UPTAKE, RETENTION AND ELIMINATION OF CYSTS OF THE TOXIC DINOFLAGELLATE ALEXANDRIUM SPP. BY THE BLUE MUSSEL, MYTILUS EDULIS

FIONA MORAG HARPER

UPTAKE, RETENTION AND ELIMINATION OF CYSTS OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM* SPP. BY THE BLUE MUSSEL, *MYTILUS EDULIS*.

by

Fiona Morag Harper

A thesis submitted to the
School of Graduate Studies
in partial fulfillment of the
requirements for the degree of
Master of Science.

Department of Biology
Memorial University of Newfoundland

November, 1997

St. John's Newfoundland

Abstract

Cysts of the toxic dinoflagellate *Alexandrium* spp. have been implicated in incidents of paralytic shellfish poisoning. There is a paucity of data concerning the possible effects the benthic resting cyst may have upon bivalve biology, although the effects of motile, vegetative *Alexandrium* cells on bivalve physiology have been extensively studied. The research presented in this thesis examines the role mussels may play in recirculating dinoflagellate cysts within an aquaculture site, the impact of passage through the bivalve gut on the viability of cysts, and the retention time of the cysts within the bivalve digestive tract.

Mussels from the top and bottom of mussel socks were similarly contaminated with cysts of several dinoflagellate species (*Scrippsiella* sp., *A. fundyense*, putative *A. ostenfeldii*) as there was active sediment resuspension within the site. *Scrippsiella* sp. cysts dominated the dinoflagellate cyst population of the sediment, however fewer cysts of this species were egested. The composition of the faecal material collected from mussels contaminated in the field and in the laboratory suggests putative *A. ostenfeldii* cysts were not digested since intact cells of this species were dominant in the faeces. The estimated 8% daily replenishment rate of the bottom sediment *A. ostenfeldii* hypnozygotes by faecal pellet deposition is a considerable contribution to the maintenance of dinoflagellate cyst populations. As well, putative *A. ostenfeldii* cysts were viable following passage through the acidic bivalve gut. The thick-walled dinoflagellate cysts egested in faeces were of similar viability as cysts found in sediments, germinating in about 10 - 14 days. The estimated gut retention time of these cysts was 9 h.

The egestion of viable cysts from mussels could have serious implications for current aquaculture practice. The transport of contaminated bivalves among sites may result in the transfer of viable cysts, capable of germinating and seeding a toxic dinoflagellate bloom in a previously uncontaminated area.

Acknowledgements

I would like to acknowledge the advice and guidance of my supervisor, R. J. Thompson, and supervisory committee, D. Deibel and P. Dabinett. Their direction and encouragement were fundamental to the completion of this study.

The technical assistance provided by E. Hatfield, C. McKenzie and the Hann family was invaluable. Special thanks go to E. Hatfield and C. McKenzie for their insight into the trials and tribulations of studying *Alexandrium*.

I would like to recognize the computer expertise provided by S. Bettles, P. Matthews, and M. Riehl. Statistical advice was provided by H. Chen and D. Schneider. As well, thanks are extended to the OSC dive team and D. McKenzie for sample collection and transport.

I am grateful for the emotional support and physical sustenance provided by the following: T. Avery, S. Bettles, A. Bochdansky, R. Evans, L. Fogt, J. Hunter, A. Johansen, T. Manning, J. Perez, C. Rice, N. Richoux, and K. Smedbol. M. Riehl deserves special thanks for the Friday night BBQs which were often my only opportunity to eat well and socialize.

Finally, I would like to thank my family for their support and constant telephone calls of encouragement. My fiancé Brent is responsible for the completion of this work and my sanity. Who else would have driven me to the lab at 4 a.m. to sample mussel faeces? I am eternally grateful for his love, patience and technical assistance.

Funding for this study was provided by an NSERC Strategic Grant to R. J. Thompson *et al.* and a graduate student fellowship from Memorial University of Newfoundland.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ADW - Ashed dry weight

DW - Dry weight

FSW - Filtered seawater

HCS - High concentration sediment

LCS - Low concentration sediment

PSP - Paralytic shellfish poisoning

S.D. - Standard deviation

In memory of Sandra Kerr & Trisha Wyman

CHAPTER I

Introduction

1.1 Paralytic Shellfish Poisoning and Bivalve Molluscs

Paralytic shellfish poisoning (PSP) is a global problem caused by the consumption of contaminated shellfish which can result in human illness and death. It is a serious public health risk that presents an economic threat to the commercial harvest of both wild and cultured bivalve populations. It is important to understand the nature and causes of bivalve toxicity as they continue to pose serious problems to harvesters, seafood processors, consumers and regulatory agencies. Efficient monitoring programs have helped to ensure product safety and minimize disruption of harvesting (see review by Shumway 1990). To prevent toxicity to humans in Canada, an estimated $3.3 million is spent annually on product monitoring and analysis on both the east and west coasts by the Department of Fisheries and Oceans (Cembella & Todd 1993; Todd 1995).

PSP toxins are produced by certain genera of marine dinoflagellates, including *Alexandrium* spp. (Halim) emend Balech, *Gymnodinium catenatum* Graham, and *Pyrodinium bahamense* var. *compressum* (Böhm) Steidinger, Tester et Taylor (Hall & Reichardt 1984; Taylor 1984; Oshima *et al.* 1990). In temperate waters, the dinoflagellate *Alexandrium* (classified formerly as *Gonyaulax* spp. or the *Protogonyaulax catenella/tamarensis* species complex) is frequently implicated in incidents of PSP

toxicity (Taylor 1984; Shimizu 1987). Among the twenty-two species in the genus *Alexandrium* (Balech 1985), eight have been documented as sources of accumulated toxins (see review by Shumway 1990), the most studied species being *A. tamarense* (Lebour) Balech, the organism responsible for PSP in many parts of the world. *Alexandrium fundyense* (Lebour) Balech (formerly known as *Protogonyaulax tamarensis* or *Gonyaulax tamarensis*) is the dinoflagellate species primarily responsible for PSP in eastern Canada and the United States (Taylor 1984). *Alexandrium fundyense* is distinguished from *A. tamarense* by the absence of a distinctive ventral pore located on the 1' apical plate found on the latter species (Balech 1985). Recently it has been suggested by Anderson *et al.* (1994) that these two species may be varieties of a single species based upon toxin composition, allozyme electrophoresis and sexual compatibility. As this proposed change in taxonomy has yet to be confirmed, the two forms will be considered as separate species in this thesis.

The PSP toxins are a suite of tetrahydropurine neurotoxins composed of saxitoxin (STX) and at least 21 naturally occurring analogues (Oshima *et al.* 1993). Saxitoxin blocks transmission across neuromuscular synapses by inhibiting the inward flow of sodium ions to the axon (Steidinger & Baden 1984). Both the toxicity and toxin composition of toxigenic *Alexandrium* species have been shown to vary considerably among isolates from different locations (Maranda *et al.* 1985; Oshima *et al.* 1990; Anderson *et al.* 1994). The toxicity of isolates of *A. fundyense* from coastal regions of the northwest Atlantic ranges from below detection levels to > 100 pg STX eq $cell^{-1}$ and

may vary by an order of magnitude, depending upon culture conditions (Maranda *et al.* 1985, Cembella *et al.* 1988; Anderson *et al.* 1994).

Bivalves accumulate PSP toxins by ingestion of toxic dinoflagellates during suspension-feeding (Shumway *et al.* 1987, Shumway 1990). Toxin accumulation may depend upon several factors: filtration rate, the toxicity and concentration of the dinoflagellate cells, and a particular species' capacity for selective ingestion and/or assimilation. Although the sensitivity of invertebrates to saxitoxin is much lower than that of vertebrates, the degree of sensitivity has been shown to be species-specific (Twarog & Yamaguchi 1974). Among other factors, the ability of bivalve species to accumulate toxin may be a result of differing sensitivities of the nervous system of each species to saxitoxin, as suggested by Twarog and Yamaguchi (1974).

Extensive research has been conducted on the impact of motile, vegetative toxic dinoflagellate cells upon bivalve physiology and feeding behaviour. Exposure to *A. tamarense* cells can elicit a wide range of physiological responses in numerous bivalve species: differential shell valve closure, erratic heart rate, increased oxygen consumption, altered filtration rate and production of mucus-like material (Shumway *et al.* 1985a, 1987; Shumway & Cucci 1987; Gainey & Shumway 1988; Bricelj *et al.* 1990; Marsden & Shumway 1992, 1993). *Alexandrium tamarense* can also reduce byssus production in the blue mussel, *Mytilus edulis* Linnaeus (Shumway *et al.* 1987). Reduced tissue growth rates, absorption efficiencies and clearance rates have also been documented (Bricelj *et al.* 1990; 1993). Responses are generally species-specific and may act to protect bivalves

from the effects of the toxins (Marsden & Shumway 1993). A laboratory study (Shumway & Cucci 1987) exposing bivalves to bloom concentrations of *A. tamarense* (5 x 10^5 cells l^{-1}) documented 75% mortality in *M. edulis* from areas free of PSP, while *M. edulis* from Maine, a region of recurrent toxic blooms, did not experience any mortality. Twarog and Yamaguchi (1974) speculated that bivalves which are periodically exposed to toxic blooms may have developed mechanisms to ingest the toxic microalgae without adverse effects. In the absence of other microalgae, juvenile mussels have been reported to utilize toxic *A. fundyense* cells effectively as a food source (Bricelj *et al.* 1993).

Although the effects of motile, vegetative *Alexandrium* cells on bivalve behaviour and physiology have been extensively studied, there remains a paucity of data concerning the possible effects the benthic resting stage of *Alexandrium* may have upon bivalve biology. The motile, vegetative stages pose only a seasonal threat to bivalve harvest. In contrast, cysts are resident in sediments year-round. The research presented in this thesis examines the role mussels may play in recirculating cysts within an aquaculture site, the impact of passage through the bivalve gut on the viability of cysts, and the residence time of the cysts within the bivalve digestive tract.

1.2 Life cycle of *Alexandrium*

Many marine phytoplankton species produce dormant cysts (hypnozygotes) during their life histories. More than 80 species of marine dinoflagellates are known to produce cysts (Matsuoka *et al.* 1989). The alternation of generations between motile

vegetative cells and dormant resting cysts enables dinoflagellate populations to inhabit both the benthos and pelagos. Population growth occurs through binary division, but a transition to sexual reproduction results in the formation of gametes, which fuse. In *A. tamarense*, conjugation can occur between anisogamous (Turpin *et al.* 1978) or isogamous gametes (Anderson 1980; Fritz *et al.* 1989), giving rise to a planozygote (Fig. 1.1). A large, longitudinally biflagellate motile cell, the planozygote does not divide, but continues swimming for several days before transforming into a thick-walled resting cyst, or hypnozygote, which sinks to the sediments (Anderson 1980). *Alexandrium tamarense* cysts have an endogenous germination rhythm (Pfiester & Anderson 1987). Following an obligate dormancy period, the hypnozygote can remain in a quiescent state until temperature cues apparently trigger excystment (Anderson & Morel 1979; Anderson 1980). Alternatively, an annual endogenous "clock" has been proposed, restricting germination to a particular time of year (Anderson & Keafer 1987). This mechanism may explain germination of cysts in deep water sediments which are not exposed to exogenous changes in environmental conditions. Upon excystment, the hypnozygote releases a large, posteriorly-biflagellate planomeiocyte (Anderson & Wall 1978) which then divides to produce vegetative cells, the inoculum for subsequent blooms (Steidinger 1975; Dale 1977; Anderson & Wall 1978; Anderson 1980).

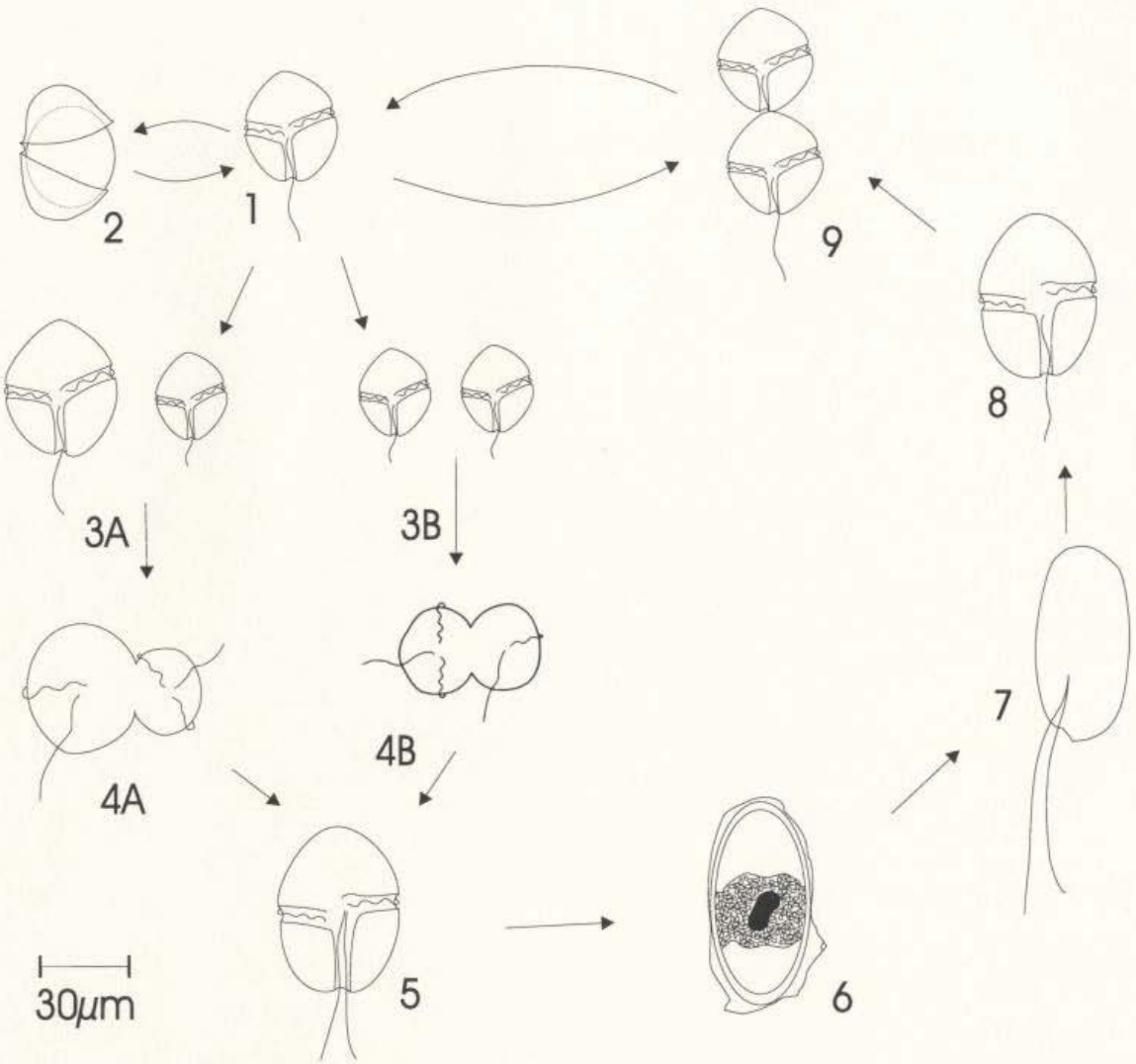

Fig. 1.1: Lifecycle diagram of the toxic dinoflagellate *Alexandrium tamarense*. Stages are as follows: (1) vegetative, motile cell; (2) temporary cyst; (3A) anisogamous "female" and "male" gametes; (3B) isogamous gametes (4A & B) fusing gametes; (5) swimming zygote or planozygote; (6) resting cyst or hypnozygote; (7 & 8) motile, germinated cell , or planomeiocyte; and (9) pair of vegetative cells (adapted from Anderson *et al.* 1995).

1.3 The role of *Alexandrium* hypnozygotes

Two types of cysts can be formed by dinoflagellates: temporary cysts and resting cysts. Temporary cysts are thin-walled, nonmotile, athecate cells, formed during vegetative growth as a result of short-term changes in ambient conditions (Anderson & Wall 1978). Absent from sediments, these cells regain motility within several days under favourable environmental conditions (Dale 1977). Temporary cysts are not considered in this thesis, therefore the use of term "cyst" here refers to the resting stage or hypnozygote.

Several functions have been attributed to dinoflagellate resting stages. Prakash (1967) established a relationship between detectable benthic cyst populations and historic shellfish toxicity sites, leading to the suggestion that toxic dinoflagellate blooms arise from benthic resting cysts. Certain locations which accumulate cysts as a result of selective deposition due to hydrographic and sedimentary processes serve as "seed beds", producing motile cells which then initiate blooms (Wall 1971; Steidinger 1975; Anderson & Morel 1979; Tyler *et al.* 1982). Other functions attributed to cysts are survival under adverse environmental conditions unfavourable for vegetative cells, species dispersal and protection against predation (Wall & Dale 1968; Dale 1983; Heaney *et al.* 1983). Encystment is often triggered by adverse conditions, particularly nutritional deficiencies (Blanco 1995).

Hypnozygotes formed at the end of *Alexandrium* blooms have been implicated as direct vectors of toxin transfer to bivalves (Dale *et al.* 1978; White & Lewis 1982). Blooms of vegetative, toxic dinoflagellates explain toxicity peaks in bivalves during

spring and summer months. However, high toxin levels have been reported during the winter which cannot be accounted for by blooms. Cembella and Shumway (1995) reported a winter toxicity peak in the surf clam, *Spisula solidissima* Dillwyn, which cannot be solely explained by toxin bioconversion since the results indicate a rise in both PSP toxin concentration and total body toxicity. This apparent discrepancy may be accounted for by the ingestion of toxic hypnozygotes.

The benthic resting cyst hypothesis for contamination of bivalves was first proposed by Bourne (1965) as an explanation for slow detoxification rates in scallops during winter months. Bivalves inhabiting regions with bottom sediments containing toxic cysts cannot avoid encountering these cells, and *A. tamarense* cysts have been reported in the gut of the sea scallop, *Placopecten magellanicus* Gmelin (Shumway *et al.* 1987). The abundance of *A. fundyense* cysts in sediments has been positively correlated with maximum recorded levels of PSP toxins in *M. edulis* (Schwinghamer *et al.* 1994).

The toxicity of the resting cyst of *Alexandrium* spp. relative to the motile vegetative cell has been debated in the literature. Early work by several researchers (Dale *et al.* 1978; Yentsch & Mague 1979; White & Lewis 1982) showed that the resting stage was more toxic than the vegetative cell, although these studies compared the toxicity of natural populations of hypnozygotes in sediments with that of cultured vegetative cells. Cultured *Alexandrium* vegetative cells are often less toxic than natural populations from the same region (Cembella & Therriault 1987; Cembella *et al.* 1988). More recently, Cembella *et al.* (1990) compared the toxin composition of naturally occurring

hypnozygotes and vegetative cells from the lower St. Lawrence estuary and reported that, in general, hypnozygotes were less toxic than motile cells. While some variation in toxicity may exist, depending upon geographical location, there is a consensus that *A. tamarense* cysts contain PSP toxins.

