

**RESEARCH CONTRACT  
MAS 2 CT94-0100**

**INTERIM REPORT**

**IMPACT ON NON TARGET ORGANISMS OF ANTI-MARINE WOOD-  
BORER TREATMENTS**

**Evaluation of stress imposed by treatments on organisms from test sites**

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SOLETCHNIK, P. GRAS, L. ADESSI**

**IFREMER/URAPC/  
Shellfish Research Laboratory,  
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**Report prepared  
by  
Dr. P. GOULLETQUER**

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## Introduction

In European waters, significant economic damages to wooden structures are caused by bivalves molluscs and crustaceans. Treatments like copper-chrome-arsenic mixtures (CCA) were designed to prevent settlement of these wood borer species. However, limited and conflicting evidence regarding the toxicity to non-target organisms are available in the literature. This report aims to provide insights regarding the CCA leachates' toxicity to an important aquaculture species, the blue mussel *Mytilus edulis* for which much information on normal and stressed physiology is available for purposes of comparisons. The mussels are usually considered as a particularly suitable species for biological monitoring (Smaal and Widdows, 1994). To address the CCA leachate issue, two approaches have been developed by using a biological early warning system (Baldwin and Kramer, 1994) and by assessing the mussel scope for growth (SFG).

The biological early warning system concerns a valvometer monitoring continuously the valve movement response. Under normal conditions, mussels have their shells open for respiration and feeding processes. They close their shells under natural or anthropogenic stress for an extended period of time as an escape behavior response. They might also increase the valve movement activity when stressed by specific pollutants (Kramer et al., 1989). Therefore, the valve movement response has been used to study a number of pollutant effects, like trace metals and trace organics (Davenport, 1977; Sloof et al., 1983).

The scope for growth (SFG) is an integrated physiological parameter reflecting the energy balance between energy acquisition (feeding - absorption) and catabolism, mainly due to respiration. This parameter has been widely used to assess environmental quality and test pollutant effects (Bayne, 1976; Bayne et al., 1985; Widdows et al., 1990).

Relationships between both approaches will be developed to improve understandings of CCA effects on the mussel physiology.

## I. Material and Methods (task 3, sub-task 3.3)

### I.1 Mussel and Oyster Population

Blue mussels, *Mytilus edulis* L. were collected in February 1996 within the intertidal rearing area at the near vicinity of Boyardville, Oleron Island (France). The two sampled populations concern mussels reared directly on CCA treated wooden poles and on 'control' poles. The first mussel batch was reared during more than 8 months on treated poles. Moreover, oysters (88mm, total weight=75g) naturally settled on these poles, were collected for similar comparisons.

After being individually sorted and cleaned, the mussels (length=35mm) were kept in a closed system with filtered seawater at 29.8ppt and 13.5°C. Phytoplanktonic algae (*Skeletonema costatum*, *Isochrysis galbana*) were used to feed the mussels.

The **CCA treated poles' mussel and oyster populations** were kept in a tank in similar environmental conditions.

The **control** population was then equally distributed into 4 tanks representing the following modalities : Control (0), Treatment 1=10kg/m<sup>3</sup>, Treatment 2 = 19kg/m<sup>3</sup>, Treatment 3 = 44kg/m<sup>3</sup>. Five treated wooden blocks were deployed into each 30 l tank containing 80 mussels to induce a continuous exposure to CCA (table 1). Mortality rates were estimated by count on a daily basis.

	SURFACE (cm <sup>2</sup> )	VOLUME (cm <sup>3</sup> )	TOTAL WEIGHT (g)
Control	186.1	112.5	69.45
Treatment 1	179.4	108.1	58.8
Treatment 2	195	120	63.4
Treatment 3	203.1	130.4	84.2

Table 1: Characteristics of the wooden blocks deployed for each modality.

## I.2. Experimental protocols

Two types of experimental protocols were carried out on these mussels to assess short-, mid-, and long-term CCA effects.

### I.2.1. Valve closure using a Valvometer

The first protocol is based upon the use of an electronic valvometer that records continuously the filtering activity by measuring valve closure. At each set, eight mussel activity was individually recorded. The valvometer was deployed in a 10 l tank filled with filtered seawater and 0.5 l of phytoplankton. The monitoring of the mussel filtering activity was recorded every 5 seconds during at least 5 hours. Several modalities were tested. In addition of testing the previously cited mussel batches after one week of exposure, the short term effect of leachate from treated wood was also tested by adding one block per treatment during a monitoring of control mussels. The table 2 summarizes the tested modalities :

	Modality	Experimental Time exposure to CCA	Records (n)
Field population	Treated poles	> 8 months	16
	Control	> 8 months	22
Laboratory population	Control	> 1 week	5
	Treatment 1	> 1 week	9
	Treatment 2	> 1 week	7
	Treatment 3	> 1 week	15
	Control	< 24 h	28
	Treatment 1	< 24 h	15
	Treatment 2	< 24 h	14
	Treatment 3	< 24 h	24

Table 2: Modalities and record number of continuous measurement of filtering activity by using an electronic valvometer.



### 1.2.2. Ecophysiological studies

The second protocol was developed to assess quantitatively the mussel filtering, ingestion and assimilation rates following the exposure to various CCA levels. Moreover, respiration rates were estimated on the same mussels in order to eventually calculate the mussel net energy balance or scope for growth (SFG). Experiments were carried out at various exposure time using mussels from the control, CCA10, CCA19 and CCA44 kg.m<sup>3</sup> treatments. The field population was also tested at the beginning and the completion of the experiment.

	Modality	Experimental Time Day N°
Field population	Treated poles	4, 11, 12
	Control	4, 11, 12
Laboratory population	Control	5, 8, 9, 10, 11
	Treatment 1	5, 8, 9, 10, 11
	Treatment 2	5, 8, 9, 10, 11
	Treatment 3	5, 8, 9, 10, 11

Table 3. Ecophysiological study on mussels exposed to various CCA concentrations. The mussels were subjected to a CCA stress at day 0 for 12 days. Day number represents the timing when physiological studies were carried out.

### Experimental setup

The experimental setup was described by Soletchnik *et al.* (1996) and included a 3 m<sup>3</sup> tank and 21 individual chambers (Fig. 1). The tank was filled on a daily basis with 1 µm filtered seawater, completed with phytoplanktonic algae (*Skeletonema costatum*, *Isochrysis galbana*) to feed the mussels. The tank was continuously homogenized to induce constant seawater characteristics throughout the daily experiment. Nineteen chambers were used with mussels from the control, 1, 2, 3 treatments or from the field population, for individual physiological measurements. Meanwhile, three chambers without mussels were used as control to estimate inflow seawater characteristics. Although mussels were not directly exposed to CCA during the measurement time in the experimental chamber, any physiological significant difference among the treatments was considered as a result of the previous accumulated exposure to CCA.

During the physiological measurements, seawater temperature and salinity was near 14°C and 31ppt respectively. Several seawater characteristics were estimated at the inflow and outflow of physiological chambers, including particulate and organic matter, chlorophyll a and pheopigments, and carbon-hydrogen-nitrogen (CHN). Total particulate matter (TPM) was estimated by filtering seawater on Whatman GF/C filters and then dried at 60°C before weighing. Particulate organic (POM) and inorganic matter (PIM) were estimated by weighing after ignition at 450°C (Razet *et al.*, 1990). Chlorophyll a concentrations were analyzed using a Jenway fluorimeter (Yentsch and Menzel, 1963). Following seawater filtration on Whatman GF/F, CHN analyses were carried out by combustion using a CHNS/O 2400 Perkin-Elmer analyzer.

Three mussels were deployed per experimental chamber, while feces and pseudofeces were collected at the completion of the daily experiment. Feces production was delayed by

considering the gut transit time (35 min) (Soletchnik *et al.*, 1996). Once the experiment completed, the mussels were shucked, frozen and then freeze-dried for 36 hours. Dry meat (DMW) was individually weighed to the nearest 0.01 mg. Dry shell (DSW) was weighed to the nearest 0.1g.

### Physiological functions

Physiological measurements were estimated on 4 replicates per treatment on a daily basis. Clearance rates ( $l \cdot h^{-1}$ ) were estimated using the total sea water volume flowing through the experimental setup to allow precise particulate matter concentration estimates.

$$CLEAR = Vol \times (E_i - E_o) / E_i / t \times 60$$

where, Vol represented the total volume of seawater collected during the experimental time;  $E_i$  and  $E_o$  were either the organic matter, chlorophyll and pheopigments, or carbon concentration at the seawater inflow seawater and experimental chambers outflow, respectively. Similarly, consumption rates ( $mg \cdot h^{-1}$ ) (CONSU) were calculated as follow :

$$CONSU = Vol \times (E_i - E_o) / t \times 60$$

Moreover, ingestion (ING) ( $mg \cdot h^{-1}$ ) and assimilation (ASS) ( $mg \cdot h^{-1}$ ) rates were calculated as follow :

$$ING = CONSU - pf$$

$$ASS = ING - f$$

where pf and f are the pseudofeces and feces production, respectively.

Following the filtration measurements, mussels were transferred to an experimental chamber to assess respiration rates (RESP) ( $\mu mole \cdot O_2 \cdot h^{-1}$ ) using a WTW oxygen probe.

Physiological estimates (e.g., respiration and clearance rates) were standardized to 1g dry meat weight using the following allometric relationship (Bayne and Newell, 1983):

$$V_{std} = (DMW_{std} / DMW_{exp})^b \times V_{exp}$$

where  $V_{exp}$  is the measured clearance (CLEAR) or respiration (RESPI) rates and ( $V_{std}$ ), the standardized value. The allometric coefficient b was 0.75 and 0.67 for respiration and clearance rates, respectively (Hawkins *et al.*, 1990; Hawkins and Bayne, 1992).

### Energy budget

Energetic conversion factors were  $20 J \cdot mg^{-1}$ ,  $0.45 J \cdot \mu mole^{-1}$  for the particulate organic matter and oxygen respectively. The phytoplankton estimates were converted to carbon by using a 50 coefficient (Strickland, 1960; Ahlgren, 1983), then to organic matter with a 2.14 factor (Widdows *et al.*, 1979).



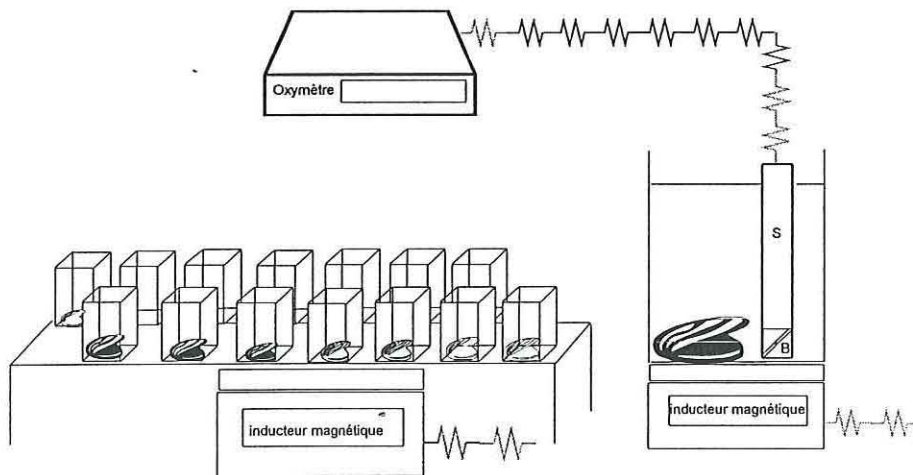
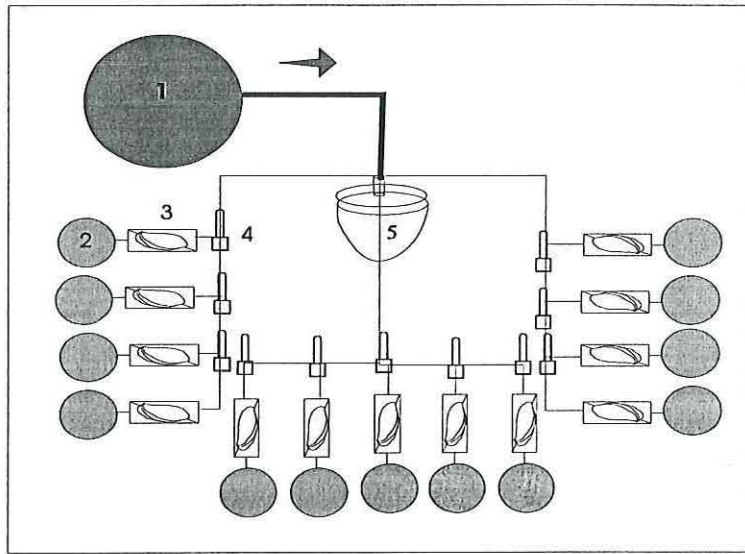


Figure 1: Experimental set-up for feeding processes (A) and respiration rate assessment (B). 1, 3m<sup>3</sup> tank; 2, output seawater collecting container; 3, experimental individual chamber; 4, flowmeter; 5, chamber to homogenize and distribute seawater to individual chamber.

Eventually, the scope for growth (SFG) ( $J.h^{-1}$ ) for a standard individual was calculated following :

$$SFG = E_{ASS} - E_{RESP}$$

All statistical tests and procedures used in the analyses and presentation of data were undertaken using STATGRAPHICS Plus, Version 7.0.

