Bol. Inst. Esp. Oceanogr. 15 (1-4). 1999: 337-341 **BOLETÍN** INSTITUTO ESPAÑOL DE OCEANOGRAFÍA
ISSN: 0074-0195

Nitrogen and phosphorus in *Ulva* sp. in the Galician Rias Bajas (northwest Spain): Seasonal fluctuations and influence on growth

R. Villares, X. Puente and A. Carballeira

Área de Ecoloxía, Facultade de Bioloxía, Universidade de Santiago de Compostela. 15706 Santiago de Compostela (A Coruña), Spain

Received September 1997. Accepted May 1998.

ABSTRACT

Coastal eutrophication has given cause for increasing concern. The Galician rias are ecosystems very sensitive to this phenomenon. In the present paper, we evaluate the possible nutrient limitation in *Ulva* sp., a green-tide alga. Variations in tissue nitrogen (N) and phosphorus (P), as well as growth rate, were determined over a complete seasonal cycle for *Ulva* sp. collected in the Rias Bajas (northwest Spain). Minimum levels of both nutrients were reached in spring and summer and maximun in winter. The low tissue P concentrations were striking. There was a parallelism between the evolution of the growth rate and nutrient contents. The most significant correlation found between growth rate and tissue P, and the equations established by stepwise multiple regression procedures, suggest that P may play a more important role than N in the limitation of productivity of *Ulva* sp. in the Galician Rias Bajas.

Key words: *Ulva*, eutrophication, nutrients, nitrogen, phosphorus, growth, Galicia.

RESUMEN

Nitrógeno y fósforo en* Ulva *sp. en las Rías Bajas gallegas: cambios estacionales e influencia en el crecimiento

La eutrofización costera es un problema de creciente preocupación y las rías gallegas son ecosistemas sensibles a este fenómeno. En este trabajo se intenta evaluar la posible limitación nutritiva en Ulva *sp., un alga que forma parte de las denominadas mareas verdes. Se realizaron análisis de nitrógeno (N) y fósforo (P), así como bioensayos de crecimiento en* Ulva *sp. recogida mensualmente en cuatro rías de la costa gallega durante un año. Los niveles mínimos de ambos nutrientes se registraron en primavera y verano y los máximos en invierno, destacando las bajas concentraciones de P encontradas. Se observó un paralelismo entre la evolución de las tasas de crecimiento de* Ulva *y las concentraciones de nutrientes en el talo a lo largo del ciclo anual estudiado. La mayor correlación encontrada entre tasa de crecimiento y concentración de P, y las ecuaciones establecidas por el procedimiento de regresión múltiple paso a paso, indican que el fósforo parece jugar un papel más importante que el nitógeno en la producción de* Ulva *sp. en las rías bajas gallegas.*

Palabras clave: Ulva, *eutrofización, nutrientes, nitrógeno, fósforo, crecimiento, Galicia.*

INTRODUCTION

Littoral proliferation of green macroalgae, known as green tides, is well known all over the world (Morand and Briand, 1996). In Europe, typical cases are found in the Northern Adriatic (Runca *et al.*, 1996; Viaroli *et al.*, 1996; Solidoro *et al.*, 1997), coast of Brittany (Ménesguen and Piriou, 1995), Baltic Sea (Pihl *et al.*, 1996; Lyngby and Mortensen, 1994; Bonsdorff *et al.*, 1997); see Schramm and Nienhuis (1996) for a European review. Coastal eutrophication can generate many harmful effects on the ecosystem, such as changes in benthic algal vegetation (Munda, 1993), seagrass decline (Short and Burdick, 1996), and changes in the zoobenthic communities (Norkko and Bonsdorff, 1996; Pihl *et al.*, 1995), as well as having an impact on recreational activities at affected beaches.

This phenomenon has been related to excessive nutrient enrichment of the aquatic environment (Rosenberg, 1985; Valiela *et al.*, 1997; Bonsdorff *et al.*, 1997). The shape of the Galician rias reduces the renovation of the water body, promoting eutrophication, mainly in those known as Rias Bajas, the most densely populated ones. Irritating effects of macroalgal proliferations on bivalves have been reported by Niell *et al.* (1996) and Rodríguez *et al.* (1987) in the inner part of the rias during summer.

