*Aquat. Living Resour.*, 1996, **9**, 95-102

# Effects on phytoplankton growth of dissolved substances produced by fish farming

Geneviève Arzul [1], Alejandro Clément [2] and Anne Pinier [3]

[1] *IFREMER, Centre de Brest, Direction de l'Environnement littoral, B.P. 70, 29280 Plouzané, France. E-mail: genevieve.arzul@ifremer.fr*

[2] *INTESAL, Pedro Montt 160, Puerto Montt, Chile.*

[3] *UBO, Laboratoire de Chimie marine, B.P. 809, 29285 Brest Cedex, France.*

Received October 2, 1995; accepted January 10, 1996.

Arzul G., A. Clément, A. Pinier. *Aquat. Living Resour.*, 1996, **9**, 95-102.

**Abstract**

Fish Farming enriches the water column in dissolved organic and inorganic material. These materials excreed by the fish, elutriate from uneaten food and derive from other polluting substances. Ratios of the organic/inorganic forms of substances (for example *N* and *P*) are thus modified. Such perturbations in turn affect phytoplankton development. From *in vitro* analyses and tests of water taken from a turbot rearing tank, we show the changes in seawater chemical composition produced as well as the way growth was modified in three phytoplankton species: the diatom, *Chaetoceros gracile*, and the dinoflagellates, *Gymnodinium* cf. *nagasakiense* and *Alexandrium minutum*. Elutriates from the granules used for feed (0.1 to 100 mg.l$^{-1}$) were found to be stimulating, particularly for *Gymnodinium*. *Alexandrium* was strongly inhibited both by turbot-tank water diluted 10-fold with coastal seawater and by diluted faeces elutriates. Urea added at low concentrations (1 to 2.5 $\mu$mol.l$^{-1}$) inhibited growth in *Alexandrium* and *Chaetoceros*, but at 1 $\mu$mol.l$^{-1}$ it stimulated growth in *Gymnodinium*. The antibiotic, Oxytetracycline was complex in its effects, with growth inhibition being observed in *Chaetoceros* and *Gymnodinium*, but stimulation in *Alexandrium* (at 0.2 mg.l$^{-1}$). In general, we found fish farming to allow phytoplankton growth, except for *Alexandrium* in a few cases. Additions to the water from fish farming may regulate growth in some species. As such additions act differently on growth in different species, fish farming is likely to markedly modify species composition in its vicinity.

**Keywords:** Antibiotic, faeces, feed, fish farming, growth regulation, phytoplankton, urea.

*Effets des substances dissoutes produites par l'aquaculture sur la croissance du phytoplancton.*

**Résumé**

L'aquaculture enrichit la colonne d'eau en substances dissoutes organiques et inorganiques. Ces substances proviennent des excrétions de poissons, des lessivages d'aliments non consommés, et d'autres substances polluantes. Le rapport des substances organiques/inorganiques (par exemple *N* et *P*) est ainsi modifié. En retour, ces perturbations affectent le développement du phytoplancton. A partir d'analyses et de tests *in vitro* sur l'eau prélevée dans un bassin d'élevage de turbot, nous avons montré les modifications produites dans la composition chimique de l'eau de mer, et la croissance de trois espèces phytoplanctoniques : la diatomée *Chaetoceros gracile*, et les dinoflagellés *Gymnodinium* cf. *nagasackiense* et *Alexandrium minutum*. Les lessivages de granulés utlisés comme aliments (0,1 à 100 mg.l$^{-1}$) se sont montrés stimulants en particulier pour *Gymnodinium*, *Alexandrium* était fortement inhibé à la fois par l'eau du bassin d'élevage diluée 10 fois avec une eau côtière, et par des lessivages de fécès dilués. L'urée ajoutée à faibles concentrations (1 à 2,5 $\mu$mol.l$^{-1}$) inhibe la croissance d'*Alexandrium* et de *Chaetoceros*, mais elle stimule la croissance de *Gymnodinium*. L'oxytétracycline utilisée comme antibiotique produit des effets complexes, avec une inhibition de croissance de *Chaetoceros* et *Gymnodinium*, mais une stimulation d'*Alexandrium* (à 0,2 mg.l$^{-1}$). D'une façon générale, nous avons trouvé que l'aquaculture permet la croissance du phytoplancton à l'exception d'*Alexandrium* dans quelques cas. Les rejets de fermes

aquacoles peuvent agir comme régulateur de croissance du phytoplancton. En agissant différemment selon les espèces, les apports provenant d'aquaculture sont susceptibles de modifier la composition floristique des populations phytoplanctoniques dans le voisinage des sites aquacoles.

**Mots-clés :** Antibiotique, fécès, aliment, aquaculture, régulation de croissance, phytoplancton, urée.

## INTRODUCTION

Fish farming is currently expanding world-wide. This has stimulated a vast amount of research on its effects on the environment. Initially much of this research aimed at selecting the best environmental conditions for fish development, the best feed, and avoidance of fish disease particularly through use of antibiotics. The resistance of the environment to the stress imposed by fish farming is a relatively more recent preoccupation, with most studies hitherto being carried out on benthic components (Fenchel and Riedl, 1970; Pearson and Rosenberg, 1978; Black and Gowen, 1988; Gowen *et al.*, 1990). Few studies have considered pelagic disturbance, particularly phytoplankton development, as modified by fish farming.