1.4 Thesis Objectives

The objectives of this thesis were to investigate the uptake, retention and elimination of *Alexandrium* hypnozygotes by a bivalve mollusc. The blue mussel, *Mytilus edulis*, was chosen for study as it is an important aquaculture species in the Maritimes and Newfoundland. Commonly used as a sentinel organism to monitor PSP, *M. edulis* is resistant to PSP toxins and is capable of sequestering large amounts of toxin during toxic algal blooms (Twarog *et al.* 1972; Twarog & Yamaguchi 1974). It rapidly accumulates and eliminates toxins from its tissues (Medcof *et al.* 1947) at rates faster than in other bivalves (Gainey & Shumway 1988).

Mussel aquaculture is an expanding industry in Newfoundland. The first documented case of PSP in Newfoundland occurred on 25 September 1982 at Harbour Grace, Conception Bay (Fig. 1.2) (White & White 1985), although it has been suggested that *A. fundyense* is indigenous to Newfoundland. Subsequent surveys of coastal sediments around the Province revealed contamination by *A. fundyense* cysts in many areas (McKenzie 1993, 1994; Schwinghamer *et al.* 1994). One former mussel farm, located in Barred Island Cove, Notre Dame Bay, now permanently closed to aquaculture

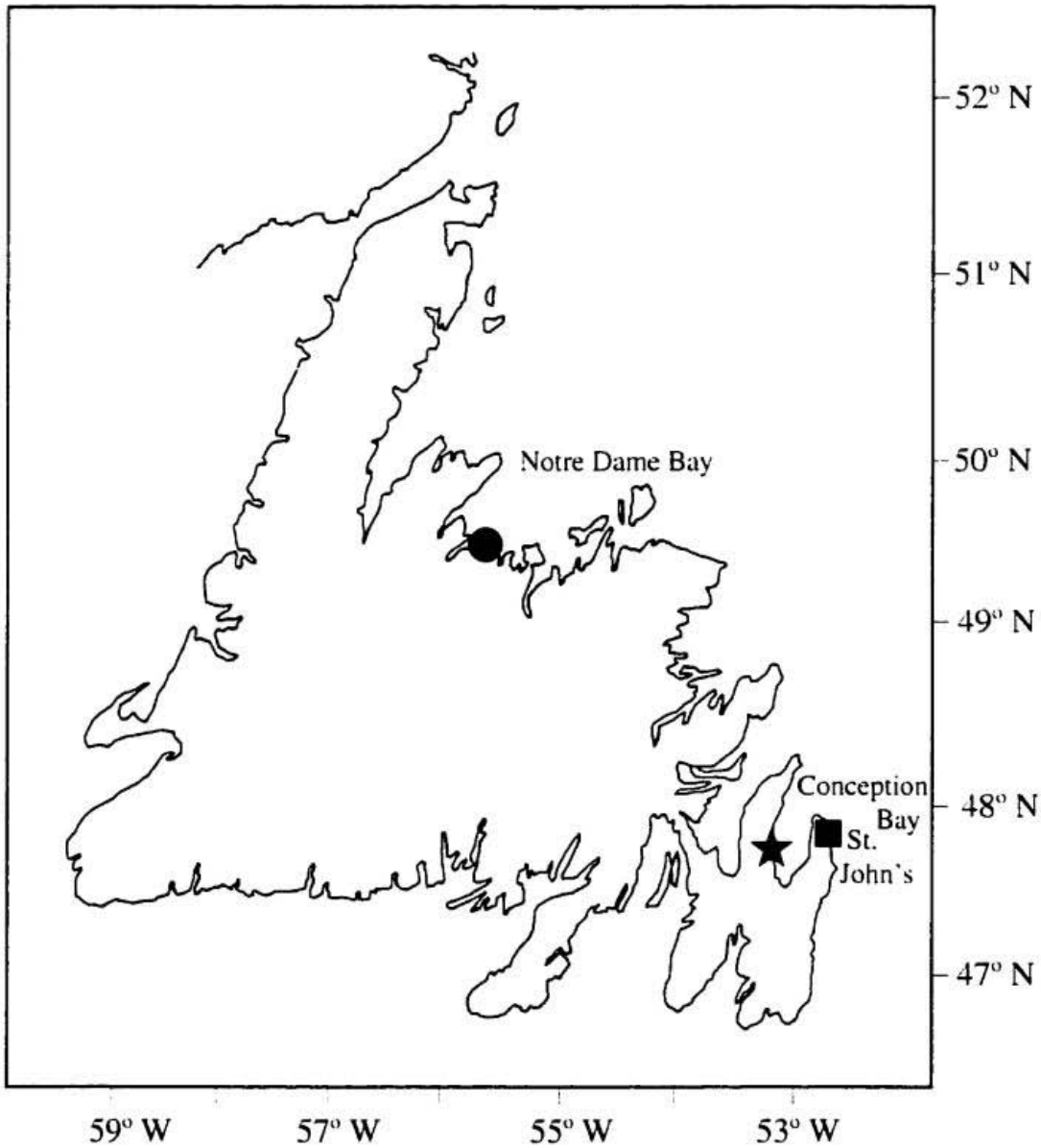

Fig. 1.2: Map of Newfoundland indicating locations of the study site, Barred Island Cove (●) and the site of the first recorded PSP incident in the Province, Harbour Grace (★).

due to consistent PSP contamination, has been the focus of ongoing research (Fig. 1.2). Sediment analysis at this site, a cove with two barrier islands and a shallow sill, revealed *A. fundyense* cyst concentrations ranging from 20 to > 1000 cysts cm^{-2} and frequent sediment resuspension as the result of strong winds (McKenzie 1993; Schwinghamer *et al.* 1994). Sediment core samples and mussels collected from established socks at this site were used to conduct the laboratory studies described in this thesis.

This thesis is divided into five chapters. The role mussels may play in maintaining populations of *Alexandrium* within an aquaculture site was examined by comparing the dinoflagellate cyst composition of faeces from naturally contaminated mussels with the distribution of cysts in the underlying sediment (Chapter II). The faecal material collected was then used to determine the viability of *Alexandrium* cysts after passage through the digestive tract of mussels (Chapter III). Mussels were artificially contaminated with sediment containing *Alexandrium* hypnozygotes in the laboratory to determine the gut retention time and compare pre- and post-ingestion selection of the cysts (Chapter IV). The results were then summarised and discussed in context with the current literature, suggesting avenues for further investigation (Chapter V).

Two species of mussels, *M. edulis* and *M. trossulus* (Gould 1850), co-exist in Newfoundland. Mussels used in this study were identified as *M. edulis* based upon morphological characteristics.

CHAPTER II

The role of the mussel in recirculating dinoflagellate cysts

2.1 Objectives

Accumulations of benthic toxic dinoflagellate cysts have been observed and studied in various marine environments: offshore trenches and depressions, fjords, and the shallow coastal embayments often selected for shellfish aquaculture operations (Dale 1976; Dale *et al.* 1978; Anderson & Morel 1979; White & Lewis 1982). The concentration of filter-feeding bivalves in an area such as an aquaculture site may facilitate the maintenance of a seed bed of *Alexandrium* hypnozygotes by concentrating transient vegetative cells or resuspended cysts. Therefore, the objective of the present study was to examine the role the mussel *Mytilus edulis* may play in recirculating dinoflagellate cysts within an aquaculture site contaminated with *A. fundyense* resting stages. Specifically, the location of mussels within the site and in the water column was assessed with respect to contamination by *Alexandrium* hypnozygotes, as measured by the concentrations of cysts in faecal material. Sediment samples were analysed to determine the concentrations of dinoflagellate resting stages within the bottom sediments and to compare these with cyst concentrations in mussel faeces. Finally, the biodeposition rate of dinoflagellate cysts was estimated as an index of the rate at which resuspended cysts are returned to the sediment surface by cultured mussels.

2.2 Materials and Methods

2.2.1 Mussel sampling and faecal pellet collection

Mussels were sampled in September and October 1996 from the top and bottom of mussel socks previously established at the study site, Barred Island Cove, Notre Dame Bay, Newfoundland (Fig. 2.1). Specific socks within the site were selected for study based upon previous estimates of *Alexandrium fundyense* cyst concentrations (McKenzie 1993), and differing bottom depths (Fig. 2.2). Since a bloom of *A. fundyense* vegetative cells occurred between the September and October sampling dates (McKenzie *et al.* 1997), the samples from September were termed pre-bloom and the samples from October were termed post-bloom.

On September 24, twenty-nine *Mytilus edulis* (mean shell length 63.5 mm ± 10.2 S.D.) were collected from socks 1, 2 and 4 and transported on ice in the dark to the Ocean Sciences Centre, Logy Bay. The transport time was approximately 8 h, during which there were no mortalities. Upon arrival, mussels were scrubbed free of epibionts and placed in individual 1 l containers with 700 ml UV-sterilized, filtered (1.0 μm) seawater (FSW) at 15°C for collection of faecal material. Following a 15 h incubation period, the mussels were removed and the faecal pellets were carefully collected with a large bore pipette and expelled into 7 ml glass vials containing 3.5 ml FSW. The total volume of the faecal material was measured to the nearest 0.1 ml using a graduated pipette. The vials were then stored in the dark at 4°C until processing. No pseudofaeces production was observed.

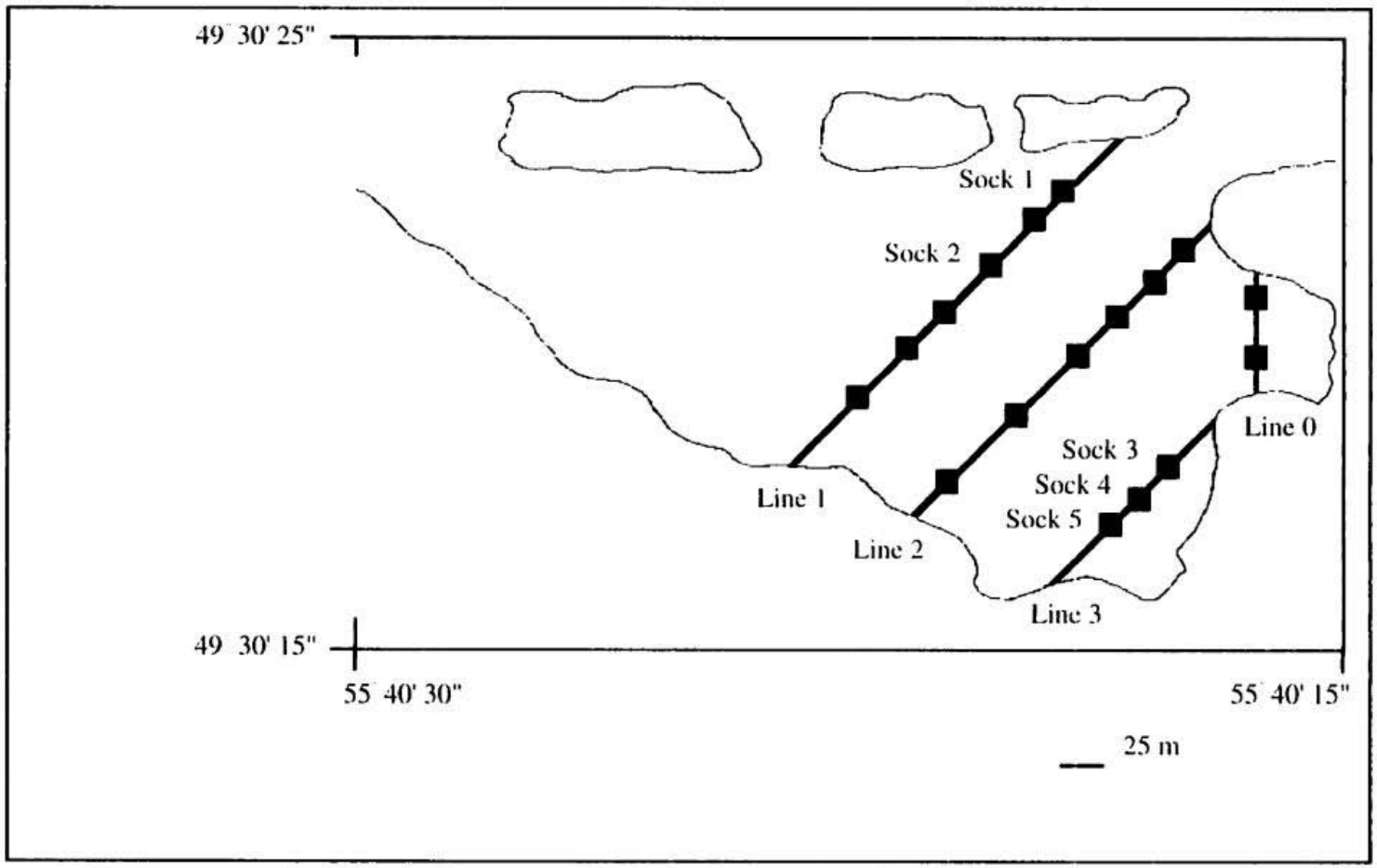

Fig. 2.1: Barred Island Cove sampling site, shown with the location of the transects (⁻) and mussel socks (■). Distance is indicated with the scale bar.

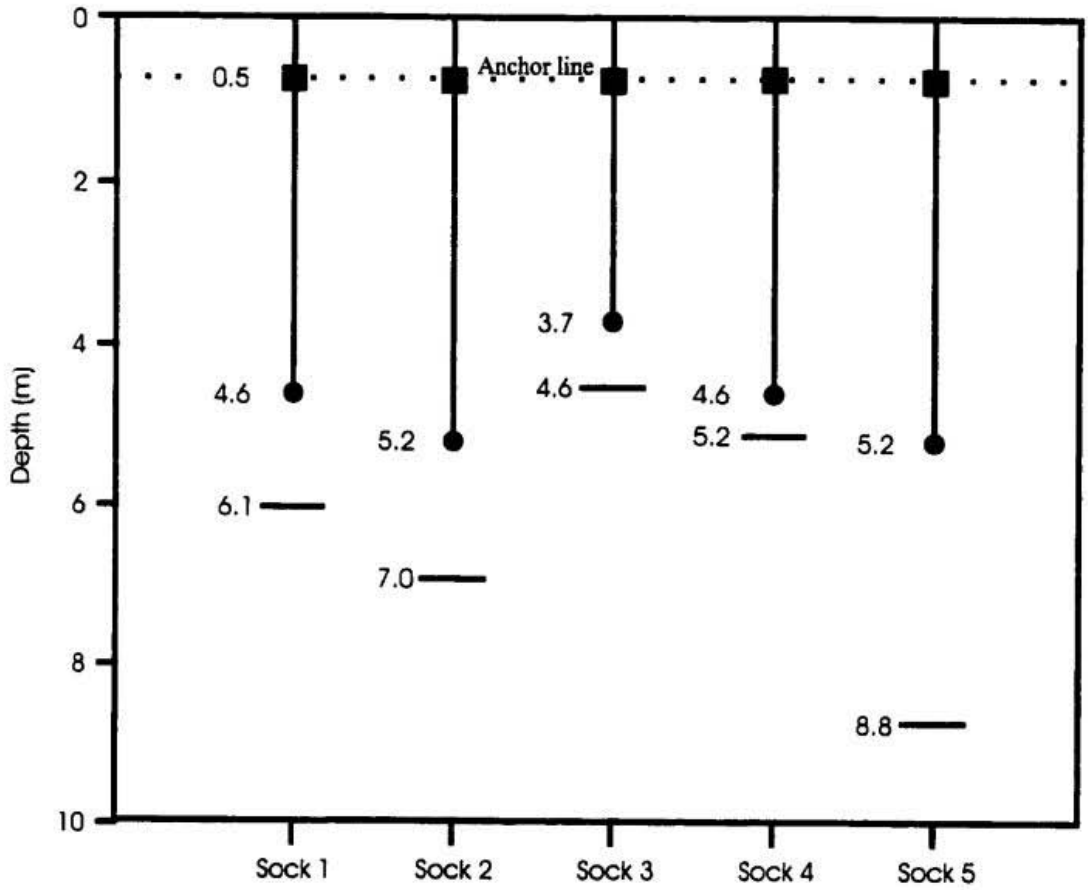

Fig. 2.2: Diagram depicting sampling depths (■ top of sock; ● bottom of sock) and bottom depths (—) for specific mussel socks established at the study site, Barred Island Cove, Notre Dame Bay, Newfoundland.

On October 29, four *M. edulis* (mean shell length 43.6 mm ± 6.7 S.D.) were sampled from the top and bottom of each of socks 1, 2, 3, and 5. Faecal pellets were collected on-site as follows: after being scrubbed free of epibionts, individual mussels were placed in plexiglass cylinders (diameter = 70 mm; height = 75 mm), filled with 200 ml surface water. Cylinders were sealed with a rubber stopper, leaving an air pocket, then submerged for a 19 h incubation period. The faecal material was then collected, transported to the laboratory and stored as described above.

Although the protocols for faecal collection were different in the two sampling dates, the experiments are comparable. In September, the faecal pellets were collected from mussels after a transport time interval of 8 h, whereas in October faeces were collected within one hour. Since the mussels remained closed during transport, there was no egestion of faeces. Prolonged retention of the material within the digestive tract does not appear to have affected the egestion of intact dinoflagellate resting stages as more cysts were recovered in mussel faeces in September than in October.

2.2.2 Determination of minimum incubation time

Although the gut retention time for *Alexandrium* cysts has not yet been determined for any bivalve (Scarratt *et al.* 1993 proposed a 12 h minimum), 15 h was considered sufficient time for complete gut evacuation based upon results of a preliminary examination of faecal and gut contents of sixteen mussels sampled from the study site on June 19, 1996. Mussels were incubated for 15 h and the faecal material

collected in the same manner as described for October. After the mussels were transported on ice to the laboratory, the entire digestive gland-stomach mass of each mussel was dissected out and the stomach contents removed with a micropipette. This material was suspended in FSW and inspected microscopically (see Section 2.2.4). Although intact *A. fundyense* resting stages were present in the faecal material collected, no intact dinoflagellate cysts of any species were found in the stomach contents, therefore all cells were presumed to have been evacuated.

2.2.3 Preparation of faecal material for examination: testing three methods of dispersal

Faeces produced by *Mytilus edulis* are mucus-bound pellets, composed of well-digested material mixed with partially digested material which may have been rejected during the sorting process within the stomach (Reid 1965; Widdows & Bayne 1971; Thompson & Bayne 1972). Three methods were tested to determine the most effective technique for complete dispersal of the faecal pellet contents: vortexing, repeated aspiration through a micropipette and sonication. Vortexing a faecal sample for up to ten minutes at the highest speed available was ineffective in disaggregating faecal pellets. Repeated aspiration through a 1 ml micropipette tip successfully fragmented the pellets into clumps, but these agglomerations were too dense to permit examination of the contents. Gentle sonication resulted in a homogenous suspension of faecal material, although it was necessary to determine the precise conditions to ensure there was no destruction of cysts (Anderson *et al.* 1984). For this purpose, two faecal samples,

suspended in filtered seawater in 7 ml glass vials, were sonicated for five minute intervals in an ice-water (4°C) sonicating bath (Cole Parmer 8851). Following each five minute period, 300 μL of each sample was examined using phase and epifluorescence microscopy. The number of *Alexandrium fundyense* cysts present was determined and the physical state of each cyst was assessed based upon the condition of the cell wall and cellular contents. Counts of *A. fundyense* cysts in the suspension remained constant for both samples after 30 minutes of sonication. Thirty minutes of sonication did not appear to have any detrimental effects upon *A. fundyense* cysts, nor did forty-five minutes of sonication, the maximum length of time tested.

