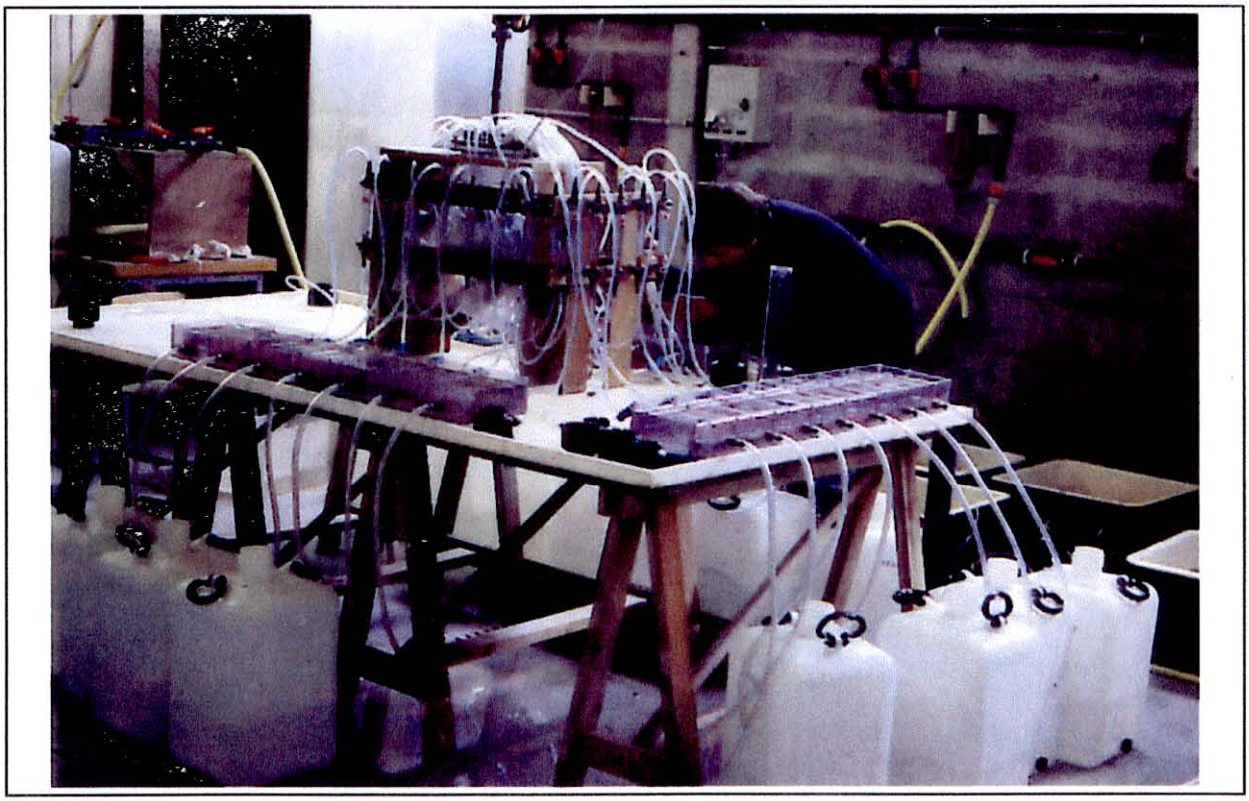
RESEARCH CONTRACT MAS 2 CT94-0100

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

Evaluation of stress imposed by treatments on organisms from test sites



P. GOULLETQUER, P. GEAIRON, , N. FAURY, D. RAZET, P. SOLETCHNIK, L. ADESSI, S. TAILLADE, P. GRAS,
IFREMER - LCPC
Shellfish Research Laboratory of Poitou-Charentes,
La Tremblade, B.P. 133, 17390 La Tremblade

Final Report

Contract Number : MAST2-CT-94-100
This work was undertaken with the financial support of the Marine Science and Technology Programme (MAST-III) of the European Union, under contract MAS2-CT94-0100.

IFREMER

December 1997

RESEARCH CONTRACT MAS 2 CT94-0100

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

Evaluation of stress imposed by treatments on organisms from test sites

P. GOULLETQUER, P. GEAIRON, , N. FAURY, D. RAZET, P.

SOLETCHNIK, L. ADESSI, S. TAILLADE, P. GRAS

IFREMER - LCPC
Shellfish Research Laboratory of Poitou-Charentes,
La Tremblade, B.P. 133, 17390 La Tremblade

Final Report

Contract Number : MAST2-CT-94-100

December 1997

This work was undertaken with the financial support of
the Marine Science and Technology
Programme (MAST-III) of the European Union,
under contract MAS2-CT94-0100.

TABLE OF CONTENTS

SUMMARY

Experiments were carried out to assess the copper-chrome-arsenic (CCA) treatment effects on the Blue mussel *Mytilus edulis* physiology. Experimental design included two complementary approaches: 1) scope for growth estimates based upon the energy balance between respiration energy loss and energy gained from feeding processes, 2) a biological early warning system for valve behavior monitoring under CCA exposures. Various temperature range (seasonal effect), physiological status (mature & immature mussels) as well as CCA concentrations (from 0 to 48kg/m^3) and time exposure were tested.

Significant mortality rates for the experimental mussel populations were recorded in all experimental modalities but the 2kg/m^3 CCA treatment concentration. Overall results showed that exposure to CCA treated woods induced significant effects on mussel valve behavior as well as on their physiology. Besides that, the wood treatment completion (e.g., dryness), affecting leaching rates, was critical when assessing precisely CCA induced effects. In severe environmental conditions (limited seawater renewal rate), CCA exposure led to mussel dry meat weight decrease, and eventually to high mortality rates. However, in less stressful conditions with a daily complete seawater renewal rate, significant mortality rates were observed, although without significant dry meat weight decrease. Therefore, the dry meat weight variable is not sufficient to assess CCA physiological impacts on a mid-term basis.

The use of valvometers, as biological ealy warning system recording continuously valve aperture, demonstrated that mussels are able to detect and change their valve behavior at a very low CCA concentration exposure below the 10kg/m^3 (treatment) CCA concentration. These results confirmed literature data on several metal effects on mussels. Meanwhile, valve behavior is increasingly disrupted with concomitant increased CCA concentration up to definitive valve closure for the highest concentration. Individual response showed a large variability likely due to initial physiological status. The initial valve opening behavior change, when exposed to limited CCA concentration, was likely to result in chemical bioaccumulation since mussels kept a significant filtering activity, changing only frequency and valve opening range. Abnormal and irregular behavior trends were recorded for mid-range CCA concentrations while permanent valve closure likely led to mussel anoxia for highest CCA concentrations, and eventually death.

With regard to the main physiological functions, experiments demonstrated that CCA leachates affected primilarly the feeeding processes rather than respiration rates. Filtration activity was affected at a 2kg/m^3 CCA concentration while respiration at 8kg/m^3. For all experiments, slightly increased physiological rates were observed for limited CCA concentration exposure compared to the controls. Otherwise, decrease physiological activity were systematically reported concomitantly to increased CCA leachate concentrations. With a significant seawater renewal rate, a recovery of physiological functions was observed after three days and one day of CCA exposure for feeding functions and respiration rate, respectively. Both results demonstrated the various sensitivity of physiological functions exposed to CCA leachates.

Based on these results, several recommandations can be provided for further assessments of CCA effects. First of all, a comprehensive model estimating CCA leaching rates is critical in developing further experiments since several variables have both effects on leaching and physiology (e.g., temperature). Our approaches, namely biological early warning system and ecophysiology were complementary to provide precise insights on how mussels reacted to CCE leachates. Therefore, both approaches are necessary for further tests. Furthermore, for the first approach, it appears critical to get appropriate background knowledge on species behavior so as to distinguish between natural fluctuations in valve movements and those caused by toxicants. Time-series analysis were appropriate to distinguish CCA impacts on mussel behavior. For the second approach, we have seen that filtration rate was the first physiological function affected by chemicals, well before respiration rate. Since physiological responses vary for a single CCA leaching concentrations, the effect thresholds are likely different when considering filtration, respiration and scope for growth, and therefore should be established separately.

PART I

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, Isochryis galbana*) were used to fed 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/m3, Treatment 2 = 19kg/m3, Treatment 3 = 44kg/m3. 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^2)	VOLUME (cm^3)	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.

I.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^3 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^3 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*, *Isochryis galbana*) to fed the mussels. The tank was continuously homogeneized 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 vas near 14°C and 31ppt respectively. Several seawater characteristics were estimated at the inflow and outflow of physiological chambers, including particulate and organic matter, chlorophylll 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 ($l.h^{-1}$) were estimated using the total sea water volume flowing through the experimental setup to allow precise particulate matter concentration estimates.

$$CLEAR = Vol \times (E_i - E_o)/Ei/tx60$$

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

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

Moreover, ingestion (ING) ($mg.h^{-1}$) and assimilation (ASS) ($mg.h^{-1}$) 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) ($\mu mole\ d'O_2.h^{-1}$) using a WTW oxygen probe.

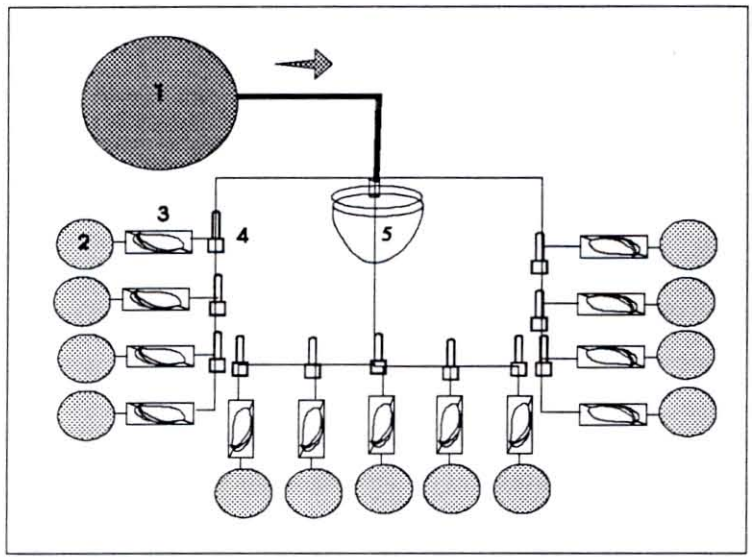
Physiological estimates (e.g., respiration and clearance rates) were standardized to 1g dry meat weight using the following allometric relationship (Bayne and Newell, 1983):

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

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 $J.mg^{-1}$, 0.45 $J.\bullet mole^{-1}$ for the particulate organic matter and oxygen respectively. The phytoplancton 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).



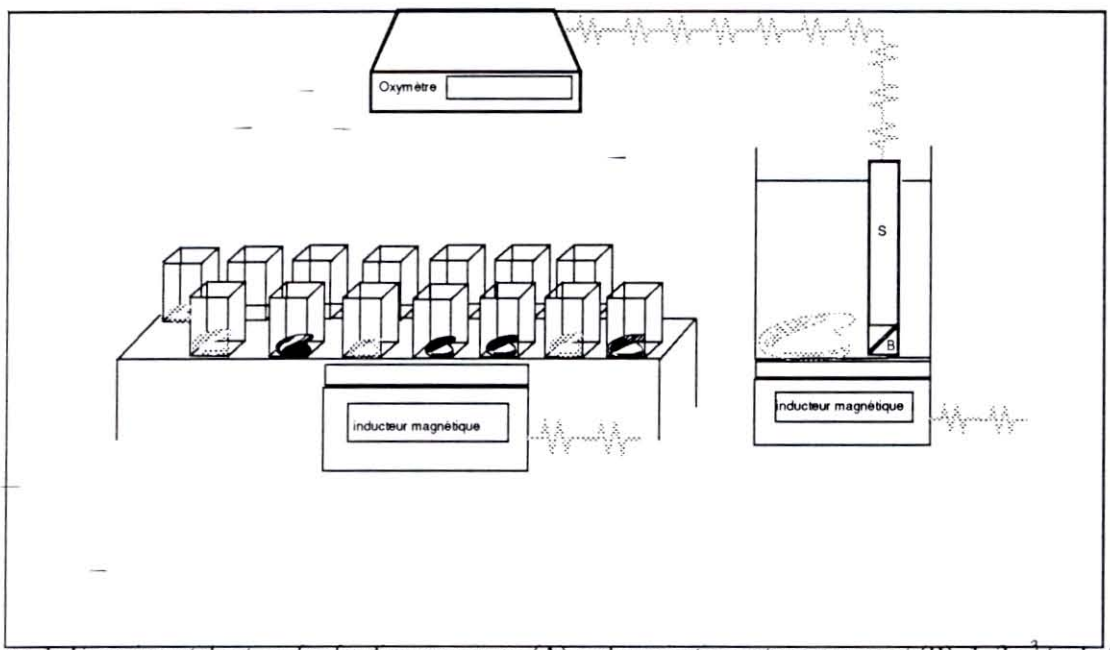


Figure 1: Experimental set-up for feeding processes (A) and respiration rate assessment (B). 1, $3m^3$ tank; 2, output seawater collecting container; 3, experimental individual chamber; 4, flowmeter; 5, chamber to homogenize and distribute seawater to individual chamber.

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

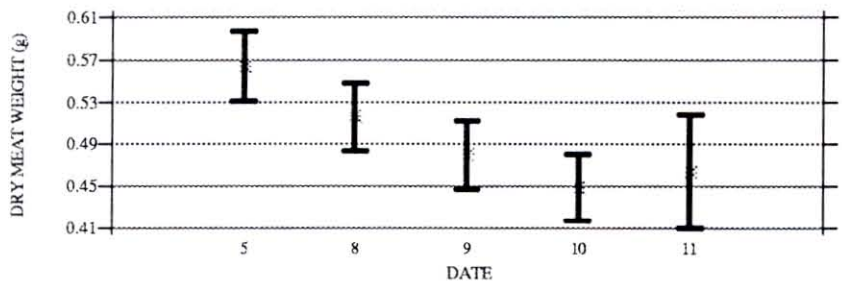
The mussel dry meat weight varied from 0.4 to 0.66g per individual during the experiment. The effects of the CCA treatment level and date were tested using a 2 way ANOVA (Table 4). Both effects were highly significant showing a dry meat weight decrease during the course of the experiment and the CCA 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
Main effects					
A:Date	0.129939	4	2.84435	7.59	0.0001
B:Type	0.21015		1.54454	4.12	0.0104
Interactions	0.146807	12	0.0122339	1.37	0.2044
AB					
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.



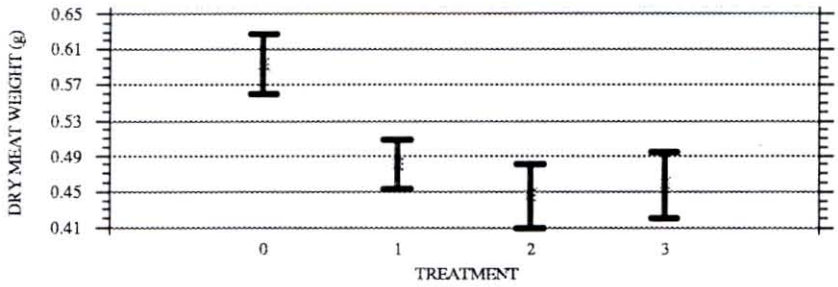


Figure 2: 2-Way ANOVA results. Effects of the 2 factors 'CCA Treatment' and 'Date' on the dry meat weight changes. Mean values and 95% LSD intervals.

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^3 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^3 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.m3 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.

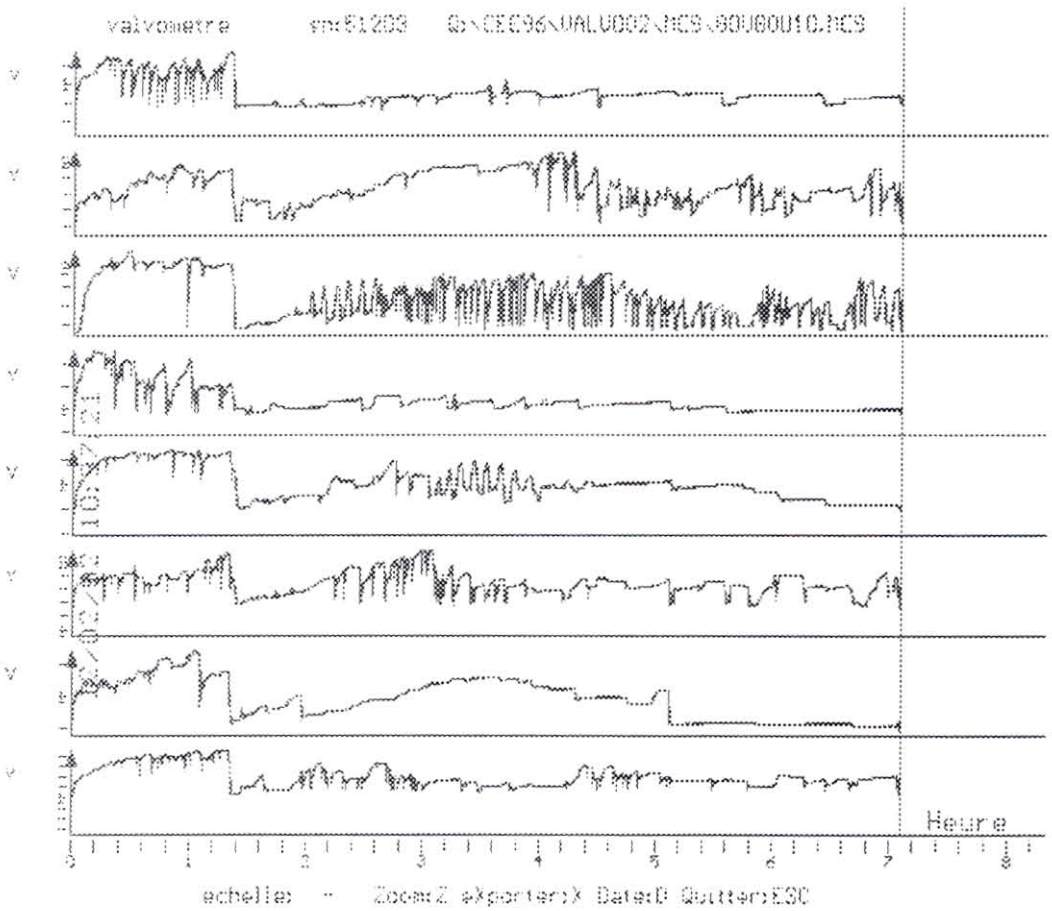


Figure 3: Example of a valvometer recording on eight individual mussels. A drastic valve closure followed the introduction of a 44kg.m^3 treated wooden block at minute 80'.