However, fish farming enriches the organic and inorganic material in the water column, through the addition of faeces, excretion, uneaten food, desquamation, mucus, vitamins and therapeutic agents (Gowen and Bradbury, 1987; Samuelsen, 1989). Various degrees of organic matter mineralisation increase levels of potential organic nutrients, such as peptides and amino acids sources of organic phosphorus and nitrogen, urea, carbohydrates and mineral nutrients (Johnsen *et al.*, 1993).

The role of site selection, especially in relation to flushing rate, is crucial for the dilution and distribution of added substances, and their consequent impacts on the phytoplankton.

Although phytoplankton grows autotrophically, utilising mineral nutrients, vitamins and trace elements, the heterotrophic properties of phytoplanktonic cells are also important, and much use is made by some phytoplankton species of organic molecules, especially amino acids (Flynn, 1990). Consequently, aquaculture may provide stimulating factors for phytoplankton growth and this paper will investigate the efects, *in vitro*, of selected pollutants characteristic of fish-farm production on three selected phytoplankton species.

The tests were performed in an experimental breeding pool of turbot *Psetta maxima*, on:

1. *Chaetoceros gracile*, a common, non-toxic diatom;

2. *Gymnodinium* cf. *nagasakiense*, an ichthyotoxic dinoflagellate forming toxic blooms in the Mediterranean Sea, the Atlantic and the North Sea (Partensky *et al.*, 1991) and

3. *Alexandrium minutum*, a toxic dinoflagellate for shellfish consumers. This alga produces paralytic shellfish poisoning (PSP) and blooms in various coastal areas (Erard-Le Denn, 1995).

This work has followed from Nishimura's work (1982) whose results showed the stimulating effect of yellowtail food and faeces elutriates, and seawater from the cages, on *G. aureolum* growth. We extended the comparison of these effects to *C. gracile*, *G.* cf. *nagasakiense* and *A. minutum*, and some effects are also investigated according to particular chemical species provided by aquaculture: nutrients, urea, organics. The object of this study has been to investigate factors, other than the major inorganic nutrients, which regulate phytoplankton growth rate.

## MATERIALS AND METHODS

Turbot tank seawater (TS) was taken from a fish tank. The tank was of volume 250 l, kept at 12°C, and seawater was pumped into it from the nearby Rade de Brest (Brittany, France), allowed to settle and high-pressure filtered through sand. This water was continuously passed through the tank at 125 $l.h^{-1}$. In this tank were kept 15-g turbot, *Psetta maxima*, approximately 5-months old, corresponding to a total mass of 3 kg of fish in the 250 l. Feed (35 g dry granule per day) was added twice daily in batches at 10.00 and 16.00, and 5% of the food was considered to escape consumption and thus to constitute direct, environmental enrichment (Warrer-Hansen, 1982, in Gowen and Bradbury, 1987). This estimation does not concern the experimental tank where dry pellets are entirely consumed, but wastage of a sea-cage farm (Dosdat, pers. comm.). The composition of the dry granules was 16% lipids, 8% total nitrogen and 52% proteins.

An antibiotic; oxytetracycline, was added in the tank seawater (2 $mg.l^{-1}$) in case of infections desease.

*Biological tests*

Bioassays on microalgae (non-axenic cultures) were performed with turbot tank seawater (TS), feed and faeces elutriate, urea and antibiotic added separately to reference seawater (RS) pretreated as in Gentien and Arzul (1990). Filtration through 0.22 $\mu$m Falcon Easy Flow™ filters was used a sterile technique. The cultures were grown until stationary phase was reached. Culture conditions were controlled at 18°C, 50 $\mu mol.l^{-2}.s^{-1}$, 12 h light–12 h dark. Each test was duplicated at least.

*C. gracile* was grown in 40 ml glass tubes adapted to direct fluorometric measurement in a

Turner fluorometer. Growth was estimated daily by chlorophyll measurement after calibration at the beginning and the end of each experiment. One fluorescent unit equals $10^6$ diatom cells per litre. Precision was about 85%. Stationary phase was reached after 8 days.

*G.* cf. *nagasakiense* and *A. minutum* were grown in flasks in 250 and 125 ml respectively. Each population was counted every three days by Coulter Counter (Coultronics). Calibration was performed once during each by microscopic count. Precision varied from 80 to 99% according to the age of the cultures. Stationary phase was reached after 18 to 21 days.

To avoid any influence of enrichment on the growth rate estimates, results were compared, for each experiment, with controls enriched with inorganic nutrients ($NaH_2PO_4$, $NaNO_3$) as in tested samples and optimum amounts of trace elements (vitamins plus metals) as in *f*/2 (Nakamura *et al.*, 1988). Because of the simultaneous uptake of both nitrogen forms, ammonium and nitrate (Quéguiner *et al.*, 1986), ammonium ($NH_4Cl$) was added to controls at the same level as in the tests.