2.2.4 Examination of faecal contents

The contents of the unpreserved faecal pellets were identified and enumerated by direct cell counting. Although this approach is laborious and time-consuming, it yields the most accurate information about the composition and condition of phytoplankton (Sakshaug 1990). An electronic particle counter could not be used for enumeration of the cells in the faecal material in this study, as the cysts were approximately the same size (40 μm diameter) and shape, and thus could not be distinguished with this method.

After disaggregating the faecal contents and freeing cysts from other detritus by sonication for thirty minutes in an ice-water bath, triplicate counts were made of dinoflagellate cysts in each faecal sample. From a homogeneous suspension, 200μL was dispensed into each of three Utermöhl settling chambers and settled for five minutes. The

entire surface of each chamber was examined using phase and epifluorescence microscopy on a Zeiss Inverted Microscope at magnifications of 200x and 400x. The counting error of this method, estimated by five replicate counts of a single sample, which contained 494 *Alexandrium ostenfeldii* putative cysts mg^{-1} ashed dry weight (ADW), was 20% (coefficient of variation: CV). When standardized for carbon content, the same sample contained 1.09 putative *A. ostenfeldii* cysts $\mu g\ C^{-1}$ and the CV was again 20%. The technical error in this counting method is comparable to the 20% CV counting error reported by Ishikawa and Taniguchi (1994) for enumeration of *Scrippsiella* spp. cysts in sediment.

The cysts of four dinoflagellate species (*Scrippsiella* sp., *Alexandrium fundyense*, and two unknown cyst types) were identified based upon their physical appearance under the light microscope (Chapter 3) and enumerated for each faecal sample. Preliminary examinations of sediment cores and mussel faeces from the study site revealed the presence of two unknown cyst types. The first is referred to as the "Grey" cyst because of its grey appearance under phase microscopy. The second cyst type numerically dominated the dinoflagellate cyst population in the sediment samples. It was not possible to determine the dinoflagellate species to which these two cyst types belonged. Germination studies conducted later (Chapter 3) suggested that the unidentified cysts of the second species are the resting stages of *Alexandrium ostenfeldii* (Paulsen) Balech et Tangen, a potentially toxic dinoflagellate species which has been observed in the Gulf of St. Lawrence (Levasseur *et al.* 1996), and in Nova Scotian coastal embayments

(Cembella *et al.* 1998). Grey cysts did not germinate under culture conditions and therefore remain unidentified in this study.

2.2.5 Faecal pellet organic and inorganic content for standardization of cyst abundance

To facilitate comparison between mussels from different locations and of different sizes, cyst abundance values were expressed per μg C and per mg ADW of faeces, as measures of organic and inorganic content of faeces respectively.

Duplicate samples of 0.7 - 1.2 ml homogeneous faecal suspension were filtered onto preweighed, preashed (450°C) Whatman GF/C filters (25 mm) to determine the carbon content of the faecal pellets. Duplicate filters for ADW determinations were similarly prepared, then rinsed with 10 ml 3% ammonium formate to remove salts. The remaining faecal suspension was used for sample blanks as seawater samples were not collected from the study site. This suspension was filtered through a 0.45 μm cellulose acetate filter which retained all cellular material and debris. Sample blanks were then prepared as above for the different analyses.

All filters were oven-dried to constant weight at 60°C. Organic carbon was measured by combustion in a Perkin Elmer Model 2400 elemental analyser. ADW filters were combusted at 450°C for 12 hrs, cooled in a desiccator and reweighed. Weights were determined within 1 μg using a Mettler UM3 microbalance.

2.2.6 Sediment core collection, concentration and examination

On September 24 and October 29, 1996, four sediment cores were collected by hand by SCUBA divers within a 1 m^2 area beneath each mussel sock in the study site. The top 6-8 cm of sediment and 2-3 cm of overlying water was collected using a modified 50 ml polycarbonate centrifuge tube with a cross-sectional area of 5.92 cm^2 (McKenzie 1993, 1994). Cores were transported on ice in the dark to the laboratory, where the top 3 cm of sediment and the overlying water were transferred to clean 50 ml centrifuge tubes, then stored in the dark, unpreserved, at 4 °C prior to processing. The top 3 cm fraction, rather than the flocculent surface layer, was analysed for the presence of dinoflagellate cysts (Anderson & Wall 1978; Dale *et al.* 1978; Lewis *et al.* 1979) because White and Lewis (1982) demonstrated that as many *Alexandrium tamarense* (= *Gonyaulax excavata*) cysts were present in the 3 - 4 cm fraction as at the surface.

The sediment was sonicated for two bursts, each 45 seconds, with a Branson 250 Probe Sonifier, with power set at level 4 to ensure adequate dispersal of detritus. The sample was then fractionated to separate and concentrate dinoflagellate resting stages following the density-gradient technique of Schwinghamer *et al.* (1991). In brief, following sonication, the sediment was sieved through 80- and 20-μm Nitex nylon sieves, retaining the material on the 20-μm sieve. A step gradient was then formed by injecting "light" and "dense" solutions composed of a non-toxic, aqueous colloidal silica suspension (Nalco 1060), made isosmotic with seawater of salinity 32‰ using sucrose, beneath the sieved cyst suspension in a 50 ml centrifuge tube. The tubes were then

centrifuged at 1469 g for 30 min at 4°C and the concentrated layer of dinoflagellate cysts formed at the interface between the light and dense solutions was removed for examination.

Cysts of the four dinoflagellate species in the mussel faecal pellets were enumerated in triplicate for each sediment core by examining the concentrated cyst layer using phase and epifluorescence microscopy (Section 2.2.4). Each count was performed on 100 - 200 μL of the homogenous cyst slurry, the volume settled for each core being adjusted to yield a total of 100 - 300 cysts per slide. It is recommended that at least 100 algal cells be counted to give a 95% confidence interval of the estimate within ± 20% of the mean value (Venrick 1978, with reference to Lund *et al.* 1958). The counting error (CV), estimated by five replicate counts of *A. ostenfeldii* cyst concentrations in one sediment core which contained 156 *A. ostenfeldii* cysts cm^{-3}, was 14%. Results are expressed as number of cysts per cm^3, from the top 3 cm of sediment.

2.2.7 Statistical analysis

The numbers of *Scrippsiella* sp., *Alexandrium fundyense, A. ostenfeldii* and Grey cysts in faeces of *Mytilus edulis* sampled from the top and bottom of mussel socks within the study site were analysed by two-way nested analysis of variance with the sock, position of mussel on the sock and the individual mussels as treatments. Separate analyses were performed for the abundance of each cyst type present in mussel faeces,

expressed per mg ADW and per μg of faecal carbon. September and October data were examined separately.

The variability within and between the four sediment cores sampled beneath each sock and between socks was analysed by nested one-way analysis of variance with the sock and the core, nested within the sock, as treatments. Analyses were performed separately for September and October data, and for each cyst species, expressed as the total number present per cm^3 of sediment.

Where the residuals of statistical analyses were not normally distributed, the p values were randomized, since randomization does not assume a normal distribution of residuals. The criterion for statistical significance in all analyses was $p \leq 0.05$.

2.3 Results

2.3.1 Analysis of dinoflagellate cyst counts in mussel faeces

In the two-way nested analysis of variance, the numbers of *Scrippsiella* sp., *Alexandrium fundyense*, *A. ostenfeldii* and Grey cysts present in the mussel faeces, per mg ADW, in September from the top of socks 1, 2 and 4 were similar to the numbers in faeces of mussels collected from the bottom of the socks ($p > 0.05$; Figs. 2.3 - 2.6). The same results were obtained for the numbers of *Scrippsiella* sp., *A. fundyense*, and Grey cysts per μg C in mussel faeces ($p > 0.05$; Figs. 2.7 - 2.9). Examination of individual socks using t-tests of mean cyst counts for each mussel revealed that there were more *A. ostenfeldii* cysts per μg C egested from mussels sampled from the bottom of socks 2 ($p =$

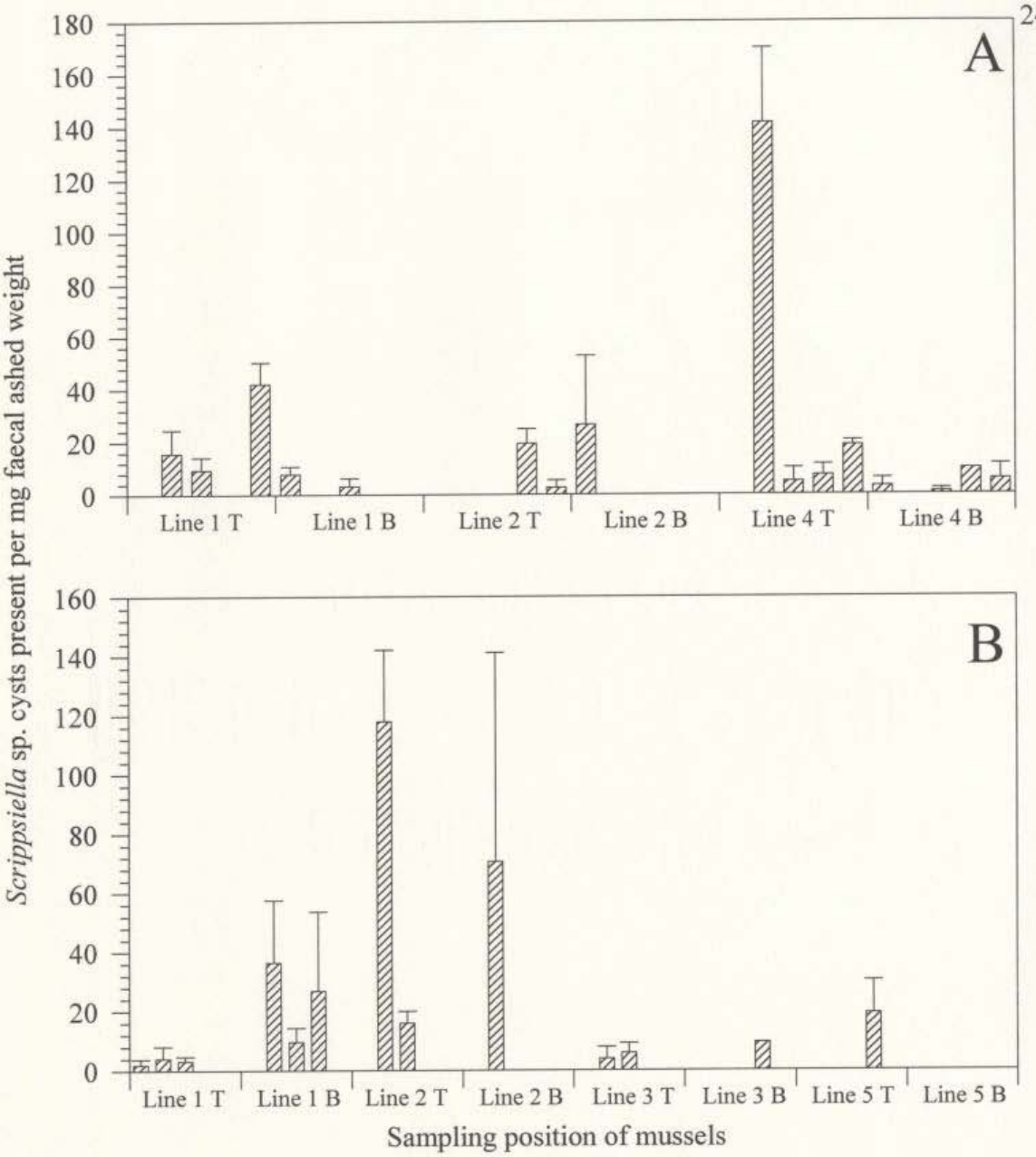

Fig. 2.3: Number of *Scrippsiella* sp. cysts present per mg faecal ashed weight from mussels sampled in September (A) and October (B) from various locations. T = top of mussel sock; B = bottom of the sock. Each solid bar represents the mean (n = 3) count per mussel, vertical bars represent the standard error of the mean.

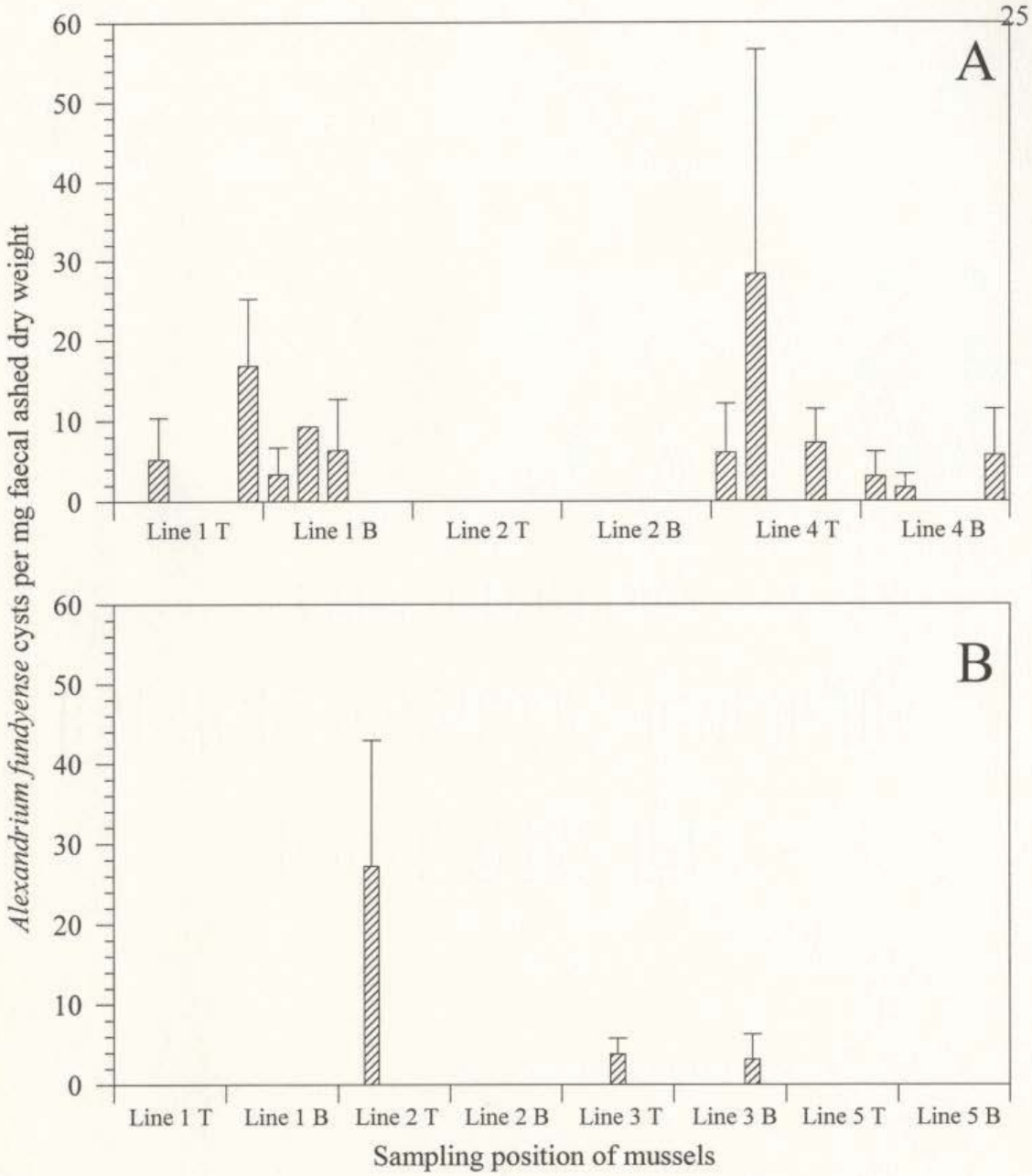

Fig. 2.4: Number of *Alexandrium fundyense* cysts present per mg faecal ashed weight from mussels sampled in September (A) and October (B) from various locations. T = top of mussel sock; B = bottom of the sock. Each solid bar represents the mean (n = 3) count per mussel, vertical bars represent the standard error of the mean.

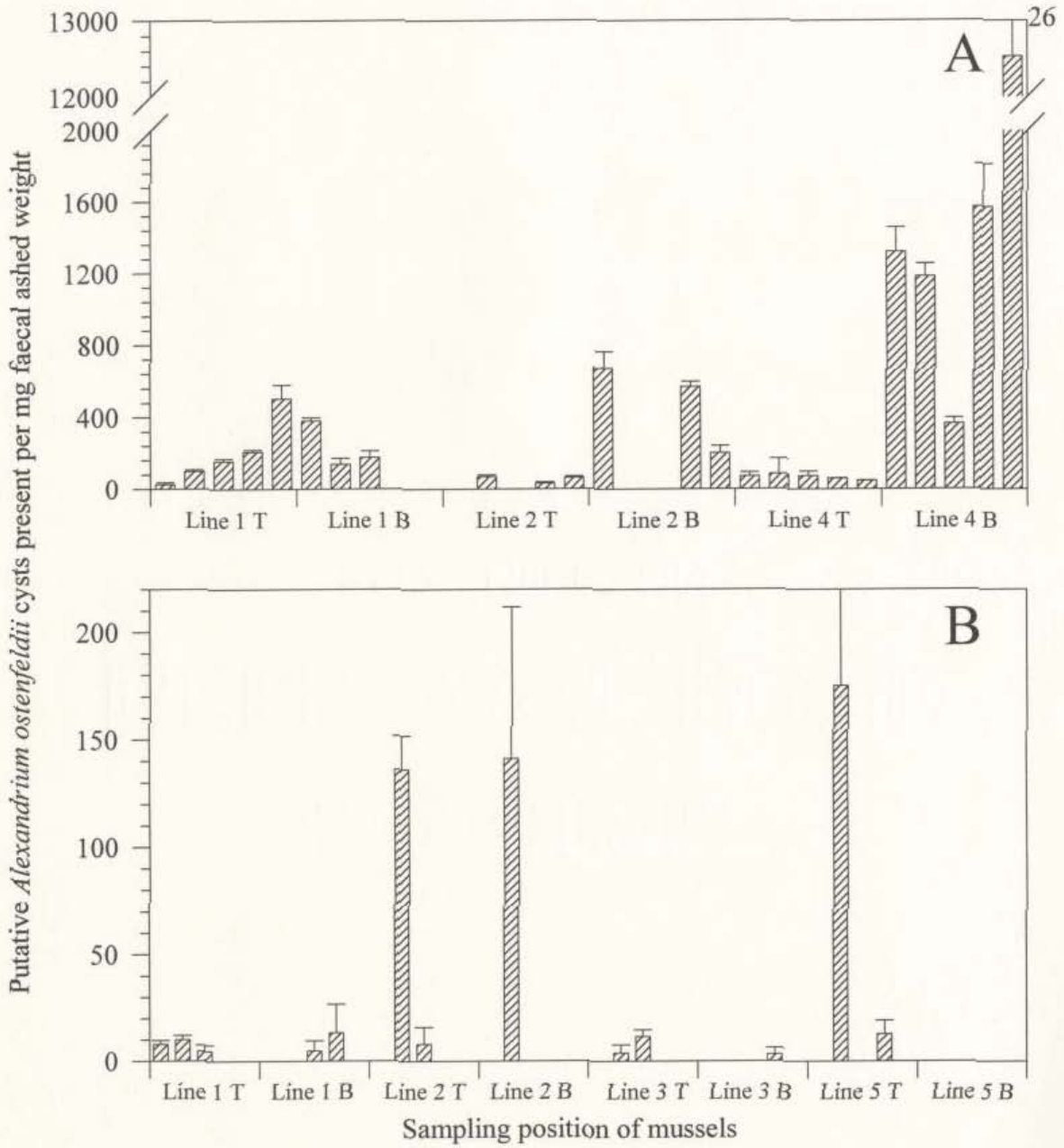

Fig. 2.5: Number of putative *Alexandrium ostenfeldii* cysts present per mg faecal ashed weight from mussels sampled in September (A) and October (B) from various locations. T = top of mussel sock; B = bottom of the sock. Each solid bar represents the mean (n = 3) count per mussel, vertical bars represent the standard error of the mean.

