations were observed, but in general they showed a pattern of increase until February or March followed by a decrease to May. For both isofloridoside and floridoside, a significant increase were evident from February to March (Figs. 46 and 47, Appendix II).

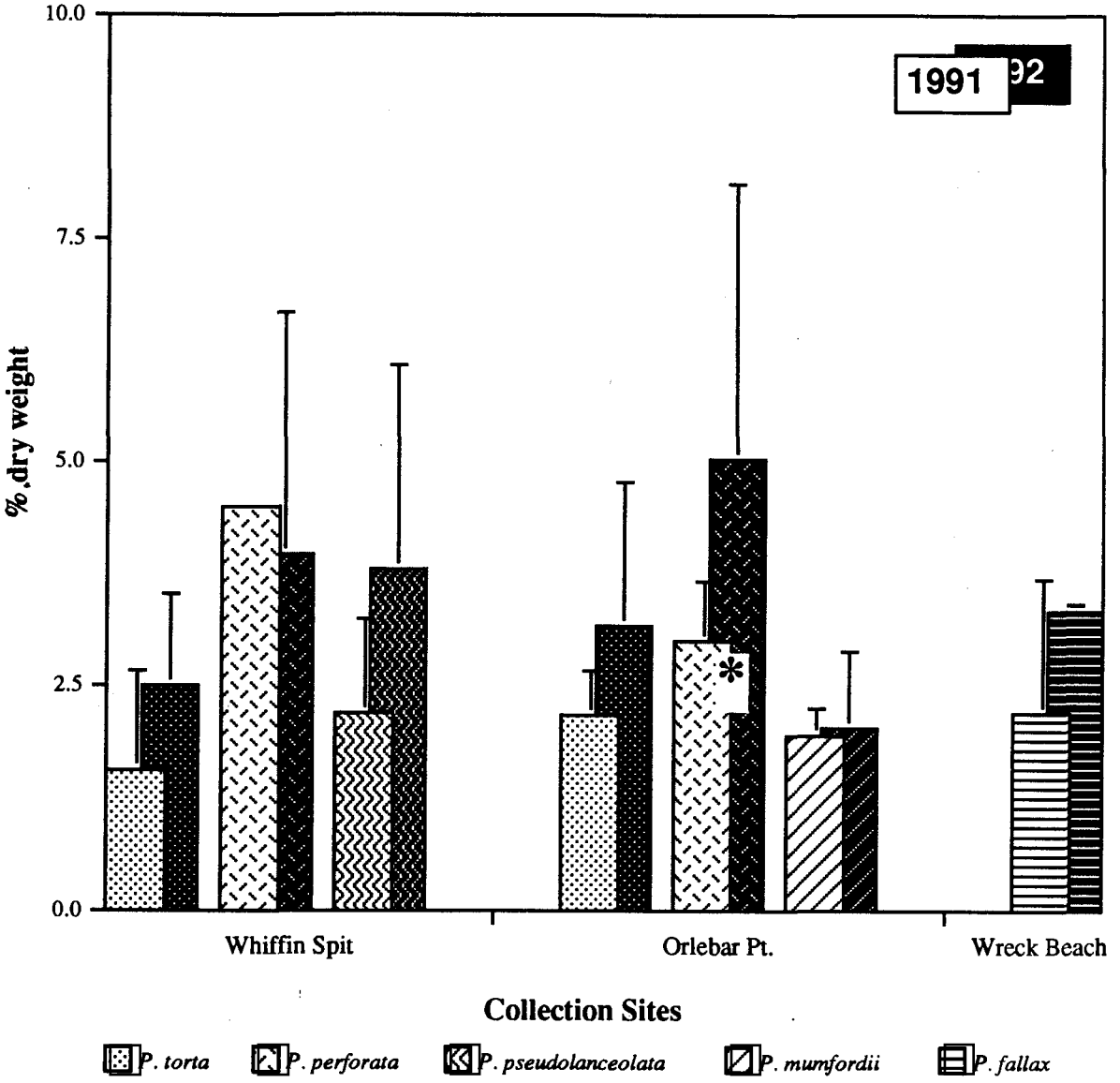


Figure 42. Isofloridoside content in *Porphyra* spp. at the three sites. The values shown are averages of all collections in one year with their standard deviations (*P. perforata* from Whiffin Spit, 1991 has no standard deviation). Bars with an asterisk (*) indicate significant differences between the two years ($p \leq 0.05$).

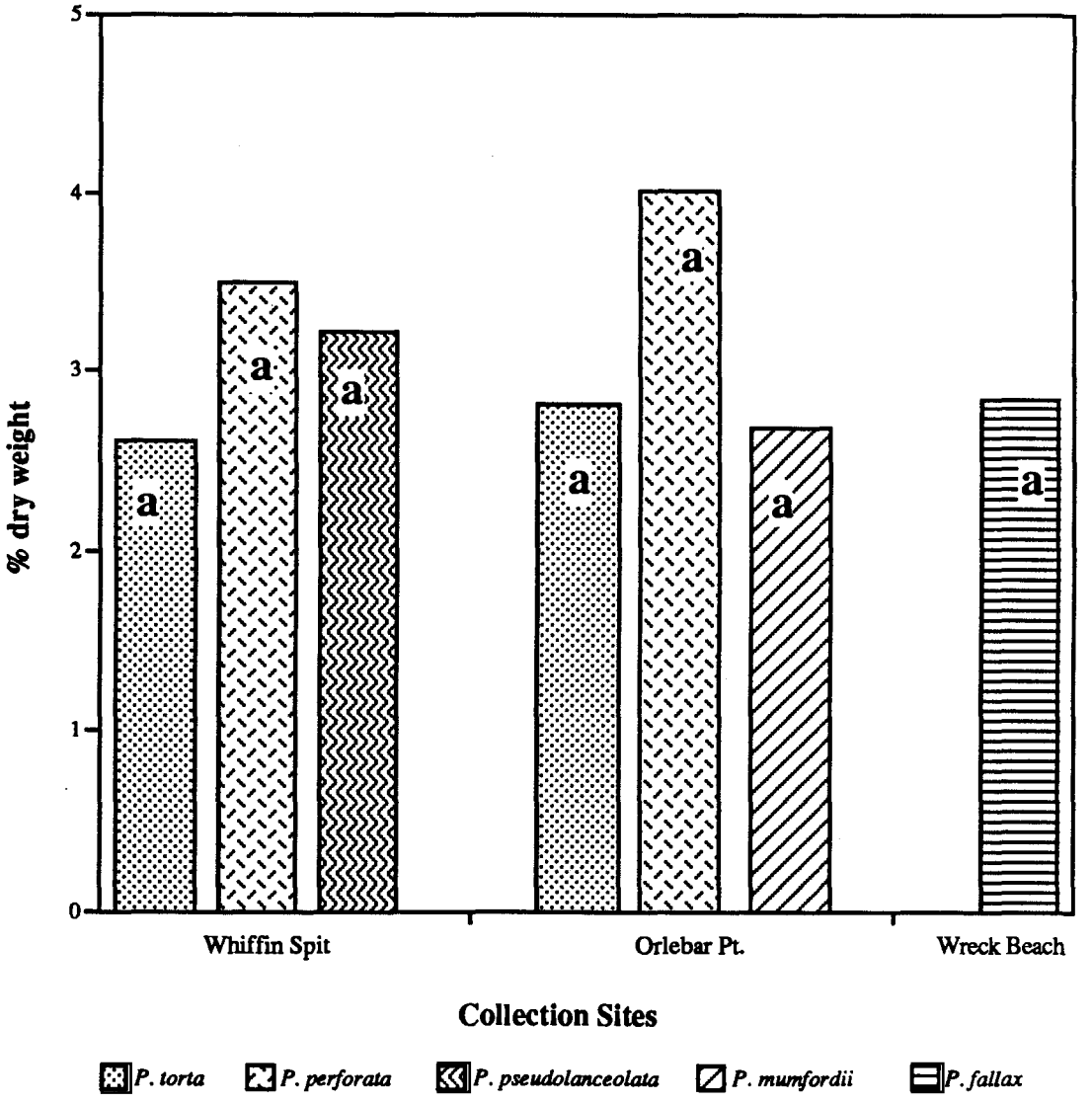


Figure 43. Average isofloridoside content in *Porphyra* spp. both 1991 and 1992 at the three sites. The bars shown are least square mean values of all collections. Bars with the same letter are not significantly different ($p > 0.05$).

