The Accumulation, Biotransformation and Elimination of Paralytic Shellfish Toxins in *Mytilus edulis* as a Function of Prior Seasonal Exposure to Natural Blooms of *Alexandrium excavatum*

Hanadie A. Chebib

A Thesis

in

The Department

of

Biology

Presented in Partial Fulfilment of the Requirements
for the Degree of Master of Science at
Concordia University
Montreal, Quebec, Canada

August, 1992

ISBN 0-315-84714-X

ABSTRACT

The Accumulation, Biotransformation and Elimination of Paralytic Shellfish Toxins in *Mytilus edulis* as a Function of Prior Seasonal Exposure to Natural Blooms of *Alexandrium excavatum*

Hanadie A. Chebib

In a transplant experiment, two geographically distinct populations of *Mytilus edulis* with different histories of contamination by Paralytic Shellfish Poisoning (PSP) toxins were exposed to natural blooms of the toxic dinoflagellate *Alexandrium excavatum*. It is hypothesised that the biochemical response of the mussels to PSP toxins is a function of prior exposure to the toxins, permitting chronically exposed mussels to exploit the toxic organism as a food source.

Transplanted mussels were suspended in cages from the quai at the site of the experiment. Mussel and *Alexandrium* cells samples were collected periodically and their PSP toxin concentration and composition analysed by High Performance Liquid Chromatography (HPLC). The mussels encountered two successive blooms of *A. excavatum* differing in duration, maximum cell concentration and toxicity per cell. The shorter first bloom displayed cell concentrations an order of magnitude greater than the second bloom, but the toxicity of the cells increased by a factor of four during the latter. The two populations displayed different PSP toxin accumulation and elimination patterns during and after the first but not the subsequent bloom. During the first bloom, the maximum toxin concentration of chronically exposed mussels was twice that of the

pristine mussels, but in terms of toxicity the difference was less pronounced. However, during the second bloom, these differences had dissapeared.

Putative toxin transformation was examined in the two populations, based on comparisons of toxin profiles in *Alexandrium* cells and in mussel digestive glands and by comparison of temporal changes in toxin epimeric ratios in the two mussel populations. Prior exposure to toxic *Alexandrium* blooms appeared to have an effect on transformation of PSP toxins. During the first bloom, the toxin patterns of pristine mussels ressembled those of the *Alexandrium* cells, but following a first exposure, the two mussel groups had comparable toxin pattens.

In general, mussels which had not been pre-exposed to PSP toxins, seemed to accumulate less toxin on a total molar basis in the presence of high *Alexandrium* cell concentrations, but they contained a higher proportion of highly toxic derivatives, and detoxified more rapidly than previously exposed mussels. The results tend to support the hypothesis that the initial differences between the two mussel groups were physiological adaptations induced by prior seasonal exposure to PSP toxins rather than the consequence of genotypic mechanisms.

To Professor M. Novak,

ACKNOWLEDGEMENTS

I acknowledge the continuous support and invaluable guidance provided by my supervisors Dr. P.D. Anderson and Dr. A.D. Cembella, and by the members of the examining committee, throughout this project.

I also extend my gratitude to Professor P.J. Albert and Environment Canada (St. Lawrence Centre) for the use of their facilities, and to B. Huppertz, I. St. Pierre and R. Katkhouda for their technical assistance in various aspects of the project.

This experiment was made possible by the support provided by the Canadian Department of Fisheries and Oceans, specifically through the Biological Oceanography Division of the Maurice Lamontagne Institute.

Finally, appreciation must go to my family and friends for their unwavering support throughout the course of my studies.

TABLE OF CONTENTS

LIST OF FIGURES x

LIST OF TABLES xiv

INTRODUCTION 1
- General description of paralytic shellfish poisoning 1
- Dinoflagellate blooms and taxonomy of *Alexandrium* species 1
- Paralytic shellfish poisoning toxins 5
- Detection methods for PSP toxins 6
  - A. Assays of PSP toxins 6
  - B. Chemical and instrumental detection methods 8
- Chemical properties of PSP toxins 9
- Effects of PSP toxins on mollusks 12
  - A. General physiology 12
  - B. Kinetics of PSP toxin in mollusks 13
- Significance of PSP contamination 15
- Purpose and hypothesis of the study 16

MATERIALS AND METHODS 18
- A. Sample collection 18
  - 1. Field study of *in situ* mussel populations (1987) 20
  - 2. Simultaneous mussel transplantation experiment (1990) 20
    - a. Cell density estimates 21
    - b. Sampling strategy 21
      - i. Mussel sampling frequency 21
      - ii. Phytoplankton samples 22
      - iii. Water samples 23
- B. Toxin extraction 27
  - 1. Mussels 27
  - 2. Toxic phytoplankton samples 28
- C. HPLC analysis 29
  - 1. Toxin separation and detection 29
  - 2. Toxin identification and measurment 30
  - 3. Toxin hydrolysis 32
- D. Statistical analysis 36
  - 1. Variances within and among the populations 36
  - 2. Comparative testing 36
  - 3. Toxin kinetics 38
  - 4. Putative biotransformation 39

RESULTS 40
I. Field study of *in situ* mussel populations (1987) 40
1. Total PSP toxin concentration and toxicity 40
2. Bioassay and HPLC Correlation 44
3. Variation in PSP toxin concentration 51
4. Mussel weights 51
II. Simultaneous mussel transplantation experiment (1990) 51
A. *Alexandrium* cells 51
B. Mussels 58
1. PSP toxin accumulation and elimination 58
a. Toxin concentration and toxicity 58
b. Kinetics of PSP toxin accumulation and elimination 60
i. Comparisons between populations 61
ii. Comparisons between blooms 62
2. Toxin compositional changes 62
a. Toxin composition in mussel and *Alexandrium* 62
b. Changes in relative toxin composition 63
i. Comparisons between populations 63
ii. Comparisons between blooms 63
c. Kinetics of toxin ratio changes 76
i. Comparisons between populations 76
ii. Comparisons between blooms 77
3. Anatomical distribution of toxin in the mussels 77
a. Comparison between populations 77
b. Comparison between tissues 83
4. Mussel weights and shell dimensions 83

DISCUSSION 89
A. Simultaneous mussel transplant experiment (1990) 89
1. *Alexandrium* cells 89
2. Kinetics of toxin uptake and accumulation 90
3. Putative biotransformation 92
4. Anatomical distribution of toxins 96
B. Field study of *in situ* mussel populations (1987) 98
1. Mussels 98
2. *Alexandrium* cells 99
C. Source of variation 100
D. Mussel weight and size-specific factors 101
E. Bioassay and HPLC correlations 103

SUMMARY AND RECOMMENDATIONS FOR FUTURE STUDIES 105

REFERENCES 107

APPENDIX 118
Bioassay data 118

ANNEX I 135

ANNEX II 163

LIST OF FIGURES

Fig. 1: Schematic represetnation of the ventral view of an *Alexandrium excavatum* cell (scale 1cm = 3.5μ) 3

Fig. 2a: Basic chemical stucture of PSP toxins showing the sites of radical groups (R1, R2, R3 and R4) that distinguish analogues 10

Fig. 2b: The three primary subgroups of PSP toxins:
- Carbamate: Saxitoxin (STX), neosaxitoxin (neoSTX), Gonyautoxins 1-4 (GTX_{1-4});
- N-Sulfocarbamoyl: B_{1-2} and C_{1-4}
- Decarbamoyl: (de-STX, de-neoSTX, de-GTX_{1-4}) 10

Fig. 3: Map of lower St. Lawrence region. Points of interest: Trois Pistoles, Metis, Cap Chat, the Magdalen Islands and Pointe-aux-Cenelles (experiment site) 18

Fig. 4: Measurment of water turbidity as given by the exctinction coefficient (K) and of water temperature (Degrees Celcius °C) and salinity (‰) at 0, 3 and 7 metres at the Pointe-aux-Cenelles experiment site 25

Fig. 5: Percentage decrease in C-toxins (C_{1-4}) and percentage increase in Gonyautoxins 2 and 3 (GTX) in mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD) 34

Fig. 6: Total PSP toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$ tissue) of mussels collected from the inter-tidal zone at Trois Pistoles, Metis and Cap Chat from June to September 1987 41

Fig. 7: Correlation of bioassay and HPLC toxicity results (μgSTXeq $100g^{-1}$ tissue) for the mussels collected from Cap Chat in the summer of 1987. Pearson correlation coefficient (r^2) and fitted linear regression for the correlation, are contrasted to an ideal 1:1 correlation curve 45

Fig. 8: Relative composition of PSP toxins in the mussels collected from the inter-tidal zone at a. Trois Pistoles, b. Metis and c. Cap Chat from June through September 1987 47

Fig. 9a: Total concentration (nmol g^{-1}) of PSP toxins in the digestive glands of mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD) and *Alexandrium* cell density at 3m depth in the water column from June to November 1990.
Inset: Concentration for August to November 1990 at 10X magnification 52

Fig. 9b: Total toxicity in the digestive glands of mussels transplanted from Cap Chat (CAP) and from the Magdalen Islands (MAD) (μgSTXeq g^{-1}) and total PSP toxicity in the water (μgSTXeq L^{-1}) at 3m depth from June to November 1990.
Inset: Toxicity for August to November 1990 at 10X magnification 54

Fig. 10: Percent molar composition of toxin in the *Alexandrium* cells for each sample collected during each of the blooms at the experiment site of the IML 56

Fig. 11: Percent molar composition of PSP toxin in *Alexandrium* cells (ALEX) during the two blooms and in the digestive glands of mussels transplanted from Cap Chat (CAP) and from the Magdalen Islands (MAD) during the various phases of toxin accumulation and elimination (n=number of samples collected during the phase) 64

Fig. 12: Percent molar composition of toxin in the digestive glands of mussels transplanted from a. Cap Chat and b. the Magdalen Islands and collected at each sampling interval during the two blooms 66

Fig. 13: Ratio of individual toxin concentration (C_x, GTX_4, GTX_1, GTX_3, GTX_2, neoSTX and STX) in the digestive glands of the mussels transplanted from Cap Chat (CAP/Alex) and from the Magdalen Islands (MAD/Alex) over the individual toxin concentration in *Alexandrium* cells during a. Bloom I and b. Bloom II 69

Figure 14: Ratio of toxin epimers: a. GTX_4:GTX_1; b. GTX_3:GTX_2 and c. neoSTX:STX for each bloom phase in the digestive glands of the mussels transplanted from Cap Chat (CAP) and from the Magdalen Islands (MAD) to the experiment site 72

Fig. 15: Changes in the absolute concentration of individual toxins: C_x, GTX_4, GTX_1, GTX_2, GTX_3, neoSTX and STX, during a transient (overlap of post-bloom I and inter-bloom) in the digestive glands of mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD) 78

Fig. 16: Toxin concentration (nmol g^{-1})(top) and toxicity (μgSTXeq g^{-1}) (bottom) in the digestive glands and in the other tissues of the mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD) for each sample collected during the end phase of the experiment (October 1-29) 81

Fig. 17: Histogram showing the percent molar toxin composition in the digestive glands and in the other tissues of the mussels transplanted from a. Cap Chat and b. the Magdalen Islands and collected during the end phase of the experiment (October 1-29) 84

Fig. 18: Curvilinear regression of shell height (cm) to digestive gland weight (g) and to total weight (g) of mussels transplanted from Cap Chat (CAP) and from the Magdalen Islands (MAD) to the experiment site 87

Fig. 19: Mouse bioassay results (μgSTXeq 100 g^{-1} tissue) for mussels collected from Magdalen Islands, Trois Pistoles, Metis and Cap Chat (1984-1989) as part of the surveillance program of the Canadian Department of Fisheries and Oceans 119

Fig. 20 a: C_x concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD) to the experiment site from June to November 1990.
Inset: Concentration for August to November 1990 at 10X magnification 121

Fig. 20 b: GTX_4 concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD) to the experiment site from June to November 1990.
Inset: Concentration for August to November 1990 at 5X magnification 123

Fig. 20 c: GTX_1 concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD) to the experiment site from June to November 1990.
Inset: Concentration for August to November 1990 at 10X magnification 125
Fig. 20 d: GTX_4 concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD) to the experiment site from June to November 1990.
Inset: Concentration for August to November 1990 at 4X magnification 127
Fig. 20 e: GTX_2 concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD) to the experiment site from June to November 1990.
Inset: Concentration for August to November 1990 at 5X magnification 129
Fig. 20 f: neoSTX concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD) to the experiment site from June to November 1990.
Inset: Concentration for August to November 1990 at 10X magnification 131
Fig. 20 g: STX concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD) to the experiment site from June to November 1990.
Inset: Concentration for August to November 1990 at 5X magnification 133

LIST OF TABLES

Table I: Absolute (MU) and relative toxicity rankings of ten PSP toxin analogues 12

Table II: Fisheries and Oceans code and geographical coordinates for the five sites from which mussel samples were obtained for the experiment 17

Table III: Composition of the mobile phases and the post-column reaction reagents 30

Table IV: Typical standard (MSPR-3 and MSPR-5) nominal retention times (mins), toxin concentration (μM) and HPLC detection limit of PSP toxin analogues 31

Table V: Toxin conversion factor used to convert the concentration of individual toxins (μM) to toxicity units (μgSTXeq g^{-1}) 32

Table VI: Coefficient of variability (CV) in toxin levels for the three mussel populations 43

ANNEX I

LIST OF TABLES

Table 1: Replicate counts (rep. 1-5) of *Alexandrium* cells in the enriched fraction (20-70μ) of the net tows used for the HPLC analysis of PSP toxins in the phytoplankton; dilution factor of the cells on each filter (dil.) and number of cells per filter (cell/filter) 135

Table 2: Concentration of PSP toxins (μM) in individual (4 and 5) and pooled (123) mussels collected from Trois Pistoles from June 15 to Sept 20, 1987 136

Table 3: Concentration of PSP toxins (μM) in individual (4 and 5) and pooled (123) mussels collected from Metis from June 15 to Sept 20, 1987 137

Table 4: Concentration of PSP toxins (μM) in individual (4 and 5) and pooled (123) mussels collected from Cap Chat from June 15 to Sept 20, 1987 138

Table 5: Total mussel wet weight (g) of the individual mussel samples (4 and 5) and pooled mussels (123) collected from Cap Chat (CC), Metis (MT) and Trois Pistoles (TP) from June to September 1987 139

Table 6: Concentration of *Alexandrium* cells (cells L^{-1}) in the Niskin bottle water samples collected at 0 3, and 7 meters at the experiment site from May to November 141

Table 7: Relative contribution of dominant genus and of *Alexandrium* to the total phytoplankton assemblage collected in the 20μ mesh size net tow of the water column at the experiment site from June to October 142

Table 8: Concentration of PSP toxins in the *Alexandrium* cells (μM) during each of the blooms at the experiment site 143

Table 9: Concentration of PSP toxins in the digestive glands of CAP mussels (μM) during the experiment, as obtained from the HPLC analysis of the mussel extracts 144

Table 10: Concentration of PSP toxins in the digestive glands of MAD mussels (μM) during the experiment, as obtained from the HPLC analysis of the mussel extracts 146

Table 11: Concentration of PSP toxins (μgSTXeq $100g^{-1}$ tissue) in total body weight of CAP mussels 148

Table 12: Concentration of PSP toxin (μgSTXeq $100g^{-1}$ tissue) in total body weight of MAD mussels 150

Table 13: Mussel shell length and height (cm), digestive gland (dig.gl.), tissue and total mussel weight (g) and ratio of digestive gland weight to total mussel weight for CAP mussels 152

Table 14: Mussel shell length and height (cm), digestive gland (dig.gl.), tissue and total mussel weight (g) and ratio of digestive gland weight to total mussel weight for MAD mussels 157

Table 15: Concentration of PSP toxin (μM) in non-visceral tissues of CAP mussels in the end phase of the experiment (Oct 1-Oct 29) 162

Table 16: Concentration of PSP toxin (μM) in non-visceral tissues of MAD mussels in the end phase of the experiment (Oct 1-Oct 29) 162

ANNEX II

LIST OF TABLES

Table 1: Mean and standard error in temperature and salinity values for 0m, 3m and 7m and at 3m for the two halves of the season and *p*-value giving the level of significance of a Student's T-test comparing temperature and salinity pairs 163

Table 2: Student's t-test comparing the means of the PSP toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$) in CC vs MT, CC vs TP and MT vs TP mussels during the entire season (n=15) 164

Table 3: Multi-factor ANOVA comparing the variances in the concentration (nmol g^{-1}) and toxicity (μgSTXeq g^{-1}) of PSP toxins in CC vs MT, CC vs TP and MT vs TP mussels during the entire season 165

Table 4: One-way ANOVA comparing the variance in the concentration (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$) of PSP toxins in individual vs pooled mussels collected from a. CC, b. MT and c. TP 166

Table 5: Student's t-test comparing the means of the PSP toxin concentrations (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$ tissue) in CC vs MT, CC vs TP and MT vs TP mussels a. prior to (n=9) and b. during the peak in toxin concentration (n=6) 169

Table 6: Student's t-test comparing the means of the relative PSP toxin concentrations (%molar) in CC vs MT, CC vs TP and MT vs TP mussels a. prior to (n=9) and b. during the peak in toxin concentration (n=6) 170

Table 7: Total weights (g, S.E.) of the mussels collected at the inter-tidal zone at Cap Chat (CC), Metis (MT) and Trois Pistoles (TP) and *p*-value giving the level of probability of difference in the weights of mussels collected from the three sites based on a Student's t-test 171

Table 8: K-S analysis comparing PSP toxin concentrations (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$ tissue) in CAP and MAD mussels and in the *Alexandrium* cells during bloom I (n=5) vs bloom II (n=9) 172

Table 9: Discriminant analysis of the concentration (nmol g^{-1}) of individual toxins in *Alexandrium* cells during bloom I vs bloom II 173

Table 10: Wilcoxon Signed-Ranks test comparing the relative PSP toxin concentration (arcsin %molar) of individual toxins in CAP and MAD mussels and in *Alexandrium* cells during bloom I (n=5) compared to bloom II (n=9) 174

Table 11: K-S analysis comparing PSP toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq g^{-1} tissue) in CAP vs MAD mussels during a. the entire season (n=45), b. bloom I (n=5), c. inter-bloom (n=10), d. bloom II (n=9), e. post-bloom II (n=8), f. end phase (n=9) and g. transient stage (n=9) 175

Table 12: Discriminant analysis of concentration (nmol g^{-1}) of individual PSP toxins in CAP vs MAD mussels during each phase of the experiment 177

Table 13: Linear Regression analysis (Y=aX+b) of the rates of a. toxin accumulation and b. elimination of PSP toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq g^{-1}) in the digestive glands of 1. CAP and 2. MAD mussels during i. bloom I and ii. bloom II 178

Table 14a: Linear regression analysis (Y=aX+b) for the rate of decrease in toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq g^{-1} tissue), as given by the percentage of the peak toxin concentration and toxicity in the digestive glands of CAP and MAD mussels following each bloom (I and II) for the elimination of 85% of the total toxin content in each mussel group 182

Table 14b: Level of significance of the pair-wise comparison of the slopes of toxin elimination for CAP vs MAD mussels during the first and second blooms, and between the first and second bloom for CAP and MAD mussels. 182

Table 15: Student's t-test comparing the slopes of increase and decrease of the concentration (nmol g^{-1}) and toxicity (μgSTXeq g^{-1}) of PSP toxins in CAP vs MAD mussels during a. bloom I and b. bloom II 183

Table 16: Student's t-test comparing the slopes of increase (a) and decrease (b) in the concentration (nmol g^{-1}) and toxicity (μgSTXeq g^{-1} tissue) of PSP toxins during bloom I vs bloom II in CAP and MAD mussels 184

Table 17: Wilcoxon Signed-Ranks test comparing the relative PSP toxin concentration (arcsin %Molar) of individual toxins in CAP vs MAD mussels and in *Alexandrium* cells vs CAP and MAD mussels during a. bloom I (n=5) and b. bloom II (n=9) 185

Table 18: Wilcoxon Signed-Ranks test comparing the relative PSP toxin concentration (arcsin %Molar) of individual toxins in CAP vs MAD mussels during a. the entire experiment (n=45), b. the inter-bloom phase (n=10), c. the post-bloom II (n=8) and d. the end phase (n=9) of the experiment 186

Table 19: Linear Regression analysis (Y=aX+b) for the rates of changes in toxin ratios in the digestive glands of 1. CAP and 2. MAD mussels for a. the accumulation and b. the elimination phases of i. bloom I, ii. bloom II and iii. the inter-bloom 187

Table 20: Student's t-test comparing the slopes of changes in the toxin epimer ratios during the accumulation and elimination of PSP toxins in CAP vs MAD mussels during a. bloom I, b. bloom II and c. the inter-bloom 190

Table 21: Student's t-test comparing the slopes of changes in toxin epimer ratios during a. the accumulation and b. the elimination phases of bloom I to that of bloom II in CAP and MAD mussels 191

Table 22: Percentage of mean PSP toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$) found in non-visceral tissues of CAP and MAD mussels during the end phase (Oct 1-Oct 29). *P*-value giving the level of significance for a Student's t-test comparing the concentrations 192

Table 23: K-S analysis comparing PSP toxin concentrations (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$ tissue) a. in the non-visceral tissues of CAP vs MAD mussels and b. in visceral vs non-visceral tissues of CAP and MAD mussels, during the end phase of the experiment (n=9) 193

Table 24: Discriminant analysis of the concentration (nmol g^{-1}) of individual toxins a. in non-visceral tissues of CAP vs MAD mussels and b. in visceral vs non visceral tissues during the end phase 194

Table 25: Wilcoxon Signed-Ranks test comparing the relative PSP toxin concentration (arcsin %Molar) of individual toxins a. in non-visceral tissues of CAP vs MAD mussels and b. in visceral vs non-visceral tissues in CAP and MAD mussels during the end phase (n=9) 195

Table 26: Mean weights (g) of the digestive glands (D.G. WT), other tissues (TIS. WT) total mussel (TOT. WT), and mean shell height and length (cm) for CAP and MAD mussels. *P*-values give the level of significance of the F-ratio of variance within each group and the T-test of difference of the means among the groups 196

INTRODUCTION

GENERAL DESCRIPTION OF PARALYTIC SHELLFISH POISONING

Many shellfish poisonings occur as a direct result of toxic phytoplankton blooms, commonly known as "red tides". Through exposure to these blooms, mollusks can become vectors of human intoxication by filter-feeding on the harmful algae and accumulating their toxins within their tissues. Among the various types of shellfish poisoning of algal origin, incidents of paralytic shellfish poisoning (PSP) are the most widely reported worldwide. The present study compares aspects of the transfer kinetics of PSP toxins from toxic dinoflagellates to mussel populations with different histories of seasonal exposure to these toxins.

DINOFLAGELLATE BLOOMS AND TAXONOMY OF *ALEXANDRIUM* SPECIES

There is no general agreement on the higher level categorization of dinoflagellates. Taylor (1985) classified them in the animal kingdom as protozoa in the order Dinoflagellida, whereas they were classified in the plant kingdom by Leedale (1974) (Class: Dinophyceae), and also by Dodge (1984), who placed them in the division Pyrrhophyta. It is perhaps most informative to consider dinoflagellates as protists, in view of their plant-like features.

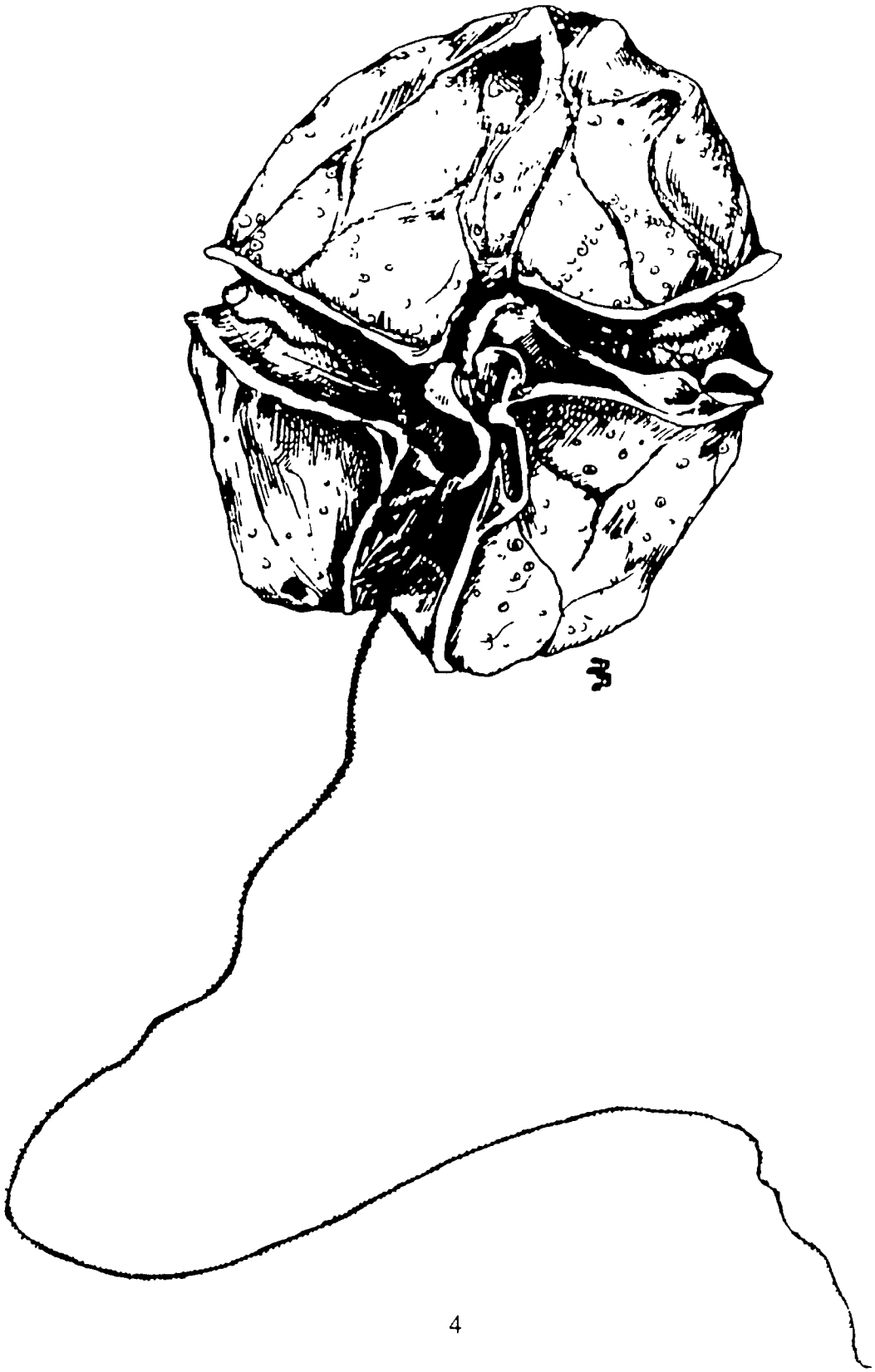
The organisms primarily responsible for PSP outbreaks in the northern hemisphere are free-living marine dinoflagellates of the genus *Alexandrium*. Since the main means of reproduction of species within this genus is asexual, taxonomists have to rely on other attributes for their classification. Characteristics used have included biogeographical

distribution, morphology of the vegetative cells and sexual cysts (hypozygotes), and biochemical properties, such as toxin and isozyme electrophoretic patterns, and nucleic acid sequencing (Turpin et al., 1978; Schoenberg and Trench, 1980; Hayhome and Pfiester, 1983; Cembella and Taylor, 1985; Cembella et al., 1987; Scholin and Anderson, 1993).

Species of the genus *Alexandrium* are armoured, i.e., the cells are covered by cellulose plates which form a protective theca. Attempts have been made to divide the species using morphological criteria. These include the shape, size and chain-forming capability of the cells, the position and shape of the thecal plates, and the disposition of the pores on those plates. About thirty species have been identified following these criteria (Steidinger, 1990), although species-level discrimination remains problematic. The species which blooms in the lower St. Lawrence estuary, *Alexandrium excavatum*, forms angular cells of 30 to 40 μm diameter (Balech, 1985) (Fig. 1). The genus *Alexandrium* includes all of the *Protogonyaulax tamarensis/catenella* group complex, previously identified as species of *Gonyaulax* (Steidinger, 1990; Steidinger and Moestrup, 1990).

Figure 1: Schematic representation of the ventral view of an *Alexandrium excavatum* cell (scale 1cm=3.5μm)

Scale: 1cm=3.5μ



It is likely that toxic dinoflagellate blooms have been occurring since this group evolved some hundreds of million years ago: the first evidence suggesting dinoflagellate blooms -and presumably shellfish poisoning, comes from analyses of sediments dating back to prehistoric times which revealed high concentrations of *Gymnodinium catenatum* cysts (Dale et al., 1993). Recently, phytoplankton blooms resulting in shellfish poisoning outbreaks gained importance due to their negative impact on the fishing and tourism industries. The *in situ* environmental factors which induce toxic phytoplankton blooms can only be speculated upon. Environmental conditions resulting in increased cell division as well as convergent migration and transport of cells have been suggested as plausible causes of blooms (Steidinger, 1983).

PARALYTIC SHELLFISH POISONING TOXINS

Two theories for the source of PSP toxins have been put forward. One theory states that PSP toxins are synthesised in the nucleus of the dinoflagellates from amino acid precursors (Anderson and Cheng, 1987; Doucette and Anderson, 1993; Shimizu et al., 1984 and 1985), they may even be involved in the metabolism of nucleic acid (Mickelson and Yentsch, 1979). The second theory suggests that PSP toxins are synthesised by bacteria within the toxic dinoflagellate cells (Kotaki et al., 1985; Kodama et al., 1989).

DETECTION METHODS FOR PSP TOXINS

PSP toxicity was first associated with dinoflagellates in the mid thirties, but the toxins were not isolated from the dinoflagellates for another twenty years (Schantz, 1986). Since their discovery several procedures have been developed for their detection and quantification.

A. Assays of PSP toxins

The first assay for PSP toxins, was based on a mouse bioassay developed by Sommer and Meyer (1937) and this assay is still widely used today. This procedure was standardized by the Association of the Official Analytical Chemists (A.O.A.C.) in 1958, and was adopted as an official PSP assay method in 1965 (A.O.A.C, 1984). The standard unit of measurement of toxin potency, the mouse unit (MU), has been defined as the minimum amount of toxin required to kill a 20-gram mouse in 15 minutes, following an intraperitoneal injection of one millilitre of tissue extract (Sommer and Meyer, 1937).

Paralytic shellfish poisonings of humans in California allowed Meyer (1953) to estimate that the minimum lethal toxin dose in humans is approximately 20,000 MU. Nevertheless, Prakash et al. (1971) stated that as little as 200μg (=1,000 MU) of PSP toxin have caused death. Other sources (Tennant et al., 1955; Bond and Medcof, 1958) estimated the lethal limit at 3,000 MU of PSP toxin. These apparent discrepancies in the lethal dosage may be explained by differences in the foods or liquids consumed with the

shellfish, and the age and health of the victim. The former variables are known to dramatically alter the absorption rate of toxins in the digestive tract.

The maximum PSP toxin concentration detected in mussel samples has been registered at 5,000μg g^{-1} of tissue (25,000 MU) (Schantz et al., 1957), a level which may reflect the upper limit of shellfish tolerance. Mouse units have been standardized to saxitoxin (STX) equivalent units (μgSTXeq): one MU is equal to 0.2μgSTXeq (Sommer and Meyer, 1937; Schantz et al., 1958). One shortcoming of the mouse bioassay procedure is its high threshold of detection at 40μgSTXeq $100g^{-1}$ tissue (W.H.O., 1984), a level that may cause discomfort in humans. Moreover, this test approach requires a large number of mice, which is an increasingly important drawback, given the opposition by animal rights groups to the use of mammalian bioassays. Thus, more acceptable bioassay procedures have been sought. Towards this end, a fly bioassay has been developed (Sieger et al., 1984 and Ross et al., 1985) whereby the toxin extracts are injected into the insect and the time lapse to death is determined. Two other assays take advantage of the Na^+ flux inhibition properties of PSP toxins in eukaryote cells: a culture tissue assay (Kogure et al., 1988) and a nerve binding assay (Davio and Fontenlo, 1984). Finally, immunoassays have also been developed for PSP toxins to include radioimmunoassays (RIA) (Carlson et al., 1984) and enzyme-linked immunosorbent assays (ELISA) (Chu and Fan, 1985; Cembella and Lamoureux, 1990; Sako et al., 1993).

B. Chemical and instrumental detection methods

The above considerations have led to the development of chemical detection of toxins. Nevertheless, there is concern about the effectiveness of such methods in reflecting the combined dosage of toxins that may be ingested. A simple chemical assay for PSP toxins has been developed (Bates and Rapoport, 1975) whereby the toxins are oxidized with hydrogen peroxide and fluorescent products are measured by spectrofluorometry. Gershey et al. (1977) modified this technique by adding biacetyl to the extracts which yields coloured derivatives of the oxidized toxins.

Individual PSP toxins may be identified and quantified by several analytical techniques, including low pressure column chromatography, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), electrophoresis and mass spectometry (MS). Due to its high sensitivity and time efficiency, HPLC is the most commonly employed method (Sullivan and Iwaoka, 1983; Sullivan and Wekell, 1984). The combination of HPLC with fluorometric detection (Buckley et al., 1978 and Jonas-Davies et al., 1984) has proved to be a cost and time efficient procedure for the separation and detection of PSP toxins. Detection is achieved through post-column oxidation of PSP toxins to fluorescent derivatives (Sullivan, 1990; Shoptaugh et al., 1981; Sullivan et al., 1985; Sullivan et al., 1988). In addition to being a more precise technique than the mouse bioassay, the HPLC method is highly sensitive (5μgSTXeq $100g^{-1}$). Since such low concentrations are not hazardous to human health, the ability to monitor toxins at these levels allows a measure of safety. Thus, the fluorometric-HPLC technique may be highly useful in a PSP regulatory programme

CHEMICAL PROPERTIES OF PSP TOXINS

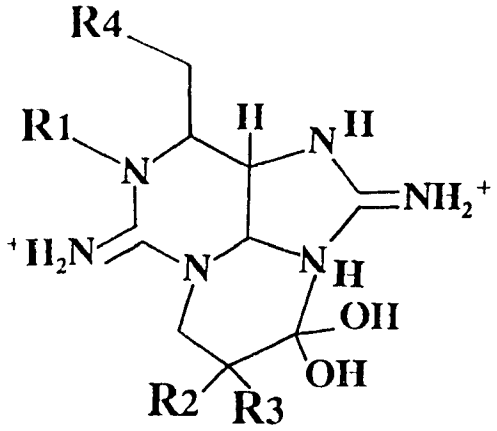
The structure of PSP toxins and their chemical properties have been elucidated using nuclear magnetic resonance (NMR), MS and infrared spectroscopy (IR) (Schantz, 1984 and 1986; Shimizu, 1986; Hall and Reichardt, 1984; Sullivan et al., 1988; Hall et al., 1990) (Fig. 2a and 2b).

PSP toxins are tetrahydropurine derivatives that can be separated chromatographically using cationic resins (Schantz, 1986). Saxitoxin (STX) includes two guanidinium groups (pK_a=8.22 and 11.28), which determine binding of STX to carboxylate cation resins at physiological pH. Neosaxitoxin (neoSTX) differs from STX in having a third dissociable group (pK_a=6.75). At physiological pH, gonyautoxins 1-4 (GTX_{1-4}), and $B_{1,2}$ are able to bind to cation resins, however, these analogues dissociate more readily due to their lower charge. The C-toxin derivatives are not charged under the same pH conditions and consequently do not bind to the cations. The PSP toxin analogues can be separated into two major groups: the low toxicity sulfamate toxins, which include B- and C-toxins ($B_{1,2}$ and C_{1-4}), and the highly potent carbamate toxins, which include the gonyautoxins (GTX_{1-4}), neoSTX, and STX. While the mechanism of action of all the analogues is the same (i.e. Na^+ channel blocking activity), their potencies, based on mouse bioassay analyses, differ between and within the groups (Hall and Reichart, 1984 and Hall, 1982). Table I provides estimates of the respective potencies of PSP toxins. Some researchers claim that neoSTX and STX are the most potent analogues, whereas others rank STX and GTX_3 as the most potent (in Steidinger, 1983).

Figure 2a: Basic chemical structure of PSP toxins showing the sites of radical groups (R1, R2, R3 and R4) that distinguish analogues

Figure 2b: The three primary subgroups of PSP toxins:

- Carbamate: Saxitoxin (STX), neosaxitoxin (neoSTX), Gonyautoxins 1-4 (GTX_{1-4});
- N-Sulfocarbamoyl: B_{1-2} and C_{1-4}
- Decarbamoyl: (dc-STX, dc-neoSTX, dc-GTX_{1-4}).



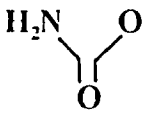
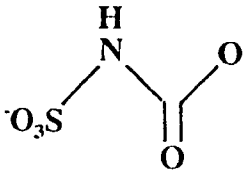
R1	R2	R3	CARBAMATE	N-SULFO-CARBAMOYL	DE-CARBAMOYL
H	H	H	STX	B_1	dc-STX
OH	H	H	neoSTX	B_2	dc-neoSTX
OH	H	OSO_3^-	GTX_1	C_3	dc-GTX_1
H	H	OSO_3^-	GTX_2	C_1	dc-GTX_2
H	OSO_3^-	H	GTX_3	C_2	dc-GTX_3
OH	OSO_3	H	GTX_4	C_4	dc-GTX_4
		R4			-OH

Table I: Absolute (MU) and relative toxicity rankings of ten PSP toxin analogues.

	ABSOLUTE TOXICITY		RELATIVE TOXICITY	
TOXIN	SULLIVAN[1]	SCHANTZ[2]	SULLIVAN[1]	HALL[3]
STX	2200	5500	1	1
neoSTX	2050	5000	0.93	1
GTX1	2000		0.91	0.73
GTX2	1200	2500	0.55	0.42
GTX3	2000	2000	0.91	0.67
GTX4	1800		0.82	0.27
C1	25	600	0.01	0.02
C2	250	60	0.11	0.02
B1	150		0.07	0.05
B2	175		0.08	0.09

Adapted from: [1]Sullivan et al., 1988, [2]Schantz, 1986 and [3]Hall, 1982.

EFFECTS OF PSP TOXINS ON MOLLUSKS

A. General physiology

Limited work has been carried out on the physiological effects of PSP toxins on mollusks. Several physiological activities seem to be affected by the initial ingestion of toxic *Alexandrium tamarensis* (*Protogonyaulax tamarensis*) (Shumway et al., 1985 a; Shumway and Cucci, 1987 and Cucci et al., 1985). Among the symptoms observed, closure of shell valves, retraction of the mantle, production of a mucus like material, increase in pseudofaeces production and decrease in byssus production, were the most obvious. Other changes included a decrease in filtration and heart rates, and an increase

in O_2 consumption. All these activities returned to normal following the exposure to toxic phytoplankton. These findings support the suggestion that repeated exposures to PSP toxins impart physiological adaptations to mollusks that may persist and become a genetic selection factor. Genetic selection would be witnessed by increased resistance to toxins. The work of Twarog et al. (1972) lends support to the genetic selection hypothesis; the authors reported variation in the mussel nerve response to PSP toxins between populations with apparent differences in exposure histories to toxic phytoplankton. Twarog and Yamaguchi (1975) and Shumway and Cucci (1987) suggested that the ability of certain species to accumulate toxins is the result of differing sensitivities of the nervous system to the PSP toxins, these being species specific. But Winter (1973) found that physiological activities of mussels were affected in a similar manner when they were exposed to high densities of non-toxic phytoplankton.

B. Kinetics of PSP toxin in mollusks

In the course of filter feeding on toxic phytoplankton, mollusks ingest the toxins contained within the algal cells. Upon digestion of the dinoflagellate cells, the toxins are released into the alimentary tract of mussels, then assimilated and stored primarily in the hepatopancreas. Some secondary accumulation of toxins occurs in the gills, the mantle the gonads and the foot (Bricelj et al. 1990 a and b). In a laboratory study, the latter authors demonstrated that the accumulation of toxin in mussel tissues follows a Michaelis Menten type of curve, whereby the maximum concentration may reflect the tolerance level of the mussels ($4.5 \times 10^4 \mu gSTXeq\ 100g^{-1}$ of tissue). In the presence of

high concentrations of toxic cells, the initial rate of increase of toxicity in mussels is logarithmic and can result in contamination levels that exceed the allowable limit for human consumption (80μgSTXeq 100g^{-1}) in less than an hour.

Price and Lee (1971) have suggested that PSP toxins are bound to mussel tissues by simple reversible ionic bonds. The release of significant quantities of toxin from the digestive gland can be detected within 24 hours after the environment becomes toxin-free The rate of release of PSP toxins is dependant on the specific toxin analogues present in the dinoflagellate strain and on the contaminated shellfish species (Fremy et. al, 1991; Bricelj et al. 1990 a and b).

Some evidence of biotransformation of PSP toxins by mollusks has been reported. The composition of toxin analogues in *Mytilus edulis* was compared to that of *Alexandrium minutum* (Ledoux et al., 1993) and *Alexandrium fundyense* used in toxin uptake experiments (Bricelj et al., 1990 a) as ways of studying toxin biotransformation. In both cases, toxin analogues in mussels differed from those in the phytoplankton. These findings lead to the conclusion that mussels are capable of metabolizing toxins and converting them to their respective derivatives, as was previously reported for other shellfish species (Oshima et al., 1987; Sullivan et al., 1983a and Shimizu and Yoshioka, 1981). The work of the latter groups revealed that relative increases in carbamate toxins were offset by proportional decreases in the sulfamate analogues. This temporal pattern suggests three possibilities: 1. that the low-toxicity sulfamates are transformed to high-toxicity carbamates by the shellfish; 2. that the carbamate toxins are selectively retained

and 3. that the sulfamate toxins are selectively eliminated. However, Sullivan et al. (1983 b) did not detect enzymatic conversions of PSP toxins in *Mytilus edulis*.

SIGNIFICANCE OF PSP CONTAMINATION

The impact of PSP on the worldwide fishing industry has resulted in the establishment of monitoring programs, primarily to regulate shellfish marketing and ensure public safety and eventually to alleviate the problem. Governmental regulatory agencies, including those in Canada and the United States, permit shellfish to be marketed when PSP contamination levels are below 400MU (80μgSTXeq 100g^{-1}) of edible shellfish tissue, a level considered safe for human consumption (A.O.A.C, 1984).

Several attempts at finding strategies to enhance toxin elimination from harvested shellfish have been made with varying degrees of success. Aalvik and Framstad (1981) advocate the simple transfer of contaminated mussels to sites free of toxic organisms. Other researchers have found that PSP contamination may be lessened by exposing mussels to ozone or sodium hypochlorite before harvesting (Blogoslawski, 1988; Blogoslawski and Stewart, 1978 and Chin, 1970). Nishitani et al. (1984) noted the disruptive effect of an *Alexandrium* endoparasite on *Alexandrium* blooms, and considered adding it to the medium. None of these processes are used on a commercial scale because their potential for adverse environment effects has yet to be assessed and their efficacy is doubtful.

PURPOSE AND HYPOTHESIS OF THE STUDY

The principal objective of the present research was to compare aspects of the kinetics of toxin accumulation, elimination and biotransformation, among transplanted mussel populations with different histories of exposure to *Alexandrium excavatum*. This objective required that an initial survey of several sites be conducted to locate a mussel population with a reliable history of seasonal exposure to toxic dinoflagellate blooms. The other population was obtained from a mussel culture site with no known record of exposure. Observation of the contamination patterns of mussels collected at various sites allowed the identification of the site-specific characteristics of shellfish with a likely history of PSP exposure.

The project is deemed to be unique because toxin kinetics are examined in transplanted mussels subject to *in situ* blooms. In laboratory studies with cultured algae, Shumway et al. (1985) have suggested that shellfish periodically exposed to toxic phytoplankton blooms, may develop mechanisms permitting them to exploit these organisms as food with no ill effects. Conceivably, through contact with the toxins, mussels could increase their tolerance; thereby accumulating more toxin, and may inhibit their ability to convert sulfamate toxins to carbamate derivatives prior to elimination. Experienced mussels may be expected to reflect the presence of less toxic derivatives through metabolism related-processes whereas highly toxic analogues would tend to prevail in naive mussels.

MATERIALS AND METHODS

A. SAMPLE COLLECTION

Mussel samples (*Mytilus edulis*) were collected from four sites with varying histories of exposure to PSP toxins. One set of samples was obtained from a mussel culture station on the Magdalen Islands (MAD) in the Gulf of St. Lawrence; the other samples were collected from the intertidal zones at Trois Pistoles (TP), Metis (MT), Cap Chat (CC: abbreviation for the *in situ* experiment and CAP: abbreviation for the mussel transplant experiment), along the south shore of the lower St. Lawrence estuary (Fig. 3). These areas correspond to the following area codes shown in table II, as designated by the Department of Fisheries and Oceans of Canada.

