



## Nutrient uptake rates by the alien alga *Undaria pinnatifida* (Phaeophyta) (Nuevo Gulf, Patagonia, Argentina) when exposed to diluted sewage effluent

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### Abstract

In the early nineties, *Undaria pinnatifida* has been accidentally introduced to Nuevo Gulf (Patagonia, Argentina) where the environmental conditions would have favored its expansion. The effect of the secondary treated sewage discharge from Puerto Madryn city into Nueva Bay (located in the western extreme of Nuevo Gulf) is one of the probable factors to be taken into account. Laboratory cultures of this macroalgae were conducted in seawater enriched with the effluent. The nutrients (ammonium, nitrate and phosphate) uptake kinetics was studied at constant temperature and radiation (16 °C and 50  $\mu\text{E m}^{-2} \text{s}^{-1}$  respectively). Uptake kinetics of both inorganic forms of nitrogen were described by the Michaelis–Menten model during the surge phase (ammonium:  $V_{\text{max}}^{\text{sur}}: 218.1 \mu\text{mol h}^{-1} \text{g}^{-1}$ ,  $K_s^{\text{sur}}: 476.5 \mu\text{M}$  and nitrate  $V_{\text{max}}^{\text{sur}}: 10.7 \mu\text{mol h}^{-1} \text{g}^{-1}$ ,  $K_s^{\text{sur}}: 6.1 \mu\text{M}$ ) and during the assimilation phase (ammonium:  $V_{\text{max}}^{\text{ass}}: 135.6 \mu\text{mol h}^{-1} \text{g}^{-1}$ ,  $K_s^{\text{ass}}: 407.2 \mu\text{M}$  and nitrate  $V_{\text{max}}^{\text{ass}}: 1.9 \mu\text{mol h}^{-1} \text{g}^{-1}$ ,  $K_s^{\text{ass}}: 2.2 \mu\text{M}$ ), with ammonium rates always higher than those of nitrate. Even though a net phosphate disappearance was observed in all treatments, uptake kinetics of this ion could not be properly estimated by the employed methodology.

### Introduction

Nutrient availability is an important factor in determining the species composition of seaweed communities from shallow coastal waters (Twilley et al., 1985; Borum, 1996). The proliferation of opportunistic macroalgae in estuaries and coastal waters has been attributed to eutrophication related to high loadings of nitrogen derived from industrial and urban sewage effluents (Valiela et al., 1992). Nitrogen is the limiting element for primary production in Nuevo Gulf (Charpy et al., 1982); nevertheless, residual waters enriched in this nutrient are discharged into it. Until the year 2001, approximately 8000  $\text{m}^3 \text{day}^{-1}$  of a secondary treated sewage (plant treatment efficiency

~50%) was discharged into the waters of the gulf from Puerto Madryn city (Fig. 1). This situation determined the appearance of eutrophication signs on the coastal waters adjacent to Puerto Madryn, previously reported by other authors (Gil, 2001; Diaz et al., 2002).

One of the possible adverse results of eutrophication is the deposition of a high biomass of seaweeds in coastal areas. In Puerto Madryn, nearly 1500 tn (wet weight) of macrophytes, are monthly drifted on to 4.5 km of recreational beaches (Piriz et al., 2003). Before 1992, the mats were mainly composed by chlorophytes of the genus *Ulva* spp. and *Codium* spp. followed by phaeophytes of the genus *Dictyota* sp., and various species of Rhodophyta (Piriz et al., 2003). In 1992, the first specimens of alien algae were ob-