## II. RESULTS

### II.1. Mussel Population

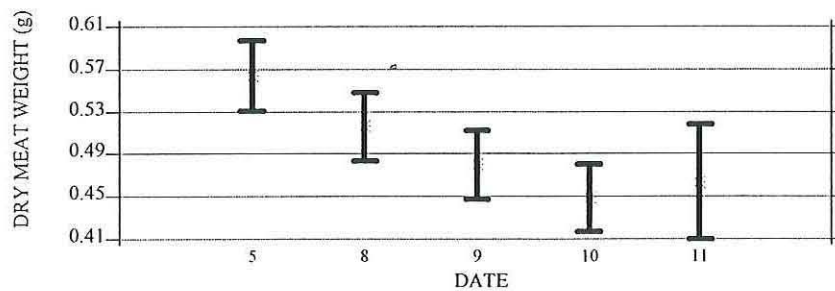
#### II.1.1. Dry meat weight

The mussel dry meat weight varied from 0.4 to 0.66g per individual during the experiment. The effects of the CCA treatment level and date were tested using a 2 way ANOVA (Table 4). Both effects were highly significant showing a dry meat weight decrease during the course of the experiment and the CCA treatment level (Fig. 2). Although the control population showed a slight decline, the stronger effect was concomitant to increased CCA levels. The interaction between both effects was not significant.

Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
<b>Main effects</b>					
A:Date	0.129939	4	2.84435	7.59	0.0001
B:Type	0.21015		1.54454	4.12	0.0104
<b>Interactions</b>					
AB	0.146807	12	0.0122339	1.37	0.2044
Residual	0.544113	61	0.00891988		
Total (Corrected)	1.00764	80			

All F-Ratios are based on the residual mean square error

Table 4 : 2-way ANOVA results of Dry meat weight function of CCA Treatment and Time.





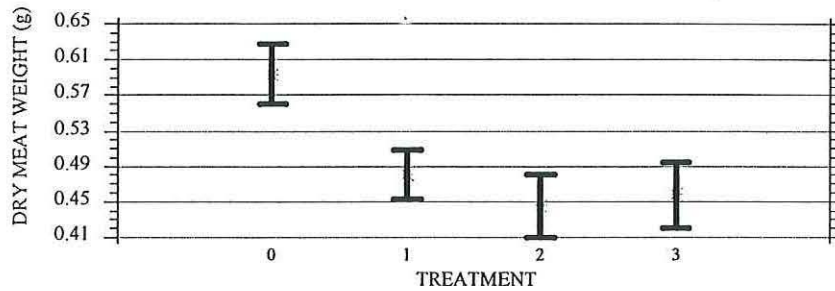


Figure 2: 2-Way ANOVA results. Effects of the 2 factors 'CCA Treatment' and 'Date' on the dry meat weight changes. Mean values and 95% LSD intervals.

A multiple comparison procedure (LSD) estimated a significant difference at 95% confidence level between the control population and the 3 CCA treatments. A multiple range test (LSD) separated the control from the 3 treatments. Therefore, ecophysiological functions are logically presented using the standardized 1g individual.

### II.1.2. Mortality rate

No mortality rate was reported for the control population during the course of the experiment (Table 5). In contrast, mortality occurred after 5 days of exposure to all CCA concentrations, with the highest daily rate (7%) in treatment 3. Significant mortality was observed after Day 8 and 9 of exposure for treatment 2 and 1, respectively.

Exposure Time	Daily Mortality Rate (%)			
	Control	Treatment 1	Treatment 2	Treatment 3
0	0	0	0	0
4	0	0	0	0
6	0	1.5	1.5	7
8	0	4	0	6.3
9	0	0	8.1	4.5
10	0	6	15	-

Table 5: Daily mussel mortality (%) rate during the experiment for the control and treatment 1, 2, 3 groups.

### II.2. Valvometer

Several examples of continuous monitoring aimed to describe short-term CCA effects on mussels are presented on figures 3 to 7. Although the statistical treatment of these records will be more deeply studied and presented in the final report, several observations can be already drawn on valve closure behavior.

The control mussels showed a very active behavior with numerous and fast valve movements ranging from 0.1 to 0.6mm (Fig. 4). The mussels valves were continuously open during the entire experiment with a highly variable activity.

As soon as the 10kg.m<sup>3</sup> wooden block was added, mussels detected the presence of chemicals and reacted by reducing the valve opening (Fig. 5). However, this behavior was not

observed systematically and several mussels show no significant reaction. Following the CCA block introduction, mussels changed their valve opening behavior by reducing the opening frequency and intensity. The changes were more significant after 3 hours of exposure.

Mussels were systematically affected by the introduction of a 19kg.m<sup>3</sup> wooden block. However, no mussel reacted by closing permanently their valves. The valve opening behavior was severely affected showing a significant closure as soon as the block was introduced (Fig. 6). Then, most of the mussels showed a reduced and irregular activity. However several mussels recovered a similar valve opening as previously, although with a different frequency. After one hour of exposure, several alive mussels showed a drastically reduced and an almost constant valve opening (Fig. 6).

By introducing a 44kg.m<sup>3</sup> treated wooden block, more drastic responses were observed with a full valve closure for several mussels (Fig. 3, 7). However, this closure lasted less than a minute, then mussels recovered a significant but abnormal activity. Then, shell valves were systematically less open compared to their status prior to the block introduction. Although not systematically observed, several mussels showed a permanent valve closure after 4 hours of exposure, until the end of this experiment.

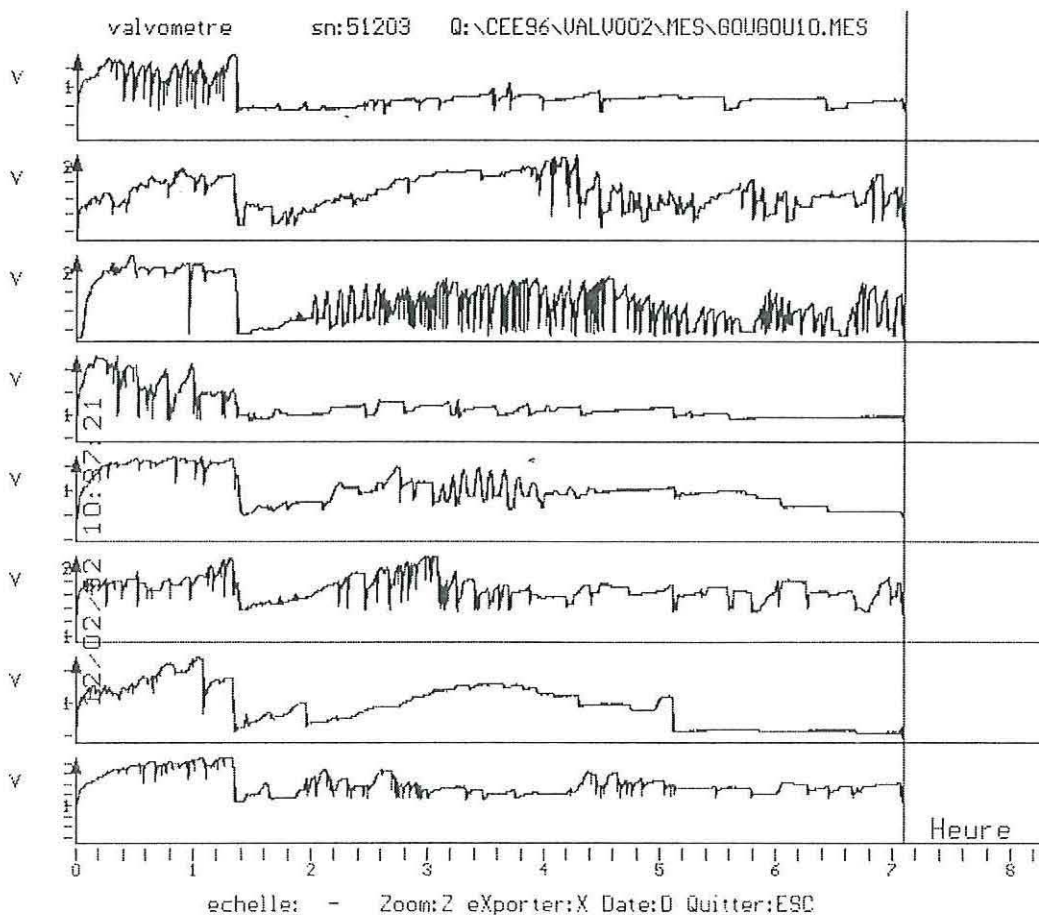


Figure 3: Example of a valvometer recording on eight individual mussels. A drastic valve closure followed the introduction of a 44kg.m<sup>3</sup> treated wooden block at minute 80'.