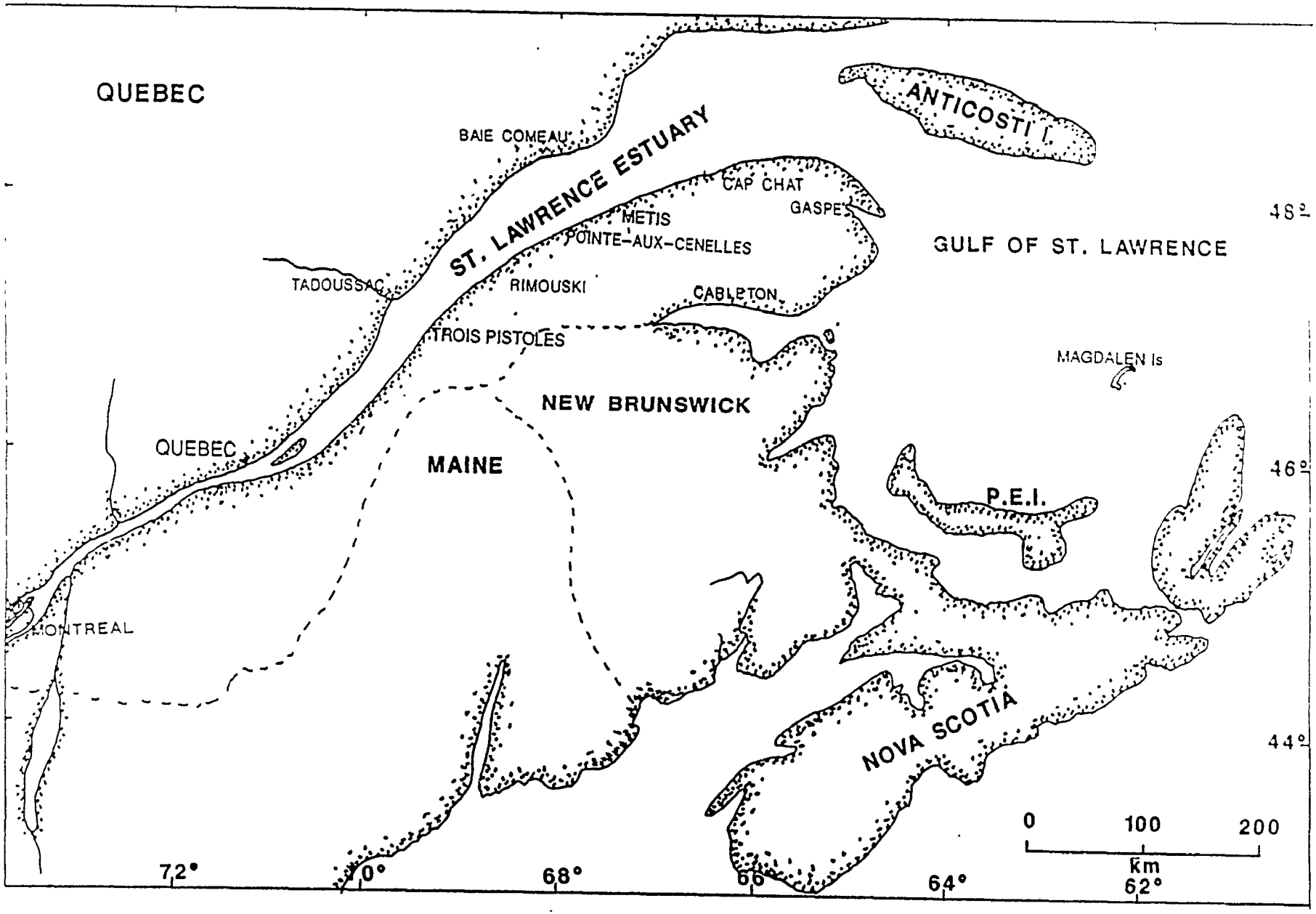
Table II: Fisheries and Oceans sector code and geographical coordinates for the four sites from which mussel samples were obtained for the experiment.

Site	Code	Geographical Coordinates
Magdalen Islands (MAD)	A-14.1	47°24' N Equator 61°50' W Greenwich
Trois Pistoles (TP)	B-3.1.2	48°08' N Equator 61°02' W Greenwich
Metis (MT)	B-6.3	48°59' N Equator 67°00' W Greenwich
Cap Chat (CC and CAP)	B-10.1.2	49°06' N Equator 66°42' W Greenwich

Bioassay data were provided through the inspection division of the Department of Fisheries and Oceans in Quebec. These were based on the A.O.A.C. hot HCl toxin extraction technique from whole mussel homogenates, intraperitoneal injection of 1 ml extract into a 20g mouse, and recording of times of death.

Figure 3: Map of lower St. Lawrence region.

Points of interest: Trois Pistoles, Metis, Cap Chat, the Magdalen Islands and Pointe-aux-Cenelles (experiment site).


QUEBEC
BAIE COMEAU
ST. LAWRENCE ESTUARY
ANTICOSTI I.
CAP CHAT
GASPE
METIS
POINTE-AUX-CENELLES
GULF OF ST. LAWRENCE
TADOUSSAC
RIMOUSKI
CABLETON
TROIS PISTOLES
MAGDALEN Is
NEW BRUNSWICK
QUEBEC
MAINE
P.E.I.
MONTREAL
NOVA SCOTIA
0
100
200
km
72°
70°
68°
66°
64°
62°
48°
46°
44°

1. Field study of *in situ* mussel populations (1987)

During the summer of 1987 (June 15-September 20), batches of five adult mussels each were collected weekly from the intertidal zone at TP, MT and CC and stored frozen at -20°C upon arrival. In 1989, the whole soft tissue extracts of two mussels from each batch were analyzed individually, whereas equal volumes of extract from the other three mussels were pooled for analysis.

2. Simultaneous mussel transplantation experiment (1990)

For the mussel transplant experiment, 250 adult mussels were collected from a natural population inhabiting the intertidal zone at Cap Chat and transported in seawater to the experimental site. Another group of 250 two year old mussels from a suspended culture site in the Magdalen Islands were shipped on ice to the experiment site. Four mussels were selected randomly from each of the two populations and used as reference specimens for toxicity determination at time zero. Within 24 hours of their arrival at the site of the experiment, the remaining mussels of each population were placed in tandem wire-mesh cages (vol=0.650m^3; mesh size=1cm) and suspended from the dock at the Maurice Lamontagne Institute (IML), Pointe-aux-Cenelles, Quebec. The cages were submerged at a depth of 3m below the mid-point of the tidal cycle and anchored at the bottom of the water column (7m depth). A period of one week was allowed for both groups to acclimate to their new environment prior to commencement of sub-sampling.

a. Cell density estimates

The density of *Alexandrium* cells in the water column adjacent to the cages was estimated at the start of the experiment. These estimates were based on cell counts of the vertical net tows (20μ mesh) collected bi-weekly. These were used to establish the initiation of the first *Alexandrium* bloom. The volume of water filtered by the net was calculated by assuming a cylindrical column of water with a radius equal to that of the opening of the net (r=0.25m) and a height equal to that of the water column (h=7$\pm$1m) ($\pi x r^2 x h = 1{,}374\ m^3$); the filtered particulate fraction ($\geq 20\mu m$) was collected in a $0.2m^3$ cod-end, giving a concentration factor of 6,872x. The number of cells in a 0.1ml Palmer-Maloney chamber was counted under an optical microscope (250X) and multiplied by the concentration factor to estimate the number of cells per litre in the water column.

b. Sampling Strategy

i. Mussel sampling frequency

A strategy was developed to optimize the sampling effort and to ensure that the frequency of sampling was highest during the more critical stages of the experiment. Two considerations were used in this determination. One was the dinoflagellate density in the water column and the other, the concentration of toxin in mussel samples analyzed from the previous interval. The initial sampling frequency of once a week was doubled when the cell counts rose to 50 cells l^{-1} or when the toxicity of the most recent sampled mussels surpassed 40μgSTXeq $100g^{-1}$ tissue. Sampling was increased to three times a

week when the *Alexandrium* cell count exceeded 5000 cells l^{-1} or when the toxicity exceeded 80μgSTXeq 100g^{-1} tissue. This sampling frequency was continued for two weeks beyond the time when the cell counts or the toxicity fell below these arbitrary levels.

An analysis of *Alexandrium* cell counts and toxicity data showed that two blooms occurred during the course of the mussel transplant experiment in 1990. In accordance with the above sampling strategy, mussels were sub-sampled three times a week during, and for the two weeks following, each of the blooms (bloom I, post-bloom I, bloom II and post-bloom II). Over the four week period separating the blooms (inter-bloom), and for the last six weeks of the experiment (end phase), the sampling frequency was maintained at twice a week. A total of 45 sub-samples were obtained from each group.

ii. Phytoplankton samples

In addition to the samples collected for the crude cell density estimates, vertical net-tow samples were collected from the immediate vicinity of the cages and used for two purposes: to determine the relative density of *Alexandrium* cells and to concentrate them for chemical analysis of their toxin composition.

For the determination of relative phytoplankton species composition, 20mls samples of the net-tow material were collected weekly and preserved in 4.5% formalin. The dominant species composition was determined microscopically by calculating the ratio of each species to the total phytoplankton abundance in a sub-sample of 200 phytoplankton cells selected at random. Dominant species were defined arbitrarily as

constituting at least 40% of the total identifiable phytoplankton counts in the $\geq 20\mu$ mesh fraction. The remainder of the net tow sample was used for HPLC analysis of PSP toxins. The net-tow sampling frequency was adjusted according to the concentration of *Alexandrium* cells in the water column. Once the *Alexandrium* cell concentration rose to 1000 cells l^{-1}, net-tow samples were collected at the same frequency as the mussel samples and used similarly for HPLC analysis. For toxin analysis, the phytoplankton samples were passed sequentially through a series of 150, 73 and 20μ mesh sieves to obtain an *Alexandrium*-enriched cell fraction retained on the finest mesh. This fraction was transferred to a 20ml scintillation vial. A 2ml sub-sample of cell concentrate was diluted (1:10) by adding 2mls of 40% formalin and 16mls of filtered seawater (0.2μ). The *Alexandrium* cell density in this fraction was counted in a Palmer-Maloney chamber (0.1ml) under a phase-contrast microscope (200x). Five replicate counts were averaged to determine the total number of *Alexandrium* cells in each sample used for HPLC analysis (Table 1 in Annex I).

iii. Water samples

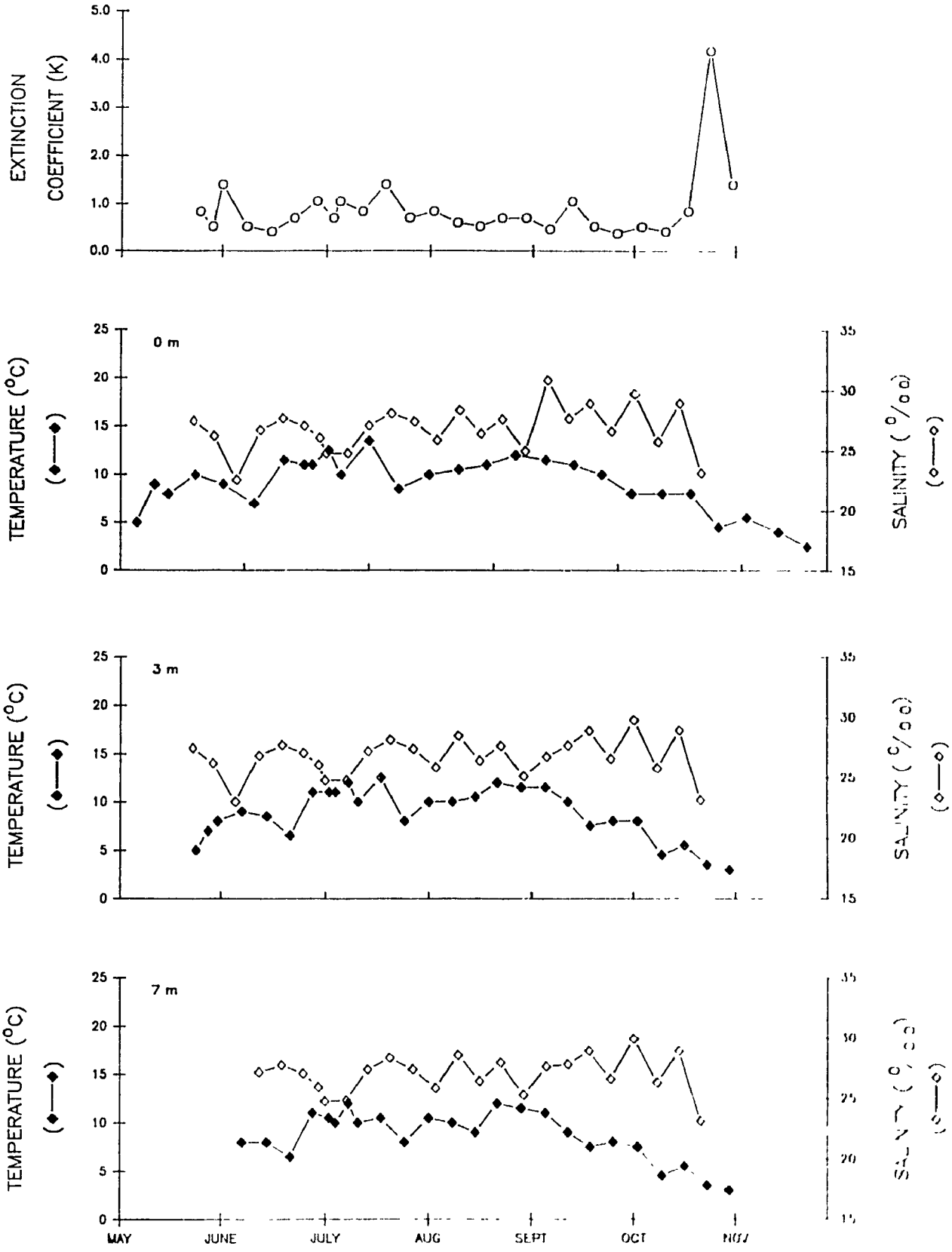
Water samples were collected in Niskin bottles at three depths (0, 3, and 7m). *Alexandrium* cell counts at each depth interval were obtained by sedimenting a 200ml sub-sample in a graduated cylinder for 4 days, then removing the top 180ml of supernatant seawater using a Pasteur pipette attached to a vacuum system. The remaining 20ml sedimented fraction (10:1 concentration) was vortex-mixed to re-suspend the cells. A 5ml inverted microscope sedimentation chamber was filled and allowed to

settle for 12 hours. When cell counts were low, the total phytoplankton content of the chamber was counted; when high cell concentrations occurred, the count was limited to an area of $1cm^2$, corresponding to a 1.02ml volume.

Various other water column variables were also determined weekly from samples collected in Niskin bottles at the site of the experiment. Water temperature was recorded for each of the three depths following a one minute period for equilibration of the thermometer in the Niskin bottle. A 200ml aliquot was transferred to salinity bottles and the salinity was measured subsequently using a "Guildine Autosal 8400" salinometer. This device measures the conductivity of a sample and compares it to the conductivity of standards of predetermined salinity in accordance with the 1978 UNESCO practical salinity formula (Strickand and Parsons, 1972). The turbidity of the water column was measured using a Secchi disk. The extinction coefficient (K) for water column turbidity was calculated as 2.1/Secchi depth.

These values are presented in Figure 4. No differences were observed in temperature or in salinity among the three depths. A mean difference of 2°C was observed between the first and the second part of the season (including bloom I and bloom II respectively), but no significant differences in salinity were observed during this period of the experiment (Table 1 in Annex II).

Figure 4: Measurements of water turbidity as given by the extinction coefficient (K) and of water temperature (°C) and salinity (°/$_{oo}$) at 0, 3, and 7 metres at the Pointe-aux-Cenelles experiment site.


EXTINCTION COEFFICIENT (K)
5.0
4.0
3.0
2.0
1.0
0.0
0 m
3 m
7 m
TEMPERATURE (°C)
(◆——◆)
SALINITY (°/oo)
(◇——◇)
25
20
15
10
5
0
35
30
25
20
15
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV

B. TOXIN EXTRACTION

1. Mussels

In the 1987 experiment, for each sampling date and site, five mussels were collected and excised soft-tissue was weighed individually. De-ionised water was added to the tissue of each individual mussel in a proportion of one ml g^{-1}. Each sample was homogenized separately using a tissue homogenizer (IKA Labortechnik Ultraturrax) until a viscous suspension was obtained. Five mls of homogenate were removed for purposes not related to this experiment. A volume of 0.2N acetic acid equivalent to that of the de-ionized water was added to the remainder of the suspension. The preparation was again homogenized. Five mls of this homogenate were stored at -20°C until further treatment.

For the mussel transplant experiment, four mussels were removed from each cage at each sampling interval and stored whole at -80°C until dissected (within two weeks). Dissection involved the removal of the digestive gland-stomach complex of each of the four mussels. These tissues were combined and treated subsequently as a composite sample. Although mitigated by the requirements to detect trace levels of PSP and limit the number of samples analyzed, the latter step is recognized now by the author as affecting the statistical inferences from the experiment since the combined samples cannot be regarded as true individual replicates. The rest of the soft tissues were discarded except for the last month of the experiment. During this latter period, the remaining tissues of the four mussels of each sub-sample were combined and analyzed for their toxins separately from the digestive glands. The height and length of the shells were

measured using a Vernier micrometer. The combined wet weight of the four digestive glands, and the wet weight of the residual (in the end phase) tissues were recorded. To each sample, a volume of 0.1N acetic acid equivalent to the weight of the sample was added and the mixture was homogenized. A 5ml volume of the homogenate was stored at -20°C until further treatment.

The homogenates were subsequently thawed and centrifuged in a high-speed centrifuge at 3,000xg for 20 minutes at 4°C. This treatment resulted in a clarified supernatant. A 0.5ml volume of supernatant was transferred to an ultrafiltration cartridge (Millipore 10,000 NMWL PLGC Ultrafree MC) and spun at 2,000xg for 50 mins at 4°C to obtain at least 0.18ml of filtrate. In the case of the mussels collected *in situ* in 1987 at TP, MT and CC, equal volumes of filtrate from three individual mussels per sampling date were combined for analysis, whereas filtrates from the other two individuals were analyzed separately. This was done to investigate the variation in toxin concentration among individuals collected on the same date from the same site. The filtrates were transferred to 0.1ml HPLC autosampler vial inserts, and stored at -20°C prior to injection into the HPLC.

2. Toxic Phytoplankton samples

Sixteen mls of the *Alexandrium*-enriched cell fraction collected from net tows (20μ) was filtered under low vacuum through a 25mm Whatman GF/F glass-fibre filter to dryness. The cell mat on the filter was scraped and the cells were transferred to a 1.5ml plastic vial and stored at -20°C. To extract the toxins from the cells, 1ml of

0.03N acetic acid was added to each vial. While in an ice bath, the mixtures were subjected to ultrasonication in six bursts totalling one minute, using an ultrasonicator equipped with a microprobe. Subsequently, the sample was centrifuged at 2,000xg for 10 minutes at 4°C. Each sample was then filtered through a syringe-mounted 0.45μ HA Millipore nitrocellulose membrane, and transferred to individual HPLC autosampler vials prior to analysis.

C. HPLC ANALYSIS

1. Toxin separation and detection

Separation of PSP toxin components from the mussel and phytoplankton samples was achieved by reverse-phase ion-pair HPLC. The equipment consisted of a Varian 5000 Liquid Chromatograph coupled with a Varian 9090 autosampler, a Kratos PCRS-520 post-column reaction module, a Perkin-Elmer LS-4 fluorescence spectrophotometer and a Waters Baseline® computerized data integrator. Ten μl of toxin extract were sampled automatically and injected onto a polystyrene divinylbenzene resin column (Hamilton PRP-1, 10μ particle size; 4.1x150mm i.d.). The analytical column, equipped with a PRP-1 resin (10-20μ particle size) pre-column cartridge, was maintained at 35°C in the post-column reactor. Toxin separation was effected by binary gradient elution, with heptane and hexane sulfonate in mobile phases A and B serving as ion-pair reagents (Table III). A post-column oxidation reaction rendered the products detectable by fluorescence (excitation:340nm; emission:400nm bandwidth$\pm$10nm), according to the method of Sullivan and Wekell (1986). The reaction involved the post-column mixing

of an oxidant (periodic acid) with the column eluent, followed by heating at 90°C in a 1ml Tefzel reaction coil, and continuous neutralization of the mixture with nitric acid (0.75N) prior to its detection by fluorescence. A dual-head Eldex Slo Syn SS80 piston pump was used to deliver the oxidant (0.3-0.5ml min^{-1}) and the acid (0.1-0.3ml min^{-1}).

Table III: Composition of the mobile phases and the post-column reaction reagents

Solution	Chemical Composition	pH (±0.02)
Phase A	1.5mM ammonium phosphate buffer 1.5mM hexane sulfonate 1.5mM heptane sulfonate	6.70
Phase B	6.25mM ammonium phosphate buffer 1.5mM hexane sulfonate 1.5mM heptane sulfonate 25% vol. acetonitrile	7.00
Oxidant	5mM periodic acid 100mM sodium phosphate buffer	7.80

2. Toxin identification and measurement

Individual PSP toxin components (Fig. 2b) in the samples were identified by comparing their retention times with those of standard reference toxins. Comparison of peak areas between unknowns and standards provided a quantitative measure of each toxin component. PSP toxin secondary standards for routine chromatography were prepared from purified dinoflagellate extracts and calibrated to a primary standard (MS-33; J. Sullivan, U.S.F.D.A., Seattle, WA.) (Table IV).

Table IV: Secondary standard (MSPR-3 and MSPR-5) nominal retention times (mins), toxin concentration (μM) and HPLC detection limit of PSP toxin analogues.

Toxin	R.T. (mins)	MSPR-3 [μM]	MSPR-5 [μM]	Limit (μM)**
Cx*	2.0 ± 0.5	8.54	24.60	0.01
GTX4	7.3 ± 1	1.99	8.87	0.02
GTX1	9.4 ± 1	2.58	22.13	0.03
GTX3	10.7 ± 0.5	2.91	3.14	0.002
GTX2	11.8 ± 1	3.75	1.53	0.005
neoSTX	15.9 ± 0.5	24.27	19.08	0.02
STX	16.9 ± 0.5	7.13	17.70	0.02

* represents an epimeric mixture of toxins C_1 and C_2.
** defined as two times the maximum baseline noise; 10μl injection.

A Waters Millipore Baseline® 810 chromatography workstation integrator was linked to an NEC PowerMate2 APV IV computer programmed to convert the toxin peak areas into concentration units. The molar concentrations (μM) of the toxins were converted to toxin concentrations (nmol g^{-1} for mussel tissue or fmol $cell^{-1}$ for *Alexandrium*) and to toxicity units (μgSTXeq $100g^{-1}$ for mussels and pgSTXeq $cell^{-1}$ for *Alexandrium*) using the published toxin-specific conversion factors of mouse units (MU) to μgSTXeq (Table V) (Boyer et al., 1986), assuming 0.18μgSTXeq per MU (Schantz et al., 1958).

Table V: Toxin conversion factor used to convert the concentration of individual toxins (μM) to toxicity units (μgSTXeq μmol^{-1}) (from Sullivan and Wekell, 1986).

Toxin	Conversion factor
Cx	47
GTX4	346
GTX1	346
GTX3	291
GTX2	182
neoSTX	364
STX	364

The total concentration (nmol g^{-1}) of toxin in the mussels was calculated by multiplying the analyzed molar concentrations (μM) by the toxin extraction volume (ml) and by the reciprocal of the wet weight of the tissue (g) (1). The toxicity (μgSTXeq g^{-1}) was obtained by multiplying the total concentration value by the toxicity conversion factor.

$$\text{nmol g}^{-1} = \mu\text{M} \times \text{extraction vol.(L)/tissue weight (g)} \quad \ldots\ldots\ldots\ldots \quad (1)$$

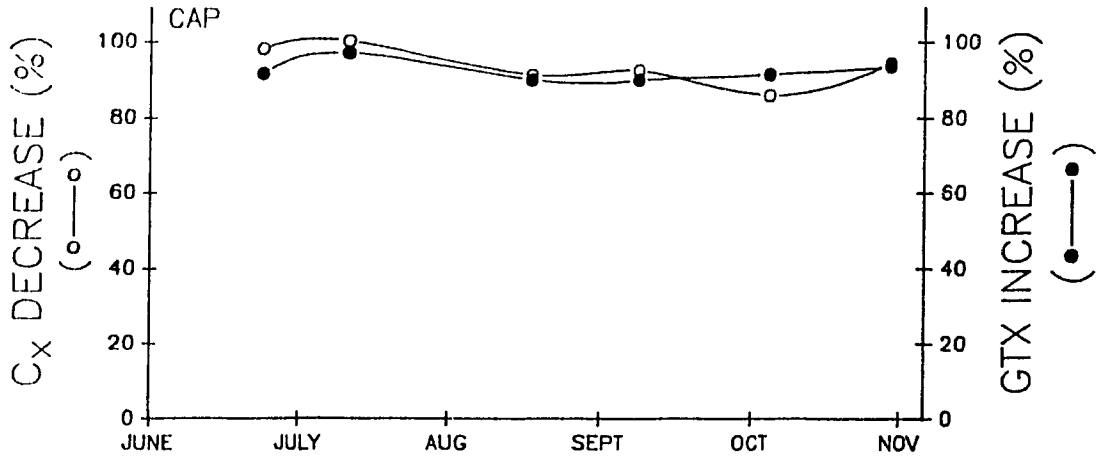
$$\mu\text{gSTXeq g}^{-1} = \text{nmol g}^{-1} \times \text{conversion factor (F) } (\mu\text{gSTXeq } \mu\text{mol}^{-1}) \quad \ldots\ldots \quad (2)$$

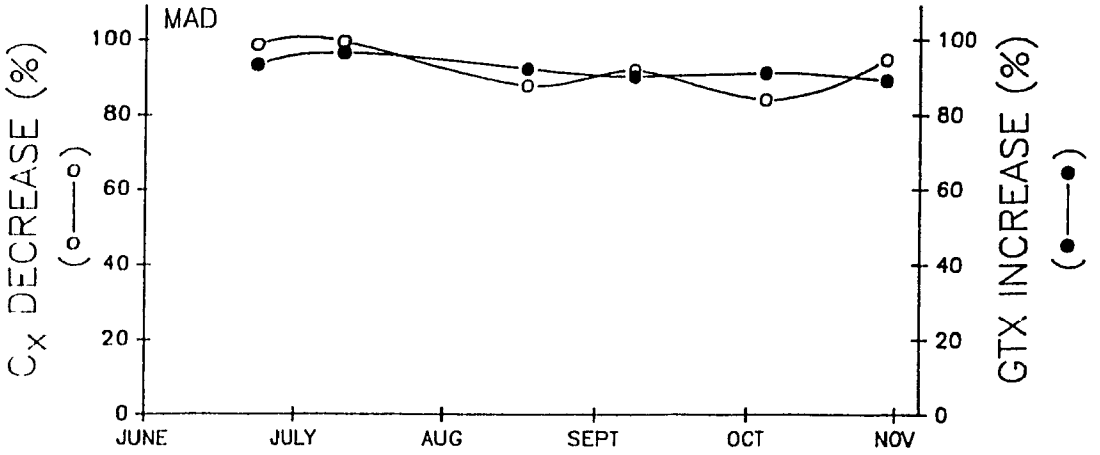
3. Toxin hydrolysis

In order to correct for co-eluting fluorescent artifacts which might interfere with the determination of C-toxins, samples were hydrolysed 0.4N HCl in a 1:1 volume of acid of sample extract for 10 minutes at 100°C. This treatment converts the labile N-sulfocarbamoyl (C_x) toxins to their respective gonyautoxin analogues (Fig. 2b) (Proctor

et al., 1975). Weak acid hydrolysis of the mussel extracts from the CC, MT and TP stations showed that C-toxin concentrations may be overestimated from unhydrolyzed samples due to fluorescent artifacts from peak areas, resulting in a maximum total error in toxicity (μgSTXeq g^{-1}) of 5.1% for CC mussels, 9.0% for MT mussels and 21.4% for TP mussels. Thus, in the transplant experiment, six representative samples were chosen from CAP and MAD mussels at different times throughout the experiment and hydrolysed to calculate a correction factor. A sample was taken immediately preceding the onset of toxin peaks in mussels. Also, a sample was taken at the peak toxin concentrations during each bloom. An additional two samples were used as representative of the end phase where the toxin concentration in each of the two populations remained unchanged. Figure 5 shows the percentage decrease in C_x and corresponding increase in the concentration of GTX_{2+3} following hot acid hydrolysis for the dates selected (abscissa). Concentrations of C-toxin samples were extrapolated using this graph. Reported data herein represent the corrected values.

Figure 5: Percentage decrease in C-toxins (C_3) and percentage increase in Gonyautoxins 2 and 3 (GTX) in mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD).


CAP
C_X DECREASE (%)
(o——o)
GTX INCREASE (%)
(●——●)
0
20
40
60
80
100
JUNE
JULY
AUG
SEPT
OCT
NOV


MAD
C_X DECREASE (%)
(o——o)
GTX INCREASE (%)
(●——●)
0
20
40
60
80
100
JUNE
JULY
AUG
SEPT
OCT
NOV

D. STATISTICAL ANALYSIS

All statistical analyses were performed through statistical software: Statgraphics[R] V.5, Statistical Graphics Corp. or a spreadsheet software: Lotus 123[R] Development Corp. For the purpose of this study, statistical significance was based on probabilities of $p \leq 0.05$ unless otherwise stated.

1. Variances within and among the populations

Toxin concentrations were determined from individual samples as well as from pooled ($n=3$) samples from the mussels collected in CC, MT and TP, in 1987. The data for each toxin were subjected to a one-way analysis of variance (ANOVA) to detect differences in the variances in the levels of individual toxins between the three populations. The level of significance for the F-ratio, given by the *p*-value and the Sum of Squares (SS) and the Mean Square (MS) values are presented with the results. The coefficient of variation (CV) for each variable was calculated as the ratio of the square root of the standard deviation to the toxin mean ($\sqrt{s}/x$).

2. Comparative testing

A Student's T-test of the difference between the mean concentrations (nmol g^{-1}) of individual toxins and total concentration and toxicity (μgSTXeq $100g^{-1}$ tissue) as well as the mean relative molar (%Molar) toxin concentrations in CC, MT and TP mussels was carried out.

Two sample Kolmogorov-Smirnov (K-S) tests were performed on the individual toxin concentrations as well as on the total toxin concentration (nmol g^{-1}) and on the total toxicity of mussels (μgSTXeq g^{-1}) to compare the distribution of the toxin concentrations of the populations, where DN represents the maximum absolute deviation between the two cumulative distribution functions. A pair-wise comparison of toxin concentration of CC, MT and TP mussels before and during the peak toxin concentration was performed as well. Toxin concentration in the digestive glands of CAP and MAD mussels were compared during each of the bloom phases. Differences between the two blooms were also determined by the comparing toxin concentrations in CAP and MAD mussels and in the *Alexandrium* cells during the first and the second bloom. Differences in toxin concentration were established between the two tissue fractions in each of the two mussel groups during the end phase.

Discriminant analysis was carried out to obtain a function of the concentration (nmol g^{-1}) of the individual toxins of CC, MT and TP mussels prior to (n=6) and during (n=9) the peak in toxin concentration in the mussels. In addition, toxin concentrations in CAP and MAD mussels were compared during the different phases of the experiment, including the non-visceral tissue fraction in the end phase, to determine the most significant toxin discriminating between the groups during each phase. The toxin composition data for the toxic phytoplankton cells were also subjected to discriminant analysis, in order to identify which toxins differentiated between the two blooms.

A Wilcoxon Signed-Ranks test comparing the medians of the arcsine transformed relative molar toxin composition in the digestive glands of CAP and MAD mussels was

carried out for each phase of the experiment, and for the different tissue fractions in the end phase. Furthermore, the relative molar toxin composition in the two mussel groups were compared with those in the *Alexandrium* cells. The Z-value gives the average rank of differences about the medians.

3. Toxin kinetics

The kinetics of toxin accumulation and elimination in the mussels were described through linear regression analyses of the natural logarithmical transformed toxin concentrations as a function of time. The accumulation phase was defined as commencing at the lowest toxin concentration preceding an increase (t=0) and ending at the peak toxin concentration during the bloom (t=N). The elimination phase was defined as the time period between the peak toxin concentration during the bloom (t=0) and the point at which the rate of decrease of the concentration levelled off (t=N), i.e., did not decay exponentially. The probability of an event (accumulation or elimination) being significant was based on the ratio of the slope of the regression line to the standard error of the slope, giving a specific Student's T-test value, i.e., a comparison of the slope of either accumulation or elimination to a slope of 0, i.e., where no net accumulation or elimination occurred over time.

A Student's T-test was used to compare the toxin kinetics in CAP and MAD mussels during each of the two blooms. It was based on a comparison of the slopes of either accumulation or elimination of toxin as a function of time. Toxin elimination in CAP and MAD mussels was further examined through the regression analysis of the

relative amount of total toxin remaining in the digestive gland (up to about 15% of the maximum) as a function of time. In both cases, the rates of toxin elimination following each bloom were compared between the two populations and between the two blooms for each population.

4. Putative biotransformation

Differences in toxin composition which may be attributable to biotransformation and/or toxin specific retention, were determined by observing the changes in toxin pair ratios. The ratios of epimeric pairs GTX_4:GTX_1 and GTX_3:GTX_2 as well as neoSTX:STX were calculated. Regression equations representing shifts in these ratios during the accumulation and elimination phases for both blooms, as well as during the inter-bloom period were computed. The probabilities of the toxin ratios changing over time were based on a comparison of the slope of the regression line to zero.

RESULTS

I. Field study of *in situ* mussel populations (1987)

1. Total PSP toxin concentration and toxicity

Toxin levels in mussels collected from TP, MT and CC in 1987, were highest in the latter two populations (Fig. 6) (Tables 2, 3 and 4 in Annex I). The mean toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$) of CC mussels over the 15 week sampling period were not significantly different from that of MT mussels, but TP mussels were found to be less toxic than the other two groups (Table 2 in Annex II). In 1987, the mean toxin concentration of CC mussels throughout the season was 21.3nmol g^{-1} (S.E.=23.5 n=15) with a corresponding mean toxicity of 369μgSTXeq $100g^{-1}$, and a peak of 1821μgSTXeq $100g^{-1}$ tissue. The mean PSP toxin concentration at MT was 14.5nmol g^{-1} (S.E.=14.3 n=15) equivalent to a mean toxicity of 248μgSTXeq $100g^{-1}$; the peak toxicity was found to be 1372μgSTXeq $100g^{-1}$ tissue. In contrast, the average toxin concentration of the TP mussels throughout the season was only 6.4nmol g^{-1} (S.E.=4.16 n=15), giving a mean toxicity of 67μgSTXeq $100g^{-1}$, and a peak toxicity of 209μgSTXeq $100g^{-1}$ tissue.

To examine the differences in the concentration (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$ tissue) of PSP toxins among the three sites, a multi-factor ANOVA was performed. The variance in the toxin concentrations between the different populations were significantly greater than those within each population for all toxins except GTX_4 ($p \leq 0.10$) (Table 3 in Annex II). The mean coefficient of variation was calculated for the mussels at the three sites (Table VI)

Figure 6: Total PSP toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$ tissue) of mussels collected from the inter-tidal zone at Trois Pistoles, Metis and Cap Chat from June to September 1987.

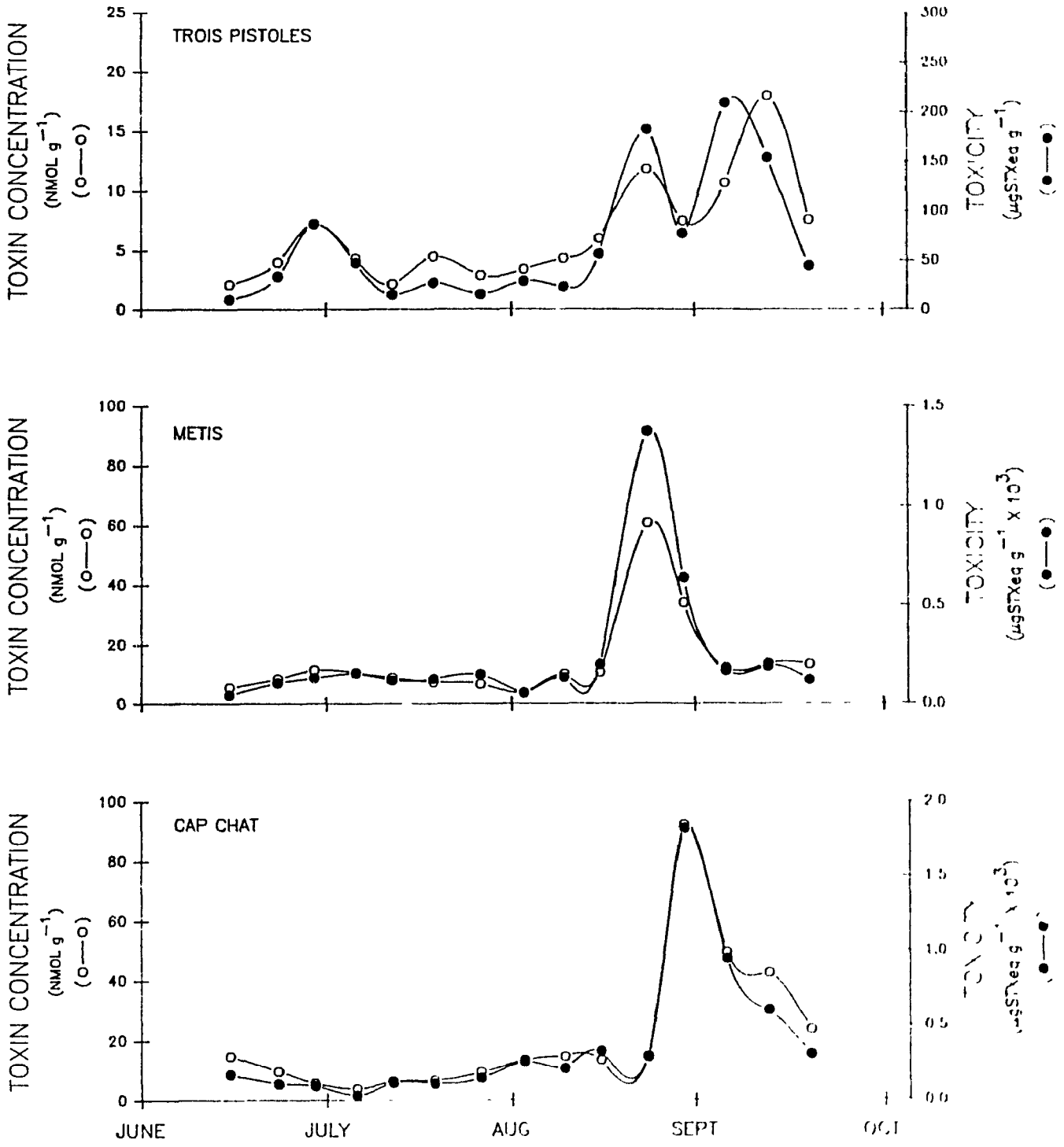

TROIS PISTOLES
METIS
CAP CHAT
TOXIN CONCENTRATION
(NMOL g^{-1})
(o—o)
TOXICITY
(μgSTXeq g^{-1})
(●—●)
TOXICITY
(μgSTXeq g^{-1} x 10^{3})
JUNE
JULY
AUG
SEPT
OCT

Table VI: Coefficient of variation (CV) in toxin concentration (nmol g^{-1}) for the three mussel populations.

Toxin	CV
Cx	78.27
GTX4	158.34
GTX1	351.81
GTX3	147.19
GTX2	198.39
neoSTX	150.16
STX	159.89
Total	97.67
Toxicity (μgSTXeq $100g^{-1}$)	124.49

In order to determine whether the variation in toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$) among the mussels was greater than that within each group, a one-way ANOVA was carried out on the individual mussels (specimens 4 and 5) in parallel to the pooled mussels (specimens 1,2 and 3) for each population. The F-ratios of these analyses were not significant, i.e., the differences within each of the groups were not greater than the differences between the three populations, except for GTX_1 and neoSTX in MT and for neoSTX in TP -these toxins were found only in trace levels in the mussels (Table 4 in Annex II).

No significant differences were observed in the concentration of PSP toxins between CC and MT mussels, except for GTX_4, which was found in higher concentrations in CC mussels during the peak in toxicity. The total toxin level (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$) were also similar in CC and MT mussels prior to and during

during the peak in toxicity. However, the test revealed that the total toxin concentration and toxicity of MT and TP mussels were different prior to, but not during, the peak in toxicity. The overall toxin concentration and toxicity of TP mussels was different from that of CC mussels both prior to and during the peak toxicity (Table 5 in Annex II).

2. Bioassay and HPLC correlations

The correlation between toxicity determined by bioassay and HPLC was high (Pearson correlation coefficient, $r^2=0.93$, $p=0.00$), but toxicity was consistently higher when determined directly by mouse bioassay than when calculated from HPLC data using toxin-specific conversion factors, as shown by the displacement of the curve from a theoretical slope of 1 (Fig. 7).

3. Variation in PSP toxin concentration

The relative molar composition of toxins in mussels from TP, MT and CC are reported in Figures 8a, b and c respectively. The composition of all toxins except GTX_4 prior to the peak and GTX_4 and STX during the peak in toxicity were significantly different between CC and TP mussels. Metis and TP mussels had a similar toxin composition prior to and during the peak in toxicity, except for GTX_1 and neoSTX which were found only in slight amounts in TP mussel samples, both prior to and during the peak. Cap Chat and MT mussels contained similar proportions of all toxins (Table 6 in Annex II).

Figure 7: Correlation of bioassay and HPLC toxicity results (μgSTXeq $100g^{-1}$ tissue) for mussels collected from Cap Chat in the summer of 1987. Pearson correlation coefficient (r^2) and fitted linear relationship for the correlation are compared to an ideal 1:1 slope.