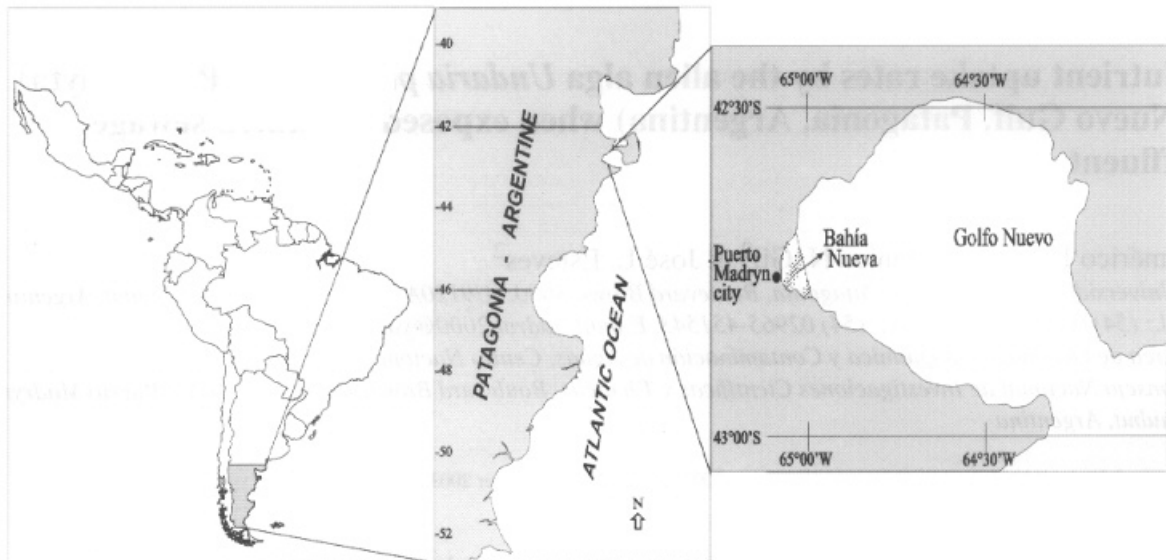


Figure 1. Map showing the location of Nueva Bay and Puerto Madryn city.

served in Nueva Bay, and were identified as *Undaria pinnatifida* (Harvey) Suringar (Phaeophyta) (Piriz & Casas, 1994), also known as Wakame in Japan. The presence of a microscopic gametophyte as part of this algae life cycle, would have allowed human assisted introduction into the gulf (e.g., ballast water). Since then, it rapidly expanded via spore dispersion by marine currents and drifting of sporophytes within the system, showing a good acclimatization to its environmental conditions (Casas & Piriz, 1996) and a high capacity to colonize different kinds of substrates. It may be found in infralitoral areas (up to 22 m depth) (Casas G., pers. commun.) as well as in tidal pools. Therefore, the efforts to eradicate this kelp have been considered useless (Casas & Piriz, 1996). *U. pinnatifida* is commercially exploited in several parts of the world (FAO, 2002) and its presence in Nuevo Gulf generates an attractive situation for those interested in harvesting it. In spite of its importance as a valuable marine resource, very little is known about the environmental conditions that have favoured its expansion. Among others, the influence of the sewage discharge is one of the probable factors to be taken into account. Considering this scarcity of information, this study determines the ammonium, nitrate, and phosphate uptake rates of *U. pinnatifida* in laboratory, as it would occur near the site of the effluent discharge.

## Materials and methods

### Material collection

In December 1999 sexually immature *U. pinnatifida* specimens were collected at 10 m depth by SCUBA diving in Nueva Bay. The individuals were placed in plastic containers with seawater, and once in the laboratory they were blotted with absorbent paper towel for not more than 3 min. Then, 5 g wet weight (approximately 1.2 g dry weight) aliquots were obtained, placed in individual containers with filtered seawater (0.45  $\mu\text{m}$  Millipore filters), and left in dark conditions at 16 °C during 12 hours to deplete exhaust the nutrient content within the cells.