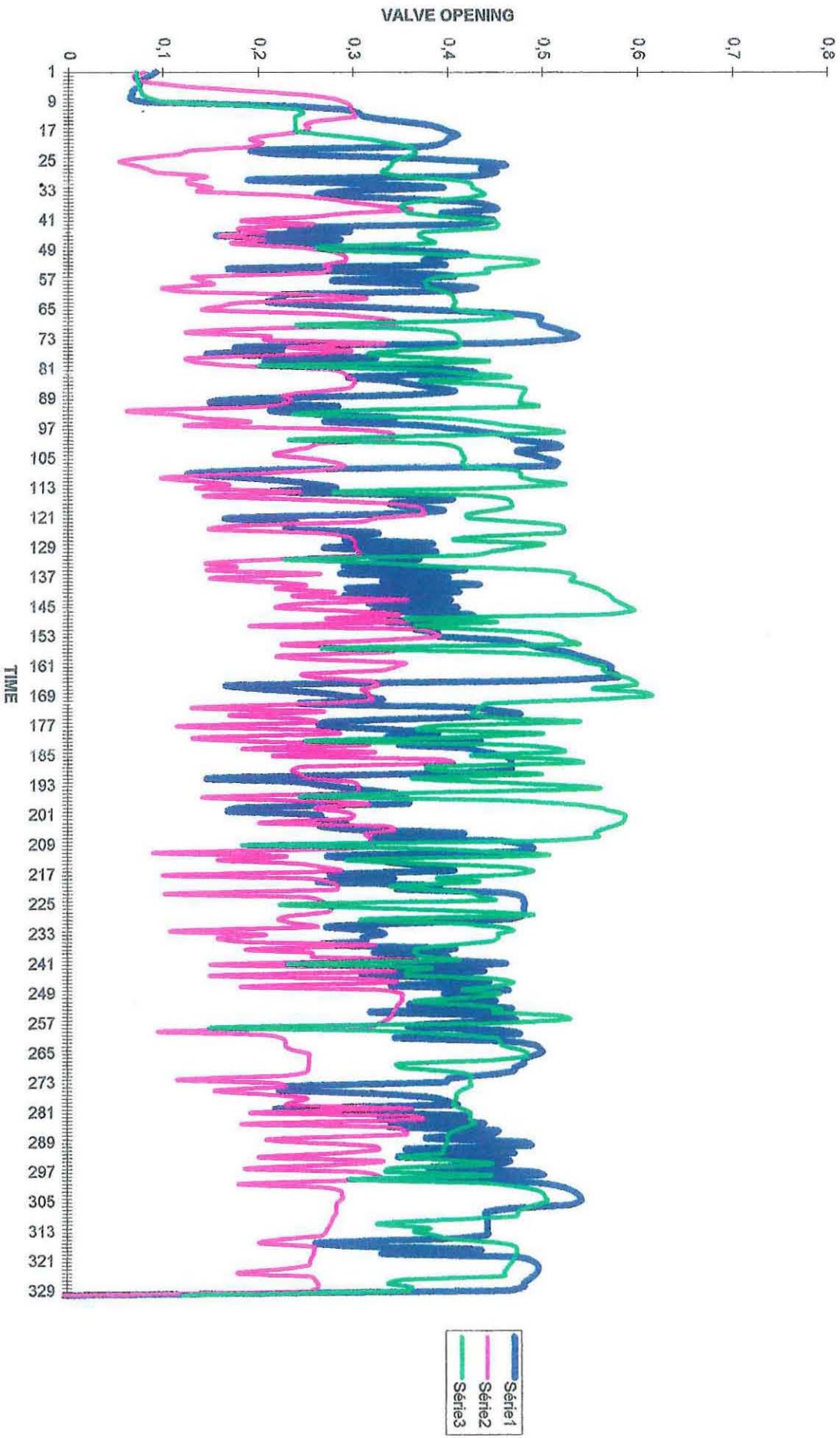


Figure 4 : Example of a valvometer recording on control musssels

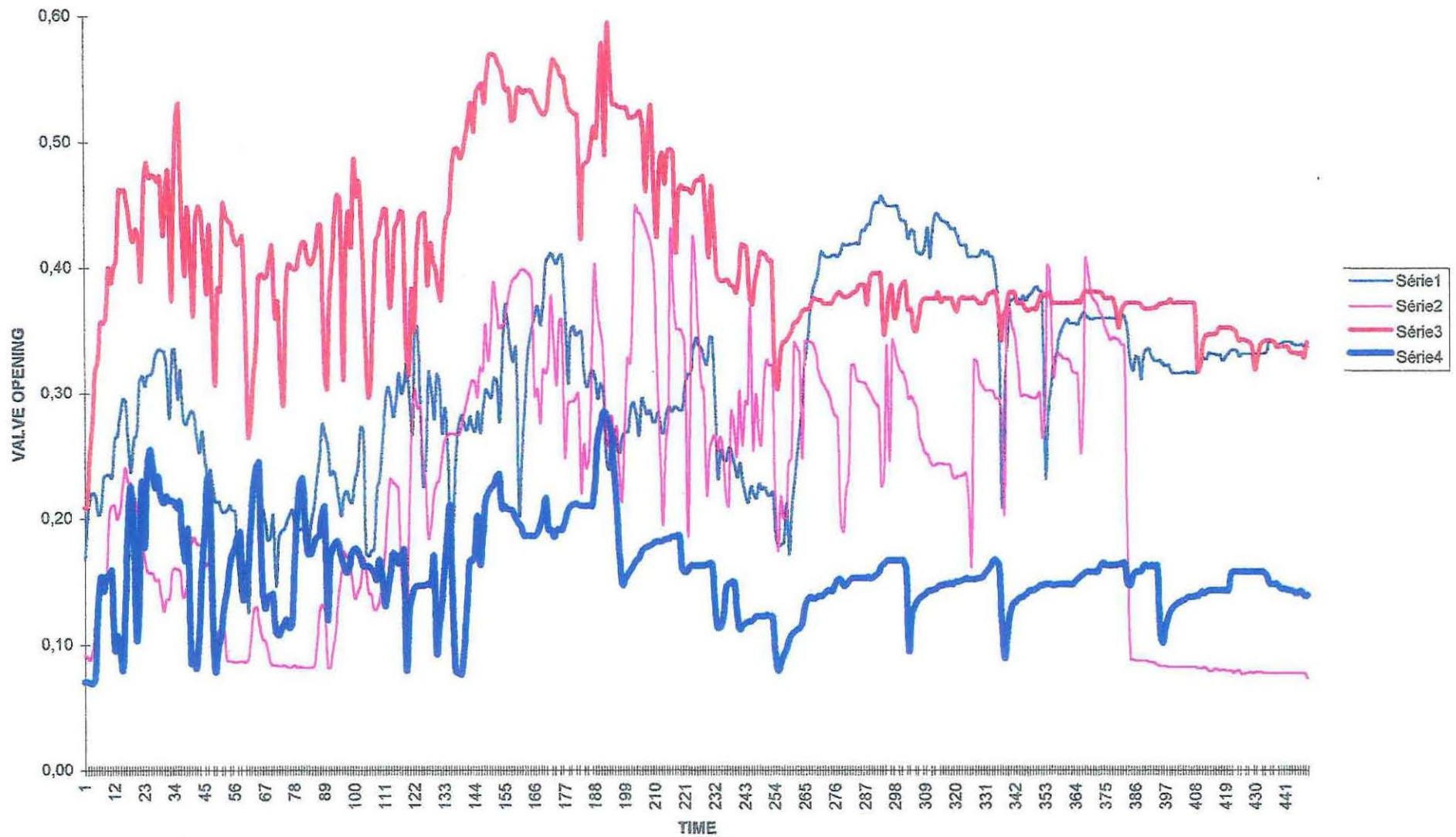


Figure 5 : Example of a valvometer recording on mussels valve activity. After 45 minutes, a CCA treated block ( $10\text{Kg.m}^3$ ) was permanently introduced at the near valvometer vicinity.



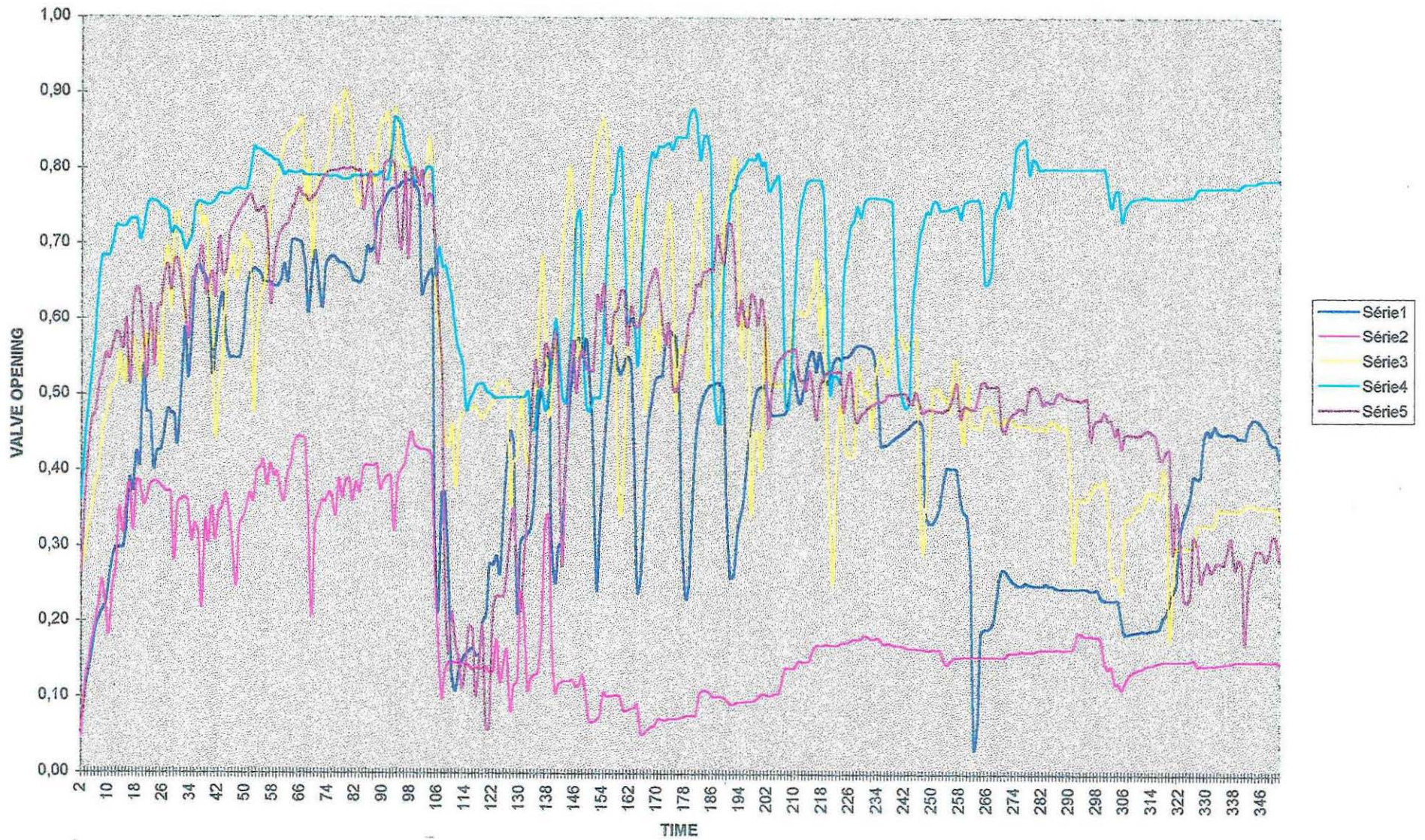


Figure 6 : Example of a valvometer recording on mussels valve activity. After 106 minutes, a CCA treated block ( $19\text{Kg.m}^3$ ) was permanently introduced at the near valvometer vicinity.



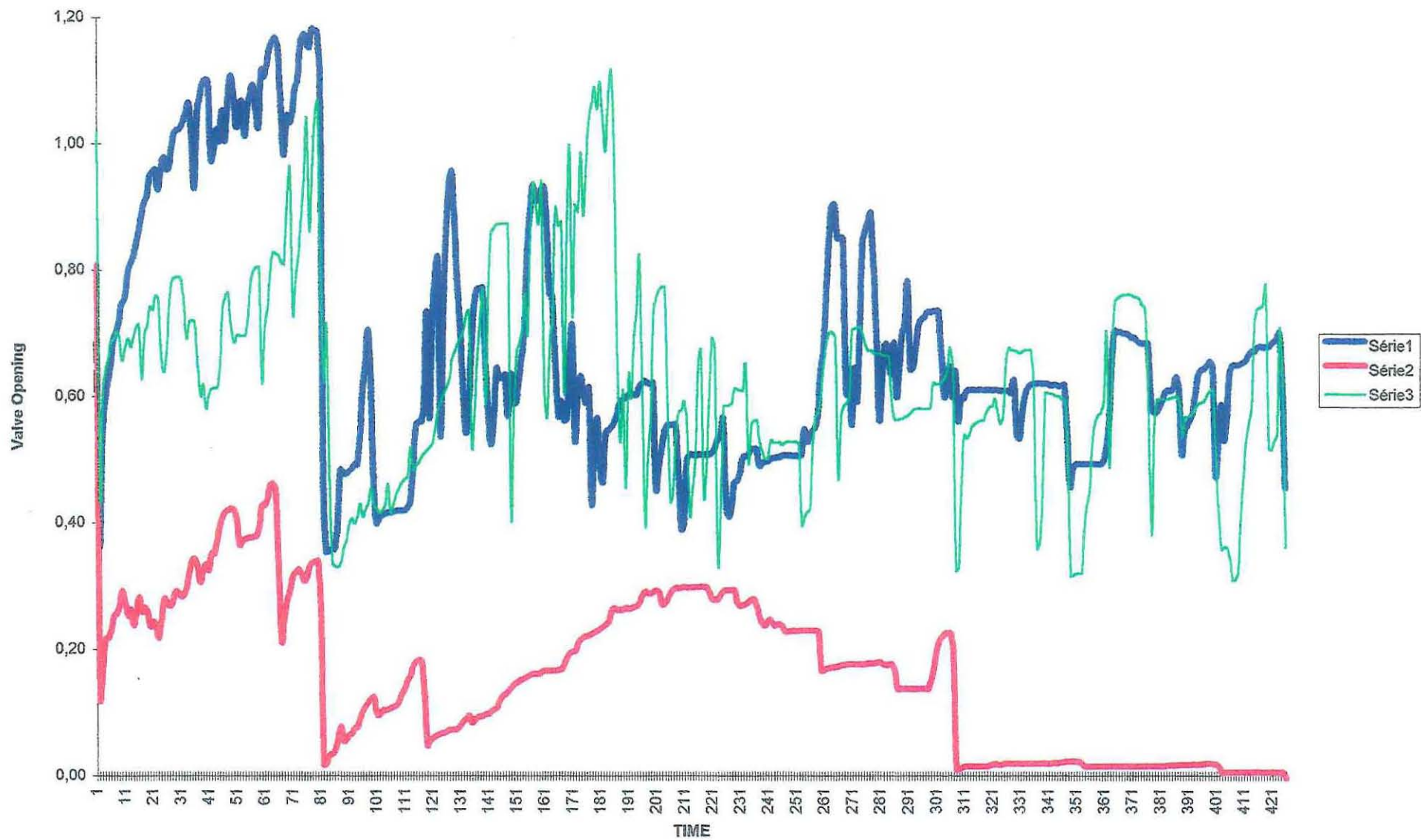


Figure 7 : Example of a valvometer recording on mussels valve activity. After 81 minutes, a CCA treated block ( $44\text{Kg.m}^3$ ) was permanently introduced at the near valvometer vicinity.



## II.3. Ecophysiology

Feeding processes were assessed and compared using 3 independent variables (i.e., particulate organic matter, chlorophyll a and CHN analysis) so as to strengthen the data output and results.

### II.3.1 Effects of CCA treatments on the mussel physiology : laboratory population

#### 2.3.1 Feeding Processes calculated with Particulate Organic Matter

The ANOVA results for the clearance rate of a standardized mussel are presented on table 6 and figure 8. Both effects of date and CCA treatment affected significantly the standardized clearance rate. Although limited, the clearance rate tended to increase with time, varying from 0.5 to 1.2 l.h<sup>-1</sup>. The main difference resulted from data at day 8. The clearance rate decreased concomitantly to the increasing CCA treatment level with the most significant decline for treatment 3 (0.67l.h<sup>-1</sup>). In the later case, clearance rates were halved compared to the control. The clearance rates were similar between the control and treatment 1 ranging between 1.3 to 1.35 l.h<sup>-1</sup>.

Analysis of Variance -Type III Sums of Square

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Date	11.3774	4	2.84435	7.59	0.0001
B:Type	4.633613	3	1.54454	4.12	0.0104
Residual	29.9894	56	0.374812		
Total (Corrected)	37.9227	63			

All F-Ratios are based on the residual mean square error

Table 6: Two-way analysis of variance for the 1g standardized mussel clearance rate. Factors are 'date' and 'type' of CCA treatment.

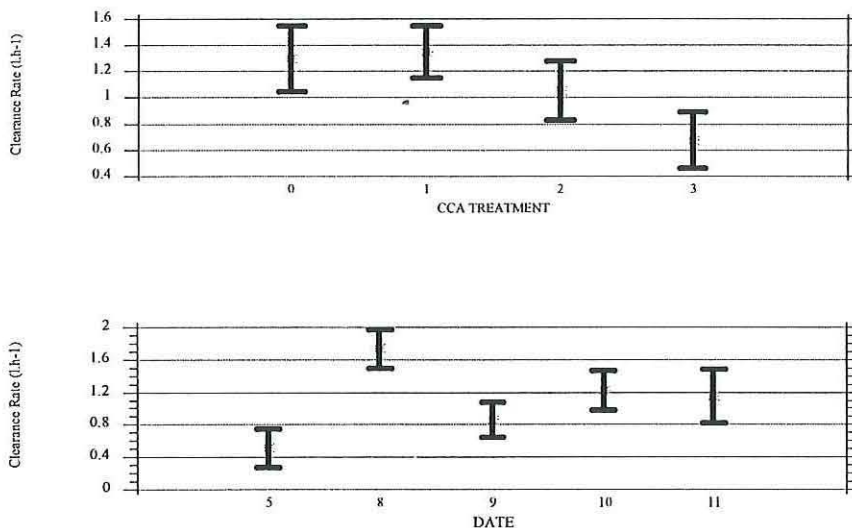


Figure 8: Results of 2 way- analysis of variance of clearance rates with the 'CCA treatment' and 'Date' factors mean values and 95% confidence intervals. Calculations are based on particulate organic matter values. (Control (0), CCA treatment : 10 (1), 19 (2), 44 (3) kg.m<sup>3</sup>).