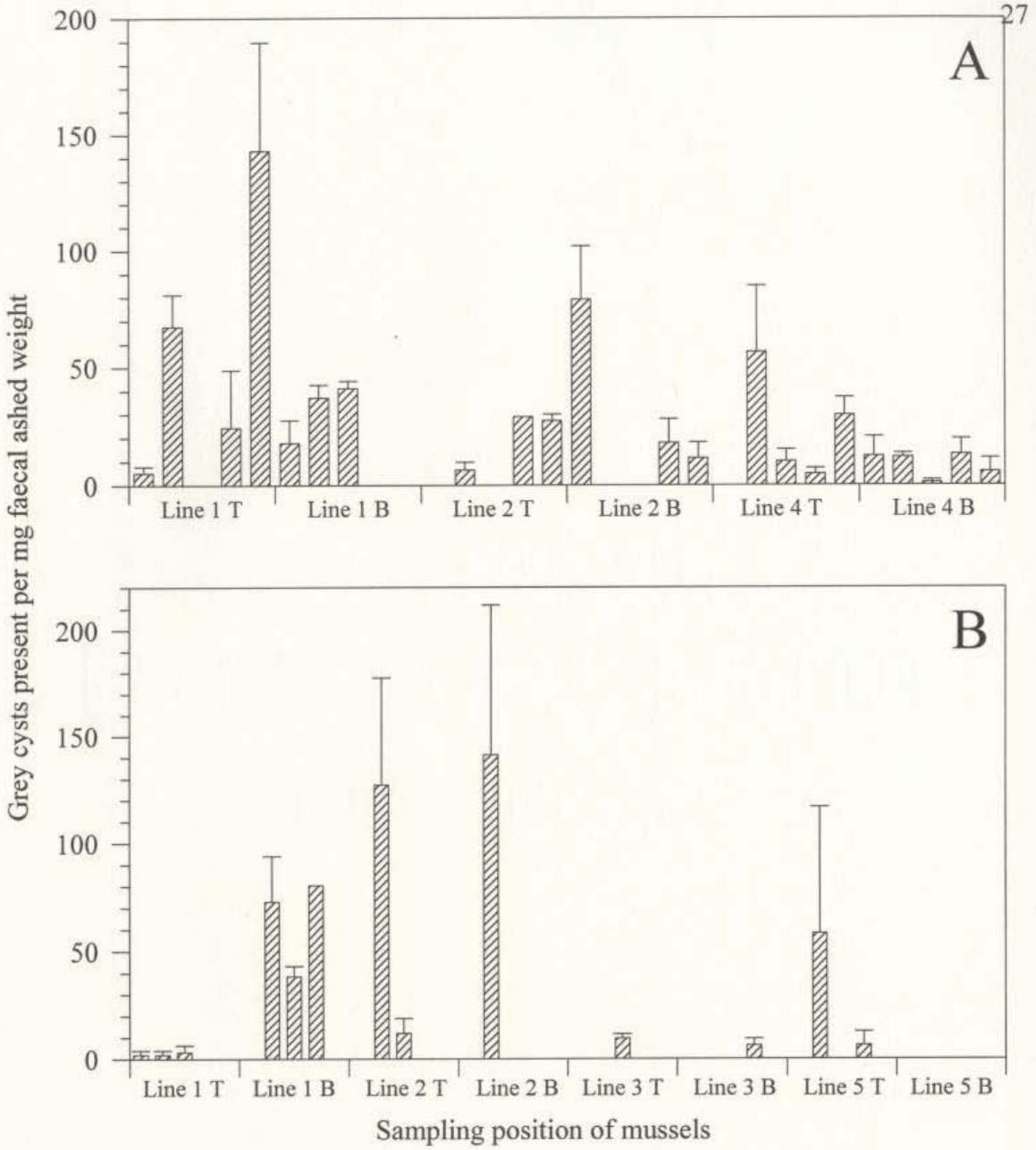

Fig. 2.6: Number of Grey cysts present per mg faecal ashed weight from mussels sampled in September (A) and October (B) from various locations.
T = top of mussel socks; B = bottom of the sock. Each solid bar represents the mean (n = 3) count per mussel, vertical bars represent the standard error of the mean.

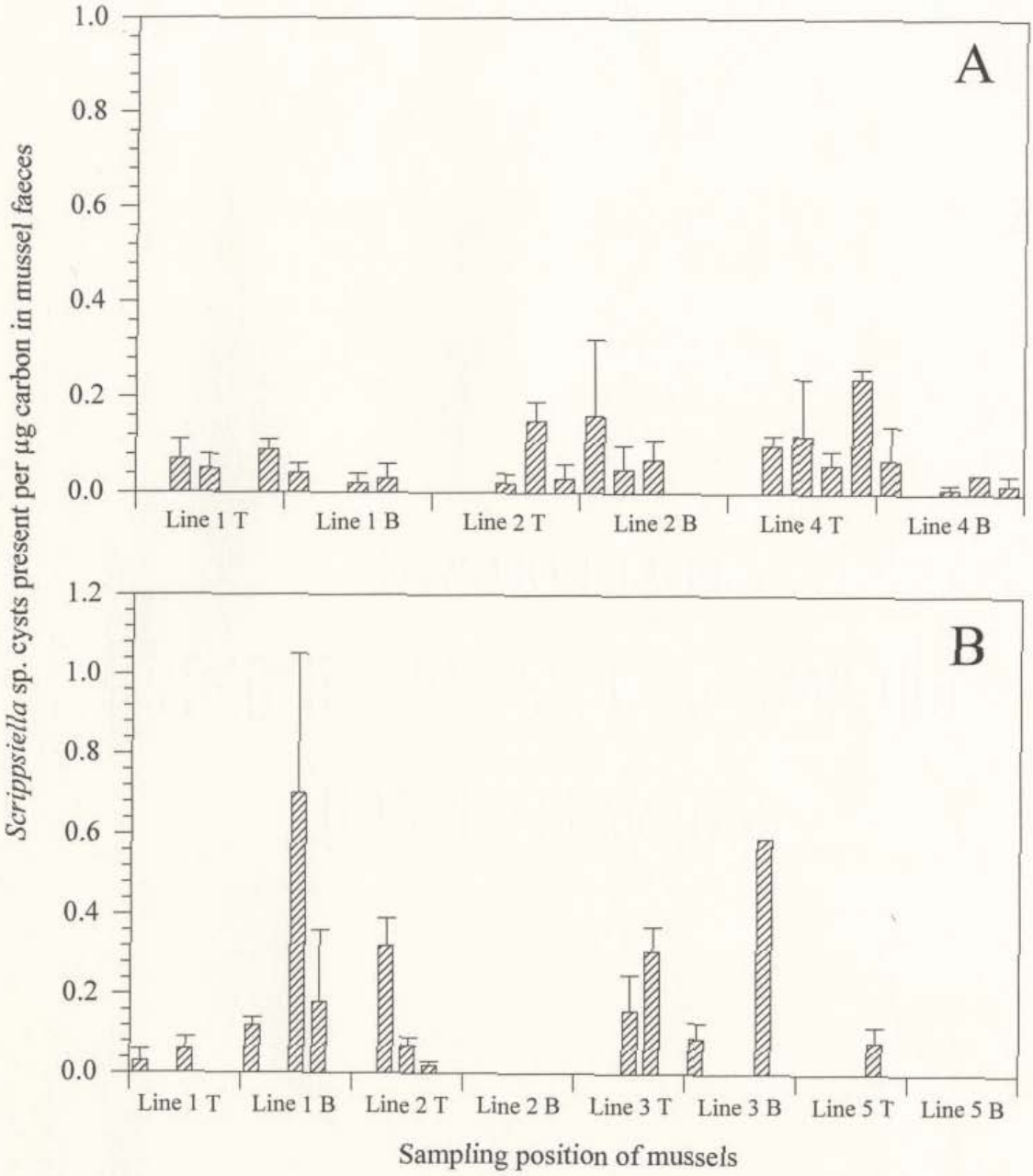

Fig. 2.7: Number of *Scrippsiella* sp. cysts present per µg carbon in faeces of mussels sampled in September (A) and October (B) from various locations.
T = top of mussel sock; B = bottom of the sock. Each solid bar represents the mean (n = 3) count per mussel, vertical bars represent the standard error of the mean.

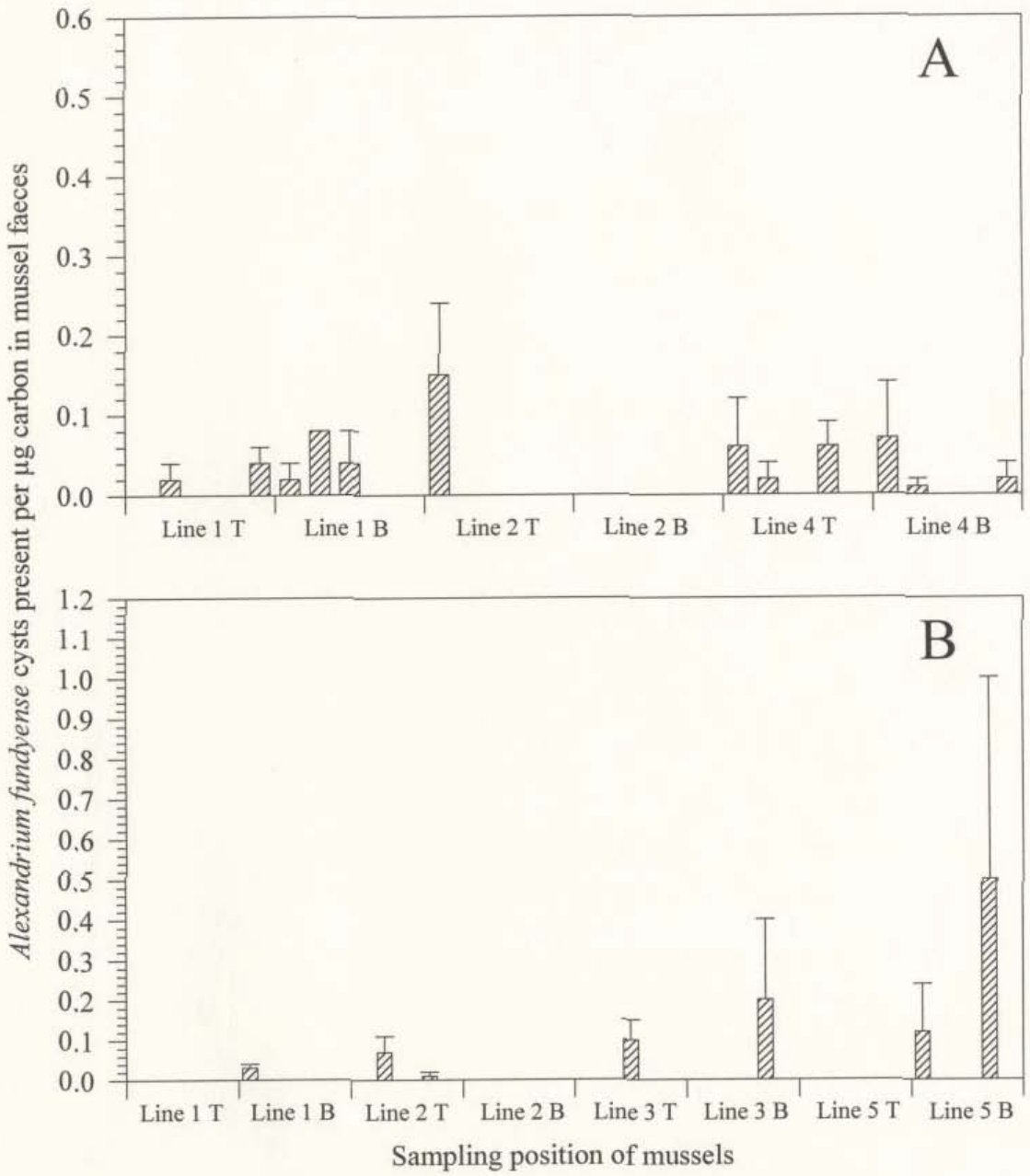

Fig. 2.8: Number of *Alexandrium fundyense* cysts present per µg carbon in faeces of mussels sampled in September (A) and October (B) from various locations. T = top of mussel sock; B = bottom of the sock. Each solid bar represents the mean ($n = 3$) count per mussel, vertical bars represent the standard error of the mean.

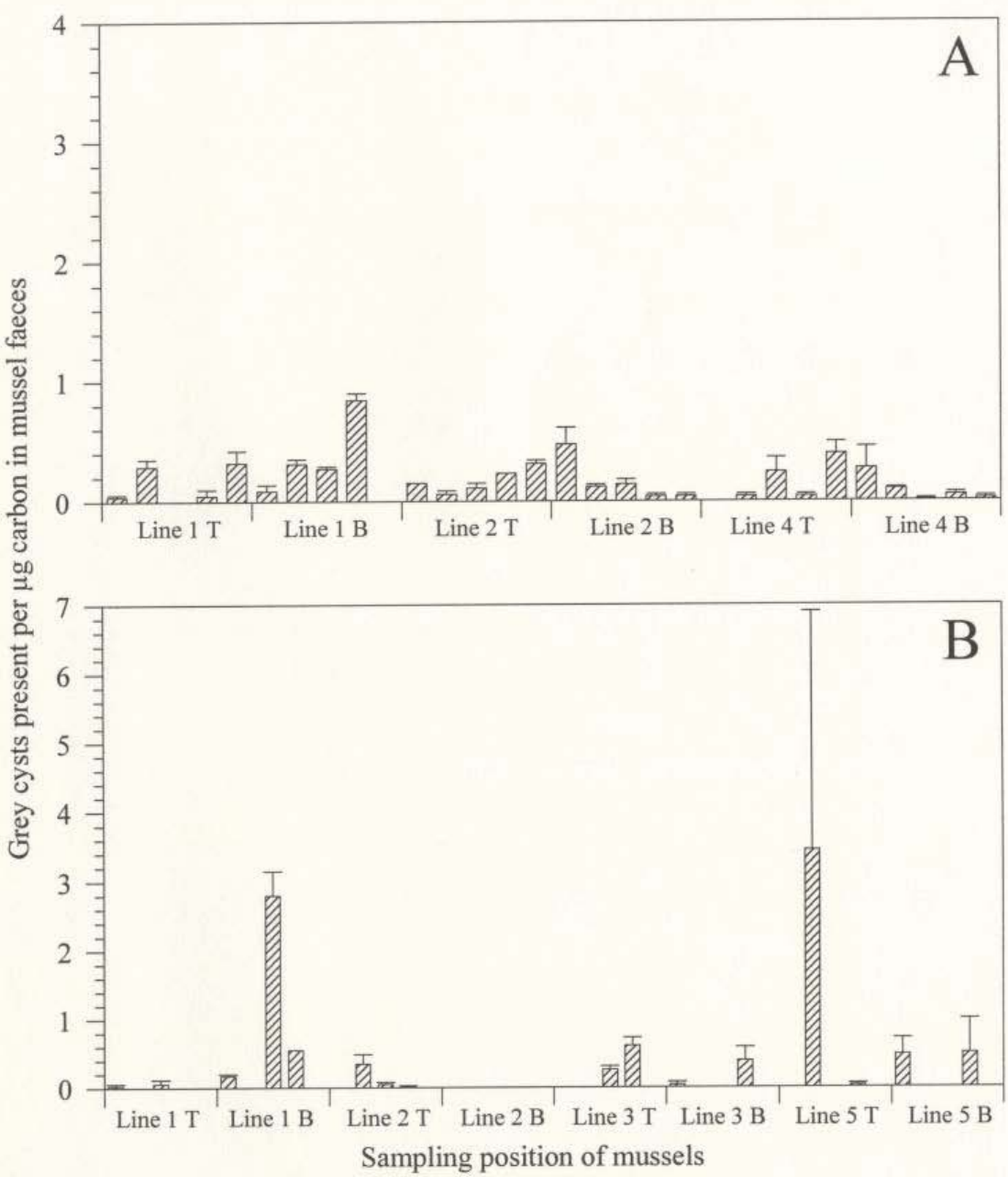

Fig. 2.9: Number of Grey cysts present per µg carbon in faeces of mussels in September (A) and October (B) from various locations.

T = top of mussel sock; B = bottom of the sock. Each solid bar represents the mean (n = 3) count per mussel, vertical bars represent the standard error of the mean.

0. 0477) and 4 ($p < 0.03$) than from the top of the socks (Fig. 2.10). There were no significant differences for sock 1.

In October, the numbers of *Scrippsiella* sp., *A. fundyense, A. ostenfeldii* and Grey cysts, standardized per mg ADW and per μg C, were similar in the faeces of mussels collected from the top and bottom of mussel socks 1, 2, 3 and 5 ($p > 0.05$; Figs. 2.3 - 2.10).

Faeces collected from socks in September generally contained more *A. ostenfeldii* cysts than faeces collected in October. The number of *A. ostenfeldii* cysts defaecated from mussels sampled from the bottom of sock 4 in September was at least thirty times greater than that defaecated from mussels from the top of the same sock (Figs. 2.5 & 2.10).

2.3.2 Analysis of dinoflagellate cyst concentrations in sediment beneath mussel socks

To examine the variability in composition within and between sediment cores beneath each mussel sock and the variability between socks, core contents were analysed using a nested one-way ANOVA. In all analyses the concentrations of *Scrippsiella* sp., *Alexandrium fundyense, A. ostenfeldii* and Grey cysts per cm^3 of sediment were significantly different between the four cores beneath each sock ($p < 0.03$), therefore the variability due to cores beneath each mussel sock was tested by separate one-way ANOVA.

