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Preservation of Marine Environment in the South of Latin America Aquaculture extension and phytoplankton development

INTERNATIONAL WORKSHOP

Preservation of marine environment in the South of Latin America : Aquaculture extension and phytoplankton development

Financial support :

ECONOMICAL EUROPEAN COMMUNITY
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8 & 9 April 1999, Puerto Montt, Chile

Organization :

Instituto Tecnológico del Salmón, S.A. (**INTESAL S.A.**) Chile
Institut Français pour l'Exploitation de la Mer (**IFREMER**) France
Instituto de Fomento Pesquero (**IFOP**) Chile

PREFACE

The workshop presents the synthesis of results obtained in the project « *Marine phytoplankton growth and animal excretion* », Action n° C96U03 sustained by both co-operation committees : ECOS in France and CONICYT in Chile, and the preliminary results obtained during the first year of the project entitled : « *Aquaculture management and ecological interaction of noxious phytoplankton developments in the south of Latin America* », contract n° IC18-CT97-0157, with the financial support of EEC.

Both projects have a common objective : to elucidate the importance of aquaculture impact on environment, and contribute to a best understanding of the interactions, in view of a possible prevention from irreversible umbalance in the phytoplanktonic populations.

Considering the probable impact of different aquacultural activities on environment, and more precisely noxious phytoplankton species with feed back consequences, we intend a study of a concerned area according to several compartments : benthos, water masses, seston and dissolved substances especially provided by animal excretions. The particularity of extreme South America is taken into account with respect to the consequences of UV-b irradiation on dissolved organic substances and the development of a PSP producer : *Alexandrium catenella*.

ORGANIZING COMMITTEE

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- Geneviève Arzul,
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Acknowledgments

We are grateful to A.L. Le Velly for page setting and P. Bodénès for cover composition.

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Geneviève Arzul (IFREMER, France), Coordinator of the projects, and
Miriam Seguel (IFOP, Chile)

Abstract

The projects « Phytoplankton growth and dissolved substances provided by marine animal excretions » , and « Aquaculture management and ecological interactions of noxious phytoplankton developments in the south of Latin America : AQUATOXSAL » are complementary

The first presents as a fundamental investigation with aim to verify the influence of excretions from bivalves and fish, on the *in vitro* regulation of phytoplankton.

The second requires applied science, and considers the *in vivo* effect of aquaculture on marine environment, in the different aspects. Benthic and pelagic compartments are studied, and the consequences on noxious phytoplankton selection.

The management of both projects is due to ECOS (France) and CONICYT (Chile) Committees, and the European Community, DG XII, in the INCO-DC Program. In the case of AQUATOXSAL. This is an international project between Chile, Argentine, Germany and France : these countries have similar problems concerning environment and phytoplankton outbreaks, and a common interest in the investigations.

First Project :

Animal excretions and phytoplankton : works with the French-Chilean Cooperation, through the funding for missions. Duration : from January 1997 to December 1999 : 3 years. This seminar constitutes the closure of the project which concrete outcoming will be a published manuscript.

The participants are :
- IFREMER in France
- INTESAL in Chile
- IFOP in Chile.

The studies are based on bioassays in laboratory. The algae, as animals used for excretions, are selected according to the environmental observations in each country.

Second Project :

AQUATOXSAL : sustained by the European Community.
duration : from January 1998 to June 2001 : 42 months.

The participants are :

- IFREMER- Brest, France : coordinator
- IFM- Kiel, Germany
- INIDEP- Mar del Plata, Argentine,
- INTESAL- Puerto Montt, IFOP- Puerto Montt and IFOP- Punta Arenas, Chile.

The objectives of the study are :

- to know the relation between aquaculture and phytoplanktonic populations,
- to define the limit of exploitation in the fish farming areas,
- to evaluate the selective effect of UV radiations on the phytoplankton blooms.

These studies require the knowledge of the global effect of aquaculture on the environment : benthic and pelagic compartments.

Complementary investigations are conducted :

- bioassays with elutriates of various fish granules : an assay for selection of the less disturbing input, in phytoplankton growth,
- evaluation of the importance of the photomineralization of organic matter under UV-b radiation,
- evaluation of the effect of UV-b on the production of Paralytic Shellfish Poisoning (PSP) by the toxic dinoflagellate *Alexandrium minutum*.

The expected results :

- to prove animal excretions and human activities can be involved in the phytoplankton selection,
- identification of an environmental parameter reflecting the alteration stage of fish farm areas, for preservation of natural resources in relation with coastal marine exploitation.

Environmental characteristics and hydrological particularities of the fjords and channels ecosystems in South America

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Financial support : EEC

Abtract

This study is a part of the pluridisciplinary project sustained by the European Community, with participation of France, Germany, Argentine and Chile. This presentation is a synthesis of the results concerning oceanographic and environmental studies in the fish farms areas, obtained until this second year.

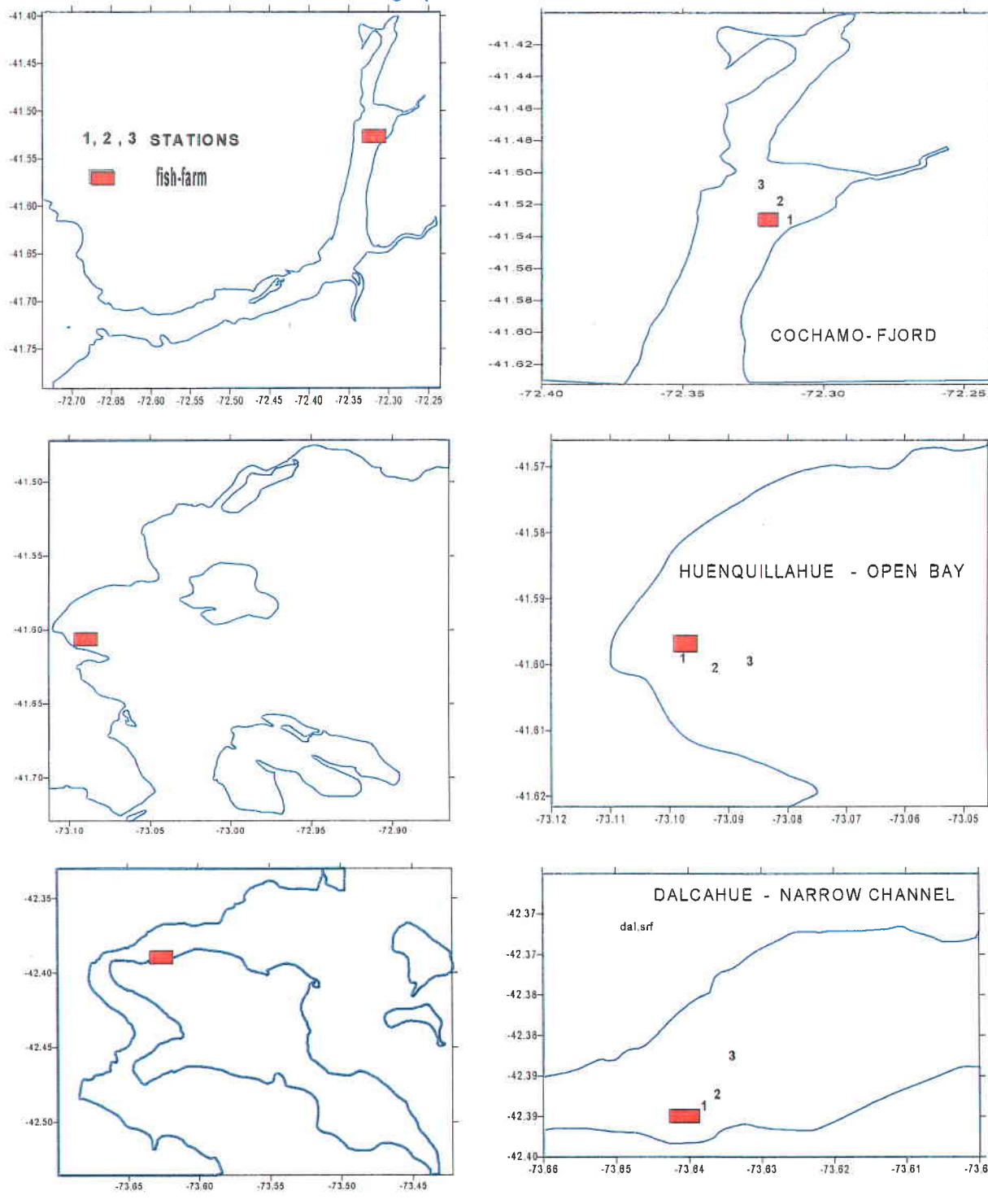
The fundamental objective at this stage is to generalise the methods for environment to the marine area. The specific objectives are to develop and apply mathematical models describing particles distribution around the fish farms.
Hydrology, sedimentation and spacial particles distributions, as nutrient analyses in the water column were studied. The nutrients : nitrite, nitrate, urea, ammonium, phosphate, silicate, Dissolved Organic Nitrogen (DON) and Dissolved Organic Phosphorus (DOP) are measured at several depths in different stations, including unimpacted stations (control).

Complementary information were obtained with total and organic seston charge of the samples.
Nitrite and nitrate did not constitute clear signals of disturbance in the water column in the fish farm areas. These measurements constitutes unnecessary expense.
Our preliminary results showed ammonium was obviously higher in the vicinity of fish farms than in control stations. So we propose to follow the spatial and temporal distribution of ammonium around the cages, taking into account the excretion rate and salmonid biomass.

Dissolved phosphate was not significatively different in the aquaculture sites, compared to control stations, and this was surprising, if we consider the published results. This can be due to the important dilution of the water masses by currents, and to other physical, chemical and biological processes. More, in this case it is well known the main phosphorus input constitutes sediment particles.

The seston charge was higher in fjords, probably due to continental water inputs.

Geographic locations of fish -farms



Figures 1 : Selected areas for the study

Selected fish farms :

The selection of areas was function of their oceanographic features.

In the Reloncavi Fjord, Cochamo site, the fish farm (Aguas Claras Company) is located in very stratified and deep waters, with salinity between 2 to 20 in the surface layer. The maximal depth in the fjord is around 300 m. The residual current is towards downstream for the superficial waters. As for the most part of the Chilean fjords, there is no sill in the head of the fjord, and this allows an adequate renewal of deep waters. *Skeletonema costatum* grows usually permanently in the surface layer and *Dinophysis acuta* used to grow and produce Diarrhetic Shellfish Poisoning (DSP), but since 1986 it has not been observed any more. In relation with fish farming in this fjord, there is at least 20 fish farms, with an annual production in the order of 10.000 ton. Many companies used this low salinity fjord has a smolts production farm, but for our experiment we choose an adult grow out trout farm.

In the Seno Reloncavi area, near Puerto Montt, the Huenquillahue fish farm belongs to Robinson Crusoe company (former Aquasur Fisheries). This area is a more open bay, with some effects of anthropogenic inputs from the Puerto Montt city and agriculture. It presents a medium stratified water column with salinity range around 25 to 32, temperatures around 8 and 19°C, and spring summer seasons with a 6 m pycnocline. The depth is about 30 to 40 m. The phytoplankton composition is constituted of different species of diatoms, dinoflagellates and nanoplankton. The annual production of this site is around 2000 ton of Atlantic Salmon.

Along the east side of the Chiloe Archipelago, the coast presents numerous island, channels, bays and inlets, which have been used for aquaculture, *i.e.,* fish farming and mollusk. The Dalcahue channel, was selected as the third site, which has a 20 m deep, with mixed waters due to tidal currents and lower freshwater inputs. The salinity is around 28 to 33 and temperatures from 9 to 14°C. The phytoplankton composition is mainly diatoms. The annual production of this site is around 2.200 ton of Atlantic Salmon.

The fish farms in Chile are usually many sets or modules, and each set has between 10 and 40 cages, therefore we specified the number of set per fish farm in table 1.

Fish farm	Oceanographic conditions	Number of platform	Biomass (ton) march	Salmonid Specie
Est.Reloncavi Cochamo	Stratified fjord	4 set	481	rainbow trout
Seno Reloncavi Huenquillahue	Open bay	2 set	861	atlantic salm.
Ca. Dalcahue San Javier	Well mixed channel	2 set	900	atlantic salm.

Table 1 : General Description of fish farm and biomass

Sampling

The water was sampled at discrete depths (see Table 2) in the 3 fish farms, selecting strong (C1, H1, D1), medium (C2, H2, D2) and unimpacted (C3, H3, D3) stations. In Dalcahue fish farm, as well mix water column was evident we sampled only 2 levels, instead of 3. Sampling was realised with Niskin bottles and duplicated.

At each fish farm and vicinity we sampled 3 stations, one as close as we can reach the cages with the vessel. C1 was 5 m approximately from the cages, C2 at 20 m from the cages and C3 more than 100 m from the cages. Usually we did not sampled in a fixed depth, as SLAPS data were available in real time. Therefore, we intended to sample when particles or other event were detected.

The nutrients samples were filtered immediately after sampling, stored in ice onboard (no more than 18 h) and frozen (-20°C) until chemical analyses in the laboratory of Universidad Austral de Chile, following the Strickland and Parsons protocol. The analysed dissolved parameters were : nitrite, nitrate, ammonia, urea, dissolved organic nitrogen (DON), phosphate, dissolved organic phosphate (DOP) and Silicate.

For the phytoplankton study, the seawater was collected at the depth specified in Table 2, and the samples were fixed with lugol solution.

Fish farms	Stations	Depths (m)	dates 1998
COCHAMO	C1	0, 6 , 9	March 5
	C2	0, 3.5, 9	March 5
	C3 (control)	0, 4, 10	March 5
HUENQUILLAHUE	H1	0, 5, 10	March 6
	H2	0, 8, 15	March 6
	H3 (control)	0, 8, 15	March 6
DALCAHUE	D1	0, 15	March 9
	D2	0, 15	March 9
	D3 (control)	0, 15	March 9

Table 2 : Sampling location and general specifications at the fish farm

Conclusions

The distribution of particles on the fish farms depends on the density field, fish farm biomass, distribution of sets or modules and evidently the currents, among others factors. This subject will be addressed by the French team.

The surface layer of the water column with important fresh water inputs has a lower proportion of organic seston, *i.e.,* had more abundant sediment than the bay and channel sites. Implications with fish farming, up to now have not been analyse more clearly during this period of the project.

Preliminary results within the EU- Project AQUATOXSAL

Effects of a Salmon Farm in the Dalcahue Channel (Isla Chiloe) on the Sediment Biogeochemistry and the Benthos
Marine video presentation

Levent Piker and Peter Krost, (IFM Kiel, Germany)

Financial support : EEC

Abstract

The first results reflect a hypertrophicated area beneath the study site, an extensive used fish farm producing about 2.000 tons of Atlantic Salmon per year.

Hydrography in the fish farm area showed a well mixed water column and an influenced oxygen profile by respiration of the salmons.

Current in the Dalcahue Channel was almost completely determined by tidal forces, i.e. flow-off (SW-direction) and inlet (NE-direction) of water masses. Mean current velocity was 2.86 cm s^{-1} with maximal values of 24.6 cm s^{-1}.

The biogeochemistry was characterised by steep vertical gradient of oxygen, sulfide and redox potential due to the organically enriched sediment. The sediment beneath the fish farm was almost completely anoxic and sulfidic showing oxygen penetration restricted to the upper 3 mm sediment.

Microbiologically mediated producing processes dominated the biogeochemistry in this sediment particularly the dissimilatory sulfate reduction, showing high rates of sulfide production (165 mmol m^{-2} d^{-1}).

The carbon and nitrogen content is influenced by the fish farm significantly. However, the sediment is only affected down at 10 cm depth and the impact is limited to a very restricted area, at least in a southern direction.

The sediment condition and the benthos in the surrounding of the fish farm could clearly be distinguished by UW-video in (1) a polluted area beneath the fish farm, (2) a transition zone and (3) an unpolluted area.

Selected fish farm characteristics

The fish farm chosen for detailed research belongs to the company "Multiexport Ltda". It is a salmon farm producing approximately 2000 t of salmon per year. The fishfarm itself consists of two flotillas, set 1 and set 2, each consisting of 20 cages, 10 on either side of a central gangway. Each cage is a cube of 15 m length, 15 m width and approx. 15 m depth, thus the total length of each set is - according to a width of about 1 m for the gangway between the cages - approx. 160 m while the total width is approx. 30 m. Water depth underneath the cages is 20 to 28 m.
As a protection against sea lions, which are very well capable to destroy the net when trying to bite into a salmon, the whole structure of each set is surrounded by a predator net with a mesh size of 10 cm. This predator net also covers the seafloor.

Scientific equipment

GPS - Navigation was performed by a "Garmin 45" GPS system. The accuracy was, due to an excellent reception of a 6 to 8 satellites, high, i.e. usually in the range of ± 30 m (69 ft).

An **echosounder** of the type eagle was used for the bathymetric survey.

Underwater Video was performed by an enclosed Sony handicam type TR 820, released from the surface and controlled by an external monitor.

Oxygen profiles in the water column were made by a WTW oxygen sensor (Oxi 192).

Temperature profiles in the water column were also made by the WTW oxygen sensor.

Salinity profiles in the water column were made by a WTW conductivity sensor (LF 191).
The water depth was measured by a pressure sensor.

Oxygen, Salinity, temperature and depth of the vertical profiles were continually recorded in a **data logger** type Squirrel (Grant).

Sulfide and oxygen microelectrodes (van Gemerden, University of Groningen, Netherlands), a pico-amperemeter and a mV-meter (Dr. Dasch Consulting GbR).
Pt-electrodes (Mettler-Toledo, Steinbach) and a pH/mV-meter (WTW, Weilheim) to determine **Eh and pH.**

Results

Bathymetry in the fish farm area is presented on figure 1. Depth beneath the cages is about 22-28 m.

The main results in the water column analyses are presented here.
The currents (fig. 2) vary according to the tide coefficient, the highest spring tide velocity was : 23 cm s^{-1}, and the lowest about 1 cm s^{-1}.

The temperature and salinity profiles showed an unstratified water column. In March, temperatures ranged between 15 °C in surface, declined to 13 °C in the upper 4 m, and stayed constant to the bottom. In July, they were about 11 °C. Salinity increased slightly with water depth from 31 to 32.

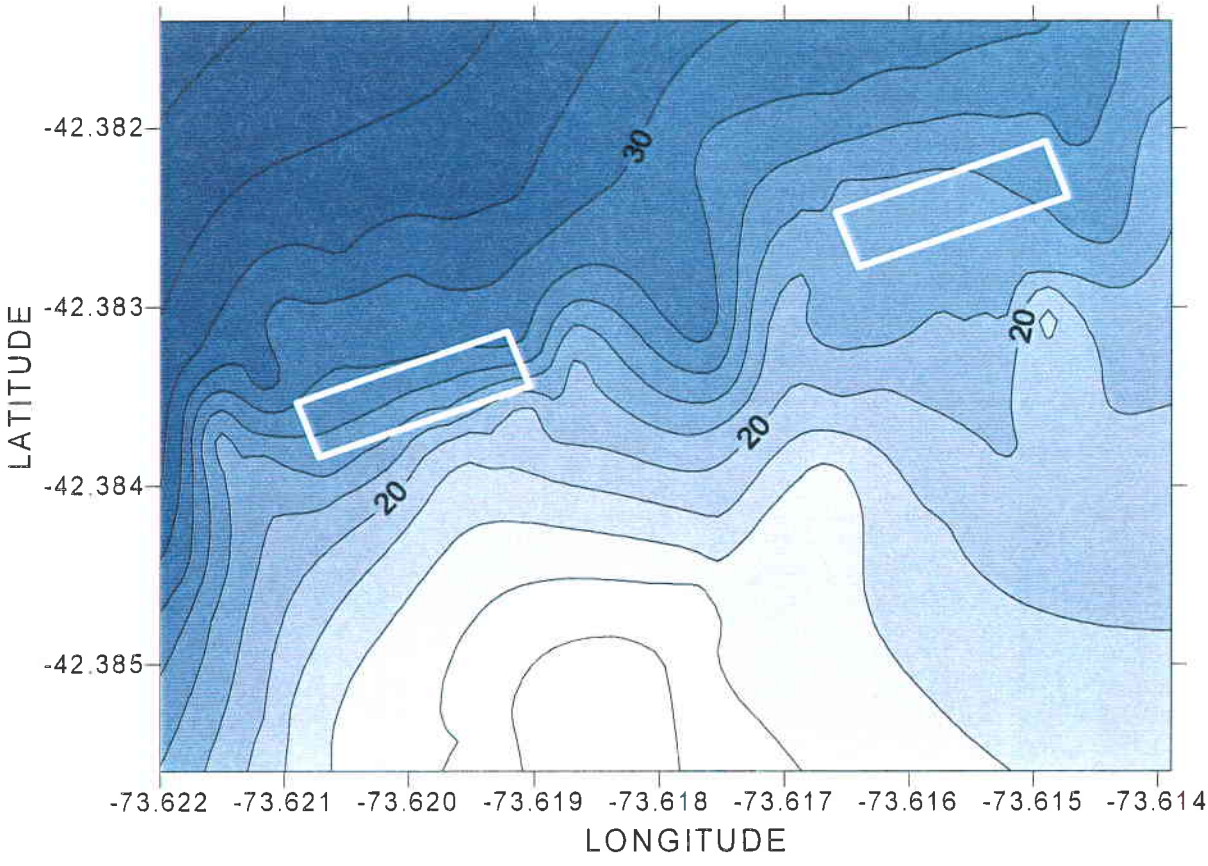


Figure 1 : Bottom topography underneath and around the sea cages of the Multiexport salmon farm set 1 (western) and set 2 (eastern).

Concentration of oxygen rised from surface to a water depth of 5 to 7 m, reaching a maximum by photosynthetic activity in this horizon as high as 3.5 to 3.7 mg dm^{-3}, followed by a decrease. The vertical profiles indicated 16 mg dm^{-3} in sub-surface, to 14 mg dm^{-3} in the bottom.
Physico-chemical measurements in the sediment were realised by diving, and cores were analysed in laboratory.

Redox potential decreased from 0 mV in the overlying water, to –250 mV in 1.75 cm depth, reflecting a narrow transit in zone from suboxic to anoxic conditions. PH dropped in the same horizon from 7.2 to 6.8 (Fig. 3).

Dissolved oxygen and total sulfide : vertical sediment profile obtained by a microsensor showed penetration of dissolved oxygen down to 2.5 mm sediment depth, reflecting highly active oxygen consuming processes (Fig. 4).
Total sulfide concentration in the porewater increased slightly in the upper 10 mm from 0.1 µM to 3.3 µM followed by an exponential rise up to 4 mM in a sediment depth of 4.5 cm.
CHN – analysis : considering the first results on sediment types and benthos zones in the fish farm surrounding it had been expected that the enriched sediments under the net cages should show enhanced carbon/nitrogen (C/N) ratios.

The results revealed contents of organic carbon (C_{org}) and total nitrogen (N) up to 20 times higher in the sediment beneath the fish farm than in the adjacent sediment (Fig. 5).

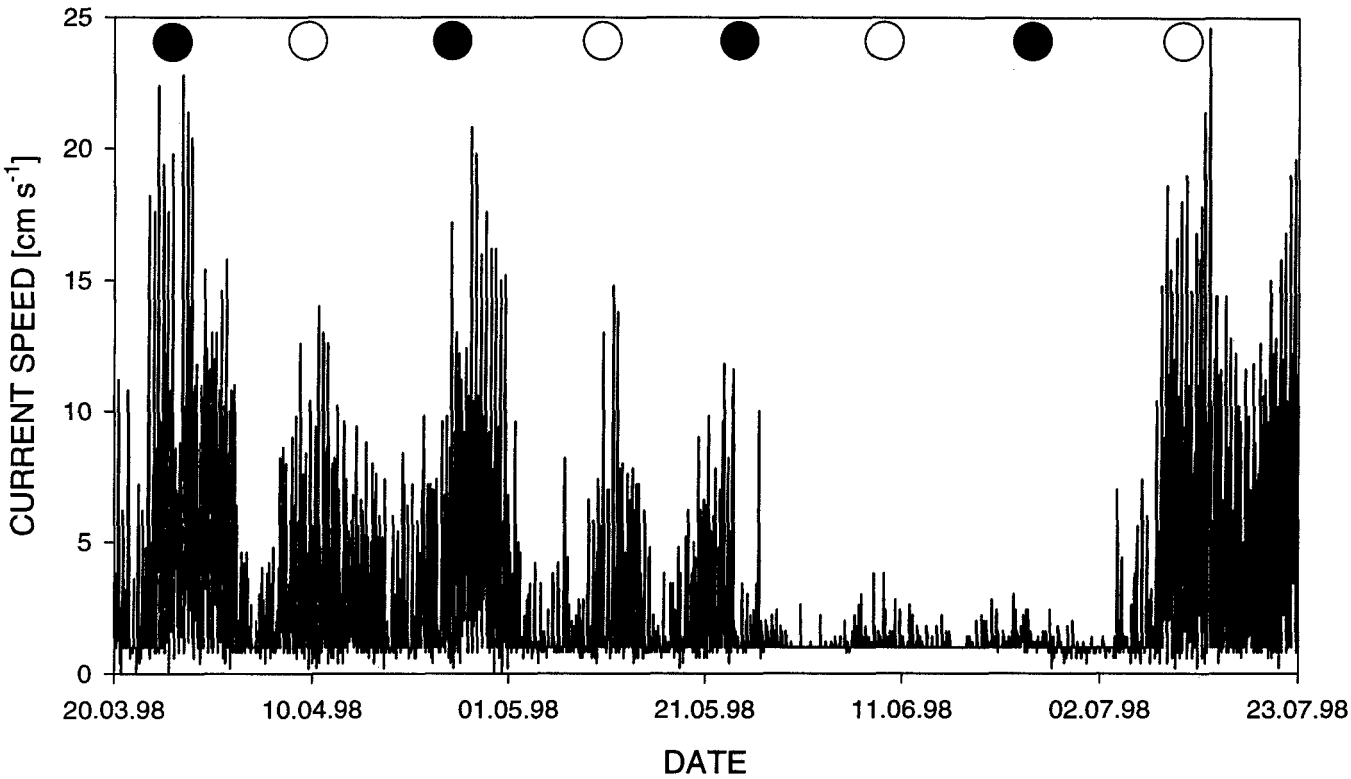


Figure 2 : Current speed during the whole period of first current measurements in 5 m water depth. Full moon and new moon (spring tides) are indicated by white and black circles.

Benthic status (underwater video)
Three zones with different benthic conditions could clearly be identified and documented by the use of underwater video :

A polluted area underneath the fish farm appeared almost completely azoic. The sediment is anoxic and sulfidic. Nevertheless, a considerable bioturbation takes place in this area caused by large number of the demersal fish (Blennidae), which probably destroy the bacterial mats and continuously remix the sediment surface in search of food.

Intermediate station with a large number of small snails (*Nassarius spp.*), equaling 22.400 individuals m^{-2} ; also the video showed extremely high densities of these predating and carrion eating animals. This area shows the appearance of white bacterial mats, possibly by the mat forming sulphide oxidizing bacteria of the genus *Beggiatoa* or copiotrophic bacteria (50 % of the surface).

The (probably) unpolluted situation which was found at the Control station.

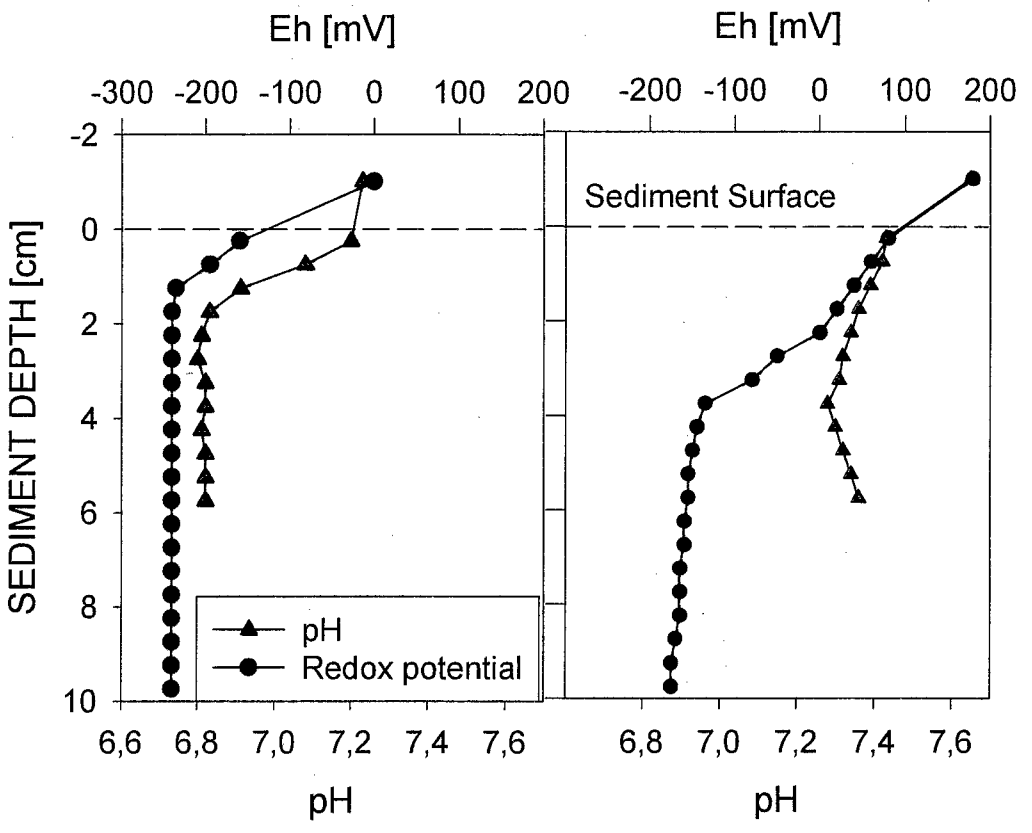


Figure 3 : Vertical profiles of redoxpotential and pH in the sediment beneath the fish farm (left) and at station 4.

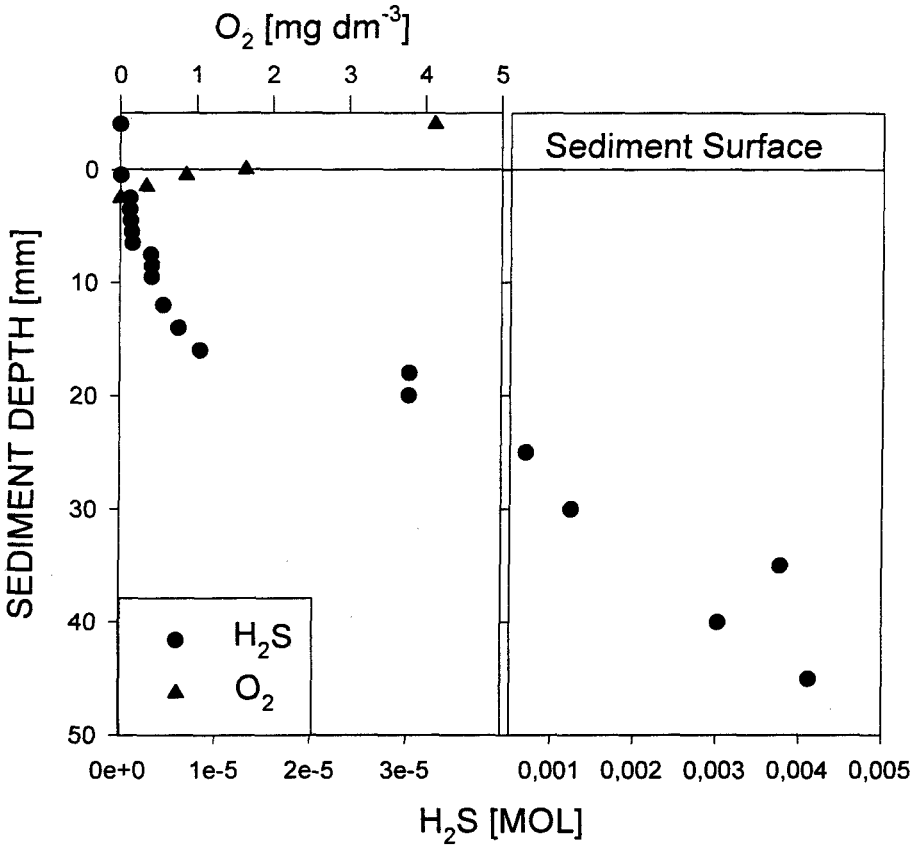


Figure 4 : Vertical sediment profiles of dissolved O_2 and H_2S.

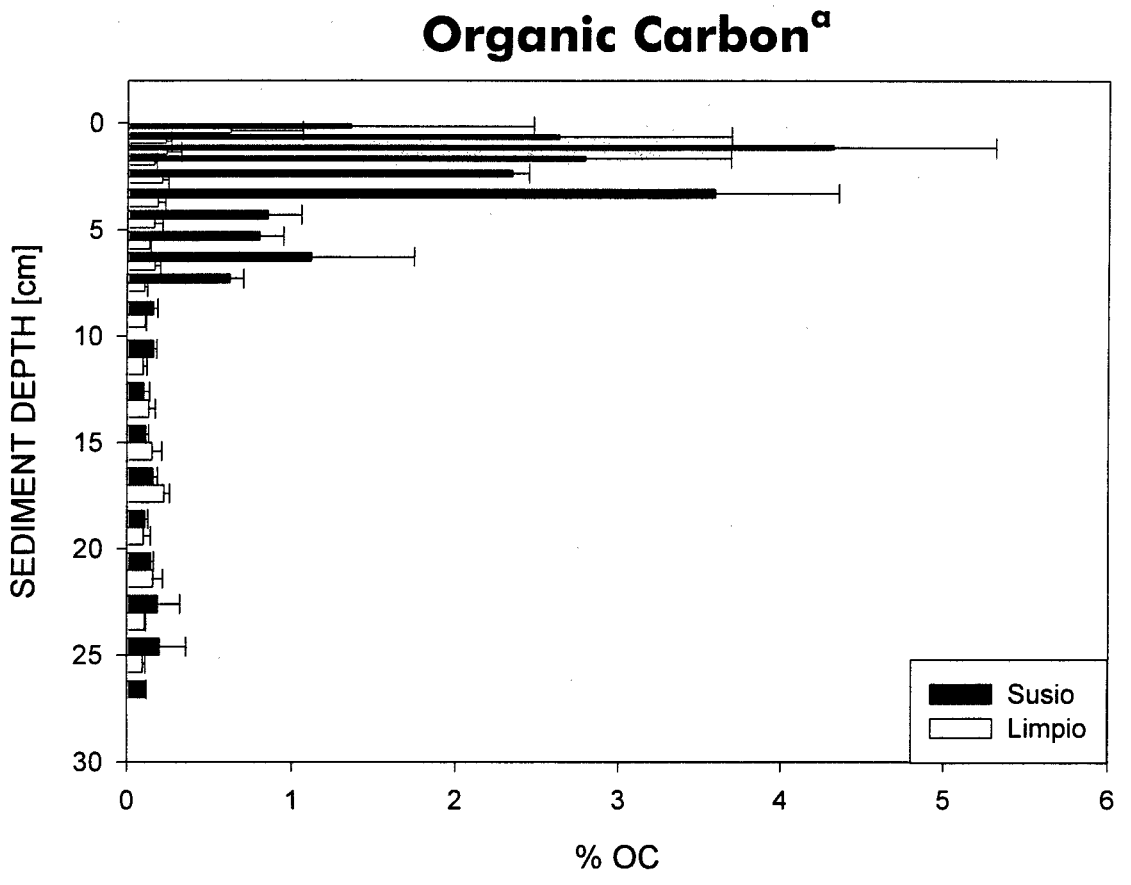


Figure 6 : Vertical profile of nitrogen and carbon contents in the sediment beneath the fish farm ("susio" : black bars) and in an adjacent sediment ("limpio" : white bars).