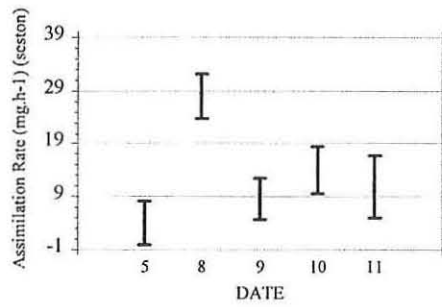
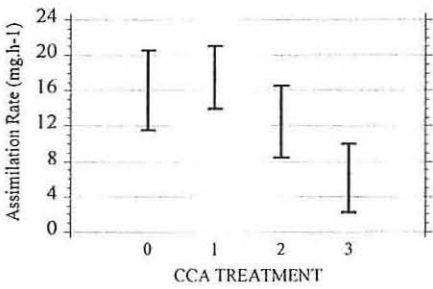
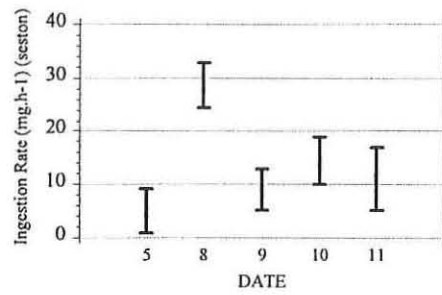
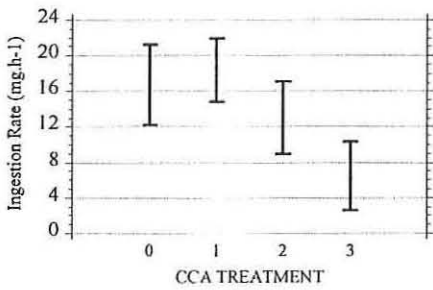
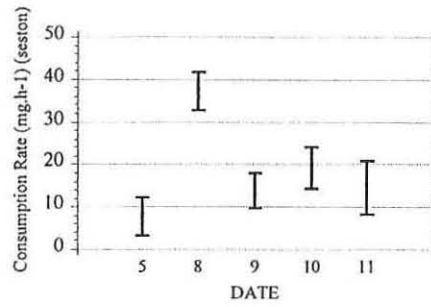
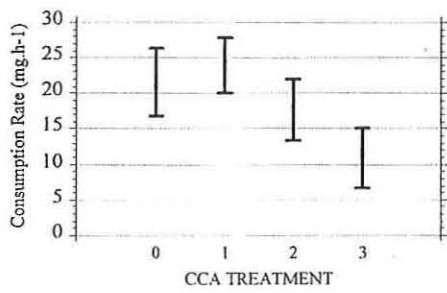


Figure 9: Results of 2 way- analysis of variance of consumption, ingestion and assimilation rates with the 'CCA treatment' and 'Date' factors mean values and 95% LSD confidence intervals. Calculations are based on particulate organic matter values. (Control (0); CCA Treatment, 10 (1), 19 (2), 44 (3) kg.m<sup>3</sup>).

Moreover, the effect of 'CCA treatment' and 'date' on consumption, ingestion and assimilation rates were all significant and similar to the effects on clearance rates. The ANOVAS' results are presented on table 7 and figure 9.



Consumption Rates - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Date	6886.91	4	1721.73	12.4	0.0000
B:Type	1647.77	3	549.257	3.96	0.0125
Residual	7774.29	56	138.827		
Total (Corrected)	16702.9	63			

All F-Ratios are based on the residual mean square error

Ingestion Rates - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Date	4520.54	4	1130.4	9.54	0.0000
B:Type	1359.42	3	453.14	3.83	0.0145
Residual	6630.67	56	118.405		
Total (Corrected)	12807.1	63			

All F-Ratios are based on the residual mean square error

Assimilation Rates - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Date	4545.76	4	1136.44	9.6	0.0000
B:Type	1260.95	3	420.316	3.55	0.02
Residual	6627.07	56	118.341		
Total (Corrected)	12718.0	63			

All F-Ratios are based on the residual mean square error

Table 7: Overall results of 2-way ANOVAs concerning the 'CCA treatment', 'Time' effects on consumption, ingestion and assimilation rates of a 1g standardized mussel. Calculations were based on particulate organic matter.

This similar pattern demonstrated that CCA treatments affected primarily the clearance activity. The significant differences in consumption, ingestion and assimilation rates resulted mainly from this initial difference.

### 2.3.2. Feeding Processes calculated with the Chlorophyll a and pheopigments concentrations

A 2-way ANOVA was performed on clearances rates' data calculated with chlorophyll a and pheopigments analysis. Clearance rates were significantly affected by both factors ('CCA Treatment' and 'Time' (Table 8, Figure 10). Mean values reached 1.64, 1.80, 1.12, 0.9 l.h<sup>-1</sup> for the control and treatment 1, 2 and 3, respectively. However for the 'Time' effect, the difference resulted mainly from day 9 showing significantly higher clearance rates. At day 11, clearance rates were reduced and showed a higher variability. Comparisons of mean values by using LSD multiple range test resulted in grouping results from days 5, 8, 10 and 11, and a second group with results from day 9.

Clearance Rates - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	22.33	4	5.58	12.38	0.0000
B: Type	8.71	3	2.90	6.44	0.0007
Residual	28.42	63	0.45		
Total (Corrected)	59.03	70			

All F-Ratios are based on the residual mean square error

Table 8 : Overall results of 2-way ANOVA concerning the 'CCA treatment', 'Time' effects on clearance rates of a 1g standardized mussel. Calculation are based on chlorophyll a and pheopigments analysis.

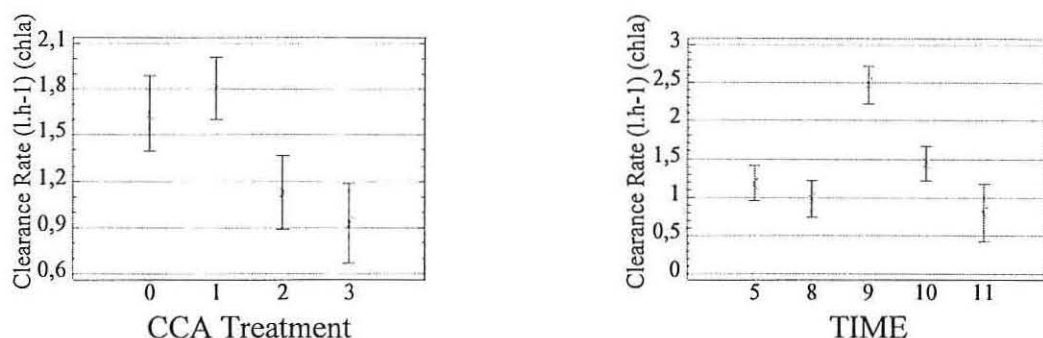


Figure 10: 2-Way ANOVA results. Effects of 'CCA treatment' and 'Time' on clearance rates mean values. Confidence intervals are at 95% LSD.

In contrast, clearance rates were significantly affected by the CCA treatment, and decreased with concomitant increased CCA treatments. For example, the clearance rate from control mussels were two fold greater than those from treatment 3. The same multiple range test performed on CCA treatment resulted in 2 distinct groups : control and treatment 1, and treatments 2 and 3.

**Consumption Rates - Analysis of Variance -Type III Sums of Square**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	210839	4	52709.9	7.8	0.0000
B: Type	163312	3	54437	8.06	0.0001
Residual	425622	63	6755.9		
Total (Corrected)	767413	70			

All F-Ratios are based on the residual mean square error

**Ingestion Rates - Analysis of Variance -Type III Sums of Square**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	189072	4	47268	7.63	0.0000
B: Type	137025	3	45675	7.37	0.0003
Residual	390450	63	6197.6		
Total (Corrected)	687744	70			

All F-Ratios are based on the residual mean square error

**Assimilation Rates - Analysis of Variance -Type III Sums of Square**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	141979	4	35494.8	6.55	0.0002
B: Type	102832	3	34277.4	6.32	0.0008
Residual	341609	63	5422.36		
Total (Corrected)	560309	70			

All F-Ratios are based on the residual mean square error

Table 9: Overall results of 2-way ANOVA concerning the 'CCA treatment', 'Time' effects on consumption, ingestion and assimilation rates of a 1g standardized mussel. Calculation are based on chlorophyll a and pheopigments analysis.

With regards to consumption, ingestion and assimilation rates, ANOVA's confirmed in a similar way the 'CCA Treatment' effect (Fig. 11). Assimilation rates for control were 5 fold greater than those from treatment 3, with 112 and 25  $\mu\text{g}\cdot\text{h}^{-1}$ , respectively. These rates decreased significantly with increasing time, reaching the lowest values at day 11 ( $24\mu\text{g}\cdot\text{h}^{-1}$ ). In contrast to the clearance rates' results, a drastic decline was observed over time from 159 to



24  $\mu\text{g}\cdot\text{h}^{-1}$ . A food level decrease over time and a physiological adaptation may explain this pattern.

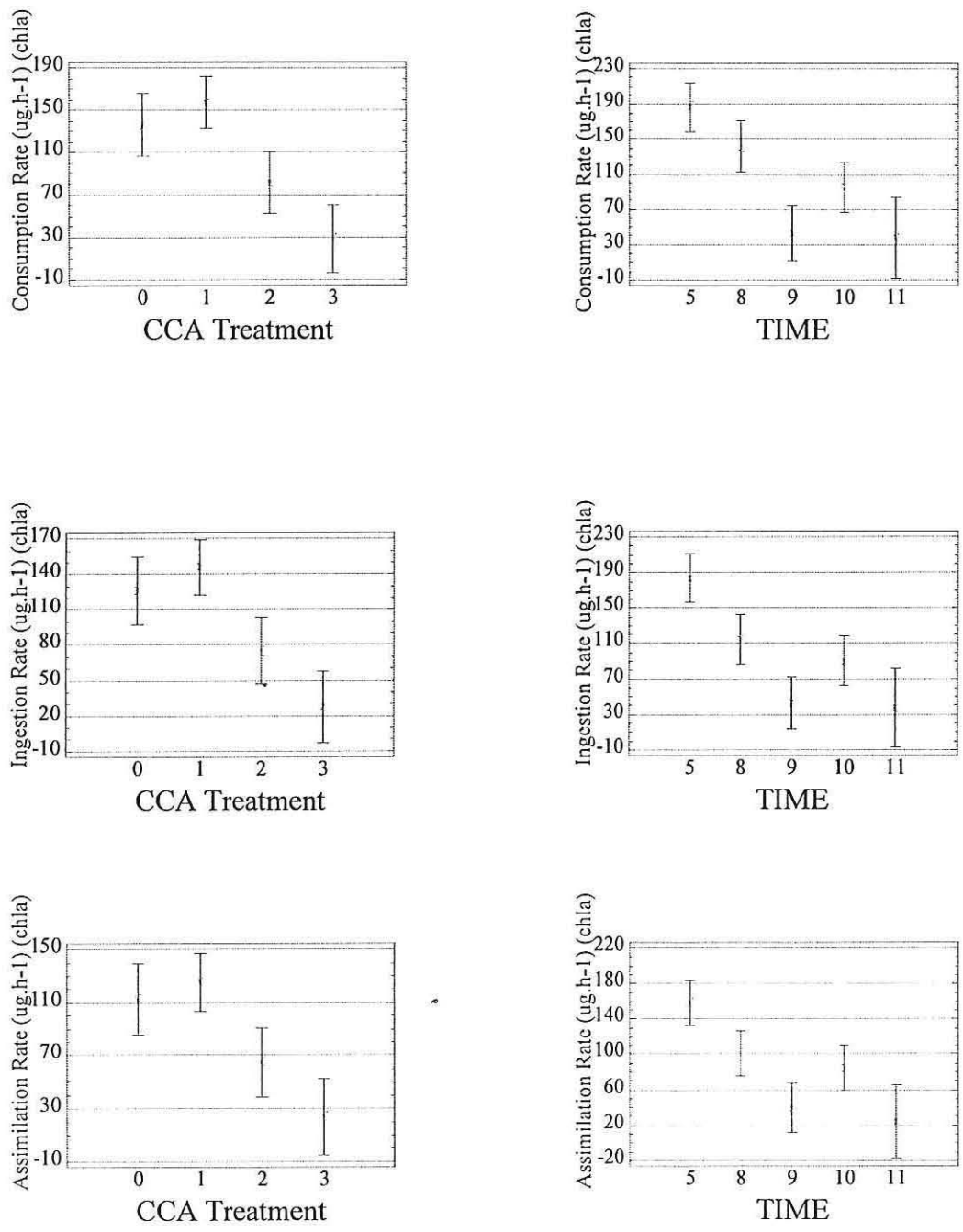


Figure 11: 2-way ANOVAs results of consumption, ingestion and assimilation rates function of the 2 factors 'CCA treatment' and 'Time'. Calculations were based on chlorophyll a and pheopigments analysis.