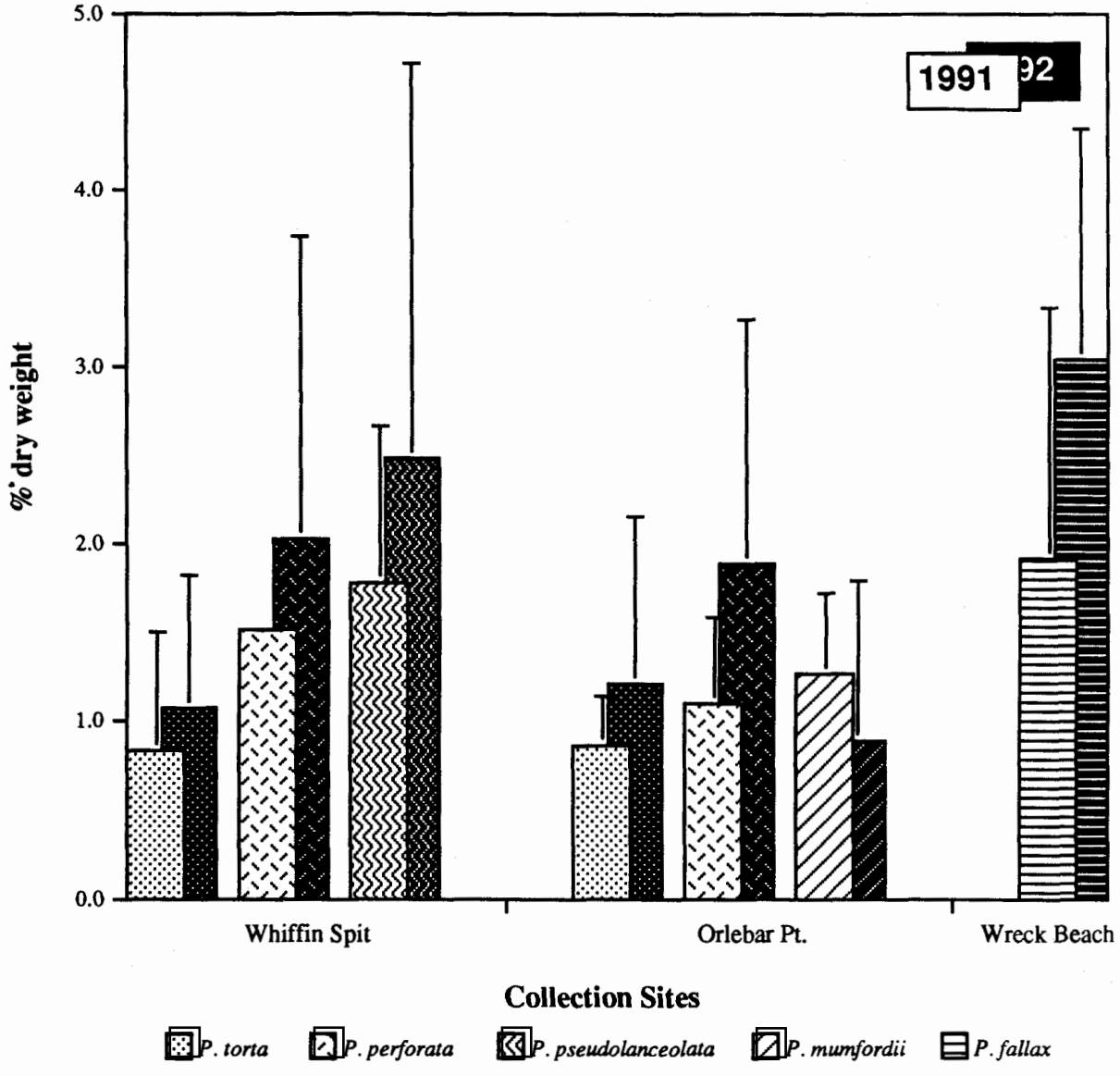


Figure 44. Floridoside content in *Porphyra* spp. at the three sites. The values shown are averages of all collections in one year with their standard deviations (*P. perforata* from Whiffin Spit, 1991 has no standard deviation). Bars with an asterisk (*) indicate significant differences between the two years ($p \leq 0.05$).

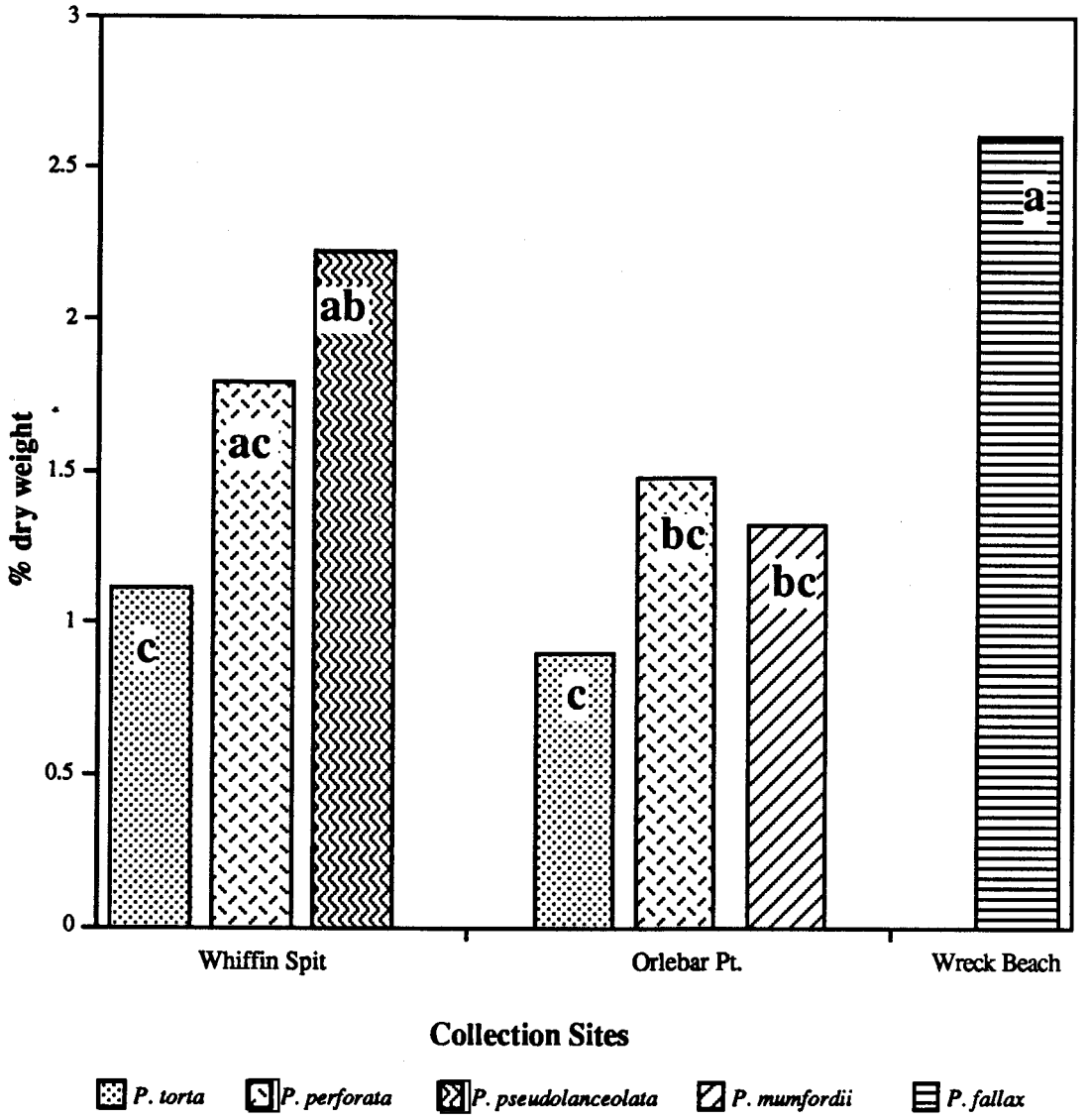


Figure 45. Average floridoside content in *Porphyra* spp. both 1991 and 1992 at the three sites. The bars shown are least square mean values of all collections. Bars with the same letter are not significantly different ($p > 0.05$).