TS, feed and faeces elutriates used as additions in the experiments, were autoanalysed for inorganic ammonium, nitrate and phosphate following Tréguer and Le Corre (1976). Total nitrogen and phosphorus were estimated in samples by the same method, but after sample irradiation by UV for 14 h. Organic nitrogen and phosphorus were then determined by subtracting total values from inorganic values. Urea was determined by the automated method of Aminot and Kerouel (1982).

*Turbot tank seawater (TS)*

TS was tested undiluted, and both 50% and 10% diluted in reference seawater (RS).

*Feed elutriate*

25 g of ground granules were suspended in 250 ml RS using magnetic stirring and soluble substances were extracted 2 h later by centrifugation. The supernatant was completed so as to have a solution of 50 g equivalent dry granule $l^{-1}$ in RS (stock solution S1). Considering the low level of the uneaten food and the high dilution in the actual conditions, the initial solution was diluted from 1/500 to 1/500 000 in RS, corresponding to: 100, 10, 1 and 0.1 $mg.l^{-1}$ dry granule.

*Faeces elutriate*

Faeces were collected during 24 h in an underlying collector, and maintained in contact with seawater. Soluble substances were isolated by centrifugation in 300 ml supernatant (stock solution S2). We consider this volume diluted within the total aqueous volume of the pool: 3000 $l.d^{-1}$, and the fractions tested by dilution of the initial solution in RS were: 200, 100 and 50 $\mu l.l^{-1}$.

*Urea*

The effect of urea was tested at 1 and 2.5 $\mu mol.l^{-1}$ in RS enriched as in the TS by comparison with the same feature without urea addition.

**Table 1.** – Chemical composition of the media tested in bioassays in $\mu mol.l^{-1}$ (DON: Dissolved organic Nitrogen; DOP: Dissolved organic Phosphorus).

| | $N(NO_2 + NO_3)$ | $N(NH_4)$ | $P(PO_4)$ | DON | DOP | Urea |
|---|---|---|---|---|---|---|
| *Reference seawater (RS)* | 0.8 | 0.3 | 0.23 | 6.9 | 0.24 | 0.4 |
| *Turbot tank seawater (TS)* | | | | | | |
| 100% | 19.1 | 9.8 | 0.81 | 9.9 | 0.28 | 1.1 |
| 50% in RS | 9.9 | 5.1 | 0.52 | 8.4 | 0.26 | 0.7 |
| 10% in RS | 2.6 | 1.2 | 0.29 | 7.2 | 0.24 | 0.5 |
| *Feed elutriate (S1) in RS* | | | | | | |
| S1 $(500)^{-1}$ eq. to 100 $mg.l^{-1}$ | 18.8 | 0.3 | 3.41 | 94.1 | 1.79 | 0.4 |
| S1 $(5\,000)^{-1}$ eq. to 10 $mg.l^{-1}$ | 2.6 | 0.3 | 0.54 | 15.6 | 0.39 | 0.4 |
| S1 $(50\,000)^{-1}$ eq. to 1 $mg.l^{-1}$ | 1.0 | 0.3 | 0.26 | 7.2 | 0.25 | 0.4 |
| S1 $(500\,000)^{-1}$ eq. to 0.1 $mg.l^{-1}$ | 0.8 | 0.3 | 0.23 | 7.0 | 0.24 | 0.4 |
| *Faeces elutriate (S2) in RS* | | | | | | |
| S2 2 $(10\,000)^{-1}$ eq. to 200 $\mu.l^{-1}$ | 2.5 | 4.0 | 0.63 | 12.4 | 27.00 | 0.9 |
| S2 $(10\,000)^{-1}$ eq. to 100 $\mu.l^{-1}$ | 1.7 | 2.2 | 0.43 | 9.6 | 13.62 | 0.7 |
| S2 0.5 $(10\,000)^{-1}$ eq. to 50 $\mu.l^{-1}$ | 1.2 | 1.2 | 0.33 | 8.3 | 6.93 | 0.5 |
| *Urea* | | | | | | |
| 2 $\mu mol.l^{-1}$ in RS* | 19.1 | 9.8 | 0.81 | 9.9 | 0.28 | 2 |
| 1 $\mu mol.l^{-1}$ in RS* | 19.1 | 9.8 | 0.81 | 9.9 | 0.28 | 1.1 |
| *Antibiotic* | | | | | | |
| 2 $mg.l^{-1}$ in RS* | 19.1 | 9.8 | 0.81 | 9.9 | 0.28 | 1.1 |
| 0.2 $mg.l^{-1}$ in RS* | 19.1 | 9.8 | 0.81 | 9.9 | 0.28 | 1.1 |

RS*: Reference seawater enriched as in turbot tank seawater.

*Antibiotic*

Oxytetracycline was tested at 0.2 and 2 mg.l$^{-1}$ in RS enriched as TS.

