A TEST OF THE CARBON/NUTRIENT BALANCE HYPOTHESIS: INTRASPECIFIC VARIATION OF SECONDARY METABOLITE PRODUCTION IN THE RED ALGA *PORTIERIA HORNEMANNII*

BY

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Title: A test of the carbon/nutrient balance hypothesis: intraspecific variation of secondary metabolite production in the red alga *Portieria hornemannii*.

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Intraspecific chemical variation in secondary metabolite production has been noted for a variety of plants in marine and terrestrial environments. Studies of intraspecific variation focus on two hypotheses to account for qualitative and quantitative variation: the optimal defense and carbon/nutrient balance hypothesis. In this study, the predictions of the carbon/nutrient balance hypothesis were tested by manipulating the nutrient resources available to the tropical marine red alga *Portieria hornemannii*, which is known to exhibit remarkable variability in monoterpene biosynthesis in regions throughout the Pacific. The following questions were addressed: 1) Is there site-to-site variability in secondary metabolite production in *P. hornemannii* on Guam?, 2) Can this variation be correlated with internal nitrogen and phosphorous stores in the seaweed?, 3) Is it possible to influence monoterpene production in *P. hornemannii* by altering the resources available to the thalli? Ochtodene and triglyceride concentrations differed significantly among *P. hornemannii* populations collected from six sites on Guam. Internal nitrogen and

phosphorous concentrations were not correlated with the observed variation in chemistry. Enhanced nitrogen and phosphorous fertilization experiments conducted in the field and laboratory failed to change ochtodene concentrations in the algae, while triglyceride concentrations increased significantly in the combined nitrogen and phosphorous treatment of the field experiment. The results of the shaded laboratory experiment indicate that light may be a factor influencing monoterpene biosynthesis. Other evidence from the unshaded laboratory experiment suggests that the differences observed in ochtodene concentration among the algae may be a result of temporal variation.

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INTRODUCTION

Intraspecific chemical variation in secondary metabolite production has been noted for a variety of plants in marine and terrestrial environments (Hay and Steinberg 1992). Recent studies concerning intraspecific variation in the production of secondary metabolites by plants focus mostly on two hypotheses that attribute this variation to phenotypic responses: the optimal defense (Rhoades 1985, Littler et al. 1986, Nitao and Zangerl 1987, Hay et al. 1987, Meyer and Paul 1992) and carbon/nutrient balance hypotheses (Bryant et al. 1983, Price et al. 1989, Fajer et al. 1989, Ilvessalo and Tuomi 1989, Yates and Peckol 1993). The optimal defense hypothesis suggests that, in the case of secondary metabolites that act as defenses, the allocation of resources to plant secondary chemistry from growth is dependent upon the susceptibility of different plant parts to predation and the foraging behavior and density of predators (Rhoades 1979, Meyer and Paul 1992, Tuomi 1992). The carbon/nutrient balance hypothesis suggests that the allocation of resources to the production of secondary metabolites in a plant is dependent primarily upon the resource regime available (Bryant 1987, Baldwin and Ohnmeiss 1994, Baldwin et al. 1994). Both hypotheses have been tested in the terrestrial environment whereas very little work has been conducted in the marine environment (Bryant et al. 1983, Coley et al. 1985, Bryant 1987, Bryant et al. 1987a,b, Fajer et al. 1989, Meyer and Paul 1992, Yates and Peckol 1993, Baldwin et al. 1994). Studies of

the carbon/nutrient balance hypothesis in the marine environment have focused on the temperate brown alga *Fucus vesiculosus* (Ilvessalo and Tuomi 1989, Yates and Peckol 1993).

The carbon/nutrient balance hypothesis contends that the concentration of secondary metabolites produced in a plant will vary quantitatively with resource availability, i.e. carbon and nitrogen (Bryant et al. 1983, Price et al. 1989). For plants that produce carbon-based secondary metabolites, nutrient stress should result in a decrease in growth rate and an increase in the concentration of secondary metabolites, while carbon stress should result in a decrease in growth rate accompanied by a decrease in the concentration of secondary metabolites. For plants that produce nitrogen-based secondary metabolites, nutrient stress should result in a decrease in growth rate and the concentration of secondary metabolites, while carbon stress should result in a decrease in growth rate and an accumulation of secondary metabolites (Bryant et al. 1983).

Resource enhancement and depletion experiments using carbon, nitrogen and combined nitrogen and phosphorous treatments conducted with plants that synthesize carbon-based compounds such as polyphenolics and tannins exhibit results well within the predictions of the carbon/nutrient balance hypothesis (Coley et al. 1985, Bryant et al. 1987a, Yates and Peckol 1993, Baldwin and Ohnmeiss 1994, Baldwin et al. 1994, Folgarait and Davidson 1994, Hartley et al. 1995), while studies with plants that produce monoterpenes, a different class of carbon-based secondary metabolites, have reported results to the contrary (Bryant et al. 1987b, Lerdau et al. 1994, Hartley et al. 1995). Experiments conducted with plants that produce monoterpenes did not result in

quantitative changes of secondary metabolites; instead qualitative changes were observed in monoterpene biosynthesis (Mihaliak and Lincoln 1985, Mihaliak et al. 1987, Lerdau et al. 1994, Hartley et al. 1995). Another study has shown that nutrient enhancement with phosphorous, in the absence of nitrogen, resulted in an increase in growth and condensed tannin concentrations which is inconsistent with the predictions of this hypothesis (Bryant et al. 1993). Therefore, the predictions of the carbon/nutrient balance hypothesis may not apply to all classes of secondary metabolites produced by plants.

Tropical marine waters around coral reefs can be considered to be nutrient-limited (Parsons et al. 1984a, Lapointe 1987, Lapointe et al. 1992a, Littler et al. 1992) or, a closed nutrient system where excess nitrogen and phosphate are not available for opportunistic algal uptake (Atkinson 1988). Noting the nutrient regime in this environment, the carbon/nutrient balance hypothesis predicts that the majority of secondary metabolites produced by marine algae should be carbon-based. This trend is reflected in the algae so far investigated in the marine natural products literature (Faulkner 1984, 1986, 1987, 1990, 1991). Cyanobacteria and a few red algal species produce nitrogen containing compounds in detectable quantities. The availability of nutrients has been shown to affect the primary productivity of marine algae (Lapointe 1985, Lapointe 1987, Carpenter et al. 1991, Bulthius et al. 1992, Hansson 1992, Fong et al. 1993), therefore, it seems reasonable to suggest that nutrient availability has a role in the production of secondary metabolites.

The branching red alga *Portieria hornemannii* (Lyngbye) Silva (Order Gigartinales, Family Rhizophyllidaceae) was selected to test the carbon/nutrient balance

hypothesis because it exhibits notable site-to-site variation in secondary metabolite production (Paul et al. 1987, Coll and Wright 1989, Wright et al. 1990, Fuller et al. 1992) and has been shown to produce halomon (Figure 1a), a potent biomedical target for cancer research (Fuller et al. 1992, Fuller et al. 1994). The acyclic monoterpene, halomon (6(R)-bromo-3(S)-(bromomethyl)-7-methyl-2,3,7-trichloro-1-octene) was first isolated from a sample of *P. hornemannii* collected in Chanaryan, Batan Island, Philippines in April 1986 (Fuller et al. 1992). This compound has exhibited unique selective antitumor activity in the National Cancer Institute's human tumor and disease oriented *in vitro* screen (Fuller et al. 1992). To date, halomon has only been isolated from one other collection of *P. hornemannii*, a recollection from Chanaryan in April 1992 (Fuller et al. 1994). Multiple other collections from Chanaryan and a variety of locations in the Pacific have not yielded this compound in detectable amounts; instead an array of related halogenated acyclic and cyclic monoterpenes have been reported (Fuller et al. 1992, Fuller et al. 1994). This remarkable site-to-site chemical variation has been documented for collections at various sites in Hawaii, Australia, Japan and the Philippines (Burreson et al. 1975a, Paul et al. 1987, Coll and Wright 1989, Wright et al. 1990, Wright et al. 1991, Fuller et al. 1992). Different sets of major secondary metabolites have been isolated from samples collected from sites separated by as few as 10 km (Burreson et al. 1975a, Wright et al. 1990).

Portieria hornemannii can be found in a variety of habitats on Guam: on reef flats (e.g. Anae Island) and on the reef slope from 3 m (e.g. Anae Island, Gun Beach) to 35 m (e.g. Iates Point). Preliminary studies of these populations of *P. hornemannii*

Figure 1. Chemical structures of halomon (a) and ochtodene (b). Halomon was isolated from *Portieria hornemannii* collected in Chanaryan, Batan Island, Philippines (Fuller et al. 1992). Ochtodene is the major secondary metabolite isolated from collections on Guam (Paul et al. 1987).

suggest that ochtodene (Figure 1b) is the major secondary metabolite produced by this alga on Guam with several acyclic monoterpenes as minor metabolites (Paul et al. 1987). Ochtodene has been shown to act as a feeding deterrent towards herbivores (Paul et al. 1987, Paul et al. 1990, Paul et al. 1992). Site-to-site variation and the influence of nutrient availability on secondary metabolite production were not previously examined in populations of *P. hornemannii* on Guam. In this study the following questions were addressed: 1) Is there site-to-site variation in secondary metabolite production in *P. hornemannii* on Guam?, 2) Can this variation be correlated with internal nitrogen and phosphorous stores in the seaweed?, 3) Is it possible to influence monoterpene production in *P. hornemannii* by altering the resources available to individual thalli?

MATERIALS AND METHODS

Collection and Study Sites

Samples of *Portieria hornemannii* (formerly *Chondrococcus hornemannii* Silva et al. 1987) were collected from six sites on Guam (Figures 2 and 3): three sites on the leeward side, Double Reef (13°36'N, 144°50'E), Gun Beach (13°31'N, 144°48'E), and Anae Island (13°23'N, 144°38'E) and three sites on the windward side of the island, Janum (13°33'N, 144°56'E), Pago Bay (13°25'N, 144°48'E), and Tagachang (13°24'N, 144°47'E). At the northern-most sites, Double Reef and Janum, nutrient-rich fresh water leaks onto the reef from the Guam aquifer (Matson 1991). Seaweed was collected between 3 - 10 m on the inner reef at Double Reef and 0.3 - 8 m at Janum. Anae Island and Tagachang are the southern-most sites. They are located near more residential development relative to the northern-most sites and receive some runoff during the rainy season. Anae Island is approximately 450 m offshore in the southwest with a small fringing reef on its eastern side from which *P. hornemannii* was collected between depths of 0.3 -10 m. Seaweed was collected from Tagachang between depths of 5 - 10 m. Gun Beach and Pago Bay are sites with commercial or residential development directly above or on the beach that contribute considerable runoff to the reefs during the rainy season. Nutrient-rich fresh aquifer water also leaks onto the reef at Gun Beach (Matson 1991). *P. hornemannii* was collected from the northern end of the reef in 0.3 - 8 m. Fresh water

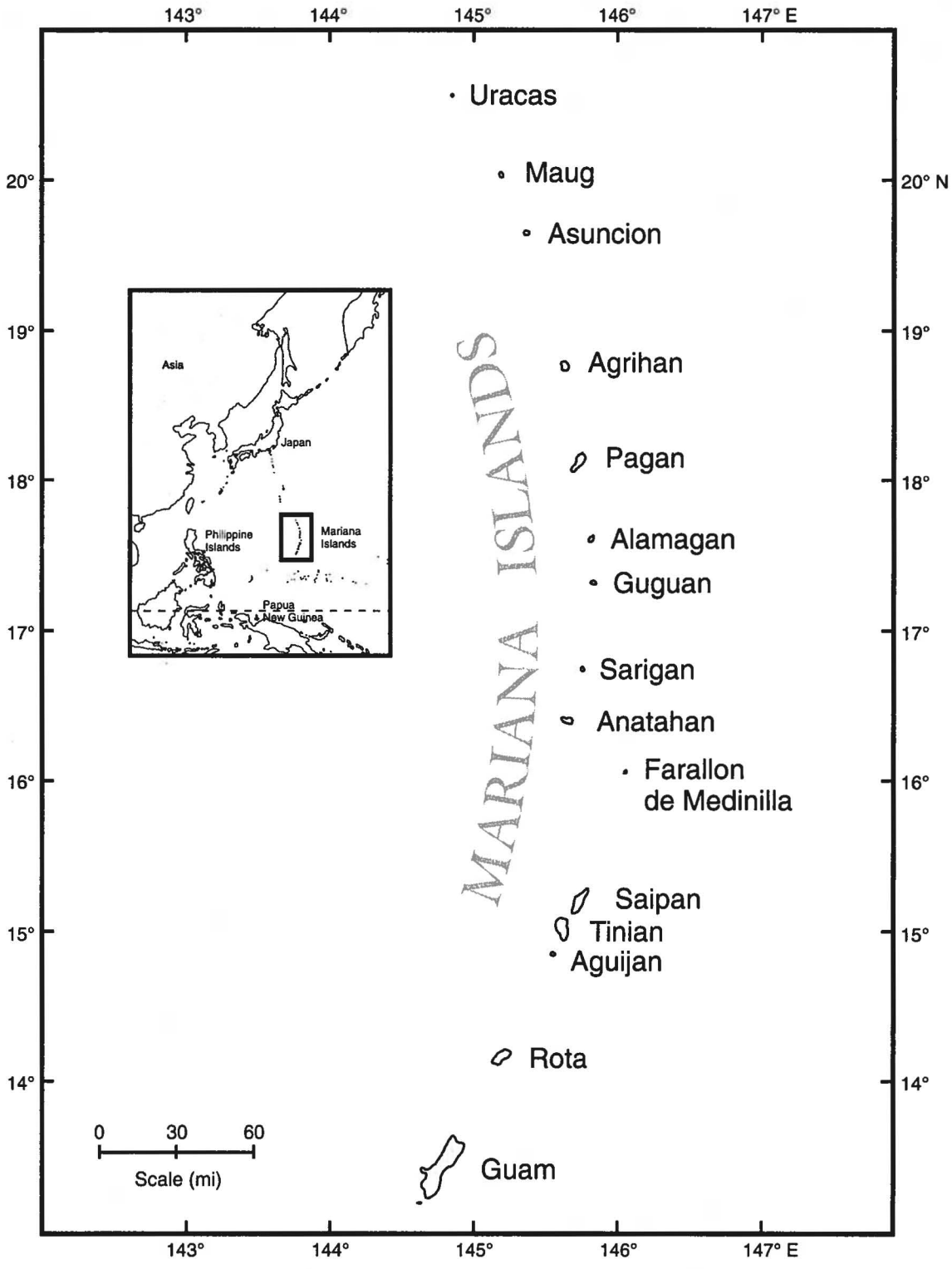

Figure 2. Map of the Mariana Islands.

Figure 3. Collection and study sites on Guam. *Portieria hornemannii* was collected from Anae Island (0.3 - 10 m), Double Reef (3 - 10 m), Gun Beach (0.3 - 8 m), Janum (0.3 - 8 m), Pago Bay (1.5 - 10 m) and Tagachang (5 - 10 m) for site-to-site variation. The *in situ* fertilization experiment was conducted at the north end of Gun Beach at depths of 3 - 5 m. Algae were collected from Anae Island for the shaded and unshaded laboratory fertilization experiments.

from the Pago River empties into Pago Bay at the southern end of the bay. Seaweed was collected at depths of 1.5 - 10 m at the northern end of the bay.

The *in situ* fertilization experiment was conducted at the north end of Gun Beach at depths of 3-5 m. All the *Portieria hornemannii* used in the laboratory fertilization experiments were collected from the small fringing reef on the eastern side of Anae Island.

Internal Nutrient Analysis

Prior to analysis, *Portieria hornemannii* samples for the site-to-site variation study and fertilization experiments were sorted to remove any epiphytes, rinsed with seawater, and blotted dry. Three to 30 mg samples (dry mass) were oven-dried at 62°C for 24 h, stored in tightly closed vials, and submitted to the College of Agriculture and Life Sciences at the University of Guam for analyses of internal nutrients. *P. hornemannii* tissue was analyzed for total Kjeldahl nitrogen (TKN) (TKN in Soil/Plant, Quickchem Method 13-107-06-2-D) and total phosphorous in a Kjeldahl digest (TKP) (Total Phosphorous in Kjeldahl Digests, Quickchem Method 13-115-01-1-B) on an automated ion analyzer (Lachat Instruments, Milwaukee, WI).

Data for TKN and TKP were collected as the percent yield of nitrogen and phosphorous from total dry mass of the plant. Percentages were arcsin-squareroot or log transformed. Bartlett's test of equal variance was used to test for homogeneity of variances. Transformed data were compared by One-Way ANOVA when the assumption

of homogeneity of variances was met. Tukey's Honestly Significant Difference (HSD) pairwise comparison of means test was used to determine which data were different ($p<0.05$). When the data did not meet the requirements for an ANOVA, means were compared by a non-parametric Kruskal-Wallis Test. Nitrogen (N):phosphorous (P) ratios were calculated and means were compared by a non-parametric Kruskal-Wallis Test (Sokal and Rohlf 1981). Statistix 4.0 (Analytical Software, St. Paul, Minnesota, USA) was used for all statistical analyses.

Chemical Analysis

The remainder of the cleaned algal tissue from each sample that was not used for analysis of internal nutrients was ground in a blender in a small amount of methanol and immersed in 1:1 dichloromethane (DCM)/methanol (MeOH). Bulk collections and the individual thalli were extracted thoroughly in 1:1 DCM:MeOH over a 72 h period and then partitioned between hexane and water to remove salts and water-soluble components. The solvents were evaporated to yield a crude extract, which was then weighed to constant weight. The remaining algal material was dried to constant weight in an oven at 62°C to obtain estimates of the natural yields of the organic extracts. Individual samples were further partitioned through a Florisil (4 - 6 mg) column with 20% ethyl acetate/hexane to remove chlorophyll pigments from the extracts before quantitative analysis.

The crude extract of the bulk collections was partitioned by normal phase silica gel vacuum-flash chromatography in a 5 - 20% hexane/ethyl acetate gradient. Qualitative separation was achieved by preparative normal phase high performance liquid chromatography (HPLC). The HPLC system consisted of a Waters 501 HPLC pump and a R401 differential refractometer. The column was an Alltech Econosil 10U silica column (25 cm x 10 mm). Samples were injected on the column through a 2 ml loop. The compounds were separated in pure hexane or 5% ethyl acetate/hexane solvent systems.

Individual thalli were analyzed on a quantitative HPLC instrument with an integrator in 100% hexane. This system consisted of a Beckman model 110B solvent pump, model 156 refractive index detector and model 427 integrator. The analytical column was an Alltech Spherisorb S-5-W silica 5U column (25 cm x 4.6 mm). One-mg samples were injected onto the column in 10 µl through a 20 µl loop. A standard curve was obtained for the mixture of ochtodene and "isoochtodene" and for Peak 1 (Figures 4 a and b). The chemical structure for ochtodene was confirmed by comparison with previously reported chemical shifts for ^{1}H nuclear magnetic resonance spectroscopy (NMR) (McConnell and Fenical 1978, Paul et al. 1987). Similar techniques were used to investigate minor compounds.

Organic extract yields were calculated from dry mass of extract and algal material. The mass of pure compound per injection was calculated from the standard curve and converted to percent yield of pure compound from the total dry mass. Statistical analyses of the organic compound yields, dry mass were carried out as described for the internal

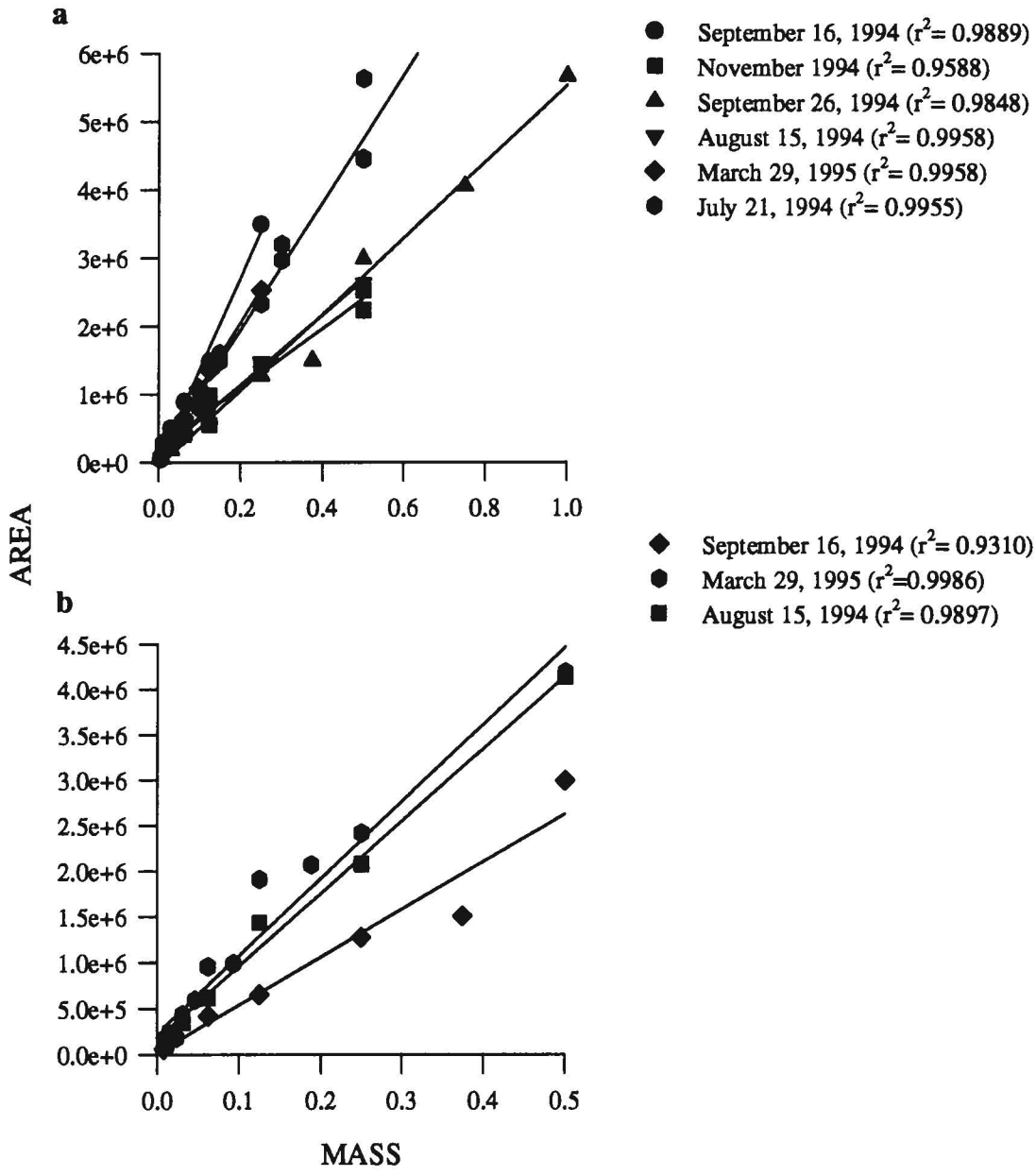

Figure 4. Standard curves are shown for samples of ochtodene (a) and Peak 1 (b) in 100% hexane on an analytical Beckman HPLC. Area is regressed with mass of pure compound.

nutrient analysis data. The mean number of minor peaks observed between the two major metabolites on the analytical HPLC traces was calculated for each individual. The major secondary metabolite concentrations were regressed with the internal nutrient levels of each thallus. Adjusted r^2 values are reported for the goodness of fit for each regression. R^2 measures the proportion of variance in the dependent data set and increases as new independent variables are added. Adjusted r^2 is adjusted for the number of independent variables in the data set and corrects for the inherent error in the calculation of r^2 (Sokal and Rohlf 1981).