This paper presents preliminary data on seasonal variation of nutrient contents in *Ulva* sp. along the western coast of Galicia (northwest Spain) and how nitrogen and phosphorus tissue levels control algal growth.

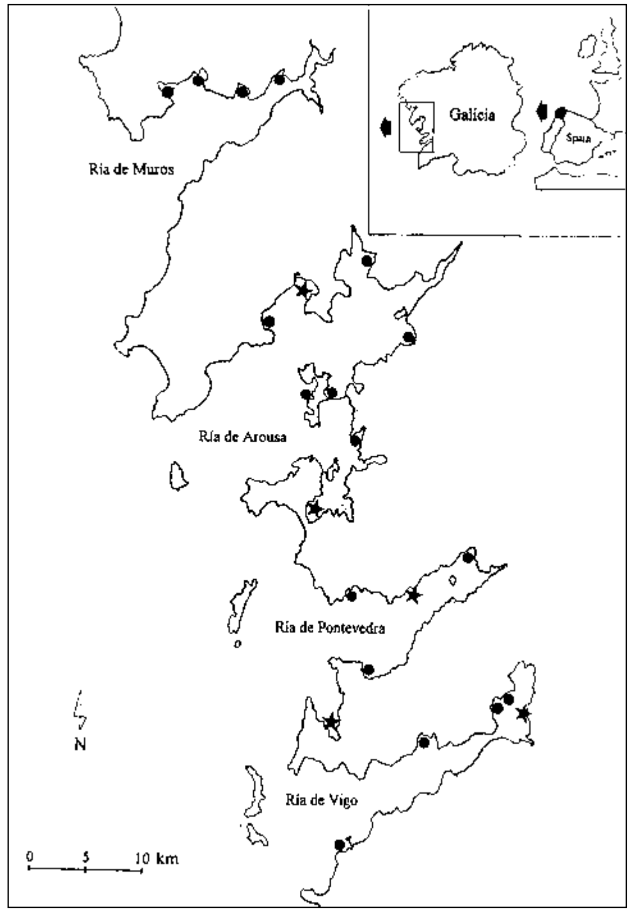

Figure 1. Location of sampling sites. Stars show the places where *Ulva* sp. was collected for growth bioassays

MATERIALS AND METHODS

Monthly samples (between March 1994 and February 1995) of free floating *Ulva* sp. fronds were collected from 22 stations along the four Rias Bajas (Muros, Arousa, Pontevedra and Vigo), on the west coast of Galicia (42°-43° N, 9° W) (figure 1). Fresh, epiphyte-free fronds were transported to the laboratory at 4 °C and frozen until analysis. Fronds were thoroughly washed with distilled water, dried at 55 °C and ground into fine powder using a mortar and pestle.

Total nitrogen in *Ulva* fronds was analysed in a LECO 1000 Elemental Analyzer (CHN). To determine total phosphorus, samples were ashed in crucibles in a mufla furnace at 550 °C, extractions were obtained with 2N HCl heating at 200 °C and brought to 50 ml with distilled water; vanadate-molybdate reagent for phosphate determination was added to these extracts and measured colorimetrically at 405 nm (Chapman and Pratt, 1981). Certified reference material (hay powder, CRM-129, Community Bureau of Reference) was used to get a proper extraction.

We measured growth rates of *Ulva* sp. from five locations sampled fortnightly from April to November. Growth rate was estimated in the laboratory from the increase in surface area of discs (17 mm in diameter) cut from free-floating thalli with a sharpened brass tube and incubated in Pyrex flasks with 400 ml of seawater. The latter was collected once in an open coastal zone far from any sewage discharge, filtered through Whatman GF/C filters and kept at 4 °C in total darkness throughout the experiment. Two replicates were used for each sampling site. Twenty discs were introduced into each flask. The area of the discs was calculated from two perpendicular diameters. Relative growth rate (μ) was calculated assuming an exponential function $\mu = 100\ (ln\ A_t\ \ ln\ A_0)\ t^{-1}$, where A_0 was the initial area and At the area of the disc after 9 days. Damaged discs were discarded. Incubation was carried out at 17 °C (12:12 light:dark) using warm white fluorescent tubes as a light source which supplied a photon flux density of about 80 μmol photons m^{-2} s^{-1}. Seawater was replaced every 3 days.