*Table* 1 summarises the chemical composition of the media tested in bioassays.

Growth rate $\mu_2$ of each cultured phytoplankton was calculated by using Fukazawa's formula (1980): $\mu_2 = (\ln N_2 - \ln N_1) \cdot [(t_2 - t_1) \ln 2]^{-1}$, $N_1$ and $N_2$ being the number of cells counted respectively at the days $t_1$ and $t_2$, $\mu_2$ corresponding to the number of division per day (d$^{-1}$). Final results are expressed in $\mu_2^{-1}$ days for estimation of the time necessary for a cell to divide in each culture. It is the doubling time (D), presented on *table* 2.

## RESULTS

The doubling time D calculated in experimental treatments are compared in *figure* 1. Expressed as $D\% = 100\,(D_{test} - D_{control})\,D_{control}^{-1}$, results indicate significant stimulation or inhibition when absolute difference exceeds 20%.

### Growth in water from the turbot tank

*Table* 1 shows that the inorganic nutrients were 28.9 µmol.l$^{-1}$ total N and 0.81 µmol.l$^{-1}$ total P in the turbot tank-seawater (TS). The inorganic N/P ratio was higher, 35.7, in TS compared with only 4.8 in RS. Organic N and P were only slightly more concentrated in TS, respectively 9.9 and 0.28 µmol.l$^{-1}$, than RS, respectively 6.9 and 0.24 µmol.l$^{-1}$. The concentration

**Table 2.** – Doubling time D expressed in days. Columns D%: $100\,(D_{test} - D_{control})D_{control}^{-1}$.

| Bioassays | *Chaetoceros gracile* | | *Gymnodinium* cf. *nagasakiense* | | *Alexandrium minutum* | |
|---|---|---|---|---|---|---|
| | D | D% | D | D% | D | D% |
| Accuracy to within | 8% | | 17% | | 18% | |
| *Turbot tank seawater (TS)* | | | | | | |
| 100% TS | 1.1 | −31 | 3.8 | +5 | 8.4 | +236 |
| Control for 100% | 1.6 | | 3.6 | | 2.5 | |
| 50% TS | 1.8 | +6 | 4.3 | 0 | 11.1 | +327 |
| Control for 50% | 1.7 | | 4.3 | | 2.6 | |
| 10% TS | 2.9 | +11 | 3.2 | +7 | 19.2 | +638 |
| Control for 10% | 2.6 | | 3.0 | | 2.6 | |
| *Feed elutriate (S1)* | | | | | | |
| S1 (500)$^{-1}$ eq. to 100 mg.l$^{-1}$ | 1.8 | +12 | 1.9 | −30 | 3.0 | −51 |
| Control for 100 mg.l$^{-1}$ | 1.6 | | 2.7 | | 6.1 | |
| S1 (5000)$^{-1}$ eq. to 10 mg.l$^{-1}$ | 2.3 | −11 | 1.9 | −42 | 3.0 | −9 |
| Control for 10 mg.l$^{-1}$ | 2.6 | | 3.3 | | 3.3 | |
| S1 (50000)$^{-1}$ eq. to 1 mg.l$^{-1}$ | 2.6 | 0 | 2.0 | −39 | 4.4 | +33 |
| Control for 1 mg.l$^{-1}$ | 2.6 | | 3.3 | | 3.3 | |
| S1 (500000)$^{-1}$ eq. to 0.1 mg.l$^{-1}$ | 3.6 | +38 | 2.2 | −33 | 2.7 | −18 |
| Control for 0.1 mg.l$^{-1}$ | 2.6 | | 3.3 | | 3.3 | |
| *Faeces elutriate (S2)* | | | | | | |
| S2 2 (10000)$^{-1}$ eq. to 200 µl.l$^{-1}$ | 7.3 | +74 | 2.5 | −77 | – | |
| Control for 200 µg.l$^{-1}$ | 4.2 | | 10.8 | | 12.2 | |
| S1 (10000)$^{-1}$ eq. to 100 µl.l$^{-1}$ | 17.0 | +467 | 3.0 | 0 | – | |
| Control for 100 µl.l$^{-1}$ | 3.0 | | 3.0 | | 12.9 | |
| S2 0.5 (10000)$^{-1}$ eq. to 50 µl.l$^{-1}$ | 15.1 | +481 | 3.6 | +29 | – | |
| Control for 50 µl.l$^{-1}$ | 2.6 | | 2.8 | | 17.9 | |
| *Urea* in Reference seawater (RS)* | | | | | | |
| 2.5 µmol.l$^{-1}$ | 2.7 | +69 | 3.2 | −11 | 7.0 | +180 |
| 1 µmol.l$^{-1}$ | 3.5 | +119 | 1.5 | −58 | 6.9 | +176 |
| 0.4 µmol.l$^{-1}$ (control) | 1.6 | | 3.6 | | 2.5 | |
| *Antibiotic* in Reference seawater (RS)* | | | | | | |
| 2 mg.l$^{-1}$ | 4.3 | +65 | 7.1 | +36 | 2.6 | −10 |
| 0.2 mg.l$^{-1}$ | 4.8 | +85 | 4.3 | −17 | 2.3 | −21 |
| RS* | 2.6 | | 5.2 | | 2.9 | |

RS*: Reference seawater enriched as in turbot tank seawater.

of urea was 1.1 $\mu$mol.l$^{-1}$ in TS, compared with 0.4 $\mu$mol.l$^{-1}$ in RS.