### Experimental design

Two liters capacity Erlenmeyers were used in all the experiments. Each Erlenmeyer was filled with an initial volume of 1.4 l culture medium, made by a mixture of seawater (aged during 10 weeks in the dark at 15 °C and filtered through 0.45  $\mu\text{m}$  Millipore filters) and different proportions of sewage (2.5, 5, 10 and 20% v/v). Final salinities after seawater dilution ranged between 33 and 27 psu. The sewage was collected in the mouth of the effluent, filtered, sterilized in autoclave during 20 min under 1.5 atmospheres, and preserved in a refrigerator during 12 h previous to its use. After that, pieces of *Undaria* were placed in each Erlenmeyer. The four treatments were conducted

by triplicate. Additionally, one control was prepared for each treatment with the corresponding culture medium but without the algae. In all the Erlenmeyers, the culture medium was supplied with air pumped through a Tygon<sup>®</sup> hose at 0.3 l min<sup>-1</sup> flow rate, irradiated with 18W/10 Phillips fluorescent lamps at 50  $\mu\text{E m}^{-2} \text{s}^{-1}$ , and kept at 16 °C. Forty milliliters culture medium sub-samples were collected with a syringe at 0, 30, 60, 90, 120, 360 and 480 min after the beginning of each experiment. The syringe was rinsed twice with 5 ml culture medium before each sample collection. Each sub-sample was filtered, and the ammonium, nitrate+nitrite, and phosphate concentrations were determined immediately according to Strickland & Parsons (1972) methods. Nitrate+nitrite analyses were determined together in all cases and they will be referred as nitrate analyses. In all cases, the measurement of nutrient concentration was corrected to account for the variation in volume due to the sub-sample collection throughout the experiments. On the other hand, to account for any possible changes not related to the plant's uptake kinetics, variations measured at each time in the control were discounted. Corrected concentrations accounting both factors will be expressed as 'S'.

#### Uptake rates and kinetic constants determination

Nutrient uptake rates were estimated using the following equation:

$$V = (S_t - S_{t+\Delta t}) / (W \cdot \Delta t),$$

where  $V$  = uptake rate ( $\mu\text{mol h}^{-1} \text{g}^{-1}$ );  $t$  = time (h);  $\Delta t$  = time interval (h);  $S_t$  = initial concentration of the ion at time  $t$  ( $\mu\text{M}$ );  $S_{t+\Delta t}$ : final concentration of the ion at time  $t + \Delta t$  ( $\mu\text{M}$ );  $W$  = dry weight (g). Ammonium and nitrate  $V_{\text{sur}}$  were estimated for the first 30 min, while  $V_{\text{ass}}$  was calculated between 60–90 min after initial exposure for ammonium and between 90–270 min for nitrate. The observed values of  $V^{\text{sur}}$  and  $V^{\text{ass}}$  were plotted against the average ion concentration at each time interval. The Michaelis–Menten function was fitted to the data by least-square regression:

$$V = V_{\text{max}}S / (K_s + S),$$

where  $V$  = uptake rate ( $\mu\text{mol h}^{-1} \text{g}^{-1}$ );  $V_{\text{max}}$  = maximum uptake rate ( $\mu\text{mol h}^{-1} \text{g}^{-1}$ );  $S$  = ion concentration ( $\mu\text{M}$ );  $K_s$  = half saturation constant ( $\mu\text{M}$ ). The initial slopes of the hyperbolic curves were calculated using the index of Healey (1980) ( $V_{\text{max}}/K_s$ ). Uptake

rates of phosphate were calculated after 210 min of initial exposure. Values between 210 and 360 min were employed to evaluate uptake kinetics.

## Results

Ammonium and nitrate concentrations decreased in all cultures; reduction in controls was low (<2 and <1  $\mu\text{mol h}^{-1}$ , respectively).

Uptake rates ( $V_{\text{sur}}$  and  $V_{\text{ass}}$ ) of both nitrogen compounds exhibited saturation kinetics, such as that predicted by the Michaelis–Menten model (Figs 2, 3). Kinetic parameters and Healey indexes are shown in Table 1. For both nitrogen forms,  $V_{\text{sur}}$  was always higher than  $V_{\text{ass}}$ . This difference was more remarkable at high substrate concentrations than at low ones.

Phosphate concentration in the controls showed a slight increase (<0.3  $\mu\text{mol h}^{-1}$ ). In the cultures, this ion increased during the first 90 minutes and then decreased (never below 3  $\mu\text{mol}$ ) until the end of the incubation period in all treatments. Hence, initial uptake rates could not be evaluated. A linear tendency ( $V = 0.10S$ ;  $R^2 = 0.64$ ) was observed when rates (estimated in the interval 210–360 min), where plotted against substrate concentrations (Fig. 4).