Site-to-Site Variation

One bulk sample and between 14-17 individual samples were collected per site between August 25 and 31, 1994. Seaweed for the bulk extraction was collected haphazardly over the reef. Bulk extractions were carried out to obtain milligram quantities of pure compounds for identification by NMR and to use for calibration curves on the analytical HPLC. The samples collected to observe individual variation were collected at least 0.5 m apart. Five individuals of *Portieria hornemannii* collected from each site that were extracted for secondary chemistry were also analyzed for TKN and TKP as previously described. The mean yields of internal nitrogen and phosphorous stores were arcsin-squareroot transformed and compared by Kruskal-Wallis Test for differences among sites (Appendix I). Chemical extractions were carried out within 24 h of collection as previously described. Data analysis was carried out as previously

decribed. Chemical data were log transformed (Appendix II - VII). The mean number of peaks in the analytical HPLC traces was determined for each individual thallus (Appendix VIII - XIII).

In situ Fertilization Experiment

A field nutrient enrichment experiment was conducted at Gun Beach between May 20 - June 27, 1994. Toroidal seep-through bags of fertilizer were attached to metal screens (30 cm x 30 cm, center hole 12 cm x 12 cm) by cable ties. The screens were fastened to the reef by masonry nails at depths of 3 - 5 m around the base of individual *Portieria hornemannii* thalli. Commercially available agricultural fertilizers were used for nitrogen (Par Ex ID with isobutylidenediurea (IBDU), 34-0-0, time release), phosphorous (Triple Super Phosphate (TSP), 0-45-0) and combined (34-45-0) enhancement of the algae. The bags on the control screens were filled with dried sand previously collected from Gun Beach. A total of 96 screens were attached to the reef, 24 of each treatment and 24 control plates. During the 5 weeks of the experiment the treatment bags were replaced by divers by removing the depleted bags and attaching full bags to the screens with cable ties every 7 to 9 days.

Ambient reactive phosphate concentrations were measured around the treatment and control plates at the time the screens were placed by the plants, within 24 h and every 48 h until the fertilizer bags were replaced. Three hundred-ml water samples were collected in empty, acid washed nalgene bottles within 20 cm of the base of ten plants

from the first ten of each treatment and control plates divers encountered, varying with each sampling day. Samples were transported from Gun Beach to the Marine Laboratory in coolers and stored in the refrigerator until analysis. Phosphate concentrations were determined by reaction with a molybdic acid-ascorbic acid-trivalent antimony solution, followed by spectrophotometric analysis at 880 nm as described by Parsons et al. (1984b). A standard curve was obtained for reactive phosphorous for 5 cm and 1 cm cells (Appendix XIV). Ambient reactive phosphate concentrations were compared by a Kruskal-Wallis Test (Appendix XV) (Sokal and Rohlf 1981).

After 5 weeks, approximately half of the fertilized and control individuals were recovered from the reef for analysis of internal nutrients and secondary metabolites according to the procedures previously described. The remaining individuals died during the experiment. Internal nutrient data were arcsin-squareroot transformed (Appendix XVI). Chemistry data were log transformed (Appendix XVII - XX). The mean number of minor peaks was calculated for the control and each treatment (Appendix XXI - XXIV).

Laboratory Fertilization Experiments

An enhanced nutrient experiment was conducted at the University of Guam Marine Laboratory in shaded flow through tanks from August 25 - September 13, 1994. Thirty *Portieria hornemannii* individuals attached to rocks were collected from Anae Island by hammer and chisel on August 25, 1994 and transported back to the laboratory

in coolers containing seawater. Ten individuals (initial field controls) were analyzed for internal nutrients and secondary metabolites as previously described. Twenty of the algae were weighed while still attached to the rocks and then placed in separate, covered, flow-through tanks arranged in 2 rows of 10. Three treatments (enriched nitrate, enriched phosphate, and combined) and control tanks were assigned with a random numbers table. The seawater in the tanks was drawn from Pago Bay and UV-filtered to limit the growth of epiphytes.

'Pulsed' nutrient enrichment according to the methods of Lapointe (1987) and Lapointe and O'Connell (1989) was used for the fertilization of individual *Portieria hornemannii* for eighteen days. Nutrients were added to the treatment tanks in the evening, after the seawater flow was stopped, at approximately 6 times the concentration of the seawater being drawn from Pago Bay, nitrate (50 μM) in the form of $NaNO_3$ and phosphate (10 μM) in the form of NaH_2PO_4. Seawater flow was restored during daylight hours to prevent the water in the tanks from getting too warm. Ambient nutrient concentrations were measured in each tank periodically during the experiment in the evening after adding the 'pulse' treatments and in the morning before restoring seawater flow, to monitor nutrient depletion overnight. Nitrate concentrations were determined by nitrate reduction by shaking with cadmium followed by spectrophotometric analysis at 543 nm according to the methods of Jones (1984). A standard curve was obtained for reactive nitrate for a 5 cm and 1 cm cell (Appendix XXV). Absorption was regressed with nitrate concentration. Phosphate concentrations were determined as previously

described. At the end of the experiment the individuals of *Portieria hornemannii* were removed from the tanks and weighed while still attached to the rocks. Seaweed was prepared for internal nutrient and chemical analyses as previously described. Quantum flux density (μM quanta/m^2/s) was measured over 10 h (08:00 - 18:00) in 2 empty covered and 2 open tanks with a non-submersible model LI-190 SA quantum sensor (LI-COR 1000 Datalogger; LI-COR, Lincoln, Neb.) on a sunny day (February 21, 1995) to estimate the difference in light availability to shaded versus unshaded tanks.

Data were analyzed for significant differences among the control and three treatments as described for the *in situ* fertilization experiment. Nutrient depletion data were arcsin-squareroot transformed and compared by Kruskal-Wallis Test (Appendix XXVI and XXVII). The growth of *Portieria hornemannii* during the experiment was estimated by calculating the change in wet mass (final - initial). Percent growth data were log transformed and compared by One-Way ANOVA (Appendix XXVIII). Internal nutrient data were arcsin-squareroot transformed (Appendix XXIX). Percent yields of the extracts and major secondary metabolites were arcsin-squareroot transformed (Appendix XXX). The mean number of minor peaks was calculated for each individual thalli (Appendix XXXI - XXXIV).

A second laboratory fertilization experiment was conducted from February 13 - 24, 1995 using 20 unshaded tanks. Thirty individuals of *Portieria hornemannii* attached to rocks were collected from Anae Island. The experimental procedure was repeated as described above using open tanks. The duration of the experiment was

reduced to 11 days from 18 days. A second set of field controls (n=10) was collected on February 28, 1995 at the termination of the experiment. Samples were prepared and analyzed as described above.

The % change in wet mass (Appendix XXXV), mean number of minor peaks (Appendix XXXVI - XXXXI) and major secondary metabolite concentrations were regressed with the internal nutrient concentrations of the algae as previously described. Internal nutrient data were arcsin-squareroot transformed (Appendix XXXXII). TKN data were compared by One-Way ANOVA while TKP data were compared by Kruskal-Wallis Test. Percent yields of the extracts and major secondary metabolites were arcsin-squareroot transformed (Appendix XXXXIII - XXXXV).

RESULTS

Chemical Analysis

Two major peaks were observed in the analytical HPLC traces of all *Portieria hornemannii* extracts analyzed. The ^{1}H NMR trace (Figure 5) of the first major peak (retention time = 1.38 to 1.45 minutes) suggested that this compound is a triglyceride (peak 1) composed primarily of saturated fatty acids (evidenced by no olefinic protons in the 5.0 - 5.5 ppm range). The second major peak (retention time = 3.30 to 7.14 minutes) was a mixture of ochtodene and a structurally related compound, "isoochtodene". NMR analysis of the analytical extracts from the site-to-site variation study indicate that "isoochtodene" is at least 5% of the extracts from the *P. hornemannii* individuals collected from all sites. NMR analysis of the extracts from the control and treated algae from the *in situ* fertilization experiments were essentially triglyceride and ochtodene. The separation of "isoochtodene" from ochtodene by HPLC has proven to be difficult due to similar polarities and pure "isoochtodene" has not been available for characterization by structural determination. Although the second peak is a mixture it will henceforth be referred to as ochtodene . The retention time of ochtodene on the silica column varied depending upon the temperature in the laboratory. Retention time was optimized to 7 minutes when the temperature in the laboratory was kept at 23°C or cooler. Both ochtodene and triglyceride concentrations were analyzed quantitatively for site-to-site variation and changes due to enhanced fertilization in field and laboratory experiments.

Figure 5. ^{1}H NMR for the first major peak on the Beckman HPLC traces. A triglyceride composed primarily of saturated fatty acids.

Site-to-Site Variation

Portieria hornemannii showed significant site-to-site variation in mean algal dry mass (Figure 6a, p=0.0241), extract yield (Figures 6b, p=0.0001), ochtodene (Figure 6c, p<0.0001) and triglyceride (Figure 6d, p=0.0287) yields. The mean algal dry mass of the samples collected from Double Reef was approximately twice that of the samples collected from the other five sites (Figure 6a). The samples collected from Anae Island and Pago Bay exhibited a significantly greater extract yield than the samples collected from Double Reef. The extracts from Anae Island exhibited the highest mean yield of ochtodene, 1.11% $\pm$ 0.2 of the total dry mass (Figure 6c). Ochtodene yields in the Pago Bay and Gun Beach extracts were 0.63% $\pm$ 0.1 and 0.48% $\pm$ 0.03 of the total dry mass. The samples collected from Double Reef, Janum, and Tagachang exhibited the lowest yields, 0.30% $\pm$ 0.05, 0.26% $\pm$ 0.05 and 0.20% $\pm$ 0.03 of total dry mass, respectively. Tagachang samples, which had the lowest yield of ochtodene, exhibited the highest yield of triglyceride, 0.64% $\pm$ 0.1 of total dry mass. The samples collected from Double Reef had a significantly lower yield of triglyceride than samples collected from Tagachang.

There was no significant difference observed in the internal nitrogen (Figure 6e, p=0.5620) or phosphorous (Figure 6f, p=0.3952) stores among sites. The mean value for internal nitrogen for all the samples analyzed was approximately 1.6% of the dry mass. The mean value for internal phosphorous for all the samples was 0.15% of total dry mass. The N:P ratios of the *Portieria hornemannii* thalli collected from the Anae Island (21:1), Pago Bay (23:1), Gun Beach (38:1), Janum (32:1) and Tagachang (19:1) are higher than the algae collected from Double Reef (12:1). There was no

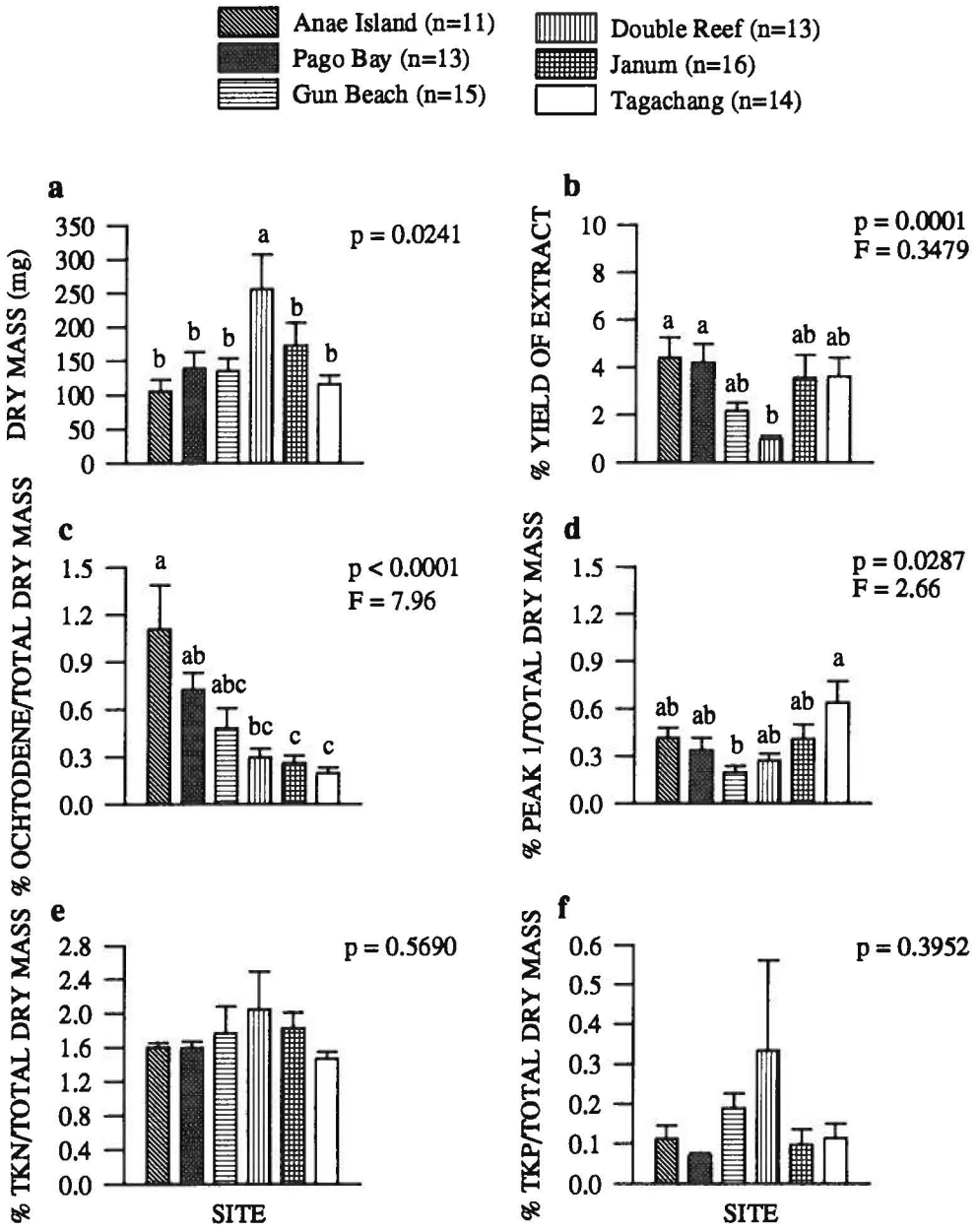

Figure 6. Histogram bars represent mean algal dry mass (a), extract (b), ochtodene (c), Peak 1 (d), TKN (e) and TKP (f) yields + one standard error for *Portieria hornemannii* collected from Anae Island, Pago Bay, Gun Beach, Double Reef, Janum and Tagachang August 25 - 31, 1994. Alga size, TKN and TKP data were analyzed using Kruskal-Wallis Test. Data for the yields were analyzed by One-Way ANOVA and by Tukey's HSD to compare means. Identical letters above bars indicate similar means ($p>0.05$). n=number of samples analyzed per site. n=5 for TKN and TKP analyses for all sites.

correlation observed between the yields of either ochtodene and internal nitrogen or phosphorous, or triglyceride and internal nitrogen and phosphorous for each site (Figures 7 and 8) or the pooled data set (Figure 9).

In addition to the quantitative differences noted above, the extracts of the *Portieria hornemannii* individuals exhibited qualitative differences. The number of minor peaks, henceforth referred to as compounds, observed in the analytical HPLC traces for the extracts in 100% hexane varied considerably within and among sites (Table 1). The extracts of the majority of the samples collected from Janum and Tagachang contained essentially ochtodene and triglyceride. Those extracts from Anae Island, Pago Bay and Double Reef had the largest mean number of compounds per extract, 8, 7 and 7, respectively. Anae Island samples were qualitatively different from those collected at the other sites exhibiting a minimum number of 5 minor compounds per trace, while the extracts from the other sites had a minimum of 2. The maximum number of compounds did not vary from site-to-site. ^{1}H NMR traces of the minor peaks suggest that a number of them may be acyclic monoterpenes.

In situ Fertilization Experiment

Analysis of water samples for reactive phosphorous taken during the *in situ* fertilization experiment showed that mean phosphate concentrations around the TSP and combined treated algae varied, but were generally higher than the concentrations around the control and IBDU treated algae on most days when water samples were taken (Table 2). Phosphate concentrations were significantly higher on Day 0

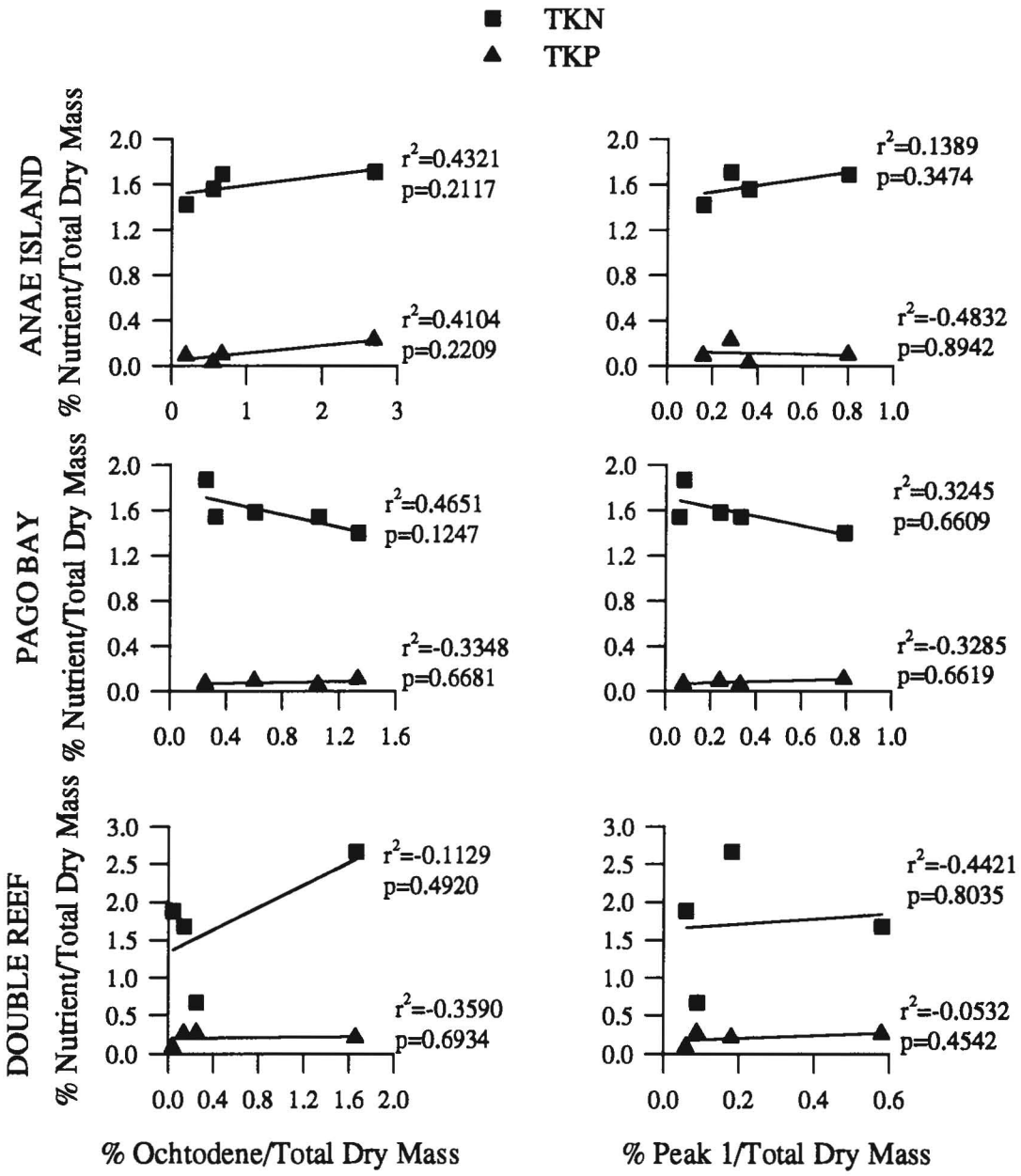

Figure 7. Ochtodene and Peak 1 yields regressed with internal nitrogen and phosphorous yields for *Portieria hornemannii* collected from Anae Island, Pago Bay and Gun Beach for site-to-site variation. Adjusted r^2 are shown for each site.

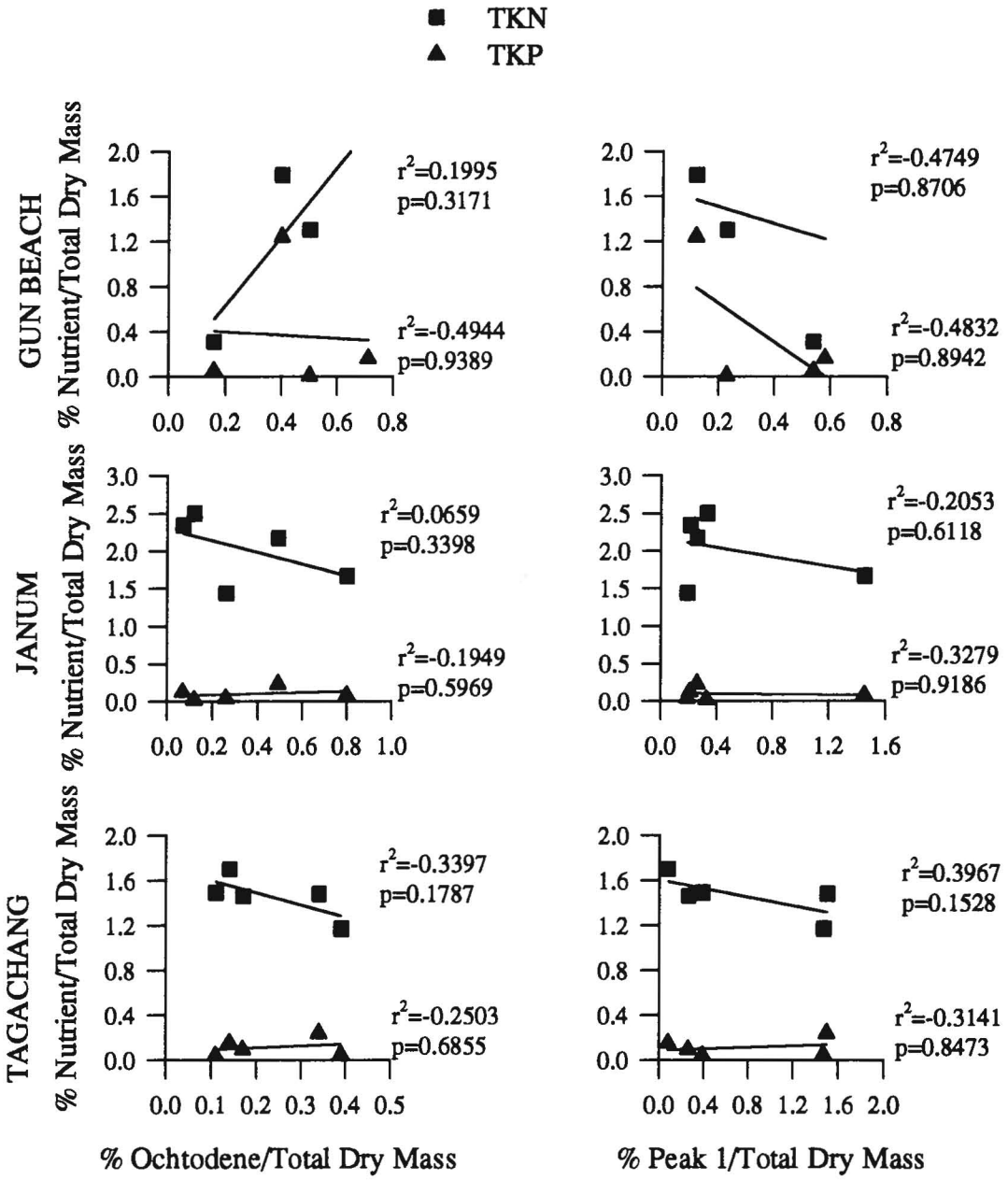

Figure 8. Ochtodene and Peak 1 yields regressed with internal nitrogen and phosphorous yields for *Portieria hornemannii* collected from Double Reef, Janum and Tagachang for site-to-site variation. Adjusted r^2 is shown for each site.