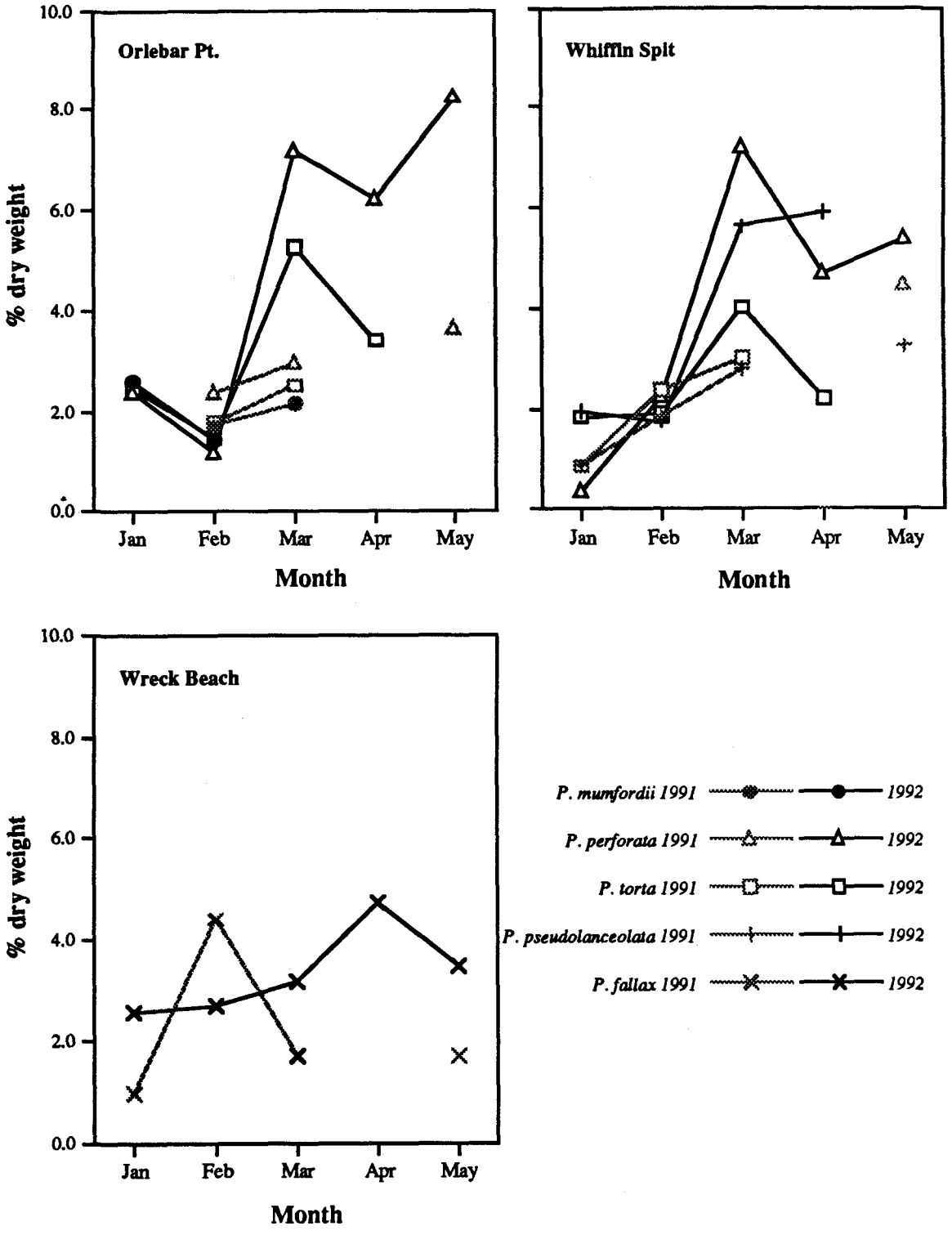


Figure 46. Changes in isofloridoside content of *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

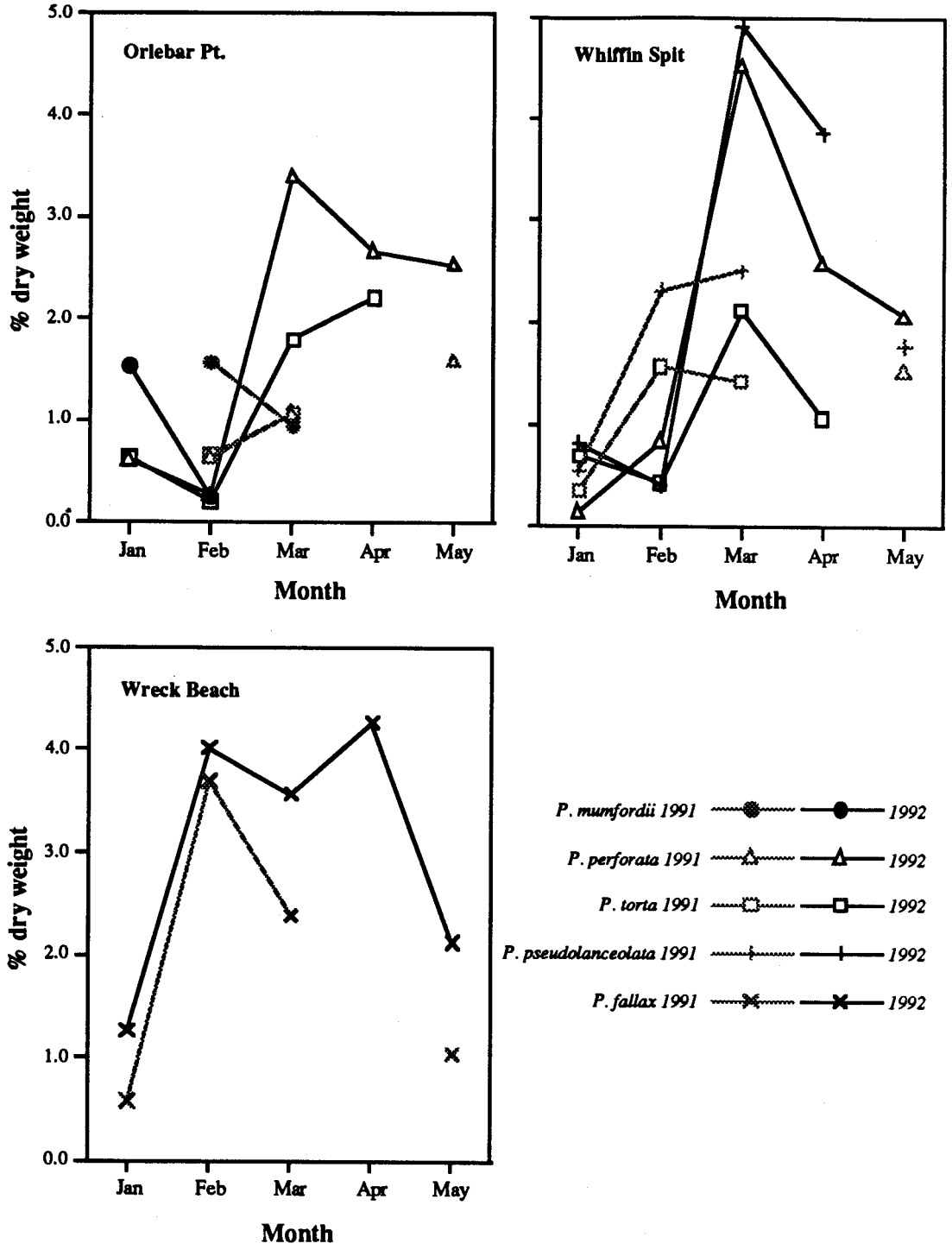


Figure 47. Changes in floridoside content in *Porphyra* spp. collected January to May, 1991 and 1992, at the three collection sites.

IV. DISCUSSION

OCCURRENCE OF *PORPHYRA*

The vertical distribution of *Porphyra* spp. in this study was in agreement with that given by Conway *et al.* (1975), Conway and Cole (1977), and Lindstrom and Cole (1992), namely that *P. torta*, *P. pseudolanceolata*, and *P. mumfordii* are high-intertidal forms, whereas *P. perforata* and *P. fallax* are mid to high intertidal forms. The variations in vertical distribution among species reflect the abilities of different species to tolerate unfavorable environmental conditions (see Kain and Norton, 1990). As high or mid-intertidal forms, the *Porphyra* spp. under study here experience varying degrees of exposure to air and attendant desiccation.

These species also showed variations in year to year occurrence. These variations in occurrence could be due to differences in environmental conditions in 1991 and 1992, where 1992 had a mild winter. As Table 2 shows, the algae were exposed to higher air temperatures in 1992 than in 1991, which could result in more stressful conditions when emerged. The reason for the delay in occurrence of *P. perforata* until April 1991 at Whiffin Spit is not known. It could be due to several reasons-temperature, tide level, or substratum availability for this species to grow from January through March.

SEAWATER NUTRIENTS

The seasonal changes in seawater nitrate at the three collection sites showed a substantial decrease from January, winter, through May, late spring (Fig. 5), a pattern which is in agreement with that reported in BC waters (Wheeler and Srivastava, 1984), and other coastal waters of North temperate regions (Chapman and Craigie, 1977; Jackson, 1977; Buggeln, 1978; Wheeler, 1978; Wheeler and North, 1980).

The seasonal changes in seawater nitrate as well as the other three nutrients recorded in this study should be taken with caution since the samples were only collected monthly.

TOTAL CARBON AND NITROGEN

The irregular fluctuations in total tissue carbon observed in this study (Fig. 13) were also reported in brown algae *Macrocystis integrifolia* and *Nereocystis luetkeana* from Bamfield, BC (Rosell and Srivastava, 1985).

The high concentration of tissue nitrogen in winter and decrease through spring and early summer observed in this study (Fig. 14) coincided with the changes in seawater nutrients, especially nitrate. These results suggested that the species under study accumulated nitrogen reserves in winter, when relatively high concentrations of nitrogen are present in seawater, and then utilized the reserves to support growth when the supplies of the nutrients in the water become depleted. This phenomenon has been

also noted for many macrophytes (e. g., *P. yezoensis*, Noda, 1971; Ji , *et al.*, 1981; *Gracilaria* sp., Penniman and Mathieson, 1987; and several brown algae, Asare and Harlin, 1983; Rosell and Srivastava, 1985).

The seasonal changes in C/N (by atom) values reported here (Fig. 15) were due more to a decline in total nitrogen than an increase in total carbon as has also been found in brown algae (Probyn and Chapman, 1983; Rosell and Srivastava, 1985).

TISSUE INORGANIC NUTRIENTS

While seawater nitrate decreased markedly from the level seen in January (Fig. 5), the levels of tissue nitrate, at least in some samples, e. g. *P. perforata*, Whiffin Spit (1992), showed an initial increase before decreasing (Fig 16). The delay in decrease of tissue nitrate suggested that *Porphyra* species studied are capable of accumulating nitrogen in the form of nitrate, when nitrogen concentration in seawater is still high. This accumulation mechanism was also reported in *P. perforata* (Thomas and Harrison, 1985), and other red algae, *Gracilaria tikvahiae* (Bird *et al.*, 1982) and *Chondrus crispus* (Asare and Harlin, 1983). Some reports have shown the capability of several brown algae to accumulate nitrate in their tissue in much higher concentration than in seawater (Chapman and Craigie, 1977; Chapman et al., 1978; Wheeler and Srivastava, 1984).