## Discussion

### Nitrogen

The saturation kinetics in ammonium and nitrate uptake rates, was previously observed in other macroalgae studies. Examples of this are the ammonium uptake kinetics reported for sexually mature and immature *U. pinnatifida*, *Hinchsia sordida* (Harvey) Clayton, *Polysiphonia decipiens* Montagne, and *Ulva* sp. (Campbell, 1999), and the nitrate uptake kinetic observed by Gerard (1982) and Kopczak (1994) in *Macrocystis pyrifera* (L.) C. Agardh.

Nitrogen uptake in macroalgae is a diffusive process (Lobban et al., 1985; Taylor & Rees, 1999), controlled by small pools of intracellular ammonium (Lobban et al., 1985; Taylor & Rees, 1999). Since a feedback control may be exerted as the pools are filled, a decline in uptake is generally observed (Pedersen, 1994; McGlathery et al., 1996). Assimilation is known to be catalyzed by enzymatic activity and has been related to facilitated diffusion or active transport (Lobban et al., 1985). It represents the rate of aminoacids synthesis within the algae (McGlathery et al., 1996).

Table 1. Kinetic parameters for ammonium and nitrate uptake for *U. pinnatifida*. Parameters were derived from non-linear regressions of raw data using the Michaelis-Menten function; coefficients of determination ( $R^2$ ) of this function and Healey indexes are shown. ( $V_{\max}$ :  $\mu\text{mol h}^{-1} \text{g}^{-1}$ ;  $K_s$ :  $\mu\text{M}$ ).

Nutrient ( $\mu\text{M}$ )	Surge uptake ( $V_{\text{sur}}$ )					Assimilation uptake ( $V_{\text{ass}}$ )				
	$V_{\max}$	$K_s$ index	Healey index	$n$	$r^2$	$V_{\max}$	$K_s$ index	Healey index	$n$	$r^2$
Ammonium	218.1	476.5	0.46	20	0.54	135.6	407.2	0.33	18	0.61
Nitrate	10.7	6.1	1.76	16	0.71	1.9	2.2	0.87	21	0.79

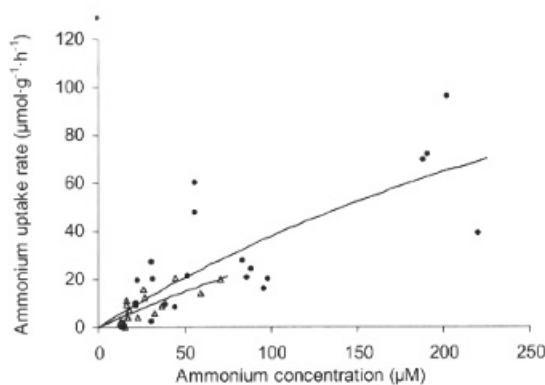


Figure 2. Rate of uptake of ammonium as a function of initial substrate concentration for *U. pinnatifida*. Surge uptake ( $V_{\text{sur}}$  ●) was measured over the initial 30 min after the experiments were initiated. Assimilation uptake ( $V_{\text{ass}}$  △) was measured over the 60–90 min after the experiments were initiated. Curves were fitted by use of the Michaelis-Menten equation. Hyperbolic parameters are shown in Table 1.

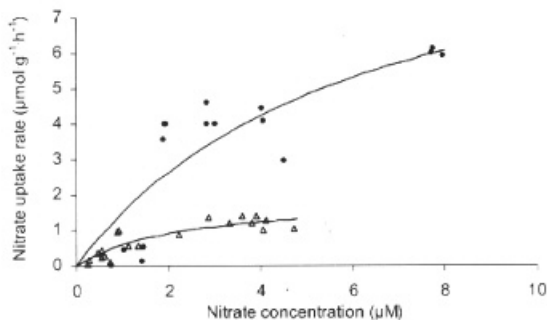


Figure 3. Rate of uptake of nitrate as a function of initial substrate concentration for *U. pinnatifida*. Surge uptake ( $V_{\text{sur}}$  ●) was measured over the initial 30 min after the experiments were initiated. Assimilation uptake ( $V_{\text{ass}}$  △) was measured over the 90–270 min after the experiments were initiated. Curves were fitted by use of the Michaelis-Menten equation. Hyperbolic parameters are shown in Table 1.

