

Nutrient enrichment of Cabo Frio (Brasil) sea water for phytoplankton mass production

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Abstract

Natural phytoplankton of Cabo Frio area was grown in 42 m-deep artificially upwelled seawater enriched with increasing concentrations of nitrogen or phosphorus. Respective values allowing maximum biomass, maximum uptake of initial reserve and maximum yield coefficient are rather conflicting. Notwithstanding, respective values of 75 μg at l^{-1} nitrogen and 5 μg at l^{-1} phosphorus, and therefore N:P = 15, appeared to be the best compromise for initial nutrient levels.

Introduction

In the Cabo Frio area, deep nutrient-rich waters are naturally upwelled along the slope of the continental shelf and then transferred to the coast (Moreira da Silva & Rodriguez 1966). In these deep waters, an integrated project of aquafarming was established (Moreira da Silva, 1969, 1971) which paralleled the one of Saint Croix (Gerard & Worzel, 1967; Roels *et al.*, 1971). However, because this phenomenon of upwelling is seasonal and the waters reaching the coast are impoverished during transfer on the shelf by mixing (Kempf *et al.*, 1974), a nutrient supplement should be added to the waters to allow a reasonable algal cell density for practical rearing of shellfish (Gonzalez-Rodriguez, 1982).

The mass production of marine algae has hitherto been done with little attention to the cost of nutrients. This was so because either (i) the cultures were established at a laboratory or pilot plant size with the aim to support a high production rate (e.g. Ukeless, 1965; Helm *et al.*, 1979; Spectorova *et al.*, 1981/1982; Trotta, 1981) or (ii) the nutrients were provided by low-cost wastes (Ryther *et al.*, 1972; De Pauw & De Leenheer, 1979). Nutrients from wastes

are quite cheap, indeed, since they are a byproduct of water reclamation. However, microbial and/or chemical contamination are uncontrolled and this has thus far prevented their use on a large scale. Thus, the use of inorganic compounds for enriching seawater is still the most convenient and practical way.

Low-cost industrial-grade compounds for preparing enrichment mixtures has long been used (Loosanoff & Engle, 1942), but little attention, if any, has been paid to the efficiency of biomass production with regard to the added nutrient concentrations. In other words, losses of nutrients resulting from luxury uptake and subsequently from unbalanced respective nutrient concentrations have been neglected. Such processes have nevertheless some importance when huge quantities of chemicals are needed to enrich a large body of water. The experiments of Gross *et al.* (1949/1950) are demonstrative with respect to this problem: for supplementing the natural nutrient reserve of a Scottish loch, totalling 61.4 ha (= 152 acres) and $3 \cdot 10^6$ m³ seawater, they had to distribute 1821 kg NaNO₃ and 105 kg Ca(PO₄)₂ per spike, which added only 7.1 μg at N and 0.4 μg at P l^{-1} . Thus, with such an order of magnitude of seawater volume, that could not be at

all unrealistic for future marine aquafarming, the loss of $10 \mu\text{g at l}^{-1}$ nitrogen will amount to several tens of metric tons of nitrate wasted every year. Hence, stating the right concentrations of added nutrients is of critical importance.

The research data reported here have been collected with the aim of assessing the nutrient concentrations which allow for the best compromise between a high algal growth rate and a low nutrient waste.

Material and methods

Seawater of -42 m was collected at the station 'Saco do Ingles' (Fig. 1), by using the artificial upwelling system of 'projeto Cabo Frio' (Moreira da Silva, 1971). Dates of sampling, physical and chemical characteristics of sampled waters are given in Table 1. Seawater was filtered with a $250 \mu\text{m}$ mesh and then 15-l aliquotes were distributed in 20-l glass carboys.

Nutrient enrichments were made as follows: (i) fixed phosphorus concentration: $5 \mu\text{g at l}^{-1}$, and increasing nitrogen concentrations: 10, 20, 30, 50, 75, 100 and $200 \mu\text{g at l}^{-1}$; (ii) fixed nitrogen concentration: $100 \mu\text{g at l}^{-1}$ (as determined by i-set experiments), and increasing phosphorus concentrations: 0.5, 1.0, 1.25, 1.7, 2.5, 5.0, 6.7, 10.0 and $20.0 \mu\text{g at l}^{-1}$. Nitrogen was added as NaNO_3 and phosphorus as K_2HPO_4 . Since preliminary experiments demonstrated that a chelator is needed for a good nutrient uptake, all cultures were supplemented with $10 \mu\text{M l}^{-1}$ EDTA.

Surface phytoplankton was used as inoculum. Seawater (1000 l) was filtered on $175 \mu\text{m}$ and $25 \mu\text{m}$ meshes. Then, harvested algal cells were distributed in differentially-enriched carboys, in order to obtain an initial biomass of ca. $5 \mu\text{g l}^{-1}$ chlorophyll-*a*.