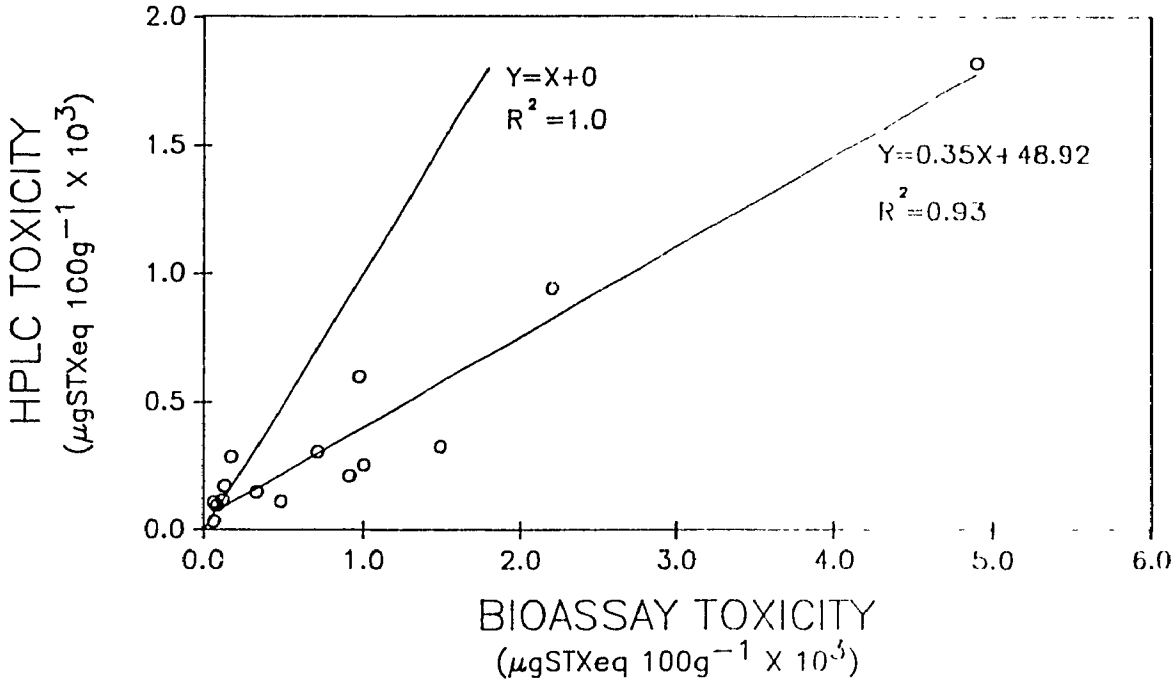
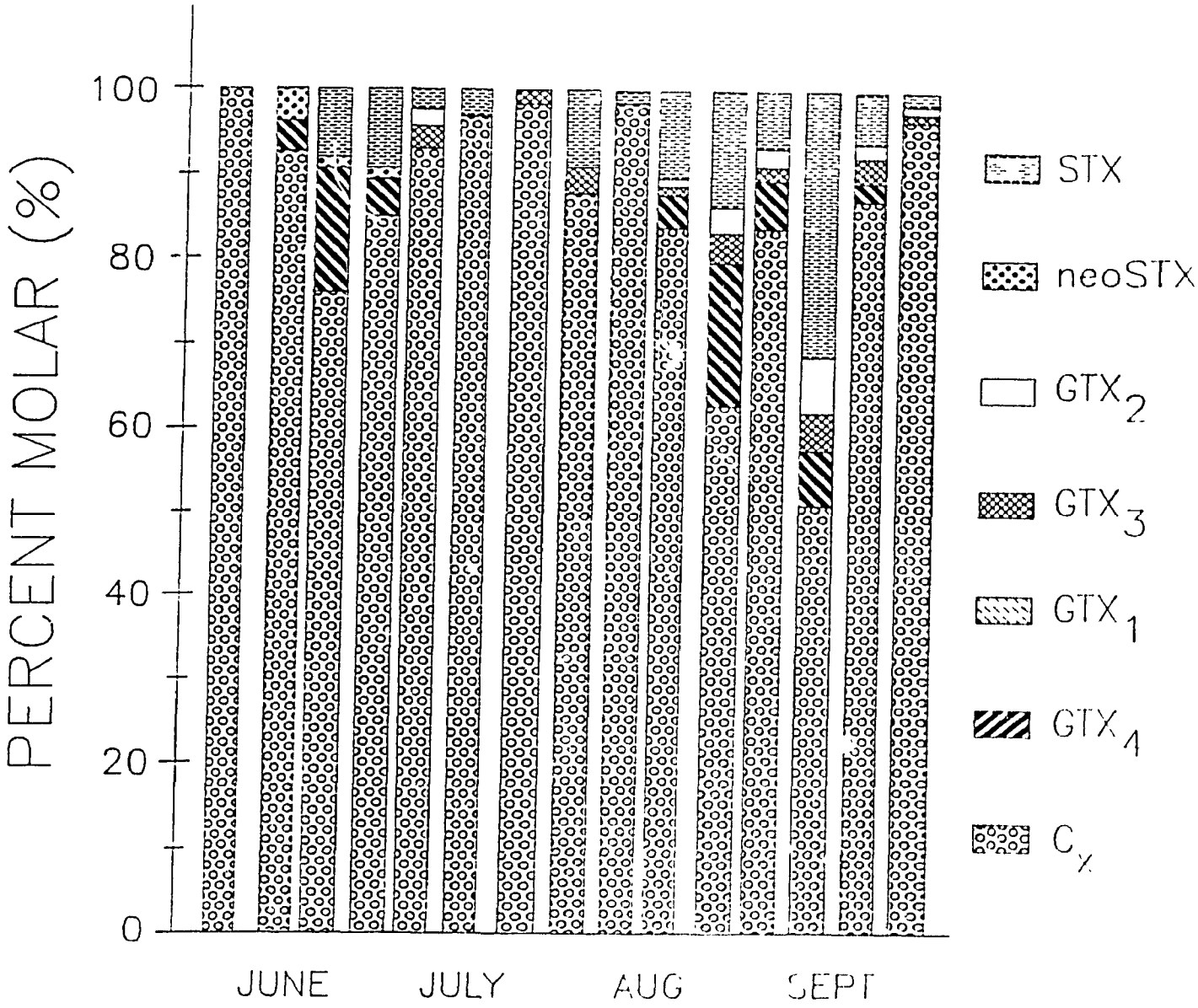
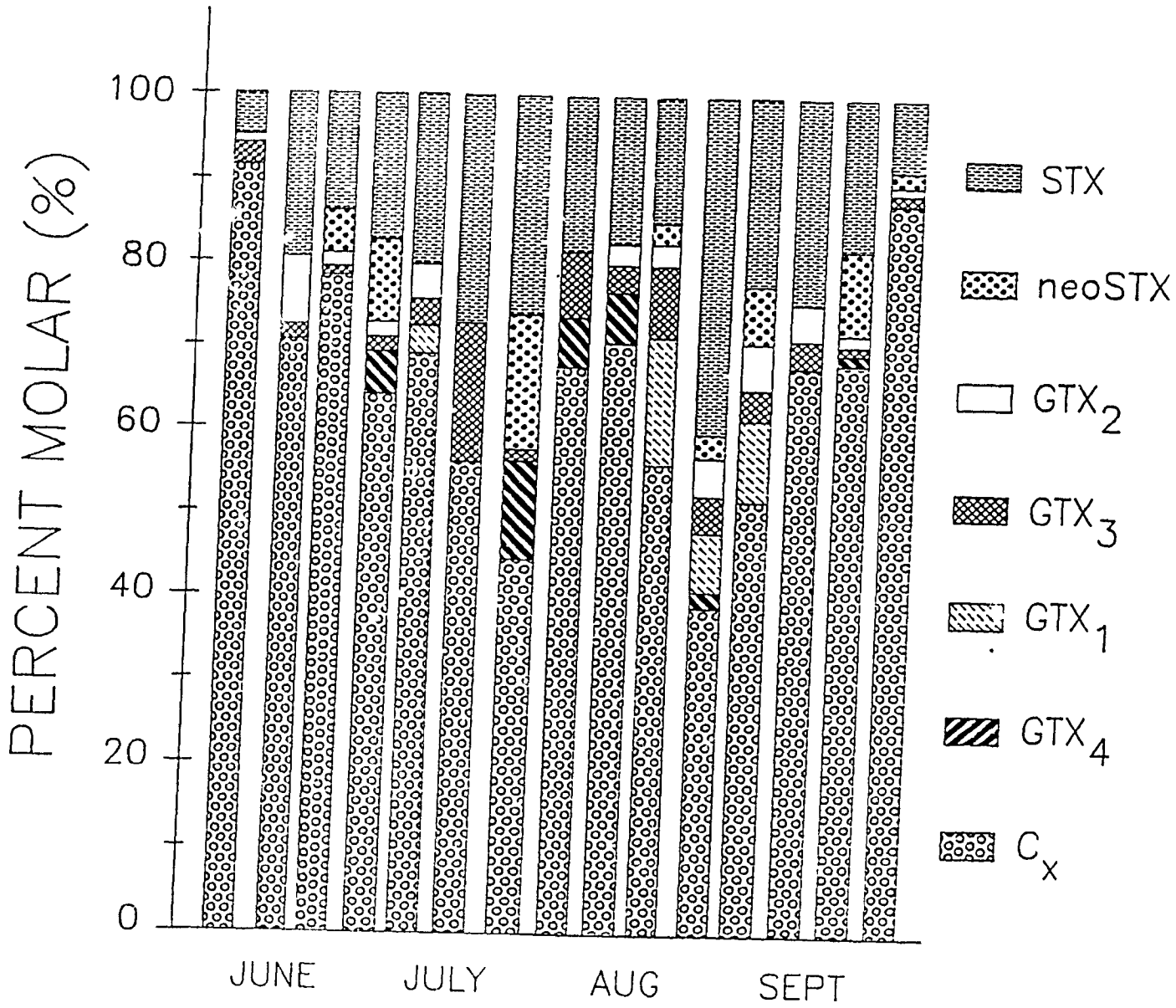

2.0
1.5
1.0
0.5
0.0
HPLC TOXICITY
(μgSTXeq 100g⁻¹ X 10³)
Y=X+0
R² =1.0
Y=0.35X+ 48.92
R² =0.93
0.0
1.0
2.0
3.0
4.0
5.0
6.0
BIOASSAY TOXICITY
(μgSTXeq 100g⁻¹ X 10³)

Figure 8: Relative composition of PSP toxins in the mussels collected from the inter-tidal zone at a. Trois Pistoles, b. Metis and c. Cap Chat from June through September 1987.

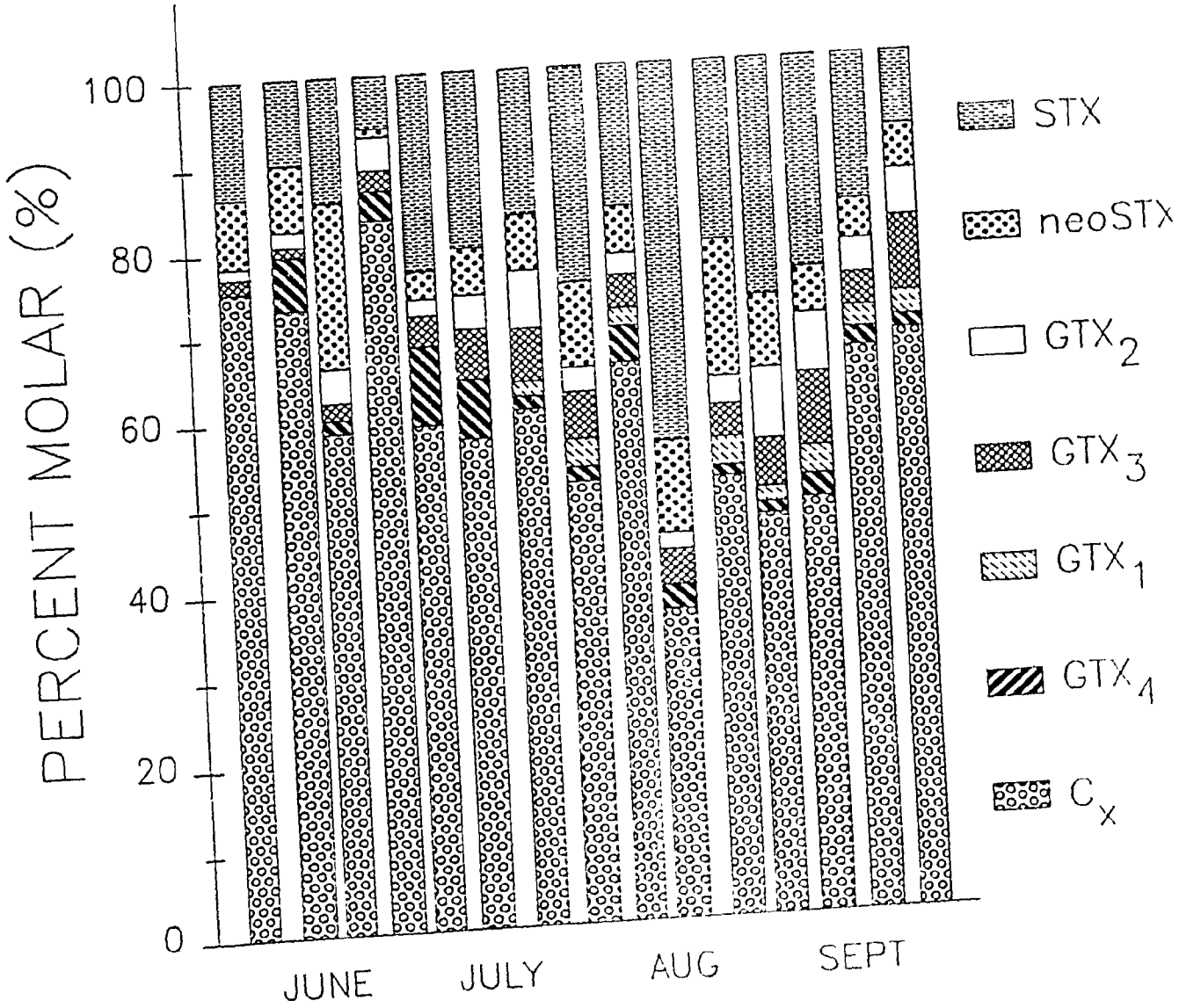
a. Trois Pistoles



b. Mells


PERCENT MOLAR (%)
100
80
60
40
20
0
JUNE
JULY
AUG
SEPT
STX
neoSTX
GTX2
GTX3
GTX1
GTX4
Cx

e. Cap Chat


PERCENT MOLAR (%)
100
80
60
40
20
0
JUNE
JULY
AUG
SEPT
STX
neoSTX
GTX2
GTX3
GTX1
GTX4
Cx

4. Mussel weights

The soft tissue wet weights of mussels collected from the inter-tidal zone at CC, MT and TP in 1987 and averaged over the sampling period are presented in Table 5 (Annex I). According to a Student's T-test, MT mussels weighed significantly more than TP mussels, which in turn weighed more than CC mussels (Table 7 in Annex II).

II. Simultaneous mussel transplantation experiment (1990)

A. *Alexandrium* cells

During the second phase of the project, two blooms of *Alexandrium excavatum* were observed. The first bloom peaked at 1.7×10^5 *Alexandrium* cells L^{-1} and occurred in late June-early July, whereas the peak cell concentration in the second bloom, which occurred in late August, was approximately an order of magnitude less (Fig. 9a) (Table 6 in Annex I). The dominant phytoplankton genera in the 20μ net tow fraction were found to be the toxic *Alexandrium* and the diatoms *Thalassiosira*, *Chaetoceros* and *Skeletonema* (Table 7 in Annex I). At the start of the second bloom, their ratio still constituted an important fraction of the available food in the water, but decreased sporadically so that, towards the end of the bloom, they represented an insignificant share of the cell ratio counts in the water column. *Alexandrium* cells exhibited a mean toxicity of 3.3pgSTXeq $cell^{-1}$ (s.d. = 4.04, n=5) during the first bloom and 13.8pgSTXeq $cell^{-1}$ (s.d. = 7.88 n = 9) during the second bloom (Fig. 9b) (Table 8 in Annex I). The low potency N sulfocarbamoyl toxins C_1/C_2 and the high potency carbamate derivatives neoSTX and STX were the dominant analogues in *Alexandrium* (Fig. 10).

Figure 9a: Total concentration (nmol g^{-1}) of PSP toxins in the digestive glands of mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD) and *Alexandrium* cell density at 3m depth in the water column from June to November 1990.

Inset: Concentration for August to November 1990 at 10X magnification.

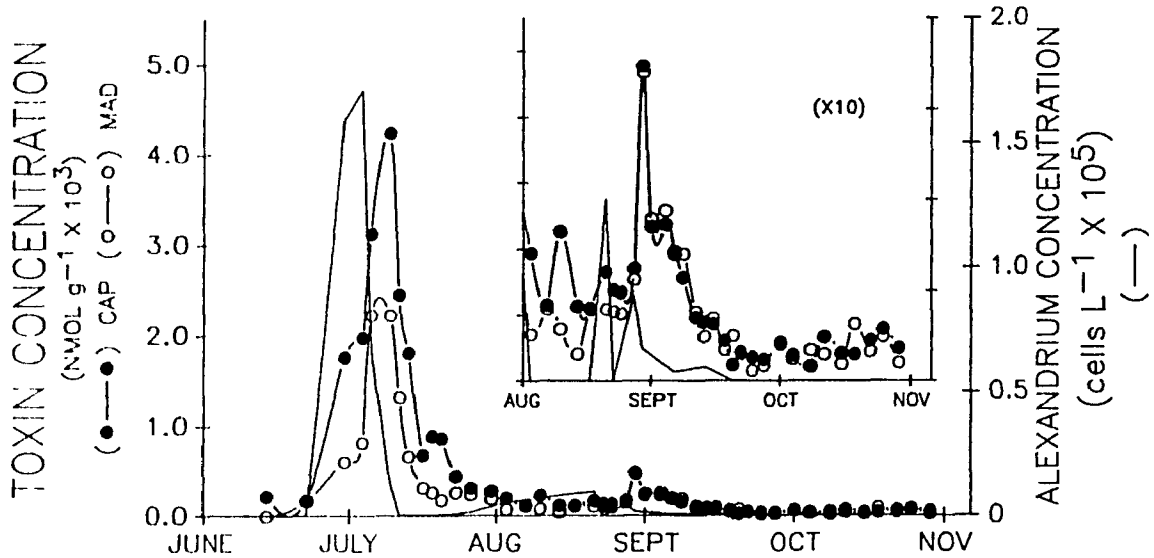

TOXIN CONCENTRATION
(NMOL g^{-1} X 10^3)
(●—●) CAP (o—o) MAD
5.0
4.0
3.0
2.0
1.0
0.0
JUNE
JULY
AUG
SEPT
OCT
NOV
(X10)
AUG
SEPT
OCT
NOV
2.0
1.5
1.0
0.5
0
ALEXANDRIUM CONCENTRATION
(cells L^{-1} X 10^5)
(—)

Figure 9b: Total toxicity in the digestive glands of mussels transplanted from Cap Chat (CAP) and from the Magdalen Islands (MAD) (μgSTXeq g^{-1}) and total PSP toxicity in the water (μgSTXeq L^{-1}) at 3m depth from June to November 1990.

Inset: Toxicity for August to November 1990 at 10X magnification.

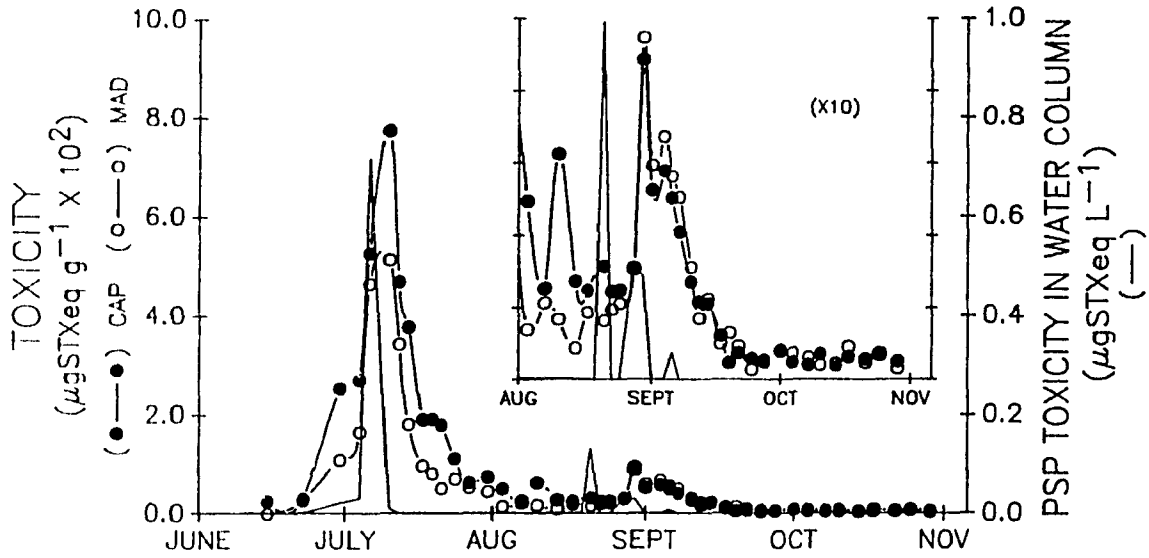
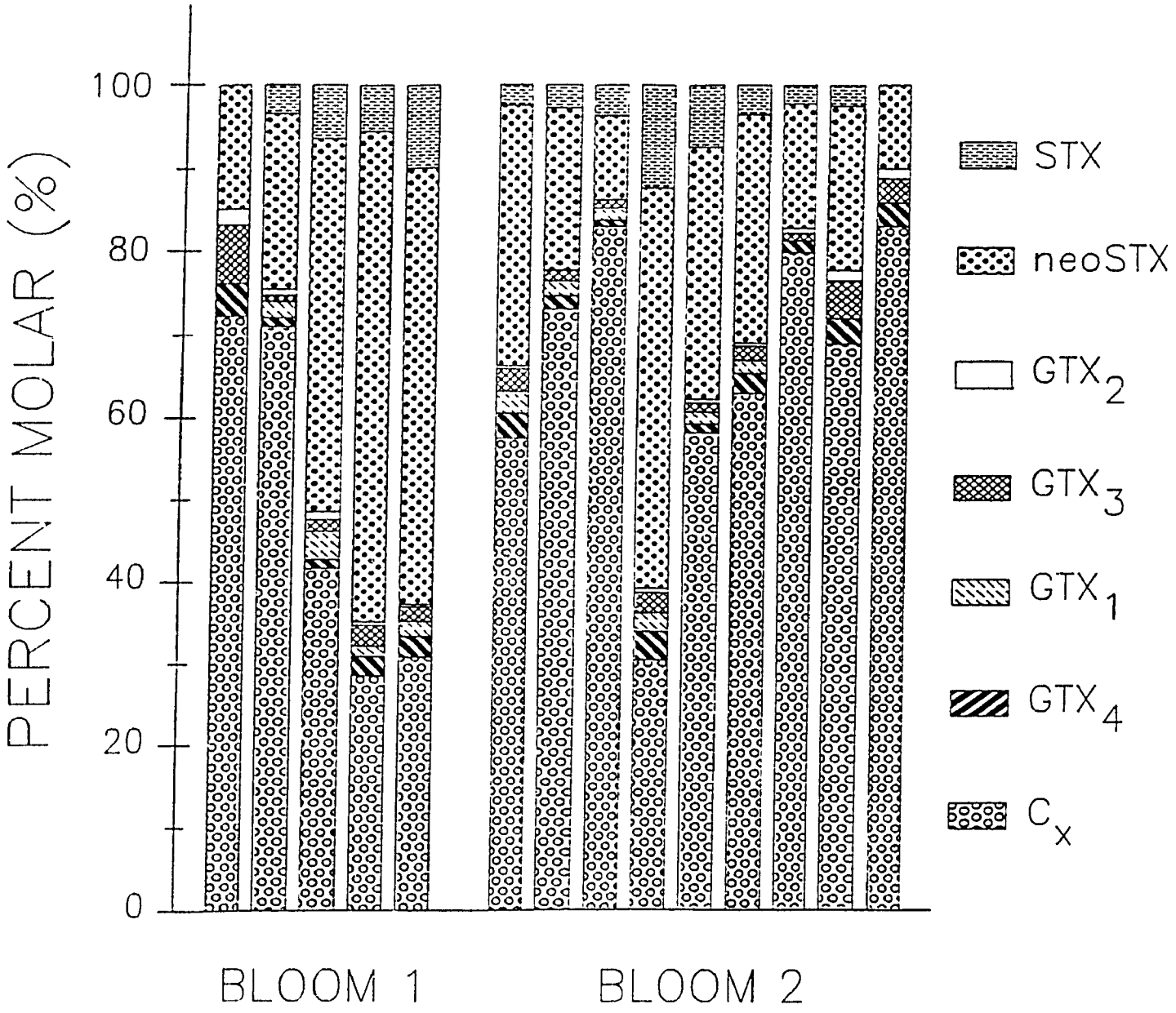

TOXICITY
(μgSTXeq g⁻¹ X 10²)
(●—●) CAP (o—o) MAD
10.0
8.0
6.0
4.0
2.0
0.0
JUNE
JULY
AUG
SEPT
OCT
NOV
(X10)
AUG
SEPT
OCT
NOV
1.0
0.8
0.6
0.4
0.2
0.0
PSP TOXICITY IN WATER COLUMN
(μgSTXeq L⁻¹)
(—)

Figure 10: Percent molar toxin composition of toxin in *Alexandrium* cells for each sample collected at each sampling interval during the two blooms at the experiment site.


PERCENT MOLAR (%)
100
80
60
40
20
0
BLOOM 1
BLOOM 2
STX
neoSTX
GTX2
GTX3
GTX1
GTX4
Cx

The concentration of each toxin analogue (fmol cell^{-1}) as well as the total toxin concentration and toxicity (pgSTXeq cell^{-1}) in the cells during each of the two blooms were compared; the concentration of GTX_1, GTX_3 and GTX_5 differed between the first and the second bloom (Table 8 in Annex II). However, there was no discrimination between the two blooms on basis of the toxin concentration (Table 9 in Annex II).

The relative concentration of the toxin analogues in *Alexandrium* cells were not significantly different between the two blooms (Table 10 in Annex II).

B. Mussels

1. PSP toxin accumulation and elimination

a. Toxin concentration and toxicity

Prior to the appearance of the first *Alexandrium* bloom, when ambient cell density was less than 100 cells L^{-1}, mussels transplanted from both the Magdalen Islands and Cap Chat accumulated low levels of PSP toxins (<300μSTXeq g^{-1} digestive gland) (Tables 9 and 10 in Annex I). During the first bloom, a delay of 6 days was observed between the maximum concentration of *Alexandrium* cells in the water column and the peak PSP toxin concentration in the digestive glands (Fig. 9a). CAP mussels exhibited a greater capacity to accumulate toxin than MAD mussels. When expressed as total PSP concentration (nmol g^{-1}), the difference was two-fold but when expressed as toxicity (μgSTXeq g^{-1}), CAP mussels were 1.5X more toxic than MAD mussels (Fig. 9b). A maximum toxin concentration of 4.23x10^{5}nmol g^{-1} digestive gland (7.73x10^{7}μgSTXeq g^{-1} digestive gland) was reached in CAP mussels during the first bloom, compared to

2.22×10^3nmol g^{-1} digestive gland ($=5.13 \times 10^2 \mu$gSTXeq g^{-1} digestive gland) in MAD mussels. During the second bloom, both mussel groups accumulated a similar toxin load at 4.76×10^3nmol g^{-1} and 4.67×10^2nmol g^{-1} for CAP and MAD, respectively. The peak toxicities reached in both mussel groups during the second bloom were also comparable at 88.50μgSTXeq g^{-1} and 94.53μgSTXeq g^{-1} digestive gland for CAP and MAD mussels respectively. A visual comparison of Figures 20a through g reveals that the concentration of C_3, GTX_1 and GTX_2 in CAP mussels was greater than that of MAD mussels during the first bloom, but only GTX_1 apparently differed between the two populations during the second bloom. All other analogues seemingly reached similar concentration levels in each populations during both blooms.

The concentration of C_3 differed between CAP and MAD mussels during the first bloom, but during the second bloom, all toxin analogues were found in equal concentrations. During post-bloom II, the concentration of GTX_4 was greater in MAD mussels than in CAP mussels. In the pre-bloom, post-bloom I and in the end phase, the concentrations of each toxin component, the combined toxin concentration (nmol g^{-1}) and total equivalent toxicity (μgSTXeq g^{-1} digestive gland) were not markedly different (Table 11 in Annex II). The concentration of toxins in CAP and MAD mussels were also compared between the two blooms (in the inter-bloom); the concentration of each toxin component and the total concentration and toxicity were found to be different (Table 8 in Annex II).

During and following the first bloom (in the inter-bloom phase), GTX_2 was the most discriminating factor between the two populations, whereas during and subsequent

to the second bloom (in the post-bloom II period), GTX_4 was the major discriminator. No significant function was observed in the end phase (Table 12 in Annex II).

The total toxicity of the mussels (μgSTXeq $100g^{-1}$ of mussel tissue), as expressed by the A.O.A.C. (1984) mouse bioassay, was estimated from the ratio of digestive gland weight of the mussels collected to total wet tissue weight of the mussels collected at the end phase of the experiment (Tables 11 and 12 in Annex I). The table of estimated total mussel weights can be found in the annex (Tables 13 and 14 in Annex I).

b. Kinetics of PSP toxin accumulation and elimination

The patterns of toxin accumulation and elimination in the digestive glands of CAP and MAD mussels for the two blooms are presented in Table 13 and 14a (Annex II). Not all the slopes of toxin accumulation during each bloom were significant, although the concentration of all toxins apparently increased during the initial phases of the two blooms (Fig. 20a-g in Appendix I). During the first bloom, the slope of the increase in GTX_1, GTX_2 and STX in CAP mussels was greater than zero, i.e., net accumulation occurred. In MAD mussels, a net accumulation of GTX_1, GTX_1, GTX_2, neoSTX and STX, as well as an increase in total toxicity was apparent. During the second *Alexandrium* bloom, MAD mussels showed an increase in total toxicity which was not observed in CAP mussels. Net toxin elimination from the digestive glands of the mussels from both populations started at the end of the first bloom, when *Alexandrium* cell numbers had plummeted to about 300 cells L^{-1}. During the immediate post bloom I period, when *Alexandrium* cell numbers decreased to <100 cells L^{-1}, a dramatic rapid

drop in the total toxin content in CAP and MAD mussels was observed. In both groups the total toxin load dropped to about 10% of the maximum within two weeks following the disappearance of the first *Alexandrium* bloom (during the inter-bloom period). The half life for total PSP toxin loss was 4.0 days for CAP mussels, but only 2.2 days for MAD mussels. During the inter-bloom (one week following the disappearance of the first bloom), there were no substantial changes in either the concentration or the toxicity of the mussels.

More than 90% of the toxin was lost within three weeks following the termination of the second bloom (post bloom II) in both mussel groups. During the end phase of detoxification (more than three weeks after the disappearance of the second bloom), there were no changes in toxin content or toxicity in either population. The toxin levels decreased drastically following both blooms, but the rates of detoxification following the second bloom appeared slower than those following the first bloom.

1. Comparisons between populations

All toxins were accumulated at a similar rate in CAP and MAD mussels during the two blooms. However, the detoxification rates of individual toxins GTX_1, GTX_3, GTX_5, neoSTX and STX, as well as the rate of decrease in the total toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq g^{-1}), differed between the two mussel groups following the first bloom. For all toxins, MAD mussels detoxified at a higher rate than CAP mussels. The rate of toxin elimination from the digestive gland following the second bloom did not differ between CAP and MAD mussels (Table 15 in Annex II).

ii. Comparisons between blooms

The rates of toxin accumulation in CAP and MAD mussels did not differ between the first and the second bloom, but the rates of detoxification of STX and of the overall total toxin concentration differed in CAP mussels (Table 16 in Annex II).

The rate of total toxin elimination to an arbitrary level of 15% of the peak in toxicity (nmol g^{-1} and μgSTX·q g^{-1} digestive gland) differed between the first and the second bloom ($p<0.1$) in CAP and MAD mussels (Table 14b in Annex II)

2. Toxin compositional changes

a. Toxin composition in mussels and *Alexandrium*

Comparison of the PSP toxin profiles of *Alexandrium* cells and mussels for the same dates during each of the two blooms revealed a similar composition. C_x and neoSTX were the major toxin components in both CAP and MAD mussels and in the dinoflagellate cells. These two toxins constituted at least 67% of the total toxin of the mussels on a relative molar basis throughout all bloom phases (Fig. 11 and 12a and b) The difference in relative toxin content between the two mussel groups and the dinoflagellate cells during each bloom, was evaluated as an indication of toxin biotransformation and/or selective uptake and retention in the mussels. CAP mussels and *Alexandrium* cells differed in their relative content of GTX_1 during the first bloom and in C_x, GTX_1 and GTX_4 during the second bloom. In contrast, MAD mussels differed from the dinoflagellates in their mean relative concentration of GTX_1 and STX during the first bloom, and in all toxins except (GTX_4 and STX) during the second bloom (Fig

II and Fig. 12a and b) (Table 17 in Annex II). A direct comparison of mussel versus cell toxin ratios show ratios different than 1 for most toxins (Fig. 13a and b).

b. Changes in relative toxin composition

i. Comparison between populations

Both mussel groups seem to have altered their toxin composition relative to that of the *Alexandrium* cells but also relative to each other when the cells are absent from the water column. These modifications tended to increase the toxicity of MAD mussels relative to that of CAP mussels. The two mussel groups differed in their relative content of C_x, GTX_1, GTX_4, neoSTX and STX during the first bloom and of GTX_4, GTX_1 and GTX_3 during the second bloom (Table 17 in Annex II). Over the entire season, the relative composition of C_x, GTX_4, GTX_1 and STX differed between the two populations. In the post bloom II period, the two groups differed in the relative composition of all their toxins, except neoSTX. Later, during the end phase, all toxins were found in similar proportions in the mussel populations (Table 18 in Annex II).

ii. Comparison between blooms

A comparison of the relative mean toxin composition during the first and the second blooms, showed differences in the relative content of C_x and GTX_3 in CAP mussels and of GTX_1 in MAD mussels (Fig. 14a, b and c) (Table 10 in Annex II).

Figure 11: Percent molar composition of PSP toxin in *Alexandrium* cells (ALEX) during the two blooms and in the digestive glands of mussels transplanted from Cap Chat (CAP) and from the Magdalen Islands (MAD) during the various phases of toxin accumulation and elimination (n = number of samples collected during each phase).

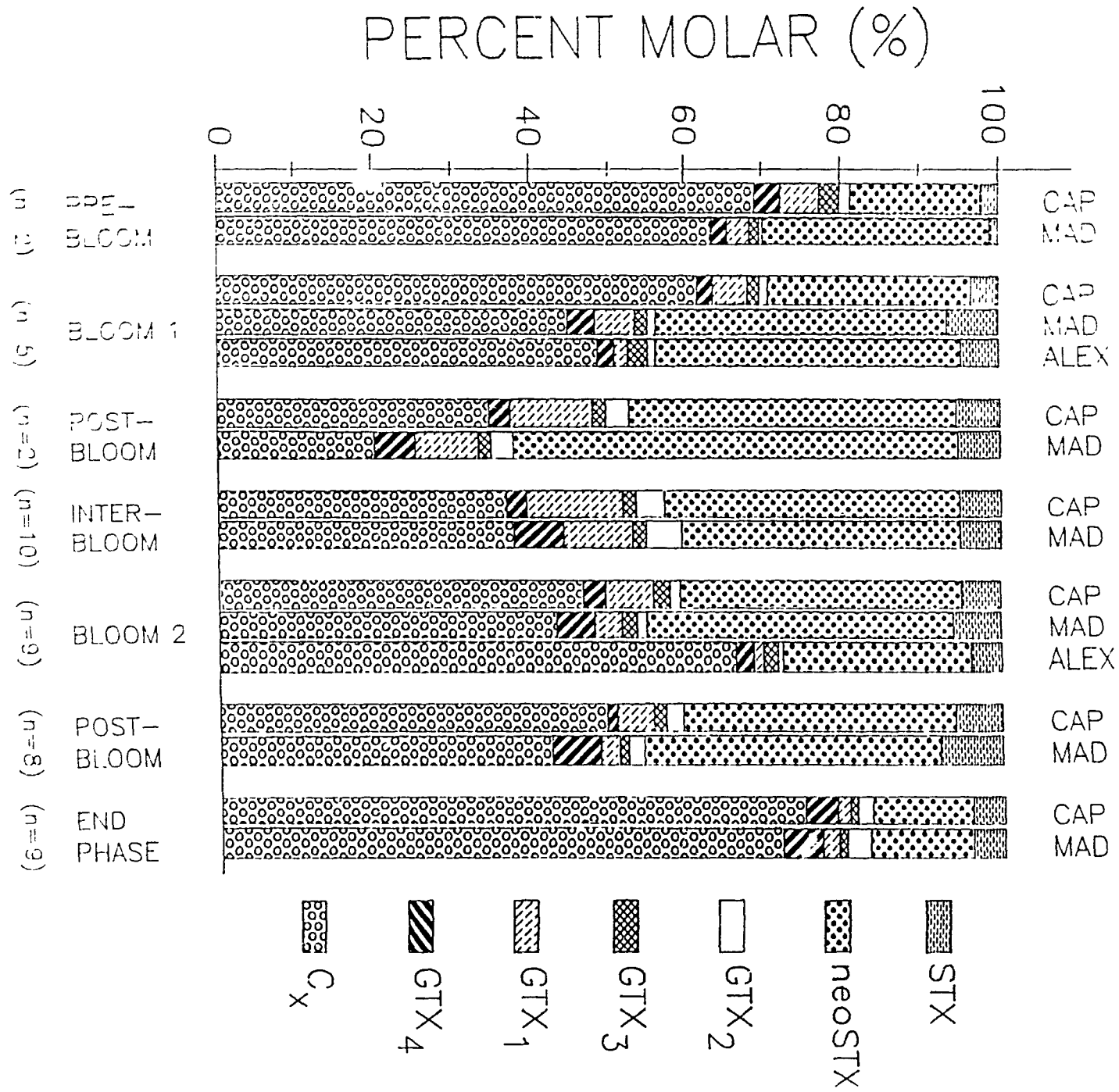
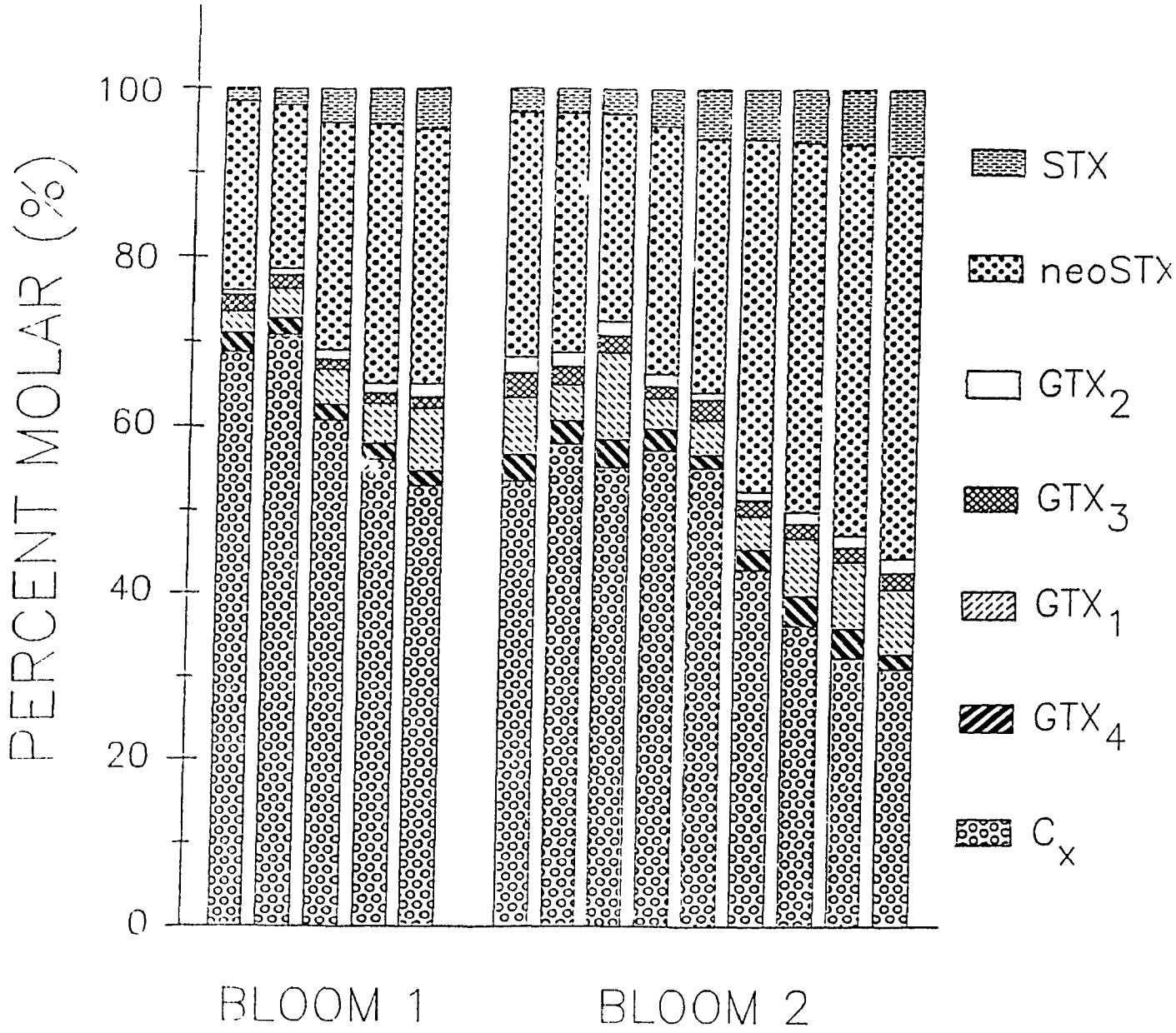

PERCENT MOLAR (%)
0
20
40
60
80
100
PRE-BLOOM (n=3)
BLOOM 1 (n=5)
POST-BLOOM (n=2)
INTER-BLOOM (n=10)
BLOOM 2 (n=9)
POST-BLOOM (n=8)
END PHASE (n=9)
CAP
MAD
ALEX
Cx
GTX4
GTX1
GTX3
GTX2
neoSTX
STX

Figure 12: Percent molar composition of toxin in the digestive glands of mussels transplanted from a. Cap Chat and b. the Magdalen Islands and collected at each sampling interval during the two blooms.

a CAP


PERCENT MOLAR (%)
100
80
60
40
20
0
BLOOM 1
BLOOM 2
STX
neoSTX
GTX2
GTX3
GTX1
GTX4
Cx

b. MAD

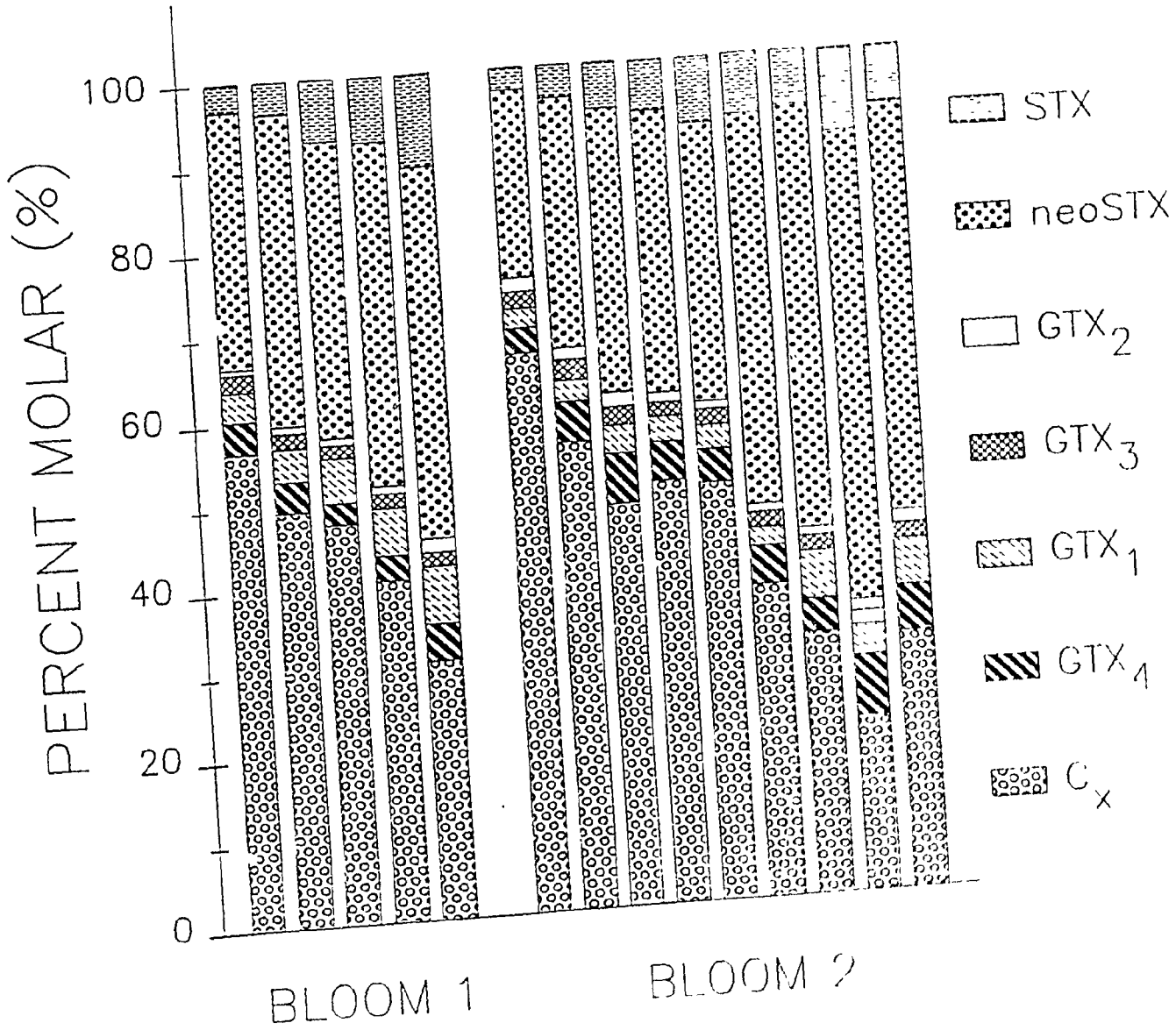
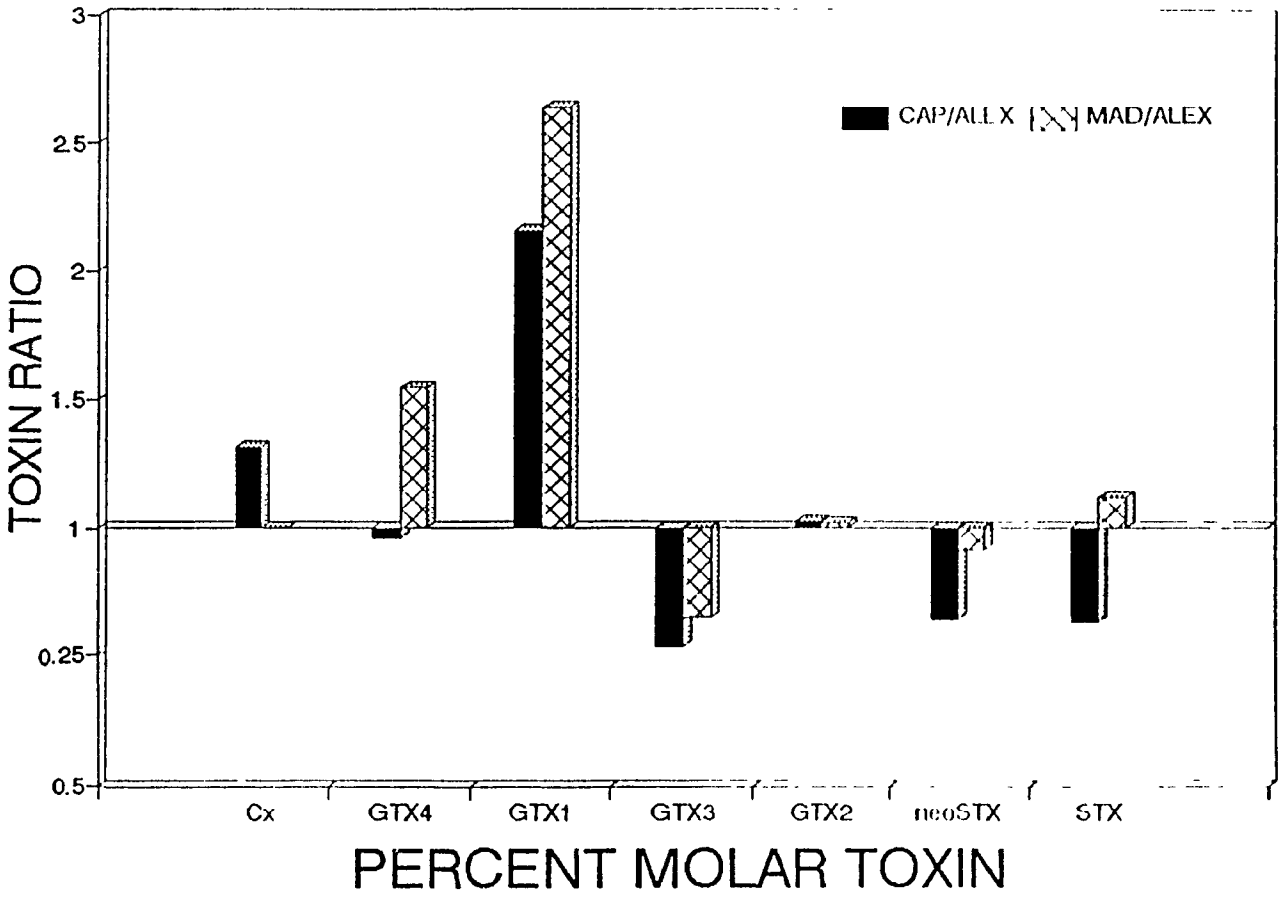