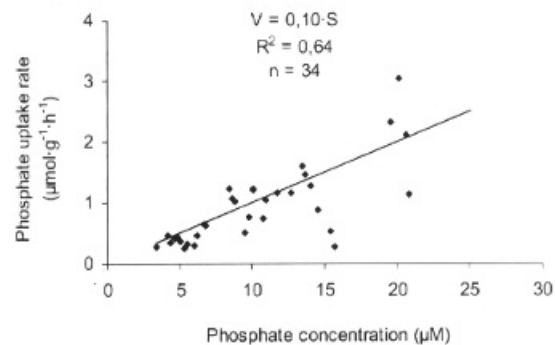


Figure 4. Relationship between rate of uptake of phosphate and the substrate concentration for *U. pinnatifida*. Uptake rates were measured over the initial 210–360 min after the experiments were initiated.

Macroalgae uptake rates of nitrogen, and those of other ions, can be influenced by several biotic and abiotic factors. Among biotic factors, different authors have mentioned the age of the individuals (or that of specific tissues) (Topinka, 1978; Harrison et al., 1986), the nutritional story, the tissue type and the inter-individual differences (Gerard, 1982; Campbell, 1999). On the other hand, common abiotic factors that can influence nutrient uptake rates are the available radiation, the temperature, the desiccation level and the ionic form of the elements (Lobban et al., 1985).

Even though the absolute values of the estimated kinetic parameters could have been influenced by seawater dilution with the sewage (33–27 psu), general tendency to saturation in uptake was not affected. This agrees with the fact that seaweeds can resist important salinity variations during short periods of time (subtidal species: 15–45 psu, intertidal species: 3–100 psu, Darley, 1982).

It has been shown that the concentration of ammonium can inhibit the nitrate uptake up by seaweeds to 50%, when they are supplied simultaneously (Lobban et al., 1985). Nevertheless, equal rates of both

Table 2. Environmental conditions near effluent discharge (early fall values) and in the laboratory.

	Effluent discharge	Laboratory Laboratory
Temperature (°C)	16	16
Salinity (psu)	32	27.0–33.0
Nitrate (μM)	4	2.2–7.4
Ammonium (μM)	120	34.3–217.5
Phosphate (μM)	11	4.0–9.0

compounds have also been reported (Haines et al., 1978). In the present study, higher concentrations of ammonium than those of nitrate were present in the culture medium. However, the obtained nitrate uptake rates were comparable to those reported in studies where nitrate was the only available nitrogen compound (Da Costa Braga & Yoneshigue-Valentin, 1996). Experimental work on nutrient uptake by seaweed is generally conducted separately for each nutrient, using specific compounds (e.g.,  $\text{NH}_4\text{Cl}$ ,  $\text{KNO}_3$ ,  $\text{KH}_2\text{PO}_4$ ) at low seawater enrichment levels. In this study, effluent is the nutrient source in the culture medium, providing higher levels than those normally found in seawater, but similar to those found near the sewage discharge point (Sotomayor M., pers. commun.) (Table 2).

Given that the values of  $K_s$  and  $V_{\max}$  are auto-correlated the slope of the initial portion of the Michaelis–Menten hyperbola, mathematically expressed as  $V_{\max}/K_m$  (Healey, 1980), can be used to compare the preference of one species for a given ion. *U. pinnatifida* from Nueva Bay showed higher values of  $V_{\text{sur}}$  and  $V_{\text{ass}}$  for ammonium than for nitrate uptake. However, according to the corresponding Healey indexes, it would have greater affinity for nitrate than for ammonium, in contrast to that reported in the literature for different macroalgae (Da Costa Braga, 1996; Pedersen et al., 1997). Further research is needed in order to explain the physiological role of this behaviour.