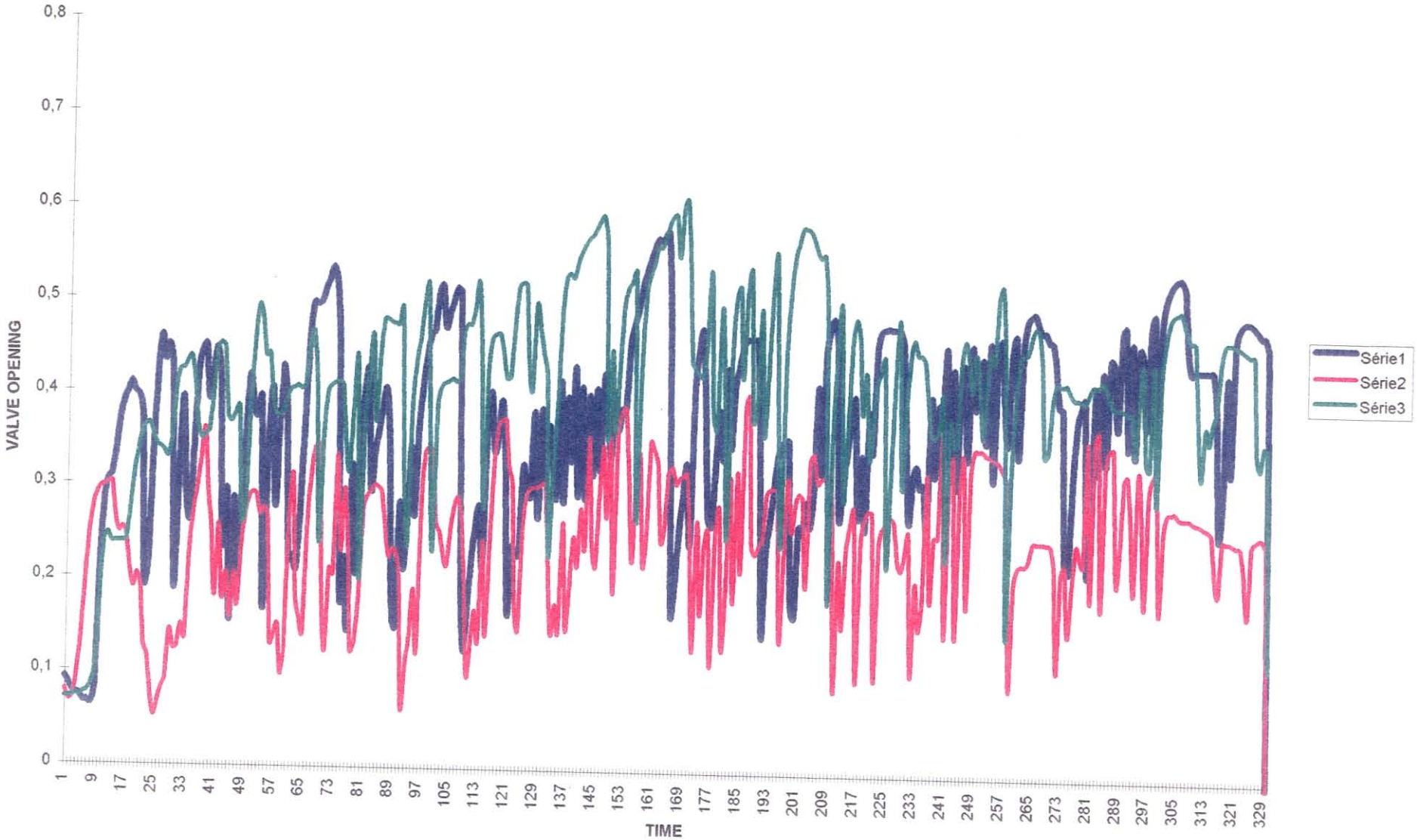


Figure 4 : Example of a valvometer recording on control mussels

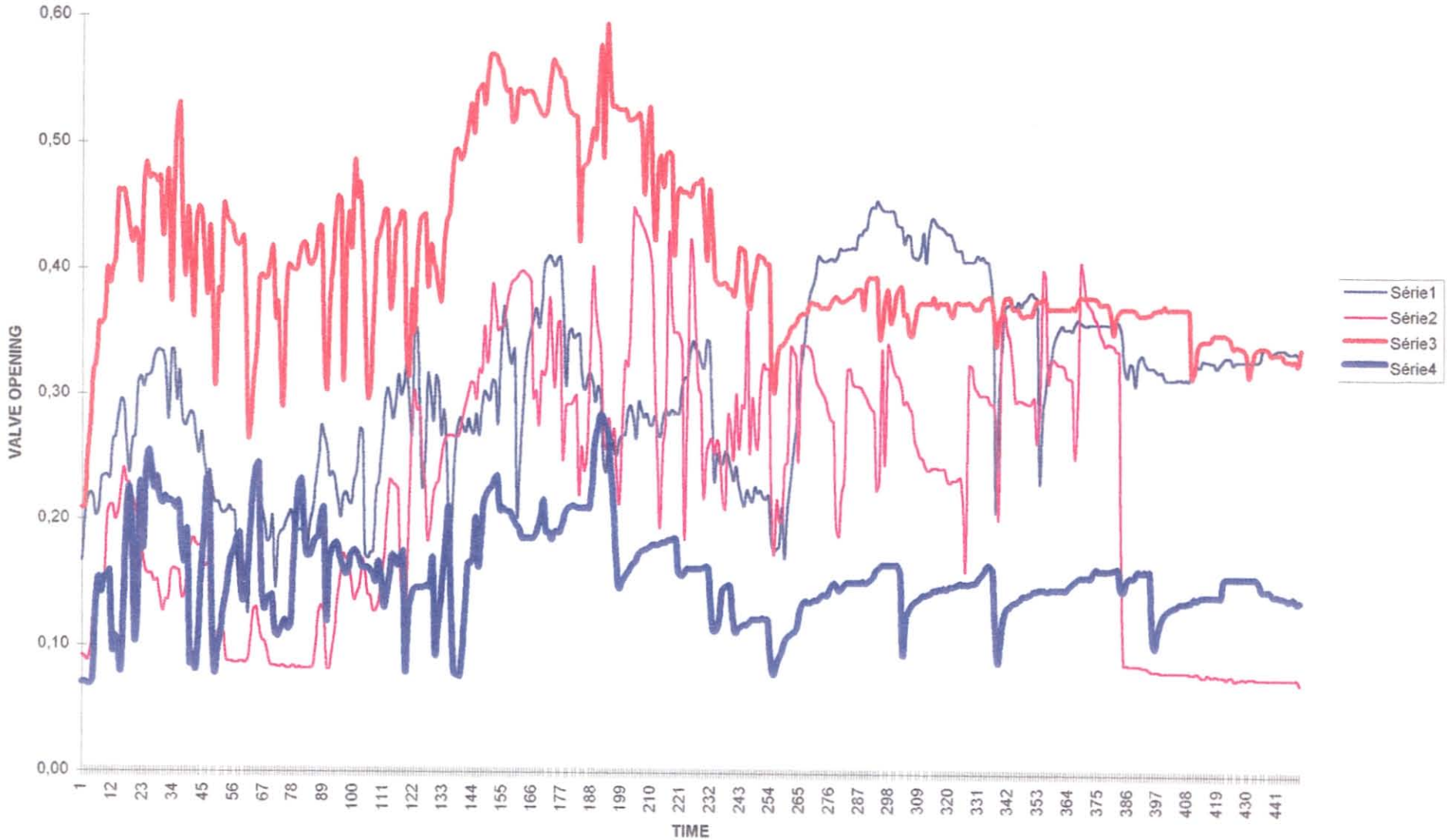


Figure 5 : Example of a valvometer recording on mussels valve activity. After 45 minutes, a CCA treated block (10Kg.m^3) was permanently introduced at the near valvometer vicinity.

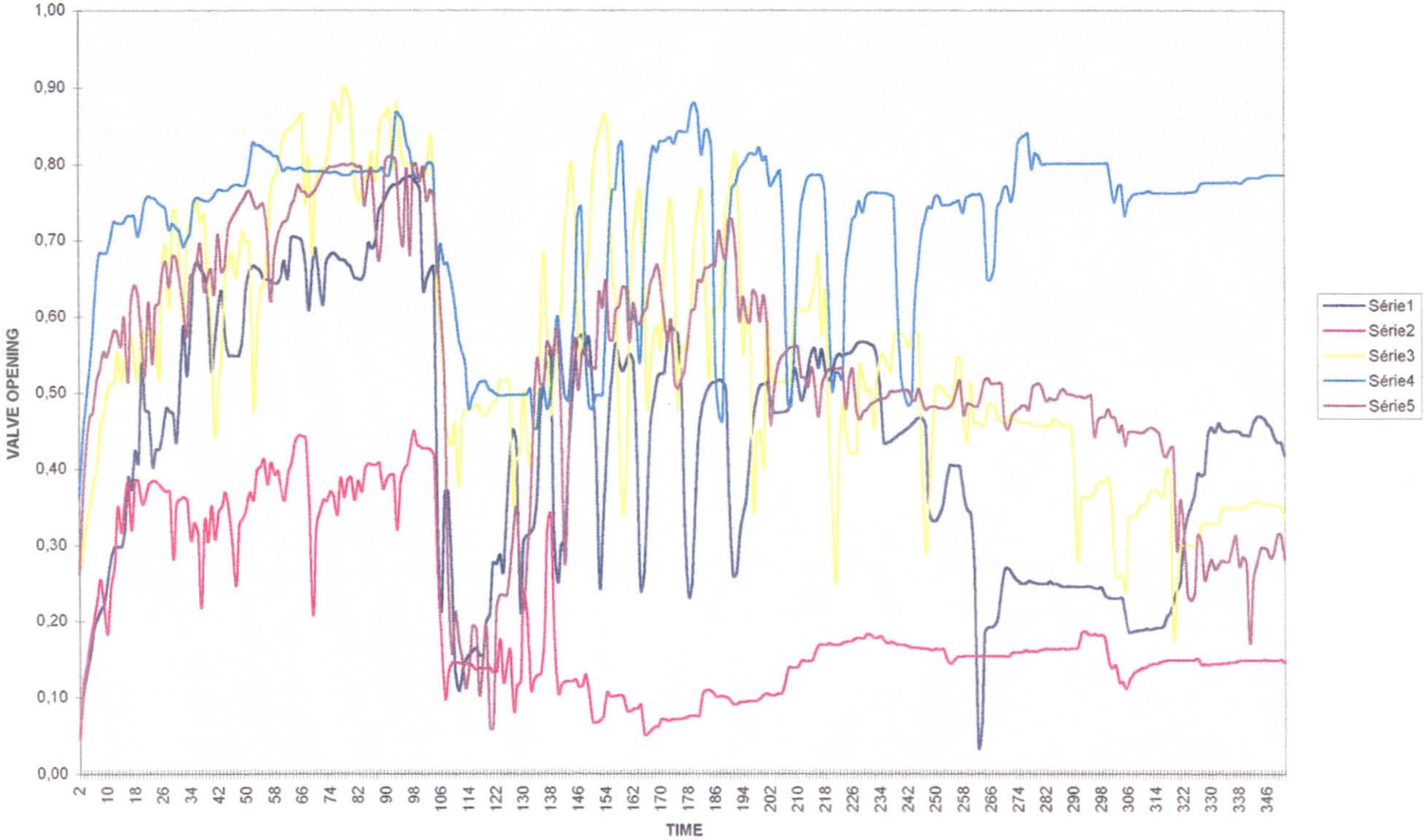


Figure 6 : Example of a valvometer recording on mussels valve activity. After 106 minutes, a CCA treated block (19Kg.m^3) was permanently introduced at the near valvometer vicinity.

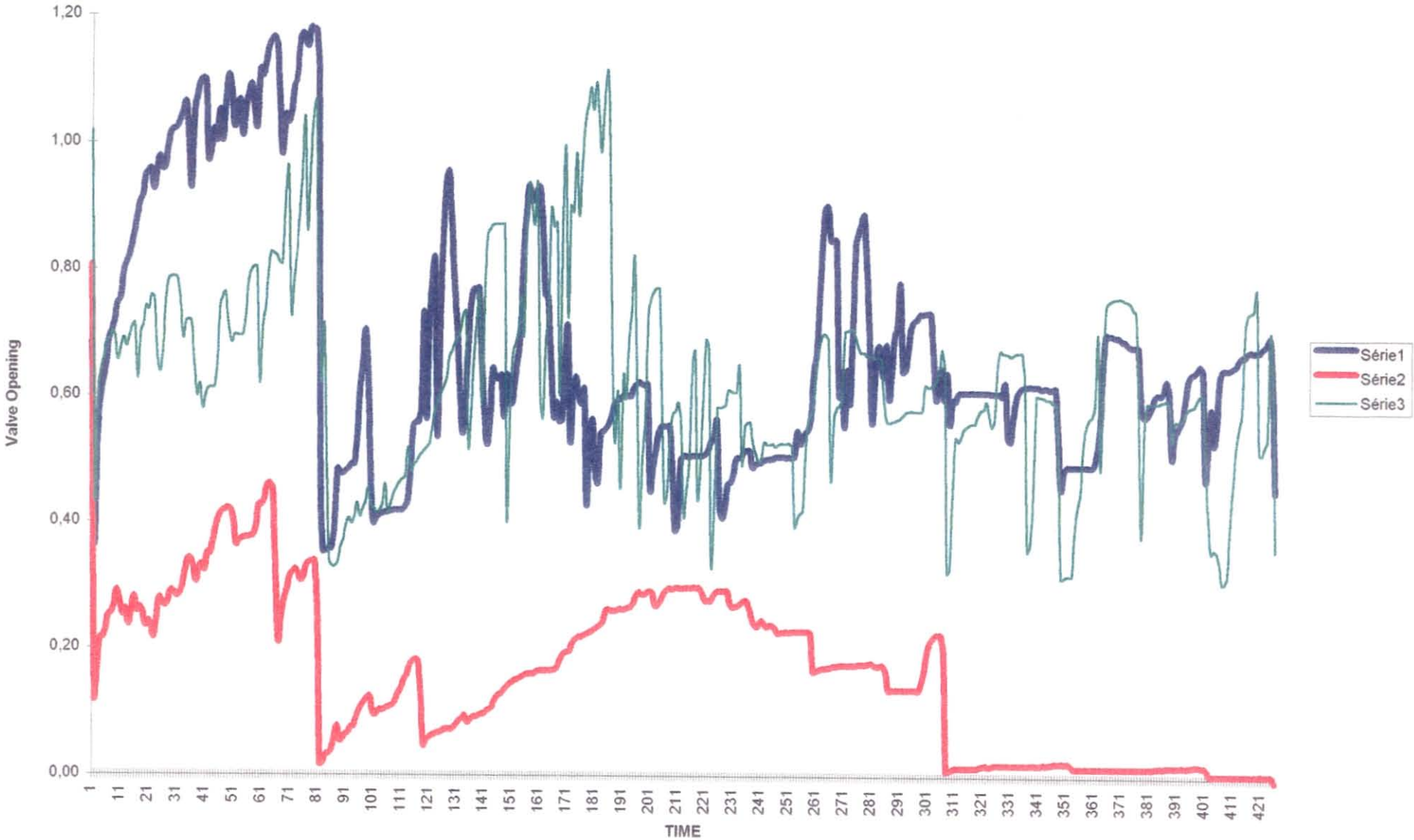


Figure 7 : Example of a valvometer recording on mussels valve activity. After 81 minutes, a CCA treated block (44Kg.m^3) was permanently introduced at the near valvometer vicinity.

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

2.3.1.1 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 $l.h^{-1}$. 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 ($0.67l.h^{-1}$). 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 $l.h^{-1}$.

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.

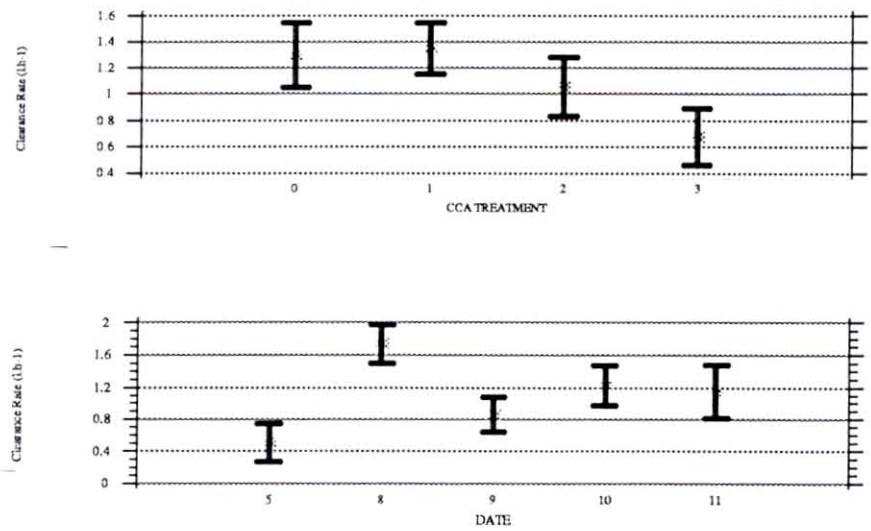


Figure 8: Results of 2 way- analysis of variance of clearance rates with the 'CCA treatment' and 'Date' factors mean values and 95% confidence intervals. Calculations are based on particulate organic matter values. (Control (0), CCA treatment : 10 (1), 19 (2), 44 (3) $kg.m^3$).