The cultures were incubated at controlled tem-

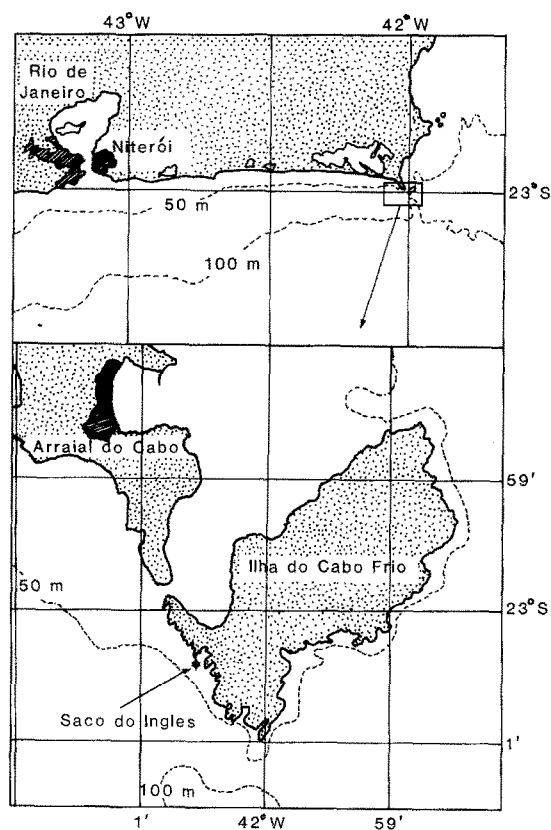


Fig. 1. Position of studied area; the star indicates the site of pumping (-42 m) of the artificial upwelling system of 'Projeto Cabo Frio'.

perature conditions ($= 19^\circ \text{C}$) and continuous artificial illumination ($= 8000 \text{ lux}$) provided by 'day-light' fluorescent tubes.

Nutrient concentrations and chlorophyll-*a* concentration were analysed daily; $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and $\text{PO}_4\text{-P}$: Strickland & Parsons (1972), $\text{NH}_4\text{-N}$: Solorzano (1969); chlorophyll-*a*: SCOR-UNESCO (1964).

Table 1. Physical and chemical characteristics of Cabo Frio sea water used for the experiments.

Set of exper.	Date of sampling	T °C	S (‰)	$\text{NO}_3\text{-N}$	$\text{NO}_2\text{-N}$	$\text{NH}_4\text{-N}$	ΔN	$\text{PO}_4\text{-P}$	$\text{SiO}_4\text{-Si}$	Chl- <i>a</i> ($\mu\text{g l}^{-1}$)
N	05-29-80	16.0	35.87	6.9	0.5	0.8	8.3	0.80	6.7	1.1
N	06-12-80	17.0	35.94	4.1	0	0.9	5.0	0.65	6.1	0.7
P	01-21-81	14.8	35.25	10.1	0.5	1.8	12.4	1.1	8.8	0.6
P	01-17-81	14.0	35.18	10.8	0.6	0.6	12.0	1.1	4.0	0.1
P	02-09-81	14.7	35.30	8.4	0.5	1.7	10.7	0.9	4.4	0.7

Results

Most cultured populations did not show any lag phase; for few others a one-day lag phase occurred. The cells divided quite fast and thus the maximum biomass was usually shown after a 3-day growth period (Fig. 2). The parallel data were consistent, indeed; except, however, for one set of phosphorus concentrations (Fig. 3). Yet even there the shape of the growth curve depicts the same model.

At increasing nitrogen concentrations and fixed phosphorus concentration ($= 5 \mu\text{g at l}^{-1}$), the biomass increased up to a maximum of ca. $65 \mu\text{g l}^{-1}$ chlorophyll-*a*, which pertained to an N:P = 20 (atom:atom). At higher nitrogen concentrations and N:P values, the total algal biomass decreased. The subsequent yield index curve (Fig. 4) depicts a continuous decrease from 1.1 to $0.4 \mu\text{g } \mu\text{g at}^{-1}$ (chlorophyll-*a*:nitrogen) within the range of nitrogen concentrations tested. However, the decrease was quite fast from the lower concentration ($10 \mu\text{g at l}^{-1} \text{NO}_3\text{-N}$) to $30 \mu\text{g at l}^{-1}$ and then slow. Within a broad range of nitrogen concentrations, namely $50\text{--}125 \mu\text{g at l}^{-1}$, the mean yield coefficient value was roughly $0.65 \mu\text{g } \mu\text{g at}^{-1}$.