PERCENT MOLAR (%)
100
80
60
40
20
0
BLOOM 1
BLOOM 2
STX
neoSTX
GTX2
GTX3
GTX1
GTX4
Cx

Figure 13: Ratio of individual toxin concentration (C_x, GTX_4, GTX_1, GTX_3, GTX_2, neoSTX and STX) in the digestive glands of the mussels transplanted from Cap Chat (CAP/Alex) and from the Magdalen Islands (MAD/Alex) over the individual toxin concentration in *Alexandrium* cells during a. Bloom I and b. Bloom II.

a. Bloom I


CAP/ALEX
MAD/ALEX
TOXIN RATIO
3
2.5
2
1.5
1
0.25
0.5
Cx
GTX4
GTX1
GTX3
GTX2
neoSTX
STX
PERCENT MOLAR TOXIN

b. Bloom II

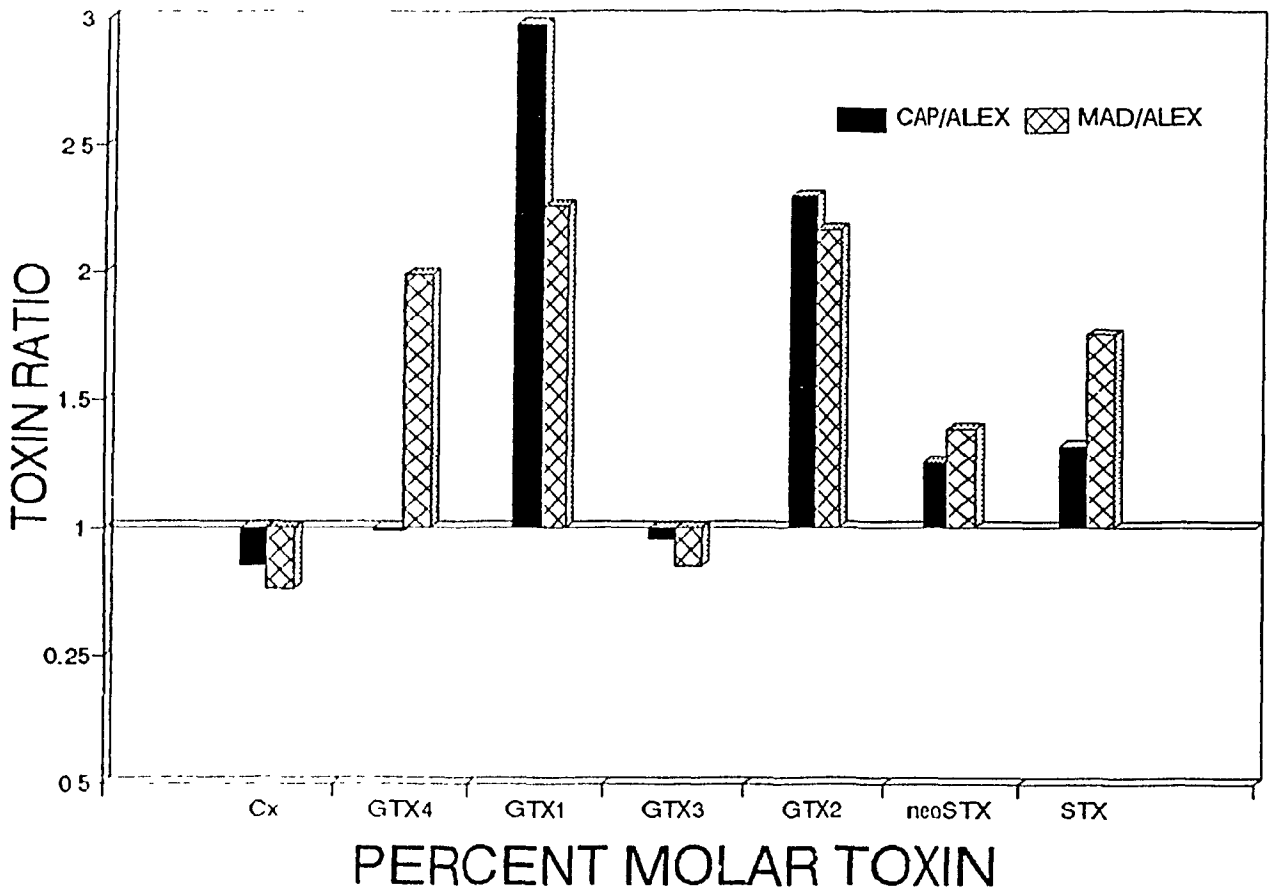
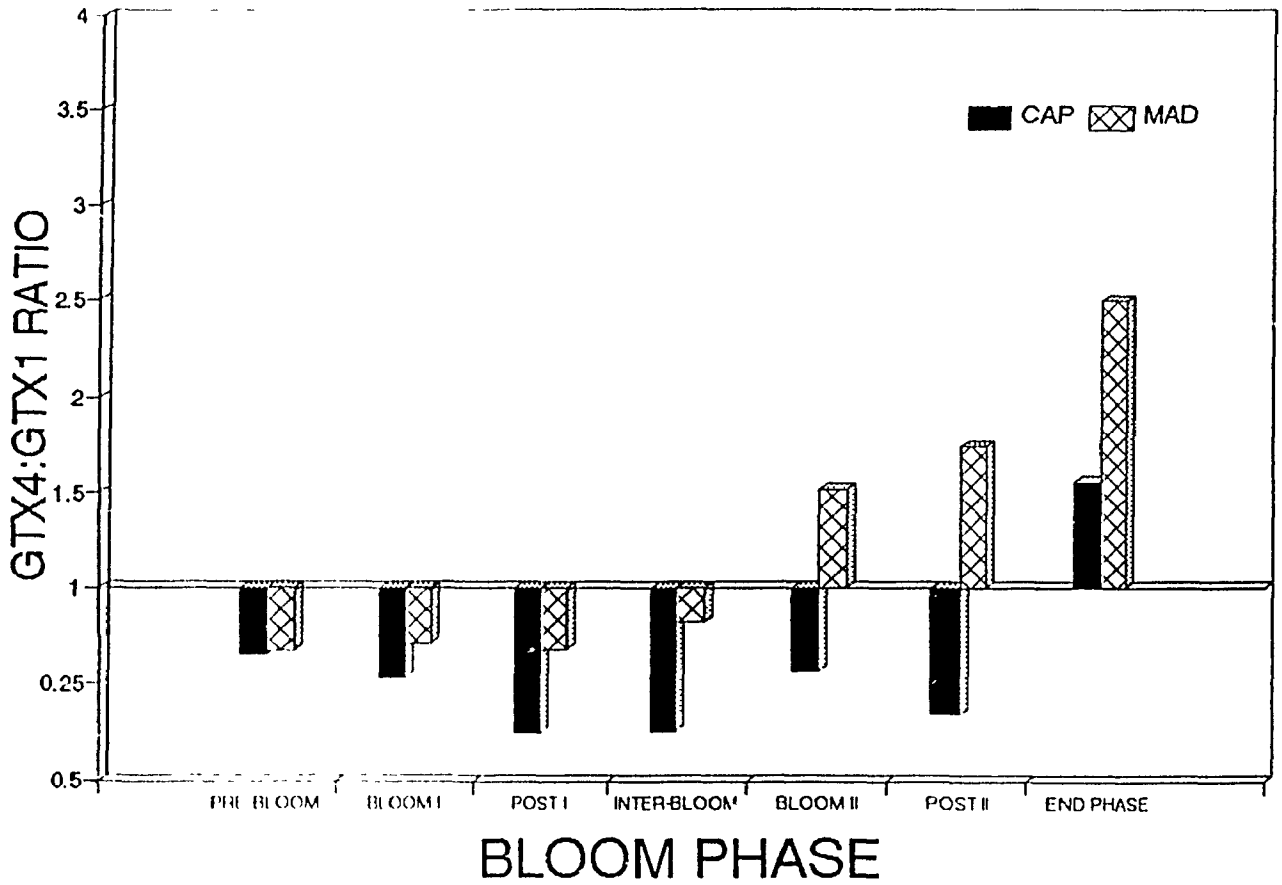
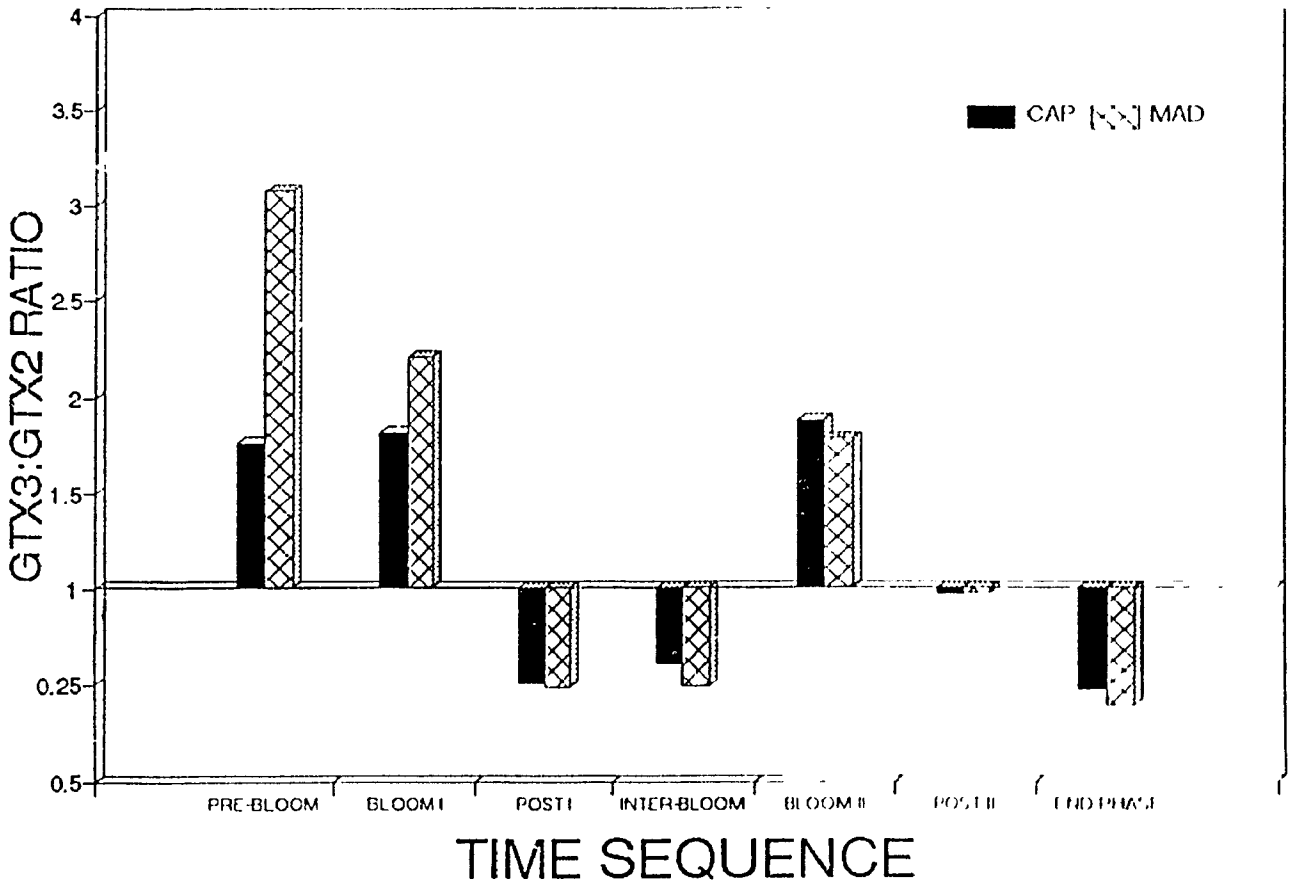

CAP/ALEX
MAD/ALEX
TOXIN RATIO
3
2.5
2
1.5
1
0.25
0.5
Cx
GTX4
GTX1
GTX3
GTX2
neoSTX
STX
PERCENT MOLAR TOXIN

Figure 14: Ratio of toxin epimers: a. GTX_4:GTX_1; b. GTX_3:GTX_2 and c. neoSTX:STX for each bloom phase in the digestive glands of the mussels transplanted from Cap Chat (CAP) and from the Magdalen Islands (MAD) to the experiment site.

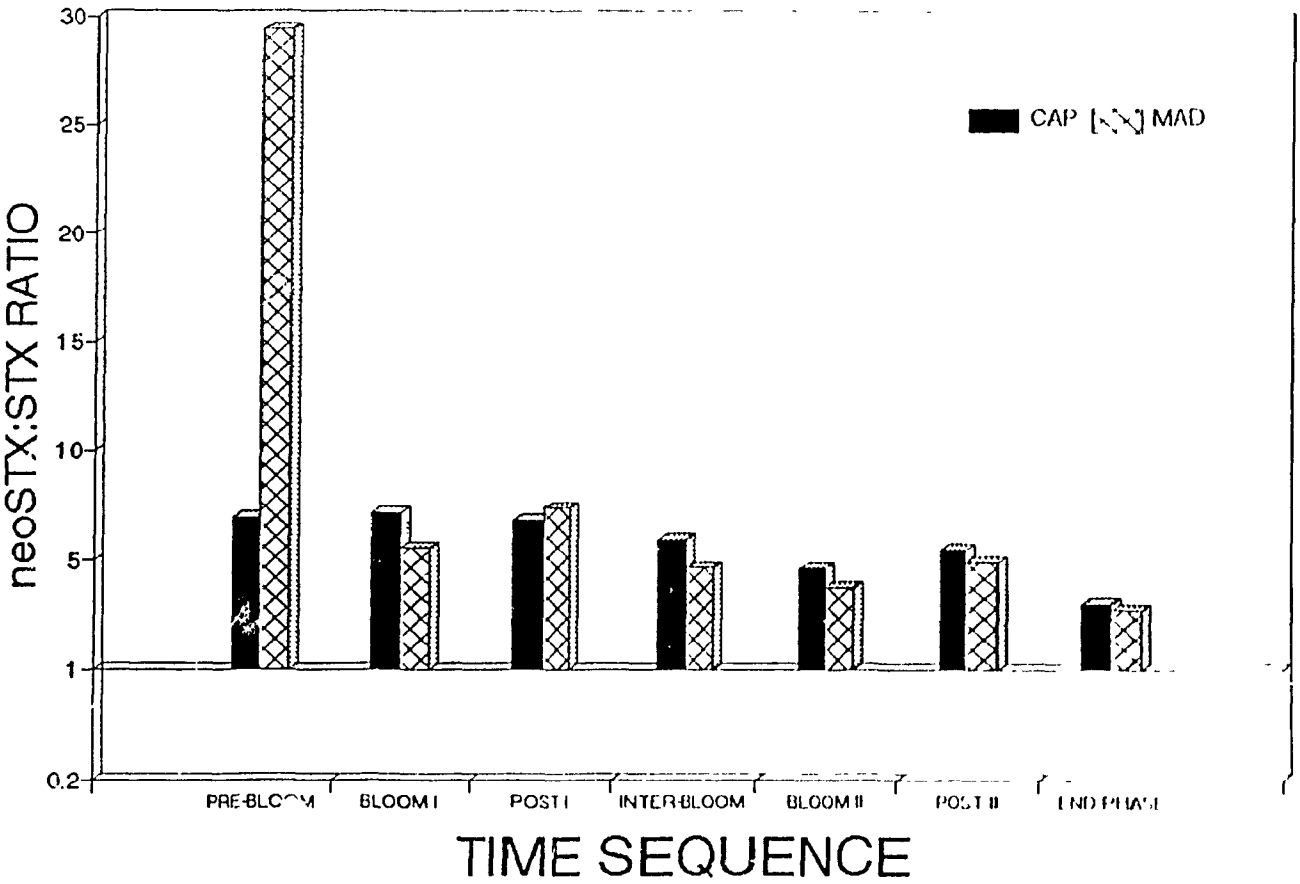
a. $GTX_4:GTX_1$


GTX4:GTX1 RATIO
4
3.5
3
2.5
2
1.5
1
0.25
0.5
CAP
MAD
PRE BLOOM
BLOOM I
POST I
INTER-BLOOM
BLOOM II
POST II
END PHASE
BLOOM PHASE

b. GTX_3:GTX_2


CAP
MAD
GTX3:GTX2 RATIO
4
3.5
3
2.5
2
1.5
1
0.25
0.5
PRE-BLOOM
BLOOM I
POST I
INTER-BLOOM
BLOOM II
POST II
END PHASE
TIME SEQUENCE

c. neoSTX:STX


neoSTX:STX RATIO
30
25
20
15
10
5
1
0.2
CAP
MAD
PRE-BLOOM
BLOOM I
POST I
INTER-BLOOM
BLOOM II
POST II
END PHASE
TIME SEQUENCE

c. Kinetics of toxin ratio changes

The toxin ratio changes in the uptake and detoxification phases of each bloom, as well as in the inter-bloom period are presented through a series of regression analyses of changes in epimer ratios as a function of time (Table 19 in Annex II). There was notable equilibration of GTX_3 to GTX_2 for both CAP and MAD mussels, and a decrease in the GTX_4:GTX_1 and neoSTX:STX ratios for CAP mussels in the uptake phase of the first bloom. The neoSTX:STX ratio decreased during the uptake phase of the second bloom, whereas STX consistently increased in both populations. During the detoxification phase following the first bloom, both mussel groups exhibited a decrease in GTX_3 relative to GTX_2 and an increase in the neoSTX relative to STX. In addition, a decrease in the GTX_4:GTX_1 ratio during that phase was observed in CAP mussels. During the detoxification phase following the second bloom, the ratio of GTX_3:GTX_2 in both mussel groups and the ratio of GTX_4:GTX_1 in MAD mussels decreased. Finally, MAD mussels showed a decrease in the ratio of neoSTX:STX during the inter-bloom.

1. Comparisons between populations

Major differences were observed between CAP and MAD mussels in the changes of the ratios of GTX_4:GTX_2 and neoSTX:STX for the detoxification phase of the first bloom and in the ratio of GTX_4:GTX_1 during the detoxification phase of the second bloom (Table 20 in Annex II).

ii. Comparisons between blooms

Differences in the GTX_4:GTX_3 ratio for both CAP and MAD mussels were observed between the two blooms. There was a marked increase in GTX_3 relative to GTX_4 in the uptake phase of the first bloom, and a decrease in GTX_3 relative to GTX_4 in the uptake phase of the second bloom. The ratios of neoSTX:STX were also different for both populations; an increase of neoSTX relative to STX was observed in the detoxification phase of the first bloom, while a decrease in that ratio was evident following the second bloom (Table 21 in Annex II).

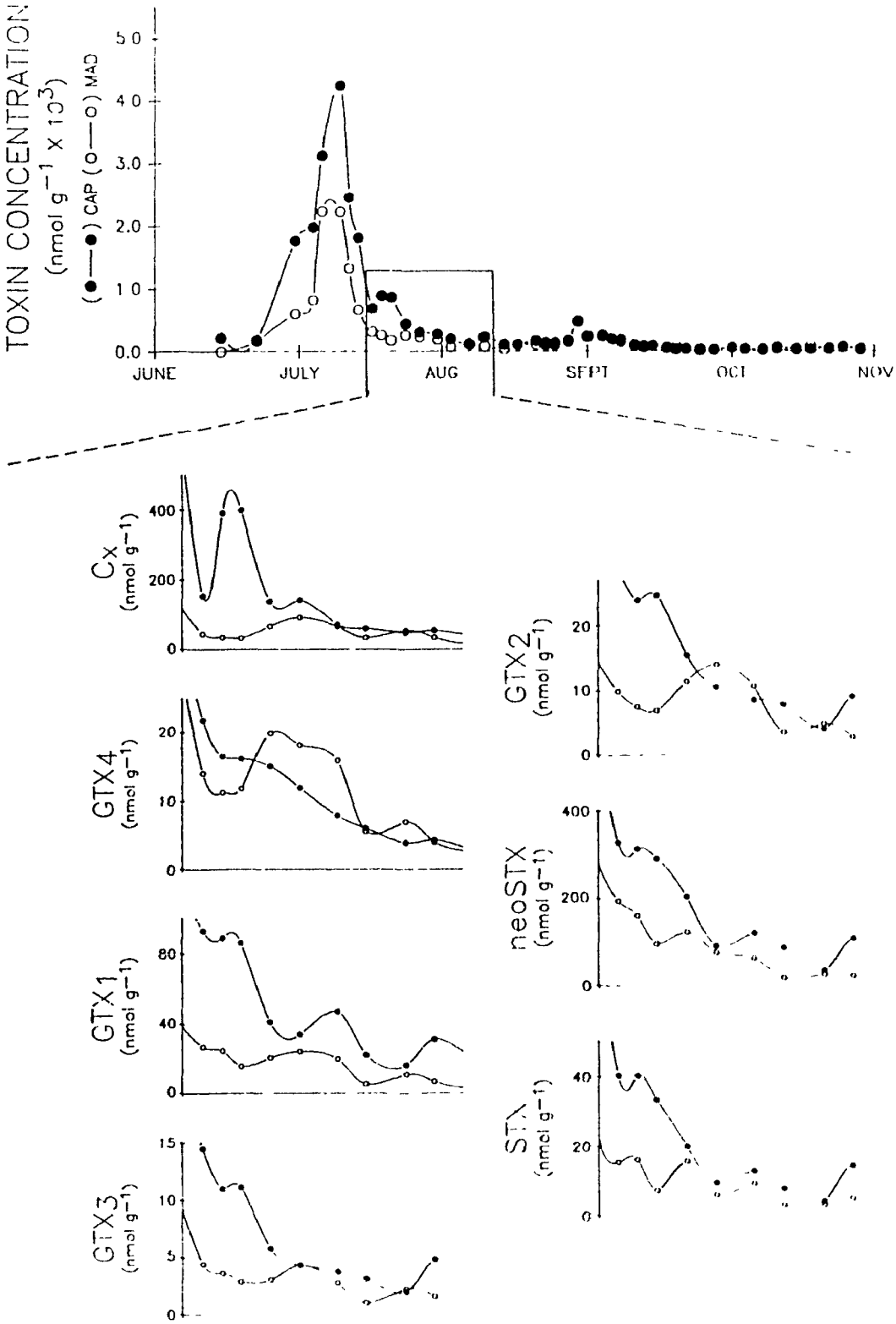
There was an observed short-lived increase in the absolute amounts of certain toxins in MAD mussels, namely GTX_4 and GTX_2 (Fig. 15). This increase occurred during the inter-bloom when *Alexandrium* cells were undetectable in the water column (transient phase). Differences in the concentration (nmol g^{-1}) of GTX_4 and GTX_1 were noted between CAP and MAD mussels (Table 11 in Annex II).

3. Anatomical distribution of toxins in the mussels

i. Comparisons between populations

The total toxin content and the relative toxin composition in the digestive glands of the two mussel populations were indistinguishable in the end phase of the experiment, however the fraction of toxin transferred from the digestive gland to the other tissues was different for the two mussels groups (Tables 15 and 16 in Annex I).

Figure 15: Changes in the absolute concentration of individual toxins: C_x, GTX_4, GTX_1, GTX_3, GTX_2, neoSTX and STX, during a transient phase (overlap of post-bloom I and inter-bloom) in the digestive glands of mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD).


TOXIN CONCENTRATION
(nmol g^{-1} X 10^{3})
(●—●) CAP (o—o) MAD
5.0
4.0
3.0
2.0
1.0
0.0
JUNE
JULY
AUG
SEPT
OCT
NOV
Cx
(nmol g^{-1})
400
200
0
GTX4
(nmol g^{-1})
20
10
0
GTX1
(nmol g^{-1})
80
40
0
GTX3
(nmol g^{-1})
15
10
5
0
GTX2
(nmol g^{-1})
20
10
0
neoSTX
(nmol g^{-1})
400
200
0
STX
(nmol g^{-1})
40
20
0

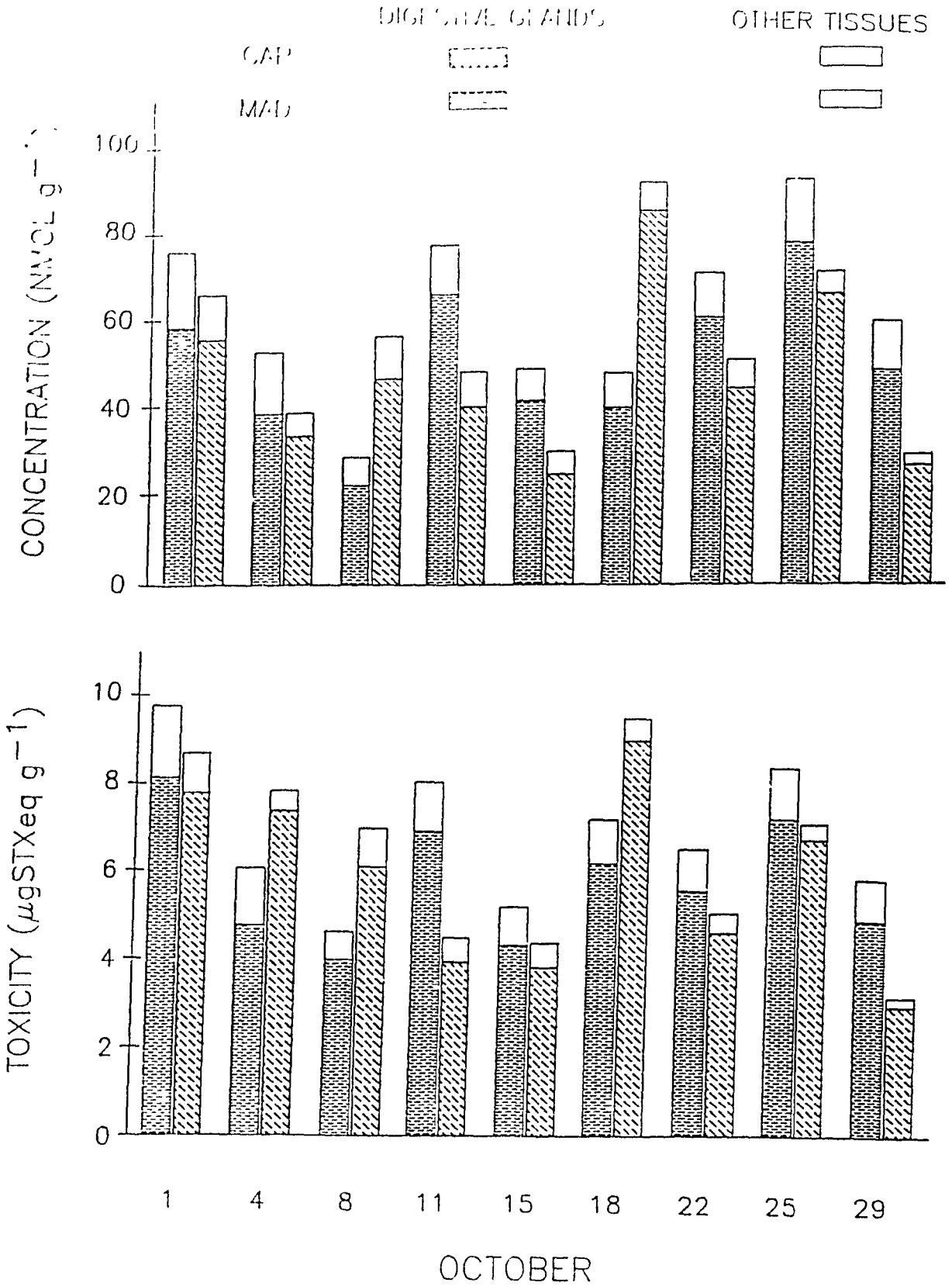
The non-visceral tissues of CAP mussels contained a greater proportion of total body toxin burden (and were more toxic) than those of MAD mussels. All PSP toxin derivatives (except GTX_1 in both CAP and MAD and GTX_4 in MAD mussels) were found outside the digestive gland at the end phase of the experiment. CAP mussel tissues contained an average of 23.63% of the total body toxin concentration, equivalent to 18.22% of the total toxicity. The non-visceral tissues of MAD mussels contained an average of 15.24%, which corresponded to 10.04% of the total toxicity (Fig. 16) (Table 22 in Annex II).

The concentration of all the toxins (except C_2) and the total toxicity differed in the non-visceral tissues of CAP and MAD mussels (Table 23a in Annex II); in the viscera, the levels of all toxins were similar (Table 11f in Annex II).

During the end phase, neoSTX and GTX_1 contributed to most of the observed differences in toxin concentration in the non-visceral tissues (Table 24a in Annex II); whereas GTX_3 and C_2 were apparently the major discriminators in the viscera, although the function was not significant (Table 12 in Annex II).

No differences were observed in the relative proportions of toxins in the visceral and the non-visceral tissues between CAP and MAD mussels (Tables 18d and 25a in Annex II).

Figure 16: Toxin concentration (nmol g^{-1})(top) and toxicity (μgSTXeq g^{-1})(bottom) in the digestive glands and in the other tissues of the mussels transplanted from Cap Chat (CAP) and the Magdalen Islands (MAD) for each sample collected during the end phase of the experiment (October 1-29).


DIGESTIVE GLANDS
OTHER TISSUES
CAP
MAD
CONCENTRATION (NMOL g⁻¹)
100
80
60
40
20
0
TOXICITY (μgSTXeq g⁻¹)
10
8
6
4
2
0
1
4
8
11
15
18
22
25
29
OCTOBER

ii. Comparison between tissues

Marked differences between the two tissue fractions in CAP and MAD mussels were found for all analogues except GTX_4 and GTX_1 in CAP mussels (Table 23b in Annex II). GTX_4 and STX discriminated between the two tissue components in CAP mussels, whereas GTX_3 and GTX_2 were the major discriminators in MAD mussels (Table 24b in Annex II).

The relative toxin composition of most toxins also differed for both mussel groups between the two tissue fractions (Figs. 17a and b). Significant differences in the relative content of all toxins were observed between the two tissue fractions, except for STX in CAP and MAD, and neoSTX in MAD mussels (Table 25b in Annex II).

4. Mussel weights and shell dimensions

CAP mussels were significantly smaller and weighed less than MAD mussels (Table 26 in Annex II). The digestive gland weight to total body weight ratio from which the total weights of mussels were estimated was 0.120 (S.E.=0.008) for CAP mussels and 0.101 (S.E.=0.009) for MAD mussels (Tables 14 and 15 in Annex I).

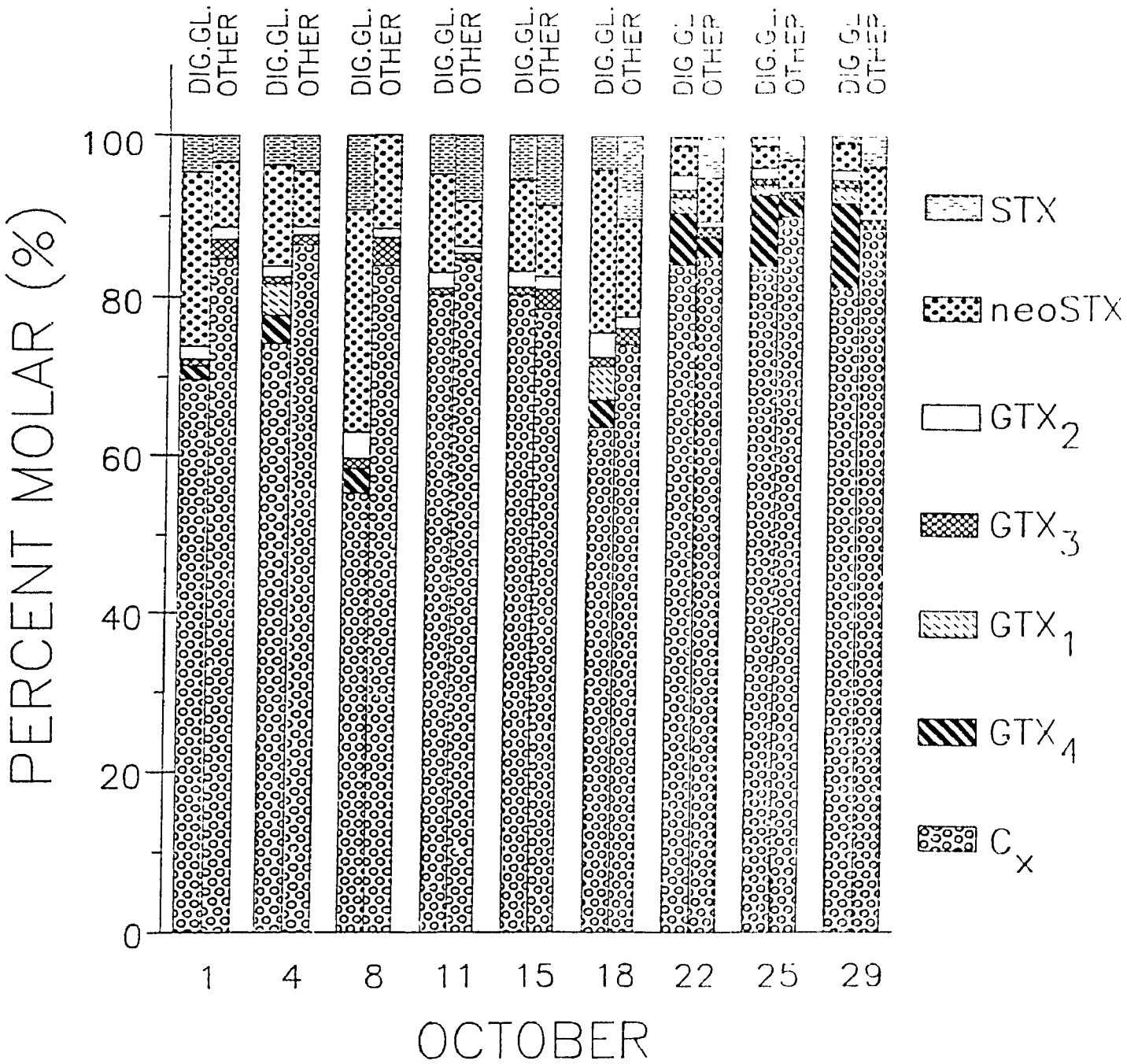
The relationship between digestive gland weight and calculated total weight (Y,g) to shell height (X,cm) for the two populations are presented in graphs 17a and b. The regression lines are given in the form of equation 3.

$$Y = \text{intercept } X^{\text{slope}(SE)} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

There was more variability in body weight and in digestive gland weight for a given shell height in MAD mussels than in CAP mussels

Figure 17: Histograms showing the percent molar toxin composition in the digestive glands and in the other tissues of the mussels transplanted from a. Cap Chat and b. the Magdalen Islands and collected during the end phase of the experiment (October 1-29).

a. CAP


DIG.GL.
OTHER
PERCENT MOLAR (%)
100
80
60
40
20
0
1
4
8
11
15
18
22
25
29
OCTOBER
STX
neoSTX
GTX2
GTX3
GTX1
GTX4
Cx

b MAD

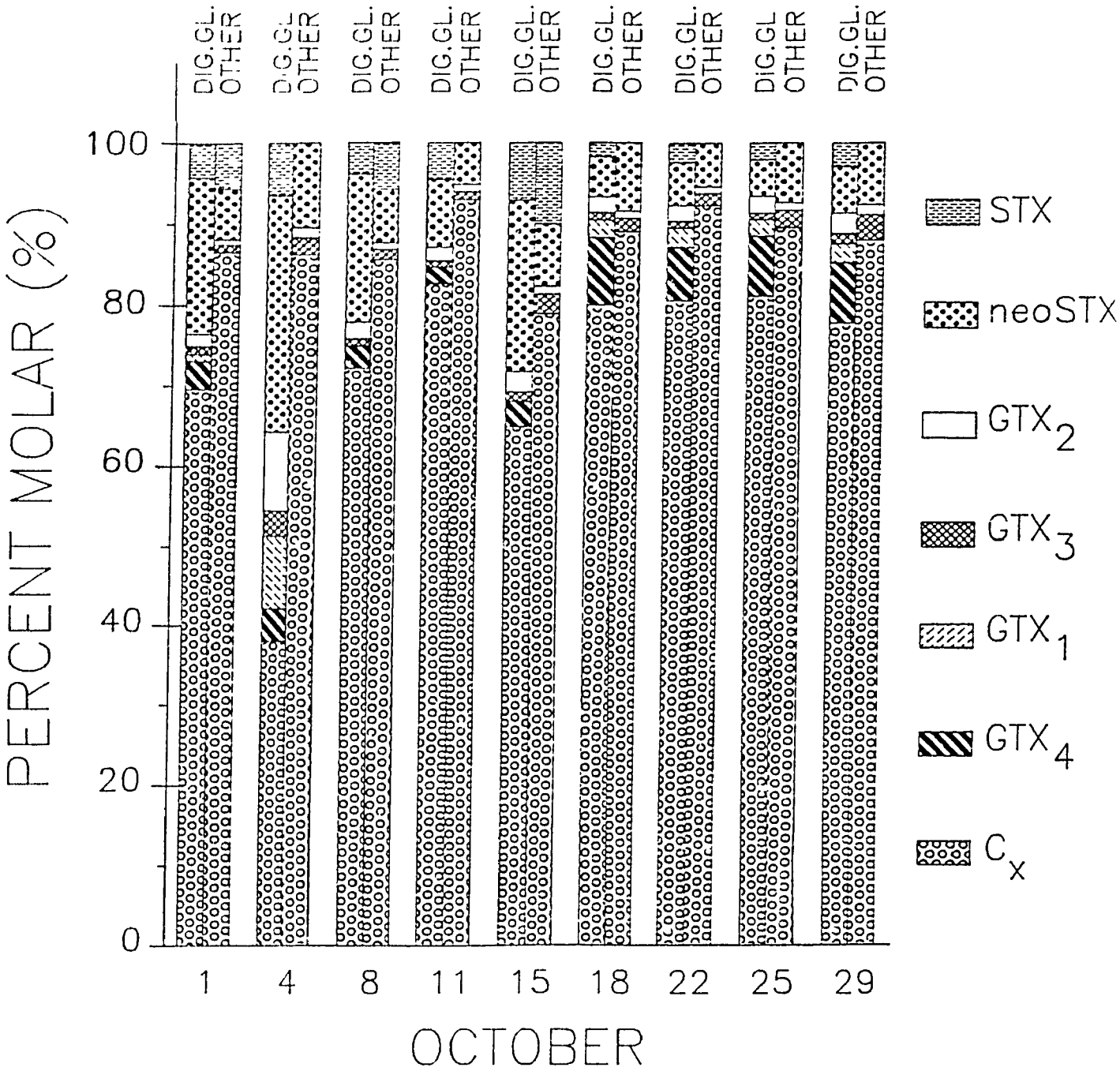
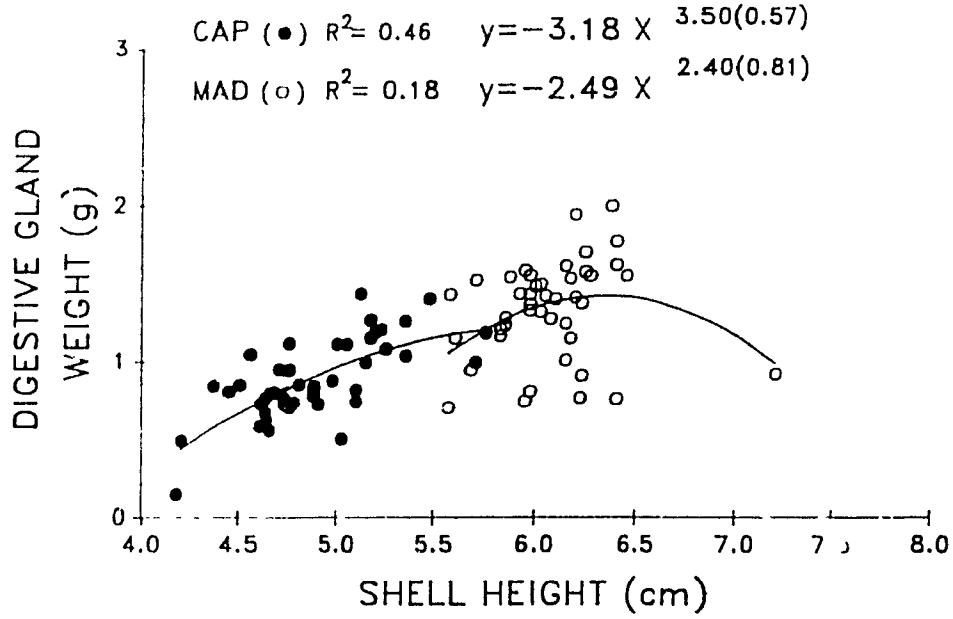
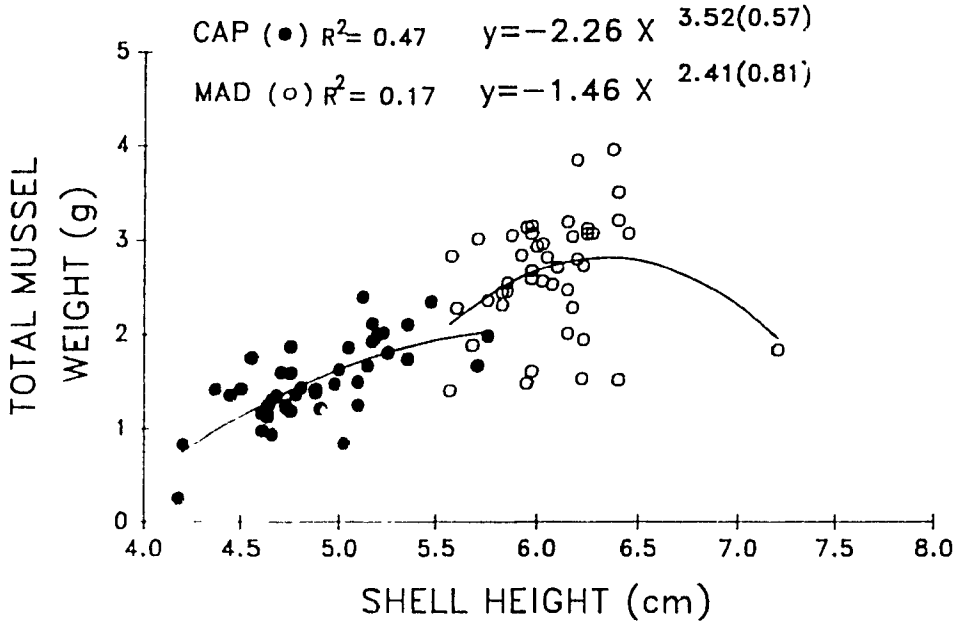

DIG.GL.
OTHER
PERCENT MOLAR (%)
100
80
60
40
20
0
1
4
8
11
15
18
22
25
29
OCTOBER
STX
neoSTX
GTX2
GTX3
GTX1
GTX4
Cx

Figure 18: Curvilinear regression of shell height (cm) to digestive gland weight (g) and to total weight (g) of mussels transplanted from Cap Chat (CAP) and from the Magdalen Islands (MAD) to the experiment site.