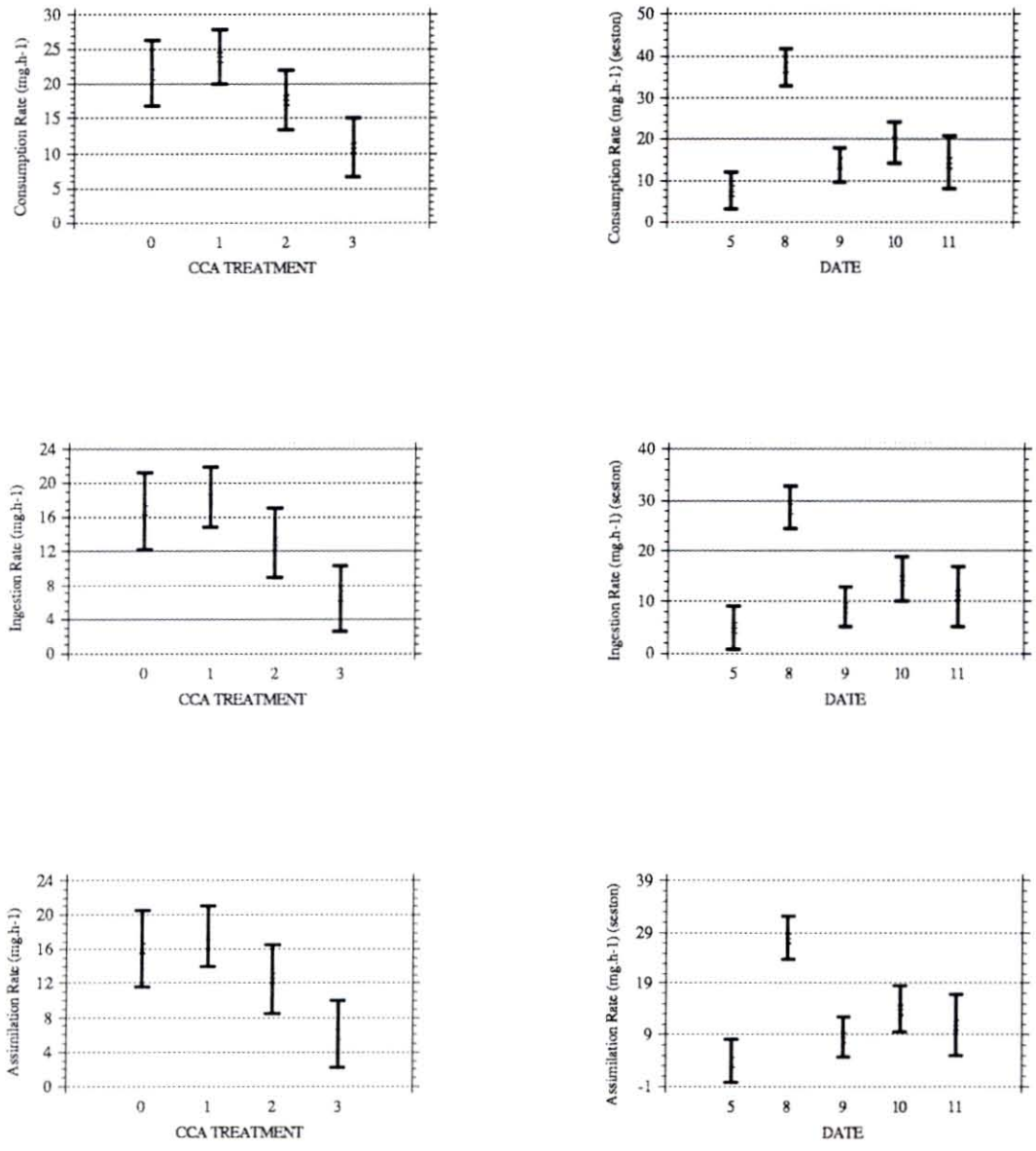


Figure 9: Results of 2 way- analysis of variance of consumption, ingestion and assimilation rates with the 'CCA treatment' and 'Date' factors mean values and 95% LSD confidence intervals. Calculations are based on particulate organic matter values. (Control (0); CCA Treatment, 10 (1), 19 (2), 44 (3)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

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.

2.3.1.2 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 $l.h^{-1}$ 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.

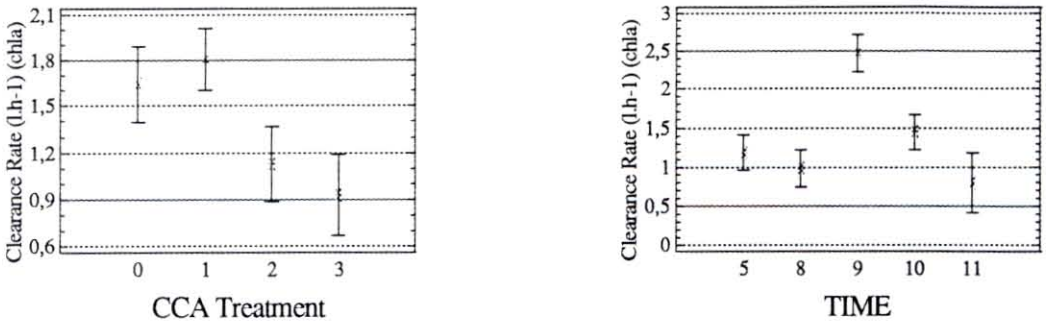


Figure 10: 2-Way ANOVA results. Effects of 'CCA treatment' and 'Time' on clearance rates mean values. Confidence intervals are at 95% LSD.

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 $\mu g.h^{-1}$, respectively. These rates decreased significantly with increasing time, reaching the lowest values at day 11 ($24\mu g.h^{-1}$). In contrast to the clearance rates' results, a drastic decline was observed over time from 159 to 24 $\mu g.h^{-1}$. A food level decrease over time and a physiological adaptation may explain this pattern.

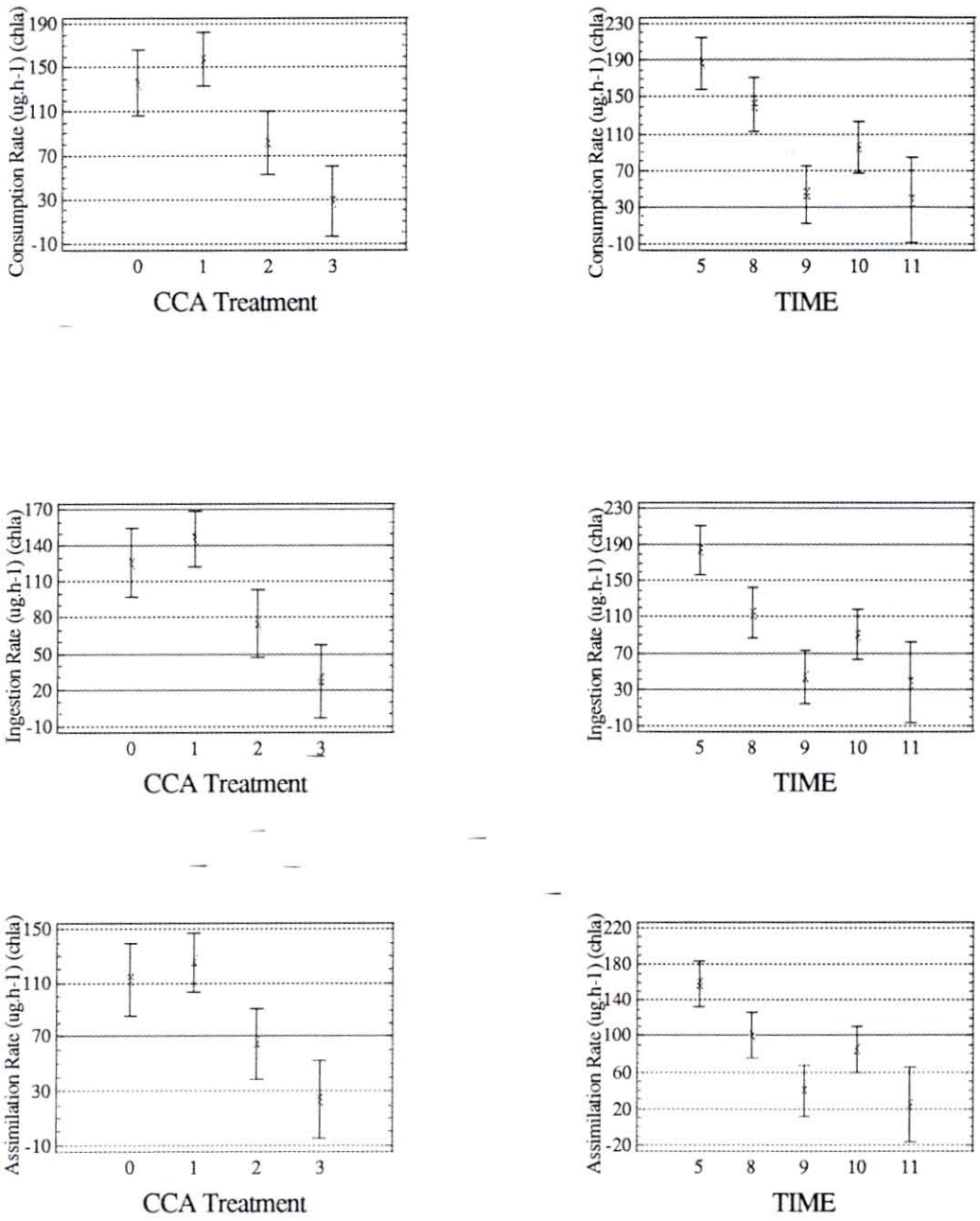


Figure 11: 2-way ANOVAs results of consumption, ingestion and assimilation rates function of the 2 factors 'CCA treatment' and 'Time'. Calculations were based on chlorophyll a and pheopigments analysis.

2.3.1.3 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

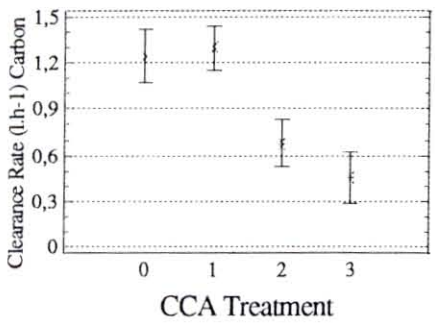
Assimilation Rates - Analysis of Variance -Type III Sums of Square

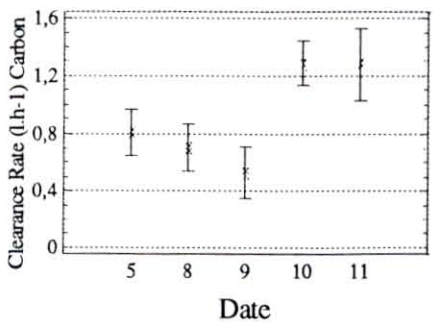
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Date	178.339	4	44.5848	10.22	0.000
B: Type	109.045	3	36.3484	8.33	0.0001
Residual	274.8	63	4.3619		
Total (Corrected)	554.73	70			

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 $l.h^{-1}$ 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 $kg.m^3$). 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.





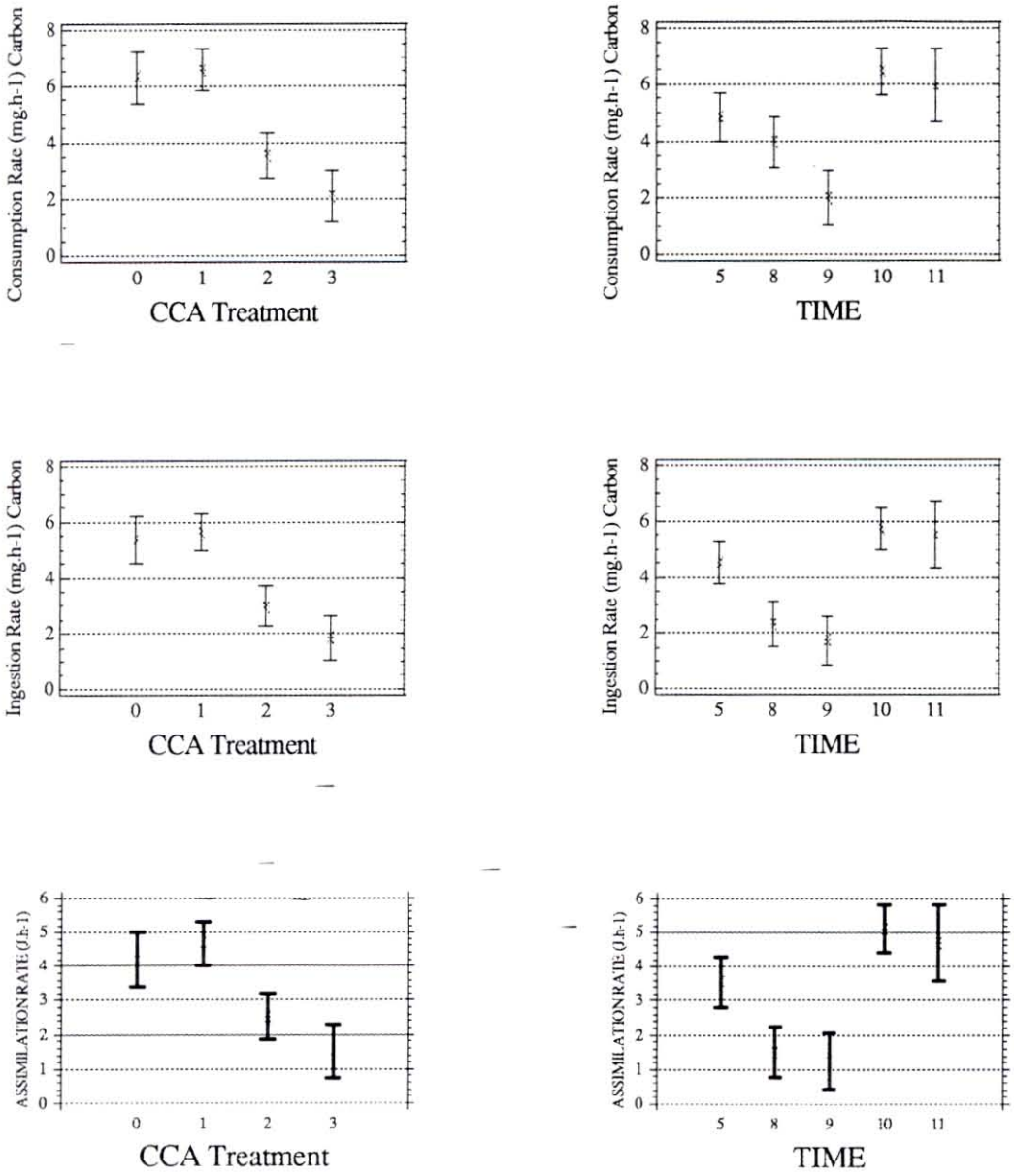


Figure 12: 2-way ANOVAs results of clearance, consumption, ingestion and assimilation rates function of the 2 factors 'CCA treatment' and 'Time'.

2.3.2 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^3 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.

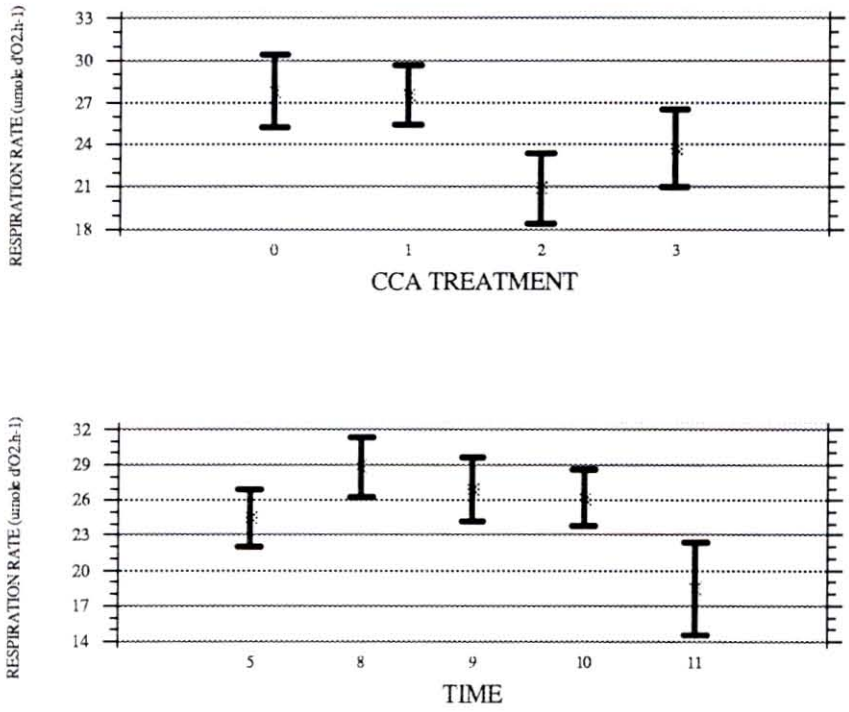


Figure 13. Respiration rates of the mussel population. Effects of CCA treatment and Time on mean values. 95% LSD confidence intervals.

2.3.3. Scope For Growth

2.3.3.1 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

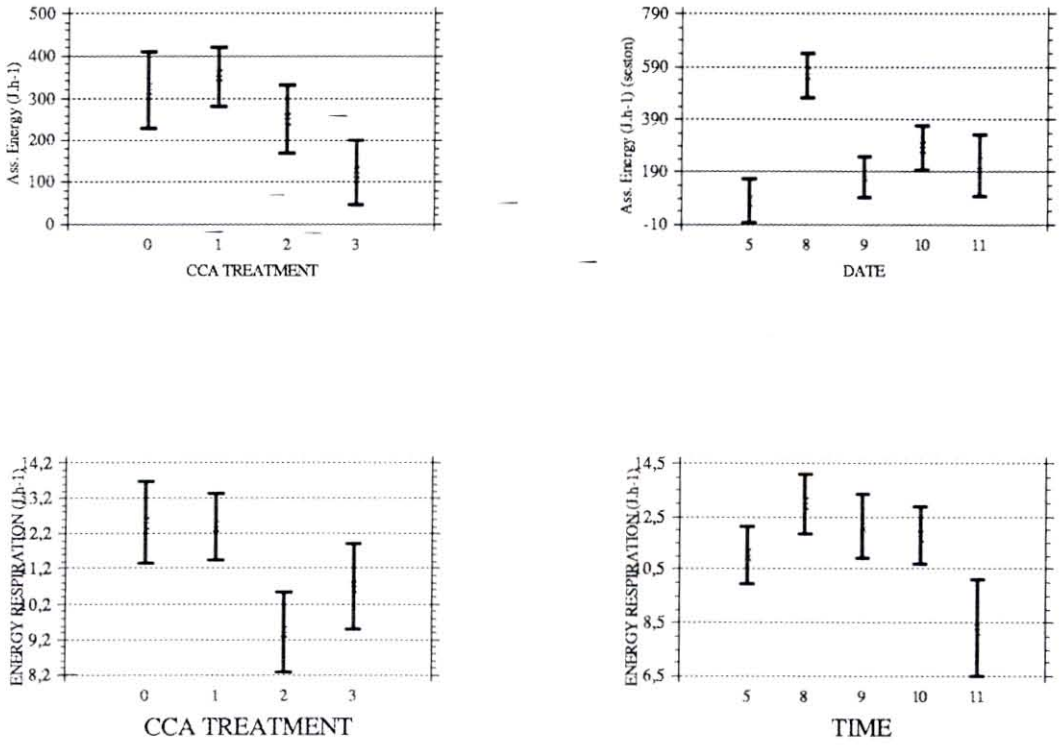
Scope for Growth - Analysis of Variance -Type III Sums of Square

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 $J.h^{-1}$ 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.