### 2.3.2. Feeding Processes calculated with CHN analysis

The clearance rates' results, based on carbon concentrations, are presented in table 10 and figure 12. In these computations, both effects were significant showing increasing clearance rates with time and reduced clearance rates with higher CCA concentrations.

Clearance Rates - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	6.258	4	1.565	7.24	0.001
B: Type	8.872	3	2.957	13.69	0.000
Residual	13.607	63	0.216		
Total (Corrected)	29.02	70			
All F-Ratios are based on the residual mean square error					
Consumption Rates - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	167.992	4	41.9979	7.12	0.0001
B: Type	236.324	3	78.7746	13.35	0.000
Residual	371.755	63	5.909		
Total (Corrected)	765.697	70			
All F-Ratios are based on the residual mean square error					
Ingestion Rates - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	183.242	4	45.8104	9.45	0.000
B: Type	172.347	3	57.4491	11.85	0.000
Residual	305.464	63	4.84863		
Total (Corrected)	651.698	70			
All F-Ratios are based on the residual mean square error					
Assimilation Rates - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	178.339	4	44.5848	10.22	0.000
B: Type	109.045	3	36.3484	8.33	0.0001
Residual	274.8	63	4.3619		
Total (Corrected)	554.73	70			
All F-Ratios are based on the residual mean square error					

Table 10: Overall results of 2-way ANOVA concerning the 'CCA treatment', 'Time' effects on consumption, ingestion of a 1g standardized mussel. Calculations are based on CHN analysis.

Clearance rates ranged from 0.46, 0.68, 1.30, 1.24 l.h<sup>-1</sup> for treatments 3, 2, 1, and control respectively (Fig. 12). Therefore, the control mussels were 3 fold more active than those affected by the treatment 3 (44 kg.m<sup>3</sup>). The multiple range test (LSD) discriminated the 2 groups: control and treatment 1, and treatments 2, 3. With regard to the 'Time' effect, the increased values for date 10 and 11 might result from a various seawater quality.



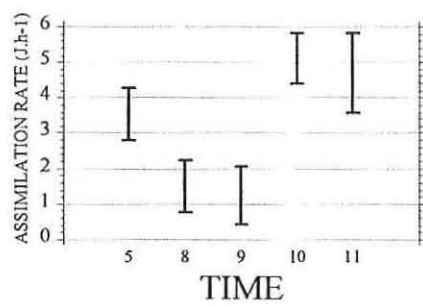
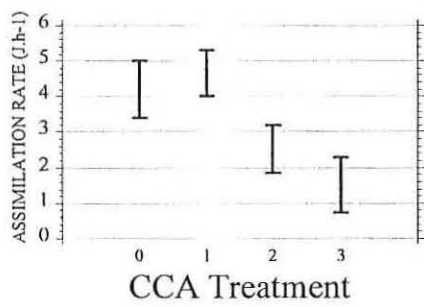
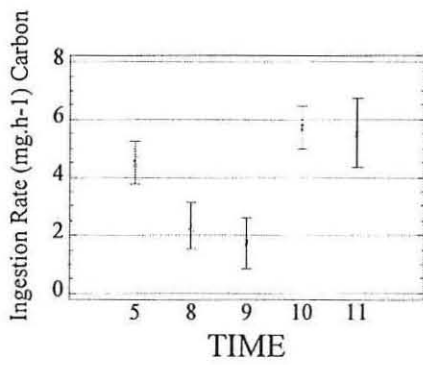
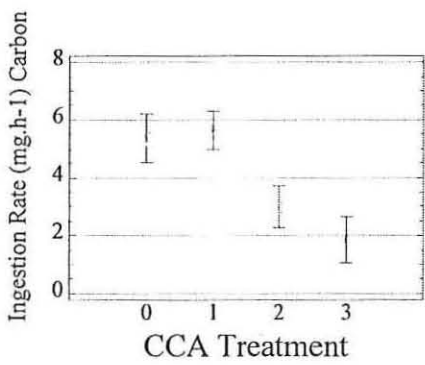
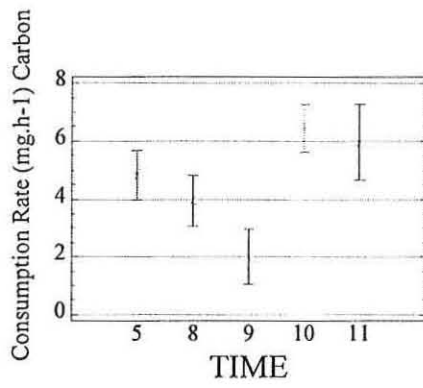
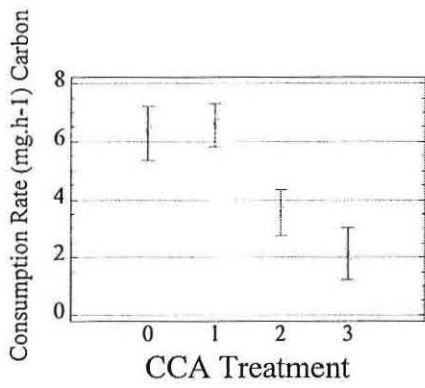
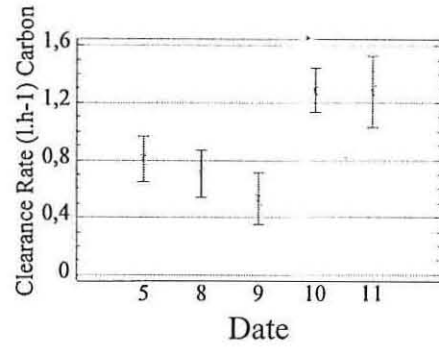
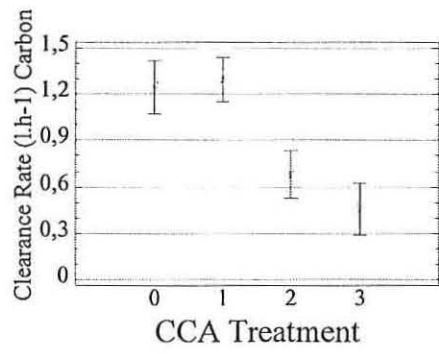


Figure 12: 2-way ANOVAs results of clearance, consumption, ingestion and assimilation rates function of the 2 factors 'CCA treatment' and 'Time'.

## 2.4 Respiration Rates

The main results concerning the respiration rates are presented on table 11 and figure 13. The two factors 'CCA treatment' and 'date' affected significantly the mussel respiration rate. Three groups emerged from the 2 way ANOVA analysis and the multiple range tests: the control and treatment 1, treatments 1&3, and treatments 2&3. The 10kg/m<sup>3</sup> treatment did not affect significantly the respiration rate in contrast to the later group showing a reduced catabolism activity. Although significant, this more progressive trend demonstrated that respiration rates were less affected than clearance rates by the CCA treatments.

Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Date	529.787	4	132.447	2.69	0.0388
B:Type	535.065	3	178.355	3.63	0.0176
Residual	3098.01	63	49.1747		
Total (Corrected)	3986.24	70			

All F-Ratios are based on the residual mean square error

Table 11 : ANOVA on Respiration rates (umole d'02.h-1) affected by 'CCA Treatment' and 'time'

With regard to the 'date effect', the figure 13 showed that respiration rates were more variable and reduced at day 11 compared to the previous dates. The data at day 11 are mainly responsible for the significant difference. No significant difference was observed between day 5 and day 10 per treatment.

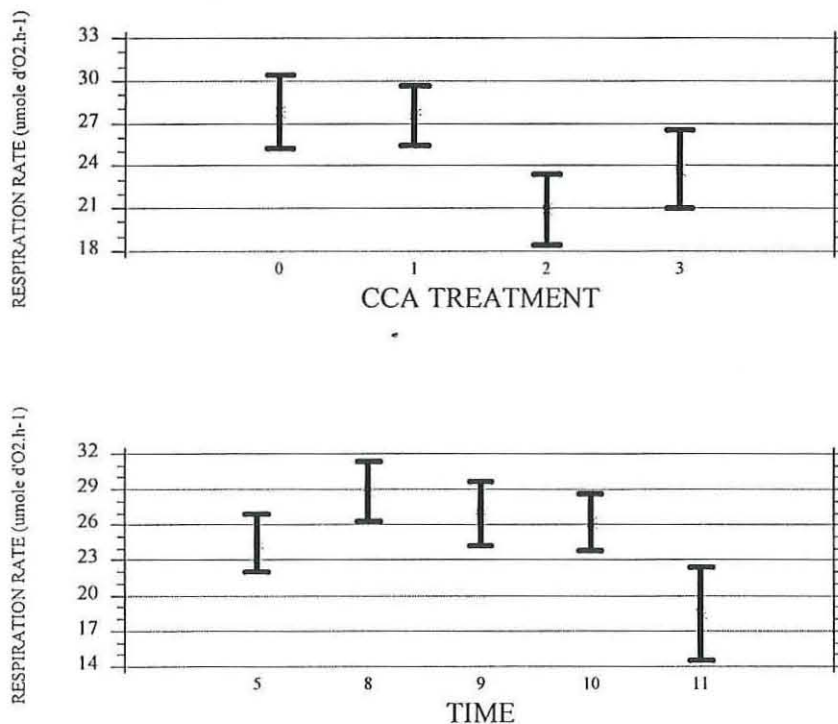


Figure 13. Respiration rates of the mussel population. Effects of CCA treatment and Time on mean values. 95% LSD confidence intervals.



## 2.5 Scope For Growth

### 2.5.1. Scope for Growth (Particulate Organic Matter)

Based on the previous results, the Scope for Growth can be estimated (Table 12, Fig. 14). In a similar way, both factors 'CCA Treatment' and 'Date' affected significantly the mussel SFG (Table 12).

Energy from Assimilation - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Date	1.818E6	4	454576	9.6	0.0000
B:Type	504379.0	3	168126	3.55	0.02
Residual	2.65E6	56	47336.2		
Total (Corrected)	5.087E6	63			

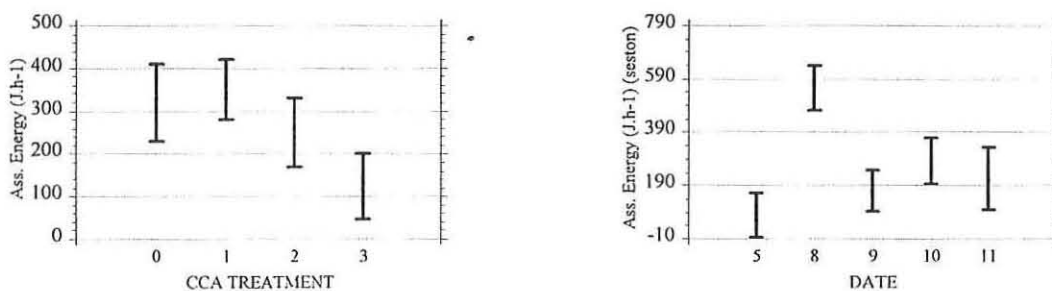
All F-Ratios are based on the residual mean square error

Scope for Growth - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Date	1.086E6	4	451521	9.57	0.0000
B:Type	494626	3	164875	3.5	0.0213
Residual	2.6416E6	56	47172.9		
Total (Corrected)	5.0563	63			

All F-Ratios are based on the residual mean square error

Table 12: ANOVAs' results of the Assimilation (energy) and Scope for Growth estimates affected by CCA treatment and time. Calculations are based on particulate organic matter concentrations.

The figure 14 demonstrated that the scope for growth declined concomitantly to the increased CCA treatment, with 112, 239, 338, and 307 J.h<sup>-1</sup> for treatment 3, 2, 1 and control respectively. The multiple range test (LSD) discriminated 2 groups: treatments 3&2, and treatments 2, 1 and control. In contrast, the main differences during the course of the experiment resulted from the highest assimilation rate observed at day 8. The Scope for Growth tended to increase when considering all the experimental days but day 8. This resulted from both reduced respiration and increased assimilation rates.