Conclusion

Fish farming activity produces modifications in the benthic sector immediately beneath the cages, but after intermediate status, the recover in quality is rapidly observed, geographically, in the surroundings. The recover in time is longer and necessitates optimal conditions in current velocity, cages disposition. Disturbance in physico-chemical qualities of sediment are higher in areas supporting long time exploitation. These conditions will be considered in discussion.

Sestonic disturbance in the surroundings of fish farms

Michel Lunven, Patrick Gentien (IFREMER, France) and Alejandro Clément

Financial support : EEC

Abstract

The pelagic compartment study focussed the particles charge in three fish farms located in different hydrological situations : homogene (channel), medium-stratified (bay) and marked-stratified (fjord) water column.

The use of the particle size analyser, with additional probes for salinity, temperature, density and chlorophyll (fluorescence) measurements, gave results in real time and, owing to water sampling for calibrations, profiles and hydrological parameters mapping were realised. Sestonic charge was relatively low, about 3.5 mg l^{-1}.

The particles distribution was very dependant on the current and pycnocline, completely located in the upper layer (5-6m) in the case of the fjord. Seston from the fish farm presented a predominance of large particles (< 210 µm) and high organic proportion. This composition influenced the sedimentation rate results.

Fluorescent particles were mainly 10 µm-sized in unimpacted areas, and this relationship was not observed in fish farms vicinity.

Material and methods

The selected fish farms were located in sites with different hydrological characteristics. Figure 1 presents the Cochamo fish farm in the Reloncavi Fjord, with deep and stratified water. Huenquillahue plant is located in the Puerto Montt Bay, medium stratified, and Dalcahue fish farm is located in a 30 m-deep channel on the east side of Chiloe Island.

Figure 2 presents the equipment for particles detection and analyses : the underwater particle size analyser Cilas 925, developed by Cilas and IFREMER under Euromer. This analyser measures particles *in situ* with real time size characterisation of particles suspended in the water, using laser diffraction. Suspended particles pass through an incident laser beam. A diffraction pattern is produced and analysed by the instrument. The particles remain in the media, thus allowing the utmost accuracy in particle characterisation.

Additional measurements were performed by probes. Data registered and transmitted continuously to the deck on the boat, were immediately stored and displayed (computer and corresponding equipment). The parameters such obtained were : density and size of particles, chlorophyll fluorescence of vegetal particles, conductivity (giving salinity), temperature, pressure (giving depth), and light measurement.

Seawater were sampled with Niskin bottles, according to the characteristics of the situation : structure of the water column, turbidity. Discrete samples allowed calibration of the probes, specific analyses of phytoplankton, and chemical analyses following standard colorimetric methods. Sampling was performed all around the cages, at two depths and at few metres from the farm.

Results

The hydrological structure of the studied areas are presented on figure 3. As can be observed, Cochamo site stratification is mainly due to the low salinity in the upper layer. Salinity variations are detected at 0- 10 m, and temperature variations at 0-20 m. The stratification in Huenquillahue is observable around 15 m depth, and Dalcahue vertical homogeneity is remarkable.

Cochamo fish farm

The study of the fjord shows strong gradients. On figure 4 the longitudinal variations in surface temperature and salinity reveal the influence of continental spring water input. Desaulted and cold waters correspond to low fluorescence of particles, in the vicinity of the main continental water input, and to the higher particles total load. The influence of the fish farm can be detected by the higher particles load.

Figure 5 presents the vertical stratification of the water in the fjord : high temperatures and lower salinity in surface, associated with fluorescence and total particles load. The pycnocline above 10 m, constitutes a barrier for particles diffusion.

Figure 6 summarises the vertical variations in these parameters, in a control station and in the fish farm (impacted station). The influence of fish farming is detectable on the vertical variations of temperature, which present irregularities near the bottom of the cages (15 m). In the fish farm, fluorescence of particles load is also modified at this depth : peaks in fluorescence are blunted, and particles abundance forming two peaks, is higher than in control station. According to Alejandro Clement observations, the upper peak in fluorescence is due to diatom cells, and the deeper to dinoflagellates cells.

The size of particles distribution along a transect in the vicinity of the fish farm is presented on figure 7. The 10-70 µm particles are abundant beneath the cages. The 70-210 µm particles are detected under the pycnocline, mainly under the fish farm area. The particles larger than 210 µm are distributed both in the deeper layers near the bottom, and in the superficial layers mainly near the cages.

Dalcahue fish farm

Figure 8 summarises the surface data. The main gradient is longitudinal, and the influence of the fish farm is detected by lower fluorescence and higher particles quantities.

The vertical profiles presented on figure 9, show an impact of the fish farm in a way similar to Cochamo : the fluorescent peak is blunted and particles load increases near the cages.

Figure 10 shows the vertical section near the Dalcahue fish farm, along a longitudinal transect from the cages. The role of current is conclusive in the structure of water masses. The influence of the fish farm appears for results in fluorescence, higher in the water before passing through the cages, and the particles more abundant after. The particles load is high in the bottom layer under and in the vicinity of the fish farm, in relation with bioturbation.

The size of particles are presented on figure 11. The particles larger than 210 µm are predominant, and located under the cages.

The comparison of particles size and their fluorescence was studied, and a relationship can be obtained between load in particles smaller than 10 µm and fluorescence intensity. The relationship is obvious in general data at the control station, and disappears completely in the impacted stations. It is interesting to observe the recover of the correlation 70 m away from the fish farm, corresponding to the unimpacted zone.

The sedimentation experiment realised in Dalcahue channel revealed the particular behaviour of the particles coming from fish farms.

Fig 12a presents the particle size distribution underneath the cage in Dalcahue area : a typical spectra with high organic content. Large size particles are mainly due to large aggregates. This sample was enclosed in the measurement cell of the grain size analyser.

Fig 12b shows the sedimentation time series for the small size particles which sediment regularly. Fig 12c shows the sedimentation times series for large size particles. Despite the oscillation in the signal which is not explained, there is not apparent sedimentation of the large size particles. This confirms large size particles have low density and are organic aggregates.

Relative sedimentation rates have been calculated by regression on the linear part of each curve. The results are plotted on fig 12d and we can observe a difference between some classes. Particles between 15-30 µm seems to be denser than the 30-36 µm class.

Conclusion

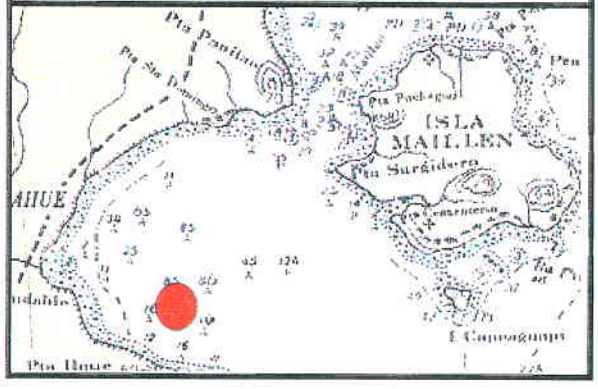
The results indicate :

1. Particle amounts were relatively low at the period of the mission, compared to estuarian results obtained in other works. The maximal sestonic charge measured during the mission was 3.5 $mg.l^{-1}$.
2. Particles were more abundant around and under the cages, and this indicated their origin.
3. Their diffusion depends on the hydrological and dynamical characteristics of the water mass. Stratification constituted an efficient barrier to vertical diffusion, *i.e.* sedimentation (excepted in fjord Reloncavi for large particles), while currents ensure the lateral transport.
4. The fluorescence property of the lower-sized particles (<10 µm) revealed they are chlorophyllian cells. High amounts of chlorophyllian particles were detected in the vicinity of the picnoclyne (Cochamo), in distant accumulation areas (Huenquillahue) or around the cages (Dalcahue).
5. The study of sedimentation rates and measures of organic content of seston revealed the predominance of organic compound, in accordance with their waste origin.

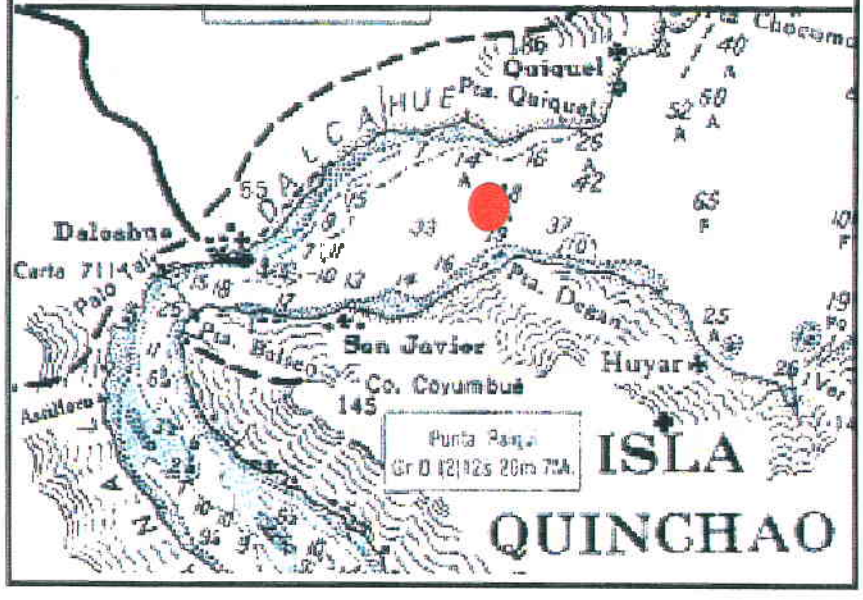
These main observations lead to consider, even in low sestonic areas, the importance of accumulation areas, as they constitute an obstacle to dispersion of detritical particles. Degradation of dense substances by chemical (oxidation, photodegradation) or biological (bacterial, zooplanktonic) processes, provides nutrient substrate for the chlorophyllian production. The fluorescence-particle size relationship could constitute an interesting parameter for indication of impacted area and perhaps chlorophyllian genus.

3 STUDIED AREA

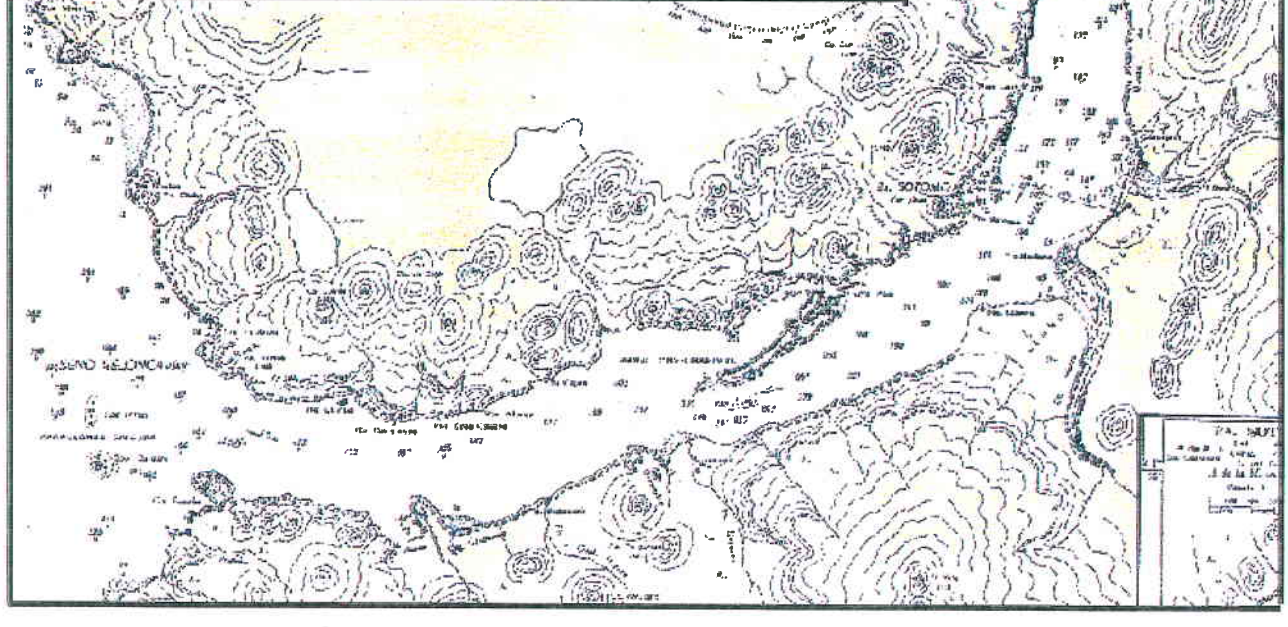
HUENQUILLAHUE FISH FARM



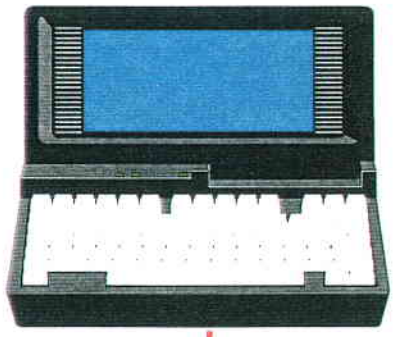
DALCAHUE FISH FARM



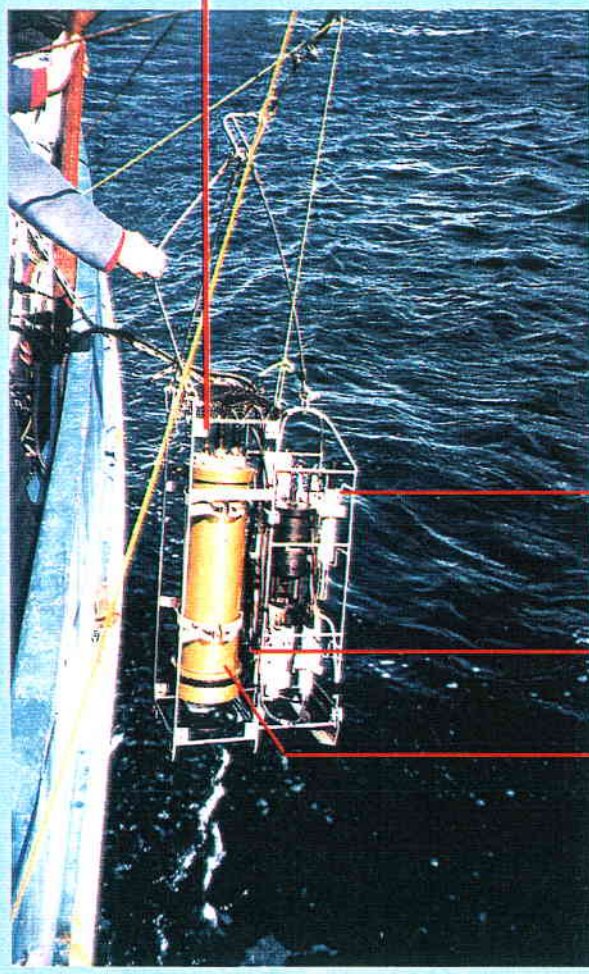
COCHAMO FISH FARM

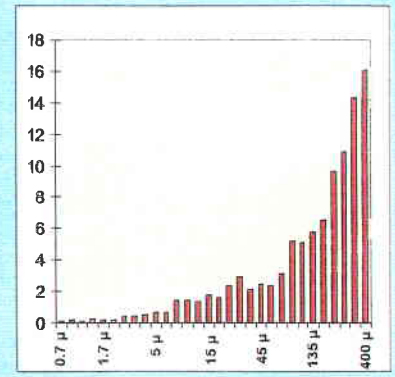


Granulometric Profiler



Real Time Data Acquisition of :
- Depth
- Temperature
- Salinity
- Light Intensity
- Fluorescence
- Particle Load
- Size distribution of particles
  30 classes 0.7 - 400 µm
  (@ 2 seconds)





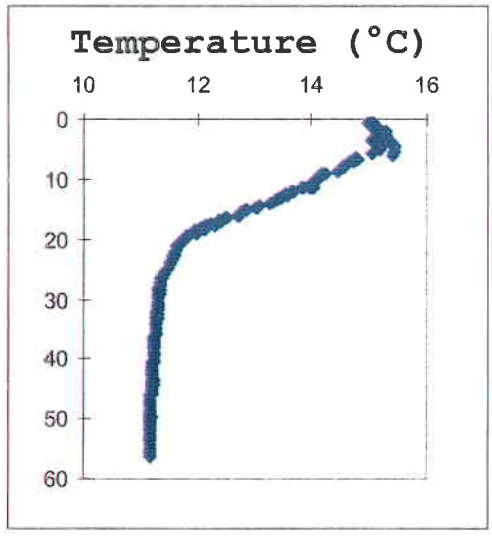
SBE 25 CTD probe

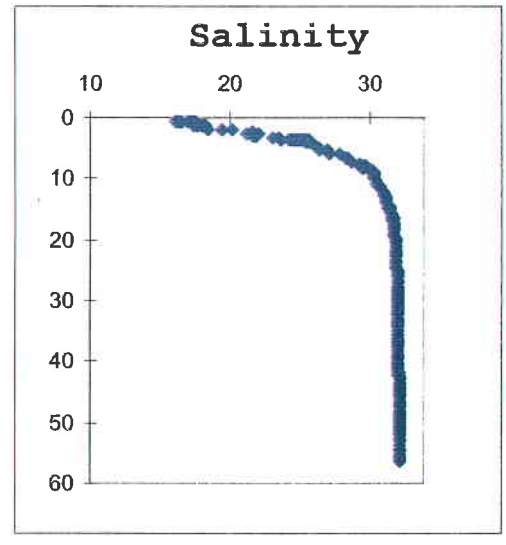
Particle Size Analyzer

Measurement Cell

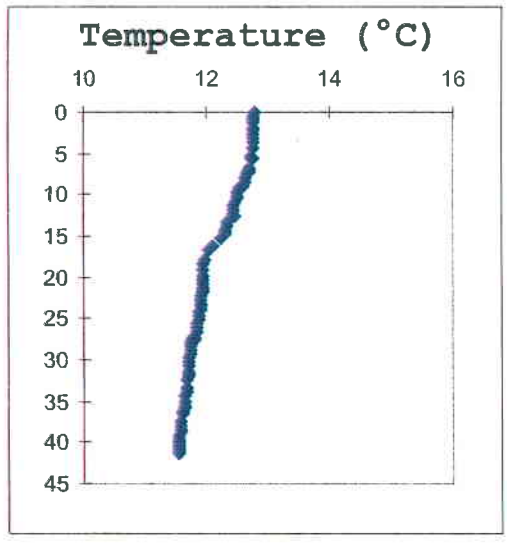
Temperature and Salinity profiles in the three control stations

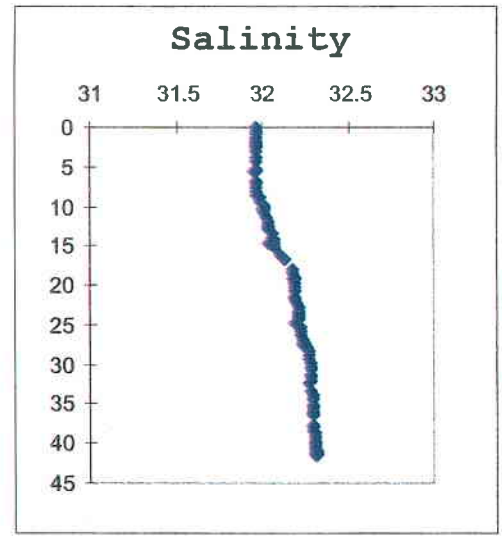
Cochamo



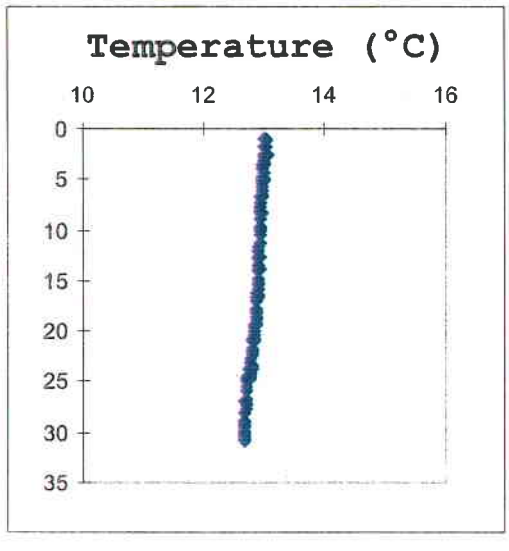


Huenquillawe





Dalcahue



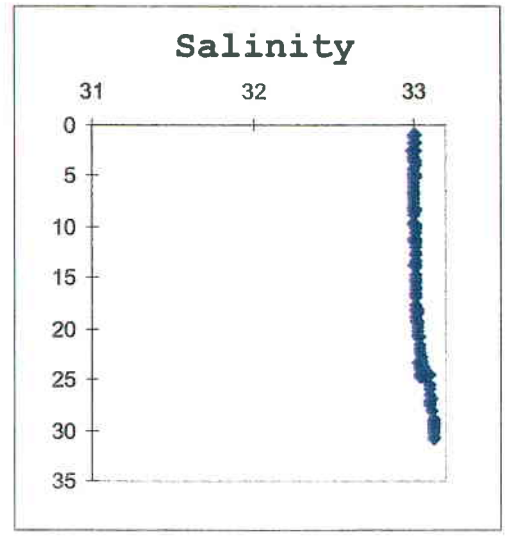


Figure 4. Surface distribution in Reloncavi fjord and Cochamo farm

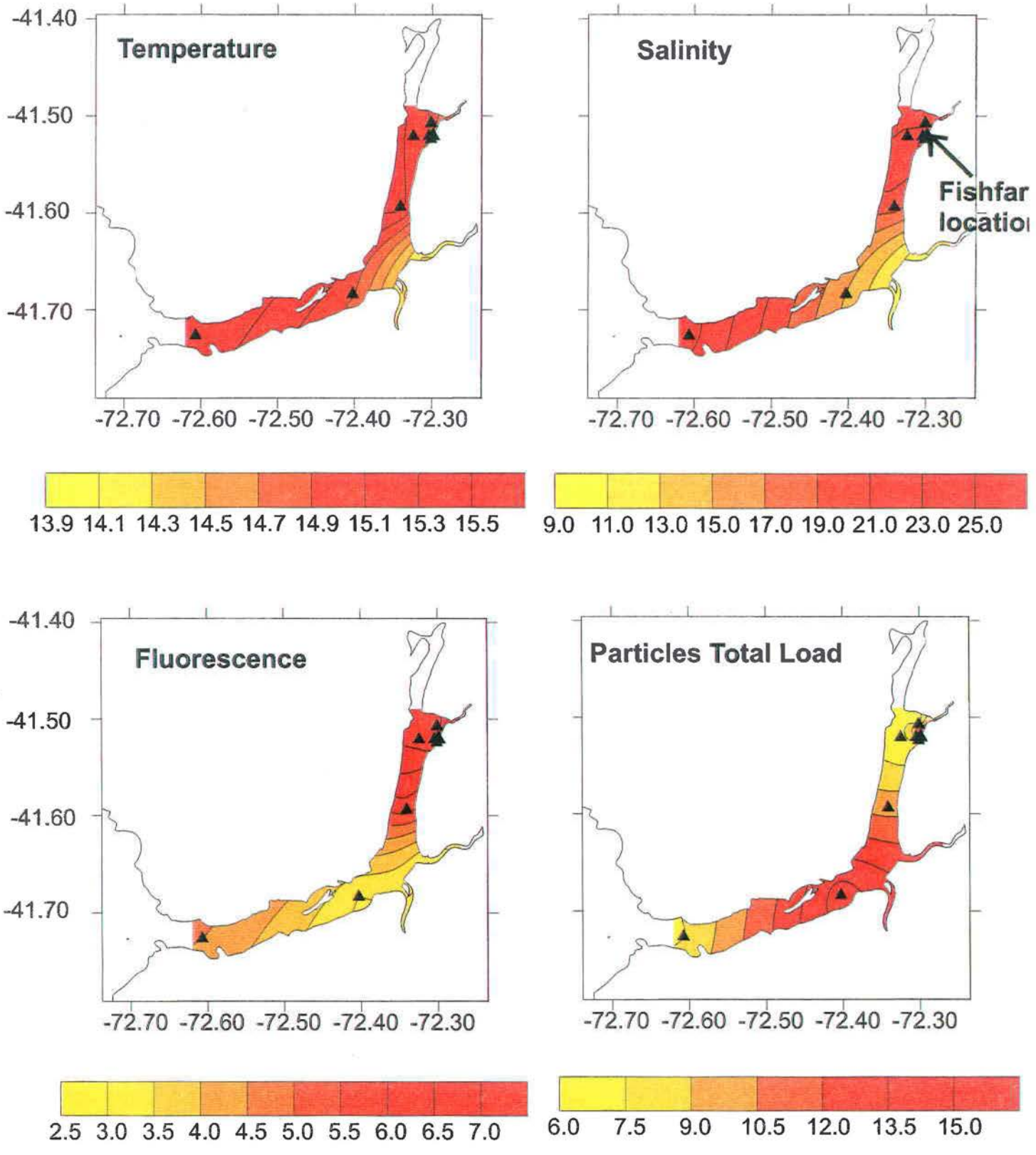
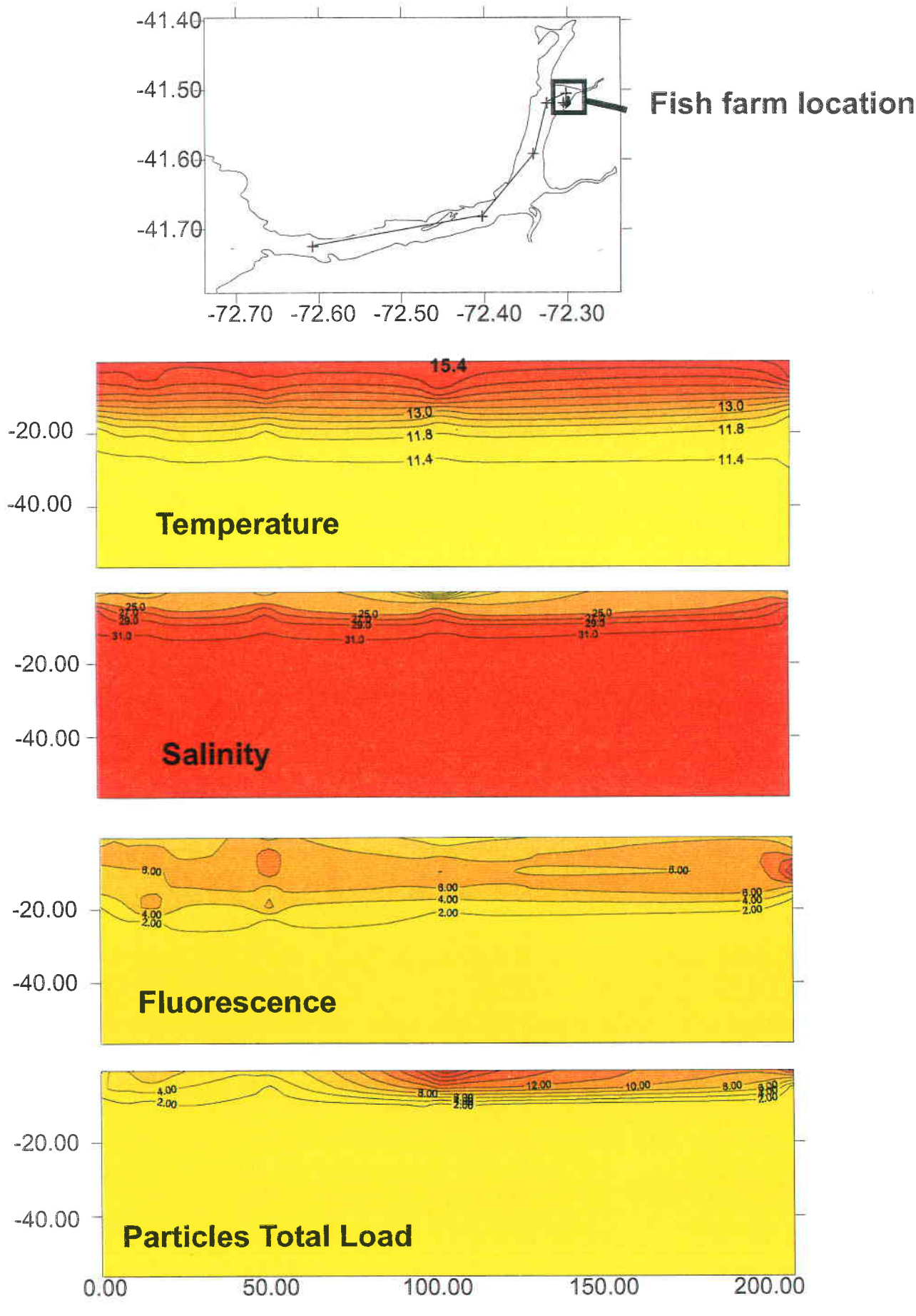
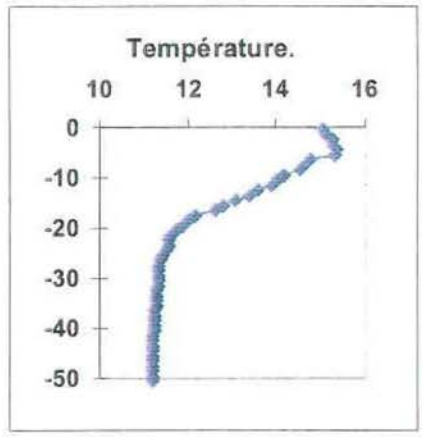


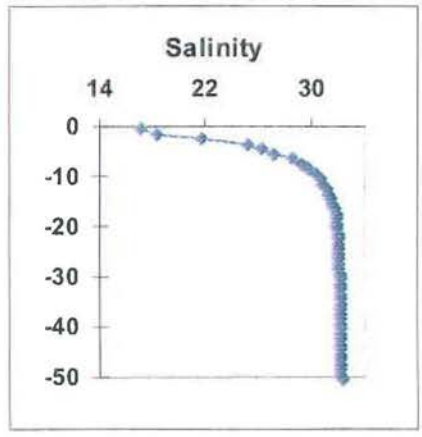
Figure 5. Vertical section along the Reloncavi fjord

