Aquat. Living Resour. 12 (5) (1999) 335–342

Limiting nutrients of oyster pond seawaters in the Marennes-Oléron region for *Haslea ostrearia*: applications to the mass production of the diatom in mesocosm experiments

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Received February 26, 1999; accepted September 6, 1999

Abstract — Bioassays were carried out with the 'blue diatom' *Haslea ostrearia* Simonsen, which is responsible for oyster greening during the fattening period of *Crassostrea gigas* Thunberg in oyster ponds. Samples of seawater were taken from two oyster ponds: one without oysters and the other with 20 oysters per m^2, maximal density allowed by the French AFNOR norm for 'refinement'. The aims were to clarify the nutrient requirements of this diatom, also to elucidate the eventual influence of *C. gigas* at this density on the seawater fertility and to envisage the mass production of this diatom by pond fertilization. Examination of cell numeric densities at the end of bioassays allows us to conclude that silicate was the first limiting nutrient, closely followed by phosphate. Chlorophyll *a* concentrations led to different conclusions: phosphate was the first limiting factor, but after the seawater storage period in ponds, seawater quality evolved to a deficiency of nitrogen. Silicate addition increased cell division rate, and silicate depletion increased chl *a* synthesis for this species. Examination of nutrient assimilation ratios confirms that *H. ostrearia* requires a large amount of silicon. From these results, it was possible to prepare a N + P + Si simplified medium which has been tested in laboratory and field mesocosm conditions. In both conditions, similar results were observed: a significant increase in *H. ostrearia* cell concentrations and consequently an evolution up to the greening stage. Applications of this work are numerous; the principal permits us to envisage the production of this species in 25-m^3 ponds, with the aim of allowing constant production of the greening phenomenon. © 1999 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

Limiting nutrients / Bacillariophyceae / *Haslea ostrearia* / bioassay / mesocosm experiment / oyster ponds

Résumé — Facteurs nutritionnels limitants d'*Haslea ostrearia* dans les eaux des claires ostréicoles de la région de Marennes-Oléron : applications à la production de masse de la diatomée par des expérimentations en mésocosmes. Des bioessais ont été réalisés avec la « navicule bleue » *Haslea ostrearia* Simonsen, qui est responsable du verdissement des huîtres durant la période d'affinage de *Crassostrea gigas* Thunberg dans les claires. Des échantillons d'eau de mer ont été prélevés dans deux claires : une sans huîtres et l'autre avec 20 individus au m^2, densité d'affinage maximale autorisée par la norme française Afnor. Les buts étaient de clarifier les besoins nutritionnels de cette diatomée, de mettre en évidence l'influence de *C. gigas* sur la fertilité de l'eau des claires, et d'envisager la production de masse de cette diatomée par fertilisation des bassins. À la fin des bioessais, l'examen des densités numériques en cellules permet de conclure que les silicates seraient le premier facteur nutritionnel limitant, suivis des phosphates. Les concentrations en chlorophylle-*a* obtenues conduisent à des conclusions différentes : les phosphates seraient le premier facteur limitant, cependant après séjour de l'eau dans les claires, la qualité de l'eau évolue vers un déficit en azote. Pour cette espèce, il semble que l'ajout de silicates augmente le taux de division des cellules, alors que la déficience en silicates conduit à l'augmentation de la synthèse de chlorophylle *a*. L'estimation des rapports d'assimilation en nutriments confirme les besoins importants en silice pour *H. ostrearia*. De ces résultats, il a été possible d'élaborer un milieu nutritif simplifié N+P+Si qui a été testé en conditions de laboratoire et de milieu extérieur en mésocosmes. Dans les deux cas, les résultats observés sont similaires, une augmentation significative des concentrations en cellules d'*H. ostrearia* et donc une évolution vers le phénomène de verdissement. Les applications de ce travail sont nombreuses ; la principale étant de permettre la production de masse de cette espèce en bassin de 25 m^3 dans le but de s'affranchir des conditions aléatoires régissant le phénomène de verdissement. © 1999 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

Nutriments limitants / Bacillariophyceae / *Haslea ostrearia* / bioessai / expérience en mésocosmes / claires à huîtres

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1. INTRODUCTION

The present study was carried out in the large oyster breeding area, the Marennes-Oléron Bay, located on the south-west coast of France (*figure 1*), more exactly in salt marsh areas. Oyster farmers use a special culture technique, which consists in immersing oysters in ponds for 'refinement' (fattening). These basins (400 m^2 in area and 0.6 m deep) are used to fatten and green the oysters [35]. Oyster fattening is achieved through the great richness in phytoplankton, and oyster greening is the result of the proliferation of the diatom *Haslea ostrearia* which is responsible for the greening of the gills after secretion by this microalgae of a blue hydro-soluble pigment called 'marennine' [28].

Fattening and greening are the final stages of oyster rearing, which are a guarantee of the product quality and represent a large economical importance. But the proliferation of this diatom in ponds depends on the combination of many factors poorly understood, which give to the phenomenon an unpredictable character.