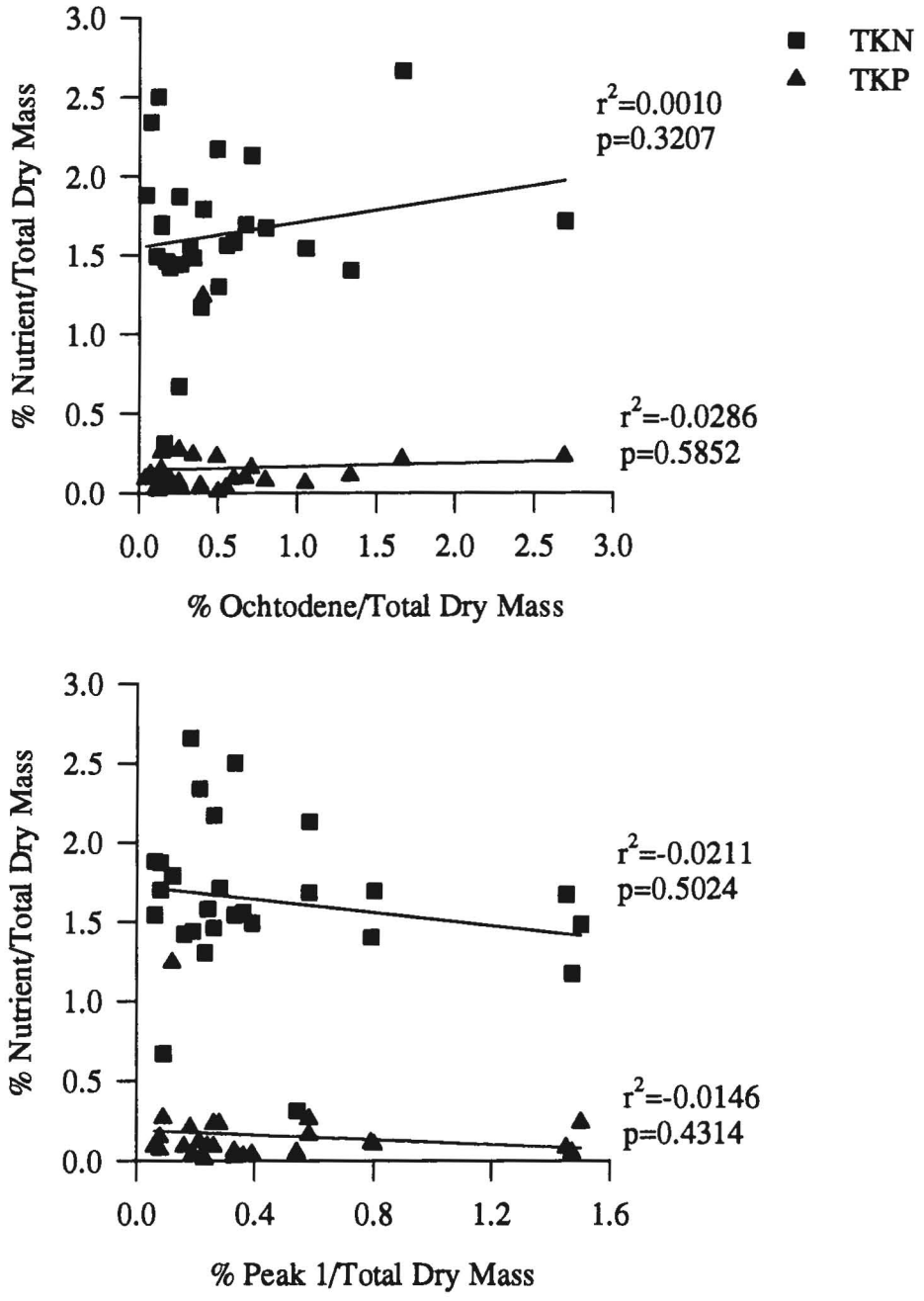

Figure 9. Ochtodene and Peak 1 yields regressed with internal nitrogen and phosphorous yields for *Portieria hornemannii* collected for site-to-site variation. The regression includes the data sets for all six sites. Adjusted r^2 vales are shown.

Table 1. Mean ± 1 S.D. , minimum and maximum number of peaks observed for the HPLC fractions analyzed for site-to-site variation in the secondary chemistry of *Portieria hornemannii* on the Beckman analytical HPLC with integrator in 100% hexane. n = number of samples analyzed. The number of individuals exhibiting the minimum and maximum number of peaks shown is indicated in ( ).

Site	n	Mean ± S.D.	Number of Peaks	
			Minimum (# Observed)	Maximum (# Observed)
Anae Island	11	7 ± 1	5(2)	9(2)
Pago Bay	13	8 ± 2	2(1)	11(1)
Double Reef	13	7 ± 3	2(1)	12(1)
Gun Beach	15	6 ± 2	2(3)	9(2)
Janum	16	4 ± 3	2(8)	11(1)
Tagachang	14	4 ± 2	2(4)	8(1)

Table 2. Mean phosphate concentrations (μM) ± 1 s.e. of seawater samples taken around the control, IBDU, TSP and combined treated *Portieria hornemannii* over time during two fertilization periods of the *in situ* fertilization experiment conducted at Gun Beach. Phosphate concentrations were analyzed by a Kruskal - Wallis Test.

Treatment		May 20 - May 28 Day 0*	Day 1	Day 5*	Day 7	Day 9	May 31- June 8 Day 0	Day 2	Day 5	Day 7
Control	x	1.25±0.34	6.91±6.66	0.41±0.65	0.36±0.06	0.65±0.22	11.70±7.94	2.33±1.58	0.78±0.31	0.39±0.15
	n	6	7	9	10	6	9	9	9	10
IBDU	x	0.65±0.45	1.12±0.38	0.57±0.07	0.51±0.07	3.30±0.72	18.30±11.14	0.45±0.18	1.03±0.41	0.84±0.54
	n	6	4	10	10	5	8	10	9	10
TSP	x	6.37±1.43	5.67±4.56	1.23± 0.55	1.75±0.81	1.73±0.36	23.10 ±8.44	2.61±0.70	0.39±0.15	0.88±0.45
	n	7	8	9	11	6	11	10	9	10
Combined	x	8.05±2.35	13.79±0.46	1.65±0.72	1.28±0.62	3.15±1.31	20.59±7.87	3.63±2.14	2.26±1.74	0.99±0.73
	n	6	6	10	10	7	9	9	9	8

* Indicates significant difference ($p < 0.05$) in phosphate concentration among treatments for the day.

(p=0.0034) and Day 5 (p=0.0013) of the samples collected between May 20-May 28. Concentrations of the reactive phosphates were always highest in the first 5 days. The fertilizer bags were replaced by day 7 or 9 when the concentrations around the treated plates dropped to levels observed around the control plates.

Mean algal dry mass (Figure 10a, p=0.1475) and ochtodene (Figure 10c, p=0.3531) concentration did not differ significantly among treatments. Organic extract (Figure 10b, p=0.0046) and triglyceride (Figure 10d, p=0.0269) yields for the combined treated algae were significantly higher than for the control and other two treatments. The mean extract yield of the combined treated algae was 14.53% $\pm$ 2.2 of the total dry mass, twice that observed for the control, IBDU and TSP treatments. The extract yields for the *Portieria hornemannii* extracted for the site-to-site variation portion of this study were also considerably lower, ranging from 1 to 4% of total dry mass (Figure 6b). The mean yield of triglyceride was 4.32% $\pm$ 0.9, double that of the control, IBDU and TSP treatments. The mean yields of triglyceride for all *P. hornemannii* used in the field experiment were considerably higher compared with the algae extracted from Gun Beach for the site-to-site variation study which had a mean yield of 0.27% $\pm$ 0.04 (Figure 6d).

Due to the fact that many of the algae collected at the end of the field experiments were very small, two or three small samples of the same treatment were pooled for internal nutrient analysis to increase the sample size. At least three individual samples were analyzed per treatment, however in some cases the number of samples per treatment is less because the phosphorous concentrations of some individual *Portieria hornemannii*

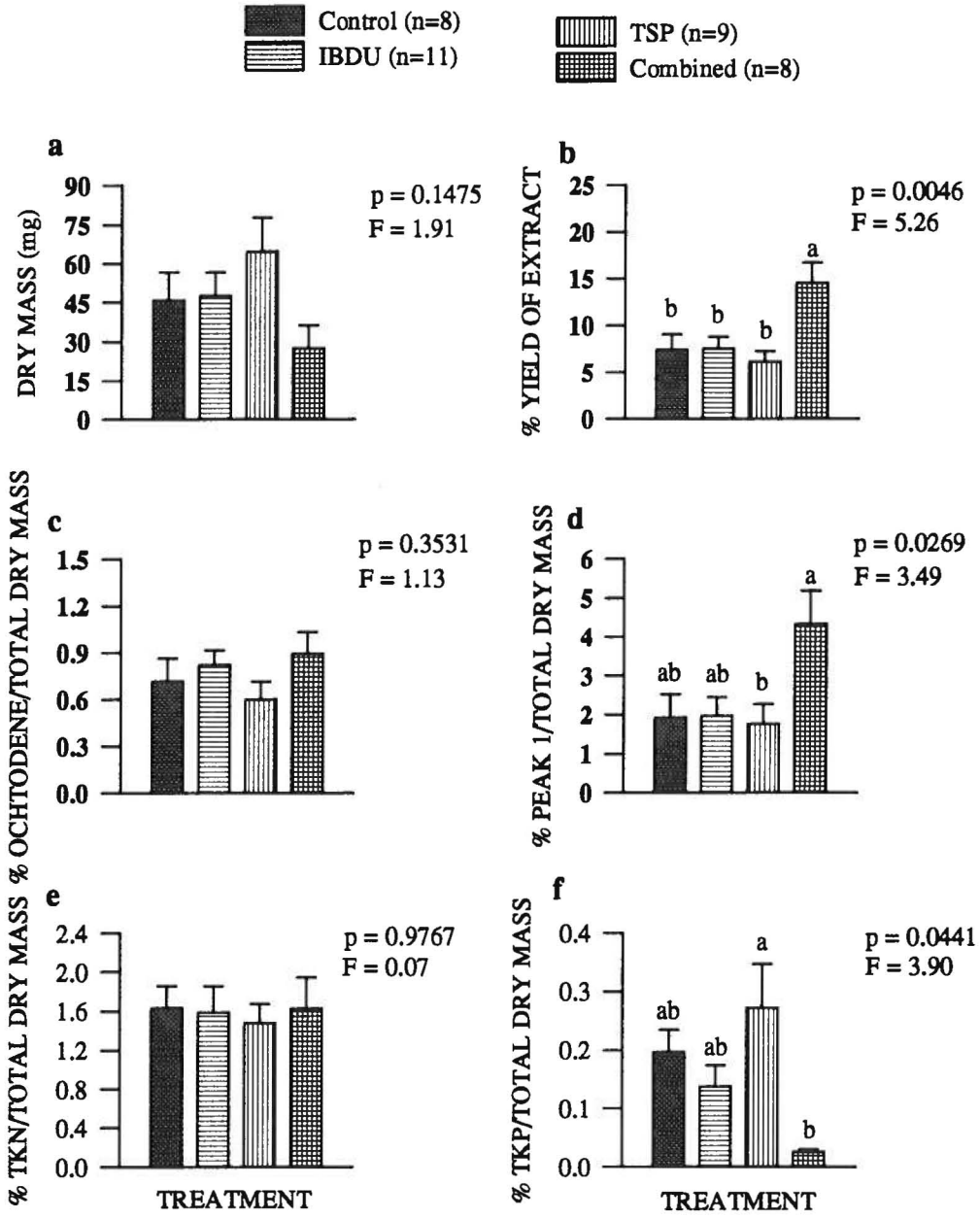

Figure 10. Histogram bars represent mean algal dry mass (a), extract (b), ochtodene (c), Peak 1 (d), TKN (e) and TKP (f) yields + one standard error for control, IBDU, TSP and combined treatment *Portieria hornemannii* used for the *in situ* ferilization experiment conducted at Gun Beach. Data were analyzed by One-Way ANOVA and by Tukey's HSD to compare means. Identical letters above bars indicate similar means ($p>0.05$). n=number of samples per treatment. n=5 for TKN IBDU and TSP treatments and TKP IBDU treatment; n=4 for TKN control and combined treatments and TKN IBDU treatment; n=3 for TKN control treatment; n=2 for TKN combined treatment. Small sample were combined for internal nutrient analysis.

were below detectable limits. No significant difference was observed in internal nitrogen content among treatments (Figure 10e, p=0.9767). The average for all treatments was approximately 1.6% of the dry mass. Significant differences were observed in internal phosphorous content (Figure 10f, p=0.0441). The individuals fertilized using the combined treatment were significantly lower in total phosphorous content. The internal phosphorous concentration of 2 of the 4 combined treatment samples analyzed was below the detection level of the instrument. The N:P ratios of the control (10:1) and TSP (9:1) treated algae were low, whereas the IBDU (35:1) and combined (80:1) treated algae were very high. The N:P ratio of the control and treated algae in the field experiment varied from the N:P ratio observed for the Gun Beach collection (21:1) in the site-to-site variation study. No correlation was observed between the yields of triglyceride and internal nitrogen or phosphorous (Figures 11 and 12). A weak negative correlation is observed for the yields of ochtodene and internal nitrogen in the TSP treated algae, r^2=0.7434 (p=0.0381). A weak positive correlation is observed for ochtodene and internal nitrogen yields for the combined treated thalli, r^2=0.9302 (p=0.1196) (Figure 12). The small sample size (n=2) for the total phosphorous analysis of the combined treatment did not allow for regression analysis.

The extracts from the algae used in the field experiment in June 1994 were less complex than those from the *Portieria hornemannii* thalli collected from Gun Beach for the site-to-site variation study in August 1994. There was some variability in the number of minor compounds among and within treatments; means only varied between

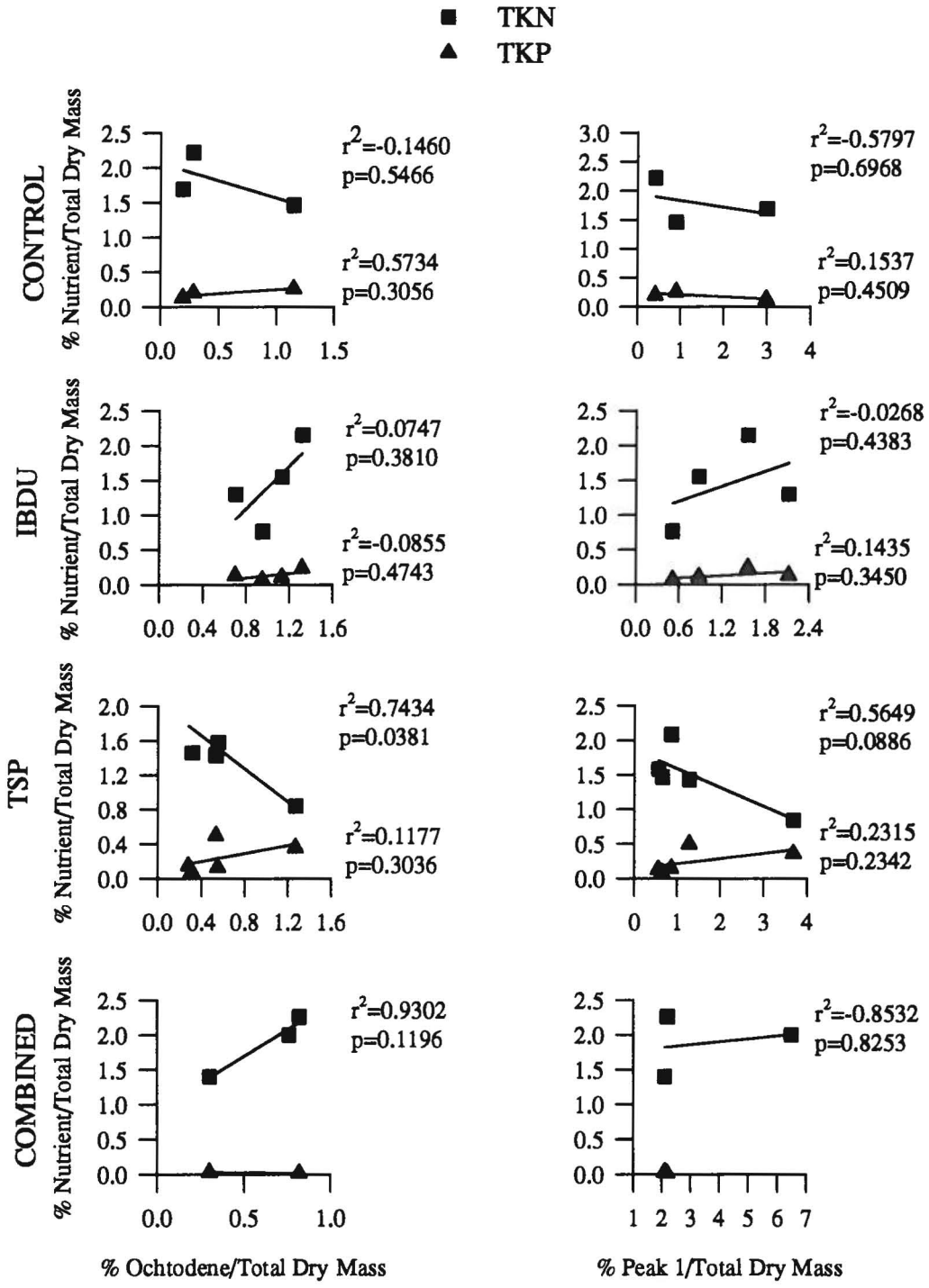

Figure 11. Ochtodene and Peak 1 yields regressed with internal nitrogen and phosphorous yields for the control and treated *Portieria hornemannii* used in the field fertilization experiment conducted at Gun Beach. Adjusted r^2 values are shown for the control and each treatment. The small sample size for the combined treatment (n=2) did not allow for regression analysis.

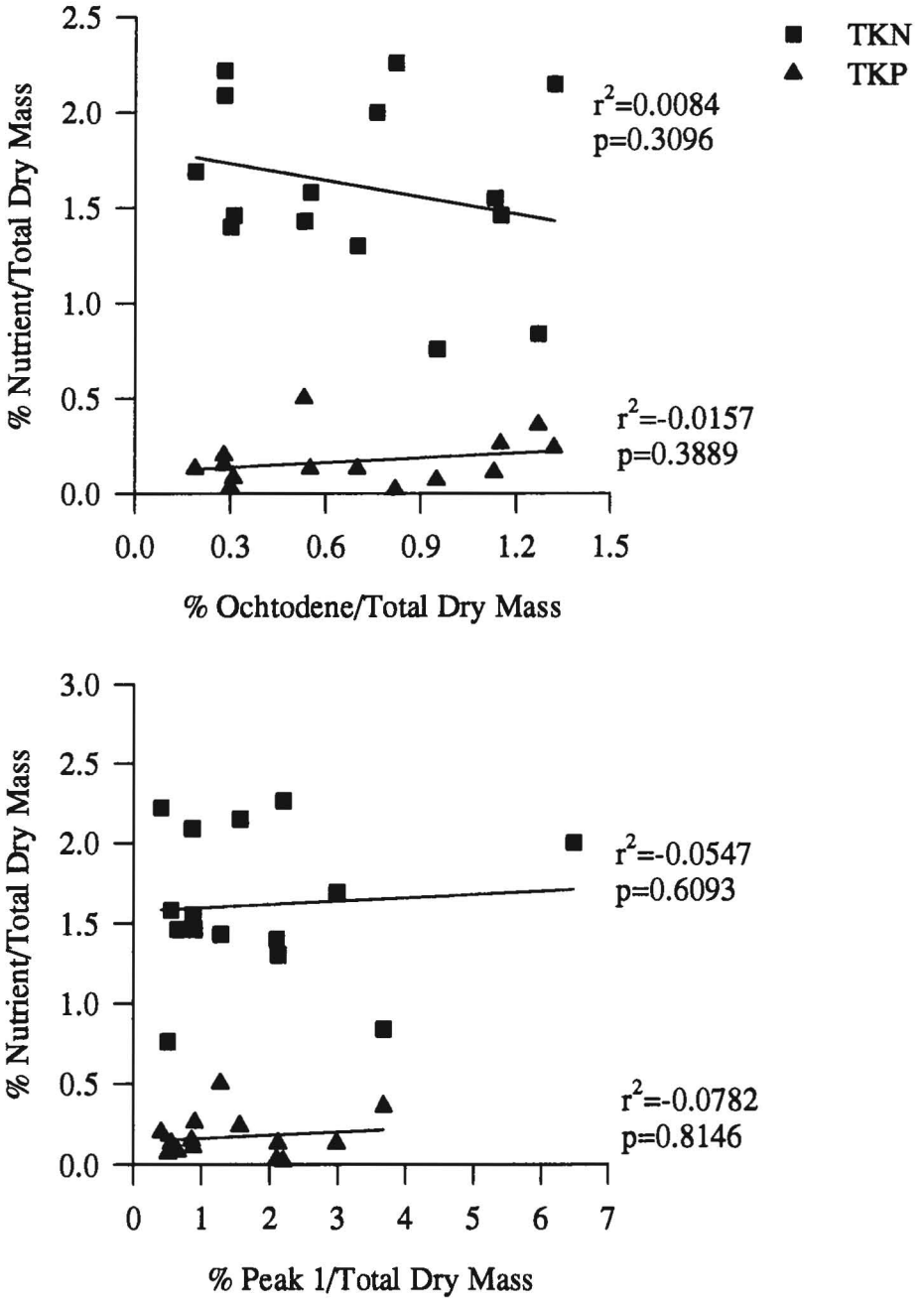

Figure 12. Ochtodene and Peak 1 yields regressed with internal nitrogen and phosphorous yields for *Portieria hornemannii* collected for the field experiment conducted at Gun Beach. The regression includes the data sets for the control and and three treatments. Adjusted r^2 values are shown.

3 and 5 among treatments with small standard deviations (Table 3). The minimum and maximum number of compounds did not vary to any degree among treatments.

Laboratory Fertilization Experiments

The light intensity on a sunny day available to the empty, shaded tanks used in the laboratory experiment conducted August-September 1994 during daylight hours (08:00 - 18:00) ranged from 60 - 456 μM, while the light intensity available to the unshaded tanks in the experiment conducted in February 1995 ranged from 60 - 2250 μM, a five fold increase (Figure 13). NO_3^- and $H_2PO_3^-$ were depleted from the control and enhanced nutrient treated tanks during the evening "pulse" (Figure 14). Nutrients in clean tanks without *Portieria hornemannii* were not depleted.

No significant difference was observed in growth (change in wet mass) of *Portieria hornemannii* among treatments in the laboratory experiment conducted in September - August 1994 (Figure 15, $p=0.6413$). The algae in all treatments showed an increase in wet mass with high variability within treatments. Mean algal dry mass (Figure 16a, $p=0.0003$), extract yield (Figure 16b, $p=0.0012$), ochtodene (Figure 16c, $p<0.0001$) and Triglyceride(Figure 16d, $p<0.0001$) yields differed significantly between the initial field control and the algae analyzed for the control and treatments in the shaded experiment. The algae collected for the field controls were smaller, but yielded significantly more extract. The ochtodene and triglyceride yields were $1.11\% \pm 0.3$ and $0.41\% \pm 0.07$, respectively, almost 4 times greater than for the algae kept in the tanks for 18 days. No significant difference was observed in mean algal size, extract yield,

Table 3. Mean ± 1 S.D., minimum and maximum number of peaks observed for the extracts for the *in situ* fertilization of *Portieria hornemannii* at Gun Beach on the Beckman analytical HPLC with integrator in 100% hexane. n = number of samples analyzed. The number of individuals with the minimum and maximum number of peaks shown is indicated in ( ).