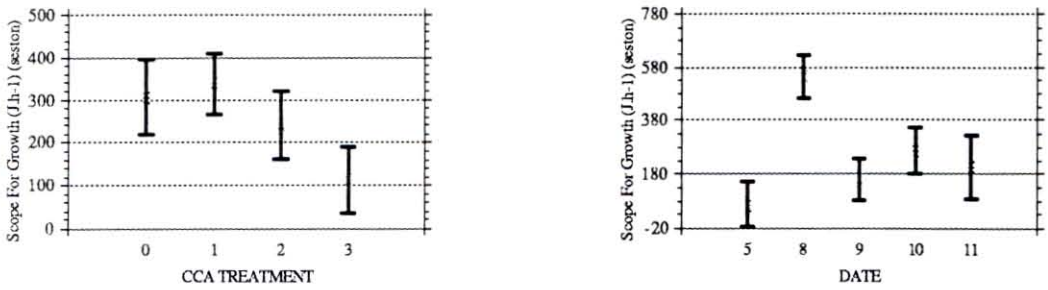


Figure 14: ANOVA mean values of energy gain from assimilation, energy expenditure from respiration and the energetic balance of mussels affected by CCA treatments and time. Calculations were based on particulate organic matter.

2.3.3.2 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
Main effects					
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.

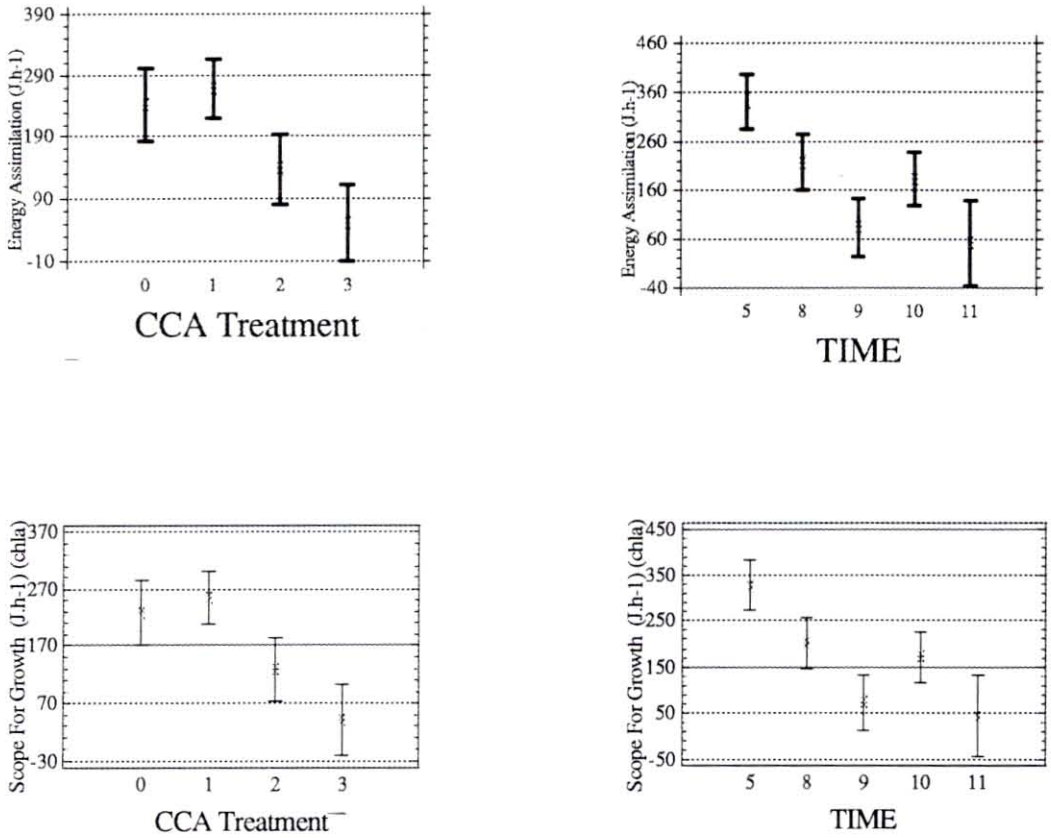


Figure 15: ANOVA mean values of energy gain from assimilation, energy expenditure from respiration and the energetic balance of mussels affected by CCA treatments and time. Calculations were based on chlorophyll a and pheopigments.

2.3.3.3 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
Main effects					
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			

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	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			

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.

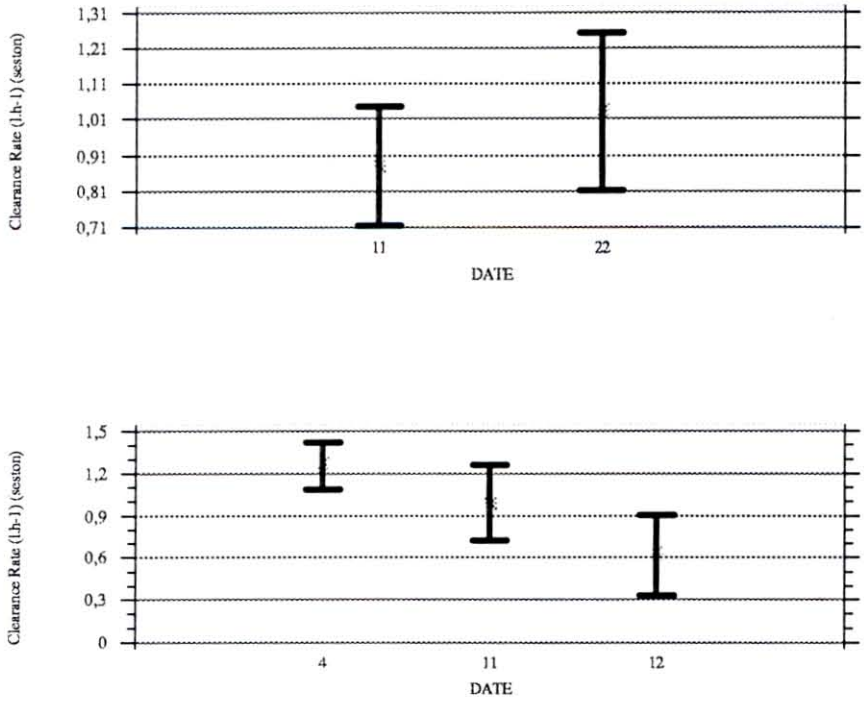


Figure 17: 2-way ANOVA results of clearance rates ($l.h^{-1}$) function of CCA Treatment (11, treated poles; 22 non treated poles) and time. Mean values and 95% LSD confidence intervals.

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	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 µmole d'O_2.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 Chlorophylll 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.

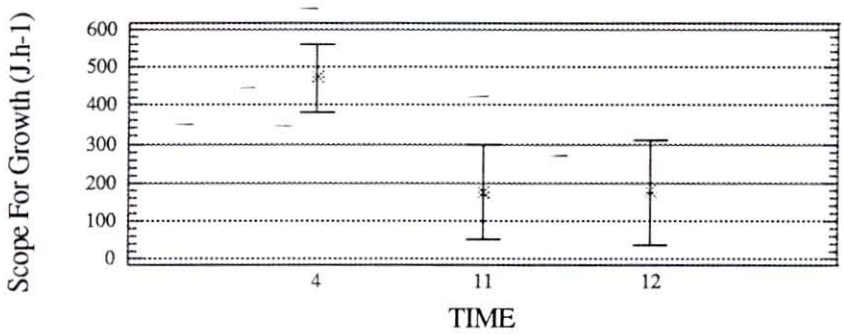


Figure 18. 2-way ANOVA results of Scope for Growth ($J..h^{-1}$) function of time. Mean values and 95% LSD confidence intervals.

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.

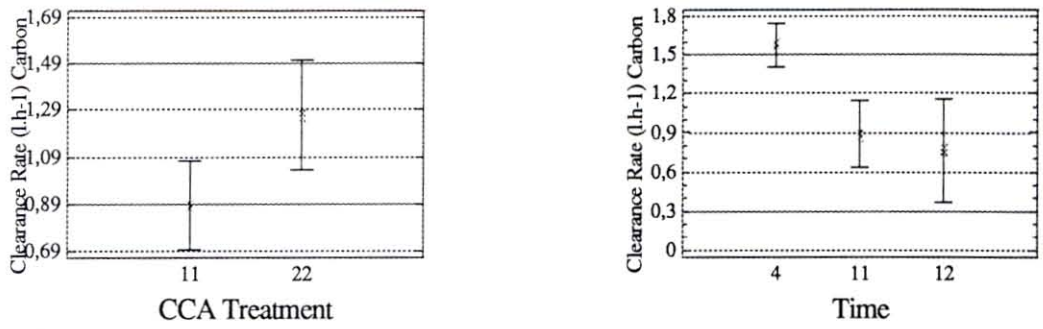


Figure 19. 2-way ANOVA results of clearance rates ($l..h^{-1}$) function of CCA Treatment (11, treated poles; 22 non treated poles) and time. Mean values and 95% LSD confidence intervals.

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 1g 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 0.9l.h^{-1} and 1.28l.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.

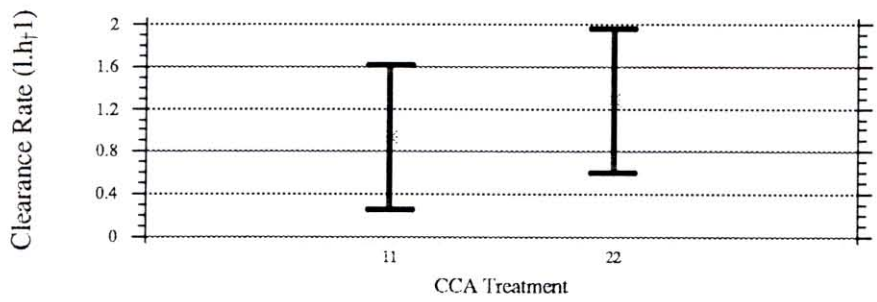


Figure 20. One-way ANOVA results concerning the effect of 'CCA treatment' on oyster clearance rates.

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.

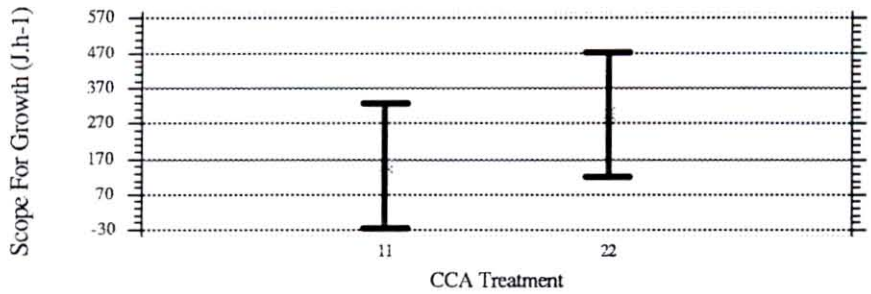


Figure 21. Mean values of Scope for Growth between control and oysters living on CCA treated poles. Marks are 95% LSD confidence intervals.

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^{-1} for the CCA modality and control respectively. In contrast, no significant difference was observed between SFG estimates (P-value>0.05) (Fig. 22).

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

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.

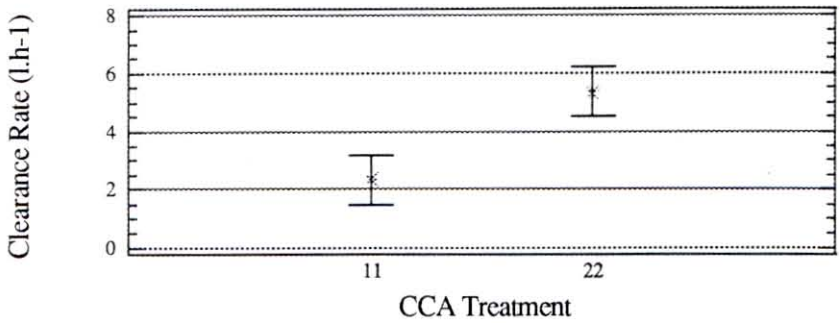


Figure 22. Clearance rate mean values of oysters living on CCA treated poles (11) and control (22).

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 4.511.h^{-1} for the oysters living on CCA treated poles and on 'control' poles respectively.

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

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

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

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		
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		
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		
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.

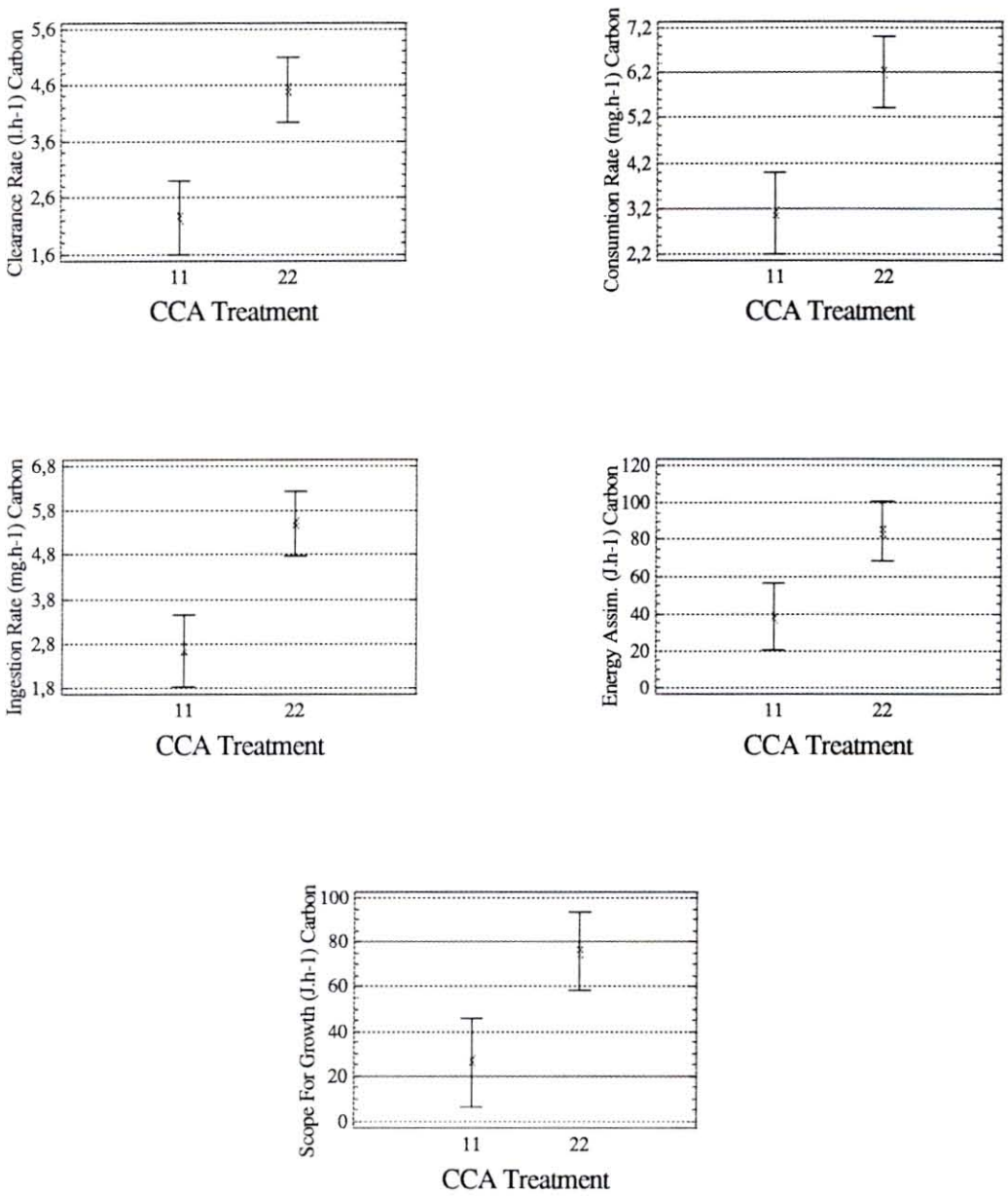


Figure 23. Mean values of One way ANOVAS on oysters living on CCA treated and control poles. Clearance, consumption, ingestion rates and energy estimates are compared.

III. 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 $10kg.m^3$ 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 $300\mu g.l^{-1}$ copper concentration was reported as the lethal concentration for 50% mortality (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 occured 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 $44kg.m^3$ 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 ($10kg.m^3$) 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., $44kg.m^3$) and non toxic ($10kg.m^3$) 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, repiration 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 undercontrol, 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 $10kg.m^3$ CCA treatment appeared the less stressful for the mussel population and therefore will be the focus of the next series of experiments.

References

Abel P.D. (1976). Effects of some pollutants on the filtration rate of *Mytilus edulis*. Mar. Poll. Bull., 7:228-231.

Adema D.M.M., S.I. Swaaf-Mooy, and P. Bais (1972). Laboratorium-onderzoek over de involoed van koper mossolen (*Mytilus edulis*). T.N.O.-nieuws, 27:482-486.

Ahlgren (1983). Comparison of methods for estimation of phytoplankton carbon. Arch. Hydr. Biol., 98:489-508.

Akberali H.B. and M.J. Earnshaw (1982). The mechanism of calcium transport in mitochondria from different tissues in the bivalve mollusc *Mytilus edulis* L.. Cell Calcium, 3:55-66.

Akberali H.B. and E.R. Trueman (1985). Effects of environmental stress on marine bivalve molluscs. In: Advances in Marine Biology Eds. J.H.S. Blaxter, F.S. Russel, S.M. Yonge, V. 22:101-198.