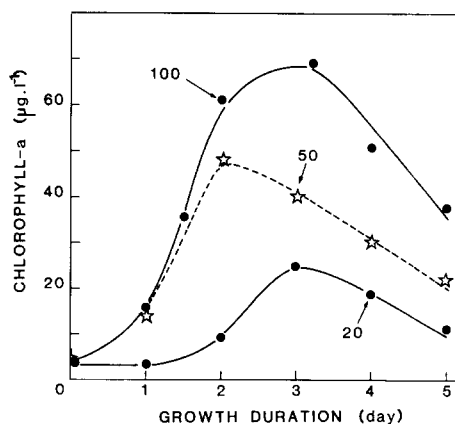


Fig. 2. Increase of chlorophyll-*a* content versus growth duration, in 20, 50 or $100 \mu\text{g at l}^{-1}$ nitrogen-enriched cultures.

As a whole, the maximum algal biomass was quite higher at increasing phosphorus concentrations, except for one set of experiments (Fig. 3). As a matter of fact, maximum recorded values reached roughly $92 \mu\text{g l}^{-1}$ chlorophyll-*a*; that is half more than the value obtained with the set of nitrogen-varying experiments. But for the unique couple of

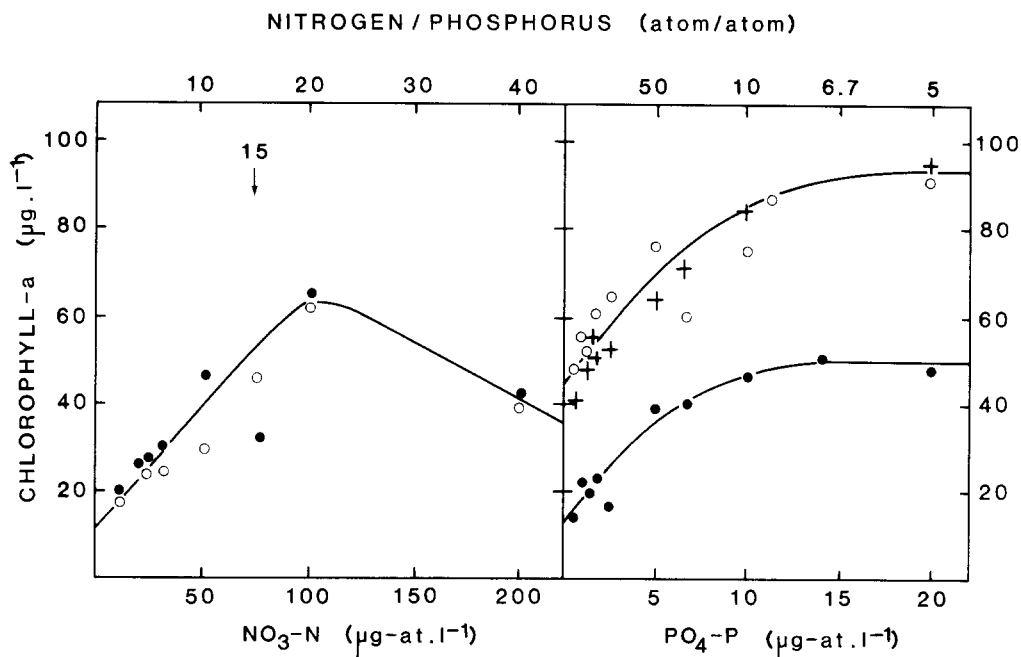


Fig. 3. Algal biomass, as chlorophyll-*a* l^{-1} , yielded at respective increasing concentrations of nitrogen or phosphorus by Cabo Frio phytoplankton. Filled circles, open circles and crosses depict respective parallel experiments.

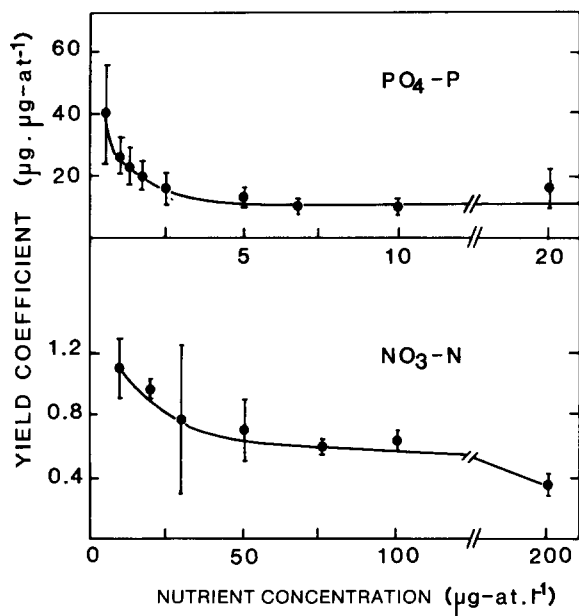


Fig. 4. Yield index, as μg chlorophyll-*a* produced per μg at of nutrient taken up, of multispecies Cabo Frio phytoplankton grown at increasing concentrations of nitrogen or phosphorus. Vertical bars depict the mean standard deviation.

phosphorus and nitrogen concentrations used with both the experiments, namely $\text{N} = 100 \mu\text{g at l}^{-1}$ and $\text{P} = 5 \mu\text{g at l}^{-1}$, the respective crops were similar: ca. 65 and $70 \mu\text{g l}^{-1}$ chlorophyll-*a*.