Treatment	n	Mean ± S. D.	Number of Peaks	
			Minimum (# Observed)	Maximum (# Observed)
Control	8	3 ± 1	2(1)	5(2)
IBDU	11	5 ± 2	2(3)	8(1)
TSP	9	3 ± 1	2(1)	5(1)
Combined	8	4 ± 2	3(3)	7(1)
Gun Beach August 1994	15	6 ± 2	2(3)	9(2)

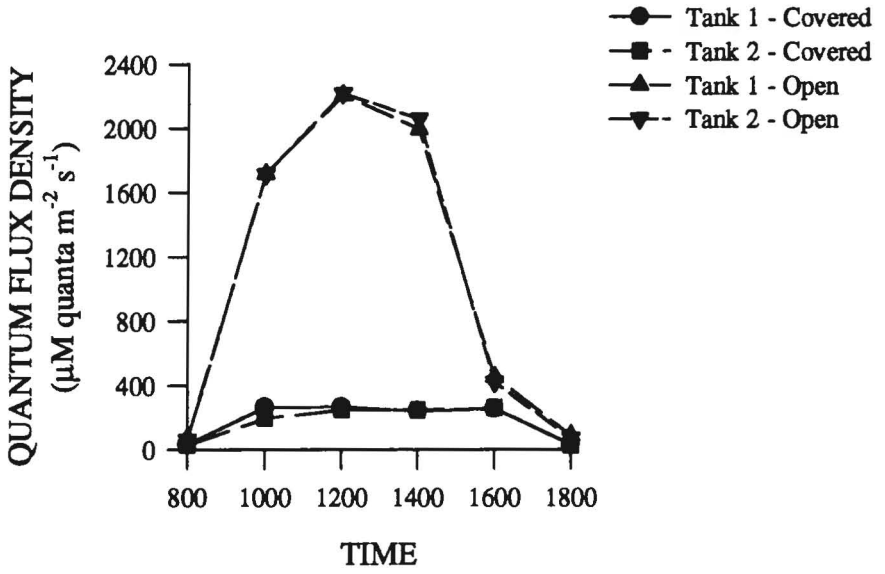

Figure 13. Quantum flux density (μM quanta m^{-2} s^{-1}) measured in empty covered and open tanks used to keep the individual *Portieria hornemannii* in the shaded and unshaded fertilization experiments. Measurements were taken over 10 hours (08:00-18:00) on a sunny day (February 21, 1995) using a non-submersible model LI-190 SA quantum sensor.

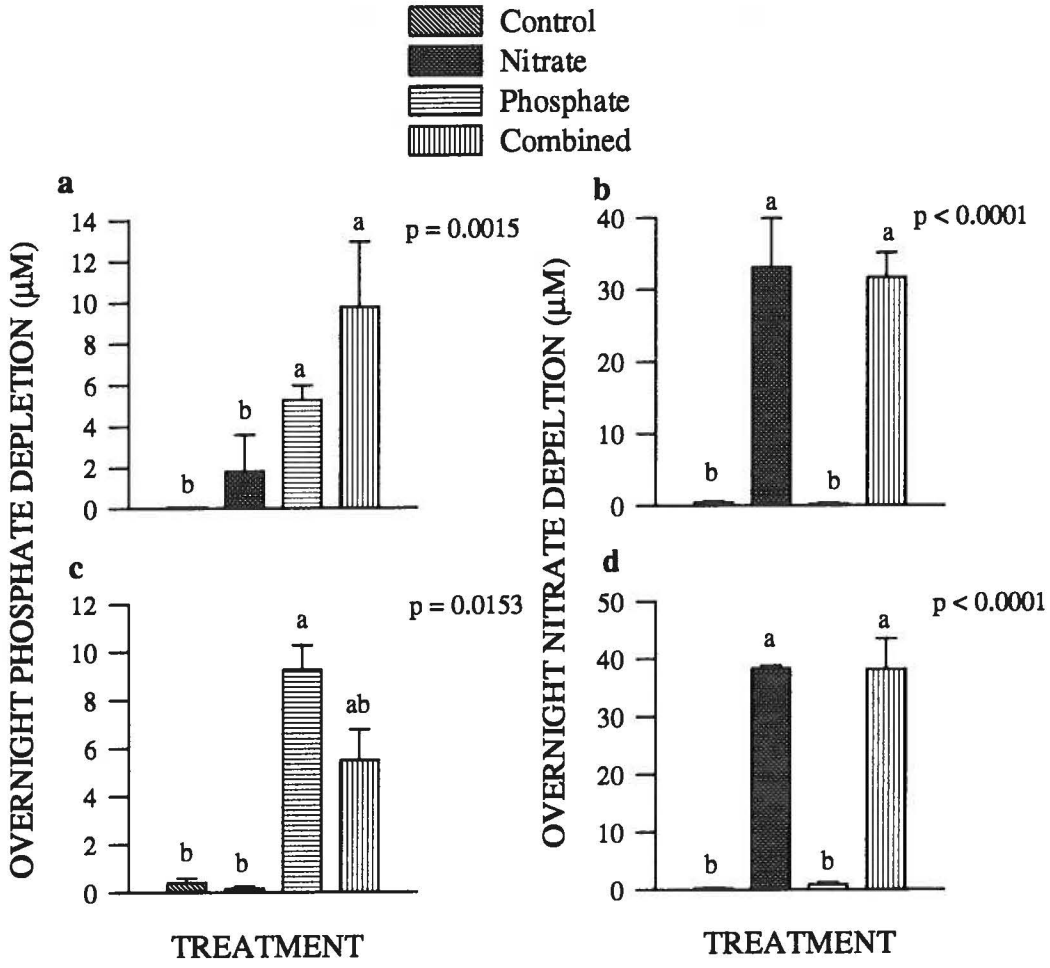

Figure 14. Phosphate (**a**, **c**) and nitrate (**b**, **d**) depleted from the laboratory tanks by the control and treated *Portieria hornemannii* on June 9-10,1994 (**a**, **b**) and June 15-16, 1994 (**c**, **d**). Depletion data were analyzed using Kruskal-Wallis Test. Identical letters above bars indicate means that do not differ ($p>0.05$). $n = 5$ for all treatments.

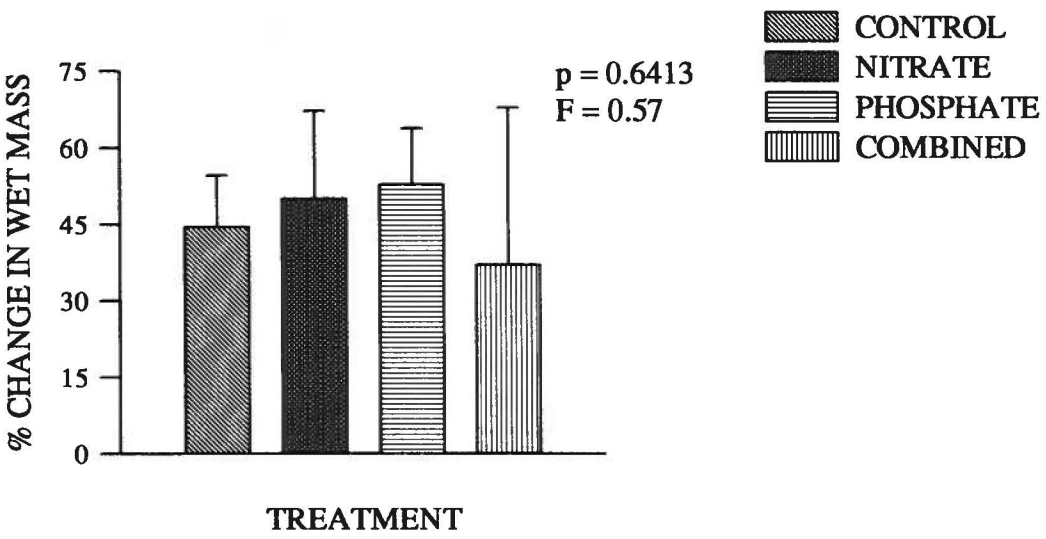

Figure 15. Histogram bars represent % change in wet mass for control and treated *Portieria hornemannii* in the shaded laboratory experiment conducted August - September 1994. The algae was weighed prior to fertilization and at the end of the experiment before internal nutrient analysis and extraction. Data was log transformed and analyzed by One-Way ANOVA. There was no difference observed in growth among treatments.

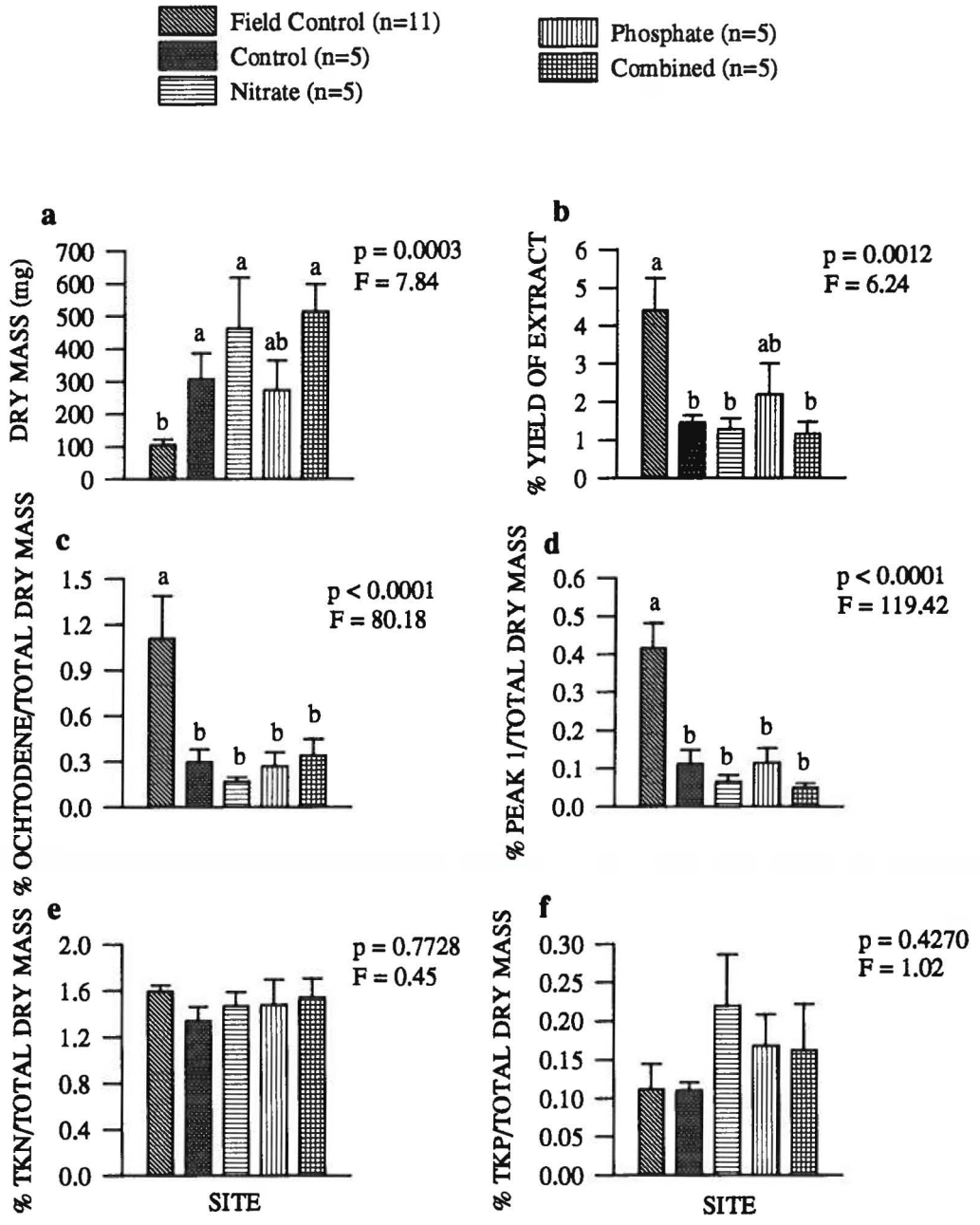

Figure 16. Histogram bars represent mean algal dry mass (a), extract (b), ochtodene (c), Peak 1 (d), TKN (e) and TKP (f) yields + one standard error for *Portieria hornemannii* used in the shaded laboratory fertilization experiment conducted in August - September 1994. Data for yields were analyzed by One-Way ANOVA and by Tukey's HSD to compare the means. Identical letters above bars indicate means that are not significantly different ($p>0.05$). n=5 for TKN and TKP field control samples: n=4 for TKN phosphate treatment and TKP control and combined treatments; n=3 for TKN nitrate treatment.

ochtodene and triglyceride concentrations among control and various treatment algae used in the experiment. No significant difference was observed for internal nitrogen (Figure 16e, p=0.7728) and phosphorous (Figure 16f, p=0.4270) content among the field control, laboratory control and various fertilization treatments. The mean internal nitrogen concentration for all *P. hornemannii* analyzed was 1.6% of the total dry mass. The N:P ratio of the field control algae (21:1) was higher than the control (13:1), nitrate (9:1), phosphate (10:1) and combined (14:1) treated thalli. No correlation was observed between the yield of either ochtodene and internal nitrogen or internal phosphorous, or triglyceride and internal nitrogen or internal phosphorous content (Figures 17 and 18). A weak correlation was observed between the yields of triglyceride and internal phosphate for the nitrate treated algae, r^2=0.9792 (p=0.0651) (Figure 18).

Qualitative analysis of the number of minor compounds in the analytical HPLC traces suggested that the extracts were complex with high variability within and among treatment and control algae (Table 4). The mean number of compounds in a trace was between 7 and 9 with small standard deviations. The minimum number of compounds was at least 4 and varied considerably among treatment, while the maximum number if peaks was about the same for all treatments. The extracts of the algae for the shaded experiment exhibited similar complexity to the field controls.

The *Portieria hornemannii* used in the unshaded laboratory fertilization experiment conducted February 1995 did not exhibit significant differences in growth among treatments (Figure 19, p=0.9391). Mean algal dry mass (Figure 20a, p=0.0420)

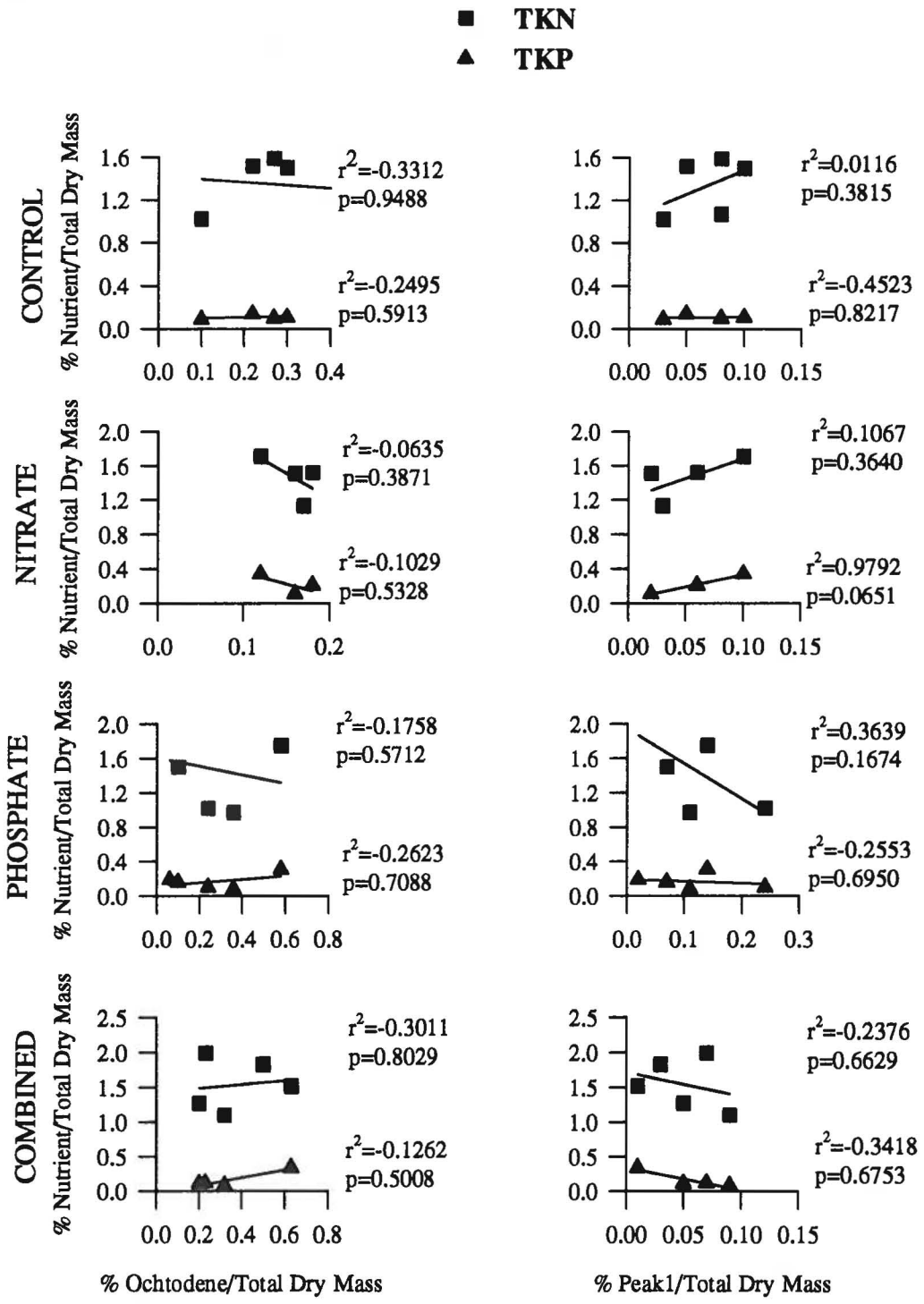

Figure 17. Ochtodene and Peak 1 yields regressed with internal nitrogen and phosphorous yields for the control and treated *Portieria hornemannii* used in the unshaded laboratory experiment conducted August - September 1994. Adjusted r^2 values are shown.

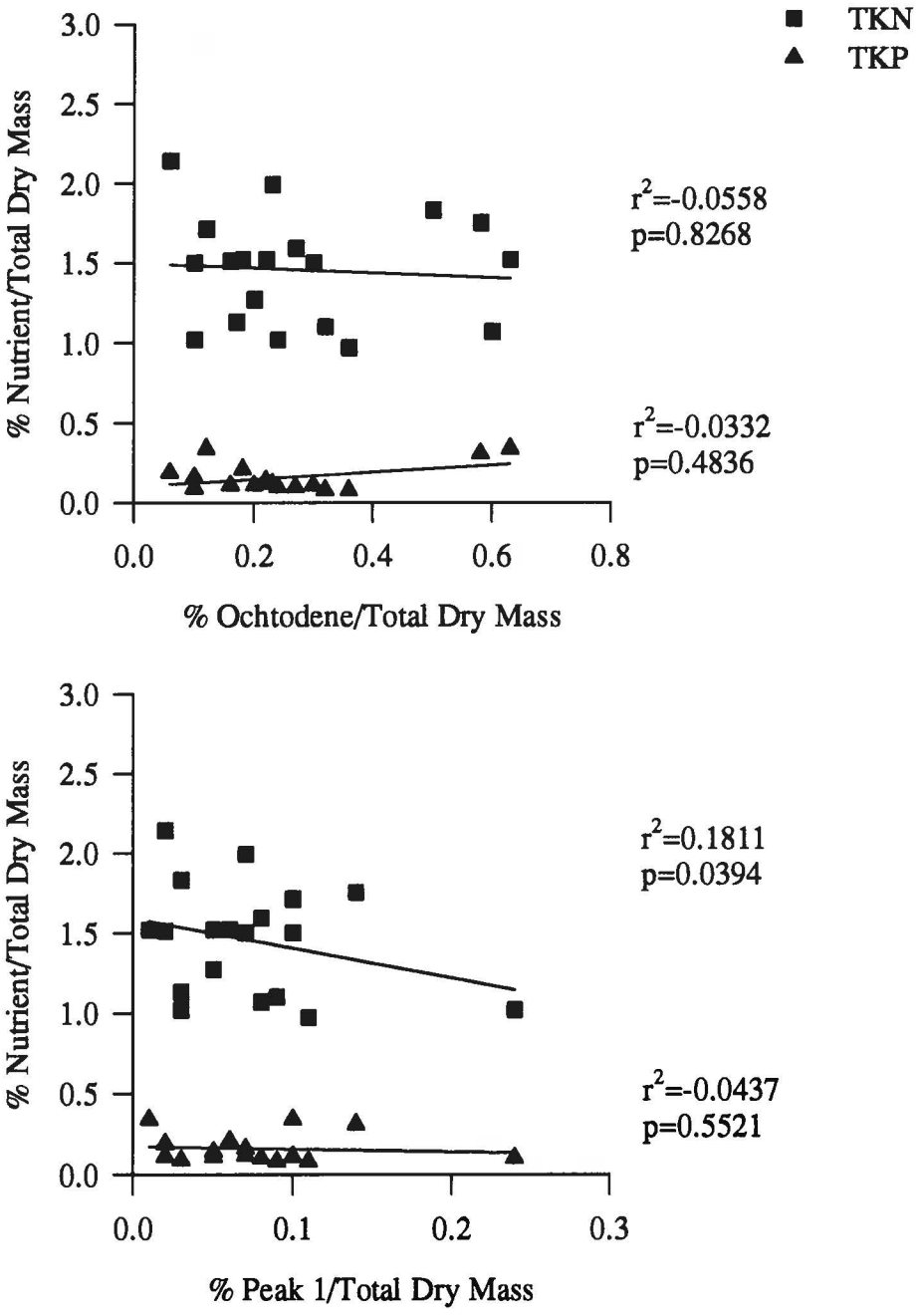

Figure 18. Ochtodene and Peak 1 yields regressed with internal nitrogen and phosphorous yields for *Portieria hornemannii* used in the shaded laboratory experiment conducted August - September 1994. The regression includes the sets for the control and three treaments. Adjusted r^2 are shown.

Table 4. Mean ± 1 S.D., minimum and maximum number of peaks observed for the extracts for the shaded laboratory fertilization experiment conducted August - September 1994. *Portieria hornemannii* extracts were analyzed on the Beckman analytical HPLC with integrator in 100% hexane. n = number of samples analyzed. The number of individuals with the minimum and maximum number of peaks shown is indicated in ( ).

