Tissue Nitrogen- and Phosphorus-Content of *Gracilaria coronopifolia* **J. Agardh from Iligan Bay in Relation to the Seawater Nutrient Concentration**

PATRIA ROSALES-APAO WINFRID SCHRAMM

Abstract

Tissue nitrogen- and phosphorus-content of Gracilaria coronopifolia J. Agardh were analysed from samples collected in Iligan Bay (Initao, Misamis Oriental, Northern Mindanao, Philippines) over a 20-month period.

The samples were oven-dried (24 h at 70 ° C) and ~ound to fine powder for nitrogen analysis employing a Carlo Erba C/H/N Elemental Analyser. Tissue ^phosphorus content was measured photometrically as phJsphate following digestion in a concentrated HCl of combusted materials (5 h at 550 ° C).

Tissue nitrogen-and phosphorus-content (0.7 to 2.76 % *g dw and 0.03 to·0.26* % *g dw, respectively) varied throughout the collecting period without showing distinct seasonal trends.*

The *results may show that nutrient acquisition and -primary productivity of seaweeds are not solely a function of the external nutrient concentration, but are influenced by time dependent processes such as nutrient accumulation and internal nutrient levels, adaptatjon and physiological state of the seaweeds.*

Introduction

t present much of the Philippine seaweed production for export and processing for phycocolloid are derived from the cultivation of Eucheuma, the leading economically important seaweed. However, processing for phycocolloid are derived from the cultivation of *Eucheuma,* the leading economically important seaweed. However, other genera are identified of importance for their chemical constituents and potential for culture such as *Gracilaria* (frono & Ganzon-Fortes, 1981, 1988; Legasto, 1988).

Although. technology for pond culture of *Gracilaria* is available from Taiwan (Shang 1976, Yang & Wang 1983), this has not been widely practised in the Philippines (e.g. Hurtado-Ponce, 1990). Thus much of the seaweeds utilized for production are derived from the natural stock from the wild.

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Despite continuous commercial utilization and increasing demand for phycocolloid, in the Philippines little basic information is available on the be or environmental conditions, as for example nutrient availability w_{th} ; of the structurity and chamical quality \hat{a} affect productivity and chemical quality of *Gracilaria*. **• A Constant** which

Among the environmental factors affecting algal growth and productivity, nutrients play an important role (e.g. Ryther & Dunstan 1971, 1981, ity, nutrient play and important r-0le (e.g. Rosenberg *et al.*, 1984). This may apply especially in the tropics where the nutrients in offshore waters are said to be low, therefore limiting
hytoplankton production (DeBoer 1981, Smith 6, Atl.: nytopiankton production (DeBoer 1981, Smith & Atkinson, 1984; Dawes
987).

It has been demonstrated that seaweeds in temperate waters take up nutrients proportional to the seawater concentrations as documented by seasonal changes in internal tissue nitrogen- and phosphorus- content of seaweeds (Chapman & Craigie, 1977, Hanisak 1979, Asare & Harlin 1983, Kornfeldt 1982, Asare & Harlin, 1983). seaweeds (Chapman & Craigie, 1977, Hanisak 1979, Asare & Harlin 1983,

In the Philippines, no studies had been done on the internal nutrient concentration in relation to the external nutrient concentration, therefore this study. In addition to characterization of the internal nutrient concentration, the productivity of the macrophyte is described..

This paper provides background data on nutrient condition in the natural habitat and. on variation of internal nitrogen- and phosphoruscontent of *Gracilaria coronopifolia* J. Agardh from Iligan Bay. In addition the importance of internal nitrogen and phosphorus to the productivity of the species is discussed.

Materials.and Methods

The algae were collected from the wild at about 2 m in Initao, Misamis Oriental, Northern Mindanao, Philippines (Figure. 1) during a 20-month period from April 1987 to November 1988. .