The yield coefficient pertaining to phosphorus obeyed the same model as nitrogen (Fig. 4). From trace values to $2.5 \mu\text{g at l}^{-1}$ phosphorus, the yield coefficient values decreased sharply from 40 to $15 \mu\text{g at l}^{-1}$; then the decrease was light. At $5 \mu\text{g at l}^{-1}$ phosphorus, the yield coefficient was practically at the minimum value: $10 \mu\text{g at l}^{-1}$.

At increasing nitrogen concentrations and fixed phosphorus concentration, the nitrogen consumption was at a maximum and near to exhaustion, i.e. 95% of the initial reserve, up to $85 \mu\text{g at l}^{-1}$; the relative consumption was then lower and lower (Fig. 5). Meanwhile, the relative phosphorus consumption was at a maximum, also at roughly 95% from $90 \mu\text{g at l}^{-1}$ and higher concentrations. On the contrary, at increasing phosphorus concentrations and fixed nitrogen concentration, the nitrogen consumption was not at all achieved; a maximum of 60% of the initial reserve was taken up, even when phosphorus was present at largely excess concentration (Fig. 6). The phosphorus reserve was taken

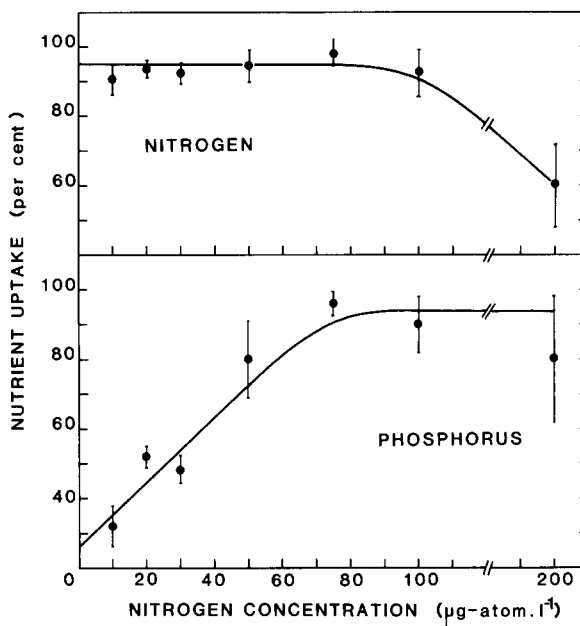


Fig. 5. Nitrogen and phosphorus taken up, as per cent of initial reserve, by Cabo Frio natural phytoplankton grown at fixed phosphorus and increasing nitrogen concentrations. Vertical bars indicate the mean standard deviation.

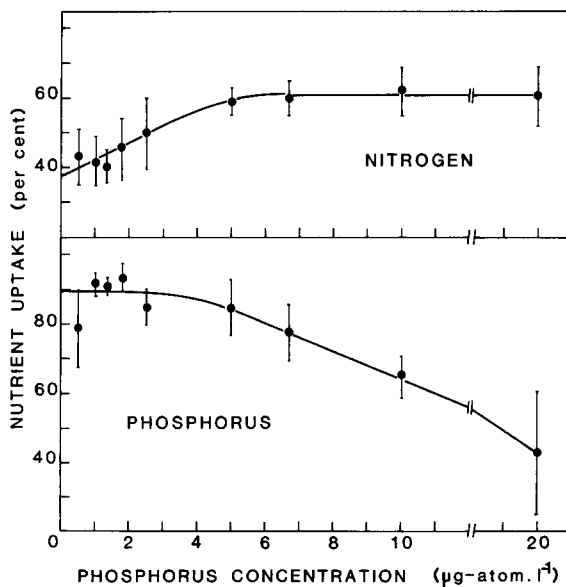


Fig. 6. Nitrogen and phosphorus taken up, as per cent of initial reserve, by Cabo Frio natural phytoplankton grown at fixed nitrogen and increasing phosphorus concentrations. Vertical bars indicate the mean standard deviation.

up at a higher rate: here again at ca. 90%; however that occurred only when the medium concentration did not exceed ca. $3 \mu\text{g at l}^{-1}$. At higher concentrations a great part of the initial reserve was left free from uptake and thus wasted.

The initial phytoplankton populations contained a large array of species; a typical set showed *Skeletonema costatum* (26% of total cell density), *Melosira moniliformis* (18.5%), *Nitzschia* sp. (7%), *Nitzschia panduriformis* (3.7%), *Asterionella glacialis* (3.7%), *Diploneis* sp. (3.7%) and *Thalassiosira* sp. (3.7%). During growth, some initial important species such as *M. moniliformis* disappeared. On the contrary, some others which were scarce took the upper hand. Ultimately, the Chaetoceros and Pseudonitzschia dominated the algal crop. More details will be published in a paper on this subject.