Algal bioassays are an important tool for monitoring seawater quality and capacity to sustain an algal biomass. Algal growth potential tests (AGP-tests) have been widely applied [3, 15, 21, 23, 25, 29]. Nutrient-enrichment bioassays have also been applied to marine systems for experimentally testing nutrient limitation. This method is characterized by using one or more nutrients which are added to a volume of seawater to study the effect on algal growth [13].

Maestrini and Robert [22] and Robert et al. [34] showed that in the oyster ponds of the Bay of Bourgneuf (Vendée, France) the first limiting nutrient was nitrogen for three most abundant diatom species (*H. ostrearia* Simonsen, *Phaeodactylum tricornutum* Bohlin, *Skeletonema costatum* Cleve).

The principal aim of this study is to characterize which nutrient(s) limit the growth potential of *H. ostrearia* in this area. Nutrient needs for this species are estimated from in vitro bioassays and an enrichment mixture with nitrates, phosphates and silicates is elaborated and tested in laboratory and field conditions. The short-term purpose consists of oyster pond fertilization with the simplified enriched formula to further *H. ostrearia* development in ponds and then obtain greening phenomenon.

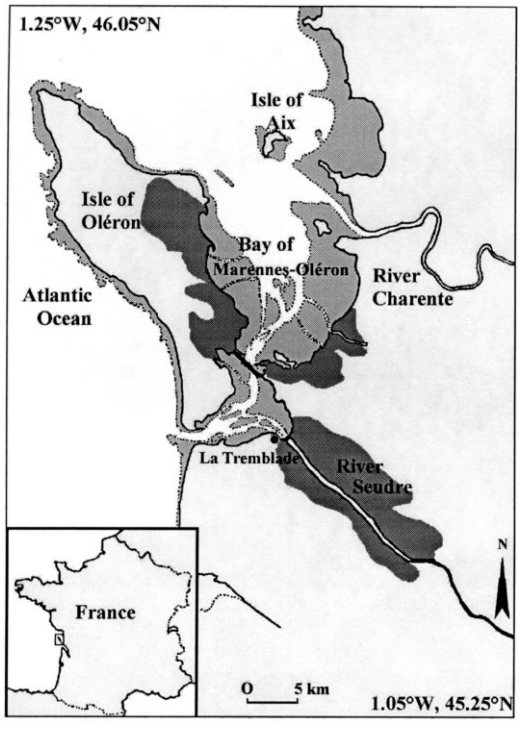

Figure 1. The Bay of Marennes-Oléron (France) and location of experimental oyster ponds near La Tremblade. ■: oyster pond area; ●: experimental oyster pond location; ----: lowest tide limit.

2. MATERIALS AND METHODS

2.1. Bioassays

Seawater samples were collected in two oyster ponds of the experimental marsh of the Ifremer Laboratory near La Tremblade (*figure 1*): in the first one, 20 oysters per m^2 (maximal density allowed by the AFNOR norm [1] during oyster fattening) were deployed on the sediment; in the other one, no oysters were added. The ponds were naturally supplied with seawater by a small canal from the Marennes-Oléron Bay, near the Seudre River estuary. The seawater renewal occurs at spring tides. In 1996, eight sampling dates were selected according to the tidal cycle: four during the renewal (t_0) and four before the following renewal after starvation of the seawater in ponds (t_{+10}).

From each pond 25-L of seawater were collected for bioassays, and filtered on Millipore cellulose–acetate filters (0.45 μm). Aliquots (80 mL) were stored in polypropylene bottles for nitrite, nitrate, silicate and phosphate concentration analyses, which were carried out with a SKALAR continuous-flow auto-analyser by using standard methods [40]. For ammonia the manual method of Solorzano [39] and Koroleff [16] modified by Grashoff and Johansen [12] was used.

Vessels used for bioassays were sterilized to reduce bacterial contamination sources; samples of 300 mL of the filtered seawater were distributed into eight 500-mL Erlenmeyer flasks for each pond. Autoclaving of seawater was not performed because of both P and Fe precipitation (Fiala in [21]) and also the NH_4 release [21]. Enrichment experiments were carried out in triplicate and seven different combinations using nitrates, silicates and phosphates were added to samples (N, P, Si, N + P, N + Si, P + Si, N + P + Si) for comparisons with an non-enriched control. As Maestrini et al. [24] recommended, initial concentrations were assumed to be low enough not to change the ecophysiological adaptation of test alga and to be high enough to sustain an algal biomass significantly higher

Table I. Means and 95 % confidence interval of initial nutrients concentrations (μM) and ΣN/P, ΣN/Si, Si/P ratios of oyster pond seawater at different sampling periods.