In *C. gracile* grown in 100% TS, *table* 2 shows that D was 1.1.d. In progressively lower concentrations of TS (increasing dilutions) D progressively increased, relative to control values.

In *G.* cf. *nagasakiense* grown in TS at all dilutions tested, D was not significantly different from control values.

Growth in *A. minutum* was reduced by TS. D was from 8 to 19 days in dilutions of TS in RS from 100 to 10%. D was negatively related to TS concentration over this range.

### Feed elutriates

*Table* 1 shows the enrichment of the media produced by addition of feed elutriates. This enrichment represented an N/P ratio from 5.6 to 4.8 for the inorganic constituents released by feed pellets. Release of organic constituents, particularly of N, is likely also to have been important.

In *C. gracile*, a value of D, 1.8 days, was found at 100 mg.l$^{-1}$, as well as in the corresponding control. Feed elutriates affected D differently when added at different levels. Addition at the lowest level, corresponding to 0.1 mg.l$^{-1}$ dry matter, slightly inhibited growth relative to that in the control. In *G.* cf. *nagasakiense,* D was reduced by elutriate addition at all levels tested, with a maximum reduction (relative to the control) of 42%, at 10 mg.l$^{-1}$.

In *A. minutum*, D was reduced by 51% at 100 mg.l$^{-1}$ elutriate addition, while smaller additions had lesser effects, the addition of 1 mg.l$^{-1}$ slowing growth.

### Faeces elutriates

The inorganic N/P ratio, from 10.3 to 7.3 in S2 enriched media, was in general higher than in S1, resulting from the high proportion of ammonia excreted by fish.

Additions of S2 to cultures of *C. gracile* gave higher values of D than did controls indicating that faeces elutriate reduced growth rate in this species.

In contrast, S2 additions to cultures of *G.* cf. *nagasakiense* stimulated growth strongly, with 200 $\mu$l.l$^{-1}$ giving a D value less than one quarter that in control. The effect did not appear at 100 $\mu$l.l$^{-1}$, however, and at 50 b$\mu$l.l$^{-1}$ even reduced the growth rate.

Growth in *A. minutum* was completely stopped by all S2 concentrations tested.

### Urea

At the concentrations tested, urea inhibited growth in both *C. gracile* and *A. minimum*, but it stimulated growth in *G.* cf. *nagasakiense,* significantly at 1 $\mu$mol.l$^{-1}$.

### Oxytetracycline

At the concentrations tested, the antibiotic, Oxytetracycline, depressed growth in *C. gracile*, had a concentration-dependent effect on *G.* cf. *nagasakiense*, and stimulated growth in *A. minutum.*

## DISCUSSION

Cell division took place in all trials except for some carried out on *Alexandrium minutum.* In the experimental treatments, the longest doubling time found in *Chaetoceros gracile* was 17 days with 100 $\mu$l.l$^{-1}$ of added faeces elutriate. The experimental treatments also gave fairly rapid growth in *Gymnodinium* cf. *nagasakiense*, for which the maximum observed value of D was 7.1 days with 2 mg.l$^{-1}$ of the antibiotic Oxytetracycline added to the medium.

Under all experimental treatments with the exceptions of feed elutriate and antibiotic treatments, *A. minimum* growth showed important inhibition, compared with control. D was never observed to exceed 19.2 days. In the urea and tank seawater treatments, growth was slowed down, however, and all faeces elutriate treatments stopped growth and development completely, with, furthermore, marked disappearance of cells.

Except for that of *G.* cf. *nagasakiense*, with addition of feed elutriate, growth in the dinoflagellates showed a complex relationship with the treatments, confirming the findings of Bonin and Maestrini (1981) and of Paerl (1988), that some, but not all, dissolved organic substances stimulate growth in some algae. The present results indicate that urea acts adversely on *A. minimum* and *C. gracile*, but that 1 $\mu$mol.l$^{-1}$ is beneficial for *G.* cf. *nagasakiense.* The medium with 200 $\mu$l.l$^{-1}$ faeces elutriate added contained 0.9 $\mu$mol.l$^{-1}$ urea and showed a similar effect. According to Roon and Levenberg (1968, cited in Bonin and Maestrini, 1981), however, urea can represent a significant source of nitrogen for phytoplankton species containing urease or ureamidolase.