Treatment	n	Mean ± S. D.	Number of Peaks	
			Minimum (# Observed)	Maximum (# Observed)
Control	5	8 ± 2	6(1)	10(1)
Nitrate	5	9 ± 2	5(1)	10(2)
Phosphate	5	7 ± 3	4(1)	10(2)
Combined	5	9 ± 1	8(2)	10(1)
Field Controls	11	7 ± 1	5(2)	9(2)

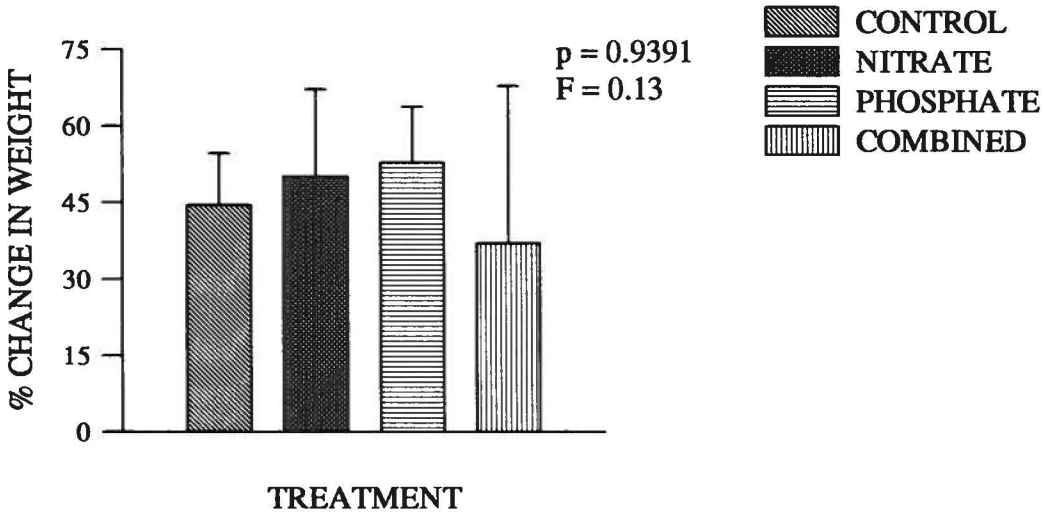

Figure 19. Histogram bars represent % change in wet mass for control and treated *Portieria hornemannii* in the unshaded laboratory experiment conducted February 1995. The algae was weighed prior to nutrient analysis and extraction. Data was log transformed and analyzed by a One-Way ANOVA. There is no significant difference observed in growth between treament. Noticeable variation is observed within treatment.

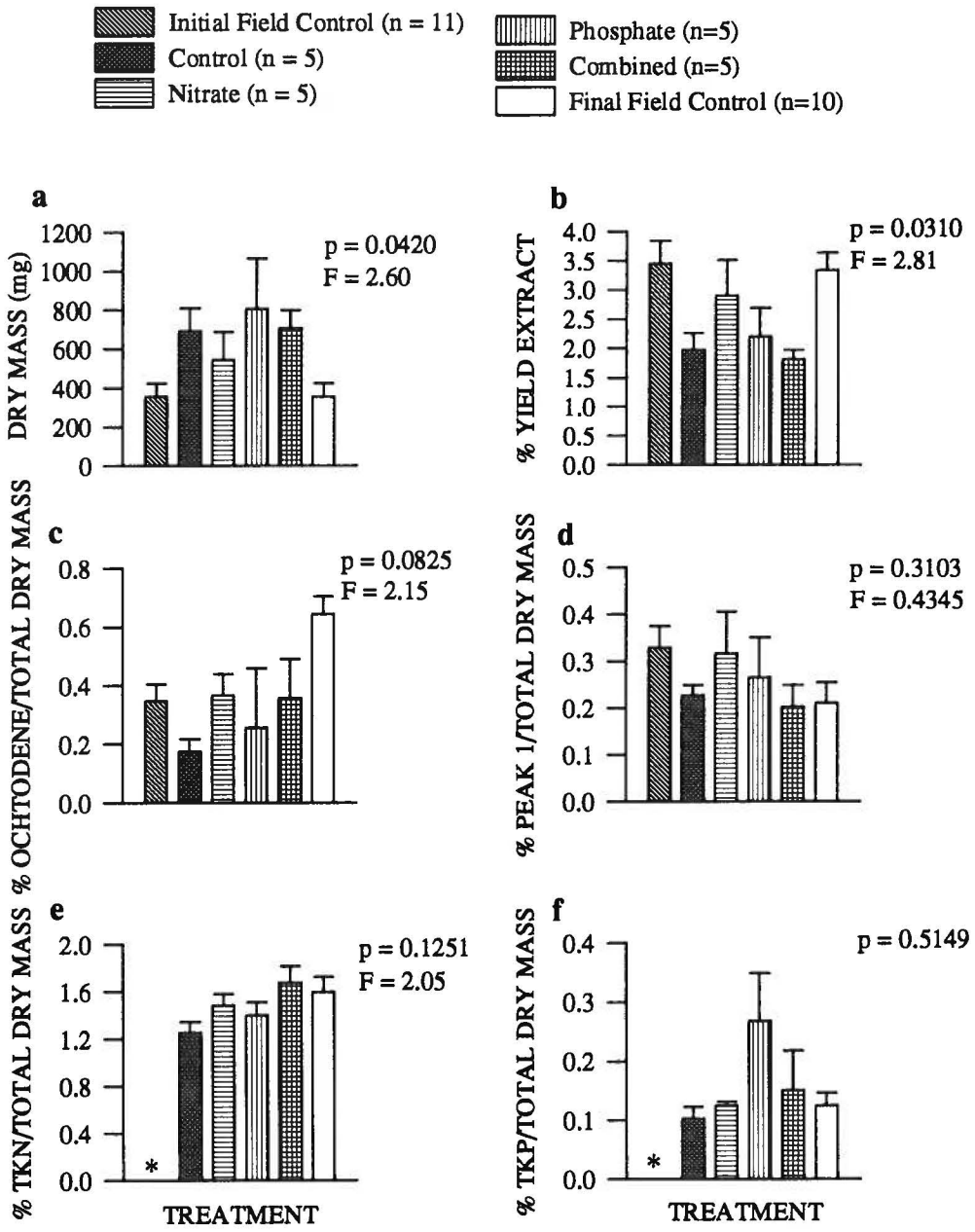

Figure 20. Histogram bars represent mean algal dry mass (a), extract (b), ochtodene (c), Peak 1 (d), TKN (e) and TKP (f) yields + one standard error for *Portieria hornemannii* used in the unshaded laboratory fertilization experiment conducted in February 1995. Data for algal dry mass, extract, ochtodene, Peak 1 and TKN yields were analyzed by One-Way ANOVA. Tukey's HSD comparison of means did not indicate differences among the dry mass and extract yield means. Data for TKP yields were analyzed by Kruskal-Wallis Test. n=5 for TKN and n=4 for TKP analyses for all treatments. * Samples collected for the initial field control were not analyzed for internal nutrients.

and extract yield (Figure 20b, p=0.0310) were significantly different between the field controls and the algae kept in the laboratory tanks. Tukey's HSD test for comparison of means for both algal dry mass and extract yield failed to show a difference in means among treatments (rejection level p>0.05). The *P. hornemannii* collected as field controls from Anae Island were approximately one half the size of the individuals at the end of the experiment. The extract yields of the February 9 and February 28, 1995 field controls and the nitrate treatment were high, 3.25% $\pm$ 0.42, 3.48% $\pm$ 0.81 and 2.89% $\pm$ 0.62, respectively. The yields of ochtodene (Figure 20c, p=0.0825) and Triglyceride (Figure 20d, p=0.3103) did not differ significantly among the treatments and controls. A potential trend in temporal variability was detected in ochtodene production. The field controls collected on February 28, 1995 exhibited a mean ochtodene yield 0.57% $\pm$ 0.09, twice that of the field controls collected February 9, 1995 and the control and treatment algae used for the experiment.

No significant differences were observed for the internal nutrient stores of nitrogen (Figure 20e, p=0.1251) and phosphorous (Figure 20f, p=0.0310) among the control and the treated algae. Mean values for the internal nitrogen stores ranged between 1.2 and 1.6%. Increased stores of phosphate were observed in the phosphate treated algae of 0.27% $\pm$ 0.08 compared with 0.1% seen for the other treatments. Field controls collected on February 9, 1995 from Anae Island were not analyzed for internal nutrient stores. The N:P ratio of the combined treated algae (41:1) was above the Redfield ratio while the N:P ratios of the control (13:1), nitrate treated (12:1), phosphate treated (9:1) and field control (13:1) algae were lower. No correlation was observed

between the yields of either ochtodene and internal nitrogen or internal phosphorous, or triglyceride and internal nitrogen content (Figures 21 and 22). Potential positive correlations were observed between triglyceride and internal phosphorous yields for the nitrate and phosphate treated algae, r^2=0.7088 (p=0.1023) and r^2=0.7954 (p=0.0707), respectively, similar to that observed in the nitrate treatment of the shaded experiment (Figure 21). The mean number of minor compounds observed in the HPLC traces for the extracts for the field controls, laboratory control and treatments was consistently 9 and 10 with a small standard deviation. The extracts from these algae were complex, consistent with all other extracts of *Portieria hornemannii* collected from Anae Island throughout this study.

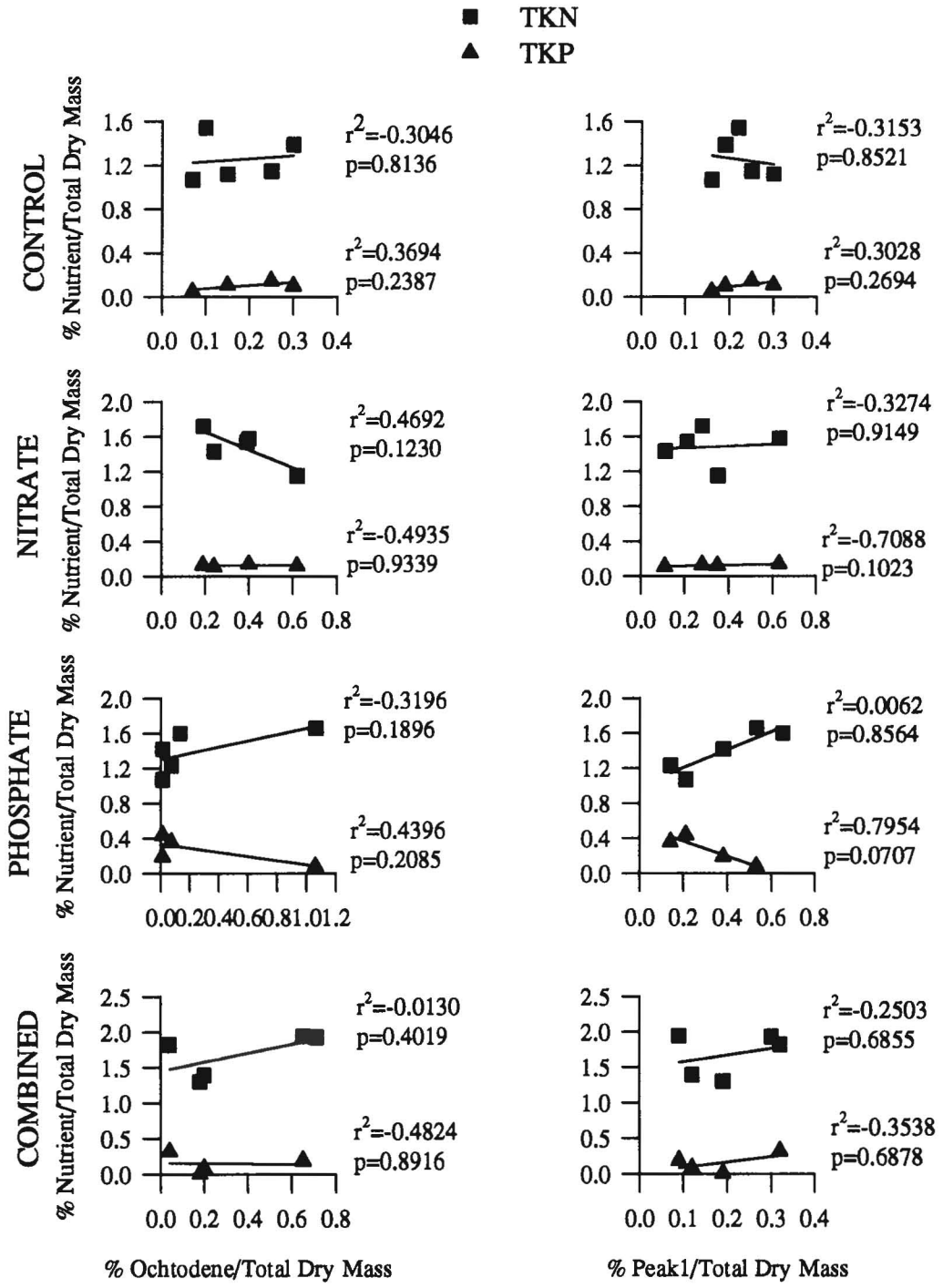

Figure 21. Ochtodene and Peak 1 yields regressed with internal nitrogen and phosphorous yields for the control and treated *Portieria hornemannii* used in the unshaded laboratory experiment conducted February 1995. Adjusted r^2 values are shown.

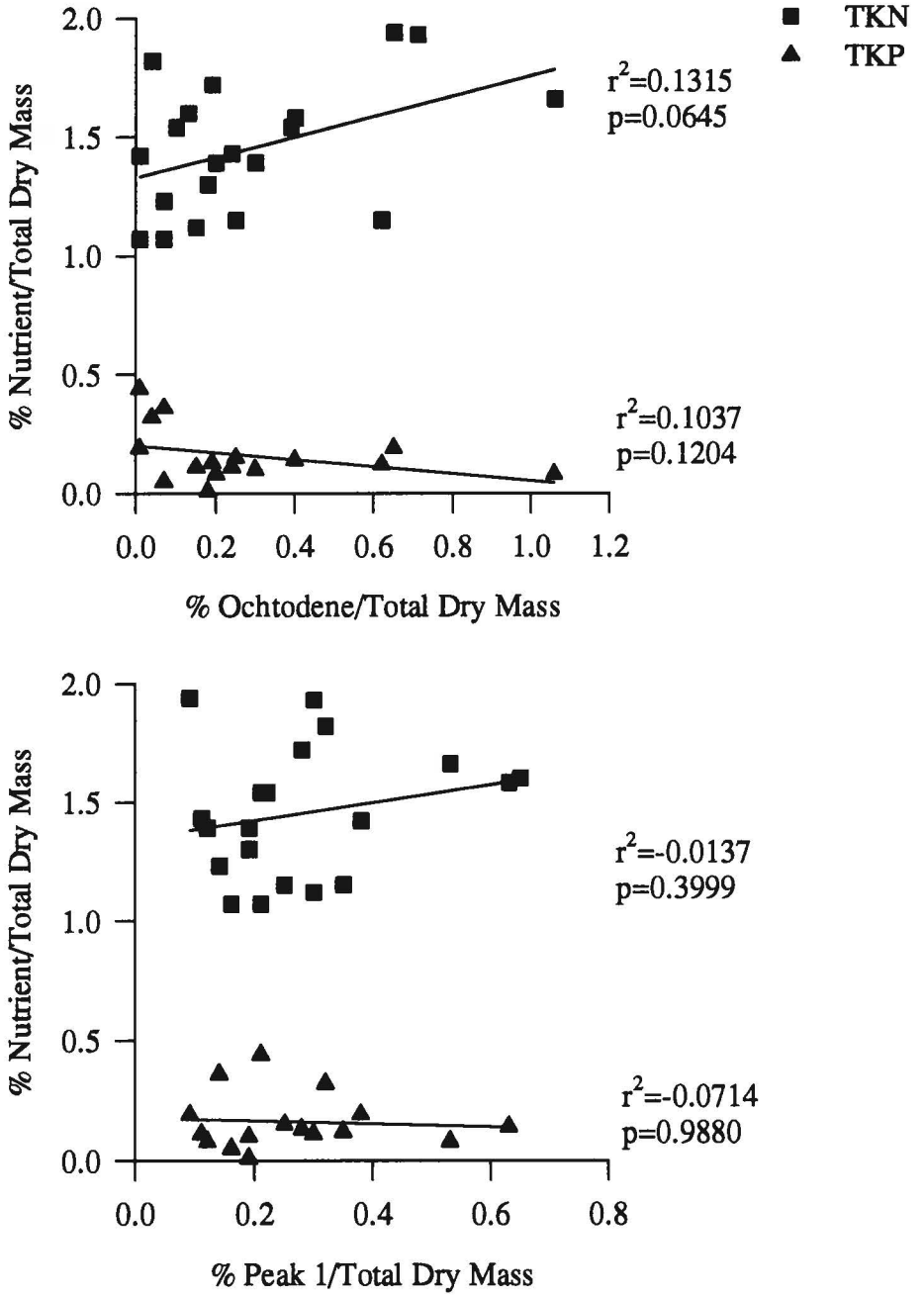

Figure 22. Ochtodene and Peak 1 yields regressed with internal nitrogen and phosphorous yields for *Portieria hornemannii* used in the unshaded laboratory experiment conducted February 1995. The regression includes the data sets for the control and three treaments. Adjusted r^2 are shown.

DISCUSSION

Collections of *Portieria hornemannii* from Anae Island, Pago Bay, Double Reef, Gun Beach, Janum and Tagachang on Guam exhibited significant site-to-site quantitative variation in ochtodene and triglyceride biosynthesis (Figures 5c and 5d). Quantitative variation in the production of similar compounds between sites has been noted for collections of *P. hornemannii* in Hawaii and Australia, but these studies did not examine individual thalli to account for natural variation in algal populations (Burreson et al. 1975a,b, Coll and Wright 1989, Wright et al. 1991). Remarkable qualitative variation has been well documented for *P. hornemannii* acyclic and cyclic monoterpene biosynthesis throughout the Pacific (Burreson et al. 1975a,b, Coll and Wright 1989, Wright and Coll 1990, Wright et al. 1991, Fuller et al. 1992, Fuller et al. 1994). Although the chemistry for the minor compounds has not been carefully investigated in this study, qualitative differences in the number of potential minor compounds are seen among sites by analytical HPLC (Table 1).

Ochtodene and triglyceride concentrations were neither positively nor negatively correlated with internal nitrogen or phosphorous stores at individual sites or as an overall data set (Figures 6e and 6f). Previous studies with the marine brown alga *Fucus vesiculosus* showed a negative correlation between intracellular nitrogen concentrations and polyphenolic content (Ilvessalo and Tuomi 1989). Thalli collected from low-N sites had increased phenolic levels compared with thalli collected from high-N sites. Because of the correlation of internal nitrogen stores with polyphenolic

concentrations, nutrient availability was proposed to account for the geographic variability in secondary metabolite biosynthesis in *F. vesiculosus*, whereas there is no evidence to support this hypothesis for the site-to-site variability in monoterpene concentration observed for *P. hornemannii* populations on Guam.

The significant differences in ochtodene production in *Portieria hornemannii* may be due to genetic variability or ecophenotypic effects in populations among sites. Variation in monoterpene production has been shown to be under strong genetic control in many species of terrestrial plants (Zavarin et al. 1990). Red algal spores rely on currents for transport to potential settling areas (Lobban and Harrison 1993). Different genetic populations of *P. hornemannii* may arise at various sites on Guam depending upon current patterns transporting algal spores. Monoterpenes have also been shown to be induced by herbivory in terrestrial environments (Lerdau et al. 1994). The effect of herbivory on monoterpene production was not addressed in this study, but should be considered as a possible factor in the site-to-site variability of ochtodene production for *P. hornemannii* on Guam.

Alternatively, variation could result depending upon the phenotypic development stage of a population. Considerable variability has been seen in the density of *Portieria hornemannii* at different sites around Guam, suggesting that there is seasonal variation in settlement for this alga. Therefore, algae collected during the site-to-site variation study may have been at different stages of development. This is further suggested by the quantitative differences in the ochtodene concentrations observed between the *P. hornemannii* collected in August 1994 from Gun Beach for

site-to-site variation (0.4% dry mass) (Figure 6c) and the thalli used in the field experiment conducted in June 1994 (0.6 - 0.9% dry mass) (Figure 10c). Similar differences were also observed for the February 9, 1995 (0.3% dry mass) and February 29, 1995 (0.6% dry mass) (Figure 16c) field controls used in the unshaded laboratory. Temporal variation in monoterpene biosynthesis has been suggested from bulk extracts from different collections from the Great Barrier Reef and the Philippines (Coll and Wright 1989, Fuller et al. 1992, Fuller et al. 1994) and may be due to age differences of the thalli (Brun et al. 1991). Monoterpene concentrations have been shown to vary with age in *Mentha xpiperita*, depending upon the developmental stage of the storage structures (Brun et al. 1991) which are produced early in the life history of terrestrial plants (Falk et al. 1990, Lerdau et al. 1994).

Studies of the carbon/nutrient balance hypothesis in the marine environment have focused on the temperate brown alga, *Fucus vesiculosus*. The polyphenolics produced by this alga were reported to accumulate under nitrogen deficiency, while carbon content did not seem to influence secondary metabolite production. From these results it was hypothesized that external resource availability could modify the accumulation of polyphenolic compounds (Ilvessalo and Tuomi 1989). A more direct test of the carbon/nutrient balance hypothesis with *F. vesiculosus* (Yates and Peckol 1993) has shown that polyphenolic production is inversely proportional to nitrogen availability, suggesting that algae that produce polyphenolics should respond to changes in nutrient availability as predicted by the carbon/nutrient balance hypothesis.

In this study with the red alga, *Portieria hornemannii*, neither triglyceride nor ochtodene concentrations decreased due to the enhanced nitrogen and phosphorous regimes in the field, shaded or unshaded laboratory experiments. Triglyceride yields were significantly increased across all treatments in the field experiment compared with those algae extracted for site-to-site variation and the laboratory experiments. Studies with the diatoms *Chaetoceros gracilis* and *Phaetodactylum tricornutum* reported that nutrient stress increased triglyceride concentration (Parrish and Wangersky 1987, Lombardi and Wangersky 1991), while another study with seven species of microalgae reported that not all species accumulated lipids under N-depletion (Reitan et al. 1994). In some cases, either no correlation was shown or total lipid concentration decreased. Increased triglyceride yields across all treatments suggests that there may have been some cross fertilization among treatments due to mixing. High N:P ratios in the IBDU and combined treated algae compared with the controls and TSP treated algae suggest that localized fertilization of *P. hornemannii* occurred, therefore it is unclear if there was significant cross fertilization among treatments.

The response of monoterpene biosynthetic pathways in terrestrial plants to changes in nutrient availability has varied considerably (Mihaliak and Lincoln 1985, Muzika et al. 1989, Lerdau et al. 1994, Hartley et al. 1995). In some cases, increased monoterpene concentrations were correlated with decreased nitrogen availability, while some found the opposite relationship and others saw no effect at all (Lerdau et al. 1994). A recent study with Sitka spruce (*Picea sitchensis*) seedlings, which

produce polyphenolics, tannins and monoterpenes, showed that polyphenolic and tannin concentrations varied within the predictions of the carbon/nutrient balance hypothesis, while monoterpene concentrations did not vary at all during the time frame of the experiment (Hartley at el. 1995).

Physiological studies of terrestrial plants that produce monoterpenes have shown that monoterpenes are synthesized and stored in discrete cells called glandular tubules, resin ducts or resin cavities (Falk et al. 1990, Brun et al. 1991, Lerdau et al. 1994). Plants require these specialized storage cells to prevent auto-cytoxicity (Lerdau et al. 1994). The variable response of monoterpene biosynthesis to nutrient stress has been suggested to depend more on how resource availability limits photosynthate supply which controls the differentiation of storage cells in the plant than on the availability of excess carbon for monoterpene biosynthesis (Lerdau et al. 1994). Red algae are also known to produce vesicles for storage (Lobban and Harrison 1993), therefore the same physiological considerations may need to be taken into account when investigating the affect of nutrient availability on monoterpene biosynthesis in red algal species.

An increase in the production of storage cells and/or monoterpene concentrations resulting from an increase in nutrient availability may only occur if the algae are nutrient-limited at the time of fertilization (Mihaliak and Lincoln 1985). N:P ratios for *Portieria hornemannii* collected for the site-to-site variation, study, except at Double Reef, and as field controls for the shaded laboratory experiment are high suggesting that some populations of this alga on Guam are P-limited. All thalli

extracted for the site-to-site variation study and the nutrient enrichment experiments exhibited a steady mean nitrogen content of 1.6%, whereas differences were observed in internal phosphorous concentrations. This observation is consistent with the hypothesis that tropical algae in carbonate-rich waters are P-limited unlike temperate species in siliciclastic waters, which are N-limited (Littler et al. 1991, Lapointe et al. 1992a, Littler et al. 1992, Lobban and Harrison 1993, Rietan et al. 1994, Chopin et al. 1995).