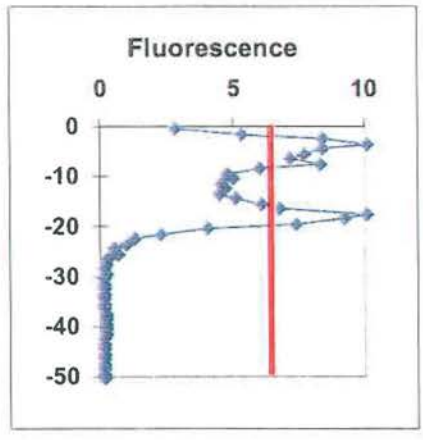


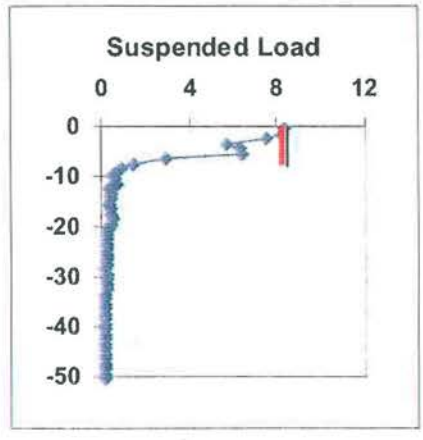
TYPICAL PROFILES IN COCHAMO AREA

CONTROL STATION

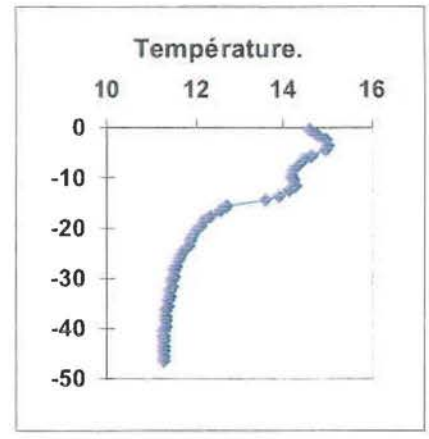


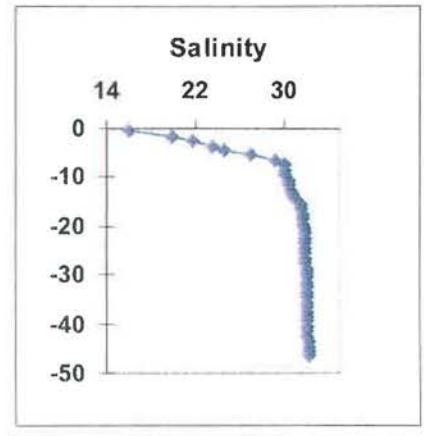


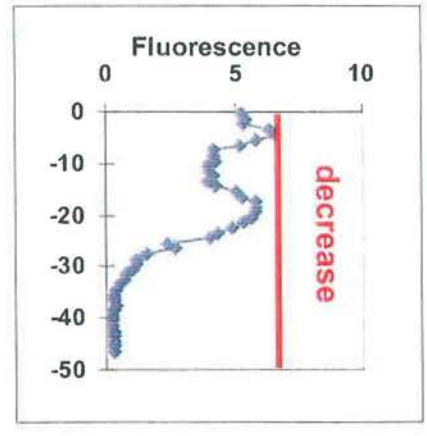


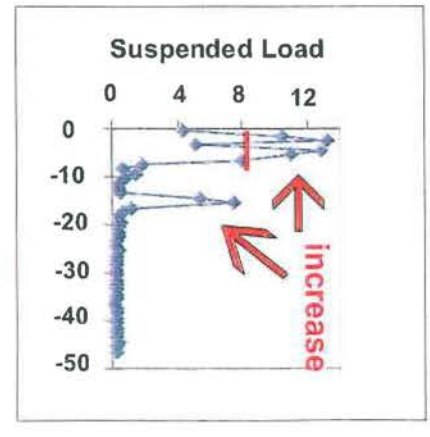


IMPACTED STATION



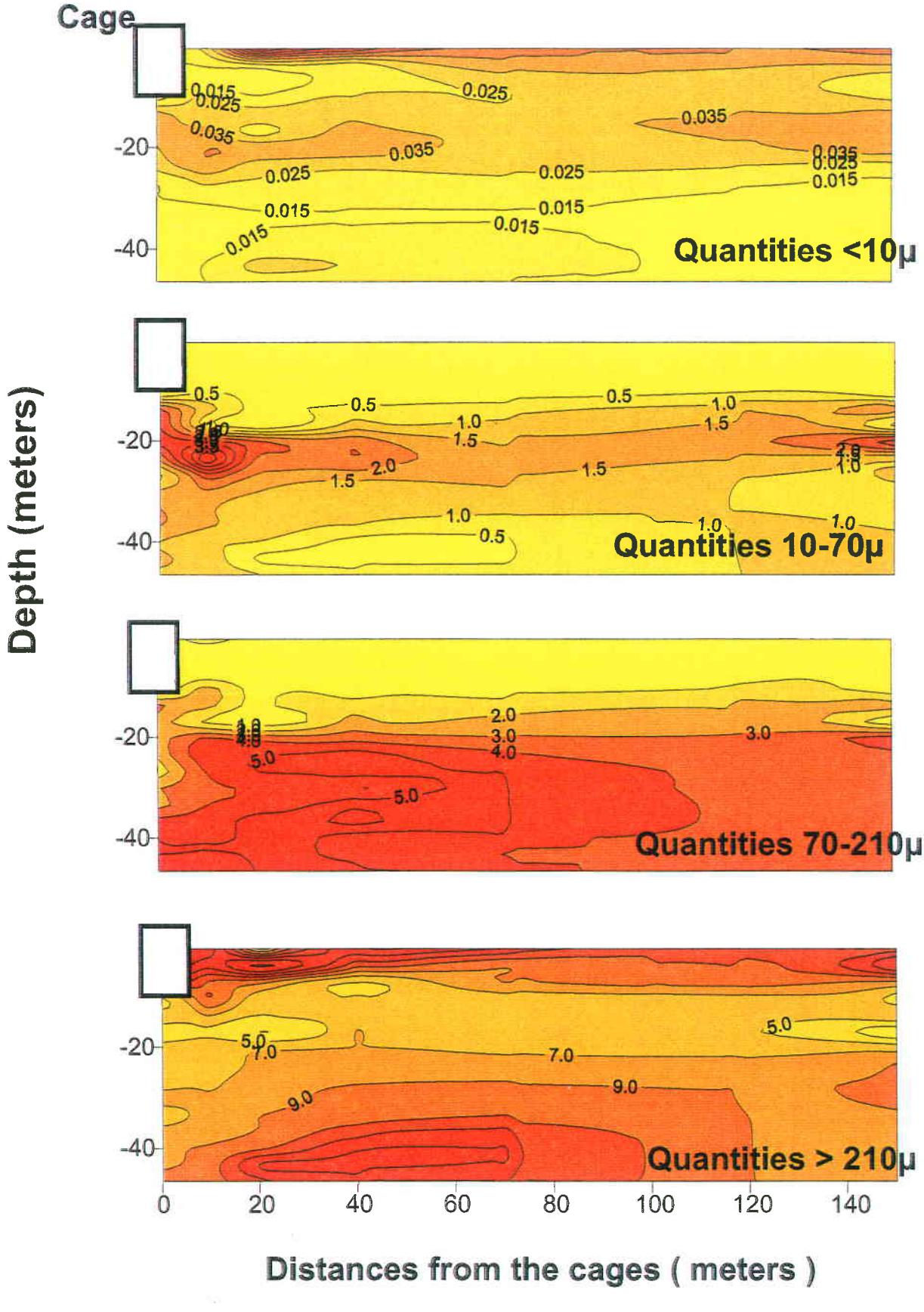




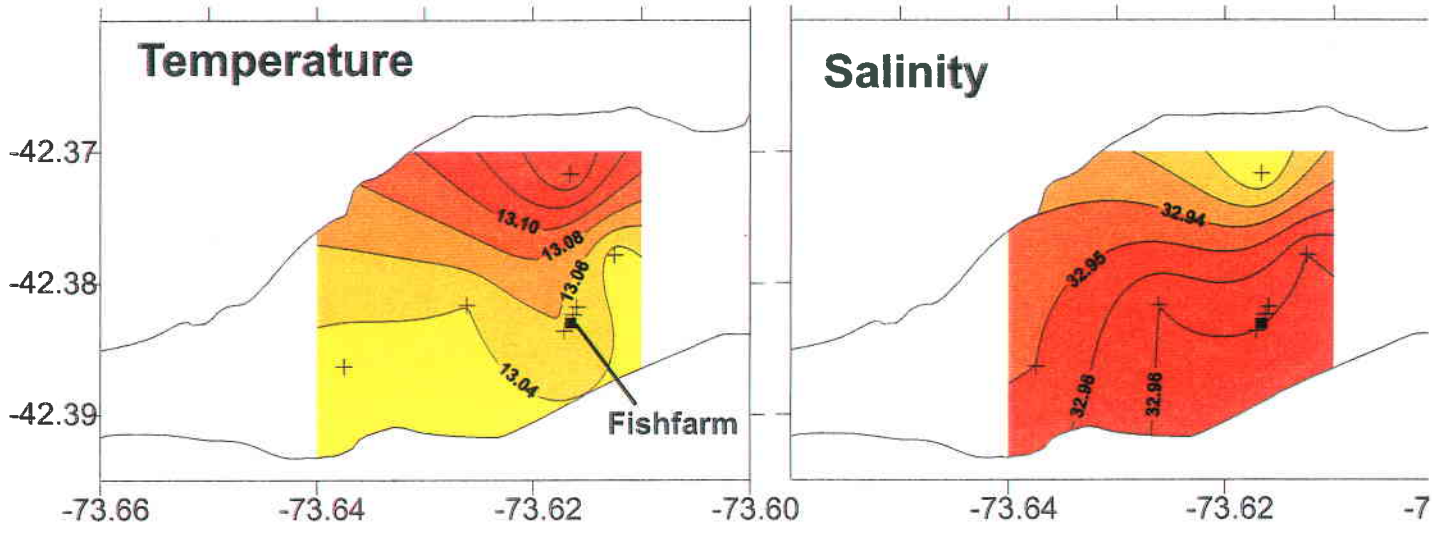


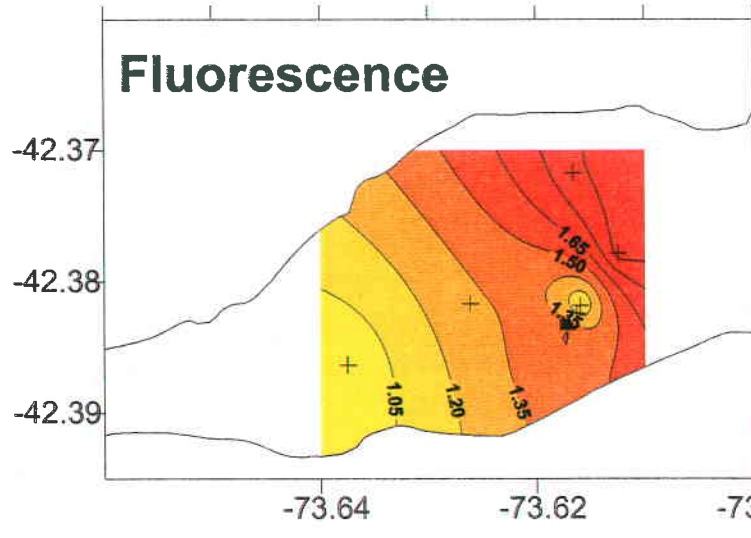
COCHAMO FISHFARM - 5 March 1998

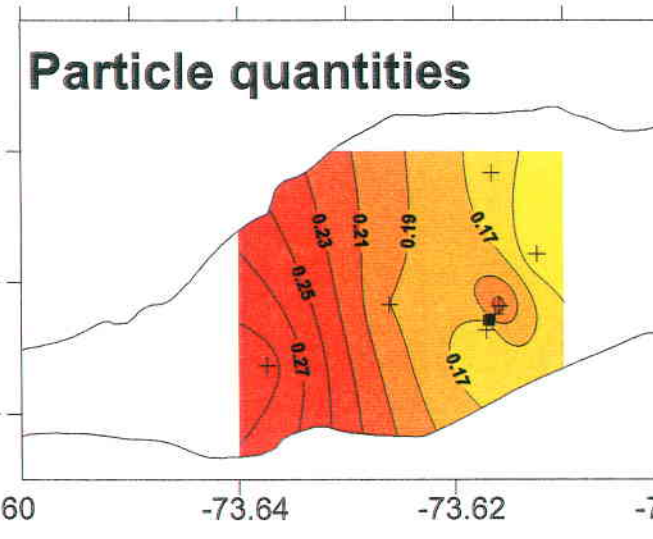
Vertical transect in Cochamo area with particle size distribution



Surface data distribution in Dalcahue area

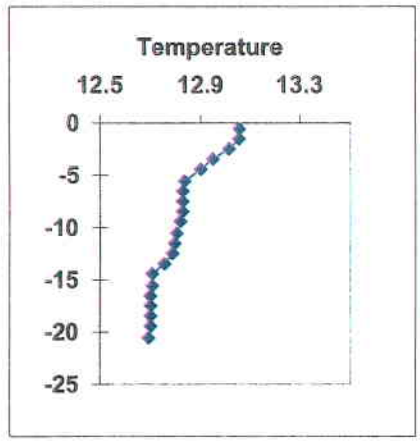


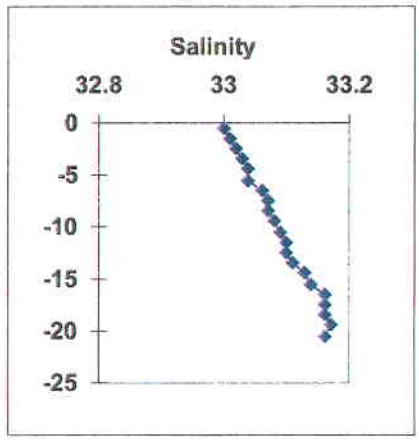


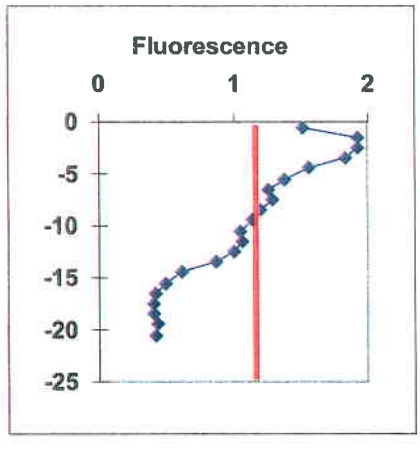


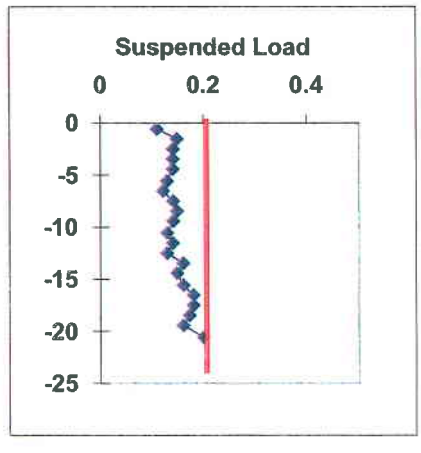
TYPICAL PROFILES IN DALCAHUE AREA

CONTROL STATION

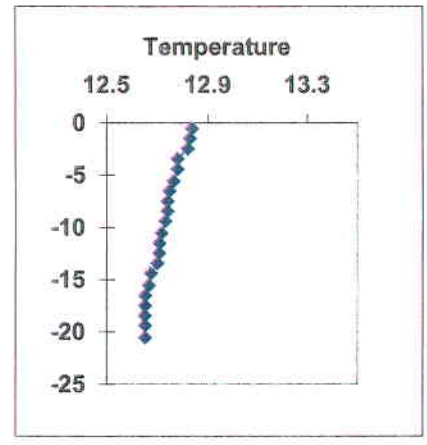


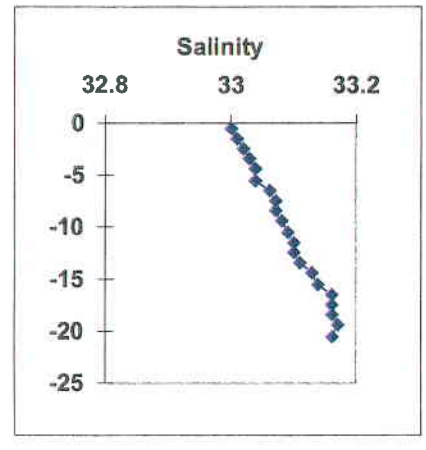


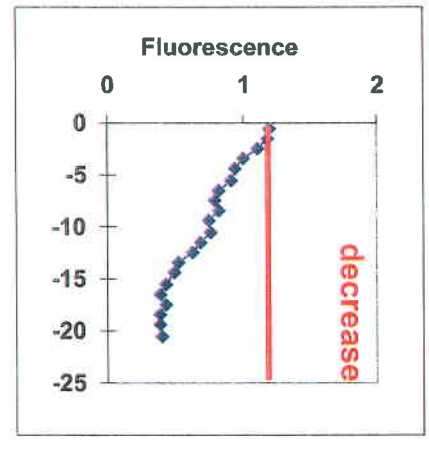


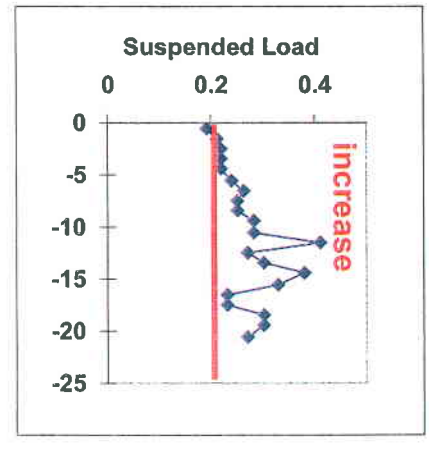


IMPACTED STATION

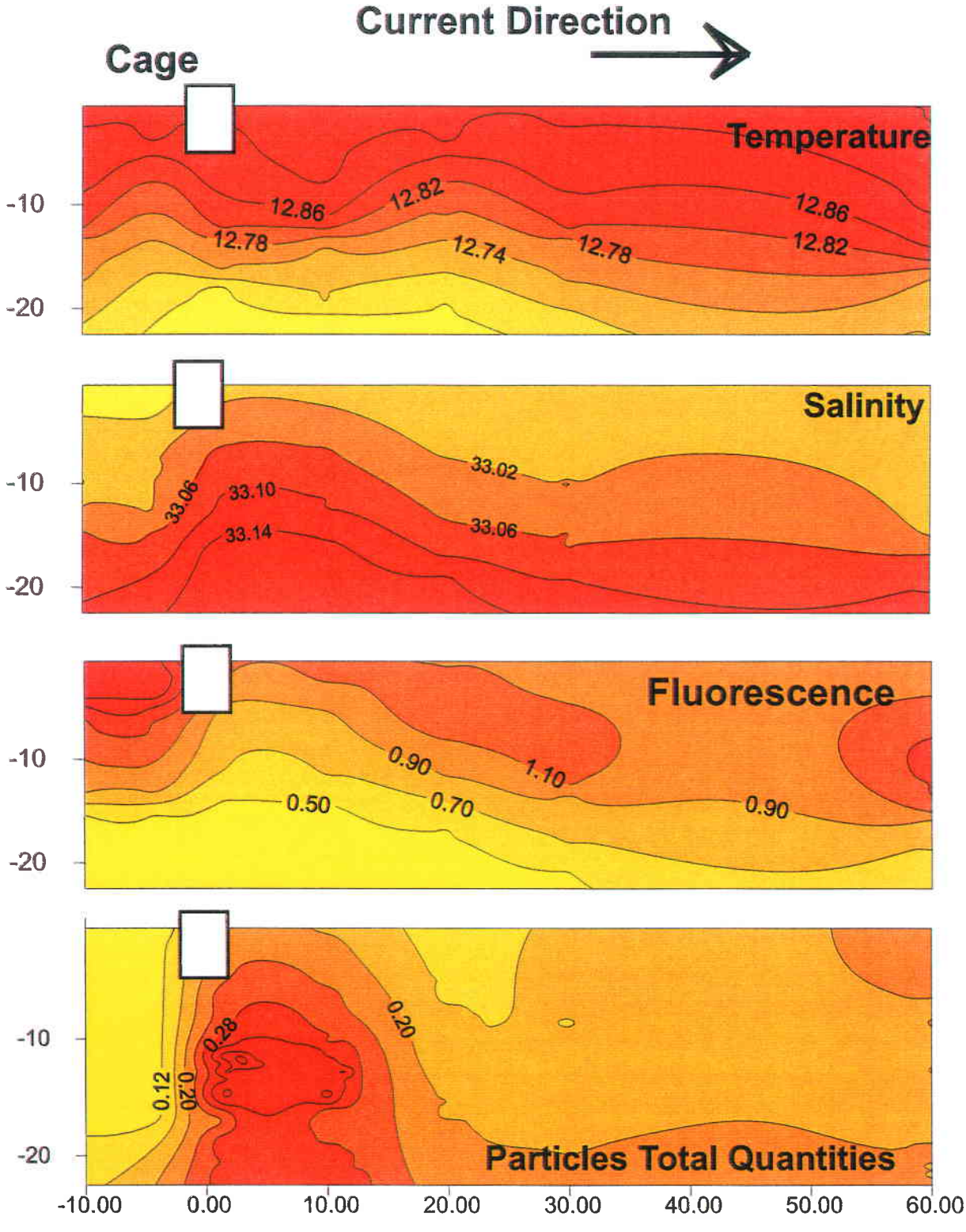








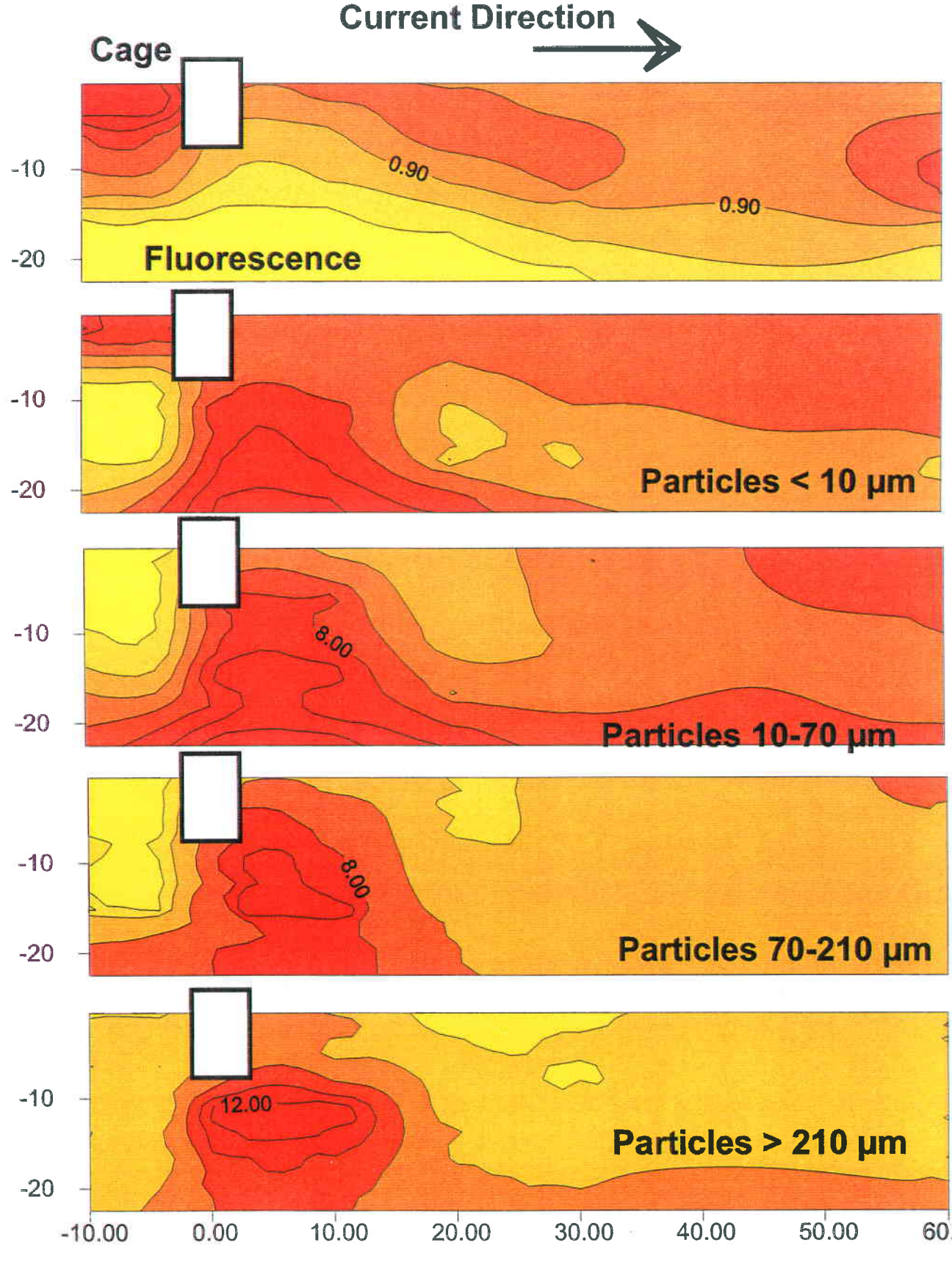
Vertical section near the Dalcahue Fishfarm



DISTANCE FROM THE CAGE (meters)

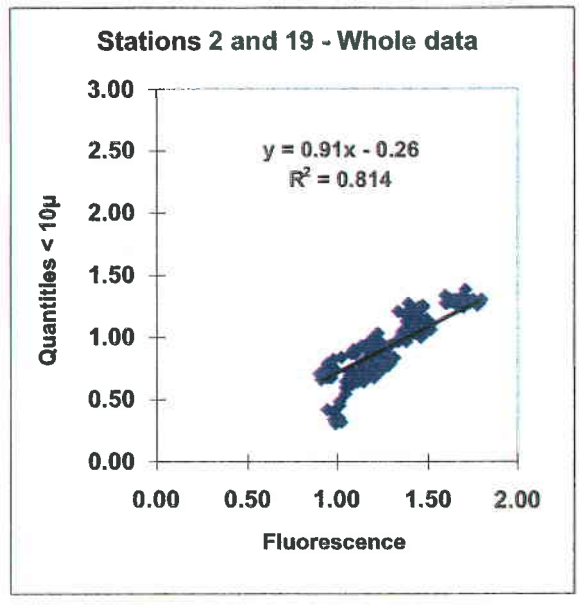
Vertical transect in Dalcahue area.

Fluorescence and quantities of particles near the cages

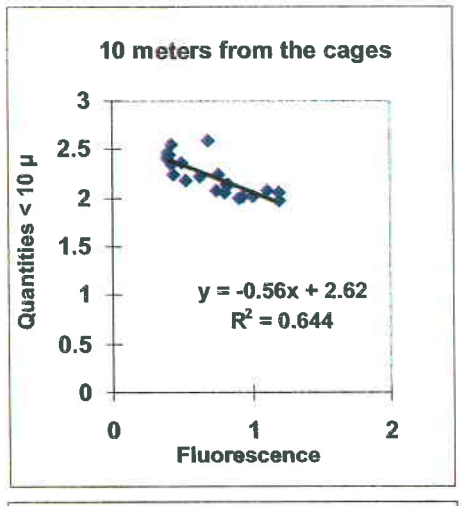


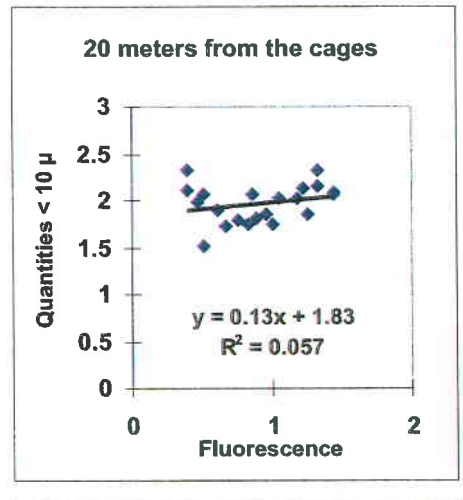
Relationship between particles<10µm and fluorescence in Dalcahue

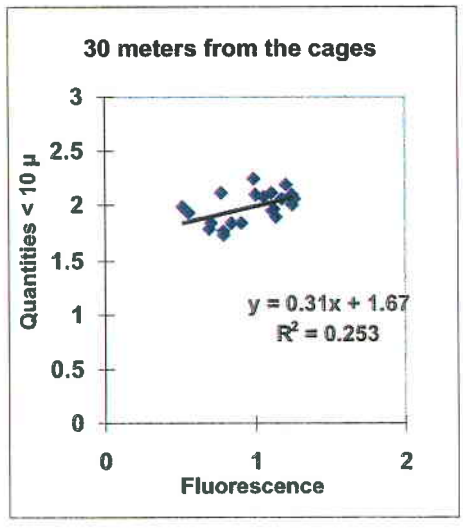
CONTROL STATIONS

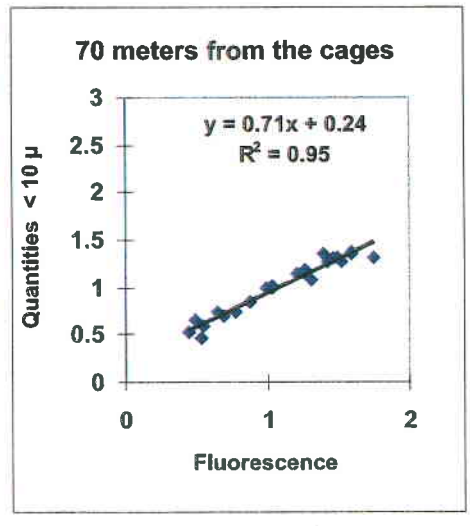


IMPACTED STATIONS

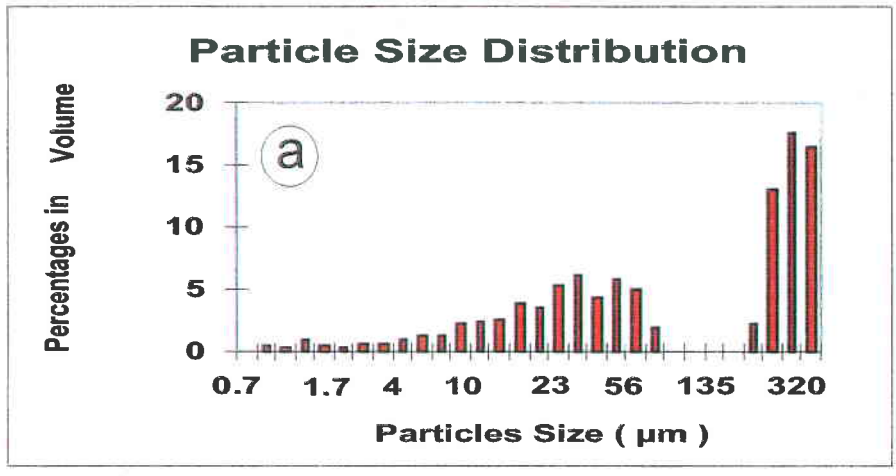


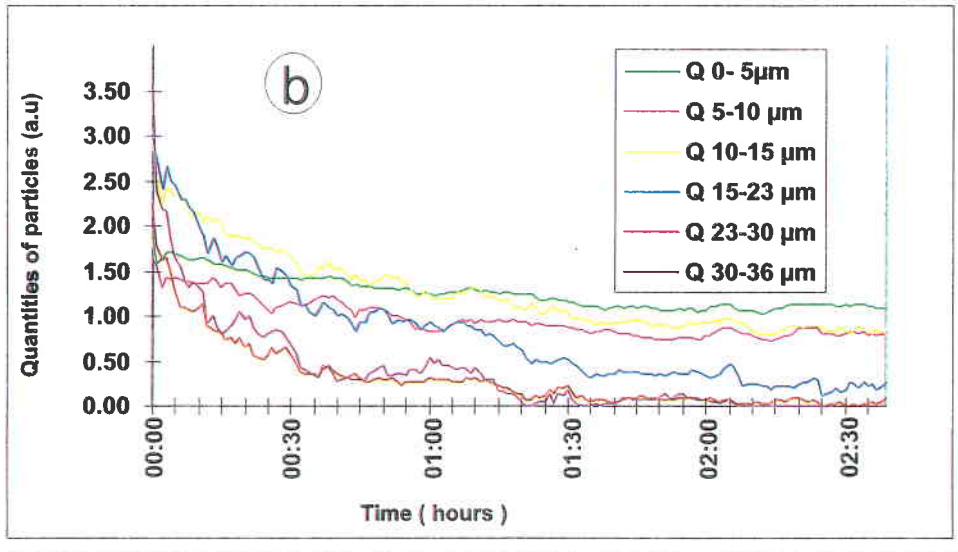


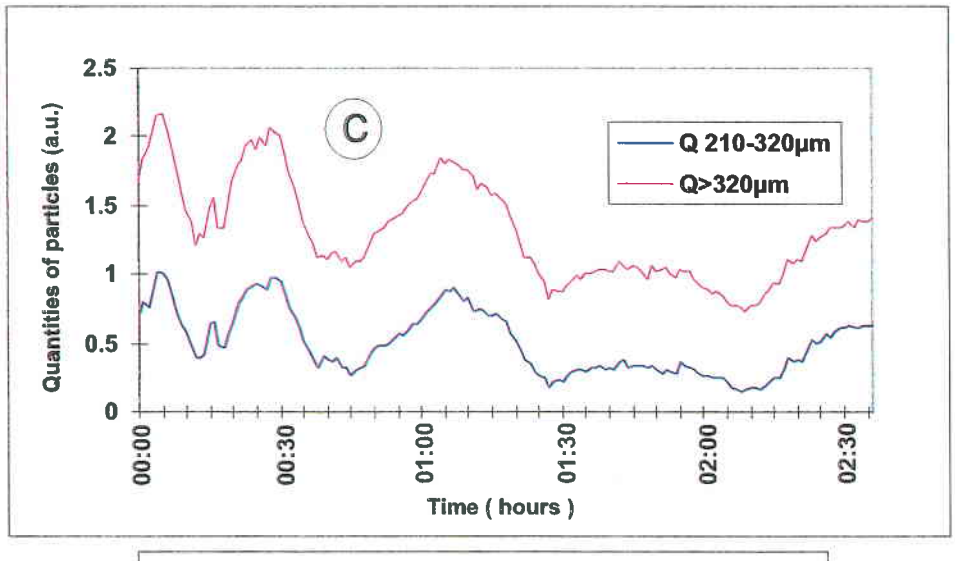


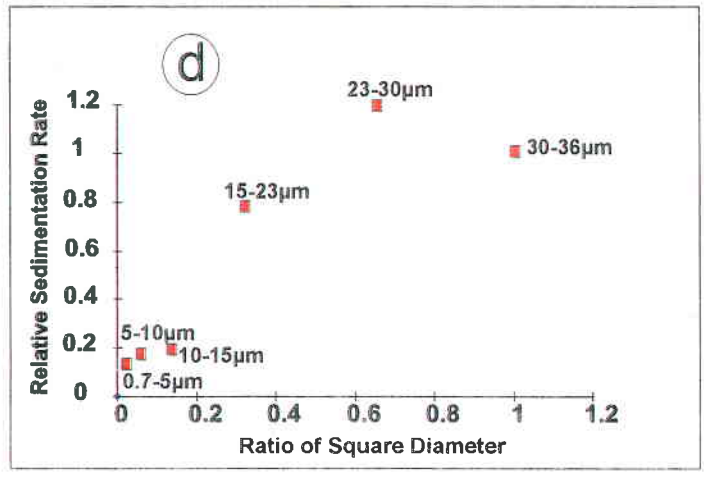


DALCAHUE - SEDIMENTATION EXPERIMENT









Model of organic particles sedimentation, for prediction of environmental effects of salmon culture

Dr Martin Hevia (IFM Kiel, Germany)

Salmon breading in coastal areas brings considerable quantities of particular organic wastes, mainly constituted of faeces and aliment losses. More, therapeutants added to aliment are scattered into the environment. It is well known that organic wastes accumulation on the marine bottom produces physical and chemical changes in the sediment, and disturbs benthic communities in the vicinity of the salmon farm. Some chemical modifications in the sediment, increase organic carbon, nitrogen and phosphate amounts, important oxygen losses and consequently, low oxido-reduction potential. Changes in benthic macrofauna abundance and specific composition are observed, as occasionally unstocked zones straight beneath the cages. Waste accumulation on the bottom can have implications in the viability of salmon culture. Gases emanation such as methan and H_2S originated in anoxic sediments, can impair salmon health or notably lower the yield of production, involving plant closure in some cases. In repeated occurrence, this undesirable ecological change implicates unavoidable negative consequences.

In proportion to salmon culture extension, problems in production require detailed examination. Modelling particular organic matter in Dalcahue channel was used for evaluation of a fish farm ecological effects, taking into account numerous factors. In short, the model is based on a simple relationship between depth (bathymetry in the sector), particles sedimentation rate (pellets and faeces or distincts fractions), current-metry (speed and direction registered during a representative time), specifically for each area and production data (feed distribution per time unit etc.) among others. This model constitutes a useful practical tool for productors in environment management.

Simulation with this model allows calculation of dispersal and the corresponding sedimentation rate on the bottom, in g C m^{-2} d^{-1} for particular organic wastes from salmon farm. The model can be applied as a practical tool for selection of new sites of plant production, as for prediction and prevention from environmental damages on the bottom beneath the cages in the selected areas. More, a simulation can be used for estimating the optimal location of next cages (producing lower environment impact), stress and desease prevention (lowering the treatment cost), leading to production increase.

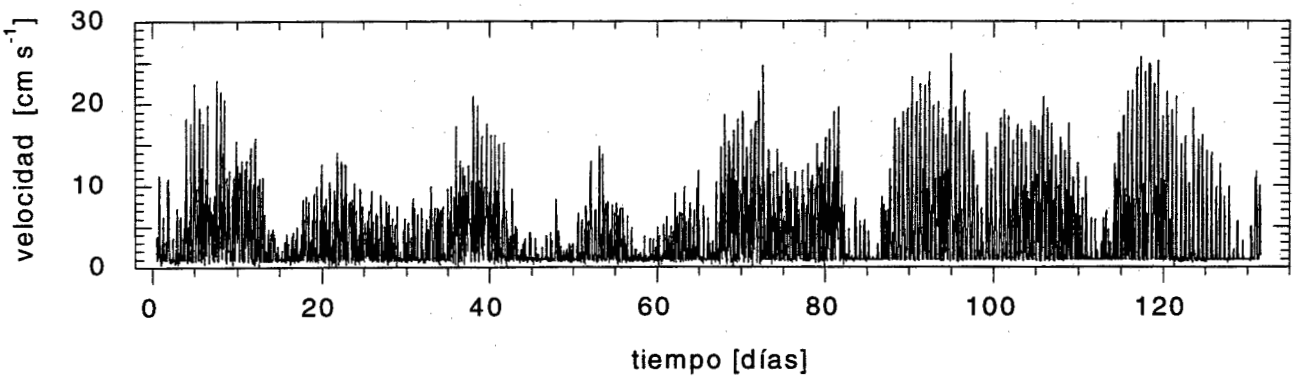


Figure 1: Time serie of current velocity. The data were registered every 30 min interval ; n= 6294.