Samples from different individuals were combined and after removal of Samples from different individuals were combined and after removal of
ther attached algae and debris, weighed, oven-dried (24 h at 65 - 70) Centigrade) and finally ground to fine powder with a mortar and pestle The ground material were kept in polypropylene vials in dessicator $^{\text{u}}$

further analysis.
Itssue nitrogen content was measured employing a Carlo Erba C/H/N . The neutrogen content was measured employing a Carlo Erba C/H/N Elemental Analyzer at the Institute for Marine Sciences, Kiel, Germany. Total phosphorus in the tissue was determined as phosphate ϵ and ϵ ϵ ϵ ϵ with concentrated HCl of combusted material (5 hours at 550°Centigrade) and subsequent dilution with distilled water (Schramm & Booth, 1981). Aliquots were taken for spectrophotometric analysis according to Grasshoff

Figure 1. Location of sampling station (Initao) in Iligan Bay, Mindanao, Philippines.

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al. (1983). Two to three replicate analyses were made from the combinent and the combinent of the combinent and combinent an

samples.
In order to relate the internal nutrient content with the external source,
seawater samples were collected from the same site. A 100-ml plastic
seawater samples were collected from under or water in the also like seawater samples were collect bottom water or water in the algal bed. Three
syringe was used to collect bottom water of water completesyringe was used to collected each time. Water samples were transferred plicate samples were collected each time. Water samples were transier
to separate polypropylene containers and were brought in a cooling b
to separate polypropylene containers and were brought in a cooling b to the laboratory for analysis within six hours. Water samples were analysed for inorganic nitrate, nitrite, ammonium, phosphate and *urea* following standard methods (Grasshoff *et al.* 1983). Absorbance readings were made with a double beam spectrophotometer (SHIMADZU 150).

Results

Concentrations of inorganic nitrate, nitrite, ammopium and phosphateion as well as urea in the seawater varied, however, did not show a seasonal trend throughout the sampling period (Figure 2,3;Table -1). Although ammonium and urea.were not determined during the first 8 months *of* the study period, we may therefore assume that the concentrations of these nitrogen sources have been similar to those of the succeeding months.

Tissue N- and P-content of *Graci/aria coronopifolia* varied considerably, however, without showing seasonal trends during the 20-month sampling period (Figure 4, Table 2). Tissue N- content values ranged from 0.7 to 2.67 % g dry weight, with the lowest and the highest values observed in the months of March and November 1988, respectively. On average, tissue Ncontent was 1.35% g dry weight. Tissue P-content, on the other hand, varied from 0.03 to 0.26% g dry weight with the highest values observed in

ecember.
Tissue N has a low correlation (0.389, P>0.05) with nitrate concentration Tissue N has a low correlation (0.389, P>0.05) with research the water whereas tissue P-content and phosphate concentration the water whereas tissue P-content and μ rose μ -
egatively correlated (-0.86, P> 0.05).

ively correlated (-0.86, P> 0.05).
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zal species

Discussion -*DISCUSSION*

Trop· *¹*• $\frac{1}{2}$ is a matrix nutrience of the matrix often point in the matrix often point $\frac{1}{2}$ and $\frac{1}{2}$ are very often point $\frac{1}{2}$ and $\frac{1}{2}$ are very often point in the matrix of the matrix of the matrix of th Tropical marine waters are usually considered as P
rticularly in offshore areas, nitrogen as well as phosphorus are ver rly in offshore areas, nitrogen as well as phosphorus are in the factors for primary production. the limiting factors for primary production.
On the other hand, it is well known from temperate areas that nutrient

conditions in the offshore pelagic systems may considerably differ from benthic systems, particularly from phytobenthic communities, where as ^a result of rapid regeneration of nutrients in the sediment system and through the activity of the associated epifauna and microorganisms (e.g. Nowicki & Nixon, 1985), seawater nutrient concentration in the plant canopy are significantly higher compared to the surface water.

In the Baltic Sea, for example, in *Fucus* or red-algal communities nutrient levels are mostly beyond growth saturating levels, even in early summer, when in the water column of the offshore areas nutrient concentrations usually become limiting for primary production (Schramm *et al.* 1988). Similar observations were made in subtropical *Cladophora* beds (Schramm & Booth 1981) and tropical coral reef flats or seagrass communities (Schramm,

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unpublished data).

Also in our study area at Initao, the nutrient concentrations were in the range determined in other locations in the Philippines (e.g. Horstman, 1980, Dy, 1984, Bodungen et al. 1985) and slightly higher than in the offshore water, although considerably lower than those known for coastal waters of higher latitudes (e.g. Wheeler & North, 1980; Asare & Harlin, 1983, Schramm et al., 1988, Lyngby 1990).