CAP (•) R2= 0.46 y=−3.18 X 3.50(0.57)
MAD (o) R2= 0.18 y=−2.49 X 2.40(0.81)
DIGESTIVE GLAND WEIGHT (g)
SHELL HEIGHT (cm)
0
1
2
3
4.0
4.5
5.0
5.5
6.0
6.5
7.0
8.0


CAP (•) R2= 0.47 y=−2.26 X 3.52(0.57)
MAD (o) R2= 0.17 y=−1.46 X 2.41(0.81)
TOTAL MUSSEL WEIGHT (g)
SHELL HEIGHT (cm)
0
1
2
3
4
5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0

DISCUSSION

A. Simultaneous mussel transplant experiment (1990)

Mussels with different histories of PSP contamination seem to behave differently when exposed to blooms of toxic *Alexandrium* cells. There is a threshold limit beyond which mussels are not able to accumulate PSP toxins (Bricelj et al., 1990 a and b), which seems lower in non-previously contaminated mussels. Naive mussels accumulate toxins at a slower rate in response to an intense bloom, but eliminate them at a higher rate than chronically exposed mussels, thus remaining toxic for a shorter time. Biotransformation of toxins also seems to be a function of previous exposure, in that pre-exposed mussels tend to metabolise the toxins in such a way as to retain a greater proportion of the less toxic derivatives. Thus for a given toxin body burden, pristine mussels will be more toxic than experienced mussels. It would seem that the difference disappears following a first exposure to toxic *Alexandrium* cells. However, this experiment did not reveal conclusively whether or not this reflects a true physiological adaptation since the two blooms to which the transplanted mussels were exposed, differed in duration, cell number and cell toxicity, although not cell toxin composition.

1. *Alexandrium* cells

The *Alexandrium* cells exhibited a higher mean toxin content during the second bloom, whereas the toxin composition of the cells did not significantly differ. Therefore, there was an overall increase in toxicity per cell during the second bloom. According to Hall et al. (1990), the same *Alexandrium* strain can display variations in toxin contents

which are induced by altered physical parameters. The conditions altering cell toxin concentration and toxicity, as described by Therriault et al., (1985), include salinity, nutrients and water temperature. Variations in the above parameters can affect the formation of a bloom within the season and between consecutive seasons. It has been suggested that the cellular toxin levels in an *Alexandrium* bloom are inversely proportional to the rate of cell division in a natural environment and to cell culture age in an artificial setting (Mickelson and Yentsch, 1979; Hall, 1982; Boczar et al., 1988). Ogata et al (1987; 1989) associated an increase in toxin production with a decrease in cell growth rate. Anderson and Kulis (1993) attribute variations in toxin production in *Alexandrium* cells specifically to cell cycle phases. The cause of the lower toxicity per cell observed during the first bloom of this experiment, where the cell counts were an order of magnitude greater than during the second bloom, may have been due to differences in growth rate, mediated by *in situ* environmental factors.

2. Kinetics of toxin uptake and elimination

Peak toxin loads in mussels were obtained following a several day delay (Fig. 9) after the peak concentration in *Alexandrium* cells was attained. Laboratory experiments by Bricelj et al. (1990 a and b) performed on *Mytilus edulis* with no prior exposure to PSP toxins, indicated that the toxin levels in the *Mytilus edulis* exposed to bloom concentrations (2.6×10^5cell L^{-1}) of a high toxicity *Alexandrium* strain (65.7pgSTXeq cell $^{-1}$) exceeded the allowable limit for human consumption within one hour of exposure. Saturation levels of toxin in the mussels was reached at $4.5 \times 10^4 \mu$gSTXeq $100g^{-1}$ tissue.

Laboratory experiments allowed Prakash et al. (1963) to show that the PSP toxin concentration in clams (*Mya arenaria*) can exceed the 80μgSTXeq 100^{-1} human safety limit following a three days exposure to an artificial bloom of 1.9×10^7 *Alexandrium* cells per litre. In this experiment, maximum toxicities attained within a week of exposure, exceeded the regulatory harvest closure level by several orders of magnitude.

Mussels previously exposed to PSP seem to have a lower sensitivity to the toxins and thus accumulated toxins more rapidly and endure a higher total toxin body burden (Fig. 9). This effect is unlikely to be due simply to the use of cultured versus wild mussels, since wild mussels from a toxin-free zone accumulate less toxin than cultured mussels when exposed to the same natural *Alexandrium* bloom in a previous experiment (Carreto et al., 1986; Desbiens et al. 1990; Needler, 1949). Also, both populations behaved in a similar manner during and following the second bloom with comparable peak toxin concentrations and toxicities. Shumway and Cucci, (1987) have shown the antagonistic physiological effect of PSP toxins on mussels that were not previously exposed to *Alexandrium* blooms. It would thus seem that mussels previously exposed to PSP have evolved a mechanism which reduces their vulnerability to the effect of the toxin, and thus permit them to accumulate more toxin.

The elimination of toxins from the digestive glands of mussels is manifested as a bi-phasic event. The two phases correspond to different elimination procedures: In the first phase, the gut content is freed of unbound toxin; in the second phase, there is a gradual loss of incorporated toxin. Gestal-Otero et al. (1978) and Aalvik and Framstad (1981) describe the detoxification from mussels as a rapid decline in the first phase (73%

in 7 days) and a slower decrease during the second phase (94% in 10 days). Based on mouse bioassay results, Hurst and Gilfillan (1977) state that mussels depurate 60% of their toxin load within 12 days. Elimination of toxins also seems to be a function of previous exposure to PSP toxins; pristine mussels detoxify at a faster rate than chronically exposed mussels, and thus tend to retain toxicity for a shorter period of time.

The response of the pristine mussel population differed between the two blooms, a point also illustrated by the fact that the toxins discriminating between the two populations (CAP and MAD) are not the same in the first and the second bloom, even though the relative content of these toxins did not vary in the *Alexandrium* cells. It is apparent that MAD mussels altered their response to the toxins subsequent to their initial exposure to an *Alexandrium* bloom; i.e., physiological adaptive response conditioning and not genetic difference.

3. Putative biotransformation

Observed differences in toxin concentration in the mussels over those in the phytoplankton point to the possibility of bioconversion or selective uptake, retention or excretion of toxins from within the digestive gland of the mussels. The uptake of GTX_3 was favoured during each bloom, hinting at the high abundance of that toxin relative to GTX_3 in the *Alexandrium* cells. The increase of GTX_2 relative to GTX_3 observed in our experiment agrees with the findings of other authors. In a laboratory experiment under controlled conditions, Ledoux et al., (1993) compared the toxin profiles in pristine mussels with those of *Alexandrium* cells and found them similar under conditions of high

toxicity in the medium, but noted a shift from GTX_4 to GTX_3 following the elimination of the toxic species from the food supply of the mussels. The constant rate of decrease of neoSTX to STX in both mussels groups indicated that there is biotransformation of neoSTX to STX within the digestive glands. But selective elimination of neoSTX did not seem to occur because of the observed increase in the relative concentration of STX within the digestive glands of both mussel groups following the second bloom. The ratio of neoSTX to STX decreased faster in pristine mussels than in chronically contaminated mussels. This phenomenon suggests that pristine mussels have higher activity at a reductive enzyme capable of converting neoSTX to STX through elimination of the hydroxyl group at N-1 or that the pristine mussels have a greater binding affinity for the more highly charged STX. Chronically contaminated mussels may be unaffected by either highly potent toxin; and thus favour the equilibration of neoSTX to STX in the digestive glands (Shumway et al., 1985; Shumway and Cucci, 1987; Cucci et al, 1985). Based on the relative molar toxin composition, the sulfocarbamoyl to carbamate toxin ratio was maintained at a substantially higher level in CAP mussels than in MAD mussels throughout the first bloom and subsequent phase. This could indicate the presence of a greater proportions of undigested or partially digested *Alexandrium* cells in the digestive tract of CAP mussels as a result of continued active filtration. Alternatively, the naive MAD mussels may be more active enzymatically in the decarbamoylation necessary to convert N-sulfocarbamoyl toxins to carbamates. The differences in toxin accumulation between the two populations, which were more pronounced in terms of total toxin concentration than in terms of toxicity, suggest that toxin-specific activity is more critical

in determining the magnitude of the physiological response than total toxin body burden. The observed decrease in the ratio of neoSTX to STX in CAP and MAD mussels is in conformity with the results reported by Shimizu et al. (1984) and by Bricelj et al. (1990 a and b) working with contamination of *Mytilus* by *Alexandrium* under controlled conditions. In our experiment, the relative increase in high toxicity carbamates, e.g. STX, in mussels, as compared to the *Alexandrium*, is interpreted as evidence of biotransformation of the toxins. This was suggested previously by Anderson et al. (1989) and by Oshima et al. (1987), who compared toxin profiles of toxic mussels to those of the PSP toxin producing dinoflagellate *Gymnodinium catenatum*, and found significant amounts of carbamates in the mussels which were not detected in the dinoflagellates. The high initial proportion of labile C-toxins in the mussel digestive glands prior to the first bloom could be the result of a recent contamination from low concentrations of an *Alexandrium* population extremely rich in N-sulfocarbamoyl toxins. However, the fact that the initial toxin profile closely resembles that found at the end of the experiment, when *Alexandrium* was absent from the water column, also suggests that selective elimination of carbamate toxins by the mussels may have occurred. The difference in the C-toxin content between the cells and the mussels during the second bloom is probably due to the high residual quantities of the other toxins in the viscera of CAP and MAD mussels.

The possibility of significant biotransformation of the toxins is reflected by the changes in the ratios of the epimeric pairs (GTX_4:GTX_1, GTX_3:GTX_2) as well as the ratios of neoSTX:STX and total carbamates to sulfamates for both populations.

However, it is not possible to distinguish between selective elimination and biotransformation based solely upon the changes in toxin profiles.

The most dramatic changes in the toxin profiles were associated with the transition from the first bloom to the post-bloom phase. Epimerisation resulting in the relative increase in the GTX_3:GTX_2 and GTX_4:GTX_1 ratios is as expected, but the significant increase in the carbamate:C_x ratio, observed particularly in MAD mussels, is a more complex conversion by decarbamoylation. Thus, selective retention of carbamate toxins from the digestive glands cannot be rejected as a cause of these latter changes.

There is an indication that MAD mussels converted GTX_4 to GTX_1 rapidly as evidenced by the slight decrease in the molar concentration of GTX_4 relative to other toxins in the mussels during the first bloom. The subsequent apparent biotransformation of GTX_1 to GTX_4 in the mussels during the inter-bloom is difficult to explain given that the chemical equilibrium favours formation of the 11-α hydroxysulfate epimer. The net result of these two activities is the overall increase in GTX_4 from the pre-bloom to the inter-bloom. Following the second bloom, the ratio of GTX_4:GTX_1 was reversed, favouring the presence of GTX_4 in MAD mussels, which indicates a change in the behaviour of these mussels with respect to the presence of these toxins; again hinting at an adaptation of MAD mussels to toxic phytoplankton blooms. The similar increase in GTX_3 in MAD mussels can be attributed to physicochemical epimerisation of GTX_1 to GTX_2 in that population.

Biotransformation is also manifested by the increase in the absolute concentration of certain toxins, namely GTX_4 and GTX_3 in the digestive gland of MAD mussels. This

increase coincides with an overall toxin concentration decrease in the mussels and occurs during the inter-bloom where the cell concentration in the water is at zero. This observation constitutes the most compelling argument for biotransformation of toxins within the mussels.

4. Anatomical distribution of toxins

An analysis of the final toxin concentrations and compositions in the two mussel fractions over the final month of the experiment, yielded evidence of differences in the toxin profiles between mussel populations and among various tissue fractions. However, in order to determine the rates of toxin transfer within the mussels, the analysis should have been based, ideally, on a study of the transfer kinetics throughout the experiment.

The amount of toxin transferred from the digestive gland to other tissues differed between CAP and MAD mussels, as did the relative toxin composition outside the viscera. More toxin and greater proportions of highly toxic derivatives were exported from the digestive gland of CAP mussels than of MAD mussels.

The relative toxin composition observed in the non-visceral tissues in the end phase of this experiment cannot be assigned to preceding phases, as toxin distribution in different tissues was studied at the end of the experiment only. Bricelj et al. (1990 a) analyzed different mussel tissues for their toxin content and found that although the viscera contributed 30% of the total wet weight, this fraction contained 96% of the total toxicity during the toxin uptake phase. This contribution decreased steadily to about 60% of the total toxicity by the end of detoxification. The viscera were found to eliminate

70% of its total toxin burden in the 18 hours following the removal of toxic organisms from its diet, while only 2% of the toxin in the other tissues was eliminated in the same time interval (Bricelj et al, 1991). In light of this, it can be inferred that at least some of the toxin detected in the non-visceral tissues of CAP and MAD mussels may be residue from the first bloom. Although MAD and CAP mussels seemed to have similar patterns of uptake and detoxification during and following the second bloom, the total toxin content of the non-visceral tissues differed, with the chronically exposed mussels transferring and storing more toxin outside the digestive gland than pristine mussels. This suggests that a complete adaptation of MAD mussels to the PSP toxins requires that they be exposed to more than one bloom.

Although variations in temperature and salinity are known to alter the rate of toxin elimination in mussels (Prakash et al., 1971), they can be excluded as a potential contributing factors to the differences in the rates of toxin accumulation and elimination by the mussels. These environmental variables were not found to be significantly different between the two blooms. The effect of variations in water turbidity on toxin uptake and elimination from the mussels has not been previously investigated. Water turbidity would reflect the amount of phytoplankton and suspended sediments, and therefore should have an effect on the uptake and the release of toxin.

B. Field study of *in situ* mussel populations (1987)

1. Mussels

A preliminary screening of the PSP toxin content in mussels at various sites was carried out to identify chronically exposed versus pristine mussel populations. The sites were selected initially on the basis of the PSP toxicity classification scheme of Beaulieu and Menard (1985). The three sites retained for the screening, TP, MT and CC, were classified as low (mean $<50\mu$gSTXeq $100g^{-1}$), moderate (mean: 50-80μgSTXeq $100g^{-1}$) and high (mean $>80\mu$gSTXeq $100g^{-1}$) toxicity respectively.

The PSP toxin content differed among the three populations in both total toxin levels in the mussels, and in their relative molar composition. CC mussels were the most toxic, but for equivalent amounts of total toxin, MT mussels contained a higher proportion of carbamate derivatives than CC mussels. In view of the experiments carried out subsequently on pristine and chronically-contaminated mussels, and assuming a similar behaviour for all mussels, the results of the toxicity analysis suggest that TP mussels were pre-exposed to at least one bloom of *Alexandrium* cells. Had this not been the case, the toxicity levels would have been higher relative to the other sites. Although this deduced exposure did not occur between 1984 and 1987 (Fig. 17 in Appendix) it is nevertheless confirmed by the fact that Beaulieu and Menard (1985) classified TP as a low toxicity site. Based on the HPLC data obtained from the samples collected in 1987, this site could be mistaken as a moderate toxicity site, a discrepancy which exemplifies the inter-annual variation in the toxicity documented by Beaulieu and Menard (1985).

Thus, TP mussels were not be retained for the mussel transplant experiment, where pristine mussels were needed.

Variations in relative and absolute content of toxin in the mussels collected from the three sites can be attributed to differences in spatial and temporal factors (Beaulieu and Menard, 1985), physiological differences (Hall et al., 1990) or physical parameters (Therriault et al., 1985).

2. *Alexandrium* cells

Hall et al. (1990) showed variations in the relative toxin content of *Alexandrium* strains blooming in different regions as a result of natural or induced variations in physiological parameters. An attempt was made to infer the toxin profiles of *Alexandrium* blooms responsible for PSP toxicity at CC, MT and TP by examining the toxin composition of the mussels collected at these sites. Mussels from CC and MT showed similar toxin profiles which suggests that the same *Alexandrium* strain bloomed at both sites. The different toxin spectrum in TP mussels indicates that they were contaminated by a different *Alexandrium* strain (Alam et al., 1979 and Cembella et al., 1987). However, according to the mussel transplant experiment, the toxin patterns of the mussels do not necessarily correspond to those of the toxic phytoplankton, since the mussels may retain, biotransform or eliminate certain toxins selectively. Although the concentrations of certain toxins differed significantly among the three populations, it is unclear whether these differences, particularly between CC and TP, reflect differences

in mussel metabolism of these toxins or are due to contamination by different *Alexandrium* strains.

C. Source of variation

The observed variations in toxin patterns among the three populations, as measured by the coefficient of variability (CV), may be attributed to bloom patchiness and water turbulence, mussel clearance rates, or low toxin concentrations in the mussel samples. According to Thompson (1984), there is no seasonal variability in the clearance rates of *Mytilus edulis*. In general, clearance rates are a function of particle size distribution, density and their nutritional quality, all of which can vary from site to site.

Although the CV of total toxin concentration in TP, MT and CC mussels was high, ANOVA differentiated the three mussel populations, showing that the variance in toxin concentration between the three sites was significantly greater than that within each population. Large individual variation in toxin levels in mussels within a given site are not likely according to Prakash et al. (1971), although they have been demonstrated in Atlantic surfclams (Shumway et al., 1993) and a variety of other species (White, et al., 1993). The latter authors reported an overall CV in four shellfish species of 48.5%, compared to about 100% variability in our samples. These differences were attributed to bloom patchiness and water turbulence, since the organisms may have been collected a few hundred meters from each other. Mollusks less than 25 kilometres distant from each other were found to have a heterogenous distribution of toxins (Hall et al., 1979). Bloom patchiness and water turbulence could have been a factor in increasing the vari-

ability in toxin concentration from the mussels collected in the inter-tidal zones of CC, MT and TP, since the sampling was not limited to a small area within each site. In the mussel transplant experiment, the mussel samples were placed in tandem cages and the *Alexandrium* cells were collected from the immediate proximity, thus eliminating the potential effect of bloom patchiness as a source of variability in toxin concentration.

Variability among samples can also occur when the toxin concentration approaches the lower HPLC detection limit, as was the case of the mussels collected at the TP site. This variability was minimised in the mussel transplant experiment, by limiting the toxin analysis to the digestive gland of the mussels, thus maximising the concentration of toxins in the samples.

Spawning, which can be activated by abnormally high dinoflagellate densities (Bricelj et al., 1990 a), has an inhibiting effect on the feeding activity of *Mytilus edulis* (Newell and Thompson, 1984). Reproduction could have contributed to a high CV in the present experiments; however, the abundant and diverse phytoplankton assemblage that existed prior to our first bloom (B.G. Huppertz, personal communication), would have favoured the spawning of the mussels prior to the initiation of sampling. Under these circumstances, normal filtration rates should have been restored within a week of adaptation to the new environment.

D. Mussels weight and size-specific factors

The filtration rate of *Mytilus edulis* is reported by Ali (1970) and Jones et al. (1992) to be weight-specific; as such, small mussels would tend to accumulate more toxin

per unit weight than large mussels (Aalvik and Framstad, 1981). Thus, MT and TP mussels should have accumulated less toxin per unit weight than the larger CC mussels. It follows that, to compare the toxin concentrations quantitatively, a weight factor, reflecting the filtration rates, should be taken into consideration. This was not monitored in light of the field nature of the experiment and the objectives sought. However, given that the mussel collection was at random, it can be assumed that the results obtained are a true representation of the mussel population at the sites.

Although filtration rates tend to decrease with the increased weight of the mussel (Ali, 1970, and Jones et al., 1992), and cell ingestion rate is proportional to mussel weight (Bricelj et al. 1990 a and b), the difference in toxin accumulation between CAP and MAD mussels during the first bloom cannot be attributed to this factor alone. The difference in toxicity between the two groups was insignificant in response to the second bloom, even though the differences in digestive gland weights were still significant. A probable explanation for the difference in toxin accumulation would be that MAD mussels have adapted physiologically to the presence of PSP toxins.

Mussel weights are known to vary seasonally by a factor of four (Bayne and Worrall, 1980), and feeding status of the mussels has an impact on the weight ratio of digestive gland to total body weight (Hawkins et al., 1990). Bricelj et al. (1990 a) found that the viscera contributed 30% of the total body weight. In our experiment, data on total mussel weight was collected only during a period when the food supply in the water column was low, therefore these ratios of digestive gland weight to total body weight are

not necessarily representative of the entire season and may not be reliable for direct comparisons with the bioassay data.

The correlation of mussel weight to shell height in the mussel transplant experiment did not give an adequate fit to any known curvilinear expression. This is probably due to the fact that the sampling targeted mussels within a narrow size range, in order to minimise the variance among the mussels. It could also be attributed to the fact that the mussels were frozen and some may have been dissected prior to complete thawing, possibly resulting in an overestimate of their weights. Thus, it was not possible to reliably correlate shell dimensions to digestive gland weight, data that could have been useful in estimating total mussel toxicity, and thus comparisons with mouse bioassay toxicity.

E. Bioassay and HPLC correlations

Correlation of toxicity data from HPLC to those from mouse bioassay for the mussels collected from CC in 1987 gave a high correlation coefficient even though the two sets of results did not originate from replica of the same mussel samples. The observed overestimation of toxicity using the mouse bioassay technique, can be attributed to several factors. The toxin extraction procedures differ for the HPLC and Bioassay analyses. Bioassay samples were extracted in hot HCl, whereas HPLC samples were extracted in acetic acid. The first procedure results in more completed extraction of the toxins and enhances the conversion of N-Sulfocarbamates to carbamates, thus increasing the toxin concentration and the toxicity values. The toxin-specific conversion factors

may also contribute to an underestimation of the toxicity values using the HPLC technique, because of the lack of purified individual toxins for accurate bioassay determination. Also, the correlation was based on HPLC data originating from samples which were stored frozen for two years prior to their extraction and analysis. Although there are no reports of decrease in toxicity over time in frozen samples, this possibility can not be excluded (Shumway and Cembella, in press). Schantz et al. (1958) noted a protective effect of salt on the mice, which would cause an overestimation of toxin levels in the samples.

Sullivan et al. (1983 a) and Sullivan and Wekell (1986) compared results obtained through both techniques and found that the correlation between the two was higher for samples with low toxicity, and decreased as the toxicity reached level above 200μgSTXeq $100g^{-1}$ tissue. Park et al. (1986) also found a lower correlation of bioassay results for shellfish samples at higher toxicity levels. Our results are in contrast to those presented by the above authors in that the correlation coefficient is higher at concentrations above 200μgSTXeq $100g^{-1}$ tissue. Thus, a correlation of the data from the other populations, namely MT and TP, would not have given less reliable results because of their lower levels of toxicity.

SUMMARY AND RECOMMENDATIONS FOR FUTURE STUDIES

In summary, previous exposure to PSP toxins can affect the response of mussels, specifically by affecting the uptake, accumulation, detoxification and biotransformation kinetics. Pristine mussels accumulated less toxin during an initial intense bloom, suggesting that they have a lower threshold limit beyond which they cannot accumulate toxins, or are relatively inhibited from toxin uptake or are more efficient at toxin elimination. This discrepancy seemed to have been eliminated during the second exposure to PSP toxins. The toxicity of naive MAD mussels was relatively higher than that of chronically exposed CAP mussels, with respect to total concentration of toxins. Mussels exposed previously to PSP toxin metabolise the toxins more efficiently to retain the less toxic derivatives, whereas the naive mussels retained a higher proportion of the more toxic derivatives within their digestive glands. However, if the transformation of sulfocarbamoyl toxins to carbamate derivatives is solely a chemical process, it is possible that, with time, chronically exposed mussels evolved a mechanism blocking this reaction.

The results of these experiments suggest that the use of transplanted mussel stocks from previously uncontaminated areas may ameliorate the risk of extreme toxin accumulation levels in areas subject to episodic toxic dinoflagellate blooms. These properties can have important implications for the shellfish industry since they suggest that in the event of a toxic bloom, previously exposed mussels are likely to accumulate less of the highly toxic derivatives than would naive mussels and therefore appear to be safer for human consumption. However, they would retain the toxins for a longer period of time.

The next step in such a study would be to repeat the latter part of the experiment with the pre-exposed MAD mussels as well as new mussels from the Magdalen Islands to verify the response of the mussels. Should the two MAD populations respond similarly, it would be an indication that the adaptive mechanism is genetically induced, otherwise, it could be deduced that the response of the mussels to the toxin is purely physiological.

REFERENCES

Aalvik, B. and K. Framstad, 1981. Assay and detoxification experiments with mytilotoxin in mussels (*Mytilus edulis* L.) from Nordasstraumen, Western Norway, 1979 and 1980. Sarsia 66(2):143-146.

Alam, M.L., C.P. Hsu and Y. Shimizu, 1979. Comparison of toxins in three isolates of *Gonyaulax tamarensis* (Dinophycea). Journal of Phycology 15:106-110.

Ali, R.M., 1970. The influence of suspension density and temperature on the filtration rate of *Hiatella arctica*. Marine Biology 6:291-302.

Anderson, D.M. and T.P.O. Cheng, 1987. Intracellular localization of saxitoxins in the dinoflagellate *Gonyaulax tamarensis*. Journal of Phycology 24:17-23.

Anderson, D.M. and D.M. Kulis, 1993. Short term toxin content variability in *Alexandrium*. Proceedings of the Fifth International Conference on Toxic Marine Phyto-plankton, Newport, Rhodes Island, U.S.A. October 28-November 1, 1991. Elsevier Science Publishing Co. Inc., North Holland.

Anderson, D.M., J.J. Sullivan and B. Reguera, 1989. Paralytic shellfish poisoning in Northwest Spain: the toxicity of the dinoflagellate *Gymnodinium catenatum*. Toxicon 27(6):665-674.

Association of Official Analytical Chemists, 1984. Official Methods of Analysis, 14th ed. (Editor: Horowithz, W.), Washington.

Balech, E., 1985. The Genus *Alexandrium* or *Gonyaulax* of the *tamarensis* group. In Toxic Dinoflagellates, pp.33-38 (Editors: Anderson, D.M., White, A.W. and Baden, D.G.). Proceedings of the Third International Conference on Toxic Dinoflagellates, St. Andrews, New Brunswick, Canada, June 8-12, 1985. Elsevier Science Publishing Co. Inc., North Holland.

Bates, H.A. and H. Rapoport, 1975. A chemical assay for saxitoxin, the paralytic shellfish poison. Journal of Agricultural Food Chemistry 23:237-239.

Bayne, B.L. and C.M., Worrall, 1980. Growth and production of mussels *Mytilus edulis* from two populations. Marine Ecological Progress Series 3:317-328.

Beaulieu, J.L. and J. Menard, 1985. Study of the Quebec shellfish toxicity data (1955-1983). In: Toxic Dinoflagellates, pp.445-450 (Editors: Anderson, D.M., White, A.W. and Baden, D.G.). Proceedings of the Third International Conference on Toxic Dinoflagellates, St. Andrews, New Brunswick, Canada, June 8-12, 1985. Elsevier Science Publishing Co. Inc., North Holland.

Blogoslawski, W.J., 1988. Ozone depuration of bivalves containing PSP: Pitfalls and possibilities. Journal of Shellfish Research 7:702-705.

Blogoslawski, W.J. and M.E. Stewart, 1978. Paralytic shellfish poison in *Spisula solidissima*: Anatomical location and ozone detoxification Marine Biology 45:261-264.

Boczar, B.A., M.K. Beitler, J. Liston, J.J. Sullivan and R.A. Cattolico, 1988. Paralytic shellfish toxins in *Protogonyaulax tamarensis* and *Protogonyaulax catenella* in axenic culture. Plant Physiology 88:1285-1290.

Bond, M. and J.C. Medcof, 1958. Epidemic shellfish poisoning in New Brunswick, 1957. Canadian Medical Association Journal 79:19-24.

Boyer, G.L., J.J. Sullivan, R.J. Andersen, F.J.R. Taylor, P.J. Harrison and A.D. Cembella, 1986. Use of High-Performance Liquid Chromatography to investigate the production of paralytic shellfish toxins by *Protogonyaulax* spp. in culture. Marine Biology 93:361-369.

Bricelj, V.M., J.H. Lee, A.D. Cembella and D.M. Anderson, 1990(a). Uptake kinetics of paralytic shellfish toxins from the dinoflagellate *Alexandrium fundyense* in the mussel *Mytilus edulis*. Marine Ecology Progress Series 63:177-188.

Bricelj, V.M., J.H. Lee, A.D. Cembella and D.M. Anderson, 1990(b). Uptake of *Alexandrium Fundyense* by *Mytilus Edulis* and *Mercenaria Mercenaria* under controlled conditions. In: Toxic Marine Phytoplankton, pp.269-274 (Editors: Granéli, E., Sundstrom, B., Edler, L. and Anderson, D.M.). Proceedings of the Fourth International Conference on Toxic Marine Phytoplankton, Lund, Sweden, June 26-30, 1989. Elsevier Science Publishing Co. Inc., North Holland.

Bricelj, V.M., J.H. Lee and A.D. Cembella, 1991. Influence of dinoflagellate toxicity on uptake and loss of paralytic shellfish toxins in the northern quahog *Mercenaria mercenaria*. Marine Ecology Progress Series 74:33-46.

Bricelj, V.M., M. Greene, J.H. Lee and A.D. Cembella, 1993. Growth response and fate of dinoflagellate cells and paralytic shellfish poisoning (PSP) toxins in mussels, *Mytilus edulis*. Proceedings of the Fifth International Conference on Toxic Marine Phytoplankton NewPort, Rhodes Island, U.S.A., October 28-November 1, 1991. Elsevier Science Publishing Co. Inc., North Holland.

Buckley, L.J., Y. Oshima and Y. Shimizu, 1978. Construction of a Paralytic Shellfish Toxin Analyzer and Its Application. Analytical Biochemistry 85:157-164.

Carlson, R.E., M.L. Levet, B.W. Lee and P.E. Guire, 1984. Development of immunoassays for paralytic shellfish poisoning: A radioimmunoassay for saxitoxin. In: Seafood Toxins, pp.181-192 (Editor: Ragelis, E.P.), American Chemical Society, Washington.

Cembella, A.D. and F.J.R. Taylor, 1985. Biochemical variability within the *Proto gonyaulax tamarensis/catanella* species complex. In: Toxic Dinoflagellates, pp.55-60 (Editors: Anderson, D.M., White, A.W. and Baden, D.G.). Proceedings of Third International Conference on Toxic Dinoflagellates, St. Andrews, New Brunswick, Canada, June 8-12 1985. Elsevier Science Publishing Co. Inc., North Holland.

Cembella, A.D. and G. Lamoureux, 1993. A competitive inhibition enzyme-linked immunoassay for the detection of paralytic shellfish toxins in marine phytoplankton. Proceedings of the Fifth International Conference on Toxic Marine Phytoplankton, NewPort, Rhodes Island, U.S.A., October 28-November 1, 1991. Elsevier Science Publishing co., Inc., North Holland.

Cembella, A.D., J.J. Sullivan, G.L. Boyer, J.R. Taylor and R.J. Andersen, 1987. Variation in paralytic shellfish toxin composition within the *Protogonyaulax tamarensis/catenella* species complex; Red tide dinoflagellates. Biochemical Systematics and Ecology 15(2):171-186.

Chin, C.D., 1970. Neutralization of shellfish poison by chemical disinfectants. Toxicology and Applied Pharmacology 16:430-433.

Chu, F.S. and T.S L. Fan, 1985. Indirect enzyme-linked immunosorbent assay for saxitoxin in shellfish. Journal of the Association of Official Analytical Chemistry 68:13-16.

Cucci, T.L., S.E. Shumway, R.C. Newell and C.M. Yentsch, 1985. A Preliminary study of the effects of *Gonyaulax Tamarensis* on feeding in bivalve molluscs. In: Toxic Dinoflagellates, pp.395-400 (Editors: Anderson, D.M., White, A.W. and Baden, D.G.). Proceedings on the Third International Conference on Toxic Dinoflagellates, St. Andrews, New Brunswick, Canada, June 8-12. Elsevier Science Publishing Co. Inc., North Holland.

Dale, B., A. Madsen, K. Nordberg and T.A. Thorsen, 1993. Evidence for prehistoric and historic "blooms" of the dinoflagellate *Gymnodinium catenatum* in the Kattega-Skagerrak region of Scandinavia. Proceedings of the Fifth International Conference on Toxic Marine Phytoplankton, NewPort, Rhodes Island, U.S.A., October 28-November 1, 1991. Elsevier Science Publishing Co. Inc., North Holland.

Davio, S.R. and P.A. Fontelo, 1984. A competitive displacement assay to detect saxitoxin and tetrodotoxin. Analytical Biochemistry 141:199-204.

Desbiens, M., F. Coulombe, J. Gaudreault, A.D. Cembella and R. Larocque 1990. PSP toxicity in wild and culture blue mussels induced by *Alexandrium excavatum* in Gaspé Bay: Implications for aquaculture. In: Toxic Marine Phytoplankton, pp.459-462 (Editors: Granéli, E., Sundstrom, B., Edler, L. and Anderson, D.M.). Proceedings of the Fourth International Conference on Toxic Marine Phytoplankton, Lund, Sweden, June 26-30, 1989. Elsevier Science Publishing Co. Inc., North Holland.

Dodge, J.D., 1984. Dinoflagellate taxonomy. In: Dinoflagellates, pp.17-42 (Editor: Spector, D.L.), Academic Press, Orlando, Florida.

Doucette, G.J. and D.M. Anderson, 1993. Intracellular distribution of saxitoxin in *Alexandrium fundyense*. Proceedings of the Fifth International Conference on Toxic Marine Phytoplankton, NewPort, Rhodes Island, U.S.A., October 28-November 1, 1991. Elsevier Science Publishing Co. Inc., North Holland.

Fremy, J.M., M. Ledoux, E. Nezan, C. Piclet and H. Belvez, 1991. Evolution de la présence de toxines paralysantes dans les coquillages lors de l'épisode toxique en Aber Wrach'h (1988). Toxicorama 1:23-28.

Gershey, R.M, R.A. Neve, D.L. Musgrave and P.B. Reichardt, 1977. A colorimetric method for determination of saxitoxin. Journal Fisheries Research Board of Canada 34:559-563.

Gestal-Otero, J.J., J.M. Hernandez Cochon, O. Bao Fernandez y L. Martinez-Risco Lopez, 1978. Brote de Mitilotoxismo en la Provincia de la Coruna. Bol. Inst. Espa. Ocean. 258(4):5-29.

Hall, S., 1982. Toxins and toxicity of *Protogonyaulax* from the Northeast Pacific. 196p. Ph.D. Dissertation, University of Alaska, Fairbanks.

Hall, S. and P.B. Reichardt, 1984. Cryptic paralytic shellfish toxins. In: Seafood Toxins, pp.113-123 (Editor: Ragelis, E.P.). ACS Symposium Series 262, American Chemical Society, Washington.

Hall, S., R.A. Neve, P.B. Reichardt and G.A. Swisher Jr., 1979. Chemical analysis of paralytic shellfish poisoning in Alaska. In: Toxic Dinoflagellate Blooms, pp.345-350 (Editors: Taylor, D.L. and Seliger, H.H.). Proceedings of the Second International Conference on Toxic Dinoflagellate Blooms, Key Biscane, Florida, October 31-November 5, 1978. Elsevier Sciences Publishing Co. Inc., North Holland.

Hall, S., G. Stichartz, E. Moczydlowski, A. Ravindran and P.B. Reichardt, 1990. The Saxitoxins: Source, chemistry and pharmacology. In: Marine Toxins: Origin, Structure and Molecular Pharmacology, pp.29-65 (Editors: Hall, S. and Stichartz, G.). ACS Symposium Series, American Chemical Society, Washington.

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*. Marine Biology 105(2):197-204.

Hayhome, B.A. and L.A. Pfiester, 1983. Electrophoretic analysis of soluble enzymes in five freshwater dinoflagellate species. American Journal of Botany 70:1165-1172.

Hurst, J.W. and E.S. Gilfillan, 1977. Paralytic shellfish poisoning in Maine. Proceedings of the Tenth National Shellfish Sanitation Workshop, June 29-20, 1977. Hunt Valley Maryland, pp.152-161 (Editor: Wilt D.S.). U.S. Dept. Health, Education and Welfare.

Jonas-Davies, J., J.J. Sullivan, L.L. Kentala, J. Liston, W.T. Iwaoka and L. Wu, 1984. Semiautomated method for the analysis of PSP toxins in shellfish. Journal of Food Science 49:1506-1516.

Jones, D.H., O.G. Richards and T.A. Southern, 1992. Gill dimensions, water pumping rate and body size in mussel *Mytilus edulis* L. Journal of Experimental Marine Biology and Ecology 155:213-237.

Kodama, M., T. Ogata and S. Sato, 1989. Saxitoxin-producing bacterium isolated form *Protogonyaulax tamarensis*. In: Red Tides: Biology, Environmental Science and Toxicology, pp.363-366 (Editors: Okaichi, T., Anderson, A.D. and Nemoto, T.). Proceedings of the International Symposium on Red tides, Takamatsu, Kagawa, November 10-December 4, 1987. Elsevier Science Publishing Co. Inc., North Holland.

Kodama, M., T. Ogata, Y. Takahashi, T. Niwa and F. Matsuura, 1982. Gonyautoxin associated with RNA-containing fraction in the toxic scallop digestive gland. Journal of Biochemistry 92:105-109.

Kogure, K., M.L. Tamplin, U. Simidu and R.R. Colwell, 1988. A tissue culture assay for tetrodotoxin, saxitoxin and related toxins. Toxicon 26:191-197.

Kotaki, Y., Y. Oshima and T. Yasumoto, 1985. Bacterial transformation of paralytic shellfish toxins. In: Toxic Dinoflagellates, pp.287-292 (Editors: Anderson, A.D., White, A.W. and Baden, D.G.). Proceedings on the Third International Conference on Toxic Dinoflagellates, St. Andrews, New Brunswick, Canada, June 8-12, 1985. Elsevier Science Publishing Co. Inc., North Holland.

Ledoux, M., M. Bardouil, J.M. Fremy, P. Lassus, I. Murail and M. Bohec, 1993. Use of HPLC for toxin analysis of shellfish contaminated by an *Alexandrium minutum* strain. Proceedings of the Fifth International Conference on Toxic Marine Phytoplankton, NewPort, Rhodes Island, U.S.A., October 28-November 1, 1991. Elsevier Science Publishing co., Inc., North Holland.

Leedale, G.F., 1974. Algal Classes: Delimitations, inter-relationships and phylogeny. In: Taxonomy of Algae, pp.1-20 (Editors: Desikachary T.V. and Raja Rao, V.N.). International symposium on Taxonomy of Algae, Madras, India, December 9-16. University of Madras, Madras, India.

Maranda, L., D.M. Anderson and Y. Shimizu, 1985. Comparison of toxicity between populations of *Gonyaulax tamarensis*. Estuarine Coastal Shelf Science 21:401-410.

Meyer, K.F., 1953. Medical Progress: Food poisoning. NewEngland Journal of Medecine 249:843-852.

Mickelson, C. and C.M. Yentsch, 1979. Toxicity and nucleic acid content of *Gonyaulax excavata*. In: Toxic Dinoflagellate Blooms, pp.131-134 (Editors: Taylor, D.L. and Seliger, H.H.). Proceedings of the Second International Conference on Toxic Dinoflagellate Blooms, Key Biscane, Florida, October 31-November 5, 1978. Elsevier Science Publishing Co. Inc., North Holland.

Newell, R.I.E. and R.J. Thompson, 1984. Reduced clearance rates associated with spawning in the mussel, *Mytilus edulis* (L.) (Bivalvia, Mytilidae). Marine Biological Letters 5:21-33.

Nishitani, L., R. Hood, J. Wakeman and K.K. Chew, 1984. Potential importance of an endoparasite of *Gonyaulax* in paralytic shellfish poisoning outbreaks. In: Seafood Toxins, pp.139-149 (Editor: Ragelis, E.P.). ACS Symposium Series 262. American Chemical Society, Washington.

Ogata, T., M. Kodama and T. Ishimaru, 1989. Effect of water temperature and light intensity on growth rate and toxin production of toxic dinoflagellates. In: Red Tides: Biology, Environmental Science and Technology, pp.423-426 (Editors: Okaichi, T., Anderson, D.M. and Nemoto, T.). Proceedings of the International Symposium on Red Tides, Takamatsu, Kagawa, November 10-December 4, 1987. Elsevier Science Publishing Co. Inc., New York.

Ogata, T., T. Ishimaru and M. Kodama, 1987. Effect of water temperature and light intensity on growth rate and toxicity change in *Protogonyaulax tamarensis*. Marine Biology 95:217-220.

Oshima, Y., M. Hasegawa, T. Yasumoto, G. Hallegraef and S. Blackburn, 1987. Dinoflagellate *Gymnodinium catenatum* as the source of paralytic shellfish toxins in Tasmanian shellfish. Toxicon 25:1105-1111.

Park, D.L., W.N. Adams, S.L. Graham and R.C. Jackson, 1986. Variability of mouse bioassay for determination of paralytic shellfish poisoning toxins. Journal of the Association of Official Analytical Chemistry 69:547-550.

Prakash, A., 1963. Source of paralytic shellfish toxin in the Bay of Fundy. Journal of the Fisheries Research Board of Canada 20:983-996.

Prakash, A., J.C. Medcof and A.D. Tennant, 1971. Paralytic shellfish poisoning in Eastern Canada. Fisheries Research Board of Canada Bull. 177, Ottawa.

Price, R.J. and J.S. Lee 1971. Interaction between paralytic shellfish poison and melanin obtained from Butter Clam (*Saxidomus giganteus*) and synthetic melanin. Journal of Fisheries Research Board of Canada 28:1789-1792.

Proctor, N.H., S.L. Chan and A.J. Trevor, 1975. Production of saxitoxin by cultures of *Gonyaulax catenella*. Toxicon 13:1-9.

Ross, M.R., A. Siger and B.C. Abbott, 1985. The house fly: An acceptable subject for paralytic shellfish toxin bioassay. In: Toxic Dinoflagellates, pp.433-438 (Editors: Anderson, A.D, White, A.W. and Baden, D.G.). Proceedings on the Third International Conference on Toxic Dinoflagellates, St. Andrews, New Brunswick, Canada, June 8-12, 1985. Elsevier Science Publishing Co. Inc., North Holland.

Sako, Y., M. Adachi and Y. Ishida, 1993. Preparation and characterization of monoclonal antibodies to *Alexandrium* species. Proceedings of the Fifth International Conference on Toxic Marine Phytoplankton, NewPort, Rhodes Island, U.S.A., October 28-November 1, 1991. Elsevier Science Publishing Co. Inc., North Holland.

Schar ., E.J., 1979. Poisonous Dinoflagellates. In: Biochemistry and Physiology of the Protozoa, pp.403-420 (Editors: Levandowsky, M. and Hunter, S.H.). Academic Press, New York.

Schantz, E.J., 1984. Historical perspective on paralytic shellfish poison. In: Seafood Toxins, vol.9, pp.99-111 (Editor: Ragelis, E.P.). ACS Symposium Series 262, American Chemical Society, Washington.

Schantz, E.J., 1986. Chemistry and biology of saxitoxin and related toxins. Annals of the New York Academy of Sciences 479:15-23.

Schantz, E.J., J.D. Mold, W.D. Stanger, J. Shavel, F.J. Riel, J.P. Bowden, J.M. Lynch, R.S. Wyler, B. Riegel and H. Sommer, 1957. Paralytic shellfish poison. VI. A procedure for the isolation and purification of the poison from toxic clam and mussel tissues. Journal of the American Chemical Society 79:5230-2535.

Schantz, E.J., McFarren, E.F., Schafer, M.L., and Lewis, K.H., 1958. Purified poison for bioassay standardization. Journal of the Association of Official Analytical Chemistry 41:160-168.

Schoenberg, D.A. and R.K. Trench, 1980. Genetic variation in *Symbiodinium* (=*Gymnodinium*) *microadriatium* Freudenthal and specificity in its symbiosis with marine invertebrates. I-III Proceedings of the Royal Society of the London Series 207(B):405-460.