When antibiotics are contained in fish feed, uneaten feed or fish faeces can result in a positive relationship between the concentrations of antibiotic in the medium and of added food (Kupa Hansen *et al.*, 1991). Paerl (1988) showed growth by heterotrophic bacteria to be increased by concentrated organic matter, leading to increases in the concentrations of substances assimilable by phytoplankton. On the other hand, if an antibiotic was also present which acted deleteriously on the algae, any such stimulation might then be repressed. Our cultures were not axenic, so the present results concerning *C. gracile* and *G.* cf. *nagasakiense*, could be the result of such a complex effect. The relative absence of effect by the antibiotic on growth in

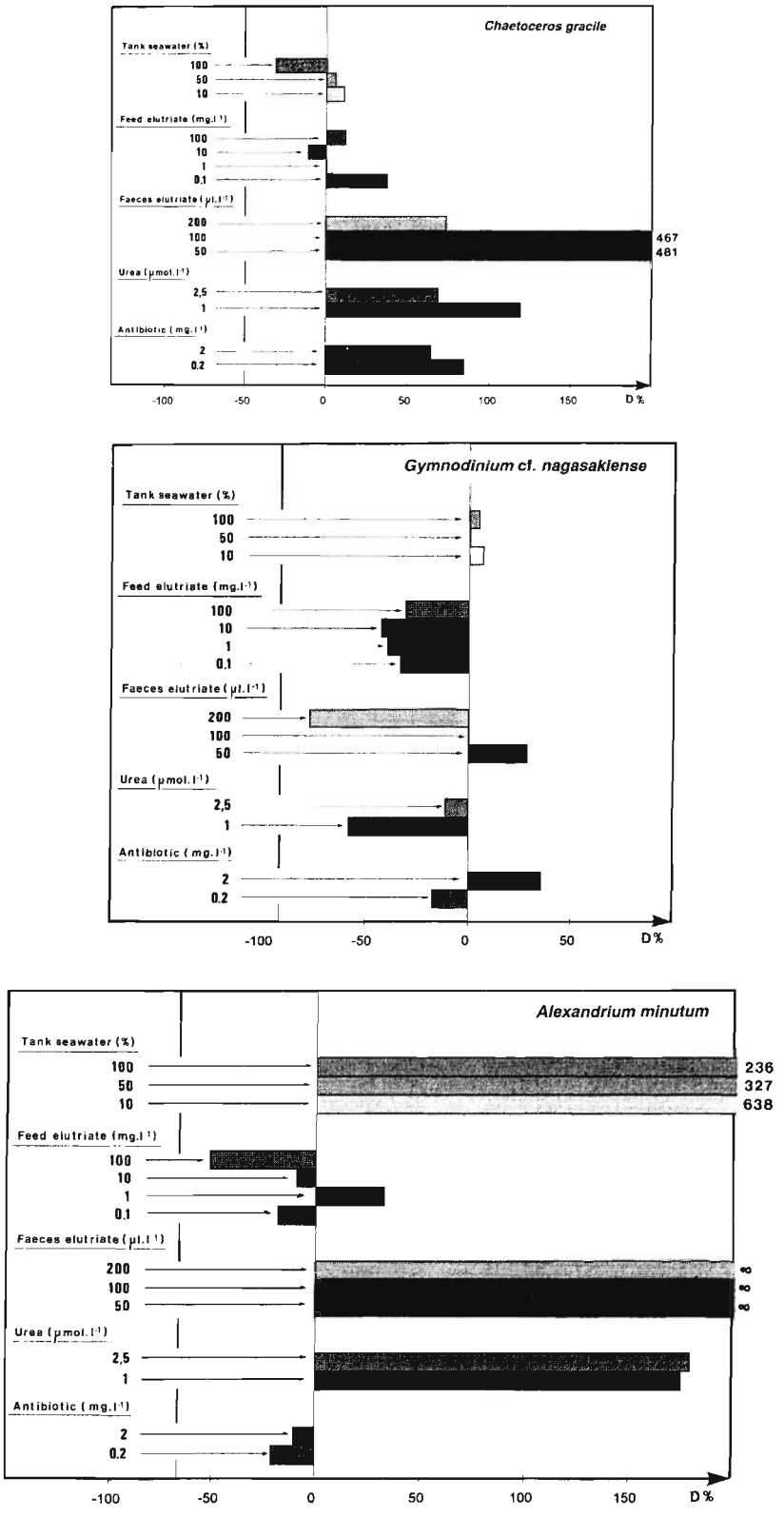


**Figure 1.** – Comparison of doubling time D (%) in each experiment for *Chaetoceros gracile*, *Gymnodinium* cf. *nagasakiense* and *Alexandrium minutum*.

*A. minimum* may reflect low sensitivity of this species to the products of bacterial degradation.

Growth in *C. gracile* was more often inhibited than stimulated (*fig.* 1a). Faeces elutriates and urea showed repressive action as did the antibiotic and so did feed elutriate at low concentration.

In *G.* cf. *nagasakiense,* over the wide range of concentrations tested, growth was stimulated to a similar degree by different concentrations feed elutriates (*fig.* 1b). These results are consistent with the observations of Takahashi and Fukazawa (1982) showing the stimulation of *Gymnodinium* sp. growth with micro-nutrients. Faeces elutriates inhibited growth when concentration was low but strongly stimulated it when high.