Albuquerque R.M. and S.M. Cragg (1995a). Fouling organisms as indicatrs of the environmental impact of marine preservative-treated wood. The International Research Group on Wood Preservation, Section 5, 26th Annual Meeting Helsingor, 14p.

Albuquerque R.M. and S.M. Cragg (1995b). Evaluation of impact of CCA - treated wood on the marine environment. The International Research Group on Wood Preservation :224-236

Amiard J.C., C. Amiard-Triquet, B. Berthet, and C. Metayer (1987). Comparative study of the patterns of bioaccumulation of essentail (Cu, Zn) and non essential (Cd, Pb) trace metals in various estuarine and coastal organims. J. Exp. Mar. Biol. Ecol., 106:73-89.

Baldwin I.G. and K.J.M. Kramer (1994). Biological early warning systems (BEWS). In: Biomonitoring of coastal waters and estuaries. CRC Press, 1-29.

Bayne B.L. (1976). Marine mussels, their ecology and physiolohy. Cambridge Univ. Press, Cambridge, pp 506.

Bayne B.L. and R.C. Newell (1983). Physiological energetics of marine molluscs. In: Mollusca, vol. 4, part 1, Wilbur K.M., A.S. Salenddin eds Academic Press, London, 407-513.

Bayne B.L., R.S. Thompson and J. Widdows (1973). Some effects of temperature and food on the rate of oxygen consumption by *Mytilus edulis* L.. In: "Effects of temperature of ectothermic organisms". W. Wieser Ed., p. 211 Springer Verlag, Berlin and New-York.

Bayne B.L., D.A. Brown, K. Burns, D.R. Dixon, A. Ivanovici, D.R. Livingstone, D.M. Lowe, M.N. Moore, A.R.D. Stebbing, and J. Widdows (1985). The effect of stress and pollution on marine animals. Praeger Press, New York, pp 384.

Brooks K.M. (1994a). Literature review and assessment of the environmental risks associated with the use of CCA treated wood products in aquaculture environments. Aquat. Envir. Sciences Report, 63p.

Brooks K.M. (1994b). References pertaining to the aquatic risks associated with CCA and ACZA treated wood held by the Western Wood Preservers' Institute. Aquat. Envir. Sciences Report, 33p.

Brown B.E. and R.C. Newell (1972). The effect of copper and zinc on the metabolism of the mussel *Mytilus edulis*. Mar. Biol., 16:108-118.

Calabrese A., J.R. MacInnes, D.A. Nelson, R.A. Greig, P.P. Yevich (1983). Effects of long-term exposure to silver or copper on growth, bioaccumulation and histopathology in the blue mussel, *Mytilus edulis*. Mar. environ. Res., 11:253-274.

Davenport J. (1977). A study of the effect of copper applied continuously and discontinuously to specimens of *Mytilus edulis* (L) exposed to steady and fluctuating salinity levels. J. mar. Biol. Ass. U.K., 57:63-74.

Hawkins A.J.S. and B.L. Bayne (1992). The mussel *Mytilus edulis*, ecology, physiology, genetic and culture. In: Physiological interrelation of production. Ed. E. Gossling Elsevier:171-212.

Hawkins A.J.S., E. Navarro and J.I.P. Iglesias (1990). Comparative allometries of gut passage time, gut content, and metabolic faecal loss in *Mytilus edulis* and *Cerastoderma edule*. Mar. Biol., 105:197-204.

Kramer K.J.M. (1994). Biomonitoring of coastal waters and estuaries. CRC Press, 327p.

Kramer K.J.M., H.A. Jennerand D. de Zwart (1989). The valve movement response of mussels: a tool in biological monitoring. Hydrobiol. 188:189:433-443.

Manley A.R. (1983). The effects of copper on the behaviour, repsiration, filtration, and ventilation activity of *Mytilus edulis*. J. Mar. Biol. Assoc. of the U.K., 63:205-222.

Martin J.L.M. (1979). Scheme of lethal action copper on mussels. Bull. Envir. Contam. and Toxico., 21:804-814.

Martin J.M., F.M. Piltz, and D.J. Reish (1975). Studies on the *Mytilus edulis* community in Alamitos Bay, California. V. The effects of heavy metals on byssal thread production. Veliger, 18:183-188.

Maung-Myint U. and P. Tyler (1982). Effects of temperature, nutritive and metal stressors on the reproductive biology of *Mytilus edulis*. Mar. Biol., 67:209-223.

Razet D., M. Héral, J. Prou, J. Legrand, J.M. Sornin (1990). Variations des productions saisonnières des biodépôts (fécès et pseudofécès) de l'huître *Crassostrea gigas* dans un estuaire macrotidal : Baie de Marennes-Oléron. Haliotis, 10143-161.

Rule J.H. and R.W. Alden III (1996). Interactions of Cd and Cu in anaerobic esturaine sediments. II. Bioavailability, body burdens, and respiration effects as related to geochemical partitioning. Envir. Toxic. and Chem., 15(4):466-4671.

Scott D.M. and C.W. Major (1972). The effect of copper (II) on survival, respiration, and heart rate in the common blue mussel *Mytilus edulis*. Biol. Bull., 143:679-688.

Slooff W., D. de Zwart and J.M. Marquenie (1983). Detection limits of a biological monitoring system for chemical water pollution based on mussel activity. Bull. Envir. Contam. Toxicol., 30:400-405.

Smaal A.C. and J. Widdows (1994). The scope for growth of bivalves as an integrated response parameter in biological monitoring. In: Biomonitoring of coastal waters and estuaries. CRC Press, 246-268.

Soletchnik P., P. Goulletquer, M. Héral, D. Razet and P. Geairon (1996). Evaluation du biln énergétique de l'huître creuse *Crassostrea gigas* en baie de Marennes-Oléron (France). Aquat. Living Res., 9:65-73.

Strickland J.D.H. (1960). Measuring the production of marine phytoplankton. Bull. Fish. Res. Board Can., 122:1-172.

Swinehart J.H. and J.H. Crowe (1980). Effects of heavy metals on amino acid and divalent cation transport in gills of bivalve molluscs. Amer. Zool., 20:735.

Van Eetvelde G., R. Orsler, G. Holland and M. Stevens (1995a). Effect of leaching temperature and water acidity on the loss of metal elements from CCA treated timber in aquatic applications. Part 1: laboratory scale investigation. The International Research Group on Wood Preservation, Section 5, 26th Annual Meeting Helsingor, 16p.

Van Eetvelde G., W.J. Homan, H. Militz and M. Stevens (1995b). Effect of leaching temperature and water acidity on the loss of metal elements from CCA treated timber in aquatic applications. Part 2: semi-industrial investigation. The International Research Group on Wood Preservation, Section 5, 26th Annual Meeting Helsingor; 195-208.

Watling H. (1981). The effects of metals on mollusc filtering rates. Trans. Roy. Soc. of South Africa, 44:441-451.

Weis J.S. and P. Weis (1992). Transfer of contaminants from CCA-treated lumber to aquatic biota. J. exp. Mar. Biol., 161:189-199.

Weis J.S. and P. Weis (1996). The effect of using wood treated with chromated copper arsenate in shallow water environments: a review. Estuaries, 19(2A):306-310.

Weis P., J.S. Weis and L.M. Coohill (1991). Toxicity to estuarine organisms of leachates from chromated copper arsenate treated wood. Arch. Environ. Contam. Toxicol., 20:118-124.

Weis P., J.S. Weis, A. Greenberg and T.J. Nosker (1992). Toxicity of construction materials in the marine environment: a comparison of chromated copper arsenate treated wood and recycled plastic. Arch. Environ. Contam. Toxicol. 22:99-106.

Viarengo A., M. Pertica,, G. Mancinelli, R. Capelli, and M. Orunesu (1980). Effects of copper on the uptake of amino acids, on protein synthesis, and on ATP content in different tissues of *Mytilus galloprovincialis* Lam.. Mar. Envir. Res., 4:145-152.

Widdows J. (1993). Marine and estuarine toxicity tests. In: Handbook of ecotoxicology, Vol 1; P. Calow Ed.Blackwell, Oxford, 145-166.

Widdows J., P. Fieth, C.M. Worrall (1979). Relationships between seston, available food, and feeding activity in the common mussel *Mytilus edulis*. Mar. Biol., 50:195-207.

Widdows J., K.A. Burns, N.R. Menon, D.S. Page, and S. Soria (1990). Measurement of physiological energetics (Scope for Growth) and chemical contaminants in mussels (*Arca zebra*) transplanted along a contaminant gradient in Bermuda. J. exp. Mar. Biol. Ecol., 138:99-117.

Widdows J., P. Donkin, M.D. Brinsley, S.V. Evans, P.N. Slkeld, A. franklin, R.J. Law, and M.J. Waldock (1995). Scope for growth and contaminant levels in North Sea mussels *Mytilus edulis*. Mar. Ecol. Prog. Ser., 127:131-148.

Widdows J. and D. Johnson (1988). Physiological energetics of *Mytilus edulis*: scope for growth. Mar. Ecol. Prog. Ser., 46:113-121.

IMPACT ON NON TARGET ORGANISMS OF ANTI MARINE WOOD BORER TREATMENTS

Evaluation of Stress imposed by treatments on organims from test sites

Part II

P. Goulletquer, P. Geairon, N. Faury, D. Razet, P. Soletchnik, P. Gras

IFREMER - LCPC
Shellfish Research Laboratory of Poitou-Charentes

Final Report
Contract Number : MAST2-CT-94-100

Report prepared by

P. Goulletquer

November 1997

This work was undertaken with the financial support of the Marine Science and Technology Programme (MAST-III) of the European Union, under contract MAS2-CT94-0100.

Part II

Introduction

Similarly to the first set of experiments, two approaches were developed to assess CCA effects on mussel physiology, including a biological early warning system, namely 'valvometer', and by assessing the mussel scope for growth (SFG) under various CCA exposures.

During the first set of experiments, significant CCA impacts on mussel physiology were reported: dry meat weight decline over time for increasing CCA exposures, behavior change of the valve closure, reduced filtering activity and increased mortality rates.

Since these experiments were carried out for CCA concentrations ranging from 10 to 44kg/m^3 (showing limited effects at 10kg/m^3 concentration), this study aims to assess more precisely the physiological disorders which might occur for reduced CCA thresholds, particularly below 10kg/m^3. The experiments should provide information on CCA thresholds which might affect only punctually the mussel physiology (short term effect) and therefore limit the long term impact on mussel growth. Moreover, one objective is to assess the mussel sensitivity to reduced CCA concentration exposures as well as to estimate recovery following the main CCA leaching period. Combined together, both approaches should provide information about their own sensitivity to detect stressful conditions for mussels.

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

A population of blue mussels *Mytilus edulis* was collected early august 1996 within the intertidal area at the near vicinity of Boyardville, Oléron Island (France). The commercial rearing population was sampled on regular wooden poles.

After being individually sorted and cleaned, the mussels (length=50mm) were kept in a semi-closed system with filtered seawater at 34.5ppt and a 21-23°C temperature range. Phytoplanktonic algae *Skeletonema costatum* and *Isochrysis galbana* were used to fed the mussels. In contrast to the first set of experiments, seawater was running through the system at a renewal rate calculated to change the whole volume every day. This was done to avoid assessment of increasing concentration effects of CCA leachates.

The mussel population was equally distributed into 7 tanks representing the following modalities: Control (wooden blocks without treatment), CCA Treatment 1=2Kg/m^3, Treatment 2=5kg/m^3, Treatment 3=8kg/m^3, Treatment 4=12kg/m^3, Treatment 5=24kg/m^3, Treatment 6=48kg/m^3. Treatment blocks were deployed into each 30l tank containing 80 mussels. The block surface for each treatment was similar to the first set of experiments to facilitate comparisons. Seawater from each tank was sampled every day for further chemical analysis. Mortality rates were estimated by counting individuals on a daily basis.

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 valvometer is a biological early warning system for the continuous monitoring of water quality, based on the computer assessment of valve movements for 8 mussels individually. The first protocol is based upon the use of two electronic valvometers that record continuously the mussel valve opening. Two valvometers were individually deployed in a 30l tank with running seawater which allowed the seawater renewal every day. The mussel valve opening monitoring was recorded every 5mn over a cumulative period of 33 days. Only one modality (Treatment 4) with mussels exposed to CCA leachates was simultaneously compared to the control population. Time-series analysis methods using Statgraphics Software• were performed on the data to assess any behavior change in terms of trends, frequency and seasonality.

I.2.2 Ecophysiological Studies

The second protocol was developed to assess quantitatively the mussel filtration, ingestion and assimilation rates following the exposure to chemicals from 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, CCA2, CCA5, CCA8, CCA12, CCA24, and CCA48 kg/m^3 treatments. The treatment concentrations were based upon the first experiment results which showed a significant impact on mussel physiology for an exposure to CCA concentration greater than $10kg/m^3$. Therefore, this selection of concentration range aimed to 1) re-assess and evaluate the toxicity below the $10kg/m^3$ threshold and 2) confirm the physiological effects of higher concentrations. Physiological functions were assessed on a daily basis over a 7 days' experiment.

The experimental setup as well as the calculations of the main functions were similar to those developed during the first experiments.

II. Results

II.1 Mussel Population

II.1.1 Dry meat weight

« CCA concentration » and « time » were the two factors tested. No significant effect was observed on the mussel dry meat weight (table 1, figure 1). In contrast to the first set of experiments, no significant weight decrease was observed over the time exposure. However, although not significant, the « control » showed the highest dry meat weight mean (2.24g • 0.08).

Table 1. Two Way ANOVA results of dry meat weight function of CCA treatment and time.

Dry Meat weight - Analysis of Variance -Type III Sums of Square

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:CCA	0.0944131	6	0.0157355	0.22	0.9691
B: Date	62,7213	6	0.0309436	0.43	0.7835
Residual	6.19881	87	0.0712507		
Total (Corrected)	6.41408	97			

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

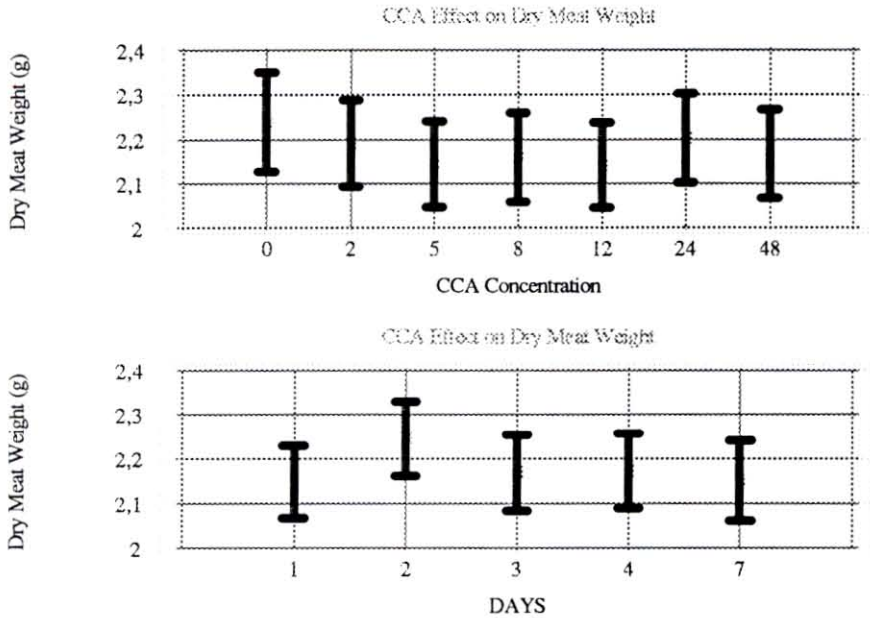


Figure 1 : 2-Way ANOVA results : CCA concentration and time effects on the mussel dry meat weight.

Moreover, the interaction between both factors was not significant. Although no significant effect on dry meat weight was observed, ecophysiological functions were still calculated using the standardized 1g individual to facilitate further comparisons.

II.1.2. Mortality rate

No mortality was observed during the first 2 days of experiments whatever the CCA concentration tested (table 2). Similarly, no abnormal mortality was recorded after 7 days for the mussel exposed to a 2kg/m^3 CCA concentration. Only at day 7, a significant mortality (40%) was observed for the 5kg/m^3 CCA concentration. Increased mortality rate was observed after day 3 with increasing concentration up to the 12kg/m^3 concentration and concomittant with increasing exposure time. Therefore, in spite of the seawater running through this open system, significant mortality rates were observed along the experiment.

In contrast, reduced mortality rates were observed for the 24 and 48 kg/m^3 CCA concentration. This abnormal trend resulted from the various dryness of the wooden blocks, therefore affecting the leaching rates. Actually, two sets of blocks were initially prepared at

different times. The highest concentration (24 and 48kg/m^3) were dryer than the lowest concentrations' blocks.

Table 2: Daily mortality rate for the mussel population exposed to various CCA concentrations (0 to 48kg/m^3) over 7 days.

Mortality Rate (daily %)						
DAY CCA Level	**0**	**1**	**2**	**3**	**4**	**7**
Control	0	0	0	0	0	0
CCA2	0	0	0	0	0	0
CCA5	0	0	0	0	0	40,38
CCA8	0	0	0	1,23	15,49	74,51
CCA12	0	0	0	4,11	26,23	63,89
CCA24	0	0	0	0	1,61	0
CCA48	0	0	0	0	0	7,14

II.2 Valvometer

Examples of continuous monitoring of valve activity for the control and exposed mussel population are presented on figure 3 & 4. The time-series analysis was carried out on one set of data concerning the CCA exposed mussel and then, a seasonal decomposition was applied to assess the periodogram, trends, seasonality and irregulars (Figure 5). A similar treatment was performed on control to facilitate comparisons. An obvious change in 'trends' was observed for the CCA exposed mussel while seasonality was quite similar for both modalities (Figure 6).

II.3 Ecophysiology

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

II.3.1 Feeding processes

2.3.1.1 Particulate Organic Matter

The ANOVA results for the filtration rate of a standardized (1g) mussel are presented in table 3 and figure 7. Both effects of time and CCA concentration affected significantly the filtration rate, which decreased concomitantly to increased CCA concentration from 0.57 to 0.2 l/h. No significant difference was observed between the control and the 2kg.m3 modality. In contrast, filtration showed higher activity rate during the first 2 days, then decreased to a reduced and constant level until the end of this experiment.