Seasonal variation in nutrient concentration in the seawater in the tropics are usually small in comparison to temperate waters,' where seasonality is triggered by key environmental factors such as light and temperature. The observed distinct, but irregular nutrient fluctuations in the seawater of Iligan Bay may have been influenced by wind regimes (monsoons), but also the influence of land run-off, precipitation, nutrient
remineralization and run-off, precipitation, nutrient remineralization and seasonal changes in biological activity in the area
could not be discussed. could not be disregarded. Tissue N - and P - content of the seaweed in the field varied throughout

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Date	$NO3-N$	$NO2$ - $NH4$ -N			Urea-N	$PO4-P$	
1987							
April	0.09	0.07					0.53
May	0.08	0.20					0.47
June	0.10	0.73					1.48
July	0.61	0.03					0.30
Aug	0.34	0.15					0.31
Sept	2.77	0.17					0.3
Oct	1.11	0.22					0.96
Nov	1.36	0.17					0.18
Dec	0.60	0.09			0.20	0.87	0.38
Dec	0.89	0.07			3.68	0.84	0.20
1988							
Jan	0.18	0.94	0.61	0.07	0.46		
Feb	0.41	0.28	4.78	0.50	0.63		
Mar	0.34	0.28	1.34	0.67	0.35		
Apr	0.14	0.13	0.67	$0.31 \cdot$	0.29		
Jun	1.84	0.06	0.35	0.54	2.05		
Aug	0.68	0.20	4.86	3.13	0.35		
Sep	1.81	0.72	1.36	1.16	0.51		
Oct	4.28	0.45	0.86	0.53	0.76		
Nov	6.56	0.17	1.84	1.51	0.46		
Total	24.19	5.13	20.55	10.13	11.0		
Mean	1.27	0.27	1.87	0.92	0.57		

Table 1. Mean nutrient concentration (umol.dm· 3+ /-SD; n=3) in seawater from the algal bed.

the sampling period without showing any seasonal trend. This is in contrast to other observations, especially for seaweeds from temperate zones, which usually show distinct changes in the tissue N-and P-content (Topinka & Robbins, 1976; Chapman & Craigie, 1977; Hoyle, 1978; Komfeldt, 1982; Rosell & Srivastava, 1985; Schramm *et al.* 1988).

Field and laboratory studies have shown the influence of the seawater nutrient concentrations on internal nutrient levels and the dependence of growth on both external and internal nutrient levels (Dawes *et al.* 1974; D~Boer *et al.,* 1978, Gagne & Mann, 1981; Bird, 1984). During periods of excess nutrient supply, seaweeds are very often capable of luxury or surplus
untake of nitrogen and of phosphorus (Chapman & Craigie 1977, Cordon uptake of nitrogen and of phosphorus (Chapman & Craigie 1977, Gordon *et al.,* 1981, Rosenberg *et al.*, 1984; Fujita, 1985; Hurd & Dring, 1990).

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Tissue N-and P-content (% g d w) of G. coronopifilia from Initao, Misamis Figure 4. Orientnal from April 1987 to November 1988.

Ammonium-nitrogen, for example, is rapidly assimilated (Rosenberg et al., 1984, Thomas & Harrison, 1987). Gracilaria tikvahiae was able to double tissue N-content within hours when ammonium was supplied (Ryther et al., 1981).

In Iligan Bay where the nutrients are low throughout the year, nutrient availability may neither be high enough for surplus uptake, nor even for saturated uptake. The tissue N - and P - contents of Gracilaria coronopifolia from Iligan Bay are considerably lower compared to Gracilaria species from temperate waters (e.g. Wallentinus, 1981, Penniman et al., 1986).

Laboratory experiments on nitrogen and phosphorus uptake by G. coronopifolia have shown that the critical tissue N-content for saturated growth is approximately 2 % g dry weight (Rosales-Apao, 1991). On average *G. coronopifolia* from the field were below the critical tissue N content for saturated growth. In some cases, the tissue N-content was even in the upper range of viable tissue N - content, i.e. the tissue N-level barely enough for algal maintenance, which was determined as 0.5-1.0 % dry

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Table 2. ISSue N- and P-content (% g dw) of field samples collected from Initao (Iligan Bay, Philippines) from April 1987 to November 1988. For each analysis several dried samples were powdered and analysed together.

weight in the laboratory (Rosales-Apao, 1991).