*A. minutum* was strongly inhibited by the products of fish culture. Even 10% of water from the turbot tank slowed growth six-fold, and faeces elutriates stopped growth and were probably lethal, suggesting an additional toxic effect (*fig.* 1c). Paerl (1988) also found that ammonium, as well as certain specific amino acids and metal chelators can suppress growth in *A. minutum*. Feed elutriate was also inhibitory, except at high concentration (100 $mg.l^{-1}$). Urea also appeared inhibitory.

Apart from possibly controlling cell size, composition or concentration when the stationary phase is reached, different substances, which, under fish-farm conditions, would represent products of feed and faeces addition, either stimulate or inhibit cell growth (Nishimura, 1982; Takahashi and Fukazawa, 1982). Particularly as natural inputs of nutrients even when of small intensity, may represent a long-term enrichment for primary production (Ross *et al.,* 1993) then either faeces or feed addition over a long period, even if at a low rate, may perturb differential phytoplankton growth rates relative to those in the natural environment.

## CONCLUSION

As well as an enrichment of phytoplankton growth, fish farming is likely to perturb both growth rates and hence species composition in the phytoplankton. In spite of the enrichment-limited and static experimental condition, these experiments suggest that phytoplankton growth may be selectively stimulated by substances released by fishfarms. Drift towards fewer, or only a single species, is likely to be one of the consequences which would also favour the likelihood of bloom formation (Takahashi and Fukazawa, 1982).

This work *in vitro* has substantiated the findings of Oviatt *et al.* (1986) that phytoplankton may be regulated by one or more limiting factors, particularly under the conditions of high enrichment. This may explain the frequent appearances of *Gymnodinium* cf. *nagasakiense* in regions of high-density fish farmin (Jones *et al.,* 1982; Mahoney *et al.,* 1990; Arzul *et al.* 1994). Different results may furthermore be expected with different phytoplankton species (Black *et al.,* 1991), or in environments where different activities, such as mussel or oyster-culture, take place. Research is required to elucidate the impacts of different aquaculture activities on different phytoplankton species.

**Acknowledgements**

We are grateful to A. Dosdat and R. Metayer from the DRV section of IFREMER, for providing the material and information about aquaculture, M. P. Crassous and A. Youenou from the DEL-Laboratoire Ecologie pélagique of IFREMER, Ph. Bassoulet of the DEL-Laboratoire de Chimie des Contaminants of IFREMER for their technical assistance. The authors would like to thank I. Jenkinson for his helpful comments.

## REFERENCES

Aminot A., R. Kerouel 1982. Dosage automatique de l'urée dans l'eau de mer : une méthode très sensible à la diacétylmonoxime. *Can. J. Fish. Aquat. Sci.* **39**, 174-183.

Armstrong F. A., J. Tibbiths 1968. Photochemical combustion of organic matter in seawater for nitrogen, phosphorus and carbon determination. *J. Mar. Biol. Assoc. U.K.* **48**, 143-152.

Arzul G., G. Bodennec, E. Erard-Le Denn, P. Gentien 1994. Fish kills and *Gymnodinium* cf. *nagasakiense* in Corsica (France). *Harmful Algae News* **8**, 7.

Berland B. R., D. J. Bonin, S. Y. Maestrini, M. L. Lizarraga-Partida, N. J. Antia 1976. The nitrogen concentration requirement of D-glucosamine for supporting effective growth of marine microalgae. *J. Mar. Biol. Assoc. U.K.* **56**, 629-637.

Black E. A., R. J. Gowen 1988. Algae: a problem for fish farmers. Aquaculture International Congress and Exposition, Vancouver Trade and convention Centre,

Vancouver, British Columbia, Canada, Sept. 6-9, 1988. Proc. Aquacult. Int. Congr. p. 54.

Black E. A., J. N. C. Whyte, J. W. Bagshaw, N. G. Ginther 1991. The effect of *Heterosigma akashiwo* on juvenile *Oncorhynchus tshawytscha* and its implication for fish culture. *J. Appl. Ichthyol.* **7**, 168-175.

Bonin D. J., S. Maestrini 1981. Importance of organic nutrients for phytoplankton growth in natural environments: implication for algal species succession. *Can. Bull. Fish. Aquat. Sci.* **210**, 279-291.

Brown J. R., R. J. Gowen, D. S. McLusky 1987. The effect of salmon farming on the benthos of a Scottish sea loch. *J. Exp. Mar. Biol. Ecol.* **109**, 39-51.

Erard-Le Denn E. 1995. Resting cysts of *Alexandrium minutum* in marine sediments: quantification by three methods. In: Harmful Marine Algal Blooms. Lassus P. *et al.*, Eds. Lavoisier, 725-730.

Fenchel T. M., R. J. Riedl 1970. The sulfide system: a new biotic community underneath the oxidized layer of marine sand bottoms. *Mar. Biol.* **7**, 255-268.