	Seawater supply to oyster ponds (t_0)		Seawater storage in oyster ponds (t_{+10})	
	+O	–O	+O	–O
NH_4	1.30 ± 0.26	0.82 ± 0.18	1.39 ± 0.25	0.83 ± 0.39
NO_2	0.32 ± 0.02	0.33 ± 0.02	0.12 ± 0.02	0.11 ± 0.02
NO_3	28.89 ± 4.67	30.86 ± 4.66	1.54 ± 0.37	1.70 ± 0.36
ΣN	30.51 ± 4.62	32.01 ± 4.67	3.04 ± 0.47	2.64 ± 0.61
SiO_3	20.41 ± 6.64	19.45 ± 4.95	8.56 ± 0.99	11.11 ± 0.99
PO_4	0.69 ± 0.17	0.49 ± 0.08	0.30 ± 0.06	0.31 ± 0.05
ΣN/P	51.1 ± 7.6	71.0 ± 6.4	11.9 ± 2.3	8.9 ± 1.7
ΣN/Si	1.9 ± 0.4	1.9 ± 0.2	0.4 ± 0.1	0.2 ± 0.1
Si/P	27.8 ± 2.1	40.3 ± 5.3	32.4 ± 5.1	46.0 ± 13.5

Seawater supply to oyster ponds: at the beginning of tidal cycle; after seawater storage in oyster ponds: the same seawater before its renewal; +O: oyster pond with *C. gigas*; –O: oyster pond without *C. gigas*.

than that sustained in non-enriched controls. The nutrient concentrations were equal to enriched seawater Provasoli medium diluted 1/3 [32]: 100 μM (NO_3), 100 μM (SiO_3) and 6.25 μM (PO_4).

The test alga *H. ostrearia* Simonsen (axenic local strain) was inoculated in sterile conditions at an initial concentration of 2 000 cells per mL after starvation for 2 d on a nutrient-poor seawater (depleting treatment) (for details see [4, 24]). The 48 Erlenmeyers sealed with cotton plugs (eight combinations in triplicate for each pond), were placed in a culture chamber, at 15 ± 1 °C at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ under 14 h light and 10 h dark. The experiment was stopped on the sixth day of culture.

Algal growth potential was estimated by cell counting on a Nageotte's haematocymeter and chlorophyll *a* concentration estimation. Aliquots (20 mL), from each Erlenmeyer, were filtered on GF/F Whatman filters to analyse chlorophyll *a* content by using the protocol described by Lorenzen [20].

2.2. Mesocosm experiments conducted in oyster pond

Mesocosms were PVC transparent cylinders (height 1 m, diameter 0.6 m, volume 170 L); the bottom was water tight to eliminate sediment influence on seawater quality. To induce a proliferation of *H. ostrearia* and the greening of the cultures, eight mesocosms were immersed in one oyster pond and filled with 170 L of the oyster pond seawater just after the renewal; in those experiments the seawater was not filtered and consequently contained its natural phytoplankton and zooplankton communities. Four initial concentrations of *H. ostrearia* were tested: 250, 500, 1 000, 2 000 cells per mL and cells were inoculated in mesocosms in presence or absence of a N + P + Si supply. Simultaneously for each mesocosm, 500 mL of seawater sample were collected and incubated at the laboratory in the culture room in the same conditions described previously except for photoperiod: 10 h light and 14 dark corresponding to the natural field experiments.

At t_o and t_{+10} d, seawater samples were collected both in mesocosm and flask conditions to estimate *H. ostrearia* cell concentrations and external marennine concentrations by using the method described by Robert and Hallet [33].

2.3. Statistical analysis

All statistical analyses (ANOVA and Student-Newman-Keuls a posteriori tests) were carried out with Sigma Stat 2.0 (Jandel Scientific Software), after determination of normality and variance homogeneity. Data were log-transformed when non-normal.

3. RESULTS

3.1. Nutrient concentrations

Analysis of nutrient concentrations of seawater samples was carried out before bioassay experiments, this first classical approach is indirect and only descriptive. Detailed nutrient mean concentrations are reported in *table I*. In all cases but ammonia, nutrient concentrations (NO_2, NO_3, SiO_3 or PO_4) decreased similarly in the two ponds during storing of seawater in ponds. Indeed, NH_4 mean concentrations were twice or three times greater in the pond containing oysters. This probably resulted, at least partly, from the oysters' excretory products. The most important decrease was observed for nitrate concentrations while nitrite, silicate and phosphate concentrations showed a limited decline.

In the beginning of the tidal cycle, comparison of seawater nutrient concentrations (*table I*) with the Redfield ΣN/P ratio [31] shows a potential P-limitation of seawater samples from the two ponds. ΣN/P mean ratio ($\Sigma N = NH_4 + NO_2 + NO_3$) was between 51.1:1