### Phosphate

In marine environments, phosphate is not considered a limiting element (Lobban et al., 1985), with few exceptions like estuaries and tropical waters (Smith, 1984). Despite there is less information about its kinetics of uptake than that for nitrogen, some authors have reported a saturation tendency (Da Costa Braga & Yoneshigue-Valentin, 1996; Schaffelke & Klumpp,

1997). In this study, the initial increase in phosphate level observed in all experiments could be related to phosphate production due to organic matter oxidation. Simultaneous production and uptake during the second phase of the experiments would determine an apparent linearity between net incorporation rates and substrate concentration (Fig. 4). This suggests that real uptake kinetics of this nutrient, can not be adequately represented by this model.

### Conclusion

Since the first notice on the presence of *U. pinnatifida* in Nuevo Gulf in 1992, it has rapidly spread its geographic distribution along more than 30 km coasts (Casas & Piriz, 1996). The discharge of the sewage into Nueva Bay would have contributed to this expansion.

The results of this study have shown that *U. pinnatifida* is capable of incorporate ammonium, nitrate and phosphate at the levels found near the sewage outfall. No inhibition in uptake has been observed, even at the highest effluent concentration tested (20% v/v). This agrees with the fact that *U. pinnatifida* has got the characteristics of an invasive species, allowing it to be adapted to different environmental conditions. Furthermore, taking into account that algae drifted onto the coast are daily removed during summer time, this alien species would be indirectly contributing to eliminate sewage derived nutrients from the bay.

Since *U. pinnatifida* is a species that rapidly uses dissolved inorganic nitrogen, its competitive abilities could be enhanced related with those species that take up nitrogen at relatively low rates. More studies on the interaction of this alien species with environmental factors, including other species, are necessary to predict and help to control this explosive dispersion.