Qualitative changes in monoterpene composition were observed during enhanced fertilization studies with the grand fir, *Abeis grandis*, in lieu of quantitative changes, suggesting that the monoterpene biosynthetic pathways in the tree may be regulated by nutrient changes (Lerdau et al. 1994). Potential qualitative differences in monoterpene biosynthesis were observed for *Portieria hornemannii* among treatments in the field and shaded laboratory experiments. These changes have not been investigated thoroughly through rigorous chemical studies due to the limited amount of extract available for analysis. Considerable variation is observed within treatments, suggesting that the response to enhanced nutrient availability may be dependent upon the genotype of the individual.

The quantitative changes observed in triglyceride yields between the shaded and unshaded experiments suggest that light is a potential factor affecting the production of triglycerides in *Portieria hornemannii*. A difference was not observed in triglyceride concentration for the controls of the shaded and unshaded laboratory experiments indicating that the difference observed is not due to temporal variation.

The lack of differences between the laboratory control and treatments suggest that light has a more profound affect on triglyceride biosynthesis than nitrogen and phosphorous. Ochtodene yields did not differ in overall concentration between the unshaded and shaded experiments. However, there was a significant decrease in the yield of ochtodene in the algae kept in the shaded laboratory tanks across all treatments compared with the initial field controls in the laboratory fertilization experiment conducted August-September 1994 suggesting that light is also a potential factor affecting ochtodene biosynthesis. Light has been shown to be a factor in cell differentiation of storage structures and monoterpene production in terrestrial plants (Brun et al. 1991, Lerdau et al. 1991).

The predictions of the carbon/nutrient balance hypothesis seem to encompass algal species such as *Fucus vesiculosus*, which produce polyphenolics and tannins. Nitrogen and phosphorous availability do not account for the site-to-site variability in ochtodene production in *Portieria hornemannii* populations on Guam. The results from the shaded laboratory experiment suggest that light may be a factor, but there is no evidence suggesting that the algae are more shaded at some sites compared to others. Some evidence suggests that ochtodene concentrations vary temporally. Further research in this area should focus upon investigations of temporal variation and the potential effects of herbivory on monoterpene production.

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APPENDIX I. Total Kjeldahl nitrogen and phosphorous in a Kjeldahl digest for *Portieria hornemanni* collected from Anae Island, Pago Bay, Double Reef, Gun Beach, Janum and Tagachang for analysis of site-to-site variation in secondary chemistry.

SITE	SAMPLE	% NITROGEN	% PHOSPHOROUS
Anae Island	3	1.42	0.09
	5	1.69	0.10
	8	1.71	0.23
	10	1.56	0.03
	12	1.60	0.11
Pago Bay	5	1.58	0.09
	6	1.54	0.06
	8	1.54	ND
	9	1.87	0.07
	13	1.40	0.06
Double Reef	1	1.68	0.26
	2	1.88	0.09
	4	0.67	0.27
	5	2.66	0.21
	9	1.92	0.11
Gun Beach	3	2.13	0.16
	7	3.71	0.20
	8	1.30	0.01
	9	1.31	0.05
	12	1.79	1.24
Janum	1	2.34	0.12
	2	1.50	0.02
	13	2.17	0.23
	15	1.67	0.08
	16	1.44	0.04
Tagachang	1	1.17	0.05
	6	1.49	0.04
	9	1.70	0.15
	12	1.46	0.09
	14	1.48	0.24

ND = not detectable.

APPENDIX II. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of *Portieria hornemannii* collected at Anae Island on August 25, 1994 for analysis of site-to-site variation in secondary chemistry and as a field control for the unshaded laboratory experiment conducted August - September 1994. Algal material, crude extracts and HPLC fractions were weighed on an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

SAMPLE	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS /INJ (mg)	% YIELD	OCHTODENE AREA	MASS /INJ (mg)	% YIELD
1	123.4	5.9	4.75	3.6	2.90	1353862	0.1495	0.43	1404793	0.2630	0.76
2	137.0	9.7	7.08	2.0	1.46	650250	0.0619	0.09	1567152	0.2916	0.43
3	161.9	5.2	3.21	4.7	2.90	595462	0.0551	0.16	284105	0.0654	0.19
4	49.1	11.2	22.81	5.8	11.81	367997	0.0268	0.32	1126659	0.2140	2.53
5	45.3	6.0	13.25	2.2	4.86	1479002	0.1650	0.80	690986	0.1371	0.67
6	75.5	5.0	6.62	3.5	4.64	1444425	0.1607	0.75	1162103	0.2202	1.02
7	67.9	2.7	3.98	1.7	2.50	1065354	0.1136	0.28	470019	0.0982	0.25
8	16.4	8.3	50.61	1.3	7.93	619919	0.2468	0.21	1836303	0.3391	2.69
9	241.3	15.4	6.38	5.5	2.23	602921	0.2290	0.52	55057	0.1123	2.28
10	110.3	6.9	6.26	3.0	2.72	1225690	0.1335	0.36	1067535	0.2035	0.55
11	78.3	5.6	7.15	2.8	3.58	1054250	0.1122	0.40	1227237	0.2317	0.83

APPENDIX III. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of *Portieria hornemannii* collected at Pago Bay on August 26, 1994 for analysis of site-to-site variation in secondary chemistry. Algal material, crude extracts and HPLC fractions were weighed on an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

SAMPLE	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS /INJ (mg)	% YIELD	OCHTODENE AREA	MASS /INJ (mg)	% YIELD
1	188.2	9.3	4.94	6.3	3.35	317966	0.0206	0.07	680038	0.1352	0.45
2	116.9	9.7	8.30	5.1	4.36	210042	0.0072	0.03	1752330	0.3243	1.42
3	165.8	10.2	6.15	10.2	6.15	1236789	0.1349	0.83	988763	0.1896	1.17
4	193.2	5.0	2.59	3.8	1.97	620983	0.0583	0.12	1395284	0.2613	0.51
5	157.3	6.8	4.32	3.6	2.29	1003859	0.1059	0.24	1405162	0.2631	0.60
6	201.9	10.9	5.40	5.4	2.68	1149075	0.1240	0.33	2140421	0.3927	1.05
7	103.3	3.9	3.78	2.3	2.23	969448	0.1004	0.22	1235456	0.2331	0.52
8	91.6	3.2	3.49	2.1	2.29	369589	0.0270	0.06	710808	0.1406	0.32
9	282.9	4.5	1.59	2.9	1.03	813041	0.0822	0.08	1317692	0.2476	0.25
10	45.6	5.4	11.84	3.9	8.55	800930	0.0807	0.69	411482	0.0878	0.75
11	88.7	3.6	4.06	2.4	2.71	786945	0.0789	0.21	789327	0.1545	0.42
12	55.7	6.6	11.85	6.6	11.85	614091	0.0575	0.68	216069	0.0534	0.63
13	209.7	13.1	6.25	7.6	3.62	189726	0.2166	0.79	1991167	0.3664	1.33

APPENDIX IV. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of *Portieria hornemannii* collected at Double Reef on August 27, 1994 for analysis of site-to-site variation in secondary chemistry. Algal material, crude extracts and HPLC fractions were weighed using an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

SAMPLE	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS/ INJ (mg)	% YIELD	OCHTODENE AREA	MASS/ INJ (mg)	% YIELD
1	61.7	6.3	10.21	2.7	4.38	1225570	0.1335	0.58	99888	0.0329	0.14
2	304.2	8.5	2.79	0.8	0.26	629837	0.0594	0.06	2135901	0.0784	0.04
3	789.6	19.0	2.53	8.1	1.08	927150	0.0964	0.10	4536558	0.8153	0.88
4	308.6	10.1	3.27	4.9	1.59	614912	0.0576	0.09	773393	0.1571	0.25
5	387.2	17.8	4.60	9.0	2.32	763427	0.0760	0.18	365701	0.7146	1.66
6	82.5	4.2	5.09	3.1	3.76	555124	0.0501	0.19	282480	0.0651	0.25
7	176.9	8.8	4.98	4.0	2.26	1016277	0.1075	0.24	913800	0.1764	0.40
8	298.8	7.4	2.48	1.4	4.69	840288	0.0856	0.04	2382235	0.4354	0.20
9	128.5	6.5	5.41	4.1	3.19	933100	0.0971	0.31	903388	0.1746	0.56
10	212.0	8.6	4.06	2.4	1.13	939422	0.0979	0.11	1646634	0.3057	0.35
11	129.7	5.6	4.32	2.0	1.54	695507	0.0676	0.10	467419	0.0977	0.15
12	264.1	17.5	6.63	6.5	2.46	1374814	0.1521	0.37	2388563	0.4365	1.07
13	116.1	9.1	7.84	4.2	3.62	528560	0.0468	0.17	395098	0.0849	0.31

APPENDIX V. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of *Portieria hornemannii* collected at Gun Beach on August 29, 1994 for analysis of site-to-site variation in secondary chemistry. Algal material, crude extracts and HPLC fractions were weighed using an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

SAMPLE	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS/ INJ (mg)	% YIELD	OCHTODENE AREA	MASS/ INJ (mg)	% YIELD
1	167.0	5.1	3.05	1.0	0.60	490426	0.0421	0.03	472528	0.0986	0.06
2	53.4	1.1	2.06	1.1	2.06	396889	0.0304	0.06	574261	0.1165	0.24
3	103.6	4.1	3.96	0.5	0.48	2555770	0.5980	0.58	1934473	0.7308	0.71
4	139.4	2.2	1.58	1.1	0.79	2131326	0.2462	0.19	1701949	0.3154	0.25
5	278.9	6.4	2.30	3.5	1.26	1986823	0.2282	0.29	1774256	0.3282	0.41
6	64.0	0.7	1.09	0.7	1.09	343739	0.0476	0.10	41998	0.0454	0.10
7	217.2	8.7	4.01	4.2	1.93	1726430	0.1958	0.38	2038085	0.3747	0.72
8	143.3	6.5	4.54	1.6	1.12	1784656	0.2030	0.23	2465256	0.4500	0.50
9	72.6	3.5	4.82	0.7	0.96	1281495	0.2810	0.54	150226	0.0836	0.16
10	121.8	3.5	2.87	0.5	0.41	1026048	0.2174	0.18	581545	0.2356	0.19
11	59.6	3.1	5.20	0.5	0.84	1223764	0.2666	0.45	398835	0.1712	0.29
12	248.7	4.2	1.69	2.5	1.01	733126	0.2892	0.12	1309717	0.9848	0.40
13	182.5	13.6	7.45	0.5	0.27	3186044	0.7546	0.41	494906	0.2124	0.12
14	96.4	3.8	3.94	1.0	1.04	1908550	0.2185	0.23	964372	0.1853	0.19
15	139.9	6.5	4.65	2.4	1.72	1493817	0.1669	0.29	324500	0.0725	0.12

APPENDIX VI. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of *Portieria hornemannii* collected at Janum on August 31, 1994 for analysis of site-to-site variation in secondary chemistry. Algal material, crude extracts and HPLC fractions were weighed using an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

SAMPLE	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS/ INJ (mg)	% YIELD	OCHTODENE AREA	MASS/ INJ (mg)	% YIELD
1	101.6	4.8	4.72	1.0	0.98	1897044	0.2170	0.21	308093	0.0696	0.07
2	115.5	6.2	5.37	3.2	2.77	1118467	0.1202	0.33	152267	0.0421	0.12
3	130.2	13.7	10.52	5.9	4.53	688200	0.0667	0.30	100950	0.0331	0.15
4	66.2	5.1	7.70	4.1	6.19	702659	0.0685	0.42	95752	0.0322	0.20
5	66.2	10.1	15.26	3.4	5.14	843340	0.0860	0.44	199854	0.0505	0.26
6	81.9	4.9	5.98	2.5	3.05	1345105	0.1484	0.45	180193	0.0470	0.14
7	26.0	10.8	41.54	4.1	15.77	654030	0.0624	0.98	111937	0.0350	0.55
8	315.9	9.1	2.88	4.9	1.55	706379	0.0689	0.11	329508	0.0734	0.11
9	108.8	5.1	4.69	0.5	0.46	2115171	0.4884	0.45	602834	0.2432	0.22
10	428.1	6.2	1.45	1.0	0.23	2534414	0.2963	0.07	2421787	0.4423	0.10
11	195.6	7.2	3.68	4.7	2.40	1070993	0.1143	0.28	579754	0.1175	0.28
12	104.9	4.5	4.29	0.6	0.57	451457	0.0744	0.09	393865	0.1694	0.19
13	289.3	10.1	3.49	5.5	1.90	1241595	0.1355	0.26	1367388	0.2564	0.49
14	411.0	9.9	2.41	6.1	1.48	1559565	0.1750	0.26	654391	0.1307	0.19
15	63.7	3.4	5.34	3.4	5.33	1245232	0.2720	1.45	337332	0.1496	0.80
16	85.2	6.5	7.63	3.9	4.58	492772	0.0424	0.19	238348	0.0573	0.26

APPENDIX VII. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of *Portieria hornemannii* collected at Tagachang on August 31, 1994 for analysis of site-to-site variation in secondary chemistry. Algal material, crude extracts and HPLC fractions were weighed using an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

SAMPLE	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS/ INJ (mg)	% YIELD	OCHTODENE AREA	MASS/ INJ (mg)	% YIELD
1	38.5	2.6	6.75	2.6	6.75	1897650	0.2171	1.47	237789	0.0572	0.39
2	145.6	5.4	3.71	4.6	3.16	823984	0.0836	0.26	317437	0.0713	0.23
3	73.8	6.1	8.27	3.7	5.01	2363084	0.2750	1.38	194401	0.0496	0.25
4	135.2	5.8	4.29	4.4	3.25	1290735	0.1416	0.46	181850	0.0473	0.15
5	139.4	4.2	3.01	1.7	1.22	2644983	0.3100	0.38	264445	0.0619	0.08
6	130.7	5.9	4.51	2.3	1.76	1918589	0.2197	0.39	265695	0.0621	0.11
7	123.4	6.7	5.43	5.3	4.30	1460135	0.1627	0.70	121065	0.0366	0.16
8	115.1	8.7	7.56	8.7	7.56	1284778	0.1409	1.07	277550	0.0642	0.49
9	191.2	5.3	2.77	2.7	1.41	630856	0.0595	0.08	490223	0.1017	0.14
10	91.3	5.3	5.81	1.0	1.10	4393409	0.0528	0.06	502424	0.1039	0.11
11	152.8	3.9	2.55	1.0	0.65	3492901	0.4155	0.27	1240695	0.0577	0.04
12	182.8	11.2	6.12	4.2	2.30	1067724	0.1139	0.26	323833	0.0724	0.17
13	64.3	4.2	7.47	1.0	1.56	3413828	0.4057	0.63	263772	0.0618	0.10
14	34.1	8.1	23.75	3.6	10.56	1291806	0.1417	1.50	93023	0.0317	0.34

APPENDIX VIII. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the *Portieria hornemannii* collected at Anae Island for analysis of site-to-site variation in secondary chemistry.

SAMPLE	Peak 1		2		3		4		5	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
1	1.39	1353862			1.66	138837	1.77	225001		
2	1.41	650250	1.45	248479	1.67	826335	1.78	277065	1.88	151445
3	1.43	595462								
4	1.41	367997	1.45	254295	1.67	213980	1.78	113002	1.88	45649
5	0.56	1479002	0.91	86460	1.17	70175				
6	1.45	1444425			1.67	360699	1.76	579170		
7	1.36	1065354							1.88	60280
8	1.44	619919			1.67	205760	1.78	291104		
9	1.39	602921	1.53	93338	1.61	1993687	1.72	252156	1.88	176942
10	1.43	1225690			1.69	70935	1.77	196446		
11	1.45	1054250			1.63	242352	1.77	462885		

SAMPLE	Peak 6		7		8		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
1	2.01	36671			2.36	313870	3.79	1404793
2	2.02	445221			2.36	385501	3.73	1567152
3	2.02	14632	3.07	14275	3.17	26239	5.97	284105
4	2.02	112660	2.30	22155	2.37	39524	3.80	1126659
5			1.51	33283	1.52	23857	2.93	690986
6	2.02	504618	2.29	279792	2.35	338114	3.84	1162103
7	2.23	46490	2.30	873372	2.80	35920	5.03	470019
8	2.01	386384			2.37	424170	3.79	1836303
9	1.95	271599	2.23	574372	2.30	99962	3.66	1806630
10	1.91	51270	2.03	98318	2.37	128878	3.75	1067535
11	2.03	614721			2.37	716435	3.82	1227237

APPENDIX IX. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the *Portieria hornemannii* collected at Pago Bay for analysis of site-to-site variation in secondary chemistry.

SAMPLE	Peak 1		2		3		4		5		6		7	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
1	1.42	317966	1.46	220469	1.56	58555	1.70	78990	1.81	109771	1.92	44456	2.06	54156
2	1.43	210042	1.47	188616	1.56	43110	1.70	31648	1.81	125151	1.72	47643	2.03	97607
3	1.42	1236789					1.69	237450	1.80	309647			2.00	317130
4	1.44	620983	1.47	280057	1.56	167650	1.70	230973	1.80	339683	1.91	97887	2.05	229081
5	1.44	1003859			1.57	158307	1.71	78821	1.82	184790	1.93	49316	2.05	68116
6	1.44	1149075			1.55	125974	1.69	297943	1.80	172492	1.90	117899	2.01	134104
7	1.46	969448					1.69	231793	1.79	309868	1.91	112858	2.04	252028
8	1.43	369589	1.47	245498	1.64	231777	1.70	191367	1.80	243911	1.91	150728	2.02	119491
9	1.43	813041			1.55	137300	1.69	80903	1.75	308383	1.89	25655	2.02	160462
10	1.43	332702	1.47	800930					1.80	12558			2.03	17765
11	1.43	786945			1.55	155532			1.75	144160	1.89	21020	2.00	62066
12	1.45	614091												
13	1.43	1893726			1.55	225617	1.69	737018	1.81	255500	1.92	205616	2.04	212988

SAMPLE	Peak 8		9		10		11		12		13		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
1	2.37	35087											3.99	680038
2	2.35	66239											3.83	1752330
3	2.21	191685	2.43	117690	2.52	420979	3.02	172665	3.12	148897			5.71	988763
4	2.34	82450	2.40	91493									3.82	1395284
5	2.37	80265											3.91	1405162
6	2.36	131240											3.70	2140421
7	2.38	471195							3.28	540562			3.85	1235456
8			2.41	231686					3.28	150016			3.87	710808
9	2.30	89153	2.36	99022					3.24	210684	3.31	104264	3.74	1317692
10													3.86	411482
11	2.30	70024											3.77	789327
12													5.93	216069
13	2.34	171988			2.63	125122					3.50	246284	3.81	1991167

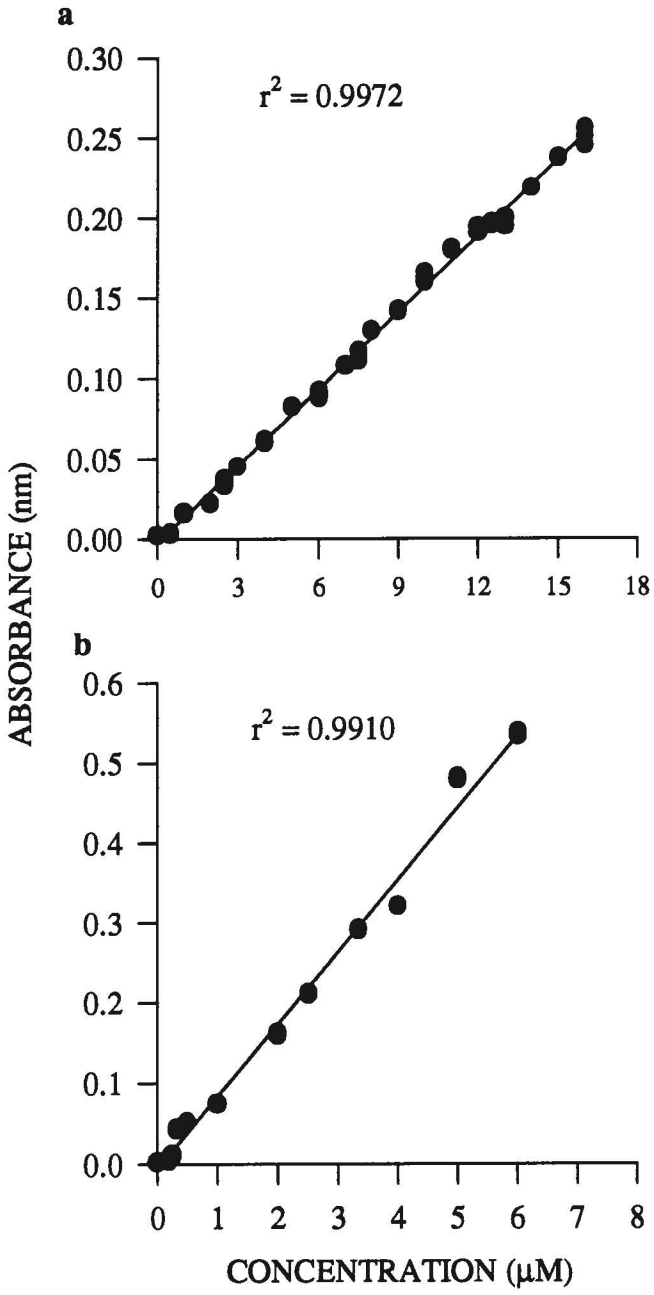
APPENDIX X. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the *Portieria hornemannii* collected at Double Reef for analysis of site-to-site variation in secondary chemistry.

SAMPLE	Peak 1		2		3		4		5		6		7		8	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
1	1.43	1225570													2.23	376373
2	1.39	629837	1.53	114683					1.84	92901	1.96	45070	2.12	44926		
3	1.35	927150	1.56	287054	1.72	90447	1.80	299063			1.93	277755	2.08	92919	2.20	125699
4	1.42	614912											2.08	384819		
5	1.42	763427			1.72	290050	1.79	116564	1.86	296312	2.00	309574	2.15	230337	2.27	152182
6	1.41	555124														
7	1.43	1016277											2.13	233225	2.34	275718
8	1.42	840288	1.60	344185			1.77	69472	1.85	183284	1.97	223243	2.13	165115	2.24	125733
9	1.42	933100					1.76	447039			1.93	254186			2.39	416257
10	1.40	939422	1.58	347732					1.84	104022	1.95	236751	2.12	92411	2.34	58894
11	1.43	695507											2.14	369251		
12	1.45	1374814	1.64	78329			1.77	50595	1.89	96549			2.15	78927	2.37	12136
13	1.43	528560														

SAMPLE	Peak 9		10		11		12		13		14		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
1													7.04	99888
2			2.55	135189									4.33	2135901
3	2.47	209833	2.55	159600									6.16	67759
4					3.29	234157	3.42	255974					6.57	773393
5			2.55	205807	2.64	161900							4.35	3965701
6													7.59	282480
7	2.78	177028	2.92	171408	3.07	226136	3.53	258219	3.70	211118	3.89	271002	7.21	913800
8			2.56	125863									4.32	2382235
9	2.78	180961	2.86	228840									5.04	903388
10			2.58	235717									4.37	1646634
11							3.35	195824	3.44	214204			6.71	467419
12	2.49	12136			2.99	13914	3.63	62486	3.76	69094	3.94	62166	7.24	2388563
13			3.59	20647	3.79	29589							7.42	395098

APPENDIX XIII b. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the *Portieria hornemannii* collected at Tagachang for analysis of site-to-site variation in secondary chemistry.