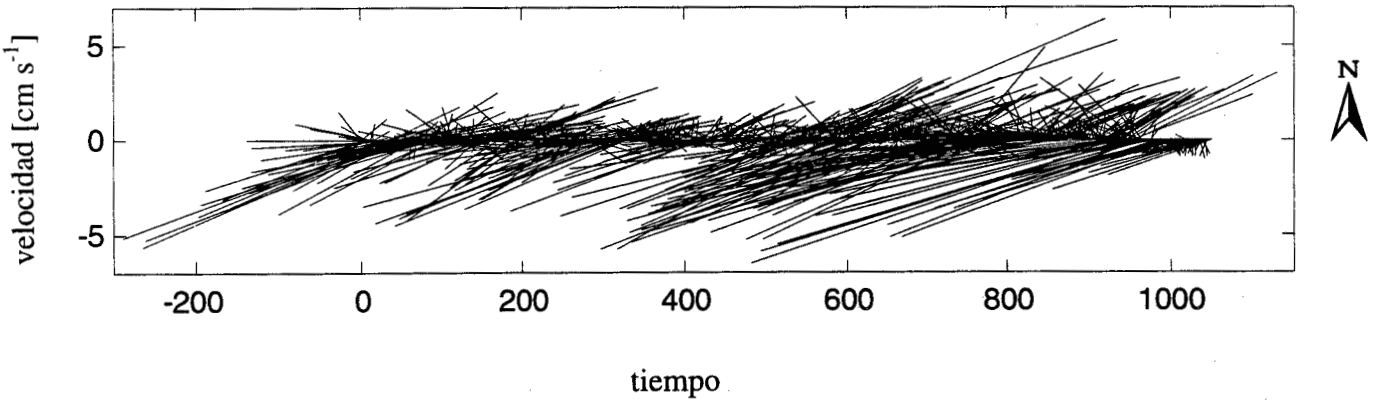


Figure 2: Time serie of current vectors. Eeach vector corresponds to three-hour mean.

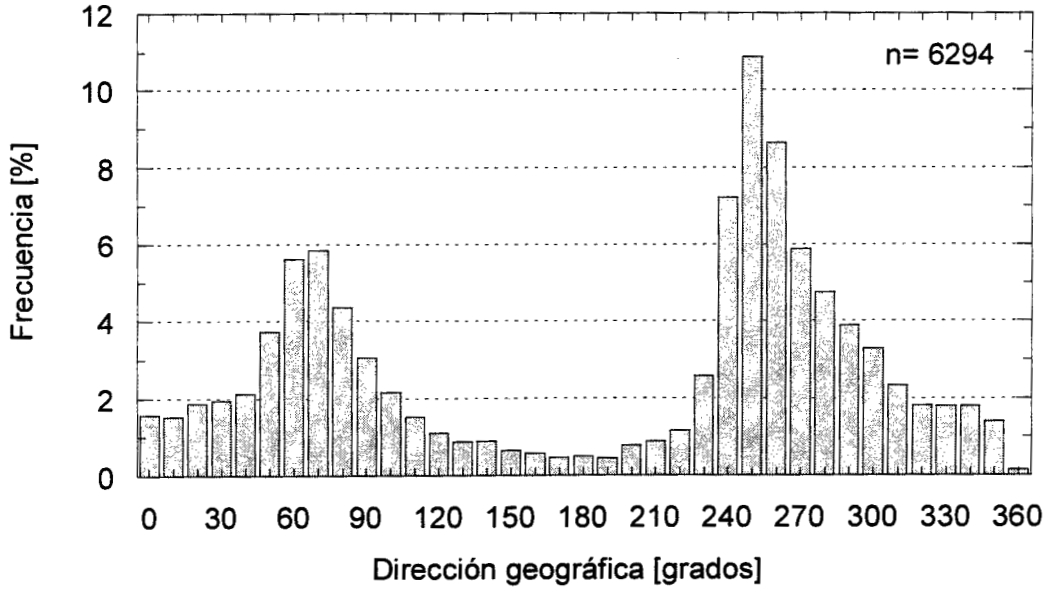


Figure 3: Histogram of current direction. The data were registered every 30 min interval.

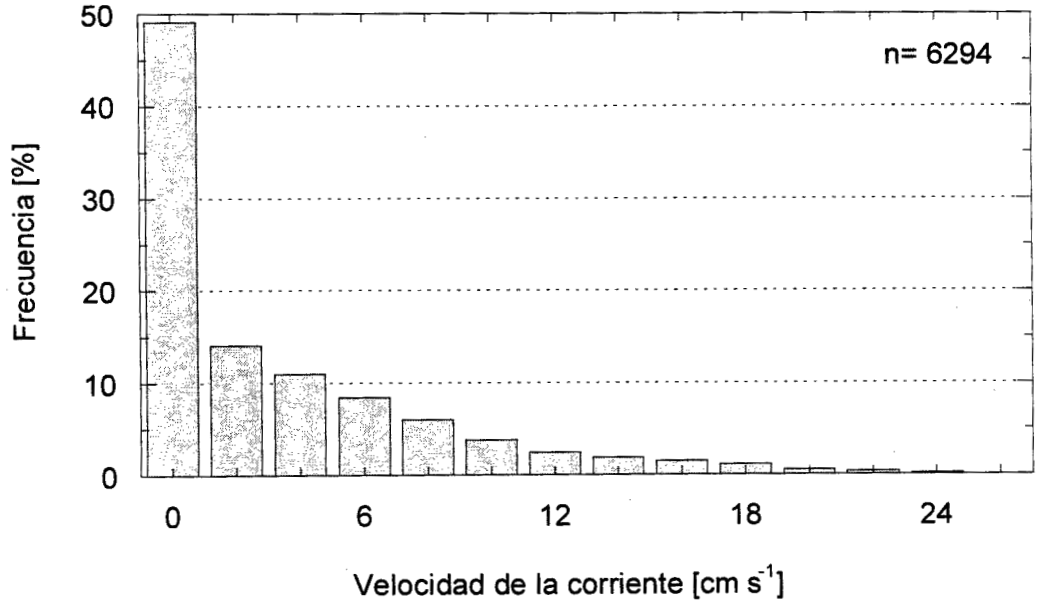


Figure 4: Histogram of current velocity. The data were registered every 30 min interval.

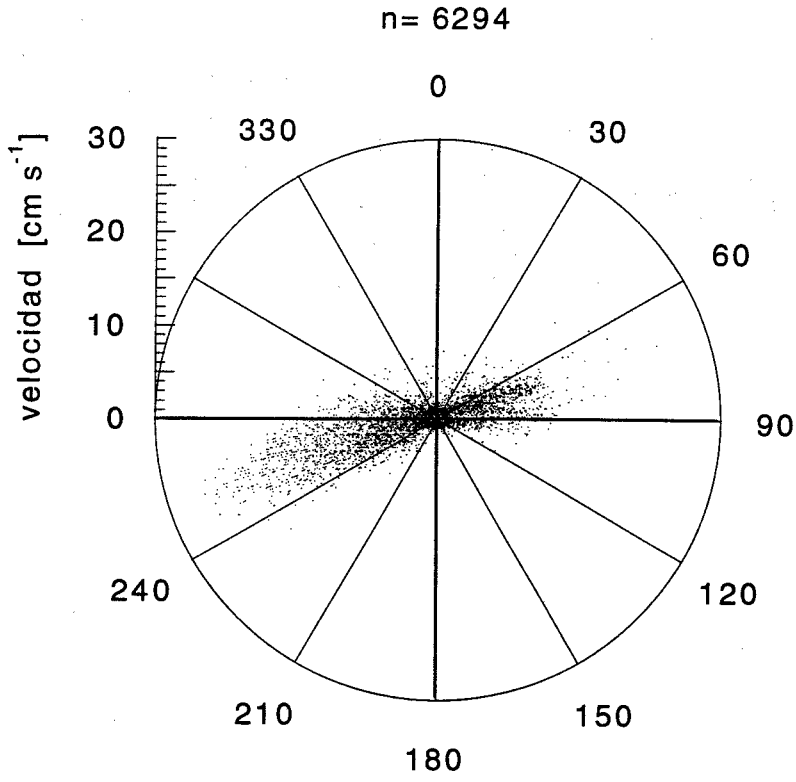
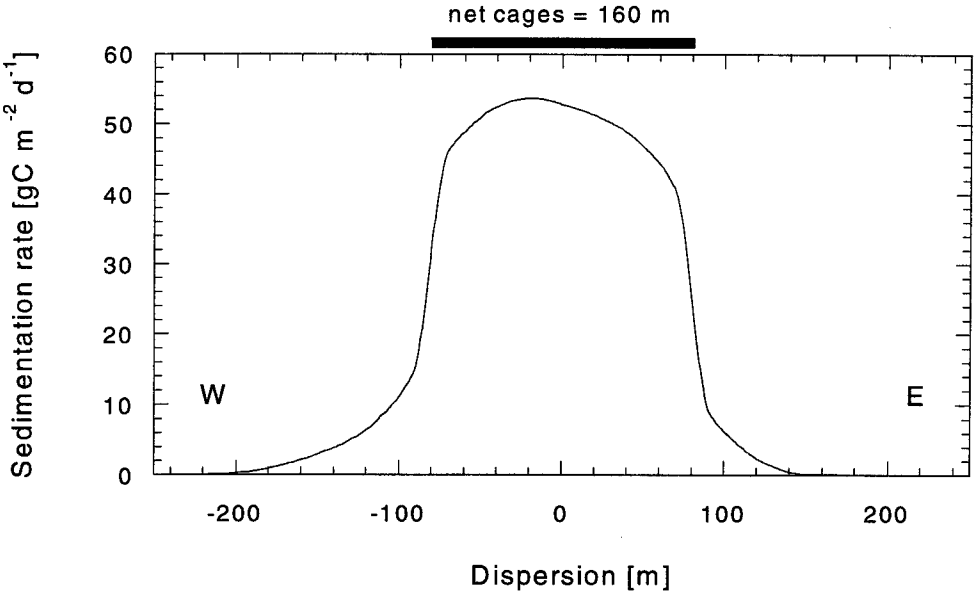
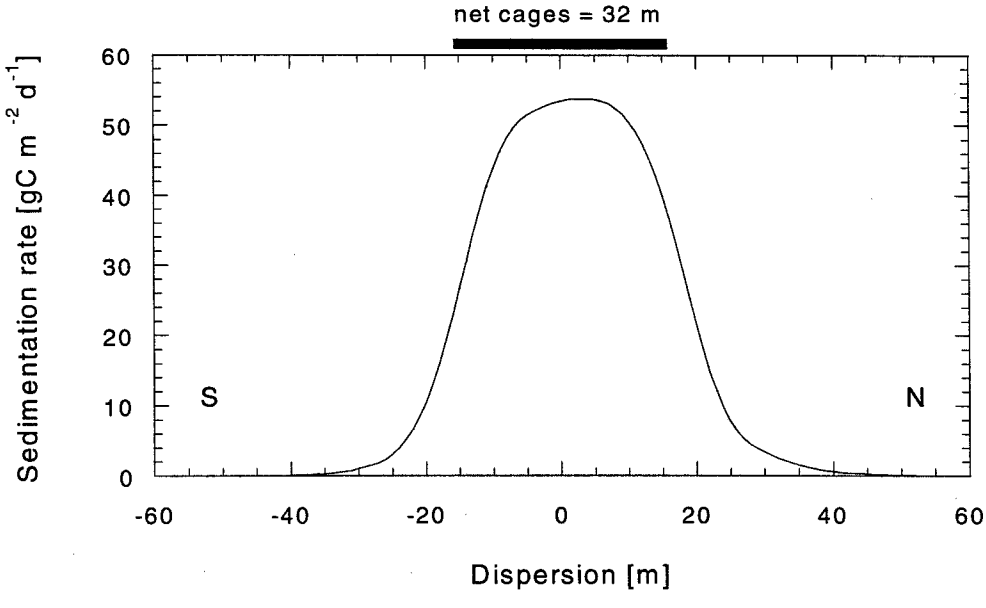


Figure 5: Polar diagram of velocity and geographical direction of current. The data were registered every 30 min interval. Each point represents the vector extremety originated in the center.


net cages = 160 m
Sedimentation rate [gC m -2 d-1]
60
50
40
30
20
10
0
W
E
-200
-100
0
100
200
Dispersion [m]


net cages = 32 m
Sedimentation rate [gC m -2 d-1]
60
50
40
30
20
10
0
S
N
-60
-40
-20
0
20
40
60
Dispersion [m]

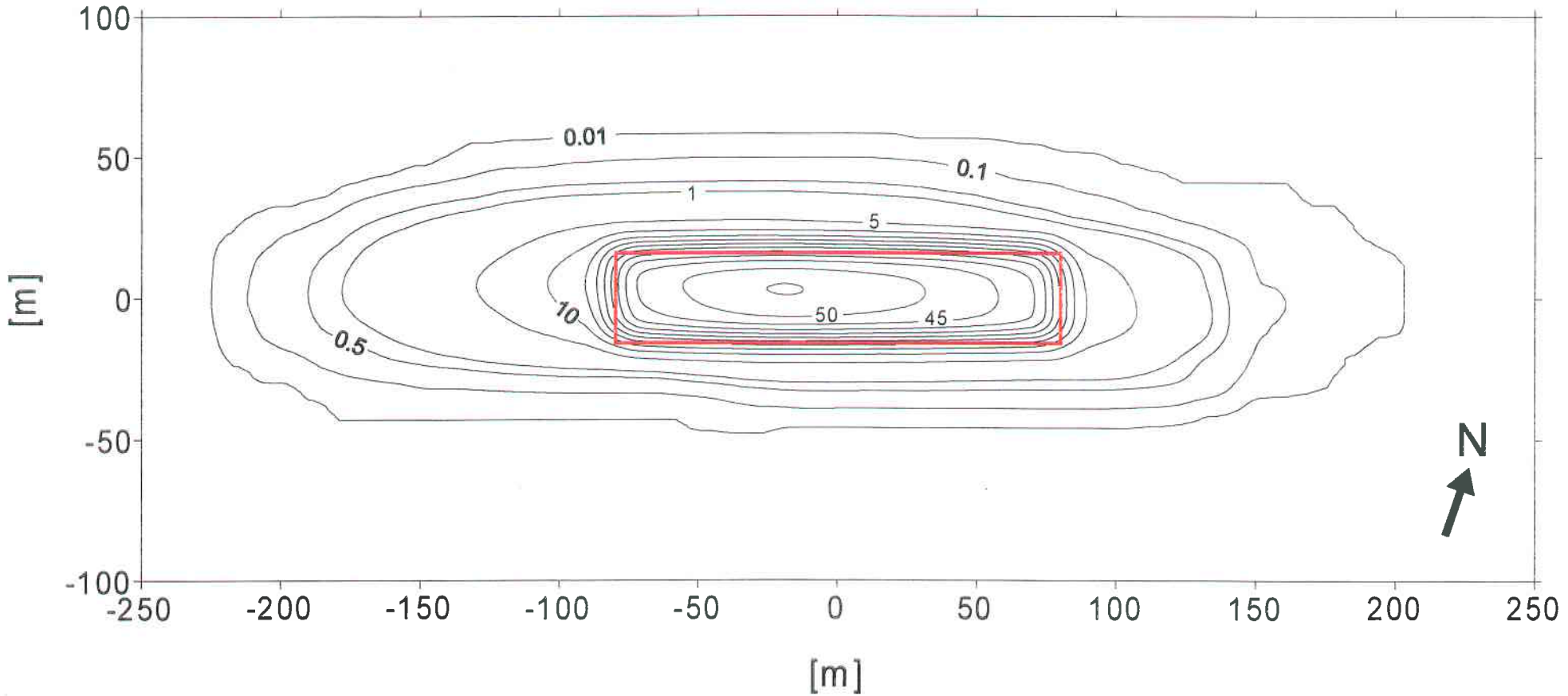


Figure 1: Dispersion of organic particles on the bottom with contour lines, indicating the sedimentation rate in g C m^{-2} d^{-1}. The red rectangle gives the position of the cages set (length 160 m)

Environmental and oceanographical characteristics of channels and fjords in South America General environmental effects and dissolved nutrients in the water column in salmon farms

Alejandro Clément*, Miriam Seguel**, Leonardo Guzman**,Gemita Pizarro**, Georgina Lembeye***, Ximena Rojas** and Martin Hevia****

* INTESAL, Chile
** IFOP, Chile
*** Universidad Austral de Chile, Chile
**** IFM of Kiel, Germany

Financial support : EEC

Abstract

The south austral area in Chile and especially the marine ecosystem, supported increasing use of the water for aquaculture during the last years. More, the recurrent toxic bloom events constitute the main topic of this study.

The first step is to describe the general characteristics of the areas where the studied fish farms are located. The channel and fjord environment in this part are the most pristine of the world, in spite of the various activities such as : sea transport, harbours, extension of coastal cities, tourism, fishing, aquaculture, and so on.

Salmon farming will go on increasing in the next years, and will necessitate more and more water masses, involving more and more wastes. Optimal diet, improvement in FC, genetic and sanitary controls are relevant measures for a lowest impact of aquaculture. As an alternative, a good knowledge of the environment and interaction with aquaculture constitute an important part of the results. The outcomings are for productors, in view of sustaining their activity. The work was applied to the water column, sediment and benthic compartment, and the last step will be later, in mathematical modelling.

The modifications and regulation in phytoplankton populations constitute an other part of the project : influence on microalgae selection (toxic or not), of chemical substances from fish farm waste and animal excretions is studied. Red tides and radiations effects are studied especially in the Magellan Strait region, by the different teams : Chilean, Argentinean, French. It is well known that UV-b radiations increase in this area, with important ecological implications.

During this seminar we hope our discussions will lead to concrete and technical contributions of each one, in view of improve methods and suggest recommendations for public and private sectors.

There is no longer any doubt about the effect of local and national growth will affect the environment and ecosystems. We have to consider the bases of technologies, society and economy in view of minimizing their impact and support the production.

Phytoplankton Distribution and HAB

Intense fish farming did not produce detectable differences neither in the phytoplankton composition nor in abundance, in a given farm area. However, the results point out influence on spatial distribution of the chlorophyllian cells.

In the Reloncavi Fjord (Table 1), a subsurface peak of pennate diatoms, dinoflagellates and nanoplankton was observed in the water column, constituting an original feature of this fjord. During the mission the toxic dinoflagellate *Alexandrium ostenfeldii* was observed in Reloncavi Fjord, particularly in the subsurface samples. This was unusual, and could be due to the lack of freshwater input during the summer (salinity 25). However, we found this potential Paralytic Shellfish Poisoning (PSP) algae either in control stations as well as fish farm station, therefore the presence of this algae is probably due to local oceanographic anomalies rather fish farming impacts.
The highest density in cells : approx. 10^6 .l^{-1} was observed in Huenquillahue area (Figure 2) , the lowest : less than 10^3.l^{-1} in Dalcahue (Figure 3). Cochamo (Figure 1) presented intermediate densities : about $0.6x10^6$.l^{-1} at the maximum of density.

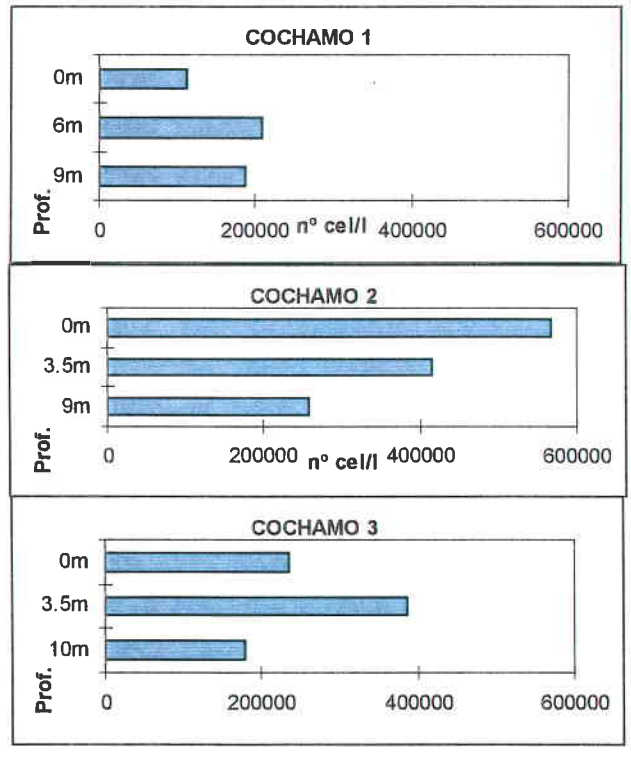


Figure 1 : Total cell number in Cochamo fish farm, Reloncavi fjord

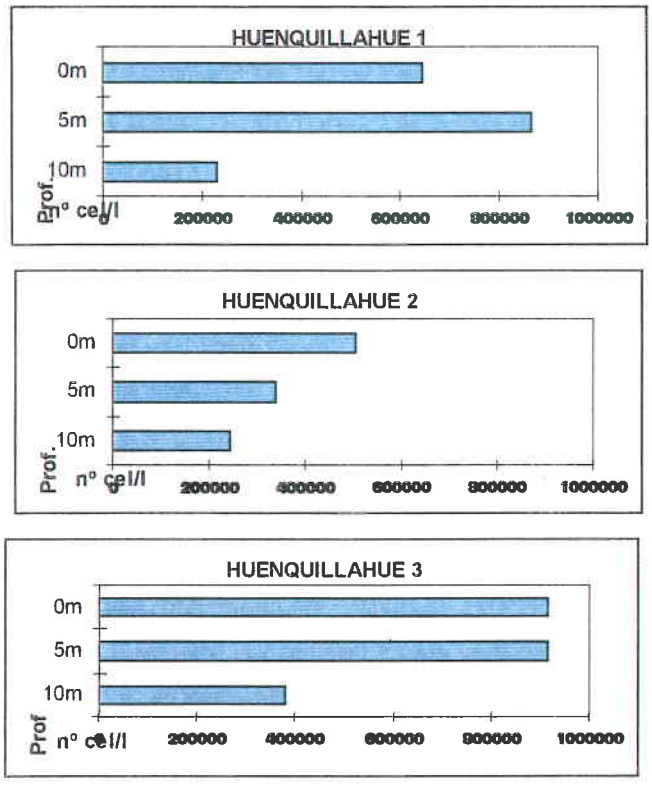


Figure 2 : Total cell number in Huenquillahue fish farm, Puerto Montt bay

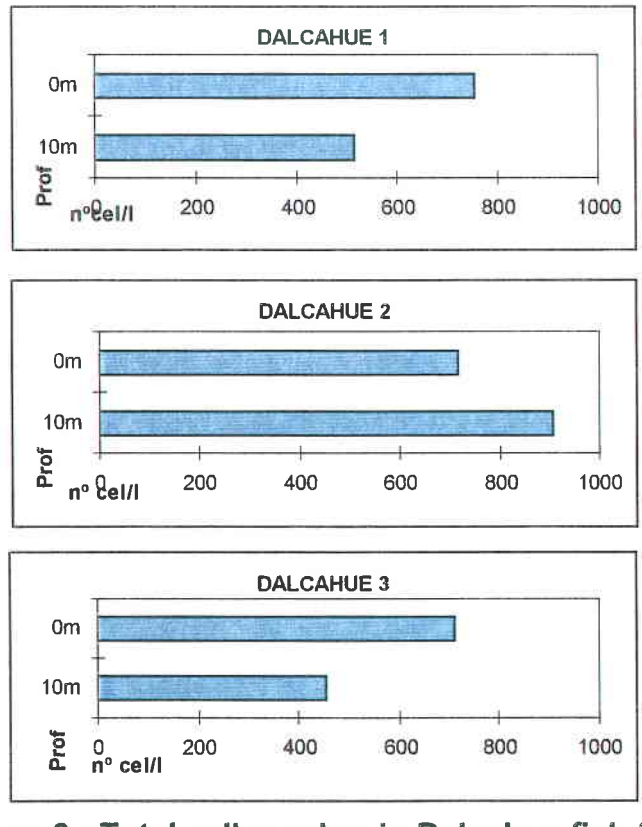


Figure 3 : Total cell number in Dalcahue fish farm

5.3.98	Data in cells/mL. These samples were collected with Ifremer mission									
STATION	C3		FJ-10		FJ-10		FJ-11		FJ-20	
DEPTH (m)	18,5 avg	std	9,5 avg	std	18,5 avg	std	12 avg	std	9,3 avg	std
Thalassiosira spp.	279	70,9	86	25,5	252	82,8	117	35,5	36	12,7
DIATOMS SUB TOTAL	**2063**		**1121**		**1286**		**2601**		**3283**	
Atecados	158	16,8	86	38,7	194	82,8	86	44,6	198	27,8
DINOFLAGEL.SUB TOTAL	**165**		**93**		**199**		**92**		**204**	
Nanoplankton	567	22,1	1166	121,0	1211	152,2	910	205,3	1450	81,3
OTHERS SUB TOTAL	**567**		**1167**		**1225**		**911**		**1450**	
TOTAL PHYTOPLANKTON	**2795**		**2380**		**2710**		**3603**		**4937**	
ciliates	0	0,0	0	0,5	2	2,1	0	0,5	0	0,5
pellet	3	0,9	4	1,2	4	2,6	1	0,0	2	0,5

Table 1 : Phytoplankton results at peak of maximal fluorescence in Reloncavi fjord

Nutrients

Understanding the effect of nutrients coming from fish farm, will give an important tool to mitigate the future potential toxic red tide in this region of the world.

Ammonia, urea, nitrite, nitrate, phosphate, silicate, DOP and DON results were analysed and compared according to the sampling location.

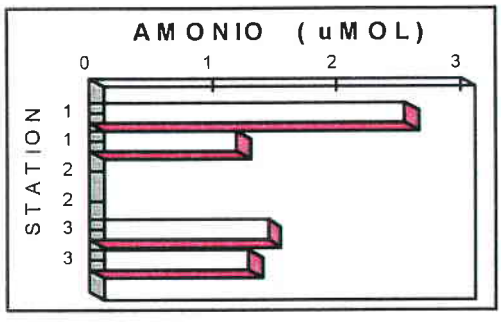
Surprisingly, only ammonia showed a significant difference between fish farms stations and the control. This could be the consequence of high dilution of the flux of nutrients in fish farm sites, due to currents. Others additional physical, chemical and biological process could interfere : photodegradation, bacterial mineralization.

Urea was not the best chemical index to evaluate the dissolved nitrogen impact of fish farming : there was no clear signal between fish farm stations and control, and the chemical analysis is delicate. From this point of view, the measurement of urea was not practical neither straightforward in this experiment.

Phosphate data in all fish farm stations, were not different from control stations. It is well know that particulate phosphate from fish farming is a relevant input into the water column and sediments, may be this is the explanation.

Therefore, we propose to follow more closely the spatial and temporal distribution of ammonia in a fish farm area, and moreover, to correlate fish biomass per cage and the ammonia concentration in integrated sample (0 to 10 m) per cage.

Dalcahue



Cochamo

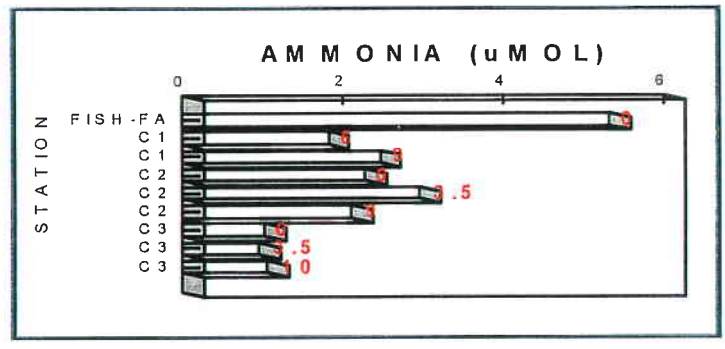


Figure 2 : Histograms of ammonium values (µM N-NH_4) in two selected areas

Conclusion

The nutrients results shows that variations in **Ammonia (NH4+)** concentration constitute a signal of environmental impact in the water column, due to fish farming. This impact is more remarkable in the surface water rather than in underlying water.

Urea did not appeared as a good indicator of the environmental impact of fish farms in the water column. This could be attributed to the low concentrations resulting of important dilutions, and difficulties in chemical analysis. Therefore we believe it was not a useful practical chemical tool to assess the impact of fish farming in this case.

In general, nitrite and nitrate did not showed clear signal of environmental impact of fish farming in the water column.

However, we observed a signal in the **N/P ratio,** [(N-NH_4+N-NO_2+N-NO_3)/P-PO_4] in the comparison of fish farms and control stations. Surface waters of fish farms has the higher values between 13.4 and 14.8 excluding DOP and DON.

Therefore, the qualitative nutrients impact, in the water column, due to fish farming was moderate to low. Only ammonia showed increase in a fish farm site. The rates and mass balance of this nitrogen input into the inland sea marine environment should be studied in the short term.

1998 has been very unusual from the HAB point of view : Until December of 1998, at least 4 different diatoms blooms impacted fish farming. The most important issue is the presence in the X region of *Alexandrium catenella*. This extremely toxic dinoflagellate usually blooms in southern waters, but now its presence is in the northern inland sea of Chile. Therefore, is a potential risk for mariculture and fisheries.

Effect on phytoplankton growth of substances excreted by marine animals

Miriam Seguel, Chilean coordinator of CONICYT project, G. Arzul and A. Clément

Financial support : ECOS-CONICYT

Abstract

The explosive growth of one phytoplanktonic species is commonly known as red tide. The factors which trigger this growth are not clearly understood. However, the increment of nutrients in the marine environment could be one of the factors which would be caused the growth of certain species. Aquaculture is one of the activity which is continuously adding nitrogen compound such as ammnonium and urea coming from the excretions of the marine organisms. So, it could be assumed that the high concentrations of this organisms could produce changes in the N/P ratio. The objective of this study is to evaluate whether the substances coming from the excretions of marine organisms have a selective effect on the growth of some phytoplanktonic species which are red tide former in different part of the world.

Biossays were carried out with different marine organisms excretions : sea bass (*Dicentrarchus labrax*) cultured in tank ; Atlantic salmon (*Salmo salar*) from a net pen reared system, and the mussels (*Mytilus chilensis*) reared under laboratory conditions. The phytoplanktonic species usually cultured in laboratory are the following : the dinoflagellate *Gymnodinium mikimotoi*, *Alexandrium minutum*, *Alexandrium catenella* ; the raphidophycea *Heterosigma akashiwo* and the diatom *Chaetoceros gracilis*.

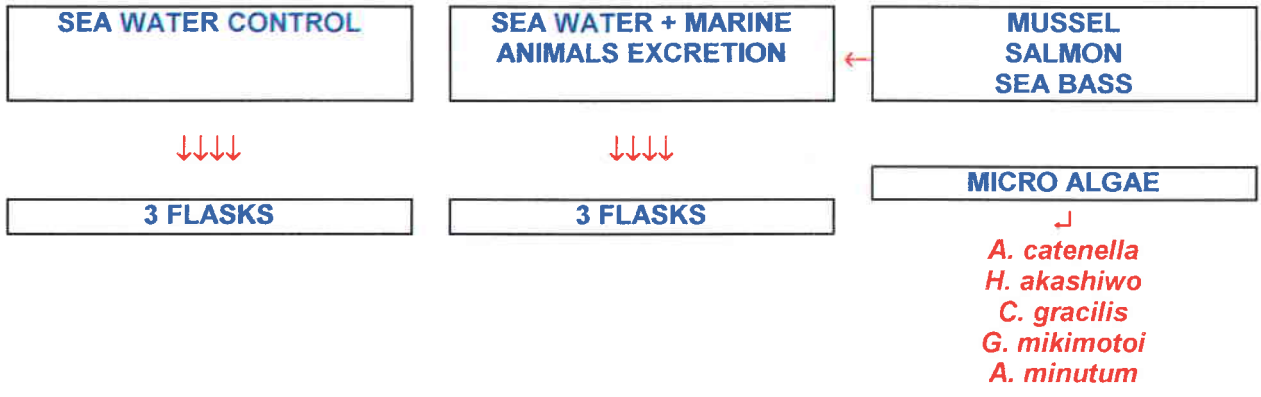
The seawater with salmon excretion produced an inhibition on the growth of *Heterosigma akashiwo* and there was no significant effect on the growth of *A. catenella*. The mussel excretion produces an increment on the growth of both above mentioned planktonic species. The sea bass excretion produces different effect on the distinct species studied : a stimulation on *A. minutum* and. *C. gracilis*, inhibition in *H. akashiwo* and no effect on *G. mikimotoi*.

OBJECTIVE

To evaluate whether the substances coming from the excretion of marine organisms have a selective effect on the phytoplanktonic species which are red tide former.

The phytoplankton was studied in monospecific cultures realized in laboratory controled conditions, according to the following scheme :

MATERIAL AND METHODS



In every case the chemical composition of the seawater modified by animal excretions was analysed :

MINERAL NUTRIENTS CONCENTRATION IN THE SEAWATER WITH ANIMALS EXCRETIONS AND THE CONTROL

		NH4 (µM)	NO3 (µM)	PO4(µM)	N/P(µM)
MUSSELS	CSW	0.3	3.9	2.0	2.1
	ESW	19.7	3.9	2.0	11.8
SALMON	CSW	0.7	0.9	0.2	8
	ESW	4.1	0.9	0.2	24
SEA BASS	CSW	0.3	4.2	2.3	1.9
	ESW	46.5	4.2	2.3	22

CSW : CONTROL

ESW : SEAWATER+ MARINE ANIMALS EXCRETION

CONCLUSIONS

- The marine animals excretions produce a different effect on the microalgae tested.
- The sea bass excretion produces an inhibition on the growth of *H. akashiwo*, and stimulation on *C. gracilis* and *A. minutum*, and no effect in *G. mikimotoi*.
- The excretion of mussels produce a stimulation on the growth of *H. akashiwo* and *A. catenella*.
- The seawater with salmon excretion produces an inhibition on the growth of *H. akashiwo* and no effect on *A. catenella*.

Active substances from excretions, regulating phytoplankton growth

Geneviève Arzul, M. Seguel and A. Clément.