Although sampled within a one square metre area, the dinoflagellate cyst compositions of the four cores taken beneath each sock were highly variable in both

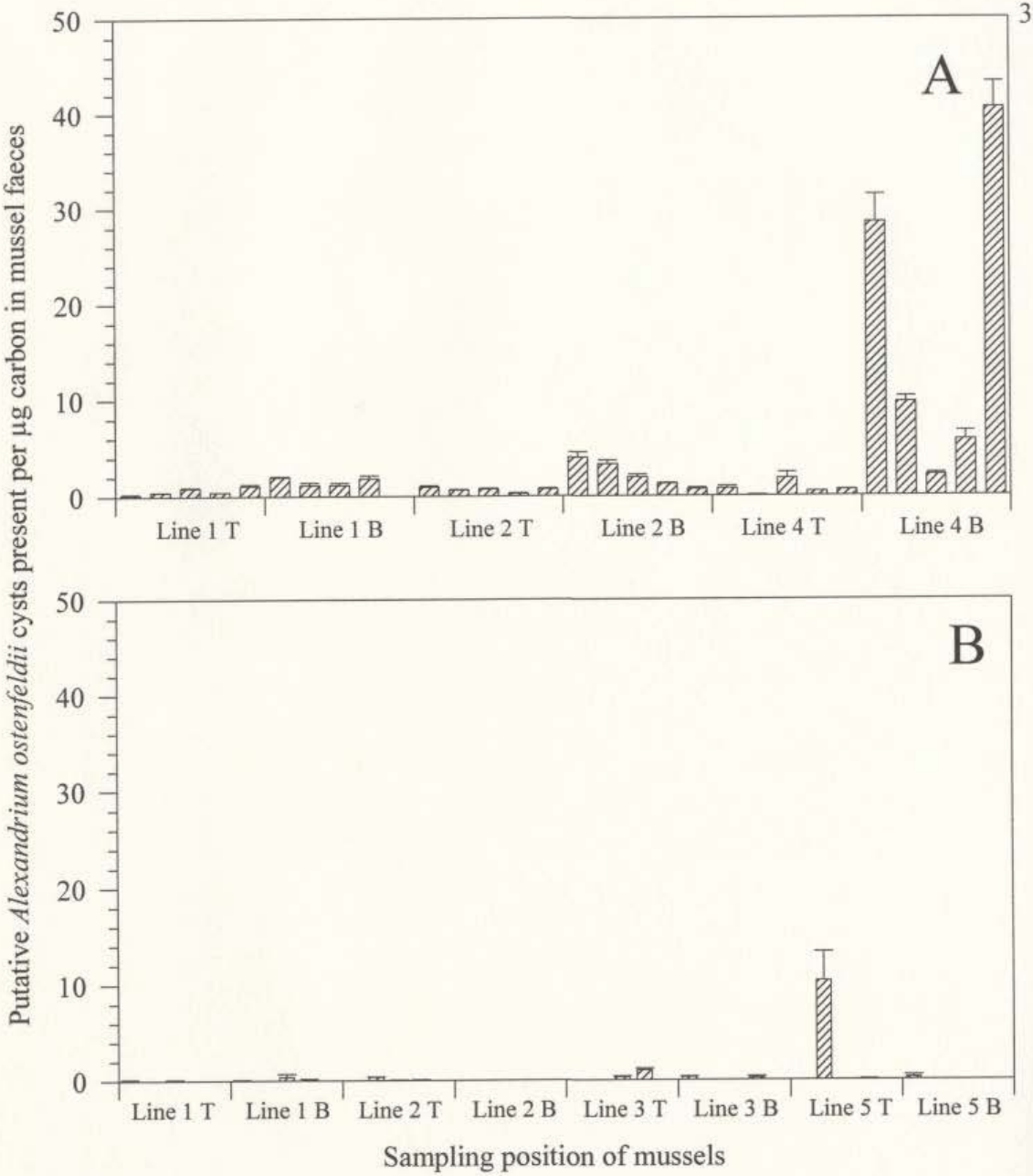

Fig. 2.10: Number of putative *Alexandrium ostenfeldii* cysts present per µg carbon in faeces of mussels sampled in September (A) and October (B) from various locations. T = top of mussel sock; B = bottom of the sock. Each solid bar represents the mean (n = 3) count per mussel, vertical bars represent the standard error of the mean.

September and October (Table 2.1). The concentrations of *Scrippsiella* sp. hypnozygotes varied between all sediment cores beneath each mussel sock, ranging from 265 to 1665 cysts cm^{-3} in September and from 76 to 610 cysts cm^{-3} in October (Fig. 2.11). Concentrations of *A. fundyense* (Fig. 2.12) and *A. ostenfeldii* (Fig. 2.13) in cores beneath sock 2 were consistent for both sampling dates, however beneath sock 3 concentrations of the same species were highly variable. Prior to the bloom of *Alexandrium* vegetative cells in September, *A. fundyense* cyst concentrations in the sediment varied from 3 to 371 cysts cm^{-3} while *A. ostenfeldii* cyst concentrations ranged from 13 to 183 cysts cm^{-3}. Post-bloom concentrations of *A. fundyense* cysts in the sediment ranged from 1 to 214 cysts cm^{-3} and concentrations of *A. ostenfeldii* cysts varied from 14 to 581 cysts cm^{-3}. With the exception of the sediment samples beneath sock 1 in September (2 - 25 cysts cm^{-3}), Grey cysts were rare (<11 cysts/cm^3) or absent from sediment beneath each sock (Fig. 2.14).

Statistical comparisons between dinoflagellate cyst concentrations in sediment from September and October were not possible due to the variability between cores sampled beneath the same mussel sock, so general trends are presented. From September to October, the number of *Scrippsiella* sp. resting stages in the sediment decreased, as did the abundance of *A. fundyense* cysts, with the exception of sock 3 beneath which *A. fundyense* concentrations increased. The concentration of putative *A. ostenfeldii* hypnozygotes beneath the mussel socks also decreased from September to October for socks 1 and 2, but tended to increase under socks 3 and 4. Grey cysts, present in all cores

Table 2.1: P values from one-way analysis of variance comparing the variability between sediment cores beneath each mussel sock using mean cyst counts per core for each cyst species. NS = not significant.

Cyst species	Sock	September	October
Scrippsiella sp.	1	0.0001	0.0002
	2	0.0001	0.0001
	3	0.0001	0.0023
	4	0.0009	0.0001
Alexandrium fundyense	1	0.0001	NS
	2	NS	NS
	3	0.0037	0.001
	4	NS	0.0016
putative *Alexandrium ostenfeldii*	1	NS	0.0117
	2	NS	NS
	3	0.0001	0.0001
	4	NS	NS
Grey cyst	1	NS	NS
	2	NS	0.0126
	3	NS	---
	4	---	---

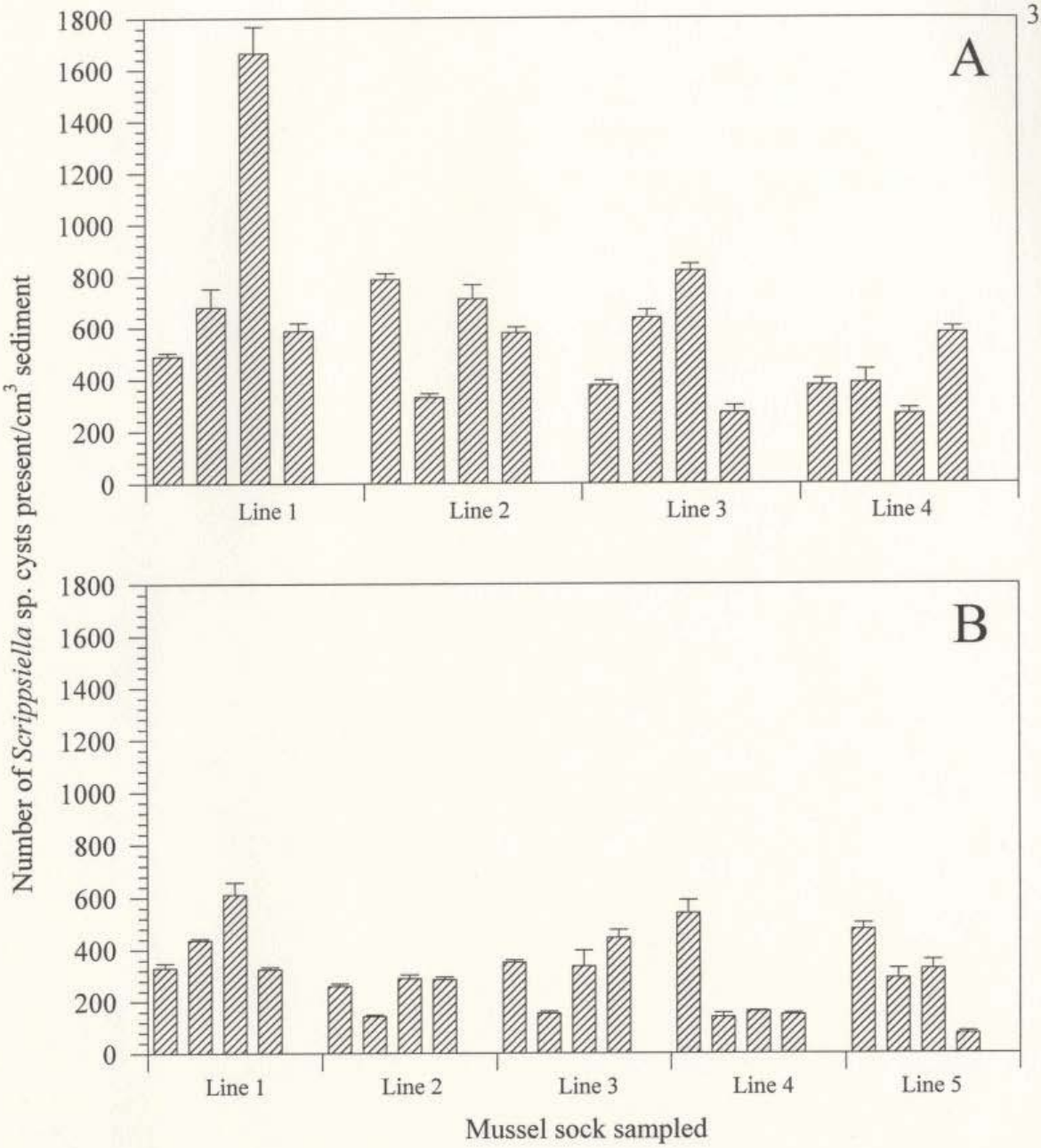

Fig. 2.11: Number of *Scrippsiella* sp. cysts present per cm^3 sediment sampled beneath mussel socks in September (A) and October (B). Each solid bar represents the mean count per core, vertical bars represent the standard error of the mean.

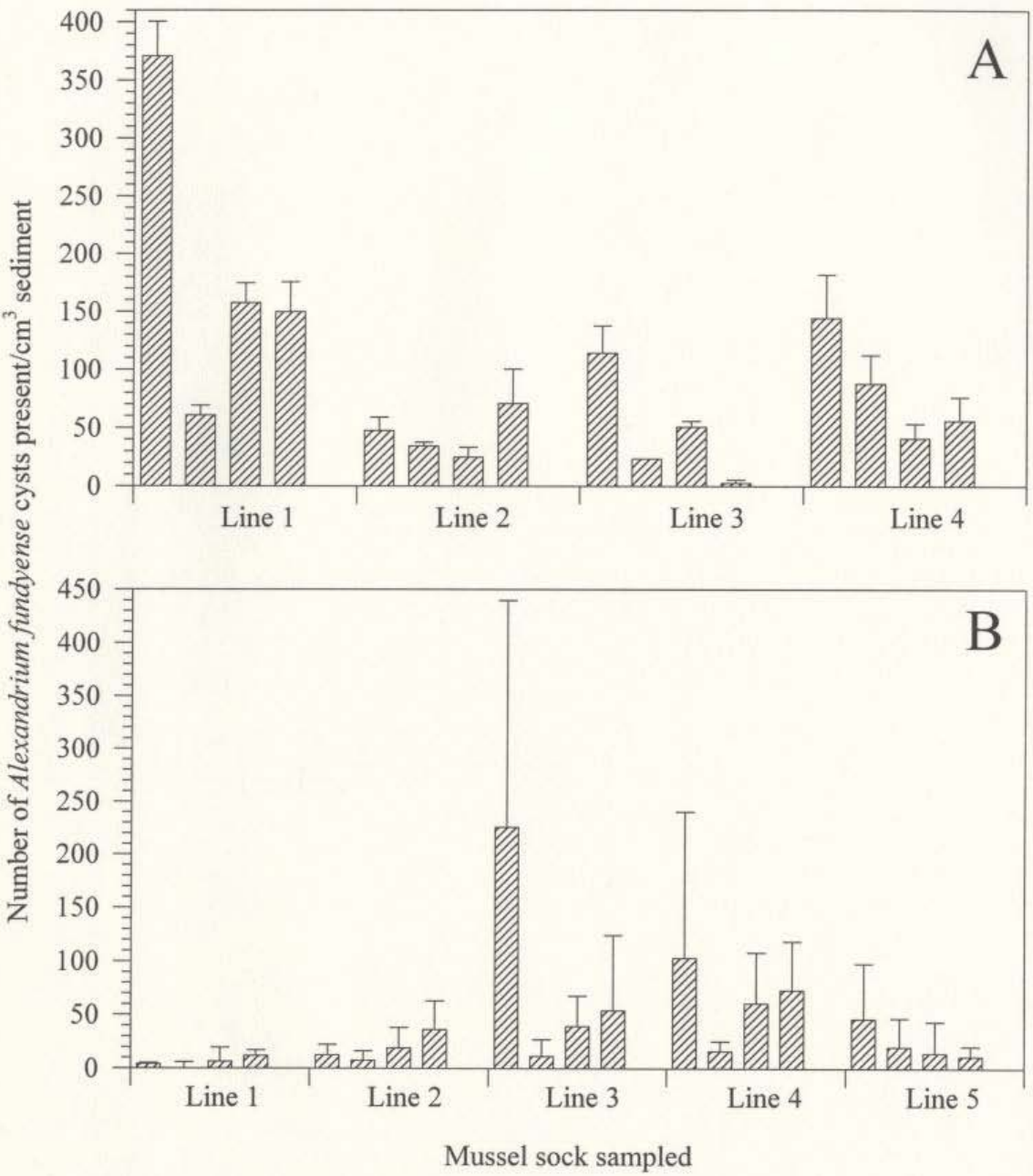

Fig. 2.12: Number of *Alexandrium fundyense* cysts present per cm^3 sediment sampled beneath mussel socks in September (A) and October (B). Each solid bar represents the mean count per core, vertical bars represent the standard error of the mean.

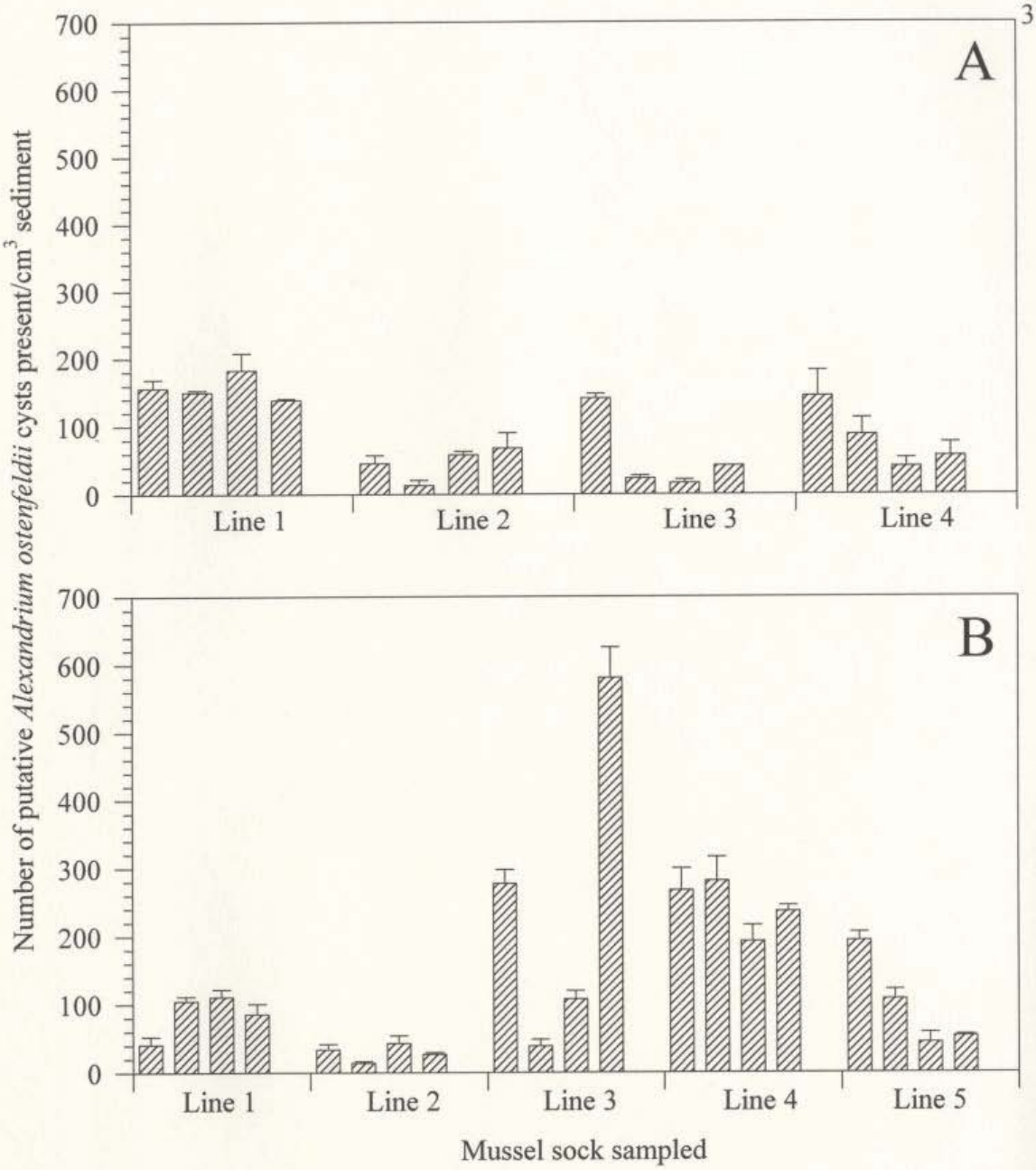

Fig. 2.13: Number of putative *Alexandrium ostenfeldii* cysts present per cm^3 sediment sampled beneath mussel socks in September (A) and October (B). Each solid bar represents the mean count per core, vertical bars represent the standard error of the mean.