SAMPLE	Peak 7		8		9		10		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
1					3.34	215286	4.37	348453	5.31	237789
2									6.09	317437
3									5.42	194401
4									6.27	181850
5									5.48	264445
6									5.24	265695
7									6.50	121065
8									5.76	277550
9	2.80	170728							5.17	490223
10									4.42	502424
11									4.43	1240695
12									5.33	323833
13	2.85	16169	2.95	12857	3.08	19857			4.44	263772
14									5.05	93023



APPENDIX XIV. Standard curve for phosphate on the Beckman DU-65 Spectrophotometer for a 1 cm (a) and 5 cm (b) cell.

APPENDIX XV a. Phosphorous concentrations (μM) of the seawater taken around the control and IBDU treated *Portieria hornemannii* over time during two periods of the *in situ* fertilization experiment conducted at Gun Beach.

	SET A					SET B			
TREATMENT	Day 0	Day 1	Day 5	Day 7	Day 9	Day 0	Day 2	Day 5	Day 7
Control	2.26	0.27	0.46	0.66	0.18	1.98	0.51	0.55	0.13
	0.45	0.67	0.21	0.53	0.32	0.82	0.09	0.37	0.37
	0.52	0.15	0.47	0.33	0.26	0.76	0.38	1.16	0.13
	1.40	0.06	0.05	0.58	0.10	57.64	12.68	0.74	0.27
	1.86	0.50	0.67	0.29	0.21	1.45	1.49	1.06	1.28
	0.98	35.76	0.46	0.62	0.64	8.15	0.51	0.70	0.16
		0.26	0.41	0.74		33.82	0.31	0.19	0.12
			0.65	2.38		0.63	3.66	0.85	0.21
			0.36	0.41		0.06	1.38	1.43	0.28
				0.74					0.94
IBDU	0.21	0.09	0.46	1.53	0.42	0.24	1.20	1.49	1.17
	0.18	0.80	0.70	2.11	0.50	1.87	0.31	0.99	0.69
	0.23	0.09	0.73	0.33	0.34	67.39	0.02	1.53	0.17
	0.62	1.48	0.30	0.90	0.35	62.04	0.52	0.58	0.18
	0.23		0.61	0.45	0.22	0.30	0.86	1.74	0.21
	1.74		0.13	0.36		14.71	0.02	0.19	4.86
			0.61	19.83		0.09	0.35	1.21	0.56
			0.56	2.34		18.09	0.35	1.41	0.17
			0.91	0.33			0.42	0.13	0.30
			0.44	2.59			0.40		0.13

APPENDIX XV b. Phosphorous concentrations (μM) of the seawater taken around the TSP and combined treated *Portieria hornemannii* over time during two periods of the *in situ* fertilization experiment conducted at Gun Beach.

	SET A					SET B			
TREATMENT	Day 0	Day 1	Day 5	Day 7	Day 9	Day 0	Day 2	Day 5	Day 7
TSP	12.13	0.67	0.17	1.57	0.42	5.25	0.35	0.42	0.32
	2.70	2.18	1.62	1.45	2.09	1.50	0.49	0.76	0.56
	10.69	0.62	0.68	0.40	0.50	0.80	6.24	0.28	0.52
	13.84	27.67	7.30	2.88	0.74	15.72	1.46	0.52	0.38
	1.37	0.17	1.11	1.13	0.59	35.94	1.36	0.19	0.52
	0.68	37.25	0.59	2.30	0.36	34.16	0.39	0.48	3.73
	0.92	0.42	1.26	0.68		36.84	0.09	0.32	0.38
		18.51	0.76	1.71		39.60	0.33	0.27	1.80
			1.39	1.02		46.69	0.53	0.27	0.39
				3.55		36.41	14.84		0.21
				0.84		1.24			
Combined	9.03	0.69	0.49	2.66	0.24	5.74	3.64	0.18	0.18
	4.14	0.78	6.04	1.76	0.77	27.33	0.94	0.35	1.01
	6.83	1.25	0.38	5.41	0.18	19.08	16.18	0.30	0.55
	1.64	25.32	1.05	0.27	0.13	17.20	6.56	14.52	0.30
	9.31	2.44	0.58	1.53	0.77	10.91	0.93	0.24	0.12
	3.73	0.57	0.49	2.18	0.13	26.94	1.77	0.32	0.27
			0.82	0.59	0.01	21.63	2.31	1.84	5.29
			0.33	1.01		34.52	0.01	2.37	0.18
			1.85	12.57		21.93	0.30	0.23	
			0.64	1.44					

APPENDIX XVI. Total Kjeldahl nitrogen and phosphorous in a Kjeldahl digest for control, IBDU, TSP and combined treated *Portieria hornemannii* collected for the *in situ* fertilization experiment conducted at Gun Beach.

TREATMENT	STATION	% NITROGEN	% PHOSPHOROUS
Control	3	1.15	ND
	12	2.22	0.20
	25	1.69	0.13
	6,28	1.46	0.26
IBDU	47	2.17	ND
	53	2.15	0.24
	55	1.55	0.11
	50,59	1.30	0.13
	34,52,54	0.76	0.07
TSP	62	0.84	0.36
	63	2.09	0.15
	88	1.46	0.08
	72,84	1.58	0.27
	76,81	1.43	0.50
Combined	97	1.40	0.03
	113	2.26	0.02
	114	2.00	ND
	90,107,110	0.83	ND

ND = not detectable.

APPENDIX XVII. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of the unfertilized (control) *Portieria hornemannii* collected from the *in situ* fertilization experiment conducted at Gun Beach. Algal material, crude extracts and HPLC fractions were weighed on an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

STATION	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS/ INJ (mg)	% YIELD	OCHTODENE AREA	MASS/ INJ (mg)	% YIELD
3	38.0	2.9	7.63	1.7	4.47	901899	0.1035	0.46	296375	0.0675	0.30
4	10.3	2.2	21.36	1.8	17.48	2682654	0.3146	5.50	280175	0.0647	1.13
5	17.3	2.2	12.72	1.6	9.25	2141221	0.2474	2.29	296300	0.0675	0.62
6	99.3	7.2	7.25	4.7	4.73	891425	0.0919	0.44	1234805	0.2330	1.10
10	29.1	5.1	17.53	2.7	7.28	1829518	0.2086	1.94	458748	0.0962	0.89
12	66.0	3.4	5.15	2.4	3.64	1055949	0.1124	0.41	345798	0.0763	0.28
25	34.5	4.3	12.46	2.6	7.54	3344488	0.3970	2.99	59568	0.0258	0.19
28	73.3	4.1	5.59	2.1	2.87	3609132	0.4673	1.34	2285001	0.4182	1.20

APPENDIX XVIII. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of the IBDU fertilized *Portieria hornemannii* collected from the *in situ* fertilization experiment conducted at Gun Beach. Algal material, crude extracts and HPLC fractions were weighed on an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

STATION	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS/ INJ (mg)	% YIELD	OCHTODENE AREA	MASS/ INJ (mg)	% YIELD
32	17.4	1.9	10.92	1.8	10.35	1427047	0.1586	1.64	144040	0.0354	0.37
33	35.1	6.0	17.09	3.8	10.83	4379940	0.5258	5.69	508884	0.1100	1.19
34	89.1	4.5	5.06	2.5	2.81	1828423	0.2085	0.59	1893675	0.3492	0.98
47	19.1	2.5	13.09	1.2	6.28	1045762	0.1111	1.40	207150	0.1036	0.65
50	44.1	2.7	6.12	2.4	5.44	1302548	0.1413	0.77	555697	0.1133	0.62
51	19.0	4.5	23.68	3.4	17.90	1632885	0.1842	3.30	164460	0.0443	0.79
53	43.3	6.0	13.86	2.9	6.70	2029379	0.2335	1.56	1029717	0.1696	1.32
54	72.0	4.1	5.69	2.7	3.75	1088796	0.1165	0.44	1238100	0.2336	0.88
55	104.8	6.3	6.01	3.5	3.34	2231845	0.2624	0.88	1830665	0.3381	1.13
59	57.1	5.6	9.81	4.0	7.01	4254267	0.5012	3.51	552776	0.1128	0.79
60	21.8	3.0	13.76	1.8	8.26	2025742	0.2330	1.92	119112	0.0363	0.30

APPENDIX XIX. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of the TSP fertilized *Portieria hornemannii* collected from the *in situ* fertilization experiment conducted at Gun Beach. Algal material, crude extracts and HPLC fractions were weighed using an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

STATION	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS/ INJ (mg)	% YIELD	OCHTODENE AREA	MASS/ INJ (mg)	% YIELD
61	39.8	3.1	7.79	1.9	4.77	2614880	0.3063	1.46	245190	0.0585	0.28
62	73.8	7.1	9.62	4.5	6.10	5004968	0.6036	3.68	1089695	0.2074	1.27
63	57.3	3.2	5.59	2.5	4.36	1745266	0.1981	0.86	277582	0.0642	0.28
73	16.5	3.4	20.61	2.0	12.12	3281600	0.3892	4.72	94876	0.0320	0.39
75	37.7	4.0	10.61	3.8	10.08	1955691	0.2243	2.26	423905	0.0900	0.91
81	24.7	3.9	15.79	2.1	8.50	1361889	0.1505	1.28	265786	0.0621	0.53
84	93.4	25.9	27.73	3.0	3.21	1552660	0.1742	0.56	875257	0.1696	0.55
88	136.1	4.0	2.94	3.3	2.42	2289883	0.2659	0.65	633079	0.1269	0.31
90	101.8	7.3	7.17	3.7	3.64	1143572	0.1233	0.45	1320737	0.2482	0.90

APPENDIX XX. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of the combined (IBDU and TSP) fertilized *Portieria hornemannii* collected from the *in situ* fertilization experiment conducted at Gun Beach. Algal material, crude extracts and HPLC fractions were weighed on an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

STATION	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS/ INJ (mg)	% YIELD	OCHTODENE AREA	MASS/ INJ (mg)	% YIELD
93	14.6	3.0	20.55	1.4	9.59	784554	0.1572	1.51	133307	0.0776	0.74
97	18.3	3.9	21.31	2.7	14.75	1296975	0.1424	2.10	29783	0.0205	0.30
99	21.8	4.3	19.73	3.3	15.14	3591463	0.4278	6.48	239328	0.0575	0.87
100	30.6	3.5	11.44	3.5	11.44	2108180	0.2433	2.78	265057	0.0620	0.71
103	9.2	2.5	27.17	2.5	27.17	1712462	0.1941	5.27	217985	0.0537	1.46
113	87.5	8.0	9.14	5.2	5.94	3117624	0.3688	2.19	699881	0.1387	0.82
114	17.0	3.6	21.17	2.5	14.71	3692672	0.4404	6.48	204989	0.0514	0.76
115	20.0	5.1	25.50	3.5	17.50	3714071	0.4430	7.75	393155	0.0846	1.48

APPENDIX XXI. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the unfertilized (control) *Portieria hornemannii* collected from the *in situ* fertilization experiment conducted at Gun Beach.

STATION	Peak 1		2		3		4	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
3	1.45	983989	1.68	137331				
4	1.40	2681948						
5	1.51	2141331	1.74	211513				
6	1.51	891425						
10	1.44	1829518	1.72	212732				
12	1.46	1055949			1.79	67911		
25	1.45	3344488	1.74	125599	1.82	151600	2.13	43778
28	1.50	3609132					2.09	7363

STATION	Peak 5		6		7		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
3							6.04	296375
4							5.96	280175
5							6.02	296300
6	2.63	16357			3.23	74915	6.06	1234805
10							6.02	458748
12					3.15	37406	6.03	345789
25							6.13	59568
28	2.64	12023	2.73	12023	3.23	74445	5.93	2285001

APPENDIX XXII. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the IBDU fertilized *Portieria hornemannii* collected from the *in situ* fertilization experiment conducted at Gun Beach.

STATION	Peak 1		2		3		4		5	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
32	1.45	1427047								
33	1.39	4379940			1.66	287254			2.05	63221
34	1.36	1828423			1.63	243334	1.95	56014	2.01	43036
47	1.41	1045762								
50	1.47	1302548	1.55	119206	1.74	107788			2.03	35490
51	1.43	1632885								
53	1.44	2029376			1.69	718853			2.11	158144
54	1.41	1088796								
55	1.46	2261845							2.06	28455
59	1.42	4254267					1.79	232787	2.09	73123
60	1.48	2025742					1.75	217324		

STATION	Peak 6		7		8		9		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
32									6.10	114040
33			2.51	87641	3.11	23935	3.22	10786	5.97	508884
34	2.49	43585	2.58	34520	3.07	108319			5.72	1893675
47									6.02	207150
50	2.63	15725	2.72	15779			3.24	59762	6.16	555697
51									6.06	164460
53	2.60	137314	2.69	134056	3.19	263349			6.12	1029717
54	2.54	113209	2.63	106274	3.14	253737			5.81	1238100
55	2.60	12198	2.69	7181	3.19	55983			5.92	1830665
59	2.52	54571			3.14	32698			5.98	552776
60									6.07	119112

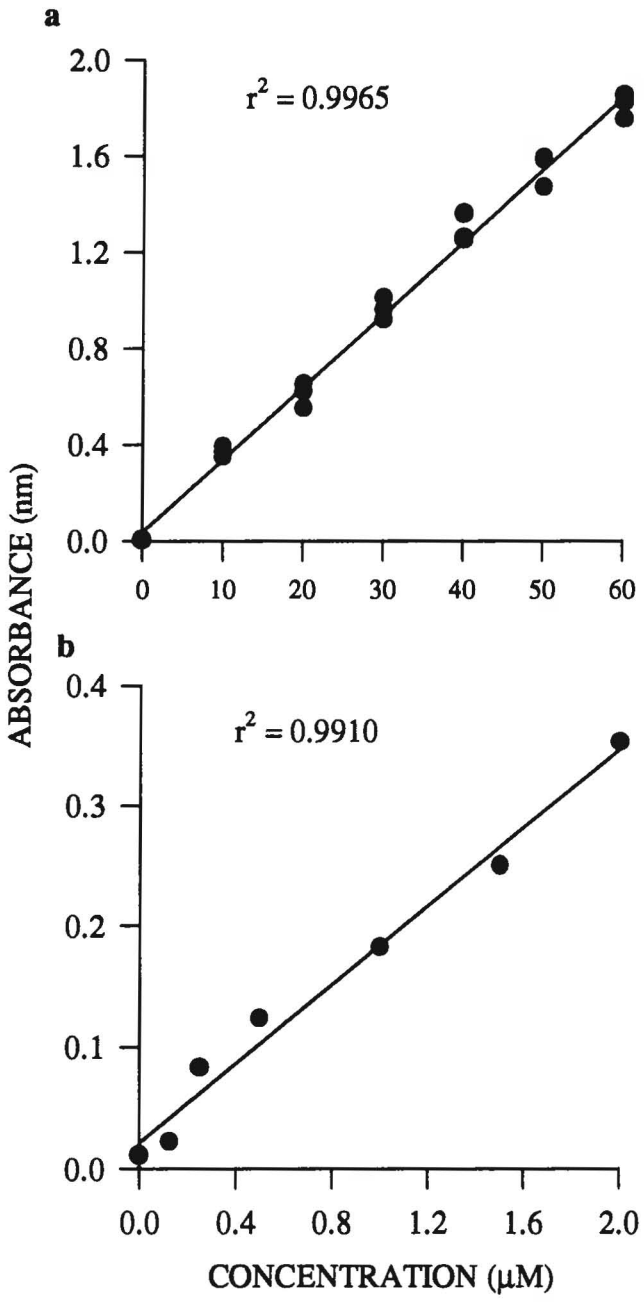
APPENDIX XXIII. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the TSP fertilized *Portieria hornemannii* collected from the *in situ* fertilization experiment conducted at Gun Beach.

STATION	Peak 1		2		3		4		5	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
61	1.48	2614880					1.80	31849		
62	1.46	5004968			1.73	278870				
63	1.48	1745266							2.12	95467
73	1.38	3281600	1.65	114577	1.74	236944				
75	1.45	1955691			1.72	191750				
81	1.42	1361889					1.79	95750		
84	1.53	1552660					1.79	305872		
88	1.46	2289883					1.79	13219	1.99	5423
90	1.44	1143572			1.72	204923	1.74	272780	2.04	294216

STATION	Peak 6		7		8		Peak 9		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
61									6.06	245190
62			2.69	40151	3.19	36947	3.30	32993	6.01	1089695
63	2.60	50341							6.12	277582
73									6.00	94876
75									5.98	423905
81									6.10	265786
84									6.09	875257
88					3.19	60709			5.98	633079
90	2.58	571382	3.15	243059					6.01	654857

APPENDIX XXIV. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the combined (IBDU and TSP) fertilized *Portieria hornemannii* collected from the *in situ* fertilization experiment conducted at Gun Beach.

STATION	Peak 1		2		3		4		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
93	1.54	784533							6.46	133307
97	1.42	1296975			1.79	74246			6.12	29773
99	1.40	3591463	1.69	293979					5.95	239328
100	1.42	2108180	1.73	684962					6.20	265057
103	1.45	1712462	1.72	747192					6.20	217985
113	1.43	3117642	1.72	158089					6.08	699881
114	1.41	3692672	1.69	138840	1.77	219651			6.01	204989
115	1.43	3714071	1.70	146331	1.71	272951	2.09	61028	6.01	393155



Appendix XXV. Standard curve for nitrate on the Beckman DU-65 Spectrophotometer with a 1 cm (a) and 5 cm (b) cell.

APPENDIX XXVI. Phosphate concentrations for seawater sampled from the control, nitrate, phosphate and combined treated *Portieria hornemanni* tanks in the laboratory fertilization experiment conducted August - September 1994 to monitor nutrient uptake. Concentrations are reported for samples taken in the evening just after fertilization and in the morning just before water flow was restored.

		June 9 - 10, 1994		June 15 - 16, 1994	
TREATMENT	TANK #	Evening	Morning	Evening	Morning
Control	4	3.6066	1.5307	0.4846	0.3304
	6	0.1540	0.1321	0.3855	0.4295
	10	0.4381	0.3616	1.4207	0.2974
	13	0.1977	0.1868	0.8480	0.5286
	17	0.5473	0.4271	0.9361	0.4736
Nitrate	2	9.3771	0.4927	0.5507	0.3084
	3	0.1758	0.1540	0.8601	0.4405
	5	0.3725	0.3616	0.3524	0.4295
	9	0.3288	0.3069	0.4405	0.5286
	18	0.2632	0.1977	0.4405	0.3524
Phosphate	8	13.1863	7.1551	12.2975	5.8700
	11	14.8370	11.2817	13.5672	1.3326
	14	14.3291	6.9011	12.9959	5.2505
	16	14.3291	8.8692	10.5199	0.4075
	19	15.4719	11.6626	17.6939	7.9169
Combined	1	22.1380	0.3616	7.2820	0.3855
	7	13.0594	2.7216	8.2343	2.6872
	12	11.6626	6.6472	9.1866	0.9582
	15	12.1070	6.7741	13.8847	13.1228
	20	10.2659	4.8060	9.0597	3.0066

APPENDIX XXVII. Nitrate concentrations for seawater sampled from the control, nitrate, phosphate and combined treated *Portieria hornemanni* tanks in the laboratory fertilization experiment conducted August - September 1994 to monitor nutrient uptake. Concentrations are reported for samples taken in the evening just after fertilization and in the morning just before water flow was restored.

		June 9 - 10, 1994		June 15 - 16, 1994	
TREATMENT	TANK #	Evening	Morning	Evening	Morning
Control	4	0.6443	0.0911	0.0165	0.0000
	6	0.8867	0.1650	0.2092	0.0849
	10	0.4140	0.0000	0.0050	0.0000
	13	0.0601	0.0000	0.7313	0.0000
	17	0.6008	0.0723	0.0050	0.0000
Nitrate	2	35.1380	9.1899	37.8410	0.0000
	3	54.9010	9.6250	38.1520	0.1160
	5	59.9980	9.2210	38.8980	0.3024
	9	49.3390	36.4740	40.0790	0.0170
	18	57.0150	26.5920	37.2200	0.0050
Phosphate	8	0.2241	0.0001	1.7754	0.0000
	11	0.1820	0.1781	1.0420	0.0050
	14	0.1910	0.0000	0.5883	0.0000
	16	0.0821	0.0476	0.2216	0.0005
	19	0.7313	0.0370	1.0110	0.0000
Combined	1	40.9180	0.0538	31.5950	0.0290
	7	38.9600	0.0000	34.0810	0.0050
	12	36.8160	7.3254	59.3450	0.3397
	15	32.1540	7.3369	34.3610	0.0050
	20	32.4650	8.2577	31.3460	0.0070

APPENDIX XXVIII. Initial wet mass, final wet mass and % change in growth of the control, nitrate, phosphate and combined treated *Portieria hornemannii* in the laboratory fertilization experiment conducted August-September 1994. Algal material was weighed on an analytical balance.

TREATMENT	TANK	INITIAL MASS (mg)	FINAL MASS (mg)	INITIAL - FINAL	% CHANGE
CONTROL	4	6.7	12.4	5.7	85
	6	10.1	15.1	5.0	50
	10	15.6	19.9	4.3	28
	13	33.7	38.7	4.6	14
	17	4.9	6.3	1.4	29
NITRATE	2	5.2	6.4	1.2	23
	3	4.6	7.7	3.1	67
	5	9.0	18.1	9.1	101
	9	7.6	11.5	3.9	51
	18	34.8	35.0	0.2	0.58
PHOSPHATE	8	13.0	16.0	3.0	23
	11	3.5	6.5	3.0	86
	14	4.0	7.8	3.8	95
	16	56.2	66.4	10.2	18
	19	6.5	9.8	3.3	51
COMBINED	1	17.9	22.3	4.4	25
	7	16.0	23.1	7.1	44
	12	10.5	13.4	2.9	28
	15	21.1	23.6	2.5	12
	20	154.9	162.3	7.4	4.8

APPENDIX XXIX. Total Kjeldahl nitrogen and phosphorous in a Kjeldahl digest for control, IBDU, TSP and combined treated *Portieria hornemannii* collected for the laboratory fertilization experiment conducted August - September 1994.

TREATMENT	SAMPLE/ TANK	% NITROGEN	% PHOSPHOROUS
August 25, 1994	3	1.42	0.09
	5	1.69	0.10
	8	1.71	0.23
	10	1.56	0.03
	12	1.60	0.11
Control	4	1.52	0.14
	6	1.50	0.11
	10	1.59	0.10
	13	1.07	ND
	17	1.02	0.09
Nitrate	2	1.13	ND
	3	1.51	0.11
	5	1.52	0.21
	9	1.71	0.34
Phosphate	8	0.97	0.08
	11	2.14	0.19
	14	1.75	0.31
	16	1.50	0.16
	19	1.02	0.10
Combined	1	1.27	0.11
	7	1.10	ND
	12	1.99	0.08
	15	1.52	0.12
	20	1.83	0.34

ND = not detectable.