RESULTS AND DISCUSSION

Both tissue nutrients showed a clear seasonal pattern (figure 2). Minimum nitrogen values were found in summer and increased sharply towards winter. Similar patterns were found in northern Brittany (Ménesguen and Piriou, 1995), Oregon coastal waters (Wheeler and Björnsäter, 1992), the northern Adriatic Sea (Pugnetti, Viaroli and Ferrari, 1992) and southern Spain (Hernández *et al.*, 1997). Phosphorus levels in *Ulva* sp. showed a similar pattern, with the lowest concentrations in spring and early summer, and the highest in winter; however, it was not as marked as the nitrogen seasonal pattern. However, the relative increase between minimum and maximum levels of both nutrients was similar. On the contrary, Ménesguen and Piriou (1995), Wheeler and Björnsäter (1992) and Hernández *et al.* (1997) did not find a definite pattern for this nutrient for different species of *Ulva*.

Comparing seasonal tissue nutrient levels and critical contents (the concentration of a nutrient that limits maximal growth) determined under experimental conditions for *Ulva* sp. studied on the coast of Brittany by Le Bozec (1996), we observed phosphorus limitation during spring and summer in our coastal waters and a slight tendency towards nitrogen limitation in summer, as well (figure 2).

Phosphorus tissue levels found in this survey are striking, because they are clearly below those obtained by many other authors (Wheeler and Björnsäter, 1992; Ho, 1981; Ménesguen and Piriou, 1995; Hernández *et al.*, 1997) for *Ulva* spp. Some values were even lower than the subsistence concentration (the nutrient concentration that permits basal metabolism without growth) obtained by Le Bozec (1996) (figure 2b). Thus, the high values we obtained for the N:P ratio are due to these low levels of phosphorus (table I).

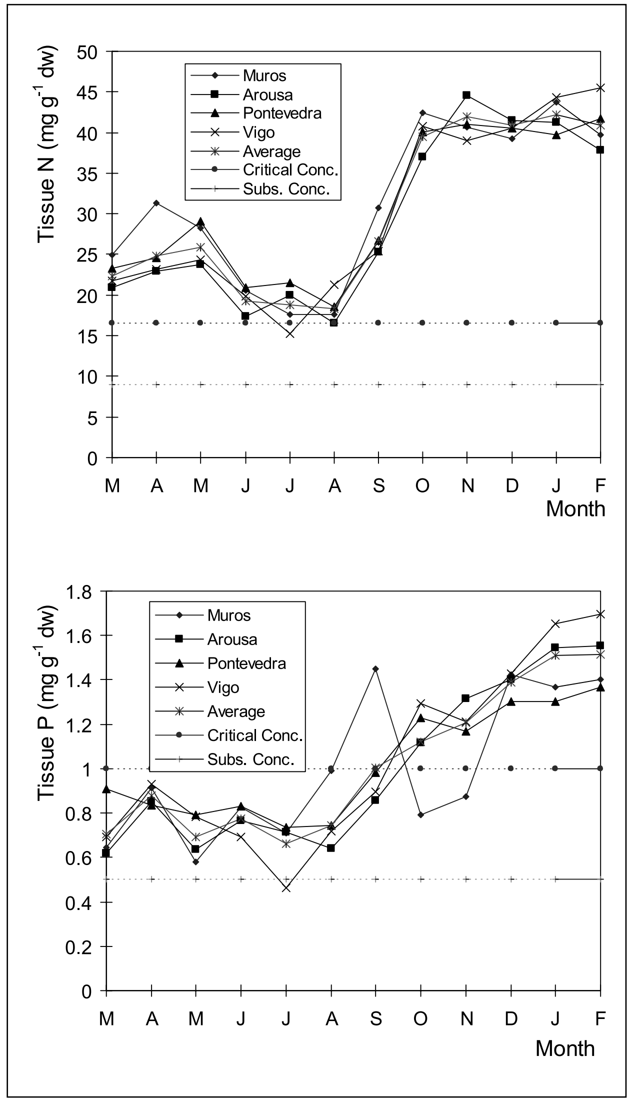

Figure 2. Seasonal evolution of tissue nutrient concentrations for *Ulva* sp. for nitrogen (a) and for phosphorus (b). Thin lines are the average of all the points of each ria, thick lines represent the whole of the littoral zone. Horizontal lines are critical and subsistence concentrations as determined by Le Bozec (1996)