Financial support : ECOS-CONICYT

Abstract

The growth of *Chaetoceros gracilis*, *Heterosigma akashiwo*, *Gymnodinium mikimotoi*, *Alexandrium minutum* and *Alexandrium catenella* was studied in seawater enriched with dissolved organic substances and mineral salts provided by excretions of marine animals : mussel, oyster, sea bass and salmon. The organic fraction was extracted by passing the seawater through Waters Sep-Pak cartridges, C18 and Florisil. This fraction showed a stimulation effect on the growth rate µ2 , excepted organic from sea bass for *C. gracilis* and from salmon for *A. catenella*, which presented inhibition effect.

According to the chemical analyses, increase in ammonium was important with oyster excretions. In our experiments this compound was stimulator for *C. gracilis*. *A. minutum* and *H. akashiwo* growth rates increased respectively with 5 and 5 to 10 µM N-NH_4, and *G. mikimotoi* was inhibited in presence of the maximal concentration analysed (50µM N-NH_4). This species and *C. gracilis*, were stimulated with 50 µM N-urea.

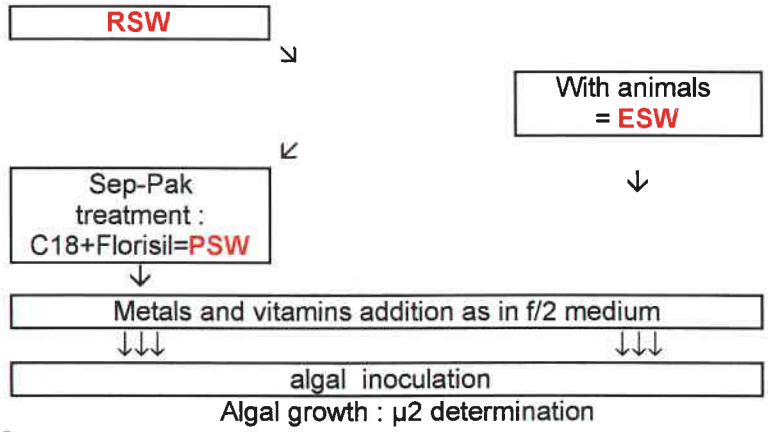
However, in our experiments, the analyses revealed urea never higher than 4 µM N-urea. These experiments confirmed the differences in algal ability to face chemical modifications in marine environment.

OBJECTIVE

To estimate the importance on algal growth, of the different substances from animal excretions : **organic and mineral**

Method

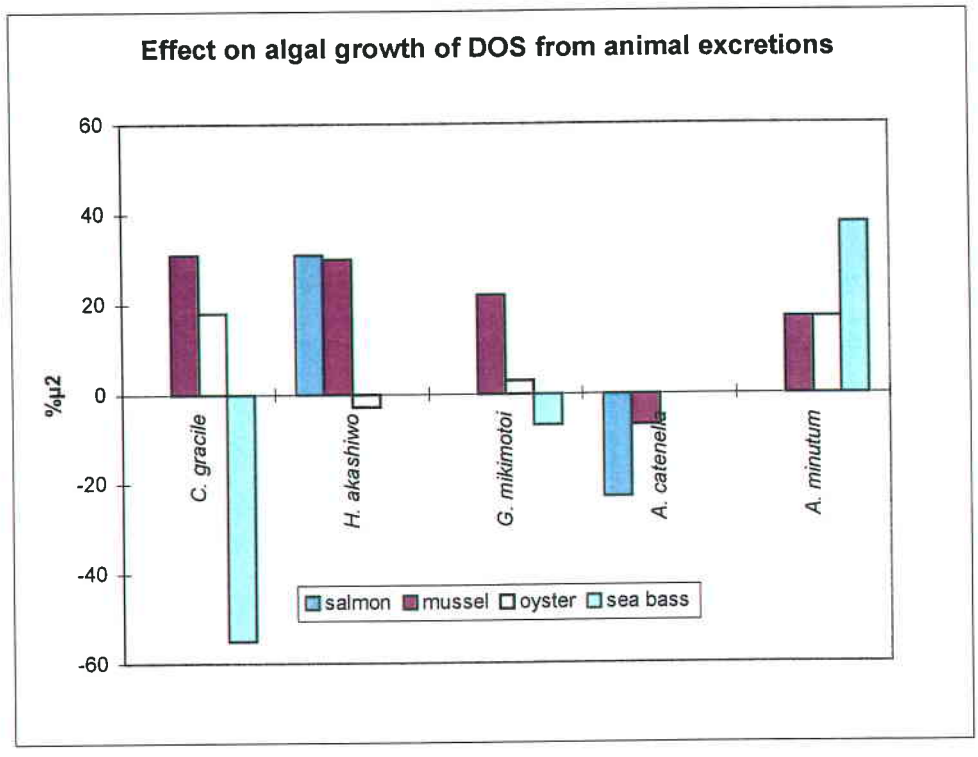
The method was based on bioassays : monospecific cultures of phytoplankton grown in controlled conditions with sea water enriched in animal excretions :



Algal growth : µ2 determination

RSW : Reference Seawater

µ2 calculated in bioassays in ESW, is compared in PSW seawater. The difference in effect is due to the substances retained on the Sep-Pak cartridges, mainly constituted of Dissolved Organic Substance (DOS). Results are presented in variations of % µ2 for each cultivated algal species.

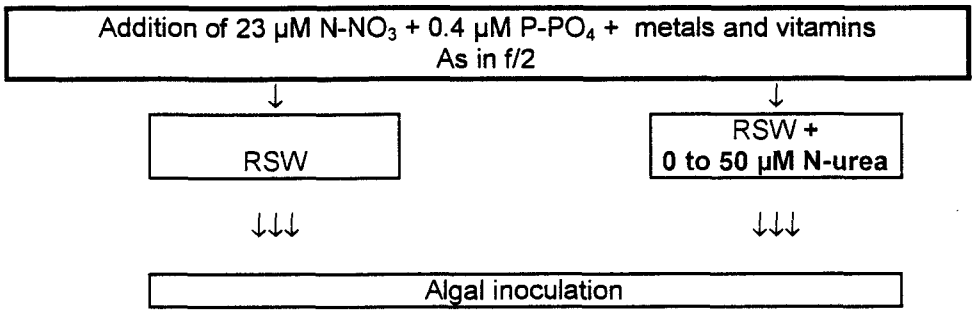


The analyses in seawater modified by animal excretions revealed in some cases, high concentrations in urea. The results of measures before/after excretion are presented in the following table :

		µM N-urea
salmon	**RSW** **ESW**	**1.76** **1.82**
sea bass	**RSW** **ESW**	**0.8** **4.0**
turbot	**RSW** **ESW**	**0.4** **1.1**

RSW : reference seawater
ESW : reference seawater with animal excretion

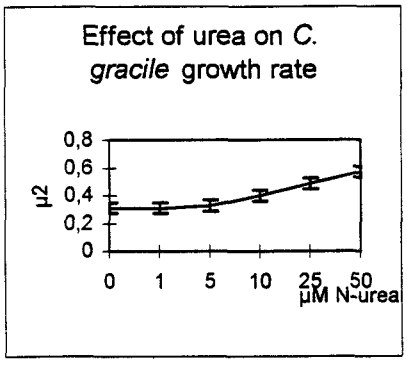
To test the effect of urea on the phytoplankton growth, this methodology was applied, based on bioassays :

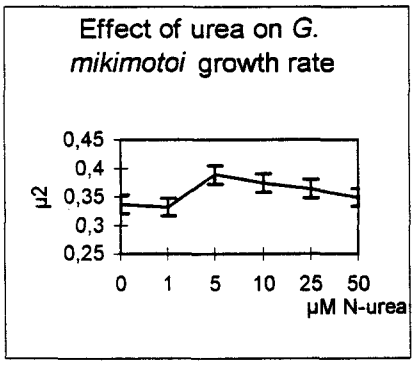


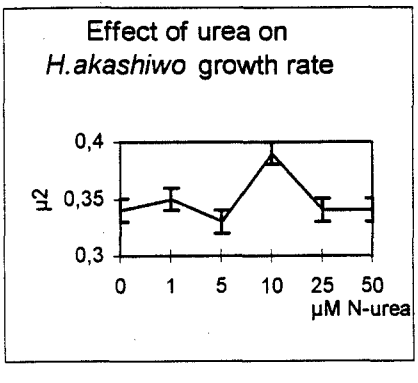
Algal growth, µ2 calculation

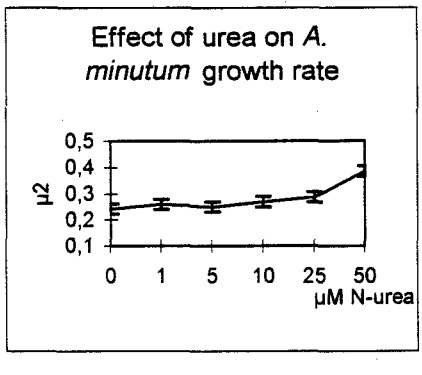
RSW = Reference seawater

The differences in µ2 growth rate are presented on the figures :





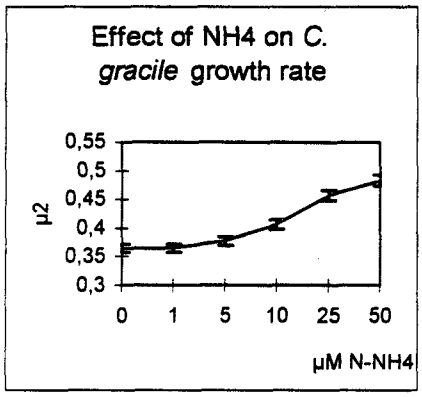


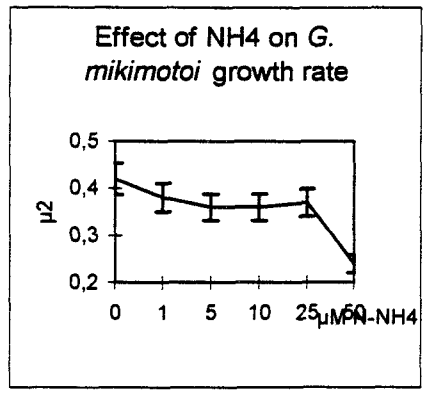


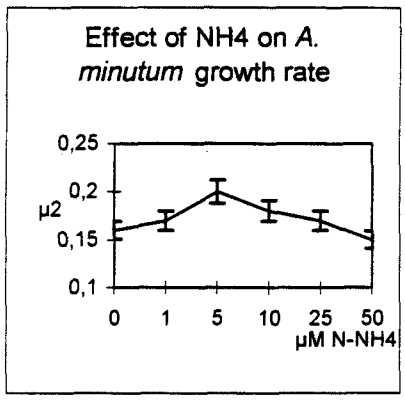
Results in chemical analyses of mineral nutrients in seawater modified by animal excretions are summarised on the following table. The difference in composition before/after excretion is mainly due to ammonium enrichment.

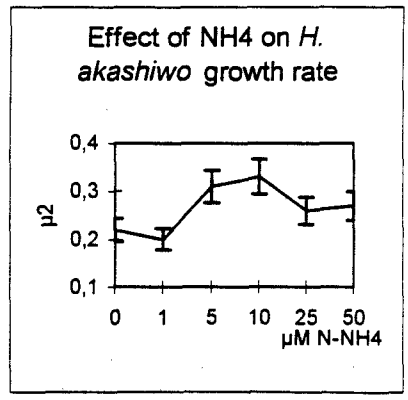
		$NH_4\mu M$	$NO_3\mu M$	$PO_4\mu M$	N/P
mussel	RSW ESW	0.3 **19.7**	0.8 3.9	0.2 2.0	5.5 11.8
oyster	RSW ESW	0.3 **32.0**	8.0 10.0	0.5 2.1	16.6 20
salmon	RSW ESW	0.7 **4.1**	0.3 0.9	0.1 0.2	10 24
sea bass	RSW ESW	0.3 **46.5**	0.7 4.2	0.1 2.3	10 22

Ammonium was the most modified parameter. The effect of ammonium was tested in bioassays, according to the urea method, and the results in phytoplankton growth rate are presented on the following figures :
Comparison of the growth rate in NH4-enriched seawater to the control is expressed as % µ2 :

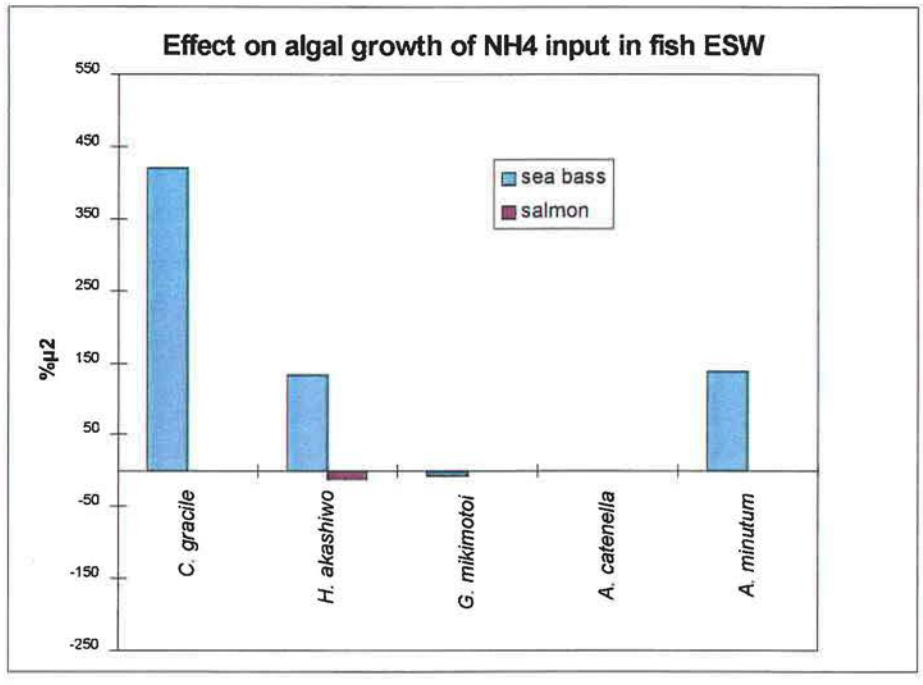








Comparison of the growth rate in NH4-enriched seawater to the controls is expressed as % µ2 :



CONCLUSION

Organic substances from animal excretions have an obvious effect on the algal growth : in major cases we observed a stimulation :

Chaetoceros gracilis,
Heterosigma akashiwo,
Gymnodinium mikimotoi,
Alexandrium minutum

Ammonium, the most important mineral associated to excretion :

- **was a potent stimulator for *C. gracilis* at high concentrations** (50 µM),
- presented a low optimal concentration for ***H. akashiwo* and *A. minutum.***

Effect of dissolved substances coming from salmon culture, on the growth of phytoplankton

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Financial support : EEC

Abstract

The salmon farming activity produces dissolved and particulate organic matter, including ammonium, uneaten food and faeces. The nitrogen compounds modify the N/P ratio in the marine ecosystem. The objective of this project is to evaluate the effect of minerals nutrients, feeds and faeces elutriate on the growth of natural phytoplankton population at different season of the year.

Biossays were preformed with phytoplanktonic population collected during spring time and summer. Different concentration of nutrients (NH_4, PO_4 y NO_3) were added in similar concentration to the observed in fish farms, faeces elutriate (8 mg/L) and feed elutriate (4 mg/L). The number of the cell was counted during seven day of culture.

In spring time, were identify 26 species, being the diatom *Skeletonema costatum* the more abundant, of less important were the prymnseiophycean *Phaeocystis sp.* and the silicoflagellates *Dictyocha sp.* and *Ebria sp.* The results show an increase in the growth of *Skeletonema costatum* in the treatment with mineral nutrients. The *Phaeocytis sp.* previously unobserved in the net pen reared system in this region show the higher number of cell in minerals nutrients and faeces. Furthermore, there was a lesser increment in *Ebria sp.* and *Dictyocha sp.* in mineral nutrients and food extract. Both species, produce fish mortality in high concentration, due to the siliceous skeletons.

In summer, were identify 30 species. The more important were diatoms such us *Chaetoceros* (90 %), *Skeletonema* (2.6 %), *Pseudonitzchia* (4.7 %), *Thalassiosira* (0.8 %) and *Detonula* (0.8 %). A lower concentration of dinoflagellates, silicoflagellates and euglenoids was noted. An increase in the growth of diatoms in all the treatment was observed with the higher value after 4 days of culture. The cultures decay after 7 day of culture. On the other hand, an important increment of a naked dinoflagellate was observed at the end of experiment, and the most important growth was observed in feed extract (30 cel/mL). This event possible indicate an heterotrophic behavior of this species.

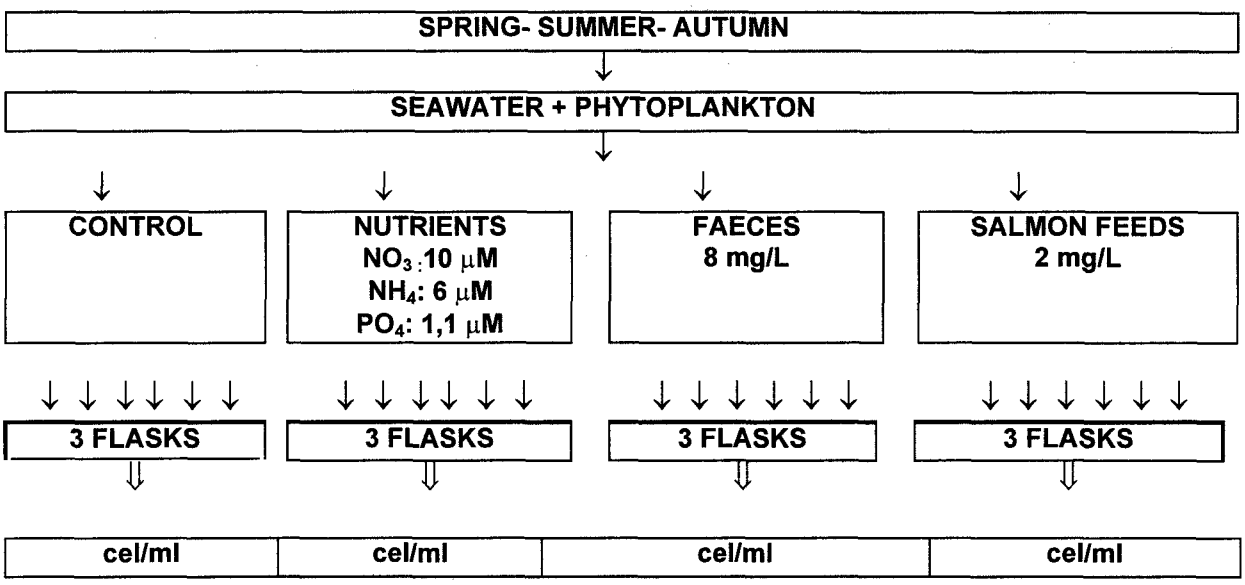
The autumn experiment will be added to this study in the near future, where a knowledge of the behaviour of the population predominated with dinoflagellates. The whole study with be usefull to understand the possible modification of phytoplanctonic structure caused by nutrients coming from the fish farming activities.

Objective

To evaluate the effects of uneaten feeds, faeces and minerals nutrients on the growth of natural phytoplanktonic population in different season of the year.

Material and Methods

To attempt this objective, bioassays on natural populations sampled in unimpacted areas (control stations) were realized, following the scheme :



The chemical composition of the different media was checked before incubation of the algae in laboratory conditions :

CHEMICAL COMPOSITION OF THE SEAWATER

SPRING

Treatment	NH_4 (µM)	NO_3 (µM)	PO_4 (µM)
control (initial)	0.43	3.9	1.97
control (final)	0.50	1.2	0.2
+ Minerals	5.75	10	1.1
+ Faeces	0.39	4.0	1.97
+ Salmon feeds	0.59	4.0	1.97

SUMMER

Treatment	NH4 (µM)	NO3 (µM)	PO4 (µM)
Control (initial)	1,5-1,6	9,1	1,9
Control (final)		2.9	0.2
+ Minerals	4.9 -5,3	11,1	2.4
+ Faeces	1,4-1,4	10.7	1.1
+ Salmon feeds	1,2-1,3	9.6	1.1

The phytoplankton composition was analysed quantitatively and qualitatively during seven days

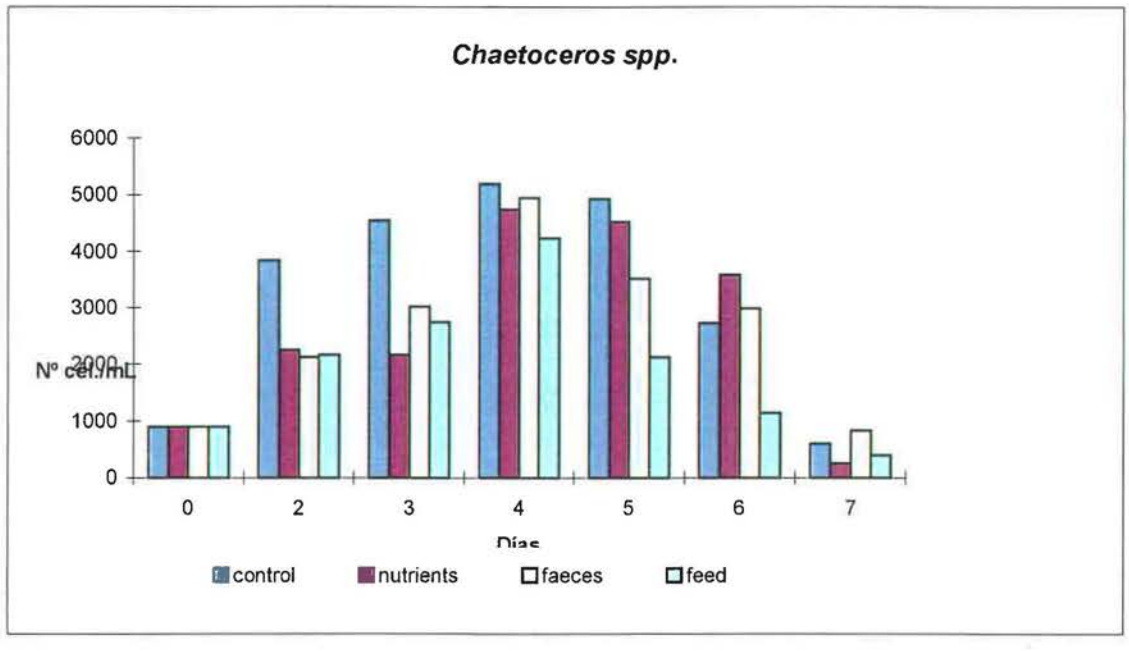
PHYTOPLANKTON COMPOSITION IN SPRING

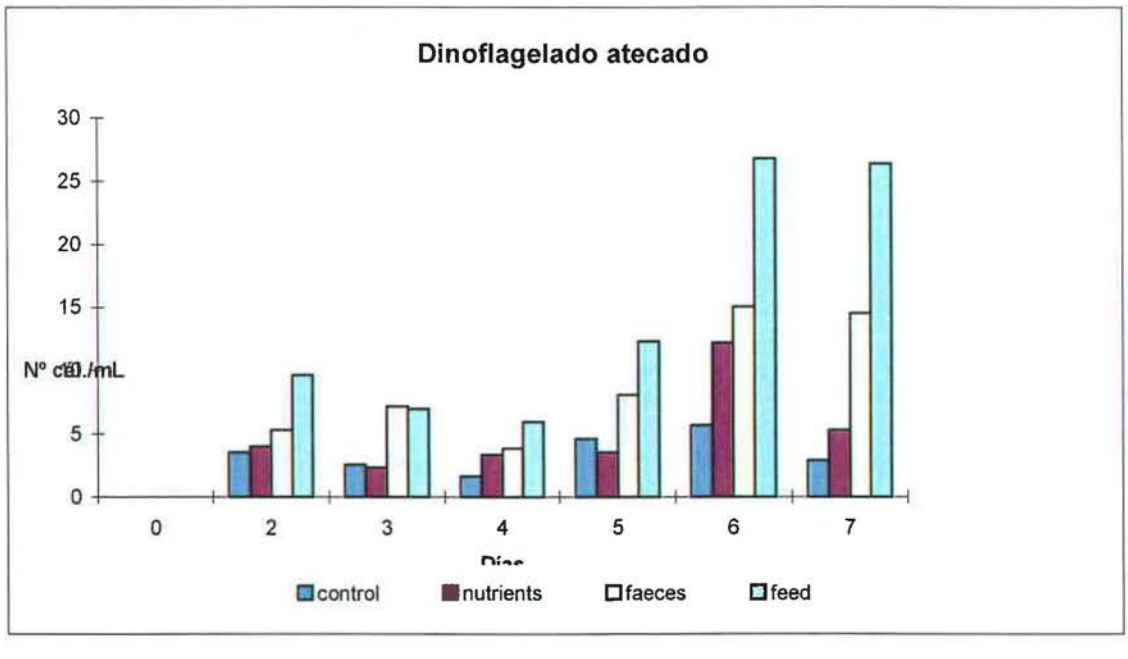
SPECIES	PERCENTAGE
DIATOMS	
Skeletonema costatum	98 %
SILICOFLAGELLATES	
Ebria sp.	0,1 %
Dictyocha	0,1 %
Total : 26 species	

PHYTOPLANKTON COMPOSITION IN SUMMER

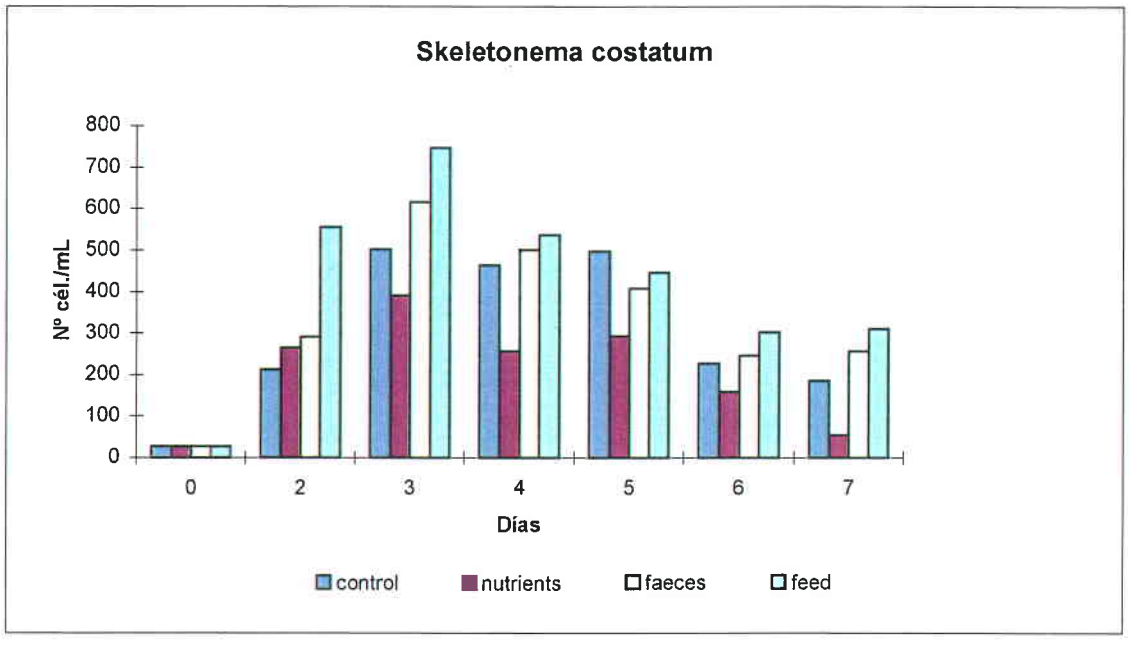
SPECIES	PERCENTAGE
DIATOMS	
Chaetoceros spp.	89,9 %
Pseudonitzschia sp.	4,8 %
Skeletonema costatum	2,6 %
Thalassiosira spp.	0,8 %
Detonula pumila	0,8 %
DINOFLAGELLATES	0,2 %
SILICOFLAGELLATES	0,1 %
Total: 30 species	

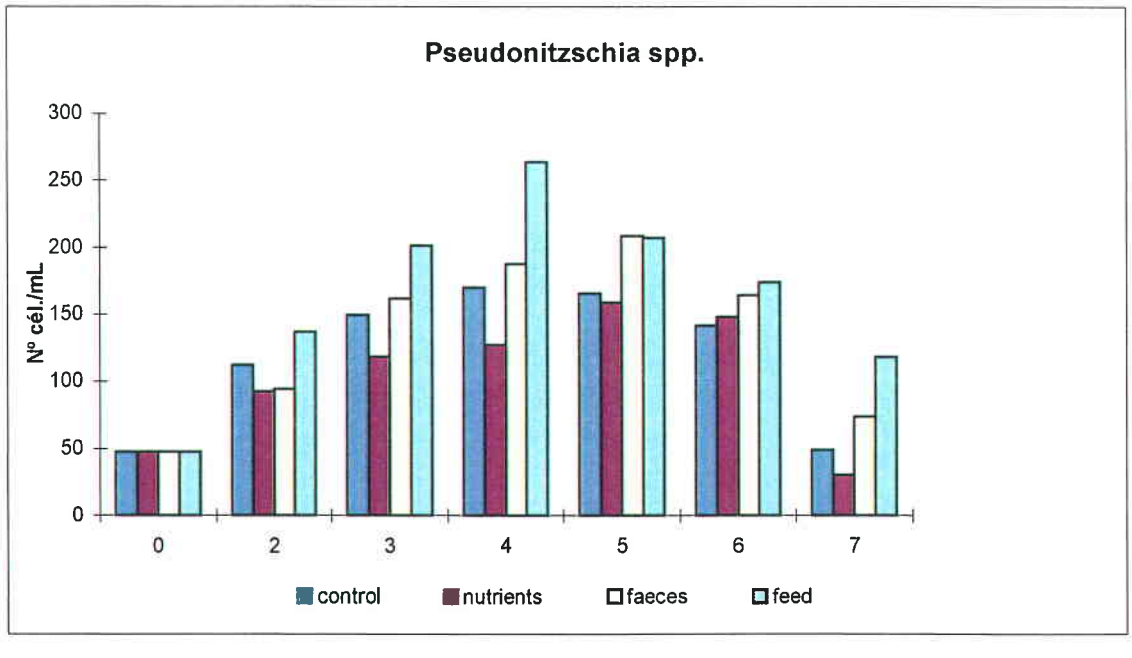
SUMMER EXPERIMENT





SUMMER EXPERIMENT





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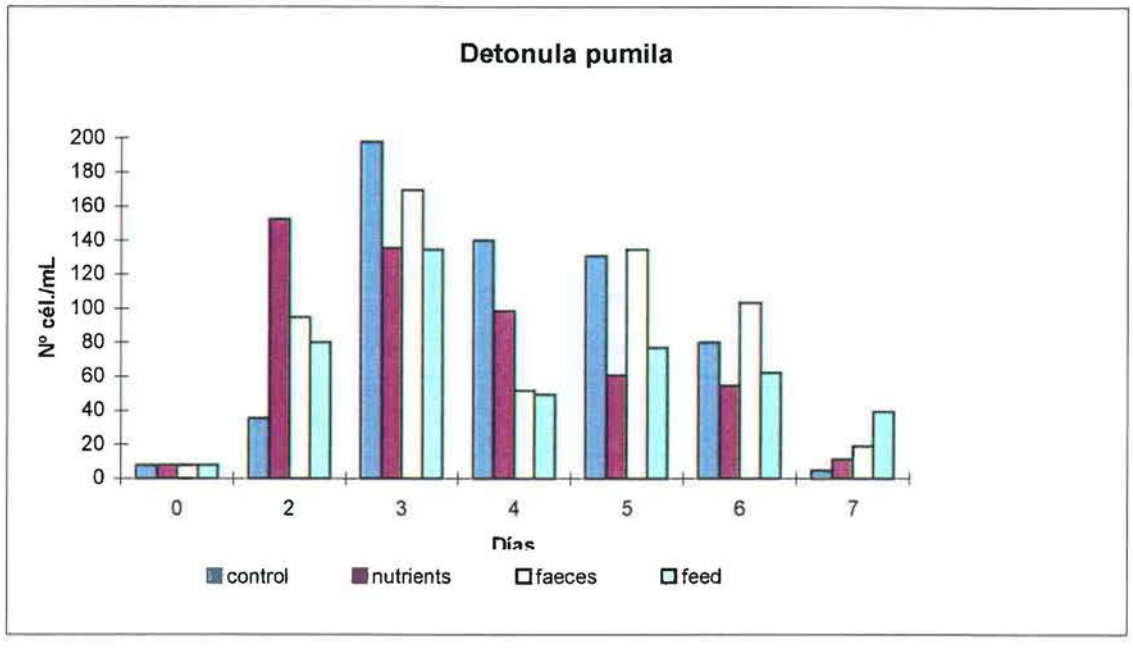