Discussion

Data obtained obviously lead to conflicting results, since the lower the nutrient concentration, the higher the yield coefficient and the lower the standing crop.

Even the best yield coefficient values we recorded are lower than the ones reported for unenriched similar waters. For instance, data reported by Charpy *et al.* (1982) for north-patagonic gulf waters range from 0.5 to $33.0 \mu\text{g } \mu\text{g at}^{-1}$ (chlorophyll-*a*: nitrogen) with most data ranging from 1.0 to $2.7 \mu\text{g } \mu\text{g at}^{-1}$. Maestrini & Robert (1981) also reported averaged yield coefficient values ranging from 1.1 to $1.9 \mu\text{g } \mu\text{g at}^{-1}$, for coastal waters of the French Atlantic Ocean. However, both these authors have used cultured unialgal strains for their experiments and, moreover, have assessed that a great deal of the algal yield was supported by a huge bulk of organic nutrients readily available to algae, what led to a great overestimation of the yield coefficient. Thus, when original data were reviewed again after further analysis of dissolved organic nitrogen, the mean yield coefficient was $1.1 \mu\text{g } \mu\text{g at}^{-1}$ (Maestrini & Robert, unpubl. data), which is indeed exactly the same value as we obtained with the lowest nitrogen enrichment: $10 \mu\text{g at l}^{-1}$. Otherwise, results obtained with phosphorus obey the same model. Hence, since low nutrient concentrations have been reported not to limit the algal growth rate (Hulburt, 1977, 1979; Goldman *et al.*, 1979; Mc Carthy &

Goldman, 1979), such low nutrient concentrations as $10 \mu\text{g at l}^{-1}$ nitrogen and $0.5 \mu\text{g at l}^{-1}$ phosphorus should be used in order to obtain the maximum algal yield and load the minimum chemical compounds, were it not a sufficient algal cell density is conversely needed for optimum shellfish food collection efficiency and subsequent optimum growth rate (Foster Smith, 1975; Møhlenberg & Riisgaard, 1978; Widdows *et al.*, 1979; Griffith, 1980).

Thus, the final nitrogen and phosphorus concentrations will be a matter of compromise (the role of EDTA will be discussed elsewhere). One should note, however, that relative uptake of nitrogen and phosphorus reserve is maximal from lowest respective concentration up to $80 \mu\text{g at l}^{-1}$ nitrogen (Fig. 5) and $3 \mu\text{g at l}^{-1}$ phosphorus (Fig. 6). Therefore, the decreasing values of respective yield coefficient at lower levels were not totally generated by waste of nutrients; some discrepancy is certainly related to the parameter used for estimating the algal biomass. As a matter of fact, all such parameters are partly governed by the nutrient status of the cells, namely as far as nutrient satiety of nutrient depletion and the nature of less available nutrient are concerned (Healey, 1975, 1978; Sakshaug, 1980; Leftley *et al.*, 1983; Maestrini *et al.*, in press).

Compromise for nitrogen:phosphorus ratio (atom:atom) (Fig. 7)

According to the maximum biomass values we obtained, the right ratio should be either 6.7 or 20. Otherwise experiments involving increasing concentrations of phosphorus indicate N:P should be lower than 21 (see i), either for a good nutrient recovery or stable phosphorus yield index. On the contrary, the set of experiments carried out with increasing nitrogen concentrations (see ii) led to the requirements of a maximum value of 7 for having a stable nitrogen yield index, a maximum value of 19 for having a good nitrogen exhaustion of initial reserve, and a minimum of 15 for allowing a maximum nitrogen uptake.

Thus, the compromised N:P value ranges between 7 and 21. Since the N:P = 15 has an especially well-known ecological importance (Redfield, 1934; Goldman *et al.*, 1979) and since this value is the mean of the two former limits, we believe it is the best compromise.