Flynn K. J., 1990. Composition of intracellular and extracellular pools of amino acids, and amino acid utilization of microalgae of different sizes. *J. Exp. Mar. Biol. Ecol.* **139**, 151-166.

Fukazawa N., T. Ishimaru, M. Takahashi, Y. Fujita 1980. A mechanism of "red tide" formation. 1. Growth rate estimate by DCMU induced fluorescence increase. *Mar. Ecol. Prog. Ser.* **3**, 217-222.

Gentien P., G. Arzul 1990. A theoretical case of competition based on the ectocrine production by *G.* cf. *aureolum.* In: Toxic Marine Phytoplankton, Granéli E. *et al.*, Eds. Elsevier, 161-164.

Gowen R. J., N. B. Bradbury 1987. The ecological impact of salmonid farming in coastal waters: a review. *Oceanogr. Mar. Biol. Ann. Rev.* **25**, 563-575.

Gowen R. J., H. Rosenthal, T. Mackinen, I. Ezzi 1990. Environmental impact of aquaculture activities. Depauw N., Billars R. Eds., Spec. Publ. Eur. Aquacult. Soc. **12**, 257-283.

Guillard R. R. L., J. H. Ryther 1962. Studies of marine planktonic diatoms. 1: *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gram. *Can. J. Microbiol.* **8**, 229-239.

Johnsen R. I., O. Grahl-Nielsen, B. T. Lunestad 1993. Environmental distribution of organic waste from a marine fish farm. *Aquaculture* **118**, 229-244.

Jones K. J., P. Ayres, A. M. Bullock, R. J. Roberts, P. Tett 1982. A red tide of *Gyrodinium aureolum* in sea lochs of the first of Clyde and associated mortalities of pond-reared salmon. *J. Mar. Biol. Assoc. U.K.* **62**, 771-782.

Kupka Hansen P., B. T. Lunestad, O. B. Samuelsen 1991. Environmental effects of antibiotics/chemotherapeutics from aquaculture. In: Aquaculture and the environment. Depauw N., Joyce J., Eds. Aquaculture Europe 1991, Dublin 10-12 June 1991. Spec. Publ. Eur. Aquacult. Soc., 176-179.

Mahoney J. B., P. Olsen, M. Cohn, 1990. Blooms of a Dinoflagellate *Gymnodinium* cf. *aureolum* in New Jersey Coastal waters and their occurrence and effects worldwide. *J. Coast. Res.* **6**, 121-135.

Nakamura Y., J. Takashima, M. Watanabe 1988. Chemical environment for red tides due to *Chattonella antigua* in the Seto Irland Sea, Japan. Part 1. Growth bioassay of the seawater and dependance of growth rate on nutrient concentrations. *J. Oceanogr. Soc. Jap.* **44**, 113-124.

Nishimura A. 1982. Effects of organic matters, produced in fish farms on the growth of red tide algae *Gymnodinium* type-65 and *Chattonella antigua. Bull. Plankton Soc. Jap.* **29**, 1-7.

Oviatt O., A. A. Keller, P. A. Sampou, L. L. Beatty 1986. Patterns of productivity during eutrophication: a mesocosm experiment. *Mar. Ecol. Prog. Ser.* **28**, 69-80.

Partensky F., P. Gentien, A. Sournia 1991. *Gymnodinium* cf. *nagasakiense*: *Gyrodinium* cf. *aureolum.* In: Le phytoplancton nuisible des côtes de France, Chap. 2, IFREMER-Brest, 63-83.

Pearl H. W. 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. *Limnol. Oceanogr.* **33**, 823-847.

Pearson T. M., R. Rosenberg 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr. Mar. Biol. Ann. Rev.*, **16**, 229-311.

Quéguiner B., M. Hafsaoui, P. Tréguer 1986. Simultaneous uptake of ammonium and nitrate by phytoplankton in coastal ecosystems. *Estuar. Coast. Shelf. Sci.* **23**, 751-757.

Roon R. J., B. Levenberg 1968. An adenosine triphosphate dependant, avidin-sensitive enzymatic cleavage of urea in yeast and green algae. *J. Biol. Chem.* **19**, 5213-5215.

Ross A. H., W. S. C. Gurney, M. R. Heath 1993. Ecosystems models of Scottish sea lochs for assessing the impact of nutrient enrichment. *J. Mar. Sci.* **50**, 359-367.

Samuelsen O. B. 1989. Degradation of oxytetracycline in seawater at two different temperatures and light intensities, and the persistence of oxytetracycline in the sediment from a fish farm. *Aquaculture* **83**, 7-16.

Takahashi M., N. Fukazawa 1982. A mechanism of "red tide" formation. II. Effect of selective nutrient stimulation on the growth of different phytoplankton species in natural water. *Mar. Biol.* **70**, 267-273.

Tréguer P., P. Le Corre 1976. Study of dissolved organic matter and nutrients in seawater. Chemical characteristics of the Biscay bay and of the upwellings in the northwest African area. Thèse dr. Univ. Brest, n° 36-37, 490 p.