The measured tissue inorganic nitrogen content, nitrate, nitrite, or ammonium, is a function of uptake from seawater,

interconversion rates between the three forms, and the incorporation of ammonium into organic nitrogen compounds, such as amino acids, proteins, nucleic acids, and other nitrogenous compounds. These activities are probably influenced by environmental conditions, especially temperature and nutrient levels in seawater. Therefore, the difference in tissue inorganic nitrogen content in the two collection years could be due to interaction of the above factors.

Except for tissue nitrate, the other three tissue nutrients, nitrite, ammonium, and phosphate did not show a clear pattern of seasonal changes (Figs. 17 to 19).

TOTAL LIPIDS AND FATTY ACIDS

Sato and Murata (1980), Shifrin and Chisholm (1981), and Tedesco and Duerr (1989) have shown that cellular lipid composition in algae is altered through manipulation of environmental conditions. Among environmental factors, temperature has been well documented to be positively correlated with the degree of saturation of fatty acids; as the temperature goes down, the degree of saturation decreases (Kayama *et al.*, 1985). Tedesco and Duerr (1989) showed that in the microalga, *Spirulina platensis*, nitrogen depletion led to an increase in storage of nonacyl lipids, but it inhibited the synthesis of polar membrane lipids, an observation that was confirmed for the macroalga *Fucus vesiculosus* (Pohl and Zurheide, 1982).

Among the lipids in *Porphyra* spp., eicosapentaenoic acid represents the major constituent of glycolipids (*P. yezoensis*, Araki *et al.*, 1986; *P. tenera*, Sato, 1971). Consequently, this fatty acid, mainly as a membrane lipid constituent, is expected to contribute to stabilization of photosynthetic lamellae, e. g. preventing molecular arrangement and mediating its photochemical function (see Benson and Shibuya, 1962). In addition to the role of lipid membranes in stabilizing cell functions (functional-protective response), Wiltens (1975) reported a physical-protective function of unsaturated fatty acids vis-a-vis desiccation.

Therefore, the significant increase in eicosapentaenoic acid in early season (Fig. 27, Appendix II) suggested the above functional- and physical-protective responses of *Porphyra* to the changes in environmental conditions, especially air temperature and degree of exposure to air (Table 2, Appendix I), whereas the decrease afterward could be due to depletion of seawater nutrients, especially nitrate (Fig. 5).

Biosynthesis of lipids in algae, including *Porphyra*, is still a matter of conjecture. The only study on *Porphyra*, so far, was on *P. yezoensis* by Kayama, *et al.* (1986), who hypothesized that the C-20 polyunsaturated fatty acids were synthesized from C-16 fatty acids by chain elongation and desaturation reactions. Whatever the detailed mechanism of eicosapentaenoic acid or palmitic acid biosynthesis may be, the results in this study showed that the two acids had a similar pattern of seasonal change (Figs. 27 and 28).

AMINO ACIDS

Porphyra sp. is well known as having a relatively high protein content, and for this reason, is utilized as a nutritious food. The content of total amino acids was reported to reach as high as 30% (dw) in *P. yezoensis* (Ji *et al.*, 1981) and 29% to 35.6% (dw) in *P. tenera* (Arasaki and Arasaki, 1983). In comparison, total amino acid content of *Porphyra* spp.in this study ranged between 19.22% (dw) for *P. fallax*, 1991 to 37.78% (dw) for *P. mumfordii*, 1992 (Fig. 29). The calculated crude protein of *Porphyra*, total N multiplied by 6.25, ranged from 23.71% (dw) for *P. perforata* at Orlebar Pt. in 1992 to 33.47% (dw) for *P. mumfordii* at Orlebar Pt. in 1992, slightly less than that of *P. yezoensis* grown in China (40%, Ji *et al.*, 1981) and Japan (48%, Noda, 1971).

The significant difference in total amino acid concentration of *P. perforata* at Whiffin Spit and *P. mumfordii* between two collection years (Fig. 29) could be due to the fact that the 1991 and 1992 values did not come from the same collection periods. The value of *P. perforata* for 1991 represents the May collection only, whereas the 1992 value was an average of January through May collections. Likewise, the value of *P. mumfordii* for 1991 was an average of February and March collections, whereas the 1992 value was an average of January and February collections.

All species showed lower free amino acids content in 1991 than in 1992, although in only two species were these differences

significant (Fig. 31). This difference in the free amino acids between the two years could be due to lower temperatures in 1991 (Table 2). Laycock *et al.* (1981) reported that in the red alga *Chondrus crispus* low temperature stimulated accumulation of dipeptides. It is possible, therefore, that in the samples of *Porphyra* in 1991 less amino acids were in free form. This possibility is supported by the calculated content of free amino acids as percent of total amino acids. This value ranged from 2.0% for *P. perforata* at Orlebar Pt. to 4.8% for *P. torta* at Orlebar Pt. in 1991, and from 6.5% for *P. mumfordii* to 14.3% for *P. fallax* in 1992.

The seasonal changes in concentrations of total amino acids as well as free amino acids in this study did not show a clear pattern (Figs. 33 and 34), but at least a relatively high or even an increase in concentrations of both forms of amino acids was evident early in the season. These results suggested that *Porphyra* samples had the capability to accumulate amino acids as nitrogen reserves when relatively abundant nitrogen was available in seawater. Oohusa *et al.* (1977) reported that *P. yezoensis* could accumulate amino acids, which represent over 40% of its nitrogen reserves.

The decrease in total and free amino acid concentration of most samples at the end of the growing season suggested that both amino acids and proteins represent a utilizable nitrogen pool in *Porphyra*, which is used immediately when seawater nutrients are

depleted. This phenomenon has been shown for *Gracilaria* spp. (Bird, *et al.*, 1982; Rosenberg and Ramus, 1982).

The differences in the composition of total amino acids between the two collection years (Figs. 35 and 36) are not considered important since amino acids that were dominant in one year were still present in moderate concentrations in the other year, and vice versa. The 1991 samples were dominated by methionine, alanine, and tyrosine, whereas the 1992 samples were dominated by alanine, arginine, and glutamic acid.

. Interestingly, the content of taurine, the amino acid having therapeutic activity, ranged from 0.3% (dw) for *P. fallax* at Wreck Beach, 1991 to 1.6% (dw) for *P. perforata* at Whiffin Spit, 1992, compared to about 1.4% (dw) for various species of *Porphyra* in Japan (Noda *et al.*, 1975).

The range of total amino acid content in *Porphyra* spp. studied here was comparable to that of the Japanese species, *P. yezoensis* (Ji, *et al.*, 1981) and *P. tenera* (Arasaki and Arasaki, 1983). The desirable flavor of *P. tenera*, a Japanese species, has been attributed in part to glutamic acid, alanine and glycine as major constituents (Tsuchiya and Suzuki, 1955). These amino acids were relatively dominant in *Porphyra* species in the present study (Figs. 35 and 36), and hence these species are likely to have an acceptable flavor.

PORPHYRAN

Porphyran, the major mucilaginous cell wall carbohydrate in *Porphyra* spp., has been suggested to function as a cushion against physical buffeting, e. g. wave action (Eppley and Cyrus, 1960) and to provide protection against desiccation (see McCandless, 1981). These suggestions might explain why porphyran content in some samples was significantly higher in 1992, when the temperature was higher, than in 1991 (Fig. 39, Table 2). A relatively higher porphyran content was also noted for *P. torta* at Orlebar Pt. than for the same species at Whiffin Spit, which once again confirms that porphyran content varied as an ecological effect (with probability of least squares means closed to a critical value, 0.0694). Species that showed high porphyran content, e. g. *P. torta* at Orlebar Pt., could be considered as a source of agar since alkali pretreatment changes the sulfated portion into anhydrogalactose, producing agar for food use (Yaphe, 1984).

FLORIDOSIDES

Since all studies so far dealt with floridoside, but not isofloridoside, the following discussion is only focused on floridoside. While the factors affecting the biosynthesis of floridoside are still unknown, environmental factors, such as salinity (Reed et al., 1980; Wincke and Läuchli, 1981 and Macler, 1988), nitrogen level in medium (Macler, 1986), and the key floridoside biosynthesis enzyme, floridoside phosphate synthase

(Meng and Srivastava, 1990, 1993), have been shown to affect floridoside level in red algae. In addition, as a photosynthetic product, it would be expected that floridoside production will be influenced to some extent by parameters that control photosynthesis, such as temperature, nutrients and light. The initial increase in floridoside content early in the season (Fig. 47), therefore, could be a result of temperature increase as the season progressed and nutrient concentration in seawater, which was still relatively high (Figs. 5 and 6). This temperature effect, either indirectly through photosynthetic rate or directly through enzymatic activity enhancement could result in an increase in floridoside content (Meng and Srivastava, 1990, reported that the activity of floridoside phosphate synthase was regulated and positively correlated to temperature). The nutrient availability, especially nitrogen, has been shown to affect partitioning of fixed carbon into floridoside, agar, and floridean starch. For example, in the red alga *Gelidium coulteri*, an increase in nitrogen was shown to stimulate floridoside synthesis (Macler, 1986). In addition, as an intertidal species, the *Porphyra* spp. studied here are subject to marked changes in salinity when exposed at low tide. This increase in salinity at low tide has been related to the accumulation of floridoside *via* degradation of floridean starch as an osmotic regulation response in *P. umbilicalis* (Wincke and Läuchli, 1981). However, in March or April and subsequently, a decrease in floridoside content was observed. This decrease in floridoside content could be due to nutrient

depletion in seawater at this time (Figs. 5 and 6). Macler (1986) reported that nitrogen starvation in *G. coulteri* led to a large loss of photosynthetic pigments and cellular protein, which in turn decreased the rate of photosynthetic carbon fixation.