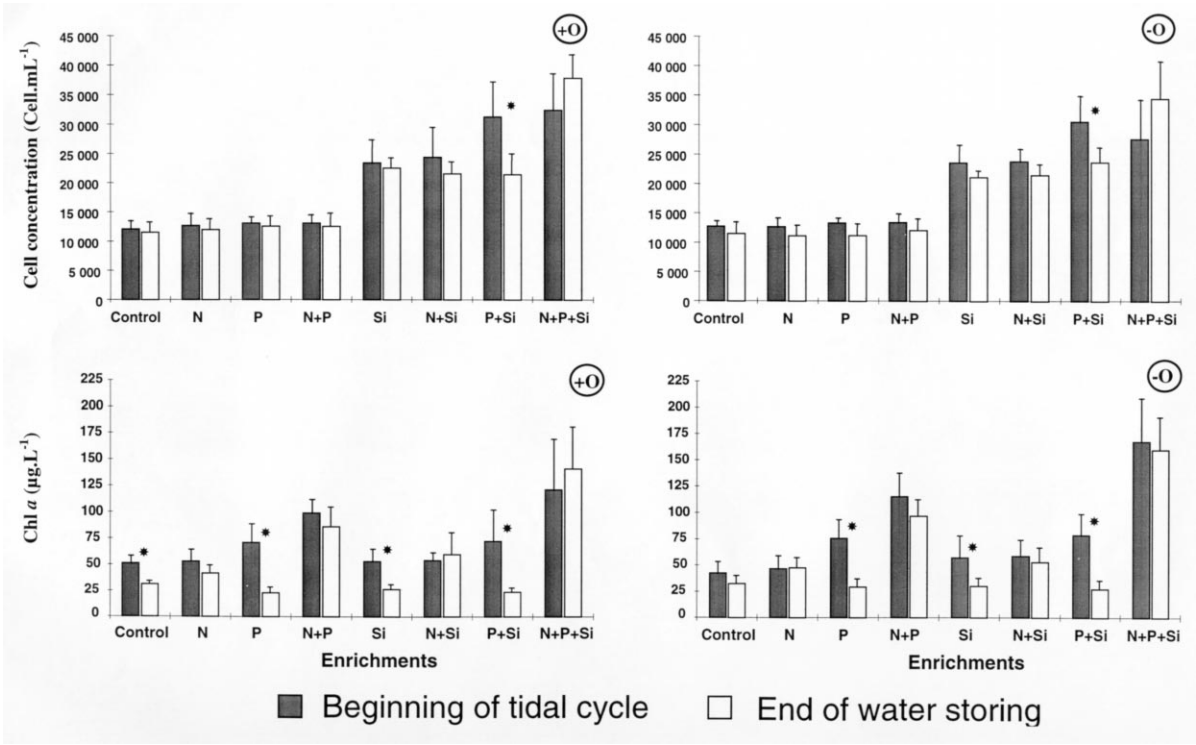

Figure 2. Algal growth potential (AGP) expressed with the two selected estimators (cell and chlorophyll *a* concentrations) of *Haslea ostrearia* on the sixth day (beginning of the stationary phase) obtained with seawater samples from oyster ponds studied at the two sampling periods. +O: oyster pond with 20 oysters per m^2; –O: oyster pond without oysters. Data are mean ± 95 % confidence interval (n = 4). Stars indicate statistically significant differences at P = 0.05 after SNK a posteriori tests between beginning of tidal cycle and end of water storing.

for the pond with immersed oysters and 71.0:1 for the other pond.

For Si/P ratio the same potential P-limitation was observed (Si/P > 16). However, silicic acid showed lower concentrations than ΣN (ΣN/Si > 1:1). After the neap tide period, approximately 10 d, a notable evolution of the proportions between nutrient concentrations was observed: ΣN/P tended to be lower than the Redfield ratio of 16:1, especially in oyster ponds without oysters, indicating a possible N-limitation of the algal growth potential. ΣN/Si ratio was respectively 0.4:1 and 0.1:2 in the two ponds, which is in agreement with the N-limitation previously observed.

3.2. Bioassay experiments

The descriptive approach is not sufficient to describe the potential limitation observed, and therefore differential enrichment experiments are necessary to explain the role of N, P and Si in limiting growth potential of *H. ostrearia* and to specify respective nutrient contribution to this limitation.

Algal growth potential (AGP) of *H. ostrearia* was similar for each sampling periods [supply of seawater (spring tides) and after seawater storage (neap tides)]. Therefore, the four dates from each sampling period were combined for statistical analysis.

Bioassay results are displayed in *figure 2* for the two AGP estimators chosen. After 6 d of culture, comparisons of the cell concentrations obtained from seawater at the two sampling stages showed no significant difference for each nutrient combination, except for the P + Si enrichment. Moreover, for each pond, and independently of the seawater origin, two or three groups were statistically isolated (P < 0.05): 1) [control, N, P, N + P] and [Si, N + Si, P + Si, N + P + Si] for seawater samples collected after renewal in ponds; 2) [control, N, P, N + P], [Si, N + Si, P + Si] and [N + P + Si] for seawater samples collected after storage in ponds. These results demonstrate that Si supply significantly increased cell numbers. Therefore, the *H. ostrearia* growth potential was Si-limited, both at the beginning and at the end of the neap tide phase. However, it should be noted that in all cases, except the N + P + Si enrichment, cell numbers obtained were lower following the 10-d seawater storage in ponds.

The results are different if the AGP estimator is chlorophyll *a* (*figure 2*). For the two ponds, bioassays showed a P-limitation of *H. ostrearia* AGP obtained at the spring tide; a N-limitation was observed after seawater storage in the ponds. Statistical analysis confirmed those results, which are in agreement with the above observations on nutrient ratios (*table II*). No statistical difference was observed between seawater

Table II. Results of two-way ANOVA on cell density of the control Erlenmeyer flasks.