Scholin, C.A. and D.M. Anderson, 1993. Population analysis of toxic and non-toxic *Alexandrium* species using ribosomal RNA signature sequences. Proceedings of the Fifth International Conference on Toxic Marine Phytoplankton, NewPort, Rhodes Island, U.S.A., October 28-November 1, 1991. Elsevier Science Publishing co., Inc., North Holland.

Shimizu, Y., 1986 , Chemistry and biochemistry of saxitoxin analogues and tetrodotoxin. Annals of the New York Academy of Sciences 479:24-31.

Shimizu, Y. and M. Yoshioka, 1981. Transformation of paralytic shellfish toxins as demonstrated in scallop homogenates. Science 212:547-549.

Shimizu, Y., M. Kobayashi, A. Genenah and N. Ichihara, 1984. Biosynthesis of paralytic shellfish toxins. In: Seafood Toxins, pp.151-160 (Editor: Ragelis, E.P.). ACS Symposium Series 262, American Chemical Society, Washington.

Shimizu, Y., S. Gupta, M. Norte, A. Hori, A. Genenah and M. Kobayashi, 1985. Biosynthesis of paralytic shellfish toxins. In: Toxic Dinoflagellates, pp.271-274 (Editors: Anderson, A.D, White, A.W. and Baden, D.G.). Proceedings on the Third International Conference on Toxic Dinoflagellates, St. Andrews, New Brunswick, Canada, June 8-12, 1985. Elsevier Science Publishing Co. Inc., North Holland.

Shoptaugh, N.H., P.W. Carter, T.L. Foxall, J.J. Sasner, Jr. and M. Ikawa, 1981. Use of fluorometry for the determination of *Gonyaulax tamarensis* var. *excavata* toxins in New England Shellfish. Journal of Agricultural Food Chemistry 29:198-200.

Shumway, S.E. and T.L. Cucci, 1987. The effects of the toxic dinoflagellate *Proto gonyaulax tamarensis* on the feeding and behaviour of bivalve molluscs. Aquatic Toxicology 10:9-27.

Shumway, S.E. and A.D. Cembella, in press. The impact of toxic algae on scallop culture and fisheries.

Shumway, S.E., T.L. Cucci, L. Gainey and C.M. Yentsch, 1985. A preliminary study of the behavioral and physiological effects of *Gonyaulax tamarensis* on bivalve molluscs. In: Toxic Dinoflagellates, pp.389-394 (Editors: Anderson A.D., White, A.W. and Baden, D.G.). Proceedings of the Third International Conference on Toxic Dinoflagellates, St. Andrews, New Brunswick, Canada, June 8-12, 1985. Elsevier Science Publishing Co. Inc., North Holland.

Shumway, S.E., K. Geib, J. Barter and S. Sherman-Caswell, 1993. Toxin uptake and retention by surfclams (*Spisula solidissima*) on Georges Bank. Proceedings of the Fifth International Conference on Toxic Marine Phytoplankton, NewPort, Rhodes Island, U.S.A., October 28-November 1, 1991. Elsevier Science Publishing Co. Inc., North Holland.

Sieger, A., B.C. Abbott and M. Ross, 1984. Response of the house fly to saxitoxins and contaminated shellfish. In: Seafood Toxins, pp.193-195 (Editor: Ragelis, E.P.). ACS Symposium Series 262, American Chemical Society, Washington.

Sokal, R.R. and R.J. Rohlf, 1981. Biometry, 2nd ed. W.H. Freeman & Co., San Fransisco.

Sommer, H. and K.F. Meyer, 1937. Paralytic shellfish poisoning. Archives of Pathology 24:560-598.

Steidinger, K.A., 1983. A re-evaluation of toxic dinoflagellate biology and ecology. Progress in Phycology Research 2:147-188.

Steidinger, K.A., 1990. Species of the *tamarensis/catenella* group of *Gonyaulax* and the Fucoxanthin derivative-containing Gymnodinioids. In: Toxic Marine Phyto plankton, pp.11-16 (Editors: Granéli, E., Sundstrom, B., Edler, L. and Anderson, D.M.). Proceedings on the Fourth International Conference on Toxic Marine Phytoplankton, Lund, Sweden, 1989. Elsevier Science Publishing Co. Inc., North Holland.

Steidinger, K.A. and O. Moestrup, 1990. The taxonomy of *Gonyaulax*, *Pyrodinium*, *Alexandrium*, *Gessnerium*, *Protogonyaulax* and *Goniodoma*. In: Toxic Marine Phytoplankton, pp.522-523 (Editors: Granéli, E., Sundstrom, B., Edler, L. and Anderson, D.M.). Proceedings of the Fourth International Conference on Toxic Marine Phytoplankton, Lund, Sweden, June 26-30, 1989. Elsevier Science Publishing Co. Inc., North Holland.

Strickand, J.H. and T.R. Parsons, 1972. Practical Handbook of Sea Water Analysis, 2nd ed. Ottawa Fisheries Research board of Canada 310p. Fisheries Research Board of Canada, Bulletin, n.167.

Sullivan, J.J., 1990. High-Performance Liquid Chromatographic method applied to paralytic shellfish poisoning research. In: Marine Toxins. Origin, Structure and Molecular Pharmacology, pp.67-77 (Editors: Hall, S. and Strichartz G.). ACS Symposium Series, American Chemical Society, Washington.

Sullivan, J.J. and W.T. Iwaoka, 1983. High Pressure Liquid Chromatographic determination of toxins associated with paralytic shellfish poisoning. Journal of the Association of Official Analytical Chemists 66:297-303.

Sullivan, J.J. and M.M. Wekell, 1984. Determination of paralytic shellfish poisoning toxins by High Pressure Liquid Chromatography. In: Seafood Toxins, pp.197-206 (Editor: Ragelis, E.P.). ACS Symposium Series 262, American Chemical Society, Washington.

Sullivan, J.J. and M.M. Wekell, 1986. The application of High Performance Liquid Chromatography in a paralytic shellfish poisoning monitoring program. In: Seafood Quality Determination, pp.197-205 (Editors: Kramer, D.E. and Liston, J.). Proceedings of an International Symposium Coordinated by the University of Alaska. Sea Grant College Program, Anchorage, Alaska.

Sullivan, J.J., M.G. Simon and W.T. Iwaoka, 1983(a). Comparison of HPLC and mouse bioassay methods for determining PSP toxins in shellfish. Journal of Food Science 48:1312-1314.

Sullivan, J.J., W.T. Iwaoka and J. Liston, 1983(b). Enzymatic transformation of paralytic shellfish poisoning toxins in the Littleneck clam *Protothaca stamina*. Biochemical and Biophysical Research Communications 114:465-472.

Sullivan, J.J., J. Jonas-Davies and L.L. Kentala, 1985. The determination of PSP toxins by HPLC and autoanalyzer. In: Toxic Dinoflagellates, pp.275-280 (Editors: Anderson, D.M., White, A.W. and Baden, D.G.). Proceedings on the Third International Conference on Toxic Dinoflagellates, St. Andrews, New Brunswick, Canada, June 8-12, 1985. Elsevier Science Publishing Co. Inc., North Holland.

Sullivan, J.J., M.M. Wekell and S. Hall, 1988. Detection of paralytic shellfish toxins. In: Handbook of Natural Toxins (vol. 3) Marine Toxins and Venoms, pp.87-106 (Editor: Tu, A.T.). Marcel Dekker, Inc., New York.

Taylor, F.J.R., 1985. The taxonomy and relationships of red tide flagellates. In: Toxic Dinoflagellates, pp.11-26 (Editors: Anderson, D.M., White, A.W. and Baden, D.G.). Proceedings on the Third International Conference on Toxic Dinoflagellates, St. Andrews, New Brunswick, Canada, June 8-12, 1985. Elsevier Science Publishing Co. Inc., North Holland.

Tennant, A.D., J. Naubert and H.E. Corbeil, 1955. An outbreak of paralytic shellfish poisoning. Canadian Medical Association Journal 72:436-439.

Therriault, J.C., J. Painchaud and M. Levasseur, 1985. Factors controlling the occurrence of *Protogonyaulax tamarensis* and shellfish toxicity in the St.Lawrence estuary: Freshwater runoff and the stability of the water column. In: Toxic Dinoflagellate Blooms, pp.141-146 (Editors: Taylor, D.L. and Seliger, H.H.). Proceedings of the Second International Conference on Toxic Dinoflagellate Blooms, Key Biscane, Florida, October 31-November 5, 1978. Elsevier Science Publishing Co. Inc., North Holland.

Thompson, R.J., 1984. The reproductive cycle and physiological ecology of the mussel *Mytilus edulis* in a Subarctic non-estuarine environment. Marine Biology 79:277-288.

Turpin, D.H., P.E.R. Dobell and F.J.R. Taylor, 1978. Sexuality and cyst formation in Pacific strains of the toxic dinoflagellate *Gonyaulax tamarensis*. Journal of Phycology 14:235-238.

Twarog, B.W., T. Hidaka and H. Yamaguchi, 1972. Resistance to tetrodotoxin and saxitoxin in nerves of bivalve molluscs. Toxicon 10:273-278.

White, A.W., S.E. Shumway, J. Nassif and D.K. Whittaker, 1993. Variation in levels of paralytic shellfish toxins among individual shellfish. Proceedings of the Fifth International Conference on Toxic Marine Phytoplankton, NewPort, Rhodes Island, U.S.A., October 28-November 1, 1991. Elsevier Science Pubirshing Co. Inc., North Holland.

Winter, J.E., 1973. The filtration of *Mytilus edulis* and its dependence on algal concentration measured by a continuous automatic recording apparatus. Marine Biology 22:317-328.

World Health Organization, 1984. Aquatic (marine and freshwater) biotoxins. W.H.O., Geneva. Environmental Health Criteria (37).

APPENDIX

BIOASSAY DATA

Mouse bioassay data (Table 1 Appendix I) for the six-year period of 1984-1989 for mussels collected from the Magdalen Islands (MAD), and from the intertidal zone at Cap Chat, Metis, Trois Pistoles are presented in Figure 19. These data identify CC as the most toxic site, consistently showing at least one PSP toxin peak per year over the six years for which detailed data are available (Department of Fisheries and Oceans, Inspection Branch), with peak toxicities ranging between 1800 and 5200μgSTXeq 100g^{-1} tissue. Metis is a moderately contaminated site with sporadic peaks of high toxicity; when mussels exceeded the safety limit for human consumption of 80μgSTXeq 100g^{-1} tissue every year (except 1985), and peak toxicities of up to 470μgSTXeq 100g^{-1} tissue. Trois Pistoles mussels showed an unusually high peak toxin level of 320μgSTXeq 100g^{-1} tissue in 1987, but this site is usually characterised as a low-toxicity zone. In Magdalen Islands mussels, toxicity did not exceed the detection limit (42μgSTXeq 100g^{-1}) over the two years covered by the available data.

Figure 19: Mouse bioassay results (μgSTXeq 100 g^{-1} tissue) for mussels collected from the Magdalen Islands, Trois Pistoles, Metis and Cap Chat (1984-1989) as part of the surveillance program of the Canadian Department of Fisheries and Oceans.

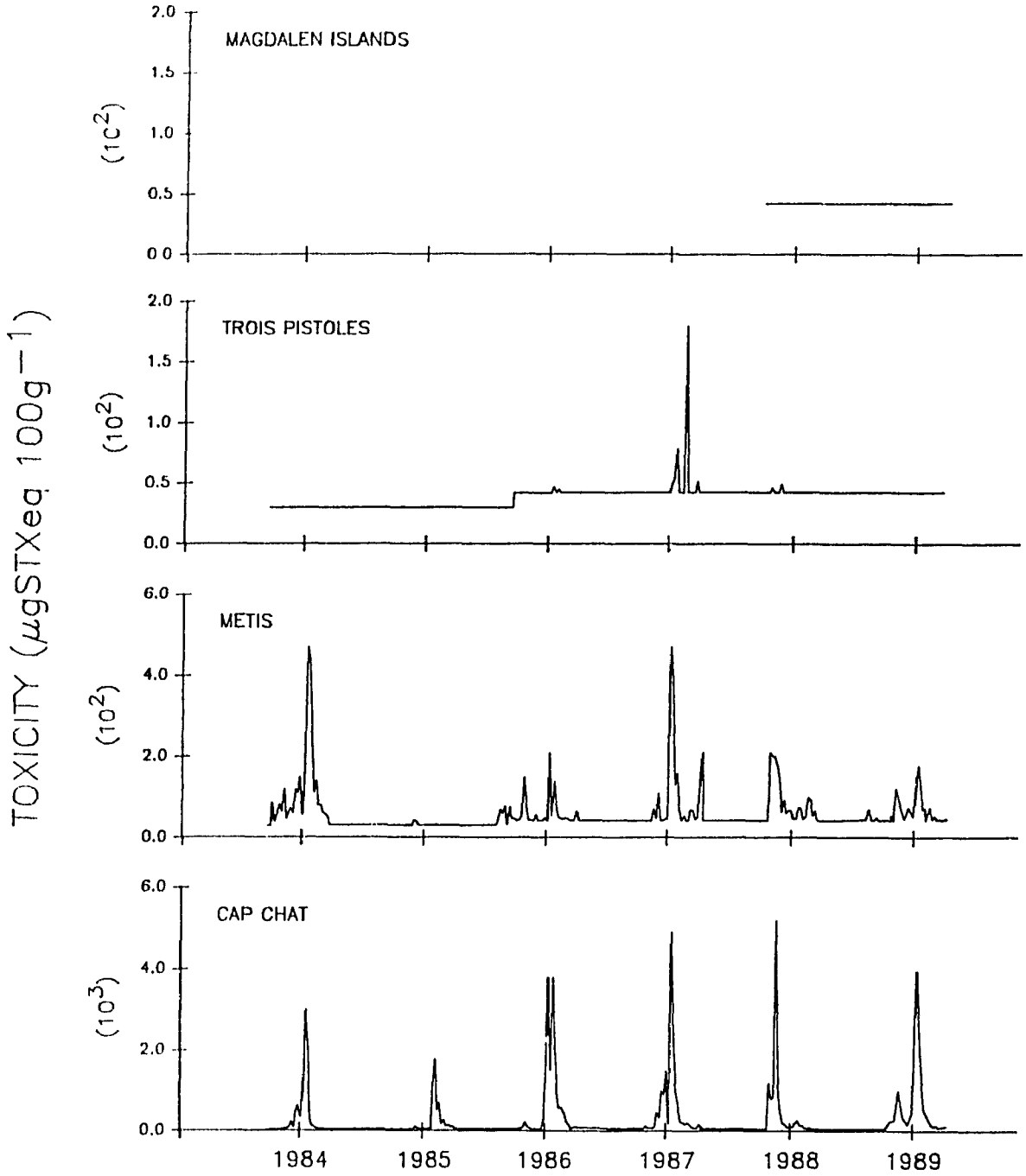

TOXICITY (μgSTXeq 100g⁻¹)
MAGDALEN ISLANDS
(10²)
2.0
1.5
1.0
0.5
0.0
TROIS PISTOLES
(10²)
2.0
1.5
1.0
0.5
0.0
METIS
(10²)
6.0
4.0
2.0
0.0
CAP CHAT
(10³)
6.0
4.0
2.0
0.0
1984
1985
1986
1987
1988
1989

Figure 20a: C_x concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Capucin (CAP) and the Magdalen Islands (MAD) to the experimental site from June to November 1990.

Inset: Concentration for August to November 1990 at 10X magnification.

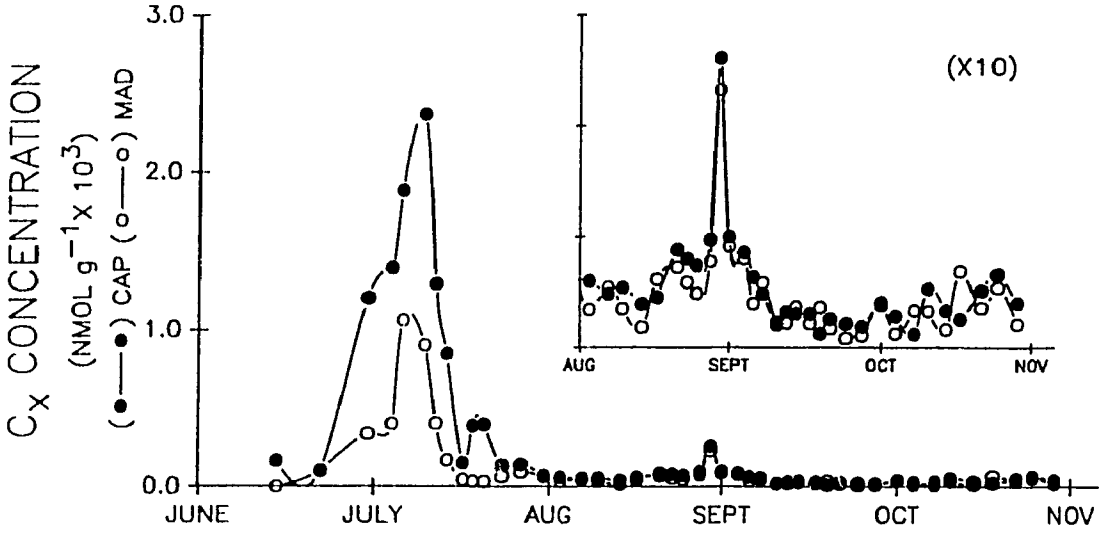

C_X CONCENTRATION
(NMOL g^{-1} X 10^3)
(●——●) CAP (o——o) MAD
3.0
2.0
1.0
0.0
JUNE
JULY
AUG
SEPT
OCT
NOV
(X10)
AUG
SEPT
OCT
NOV

Figure 20b: GTX_4 concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Capucin (CAP) and the Magdalen Islands (MAD) to the experimental site from June to November 1990.

Inset: Concentration for August to November 1990 at 5X magnification.

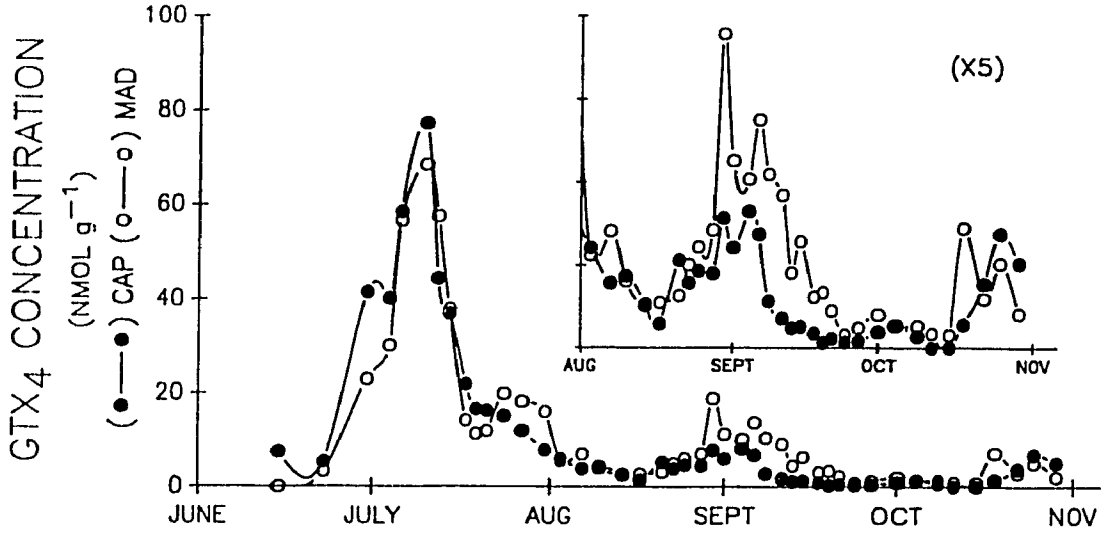

GTX4 CONCENTRATION
(NMOL g-1)
(●—●) CAP (o—o) MAD
100
80
60
40
20
0
JUNE
JULY
AUG
SEPT
OCT
NOV
(X5)
AUG
SEPT
OCT
NOV

Figure 20c: GTX_1 concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Capucin (CAP) and the Magdalen Islands (MAD) to the experimental site from June to November 1990.

Inset: Concentration for August to November 1990 at 10X magnification.

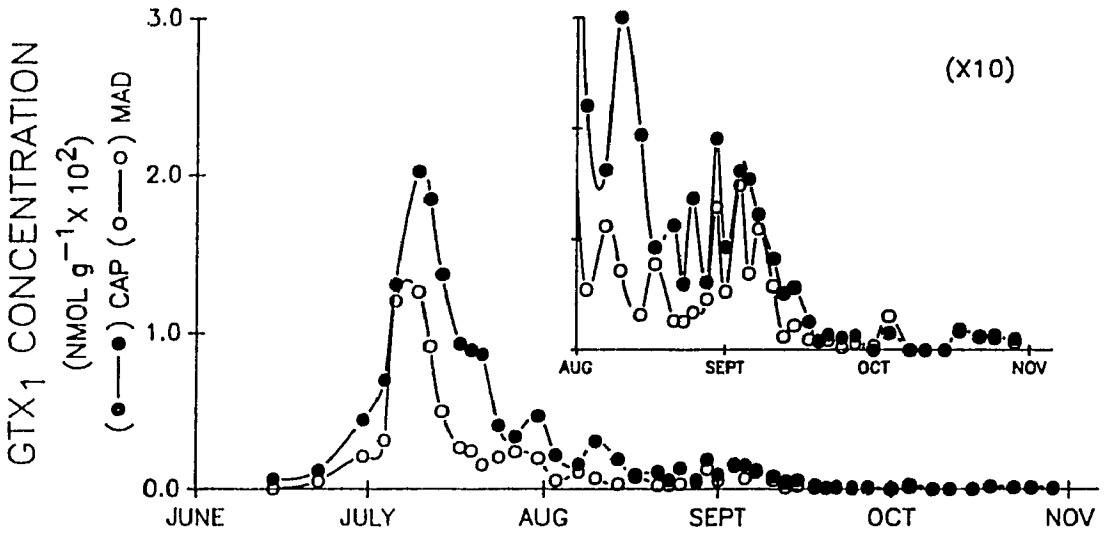

GTX1 CONCENTRATION
(NMOL g-1 X 10^2)
(●—●) CAP (o—o) MAD
3.0
2.0
1.0
0.0
JUNE
JULY
AUG
SEPT
OCT
NOV
(X10)
AUG
SEPT
OCT
NOV

Figure 20d: GTX_3 concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Capucin (CAP) and the Magdalen Islands (MAD) to the experimental site from June to November 1990.

Inset: Concentration for August to November 1990 at 4X magnification.

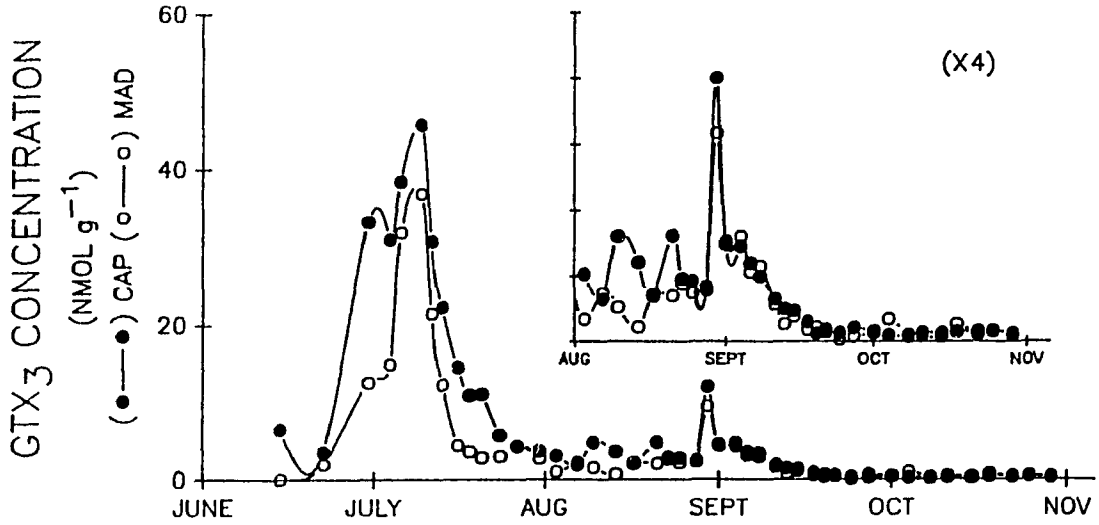

GTX3 CONCENTRATION
(NMOL g-1)
(●——●) CAP (o——o) MAD
60
40
20
0
JUNE
JULY
AUG
SEPT
OCT
NOV
(X4)
AUG
SEPT
OCT
NOV

Figure 20e: GTX_2 concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Capucin (CAP) and the Magdalen Islands (MAD) to the experimental site from June to November 1990.

Inset: Concentration for August to November 1990 at 5X magnification.

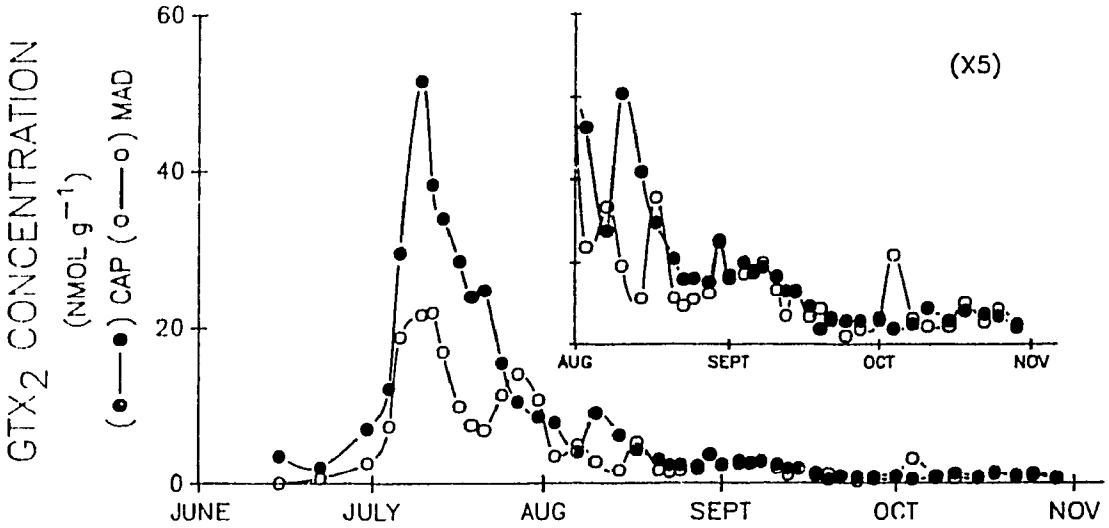

GTX2 CONCENTRATION
(NMOL g-1)
(●—●) CAP (o—o) MAD
60
40
20
0
JUNE
JULY
AUG
SEPT
OCT
NOV
(X5)
AUG
SEPT
OCT
NOV

Figure 20f: neoSTX concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Capucin (CAP) and the Magdalen Islands (MAD) to the experimental site from June to November 1990.

Inset: Concentration for August to November 1990 at 10X magnification.

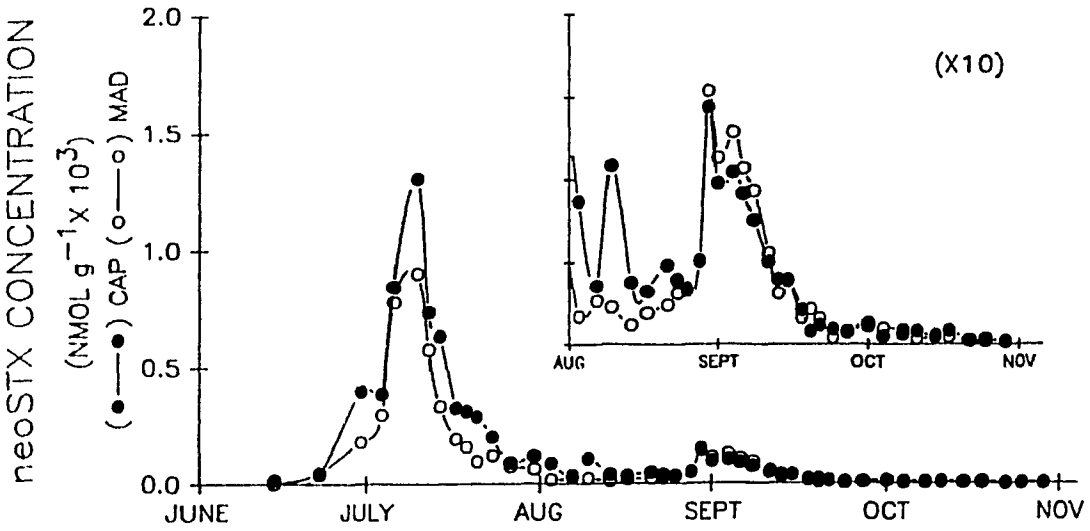
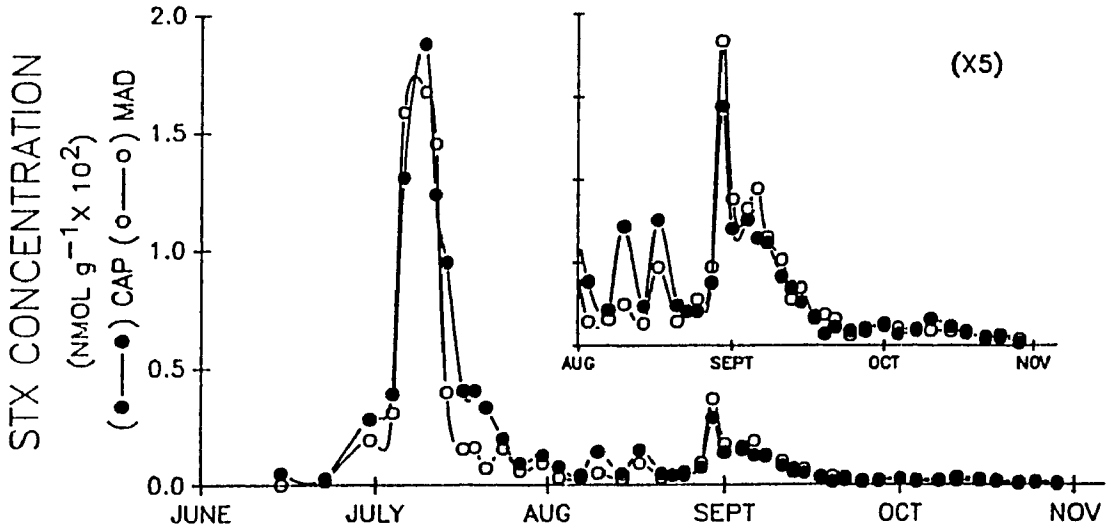

neoSTX CONCENTRATION
(NMOL g^{-1} X 10^{3})
(●——●) CAP (o——o) MAD
2.0
1.5
1.0
0.5
0.0
JUNE
JULY
AUG
SEPT
OCT
NOV
(X10)
AUG
SEPT
OCT
NOV

Figure 20g: STX concentration (nmol g^{-1}) in the digestive glands of the mussels transplanted from Capucin (CAP) and the Magdalen Islands (MAD) to the experimental site from June to November 1990.

Inset: Concentration for August to November 1990 at 5X magnification.


STX CONCENTRATION
(NMOL g^{-1} x 10^2)
(●——●) CAP (o——o) MAD
2.0
1.5
1.0
0.5
0.0
JUNE
JULY
AUG
SEPT
OCT
NOV
(X5)
AUG
SEPT
OCT
NOV

ANNEX I

Table 1: Replicate counts (rep. 1-5) of *Alexandrium* cells in the enriched fraction (20-70μ) of the net tows used for the HPLC analysis of PSP toxins in the phytoplankton; dilution factor of the cells on each filter (dil.) and number of cells per filter (Cells/filter).

		Cell count in enriched fraction of net tow					
Date	dil	rep 1	rep 2	rep 3	rep 4	rep 5	Cells/filter
28 Jun	1/100	213	225	191	197	208	3308800
03 Jul	1/100	741	601	573	642	652	10268800
05 Jul	1/10	946	1075	1073	944	1184	1671040
09 Jul	1/100	567	604	623	643	557	9584000
10 Jul	1/100	981	952	971	988	961	15529600
20 Aug	1/10	316	314	306	302	323	499200
22 Aug	1/10	159	150	126	138	162	235200
24 Aug	1/10	156	154	166	147	157	249600
27-Aug	1/10	1830	1701	1807	1604	1730	2775040
29 Aug	1/10	224	238	221	210	219	355840
31 Aug	1/10	29	25	35	27	32	47360
03 Sep	1/10	97	89	103	115	101	161600
06 Sep	1/10	68	66	49	53	59	94080
07 Sep	1/10	43	51	38	51	47	73600

Table 2: Concentration of PSP toxins (μM) in individual (4 and 5) and pooled (123) mussels collected from Trois Pistoles from June 15 to Sept 20, 1987.

	Concentration (μM)							
SAMPLE	C1	GTX4	GTX1	GTX3	GTX2	neoSTX	STX	TOTAL
15 Jun 4	0.66	0.00	0.00	0.00	0.00	0.00	0.00	0.66
5	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.50
123	0.66	0.00	0.00	0.00	0.00	0.00	0.00	0.66
23 Jun 4	0.55	0.00	0.00	0.00	0.00	0.00	0.00	0.55
5	1.45	0.19	0.00	0.00	0.00	0.21	0.00	1.85
123	0.92	0.00	0.00	0.00	0.00	0.00	0.00	0.92
29 Jun 4	1.74	0.00	0.00	0.00	0.00	0.00	0.15	1.89
5	0.95	0.50	0.00	0.00	0.00	0.06	0.13	1.64
123	1.90	0.33	0.00	0.00	0.00	0.00	0.20	2.43
06 Jul 4 5	1.34	0.00	0.00	0.00	0.00	0.05	0.10	1.49
123	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.53
	1.11	0.23	0.00	0.00	0.00	0.00	0.37	1.70
12 Jul 4 5	0.59	0.00	0.00	0.00	0.00	0.00	0.00	0.59
123	0.63	0.00	0.00	0.06	0.04	0.00	0.06	0.80
	0.46	0.00	0.00	0.00	0.00	0.00	0.00	0.46
19 Jul 4 5	1.58	0.00	0.00	0.00	0.00	0.00	0.16	1.75
123	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.60
	1.37	0.00	0.00	0.00	0.00	0.00	0.00	1.37
27 Jul 4	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.33
5	0.80	0.00	0.00	0.00	0.00	0.00	0.00	0.80
123	1.17	0.00	0.00	0.06	0.00	0.00	0.00	1.23
03 Aug 4	0.75	0.00	0.00	0.02	0.00	0.00	0.07	0.84
5	0.81	0.00	0.00	0.07	0.00	0.00	0.21	1.09
123	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.97
10 Aug 4	1.38	0.00	0.00	0.00	0.00	0.00	0.07	1.44
5	0.96	0.00	0.00	0.00	0.00	0.00	0.00	0.96
123	1.17	0.00	0.00	0.00	0.00	0.00	0.00	1.17
16 Aug 4 5	1.48	0.00	0.00	0.00	0.00	0.00	0.11	1.59
123	1.04	0.17	0.00	0.03	0.00	0.00	0.17	1.41
	1.72	0.00	0.00	0.02	0.05	0.00	0.25	2.03
24 Aug 4	1.84	0.88	0.00	0.11	0.08	0.00	0.35	3.25
5	2.05	0.23	0.00	0.12	0.10	0.00	0.48	2.99
123	2.34	0.58	0.00	0.11	0.13	0.00	0.52	3.69
30 Aug 4	1.84	0.49	0.00	0.08	0.09	0.00	0.36	2.85
5	1.47	0.00	0.00	0.00	0.00	0.00	0.00	1.47
123	1.84	0.00	0.00	0.05	0.06	0.00	0.16	2.12
06 Sep 4	1.73	0.39	0.00	0.21	0.44	0.00	1.64	4.30
5	1.32	0.36	0.00	0.08	0.17	0.00	0.85	2.78
123	1.14	0.00	0.00	0.10	0.05	0.00	0.44	1.73
13 Sep 4	5.40	0.00	0.00	0.41	0.00	0.00	0.00	5.81
5	4.94	0.00	0.00	0.04	0.14	0.00	1.18	6.30
123	2.09	0.14	0.00	0.03	0.05	0.00	0.00	2.31
20 Sep 4 5	2.50	0.00	0.00	0.06	0.08	0.00	0.13	2.77
123	1.79	0.00	0.00	0.00	0.00	0.00	0.00	1.79
	1.58	0.00	0.00	0.00	0.00	0.00	0.00	1.58

Table 3: Concentration of PSP toxins (μM) in individual (4 and 5) and pooled (123) mussels collected from Metis from June 15 to Sept 20, 1987.

	Concentration (μM)							
SAMPLE	C1	GTX4	GTX1	GTX3	GTX2	neoSTX	STX	TOTAL
15 Jun 4	0.56	0.00	0.00	0.04	0.00	0.00	0.00	0.60
5	1.33	0.00	0.00	0.00	0.00	0.00	0.00	1.33
123	2.26	0.00	0.00	0.04	0.05	0.00	0.43	2.79
23 Jun 4	1.99	0.00	0.00	0.09	0.12	0.00	0.51	2.76
5	1.70	0.00	0.00	0.00	0.13	0.00	0.61	2.44
123	1.73	0.00	0.00	0.05	0.32	0.00	0.36	2.46
29 Jun 4	4.23	0.00	0.00	0.07	0.14	0.86	0.40	5.70
5	1.21	0.00	0.00	0.00	0.00	0.00	0.33	1.54
123	2.16	0.00	0.00	0.04	0.07	0.00	0.35	2.61
06 Jul 4	3.74	0.00	0.00	0.04	0.09	0.60	0.58	5.04
5	1.67	0.00	0.00	0.04	0.11	0.49	0.44	2.75
123	1.01	0.28	0.00	0.05	0.00	0.00	0.43	1.77
12-Jul 4	1.19	0.00	0.00	0.06	0.00	0.00	0.37	1.61
5	1.09	0.00	0.00	0.06	0.13	0.00	0.41	1.68
123	2.68	0.00	0.39	0.11	0.20	0.00	0.51	3.88
19 Jul 4	1.80	0.00	0.00	0.13	0.00	0.00	0.71	2.65
5	0.88	0.00	0.00	0.11	0.00	0.00	0.50	1.48
123	0.96	0.00	0.00	0.89	0.00	0.00	0.50	2.34
27 Jul 4	0.87	0.67	0.00	0.04	0.00	0.00	0.33	1.92
5	0.41	0.00	0.00	0.00	0.00	0.00	0.47	0.89
123	1.25	0.00	0.00	0.07	0.00	1.49	0.23	3.04
03-Aug 4	0.82	0.00	0.00	0.27	0.00	0.00	0.31	1.40
5	0.64	0.19	0.00	0.00	0.00	0.00	0.25	1.09
123	0.66	0.00	0.00	0.04	0.00	0.00	0.07	0.77
10 Aug 4	1.88	0.14	0.00	0.06	0.00	0.00	0.47	2.55
5	2.04	0.39	0.00	0.13	0.14	0.00	0.45	3.14
123	2.25	0.00	0.00	0.11	0.09	0.00	0.61	3.06
16 Aug 4	1.61	0.00	0.00	0.41	0.00	0.00	0.27	2.29
5	0.99	0.00	1.63	0.10	0.09	0.25	0.47	3.53
123	1.85	0.00	0.00	0.12	0.16	0.00	0.54	2.68
24 Aug 4	9.94	0.38	1.46	1.06	0.94	0.00	9.10	22.88
5	4.98	0.17	0.00	0.60	0.72	0.17	6.98	13.63
123	5.69	0.42	2.31	0.65	0.68	1.14	4.74	15.64
30 Aug 4	4.45	0.00	0.00	0.44	0.30	0.64	2.82	8.66
5	4.14	0.00	2.87	0.31	0.51	0.47	1.57	9.87
123	5.12	0.00	0.00	0.25	0.67	0.72	1.55	8.32
06 Sep 4	2.11	0.00	0.00	0.09	0.16	0.00	0.66	3.02
5	2.33	0.00	0.00	0.11	0.09	0.00	0.96	3.49
123	2.46	0.00	0.00	0.14	0.19	0.00	0.90	3.69
13 Sep 4	1.35	0.00	0.00	0.03	0.00	0.21	0.52	2.11
5	2.44	0.11	0.00	0.03	0.06	0.38	0.57	3.59
123	3.75	0.00	0.00	0.06	0.12	0.55	0.69	5.17
20 Sep 4	4.77	0.00	0.00	0.09	0.12	0.33	0.66	5.98
5	2.32	0.00	0.00	0.03	0.00	0.00	0.00	2.35
123	2.41	0.00	0.00	0.03	0.00	0.00	0.42	2.85

Table 4: Concentration of PSP toxins (μM) in individual (4 and 5) and pooled (123) mussels collected from Cap Chat from June 15 to Sept 20, 1987.

	Concentration (μM)							
SAMPLE	Cx	GTX4	GTX1	GTX3	GTX2	neoSTX	STX	TOTAL
15 Jun 4 5	3 72	0 00	0 00	0 04	0 00	0 32	0 23	4.31
123	1 31	0 00	0 00	0 07	0 07	0 23	0 54	2 21
	4 27	0 00	0 00	0 05	0 05	0 35	0 58	5 30
23 Jun 4 5	0 84	0 31	0 00	0 03	0 03	0 28	0 21	1.70
123	3 20	0 00	0 00	0 03	0 07	0.27	0 29	3 85
	2 41	0 00	0 00	0 03	0 04	0 00	0 28	2 76
29 Jun 4	0 82	0 00	0 00	0 04	0 05	0 36	0 19	1 46
5	0 71	0 00	0 00	0 02	0 05	0 30	0 24	1.33
123	1 21	0 10	0 00	0 04	0 09	0 21	0 23	1.87
06 Jul 4 5	0 75	0 00	0 00	0 02	0 04	0.02	0 09	0.92
123	1 17	0 00	0 00	0 02	0.05	0 00	0.05	1.29
	0 67	0 09	0 00	0 02	0 03	0 00	0 05	0 86
12 Jul 4 5	0 80	0 17	0 00	0 06	0 00	0 00	0.15	1 18
123	0 91	0 13	0 00	0 03	0.04	0 22	0 93	2.26
	0 76	0 08	0 00	0 05	0 05	0 00	0 17	1 12
19 Jul 4 5	0 60	0 16	0 00	0 02	0 03	0 00	0 25	1.06
123	1 52	0 15	0 00	0 17	0 10	0 00	0 45	2 39
	0 82	0 00	0 00	0 14	0 08	0 28	0 31	1 62
27 Jul 4 5	1 63	0 15	0 07	0 20	0 22	0 26	0 56	3 10
123	1 32	0 00	0 05	0.13	0 15	0.00	0.32	1.97
	1 04	0 00	0 00	0 09	0 10	0 19	0.29	1.70
03 Aug 4 5	1 95	0 11	0 00	0 21	0 15	0 42	1 35	4 19
123	2 24	0 10	0 13	0 25	0.12	0.34	0.81	3.98
	1 72	0 00	0 23	0 18	0.07	0 38	0.77	3.34
10 Aug 4 5	1 76	0 21	0 00	0 16	0 08	0 26	0 76	3.23
123	3 74	0 19	0 14	0 11	0 08	0 19	0 61	5.06
	2 74	0 12	0 13	0 19	0 14	0 21	0 58	4 11
16 Aug 4 5	1 21	0 17	0 00	0 11	0 06	0 37	2 55	4.47
123	1 28	0 00	0 00	0 18	0 04	0 38	1 22	3.10
	1 50	0 17	0 00	0 18	0 10	0 44	1 38	3 77
24 Aug 4 5	1 69	0 00	0 20	0 22	0 11	1 01	0 89	4 13
123	2 48	0 00	0 00	0 00	0 11	0.25	0 66	3.49
	1 98	0 17	0 24	0 29	0 18	0 78	1.09	4.75
30 Aug 4 5	13 68	0 22	0 58	1 69	3 10	2 63	10 36	32 25
123	9 90	0 47	0 29	0 91	1.70	1 12	5 34	19 73
	5 97	0 11	0 23	0 89	0 84	1 45	3 09	12.62
06 Sep 4 5	6 18	0 25	0 20	1 64	1 29	0 63	4 37	14 57
123	5 46	0 26	0 40	0 79	0 44	0 66	2 52	10.52
	7 34	0 56	0 66	1 02	1 12	0.77	2 98	14 45
13 Sep 4 5	5 26	0 20	0 00	0 35	0 30	0 46	1 62	8 19
123	1 86	0 09	0 16	0 16	0 17	0 22	0 54	6 20
	1 05	0 18	0 36	0 37	0 42	0 37	1 67	7 42
20 Sep 4 5	2 20	0 10	0 17	0 12	0 21	0 24	0 18	3 20
123	1 20	0 00	0 00	0 42	0 12	0 00	0 24	1 98
	3 31	0 07	0 14	0 08	0 16	0 35	0 39	4 51

Table 5: Total mussel wet weight (g) of the individual mussel samples (4 and 5) and pooled mussels (123) collected from Cap Chat (CC), Metis (MT) and Trois Pistoles (TP) from June to September 1987.