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## References

- Borum, J., 1996. Shallow waters and land/sea boundaries. In Jørgensen, B. B. & K. Richardson (eds), *Eutrophication in Coastal Marine Ecosystems*. *Coast Estuarine* 52: 189–204.
- Campbell, S. J., 1999. Uptake of ammonium by four species of macroalgae in Port Phillip Bay, Victoria, Australia. *Journal of Marine and Freshwater Research* 50: 515–522.
- Casas, G. N. & M. L. Piriz, 1996. Surveys of *Undaria pinnatifida* (Laminariales, Phaeophyta) in Golfo Nuevo, Argentina. *Hydrobiologia* 326/327: 213–215.
- Charpy Roubaud, C. J., L. J. Charpy & S. Y. Maestrini, 1982. Fertilité des eaux côtières nord-patagoniques: facteurs limitant la production du phytoplancton et potentialités d'exploitation mycicole. *Oceanologica Acta* 5: 179–188.
- Dacosta Braga, A. & Y. Yonshigue-Valentin, 1996. Nitrogen and phosphorus uptake by the Brazilian Kelp *Laminaria abyssalis* (Phaeophyta) in culture. *Hydrobiologia* 326/327: 445–450.
- Darley, W. M., 1982. *Algal Biology: A Physiological Approach*. Blackwell Scientific Publications, London, 168 pp.
- Díaz, P., J. L. López Gappa & M. L. Piriz, 2002. Symptoms of eutrophication in intertidal macroalgal assemblages of Nuevo Gulf (Patagonia, Argentina). *Botanica Marina* 45: 267–273.
- FAO, 2002. *Global Captures 1970–2000*. FAO Fisheries Department, Fishery Information, Data and Statistics Unit. Fishstat plus, Universal Software for Fishery Statistical Time Series, Version 2.3.
- Gerard, V. A., 1982. *In situ* rates of nitrate uptake by giant kelp *Macrocystis pyrifera* (L.) C. Agardh: tissue differences, environmental effects and predictions of nitrogen-limited growth. *Journal of Experimental Marine Biology Ecology* 62: 211–224.
- Gil, M. N., 2001. Eutroficación: rol del nitrógeno en ecosistemas marinos costeros. Thesis. Bahía Blanca (Bs. As.), Universidad Nacional del Sur, 1657 pp.
- Haines, K. C. & P. A. Wheeler, 1978. Ammonium and nitrate uptake by the marine macrophytes *Hypnea musciformis* (Rhodophyta) and *Macrocystis pyrifera* (Phaeophyta). *Journal of Phycology* 14: 319–324.
- Harrison, P.J., L.D. Druehl, K.E. Lloyd & P.A. Thompson, 1986. Nitrogen uptake kinetics in three year classes of *Laminaria groenlandica* (Laminariales: Phaeophyta). *Marine Biology* 93: 29–35.
- Healey, E. P., 1980. Slope of the Monod equation as an indicator of advantage in nutrient competition. *Microbial Ecology* 5: 281–286.
- Kopczak, C. D., 1994. Variability of nitrate uptake capacity in *Macrocystis pyrifera* (Laminariales, Phaeophyta) with nitrate and light availability. *Journal of Phycology* 30: 573–580.
- Lobban, C. S., P. J. Harrison & M. J. Duncan, 1985. *The Physiological Ecology of Seaweeds*. Cambridge University Press, Cambridge, 242 pp.
- McGlathery, K. J., M. F. Pederson & J. Borum, 1996. Changes in intracellular nitrogen pools and feedback controls on nitrogen uptake in *Chaetomorpha linum* (Chlorophyta). *Journal of Phycology* 32: 393–401.
- Pedersen, M. F., 1994. Transient ammonium uptake in the macroalga *Ulva lactuca* (Chlorophyta): nature, regulation, and the consequences for choice of measuring technique. *Journal of Phycology* 30: 980–986.
- Pedersen, M. F. & J. Borum, 1997. Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. *Marine Ecology Progress Series* 161: 155–163.
- Piriz, M. L. & G. N. Casas, 1994. Occurrence of *Undaria pinnatifida* in Golfo Nuevo, Argentina. *Journal of Applied Phycology Forum* 10: 4.
- Piriz M. L., M. C. Eyra & C. M. Rostagno, 2003. Changes in biomass and botanical composition of beach-cast seaweeds in a disturbed coastal area from Argentine Patagonia. *Journal of Applied Phycology* 15: 67–74.
- Schaffelke, B. & D. W. Klumpp, 1997. Growth of germlings of the macroalga *Sargassum baccharia* (Phaeophyta) is stimulated by enhanced nutrients. *Proc. 8th. Int. Coral Reef Symposium* 2: 1839–1842.
- Smith, S. V., 1984. Phosphorous versus nitrogen limitation in the marine environment. *Limnology and Oceanography* 29: 1149–1160.
- Strickland, J. D. H. & T. R. Parsons, 1972. *A Practical Handbook of the Seawater Analysis*. *Journal of Fisheries Research Board of Canada*, Ottawa. Bulletin 167, 2nd edn: 310 pp.
- Taylor, M. W. & T. A. V. Rees, 1999. Kinetics of ammonium assimilation in two seaweeds, *Enteromorpha* sp. (Chlorophyceae) and *Osmundaria colensoi* (Rhodophyceae). *Journal of Phycology* 35: 740–746.
- Topinka, J. A., 1978. Nitrogen uptake by *Fucus spiralis* (Phaeophyceae). *Journal of Phycology* 14: 241–247.
- Twilley, R. R., W. M. Kemp, K. W. Staver, J. C. Stevenson & W. R. Boynton, 1985. Nutrient enrichment of estuarine submersed vascular plant communities: I. Algal growth and effects on production of plants and associated communities. *Marine Ecology Progress Series* 23: 179–191.
- Valiela, I. K., M. Foreman, D. LaMontagne, J. Hersh, P. Costa, B. Peckol, C. DeMeo-Anderson, M. d'Avanzo, C. H. Babione, J. Sham Brawley & K. Lajtha, 1992. Coupling of watersheds and coastal waters: sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. *Estuaries* 15: 443–457.