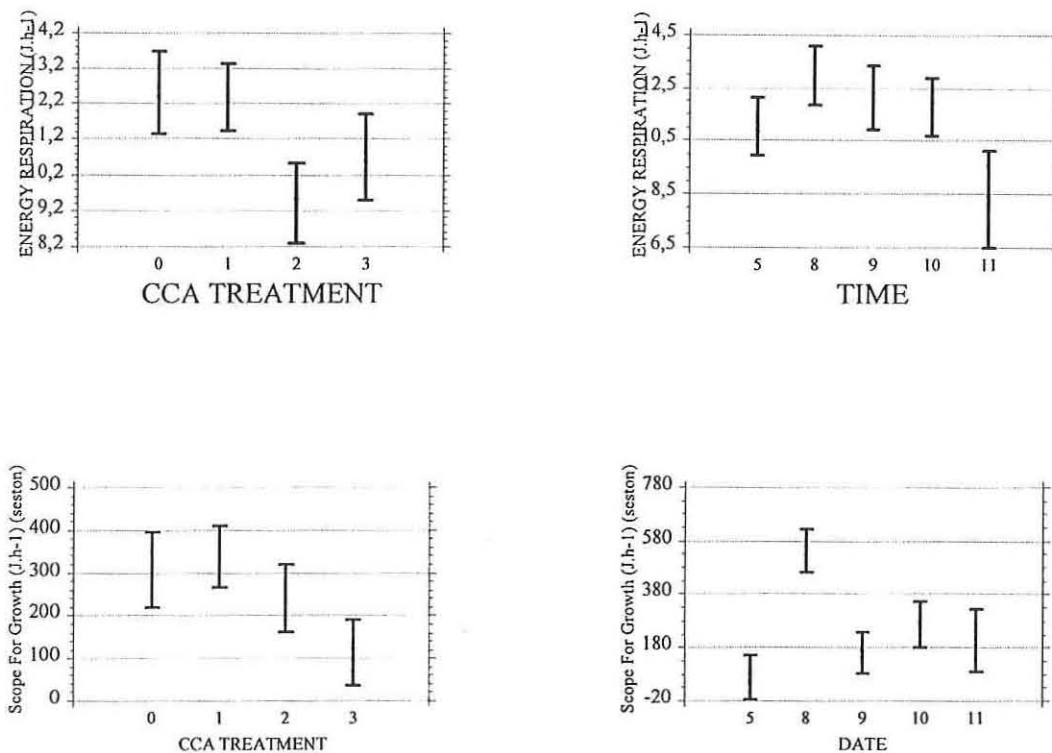


Figure 14: ANOVA mean values of energy gain from assimilation, energy expenditure from respiration and the energetic balance of mussels affected by CCA treatments and time. Calculations were based on particulate organic matter.

### 2.5.2. Scope for Growth (Chlorophyll a and Pheopigments)

By combining respiration rates values to the previous data, we can conclude that scope for growth estimates showed a similar pattern with both significant effects from 'CCA treatment' and 'time' (Table 13, Fig. 15). In treatment 3, several mussels were severely stressed showing a negative scope for growth (Fig. 15). Mean values ranged from 40.7, 129.4, 255.9, 228.9  $J.h^{-1}$ , for treatment 3, 2, 1, and control respectively. Three groups were obtained using multiple range tests: 0-1, 0-2, 2-3.

Energy Assimilation - Analysis of Variance -Type III Sums of Square

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	650207	4	162552	6.55	0.0002
B: Type	470930	3	156977	6.32	0.0008
Residual	1.5644E6	63	24832		
Total (Corrected)	2.5656E6	70			

All F-Ratios are based on the residual mean square error



Scope for Growth - Analysis of Variance -Type III Sums of Square

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
<b>Main effects</b>					
A: Date	648357	4	162089	6.55	0.0002
B: Type	460591	3	15353	6.2	0.0009
Residual	1.55E6	63	24752		
Total (Corrected)	2.55E6	70			

All F-Ratios are based on the residual mean square error

Table 13: ANOVA results of Energy assimilated and Scope for Growth (in  $J.h^{-1}$ ) function of CCA treatment and 'time'. Calculations are based on chlorophyll a and pheopigments analysis.

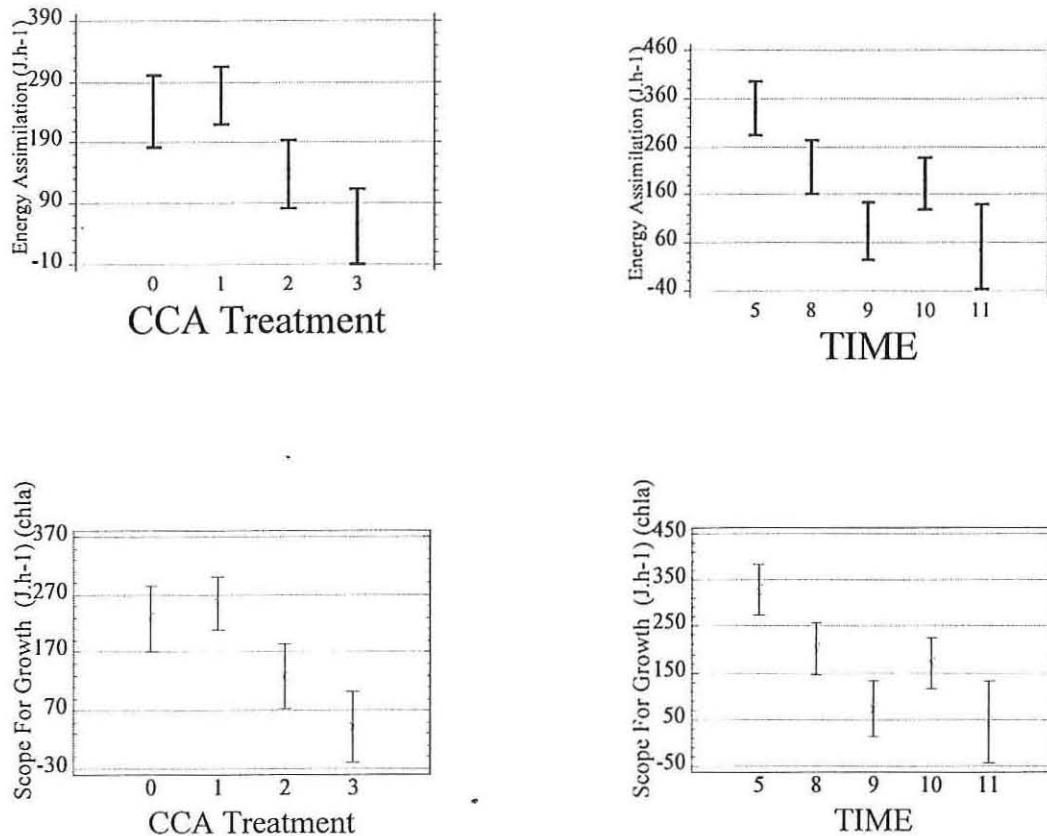


Figure 15: ANOVA mean values of energy gain from assimilation, energy expenditure from respiration and the energetic balance of mussels affected by CCA treatments and time. Calculations were based on chlorophyll a and pheopigments.

### 2.5.3. Scope for Growth (Carbon)

Scope for growth results are presented on table 14 and figure 16. As previously noted, both effects were significant. SFG mean values decreased concomitantly to increasing CCA treatments, with 70.3, 80.1, 40.6 and 21.6  $J.h^{-1}$ , for control, treatment 1, 2, 3 respectively. Two groups were distinct : control and treatment 1, and treatments 2 & 3. Actually, the 'control' SFG was on the average 3 fold higher than on treatment 3. Higher SFG values at Day 10 and 11, resulted from increased assimilated energy due to higher seawater quality.

**Energy Assimilation Analysis of Variance -Type III Sums of Square**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
<b>Main effects</b>					
A: Date	71335.7	4	17833.9	10.22	0.000
B: Type	43618.1	3	14539.4	8.33	0.0001
Residual	109920	63	1744.76		
Total (Corrected)	221892	70			

All F-Ratios are based on the residual mean square error

**Scope for Growth - Analysis of Variance -Type III Sums of Square**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
<b>Main effects</b>					
A: Date	70317.6	4	17579.4	10.32	0.000
B: Type	37237.9	3	12412.6	7.29	0.0003
Residual	107327	63	1703.61		
Total (Corrected)	212282	70			

All F-Ratios are based on the residual mean square error

Table 14: ANOVA results of Energy assimilated and Scope for Growth (in  $J.h^{-1}$ ) function of 'CCA treatment' and 'time'. Calculations are based on CHN (Carbon) analysis.

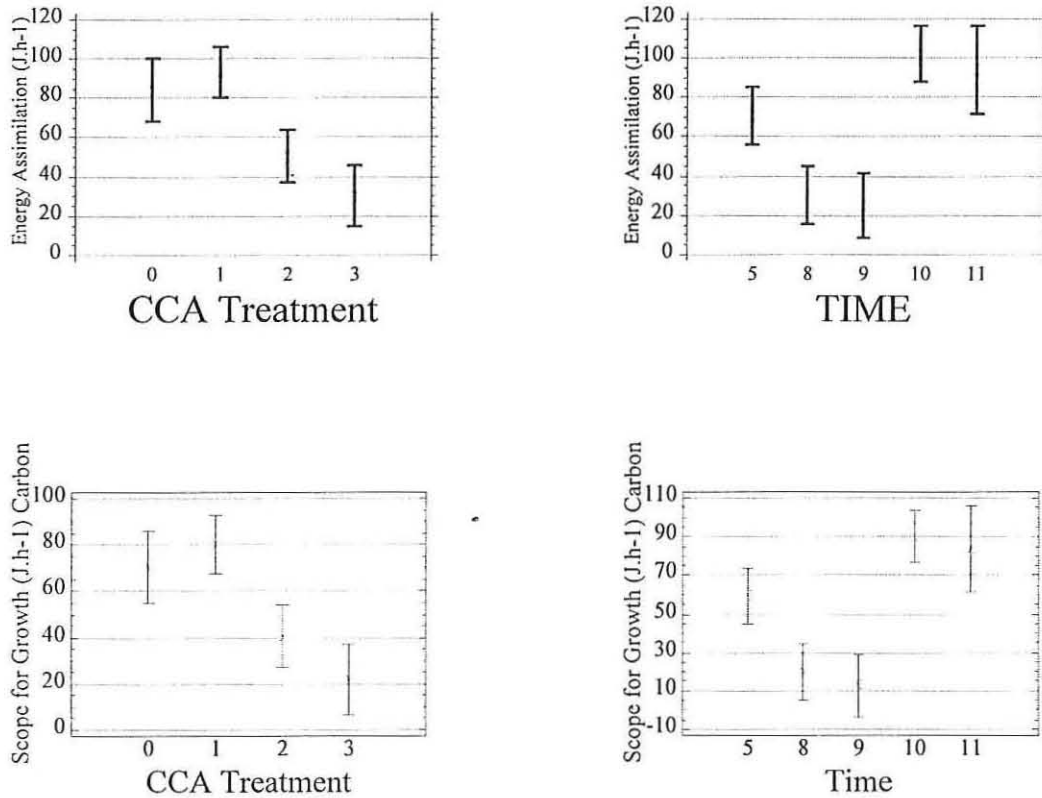


Figure 16: ANOVA mean values of energy gain ( $J.h^{-1}$ ) from assimilation, energy expenditure from respiration and the energetic balance of mussels affected by CCA treatments and time. Calculations were based on CHN (Carbon) analysis.



## II.3.2 Long term effects of CCA leachates on the mussel physiology: field population

### 2.3.2. Comparison of the mussel groups

#### 2.3.2.1 Scope for Growth (Particulate Organic Matter)

A 2-way ANOVA was performed on the clearance rates from the natural mussel population reared on treated and control (non-treated) wooden poles (Table 15, fig. 17). Although clearance rates from mussels living on CCA poles tended to be reduced and more homogeneous, no significant difference was observed between the 2 types of poles (P value=0.39). The average clearance rates varied from 0.88 and 1.03 l.h<sup>-1</sup> for the CCA treatment and control respectively. In contrast, the clearance rates significantly declined with time from 1.3 to 0.6 l.h<sup>-1</sup>. However, since variability increased with time, only the results obtained at day 4 were significantly higher than those at day 12.

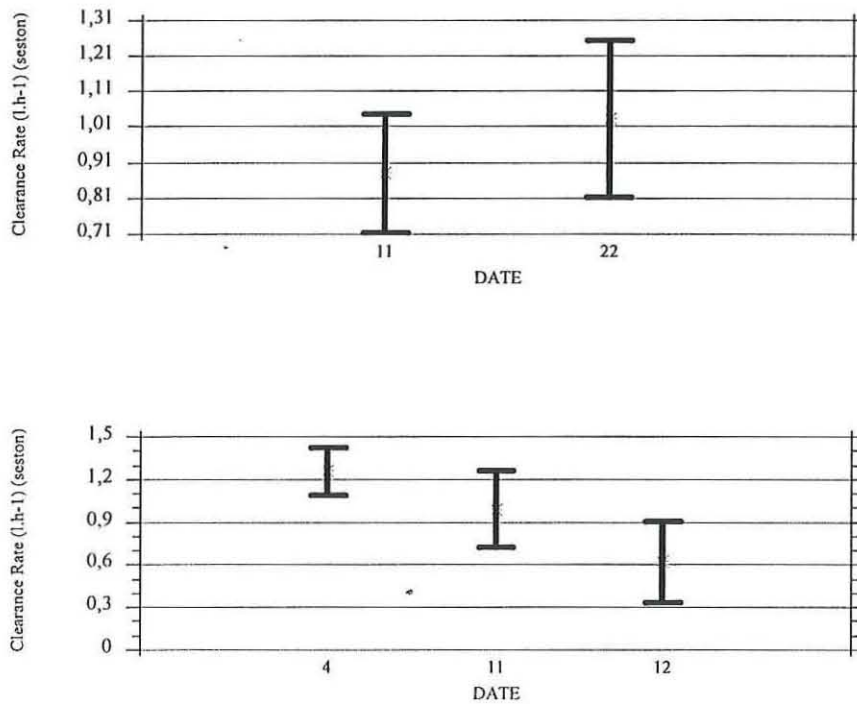


Figure 17: 2-way ANOVA results of clearance rates (l.h<sup>-1</sup>) function of CCA Treatment (11, treated poles; 22 non treated poles) and time. Mean values and 95% LSD confidence intervals.