Although its biosynthesis is still unknown, isofloridoside exhibited a similar pattern of seasonal change to that of floridoside (Figs. 46 and 47).

CONCLUSIONS

Having analyzed the results above, the following important points need to be made. Firstly, this study confirmed that *Porphyra* spp. have a relatively high protein content (see Appendix III). Moreover, species of *Porphyra* investigated here were able to accumulate nitrogen in their tissue, inorganic as well as organic forms, when nutrients were available in abundance. This capability to store nitrogen, presumably as a reserve, precludes the need to apply fertilizer in winter or early in the growing season.

Secondly, it was clear, as discussed above, that parameters, such as temperature, nutrient availability, tide level, and salinity are among the important environmental factors that influence the chemical composition of *Porphyra* spp. Eicosapentaenoic acid, palmitic acid, floridosides, porphyran, ash content and dry weight showed significant but irregular fluctuations or seasonal changes over the growing period of the *Porphyra* species. In addition to environmental factors, inter-site variations were also noted for concentrations of some chemical

constituents of *Porphyra*. The concentration of total amino acids and eicosapentaenoic acid in *P. perforata* varied significantly between samples collected at Whiffin Spit and Orlebar Pt. These observations lead to the necessity to consider the appropriate site and time of harvesting for specific utilization purposes. The effects of periodic emersion were also noted in the natural occurrence of *Porphyra* spp. in the two years, especially the species growing at a higher tide level, i. e. *P. mumfordii, P. torta,* and *P. pseudolanceolata,* suggesting the application of proper cultivation methods, either a floating system where the thalli are soaked constantly in seawater, or a pole system where the thalli are exposed to air during the period of low tide (Oohusa, 1984).

Thirdly, each species showed distinctive characteristics in containing relatively higher concentrations of certain chemical compounds compared to the other species (Appendix III). *P. torta* was high in total lipids and porphyran; *P. perforata* in taurine, eicosapentaenoic acid, and isofloridoside; *P. mumfordii* in total amino acids and palmitic acid; *P. fallax* was high in floridoside and ash. These data may be important in selection of species for cultivation when specific chemical constituents are desired.

V. POSTSCRIPT

The results in the present study along with the previous discussion provide some conclusive points which hopefully would be considered in order to utilize *Porphyra* optimally.

1. Native Pacific Northwest species of *Porphyra* under study can be promising species for commercial cultivation.
2. Understanding physiological characteristics and its responses to changing environmental variables of *Porphyra* could allow the species to be used optimally.
3. It is imperative to select an appropriate species, site, and cultivation method.

For further study on *Porphyra* biochemistry, the following items may be found useful:

1. It is desirable to elucidate the detailed effects of every single environmental factor and its interactions with other factors on the chemical composition of *Porphyra* spp. by conducting laboratory experiments in which the factor of interest is varied while the others are kept constant. Furthermore, having analyzed regression and correlation of all major environmental factors to chemical contents of *Porphyra*, the form and degree of their relations can be drawn, which provides a means of predicting the chemical compositions of *Porphyra* by analyzing environmental conditions.

2. As environmental conditions vary from year to year (as observed in this study), more than two years of replication are needed to adequately study seasonal changes.
3. Due to the fact that variations in occurrence and chemical contents occurred among the species of *Porphyra*, further study on a specific species of interest is advisable in order to obtain a more detailed characterization of the species.
4. Organoleptic tests of *Porphyra*, as a complement to chemical composition analyses, especially for food production, should be done to explore the desired taste and flavor.

REFERENCES

Amano, H. and Noda, H. (1990). Protein of protoplast from red alga *Porphyra yezoensis*. *Nippon Suisan Gakkaishi* 56:1859-64.

Anderson, N. S. and Rees, D. A. 1965. Porphyran: A polysaccharide with a masked repeating structure. *J. Chem. Soc.* 5880-7.

Anonymous 1989. Nori Farming Workshop Pamphlet. Sponsored by the Institute of Aquaculture Research Simon Fraser University. Vancouver, B. C. Canada.

Araki, S., Sakurai, T., Omata, T., Kawaguchi, A. and Murata, N. 1986. Lipid and fatty acid composition in the red alga *Porphyra yezoensis*. *Jap. J. Phycol.* 34:94-100.

Arasaki, S. and Arasaki, T. 1983. Low Calorie, High Nutrition Vegetable from the Sea to Help You Look and Feel Better. Kodansha International Press, New York.

Arnon, D. I. 1953. Growth and Function as Criteria in Determining the Essential Nature of Inorganic Nutrients. *In* Mineral Nutrition of Plants. Trong, E. (ed.). The University of Wisconsin Press. Pp. 313-341.

Aro, E and Karunen, P. 1979. Effect of changed environmental conditions on glycolipids of the mosses *Pleurozium schreberi* and *Ceratodon purpureus*. *Physiol. Plant.* 45:201-6.

Asare, S. O. and Harlin, M. M. 1983. Seasonal fluctuations in tissue nitrogen for five species of perennial macroalgae in Rhode island sound. *J. phycol.* 19:254-7.

Bean, R. C. and Hassid, W. Z. 1955. Assimilation of $C^{14}O_2$ by photosynthesizing red alga, *Iridophycus flaccidum*. *J. Biol. Chem.* 212:411-25.

Benson, A. A. and Shibuya, I. 1962. Surfactant lipids. *In* Physiology and Biochemistry of Algae. Lewin, R. A. (ed.). Academic Press. Pp. 371-83.

Bidwell, R. G. S. 1958. Photosynthesis and metabolism of marine algae. II. A survey of rates and products of photosynthesis in $C^{14}O_2$. *Can J. Bot.* 36:337-49.

Bindra, J. S. and Bindra, R. 1977. Prostaglandin Synthesis. Academic Press. Inc.

Bird, K. T., Habig, C. and DeBusk, T. 1982. Nitrogen allocation and storage patterns in *Glacilaria tikvahiae* (Rhodophyta). *J. Phycol.* 18:344-8.

Buggeln, R. G. 1978. Physiological investigations on *Alaria esculenta* (Laminariales, Phaeophyceae). IV. Inorganic and organic nitrogen in the blade. *J. Phycol.* 14:156-60.

Butler, M. L. 1936. Seasonal variation in *Chondrus crispus*. *Biochem. J.* 30:1338-44.

Chapman, A. R. O., and Craigie, J. S. 1977. Seasonal Growth in *Laminaria longicruris*: : relation with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol.* 40 : 197-205.

Chapman, A. R. O., and Craigie, J. S. 1978. Seasonal Growth in *Laminaria longicruris*: : relations with reserve carbohydrate storage and production. *Mar Biol.* 46:209-13.

Chapman, A. R. O., Markham, J. W. and Lüning, K. 1978. Effects of nitrate concentration on the growth and physiology of *Laminaria saccharina* (Phaeophyta) in culture. *J. Phycol.* 14:195-8.

Chapman, V. J. 1970. Seaweeds and Their Uses. 2nd Edition. Methuen & Co. Ltd, London.

Chapman, V.J. and Chapman, D. J. 1980. Seaweeds and Their Uses. Chapman and Hall, London.

Chu, F. E. and Webb, K. L. 1984. Polyunsaturated fatty acids and neutral lipids in developing larvae of the oyster, *Crassostrea virginica*. *Lipids* 19:815-20.

Colin, H. and Guéguen, E. 1930. La constitution du principe sucre de *Rhodymenia palmata*. *Compt. Rend. Acad. Sci.* 191:163-4.

Combaut, G., Codomier, L., and Teste, J. 1981. Seasonal chemical evolution of the alga *Cystoseira elegans*. *Phytochem.* 20:2036-37.

Conway, E. and Cole, K. 1977. Studies in Bangiaceae: structure and reproduction of the conchocelis of *Porphyra* and *Bangia* in culture (Bangiales, Rhodophyceae). *Phycologia* 16:205-16.

Conway, E., Mumford, T. Jr. and Scagel, R. F. 1975. The genus *Porphyra* in British Columbia and Washington. *Syesis* 8:185-244.

Craigie, J. S. 1974. Storage products. *In* Algal Physiology and Biochemistry, Stewart (ed.). University of California Press, Berkeley. Pp. 206-35.

Craigie, J. S., McLachlan, J. and Tocher, R. D. 1968. Some neutral constituents of rhodophyceae with special reference to the occurrence of the floridosides. *Can J. Bot.* 46:605-11.

Dawes, C. J., Lawrence, J. M., and Cheney, D. P. 1974. Ecological study of floridian *Eucheuma* (Rhodophyta, Gigartinales). III. Seasonal variation of carrageenan, total carbohydrate, protein, and lipid. *Bull. Mar. Sci.* 24:286-99.

Dawes, C. J., Stanley, N. F., and Stancioff, D. J. 1977. Seasonal and reproductive aspects of plant chemistry, and t-carrageenan from Floridian *Eucheuma* (Rhodophyta, Gigartinales). *Bot. Mar.* 20 : 137-147.