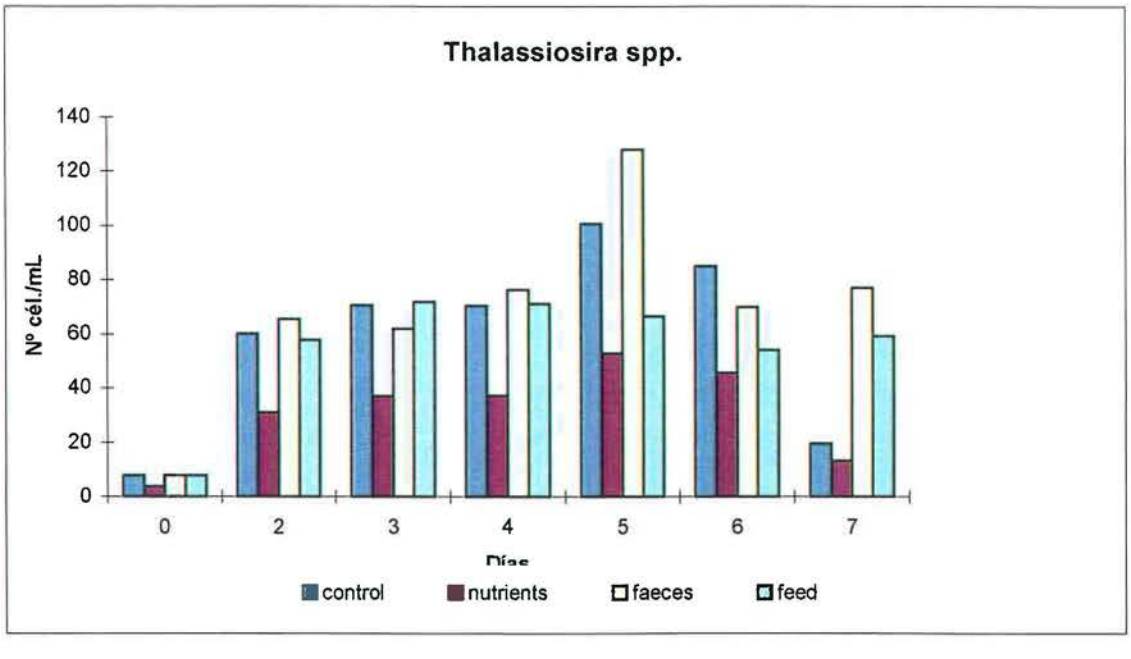

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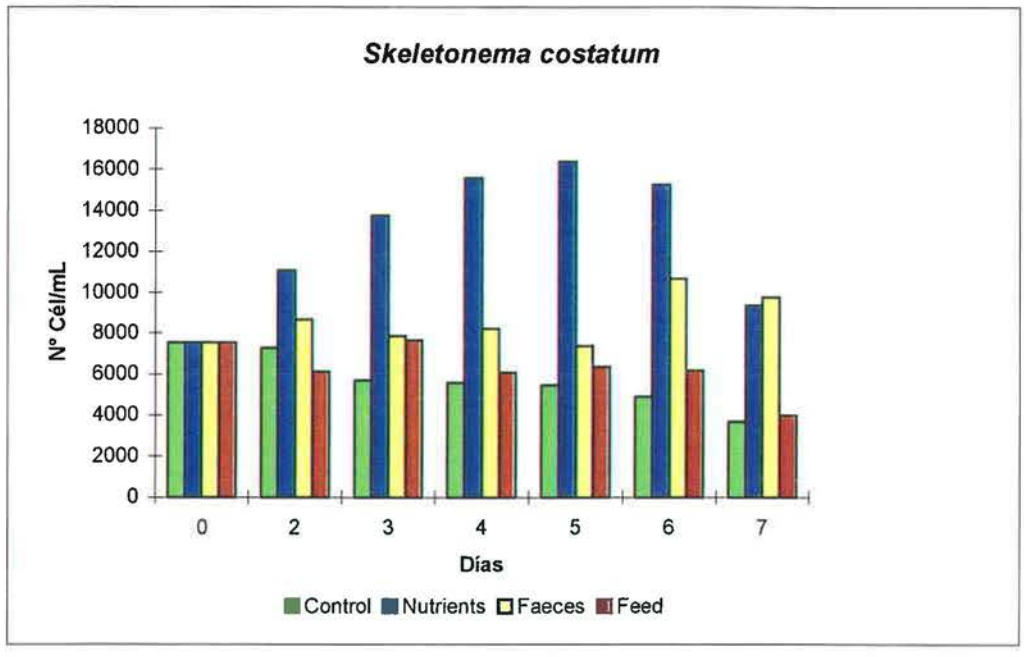

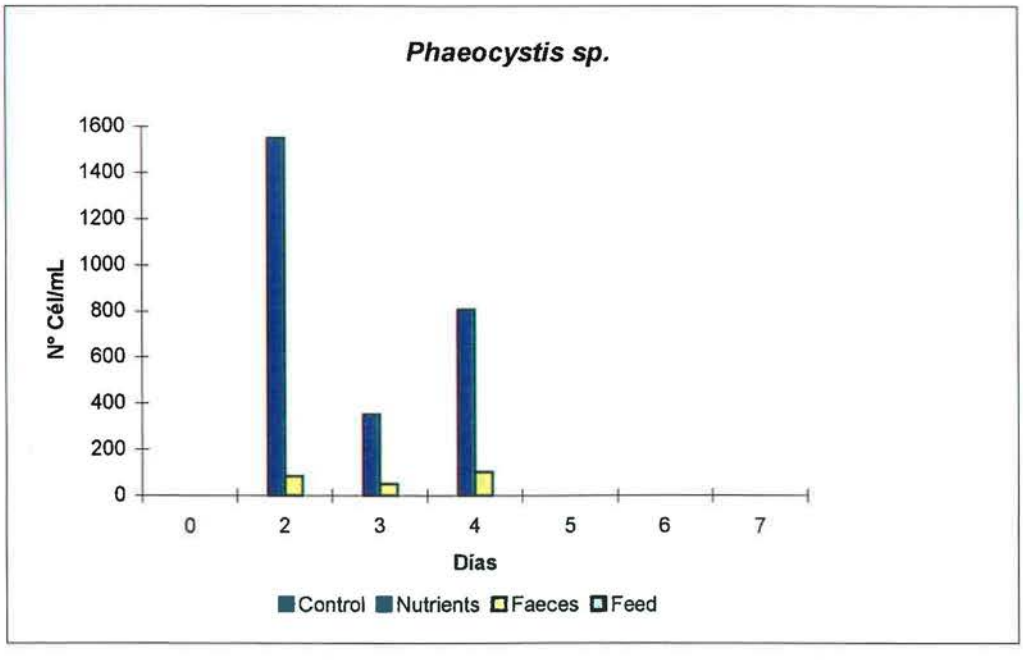

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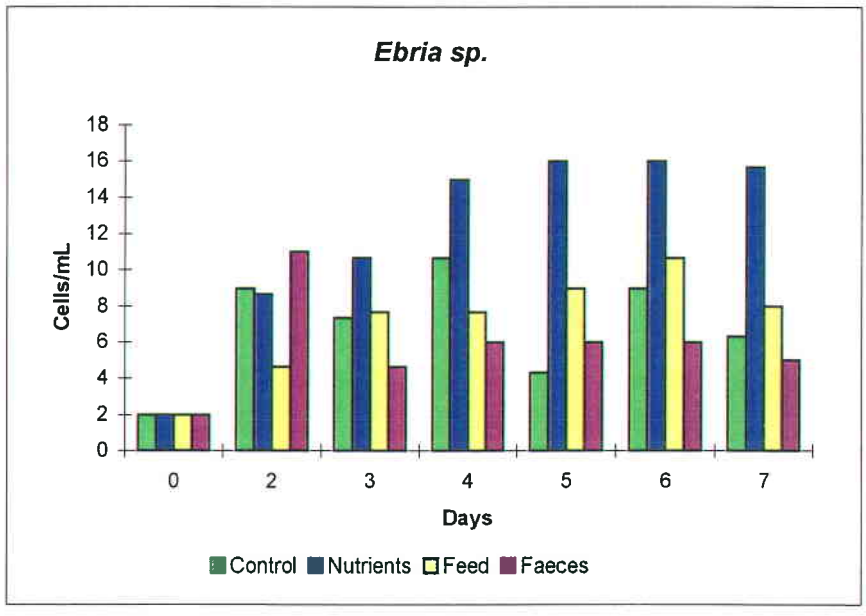

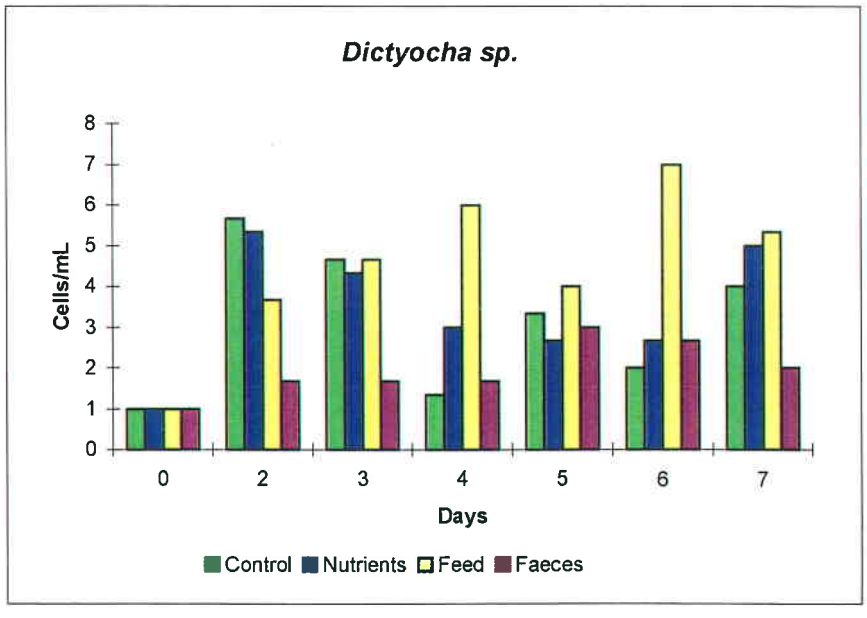

CONCLUSIONS

- In the spring experiment, there was an increase on the growth of *Skeletonema costatum* in the sea water with minerals nutrients at the similar concentration which was observed in fish farms.

- *Phaeocystis,* previously unobserved in the net pen reared system in this region area, show the higher cell concentration in minerals nutrients and faeces elutriate.

- There was an increase in the concentration of the silicoflagellates *Ebria sp* and *Dictyocha sp.* in the treatment with mineral nutrients and feed elutriated. Both species produce fish mortality at high concentration.

- In summer, there was an increase on the growth of several species of diatoms. The maximum growth was at four day of culture.

- There was an increase in the cell concentration of naked dinoflagellate after seven day of culture. The maximum cell concentration was in the feed and faeces elutriate treatment.

U-V radiation effects on phytoplankton populations

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Financial support : EEC

Abstract

Preliminary results of a field experiment to assess UV radiation and active photosynthetical radiation (PAR) on phytoplankton populations are informed. The experiment was performed between the 11th and 16th November, 1998. Design considered a completely randomized distribution, including three replicates per treatment. Treatments considered different radiation regimes and organic substances addition. Irradiation quality was established by using nylon filters (UV-b+UV-a+PAR) and astralon filters (UV-a+PAR). For organic addition salmon food was utilized. Treatment units consisted in wide mouth glasses darkened on their sides but excluding its mouths, which were covered by nylon or astralon filters.

Experiment was established on the high intertidal zone of Tres Puentes, located 5 Km North from Punta Arenas. Temperature of experimental system was maintained by a continuous seawater flux whose temperature varied between 7° and 9° C. Natural UV-a, UV-b y PAR radiation records were provided by Universidad de Magallanes and obtained approximately at 1 Km from experimental area.

Incubated phytoplankton was collected from coastal surface waters by 3-5 minutes horizontal tows by using a phytoplankton net of 22 μ. Collected sample was diluted in 30 litres of surface seawater and randomly distributed in treatment units. From each treatment unit a sample of 15 ml was collected daily for quantitative analyses and preserved with iodine solution. Cell number estimation at a specific level was done by Utermöhl method, excluding empty cells. Besides, samples for chlorophyll and nutrient estimations were collected, but results are informed in a separate paper.

Results correspond to initial phytoplankton samples (T_0), as well as for sampling for first (T_1) and last (T_5) experimental days. Initial phytoplankton (T_0) was integrated by 22 species (21 diatoms and 1 dinoflagellate) and dominated by *Chaetoceros cinctus* and *Leptocilindrus minimus* which represented 56.1 % of total phytoplankton (145,332 ± 24,585.4 cells liter^{-1}). 12 species represented between 1% and 10 % of total phytoplankton (called subdominant species). After one day of incubation, phytoplankton was different, number of dominant species increased (those with >10 % of total phytoplankton) in control units (Mann Whitney p=0,049), as well as subdominant species abundance (p=0,049) and rare species (p=0,049). If acclimmated phytoplankton (T_1) with last incubation day (T_5) are compared, none of eight variables compared presented significant differences (Kruskal Wallis P > 0,05).

Methodology

A total of six *in situ* experiments between the 28th September and the 16th November, 1998, were conducted. During this period phytoplankton incubations exposed to natural UV irradiation (UV) and photosynthetically active radiation (PAR) were performed.

The design followed a completely randomised distribution with three replicates in each of 3 treatments by using different irradiations regimes and organic additions (Fig. 1 ; Table 1).

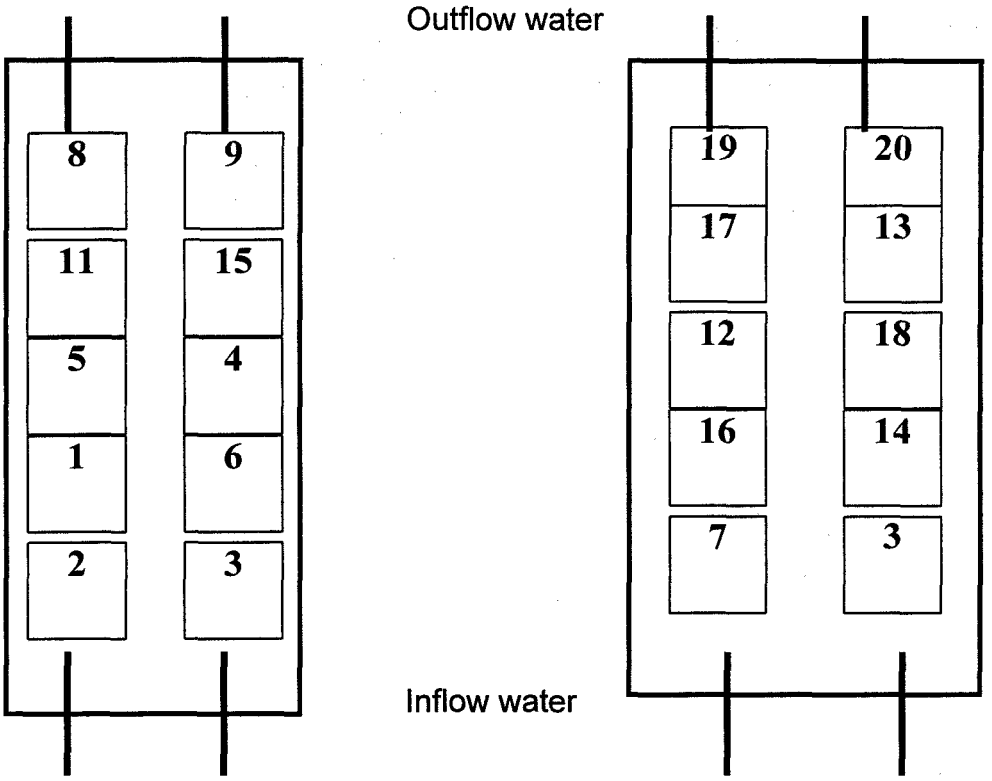

Figure 1 : Treatment disposition in each refrigerating pool (for numbers meaning see Table 1)

NUMBER	TREATMENT
1 - 2 - 3	PAR + UV-a + UV-b
4 - 5 - 6	PAR + UV-a
7 - 8 - 9	"pellet" + PAR + UV-a + UV-b
10 - 11 - 12	"pellet" + PAR + UV-a
13 - 14- 15-16	PAR + UV-a + UV-b *
16 - 17- 18-19-20	PAR + UV-a *

Table 1 : *In situ* phytoplankton incubations : number of each experimental unit according to its irradiation and organic addition treatments. PAR= photosynthetic active irradiation ; UV-a and UV-b= ultraviolet irradiation ; "pellet" = food supply used in salmoniculture.

* Experimental units orientated to Dr. Carreto's group. In this case, final filtered sample was 1-2 litres for chlorophyll plus 1-2 litres for mycosporine like pigments.

The irradiation quality was established with Nylon filters (UV-b+UV-a+PAR) and Astralon filters (UV-a+PAR). For organic additions, pellets for feeding salmon in raft cultures, were utilised. 2 grams were grained and diluted in 100 ml of filtered sea water for a period of one hour at ambient temperature. This volume was rised to 500 ml, previous filtration on GF/F filters, which correspond to 4 g l^{-1}. 1 ml of this solution corresponds to 4 mg of food supply. A volume of 1 ml of this solution was added to each of six of 18 experimental units.

The transmittance spectra for Nylon and Astralon filters to establish UV-b+UV-a+PAR and UV-a+PAR irradiations treatments, respectively, were realised in a Shimadzu UV-VIS 1203 spectrophotometer (Fig. 2).

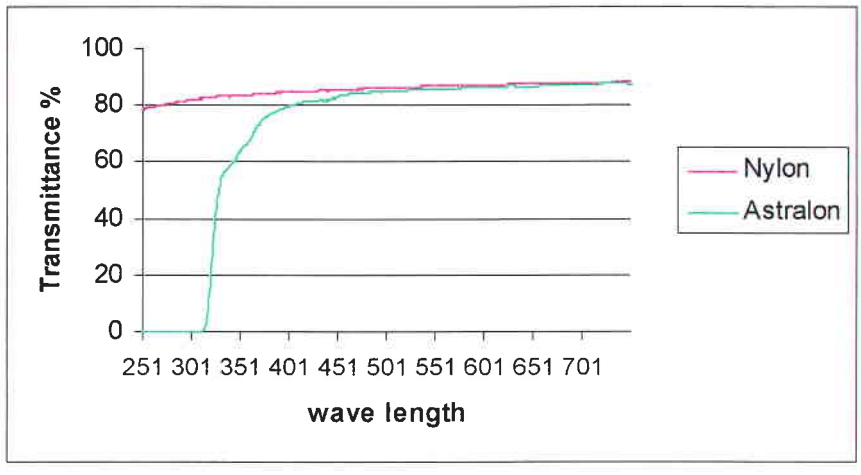

Figure 2 : Transmittance curves for Nylon and Astralon filters used in phytoplankton experiments

Excluding the first experiment that was conducted in a floating system anchored 50 m from the coastline, the rest of the experiments were laid in two black painted plastic pools established on the higher zone of a pebble-sandy beach located 5 Km North from Punta Arenas in a place called Tres Puentes. Temperature of experimental system was maintained with continuous running sea water provided by a processing plant (PESCA CHILE). Water is pumped from the coastal area and re-entered to sea through an open pipeline from which sea water was taken.

The temperature of one of the refrigerating pools was controlled by using a BENKO temperature recorder programmed to measure sea water temperature every 10 minutes. The processing plant treated this water with chlorine, but it was not in direct contact with treatment units. Since refrigerating water was continuously taken from coastal waters, sea water temperature followed the same diurnal variation pattern, although it was 0.5-1.0° C higher.

Incubated phytoplankton was collected from coastal waters by horizontal net tows and diluted with surface waters to a volume of 30 litres. This batch of water was randomly distributed to 18-20 buchner glasses vessels (Becher type) of 2 litres each. Each experimental unit was covered with black plastic excluding its mouth, where Nylon or Astralon filters were placed, to assure direct or disperse sun light and protect them from lateral illumination. Every experiment was planned for a period of six days.

Quantitative phytoplankton samples were taken every day by aid of a syringe in a volume of 15 ml and preserved with iodine lugol solution. Estimation of cell numbers was done at a specific level using Utermöhl method (1958). Empty cells were excluded from counting.

Pigments : chlorophyll was estimated on the first day (T_0) and final day (T_5), mineral nutrients : NO_3, NO_2, NH_4, Si and PO_4, total nitrogen and total phosphorus were analysed and presented in the same seminar by Gemita Pizarro.

Natural UV and PAR irradiations records were provided by the UV-a, UV-b and PAR monitoring station that belongs to Universidad de Magallanes and established about 1 Km South East from the experimental area. Also UV-b and UV-a of portable width band sensors were calibrated with recorded information at this station.

Treatment units from number 13 to 18-20 were orientated to mycosporine like pigments analyses, task that will be performed by Dr. José Carreto's group from INIDEP, Argentine. Activities at Punta Arenas included only *in situ* incubations and sea water filtrations after experiments were finished (day number 6). Filters were maintained at -20° C until its transportation to Argentine.

Phytoplankton species evolution

Quantitative phytoplankton results obtained in experiment number 6 (11 to16 November) are : (T_0) at the beginning of experiment, as well as quantitative results for the first (T_1) and last day (T_5) of field experiment. T_0 phytoplankton was integrated by 22 species, 21 diatoms and 1 dinoflagellate. From the quantitative point of view, phytoplankton was dominated by two species : *Chaetoceros cinctus* and *Leptocilindrus minimus* representing 56,1 % of total phytoplankton (145.332 ± 24.585,4 cell liter^{-1}). A group of 12 species representing between more than 1 % and less than 10 % of total phytoplankton was observed. Although no statistical analysis have been applied to data, after one day of incubation, quantitative phytoplankton seems to be different, in terms of dominant species, which increased to 4-5 species in all treatments, since when experiment was finished (T_5) dominant species were 3-4 species. Also it is clear that total number of cells at the end of the experiment seems to be different depending on applied treatment.

Replicates exposed to PAR+UV-a+UV-b irradiations showed a decrease in total number of species and particularly in total cells number. Glasses treated by PAR+UV-a showed a slight decrease in species number and a similar total quantitative phytoplankton between both periods of incubation. Replicates treated by "pellet"+PAR+UB-a+UV-b experimented a clear increase in total cells number, since species number decreased from 22 to 19 species. Total cells number increase is mainly explained by dominant species increment (*Chaetoceros cinctus*, *C. didymus* and *Leptocylindrus minimus*), which at the end of this treatment represented 73,6 % of total phytoplankton. Glasses treated by PAR+UV-a showed an increase in total cells number and a decrease in species number. At the end of the experiment (T_5), in all treatments applied, cells were in bad physiological conditions, since its pigmentation was a palish green or green yellow.

Discussion

Is it possible to define an impacted area, using the described parameters ?

The described parameters concerning the impacted benthic compartment are :

- lower pH (<7)
- lower redox potential (0mV)
- lower concentration in dissolved oxygen (0mg/l)
- increase in particular organic carbon rate (~ 1-4 %)
- presence of bacteries (sulfatoreductors) in the azoic area
- presence of *Nassarius*

The described parameters concerning the impacted pelagic compartment are :

- high amounts in large size sestonic particles (>210µm)
- lack of relationship between particles size classes and chlorophyllian fluorescence
- lower chlorophyllian fluorescence
- higher concentrations in ammonium (~ 4-6 µM N)

The water column appears less impacted than the benthic compartment, due to the high volume, influenced by the currents. In contrast, the sediment accumulates all substances of the impacted environment, and constitutes a « memory » of the passed.

The more representative parameter for aquacultural impact in the water, could be the ammonium, but this substance is also produced by other activities. The use of ^{15}N could be a technic for aquacultural waste detection. The redox potential measurement in sediment appears a good indicator of impacted area. A biological indicator such as *Nassarius*, or a bacteria : *Beggiotoa*, could be proposed.

It appears difficult to propose only one indicator now : there are numerous parameters and each case is particular.

Bioturbation constitutes a natural limitation of impact, with participation of demersal fish and *Nassarius*. Oxygen and dissolved organic substances exchanges between benthic and pelagic sectors allow improvement in the quality of environment. However, the total substances input does not decreased, and the areas with deep bottom are difficult to monitor.

The alteration of the bottom due to aquacultural activity can be observed directly (aspect, gases production), and changing site of fish farm solves the problem. Fish disease constitutes the main factor for such a decision.

A best knowledge in currents, and good choice is cages positions (with respect to particles dispersion : Martin Hevia's model) could be a good prevention from site degradation. Generally, the importance in sediment-water column exchanges are not well defined concerning gases, antibiotics and mineral substances. It will be interesting to analyse phosphate release from sediment in presence of low potential redox, under the cages.

The quality of environment where fish are growing is taken into account in the market of aquaculture, and all factors have to be considered, especially the use of antibiotics.

Can dense populations of marine animals induce modifications in phytoplanktonic populations ?

The results show the different sensibilities of the algae, to the presence of enrichment due to the animal excretions. The behaviour of the phytoplankton in our experiments can be considered as a reflect of ability to face perturbations, as f/2 medium is used in routine for the strain growth in the laboratory.

Are the results in accordance with *in situ* observations ?

The algal populations growths in presence of substances provided by aquacultural activities, reflect modifications we could observe in the field. The concentrations used in the experiments were similar to the real situations.

The experimental volume in the test on UV radiations is the only difference, and results have to be considered during a short duration time.

Can we define a relationship between algal blooms and aquaculture ?

Now we can observe an increase in phytoplankton blooms occurrence, but it is not possible to define a relationship with aquaculture. Other phenomenon seem more determinant : meteorology, climatical modifications (cf. the *Gymnodinium* sp. bloom). The substances provided by aquaculture and favourable to the algal growth could contribute to increase the blooms.

Energy plus nutrients are major factors for algal growth, but anthropogenic factors interfere, associated with aquaculture.

UV-b radiation effect on dissolved organic substances during *in vitro* experiments

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Financial support : EEC

Abstract

Dissolved organic substances from a senescent diatom culture, fish granule elutriate and fish faeces elutriate are degradable by chemical oxidation, hydrolysis and photolysis under natural irradiation.

The main substances resulting in this degradation are mineral nutrients, assimilated by chlorophyllian organisms during photosynthesis. The aim of the study is to estimate the possible sea water enrichment in mineral nutrients coming from dissolved organic substances, under *in vitro* irradiation by UV-a and UV-b (15 W, 1 mW cm^{-2}, 45 h). Total mineralisation was obtained by 900 W –lamps irradiation during 16 h.

The maximal mineralisation rate of nitrogen was obtained in the senescent diatom culture : 57.9 % (1.0µM h^{-1}), then the granule elutriate : 44.7 % and the faeces elutriate : 34.2 % (0.7 and 0.8 µM h^{-1} respectively). The more potent dissolved organic nitrogen (DON) producer was the diatom culture (26 070 µmol N g^{-1}) then the granule elutriate (1 460 µmol N g^{-1}) and finally the faeces (17 µmol N g^{-1}).

The mineralization rate for phosphorus was higher in faeces elutriate : 40 % (2 µmol P g^{-1}) than in granule : (31.4 % (45 µmol g^{-1}), and no significant in the senescent diatom culture.

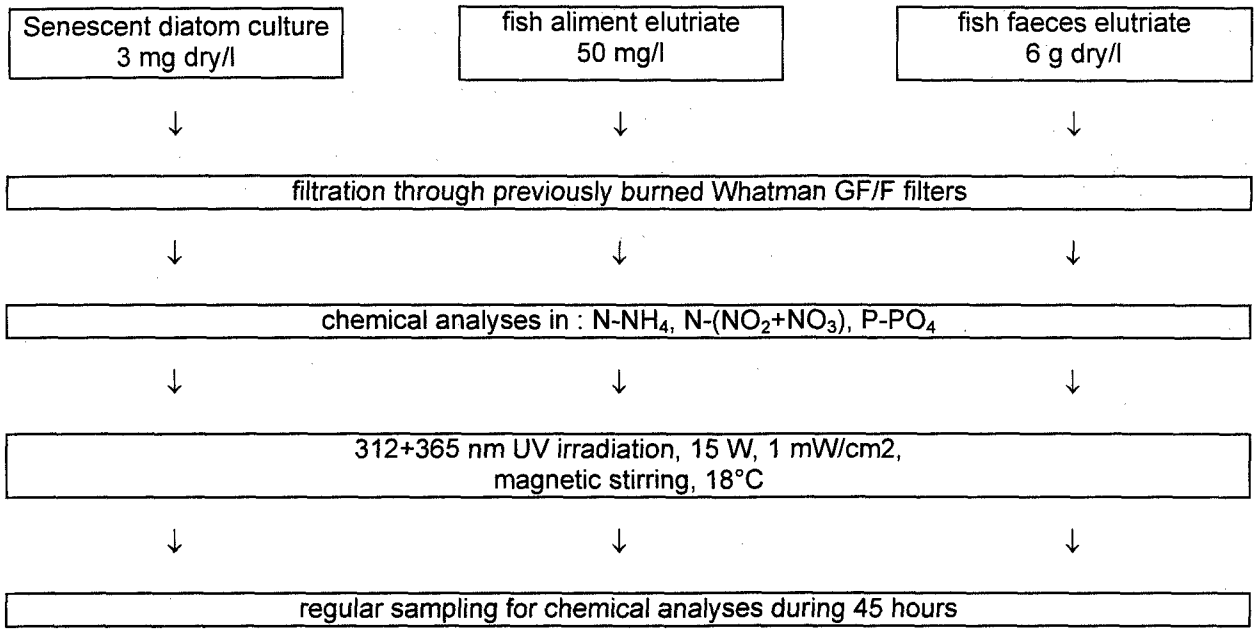
At the beginning of our experiment nitrate was the most abundant nitrogenous salt in the diatom culture (7.8 µM) and granule elutriate (7.4 µM). After 8 to 10 hours irradiation, ammonium was the most important, forming 80 % of the total mineralised nitrogen in the three solutions.

OBJECTIVE

Estimation of the possible enrichment in mineral nutrients for phytoplankton, by the way of *in vitro* photodegradation of dissolved organic substances

METHOD

The method was based on artificial UV-(a+b) irradiation of three seawater solutions, where dissolved organic substances from three different origins were diluted.



Estimation of total photomineralizable nutrients : intense UV radiation 900W Hg lamp (Haereus apparatus) during 16 hours, after addition of 0.1 % hydrogen peroxide

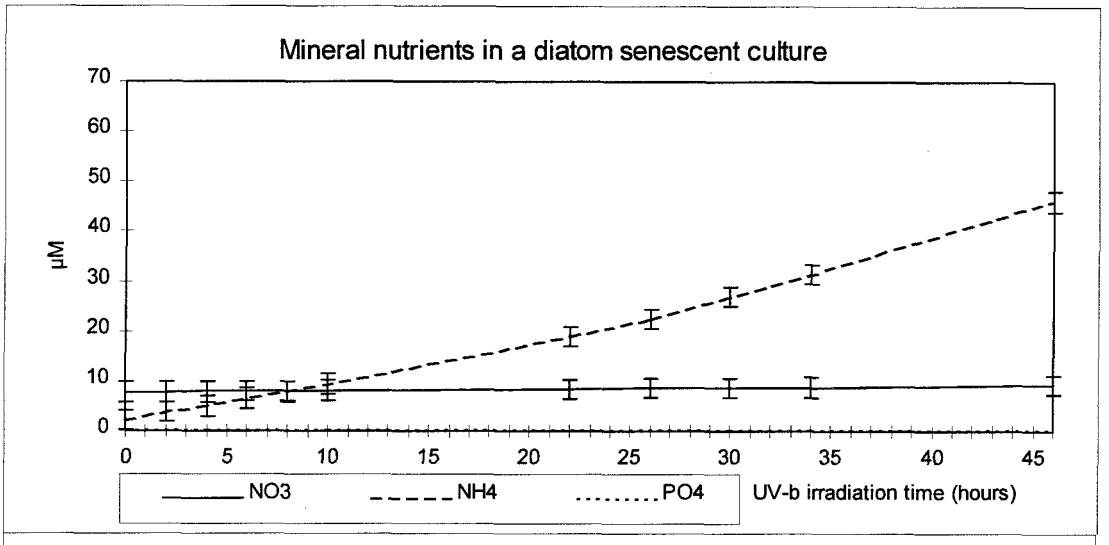
RESULTS

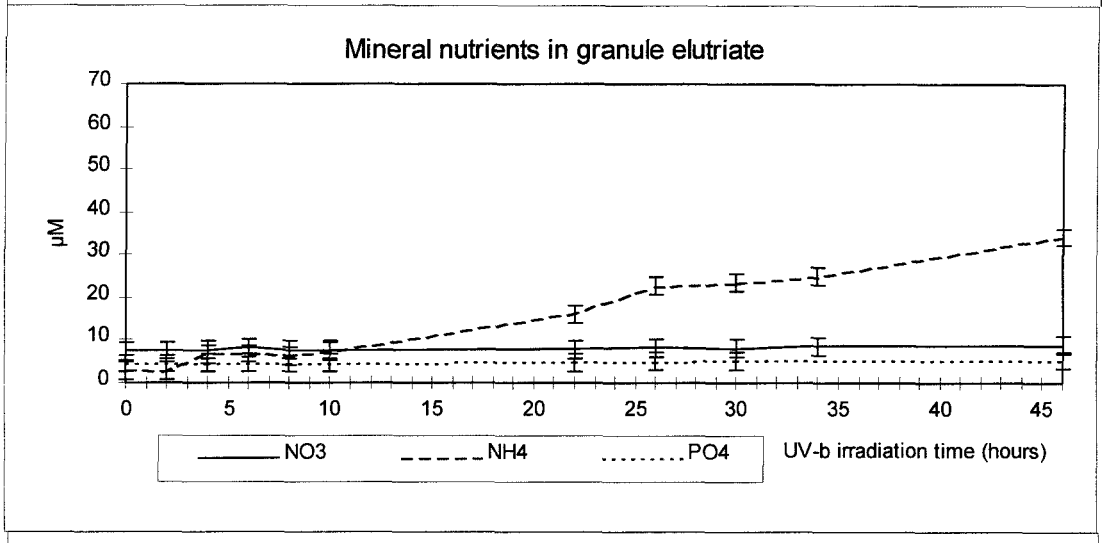
The results in chemical analyses of mineral nutrients are treated, considering :