SAMPLE		WET WEIGHT (g)		
		CC	MT	TP
15-Jun-87	4	4.34	5.32	8.16
	5	0.81	6.88	3.11
	123	5.62	3.35	3.86
23 Jun 87	4	5.9	7.78	2.00
	5	5.62	9.88	4.14
	123	3.64	5.38	3.02
29 Jun 87	4	2.34	4.17	3.76
	5	2.31	3.99	1.77
	123	4.33	3.88	4.62
06 Jul 87	4	2.46	10.66	5.66
	5	2.52	6.51	4.26
	123	2.37	6.17	4.09
12-Jul 87	4	5.39	2.96	7.65
	5	1.39	2.85	4.13
	123	0.86	2.44	3.49
19 Jul 87	4	0.61	8.83	3.87
	5	3.62	3.84	3.75
	123	3.21	7.52	2.70
27 Jul 87	4	1.69	7.55	7.75
	5	0.95	5.44	5.73
	123	2.06	4.44	2.13
03 Aug 87	4	9.1	5.28	5.97
	5	2.67	7.18	4.27
	123	5.02	5.12	3.33
10 Aug 87	4	6.18	4.55	2.62
	5	3.48	3.2	3.57
	123	3.28	6.02	3.80
16 Aug 87	4	4.08	2.55	3.30
	5	3.53	1.78	5.28
	123	4.57	5.11	2.73
24 Aug 87	4	3.33	4.61	4.47
	5	3.74	5.64	3.49
	123	4.42	3.49	3.11
30 Aug 87	4	1.84	2.7	5.59
	5	0.75	2.47	5.93
	123	2.19	1.89	3.09
06 Sep 87	4	2.65	3.53	2.64
	5	1.96	4.84	3.70
	123	3.22	3.28	3.94
13 Sep 87	4	0.82	4.2	1.99
	5	0.3	1.65	3.21
	123	0.82	2.94	2.83

Table 5 (cont'd)

SAMPLE		WET WEIGHT (g)		
		CC	MT	TP
30 Sep 87	4	0 52	10 63	2 07
	5	2 14	2 23	3.61
	1 23	3 69	1 66	3 77

Table 6: Concentration of *Alexandrium* cells (cell L^{-1}) in the Niskin bottle water samples collected at 0, 3, and 7 metres at the experiment site from May to November.

	Cell L^{-1}		
DATE	(0m)	(3m)	(7m)
24-May	0	0	0
31-May	0	0	0
07-Jun	0	0	0
14-Jun		0	0
21-Jun	0	0	0
28 Jun	122647	159314	35998
03-Jul	108235	170980	100882
05-Jul	58431	67255	13333
09 Jul	14118	12941	2745
12 Jul	588	196	490
19 Jul	980	294	0
26 Jul	1176	1471	196
02 Aug	7745	5588	2157
09-Aug	0	0	0
18-Aug	6471	10000	3922
23-Aug	490	0	392
27 Aug	2745	4706	1275
30-Aug	2157	1765	0
06 Sep	0	490	0
13 Sep	0	784	0
20 Sep	0	0	0
27 Sep	0	0	0
04 Oct	0	0	0
11-Oct	0	0	0
18 Oct	0	0	0
25 Oct	0	0	0
01-Nov	0	0	0

Table 7. Relative contribution of dominant genus and of *Alexandrium* to the total phytoplankton assemblage collected in the 20μ mesh size net tow of the water column at the experiment site from June to October.

	Percent Dominant Species (%)			
DATE	*Alexandrium*	*Thallasosira*	*Chaetoceros*	*Skeletonema*
June 7		99		
June 21		49		
June 28	13		55	
July 2	43		53	
July 5	71			
July 12		70		
July 19			93	
July 26			72	
Aug 2			86	
Aug 9			63	
Aug 16			63	
Aug 23	57			
Aug 30	1		95	
Sept 13			97	
Sept 20				39
Oct 4			64	
Oct 11			40	
Oct 18				45

Table 8: Concentration of PSP toxins in the *Alexandrium* cells (μM) during each of the two blooms at the experiment site.

	Concentration (μM)							
DATE	Cx	GTX4	GTX1	GTX3	GTX2	neoSTX	STX	TOTAL
28 Jun	2.62	0.14	0.00	0.26	0.07	0.54	0.00	3.63
03-Jul	9.63	0.11	0.30	0.09	0.09	2.89	0.43	13.54
05-Jul	32.29	0.74	2.83	1.08	0.69	35.39	4.74	77.75
09-Jul	7.55	0.61	0.31	0.68	0.07	15.91	1.40	26.53
10 Jul	82.06	6.87	4.61	4.89	0.43	142.83	36.02	267.72
20 Aug	21.13	1.14	0.97	0.98	0.06	11.74	0.81	36.85
22-Aug	17.14	0.38	0.44	0.28	0.03	4.65	0.58	23.50
24-Aug	16.63	0.15	0.31	0.19	0.02	2.06	0.70	20.06
27-Aug	20.82	2.43	1.60	1.69	0.29	33.76	8.57	69.16
29-Aug	18.74	0.31	0.51	0.34	0.11	9.90	2.39	32.31
31-Aug	6.24	0.24	0.15	0.18	0.02	2.76	0.35	9.94
03-Sep	13.50	0.24	0.00	0.15	0.07	2.60	0.35	16.92
06-Sep	6.96	0.31	0.00	0.48	0.13	2.03	0.34	10.15
07-Sep	4.23	0.15	0.00	0.15	0.06	0.53	0.00	5.11

Table 9 Concentration of PSP toxins in the digestive glands of CAP mussels (μM) during the experiment, as obtained from the HPLC analysis of the mussel extracts.

	Concentration (μM)							
DATE	C1	GTX4	GTX1	GTX3	C1X2	neoSTX	STX	TOTAL
17 Jun	5[illegible] 80	2 66	2 31	2 28	1 27	6 49	1 82	75 64
21 Jun	35 93	1 88	4 22	1 24	0 73	14 63	1.04	59.67
29 Jun	304 26	13 90	14 99	11 15	2 38	133 98	9 48	590 14
03 Jul	468 98	13 50	23 53	10 41	4 13	130 59	13 13	664 27
05 Jul	646 56	20 04	44 76	13 19	10 15	289 99	44 82	1069.50
09 Jul	819 73	26 73	69 93	15 82	17 83	451 20	64 88	1466 12
11 Jul	478 16	16 41	68 31	11 37	14 16	272 61	45 68	906 69
13 Jul	284 31	12 36	45 84	7 44	11 37	211 70	31 72	604 73
16 Jul	51 71	7 40	31 44	4 89	9 63	110 72	13 61	229.39
18 Jul	134 84	5 71	30 60	3 76	8 25	107 62	13 83	304 62
20 Jul	144 61	5 88	31 18	4 01	8 97	104 70	11 97	311.31
23 Jul	46 59	5 16	13 95	1 95	5 29	69 40	6 78	149 13
26 Jul	48 04	4 06	11 46	1 46	3 58	30 81	3 19	102 62
30 Jul	24 76	2 75	16 41	1 29	3 00	42 02	4 48	94 71
03 Aug	21 35	2 16	7 84	1 10	2 80	30 84	2 75	68 87
06 Aug	12 49	1 43	5 84	0 70	1 49	12 82	1 56	41.32
09 Aug	18 23	1 50	10 67	1 65	3 14	37 31	4 95	77.95
13 Aug	13 65	0 92	6 72	1 25	2 17	13 02	1 66	39 40
16 Aug	15 82	0 51	3 18	0 72	1 53	11 19	5 24	38 19
20 Aug	31 18	1 87	3 96	1 70	1 10	16 97	1 71	58 49
23 Aug	29 0	1 43	3 16	1 04	0 86	14 30	1 50	50 37
24 Aug	26 33	1 66	1 87	0 98	0 86	11 75	1 47	47 93
[illegible] Aug	35 06	1 63	2 19	0 90	0 82	18 19	2 74	61 53
29 Aug	8[illegible] 05	3 61	6 34	4 00	1 27	47 87	9 59	158 73
31 Aug	37 33	2 27	3 45	1 65	0 90	36 40	5 24	87 13
03 Sep	29 63	2 86	5 59	1 50	1 03	36 01	5 23	81.85
05 Sep	21 46	2 33	5 21	1 21	0 88	30 83	4 35	66 28
0[illegible] Sep	16 [illegible]3	0 98	4 21	1 03	0 98	25 95	4 26	54 15
10 Sep	11 58	0 63	3 86	0 68	0 86	17 55	2 89	37 04
1[illegible] Sep	11 43	0 43	1 80	0 53	0 68	13 88	2 43	31 17

Table 9 (cont'd).

	Concentration (μM)							
DATE	CX	GTX4	GTX1	GTX3	GTX2	neoSTX	STX	TOTAL
14 Sep	10.71	0.44	1.96	0.49	0.68	13.67	1.83	29.81
17 Sep	11.40	0.33	0.95	0.33	0.51	7.80	1.31	22.65
19 Sep	6.34	0.11	0.28	0.13	0.20	2.76	0.49	10.30
21 Sep	8.66	0.19	0.47	0.18	0.33	3.91	0.75	14.49
24 Sep	7.77	0.13	0.40	0.14	0.31	3.30	0.65	12.70
27-Sep	6.50	0.15	0.45	0.22	0.29	3.71	0.59	10.91
01 Oct	14.22	0.34	0.00	0.14	0.34	4.41	0.92	20.37
04 Oct	9.81	0.47	0.54	0.10	0.20	1.64	0.48	13.23
08 Oct	4.11	0.23	0.00	0.08	0.25	2.08	0.67	7.42
11 Oct	18.03	0.00	0.00	0.14	0.45	2.72	1.08	22.43
15 Oct	11.70	0.00	0.00	0.13	0.30	1.64	0.80	14.56
18 Oct	9.40	0.51	0.62	0.16	0.45	3.01	0.62	14.78
22 Oct	17.79	1.33	0.44	0.17	0.38	0.76	0.26	21.13
25 Oct	22.76	2.38	0.38	0.17	0.36	0.71	0.31	27.08
29 Oct	13.89	1.77	0.36	0.13	0.22	0.58	0.13	17.06

Table 10 Concentration of PSP toxins in the digestive glands of MAD mussels (μM) during the experiment, as obtained from the HPLC analysis of the mussel extracts

	Concentration (μM)							
DATE	C2	GTX4	GTX1	GTX3	GTX2	neoSTX	STX	TOTAL
12 Jun	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
21 Jun	36 06	1 11	1 66	0 68	0 22	16.47	0 54	56.75
29 Jun	117 40	7 88	7 29	4 32	0 92	62 66	6 65	207 13
03 Jul	142 37	10 60	11 08	5 23	2 62	104 95	10 91	287 75
05 Jul	368 82	19 64	41 70	11 05	6 51	270 09	54 93	772.72
09 Jul	319 09	21 20	43 44	13 01	7 65	318 85	59 06	786 30
11 Jul	137 91	19 64	31 15	7 31	7 49	196 56	49 48	449 57
13 Jul	58 28	13 04	17 09	4 21	5 81	114 99	13 62	227 03
16 Jul	15 44	4 84	9 12	1 51	3 39	66 56	5 28	106 14
18 Jul	17 38	5 62	12 16	1 79	3 76	79 45	7 99	128 17
20 Jul	16 44	5 93	7 79	1 41	3 44	47 71	3 58	86.30
23 Jul	23 25	6 98	7 17	1 05	4 02	42 91	5 50	90.88
26 Jul	33 50	6 60	8 73	1 55	5 11	27 54	2 19	85 22
30 Jul	23 39	5 67	7 01	0 97	3 81	22 30	3 29	66 45
02 Aug	13 25	1 99	1 91	0 36	1 26	6 12	1 05	24 97
06 Aug	19 49	2 52	1 00	0 79	1 79	9 51	1 14	39.24
09 Aug	13 72	1 57	2 77	0 61	1 11	8 83	1 97	30 58
13 Aug	6 50	0 91	1 12	0 24	0 60	4.19	0 96	14 52
16 Aug	21 78	0 97	2 72	0 77	1 88	6 73	3 32	38.16
20 Aug	36 15	1 58	1 32	1 05	0 85	12 06	1 47	54 47
23 Aug	21 68	1 85	0 95	0 97	0 51	11 50	1 50	38 97
24 Aug	17 90	2 23	1 25	0 81	0 60	12 53	2 05	37 37
27 Aug	27 57	2 53	1 64	0 83	0 66	18 17	3 35	54 76
29 Aug	81 53	6 63	4 51	3 34	1 30	53 88	12 87	164 06
31 Aug	32 65	4 05	1 88	1 64	0 91	40 44	6 30	87 87
03 Sep	39 16	3 22	5 42	1 75	0 94	47 02	6 01	94 03
05 Sep	14 73	5 10	2 55	1 17	1 00	39 45	7 00	71 00
07 Sep	20 80	3 71	3 88	1 22	1 06	32 89	4 62	68 18
10 Sep	51	3 36	2 11	0 62	0 73	20 18	3 75	38 25
13 Sep	2 95	1 60	0 43	0 28	0 37	10 92	1 96	23 48

Table 10 (cont'd).

	Concentration (μM)							
DATE	Cx	GTX4	GTX1	GTX3	GTX2	neoSTX	STX	TOTAL
14 Sep	13 13	2 27	0 79	0 41	0 67	13 67	2 44	33 38
17-Sep	7 75	1 09	0 34	0 19	0 36	5 70	1 16	16 59
19 Sep	12 69	1 17	0 29	0 23	0 45	7 41	1 30	23 55
21 Sep	6 27	0 81	0.32	0 15	0 31	5 67	1 17	14 68
24-Sep	4 24	0 40	0.12	0 06	0 16	1 93	0 64	7 51
27-Sep	3 91	0 44	0 22	0 09	0 20	2 62	0 76	8 24
01-Oct	13 70	0 72	0 13	0 17	0 31	3 84	0 84	19 71
04-Oct	4 74	0 51	1 15	0 39	1 23	3 62	0 82	12 46
08 Oct	16 84	0 66	0 00	0 16	0 47	4 29	0 90	23 31
11 Oct	11 58	0 31	0 00	0 09	0 23	1 17	0 64	14 02
15 Oct	5 99	0 29	0 00	0 08	0 24	1 96	0 65	9 22
18-Oct	25 25	2 65	0 71	0 29	0 57	1 59	0 55	31 59
22-Oct	13 01	1 07	0 41	0 12	0 30	0 87	0 39	16 16
25 Oct	18 85	1 77	0 48	0 18	0 45	1 09	0 45	23 27
29 Oct	7 47	0 72	0 24	0 10	0 26	0 55	0 28	9 62

Table 11: Concentration of PSP toxins (μgSTXeq $100g^{-1}$ tissue) in total body weight of CAP mussels

	Concentration (μgSTXeq $100g^{-1}$)							
DATE	C x	GTX4	GTX1	GTX3	GTX2	neoSTX	STX	TOTAL
17 Jun	77 52	25 77	22 46	18.63	6.50	66.26	18 55	235 70
21 Jun	47 78	18 41	41 32	10 20	3 76	150 65	10.70	282 83
29 Jun	554 30	140 30	151 31	94 66	12 62	1422 76	100 63	2476 58
03 Jul	641 [illegible]	135 85	236 84	88 15	21 87	1382 80	139 03	2645 76
05 Jul	866 36	197 68	441 55	109 39	52 66	3009 35	465 10	5142 08
09 Jul	1089 30	261 50	684 11	130 14	91 74	4643 51	667 72	7568 03
11 Jul	593 31	149 90	623 96	87 33	68 04	2619 76	438 98	4581 29
13 Jul	389 50	124 61	462 29	63 10	60 34	2246 12	336 50	3682.47
16 Jul	70 [illegible]	73 94	314 37	41 10	50 65	1164.52	143 12	1857.92
18 Jul	179 91	56 03	300 60	31 06	42 63	1112.08	142 94	1865 27
20 Jul	183 45	54 93	291 20	31 48	44 04	1028 63	117 60	1751 33
23 Jul	63 66	51 12	138 15	16 27	27 57	722 94	70 63	1089.34
26 Jul	64 85	40 39	113 92	12 23	18 73	322 09	33 34	605.56
30 Jul	33 56	26 66	158 86	10 50	15 27	427 98	45 67	717 51
03 Aug	27 58	20 48	74 43	8 77	14 00	308 12	27 51	480 89
06 Aug	33 30	13 29	54 77	5 51	7 34	126 58	15 44	245 23
09 Aug	24 98	14 69	104 67	13 63	16 23	385 09	51 14	610 41
13 Aug	18 08	8 94	65 43	10 27	11 13	133.34	17 01	264.16
16 Aug	20 99	4 97	31 10	5 90	7 86	115 00	53 80	239 62
20 Aug	40 65	17 98	37 98	13 76	5 55	171 40	17 30	304.59
23 Aug	36 28	13 34	20 09	8 16	4 23	140 14	14 73	237 47
24 Aug	33 97	15 [illegible] 3	46 27	7 85	4 28	117 44	14 72	240 26
27 Aug	41 40	15 28	20 58	7 12	4 05	179 65	27 04	298 39
29 Aug	130 21	26 53	64 47	34 24	6 77	511 95	102 58	866 73
31 Aug	45 [illegible]	20 53	31 21	12 56	4 30	346 37	49 84	510 52
03 Sep	39 33	37 93	54 61	12 35	5 32	370 34	53 80	563 68
05 Sep	29 14	23 30	52 09	10 14	4 65	324 24	45 79	489 35
07 Sep	33 [illegible]	9 61	41 27	8 44	5 04	267 49	43 95	398 09
10 Sep	15 [illegible]	6 04	27 73	5 53	4 42	179 14	29 55	267 66
17 Sep	14 98	4 10	1 [illegible] 32	4 21	3 47	140 66	24 66	209 37

Table 11 (cont'd)

	Concentration (µgSTXeq 100g⁻¹)							
DATE	C1	GTX4	GTX1	GTX3	GTX2	neoSTX	STX	TOTAL
14-Sep	14 19	4 33	19 02	3 98	3 50	139 90	18 62	203 54
17-Sep	14 05	3 04	8 65	2 52	2 44	74 42	12 52	117 64
19-Sep	8 36	1 05	2 75	1 05	1 00	28 15	4 97	47 34
21 Sep	11 89	1 89	4 73	1 55	1 73	41 61	7 95	71 35
24 Sep	9 90	1 21	3 73	1 14	1 53	32 60	6 39	56 48
27-Sep	8 75	1 44	4 43	1 82	1 53	28 23	6 18	52 37
01-Oct	19 28	3 39	0 00	1 16	1 77	46 31	9 71	81 62
04 Oct	12 66	4 43	5 08	0 78	0 99	16 36	4 81	45 1[illegible]
08 Oct	5 34	2 21	0 00	0 64	1 [illegible]7	20 93	6 76	37 14
11 Oct	22 59	0 00	0 00	1 11	2 18	26 42	10 52	62 83
15 Oct	16 32	0 00	0 00	1 10	1 63	17 69	8 61	45 38
18 Oct	13 14	5 28	6 41	1 41	2 36	32 57	6 75	68 01
22 Oct	22 68	12 52	4 09	1 31	1 89	7 48	2 57	52 54
25 Oct	30 64	23 55	3 72	1 41	1 87	7 39	3 50	7[illegible] 08
29 Oct	19 33	18 10	3 65	1 10	1 17	6 [illegible]5	1 35	50 95

Table 12 Concentration of PSP toxin (μgSTXeq 100g⁻¹ tissue) in total body weight of MAD mussels

	Concentration (μgSTXeq 100g⁻¹)							
DATE	C1	GTX4	GTX1	GTX3	GTX2	neoSTX	STX	TOTAL
12 Jun	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21 Jun	49.44	11.21	16.75	5.80	1.18	174.90	5.75	265.04
29 Jun	159.88	79.05	73.12	36.42	4.86	660.84	70.15	1084.32
03 Jul	189.[illegible]	103.75	108.38	43.05	13.46	1080.23	112.26	1650.35
05 Jul	497.99	195.21	414.45	92.38	34.02	2824.31	574.36	4632.72
09 Jul	4[illegible].73	336.03	433.38	106.73	39.23	3271.42	606.00	5115.52
11 Jul	189.07	198.18	314.28	62.06	39.76	2086.56	525.24	3415.14
13 Jul	79.1[illegible]	130.28	170.80	35.41	30.55	1209.00	143.16	1798.32
16 Jul	21.09	48.62	91.73	12.76	17.93	703.99	55.90	952.02
18 Jul	[illegible].93	54.61	118.12	14.62	19.22	811.65	81.67	1122.80
20 Jul	21.69	57.[illegible]9	75.66	11.49	17.59	487.37	36.57	707.96
23 Jul	31.01	68.65	70.48	8.68	20.77	443.68	56.87	700.16
26 Jul	43.[illegible]3	62.67	82.90	12.41	25.55	275.22	21.84	523.82
30 Jul	30.90	55.13	68.15	7.93	19.49	228.10	33.69	443.38
0[illegible] Aug	16.10	19.28	18.77	2.92	6.42	62.33	10.65	136.47
06 Aug	[illegible].18	[illegible].26	38.50	6.43	9.06	96.30	11.56	211.58
09 Aug	16.51	13.89	21.50	4.53	5.19	82.22	18.39	165.21
13 Aug	8.56	8.79	10.87	1.95	3.05	42.70	9.81	85.73
16 Aug	29.00	9.46	26.63	6.34	9.68	69.38	34.23	184.72
20 Aug	47.69	15.38	12.80	8.55	4.32	123.17	14.98	226.88
2[illegible] Aug	[illegible].64	17.33	8.93	7.66	2.54	113.52	14.86	192.48
24 Aug	[illegible].95	21.05	11.79	6.46	3.00	124.41	20.33	209.99
[illegible] Aug	36.13	21.59	15.91	6.82	3.39	185.98	34.29	307.41
29 Aug	108.9[illegible]	65.[illegible]5	11.34	[illegible].61	6.74	557.49	133.14	943.49
31 Aug	[illegible].61	38.86	18.10	13.22	4.60	408.68	63.71	589.79
03 Sep	[illegible].39	35.10	51.19	13.87	4.67	466.87	59.67	668.76
05 Sep	18.61	[illegible].43	[illegible].71	9.19	4.91	386.01	68.51	558.36
0[illegible] Sep	[illegible].15	36.09	37.68	9.97	5.41	336.17	47.18	499.94
10 Sep	9.63	31.[illegible]0	19.88	4.92	3.63	200.37	37.22	307.35
1[illegible] Sep	10.86	15.64	4.16	2.31	1.89	112.54	20.17	167.28
14 Sep	[illegible].15	[illegible].18	[illegible].0	3.36	3.46	140.64	25.13	219.90

Table 12 (cont'd):

	Concentration (µgSTXeq 100g⁻¹)							
DATE	Cx	GTX4	GTX1	GTX3	GTX2	neoSTX	STX	TOTAL
17-Sep	10.25	10.63	3.28	1.56	1.82	58.38	11.86	97.78
19-Sep	17.15	11.59	2.93	1.91	2.37	77.55	13.62	127.11
21-Sep	8.14	7.71	3.02	1.21	1.57	57.00	11.72	90.37
24-Sep	5.60	3.86	1.12	0.46	0.79	19.68	6.55	38.07
27-Sep	5.11	4.20	2.08	0.72	1.00	26.49	7.72	47.33
01-Oct	15.37	5.94	1.11	1.17	1.36	33.34	7.28	65.56
04-Oct	6.18	4.91	11.05	3.11	6.19	36.54	8.26	76.24
08-Oct	22.45	6.46	0.00	1.32	2.42	44.23	9.28	86.16
11-Oct	14.81	2.88	0.00	0.74	1.14	11.55	6.35	37.46
15-Oct	8.07	2.85	0.00	0.69	1.27	20.41	6.82	40.10
18-Oct	34.29	26.48	7.05	2.43	2.98	16.69	5.73	95.65
22-Oct	17.27	10.44	4.04	1.00	1.52	8.90	3.99	47.16
25-Oct	24.36	16.85	4.55	1.43	2.26	10.90	4.54	64.89
29-Oct	10.28	7.34	2.48	0.86	1.38	5.81	2.94	31.09

Table 13 Mussel shell length and height (cm), digestive gland (dig.gl.), tissue, total mussel weight (g) and ratio of digestive gland weight to total mussel weight for CAP mussels

DATE	SAMPLE	SHELL LENGTH	SHELL HEIGHT	DIG GL. WEIGHT	TISSUE WEIGHT	TOTAL WEIGHT	WEIGHT RATIO
17 Jun	1	4.2	2				
	2	4	2.2				
	3	4.5	2.3				
	4	4	2				
	MEAN	4.175	2.125	0.62		5.16*	0.120
21 Jun	1	4.1	2.2				
	2	4.7	2				
	3	5.2	2.7				
	4	4.6	2.4				
	MEAN	4.65	2.325	2.25		18.74*	0.120
29 Jun	1	5.1	2.6				
	2	4.2	2.2				
	3	4.5	2.2				
	4	4.6	2.8				
	MEAN		2.45	2.35		19.57*	0.120
03 Jul	1	4.5	2.5				
	2	6	2.5				
	3	5.2	2.4				
	4	4.4	2.3				
	MEAN	5.025	2.425	2.02		16.83*	0.120
05 Jul	1	5.2	2.7				
	2	5.2	2.4				
	3	4.4	2.5				
	4	4.8	2.2				
	MEAN	4.9	2.45	2.92		24.32*	0.120
09 Jul	1	5.2	2.5				
	2	5.2	2.4				
	3	4.9	2.4				
	4	5.7	2.6				
	MEAN	5.35	2.475	4.33		36.07*	0.120
11 Jul	1	5.1	2.5				
	2	5.5	2.3				
	3	5	2.9				
	4	4.9	2.9				
	MEAN	5.125	2.65	5.75		47.90*	0.120
13 Jul	1	4.9	2.5				
	2	5	2.6				
	3	5.2	2.5				
	4	6.3	2.8				
	MEAN	5.35	2.6	5.04		41.98*	0.120
16 Jul	1	4.2	2.1				
	2	4.9	2.3				
	3	4.7	2.1				
	4	4.9	2.4				
	MEAN	4.675	2.225	3.22		26.82*	0.120

Table 13 (cont'd):

DATE	SAMPLE	SHELL LENGTH	SHELL HEIGHT	DIGESTIVE WEIGHT	TISSUE WEIGHT	TOTAL WEIGHT	WEIGHT RATIO
18-Jul	1	4.6	2.3				
	2	5.4	2.8				
	3	5.6	2.8				
	4	5.3	2.8				
	MEAN	5.225	2.675	4.83		40.23*	0.120
20 Jul	1	5.3	2.3				
	2	5.1	2.4				
	3	4.6	2.5				
	4	5.4	2.6				
	MEAN	5.175	2.45	5.08		42.32*	0.120
23 Jul	1	4.9	2.3				
	2	4.7	1.9				
	3	5	2.4				
	4	6.1	2.9				
	MEAN	5.175	2.375	4.62		38.48*	0.120
26 Jul	1	4.3	2				
	2	4.6	2.2				
	3	4.5	2				
	4	4.6	2.3				
	MEAN	4.5	2.125	3.41		28.40*	0.120
30 Jul	1	4.6	2.4				
	2	5.3	2.4				
	3	4.3	2				
	4	4	2.4				
	MEAN	4.55	2.3	4.2		34.98*	0.120
02-Aug	1	4.1	2.3				
	2	4.4	2.1				
	3	4.1	2.1				
	4	4.9	2.4				
	MEAN	4.375	2.225	3.39		28.24*	0.120
06 Aug	1	4.6	2				
	2	4.5	2.1				
	3	4.5	2.2				
	4	4.2	1.9				
	MEAN	4.45	2.05	3.25		27.07*	0.120
09 Aug	1	5	2.8				
	2	4.5	2.3				
	3	4.4	2.3				
	4	5.1	2.4				
	MEAN	4.75	2.45	4.49		37.40*	0.120
13 Aug	1	4.7	2				
	2	4.8	2.4				
	3	4.8	2.3				
	4	4.5	2.1				
	MEAN	4.7	2.225	3.84		31.90*	0.120

Table 13 (cont'd):

DATE	SAMPLE	SHELL LENGTH	SHELL HEIGHT	DIG GL WEIGHT	TISSUE WEIGHT	TOTAL WEIGHT	WEIGHT RATIO
16 Aug	1	6.4	2.8				
	2	5.3	2.3				
	3	5.4	2.8				
	4			3.99		33.24*	0.120
	MEAN	5.7	2.633				
20 Aug	1	5	2.6				
	2	5.7	2.6				
	3	4.7	2.4				
	4	4.5	2.2	3.53		29.40*	0.120
	MEAN	4.975	2.45				
22 Aug	1	4.5	2.2				
	2	4.8	2.7				
	3	4.4	2.1				
	4	5.3	2.8	3.82		31.82*	0.120
	MEAN	4.75	2.45				
24 Aug	1	5	2.5				
	2	4.9	2.6				
	3	4.5	3.3				
	4	5.8	2.7	4.46		37.15*	0.120
	MEAN	5.05	2.775				
27 Aug	1	4.6	2.3				
	2	4.7	2.4				
	3	4.9	2.5				
	4	5	2.5	3.43		28.57*	0.120
	MEAN	4.8	2.425				
29 Aug	1	5.7	2.9				
	2	4.5	2.3				
	3	5.2	2.4				
	4	5.2	2.4	4		33.32*	0.120
	MEAN	5.15	2.5				
31 Aug	1	5.3	2.7				
	2	5.6	2.8				
	3	5.5	2.8				
	4	5.5	2.8	5.62		46.81*	0.120
	MEAN	5.475	2.775				
03 Sep	1	5	2.4				
	2	4.5	2.4				
	3	5.7	2.7				
	4	6.2	3.1	4.16		34.65*	0.120
	MEAN	5.35	2.65				
05 Sep	1	5	2.2				
	2	4.9	3				
	3	5	2.3				
	4	4.6	2.2	3.39		28.24*	0.120
	MEAN	4.875	2.425				

Table 13 (cont'd):

DATE	SAMPLE	SHELL LENGTH	SHELL HEIGHT	DIGESTI WEIGHT	TISSUE WEIGHT	TOTAL WEIGHT	WEIGHT RATIO
07-Sep	1	4.5	2.2				
	2	4.7	2.5				
	3	4.7	2.6				
	4	5	2.9				
	MEAN	4.725	2.55	2.94		24.49*	0.120
10-Sep	1	4.3	2				
	2	4.6	2				
	3	4.8	2				
	4	5	2.1				
	MEAN	4.675	2.025	3.24		26.99*	0.120
12-Sep	1	5.5	2.6				
	2	5.6	2.7				
	3	6.3	2.7				
	4	5.6	2.9				
	MEAN	5.75	2.725	4.75		39.57*	0.120
14-Sep	1	4.9	2.3				
	2	5.1	2.2				
	3	4.7	2.2				
	4	4.8	2.3				
	MEAN	4.875	2.25	3.31		27.57*	0.120
17-Sep	1	5.7	2.9				
	2	4.9	3.4				
	3	4.9	2.5				
	4	4.9	2.4				
	MEAN	5.1	2.8	2.99		24.91*	0.120
19-Sep	1	4.1	2				
	2	4.5	2.2				
	3	4.7	2.3				
	4	3.5	2.1				
	MEAN	4.2	2.15	1.99		16.58*	0.120
21-Sep	1	4.9	2.6				
	2	4.3	2				
	3	4.7	2.1				
	4	5.1	3.4				
	MEAN	4.75	2.525	2.85		23.74*	0.120
24-Sep	1	4	2.1				
	2	4.4	2.2				
	3	5	2.4				
	4	5.1	2.1				
	MEAN	4.625	2.2	2.71		22.57*	0.120
27-Sep	1	6.2	3.1				
	2	5.7	2.5				
	3	4.1	2.4				
	4	4.8	2.4				
	MEAN	5.2	2.6	4.79		39.90*	0.120

Table 13 (cont'd)

DATE	SAMPLE	SHELL LENGTH	SHELL HEIGHT	DIGESTA WEIGHT	TISSUE WEIGHT	TOTAL WEIGHT	WEIGHT RATIO
01 Oct	1	4.3	2				
	2	5.3	2.4				
	3	4.7	2				
	4	4.8	2.5				
	MEAN	4.775	2.225	2.97	24.24	27.21	0.109
01 Oct	1	4.7	2.3				
	2	4.4	2.3				
	3	4.7	2.1				
	4	4.6	2.1				
	MEAN	4.6	2.2	2.94	20.2	23.14	0.127
08 Oct	1	4.5	2.3				
	2	4.8	2.3				
	3	4.9	2.4				
	4						
	MEAN	4.633	2.333	2.52	20.08	22.60	0.112
11 Oct	1	4.3	2.1				
	2	5	2.6				
	3	4.3	2.3				
	4	5	2.4				
	MEAN	4.65	2.35	3.22	22.85	26.07	0.124
15 Oct	1	5.1	2.5				
	2	4.7	2.1				
	3	4.8	2.1				
	4	5.4	2.3				
	MEAN	4.875	2.25	3.15	25.14	28.29	0.111
18 Oct	1	4.9	2.5				
	2	5.2	2.3				
	3	5.1	2.3				
	4	5.2	2.5				
	MEAN	5.1	2.4	3.3	26.62	29.92	0.110
22 Oct	1	4.8	2.5				
	2	5	2.4				
	3	5.2	2.5				
	4	5	2.5				
	MEAN	5	2.475	4.48	27.96	32.44	0.138
25 Oct	1	4.9	2.4				
	2	4.7	2.2				
	3	4.5	2.3				
	4	4.9	2.2				
	MEAN	4.675	2.275	3.1	21.69	24.79	0.125
29 Oct	1	4.4	2.3				
	2	4.9	2.5				
	3	4.9	2.3				
	4	4.7	2.4				
	MEAN	[illegible]	[illegible]	3.11	21.88	24.99	0.124

* Calculated from weight ratio

Table 14: Mussel shell length and height (cm), digestive gland (dig.gl.), tissue, total mussel weight (g) and ratio of digestive gland weight to total mussel weight for MAD mussels.

DATE	SAMPLE	SHELL LENGTH	SHELL HEIGHT	DIG GL WEIGHT	TISSUE WEIGHT	TOTAL WEIGHT	WEIGHT RATIO
12 Jun	1	7.6	3.8				
	2	8.1	3.8				
	3	7.2	3.6				
	4	5.9	3.2				
	MEAN	7.2	3.6	3.72		36.66*	0.101
21 Jun	1	5.8	3.2				
	2	6	3.2				
	3	6	3.1				
	4	6.1	3				
	MEAN	5.975	3.125	3.35		33.03*	0.101
29 Jun	1	5.2	2.9				
	2	7	3.5				
	3	6.5	3.3				
	4	6.2	3.1				
	MEAN	6.225	3.2	3.1		30.55*	0.101
03 Jul	1	5.9	3.3				
	2	5.7	3				
	3	6	3.3				
	4	6.2	3.2				
	MEAN	5.95	3.2	3		29.56*	0.101
05 Jul	1	5.7	3				
	2	5.4	2.8				
	3	5.8	3.2				
	4	6.4	3.5				
	MEAN	5.825	3.125	4.69		46.[illegible]*	0.101
09 Jul	1	5.2	2.8				
	2	4.9	3.4				
	3	6.2	3.4				
	4	6.7	3.4				
	MEAN	5.75	3.25	4.78		47.10*	0.101
11 Jul	1	5.6	3				
	2	6	2.9				
	3	5.4	2.8				
	4	5.4	3.2				
	MEAN	5.6	2.975	4.62		45.53*	0.101
13 Jul	1	6.8	3.5				
	2	6	3.1				
	3	6.1	3.1				
	4	5.7	3				
	MEAN	6.15	3.175	5.01		49.37*	0.101
16 Jul	1	5.3	3				
	2	6.2	3.3				
	3	5.8	2.9				
	4	6.1	3				
	MEAN	5.85	3.05	4.98		49.07*	0.101

Table 14 (cont'd)

DATE	SAMPLE	SHELL LENGTH	SHELL HEIGHT	DIGESTIVE WEIGHT	TISSUE WEIGHT	TOTAL WEIGHT	WEIGHT RATIO
19 Jul	1	6.5	3.5				
	2	5	3.1				
	3	6.1	3.2				
	4	6.1	3				
	MEAN	5.925	3.2	5.76		56.76*	0.101
20 Jul	1	6.4	3.3				
	2	6.2	3.3				
	3	5.6	3.2				
	4	6.5	3.4				
	MEAN	6.175	3.3	6.16		60.70*	0.101
25 Jul	1	6.6	3.6				
	2	6.4	3.7				
	3	6.5	3.4				
	4	6.1	3.5				
	MEAN	6.4	3.55	6.5		64.05*	0.101
26 Jul	1	5.9	3				
	2	5.8	3.2				
	3	6.1	3.2				
	4	5.7	3				
	MEAN	5.875	3.1	6.18		60.90*	0.101
30 Jul	1	5.[illegible]	2.9				
	2	5.7	3.2				
	3	5.8	3.2				
	4	6.2	3.2				
	MEAN	5.85	3.125	5.15		50.75*	0.101
02 Aug	1	5	3				
	2	5.6	2.8				
	3	6.3	3.1				
	4	6.6	3.5				
	MEAN	6.05	3.1	5.71		56.27*	0.101
06 Aug	1	5.8	3				
	2	5.6	3				
	3	6	3.2				
	4	4.9	2.5				
	MEAN	5.575	2.925	5.74		56.56*	0.101
09 Aug	1	5.8	3.1				
	2	5.9	3				
	3	6.3	3.3				
	4	6.8	3.[illegible]				
	MEAN	6.2	3.225	7.8		76.86*	0.101
13 Aug	1	6.6	3.3				
	2	6.8	3.6				
	3	6.3	3				
	4	5.8	3.3				
	MEAN	6.3	3.3	8.02		79.03*	0.101

Table 14 (cont'd)

DATE	SAMPLE	SHELL LENGTH	SHELL HEIGHT	DIGGI WEIGHT	TISSUE WEIGHT	TOTAL WEIGHT	WEIGHT RATIO
16 Aug	1	6.4	3.2				
	2	5.8	3.2				
	3	6.1	3.1				
	4	5.8	3				
	MEAN	6.025	3.125	5.99		59.03*	0.101
20 Aug	1	5.9	3.3				
	2	6	3				
	3	5.8	3				
	4	6.1	3.3				
	MEAN	5.9[illegible]	3.15	6.35		63.57*	0.101
22 Aug	1	6.8	3.5				
	2	5.6	3				
	3	6.3	3.3				
	4						
	MEAN	6.233	3.267	5.52		54.39*	0.101
24 Aug	1	6.1	3.1				
	2	6.6	3.2				
	3	5.9	3.1				
	4	6.2	3.3				
	MEAN	6.2	3.175	5.67		55.87*	0.101
27 Aug	1	6.5	3.4				
	2	6.7	3.4				
	3	6.2	3.3				
	4	6.2	3.1				
	MEAN	6.4	3.3	7.1		69.96*	0.101
29 Aug	1	6.2	3.3				
	2	6.7	3.1				
	3	5.8	3.2				
	4	6.3	3.2				
	MEAN	6.25	3.2	6.33		62.38*	0.101
31 Aug	1	5.9	3.2				
	2	6.5	3.4				
	3	6	2.9				
	4	6.2	3.3				
	MEAN	6.15	3.2	6.47		63.76*	0.101
03 Sep	1	6	3.1				
	2	5.7	3				
	3	5.8	2.9				
	4	6.4	3.6				
	MEAN	5.975	3.15	6.22		61.29*	0.101
05 Sep	1	5.8	3				
	2	5.7	2.7				
	3	6	3.2				
	4	6.5	3.2				
	MEAN	6	3.025	5.94		58.53*	0.101

Table 14 (cont'd)

DATE	SAMPLE	SHELL LENGTH	SHELL HEIGHT	DIG GL. WEIGHT	TISSUE WEIGHT	TOTAL WEIGHT	WEIGHT RATIO
07 Sep	1	6.5	3.6				
	2	6.6	3.3				
	3	6.3	3.3				
	4	6.4	3.3				
	MEAN	6.45	3.375	6.22		61.29*	0.101
10 Sep	1	5.8	3				
	2	6.1	3.2				
	3	6.5	3.6				
	4	6.4	3.6				
	MEAN	6.275	3.35	6.22		61.29*	0.101
12 Sep	1	6.1	3.2				
	2	6	3.2				
	3	6.1	3.1				
	4	6.1	3				
	MEAN	6.075	3.125	5.11		50.35*	0.101
14 Sep	1	5.7	2.9				
	2	7.2	3.2				
	3	5.8	3.1				
	4	5.9	3.1				
	MEAN	6.15	3.075	4.06		40.01*	0.101
17 Sep	1	5.4	2.9				
	2	5	2.8				
	3	6.3	3				
	4						
	MEAN	5.567	2.9	2.84		27.99*	0.101
19 Sep	1	6	3.1				
	2	5.4	2.7				
	3	5.9	3.1				
	4	5.7	3.3				
	MEAN	5.675	3.05	3.82		37.64*	0.101
21 Sep	1	6.6	3.3				
	2	6.2	3.3				
	3						
	4						
	MEAN	6.4	3.3	3.07		30.25*	0.101
24 Sep	1	5.6	3				
	2	6.8	3.6				
	3	6.3	3.4				
	4	6	3				
	MEAN	6.175	3.25	4.63		45.62*	0.101
2[illegible] Sep	1	5	3.1				
	2	5.6	3				
	3	6.1	3.1				
	4	6.1	3.2				
	MEAN	5	3.1	6.11		60.21*	0.101

Table 14 (cont'd)

DATE	SAMPLE	SHELL LENGTH	SHELL HEIGHT	DIGGI WEIGHT	TISSUE WEIGHT	TOTAL WEIGHT	WEIGHT RATIO
01-Oct	1	5.7	3				
	2	6.1	3.3				
	3	6.2	3.1				
	4	5.9	3				
	MEAN	5.975	3.1	5.33	57.51	62.84	0.085
04 Oct	1	6.1	3.3				
	2	6.1	3				
	3	5.9	3.4				
	4	6.3	3.2				
	MEAN	6.1	3.225	5.63	48.49	54.12	0.104
08 Oct	1	6	3.1				
	2	6.2	3.4				
	3	5.7	3.2				
	4	6.2					
	MEAN	6.025	3.175	5.29	45.8	51.09	0.104
11 Oct	1	6.4	3.5				
	2	6.1	3.2				
	3	6.2	3.3				
	4						
	MEAN	6.233	3.333	3.67	35.11	38.78	0.095
15 Oct	1	6	3.4				
	2	5.7	3				
	3	6	3.2				
	4	6.2	3.3				
	MEAN	5.975	3.225	5.49	47.9	53.39	0.103
18 Oct	1	5.7	3				
	2	5.7	3.3				
	3	6	3.1				
	4	6	3.1				
	MEAN	5.85	3.125	4.95	43.03	48.97	0.101
22 Oct	1	5.6	3				
	2	5.7	3.8				
	3	6.1	3.9				
	4	6.5	3.2				
	MEAN	5.975	3.975	5.75	45.95	51.70	0.111
25 Oct	1	5.4	3				
	2	5.8	3				
	3	5.8	3.2				
	4	6.3	3.2				
	MEAN	5.825	3.1	4.86	43.86	48.72	0.100
29 Oct	1	5.8	3.2				
	2	6.1	3.4				
	3						
	4						
	MEAN	5.95	3.3	6.53	54.87	61.39	0.111

* Calculated from regression

Table 15 Concentration of PSP toxin (μM) in non-visceral tissues of CAP mussels in the end phase of the experiment (Oct 1-Oct 29).

	Concentration (μM)							
DATE	C1	GTX4	GTX1	GTX3	GTX2	neoSTX	STX	TOTAL
01 Oct	5.21	0.00	0.00	0.15	0.09	0.49	0.21	6.17
04 Oct	4.66	0.00	0.00	0.07	0.05	0.37	0.24	5.38
08 Oct	1.98	0.00	0.00	0.08	0.03	0.27	0.00	2.36
11 Oct	3.73	0.00	0.00	0.04	0.03	0.26	0.35	4.41
15 Oct	1.99	0.00	0.00	0.07	0.04	0.23	0.22	2.54
18 Oct	2.08	0.00	0.00	0.06	0.04	0.35	0.29	2.81
22 Oct	3.31	0.09	0.00	0.05	0.02	0.22	0.19	3.89
25 Oct	4.74	0.11	0.00	0.05	0.02	0.18	0.16	5.26
29 Oct	3.30	0.00	0.00	0.05	0.02	0.21	0.15	3.72

Table 16 Concentration of PSP toxin (μM) in non-visceral tissues of MAD mussels in the end phase of the experiment (Oct 1-Oct 29).