Table I. Summary statistics for N and P contents (mg g^{-1} dw) and atomic N:P ratio for *Ulva* sp. (n = 282). Critical Concentrations (CC) and Subsistence Concentrations (SC) as proposed by Le Bozec (1996) for *Ulva* sp.

	Range	Mean ± SD	CC	SC
Nitrogen	7.0-51.0	28.8 ± 11.5	16.6	9.0
Phosphorus	0.25-2.3	0.97 ± 0.42	1.0	0.5
N:P	27.4-177.8	69.9 ± 23.7	—	—

Figure 3 shows the monthly average for tissue nutrient concentrations and growth rate for *Ulva* sp. at the five selected locations. As a whole, a parallelism can be observed among the three data sets: lowest growth rates during summer and increasing rates during fall towards winter, a seasonal pattern which agrees with the one we found in the tissue nutrient concentrations.

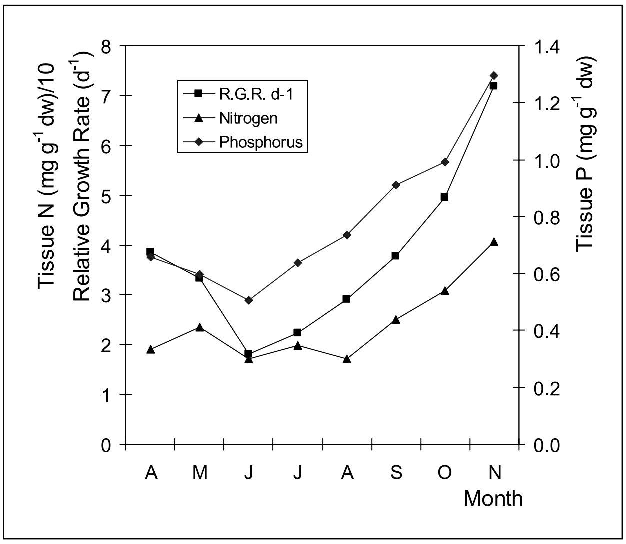

Figure 3. Seasonal evolution of growth rate (% per day) and tissue nutrient concentrations (mg g^{-1} dw) for *Ulva* sp. at the five selected locations

These results led us to ask which nutrient mainly controls algal growth. Macroalgal production is an important fraction of total primary productivity in coastal waters but it is frequently nutrient- limited. Nitrogen is generally considered the limiting nutrient for productivity of macroalgae in temperate coastal waters (Lobban and Harrison, 1994), but some authors have shown how phosphorus may also limit macroalgal production, at least during part of the year (Atkinson and Smith, 1983; Lapointe, 1987; Hernández *et al.*, 1993; Delgado, Ballesteros and Vidal, 1994; Peckol *et al.*, 1994; Vidondo and Duarte, 1995; Flores-Moya *et al.*, 1997).

We determined the relationship between tissue nutrient contents and growth rates for *Ulva* sp. The greater relationship between tissue P levels and growth rate (n = 69; r = 0.68, P < 0.001 for P; r = = 0.61, P < 0.001 for N) suggests that P plays a slightly more important role with regard to the control of growth by the nutrients. With the same aim of knowing how tissue nutrients control algal growth, we established a set of two equations (using the stepwise multiple regression procedure) that indicate the major influence of P than N on the growth of *Ulva*:

$$\mu = 11.8 + 3.08\ ln\ P \qquad r^2 = 0.46$$
$$P < 0.001 \qquad n = 69 \qquad [1]$$

$$\mu = 8.9 + 1.5\ N - 2.6\ ln\ N/P \qquad r^2 = 0.48$$
$$P < 0.001 \qquad n = 69 \qquad [2]$$

We can see in the two equations that the greater the level of tissue P, the greater the growth rate of *Ulva* sp. Therefore, we believe that, in general, P could be the limiting nutrient for primary production of *Ulva* in the western coastal waters of Galicia.