Factor	df	F	*P* value
Date	7	29.35	< 0.001 (***)
Oyster pond	1	1.17	0.287 (n.s.)
Date versus oyster pond	7	1.78	0.126 (n.s.)
Residual	32		
Total	47		

df: degrees of freedom; F: F-test value; n.s.: non-significant F-test, *** significant F-test at $P = 0.001$).

sampled at the spring tide and seawater sampled after 10 d of storage in ponds (during the neap tide) for enrichments which were complemented with N. Thus, in relation to cell numbers, we observed that most Si-enrichment treatment, maximizing cell number, presented the lowest content in chl *a* (Si, N + Si, P + Si, but not N + P + Si). In contrast, the highest content in chl *a* was observed with non-Si-enriched nutrient combinations (N, P, N + P), giving cell numbers similar to those of the controls.

3.3. Possible oyster impact on seawater fertility

Detailed nutrient mean concentrations are reported in *table I*. In all cases, except ammonia, nutrient concentrations (NO_2, NO_3, SiO_3 or PO_4) decreased similarly in the two ponds during storing of seawater in ponds. Indeed, NH_4 mean concentrations were twice or three times greater in the pond containing oysters. This probably resulted, at least partly, from the oysters' excretory products. The most important decrease was observed for nitrate concentrations while nitrite, silicate and phosphate concentrations showed a limited decline.

To show the possible influence of oyster presence on seawater quality and fertility for *H. ostrearia*, a comparison of the controls after bioassays was carried out on AGP value (cell concentration). Results of the two-way ANOVA are detailed in *table II*. Two factors of variance were chosen: date and oyster ponds. A statistically significant difference ($P < 0.001$) was observed between sampling dates, but no difference was established between the two ponds. Interaction between factor date and oyster ponds was not statistically significant.

3.4. Adjustment of a N + P + Si simplified medium for mass production

The N + P + Si combination induced high cell numbers and chl *a* concentrations. For this specific enrichment, nutrient quantities (N, P and Si) necessary to produce 10^6 cells were calculated, and the assimilation ratios of nutrients during the exponential phase of culture were estimated. Only sampling dates corresponding to seawater renewal in the oyster pond without oysters were selected because the aim was the fertilization of ponds just after renewal; the results are displayed in *table III*. Nitrogen and silicon appear to be used similarly: 2.32 and 2.30 µmol, respectively; only 0.16 µmol of phosphorus is necessary. Mean values of nutrient assimilation ratios showed N/P = 15.2:1 and Si/P = 15.5:1. Consequently a N + P + Si medium was formulated on the basis of 120 µM of silicic acid as initial concentration used for further experiments in mesocosms. So the final enrichment was developed to obtain a culture medium at the concentrations: 120 µM of $Na_2SiO_3,5H_2O$; 117.7 µM of $NaNO_3$ and 7.7 µM of $K_2HPO_4,3H_2O$.

Table III. Estimated nutrient quantity necessary to produce 10^6 cells of *Haslea ostrearia* and nutrient assimilation ratios obtained after 6 d of cultivation, on seawater samples, from the pond without oysters and at the beginning of tidal cycle (t_0).

Sampling date	Estimated nutrient quantity necessary to produce 10^6cells			Nutrient assimilation ratios	
	N (µmol)	Si (µmol)	P (µmol)	N/P	Si/P
23 January	0.97	2.33	0.12	8.1	19.4
	1.11	2.70	0.14	7.9	19.3
	1.76	2.53	0.12	14.7	21.1
21 February	3.30	3.26	0.25	13.2	13.0
	4.74	2.32	0.22	21.5	10.5
	3.66	1.93	0.15	24.4	12.9
19 March	0.39	n.e.	0.18	2.2	n.e.
	1.37	1.70	0.17	8.1	10.0
	1.18	n.e.	0.14	8.5	n.e.
17 April	3.05	1.94	0.12	25.4	16.2
	2.49	1.67	0.10	24.9	16.7
	3.76	2.62	0.16	23.5	16.4
Mean	2.32 ± 0.87	2.30 ± 0.36	0.16 ± 0.03	15.2 ± 5.3	15.5 ± 2.7

Data are means ± 95 % confidence interval, n.e.: non-estimable value.

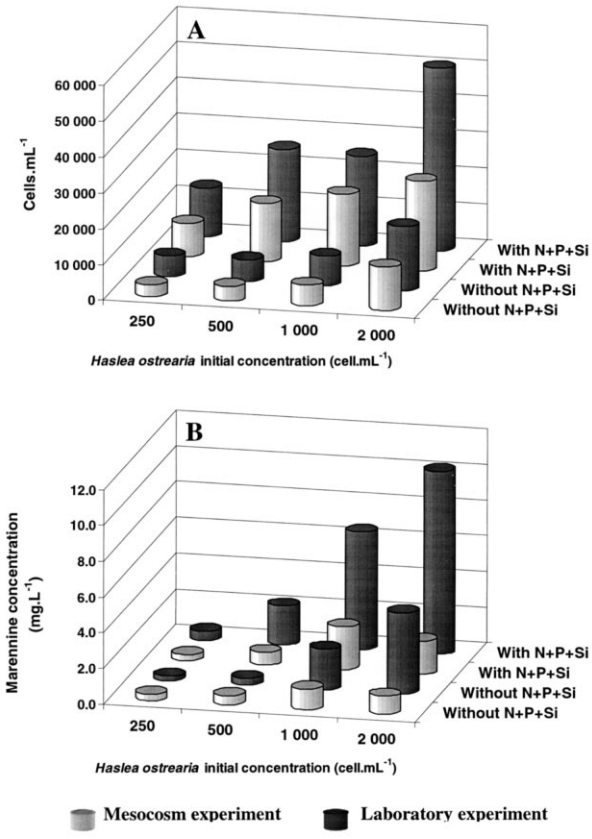

Figure 3. Cell concentration (A) and Marennine concentration (B) obtained after 10 d in mesocosms and in laboratory with different initial concentrations of *Haslea ostrearia* tested.