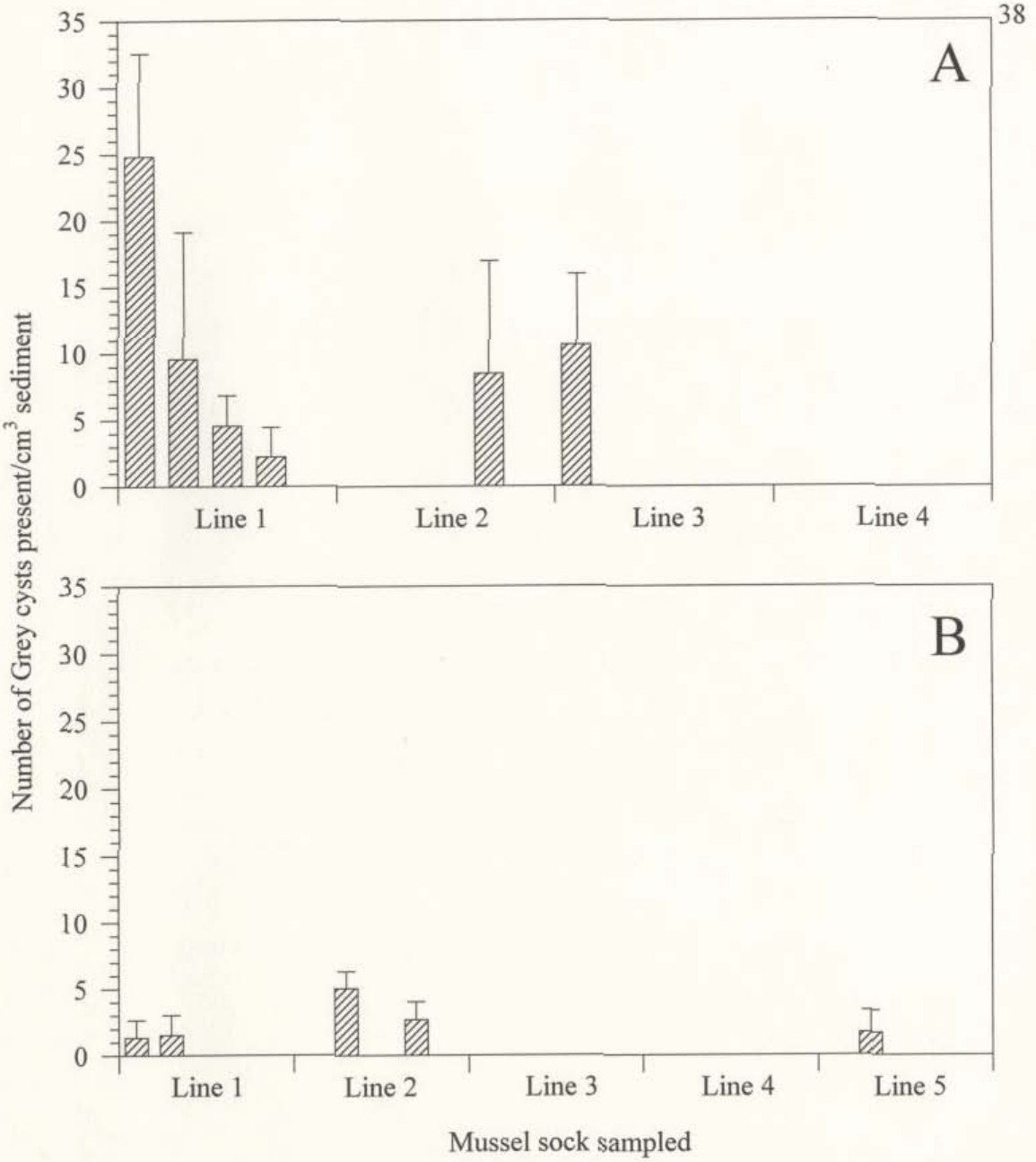

Fig. 2.14: Number of Grey cysts present per cm^3 sediment sampled beneath mussel socks in September (A) and October (B). Each solid bar represents the mean count per core, vertical bars represent the standard error of the mean.

under only sock 1 in September, were rare or absent in the sediment beneath socks 2 - 4 in September and beneath all four mussel socks in October.

2.3.3 Comparison of dinoflagellate cyst composition of faecal pellets and sediment cores

The relative proportions of the cysts of each of the four dinoflagellate species encountered in the study site were similar in the sediment in September (n = 16) and October (n = 20). On both sampling occasions, the dominant dinoflagellate cyst type in the sediment was *Scrippsiella* sp. (Fig. 2.15). In contrast, the dinoflagellate cyst composition of mussel faeces in September (n = 29) and in October (n = 32) was dominated by putative *Alexandrium ostenfeldii*. This trend is most evident in September samples: *Scrippsiella* sp. resting stages comprised 77.1% of the total dinoflagellate cysts in the sediment, whereas *A. ostenfeldii* comprised 81.1% of the cyst composition in mussel faeces.

2.4 Discussion

2.4.1 Variability of data

There was considerable variation in the precision of dinoflagellate cyst counts in mussel faeces. This can be attributed to the low counts in most of the sampling units. The spatial coverage of the core samples was too small relative to the spatial patch scale of the cysts. Fewer, larger cores should have been sampled to reduce the variability. The abundance of these cell types in the sediment underlying the mussel socks was less

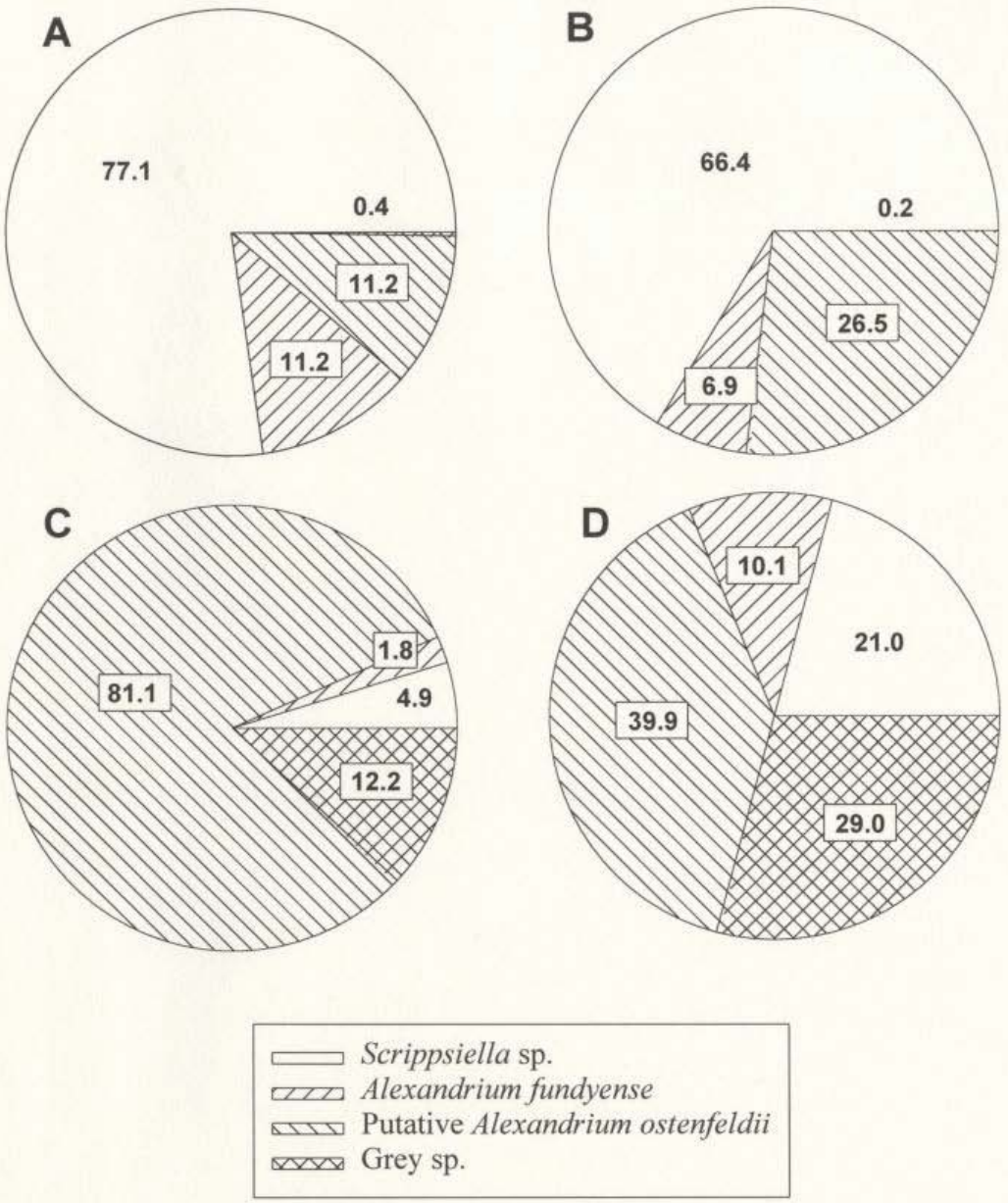

Fig. 2.15 Species composition of dinoflagellate cysts in sediment beneath mussel socks (A = September sediment, B = October sediment) and in mussel faeces (C = September faeces, D = October faeces).

ariable as cyst densities were greater. Cembella *et al.* (1988) demonstrated that the precision of triplicate counts of *Alexandrium tamarense (≈ Protogonyaulax tamarensis)* cysts from sediments in the lower St. Lawrence estuary was strongly affected by the cyst density: samples with > 500 cysts cm^{-3} had a CV between 4-10%, while the CV varied from 18-100% for samples with < 150 cysts cm^{-3}.

2.4.2 Significance of mussel position in water column with respect to cyst contamination

Vertical relaying of mussels in the water column may limit their exposure to toxic dinoflagellate cells (Desbiens *et al.* 1990). Desbiens and Cembella (1993) tested this strategy to reduce PSP contamination by positioning mussels near the bottom of the water column (13 - 15 m deep) during a bloom of vegetative *Alexandrium tamarense (≈ A. excavatum sensu* Balech) cells and raising the mussels close to the surface at times when *A. tamarense* was concentrated near the bottom of the water column. Mussels placed near the bottom were found to be the least toxic during most of the cycle. The reverse strategy was tested in this study to determine whether mussel contamination by resuspended *Alexandrium* cysts could be limited in a relatively shallow aquaculture site (5 - 11 m deep).

Contamination of *Mytilus edulis* by *Alexandrium* hypnozygotes was similar for all mussels sampled in this study, regardless of the mussel location in the water column and the depth of the sediment beneath the mussel sock. Faecal material collected from mussels at the top and bottom of mussel socks contained similar amounts of *Scrippsiella*

sp., *A. fundyense*, putative *A. ostenfeldii* and Grey cysts. The only exception to this trend occurred in September for socks 2 and 4: cysts resembling *A. ostenfeldii* were found in greater abundance in faeces (standardized by carbon content) collected from mussels sampled from the bottom of the socks than in faeces from mussels at the top. It was expected that mussels from the bottom of the socks would contain more dinoflagellate cysts than mussels from the top, due to their proximity to the sediment surface. However, sediment resuspension carries cysts throughout the site, therefore exposure to resuspended cysts cannot be minimized by careful placement of mussel arrays.

While the high contamination of mussel faeces from the bottom of socks 2 and 4 by *A. ostenfeldii* cysts was expected, as a corollary it was also expected that there would be a corresponding degree of faecal contamination by the other dinoflagellate species studied. This is based on the assumption that cysts are present in resuspended sediment and available to mussels as seston at similar concentrations to those in the underlying sediment surface layer. The densities of all four cyst types are similar (between 1.2 - 1.4 g cm^{-3}) as they all concentrate together in the density-gradient centrifugation technique (Schwinghamer *et al.* 1994). The specific gravity of *Scrippsiella trochoidea* (Stein) Loeblich III cysts ranges from 1.05 to 1.39 g cm^{-3} and that of *A. fundyense* cysts from 1.15 to 1.30 g cm^{-3} (Anderson *et al.* 1985). Faecal material from mussels from the bottom of sock 1 was not highly contaminated by putative *A. ostenfeldii* cysts, although the sediment beneath this sock contained approximately the same concentrations of cysts of this species as that below socks 2 and 4. More than one point source may be involved

in resuspension, or small-scale turbulence effects beneath the socks may account for the difference in contamination. The depth of sediment erosion may be a factor, depending upon the depth distribution of each species of cyst. White and Lewis (1982) demonstrated that there are as many *A. tamarense (= G. excavata)* cysts in the top 3 cm as in the flocculent surface layer, however it is unknown whether there are vertical gradients in abundance of cysts of other dinoflagellate species within the sediment. There is no clear relationship between the point source beneath the mussel socks and the number of dinoflagellate cysts passing through the overlying mussels.

2.4.3 Concentrations of dinoflagellate cysts in bottom sediments

The concentrations of dinoflagellate cysts in the upper 3 cm of sediment were highly variable within the square metre area sampled beneath each mussel sock. The heterogeneous distribution of *Alexandrium fundyense* and *A. ostenfeldii* cysts within a small confined area has serious implications for sampling techniques in cyst mapping and distribution studies. These studies are conducted to help assess where shellfish monitoring stations and aquaculture projects should be located (Lewis *et al.* 1979), and to determine accurately the abundance and distribution of cysts in an aquaculture site (Yamaguchi *et al.* 1995). The presence of dinoflagellate cysts in local sediments is one of several criteria suggested for assessing the susceptibility of a site to PSP, since cysts are often an early indication of a potential toxic algal bloom (Dale *et al.* 1987 as cited in Shumway 1990). Substantial differences in cyst concentrations among sediment cores

examined in this study emphasize the need to include fewer, larger cores in composite samples to obtain an accurate assessment of sediment contamination.

The dinoflagellate cyst concentrations in the sediment also varied considerably from September to October. In the interim period, a bloom of *Alexandrium* vegetative cells was observed (McKenzie *et al.* 1997). Although this bloom was attributed to the proliferation of *A. fundyense* cells, it is possible that it may have been caused by *A. ostenfeldii* vegetative cells, or that both *A. fundyense* and *A. ostenfeldii* bloomed concurrently, triggered by the same environmental conditions. Vegetative cells of both *A. fundyense* and *A. ostenfeldii* are difficult to distinguish under the light microscope, the method used by McKenzie *et al.* (1997). In material fixed in Lugol's iodine, Hansen *et al.* (1992) were unable to distinguish between the two species.

Blooms of motile, cyst-forming dinoflagellate species can increase cyst deposition in the underlying sediments (Cembella *et al.* 1988). However, with three exceptions, there were fewer cysts in the sediment post-bloom than were present pre-bloom in this study. Post-bloom concentrations of *Scrippsiella* sp. cysts decreased in the sediment beneath all the sampled mussel socks. The post-bloom abundance of *A. fundyense* hypnozygotes decreased beneath all socks except sock 3. *A. ostenfeldii* cyst concentrations post-bloom decreased beneath socks 1 and 2, however concentrations increased beneath socks 3 and 4. The concurrent accumulation of both *A. fundyense* and *A. ostenfeldii* cysts supports the hypothesis that both species bloomed at the same time. However, the differences in dinoflagellate cyst concentrations in the sediment from

September to October may not have been a result of the interim bloom, but rather of hydrodynamics: the shoreward tidal transport of cysts (Cembella *et al.* 1988) could account for the decrease in abundance beneath the more outlying socks 1 and 2, concentrating the resting stages in the shallow northeastern section of the cove (Figs. 2.1 and 2.2).

In this study, *A. ostenfeldii* cysts were more abundant in sediments than those of *A. fundyense*. The vegetative cells of *A. ostenfeldii* have been documented in the St. Lawrence estuary and gulf (Levasseur *et al.* 1996), and in Nova Scotian coastal embayments (Cembella *et al.* 1998). However, this species has not been recorded for Newfoundland waters, possibly because it is difficult to identify. Maximum concentrations of *A. fundyense* (< 375 cysts cm^{-3}) and *A. ostenfeldii* cysts (< 600 cysts cm^{-3}) in the sediment from this site are comparable with other identified seed beds in eastern Canada. The lower St. Lawrence estuary can contain 400 - 1500+ *A. excavatum* cysts cm^{-3}, depending upon the time of the year (Cembella *et al.* 1988, 1990); the southern Bay of Fundy, particularly to the east and northeast of Grand Manan Island, is rich in *A. fundyense (= G. excavata)* cysts, counts ranging from 2000 to 8000 cysts cm^{-3} (White & Lewis 1982). Sediments at Harbour Grace, Conception Bay, Newfoundland, contain between 30 and 150 *A. fundyense (= G. excavata)* cysts cm^{-3} (White & White 1985).

2.4.4 Egestion of putative Alexandrium ostenfeldii cysts in mussel faeces

Many suspension-feeding bivalves are capable of sorting particles and selectively rejecting them prior to ingestion (Kiørboe & Møhlenberg 1981; Newell & Jordan 1983) and after ingestion (Bricelj *et al.* 1984; Hawkins & Bayne 1992). Pre-ingestive particle selection occurs at the gills or labial palps (Kiørboe *et al.* 1980, 1981; Kiørboe & Møhlenberg 1981; Newell & Jordan 1983) and filtered material is rejected from the inhalent siphon as pseudofaeces (Haven & Morales-Alamo 1972). Pre-ingestive selection of particles may be correlated with factors such as cell shape, electrical charge, or chemical cues such as algal ectocrines (Ward & Targett 1989). Post-ingestive particle selection occurs during the sorting process within the stomach, and ingested material may be transported through the gut undigested, by-passing the digestive gland (Reid 1965; Widdows & Bayne 1971; Thompson & Bayne 1972). Post-ingestive sorting may be based upon differential particle size (Bricelj *et al.* 1984) and texture (Purchon 1977), among other possible factors.

The differential processing of certain species of dinoflagellate cysts in the mussel gut is suggested in the comparison of the relative dinoflagellate cyst composition of the sediment analysed in this study with the composition of faecal material collected from suspended mussel cultures. Since no pseudofaeces production was observed, it is assumed that all filtered particles were ingested. Furthermore, it is assumed that the dinoflagellate cysts in the sediment were resuspended as seston in the same proportions as they occurred in the sediment, since they are similar in size, shape and density. If this

is correct, then *Alexandrium ostenfeldii* cysts were not digested as intact cells of this species were dominant in the faeces. In contrast, although *Scrippsiella* sp. cysts dominated the dinoflagellate cyst population of the sediment, a considerably smaller proportion was egested in faecal material. This may indicate utilization of this dinoflagellate species by *Mytilus edulis*. However, this cannot be confirmed as the amounts of empty *Scrippsiella* sp. cysts egested were not determined in this study.

The egestion of *A. ostenfeldii* hypnozygotes in *Mytilus edulis* faeces requires further investigation. Size, shape and density are not apparent selection factors. It is possible that PSP toxicity may play a role. Although direct measurements of the specific toxicity of cultured vegetative cell isolates from Denmark suggest there is a low risk of PSP associated with this species (Balech & Tangen 1985; Hansen *et al.* 1992), isolates of *A. ostenfeldii* from New Zealand are highly toxic (MacKenzie *et al.* 1996).

2.4.5 Estimated biodeposition rate of cysts from suspended mussel cultures

To determine the replenishment rate of bottom sediments by *A. ostenfeldii* cysts egested in mussel faeces it is necessary to estimate the rate of biodeposition, the process by which faeces and pseudofaeces settle to the bottom (Haven & Morales-Alamo 1966). Since this study was not designed to investigate the biodeposition rates of dinoflagellate cysts, mussels were not continuously exposed to the cells of interest. Therefore values used in the calculations are to be considered estimates and may underestimate the actual

rates of biodeposition since the mussels collected in this study were isolated from the seston and were not permitted to feed continuously.