In the field, other factors can affect macroalgae growth. The temperature chosen for bioassays (17 °C), was selected because it could be considered as an average value in surface Rias Bajas water during the period of highest primary production. Minimum average temperatures in winter are about 13 °C; the low temperature, as well as the lower irradiance, can cause growth limitation in a season in during which nutrient tissue levels are well above the minimum required to sustain maximal growth.

REFERENCES

Atkinson, M. J. and S. V. Smith. 1983. C:N:P ratios of benthic marine plants. *Limnology and Oceanography* 28 (3): 568-574.

Bonsdorff, E., E. M. Blomqvist, J. Mattila and A. Norkko. 1997. Coastal eutrophication: Causes, consequences and perspectives in the archipelago areas of the Northern Baltic Sea. *Estuarine, Coastal and Shelf Science* 44 (A): 63-72.

Chapman, H. D. and P. F. Pratt. 1981. *Métodos de análisis para suelos, plantas y aguas.* Trillas. Mexico City: 195 pp.

Delgado, O., E. Ballesteros and M. Vidal. 1994. Seasonal variation in tissue nitrogen and phosphorus of *Cystoseira mediterranea* Sauvageau (Fucales, Phaeophyceae) in the Northwestern Mediterranean Sea. *Botanica Marina* 37: 1-9.

Flores-Moya, A., M. Altamirano, M. Cordero, M. E. González and M. G. Pérez. 1997. Phosphorus-limited growth in the seasonal winter red alga *Porphyra leucosticta* Thuret *in* Le Jolis. *Botanica Marina* 40: 187-191.

Hernández, I., A. Corzo, F. J . Gordillo, M. D. Robles, E. Sáez, J. A. Fernández and F. X. Niell. 1993. Seasonal cycle of the gametophytic form of *Porphyra umbilicalis*: nitrogen and carbon. *Marine Ecology Progress Series* 99: 301-311.

Hernández, I., G. Peralta, J. L. Pérez-Lloréns, J. J. Vergara and F. X. Niell. 1997. Biomass and dynamics of growth of

Ulva species in Palmones river estuary. *Journal of Phychology* 33: 764-772.

Ho, Y. B. 1981. Mineral element content in *Ulva lactuca* L. with reference to eutrophication in Hong Kong coastal waters. *Hydrobiologia* 77: 43-47.

Lapointe, B. E. 1987. Phosphorus and nitrogen limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study. *Marine Biology* 93: 561-568.

Le Bozec, S. 1996. Determination of minimal quotas in nitrogen and phosphorus and of the factor limiting the growth in *Ulva* sp. of green tides under experimental conditions. In: *Actes du 1er Colloque Interceltique d´Hydrologie et de Gestion des Eaux -Bretagne 96* (July 8-11, 1996. Rennes, France). INSA (Institut National des Sciences Appliquees): p. 116.

Lobban, C. S. and P. J. Harrison. 1994. *Seaweed Ecology and Physiology*. Cambridge University Press. Cambridge, UK: 366 pp.

Lyngby, J. E. and S. M. Mortensen. 1994. Assessment of nutrient availability and limitation using macroalgae. *Journal of Aquatic Ecosystem Health* 3: 27-34.

Ménesguen, A. and J. Y. Piriou. 1995. Nitrogen loadings and macroalgal (*Ulva* sp.) mass accumulation in Britanny (France). *Ophelia* 42: 227-237.

Morand, P. and X. Briand. 1996. Excessive growth of macroalgae: A symptom of environmental disturbance. *Botanica Marina* 39: 491-516.

Munda, I. M. 1993. Impact of pollution on benthic marine algae in the Northern Adriatic. *International Journal of Environmental Studies* 43: 185-199.

Niell, F. X., C. Fernández, F. L. Figueroa, F. G. Figueiras, J. M. Fuentes, J. L. Pérez-Llorens, M. J. García-Sánchez, I. Hernández, J. A. Fernández, M. Espejo, J. Buela, M. C. García-Jiménez, V. Clavero and C. Jiménez. 1996. Spanish Atlantic Coasts. In: *Marine Benthic Vegetation. Recent Changes and the Effects of Eutrophication* (Ecological Studies). W. Schramm and P. H. Nienhuis (eds.): 123: 263-281. Springer-Verlag. Berlin, Heildelberg.