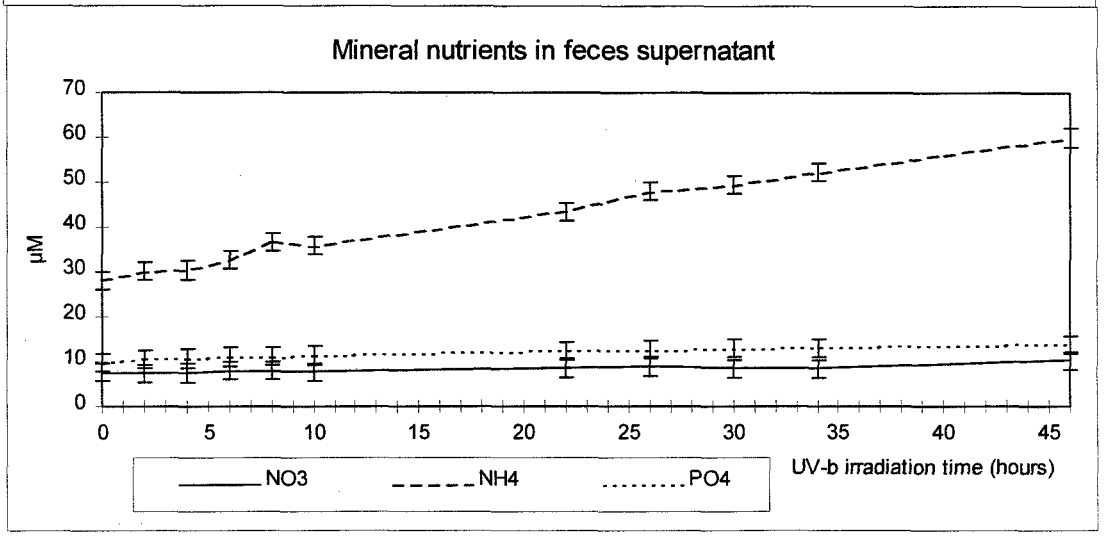
A : variation in mineral concentrations

B : variation in percentage of total mineralizable

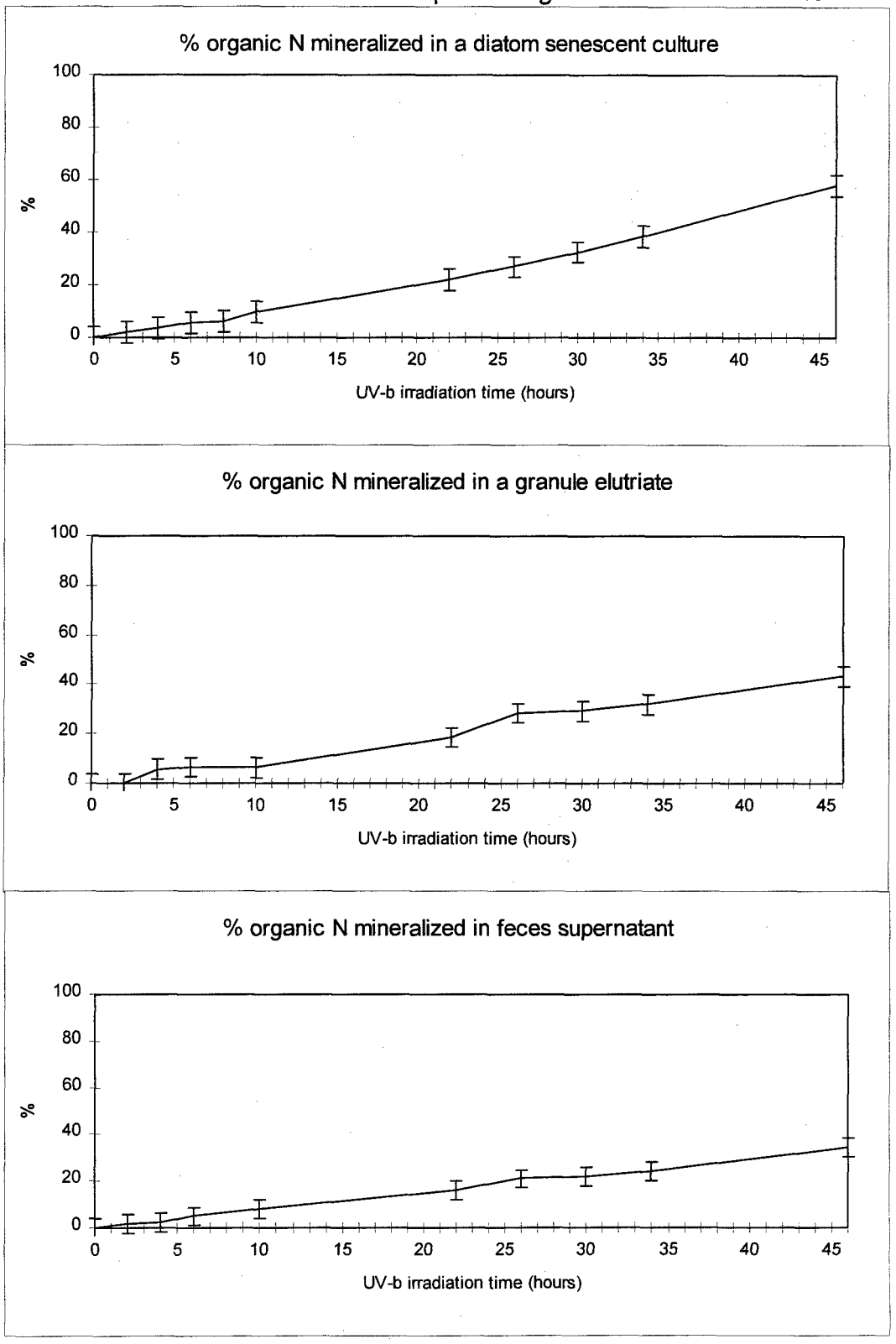
A : variation in mineral concentrations

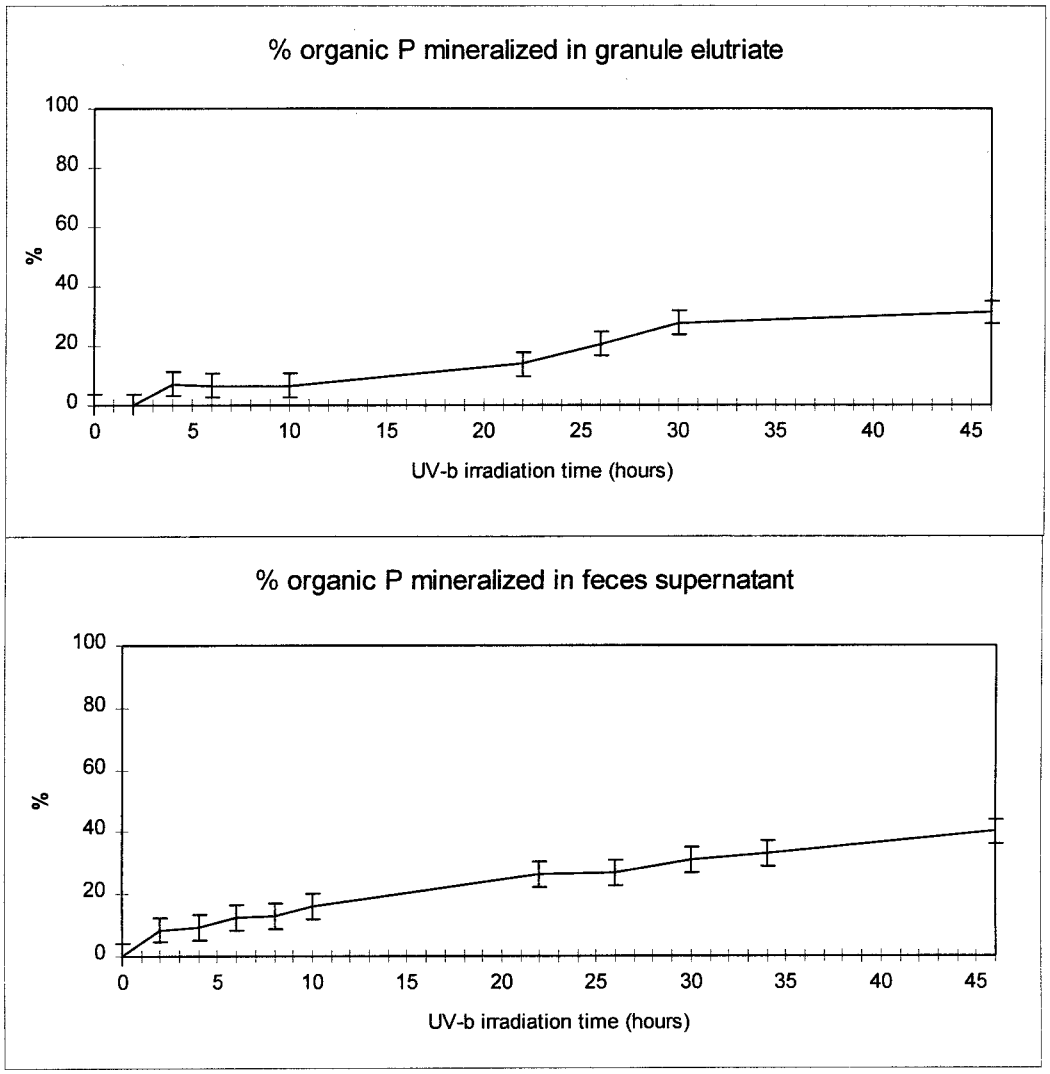






B : variation in percentage of total mineralizable





Variations in the diatom culture were not significative.

COMPARISON OF PHOTODEGRADATION RATE OF DON AND DOP in µM N and P per h, respectively.

photodegradated during 45 h ↓	senescent diatom culture 3 mg dry/l	fish aliment elutriate 50 mg/l	fish faeces elutriate 6g dry/l
DON	1.01±0.14	0.73±0.17	0.77±0.45
DOP	0	0.02±0.01	0.09±0.04

COMPARISON OF POTENTIALLY PHOTODEGRADABLE DON AND DOP

in µmol N and P per g, respectively

totally photodegradated ↓	senescent diatom culture 3 mg/l	fish aliment elutriate 50 mg/l	fish faeces elutriate 6 g dry/l
DON	26 067±0.14	1462±0.17	17±0.45
DOP	0	44.6±0.01	1.7±0.04

CONCLUSION

Photodegradation increased significantly the amounts in available mineral nutrients for vegetals, **mainly NH_4.**

Ammonium constituted **80 %** of the total mineral nitrogen after 46 h irradiation.

The **chlorophyllian cells** were the most potent and rapid provider **in mineral nitrogen**, before feed then feces elutriates.

The mineral phosphorus production was potentially higher in fish feed elutriate, but more rapid in faeces elutriate, and no significant in the phytoplankton cells.

The effect of ultraviolet – b radiation in dissolved nutrients in seawater and its relation with chlorophyll - a variation of phytoplankton assemblage

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Financial support : EEC

Abstract

A field experiment located on the intertidal zone (11^{th}-16^{th} November, 1998) whose objective was to establish the effect of natural ultraviolet B radiation (UV-B), on the concentration of active chlorophyll *a* and phaeopigments of phytoplankton assemblage, as well as dissolved nutrients in seawater (nitrates, phosphates, nitrites, silicates, total nitrogen and phosphorus).

Sea water was collected in the Strait of Magellan in a site distant 5 Km from Punta Arenas city. Sea water was placed in two litres wide mouth glasses covered by appropriated filters, considering three replicates per treatment. Two treatments of solar radiation were established: One including UV-B (with Nylon filter) and another excluding it (with Astralon filter). Besides these treatments, another was applied consistent in the addition of salmon food in each radiation treatment. Temperature varied within ±1°C and controlled by a constant water flow.

The concentration of chlorophyll *a* (Cl*a*) and nutrients were measured at the beginning (T_0) and the sixth day (final of the experiment, T_5). The determinations of Cl*a* were carried out according to Lorenzen's methodology (1972) and nutrients according to Strickland and Parsons (1968), Parsons et al. (1984) and Valderrama (1986). The results analyses were carried out by means of nonparametric Mann-Whitney test, in order to establish significant differences between control (T_0) and each measured variable during the sixth day (T_5) independently of applied treatment. It also was carried out a two factor nonparametric Analysis of Variance in order to establish the effect of radiation (with and without UV-B), food (with and without food) and its interaction, at the sixth day of experiment (T_5) for each considered variable.

The results indicate significant differences between T_0 and most of the controlled variables at T_5, excepting active chlorophyll *a* and total phosphorus. It were found non significant UV-B effects, in salmon food treatment, neither between the interaction of these two factors.

Results in relation to historical measures of UV-B radiation are discussed, including chlorophyll *a* and nutrients registered in other bays located in the Strait of Magellan, near to the place where seawater was collected for performed experiment.

Methods

The samples were collected in the incubators submitted to UV and PAR irradiations, in the same time sampling for phytoplankton species.

Pigments : chlorophyll was estimated on the first day (T_0) and final day (T_5). Water was filtered by using Whatman GF/F filters (or equivalents MFS075) and freesed under -20° C if not processed immediately. Pigment extractions were made with 90 % acetone. Extracts were kept in darkness for 24 hours at 4° C and filtered through glass fibber filters and read in a spectrophotometer Shimadzu UV-1203. Chlorophyll **a** concentrations were estimated according to Lorenzen (1972).

Nutrients : NO_3, NO_2, NH_4, Si and PO_4, and total nitrogen and total phosphorus concentrations were determined following Strickland & Parsons (1968), Parsons *et al.* (1984) and Valderrama (1986).

Results in pigments evolution

Chlorophyll **a** and phaeopigment concentrations are presented in Table 1. Active chlorophyll increased in the treatment PAR+UV-a, independently of nutrients addition ("pellets"). However, this increment was more marked in those experimental units treated with "pellets", suggesting an interactive effect between type of radiation and chlorophyll concentration. UV-b effect causing a decrease in chlorophyll concentration was evident in relation to T_0. An expectable increase in phaeopigment concentrations in all treatment units was observed when estimations were compared to T_0 concentrations, and representing approximately a 36 % of total chlorophyll. Nevertheless, it is important to point out, that phaeopigment concentrations showed a slight variation between treatment units, independently of treatments received by experimental units, as radiation quality and nutrients addition ("pellets").

T_0 11th November 1998		
	x	s.d.
Chl a mg m^{-3} (active)	5,61	0,57
Phaeopigments	1,36	0,37

T_5 16 th November 1998				
Without pellets	**UV-b+UV-a+PAR (glasses 1-2-3)**		**UV-a+PAR (glasses 4-5-6)**	
	x	s.d.	x	s.d.
Chl a mg m^{-3} (active)	5,16	1,54	5.52	0,82
Phaeopigments	3,06	0,45	3,58	0,24
With pellets	**UV-b+UV-a+PAR (glasses 7-8-9)**		**UV-a+PAR (glasses 10-11-12)**	
	x	s.d.	x	s.d.
Chl a mg m^{-3} (active)	5,16	0,62	6,59	1,34
Phaeopigments	2,81	1,23	3,63	1,36

Table 1 : Chlorophyll a and phaeopigment concentrations estimated for treatments in experiment number 6 (11-16 November).

Results in nutrients evolution

Results on dissolved nutrients concentrations and total phosphorus and total nitrogen are presented in Tables 2a-2b and 3. Independently of treatment received by experimental units, nutrient concentrations of NO_3, NO_2, NH_4 and PO_4 were similar or diminished in comparison to T_0 nutrient estimations. Only silicates concentration (Si) increased if compared to T_0, particularly in treatments UV-b+UV-a+PAR (nylon filters) probably due to glass condition of experimental units.

			NO_3			NO_2			NH_4		
	Sample	Radiation	µM NO_3	x	s.d.	µM NO_2	x	s.d.	µM NH_4	x	s.d.
T_0	0m		2,225			0,012			2,100	1,295	1,139
	Without pellets								0,490		
	0m		1,453			0,012			0,848	0,758	0,127
	With pellets								0,669		
Without	Glass 1	UV-b+UV-a+PAR	0,568	0,606	0,107	0,001	0,004	0,007	0,000	0,000	0,000
Pellets	Glass 2	UV-b+UV-a+PAR	0,727			0,012			0,000		
	Glass 3	UV-b+UV-a+PAR	0,523			0,001			0,000		
	Glass 4	UV-a+PAR	0,659	0,674	0,026	0,012	0,012	0,000	0,132	0,088	0,076
	Glass 5	UV-a+PAR	0,704			0,012			0,000		
	Glass 6	UV-a+PAR	0,659			0,012			0,132		
with	Glass 7	UV-b+UV-a+PAR	0,682	0,659	0,039	0,012	0,012	0,000	0,132	0,609	0,826
Pellets	Glass 8	UV-b+UV-a+PAR	0,682			0,012			0,132		
	Glass 9	UV-b+UV-a+PAR	0,613			0,012			1,564		
	Glass 10	UV-a+PAR	0,795	0,651	0,125	0,012	0,012	0,000	0,669	0,430	0,273
	Glass 11	UV-a+PAR	0,591			0,012			0,132		
	Glass 12	UV-a+PAR	0,568			0,012			0,490		

Table 2a : Dissolved nutrient concentrations for experiment number 6. Nitrates NO_3 ; nitrite NO_2 and ammonium NH_4

When nutrient concentrations are analysed in relation to time variable, NO_3 showed similar estimations in the four treatments.

NO_2 showed a similar concentration through time in units treated with Nylon and Astralon filters plus "pellets" and also in Astralon unit without "pellets". However, the concentration of this nutrient markedly diminished in Nylon unit (UV-b effect) without "pellets" addition.

Ammonium showed a high concentration in Astralon and Nylon experimental units including "pellets" and similar to T_0 values. On the other hand this nutrient clearly diminished in unit treated without pellets independently of radiation quality. These results are showing that pellet addition is important to understand the ammonium temporal variation. Also the natural concentration of this nutrient in surface waters is not detectable with the applied technique analysis.

PO_4 concentration values were lower in units treated without "pellets", suggesting an UV-b radiation effect in waters with low phosphates content. For a closer site where the experimental area was established, a 0,6-0,7 µM concentration in surface waters for the summer period (February) have been determined (Data Report National Scientific Commission of Italy to the Strait of Magellan, 1992). In this document are also presented data on nitrates and nitrites, ranging between 1,7-3,2 and 0,11-0,14 µM, respectively.

N and P total concentrations diminished in both treatments (with and without "pellets") when compared with T_0 estimations. Nevertheless, when time is taken in consideration, UV-b treatments presented a similar or a lower concentration than Astralon treated units. On the other hand, when comparison are made taking in consideration "pellets" addition these treated unit showed a higher or similar N and P total concentration.

			Si			PO4		
	Sample	Radiation	µM Si	x	ds	µM PO_4	x	ds
T0	0m Without pellets		0,102			0,239		
	0m With pellets		0,102			0,148		
Without Pellets	Glass 1	UV-b+UV-a+PAR	4,454	2,799	2,158	0,148	0,148	0,000
	Glass 2	UV-b+UV-a+PAR	0,358			0,148		
	Glass 3	UV-b+UV-a+PAR	3,584			0,148		
	Glass 4	UV-a+PAR	0,358	1,485	1,951	0,194	0,178	0,026
	Glass 5	UV-a+PAR	3,738			0,194		
	Glass 6	UV-a+PAR	0,358			0,148		
With Pellets	Glass 7	UV-b+UV-a+PAR	3,226	2,099	1,569	0,194	0,194	0,046
	Glass 8	UV-b+UV-a+PAR	0,307			0,148		
	Glass 9	UV-b+UV-a+PAR	2,765			0,239		
	Glass 10	UV-a+PAR	0,256	1,092	1,448	0,148	0,178	0,026
	Glass 11	UV-a+PAR	2,765			0,194		
	Glass 12	UV-a+PAR	0,256			0,194		

Table 2b : Dissolved nutrient concentrations for experiment number 6. Si Silicates and PO_4 Phosphates are included

			N total			P total		
	Sample	Treatment	µM N total	$\bar{x}$	s.d.	µM P total	$\bar{x}$	s.d.
T_0	0m w/o pellets		72,571			0,645		
	0m with pellets		56,814			0,710		
Without pellets	Glass 1	UVb+UVa+PAR	25,879	17,613	7,184	0,940	0,918	0,038
	Glass 2	UVb+UVa+PAR	14,080			0,940		
	Glass 3	UVb+UVa+PAR	12,879			0,874		
	Glass 4	UVb+PAR	28,064	24,241	3,998	0,645	0,666	0,038
	Glass 5	UVb+PAR	24,568			0,645		
	Glass 6	UVb+PAR	20,089			0,710		
With Pellets	Glass 7	UVb+UVa+PAR	23,153	25,361	7,499	0,776	0,852	0,162
	Glass 8	UVb+UVa+PAR	19,214			0,743		
	Glass 9	UVb+UVa+PAR	33,717			1,038		
	Glass 10	UVb+PAR	24,585	27,928	5,635	0.776	0.863	0.100
	Glass 11	UVb+PAR	34,433			0.841		
	Glass 12	UVb+PAR	24,764			0.973		

Table 3 : Total nitrogen and phosphorus concentrations estimated for experiment number 6

Conclusion

These results point out the effect of UV-b radiation on pigments production : if we consider the total (chla+phaeopigments a), productions under total UV-(a+b) and PAR radiations are lower than the productions under UV-a and PAR : table 4.

In the same time, ammonium provided by photomineralisation of the dissolved organic substances from pellets, is more abundant under total irradiation possibly due to unconsummed ammonium. Considering the usual low amounts in ammonium in the upper layers in this area, the role of eventual organic enrichments and their consequences on further phytoplankton developments cannot be neglected.

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	pigments in mg m^{-3}		Total in mg m^{-3}	Ammonium µM
Initial t0	Chl a : 5.61 Phaeo. A : 1.36		6.97	0
UV(a+b) + PAR	Chl a : 5.61 Phaeo. a : 1.36		8.22	0
	+ Pellets	Chl a : 5.6 1Phaeo. a : 1.36	7.97	0.6
UV-a + PAR	Chl a : 5.61 Phaeo. a : 1.36		9.10	0.1
	+ Pellets	Chl a : 5.61 Phaeo. a : 1.36	9.32	0.4

Table 4 : Summary of some data

Effect of the solar radiation on the production of pigments and mycosporine-like aminoacids

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Financial support : EEC

Abstract

It is well-known that UV (290 to 400 nm) affects the growth and survival of phytoplankton in the upper layers of the sea, inhibiting the photosynthesis, damaging DNA and different metabolic processes. However, some species develop different strategies to escape, filter, repair and face the stress.

A possible mechanism for protection against UV radiation is accumulation of substances absorbing in UV wavelengh, such as mycosporine-like aminoacids (MAA's). Although the protective function of the photosynthetic mechanism was suggested until now, the proof of its specific UV filter function is recent.

Apart from capture of light energy, carotenoids have also photoprotective functions in elimination of excessive energy accumulated in photoaccumulators (heat). In this communication we present the results concerning the evolution of the photosynthetic pigments and MAA's, in the bioassays on phytoplankton regulation by UV-b radiations, and dissolved organic substances addition in Punta Arenas with IFOP participation. The results obtained *in situ* are compared to laboratory cultures.

METHOD

The samples were collected during the *in situ* experiment in Punta Arenas, and concerned the same phytoplankton populations submitted to natural irradiation with/without dissolved organic enrichments. The cells were isolated on GF/F Whatman filters stored at –20°C before arrival in laboratory in Argentine, then maintained in liquid nitrogen until pigments and mycosporine-like aminoacids analyses. The experimental protocol is presented here after.

Sample	N° filters	Diameter filters	Volume	Date	Observations
batch1	2	25 mm	350 ml	5 october 98	Only To of Aquatoxal N°2. Aborted Experiment abortado, without final results
batch 2	6	47 mm	* **	14-17 october 1998 14-19 october 1998	Aquatoxsal N°3. Experiment during 6 days. Final filtration.Temperature increased to 15°C (cooling failure during one day)
batch 3	4	47 mm	2 lt c/u	11-16 nov 1998	Aquatoxal N°6. Experiment during 6 days. Final filtration.

* Glass 18: Astralon. Filter, problem of irradiation UV-b during the last day. Filtered volume : 1,625 liter

** Glass 13: Nylon filter. Correct. Filtered volume : 1,7 liter
Glass 14: Nylon filter. Correct. Filtered volume : 1,8 liter
Glass 15: Nylon filter. Correct. Filtered volume : 1,7 liter
Glass 16: Astralon filter. Correct. Filtered volume : 1,0 liter
Glass 17: Astralon filter. Correct. Filtered volume : 1,55 liter

RESULTS

Table 1 summarizes the results in pigments analyses, figures 2 to 4 present the corresponding chromatograms. Results in MAA's are presented on table 2, and the corresponding chromatograms on figures 5 to 7.

According to the difficulties occurred during some *in situ* experiments, only the initial composition was considered for experiment n°2.

Chlorophylle a, c1, c2, fucoxanthine, diadinoxanthine and β-carotene are characteristic of a population dominated by diatoms. Alterated chlorophylle a was revealed by presence of alomers, epimers, chlorophyllide a and phaeophorbide a, corresponding to senescent population, or submitted to grazing by zooplankton.

MAA's profil was very simple, and we observed exclusively shinorin, palitin and porphyra-334 , in contrast with results corresponding to the toxic dinoflagellates *A. tamarense*, *A. minutum* and *A. catenella*. Total MAA's and the related molar concentration in chlorophyll a were very low, and revealed either the adaptation of the alga to grow in low light intensity, or inability of the cell to accumulate MAA's. This ability seems to characterise the populations forming superficial blooms.

In spite of the experimental hindrance during incubation, experiment n°3 allowed observations in modification related to PAR+UV-a irradiation. As can be observed on figures 2b and 2c, pigment compositions of these samples are very close. However, during the same time, total and relative concentration in chlorophyll a decreased. This was clearly observed in the increasing ratios fucoxanthin/chlorophyll a and chlorophyll c2/chlorophyll a (Table 1). The MAA's profile is not modified during this time (Fig. 5b and 5c), porphyra 334 being the dominant compound, then shinorin and palithin. Their concentration and ratio with chlorophyll a increase, due to the lower chlorophyll a concentration previously mentioned.

With respect to the methodological difficulties encountered during incubation in experiment n°3, we intended a comparison of light composition effect on the pigments after six days irradiation. On figures 3a to 3d we can observe lower chlorophyll a concentration in phytoplankton submitted to PAR+ total UV, than under PAR+UV-a, in spite of the low reproductibility in duplicates (figure 3a and 3b).The relative amounts in pigments concentration followed the same pattern as above (table 1) : chlorophyll c as fucoxanthine are more stable than chlorophylle a under solar photooxidation.

The fact that samples under total solar irradiation (PAR+total UV) have higher MAA's proportions that the samples submitted to similar treatment excepted UV-b, (Figure 6a to 6d), is not a consequence of MAA's synthesis, but chlorophyll a lability.

Results in experiment n°6, after six days incubation, prove the degradation process of initial populations, due to incubation conditions. Figure 8 presents the corresponding absorbance spectra. Moreover, comparison with absorption spectrum of *A. catenella* extract shows the low MAA's concentrations in the studied samples. Pigments chromatograms with absorbance (diode rod) and fluorescence (spectrofluorometer) detections are presented on figures 4a and 4b, for samples irradiated by PAR+UV-a. On these figures we can see the low amount in chlorophyll a, and abundance in chlorophyll c1 and c2, and other unidentified chl.c (possibly results of degradation), and various phaeophorbides a. Fucoxanthin and other xanthophylls absence is also notable. This pigmentary profile is similar to the spectral analysis obtained for sediment and zooplankton fecal excretion.

The corresponding MAA's chromatogram (figure 7) present the same compounds as previously described. The only difference is in ratios. Total concentrations are low, indicating absence of pigment synthesis under the solar irradiation. This is compatible with the degradation process, and high MAA's/chlorophyll a ratio.

The results corresponding to PAR + total UV incubation (figures 4c and 4d) are close to the previous results obtained with PAR+UV-a.

CONCLUSION

At the end of these experiment we never observed phytoplankton growth. Photosynthetic pigments were degraded, especially chlorophyll a. We did not observed MAA's accumulation in the studied populations, and this is in accordance with the dependence of MAA's synthesis with photosynthesis. Algal growth inhibition seems not to be due to UR-b radiation, as we did not observed significant differences with the different treatments. This was unlike our precedent results in other experiments. The algal populations in the samples presented senescent characteristics, due to the low nutrients enrichment. These conditions : nutrients limitation, high PAR and total UV intensities, increased the photo-inhibition of phytoplankton with photo-oxidation of the pigment systems. It is important to consider the influence of nutrient concentrations in experiments carried on for several days.

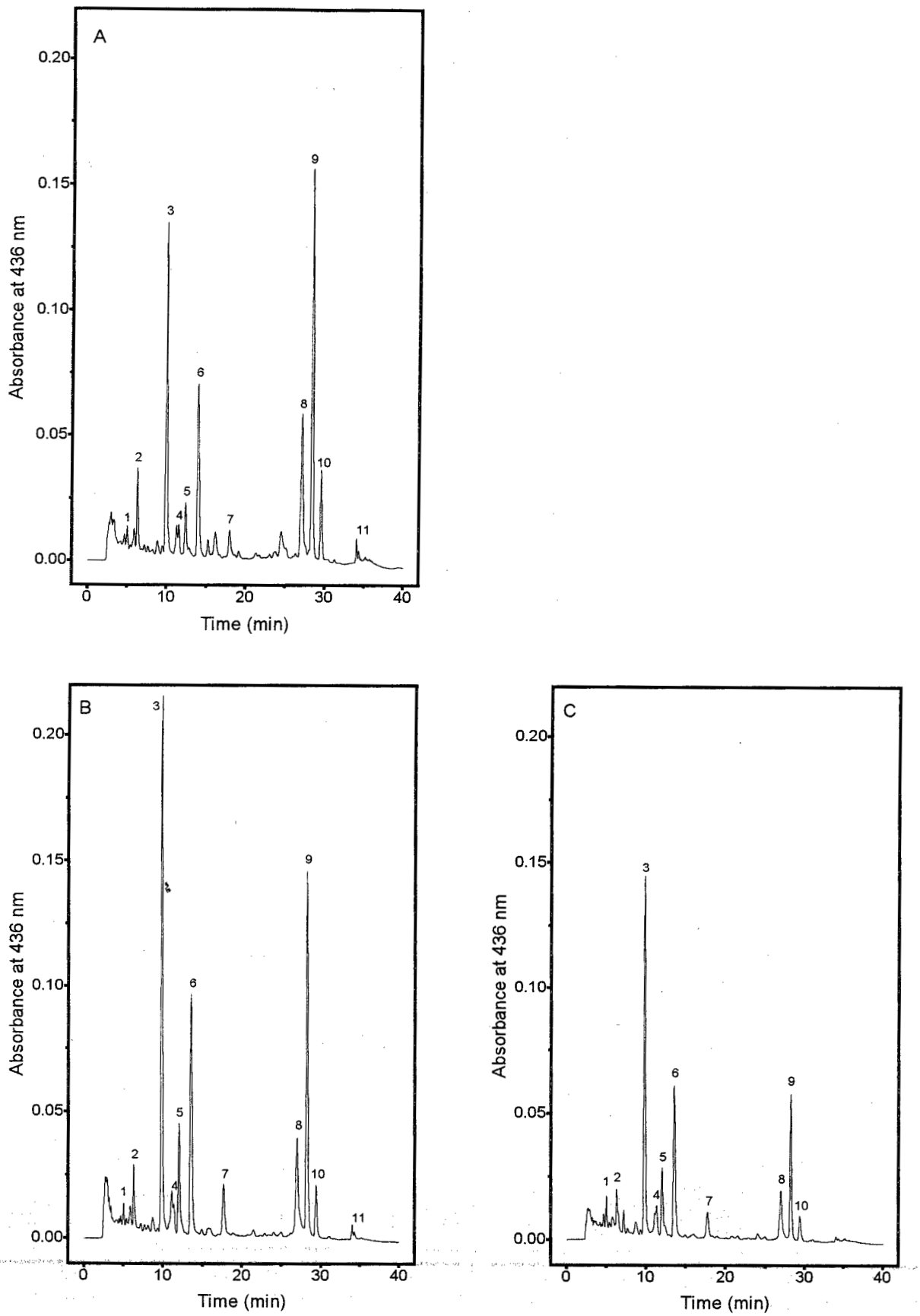


Fig. 2. Chromatogram (HPLC) of photosynthetic pigments. **A** : Time t = 0, Experiment N°2 ; **B** : Time t = day 3 (PAR+UVA), Experiment N°3 ; **C** : Time t = day 6 (PAR+UVA), Experiment N°3. Identified pigments : **1** : chlorophyllide *a*, **2** : unknown Chl *c*-like, **3** : fucoxanthin, **4**: pheophorbide *a*, **5** : Chl c_1, **6** : Chl c_2, **7** : diadinoxanthin, **8**: Chl *a* allomer, **9**: Chl *a*, **10** : Chl *a* epimer, **11** : β-carotene.

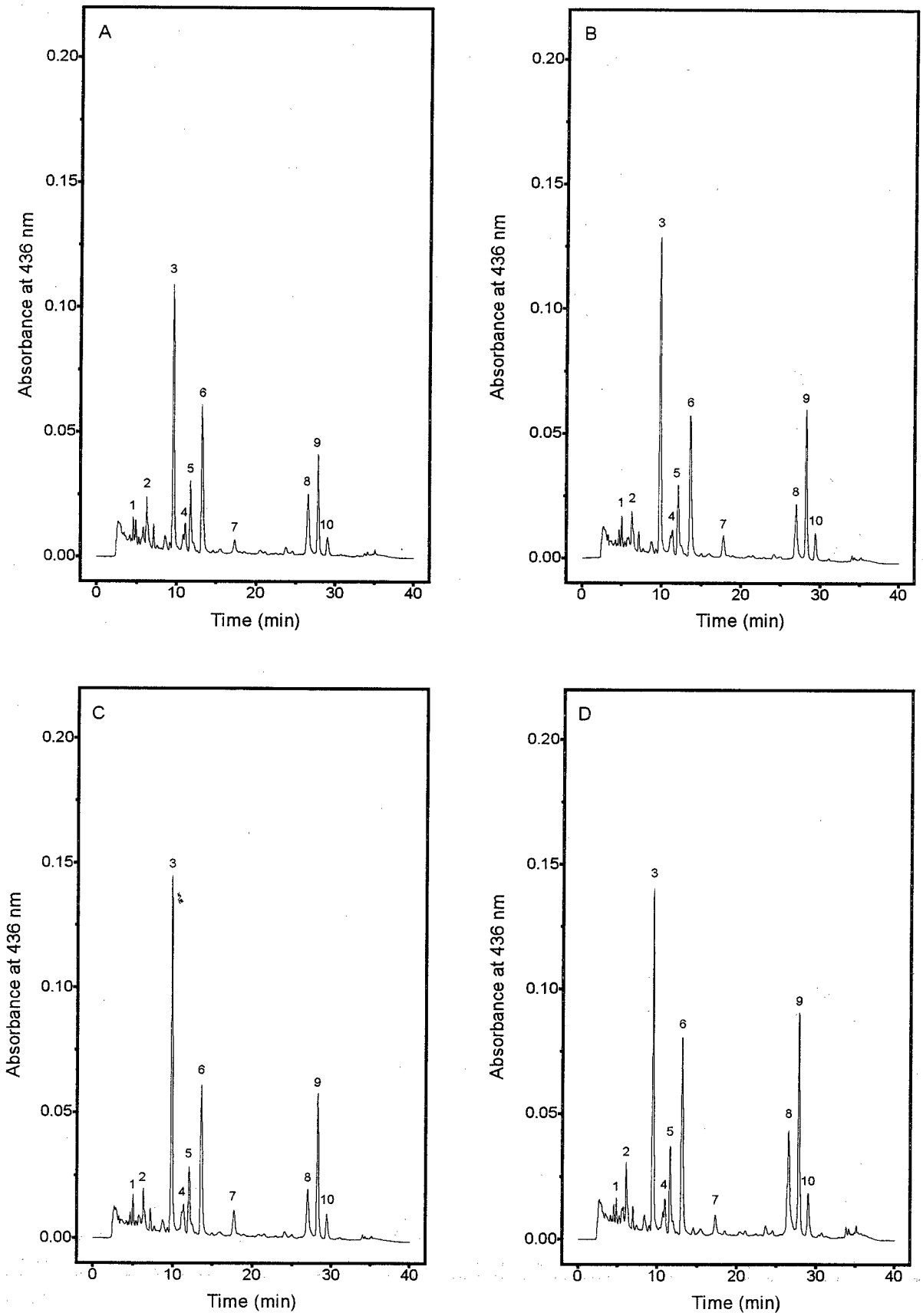


Fig. 3. Chromatograms (HPLC) of photosynthetic pigments. **A** and **B** : Time t = day 6 (PAR+UVT), Experiment N°3 ; **C** and **D** : Time t = day 6 (PAR+UVA), Experiment N°3. Identified pigments : **1** : chlorophyllide *a*, **2** : unknown Chl *c*-like, **3** : fucoxanthin, **4** : pheophorbide *a*, **5** : Chl c_1, **6** : Chl c_2, 7 : diadinoxanthin, **8** : Chl *a* allomer, **9** : Chl *a*, **10** : Chl *a* epimer.