3.5. Mesocosm experiments

Comparable results in mesocosms and in the laboratory conditions were observed (*figure 3*). However, the highest values were observed in laboratory conditions probably due to the control of photoperiod and temperature conditions.

The experimental design only allowed a three-way ANOVA without replication [37]. After 10 d of cultivation in batch conditions in the mesocosms and in laboratory conditions, the three factors: initial concentration of *H. ostrearia*, enrichment and site conditions, showed significant differences ($P < 0.05$) for the biomass and the greening estimators. The S.N.K. a posteriori tests showed a significant difference ($P < 0.05$) between initial concentrations inoculated. Two homogeneous groups could be isolated: one at 250 and 500 cell·mL^{-1} and the other at 1 000 and 2 000 cell·mL^{-1}. Significant effect of enrichment supply was observed ($P < 0.05$), and a significant difference was observed between mesocosm and laboratory experiments. For future field experiments on greening enhancement, it appears that the initial concentrations of 1 000 or 2 000 cell·mL^{-1} of *H. ostrearia* and the N + P + Si supply can be recommended.

4. DISCUSSION

Several authors [5, 31] have estimated that phytoplankton nutrient composition corresponds to an atomic ratio N/Si/P of 16:16:1, and therefore it gives an estimation of diatom requirements [10, 18]. From those values the ratio N/Si has been commonly used [6, 13, 18] to determine potential Si- or N-limitation.

Analysis of nutrient composition of oyster pond seawaters during this study showed a potential P-limitation at the beginning of each tidal cycle and a potential N-limitation after the storage period in the ponds, in comparison with the Redfield and Brzezinsky ratio. But this descriptive approach is not sufficient to assert precisely the limiting nutrient(s) for algal growth of one species, and therefore bioassays were necessary to investigate limiting nutrients of algal growth potential test by using the method of differential enrichments.

Statistical comparisons of control cell concentrations did not show any oyster impact on seawater fertility ($P > 0.05$) for *H. ostrearia* with the rearing density allowed by AFNOR norm (*table II*). This rearing density was not sufficient to demonstrate the role of filter-feeding bivalves, described by several authors with the stimulation of primary production through the supply of dissolved inorganic excreted nutrients [2, 8, 11, 14].

Haslea ostrearia cell concentrations, obtained after 6 d of cultivation on the variously enriched oyster pond seawaters, demonstrated potential Si-limitation in all cases (*figure 2*). However, both chlorophyll *a* concentrations and nutrient ratios showed a potential P-limitation of seawater at spring tides and potential N-limitation after seawater storage during neap tides. For Si-non-enriched flasks, intracellular concentrations in chl *a* were greater than for Si, N + Si and P + Si enrichment treatments. These results are in agreement with Lombardi and Wangersky [19] who reported an increase in chl *a* synthesis for a culture of *Chaetoceros gracilis* as a consequence of Si-depletion. In addition, Darley [9] reported that *Navicula pelliculosa* and *Cylindrotheca fusiformis* required silicon for mitosis. The latter species had a specific requirement for silicon in net synthesis of DNA, which is a pre-requisite for silicification in diatoms. That could explain highest cell concentrations and then highest division rates in Si-enriched flasks.

From algal bioassays, silicon requirements of *H. ostrearia* can be considered as more important than nitrogen or phosphorus, and silicon can be proposed as the first limiting nutrient for this diatom mass production in these waters. Those results differ from those of many previous studies in the 1980s which demonstrated for this diatom the role of nitrogen as first limiting nutrient [22, 34, 35]. Nevertheless, oyster farmers from the Bay of Marennes-Oléron have long observed a decrease in the frequency of the greening phenomenon, which could be explained by the Si-limitation observed. This interpretation is also in

agreement with several authors who concluded that Si-limitation has become more frequent [7, 26, 27, 36], because P and N are recycled faster than Si [17, 29]. N and P supplies have increased owing to the increase in anthropogenic activities (industrial, urban, agricultural developments, etc.), and have modified the nutrient ratios in coastal waters for at least the past two decades [38]. Ravail-Legrand [30] emphasized that nitrogen output from Charente River (*figure 1*) could represent up to 160 tons per day during autumnal and spring floods, contributing to the N-fertilization of all the bay, while Si supplies due to river terrestrial export have not evolved: Si/N ratios at the mouth of Charente were between 0.2 and 0.6. Soletchnik et al. [38], in Marenne-Oléron Bay, demonstrated a significant long-term trend for the nutrient ratios over the last two decades. By way of example, the Si/N ratio decline from 0.65 to 0.45 between 1977 and 1995 resulted from a nitrate concentration increase.