Over the 15 h incubation period in September, mussels (n = 29) egested an average of 333 *A. ostenfeldii* cysts mg^{-1} dry weight of faeces (DW). In October, mussels (n = 32) egested an average of 2.4 *A. ostenfeldii* cysts mg^{-1} DW over 19 h. Navarro (1983, as cited in Navarro & Thompson 1997) reported that biodeposition rates of ~ 53 mm shell length *Mytilus chilensis* (Hupe 1854) ranged from 10 - 38 mg DW $mussel^{-1}$ d^{-1}. Although these values are for a different *Mytilus* species than the one currently being investigated, an intermediate value was arbitrarily selected since the mussels were of the size range of *M. edulis* used in this study. Many studies have calculated biodeposition rates in terms of the deposition by all the mussels in a specified area, rather than on an individual mussel basis (e.g. Tsuchiya 1980; Jaramillo *et al.* 1992).

Using a value of 20 mg DW $mussel^{-1}$ d^{-1}, the biodeposition rate of *A. ostenfeldii* cysts by an individual mussel was 6660 cysts $mussel^{-1}$ d^{-1} in September and 47 cysts $mussel^{-1}$ d^{-1} in October. Comparison of these values with the concentrations of *A. ostenfeldii* cysts in the bottom sediments requires information on the stocking density of mussels. In Newfoundland, the average mussel farm has an estimated 30.9 mussels m^{-2} bottom (Couturier, pers. comm.), therefore in September approximately 2.1×10^5 *A. ostenfeldii* cysts m^{-2} d^{-1} were transferred to the sediment in the form of biodeposits. In October, approximately 1.5×10^3 *A. ostenfeldii* cysts m^{-2} d^{-1} were deposited on the sediment floor.

Mean *A. ostenfeldii* dinoflagellate cyst concentrations in the sediment, converted from the number of cysts present per cm^3 by integrating the 3 cm depth, were 2.55×10^6 cysts m^{-2} in September and 4.26×10^6 cysts m^{-2} in October. Therefore, approximately 8% of the bottom sediment *A. ostenfeldii* hypnozygotes were being replenished daily in September by faecal pellet deposition from the overlying mussel culture. However, in October the replenishment rate was considerably lower, as less than 1% of the cysts in the sediment were deposited in the form of mussel faeces.

The egestion of *A. ostenfeldii* hypnozygotes by *M. edulis* in faeces provides a mechanism for the recycling of dinoflagellate cysts to the sediment floor. A daily replenishment rate of approximately 8% is a considerable contribution to the maintenance of *A. ostenfeldii* cyst populations, and may alter the composition of the sediment over time, provided the egested cysts remain viable after passage through the bivalve digestive tract.

CHAPTER III

The viability of dinoflagellate cysts egested in mussel faeces

3.1 Objectives

The introduction of dinoflagellate species to previously uncontaminated areas has been attributed to the transport of viable cysts (Hallegraeff *et al.* 1990). *Alexandrium* hypnozygotes, transported in the ballast water of ships, have been successfully germinated in the laboratory to produce toxic cultures (Hallegraeff & Bolch 1992). When bivalves are transferred between aquaculture sites, there is a risk of transporting viable cysts, via the gut contents, with potential to seed future *Alexandrium* blooms. Intact *Alexandrium* cysts have been found in the gut contents of scallops (Shumway *et al.* 1987) and mussels (Schwinghamer *et al.* 1994). However, the possible effects of passage through the bivalve gut on the viability of dinoflagellate cysts have not been examined. Therefore, it was the objective of this study to assess the viability of *Alexandrium* hypnozygotes following passage through the bivalve gut. The percentage of successful germination of cysts isolated in mussel faeces was compared with the germination of cysts isolated from sediments. Germination times were also determined to facilitate comparison.

3.2 Materials and Methods

3.2.1 Preparation of sediment and faecal material

Sediment samples and faecal material collected from the study site in September and October 1996 were prepared and examined as described previously (Section 2.2). All samples were stored in the dark at 4 °C for a minimum period of six months to ensure that all cysts used in the germination study had matured beyond the obligate 4-6 month dormancy period (Anderson 1980). The samples were sonicated prior to storage. Further sonication was not necessary as the material did not re-aggregate over time.

In contrast to the abundance of putative *Alexandrium ostenfeldii* hypnozygotes in both the sediment core samples and the mussel faeces, *A. fundyense* cysts were relatively scarce. The numbers of *A. fundyense* cysts present in the faecal material were insufficient to conduct statistical analyses on viability trials. Therefore, only the viability of *A. ostenfeldii* cysts was assessed, following passage through the bivalve gut.

3.2.2 Incubation of cysts

Cysts which appeared to be *A. ostenfeldii* hypnozygotes were selected for incubation based upon their physical appearance under the light microscope: intact cell wall, prominent pigment inclusion bodies and the cell completely filled with cytoplasm. The autofluorescence of the cysts was also examined under blue-light excitation. The chlorophyll of dinoflagellate cysts fluoresces red prior to germination (Anderson & Keafer 1987).

Individual cysts were isolated with a micropipette under a dissecting microscope (Wild Makroskop, Mag. 64x) and an inverted microscope (Zeiss, Mag. 400x). To remove detritus, cysts were washed twice in FSW and deposited into 400 μl wells of polystyrene tissue culture plates (Corning 96-well micro titre) containing 250 μl of L1/10 culture medium (Guillard & Hargraves 1993). Four cysts were placed in each well because incubation of individual *A. ostenfeldii* cysts (n = 10) was unsuccessful in preliminary trials. Plates were then sealed with parafilm to prevent desiccation and incubated at 16 ± 1 °C under cool, white fluorescent bulbs (80 μmol m^{-2} s^{-1}) on a 14:10 Light: Dark cycle. Wells were examined every two days for evidence of successful germination, i.e. the presence of emergent motile vegetative cells.

Two trials were conducted, each with six plates maintained for thirty days. Although separated by a period of two weeks, the two trials were of exactly the same design. However, cysts in trial one were isolated under the dissecting microscope, whereas cysts in trial two were isolated under the inverted microscope. One plate was set up per day. On each plate, five wells were randomly designated for cysts isolated from sediment and five wells were designated for cysts isolated from faecal material. Plates were randomly allocated positions within the incubator, equidistant from the light source.

3.2.3 Statistical analysis

The number of wells per plate in which motile, vegetative cells were observed was recorded for cysts isolated from sediment and for cysts isolated from faecal material.

The data were analysed by two-way analysis of variance with the cyst source and individual plates as treatments. To compare the germination time of cysts from the different sources, the number of days required for germination in each well was analysed by two-way ANOVA with the cyst source and the individual plates as treatments. Separate analyses were performed for each trial, with $p \leq 0.05$ being the criterion for statistical significance.

3.2.4 Identification of dinoflagellate cysts and vegetative cells

Live cysts of the four dinoflagellate species described in Chapter II were photographed with a Contax 167 camera (Kodak Ektachrome 160 & 1600 slide film) on a Zeiss inverted microscope, using phase contrast and epifluorescence illumination (Figs. 3.1, 3.2). Autofluorescence of chlorophyll in the cysts was observed under blue-light excitation (Zeiss 48 77 09 filter set). Calcofluor White (Fritz & Triemer 1985) was used to stain the thecal plates of vegetative cells for epifluorescence microscopy, using ultraviolet light for excitation (Zeiss 48 77 02 filter set) (Hansen *et al.* 1992). Twenty individual Grey cysts were isolated and incubated as described previously (Section 3.2.2) to permit identification of the vegetative cells.

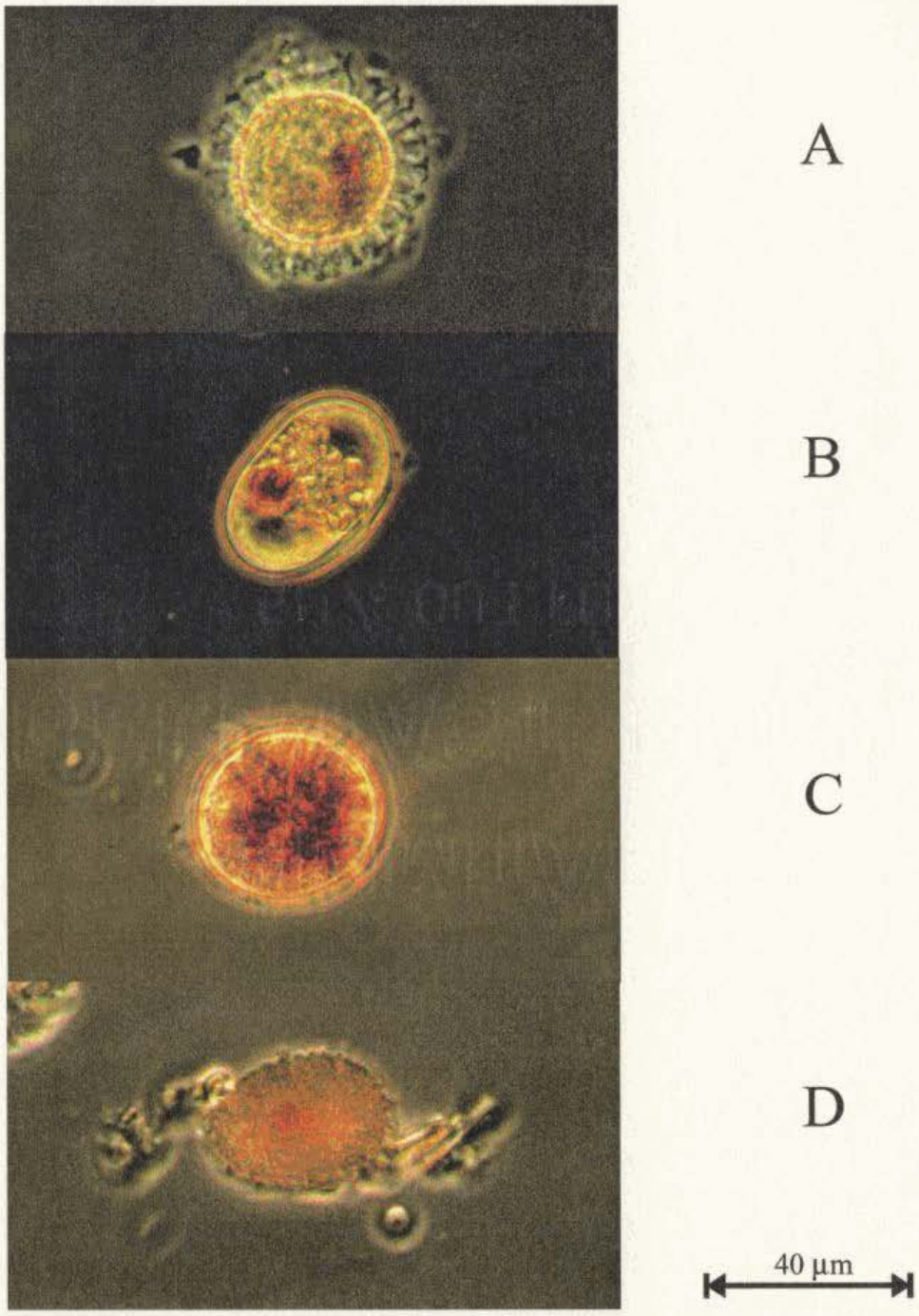

Fig. 3.1: Light micrographs of resting stages of dinoflagellate species studied. A = *Scrippsiella* sp.; B = *Alexandrium fundyense*; C = putative *Alexandrium ostenfeldii*; D = Unknown species.

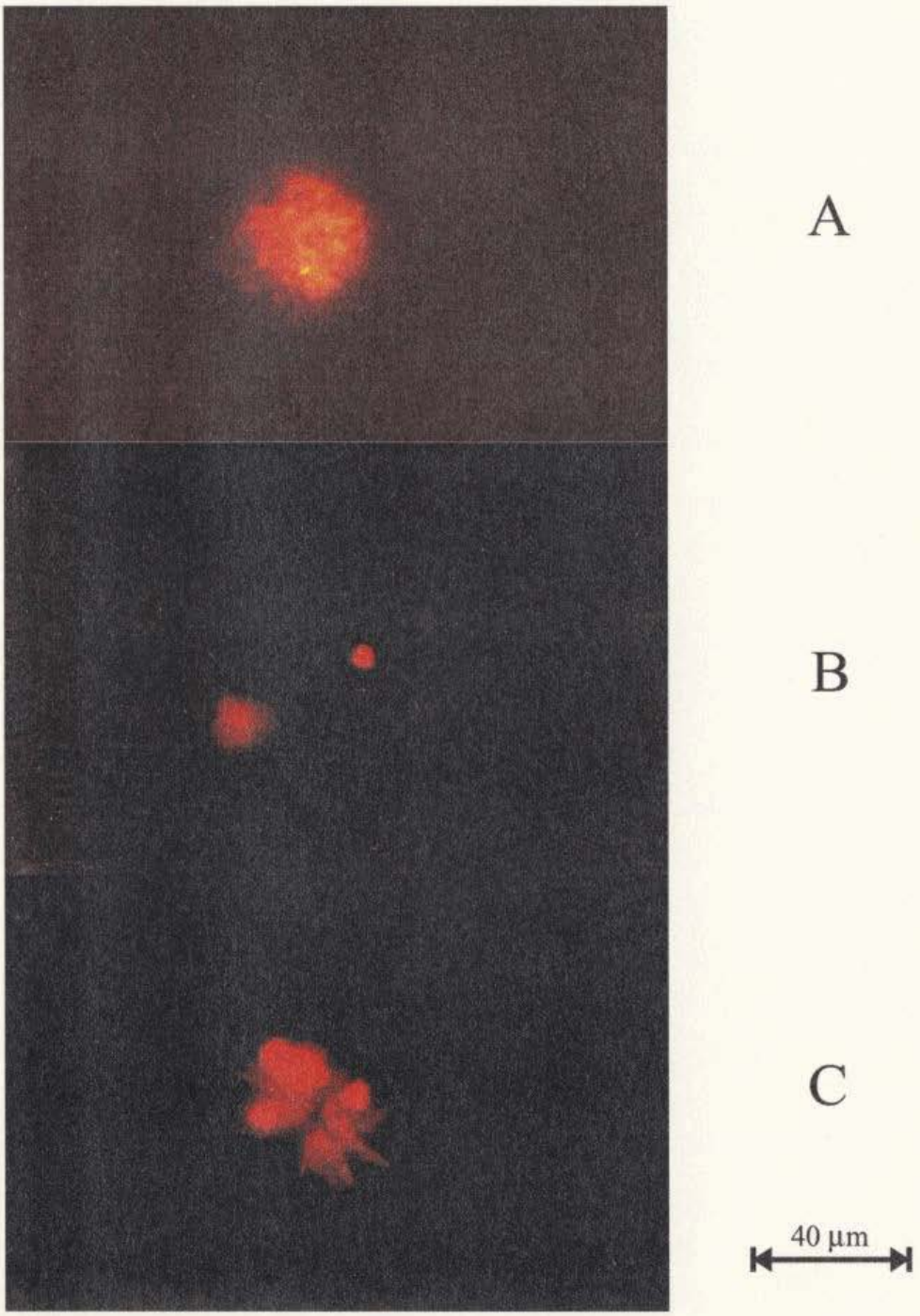

Fig. 3.2: Epifluorescence micrographs of resting stages of dinoflagellate species studied, cells as in Fig. 3.1, showing chlorophyll autofluorescence emitted under blue light excitation. A = *Scrippsiella* sp., B = *Alexandrium fundyense*, C = putative *Alexandrium ostenfeldii*. Cysts of the unknown Grey species did not autofluoresce.

3.3 Results

3.3.1 Viability and germination time of cysts isolated from sediment and mussel faeces

The number of wells which contained successfully germinated motile, vegetative cells was similar for cysts isolated in sediment and cysts isolated in mussel faeces in both trial 1 and trial 2 and there were no significant differences between the plates ($p > 0.05$ for all analyses) (Fig. 3.3). Cysts which had recently passed through the bivalve digestive tract were of similar viability as cysts resident in the upper 3 cm of sediment. However, successful germination was documented for considerably fewer wells in trial 1 than in trial 2: only 4% of wells with cysts from sediment and 37% of wells with cysts from faeces contained vegetative cells during the course of trial 1. In contrast, 60% of wells with cysts from sediment and 73% of wells with cysts from faeces contained vegetative cells during the course of trial 2. The two trials were identical in experimental design and protocol except that the cysts in trial 1 were isolated under a dissecting microscope whereas the cysts in trial 2 were isolated under an inverted microscope.

The time required for germination was similar for cysts egested in mussel faeces as for cysts isolated from sediment (Fig. 3.4). The germination time of cysts incubated in trial 1 was an average of 14 days for cysts from both sources, whereas cysts in trial 2 germinated in about 10 days. In a two-way analysis of variance, conducted for each trial separately, there were no significant differences in germination time as a result of either the cyst source or the individual culture plate ($p > 0.05$ for all analyses).