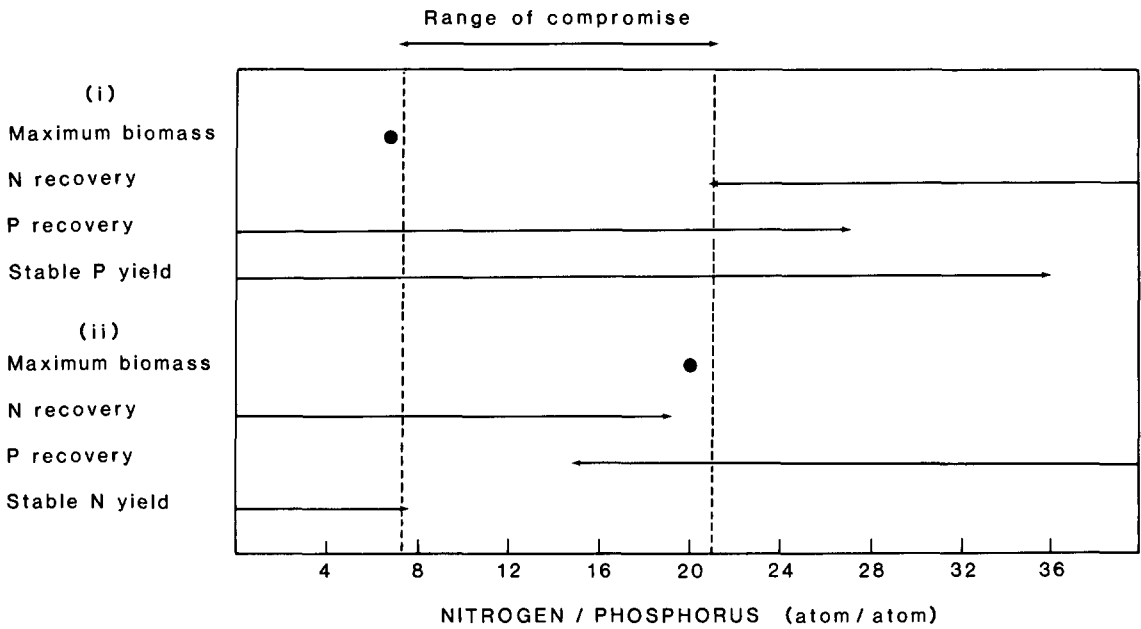


Fig. 7. Range of nitrogen: phosphorus ratio values (atom:atom) which generate maximum algal biomass, nitrogen recovery, phosphorus recovery and stable nitrogen or phosphorus yield index. (i) Set of experiments made with increasing phosphorus concentrations; (ii) Set of experiments made with increasing nitrogen concentrations.

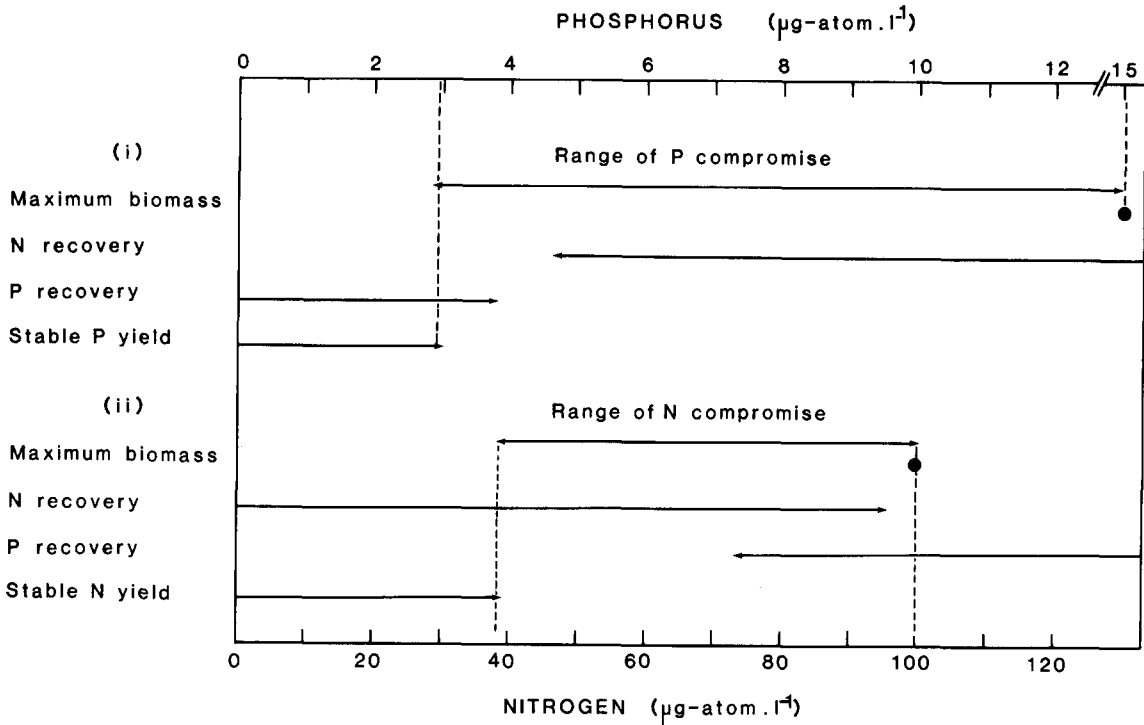


Fig. 8. Ranges of phosphorus or nitrogen concentration values which generate maximum algal biomass, nitrogen recovery, phosphorus recovery and stable nitrogen or phosphorus yield index. (i). Set of experiments made with increasing phosphorus concentrations; (ii) Set of experiments made with increasing nitrogen concentrations.