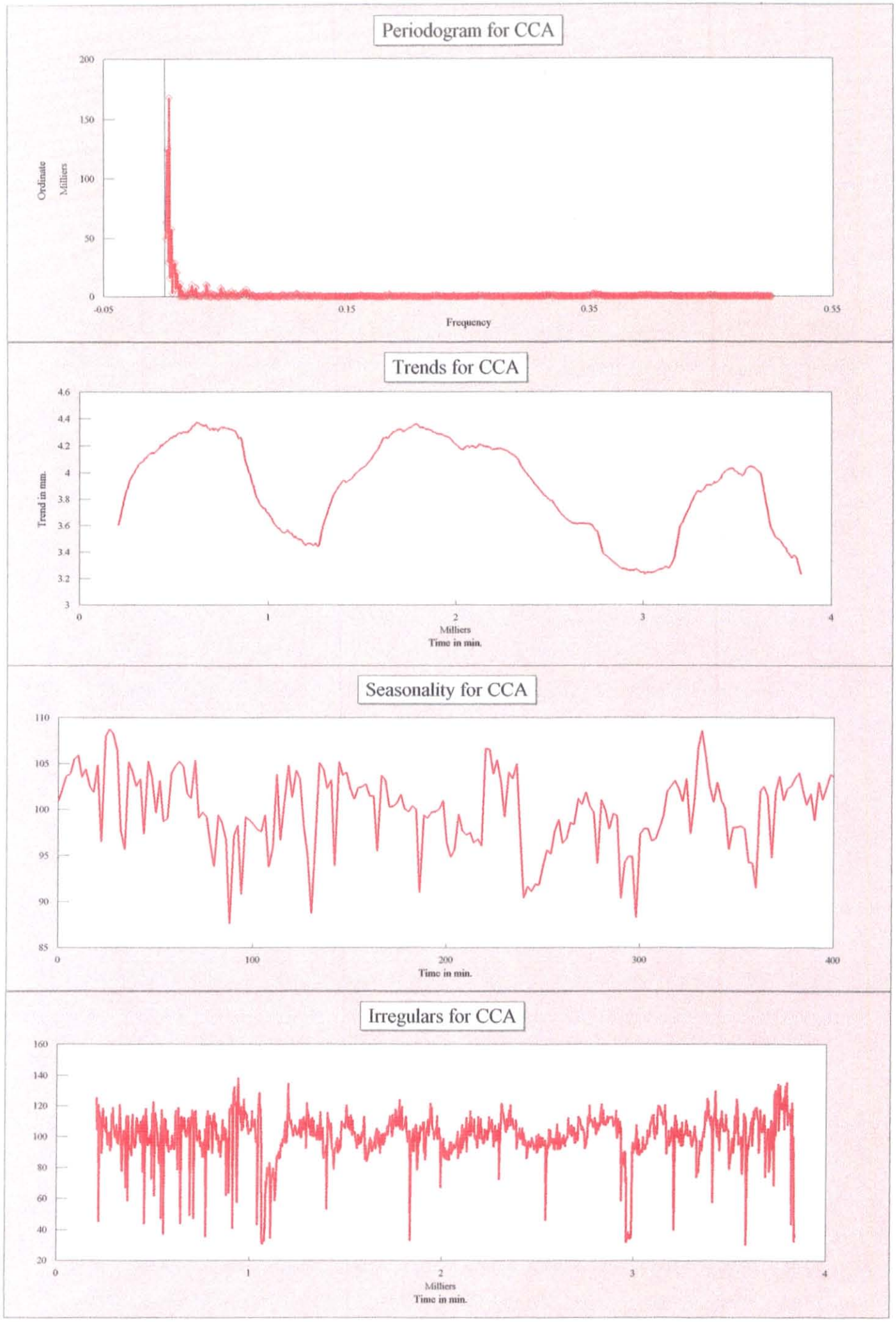


Figure 5: Time series analysis of the valve closure monitoring on CCA exposed mussels. Records were decomposed into the periodogram, trends, seasonality and irregulars.

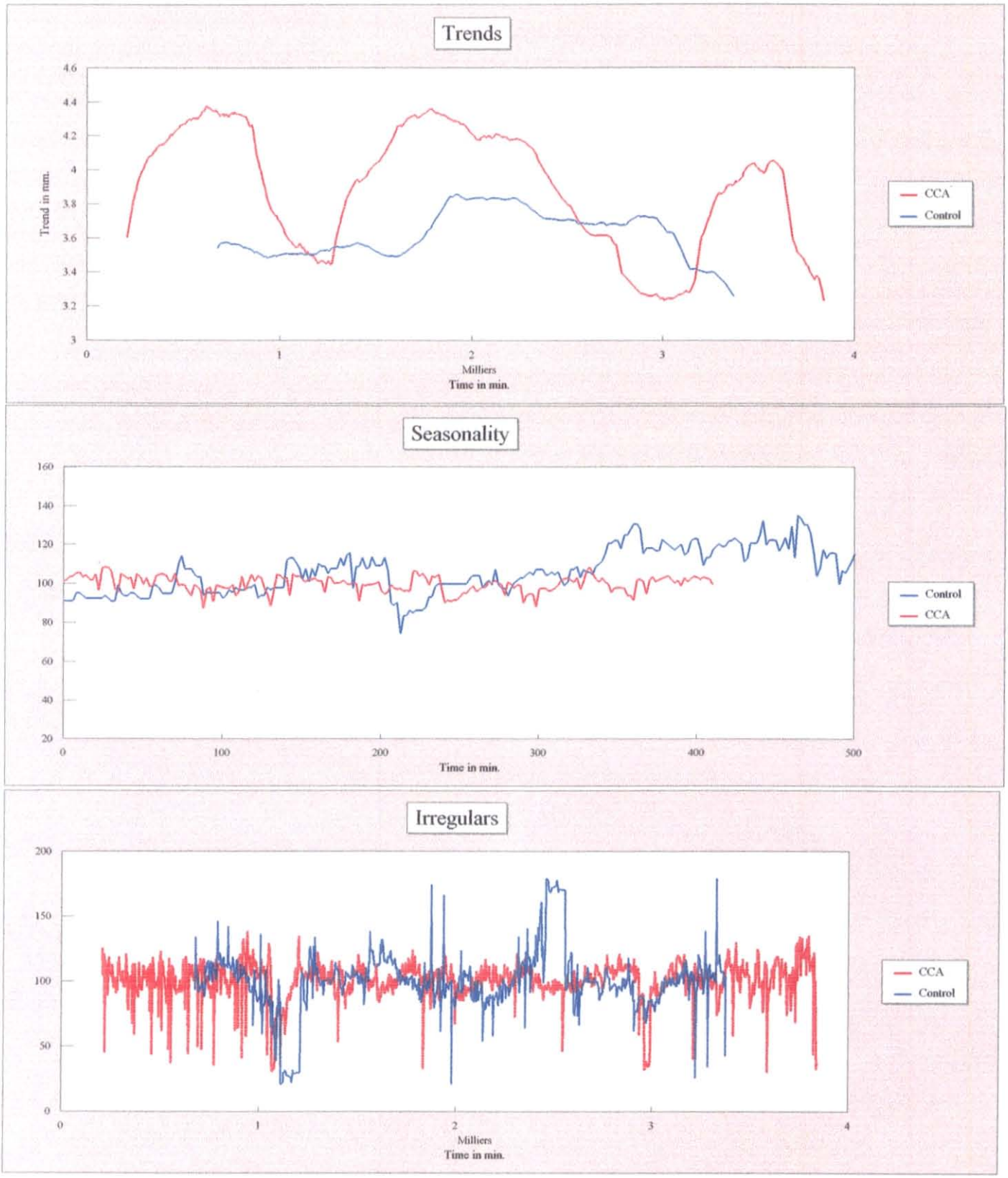


Figure 6: Comparison of the time series analysis of the valve closure monitoring on control and CCA exposed mussels.

Table 3: Two-way ANOVA results assessing CCA concentration and time effects on filtration rates. Interaction was not significant.

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

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: CCA	1.41341	6	0.235568	5.19	0.0001
B: Date	1.20824	4	0.30206	6.65	0.0001
Residual	3.76984	83	0.0454198		
Total (Corrected)	6.54803	93			

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

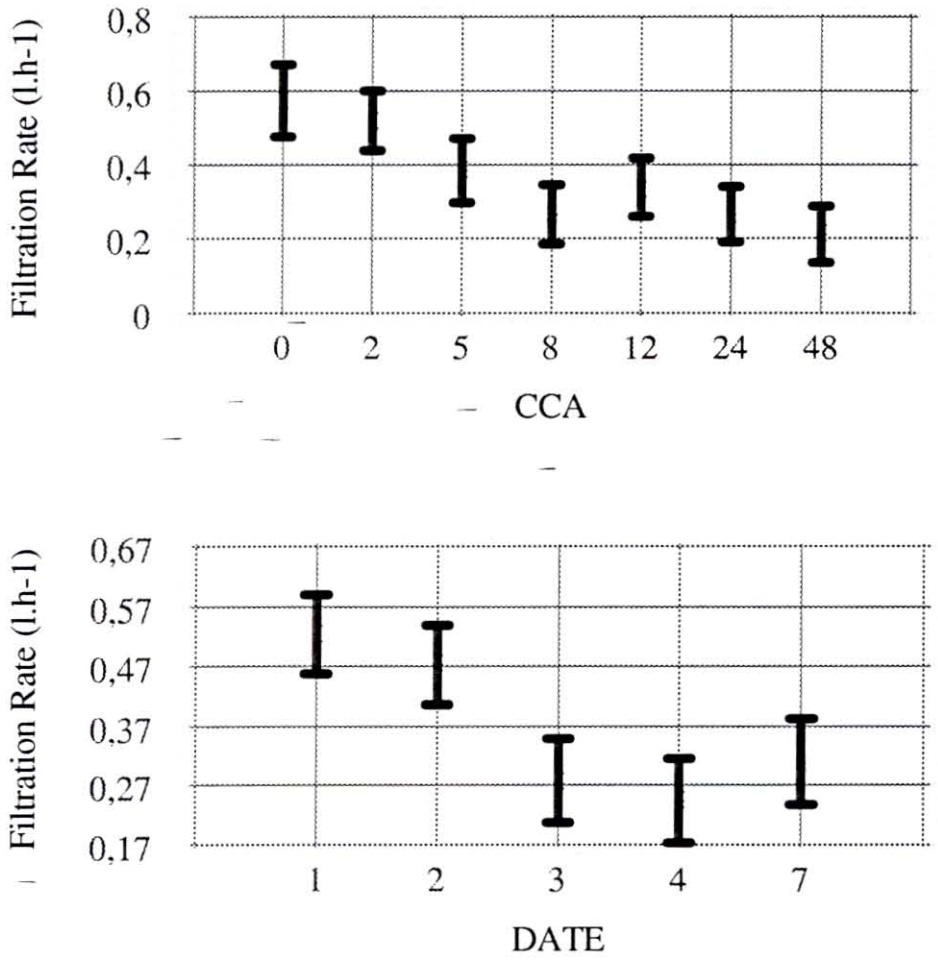


Figure 7: Results of 2 way-analysis of variance of filtration rates with the 'CCA treatment' and 'date' factors mean values and 95% confidence intervals. Calculations are based on particulate organic matter (Control (0), CCA treatment in $kg.m^3$)

Similarly, exposure time and CCA effects on consumption, ingestion and assimilation rates are presented on figure 8 and table 4. Both consumption and ingestion rates were affected significantly by the CCA exposure. Although a decreasing trend for these rates was obvious with increasing concentration, we should note that the various dryiness between the 2 sets of

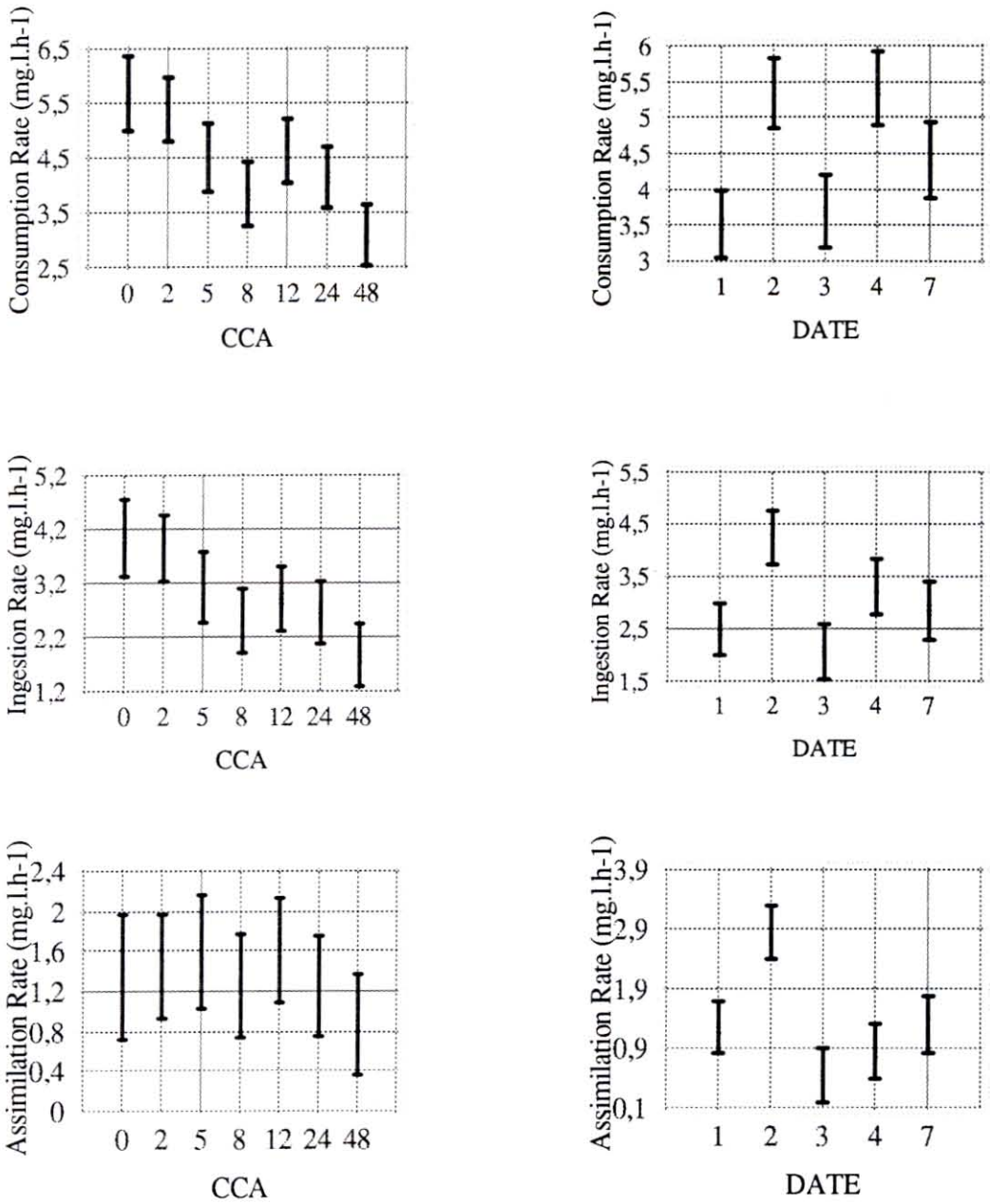


Figure 8 : Results of 2-way ANOVA testing the CCA concentration and time effects on physiological functions. No interaction significant.

blocks might have affected the results and underestimated the effects of the 24 and 48kg.m^3 on physiology.

In contrast to the previous rates, CCA leachates did not affect significantly the assimilation rates, although the lowest rates were observed for the highest CCA concentration (48kg/m^3).

All physiological functions were significantly affected by time exposure. However, no biological reason can explain this pattern but the slight daily variability of food quality.

Table 4 : Two-way ANOVA results of CCA concentration and time on the physiological functions.

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	60,2859	4	15.0715	6.36	0.0002
B: CCA	62,7213	6	10.4535	4.41	0.0006
Residual	196.571	83	2.36832		
Total (Corrected)	315.507	93			

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	53.8355	4	13.4589	5.25	0.0008
B: CCA	45.2173	6	7.53622	2.94	0.0119
Residual	212.868	83	2.56468		
Total (Corrected)	311.573	93			

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	63,462	4	15.8655	8.23	0.0000
B: CCA	5.55624	6	0.926039	0.48	0.8212
Residual	159.99	83	1.92759		
Total (Corrected)	230.532	93			

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

2.3.1.2 Chlorophyll a and pheopigments concentrations.

The ANOVA results for the filtration rate of a standardized (1g) mussel are presented on figure 9 and table 5. The results confirm the previous observations with a significant decrease concomitant to increasing CCA concentration, and stabilized rates for CCA concentrations greater than 8kg.m^3. Although not significant over the 7 days, filtration rates appeared to be significantly affected during the 3 first days, before a recovery to initial levels at day 4 and 7. The filtration rates at the 24 and 48kg/m^3 CCA concentration were quite similar to those at 8 and 12 kg/m^3, likely resulting from the various dryness for the two sets of wooden blocks. Therefore, the effects from exposure to the highest concentrations were likely underestimated.

Table 5 : Effects of CCA concentration and time on filtration rates - 2 way ANOVA results.