DeBoer, J. A. 1981. Nutrients. *In* The Biology of Seaweeds. Lobban, C. S. and Wynne, M. J. (eds.). Botanical Monographs Vol. 17. Univ. of California Press. Pp. 356-92.

DeBoer, J. A. and Ryther, J. H. 1977. Potential Yields from a Waste-Recycling Algal Mariculture System. *In* The Marine Plant Biomass of the Pacific Northwest Coast. Krauss, R. (ed.). Oregon State Univ. Press. Pp. 231-49.

Dring, M.J. 1974. Reproduction. *In* Algal Physiology and Biochemistry, Stewart (ed.). University of California Press, Berkeley. Pp. 814-837.

Duckworth, M. and Turvey, J. R. 1969a. The action of a bacterial agarase on agarose, porphyran and alkali-treated porphyran. *Biochem. J.* 113:687-92.

Duckworth, M. and Turvey, J. R. 1969b. The specificity of an agarase from a *Cytophaga* species. *Biochem. J.* 113:693-6.

Dyerberg, J., Bang, O. H., Stoffersen, E., Mancada, S. and Vane, J. R. 1978. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. *Lancet* 1978(2):117-9.

Enright, C. T., Newkirk, G. F., Craigie, J. S. and Castell, J. D. 1986. Evaluation of phytoplankton as diets for juvenile *Ostrea edulis L. J. Exp. Mar. Biol. Ecol.* 96:1-13.

Eppley, R. W. and Cyrus, C. C. 1960. Cation regulation and survival of the red alga, *Porphyra perforata*, in diluted and concentrated seawater. *Biol. Bull.* 118:55-65.

Findlay, J. A. and Patil. A. D. 1986. Antibacterial constituents of the red alga *Cystoclonium purpureum*. *Phytochem.* 25:548-50.

Fujiwara-Arasaki, T., Mino, N and Kuroda, M. 1984. The protein value in human nutrition of edible marine algae of Japan. *Hydrobiol.* 116-117: 513-6.

Fuller, S. W., and Mathieson, A. C. 1972. Ecological studies of economic red algae IV. Variations of carrageenan concentration and properties in *Chondrus crispus* Stackhouse. *J. Exp. Mar. Biol. ecol.* 10:49-58.

Germann, I., Druehl, L. D., and Hoeger, U. 1987. Seasonal variation in total and soluble tissue nitrogen of *Pleurophycus gardneri* (Phaeophyceae: Laminariales) in relation to environmental nitrate. *Mar. Biol.* 96:413-23.

Gorham, J. and S. A. Lewey. 1984. Seasonal change in the chemical composition of *Sargassum muticum*. *Mar. Biol.* 80:103-7.

Hanisak, M. D. 1979. Nitrogen limitation of *Codium fragile* ssp. *tomentosoides* as determined by tissue analysis. *Mar. Biol.* 50:333-7.

Jackson, G. A. 1977. Nutrients and production of giant kelp, *Macrocystis pyrifera*, off southern California. *Limnol. Oceanogr.* 22:979-95.

Jacques, A. G. and Osterhout, W. J. V. 1938. The accumulation of electrolytes. XI. Accumulation of nitrate by *Valonia* and *Halicystis*. *J. Gen. Physiol.* 21:767-73.

Jamieson, G. R. and Reid, E. H. 1972. The component fatty acids of some marine algal lipids. *Phytochem.* 11:1423-32.

Jensen, A. ,M. Indergaard and T. J. Holt. 1985. Seasonal variation in the chemical composition of *Saccorhiza polyschides* (Laminariales, Phaeophyceae). *Bot. Mar.* 28:375-81.

Ji, M. H., Pu, S. Z. and Niu, Z. Q. 1981. The variation in contents of various states of amino acids in *Porphyra yezoensis*. *Proc. Int. Seaweed Symp.* 10:431-5.

Johns, R. B., Nichols, P. D. and Perry, G. J. 1979. Fatty acid composition of ten marine algae from Australian waters. *Phytochem.* 18:799-802.

Kain, J. M. and Norton, T. A. 1990. Marine Ecology. *In* Biology of the Red Algae. Cole, K. M. and Sheath, R. G. (eds.). Cambridge Univ. Press. Pp. 377-422.

Kates, M. 1986. Techniques of Lipidology: Isolation, Analysis and Identification of Lipids. 2nd edition. Elsevier.

Kayama, M., Iijima, N., Kuwahara, M., Mankura, M., Araki, S. and Sakurai, T. 1985. Effect of water temperature on the fatty acid composition of Porphyra. Bull Jap Soc. Sci. Fish. 51:687.

Kayama, M., Iijima, N., Sado, T., Mankura, M., Araki, S. and Sakurai, T. 1986. *De novo* syntheses and conversion of fatty acids in *Porphyra*. *Bull. Jap. Soc. Sci. Fish.* 52:575.

Kayama, M., Imayoshi, J., Araki, S., Ogawa, H., Oohusa, T., Ueno, T. and Saito, M. 1983. Changes in the lipids of dried laver, "Nori" at different water activities. *Bull. Japan Soc. Sci. Fish.* 49:787-93.

Khotimchenko, S. V. and Svetashev, V. I. 1987. Fatty acids of marine macrophytes. *Biol. Morya* 6:3-15.

Khotimchenko, S. V. and Vaskovsky, V. E. 1990. Distribution of C_{20} polyenoic acids in red macrophytic algae. *Bot. Mar.* 33:525-8.

Kuhl, A. 1974. Phosphorus. *In*. Algal Physiology and Biochemistry. Stewart, W. D. P. (ed.). Botanical Monographs Vol. 10. Univ. of California Press. Pp. 636-54.

Langdon, C. J. and Walcock, M. J. 1981. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* Spat. *J. Mar. Biol. Ass.* 61:431-48.

Larsen, B 1978. Brown Seaweeds : analysis of ash, fiber, iodine, and mannitol. *In* Handbook of Phycological Methods Physiological and Biochemical Methods. Hellebust, J. S and Craigie, J.S (ed.).Cambridge University Press, Cambridge. Pp. 181-8.

Laycock, M. V., Morgan, K. C. and Craigie, J. S. 1981. Physiological factors affecting the accumulation of L-citrullinyl-L-arginine in *Chondrus crispus*. *Can J. Bot.* 59:522-7.

Lindberg, B. 1955. Low-molecular carbohydrates in algae XI. Investigation of *Porphyra umbilicalis*. *Acta Chem. Scand.* 9:1097-9.

Lindstrom, S. 1989. The Biology and Cultivation of *Porphyra*. A one-day nori farming workshop. Sponsored by the Institute of Aquaculture Research Simon Fraser University. November 18.

Lindstrom, S. C. and Cole, K. M. 1990. *Porphyra fallax*, a new species of Rhodophyta from British Columbia and Northern Washington. *Jap. J. Phycol.* 38: 371-6.

Lindstrom, S. C. and Cole, K. M. 1992. The *Porphyra lanceolata-P. pseudolanceolata* (Bangiales, Rhodophyta) complex unmasked: recognition of new species based on isozymes, morphology, and chromosomes and distributions. *Phycologia* 31:431-48.

Lobban, C. S., Harrison, P. J., and Duncan, M. J. 1985. The Physiological Ecology of Seaweeds. Cambridge University Press, Cambridge.

Macler, B. A. 1986. Regulation of carbon flow by nitrogen and light in the red alga, *Gelidium coulteri*. *Plant Physiol.* 82:136-41.

Macler, B. A. 1988. Salinity effects on photosynthesis, carbon allocation, and nitrogen assimilation in the red alga, *Gelidium coulteri*. *Plant. Physiol.* 88:680-4.

Macpherson, M. G., and Young, E. G. 1952. Seasonal variation in the composition of the *Fucaceae* in the maritime provinces. *Can J. Bot.* 30:67-77.

Majak, W., Craigie, J. S. and McLachlan, J. 1966. Photosynthesis in algae I. accumulation products in the rhodophyceae. *Can J. Bot.* 44:541-9.

McCandless, E. L. 1981. Polysaccharides of seaweeds. *In* The Biology of Seaweeds. Lobban, C. S. and Wynne, M. J. (eds.). Botanical Monographs Vol. 17. Univ. of California Press. Pp. 559-88.

McLachlan, J., Craigie, J. S. and Chen, L. C. M. 1972. *Porphyra linearis* Grev. - An edible species of Nori from Nova Scotia. *Proc. Int. Seaweed Symp.* 7:473-6.

Medcalf, D. G., Lionel. T., Brannon, J. H., and Scott, J. R. 1975. Seasonal variation in the mucilaginous polysaccharides from *Ulva lactuca*. *Bot. Mar.* 28:67-70.

Meng, J. X, Rosell, K., and Srivastava, L. M. 1987. Chemical characterization of floridosides from *Porphyra perforata*. *Carbohyd. Res.* 161:171-80.

Meng, J. X. and Srivastava, L. M. 1990. Extraction, assay and some properties of floridoside phosphate synthase from *P. perforata* (Rhodophyta). *J. Phycol.* 26:683-8.

Meng, J. X. and Srivastava, L. M. 1993.Variations in floridoside content and floridoside phosphate synthase activity in *Porphyra perforata* (Rhodophyta)(NOTE). *J. Phycol.* 29:82-4.