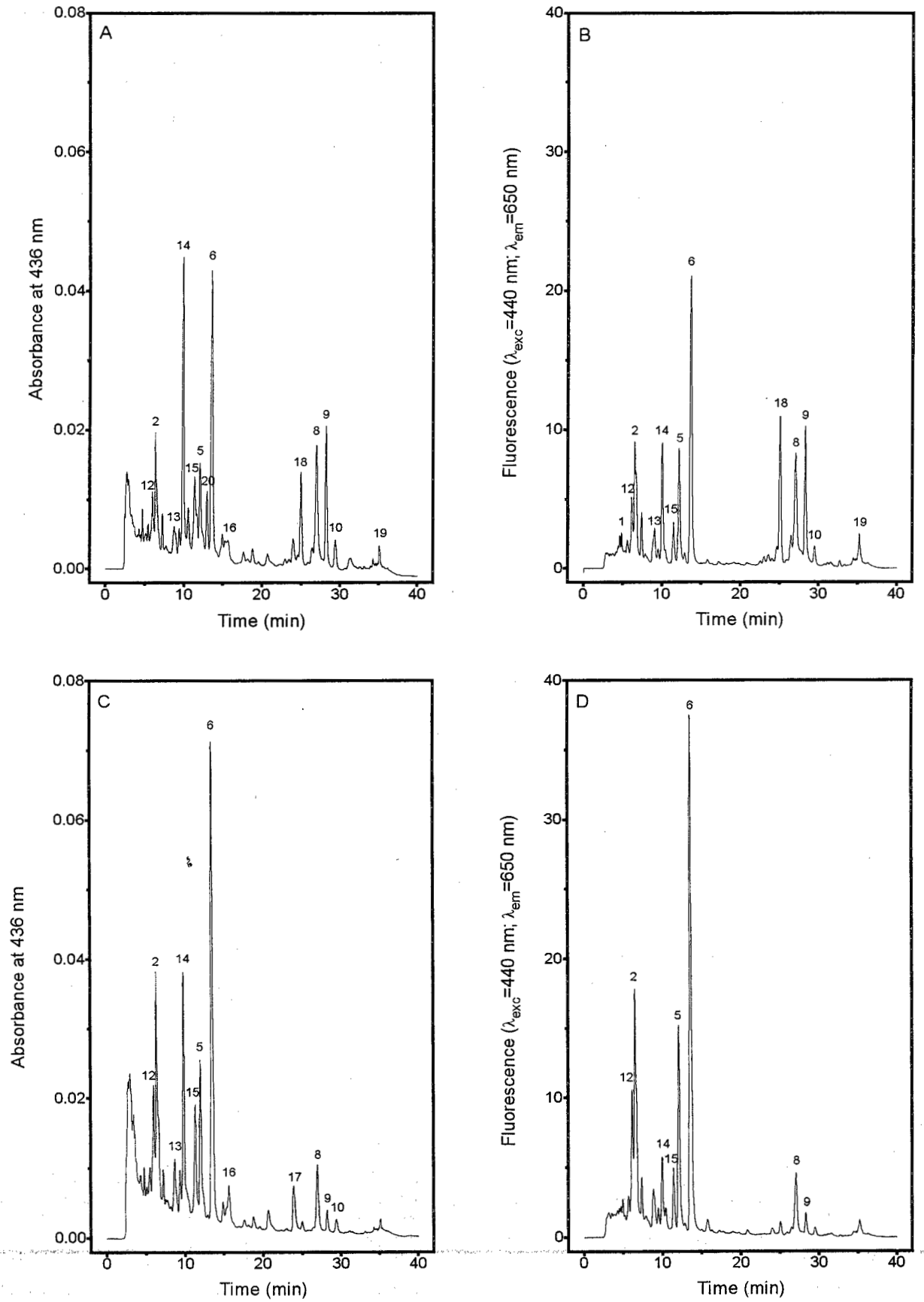


Fig. 4. Chromatograms (HPLC) of photosynthetic pigments. **A** and **B** : Time t = day 6 (PAR+UVT), Experiment N°6 ; **C** and **D** : Time t = day 6 (PAR+UVA), Experiment N°6. Identified pigments : **1** : chlorophyllide *a*, **2** : unknown$_1$ Chl *c*-like, **5** : Chl c_1, **6** : Chl c_2, **8** : Chl *a* allomer, **9** : Chl *a*, **10** : Chl *a* epimer, **12** : unknown$_2$ Chl *c*-like, **13**, **14**, **15** and **16** : unknown pheophorbide *a*-like pigments, **17** : unknown Chl derivative, **18** : Chl *b*, **19** : pyropheophytin *a*, **20** : neoxanthin.

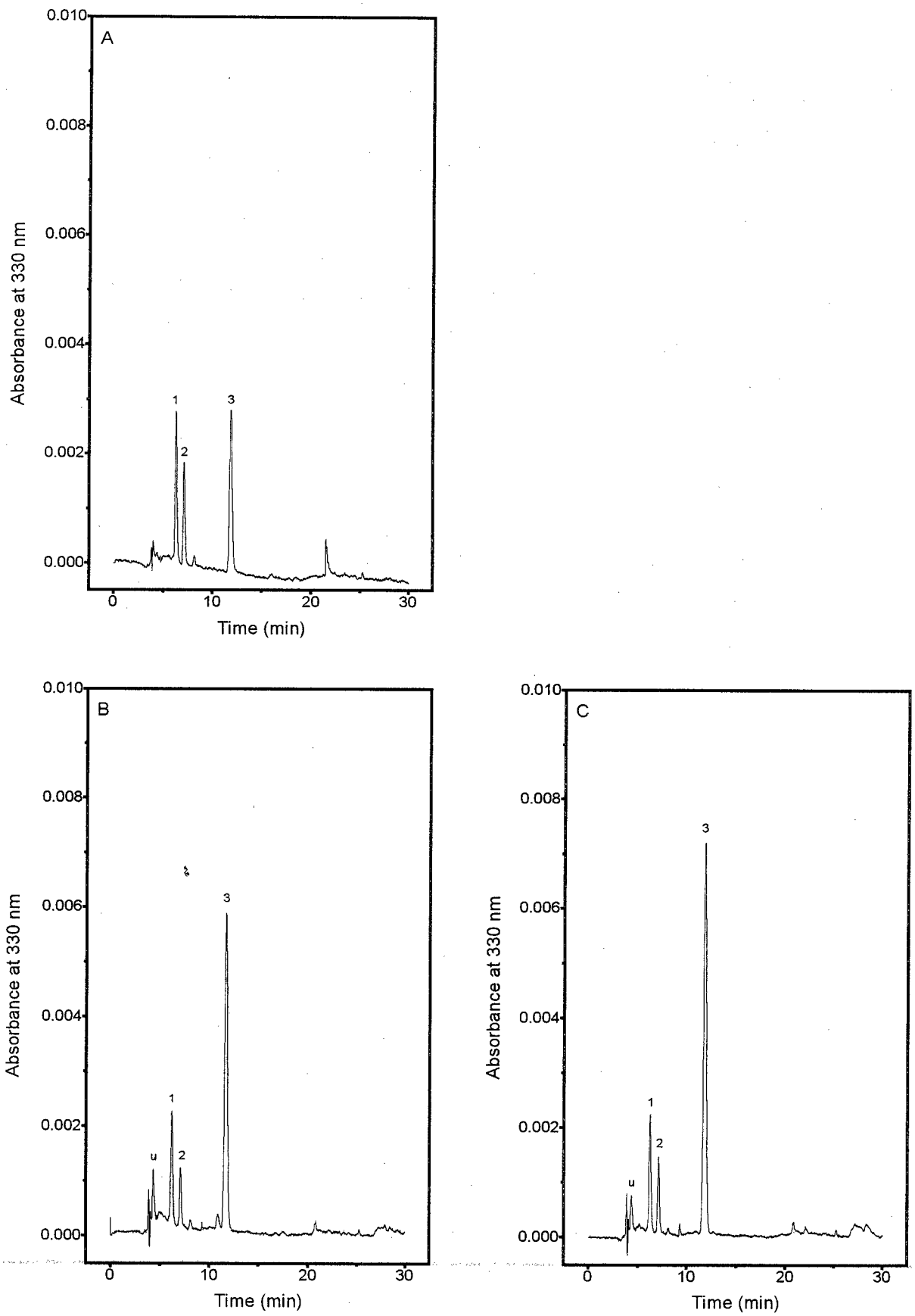


Fig. 5. Chromatograms (HPLC) of aminoacids similar to micosporines (MAA's). **A** : Time t = 0, Experiment N°2 ; **B** : Time t = day 3 (PAR+UVA), Experiment N°3 ; **C** : Time t = day 6 (PAR+UVA), Experiment N°3. Identified MAA's : **1** : shinorine, **2** : palythine, **3** : porphyra-334, **u** : unknown contaminants.

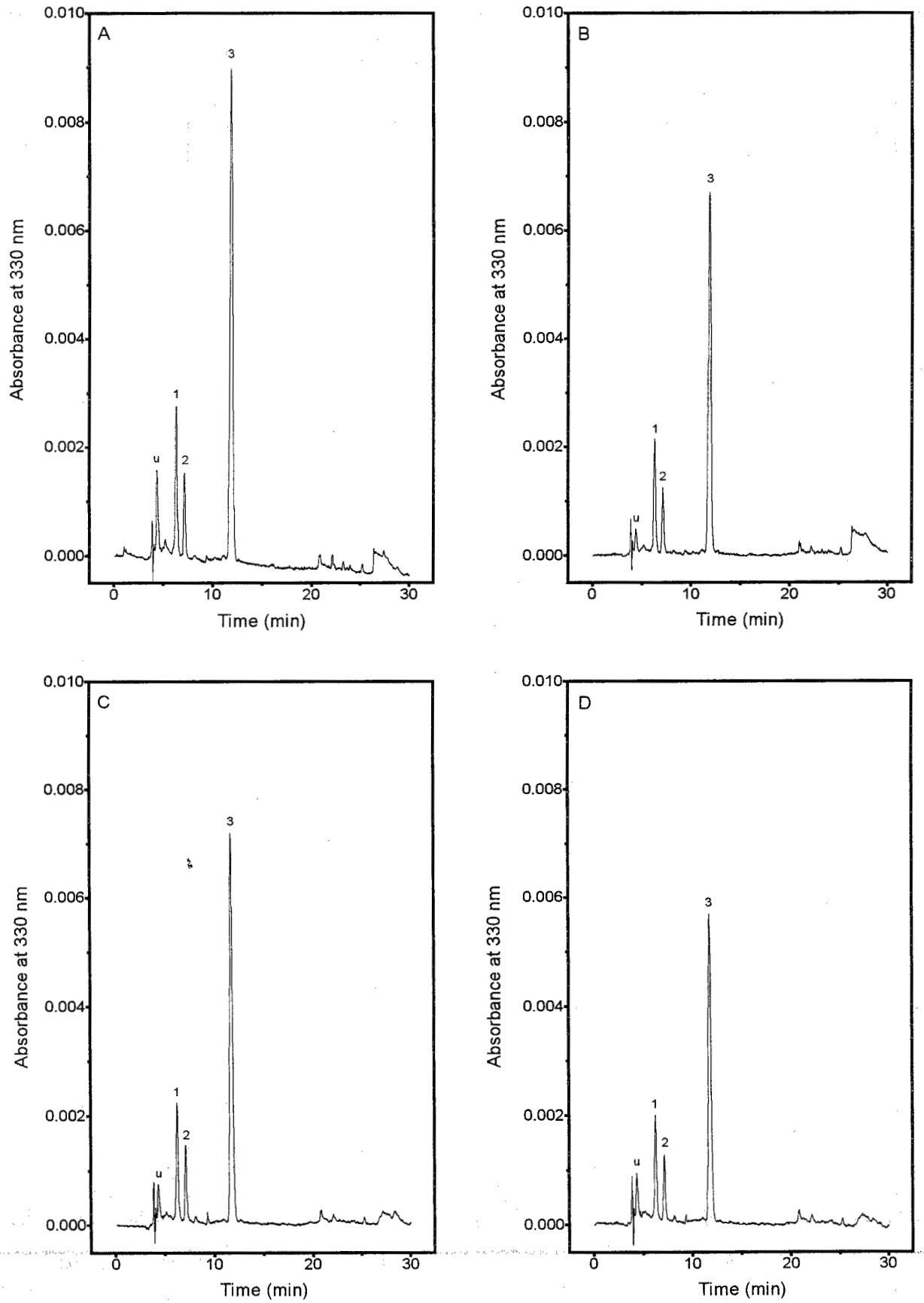


Fig. 6. Chromatograms (HPLC) of aminoacids similar to micosporines (MAA's). **A** and **B** : Time t = day 6 (PAR+UVT), Experiment N°3 ; **C** and **D** : Time t = day 6 (PAR+UVA), Experiment N°3. Identified MAA's : **1**: shinorine, **2** : palythine, **3** : porphyra-334, **u** : unknown contaminants.

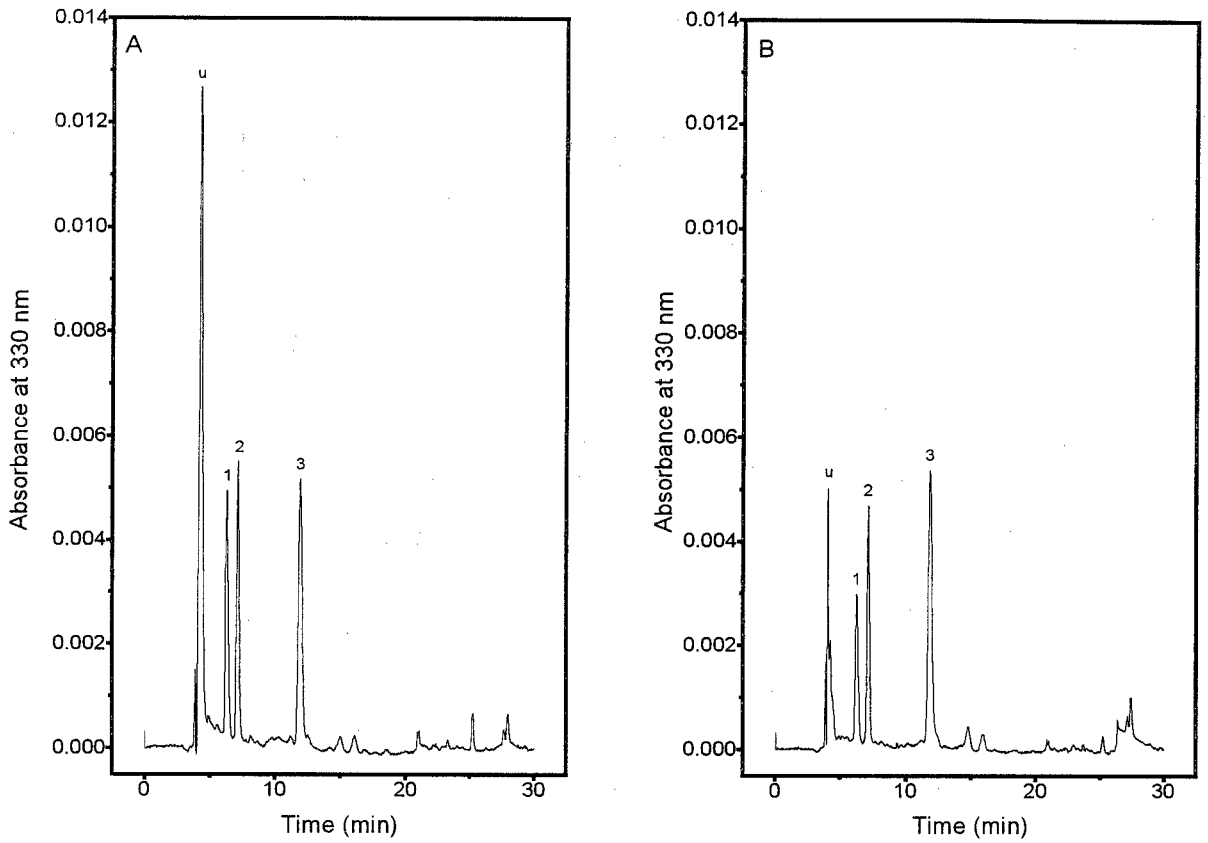


Fig. 7. Chromatograms (HPLC) of aminoacids similar to micosporines (MAA's). **A** : Time t = day 6 (PAR+UVT), Experiment N°6 ; **B** : Time t = day 6 (PAR+UVA), Experiment N°6. Identified MAA's : **1** : shinorine, **2** : palythine, **3** : porphyra-334, **u** : unknown contaminants.

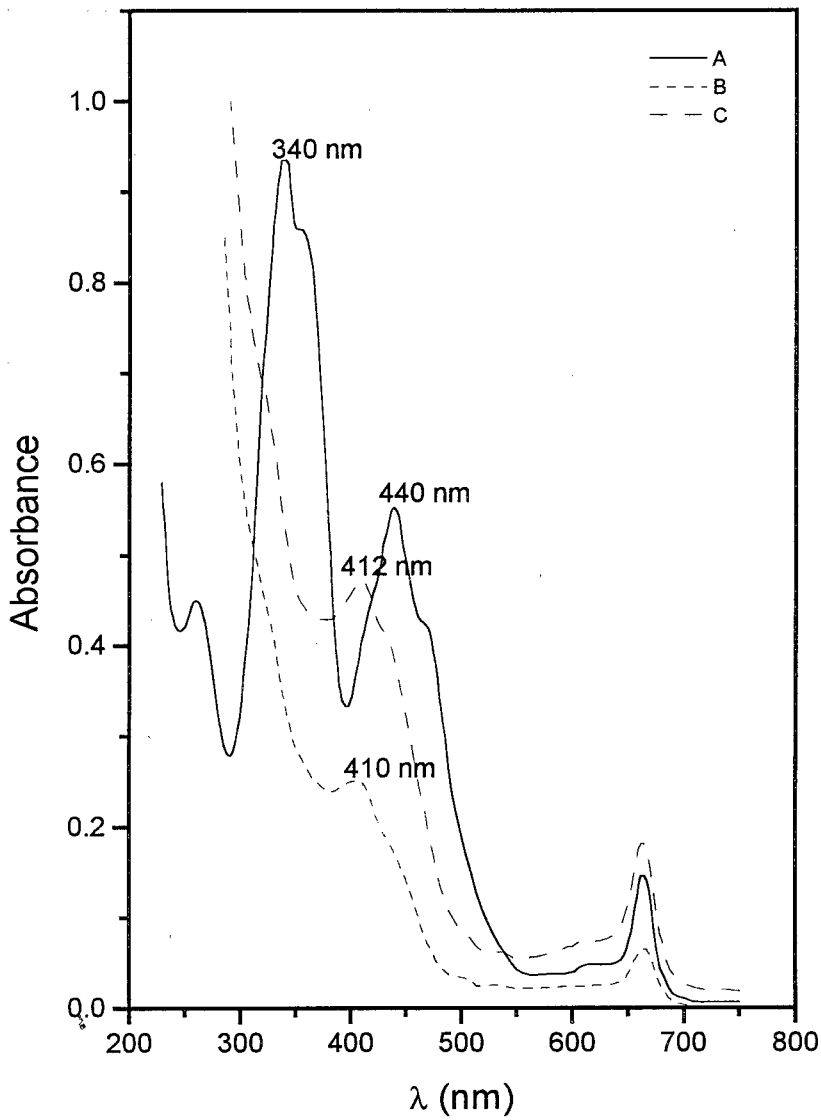


Fig.8. UV-VIS absorbance curves corresponding to methanolic extracts from : **A** : *Alexandrium catenella* (clone ACC08, Canal Costa, XI Región, Chile, 1995), **B** : sample at t = day 6 (PAR+UVA) Experiment N°6, **C** : sample at t = day 6 (PAR+UVB) Experiment N°6

	Time 0 Aquatoxsal N°2	Glass n° 13. Nylon Aquatoxsal N°3	Glass n° 14. Nylon Aquatoxsal N°3	Glass n° 15. Nylon Aquatoxsal N°3	Glass n° 16. Astralon Aquatoxsal N°3	Glass n° 17. Astralon Aquatoxsal N°3	Glass n° 18. Astralon Aquatoxsal N°3	Nylon Aquatoxsal N°6	Astralon Aquatoxsal N°6
Chlorophyllide *a*	-	0.1217	0.0592	0.0931	0.1404	0.0777	trazas	-	-
Chlorophyll *c*-like	-	-	-	-	-	-	-	nd	nd
Chlorophyll c_2-like	0.6019	0.1416	0.1016	0.0895	0.1430	0.1496	0.3269	0.1409	0.0692
Chlorophyll *a* derivado	-	-	-	-	-	-	-	nd	nd
Fucoxanthin	3.2162	1.3697	0.8987	1.1960	2.0748	1.4064	3.6460	-	-
Pheophorbide *a*	2.1129	1.0390	0.5291	1.0591	2.0812	1.0667	1.2378	0.5877	0.4069
Chlorophyll c_1	0.4591	0.3641	0.2168	0.2373	0.3484	0.3082	0.6430	0.1437	0.0796
Neoxanthin	-	-	-	-	-	-	-	0.0060	-
Chlorophyll c_2	1.6045	0.8536	0.5010	0.5397	0.8737	0.8045	1.6243	0.4803	0.2729
Pheophorbide a_3	-	-	-	-	-	-	-	-	nd
Diadinoxanthin	0.3156	0.1227	0.0703	0.1075	0.1919	0.1064	0.3916	-	-
Chlorophyll *b*	-	-	-	-	-	-	-	0.3925	-
Chlorophyll *a* allomer	3.1217	0.8393	0.5055	0.4857	0.6229	1.2096	1.8018	0.4401	0.1021
Chlorophyll *a*	7.0821	2.3730	0.7637	1.1834	1.7756	1.8886	4.9617	0.5240	0.0572
Chlorophyll *a* epimer	1.9468	0.5069	0.2358	0.3053	0.4470	0.5430	0.8944	-	0.0504
β-carotene	0.1032	trazas	trazas	trazas	trazas	trazas	trazas	-	-
Pyropheophytin *a*	-	-	-	-	-	-	-	-	nd
ΣCl.deg./Chl *a* (mol/mol)	1.01	1.06	1.74	1.64	1.85	1.53	0.79	1.96	9.78
Diadinox/Fucox (mol/mol)	0.10	0.09	0.08	0.09	0.09	0.08	0.11	-	-
Fucox/Chl *a* (mol/mol)	0.45	0.58	1.18	1.01	1.17	0.74	0.73	-	-
Chl c_1/Chl *a* (mol/mol)	0.06	0.15	0.28	0.20	0.20	0.16	0.13	0.27	1.39
Chl c_2/Chl *a* (mol/mol)	0.23	0.36	0.66	0.46	0.49	0.43	0.33	0.92	4.77

Table 1. Concentrations ($\mu g\ l^{-1}$) and ratios of photosynthetic pigments in AQUATOXSAL samples, experiments 2, 3 and 6 realized in Punta Arenas (October- November 1998).

Batch 1 : T_0 of AQUATOXSAL N° 2 experiment (aborted). 05/10/98				
Glass N°	Film	$C_{shinorine}$ ($\mu g\ l^{-1}$)	$C_{palythine}$ ($\mu g\ l^{-1}$)	$C_{porphyra-334}$ ($\mu g\ l^{-1}$)
?	?	0.1798	0.1534	0.2901
Batch 2 : day 6 of AQUATOXSAL N°3 experiment. 14/10 al 19/10/98				
Glass N°	Film	$C_{shinorine}$ ($\mu g\ l^{-1}$)	$C_{palythine}$ ($\mu g\ l^{-1}$)	$C_{porphyra-334}$ ($\mu g\ l^{-1}$)
13	Nylon	0.0724	0.0557	0.3234
14	Nylon	0.0712	0.0478	0.3712
15	Nylon	0.0587	0.0410	0.2940
16	Astralon	0.0870	0.0631	0.4894
17	Astralon	0.0609	0.0464	0.2856
18	Astralon	0.0622	0.0380	0.2790
Batch 3 : day 6 of AQUATOXSAL N°6 experiment. 11/11 to 16/11/98				
Glass N°	Film	$C_{shinorine}$ ($\mu g\ l^{-1}$)	$C_{palythine}$ ($\mu g\ l^{-1}$)	$C_{porphyra-334}$ ($\mu g\ l^{-1}$)
?	Nylon	0.0632	0.0810	0.0936
?	Astralon	0.0380	0.0674	0.0989

Table 2. MAA's concentrations in the samples corresponding to AQUATOXSAL 2, 3 and 6 experiments, realized in Punta Arenas (October-November 1998).

Batch 1 : T_0 of AQUATOXSAL N° 2 experiment (aborted). 05/10/98				
Glass N°	Film	$R_{shinorine}$ (mol.(mol Chl *a*)$^{-1}$)	$R_{palythine}$ (mol.(mol Chl *a*)$^{-1}$)	$R_{porphyra-334}$ (mol.(mol Chl *a*)$^{-1}$)
?	?	0.01	0.08	0.11
Batch 2 : Day 6 of AQUATOXSAL N° 3 experiment. 14/10 to 19/10/98				
Vaso N°	Film	$R_{shinorine}$ (mol.(mol Chl *a*)$^{-1}$)	$R_{palythine}$ (mol.(mol Chl *a*)$^{-1}$)	$R_{porphyra-334}$ (mol.(mol Chl *a*)$^{-1}$)
13	Nylon	0.08	0.09	0.35
14	Nylon	0.25	0.23	1.26
15	Nylon	0.13	0.13	0.64
16	Astralon	0.13	0.13	0.71
17	Astralon	0.09	0.09	0.39
18	Astralon	0.03	0.03	0.15
Batch 3 : Day 6 of AQUATOXSAL N°6 experiment. 11/11 to 16/11/98				
Glass N°	Film	$R_{shinorine}$ (mol.(mol Chl *a*)$^{-1}$)	$R_{palythine}$ (mol.(mol Chl *a*)$^{-1}$)	$R_{porphyra-334}$ (mol.(mol Chl *a*)$^{-1}$)
?	Nylon	0.32	0.56	0.46
?	Astralon	1.79	4.29	4.46

Table 3. MAA's and Chl a molar ratios in samples corresponding to AQUATOXSAL 2, 3 and 6 experiments, realized in Punta Arenas (October-November 1998).

The *Gymnodinium* sp. outbreak in Chile : preliminary results.

Alejandro Clément, Ximena Rojas, **Miriam Seguel** and **Geneviève Arzul**

Abstract

A large-scale fish kill developed in waters around Chiloe, Xth Region, Chile, during March and April 1999. Examination of water samples revealed that a *Gymnodinium* sp. comprised 99.9 % of the population, the density of which reached 8 to 9 million cells per litre in coloured patches.

Many factors suggest that this bloom was of oceanic origin. One possible cause of the event could be the current climate anomaly for the last 14 months in the area, with a drought and strong sunshine. It has resulted in temperatures, 15°C, 1.5°C higher than normal in the area.

Among the consequences of this bloom, it is important to mention a large mortality of salmon.

Some of the properties of this species have been studied during the bloom :

The haemolytic property : it informs on one of the action mode of the toxin (lysis). The test carried out on diluted samples showed that a concentration of 250 cells/ml showed a still non-negligible activity (18 % haemolysis in an RBC suspension of 40 million/ml).

The allelopathic property : it reveals the presence of an ectocrine secreted by the algal cell, and may explain the almost monospecific nature of the blooms. The dinoflagellate *Alexandrium catenella* was not inhibited in growth by *Gymnodinium* excretions (figure 2), in contrast the development of the diatom *Leptocylindrus minimus* was completely repressed (figure 3).

This phenomenon illustrates the importance of the climatic situation and the physical oceanographic conditions in the process of bloom dynamics

Figure 1

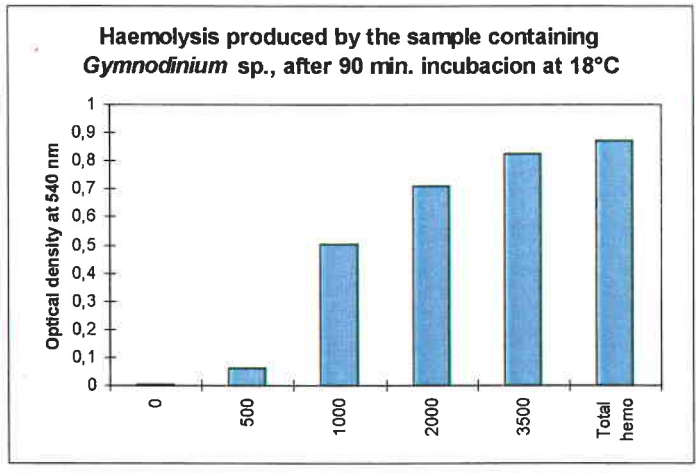
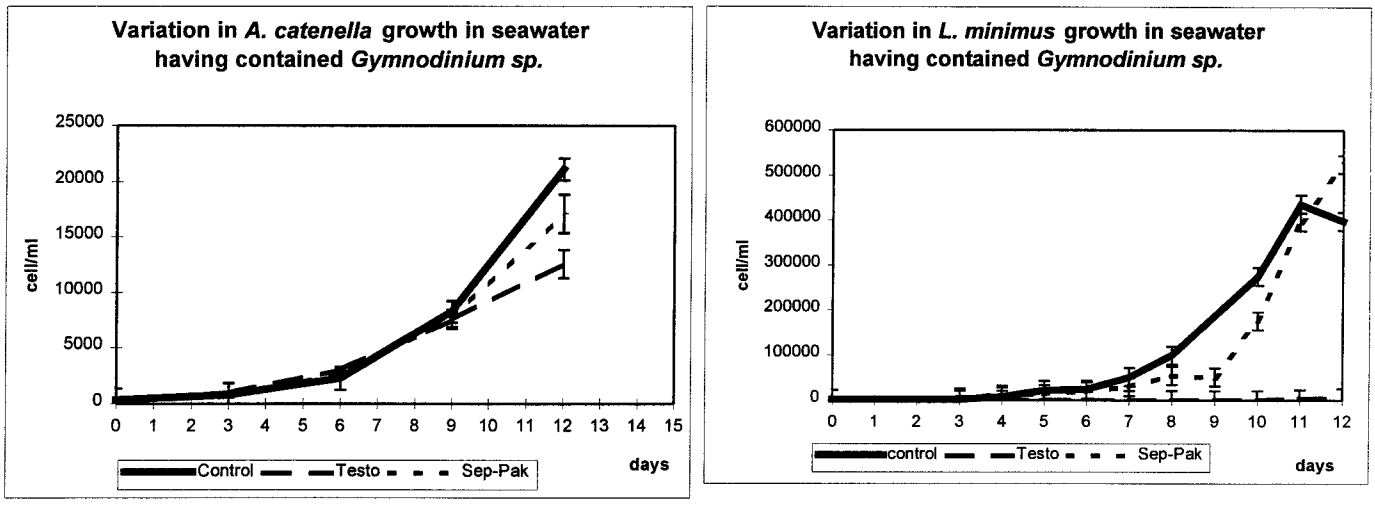


Figure 2



This event will be related in the journal "Harmul Algal Blooms" (IOC publication)

Discussion

Can we confirm the effect of UV radiations on the phytoplankton growth ?

The composition in phytoplankton populations was not modified under UV radiations. Bad meteorological conditions involved methodological difficulties and delay in the final *in situ* tests, realised under too weak UV-b radiations. The preliminary results showed 24 hours adaptations of the algae before presenting any evolution. However, chemical composition in phytoplanktonic cells presented modifications such as carotenoides production, corresponding to chlorophyll degradation.

The interest of mycosporine-like-aminoacids produced by various algae, is active protection of the cells against noxious radiations. This production, as the ability of some species to use for growth, the ammonium produced by photomineralization of dissolved organic substances, could explain the selective development of some algae. The action mode of mycosporine in prevention from radicals produced by UV-b, could be also related to the ability of some species facing allelopathic due to PUFAs. This property could be illustrated by the result in the allelopathic test concerning the growth of *Alexandrium catenella,* in water previously modified by *Gymnodinium* sp. excretions.

Can we prevent environment from disturbances due to human activities ?

Prevention from environmental disturbance due to human activities implies to define some indicators of the effects. More or less related to algal growth, we can consider all the results obtained until now.

In the water column, ammonium seems the best factor related to fish excretions. In the sediment, four parameters appear reflecting the bottom quality : granulometry, organic substances contain, redox potential, and macrobenthic animals. Sometimes bacterias constitute good indicators.

Ammonium measurement and granulometry can be realised by simple methods. The organic contain of particles is measurable either by 55°C combustion, or CHN. The necessity of methodological knowledge and good equipment, justify analyses centralisation, and would contribute to lower the cost.

A limiting value of the indicator has to be defined for tolerated impact. As the different local characteristics don't allow a generalised common value, it will be necessary to consider a percentage depending on the initial situation, for example.

The salmon farms have not identical impact on the bottom. Low energy plants present homogenous and diffuse impact. High energy plants present strong and irregular impact. Moreover, it is necessary to consider the both cases :

1. local impact in the vicinity of the production sites,

2. large-scale aspect involving long time and the whole region, more difficult to evaluate, with reference to the use of water masses, and the economical, social and environmental consequences.

The recovery of the good quality in sediment necessitates changing the site of fish farms. Now, this decision is undertaken in relation with the bad results in yield fish growth.

We believe that now the regulations in fish farming are not effective. The more effective measure is auto-regulation by the producers. We recommend to measure N and P in the water column in large time-scale to estimate the tendency to eutrophication.

We recommend also the use of *softwares* devoted to the evaluation of environmental qualities, using expert systems.

Algal bloom and aquaculture relationship ?

It is necessary to precise, now, the absence of relationship between natural phytoplankton blooms and salmon farming development. Only the *in vitro* results obtained in tests simulating the natural features, present algal development similar to bloom. From the actual case of *Gymnodinium* sp. outbreak, we can precise the natural character of the phenomenon, due to special physical and climatical conditions. The presence of aquaculture could magnify the red tide phenomenon, through the stimulation of the microalgae development. However initiation remains dependent on inoculation and we are too close to the *Gymnodiunium* sp bloom, for a proper view.

OUTCOME

The general results drawing from this workshop concern the effects of intensive aquaculture on the benthic and pelagic compartments :

- Modification of the sediment status beneath the fish farm cages with physico chemical changes : low dissolved oxygen concentration and redox potential, high sulfide and particular organic carbon composition.

- Changes in water column composition, including higher concentrations in ammonium and larger organic particles load.

- The floristic composition of the fluorescent particles corresponding to phytoplankton, can be modified during *in vitro* experiments by addition of several substances identified in fish farm wastes : feed and faeces elutriates, dissolved mineral salts and animal excretions.

- Environmental quality in term of dissolved organic substances load (from fish feed) and light wavelength irradiance (UV-a, UV-b and PAR), constitutes a complementary factor in phytoplankton regulation. Pigment composition in algal cells can be modified, and further experiments will verify this first part of the results.

The discussions focussed the possible effect of aquaculture on noxious algae developments, and prevention from disturbance due to human activities, with emphasis on auto-regulation by producers. They pointed out the importance of hydrodynamic, cages positions, geochemical characteristics of the bottom. According to the recommendations, further investigations will be conducted on sediment-water exchanges in reduced potential conditions, in impacted area.

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