Clearance Rates - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Date	1.47075	2	0.735375	4.3	0.0279
B:Type	0.1275	1	0.127503	0.75	0.3979
Residual	3.41663	20	0.170832		
Total (Corrected)	5.22783	23			

All F-Ratios are based on the residual mean square error

Table 15. Results of the 2-way ANOVA of clearance rates function of CCA Treatment and time.

Consumption, ingestion and assimilation rates confirmed these observations as well as the respiration rates with the mean values 8.66 and 8.04  $\mu\text{mole d}^{-1}\text{O}_2\cdot\text{h}^{-1}$  for CCA treatment and control respectively. Therefore, the Scope for Growth was not significantly different between the two mussel populations (Table 16).

Energy expenditure from Respiration - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Date	47.2435	2	23.62	4.12	0.0318
B:Type	2.0614	1	2.0614	0.36	0.5555
Residual	114.689	20	5.7344		
Total (Corrected)	165.5	23			
All F-Ratios are based on the residual mean square error					
SFG estimates based on Particulate Organic Matter - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Date	288372	2	144186	9.1	0.0015
B:Type	14293	1	14293.9	0.9	0.3536
Residual	316989	20	15849.5		
Total (Corrected)	649542	23			
All F-Ratios are based on the residual mean square error					

Table 16. Results of the 2-way ANOVAs of respiration and SFG function of 'CCA Treatment' and 'time'.

### 2.3.2.2 Scope for Growth (Chlorophyll a & Pheopigments concentrations)

No significant difference was observed in clearance rates using a 2-way ANOVA with 'CCA treatment' and 'time' factors ( $P > 0.05$ ) (Table 17). In contrast, the Scope for growth showed a significant decrease with time (Fig. 18).

Clearance Rates using Chlorophyll a Pheopigments Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	1.378	2	0.689	0.79	0.4609
B: Type	0.06	1	0.062	0.07	0.79
Residual	26.009	30	0.8669		
Total (Corrected)	27.50	33			
All F-Ratios are based on the residual mean square error					
Scope for Growth Rates using Chlorophyll a - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	747039	2	373520	5.86	0.0071
B: Type	10176	1	10176.8	0.16	0.6924
Residual	1.91E6	30	63790		
Total (Corrected)	2.68E6	33			
All F-Ratios are based on the residual mean square error					

Table 17. Results of the 2-way ANOVAs of clearance rates and SFG function of CCA Treatment and time.



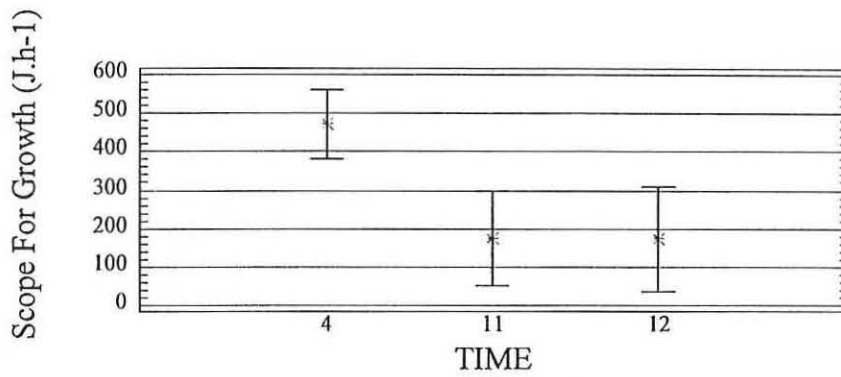


Figure 18. 2-way ANOVA results of Scope for Growth ( $J.h^{-1}$ ) function of time. Mean values and 95% LSD confidence intervals.

### 2.3.2.3 Scope for Growth (CHN-Carbon concentrations)

Although clearance rates of control mussels reached higher values, no significant difference was observed between the clearance rates of the control and mussels living on CCA treated poles (Fig. 19, Table 18). In contrast, a significant decline over time was observed while the variability increased.

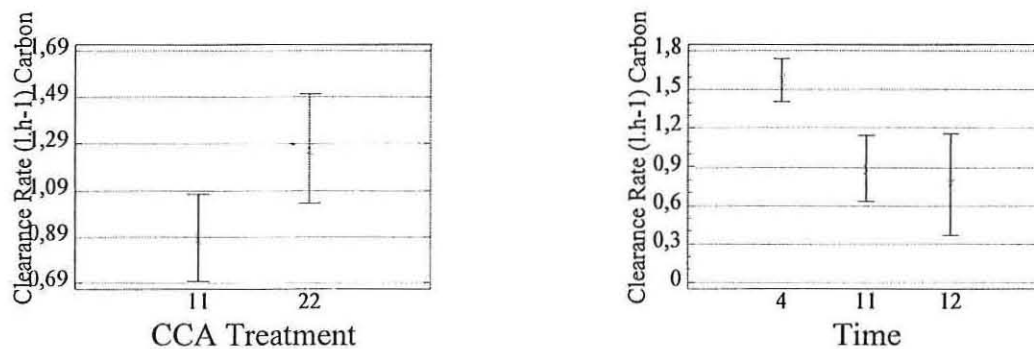


Figure 19. 2-way ANOVA results of clearance rates ( $l.h^{-1}$ ) function of CCA Treatment (11, treated poles; 22 non treated poles) and time. Mean values and 95% LSD confidence intervals.

Clearance Rates - Analysis of Variance -Type III Sums of Square

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	250.754	2	125.377	19.39	0.0000
B: Type	14.4136	1	14.4136	2.23	0.1484
Residual	155.146	24	6.4644		
Total (Corrected)	414.238	27			

All F-Ratios are based on the residual mean square error

Ingestion Rates - Analysis of Variance -Type III Sums of Square

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	211.558	2	105.779	18.96	0.0000
B: Type	15.8076	1	15.8076	2.83	0.1053
Residual	133.921	24	5.58006		
Total (Corrected)	355.358	27			

All F-Ratios are based on the residual mean square error

**Respiration rates of a Ig mussel - Analysis of Variance -Type III Sums of Square**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Type	45.441	1	45.4419	0.67	0.4207
B: Date	29.2577	2	14.6288	0.21	0.8081
Residual	2045.27	30	68.1756		
Total (Corrected)	2114.68	33			

All F-Ratios are based on the residual mean square error

Table 18. Results of the 2-way ANOVAs of clearance, ingestion and respiration rates function of CCA Treatment and time.

No significant difference was observed for the respiration rates between both modalities (Table 18). These trends are similar at the energetic level: CCA did not affect either assimilation nor the energy expenditure resulting from respiration. The only significant factor was the 'time', which affected the assimilated energy. However, this might be related to a seawater quality change (Table 19).

**Energy assimilated - Analysis of Variance -Type III Sums of Square**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	49659.3	2	24829.7	13.07	0.0001
B: Type	5373.78	1	5373.78	2.83	0.1055
Residual	45589.3	24	1899.55		
Total (Corrected)	99005.7	27			

All F-Ratios are based on the residual mean square error

**Energy expenditure from Respiration - Analysis of Variance -Type III Sums of Square**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Type	9.206	1	9.20606	0.67	0.4206
B: Date	5.9429	2	2.97147	0.22	0.8076
Residual	414.176	30	13.8059		
Total (Corrected)	428.252	33			

All F-Ratios are based on the residual mean square error

**SFG - Analysis of Variance -Type III Sums of Square**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	49818.7	2	24909.4	13.12	0.0001
B: Type	6012.37	1	6012.37	3.17	0.0878
Residual	45551.4	24	1897.98		
Total (Corrected)	99680.1	27			

All F-Ratios are based on the residual mean square error

Table 19: Results of the 2-way ANOVAs of energy assimilated, catabolized by respiration and SFG function of 'CCA Treatment' and 'time'.

### 2.3.3. Comparison of the oyster groups

#### 2.3.3.1 Scope for Growth (Particulate Organic Matter)

Oysters were tested at day 11. No significant differences in clearance and respiration rates were observed between oysters naturally collected on treated and non treated wooden poles (Table 20, Fig. 20). Clearance rate mean values reached  $0.91.h^{-1}$  and  $1.281.h^{-1}$  for oysters living on CCA treated poles and on control, respectively. A large variability was observed inducing no significant SFG's difference between both populations (CCA, mean values  $152J.h^{-1}$ , Control,  $298J.h^{-1}$ ) (Fig.21).

Clearance rate - Particulate Organic Matter - Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Type	0.231801	1	0.231801	0.38	0.5613
Residual	3.68122	6	0.613537		
Total (Corrected)	3.91302	7			

All F-Ratios are based on the residual mean square error

Table 20. Results of the 1-way ANOVAs of clearance rate function of CCA treatment.

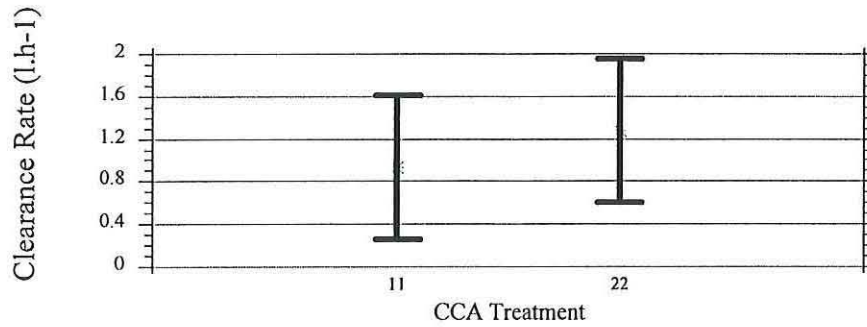


Figure 20. One-way ANOVA results concerning the effect of 'CCA treatment' on oyster clearance rates.

Respiration rates for a 1g standardized oyster. Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Type	172.64	1	172.64	1.14	0.31
Residual	1207.81	8	150.977		
Total (Corrected)	1380.45	9			

All F-Ratios are based on the residual mean square error

Energy respiration Analysis of Variance -Type III Sums of Square					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Type	9.26713	1	9.26713	0.25	0.6322
Residual	218.896	6	36.4827		
Total (Corrected)	228.163	7			

All F-Ratios are based on the residual mean square error

Table 21. Results of one-way ANOVAs on the 'CCA effect' on respiration rates and energy expenditure.

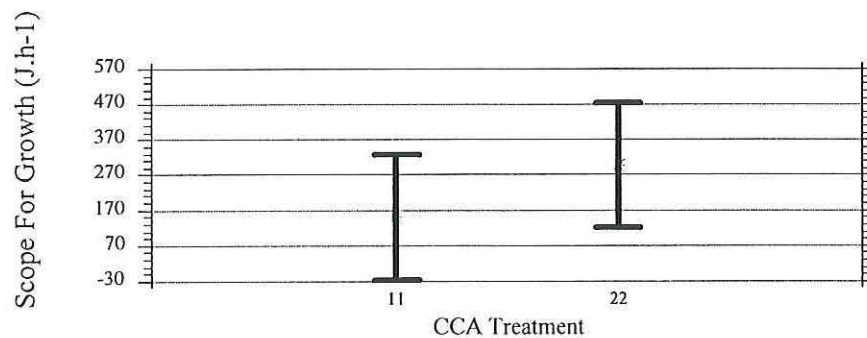


Figure 21. Mean values of Scope for Growth between control and oysters living on CCA treated poles. Marks are 95% LSD confidence intervals.



### 2.3.3.2 Scope for Growth (Chlorophyll a & Pheopigments concentrations)

The natural oyster population was tested in a similar way with regard to chlorophyll concentrations. Significant clearance rates decrease were observed for animals living on CCA treated poles, with 3.53 and 6.57l.h<sup>-1</sup> for the CCA modality and control respectively. In contrast, no significant difference was observed between SFG estimates (P-value>0.05) (Fig. 22).

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
B: Type	23.065	1	23.065	17.0	0.033
Residual	10.854	8	1.35683		
Total (Corrected)	33.919	9			

All F-Ratios are based on the residual mean square error

Table 22. CCA effect on oyster clearance rates estimated by chlorophyll estimates. One way ANOVA results.

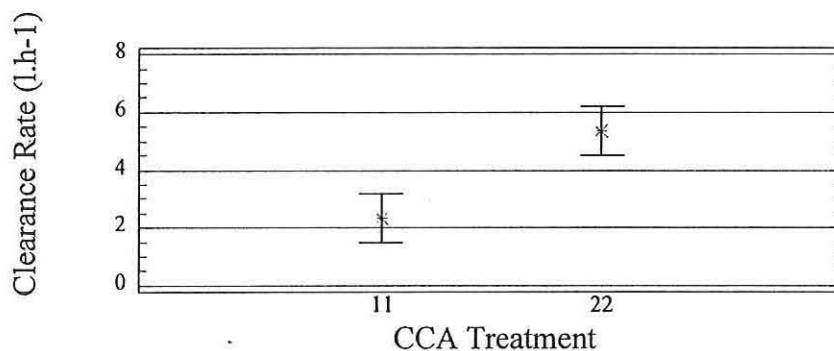


Figure 22. Clearance rate mean values of oysters living on CCA treated poles (11) and control (22).