	Concentration (μM)							
DATE	C1	GTX4	GTX1	GTX3	GTX2	neoSTX	STX	TOTAL
01 Oct	3.82	0.00	0.00	0.03	0.02	0.28	0.25	4.40
04 Oct	1.63	0.00	0.00	0.04	0.02	0.20	0.00	1.87
08 Oct	2.98	0.00	0.00	0.04	0.02	0.23	0.20	3.44
11 Oct	2.62	0.00	0.00	0.03	0.03	0.15	0.00	3.03
15 Oct	1.38	0.00	0.00	0.04	0.01	0.14	0.18	1.75
18 Oct	2.01	0.00	0.00	0.04	0.02	0.19	0.00	2.29
22 Oct	2.18	0.00	0.00	0.03	0.02	0.13	0.00	2.36
25 Oct	1.70	0.00	0.00	0.04	0.02	0.14	0.00	1.90
29 Oct	0.87	0.00	0.00	0.03	0.01	0.08	0.00	0.98

ANNEX II

Table 1: Mean and standard error in temperature and salinity values for 0m, 3m and 7m and at 3m for the two halves of the season and *p* value giving the level of significance of a student's T-test comparing temperature and salinity pairs.

Physical Factor	Mean	SE	*p* value
Temp 0m (n=27)	8.981	2.723	0.646
Temp 3m (n=27)	8.704	2.654	
Temp 0m (n=27)	8.981	2.723	0.684
Temp 7m (n=24)	8.625	2.518	
Temp 3m (n=27)	8.704	2.654	0.543
Temp 7m (n=24)	8.625	2.518	
Salinity 0m (n=25)	26.799	1.865	0.585
Salinity 3m (n=25)	26.692	1.651	
Salinity 0m (n=25)	26.799	1.865	0.475
Salinity 7m (n=22)	26.831	1.775	
Salinity 3m (n=25)	26.692	1.651	0.359
Salinity 7m (n=22)	26.831	1.775	
Temp 3m (n=16)	9.375	2.012	0.933
Temp 3m (n=11)	7.727	3.1288	
Salinity 3m (n=14)	26.478	1.4607	0.245
Salinity 3m (n=11)	26.963	1.829	

Table 2 Student's t-test comparing the means of the PSP toxin concentrations (nmol g^{-1} and μgSTXeq $100g^{-1}$ tissue) in CC vs MT, CC vs TP and MT vs TP mussels during the entire season (n = 15).

	CC vs MT		MT vs TP		CC vs TP	
Toxin	T value	*p* value	T value	*p* value	T value	*p*-value
Cx	1.024	0.323	2.832	0.013	2.476	0.027
GTX4	1.016	0.273	0.542	0.596	0.552	0.590
GTX1	1.818	0.091	2.954	0.010	0.732	0.476
GTX3	1.027	0.322	5.69	0.000	0.889	0.389
GTX2	0.704	0.493	7.169	0.000	1.043	0.315
neoSTX	1.340	0.202	23.339	0.000	0.955	0.356
STX	1.244	0.234	13.779	0.000	1.996	0.066
Total	1.172	0.261	1.698	0.112	3.590	0.003
Toxicity	0.847	0.411	2.094	0.055	2.587	0.022

Table 3: Multi-factor ANOVA comparing the variances in the concentrations (nmol g^{-1}) and toxicity (μgSTXeq g^{-1}) of PSP toxins in CC vs MT, CC vs TP and MT vs TP mussels during the entire season.

		Multi-factor ANOVA for CC, MT and TP mussels				
Toxin	Variation	df	SS	MS	F(s)	*p* value
Cx	Among grps	2	59.355	29.677	0.676	0.514
	Within grp	14	3990.708	285.051	6.496	0.000
	Among sites	2	1012.962	506.481	11.543	0.000
	Error	116	5090.025	43.880		
	Total	134	10153.050			
GTX4	Among grps	2	0.353	0.176	0.579	0.562
	Within grp	14	9.974	0.713	2.337	0.007
	Among sites	2	1.598	0.799	2.621	0.077
	Error	116	35.357	0.305		
	Total	134	47.281			
GTX1	Among grps	2	1.415	0.708	0.387	0.680
	Within grp	14	41.053	2.933	1.602	0.089
	Among sites	2	11.670	5.835	3.188	0.045
	Error	116	212.348	1.831		
	Total	134	266.487			
GTX3	Among grps	2	1.936	0.968	1.273	0.284
	Within grp	14	49.127	3.531	4.613	0.000
	Among sites	2	20.097	10.048	13.215	0.000
	Error	116	88.201	0.760		
	Total	134	159.661			
GTX2	Among grps	2	1.340	0.670	0.462	0.631
	Within grp	14	99.221	7.087	4.881	0.000
	Among sites	2	26.175	13.087	9.014	0.000
	Error	116	168.424	1.452		
	Total	134	295.160			
neoSTX	Among grps	2	2.193	1.096	0.863	0.425
	Within grp	14	72.712	5.194	4.088	0.000
	Among sites	2	53.463	26.732	21.041	0.000
	Error	116	147.374	1.270		
	Total	134	275.742			
STX	Among grps	2	45.133	22.566	1.016	0.365
	Within grp	14	1511.363	107.955	4.860	0.000
	Among sites	2	390.672	195.336	8.795	0.000
	Error	116	2576.435	22.211		
	Total	134	4523.603			
Total	Among grps	2	298.439	149.220	0.787	0.457
	Within grp	14	16465.333	1176.095	6.206	0.000
	Among sites	2	5002.699	2501.349	13.196	0.000
	Error	116	21982.993	189.509		
	Total	134	43749.464			
Toxicity	Among grps	2	126405.400	63202.700	0.754	0.459
	Within grp	14	6738356.700	481311.400	5.741	0.000
	Among sites	2	2070399.600	1035199.800	12.348	0.000
	Error	116	9350398.800	80606.026		
	Total	134	18294521.00			

Table 4 One-way ANOVA comparing the variance in the concentration (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$ tissue) of PSP toxins in individual mussels vs pooled mussels from a. TP, b. MT and c. CC during the entire season.

a. Trois pistoles

		One way ANOVA for TP mussels				
Toxin	Variation	df	SS	MS	F(s)	*p* value
Cx	Among gps	2	9.461	4.730	0.968	0.392
	Within grp	14	466.187	33.299	6.817	0.000
	Error	28	136.763	4.884		
	Total	44	612.410			
GTX4	Among gps	2	0.048	0.024	0.080	0.923
	Within grps	14	13.185	0.942	3.132	0.005
	Error	28	8.418	0.301		
	Total	44	21.651			
GTX1	Among gps	2				
	Within grps	14				
	Error	28				
	Total	44				
GTX3	Among gps	2	0.168	0.084	1.487	0.244
	Within grps	14	1.653	0.118	2.084	0.048
	Error	28	1.586	0.057		
	Total	44	3.407			
GTX2	Among gps	2	0.055	0.028	0.561	0.577
	Within grps	14	2.073	0.148	3.020	0.006
	Error	28	1.373	0.049		
	Total	44	3.501			
neoSTX	Among gps	2	0.037	0.018	1.300	0.288
	Within grps	14	0.186	0.013	0.943	0.529
	Error	28	0.398	0.014		
	Total	44	0.623			
STX	Among grp	2	0.825	0.412	0.465	0.633
	Within grps	14	37.220	2.659	3.000	0.007
	Error	28	24.816	0.886		
	Total	44	62.860			
Total	Among gps	2	18.595	9.298	1.079	0.354
	Within grps	14	785.500	56.107	6.511	0.000
	Error	28	241.301	8.618		
	Total	44	1045.396			
Toxicity	Among grps	2	4166.510	2083.253	0.849	0.438
	Within grps	14	170752.250	12196.590	4.972	0.000
	Error	28	68679.806	24.529		
	Total	44	243598.57			

Table 4 (cont'd):

b. Metts

		One-way ANOVA for MT muscle				
Toxin	Variation	df	SS	MS	F(s)	*p* value
Cx	Among grps	2	63.281	31.640	2.518	0.099
	Within grp	14	1363.761	97.412	7.753	0.000
	Error	28	351.788	12.564		
	Total	44	1778.831			
GTX4	Among grps	2	0.091	0.046	0.218	0.806
	Within grp	14	5.300	0.379	1.811	0.088
	Error	28	5.854	0.209		
	Total	44	11.245			
GTX1	Among grps	2	5.093	2.547	0.520	0.600
	Within grp	14	90.203	6.443	1.315	0.260
	Error	28	137.217	4.901		
	Total	44	232.513			
GTX3	Among grps	2	0.938	0.469	1.918	0.166
	Within grp	14	21.287	1.520	6.232	0.000
	Error	28	6.843	0.244		
	Total	44	29.067			
GTX2	Among grps	2	0.294	0.147	1.563	0.221
	Within grp	14	25.147	1.796	19.067	0.000
	Error	28	2.638	0.094		
	Total	44	28.079			
neoSTX	Among grps	2	1.994	0.997	0.751	0.481
	Within grp	14	27.128	1.938	1.459	0.192
	Error	28	37.192	1.328		
	Total	44	66.315			
STX	Among grps	2	10.637	5.318	1.272	0.296
	Within grp	14	1492.739	106.624	25.503	0.000
	Error	28	117.062	4.181		
	Total	44	1620.438			
Total	Among grps	2	87.859	43.939	1.293	0.290
	Within grp	14	8891.573	635.112	18.688	0.000
	Error	28	951.581	33.985		
	Total	44	9931.013			
Toxicity	Among grps	2	9158.900	4579.430	0.367	0.696
	Within grp	14	4817813.100	344272.370	27.763	0.000
	Error	28	347228.290	12402.439		
	Total	44	5206200.30			

Table 4 (cont'd)

c Cap Chat

		One way ANOVA for CC mussels				
Item	Variation	df	SS	MS	F(s)	*p*-value
Cs	Among grps	2	77.198	38.599	0.875	0.428
	Within grp	14	5436.306	388.308	8.801	0.000
	Error	28	1235.343	44.119		
	Total	44	6748.847			
GTX4	Among grps	2	0.310	0.155	0.753	0.480
	Within grp	14	6.716	0.480	2.332	0.028
	Error	28	5.761	0.206		
	Total	44	12.787			
GTX1	Among grps	2	0.143	0.072	0.241	0.788
	Within grp	14	13.836	0.988	3.325	0.003
	Error	28	8.324	0.297		
	Total	44	22.303			
GTX3	Among grps	2	1.694	0.847	2.103	0.141
	Within grp	14	94.124	6.723	16.698	0.000
	Error	28	11.274	0.403		
	Total	44	107.091			
GTX2	Among grps	2	4.615	2.307	1.379	0.268
	Within grp	14	185.939	13.281	7.937	0.000
	Error	28	46.852	1.673		
	Total	44	237.405			
neoSTX	Among grps	2	5.040	2.520	3.031	0.064
	Within grp	14	127.022	9.073	10.913	0.000
	Error	28	23.279	0.831		
	Total	44	155.341			
STX	Among grp	2	58.532	29.266	1.932	0.165
	Within grp	14	1964.686	140.335	9.215	0.000
	Error	28	426.415	15.229		
	Total	44	2449.633			
Total	Among grps	2	415.006	207.503	1.447	0.252
	Within grp	14	23339.090	1667.078	11.622	0.000
	Error	28	4016.260	143.438		
	Total	44	27770.356			
Toxicity	Among grps	2	2215870.600	1110785.300	2.056	0.147
	Within grp	14	9034974.300	645355.310	11.976	0.000
	Error	28	1508847.200	53887.400		
	Total	44	10768393.00			

Table 5: Student's t-test comparing the means of the PSP toxin concentrations (nmol g^{-1} and μgSTXeq $100g^{-1}$ tissue) in CC vs MT, CC vs TP and MT vs TP mussels a. prior to (n=9) and b. during the peak in toxin concentration (n=6)

a. Prior to increase in toxin concentration

	CC vs MT		MT vs TP		CC vs TP	
Toxin	t value	*p*-value	t value	*p* value	t value	*p* value
Cx	1.821	0.106	4.117	0.003	1.471	0.179
GTX4	0.371	0.720	0.482	0.643	0.491	0.636
GTX1	0.744	0.478	6.565	0.000	0.352	0.734
GTX3	2.108	0.068	2.132	0.066	0.468	0.652
GTX2	2.271	0.053	9.308	0.000	0.379	0.715
neoSTX	1.558	0.158	7.050	0.000	0.627	0.548
STX	3.554	0.158	54.703	0.000	0.791	0.452
Total	2.816	0.007	4.112	0.003	1.699	0.128
Toxicity	3.413	0.009	2.224	0.057	6.794	0.000

b. During the peak in toxin concentration

	CC vs MT		MT vs TP		CC vs TP	
Toxin	t value	p value	t value	p value	t value	p value
Cx	0.847	0.436	2.361	0.065	3.367	0.020
GTX4	-1.652	0.159	1.015	0.357	0.754	0.485
GTX1	2.185	0.081	2.946	0.032	1.003	0.362
GTX3	0.771	0.476	6.038	0.002	1.233	0.272
GTX2	0.578	0.588	6.154	0.002	1.506	0.192
neoSTX	1.139	0.306	27.885	0.000	1.303	0.249
STX	1.109	0.318	11.370	0.000	2.371	0.035
Total	1.036	0.348	1.348	0.236	4.960	0.004
Toxicity	1.138	0.307	0.651	0.544	22.646	0.000

Table 6 Student's t-test comparing the means of the relative PSP toxin concentration (%molar) in CC vs MT, CC vs TP and MT vs TP mussels a. prior to (n=9) and b. during the peak in toxin concentration (n=6).

a. Prior to the peak in toxin concentration

	CC vs MT		MT vs TP		CC vs TP	
Toxin	t value	*p* value	t value	*p* value	t value	*p*-value
C x	4.551	0.002	18.261	0.000	3.057	0.046
GTX4	0.426	0.681	0.656	0.417	1.856	0.016
GTX1	0.053	0.959	183.330	0.000	0.882	0.403
GTX3	2.123	0.067	-1.465	0.181	1.561	0.157
GTX2	1.932	0.089	8.460	0.000	1.246	0.248
neoSTX	1.119	0.296	8.155	0.000	2.048	0.075
STX	5.303	0.001	2.356	0.046	2.256	0.054

b. During the peak in toxin concentration

	CC vs MT		MT vs TP		CC vs TP	
Toxin	t value	p value	t value	p value	t value	p value
C x	2.064	0.094	8.475	0.000	3.937	0.011
GTX4	13.601	0.000	1.612	0.168	1.613	0.167
GTX1	1.748	0.141	11.072	0.000	1.088	0.326
GTX3	0.984	0.370	2.187	0.080	1.537	0.185
GTX2	0.626	0.559	5.433	0.003	1.537	0.185
neoSTX	1.866	0.121	16.806	0.000	1.956	0.108
STX	1.637	0.165	4.910	0.004	3.460	0.018

Table 7: Total weights (g, S.E.) of the mussels collected at the inter tidal zone at Cap Chat (CC), Metis (MT) and Trois Pistoles (TP) and *p*-value giving the level of probability of difference in the weights based on a student's T test

	Mussel weight		Probability of difference		
Site	(g)	S.E.	CC vs MT	MT vs TP	CC vs TP
CC (n=45)	3.03	1.83			
MT (n=45)	4.76	2.33	0.000	0.026	0.005
TP (n=45)	3.95	1.47			

Table 8: K-S analysis comparing PSP toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$ tissue) in CAP and MAD mussels and in the *Alexandrium* cells during bloom I (n = 5) vs bloom II (n = 9)

	CAP mussels		MAD mussels		*Alexandrium* cells	
Toxin	DN	*p* value	DN	*p* value	DN	*p* value
Cx	1.000	0.003	1.000	0.003	0.889	0.012
GTX4	1.000	0.003	1.000	0.003	1.000	0.003
GTX1	1.000	0.003	1.000	0.003	0.467	0.486
GTX3	1.000	0.003	1.000	0.003	0.889	0.012
GTX2	0.800	0.033	1.000	0.003	0.800	0.033
neoSTX	1.000	0.003	1.000	0.003	0.600	0.197
STX	0.889	0.012	0.889	0.012	0.578	0.334
Total	1.000	0.003	1.000	0.003	0.889	0.012
Toxicity	1.000	0.003	1.000	0.003	0.800	0.033

Table 9 Discriminant analysis of the concentration (nmol g^{-1}) of individual toxins in *Alexandrium* cells during bloom I vs bloom II.

Toxin	*Alexandrium* cells
Cx	0.405
GTX4	3.909
GTX1	1.665
GTX3	1.725
GTX2	1.145
*neo*STX	5.896
STX	2.816
Chi square value	12.138
Deg. of Freedom	7
Probability (*p*)	0.096
Centroid (Bloom I)	2.212
Centroid (Bloom II)	1.227

Table 10 Wilcoxon Signed-Ranks test comparing the relative PSP toxin concentration (arcsin %molar) of individual toxins in CAP and MAD mussels and in *Alexandrium* cells during bloom I (n=5) compared to bloom II (n=9)

	CAP mussels		MAD mussels		*Alexandrium* cells	
Toxin	*Z* value	*p* value	*Z* value	*p* value	*Z* value	*p* value
Cx	2.000	0.046	0.000	1.000	1.333	0.182
GTX4	1.733	0.083	2.133	0.033	0.267	0.790
GTX1	1.200	0.230	1.733	0.083	0.470	0.637
GTX3	2.400	0.016	1.200	0.230	0.000	1.000
GTX2	1.834	0.067	1.333	0.182	0.800	0.424
neoSTX	1.600	0.110	0.000	1.000	3.000	0.182
STX	1.600	1.096	0.133	0.894	0.601	0.548

Table II K-S analysis comparing PSP toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq g^{-1} tissue) in the CAP vs MAD mussels during a. the entire season (n=45), b. bloom I (n=5), c. inter-bloom (n=10), e. bloom II (n=9), e. post-bloom II (n=8), f. end phase (n=9) and g. the transient stage (n=9).

a. Entire season

	CAP vs MAD	
Toxin	DN	*p*-value
Cx	0.3	0.329
GTX4	0.178	0.476
GTX1	0.333	0.216
GTX3	0.2	0.329
GTX2	0.178	0.476
neoSTX	0.178	0.476
STX	0.133	0.819
Total	0.3	0.329
Toxicity	0.3	0.329

b. Bloom I

	CAP vs MAD	
Toxin	DN	*p*-value
Cx	1	0.013
GTX4	0.4	0.819
GTX1	0.6	0.329
GTX3	0.6	0.329
GTX2	0.6	0.329
neoSTX	0.4	0.819
STX	0.4	0.819
Total	0.6	0.329
Toxicity	0.4	0.819

c. Inter bloom

	CAP vs MAD	
Toxin	DN	*p*-value
Cx	0.5	0.164
GTX4	0.2	0.988
GTX1	0.6	0.055
GTX3	0.6	0.055
GTX2	0.4	0.4
neoSTX	0.5	0.164
STX	0.4	0.4
Total	0.5	0.164
Toxicity	0.5	0.164

d. Bloom II

	CAP vs MAD	
Toxin	DN	-value
Cx	0.444	0.336
GTX4	0.555	0.124
GTX1	0.555	0.124
GTX3	0.333	0.699
GTX2	0.444	0.336
neoSTX	0.333	0.699
STX	0.333	0.699
Total	0.333	0.699
Toxicity	0.333	0.699

Table 11 (cont'd)

e. Post-bloom II

	CAP vs MAD	
Toxin	DN	*p* value
Cx	0.375	0.627
GTX4	0.75	0.033
GTX1	0.25	0.964
GTX3	0.25	0.964
GTX2	0.25	0.964
neoSTX	0.25	0.964
STX	0.25	0.964
Total	0.25	0.964
Toxicity	0.25	0.964

f. End phase

	End Phase	
Toxin	DN	*p* value
Cx	0.333	0.699
GTX4	0.333	0.699
GTX1	0.333	0.699
GTX3	0.333	0.699
GTX2	0.333	0.979
neoSTX	0.333	0.979
STX	0.333	0.979
Total	0.333	0.979
Toxicity	0.333	0.699

g. Transient stage

	CAP vs MAD	
Toxin	DN	*p* value
Cx	0.556	0.124
GTX4	0.111	1.000
GTX1	0.778	0.009
GTX3	0.556	0.124
GTX2	0.444	0.336
neoSTX	0.444	0.336
STX	0.444	0.336
Total	0.556	0.124
Toxicity	0.444	0.336

Table 12 Discriminant analysis of concentration (nmol g^{-1}) of individual PSP toxins in CAP vs MAD mussels during each of phase of the experiment.

Toxin	Bloom I	Inter bloom	Bloom II	Post bloom II	End phase
C+	1.55	1.84	1.75	1.32	-1.67
GTX4	81.90	2.68	1.82	-0.49	1.23
GTX1	98.54	2.37	0.61	1.21	-1.43
GTX3	132.42	1.90	2.49	7.49	1.90
GTX2	166.73	4.03	1.75	4.34	0.25
neoSTX	143.99	0.10	0.53	-1.91	-0.54
STX	46.10	0.34	0.15	-2.77	0.22
Chi square value	40.050	15.991	19.252	19.829	2.749
Deg. of Freedom	7	7	7	7	7
Probability (*p*)	0.000	0.025	0.007	0.006	0.907
Centroid (CAP)	76.585	1.346	1.805	2.215	-0.468
Centroid (MAD)	76.585	1.346	1.805	-2.215	0.468

Table 13: Linear Regression analysis (Y = aX + b) of the rates of toxin a) accumulation and b) elimination of PSP toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq g^{-1}) in the digestive glands of 1) CAP and 2) MAD mussels during i) bloom I and ii) bloom II.

1. CAP

a. Accumulation

i. Bloom I

Toxin	Slope	S.E.	n	Intercept	S.E.	*p* value
Cx	0.025	0.031	5	7.700	0.799	0.241
GTX4	0.078	0.030	5	3.744	0.783	0.207
GTX1	0.130	0.022	5	3.855	0.214	0.005
GTX3	0.009	0.020	5	3.508	0.189	0.342
GTX2	0.161	0.037	5	2.056	0.353	0.011
neoSTX	0.082	0.041	5	5.969	0.392	0.071
STX	0.150	0.051	5	3.125	0.483	0.030
Total	0.049	0.032	5	7.535	0.305	0.110
Toxicity	0.077	0.036	5	10.152	0.339	0.060

ii. Bloom II

Toxin	Slope	S.E.	n	Intercept	S.E.	*p* value
Cx	0.104	0.055	5	4.702	0.403	0.078
GTX4	0.040	0.035	5	1.450	0.252	0.165
GTX1	0.043	0.077	5	2.123	0.562	0.307
GTX3	0.074	0.091	5	1.081	0.688	0.244
GTX2	0.016	0.034	5	0.935	0.247	0.335
neoSTX	0.109	0.066	5	3.506	0.476	0.098
STX	0.184	0.075	5	1.150	0.544	0.455
Total	0.102	0.058	5	4.799	0.423	0.088
Toxicity	0.102	0.061	5	7.699	0.446	0.096

Table 13 (cont'd)

b Elimination

i Bloom I

Toxin	Slope	S.E	n	Intercept	S.E	*p*-value
C	0.185	0.070	6	7.459	0.660	0.028
GTX4	0.143	0.016	6	4.190	0.147	0.000
GTX1	0.088	0.012	6	5.299	0.110	0.001
GTX3	0.135	0.014	6	3.708	0.132	0.000
GTX2	0.066	0.009	6	3.837	0.090	0.001
neoSTX	0.137	0.020	6	6.990	0.186	0.001
STX	0.165	0.019	6	5.153	0.179	0.000
Total	0.154	0.035	6	8.132	0.331	0.006
Toxicity	0.137	0.021	6	11.091	0.196	0.001

ii Bloom II

Toxin	Slope	S.E	n	Intercept	S.E	*p*-value
C	0.086	0.015	12	4.851	0.412	0.000
GTX4	0.136	0.013	12	2.333	0.353	0.000
GTX1	0.151	0.016	12	3.198	0.453	0.000
GTX3	0.134	0.009	12	2.133	0.257	0.000
GTX2	0.061	0.010	12	1.373	0.285	0.000
neoSTX	0.115	0.010	12	5.144	0.292	0.000
STX	0.109	0.010	12	3.267	0.274	0.000
Total	0.101	0.009	12	5.919	0.260	0.000
Toxicity	0.112	0.010	12	9.140	0.276	0.000

Table 13 (cont'd)

2. MAD
a. Accumulation
i. Bloom I

Toxin	Slope	S.E.	n	Intercept	S.E.	*p* value
Cx	0.039	0.059	5	6.069	0.565	0.277
GTX4	0.088	0.036	5	3.207	0.348	0.021
GTX1	0.142	0.056	5	3.218	0.537	0.043
GTX3	0.067	0.041	5	2.643	0.393	0.102
GTX2	0.176	0.044	5	1.287	0.423	0.014
neoSTX	0.114	0.048	5	5.410	0.459	0.049
STX	0.187	0.062	5	3.111	0.594	0.029
Total	0.086	0.051	5	6.590	0.487	0.095
Toxicity	0.114	0.048	5	9.476	0.462	0.050

ii. Bloom II

Toxin	Slope	S.E.	n	Intercept	S.E.	*p* value
Cx	0.117	0.069	5	3.897	0.503	0.094
GTX4	0.168	0.038	5	1.155	0.274	0.010
GTX1	0.163	0.046	5	0.728	0.335	0.019
GTX3	0.126	0.070	5	0.570	0.511	0.066
GTX2	0.083	0.037	5	0.301	0.272	0.058
neoSTX	0.182	0.046	5	3.073	0.339	0.015
STX	0.256	0.052	5	0.886	0.378	0.008
Total	0.145	0.059	5	4.397	0.427	0.045
Toxicity	0.174	0.050	5	7.192	0.363	0.020

Table 13 (cont'd):

b. Elimination

i. Bloom I

Toxin	Slope	S.E	n	Intercept	S.E	*p* value
Cx	0.321	0.040	6	6.564	0.379	0.001
GTX4	0.188	0.026	6	4.274	0.241	0.001
GTX1	0.190	0.035	6	4.790	0.138	0.000
GTX3	0.245	0.021	6	3.506	0.200	0.000
GTX2	0.121	0.013	6	3.201	0.123	0.000
neoSTX	0.197	0.011	6	6.728	0.104	0.000
STX	0.295	0.036	6	5.164	0.340	0.001
Total	0.234	0.018	6	7.585	0.169	0.000
Toxicity	0.240	0.013	6	10.782	0.127	0.000

ii. Bloom II

Toxin	Slope	S.E	n	Intercept	S.E	*p*-value
Cx	0.092	0.016	12	4.802	0.447	0.000
GTX4	0.100	0.012	12	3.046	0.349	0.000
GTX1	0.144	0.020	12	2.862	0.572	0.000
GTX3	0.145	0.012	12	2.203	0.338	0.000
GTX2	0.073	0.012	12	1.405	0.345	0.000
neoSTX	0.125	0.013	12	5.342	0.364	0.000
STX	0.110	0.009	12	3.471	0.249	0.000
Total	0.109	0.010	12	6.033	0.288	0.900
Toxicity	0.119	0.011	12	9.308	0.318	0.000

Table 14a: Linear regression analysis (Y=aX+b) for the rate of decrease in toxin concentration (nmol g^{-1}) and toxicity (μgSTXeq g^{-1} tissue), as given by the percentage of the peak toxin concentration and toxicity in the digestive glands of CAP and MAD mussels following each bloom (I and II) for the elimination of 85% of the total toxin content in each mussel group.

Event	Slope	S.E.	n	Intercept	S.E.	*p* value
NmolI CAP	26.725	3.678	4	91.153	8.334	0.184
NmolI MAD	28.782	3.984	4	93.838	8.910	0.019
NmolII CAP	10.508	1.306	9	88.444	10.170	0.000
NmolII MAD	9.960	1.674	9	85.715	12.964	0.001
Tox I CAP	12.701	2.616	7	80.377	13.843	0.005
Tox I MAD	14.011	3.205	7	79.129	16.958	0.007
Tox II CAP	11.600	1.068	9	92.939	8.272	0.000
Tox II MAD	11.213	1.531	9	90.322	11.857	0.000

Table 14b: Level of significance of the pair wise comparison of the slopes of toxin elimination for CAP vs MAD mussels during the first and second blooms, and between the first and second bloom for CAP and MAD mussels.

	CAP vs MAD	
Event	t value	*p* value
nmol I	0.379	0.724
nmol II	0.258	0.800
μgSTXeq I	0.317	0.758
μgSTXeq II	0.208	0.838

	Bloom I vs II	
Event	t value	value
CAP (nmol)	4.155	0.002
MAD (nmol)	4.355	0.002
CAP (μgSTXeq)	0.389	0.703
MAD (μgSTXeq)	0.788	0.445

Table 15 Student's t-test comparing the slopes of increase and decrease of the concentration (nmol g^{-1}) and toxicity (μgSTXeq g^{-1}) of PSP toxins in CAP vs MAD mussels during a. bloom I and b. bloom II

a. Bloom I

	Accumulation		Elimination	
Toxin	T value	*p* value	T value	*p* value
Cx	0.212	0.419	1.698	0.064
GTX4	1.537	0.088	1.498	0.086
GTX1	1.942	0.436	5.519	0.000
GTX3	1.265	0.437	4.347	0.001
GTX2	0.264	0.400	3.398	0.005
neoSTX	0.518	0.311	2.668	0.014
STX	0.464	0.329	3.208	0.006
Total	0.605	0.284	4.436	0.010
Toxicity	0.633	0.278	2.975	0.009

b. Bloom II

	Accumulation		Elimination	
Toxin	T value	p value	T value	p value
Cx	0.139	0.447	0.259	0.399
GTX4	2.513	0.405	2.028	0.421
GTX1	1.339	0.115	0.904	0.188
GTX3	0.436	0.339	1.420	0.086
GTX2	1.303	0.120	0.767	0.226
neoSTX	0.915	0.198	0.618	0.272
STX	0.786	0.213	0.118	0.436
Total	0.513	0.313	0.605	0.276
Toxicity	0.912	0.199	0.482	0.318

Table 16: Student's t-test comparing the slopes of a increase and b decrease in the concentration (nmol g^{-1}) and toxicity (μgSTXeq g^{-1} tissue) of PSP toxins during bloom I vs bloom II in CAP and MAD mussels

a. Toxin accumulation

	CAP		MAD	
Toxin	t value	p value	t value	p value
Cx	1.248	0.129	0.852	0.213
GTX4	0.267	0.399	1.749	0.433
GTX1	1.085	0.840	0.294	0.389
GTX3	0.680	0.261	0.725	0.248
GTX2	2.893	0.986	1.636	0.924
neoSTX	0.350	0.369	1.018	0.174
STX	0.380	0.358	0.846	0.215
Total	0.799	0.227	0.758	0.239
Toxicity	0.361	0.365	0.865	0.210

b. Toxin elimination

	CAP		MAD	
Toxin	t value	p value	t value	p value
Cx	1.387	0.094	5.336	0.005
GTX4	0.358	0.363	3.094	0.004
GTX1	1.665	0.941	1.855	0.044
GTX3	0.610	0.260	4.098	0.001
GTX2	0.392	0.351	2.670	0.009
neoSTX	1.005	0.166	4.244	0.000
STX	2.636	0.010	4.993	0.000
Total	5.768	0.000	6.033	0.000
Toxicity	1.089	0.147	5.175	0.000

Table 17 Wilcoxon Signed-Ranks test comparing the relative PSP toxin concentration (as % Molar) of individual toxins in CAP vs MAD mussels and in *Alexandrium* cells vs CAP and MAD mussels during a. bloom I (n=5) and b. bloom II (n = 9)

a. Bloom I

	CAP vs MAD		*Alexandrium* vs CAP		*Alexandrium* vs MAD	
Toxin	Z value	p value	Z value	p value	Z-value	p-value
C1	2.157	0.031	1.348	0.178	0.539	0.59
GTX4	2.157	0.031	0.27	0.787	1.888	0.059
GTX1	1.618	0.106	2.157	0.031	2.157	0.031
GTX3	2.157	0.031	1.348	0.178	0.809	0.418
GTX2	0.27	0.787	0.539	0.59	0.809	0.418
neoSTX	2.157	0.031	1.618	0.106	0.27	0.787
STX	2.157	0.031	1.348	0.178	2.157	0.031

b. Bloom II

	CAP vs MAD		*Alexandrium* vs CAP		*Alexandrium* vs MAD	
Toxin	Z value	p value	Z value	p value	Z-value	p value
C1	1.659	0.097	2.132	0.033	2.014	0.044
GTX4	2.606	0.009	1.422	0.155	2.606	0.009
GTX1	2.725	0.006	2.725	0.006	2.606	0.009
GTX3	0.829	0.407	0.237	0.813	0.237	0.813
GTX2	2.36	0.018	2.725	0.006	2.725	0.006
neoSTX	1.896	0.058	1.659	0.097	2.014	0.044
STX	1.896	0.058	1.066	0.286	1.896	0.058

Table 18: Wilcoxon Signed-Ranks test comparing the relative PSP toxin concentration (arcsin %Molar) of individual toxins in CAP vs MAD mussels during a. the entire experiment (n=45), b. the inter-bloom (n=10), c. the post bloom II (n=8) and d. the end phase (n=9) of the experiment.

a. Entire Experiment

	CAP vs MAD	
Toxin	*Z* value	*p* value
Cx	2.551	0.011
GTX4	5.079	0
GTX1	4.364	0
GTX3	0.892	0.373
GTX2	0.316	0.752
neoSTX	1.851	0.064
STX	3.07	0.002

b. Inter-bloom

	CAP vs MAD	
Toxin	*Z* value	*p* value
Cx	0.510	0.610
GTX4	2.854	0.004
GTX1	2.854	0.004
GTX3	0.714	0.476
GTX2	1.838	0.067
neoSTX	0.612	0.541
STX	0.714	0.476

c. Post-bloom II

	CAP vs MAD	
Toxin	*Z*-value	*p* value
Cx	2.31	0.021
GTX4	2.591	0.01
GTX1	2.591	0.01
GTX3	2.591	0.01
GTX2	2.45	0.014
neoSTX	1.47	0.141
STX	2.59	0.01

d. End phase

	CAP vs MAD	
Toxin	*Z* value	*p* value
Cx	0.711	0.477
GTX4	1.303	0.193
GTX1	1.258	0.208
GTX3	0.711	0.477
GTX2	0.592	0.554
neoSTX	0.118	0.906
STX	0.474	0.636

Table 19 Linear Regression analysis (Y = aX + b) for the rates of changes in toxin ratios in the digestive glands of 1. CAP and 2. MAD mussels for the a. the accumulation and b. the elimination phases of i. bloom I and ii. bloom II and iii. the inter-bloom.

1. CAP

a. Accumulation phase

i. First bloom

Toxin ratio	Slope	S.E.	n	Intercept	S.E.	*p*-value
GTX4/GTX1	0.102	0.013	5	-0.111	0.124	0.004
GTX3/GTX2	0.152	0.021	5	1.452	0.199	0.005
neoSTX/STX	0.068	0.018	5	2.545	0.176	0.003

ii. Second bloom

Toxin ratio	Slope	S.E.	n	Intercept	S.E.	*p*-value
GTX4/GTX1	0.003	0.052	5	0.672	0.376	0.958
GTX3/GTX2	0.058	0.061	5	0.146	0.444	0.408
neoSTX/STX	0.075	0.010	5	2.356	0.073	0.005

b. Elimination phase

i. First bloom

Toxin ratio	Slope	S.E.	n	Intercept	S.E.	*p*-value
GTX4/GTX1	0.056	0.014	6	-1.110	0.137	0.010
GTX3/GTX2	0.069	0.007	6	0.129	0.062	0.000
neoSTX/STX	0.028	0.009	6	1.837	0.087	0.039

ii. Second bloom

Toxin ratio	Slope	S.E.	n	Intercept	S.E.	*p* value
GTX4/GTX1	0.015	0.012	12	0.865	0.347	0.245
GTX3/GTX2	0.063	0.006	12	0.759	0.179	0.000
neoSTX/STX	0.006	0.005	12	1.877	0.132	0.230

Table 19 (cont'd)

iii. Inter-bloom

Toxin ratio	Slope	S.E.	n	Intercept	S.E.	*p* value
GTX4/GTX1	0.055	0.038	10	1.317	0.342	0.179
GTX3/GTX2	0.025	0.013	10	0.910	0.116	0.085
neoSTX/STX	0.086	0.045	10	2.429	0.412	0.092

2. MAD

a. Accumulation

i. First bloom

Toxin ratio	Slope	S.E.	n	Intercept	S.E.	*p* value
GTX4/GTX1	0.053	0.031	5	0.016	0.294	0.181
GTX3/GTX2	0.110	0.027	5	1.356	0.253	0.026
neoSTX/STX	0.073	0.021	5	2.298	0.239	0.056

ii. Second bloom

Toxin ratio	Slope	S.E.	n	Intercept	S.E.	*p* value
GTX4/GTX1	0.005	0.029	5	0.427	0.213	0.870
GTX3/GTX2	0.044	0.043	5	0.270	0.315	0.483
neoSTX/STX	0.073	0.008	5	2.137	0.059	0.003

b. Detoxification phase

i. First bloom

Toxin ratio	Slope	S.E.	n	Intercept	S.E.	*p* value
GTX4/GTX1	0.003	0.024	6	0.516	0.228	0.928
GTX3/GTX2	0.124	0.022	6	0.305	0.204	0.005
neoSTX/STX	0.09	0.029	6	1.564	0.272	0.021

Table 19 (cont'd).

ii. Second bloom

Toxin ratio	Slope	S.E.	n	Intercept	S.E.	*p*-value
GTX4/GTX1	0.044	0.015	12	0.183	0.431	0.017
GTX3/GTX2	0.072	0.004	12	0.798	0.115	0.000
neoSTX/STX	0.015	0.008	12	1.871	0.225	0.096

iii. Interbloom

Toxin ratio	Slope	S.E.	n	Intercept	S.E.	*p*-value
GTX4/GTX1	0.030	0.037	10	0.246	0.339	0.440
GTX3/GTX2	0.021	0.030	10	1.096	0.276	0.513
neoSTX/STX	0.159	0.035	10	2.612	0.316	0.002

Table 20: Student's t-test comparing the slopes of changes in the toxin epimer ratios during the accumulation and elimination of PSP toxins in CAP vs MAD mussels during a. bloom I b. bloom II and c inter-bloom.

a. Bloom I

	Accumulation		Elimination	
Toxin ratio	t value	*p* value	t value	*p* value
GTX4 GTX1	1.461	0.194	2.059	0.074
GTX3 GTX2	1.263	0.253	2.458	0.039
neoSTX STX	0.146	0.888	2.326	0.048

b. Bloom II

	Accumulation		Elimination	
Toxin ratio	t value	*p* value	t value	*p* value
GTX4 GTX1	0.137	0.896	-3.012	0.007
GTX3 GTX2	0.193	0.854	1.216	0.238
neoSTX STX	0.172	0.869	0.936	0.360

c. Inter-bloom

	Inter bloom	
Toxin ratio	t value	*p* value
GTX4 GTX1	0.477	0.639
GTX3 GTX2	0.132	0.879
neoSTX STX	1.280	0.219

Table 21 Student's t-test comparing the slopes of changes in toxin epimer ratios during a. the accumulation and b. the elimination phases of bloom I to that of bloom II in CAP and MAD mussels.

a. Accumulation

	CAP mussels		MAD mussels	
Toxin ratio	t value	p value	t value	p value
GTX4/GTX1	1.865	0.111	1.379	0.217
GTX3/GTX2	3.272	0.017	3.029	0.023
neoSTX/STX	0.338	0.747	0.018	0.986

b. Elimination

	CAP mussels		MAD mussels	
Toxin ratio	t value	p value	t value	p value
GTX4/GTX1	2.119	0.052	1.461	0.166
GTX3/GTX2	0.614	0.549	2.357	0.034
neoSTX/STX	3.267	0.006	3.776	0.020

Table 22: Percentage of mean PSP toxins concentration (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$) found in non-visceral tissues of CAP and MAD mussels during the end phase (Oct 1-Oct 29). *p*-value giving the level of significance for a student's T-test comparing the concentrations.

	Mean percent toxin in non-visceral tissues				
Toxin	CAP	SI	MAD	SI	*p* value
Cx	27.568	10.727	19.841	8.719	0.133
GTX4	1.248	2.372	0	0	0.151
GTX1	0	0	0	0	
GTX3	2.685	28.563	27.812	13.401	0.046
GTX2	11.937	7.550	5.314	2.420	0.036
neoSTX	18.739	9.174	10.431	3.501	0.034
STX	46.014	33.403	9.413	13.387	0.016
Total	23.629	7.025	15.243	5.055	0.070
Toxicity	18.215	3.454	10.038	3.527	0.009

Table 23 K S analysis comparing PSP toxin concentrations (nmol g^{-1}) and toxicity (μgSTXeq $100g^{-1}$ tissue) in a. non-visceral tissues of CAP vs MAD mussels and b in visceral vs non-visceral tissues of CAP and MAD mussels, during the end phase of the experiment (n=9).

a. CAP vs MAD

	Non visceral tissues	
Toxin	DN	*p* value
Cx	0.556	0.124
GTX4	1.000	0.000
GTX1	1.000	0.000
GTX3	0.889	0.001
GTX2	0.667	0.037
neoSTX	0.667	0.037
STX	0.667	0.037
Total	0.667	0.037
Toxicity	0.778	0.009

b. Visceral tissues vs non-visceral tissues

	CAP mussels		MAD mussels	
Toxin	DN	*p* value	DN	*p* value
Cx	0.889	0.002	1.000	0.000
GTX4	0.556	0.124	1.000	0.000
GTX1	0.556	0.124	0.567	0.035
GTX3	0.889	0.002	1.000	0.000
GTX2	1.000	0.000	1.000	0.000
neoSTX	1.000	0.000	1.000	0.000
STX	0.778	0.009	1.000	0.000
Total	1.000	0.000	1.000	0.000
Toxicity	1.000	0.000	1.000	0.000

Table 24: Discriminant analysis of the concentration (nmol g^{-1}) of individual toxins in a. non-visceral tissues of CAP vs MAD mussels and b. visceral vs non visceral tissues during the end phase

a. CAP vs MAD mussels

Toxin	Non visceral tissues
Cx	0.12
GTX4	0.71
GTX3	0.61
GTX2	0.43
neoSTX	0.73
STX	0.58
Chi-square value	13.324
Deg. of Freedom	6
Probability (*p*)	0.038
Centroid (CAP)	1.260
Centroid (MAD)	1.260

b. Visceral tissues vs non-visceral tissues

Toxin	CAP mussels	MAD mussels
Cx	-0.48	0.07
GTX4	1.51	2.17
GTX1	0.64	0.06
GTX3	0.04	4.64
GTX2	0.47	3.77
neoSTX	0.14	0.39
STX	1.52	1.19
Chi square value	31.482	33.497
Deg. of Freedom	7	7
Probability (*p*)	0.000	0.000
Centroid (dig gl)	3.185	3.475
Centroid (tissues)	3.185	3.475

Table 25 Wilcoxon Signed-Ranks test comparing the relative PSP toxin concentration (arcsin %Molar) of individual toxins in a. non-visceral tissues of CAP vs MAD mussels and b in visceral vs non-visceral tissues in CAP and MAD mussels during the end phase (n=9).

a. CAP vs MAD

	Non visceral tissues	
Toxin	Z value	p value
Cx	1.770	0.076
GTX4	1.789	0.074
GTX1		
GTX3	0.237	0.813
GTX2	0.711	0.477
neoSTX	0.474	0.636
STX	1.540	0.124

b. Visceral vs non-visceral tissues

	CAP mussels		MAD mussels	
Toxin	Z value	p value	Z value	p-value
Cx	2.488	0.013	2.725	0.006
GTX4	2.481	0.014	2.725	0.006
GTX1	2.157	0.031	2.306	0.021
GTX3	2.725	0.006	1.896	0.058
GTX2	2.725	0.006	2.725	0.006
neoSTX	2.014	0.044	1.422	0.155
STX	1.421	0.155	1.422	0.155

Table 26: Mean weights (g) of the digestive glands (D.G. WT), other tissues (TIS. WT), and total mussel (TOT. WT), and mean shell height and length (cm) of the dimension for CAP and MAD mussels. *P*-values give the level of significance of the F-test of variance within each group and the T-test of difference of the means among the groups

Mussel data	CAP mussels		MAD mussels		p value	
	mean	S.I	mean	S.I	F value	T value
D.G. WT (n=45)	3.564	1.007	5.362	1.211	0.113	0.000
TIS. WT (n=9)	23.407	2.618	47.012	6.073	0.000	0.000
TOT. WT (n=45)	29.675	8.296	52.831	11.799	0.011	0.000
Height (n=45)	4.881	0.346	6.037	0.228	0.996	0.000
Length (n=45)	2.409	0.195	3.175	0.126	0.998	0.000