Morrison, W. R. and Smith, L. M. 1964. Preparation of fatty acid methylesters and dimethylacetals from lipids with borontrifluoride-methanol. J. Lip. Res. 5:600-8.

Mumford, T. F. JR., Waaland, J. R., Dickson, L. G. and Melvin, D. J. 1985. *Porphyra* aquaculture in the Pacific North West. *J. Phycol. (suppl.)* 21:7.

Mumford, T. F., JR. and Miura A. 1988. *Porphyra* as Food : Cultivation and Economics. *In* Algae and Human Affairs. Lembi, A. and Waaland, J. R. (eds).Cambridge University Press, Cambridge, U. K. Pp. 88-117.

Mumford, T. F.,Jr. 1988. *Porphyra* Culture in the New World. Presented at Aquaculture International Congress and Exposition, Vancouver, B.C. (Canada), 6-9 Sep.

Mumford, T. F.,Jr. 1990. Nori cultivation in North America: growth of the industry. Proc. Int. Seaweed Symp. 13:89-98.

Nishide, E., Ohno, M., Anzai, H., Uchida, N. 1988. Extraction of Porphyran from *Porphyra yezoensis* UEDA F. *narawaensis* MIURA. *Nippon Suisan Gakkaishi* 54(12):2189-94.

Nisizawa, K., Noda, H., Kikuchi, R., and Watanabe, T. 1987. The main seaweed foods in Japan. *Hydrobiol.* 151/152 : 5-29.

Noda, H. 1971. Biochemical studies on marine algae-IV. relationship between chemical constituents of Laver and environmental factors in the culture grounds. *Bull. Japan. Soc. Sci. Fish.* 37:391-6.

Noda, H., Amano, H., Abo, K. and Horiguchi, Y. 1981. Sugars, organic acids, and minerals of nori, the dried laver *Porphyra sp. Bull. Jap. Soc. Sci. Fish.* 47:57-62.

Noda, H., Horiguchi, Y. and Araki, S. 1975. Studies on the flavour substances of "Nori", the dried laver *Porphyra spp.* II. Free amino acids and 5'-nucleotides. *Bull. Jap. Soc. Sci. Fish.* 41:1299-303.

O'Kelley, J. C. 1974. Inorganic Nutrients. *In* Algal Physiology and Biochemistry. Stewart, W. D. P. (ed.). Botanical Monographs Vol. 10. Univ. of California Press. Pp. 610-35.

Oohusa, T. 1984. Technical aspects of nori (*Porphyra*) cultivation and quality preservation of nori products in Japan today. *Hydrobiol.* 116/117:95-101.

Oohusa, T., Araki, S., Sakurai, T. and Saitoh, M. 1977. Physiological studies on diurnal biological rhythms of *Porphyra*. II. The growth and the contents of free and total nitrogen and carbohydrate in the thallus cultured in the laboratory. *Bull. Jap. Soc. Sci. Fish.* 43:250-4.

Peat, S. Turvey, J. R. and Rees, D. A. 1961. Carbohydrates of the red alga, *Porphyra umbilicalis*. *J. Chem. Soc.* 1590-5.

Penniman, C. A. and Mathieson, A. C. 1987. Variation in chemical composition of *Gracilaria tikvahiae* McLahlan (Gigartinales, Rhodophyta) in the Great Bay Estuary, New Hampshire. *Bot. Mar.* 30:525-34.

Pillsbury, K. S. 1985. The relative food value of and the biochemical composition of five phytoplankton diets for queen conch *Strombus gigas* larvae. *J. Exp. Mar. Biol. Ecol.* 90:221-32.

Pohl, P. and Zurheide, F. 1979. Fatty Acids and Lipids of Marine Algae and the Control of their Biosynthesis by Environmental Factors. *In* Marine Algae in Pharmaceutical Science. Hoppe, H. A., Levring, T. and Tanaka, Y. (eds.), De Gruyter, New York. Pp. 473-523.

Pohl, P. and Zurheide, F. 1982. Fat production in fresh water and marine algae. *In* Marine Algae in Pharmaceutical Science Vol. 2. Hoppe, H. A., Levring, T. and Tanaka, Y. (eds.), De Gruyter, New York. Pp. 65-80.

Probyn, T. A. and Chapman, A. R. O. 1983. Summer growth of *Chordaria flagelliformis* (O. F. Muell.) C. Ag. : Physiological strategies in a nutrient stressed environment. *J. Exp. Mar. Biol. Ecol.* 73:243-71.

Pueschel, C. M. 1990. Cell Structure. *In* Biology of the Red Algae. Cole, K. M. and Sheath, R. G. (eds.). Cambridge Univ. Press. Pp. 7-41.

Reed, R. H. and Collins, J. C. 1980. The ionic relations of *Porphyra purpurea*(Roth) C. Ag. (Rhodophyta, Bangiales). *Plant Cell Envir.* 3:399-407.

Reed, R. H., Collins, J. C. and Russell, G. 1980. The effects of salinity upon galactosyl-glycerol content and concentration of the marine red alga *Porphyra purpurea* (Roth)C.Ag. *J. Exp. Bot.* 31:1539-54.

Rimmer, M. A., Stewart, M. D., Semmens, G. L., Meikle, G. M., O'Brien, J. J., Reed, A. and Norton, J. 1988. Improved diet for baramundi larvae. *Australian Fisheries* 47:22-3.

Rosell, K. G and Srivastava, L. M. 1984. Seasonal variation in the Chemical Constituents of the Brown Algae *Macrocystis integrifolia* and *Nereocystis luetkeana*. *Can. J. Bot.* 62 : 2229-35.

Rosell, K. G. and Srivastava, L.M. 1985. Seasonal variation in total nitrogen, carbon and amino acids in *Macrocystis integrifolia* and *Nereocystis luetkeana* (Phaeophyta). *J. Phycol.* 21: 304-9.

Rosenberg, G. and Ramus, J. 1982. Ecological growth strategies in the seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva sp.* (Chlorophyceae): soluble nitrogen and reserve carbohydrates. *Mar. Biol.* 66:251-9.

Ryther, J. H. and Dunstan, W. M. 1971. Nitrogen, phosphorus and eutrophication in the coastal marine environment. *Science* 171:1008-13.

Sato, N. and Murata, N. 1980. Temperature shift-induced response in lipids in the blue-green alga, *Anabaena variabilis*. The central role of diacyl monogalactosyl glycerol in thermo-adaptation. *Biochim. Biophys. Acta.* 619:353-66.

Sato, S. 1971. Studies on glycolipids in marine algae - I. fractionation of galactolipids and composition of galactolipids in a red alga, *Porphyra tenera*. *Bull. Jap. Soc. Sci. Fish.* 37:326-32.

Shifrin, N. S. and Chisholm, S. W. 1981. Phytoplankton lipids: interspecific differences and effect of nitrate, silicate, and light-dark cycles. *J. Phycol.* 17:374-84.

Tedesco, M. A. and Duerr, E. O. 1989. Light, temperature and nitrogen starvation effects on the total lipid and fatty acid content and composition of *Spirulina platensis* UTEX 1928. *J. Appl. Phycol.* 1:201-9.

Thomas, T. E. and Harrison. P. J. 1985. Effect of nitrogen supply on nitrogen uptake, accumulation and assimilation in *Porphyra perforata* (Rhodophyta). *Mar. Biol.* 85:269-78.

Topinka, J. A. and Robbins, J. V. 1976. Effects of nitrate and ammonium enrichment on growth and nitrogen physiology in *Fucus spiralis*. *Limnol. Oceanogr.* 21:659-64.

Tsuchiya, Y. and Suzuki, Y. 1955. Free amino acids in the laver *Porphyra tenera* K. *Bull. Japan. Soc. Sci. Fish.* 20:1092-8.

Tsuji, K., Ichikawa, I., Nakagawa, Y., Matsumura, Y. and Kawamura, M. (1983). Hypocholesterolemic effects of taurocyanine or taurine on the cholesterol metabolism in white rats. *Sulfur Amino Acids* 6:239-48.

Tsuji, K., Iwao, T., Nakagawa, Y. and Seki, T. (1981). Effect of dietary taurine_on bile acid metabolism in hypercholesterolemic rats. *Sulfur Amino Acids* 4:111-9.

Turvey, J. R. and Christison, J. 1967. The enzymic degradation of porphyran. *Biochem. J.* 105:317-21.

Turvey, J. R. and Williams, T. P. 1964. Sugar sulfates from the mucilage of *Porphyra umbilicalis*. *Proc. Int. Seaweed Symp.* 4:370-3.

Waaland, J. R. and Dickson, L. G. 1983. Photoperiodic control of conchospore maturation and release in *Porphyra abbotae* and *Porphyra perforata* (Rhodophyta). *J. Phycol. (suppl.)* 19:6.

Waaland, J. R. and Dickson, L. G. 1987. Preparation of *Porphyra* protoplasts. *J. Phycol. (suppl.)* 23:13.

Waaland, J. R. and Mumford, T. F. JR. 1981. Cultivation of marine algae in Washington State USA. Abstracts of the 13th International Botanical Congress, Sydney, N.S.W., Australia, Aug. 21-28, p. 182.

Waaland, J. R., Dickson, L. G. and Carrier, B. A. 1987. Conchocelis growth and photoperiodic control of conchospore release in *Porphyra torta* (Rhodophyta). *J. Phycol.* 23:399-406.