Similarly, tissue P- content of our plant material from the wild was below the critical tissue P for saturated growth (ca. 0.3 % dry weight P), but were higher than the minimum viable tissue P levels(< 0.01 % dry weight P) as determined in laboratory experiments (Rosales-Apao, 1991).

The results suggest that average nutrient levels in Iligan Bay are not high enough to build up internal nutrient levels sufficient for continuous optimum or saturated growth of *Graci/aria coronopifolia.* Howeve concentrations are sufficient to supply nutrient above the viable level $_{\text{for}}$ concentrations are surfacent to supply matrical above the viable level for maintenance.

The results imply that for the purpose of farming or ranching of *Gracilaria coronopifolia,* as probably also for other seaweeds, information on the ranges and average levels of nutrient is advisable if not a prerequisite for the selection of a proper site. It should also be considered whether artificial re economication of the seaweeds, which is common in seaweed farming, could *fertuizing of the seaweeds, which is common in seaweed farming, could also be applied for <i>Gracilaria*.

Acknowledgment

Acknowledgment is due to the Arthur und A.A. Feindt Stiftung (Hamburg, Germany) and the DAAD (German Academic Exchange Service) for the financial support given to the authors. Also to MSU-Naawan and MSU-**Marawi (College of Fisheries) for the use of their facilities.**

References

Asare, S.0. & **M.M.** Harlin. 1983. Seasonal fluctuations in tissue nitrogen for five species of perennial macroalgae in Rhode Island Sound. *J. Phycol.* 19: 254 -257.

Bird, K.T. 1984. Seasonal variation in protein carbohydrate ratios in a subtropical estuarine alga, *Gracilaria verrucosa* and the determination of nitrogen limitation status using these ratios. *Bot. Mar.* 24: 111 - 115.

Bodungen von, B., W. Balzer, M. Bolter, G. Graf, G. Liebezeit & F. Pollehne. 1985. Chemical and biological investigations of the pelagic. system of the Hilutangan Channel (Cebu, Philippines). *The Phil. Scientist.* 22: 4 - 24.

Chapman, A.RO. & J.S. Craigie. 1977. Seasonal growth in *Laminaria longicruris:* relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol.* 40: 197 - 205.

Dawes, C. J. 1987. The biology of commercially important tropical marine algae. In: **K.T.** Bird and P.H. Benson (eds.) *Seaweed Cultivation for Renewable Resources. Developments in Aquaculture and Fisheries Science.* Vol. 16. Elsevier, Amsterdam. PP· 155 - 190.

Dawes, C.J., J.M. Lawrence, D.H. Cheney & A.C. Mathieson. 1974. Ecological studies of floridian *Eucheuma* (Rhodophyta, Gigartinales). III. Seasonal variation of carrageenan, total carbohydrate, protein, and lipid. *Bull. Mar. Sci.* 24: 286 - 299•

DeBoer, J. A. 1981. Nutrients. In: C.S. Lobban and M.J. Wynne (eds.) *The Biology*

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of Seaweeds. Blackwell Scientific Publications, Oxford. 17: 356 - 362.

DeBoer, J.A. H.J. Guigli, T.L. Israel & C.F. D'Elia. 1978. Nutritional studies of two red algae. I. Growth rate as a function of nitrogen source and concentration. /. *Phycol.* 14: 261 - 266.

Dy, D.T. 1984. Nutrient flux across the sediment - water interface and the role of marine sediments in the nutrient budget of a *Eucheuma-* farm. M. S. Thesis. University of San Carlos, Cebu City, Philippines.

Fujita, RM. 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. /. *Exp. Mar. Biol. Ecol.* 92: 283 - 301.

Gagne, J.A. & K.H. Mann.1981. Comparison of growth strategy in *Laminaria* populations living under differing seasonal patterns of nutrient availability. *Xth Intl. Seaw. Symp.* pp. _292 - 302.

Gordon, D.M., P.B. Birch & A.J. McComb. 1981. Effects of inorganic phosphorus and nitrogen on the growth of an estuarine *Cladophora* in culture. *Bot. Mar.* 24: 93 - 106.