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

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:CCA	5.66948	6	0.944913	4.55	0.0005
B:Date	1.97686	4	0.494215	2.38	0.0582
Residual	159.99	82	0.207524		
Total (Corrected)	25.0934	92			

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

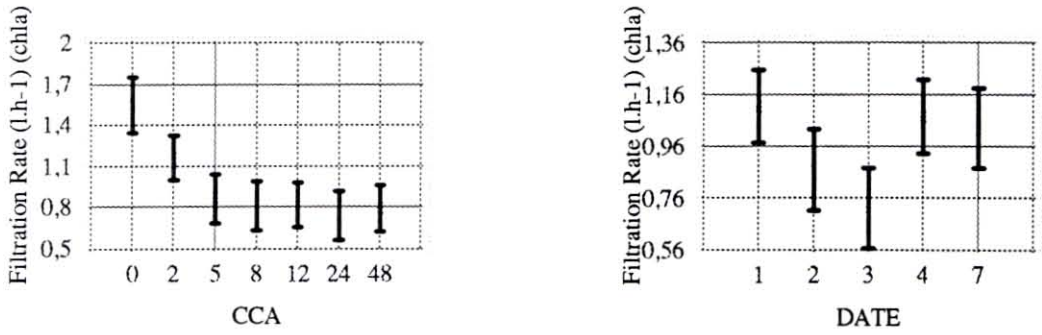
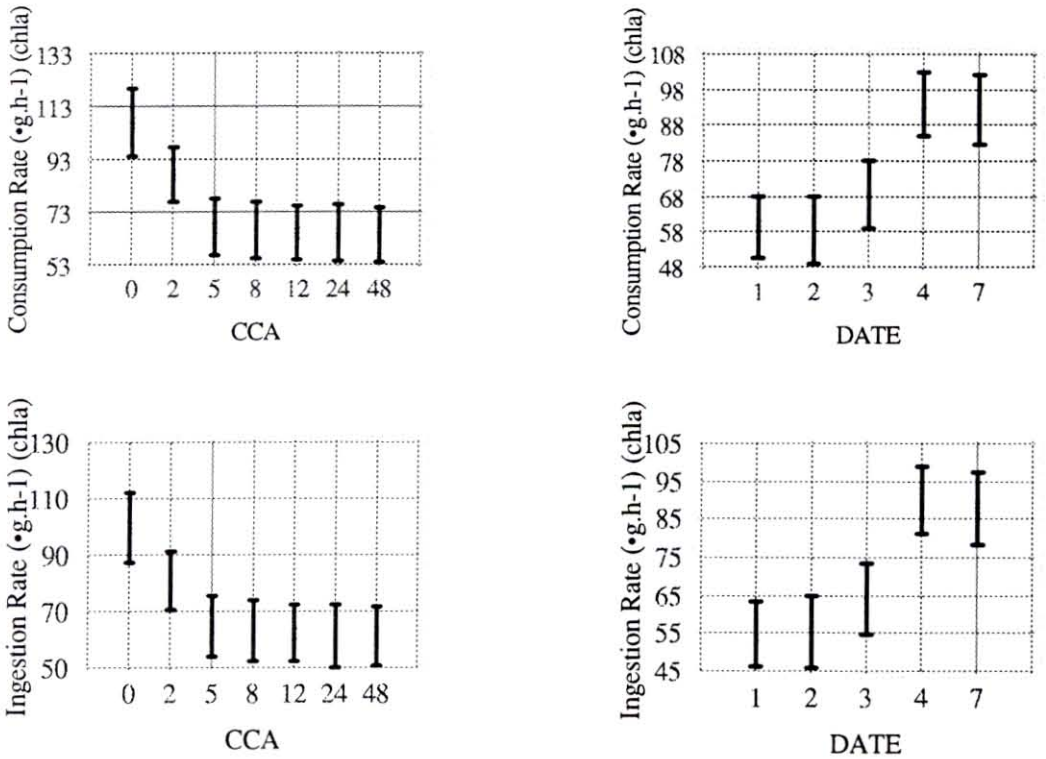


Figure 9: Filtration rates (l.h-1) calculated with chlorophyll and pheopigments estimates. Two way ANOVA results of CCA concentration and date effects.

The CCA exposure and time effects on consumption, ingestion and assimilation rates are presented on figure 10 and table 6. CCA leachates affected all physiological functions but assimilation rates which showed the highest variability. In contrast to particulate organic matter calculations, the 'time' variable affected significantly the physiological functions with an increased activity over time. By way of example, assimilation rates reached the highest values at day 4 and 7.



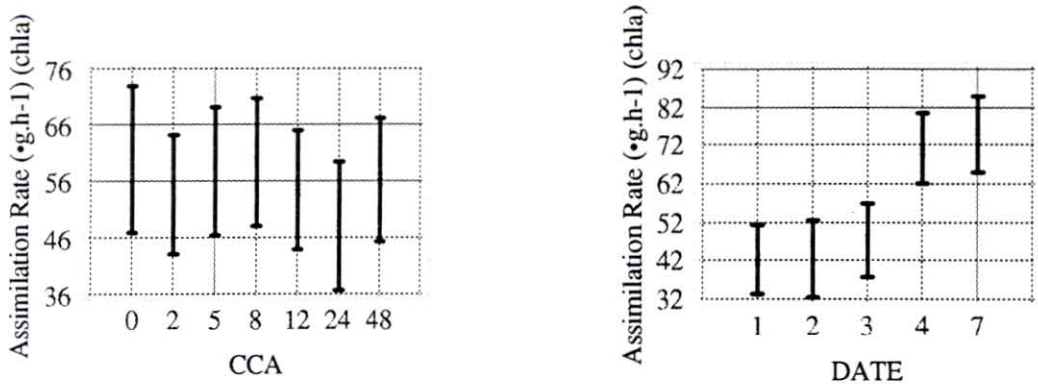


Figure 10: Results of 2-way ANOVA testing the CCA concentration and time effects on physiological functions. Calculations based upon chlorophyll a and pheopigments estimates. No interaction significant.

Table 6: Two-way ANOVA results of CCA concentration and time factors on the physiological functions.

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

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:CCA	18343.4	6	3057.23	3.87	0.0019
B: Date	22838.9	4	5709.72	7.23	0.0000
Residual	64785.7	82	790.069		
Total (Corrected)	105437.0	92			

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:CCA	14706.6	6	2451.1	3.87	0.0019
B: Date	22812.2	4	5703.05	7.34	0.0000
Residual	63686.5	82	776.665		
Total (Corrected)	100717.0	92			

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

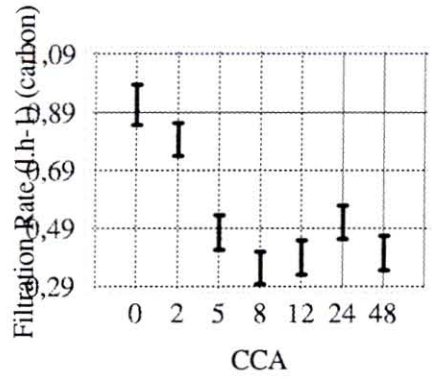
Assimilation Rates - Analysis of Variance -Type III Sums of Square

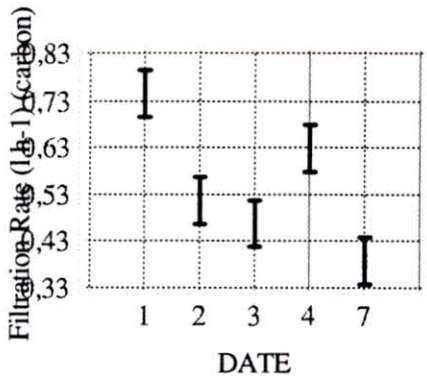
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:CCA	1288.5	6	214.75	0.25	0.9564
B: Date	19028.9	4	4757.23	5.63	0.0005
Residual	69344.6	82	845.666		
Total (Corrected)	89663.0	92			

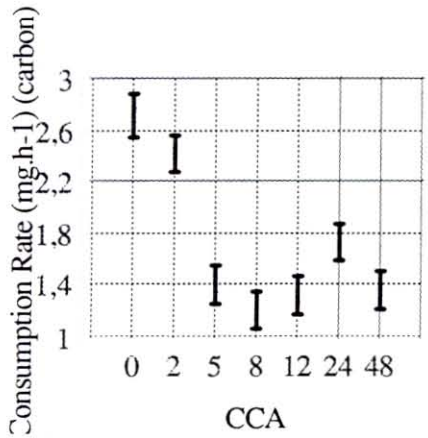
All F-Ratios are based on the residual mean square error

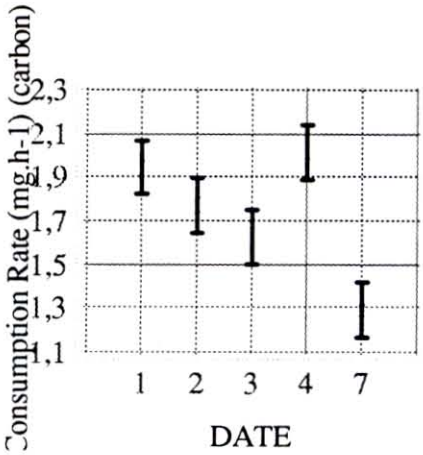
2.3.1.3 CHN analysis

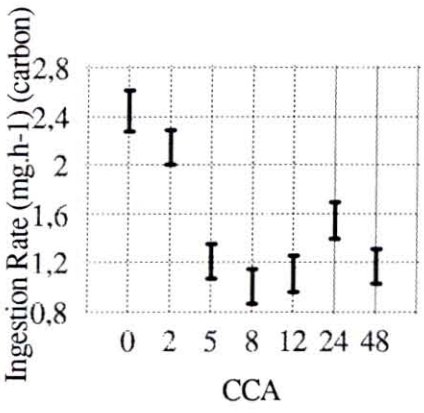
Similarly, ANOVA were performed on filtration rate estimates using carbon concentrations (Figure 11, table 8). Both factors affected significantly all physiological functions. A pattern similar to this obtained with chlorophyll computations was observed. The control and 2kg/m^3 CCA concentration showed the highest filtration rates (>0.7l.h-1). Assimilation rates were significantly different and affected by CCA increasing load up to 12kg/m^3 concentration. A strong decline for all physiological functions was recorded along the first three days. However, assimilation rates were quite similar but those at day 7 showing the lowest values.

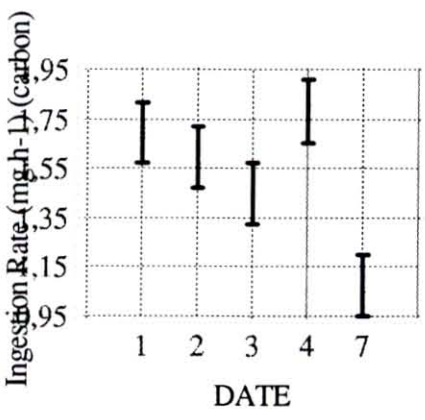












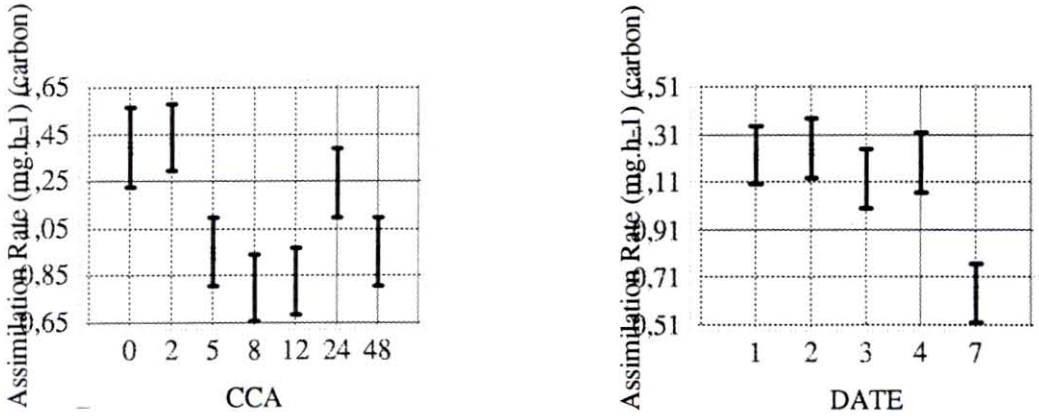


Figure 11 : Two way ANOVA results computed with carbon concentrations and testing the effect of 'CCA concentration' and 'time' on the main physiological functions.

Tableau 8 : Summary tables of 2-way ANOVA's assessing the effects of 'CCA concentration' and 'time' on consumption, ingestion and assimilation rates.

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

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:CCA	3.76365	6	0.627275	24.62	0.0000
B: Date	1.60002	4	0.400005	15.70	0.0000
Residual	2.29334	90	0.0254815		
Total (Corrected)	7.81528	100			

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:CCA	28.2941	6	4.71568	29.09	0.0000
B: Date	6.73721	4	1.6843	10.39	0.0000
Residual	14.5903	90	0.162114		
Total (Corrected)	50.1061	100			

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:CCA	24.6488	6	4.10814	25.62	0.0000
B: Date	6.15901	4	1.53975	9.6	0.0000
Residual	14.4305	90	0.160339		
Total (Corrected)	45.6089	100			

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:CCA	6.00258	6	1.00043	6.33	0.0000
B: Date	5.05119	4	1.2628	7.99	0.0000
Residual	14.223	90	0.158033		
Total (Corrected)	25.3712	100			

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

II.3.2 Respiration Rates

The results concerning the respiration rates are presented on figure 12 and table 9. Respiration rates mean values varied from 12 to 18•mole O2 per hour, respectively for 8 and 2kg/m^3 CCA concentration.

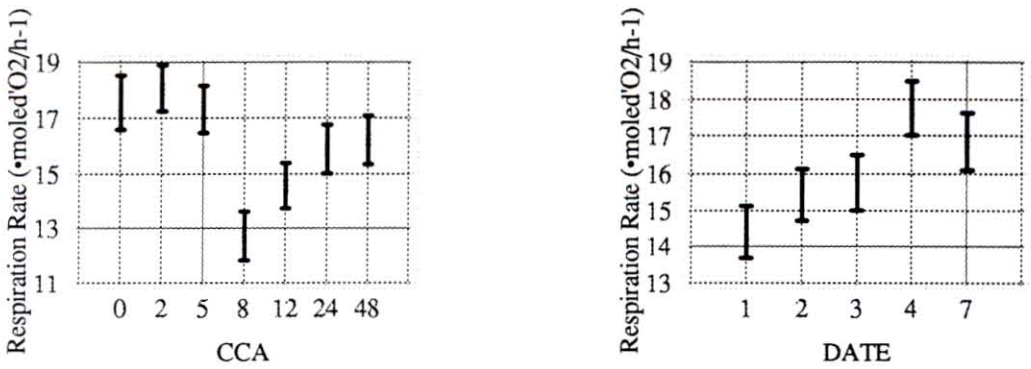


Figure 12: Two way ANOVA results of the respiration rates function of the 'CCA concentration' and 'time' factors.

The respiration rates appeared to be less affected by increasing CCA concentration than feeding processes (Figure 12). However, both variables affected significantly the respiration rates (Table 9). Actually, respiration rates were not affected by CCA leachates up to the 5kg/m^3 concentration. A recovery trend for the respiration activity was obvious following the day 1 exposure to CCA leachates.

Table 9 : Two-way ANOVA's results of the 'CCA concentration' and 'time' effects on the mussel respiration rates.

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

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:CCA	299.306	6	49.8843	9.3	0.0000
B: Date	135.913	4	33.9784	6.34	0.0002
Residual	466.462	87	5.36163		
Total (Corrected)	903.19	97			

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

II.3.3. Scope for Growth

2.3.3.1 Scope For Growth (Particulate Organic Matter)

The scope for growth (SFG) was computed using the aforementioned results based on assimilated energy and expenditure from respiration activity (Figure 13 and tables 10, 11). The figure 13 demonstrated that 'CCA' factor did not affect significantly the SFG, mainly due to the high variability of the assimilation rates. Although a decreasing trend can be reported for the highest CCA concentration.

Table 10 : Summary table of the 2-way ANOVA testing the 'CCA concentration' and 'time' effects on the estimated assimilated energy by the mussels & Scope For Growth.

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

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:CCA	2222.49	6	370.416	0.48	0.8212
B: Date	25384.8	4	6346.2	8.23	0.0000
Residual	63996.1	83	771.038		
Total (Corrected)	92212.9	93			

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

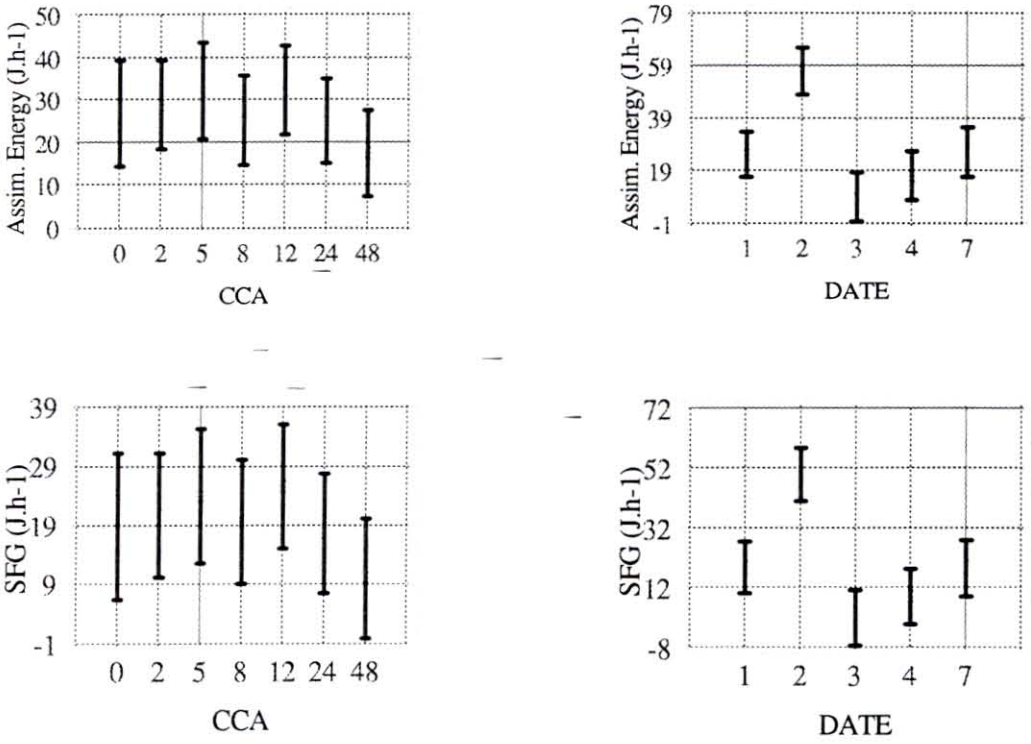


Figure 13: Scope for Growth estimates resulting from 2-way ANOVA's ('CCA concentration' and 'time' effects).

Table 11 : Summary table of the 2-way ANOVA testing the 'CCA concentration' and 'time' effects on the estimated mussel Scope For Growth.

Scope For Growth (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:CCA	2171.35	6	361.891	0.46	0.8347
B: Date	25898.4	4	6474.6	8.26	0.0000
Residual	65062.7	83	783.888		
Total (Corrected)	93691.8	93			

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

2.3.3.2 Scope for Growth (Chlorophyll a & Pheopigments)

Similarly to computations based on the particulate organic matter, Scope for Growth estimates did not show any significant differences according to CCA concentration (Figure 14, table 12). In contrast, the 'time' effect was significant and demlonstrated a recovery over time. The multiple range test (LSD) discriminated 2 groups: estimates during the three fisrt days, and days 4&7.