### 2.3.3.3 Scope for Growth (CHN concentrations)

Similar data treatments were performed on CHN estimates for clearance, consumption and ingestion rates (Table 23, Fig. 23). All estimates showed reduced rates when living on treated poles compared to control. The clearance rates mean values reached 2.25 and 4.51l.h<sup>-1</sup> for the oysters living on CCA treated poles and on 'control' poles respectively.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Type	11.3315	1	11.3315	18.65	0.0035
Residual	4.252	7	0.607		
Total (Corrected)	15.584	8			

All F-Ratios are based on the residual mean square error

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Type	21.485	1	21.486	18.65	0.0035
Residual	8.06	7	1.15		
Total (Corrected)	29.55	8			

All F-Ratios are based on the residual mean square error

**Carbon Ingestion Rates Analysis of Variance -Type III Sums of Square**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Type	18.1439	1	18.1439	18.84	0.0034
Residual	6.741	7	0.9629		
<hr/>					
Total (Corrected)	24.885	8			

All F-Ratios are based on the residual mean square error

**Carbon Assimilation Rates Analysis of Variance -Type III Sums of Square**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Type	4788.36	1	4788.36	10.38	0.0146
Residual	3230.67	7	461.524		
<hr/>					
Total (Corrected)	8019.03	8			

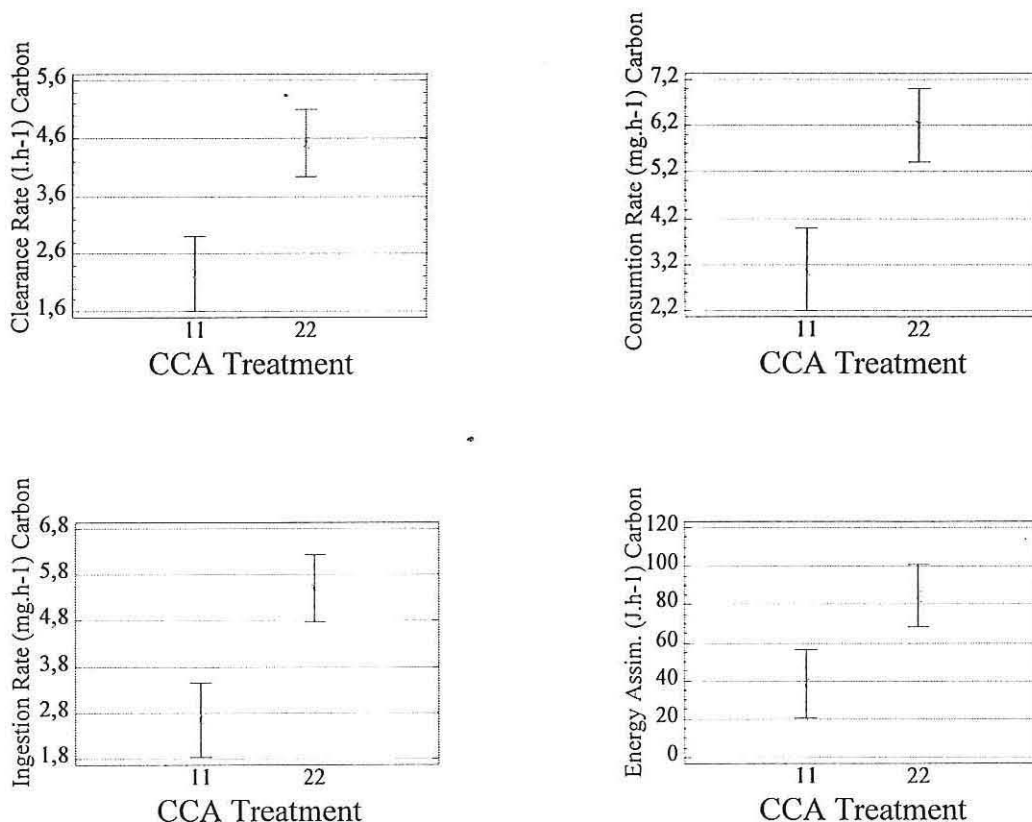
All F-Ratios are based on the residual mean square error

**SFG Analysis of Variance -Type III Sums of Square**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:Type	5438.32	1	5438.82	9.9	0.0162
Residual	3845.6	7	549.371		
<hr/>					
Total (Corrected)	9284.42	8			

All F-Ratios are based on the residual mean square error

Table 23. One way ANOVAs results of oysters' clearance, consumption, ingestion and assimilation rates living on CCA treated and non treated poles. Comparison of Scope for Growth.





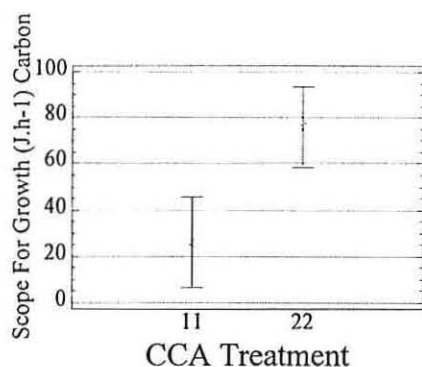


Figure 23. Mean values of One way ANOVAS on oysters living on CCA treated and control poles. Clearance, consumption, ingestion rates and energy estimates are compared.

## II. Discussion

Several literature reviews concerning the effects of using wood treated with chromated copper arsenate (CCA) have been published over the past several years (Brooks, 1994; Albuquerque and Cragg, 1995; Weis and Weis, 1996). Toxicity to estuarine organisms of CCA leachates have been widely demonstrated as well as the transfer of contaminants to the aquatic biota (Weis et al., 1991; Weis and Weis, 1992). Moreover, bioaccumulation and deleterious effects in the fouling community have been documented (Weis and Weis, 1996). The epibiota biodiversity and biomass can be considered as indicators of environmental impact of CCA (Albuquerque and Cragg, 1995b; Weis and Weis, 1996). Although several standard toxicity tests were carried out to demonstrate acute toxicity and sublethal effects, most of them concerned algae, crustaceans and gastropods. Actually, no assessment of CCA leachates effects on physiological responses of bivalves was available in the literature but long term bioaccumulation (Albuquerque and Cragg, 1995a; Weis and Weis, 1992; 1996). Meanwhile Scope for Growth is usually considered as an appropriate integrated parameter to assess impact of environmental contamination (Widdows, 1993; Smaal and Widdows, 1994). In contrast, several reviews have extensively documented the effects of copper, zinc, cadmium, lead on *Mytilus edulis* (for a review see, Akberali and Trueman, 1985). Therefore, our study represented the first effort to assess the CCA leachates effects on *M. edulis* physiological responses.

Our study facilitated assessment of CCA leachates effects on the mussel physiology. Several authors suggested that the usefulness of bivalves such as *M. edulis* as biological monitoring agent may be limited since they may fail to register short term presence of high pollutants levels by using avoidance mechanisms (Davenport, 1977; Akberali and Trueman, 1985). Actually, it must be stressed that combining monitoring surveys such as the BEWS (e.g., valvometer) and simultaneous physiological study is critical. In this case, even abnormal events like the introduction of the leachates from the 10kg.m<sup>3</sup> CCA treatment can be detected for a short period of time even though mussels remain active and likely bioaccumulating pollutants. In the other hand, in *Mytilus edulis*, the valves may be gaping and at the same time, the mussel need not necessarily be actively pumping, phenomenon that can then be assessed by clearance rates estimates (Bayne et al., 1973)

During the course of this experiment, a dry meat weight decline was observed concomitantly to increasing CCA treatments levels. This demonstrated a proportional stress effect on mussel and a negative energetic balance. We have seen that the physiological response was of several orders, including mainly the behavior change of the valve closure



or/and a reduced clearance rate activity. Ultimately, mortality was evidently the result of massive physiological disorder. With regard to mortality rates, it should be noted that continuous exposure for 7 days to a  $300\mu\text{g.l}^{-1}$  copper concentration was reported as the lethal concentration for 50% mortality ( $\text{LC}_{50}$ ) (Scott and Major, 1972; Martin et al., 1975).

All the variables considered to assess CCA leachates effects showed consistent trends, therefore confirming the overall results. Moreover, we should note that no fast physiological recovery occurred at least for treatments 2 and 3 since mussels were not exposed to CCA during the daily measurements. The valve activity pattern changed with increasing CCA levels to reach valve closure for several mussels at the  $44\text{kg.m}^3$  CCA load. The permanent valve closure induced an anaerobic catabolism that can therefore contribute to the observed significant mortality rate after several days of exposure. Meanwhile, the concomitant clearance rates' decline depicted stressful conditions, affecting the overall energy balance. Therefore, a combination of valve closure and disrupted activity are likely responsible for the increased mortality rate. In contrast low CCA ( $10\text{kg.m}^3$ ) had a limited and temporary effect on valve closure as well as on the mussel physiology. This implies that *M. edulis* discriminated between toxic (e.g.,  $44\text{kg.m}^3$ ) and non toxic ( $10\text{kg.m}^3$ ) effects of CCA leachates. Although a slight mortality rate was observed after 10 days, we can conclude that the direct effects were of limited extent until then, as demonstrated by no clearance and respiration rates significant difference between the control and the treatment 1. Later on, these effects were likely cumulative and more drastic as demonstrated by the higher variability in physiological responses and mortality rates. These physiological responses reflected physiological functions disorder.

Besides the increasing disorder resulting from increasing CCA loads, it should be noted that leachates affected preferentially the filtering activity and, at to a limited extent, the respiration rates. Although significant differences were observed in ingestion and assimilation rates, they mainly resulted from the initial discrepancy at the filtering activity level rather than at the assimilation efficacy. These results are consistent with the inhibition of respiration and filtration rates on *Mytilus edulis* by copper and zinc as described by Brown and Newell (1972), Manley (1983), Martin et al., (1975) and Davenport (1977). It has been reported that decreasing filtration rates might result from sublethal levels of heavy metals (Watling, 1981). In *M. edulis*, exposure to 0.15ppm of copper induced a 50% reduction in filtration rate (Abel, 1976) while Manley (1983) estimated this threshold at 30ppb (10.1ppb was the lower limit to detect reduction in filtration rate). Similarly, Adema *et al.* (1972) and Martin (1979) reported that prolonged exposure to copper concentration as low as 15-20ppb range can be lethal to *Mytilus edulis* and 0-20 ppb caused serious growth impairment to this species. Rule and Alden (1996) showed for *M. edulis* that a significant increasing respiration rate occurred when low copper contaminant load was added to the nearby sediment. In contrast, respiration was depressed by introducing a combined cadmium-copper mixture. Further data analysis indicated significantly depressed respiration at the highest combination Cd plus Cu treatments. Manley (1983) reported that a 0.2ppm copper concentration caused a mean reduction of 58.3% in the oxygen consumption. Moreover, Brown and Newell (1972) suggested that inhibition of the ciliary activity of the gills was responsible for the respiration rate decrease, while several authors reported that heavy metals exert inhibitory effects on heart rate (Scott and Major, 1972), byssus synthesis (Martin et al., 1975), changes in ATP content, protein synthesis, (Viarengo et al., 1980), and mitochondrial respiration and calcium transport (Akberali and Earnshaw, 1982). Copper also inhibits the influx of glycine into *Mytilus* gills (Swinehart and Crowe, 1980). These conclusions are likely related to our observations on the *Mytilus edulis* filtration and respiration activities, and then on the reduced Scope for Growth.

The analysis of the effect of CCA exposure on physiological responses was less consistent and showed several discrepancies between the considered variables. A trend might be existing or/and partially explained by a seawater quality change on a daily basis. Although the seawater quality was calibrated using fluorimetry estimates, other characteristics (e.g., carbon concentration) were not totally under control, and might have resulted in a slight bias in estimating assimilated rates over time. However, this had no effect on clearance rates' estimates as well as on our conclusions over CCA leachates' effects. Moreover, an overall pattern was observed with regard to increased variability in physiological responses over time, depicting disorders. Combined with the increased mortality rates, the responses to CCA exposure are obvious and are likely related to CCA chemicals accumulation. Further analysis of chemical concentration of CCA in mussel meat will provide more insights on the overall physiological disorders. It should be recalled that no observed effect thresholds on feeding, growth or SFG of mussels was observed for a copper body concentration up to 25µg Cu/g DMW, while the lethal body concentration was estimated to be 60µg/g DMW (Calabrese et al., 1983; Widdows and Johnson, 1988).

With regard with the long-term CCA effect on physiology, the mussel population did not show any significant difference between control and mussels living on CCA treated poles. Further analysis will demonstrate if a bioaccumulation occurred and if these mussels are harmful for the public health. In contrast to the laboratory experiments performed in a closed system facilitating bioaccumulation, the natural population was largely exposed to tide and water movements. This should be related to the conclusions from Weis and Weis (1996), who considered that the extent and severity of the effects of CCA treated wood in an estuary depends on the amount and age of the wood and the degree of dilution by water movements. Besides the lack of significant difference at the physiological level, it should be noted that the mussels were mature, showing a regular gametogenic cycle. Since Maung-Myint and Tyler (1982) have shown that continuous exposure to sublethal levels of copper and zinc suppresses gametogenesis in *M. edulis* with copper being more toxic, the mussel population was likely exposed to chemicals concentrations below these sublethal levels. However, based on the oyster physiological responses, the long term CCA effects might vary with species. Although respiration rates did not show any significant difference, clearance rates and SFGs were reduced for oysters naturally settled on CCA treated poles. This specific response should be confirmed by further experiments.

Our results have shown that CCA leachates effect directly the mussel ecophysiology mainly their filtration and respiration rates, and therefore, their Scope For Growth. Decreasing activities resulting in physiological disorders and then mortality rates are concomitant to increasing CCA treatment loads. The 10kg.m<sup>3</sup> CCA treatment appeared the less stressful for the mussel population and therefore will be the focus of the next series of experiments.

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