Grasshoff, K . ., M. Erdhardt & K. Kremling (eds.) 1983. *Methods of Seawater Analysis.* Verlag Chemie, Weinheim. 419 p.

Hanisak, M.D. 1979. Nitrogen limitation of *Codium fragile* ssp. *tomentosoides* as determined by tissue analysis. *Mar. Biol.* 50: 333 - 337.

Horstmann, U. 1980. Observations on the peculiar diurnal migration of a red tide dinophyceae in tropical shallow waters./. *Phycol.* 16: 481 - 485.

Hoyle, M. D. 1978. Agar studies in two *Gracilaria* species (G. *bursapastoris* (Gmelin) silva and G. *coronopifolia* J. Ag.) from Hawaii. II. Seasonal aspects. *Bot. Mar.* 21: 347 - 352.

Hurd, C. L. & M. J. Dring. 1990. Phosphate uptake by intertidal algae in relation to zonation and season. *Mar. Biol.* 107: 281 - 289.

Kornfeldt, R. 1982. Relation between nitrogen and phosphorus content of macroalgae and the waters of Northern Oresund. *Bot. Mar.* 25: 197 - 201.

Lyngby, J.E. 1990. Monitoring of nutrient availability and limitation using the marine macroalga *Ceramium rubrum* (Huds.) C. Ag. Aquat. Bot. 38: 153- 161.

Nowicki, B.L. & S. Nixon. 1985. Benthic nutrient remineralization in a coastal lagoon ecosystem.·*Estuaries.* 8 (28) : 182- 190.

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Penniman, C.A., A.C. Mathieson and C. E. Penniman. 1986. Reproductive Phenology and Growth of *Gracilaria tikvahiae* McLachlan (Gigartinales, Rhodophyta) in the Great Bay Estuary, New Hampshire. *Bot. Mar.* 24: 147-154.

Rosales-Apao, P. A. 1991. Nutrient requirements of selected tropical seaweeds potential for mariculture. Doctoral Dissertation, University of Kiel, Germany.

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

Ryther, J. **H., N.** Corwin, T. A. DeBusk & -L. D. Williams. 1981. Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (Mclachlan 1979). *Aquaculture.* ²⁶ : 107 -115.

Rosenberg, G., T. A. Probyn, & **K. H.** Mann. 1984. Nutrient uptake and growth kinetics in brown seaweeds: response to continuous and single additions of ammonium. /. *Exp. Mar. Biol. Ecol.* 80: 125 - 146.

ROSELL, K. & L. SRIVASTAVA. 1985. Seasonal variations in total nitrogen, carbon and amino acids in *Macrocystis integrifolia* and *Nereocystis lutkeana* (Phaeophyta). /. *Phycol.* 2 : 304 - 309.

Schramm, W. & W. Booth. 1981. Mass bloom of the alga *Cladophora prolifera* in Bermudas: Productivity and phosphorus accumulation. *Bot. Mar.* 24: 419 - 426.

Schramm, W., D. Abele, & G. Breuer. 1988. Nitrogen and phosphorus nutrition and productivity of two community forming seaweeds *(Fucus vesiculosus, Phycodrys rubens* from the Western Baltic (Kiel Bight) in the light of eutrophication processes. *Kieler Meeresforsch., Sonderh.* 6: 221 - 240.

Shang, Y. C. 1976. Economic aspects of *Gracilariaculture* in Taiwan. *Aquaculture.* $8:1 - 7.$

Thomas, T. E. & P. J. Harrison. 1987. Rapid ammonium uptake and nitrogen interactions in ffve intertidal seaweeds grown under field conditions. /. *Exp. Mar. Biol. Ecol.* 107: 1 - 8.

Topinka, J. A. & J.V. Robbins. 1976. Effects of nitrate and ammonium enrichment on growth and nitrogen physiology in *Fucus spiralis. Limnol. Oceanogr.* 21: 65⁹ - 664.

Wallentinus, 1.1981. Chemical constituents of some Balticmacroalgae in relation to environmental conditions. In: T. Levring (ed.) *Proc. Int. Seaweed Symp.* 10: 363-370•

Yang, S.-S. and C.-Y. Wang. 1983. Effect of environmental factors on *Gracilaria* cultivated in Taiwan. *Bull. Mar. Sci.* 33 (3): 759 -766.