Compromise for nitrogen and phosphorus concentrations (Fig. 8)

The maximum biomass values could readily be obtained by using 100 μg at l^{-1} nitrogen and 15 μg at l^{-1} phosphorus. However the latter value is questionable since the algal cells are capable of taking up huge quantities of unneeded phosphorus (= luxury uptake; Ketchum, 1939; Mackereth, 1953; Kuenzler & Ketchum, 1962). On the other hand, other results (see i) indicated the phosphorus concentration should be lower. Thus the whole range of concentrations, i.e. 2.8–15.0 μg at l^{-1} , should be shifted toward the lowest limit and hence the 4.7 μg at l^{-1} concentration appears to be a suitable tentative compromise.

The whole set of critical nitrogen concentrations ranges from 36 to 100 μg at l^{-1} (see ii). However, one value, namely 74 μg at l^{-1} , is close to the mean value between the extremes and allows two processes (N recovery and P recovery) to act at maximum efficiency level. Therefore it should be assessed as the best compromise for nitrogen concentration.

Best compromise for absolute and relative nitrogen and phosphorus concentrations

Thus, the separated compromise values are N:P = 15, nitrogen concentration = 74 μg at l^{-1} and phosphorus concentration = 4.7 μg at l^{-1} . When involved together these values are still in agreement indeed, since the calculated N:P value, i.e. $74:4.7 = 15.7$, is very close to the one previously stated N:P = 15) and thus could be readily employed. Nevertheless, for greater simplicity, we suggest using 75 μg at l^{-1} nitrogen and 5 μg at l^{-1} phosphorus, which in addition perfectly obeys the Redfield's ratio.

We also believe that these suggested concentrations pertain to a maximum limit, either for batch or continuous cultures, and will be useful only when harvested algal suspensions can be diluted according to specific shellfish feeding requirements and grazers' demand per unit volume of aquaculture plant. This is indeed readily met in controlled-tank aquafarming, as, for example, the 'Projeto Cabo Frio' (Braga & Druehl, 1978). For enrichment of larger-sized bodies of water, for example coastal lagoons, other processes which govern the turnover of the whole food chain will probably lead to use lower values. We are convinced they will be consist-

ent with natural nutrient reserve of such coastal waters which support a heavy traditional shellfish production (see, e.g., Robert *et al.*, 1979).

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References

- Braga, Y. Y. & L. D. Druehl, 1978. Seasonal growth and succession of tropical and introduced phytoplankton cultured in deep sea water. *Aquaculture* 14: 1–12.
- 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. *Oceanol. Acta* 5: 179–188.
- De Pauw, N. & L. De Leener, 1979. Mass culturing of marine and freshwater algae on aerated swine manure. In: E. Styczyńska-Jurewicz, T. Backiel, E. Jaspers & G. Persoone (eds.), Cultivation of fish fry and its live food. Proc. Conf. Szymbark, Poland, 23–28.06.1977, eur. Maricult. Soc., spec. Publ. 4: 441–473.
- Foster Smith, R. L., 1975. The effect of concentration of suspension and inert material on the assimilation of algae by three bivalves. *J. mar. biol. Ass. U.K.* 55: 411–418.
- Gerard, R. D. & J. L. Worzel, 1967. Condensation of atmospheric moisture from tropical maritime air masses as a freshwater resource. *Science* 157: 1300–1302.
- Goldman, J. C., J. J. McCarthy & D. G. Peavey, 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature* 279: 210–215.
- Gonzalez Rodriguez, E., 1982. La resurgence de Cabo Frio (RJ, Brésil): fertilité, facteurs nutritionnels limitant la biomasse algale; essai d'amélioration. Thèse Doct. spécialité, Univ. Aix-Marseille II, 112 pp.
- Gross, F., S. R. Nutman, D. T. Gauld & J. E. G. Raymont, 1949/1950. A fish cultivation experiment in an arm of a sea loch. 1–4. *Proc. roy. Soc. Edinb. B.* 24: 1–135.
- Griffith, R. J., 1980. Natural food availability and assimilation in the bivalve *Choromytilus meridionalis*. *Mar. Ecol. Prog. Ser.* 3: 151–156.
- Healey, F. P., 1975. Physiological indicators of nutrient deficiency in algae. *Fish. mar. Ser. tech. Rep.* 585: 1–30.
- Healey, F. P., 1978. Physiological indicators of nutrient deficiency in algae. *Mitt. int. Ver. Limnol.* 21: 34–41.
- Helm, M. M., I. Laing & E. Jones, 1979. Culture of algae for larval fish and shellfish rearing. 1. The development of a 200 l algal culture vessel at Conwy. *Fish. Res. tech. Rep., MAFF Direct. Fish. Res., Lowestoft* 53: 1–7.
- Kempf, M., J. P. Lissalde, A. Muller-Feuga, J. Valentin & F. Vallet, 1974. Conséquences biologiques de la résurgence de Cabo Frio, Brésil. 2ème Colloque int. Exploit. Océans, Bordeaux, France, Octobre 1974, 2 Bx 107, 15 pp.