Norkko, A. and E. Bonsdorff. 1996. Rapid zoobenthic community responses to accumulations of drifting algae. *Marine Ecology Progress Series* 131: 143-157.

Peckol, P., B. DeMeo-Anderson, J. Rivers, I. Valiela, M. Maldonado and J. Yates. 1994. Growth, nutrient uptake capacities and tissue constituents of the macroalgae *Cladophora vagabunda* and *Gracilaria tikvahiae* related to site-specific nitrogen loadings rates. *Marine Biology* 121: 175-185.

Pihl, L., I. Isaksson, H. Wennhage and P. O. Moksnes. 1995. Recent increase of filamentous algae in shallow Swedish bays: effects on the community structure of epibenthic fauna and fish. *Netherlands Journal of Aquatic Ecology* 29 (3-4): 349-358.

Pihl, L., G. Magnusson, I. Isaksson and I. Wallentinus. 1996. Distribution and growth dynamics of ephemeral macroalgae in shallow bays on the Swedish West coast. *Journal of Sea Research* 35 (1-3): 169-180.

Pugnetti, A., P. Viaroli and I. Ferrari. 1992. Processes leading to dystrophy in a Po River Delta lagoon (Sacca Di Goro): phytoplankton-macroalgae interactions. In: *Marine Coastal Eutrophication. Proceedings of an International Conference* (March 21-24, 1990. Bologne, Italy). R. A. Vollenweider, R. Marchetti and R. Viviani (eds.): 445-456. Elsevier. Amsterdam.

Rodríguez, E., F. Fernández, X. M. Romaris and J. P. Pazo. 1987. Valoración de una técnica de semicultivo: limpieza de algas clorofíceas en la Ensenada de San Simón. In: *Actas del II Congreso Nacional de Acuicultura* (December 1-4, 1987. Santiago de Compostela, A Coruña, Spain) (Cuadernos Marisqueros). A. Landín and A. Cerviño (eds.) 12: 359-364. Xunta de Galicia. Santiago de Compostela, Spain.

Rosenberg, R. 1985. Eutrophication, the future marine coastal nuisance? *Marine Pollution Bulletin* 16 (6): 227-231.

Runca, E., A. Bernstein, L. Postma and G. Di Silvio. 1996. Control of macroalgae blooms in the Lagoon of Venice. *Ocean and Coastal Management* 30 (2-3): 235-257.

Schramm, W. and P. H. Nienhuis. 1996. *Marine Benthic Vegetation. Recent Changes and the Effects of Eutrophication.* Ecological Studies 123. Springer-Verlag. Berlin, Heildelberg: 470 pp.

Short, F. T. and D. M. Burdick. 1996. Quantifying eelgrass habitat loss in relation to housing development and nitrogen loading in Waquoit Bay, Massachusetts. *Estuaries* 19 (3): 730-739.

Solidoro, C., V. E. Brando, C. Dejak, D. Franco, R. Pastres and G. Pecenik. 1997. Long term simulations of population dynamics of *Ulva r.* in the lagoon of Venice. *Ecological Modelling* 102: 259-272.

Valiela, I., J. McClelland, J. Hauxwell, P. J. Behr, D. Hersh and K. Foreman. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiologycal and ecosystem consequences. *Limnology and Oceanography* 42 (5,2): 1105-1118.

Viaroli, P., M. Naldi, C. Bondavalli and S. Bencivelli. 1996. Growth of the seaweed *Ulva rigida* C. Agardh in relation to biomass densities, internal nutrient pools and external nutrient supply in the Sacca di Goro lagoon (Northern Italy). *Hydrobiologia* 329: 93-103.

Vidondo, B. and C. M. Duarte. 1995. Seasonal growth of *Codium bursa*, a slow-growing Mediterranean macroalga: in situ experimental evidence of nutrient limitation. *Marine Ecology Progress Series* 123: 185-191.

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