This study has also permitted us to elaborate and test a simplified medium (N + P + Si) from the experimental estimation of nutrient needs of *H. ostrearia* by the bioassays. The three medium elements are used approximately in the same ratios as those proposed by Redfield and Brzezinski for diatom requirements: N/Si/P = 15.2:15.5:1. By using the mesocosms immersed in the oyster ponds and also in laboratory experiments, different tests of this medium were conducted. A significant bloom of *H. ostrearia* was obtained with an initial concentration of 1 000 and 2 000 cell·mL^{-1}. Biomass produced after 10 d corresponded to 20 and 15 times the natural cell concentrations observed during the natural greening observed by Robert [32]. With respect to external marennine concentrations, a maximum value of 2.4 mg·L^{-1} was observed, which was lower than for in vitro observations.

Those results are promising for the mass production in natural conditions of *H. ostrearia*. Further experiments will be carried out by using 25-m^3 mesocosms. The production in 500-L tanks of *H. ostrearia* inoculum under glasshouse will be necessary. Mesocosm fertilization with NPSi media will be carried out with 120 μM (SiO_3), 117.7 μM (NO_3) and 7.7 μM (PO_4).

Acknowledgements

This study was financially supported by the Région Poitou-Charentes, the Department of Charente-Maritime and Ifremer. We thank G. Massé, P. Rosa and Y. Chifolleau for their technical assistance.

REFERENCES

[1] AFNOR, Norme française huîtres creuses, dénomination et classification, NF V 45-056, 1985, 5 p.

[2] Asmus H., Asmus R.M., Mussel beds: limiting or promoting phytoplankton?, J. Exp. Mar. Biol. Ecol. 148 (1991) 215–232.

[3] Barbosa F.A.R., Evidence from algal bioassays of seasonal nutrient limitations in two English lakes, Hydrobiologia 188/189 (1989) 211–228.

[4] Berland B.R., Bonin D.J., Maestrini S.Y., Pointier J.-P., Étude de la fertilité des eaux marines au moyen de tests biologiques effectués avec des cultures d'algues, II-Limitation nutritionnelle et variabilité de l'inoculum, Int. Rev. Gesamten Hydrobiol. 58 (1973) 203–220.

[5] Brzezinski M.A., The Si:C:N ratio of marine diatoms: interspecific variability and effect of some environmental variables, J. Phycol. 21 (1985) 347–357.

[6] Conley D.J., Malone T.C., Annual cycle of dissolved silicate in Chesapeake Bay: implications for production and fate of phytoplankton biomass, Mar. Ecol. Prog. Ser. 81 (1992) 121–128.

[7] Conley D.J., Shelske C.L., Stroermer E.F., Modification of the biogeochemical cycle of silica with eutrophication, Mar. Ecol. Prog. Ser. 101 (1993) 179–192.

[8] Dame R.F., The role of bivalve filter-feeder material fluxes in estuarine ecosystems, in: Dame R. (Ed.), Bivalve Filter Feeders in Estuarine and Coastal Ecosystem Processes, NATO ASI Series G 33, 1993, pp. 245–269.

[9] Darley W.M., Silicon requirements for growth and macromolecular synthesis in synchronized cultures of diatom, *Navicula pelliculosa* (Brebisson) Hilse and *Cylindrotheca fusiformis* Reiman and Lewin, Ph.D. thesis, University of California, San Diego, 1969, 148 p.

[10] Del Amo Y., Le Pape O., Tréguer P., Quéguiner B., Ménesguen A., Aminot A., Impacts of high-nitrate freshwater inputs on macrotidal ecosytems, I. Seasonal evolution of nutrient limitation for the diatom-dominated phytoplankton of the Bay of Brest (France), Mar. Ecol. Prog. Ser. 161 (1997) 213–224.

[11] Doering P.H., On the contribution of the benthos to pelagic production, J. Mar. Res. 47 (1989) 371–383.

[12] Grasshoff K., Johannsen H., A new sensitive and direct method for the automatic determination of ammonia in seawater, J. Cons. Int. Explor. Mer. 34 (1972) 516–521.

[13] Hecky R.E., Kilham P., Nutrient limitation of phy-

toplankton in fresh water and marine environments: A review of recent evidence on the effects of enrichment, Limnol. Oceanogr. 33 (1988) 796–822.

[14] Kautsky N., Wallentinus I., Nutrient release from a Baltic *Mytilus*-red algal community and its role in benthic and pelagic productivity, Ophelia 1 (1980) 17–30.

[15] Klapwijk S.P., Bolier G., van der Does J., The application of algal growth potential tests (AGP) to the canals and lakes of western Netherlands, Hydrobiologia 188/189 (1989) 189–199.

[16] Koroleff K., Direct determination of ammonia in natural waters as indophenol blue, ICES, C.M. 1969/C 9, 1969, pp. 19–22.

[17] LeJehan S., Tréguer P., Uptake and regeneration Si/N/P ratios in the Indian sector of the Southern Ocean: Originality of the biological cycle of silicon, Polar Biol. 2 (1983) 127–136.