- Ketchum, B. H., 1939. The development and restoration of deficiencies in the phosphorus and nitrogen composition of unicellular plants. *J. Cell. comp. Physiol.* 13: 373-381.
- Kuenzler, E. J. & B. H. Ketchum, 1962. Rate of phosphorus uptake by *Phaeodactylum tricornutum*. *Biol. Bull.* 123: 134-145.
- Leftley, J. W., D. J. Bonin & S. Y. Maestrini, 1983. Problems in estimating marine phytoplankton growth, productivity and metabolic activity in nature: an overview of methodology. *Oceanogr. mar. Biol., Ann. Rev.* 21: 23-66.
- Loosanoff, V. L. & J. B. Engle, 1942. Use of complete fertilizers in cultivation of microorganisms. *Science* 95: 487-488.
- Mackereth, F. J., 1953. Phosphorus utilization by *Asterionella formosa* Hass. *J. exp. Bot.* 4: 296-313.
- Maestrini, S. Y. & J. M. Robert, 1981. Rendements d'utilisation des sels nutritifs et variations de l'état des cellules de trois diatomées de claires à huîtres de Vendée. *Oceanol. Acta* 4: 13-21.
- Maestrini, S. Y., D. J. Bonin & M. R. Droop, in press. Phytoplankton as indicators of sea water quality: bioassay approaches and protocols. In: L. E. Shubert (ed.), *Algae as Ecological Indicators*. Academic Press Publishing, London.
- McCarthy, J. J. & J. C. Goldman, 1979. Nitrogenous nutrition of marine phytoplankton in nutrient-depleted waters. *Science* 203: 670-672.
- Moreira da Silva, P. C., 1969. Projeto 'Cabo Frio'. *Inst. Pesq. Mar., Rio de Janeiro*, 14 pp.
- Moreira da Silva, P. C., 1971. Fertilization of the sea as a by-product of an industrial utilization of deep water. In: J. D. Costlow (ed.), *Fertility of the sea*. Gordon and Breach Scientific Publishers, 401-405.
- Moreira da Silva, P. C. & R. F. Rodrigues, 1966. Modificações na estrutura vertical das águas sobre a borda da plataforma continental por influencia do vento. *Public. Inst. Pesq. Mar., Rio de Janeiro* 35: 1-6.
- Møhlenberg, F. & H. U. Riisgaard, 1978. Efficiency of particle retention in 13 species of suspension feeding bivalves. *Ophelia* 17: 239-246.
- Redfield, A. C., 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. In: R. J. Daniel (ed.), *James Johnstone Memorial Volume*. University Press, Liverpool: 176-192.
- Robert, J. M., S. Y. Maestrini, M. Bages, J. P. Dreno & E. Gonzalez Rodriguez, 1979. Estimation, au moyen de tests biologiques, de la fertilité pour trois diatomées des eaux claires à huître de Vendée. *Oceanol. Acta* 3: 275-286.
- Roels, O. A., R. D. Gerard & A. W. H. Be, 1971. Fertilizing the sea by pumping nutrient-rich deep water to the surface. In: J. D. Costlow (ed.), *Fertility of the Sea*. Gordon and Breach Scientific Publishers: 401-415.
- Ryther, J. H., W. M. Dustan, K. R. Tenore & J. E. Huguenin, 1972. Controlled eutrophication. Increasing food production from the sea by recycling human waste. *Bio Science* 22: 144-152.
- Sakshaug, E., 1980. Problems in the methodology of studying phytoplankton. In: I. Morris (ed.), *The Physiological Ecology of Phytoplankton*. Studies in Ecology, 7. Blackwell Scientific Publications, Oxford: 57-91.
- SCOR-UNESCO, 1964. Report of SCOR-UNESCO working group 17 on Determination of photosynthetic pigments. UNESCO, Paris, 69 pp.
- Spectorova, L. V., O. I. Goronkova, L. P. Nosova & O. N. Albitskaya, 1981/1982. High-density culture of marine microalgae. Promising items for mariculture. 1. Mineral feeding regime and installation for culturing *Dunaliella tertiolecta* Bucht. *Aquaculture* 26: 289-302.
- Strickland, J. D. H. & T. R. Parsons, 1972. A practical handbook of seawater analysis, 2nd Ed., *Bull. Fish. Res. Bd. Can.* 167: 310 pp.
- Trotta, P., 1981. A simple and inexpensive system for continuous monoxenic mass culture of marine microalgae. *Aquaculture* 22: 383-387.
- Ukeles, R., 1965. A simple method for the mass culture of marine algae. *Limnol. Oceanogr.* 10: 492-495.
- Widdows, J., P. Fieth & C. M. Worrall, 1979. Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. *Mar. Biol.* 50: 195-207.

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