APPENDIX XXX. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of the control, nitrate, phosphate and combined treated *Portieria hornemannii* in the laboratory fertilization experiment conducted August-September 1994. Algal material, crude extracts and HPLC fractions were weighed on an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

TREATMENT	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS/ INJ (mg)	% YIELD	OCHTODENE AREA	MASS/ INJ (mg)	% YIELD
CONTROL	566.5	24.1	4.25	5.4	0.95	534509	0.0476	0.05	1196066	0.2262	0.22
	176.9	5.0	2.83	3.3	1.87	576945	0.0532	0.10	832281	0.1620	0.30
	252.3	5.7	2.26	2.9	1.15	730764	0.0720	0.08	1246736	0.2351	0.27
	402.3	8.5	2.11	5.4	1.34	607139	0.0566	0.08	2448857	0.4471	0.60
	138.3	2.8	2.03	2.7	1.95	1061356	0.1311	0.26	200660	0.0506	0.10
NITRATE	1010.5	11.1	1.10	4.7	0.47	715540	0.0701	0.03	1946814	0.3586	0.17
	606.0	7.9	1.30	4.7	0.78	365514	0.0265	0.02	1842781	0.3402	0.16
	275.3	6.7	2.43	4.3	1.56	496961	0.0403	0.06	565628	0.1150	0.18
	244.2	7.7	3.15	4.0	1.64	689135	0.0593	0.10	339596	0.0752	0.12
	171.5	3.9	2.27	3.4	1.98	616181	0.0580	0.12	229318	0.0557	0.11
PHOSPHATE	236.1	4.3	1.82	3.8	1.61	719170	0.0705	0.11	1170111	0.2216	0.36
	593.4	4.8	0.81	3.4	0.57	386572	0.0292	0.02	501315	0.1037	0.06
	329.2	9.3	2.83	5.2	1.58	851026	0.0860	0.14	2011131	0.3699	0.58
	138.2	3.1	2.24	2.6	1.88	448145	0.0365	0.07	211867	0.0526	0.10
	65.5	5.0	7.63	3.5	5.34	517676	0.0455	0.24	162462	0.0439	0.24
COMBINED	632.9	4.6	0.73	2.8	0.44	1052043	0.1119	0.05	220045	0.0541	0.02
	384.9	9.4	2.44	3.9	1.01	824461	0.0836	0.09	1718283	0.3183	0.32
	244.4	9.1	3.72	5.7	2.33	374885	0.0277	0.07	472528	0.0986	0.23
	618.3	9.8	1.59	6.4	1.04	256655	0.0130	0.01	3388098	0.6128	0.63
	684.9	11.1	1.62	6.4	0.93	432507	0.0349	0.03	2937154	0.5332	0.50

APPENDIX XXXI. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the control treatment *Portieria hornemannii* in the laboratory fertilization experiment conducted August - September 1994.

TANK	Peak 1		2		3		4		5		6		7		8	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
4	1.41	534509	1.83	393643	2.04	299044	2.29	249948			2.58	128655	2.67	237785		
6	1.43	576945			2.11	377932			2.39	169388			2.68	163570		
10	1.42	730764	1.87	41846	2.10	60849	2.36	27745							2.79	29323
13	1.43	607139	1.88	723435	2.12	383009			2.40	392973			2.69	144318		
17	1.42	1061356			2.10	396638	2.37	266026					2.68	211430		

TANK	Peak 9		10		11		12		13		14		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
4			3.20	159315	3.29	235607			3.98	254963			6.02	1196066
6	2.81	245628			3.37	221510	3.48	315947			4.32	453642	6.62	832281
10					3.34	43749	3.44	81394			4.23	90713	6.41	1246736
13	2.82	270580			3.39	229718	3.50	336664					6.48	2448857
17							3.44	507968					6.67	200660

APPENDIX XXXII. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the nitrate treatment *Portieria hornemannii* in the laboratory fertilization experiment conducted August - September 1994.

TANK	Peak 1		2		3		4		5	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
2	1.43	365514	1.86	71735	2.08	75792	2.35	27328	2.65	30060
3	1.43	496961								
5	1.42	715540	1.88	1038655	2.12	374022	2.42	387587	2.73	163475
9	1.43	689135	1.80	529310	2.11	204876	2.37	400181	2.66	138292
18	1.43	616181	1.87	554634			2.39	309333	2.68	175477

TANK	Peak 6		7		8		9		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
2	2.76	34892	3.31	55613	3.41	92459	4.20	22827	6.29	1842781
3			3.35	375685	3.46	238828	4.24	270948	6.53	565628
5	2.86	247562	3.43	217960	3.55	366930	4.45	470433	6.59	1946814
9	2.80	248718	3.45	511867			4.29	437116	6.67	339596
18	2.79	179377	3.36	136589	3.48	217458	4.32	267454	6.72	229318

APPENDIX XXXIII. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the phosphate treatment *Portieria hornemannii* in the laboratory fertilization experiment conducted August - September 1994.

TANK	Peak 1		2		3		4		5		6	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
8	1.45	448145					2.31	225908				
11	1.44	719170	1.87	396798	2.08	293187	2.34	237351	2.63	110829	2.74	175362
14	1.42	851026	1.85	404900	2.06	341845	2.31	283131	2.60	147574	2.71	257326
16	1.41	517676										
19	1.40	386572			2.04	44509			2.59	11661	2.70	12626

TANK	Peak 7		8		9		10		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
8					3.33	148005	4.05	229418	6.27	211867
11			3.27	158529	3.36	281406	4.10	113490	6.19	1170111
14			3.23	195765	3.32	360202	4.03	195204	6.02	2011131
16	2.91	24752					4.11	51025	6.42	162462
19			3.21	56843	3.31	44509	4.03	39149	6.19	501315

APPENDIX XXXIV. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the combined (nitrate and phosphate) treatment *Portieria hornemannii* in the laboratory fertilization experiment conducted August - September 1994.

TANK	Peak 1		2		3		4		5		6	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
1	1.43	824461			2.07	276608	2.33	252798	2.63	148703	2.74	193338
7	1.47	256655	1.87	514873	2.11	395885	2.39	39769	2.68	23206	2.82	42058
12	1.43	432507	1.87	705231	2.09	114413	2.36	43573	2.65	15572	2.77	47435
15	1.46	374885					2.30	313960	2.59	145567	2.70	170031
20	1.42	1052043			2.11	213113	2.37	347563	2.67	153480	2.79	251343

TANK	Peak 7		8		9		10		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
1			3.27	187369	3.37	306500	4.12	671861	6.22	1718283
7					3.48	213972	4.33	96587	6.37	3388098
12			3.30	82288	3.40	166951	4.16	88613	6.15	2937154
15	3.21	43124	3.30	178563			4.00	224143	6.16	472528
20			3.34	150247	3.44	250119			6.61	220045

APPENDIX XXXV. Initial wet mass, final wet mass and % growth for the control, nitrate, phosphate and combined treated *Portieria hornemannii* in the laboratory fertilization experiment conducted February 1995. Algal material was weighed on an analytical balance.

TREATMENT	TANK	INITIAL MASS (mg)	FINAL MASS (mg)	FINAL - INITIAL	% CHANGE
CONTROL	1	15.2347	19.4809	4.2462	27.87
	3	24.8749	31.7040	6.8291	27.45
	7	9.0207	12.4280	3.4073	37.77
	9	9.0302	16.4509	7.4207	82.18
	16	6.7288	9.9017	3.1729	47.15
NITRATE	2	33.6056	36.1546	2.5490	7.59
	6	10.8361	15.6849	4.8488	44.75
	10	4.6244	9.11241	4.4997	97.30
	14	12.2297	14.6657	2.4360	19.92
	16	10.1004	18.2100	8.1096	80.29
PHOSPHATE	5	15.5971	21.0507	5.4536	34.97
	11	21.3885	25.8931	4.5046	21.06
	13	21.4848	34.0691	12.5843	58.57
	17	10.3252	18.7226	8.3974	81.33
	19	8.9041	14.9643	6.0602	68.06
COMBINED	4	7.4796	10.6160	3.1364	41.93
	8	19.1546	28.9091	9.7545	50.93
	12	24.6319	27.7271	3.1952	12.97
	15	30.6165	13.7437	-16.8728	-55.11
	20	3.9840	9.3510	5.3670	134.71

APPENDIX XXXVI. Total Kjeldahl nitrogen and phosphorous in a Kjeldahl digest for the February 9 and 28, 1995 Anae Island collections and the control, IBDU, TSP and combined treated *Portieria hornemannii* collected for the laboratory fertilization experiment conducted February 1995.

TREATMENT	SAMPLE/ TANK	% NITROGEN	% PHOSPHOROUS
February 9, 1995	*	*	*
	*	*	*
	*	*	*
	*	*	*
	*	*	*
Control	1	1.54	ND
	3	1.07	0.05
	7	1.15	0.15
	9	1.39	0.10
	16	1.12	0.11
Nitrate	2	1.58	0.14
	6	1.43	0.11
	10	1.54	ND
	14	1.72	0.13
	18	1.15	0.12
Phosphate	5	1.66	0.08
	11	1.42	0.19
	13	1.60	ND
	17	1.23	0.36
	19	1.07	0.44
Combined	4	1.94	0.19
	8	1.93	ND
	12	1.39	0.08
	15	1.82	0.32
	20	1.30	0.01
February 28, 1995	1	1.67	0.17
	4	1.12	0.10
	5	1.81	ND
	7	1.56	0.08
	10	1.81	0.15

* Samples not analyzed for internal nutrients.
ND = not detectable.

APPENDIX XXXVII. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of *Portieria hornemannii* collected at Anae Island on February 9, 1995 as control for the laboratory experiment conducted February 1995. Algal material, crude extracts and HPLC fractions were weighed on an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

SAMPLE	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS/ INJ (mg)	% YIELD	OCHTODENE AREA	MASS/ INJ (mg)	% YIELD
1	621.3	29.7	4.78	19.1	3.07	709414	0.0565	0.17	1835793	0.1833	0.56
2	432.2	15.1	3.49	8.4	1.94	873603	0.0753	0.15	704978	0.0506	0.10
3	167.2	11.4	6.82	7.5	4.49	1011781	0.0912	0.41	813607	0.0633	0.28
4	247.2	15.8	6.39	9.9	1.01	1622853	0.1611	0.65	497904	0.0263	0.11
5	200.6	13.2	6.58	8.4	4.19	1152162	0.1072	0.45	860905	0.0689	0.29
6	950.8	37.6	3.96	18.1	1.90	788647	0.0656	0.13	2593290	0.2722	0.52
7	327.1	38.2	11.68	5.7	1.74	1563643	0.1544	0.27	1585987	0.1540	0.27
8	204.9	18.1	8.83	10.9	5.32	581540	0.0419	0.22	1445879	0.1376	0.73
9	246.8	13.7	5.55	8.4	3.40	794145	0.0662	0.23	860068	0.0688	0.23
10	270.6	11.9	4.40	8.4	3.10	1216583	0.1146	0.36	1826899	0.1823	0.57
11	520.6	23.6	4.53	16.7	3.21	1547051	0.1525	0.49	715742	0.0519	0.17
12	74.2	5.8	7.82	4.2	5.66	891384	0.0774	0.44	786193	0.0601	0.34

APPENDIX XXXVIII. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of the control, nitrate, phosphate and combined treated *Portieria hornemannii* collected in the laboratory fertilization experiment conducted February 1995. Algal material, crude extracts and HPLC fractions were weighed on an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

TREATMENT	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS/ INJ (mg)	% YIELD	OCHTODENE AREA	MASS/ INJ (mg)	% YIELD
CONTROL	652.3	13.0	1.99	11.2	1.72	1332337	0.1279	0.22	786078	0.0601	0.10
	1074.8	12.6	1.17	11.2	1.04	1586903	0.1570	0.16	819287	0.0640	0.07
	322.3	7.1	2.20	8.0	2.48	1305583	0.1019	0.25	1141735	0.1019	0.25
	730.1	37.1	5.08	19.2	2.63	850130	0.0727	0.19	1249323	0.1144	0.30
	674.5	15.1	2.24	13.6	2.02	1514185	0.1487	0.30	895265	0.0729	0.15
NITRATE	189.8	16.9	8.90	9.7	5.11	1294625	0.1236	0.63	933877	0.0775	0.40
	681.4	13.8	2.03	10.9	1.60	811719	0.0683	0.11	1564650	0.1515	0.24
	380.0	18.0	4.74	10.2	2.68	902078	0.0786	0.21	1499405	0.1438	0.39
	459.9	25.0	5.44	14.5	3.15	989399	0.0886	0.28	745834	0.0601	0.19
	1018.3	26.2	2.57	19.7	1.93	1010617	0.1820	0.35	1635652	0.3196	0.62
PHOSPHATE	423.6	18.7	4.42	15.2	3.59	1514578	0.1487	0.53	1535570	0.2962	1.06
	251.4	7.1	2.82	8.0	3.18	1252334	0.1187	0.38	304650	0.0036	0.01
	1682.6	24.6	1.46	18.9	1.12	720210	0.0578	0.65	1231736	0.1124	0.13
	1071.9	19.5	1.82	15.3	1.43	1081016	0.0998	0.14	568795	0.0458	0.07
	605.5	10.9	1.80	10.0	1.65	1323576	0.1269	0.21	326778	0.0062	0.01
COMBINED	821.5	27.6	3.36	14.8	1.80	653722	0.0502	0.09	1807110	0.3600	0.65
	760.5	19.1	2.51	14.8	1.95	897471	0.1562	0.30	1819946	0.3630	0.71
	960.5	21.4	2.23	11.1	1.16	1081016	0.0991	0.12	1755389	0.1739	0.20
	459.7	15.6	3.39	9.8	2.13	1511328	0.1484	0.32	431166	0.0184	0.04
	529.5	11.6	2.19	10.6	2.00	1021909	0.0923	0.19	1055466	0.0917	0.18

APPENDIX XXXIX. Individual dry mass and crude extract, HPLC fraction, peak 1 and ochtodene mass and percent yield of *Portieria hornemannii* collected at Anae Island on February 28, 1995 as control for the laboratory experiment conducted February 1995. Algal material, crude extracts and HPLC fractions were weighed on an analytical balance. Peak 1 and ochtodene mass were determined by analytical HPLC.

SAMPLE	DRY MASS (mg)	CRUDE EXTRACT MASS (mg)	% YIELD	HPLC FRACTION MASS (mg)	% YIELD	PEAK 1 AREA	MASS/ INJ (mg)	% YIELD	OCHTODENE AREA	MASS/ INJ (mg)	% YIELD
1	494.9	18.9	3.82	17.7	3.58	453568	0.0272	0.10	1374664	0.1292	0.46
2	323.8	8.9	2.75	6.9	2.13	1131868	0.1049	0.22	2469298	0.2577	0.55
3	849.6	30.1	3.54	24.1	2.84	718836	0.0576	0.16	2124044	0.2172	0.62
4	134.3	9.5	7.07	5.1	3.80	566032	0.0401	0.15	1615113	0.1574	0.60
5	327.3	18.2	5.56	13.0	3.97	1034583	0.0938	0.37	2265678	0.2338	0.93
6	628.0	17.5	2.79	16.1	2.56	1563643	0.1544	0.40	2402874	0.2851	0.73
7	109.1	1.8	1.65	3.9	3.58	581540	0.0419	0.15	1205884	0.1094	0.39
8	124.5	7.6	6.10	5.5	4.42	1028257	0.0931	0.41	1434370	0.1362	0.60
9	457.9	23.0	5.02	20.2	4.41	499395	0.0325	0.15	2035260	0.2067	0.91

APPENDIX XL. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the *Portieria hornemannii* collected at Anae Island on February 9, 1995 as control for the laboratory experiment conducted February 1995.

SAMPLE	Peak 1		2		3		4		5		6	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
1	1.41	709414	1.62	28475	1.83	1346985	1.98	343997	2.07	376121	2.35	129455
2	1.47	873603	1.64	19808	1.85	347133	1.98	393868	2.06	345857	2.25	71059
3	1.29	1011781	1.69	625877	1.77	807546	1.90	326436	2.08	39893	2.16	61808
4	1.43	1622853			1.85	474113	1.98	644219			2.29	80750
5	1.44	1152162			1.92	472085	2.06	459748	2.15	342627	2.42	32099
6	1.43	788647	1.63	70360	1.83	1376878			2.06	266326	2.32	164170
7	1.44	1563643			1.88	483007	1.96	883182			2.21	70412
8	1.44	581540	1.67	18437	1.90	1467044	2.08	409440	2.17	438163	2.44	25376
9	1.41	794145	1.62	21863	1.83	908621	1.97	723463	2.06	425970	2.29	90840
10	1.46	1216583			1.92	788399	2.08	237162	2.17	293949	2.53	70058
11	1.48	1547051			1.92	757307	1.99	1927265			2.57	78607
12	1.41	891384	1.62	74045	1.73	50987	1.83	894550	2.07	146018	2.29	30914

SAMPLE	Peak 7		8		9		10		11		Ochtodene	
	R.T.	AREA	R.T.	AREA	R. T.	AREA	R. T.	AREA	R. T.	AREA	R.T.	AREA
1			2.65	187836	3.22	79571	4.10	175332	4.43	117360	5.76	1835793
2			2.67	74157	3.11	41237	3.18	47482			5.55	704978
3	2.44	88376			3.13	83197					5.96	813607
4					3.22	112148					5.85	497904
5	2.47	68188	2.87	32281	2.94	43352	3.58	43872	5.13	59941	7.03	860950
6	2.60	51199	2.69	121081	3.19	77076	3.34	140841			5.96	2593290
7	2.60	95871	3.03	56992	3.26	62513					5.31	1585987
8	2.55	97244	2.93	31444	2.98	30372	3.06	33252	3.65	29904	7.20	1445879
9	2.66	32485	2.70	21420	2.78	22105	3.23	22195	3.31	29004	5.84	860068
10	2.90	34641	2.96	52906	3.61	37700	3.78	37497			7.00	1826899
11	2.65	189903	3.08	102647	3.16	45958	3.32	42868	3.33	95172	5.50	715742
12	2.35	55948	2.71	34795	3.04	139400	3.22	140570	4.41	70258	5.82	786193

APPENDIX XLI. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the control treatment *Portieria hornemannii* in the laboratory fertilization experiment conducted February 1995.

TANK	Peak 1		2		3		4		5		6		7		8	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
1	1.46	1332337	1.62	193663	1.71	242038	1.83	342038			2.06	247356	2.26	252187		
3	1.43	1586903	1.66	69517	1.79	54203			1.90	223295			2.16	142887		
7	1.23	1305583			1.70	464751			1.96	52646	2.03	131322	2.24	41775	2.35	51276
9	1.47	850130					1.84	1215076	1.98	156856	2.07	295034	2.25	53771	2.34	146480
16	1.45	1514185			1.71	422222	1.83	474840			2.05	258655	2.26	207327		

TANK	Peak 9		10		11		12		13		14		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
1	2.61	89663	2.71	168153			3.29	287485			4.16	123759	6.12	786078
3	2.47	147031			2.92	98029	3.13	31108	3.66	39886	4.86	109618	6.95	819287
7	2.41	77924	2.74	45969	2.80	85888	3.47	40436	3.59	54136			7.09	1141735
9	2.62	70522	2.74	214438			3.17	97969					5.66	1248323
16	2.60	80359	2.68	78826			3.28	156649	3.39	64750			6.12	895265

APPENDIX XLII. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the nitrate treatment *Portieria hornemannii* in the laboratory fertilization experiment conducted February 1995.

TANK	Peak 1		2		3		4		5		6		7		8	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
2	1.44	1294625			1.85	496635	1.97	92163	2.07	205094	2.30	187242	2.72	158074		
6	1.46	811719					1.95	805419	2.22	150624	2.30	279065	2.62	191879	2.74	232916
10	1.43	902078	1.64	25710	1.91	286704			2.06	201149	2.15	188329	2.47	81594		
14	1.45	989399			1.84	523777					2.14	145476	2.46	159205	2.89	156658
18	1.45	1010617			1.83	699846	1.97	215621	2.05	357269	2.28	196214	2.69	106052	2.75	120192

TANK	Peak 9		10		11		12		13		14		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
2					3.24	30457	3.31	56247			4.45	32179	5.83	933877
6	3.03	172384	3.09	113680	3.37	203074	3.78	182219	3.90	378242	5.72	308394	7.48	1564650
10	2.93	76999					3.58	32136	3.68	34702			6.96	1499405
14							3.63	101261					6.95	745834
18					3.19	117099	3.98	118522					5.73	1635652

APPENDIX XLIII. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the phosphate treatment *Portieria hornemannii* in the laboratory fertilization experiment conducted February 1995.

TANK	Peak 1		2		3		4		5		6		7		8	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
5	1.44	1514578			1.72	362233	1.83	1206541			2.05	396172	2.31	257583	2.59	109884
11	1.47	1252334			1.77	329031	1.86	206600	1.96	217167	2.09	175179	2.29	154131		
13	1.45	720210	1.63	71328			1.89	354858			2.05	86529	2.15	223114	2.48	145414
17	1.44	1082354														
19	1.45	1323576					1.83	611727	1.97	202570	2.04	210936	2.27	245205		

TANK	Peak 9		10		11		12		13		14		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
5	2.68	220202	3.20	105661	3.29	54172	3.36	159074					6.05	1535570
11	2.64	86819											6.33	304650
13	2.92	100229	3.00	73150	3.17	65308	3.57	44723	3.68	56464	4.90	128590	6.99	1231736
17	2.63	28840											6.09	5687515
19	2.67	118187											5.80	326778

APPENDIX XLIV. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the combined treatment *Portieria hornemannii* in the laboratory fertilization experiment conducted February 1995.

TANK	Peak 1		2		3		4		5		6		7		8	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
4	1.41	653722	1.61	24251	1.83	425593	1.97	89991	2.06	244056	2.29	48418	2.33	64710	2.62	184372
8	1.42	897471	1.63	39077	1.88	457100			2.13	203770			2.47	144366		
12	1.44	1081016	1.64	49875	1.85	1039106			2.07	232015			2.33	156797	2.61	56191
15	1.43	1511328	1.68	1110053							2.23	208864			2.56	119403
20	1.44	1021909	1.69	308887	1.81	290079			2.03	183204					2.59	61376

TANK	Peak 9		10		11		12		13		14		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
4					3.20	44648	3.27	48536			4.01	120078	5.72	1807110
8	2.89	106811	2.96	53114			3.52	46764	3.62	53356	4.80	49418	6.81	1819946
12	2.72	132243			3.23	48218	3.30	82675					6.05	1755389
15													6.09	431166
20	2.69	106786											6.08	1055466

APPENDIX XLV. Retention times (R.T.) and areas for the major (peak 1 and ochtodene) and minor compounds in the *Portieria hornemannii* collected at Anae Island on February 28, 1995 as control for the laboratory experiment conducted in February 1995.

SAMPLE	Peak 1		2		3		4		5		6		7		8	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA
1	1.45	453568					1.91	2463424			2.18	273869			2.56	198680
2	1.45	1131868	1.65	68616			1.83	1512659			2.19	73148	2.32	171268	2.59	97753
3	1.45	718836	1.63	121221	1.73	148892	1.83	2602994	2.06	312726			2.32	289374	2.60	142112
4	1.45	566032	1.63	38202			1.89	176480	2.05	41389	2.14	109657			2.47	68936
5	1.44	1034583			1.71	120360	1.83	842240	2.05	228640			2.31	106916	2.59	53035
6	1.43	734007	1.66	47555			1.83	2801151	2.06	481862			2.32	216104	2.60	126452
7	1.43	346776					1.80	88324							2.59	47181
8	1.42	1028257					1.83	405498	2.05	99191			2.26	83320	2.59	40822
9	1.45	499395			1.74	109120	1.84	2485557	2.08	288561			2.36	183564		

SAMPLE	Peak 9		10		11		12		13		14		15		Ochtodene	
	R.T.	AREA	R.T.	AREA	R.T.	AREA	R.T.	AREA	R. T.	AREA	R. T.	AREA	R. T.	AREA	R.T.	AREA
1			2.77	34606	3.00	154634	3.26	57602	3.66	47801	3.78	40413	5.24	91991	7.23	1374664
2	2.69	80545			3.12	97652									5.49	5469298
3	2.69	166643					3.22	81755	3.38	200370					6.05	2124044
4			2.91	67972	2.99	47601			3.56	50556	3.67	77385	4.89	62275	6.92	1615113
5	2.69	66470					3.31	83574							6.04	2265678
6			2.71	117939	3.14	121017					3.73	113741			5.54	2402874
7	2.69	73588	2.81	82237	3.20	59448	3.26	112670							6.02	1205884
8	2.69	76635	2.81	63541			3.28	114591							6.01	1434370
9	2.72	79881	2.80	51878			3.23	75886							5.76	2035260