Table 12 : Summary table of the 2-way ANOVA testing the 'CCA concentration' and 'time' effects on the estimated mussel Scope For Growth.

Assimilated Energy (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:CCA	5900.81	6	983.469	0.25	0.9564
B: Date	87144.8	4	21786.2	5.63	0.0005
Residual	317570.0	82	3872.81		
Total (Corrected)	410621.0	92			

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

Scope For Growth (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:CCA	6092.99	6	1015.5	0.26	0.9523
B: Date	84249.2	4	21062.3	5.47	0.0006
Residual	315888.0	82	3852.29		
Total (Corrected)	406225.0	92			

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

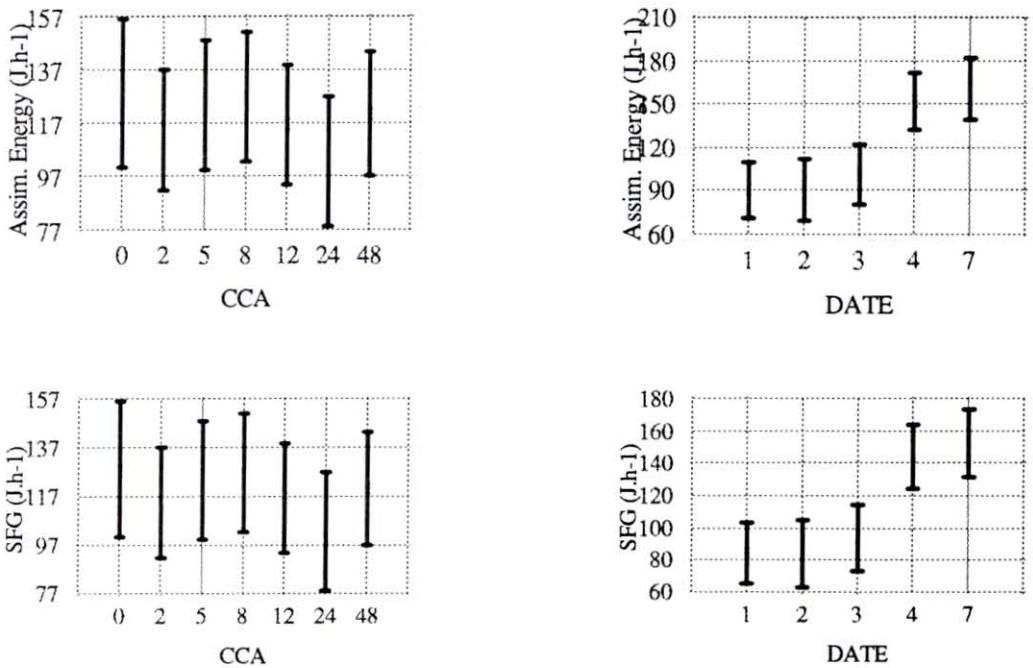


Figure 14: Scope for Growth estimates resulting from 2-way ANOVA's ('CCA concentration' and 'time' effects) (computations based on chlorophyll estimates).

2.3.3.3 Scope for Growth (Carbon)

In contrast to the two previous computations, both factors affected significantly the mussel Scope For Growth as well as the assimilated energy (tables 13 & 14, figure 15). SFG estimates were quite similar but those from day 7. With regard to the CCA concentration effect, we should note one homogeneous group including the control and the 2kg/m^3 value. SFGs were significantly reduced for greater CCA concentration while the 24 and 48kg/m^3 showed a different pattern resulting from the different dryness.

Table 13 : Summary table of the 2-way ANOVA testing the 'CCA concentration' and 'time' effects on the estimated assimilated energy (J.h-1).

Assimilated Energy (Carbon) - Analysis of Variance -Type III Sums of Square

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A:CCA	10995.8	6	1832.63	6.33	0.0000
B: Date	9252.99	4	2313.25	7.99	0.0000
Residual	26054.3	90	289.492		
Total (Corrected)	46476.0	100			

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

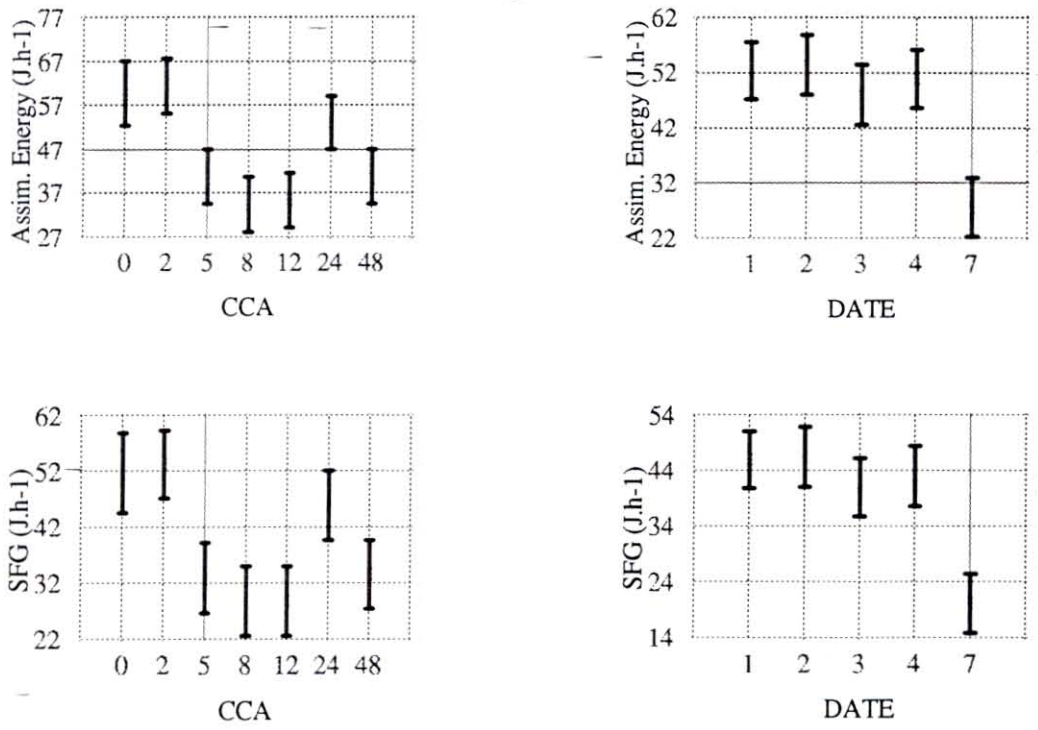


Figure 15: Scope for Growth estimates resulting from 2-way ANOVA's ('CCA concentration' and 'time' effects) (computations based on Carbon estimates).

Table 14 : Summary table of the 2-way ANOVA testing the 'CCA concentration' and 'time' effects on the estimated Scope For Growth (J.h-1).

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:CCA	9692.38	6	1615.4	5.58	0.0001
B: Date	9626.43	4	2406.61	8.32	0.0000
Residual	26038.5	90	289.317		
Total (Corrected)	45539.6	100			

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

III. Discussion

The experimental design was based upon the first set of results concerning the CCA effects on the mussel physiological activity. CCA concentrations varying from 2 to 48kg/m^3 were chosen to mainly assess the effects below the 10kg/m^3 concentration since limited effects were initially reported for that levels. The overall experimental conditions were eventually less stressful for the mussel population as demonstrated by the lack of dry meat weight decline over time. Similarly, conditions were below the LC-50 (96hours) with mortality rates reaching significant values only after day 4 for a 5kg/m^3 CCA concentration. However, the drying conditions of the wooden blocks were quiet different to those of the first set experiments as shown by the differences among the 2 to 12 kg/m^3 and the 24 and 48 treated blocks, respectively in terms of mortality rates as well as in the initial impact timing. Actually, the 2 to 12kg/m^3 treated blocks were tested before the full completion of air-drying process, therefore maximizing the leaching and resulting effects (Brooks, 1994). Moreover, these experiments were carried out at a 21-23°C temperature range compared to 13.5°C during the first set of experiments. The increased temperature is one of the most significant parameter affecting leaching rates according to Dahlgren (1975) and Brooks (1994). Moreover, Van Eetvelde et al. (1995) reported an average mean metal loss ratio of 0.67 observed with decreasing temperature (8°C/20°C). Moreover, temperature change had a significant impact on the mussel physiological activity. Therefore, a seasonal variability of leaching effects might occur in natural environment as temperature varies seasonally and should be taken into account when assessing CCA impacts.

In spite of these conditions, we should notice that no CCA effect was reported for the mussel respiration rates below the 8kg/m^3 concentration, while filtration activity was already affected at a 2kg/m^3 concentration. These results confirm those obtained during the previous experiments showing a more sensitive response for the filtration activity. Respiration rates were slightly increased at the 2kg/m^3 CCA concentration, representing phenomenon similar to those described on the hard clam when exposed to 8-13ppb copper concentration (Chen, 1995). The present study has also confirmed that the CCA leachates increase mortality and decrease physiological activity concomitantly to increased leachate concentration.

The physiological responses over time exposure showed a different pattern compared to results from the first experiments. Respiration rates were early affected and then presented a significant recovery, strongly correlated with time. Filtration activity decreased linearly during the first three days of exposure then increased by the end of this experiment. This should be

related to the higher seawater renewal rate, and likely to the initial higher leaching rates and kinetic as described for copper by Putt (1993), Van Eetvelde et al. (1995a,b). Similarly, Brooks (1994) established the following negative exponential relationship for CCA leaching rate decreasing over time: Copper loss=3.566*exp(-0.048*time in days). Moreover, the reduced dryeness of the blocks likely increased the leaching rates and then the subsequent effects on mussel physiology during the first three days. Besides leaching rates, we should note that Hummel et al. (1997) demonstrated that homeostasis is the dominant process in *M. edulis* exposed to copper. Wang et al. (1995) specified that two 2 major routes by which a metal can become available to the suspension feeding bivalves are likely, namely, dissolved uptake and particulate ingestion. Dissolved uptake occurs primarily through the gills. Specific gill transport mechanisms were indicated to maintain homeostasis (Phillips, 1976; Viarengo et al., 1985). Copper uptake and elimination can occur in several days while *M. edulis* is able to regulate this metal at high concentrations (Bryan et al., 1985; Widdows and Donkin, 1992). It seems difficult to conclude on that matter without any data on chemical concentration in mussel meat. However, we can hypothesize that chemical concentration in mussel meat reached a maximum threshold after three days of exposure when leaching was maximum, leading to the homeostasis stage or/and thereafter, to a possible early elimination, explaining the recovery trend.

Moreover, Decho and Luoma (1996) demonstrated that bivalves are capable of modifying the digestive process of food to reduce exposure to high, biologically available, Cr concentrations. These might be related to the lack of significant impact on mussel Scope for Growth during our latest experiments. Therefore, in contrast to Widdows et al. (1997), Scope for Growth might not be the most sensitive parameter to assess the CCA leachates' impacts at these chemical thresholds. Widdows et al. (1995) reported concentrations that could cause a significant SFG reduction. Recorded 'No observed effect thresholds' on feeding, growth or SFG of mussels are Cu >25µg/g dry weight (Calabrese et al., 1983; Widdows & Johnson, 1988; lethal concentration of 60µg/g Cu (Martin 1979; Widdows & Johnson, 1988). We have already reported that filtration rate was the first physiological function likely affected by chemicals well before respiration rate. Therefore, the effect thresholds are likely different when considering filtration, respiration rates and scope for growth and should be established separately.

With regard to the use of the valvometer, Borcherding (1992) proposed the valve-closure response to be utilized as an early warning system. Our experiments systematically demonstrated that valve closure behavior can be modified by exposure to chemicals well below the closure limit. Actually, Davenport and Manley (1978) reported acute toxicity threshold for *M. edulis* around 0.09-0.1ppm added copper and that mussels can register presence of very small addition of copper. Detection concentration of about 0.02ppm total copper, little more than one order of magnitude greater than the background concentration was reported by these authors. We should also note that mucus secretion increases significantly under Cu exposure (Sze and Lee, 1995). Scott and Major (1972) recorded that 0.3mg/l Cu stimulated copious mucus secretion in *M. edulis* which might result in abnormal valve response as a way to depurate the gills. Furthermore, it appears critical to get appropriate background knowledge on species behavior so as to distinguish between natural fluctuations in valve movements and those caused by toxicants (Englund and Heino, 1996). In our experiments, several replicates were carried out to address this issue. Although further tests should be done in the near future, statistical treatments such as time series analysis appeared appropriate to assess behavior changes in bivalves, particularly well below acute toxicity.

References

Borcherding J. (1992). Another early warning system for the detection of toxic discharges in the aquatic environment based on valve movements of the freshwater mussel *Dreissena polymorpha*. In: D. Neumann & H.A. Jenner (eds), Limnologie Aktuell- vol 4- The zebra mussel *Dreissena polymorpha*. Gustaf Fisher Verlag, Stuttgart, Jena, New-York:127-146.

Brooks K.M. (1994a). Literature review and assessment of the environmental risks associated with the use of CCA treated wood products in aquaculture environments. Aquat. Envir. Sciences Report, 63p.

Brooks K.M. (1994b). References pertaining to the aquatic risks associated with CCA and ACZA treated wood held by the Western Wood Preservers' Institute. Aquat. Envir. Sciences Report, 33p.

Bryan G.W., Langston W.J., Hummerstone L.G., and G.R. Burt (1985). A guide to the assessment of heavy metal contamination in estuaries using biological indicators. Occ. Publ. Mar. Biol. Assoc. UK, 4:1-92.

Calabrese A., J.R. MacInnes, D.A. Nelson, R.A. Greig, P.P. Yevich (1983). Effects of long-term exposure to silver or copper on growth, bioaccumulation and histopathology in the blue mussel, *Mytilus edulis*. Mar. environ. Res., 11:253-274.

Chen I.M. (1995). Comparison of the effects of copper on respiration and its accumulation in tissue in the hard clam *Meretrix lusoria*. Zoological Studies, 34(4):235-240.

Dahlgren S.E. (1975). Kinetics and mechanisms of fixation of Cu-Cr-As wood preservatives. Part 5. Effect of wood species and preservative composition on leaching during storage. Holzforschung, 29:84-95.

Davenport J. and A. Manley (1978). The detection of heightened sea-water copper concentrations by the mussel *Mytilus edulis*. J. mar. biol. Ass. U.K., 58:843-850.

Decho A.W. and S.N. Luoma (1996). Flexible digestion strategies and trace metal assimilation in marine bivalves. Limnol. Oceanogr., 41(3):568-572.

Englund V.P.M. and M.P. Heino (1996). Valve movement of the freshwater mussel *Anadonta anatina*: a reciprocal transplant experiment between lake and river. Hydrobiologia, 328:49-56.

Hummel H., R. Modderman, C. Amiard-Triquet, F. Rainglet, Y. Van Duijn, M. Hesservoort,, J. de Jong, R. Bogaards, G. Bachelet, M. Desprez, J. Marchand, B. Sylvand, J.C. Amiard, H. Rybarczyk, L. de Wolf (1997). A comparative study on the relation between copper and ciondition in marine bivalves and the relation with copper in the sediment. Aquat. toxicology, 38:165-181.

Martin J.L.M. (1979). Scheme of lethal action copper on mussels. Bull. Envir. Contam. and Toxico., 21:804-814.

Phillips D.J.H. (1976). The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead, and copper. I. Effects of environmental variables on uptake of metals. Mar. Biol., 38:59-69.

Putt A.E. (1993). Marine sediment toxicity study with leachate from CCA-C treated wood pilings on the benthic organisms *Amplisca abdita*. Preliminary results on work done at the Springborn laboratories. Hickson Corporation. Technical center, 3941 Bonsai Road, Conley, Georgia 30027.

Scott D.M. and C.W. Major (1972). The effect of copper (II) on survival, respiration, and heart rate in the common blue mussel *Mytilus edulis*. Biol. Bull., 143:679-688.

Sze P.W.C. and S.Y. Lee (1995). The potential role of mucus in the depuration of copper from the mussels *Perna viridis* (L.) and *Septifer virgatus* (Wiegmann). Mar. Poll. Bull., 31 (4-12): 390-393.

Van Eetvelde G., R. Orsler, G. Holland and M. Stevens (1995a). Effect of leaching temperature and water acidity on the loss of metal elements from CCA treated timber in aquatic applications. Part 1: laboratory scale investigation. The International Research Group on Wood Preservation, Section 5, 26th Annual Meeting Helsingor, 16p.

Van Eetvelde G., W.J. Homan, H. Militz and M. Stevens (1995b). Effect of leaching temperature and water acidity on the loss of metal elements from CCA treated timber in aquatic applications. Part 2: semi-industrial investigation. The International Research Group on Wood Preservation, Section 5, 26th Annual Meeting Helsingor; 195-208.

Viarengo A., M. Pertica,, G. Mancinelli, R. Capelli, and M. Orunesu (1980). Effects of copper on the uptake of amino acids, on protein synthesis, and on ATP content in different tissues of *Mytilus galloprovincialis* Lam.. Mar. Envir. Res., 4:145-152.

Wang W. X., N.S. Fisher, and S. N. Luoma (1995). Assimilation of trace elements ingested by the mussel *Mytilus edulis*: effects of algal food abundance. Mar. Ecol. Prog. Ser., 129:165-176.

Widdows J. and P. Donkin (1992). Mussels and environmental contaminants: bioaccumulation and physiological aspects. In E. Gossling (edit.). The mussel *Mytilus*: ecology, physiology, genetics and culture. Elsevier, Amsterdam:384-424.

Widdows J., P. Donkin, M.D. Brinsley, S.V. Evans, P.N. Slkeld, A. franklin, R.J. Law, and M.J. Waldock (1995). Scope for growth and contaminant levels in North Sea mussels *Mytilus edulis*. Mar. Ecol. Prog. Ser., 127:131-148.

Widdows J., C. Nasci and V. U. Fossato (1997). Effects of pollution on the scope for growth of mussels *Mytilus galloprovincialis* from the Venice lagoon, Italy. Marine Environmental Research, 43(1/2): 69-79.

Widdows J. and D. Johnson (1988). Physiological energetics of *Mytilus edulis*: scope for growth. Mar. Ecol. Prog. Ser., 46:113-121.