[18] Levasseur M.E., Therriault J.-C., Phytoplankton biomass and nutrient dynamics in a tidally induced upwelling: the role of NO_3:$Si(OH)_4$ ratio, Mar. Ecol. Prog. Ser. 39 (1987) 87–97.

[19] Lombardi A.T., Wangersky P.J., Influence of phosphorus and silicon on lipid class production by the marine diatom *Chaetoceros gracilis* grown in turbidostat cage cultures, Mar. Ecol. Prog. Ser. 77 (1991) 39–47.

[20] Lorenzen C.J., Determination of chlorophyll and phaeopigments: specrtophotometric equations, Limnol. Oceanogr. 12 (1967) 343–346.

[21] Lukavsky J., The evaluation of algal growth potential (AGP) and toxicity of water by miniaturized growth bioassay, Water Res. 26 (1992) 1409–1413.

[22] Maestrini S.Y., Robert J.-M., Rendements d'utilisation des sels nutritifs et variations de l'état des cellules de trois diatomées des claires à huîtres de Vendée, Oceanol. Acta 4 (1981) 13–21.

[23] Maestrini S.Y., Bonin D.J., Droop M.R., Phytoplankton as indicators of sea water quality: bioassays approaches and protocols, in: Shubert E. (Ed.), Algae as Ecological Indicators, Academic Press Inc., London Ltd, 1984, pp. 71–132.

[24] Maestrini S.Y., Droop M.R., Bonin D.J., Phytoplankton as indicators of sea water quality 1: prospects, in: Shubert E. (Ed.), Algae as Ecological Indicators, Academic Press Inc., London Ltd, 1984, pp. 133–138.

[25] Maestrini S.Y., Berland B.R., Bréret M., Béchemin C., Poletti R., Rinaldi A., Nutrients limiting the algal growth potential in the Po River Plume and an adjacent area, Northwest Adriatic Sea: Enrichment bioassays with the test algae *Nitzschia closterium* and *Thalassiosira pseudonana*, Estuaries 20 (1997) 416–429.

[26] Officer C.B., Ryther J.H., The possible importance of silicon in marine eutrophication, Mar. Ecol. Prog. Ser. 3 (1980) 83–91.

[27] Ragueneau O., De Blas Varelas E., Tréguer P., Quéguiner B., Del Amo Y., Phytoplankton dynamics in relation to the biogeochemical cycle of silicon in coastal ecosystem of western Europe, Mar. Ecol. Prog. Ser. 106 (1994) 157–172.

[28] Ranson G., L'absorption des matières organiques dissoutes par la surface extérieure du corps chez les animaux aquatiques, Ann. Inst. Océanogr. Paris 4 (1927) 49–174.

[29] Raschke R.L., Schulz D.A., The use of algal growth potential test for data assessment, J. Water Poll. Cont. Fed. 59 (1987) 222–227.

[30] Ravail-Legrand B., Incidences du débit de la Charente sur la capacité biotique du bassin ostréicole de Marennes-Oléron, thèse, Université de Nantes, 1993, 171 p.

[31] Redfield A.C., On the proportions of organic derivates in sea water and their relation to the composition of plankton, in: James Johnstone Memorial Volume, The University Press, Liverpool, 1934, pp. 176–192.

[32] Robert J.-M., Fertilité des eaux des claires ostréicoles et verdissement : utilisation de l'azote par les diatomées dominantes, thèse dr., Université de Nantes, 1983, 281 p.

[33] Robert J.-M., Hallet J.-N., Absorption spectrum *in vivo* of the blue pigment 'Marennine' of the pennate diatom *Navicula ostrearia* Bory, J. Exp. Bot. 32 (1981) 341–345.

[34] Robert J.-M., Maestrini S.Y., Bagès M., Dréno J.-P., Gonzalez-Rodrigez E., Estimation au moyen de tests biologiques, de la fertilité pour trois diatomées des eaux des claires à huîtres de Vendée, Oceanol. Acta 2 (1979) 275–286.

[35] Robert J.-M., Maestrini S.Y., Héral M., Rincé Y., Dréno J.-P., Becker L., Enrichissement expérimental d'eaux printanières de claires à huîtres en baie de Bourgneuf (Vendée, France) : augmentation de la biomasse et utilisation des éléments nutritifs par les algues unicellulaires, Hydrobiologia 95 (1982) 53–63.

[36] Smayda T.J., Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic, in: Toxic Marine Phytoplankton, Proc. 4th Int. Conf. Toxic Marine Phytoplankton, Elsevier Science Publishers, New York, 1990, pp. 29–40.

[37] Sokal R.R., Rohlf F.J., Biometry: The Principles and Practice of Statistics in Biological Research, 3rd ed., Freeman W.H. & Co., New York, 1995.

[38] Soletchnik P., Faury N., Razet D., Goulletquer P., Hydrobiology of the Marennes Oléron Bay, Seasonal indices and analysis of trends from 1978 to 1995, Hydrobiologia 386 (1998) 131–146.

[39] Solorzano L., Determination of ammonia in natural waters by the phenol-hypochlorite method, Limnol. Oceanogr. 14 (1969) 799–801.

[40] Strickland J.D.H., Parsons T.R., A practical handbook of sea water analysis, Bull. Fish. Res. Board Can. 167 (1972) 1–311.