Waaland, J. R., Dickson, L. G. and Duffield, E. C. S. 1984. Conchocelis growth in five Pacific North West *Porphyra* species. *J. Phycol. (suppl.)* 20:21

Waaland, J. R., Dickson, L. G. and Duffield, E. C. S. 1990. Conchosporore production and seasonal occurrence of some *Porphyra* species (Bangiales, Rhodophyta) in Washington State. *Proc. Int. Seaweed Symp.* 13:453-9.

Waaland, J. R., Dickson, L. G. and Watson, B. A. 1988. Propagation of *Porphyra* by protoplasts. *J. phycol. (suppl.)* 24:20

Waaland, J. R., Dickson, L. G., Duffield, E. C. S., and Burzycki, G. M. 1986. Research on *Porphyra* aquaculture. in Algal biomass technologies : An interdisciplinary perspective. Barclay, W. R., and Mc Intosh, R. P. (eds.). *Nova Hedwigia* 83:124-31.

Wheeler, P. A. and North, W. J. 1980. Effect of nitrogen supply on nitrogen content and growth rate of juvenile *Macrocystis pyrifera* (Phaeophyta) sporophytes. *J. Phycol.* 16:577-82.

Wheeler, P. A. and Björnsäter, B. R. 1992. Seasonal fluctuations in tissue nitrogen, phosphorus, and N:P for five macroalgal species common to the Pacific Northwest coast. *J. Phycol.* 28:1-6.

Wheeler, W. N. 1978. Ecophysiological studies on the giant kelp, *Macrocystis*. Ph.D. thesis, University of California, Santa Barbara.

Wheeler, W. N. and Srivastava, L. M. 1984. Seasonal nitrate physiology of *Macrocystis integrifolia* Bory. *J. Exp. Mar. Biol. Ecol.* 76:35-50.

Wickberg, B. 1958. Synthesis of 1-Glyceritol D-Galactopyranosides. *Acta. Chem Scand.* 12:1187-201.

Wikfors, G. H., Twarog, J. W. JR. and Ukeles, R. 1984. Influence of chemical composition of algal food sources on growth of juvenile oysters, *Crassostrea virginica*. *Biol. Bull.* 167:251-63.

Wiltens, J. S. 1975. Ultrastructural and Chlorophyll Fluorescence Responses of Intertidal Marine Algae to Desiccation. M.Sc. thesis, 85 pp. Simon Fraser Univ., Canada.

Wincke, C and Läuchli, A. 1981. Inorganic ions and floridoside as osmotic solutes in *Porphyra umbilicalis*. *Z. Pflanzenphysiol.* 103:247-58.

Wood, B. J. B. 1974. Fatty Acids and Saponifiable Lipids. *In* Algal Physiology and Biochemistry. Stewart, W. D. P. (ed.). Botanical Monographs Vol. 10. Univ. of California Press. Pp. 236-65.

Yaphe, W. 1984. Properties of *Gracilaria* agars. *Hydrobiol.* 116/117:171-86.

Appendix I. Tide level at the day of collections.

Date	Whiffin Spit		Orlebar Pt.		Date	Wreck Beach	
	Time	Height (ft)	Time	Height (ft)		Time	Height (ft)
23 Jan 1991	06.35	9.8	03.40	9.3	22 Jan 1991	03.05	7.7
	14.45	4.5	10.00	15.1		09.40	15.3
	21.10	6.3	17.30	5.1		16.50	6.0
	22.00	6.3				23.00	11.4
26 Jan 1992	06.35	10.1	03.45	9.5	28 Jan 1992	02.30	12.8
	14.25	4.6	10.00	15.3		06.25	11.7
			17.30	5.3		11.25	13.8
						19.40	4.1
21 Feb 1991	05.25	10.0	03.30	10.6	24 Feb 1991	03.25	14.1
	14.10	3.4	09.10	14.6		08.10	11.9
			16.50	3.9		12.15	13.4
						20.25	2.9
23 Feb 1992	04.55	9.9	02.35	9.1	24 Feb 1992	03.40	10.1
	12.30	4.0	08.30	14.9		09.15	14.1
	18.40	6.8	15.40	4.7		16.50	4.3
	21.25	6.5	22.40	12.9			
20 Mar 1991	03.20	9.8	01.45	9.4	22 Mar 1991	03.55	11.2
	11.30	2.5	07.15	14.3		08.35	13.6
	18.10	6.8	14.25	3.0		16.30	2.9
	20.35	6.5	21.35	13.6			
					26 Mar 1992	00.50	13.5
25 Mar 1992	04.35	9.1	04.45	11.3		06.35	11.0
	13.50	3.5	08.50	12.4		09.45	11.5
			16.35	4.8		17.50	5.1
18 Apr 1991	02.30	10.1	01.45	10.7	17 Apr 1991	01.10	10.1
	11.10	1.1	06.35	13.9		06.05	14.3
	18.45	7.0	14.00	1.5		13.35	1.3
	19.50	7.0	21.35	14.7		20.50	14.7
25 Apr 1992	02.55	7.4	00.40	13.9	24 Apr 1992	06.20	10.3
	05.20	7.5	07.25	9.7		09.15	10.7
	14.35	3.9	11.05	10.4		16.45	5.2
	22.30	7.8	17.40	6.3			
18 May 1991	02.55	9.6	02.50	11.0	19 May 1991	04.20	10.3
	11.45	1.0	07.05	12.9		08.35	11.9
	19.20	7.5	14.30	2.0		15.45	2.8
	23.05	7.3	22.10	15.4		23.20	15.4
24 May 1992	02.25	6.7	06.25	8.8	22 May 1992	04.20	10.2
	04.50	6.9	10.40	10.1		08.00	11.0
	13.15	4.0	16.30	7.0		15.10	4.4
	21.00	7.9	23.55	14.1		22.55	14.4

Source: Canadian Tide and Current Tables. Vol. 5.

Appendix II. Least square mean analysis for the effect of month to chemical contents in *Porphyra* spp.*

Factor of month	Least Square Means**									
	Total Amino Acids	Free Amino acids	Total Lipids	Eicosa penta-enoic Acid	Palmitic Acid	Porphyran	Iso-floridoside	Floridoside	Ash	Dry Weight
January	27.42 a	1.74 a	0.75 a	0.18 b	0.58 b	21.62 b	1.50 b	0.51 c	17.20 a	19.42 c
February	26.98 a	1.97 a	0.55 a	0.38 ab	0.70 a	33.97 a	2.07 b	1.29 c	14.25 b	21.24 b
March	29.18 a	2.19 a	0.72 a	0.59 a	0.73 a	27.31 ab	3.96 a	2.46 a	14.96 b	22.80 a
April	25.34 a	1.59 a	0.63 a	0.20 b	0.57 b	24.41 ab	3.86 a	2.45 ab	15.19 b	21.82 ab
May	26.25 a	1.28 a	0.59 a	0.29 b	0.48 b	31.22 ab	4.08 a	1.48 b	14.17 b	20.96 bc

* = general linear model without factor interactions.

** = In one column, numbers with the same letter are not significantly different (p = 0.05).

Appendix III : Major Chemical Compositions of Native Pacific Northwest *Porphyra* spp.*

		Chemical constituents							
Sites	Species	Total Amino Acids (%, dw)	Total Lipid (%, dw)	Eicosa - pentaenoic acid (mg. g^{-1}, dw)	Palmitic Acid (mg. g^{-1}, dw)	Porphyran (%, dw)	Iso-floridoside (% dw)	Floridoside (%, dw)	Ash (%, dw)
Whiffin Spit	*P. torta*	26.25 ± 4.80	0.48 ± 0.36	0.23 ± 0.13	0.45 ± 0.03	27.03 ± 3.10	2.02 ± 0.30	0.95 ± 0.03	14.50 ± 1.56
	P. perforata	27.83 ± 11.93	0.28 ± 0.09	0.80 ± 0.17	0.61 ± 0.03	24.70 ± 0.44	4.24 ± 0.38	1.77 ± 0.36	14.46 ± 0.34
	P. pseudolanceolata	28.01 ± 3.91	0.70 ± 0.09	0.46 ± 0.30	0.68 ± 0.11	26.05 ± 15.27	3.00 ± 1.14	2.14 ± 0.50	15.63 ± 0.67
Orlebar Pt.	*P. torta*	27.25 ± 2.95	0.87 ± 0.06	0.32 ± 0.12	0.58 ± 0.14	37.41 ± 11.89	2.66 ± 0.71	1.03 ± 0.25	14.66 ± 0.84
	P. perforata	25.55 ± 0.81	0.39 ± 0.16	0.30 ± 0.02	0.64 ± 0.15	28.63 ± 3.44	4.01 ± 1.44	1.49 ± 0.56	14.92 ± 0.68
	P. mumfordii	30.76 ± 9.92	0.56 ± 0.11	0.14 ± 0.09	0.73 ± 0.27	28.89 ± 18.23	1.99 ± 0.06	1.07 ± 0.26	13.51 ± 0.30
Wreck Beach	*P. fallax*	23.98 ± 6.73	0.58 ± 0.11	0.31 ± 0.16	0.72 ± 0.00	30.78 ± 4.24	2.76 ± 0.81	2.48 ± 0.80	17.57 ± 1.53

* Data are an average of all collections in two years ± standard deviations.