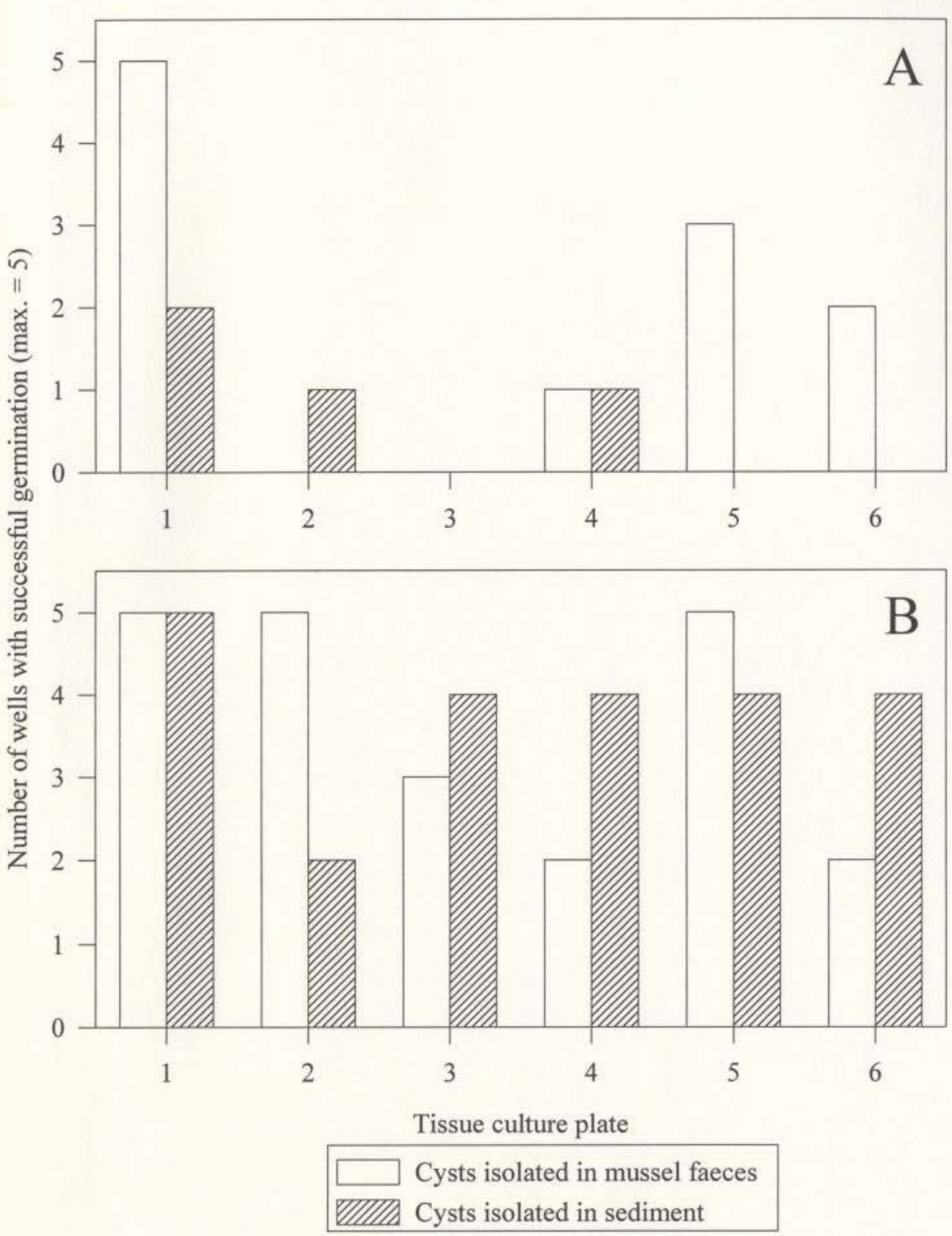

Fig. 3.3: Total number of wells in tissue culture plates which contained motile, vegetative cells of putative *Alexandrium ostenfeldii*. A = Trial 1; B = Trial 2.

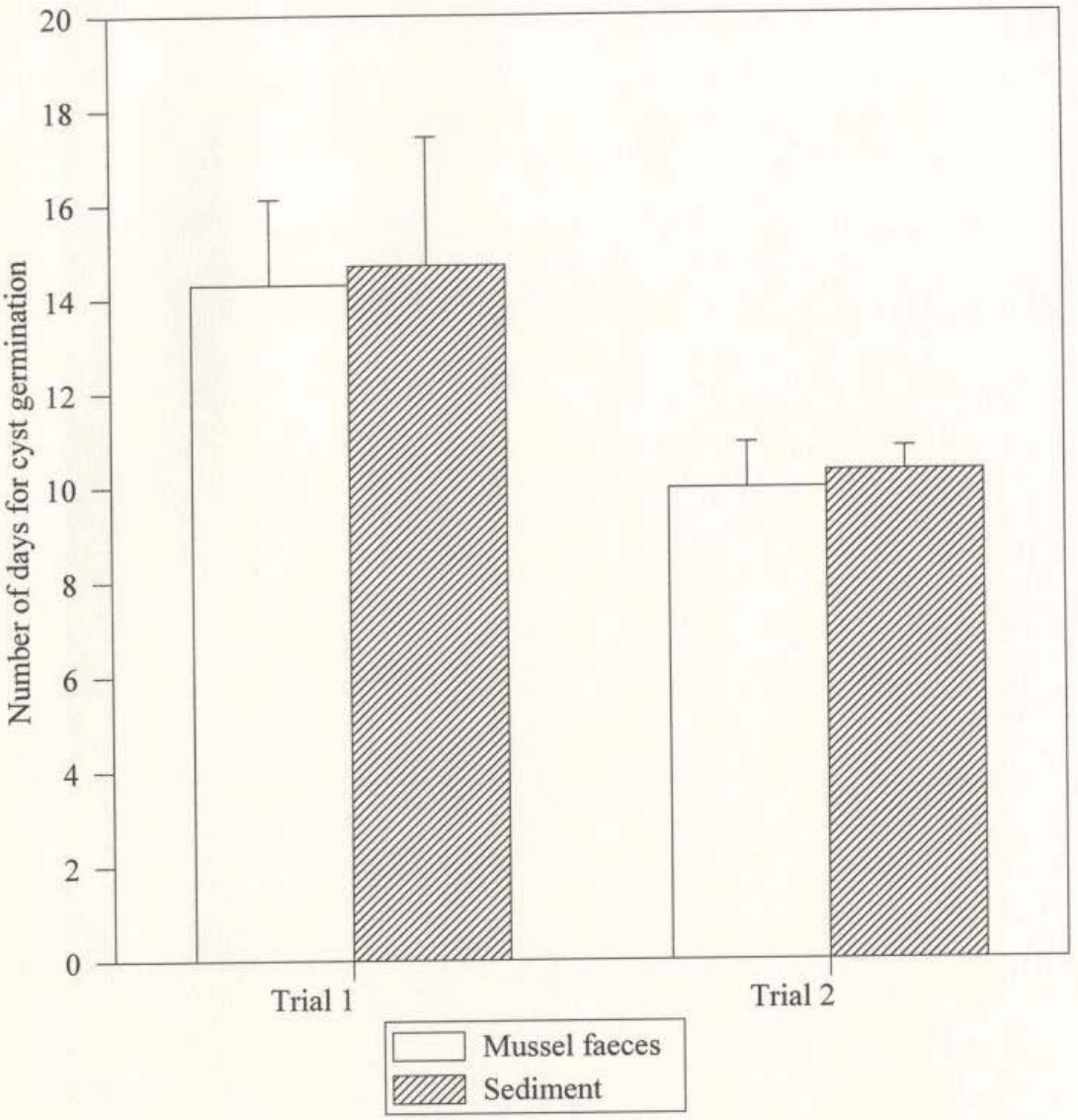

Fig. 3.4: Mean germination time for putative *Alexandrium ostenfeldii* cysts isolated in mussel faeces and in sediment, incubated under the same culture conditions.
Error bars represent the standard error of the mean.

3.3.2 Identification of Alexandrium ostenfeldii and Grey cysts

Putative *Alexandrium ostenfeldii* cysts were similar in appearance to the description given by MacKenzie *et al.* (1996): spherical cells (~ 40 μm diameter) containing one or two red-brown pigmented granules and areas of condensed, golden-brown pigmentation radiating from the centre of the cell towards the periphery (Fig. 3.1C). However, the identification of vegetative cells from these cysts was not possible. Less than twenty motile, vegetative cells survived in each well culture and repeated attempts to produce larger cultures were unsuccessful. Fluorescence microscopy of fixed cells stained with Calcofluor White (Fritz & Triemer 1985) to study the thecal morphology was difficult with the diffuse cultures. Although several cells stained optimally and fluoresced, their orientation did not permit examination of the cell thecae for the distinguishing features of *A. ostenfeldii*: the large ventral pore within the first apical plate and the wider than long shape of the sixth precingular plate (Balech & Tangen 1985). Vegetative cells were generaliy equal in length and width, with the maximum width within the 41 - 49 μm range suggested for *A. ostenfeldii* (Levasseur *et al.* 1996) (Fig. 3.5).

Grey cysts were cylindrical cells (35 - 45 μm x 30 - 35 μm) with a prominent red pigmented granule (Fig. 3.1D). The cell wall was densely covered in mucilaginous material which obscured observation of internal cell features. No chlorophyll autofluorescence was observed under blue-light excitation. Individual cysts incubated in

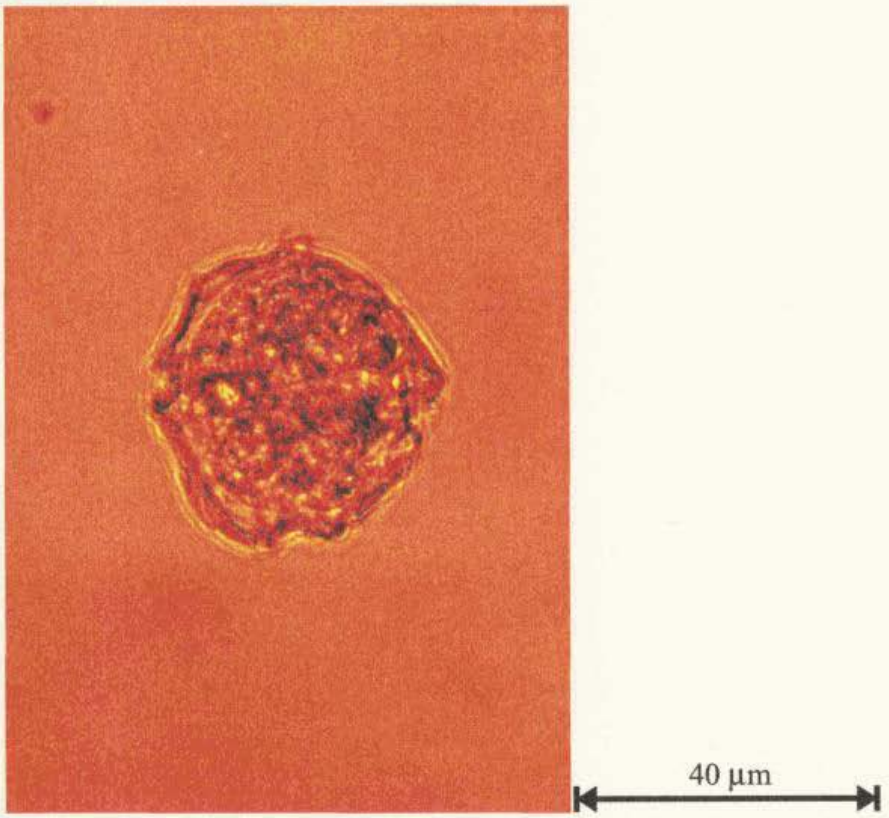

Fig. 3.5: Light micrograph of empty theca of putative *Alexandrium ostenfeldii* vegetative cell, germinated from a cyst isolated from mussel faeces.

both f2/10 and L1/10 microalgae culture media did not germinate. Therefore, the vegetative cells could not be identified.

3.4 Discussion

Dinoflagellate cysts resembling *Alexandrium ostenfeldii* passing through the digestive tract of mussels and egested as faeces were of similar viability to cysts found in the sediment. The thick-walled cysts were resistant to the extracellular digestion and the mechanical action of the crystalline style within the stomach.

The cyst wall of *Alexandrium* is composed of two layers, the exospore and the endospore (von Stosch 1973). The exospore of *A. excavatum* and *A. tamarense* is thin and resistant to both acetolysis and concentrated H_2SO_4, and the endospore probably contains cellulose (Dale 1977; Anderson & Wall 1978; Turpin *et al.* 1978). Cysts of *A. ostenfeldii* are likely to be of similar composition. Anderson and Wall (1978) suggest the cyst wall may also contain a sporopollenin-like polymer (Brooks 1971) as do other dinoflagellate cysts (Wall & Dale 1968; Wall 1975). Sporopollenin is a highly refractory compound which is very resistant to degradation by strong acids.

Dinoflagellate vegetative cells may pass through the bivalve gut unchanged and remain viable within the faecal material (Fox & Coe 1943). *A. fundyense* vegetative cells egested in *Mytilus edulis* faeces are capable of replication rates similar to those of ungrazed dinoflagellate cells (Bricelj *et al.* 1993). Mussel and scallop spat also retain

living *A. tamarense* vegetative cells in rinse water, faeces and pseudofaeces following 6 h in simulated shipment conditions (Scarratt *et al.* 1993).

The germination success of putative *A. ostenfeldii* cysts from sediment (60%) and from mussel faeces (73%) in trial 2 was comparable to the 70% germination reported for *A. ostenfeldii* isolated from sediment samples (MacKenzie *et al.* 1996). Similarly, the percentage germination ranges from 81.3 - 100% for *A. minutum* Halim (Cannon 1993), and from 27 - 90% for *A. tamarense (= G. tamarensis)*, depending upon storage and culture conditions (Anderson & Morel 1979).

Successful germination of cysts from sediment and from faeces was considerably less in trial 1 than in trial 2. The two trials were identical in experimental design and protocol, although cysts in trial 1 were isolated under the dissecting microscope whereas those in trial 2 were isolated under the inverted microscope. There may have been more contamination of the culture media with detritus and bacteria during cyst isolation under the dissecting microscope than under the inverted microscope. The growth of dinoflagellates is strongly influenced by the presence of other algae or bacteria (Martin & Martin 1987; Blanco & Campos 1988), possibly due to chemical conditioning of the growth medium.

The germination of dinoflagellate hypnozygotes is affected by many factors, including the age composition of the cyst population, temperature conditioning, light limitation, oxygen concentration and nutrient availability (Anderson & Wall 1978; Anderson & Morel 1979; Anderson 1980; Binder & Anderson 1986; Anderson *et al.*

1987; Cannon 1993; Ishikawa & Taniguchi 1994). In this study, designed to control for these factors, some cysts never germinated, although they appeared fully viable in the microscope and exhibited visible red autofluorescence under blue-light excitation. Quiescent cysts do not fluoresce red when excited with blue light until ready for germination (Anderson & Keafer 1985). Anderson *et al.* (1987) report a similar lack of germination by apparently viable *A. tamarense (= G. tamarensis)* cysts. Potential artifacts associated with sonication and isolation techniques may have affected excystment (Anderson & Morel 1979; Dale 1983; Binder & Anderson 1987), however it is unclear why some cysts do not germinate under optimal culture conditions.

Cysts from both sediment and mussel faeces germinated in about 10 - 14 days, compared with 10 days for *A. minutum* (Cannon 1993), 3-5 days for *A. tamarense (= G. excavatum)* (Fritz *et al.* 1989), and 6+ days for *A. tamarense (= G. tamarensis)* (Anderson & Morel 1979). However, *A. ostenfeldii* cysts germinate within 20 h of being isolated from sediment samples and being placed in tissue culture wells containing nutrient medium, most cysts germinating within the first 3 - 4 h (MacKenzie *et al.* 1996). Culture conditions in this study were the same as those used by MacKenzie *et al.* (1996), except that these authors incubated individual cysts in GP medium (Loeblich & Smith 1968) whereas L1/10 medium (Guillard & Hargraves 1993) was used in the present study. The two media have the same major nutrient and vitamin composition, although GP is enriched with soil extract whereas L1 is seawater enrichment which contains a number of trace elements not found in GP. Assuming the cysts incubated in this study

are *A. ostenfeldii*, differences in the obligate dormancy phase may explain the discrepancy in germination times. The germination time of another dinoflagellate cyst, *Scrippsiella trochoidea*, is also known to vary considerably under similar culture conditions, between 2.5 days (Binder & Anderson 1987) and 8 days (Ishikawa & Taniguchi 1994).

It is clear that *Alexandrium* hypnozygotes remain viable after passage through the bivalve digestive tract. The egestion of viable cysts from mussels could have serious implications for current aquaculture practice. The transport of contaminated bivalves among sites may result in the transfer of viable cysts, capable of germinating and seeding a toxic dinoflagellate bloom in a previously uncontaminated area.

CHAPTER IV

Selection and gut retention time of dinoflagellate cysts in mussels

4.1 Objectives

Mussels are selective suspension-feeders, retaining suspended particles on the ctenidia, rejecting some particles in pseudofaeces prior to ingestion, and differentially processing material within the gut. Examination of faecal material from *Mytilus edulis* contaminated with *Alexandrium* hypnozygotes in the field (Chapter II) suggests cysts resembling *A. ostenfeldii* are not digested. Since these cysts are egested intact and viable (Chapter III), further examination of differential selection of dinoflagellate cysts is required under controlled conditions.

The objective of this study was to compare pre- and post-ingestive selection by mussels of sediment contaminated with cysts of several dinoflagellate species (*Scrippsiella* sp., *A. fundyense*, putative *A. ostenfeldii*). Polystyrene beads the same size and approximate shape as the cysts were added to the sediment as control particles. Gut retention times (Taghon 1981; Bayne *et al.* 1984; Bricelj *et al.* 1984) were also determined to ascertain the minimum time required for purging potentially toxic dinoflagellate cysts from mussels.

4.2 Materials and Methods

4.2.1 Experimental mussels

Ten *Mytilus edulis* of similar size (mean shell length 68.8 ± 1.6 mm S.D.) were collected from a population near Bellevue, Trinity Bay, Newfoundland. Animals were transported on ice to the Ocean Sciences Centre and maintained in the laboratory for six weeks. They were maintained in flowing seawater (temperature = 15°C; salinity ca. 30‰) containing natural seston from Logy Bay, supplemented every second day with *Isochrysis galbana* at an approximate concentration of 50 x 10^3 cells ml^{-1} for 60 min, during which time the seawater flow was turned off.

4.2.2 Algae and sediment

Xenic cultures of *I. galbana* were grown in aerated f/2 medium in 4 l flasks (Guillard & Ryther 1962) under continuous illumination at 18 - 20°C. Cells were harvested in late exponential growth phase. Algal concentrations were determined with an electronic particle counter (Coulter Counter Multisizer) fitted with a 100 μm orifice tube. *Isochrysis galbana* was chosen because it is motile and can easily be kept in suspension using aeration.

Sediment contaminated with potentially toxic *Alexandrium fundyense* and putative *A. ostenfeldii* hypnozygotes was collected from Barred Island Cove, Notre Dame Bay, Newfoundland. In the laboratory, the sediment was sonicated, sieved and concentrated using a density-gradient centrifugation technique (Chapter II).

Concentrations of dinoflagellate cysts in the sediment were determined from triplicate counts of 200 μl sediment slurry settled onto Utermöhl chambers and examined under an inverted microscope. Samples were pooled into two groups based upon the numbers of dinoflagellate cysts present: low concentration sediment (LCS) and high concentration sediment (HCS). Non-toxic *Scrippsiella* sp. cysts (40 μm diameter), which occur in the same sediment as *Alexandrium* resting stages, were used to examine the basis of selection between dinoflagellate cysts of similar size, shape and density. Purple polystyrene beads (41μm diameter, Coulter) were added as a control to the two pools in similar concentrations such that the dinoflagellate cyst composition of LCS was 27 *Scrippsiella* sp., 10 *A. fundyense*, 55 *A. ostenfeldii*, and 377 beads per ml of sediment slurry. HCS was composed of 33 *Scrippsiella* sp., 60 *A. fundyense*, 465 *A. ostenfeldii*, and 315 beads per ml of sediment slurry. The dry weights (60°C, 3 d) of LCS and HCS sediment slurries were 3.1 mg ml^{-1} and 4.3 mg ml^{-1} respectively. The amount of organic material in both LCS and HCS was 60%, as determined by weight loss upon ignition in a muffle furnace (450°C, 12 h).

4.2.3 Experimental apparatus

A schematic representation of the experimental apparatus is depicted in Fig. 4.1. Flowing seawater from Logy Bay was collected in a 20 l header tank and supplied to an experimental tray (46 x 37 x 13 cm high) containing ten individual plastic incubation chambers (13.0 x 5.0 x 3.8 cm high). The rate of seawater inflow (15°C) into the

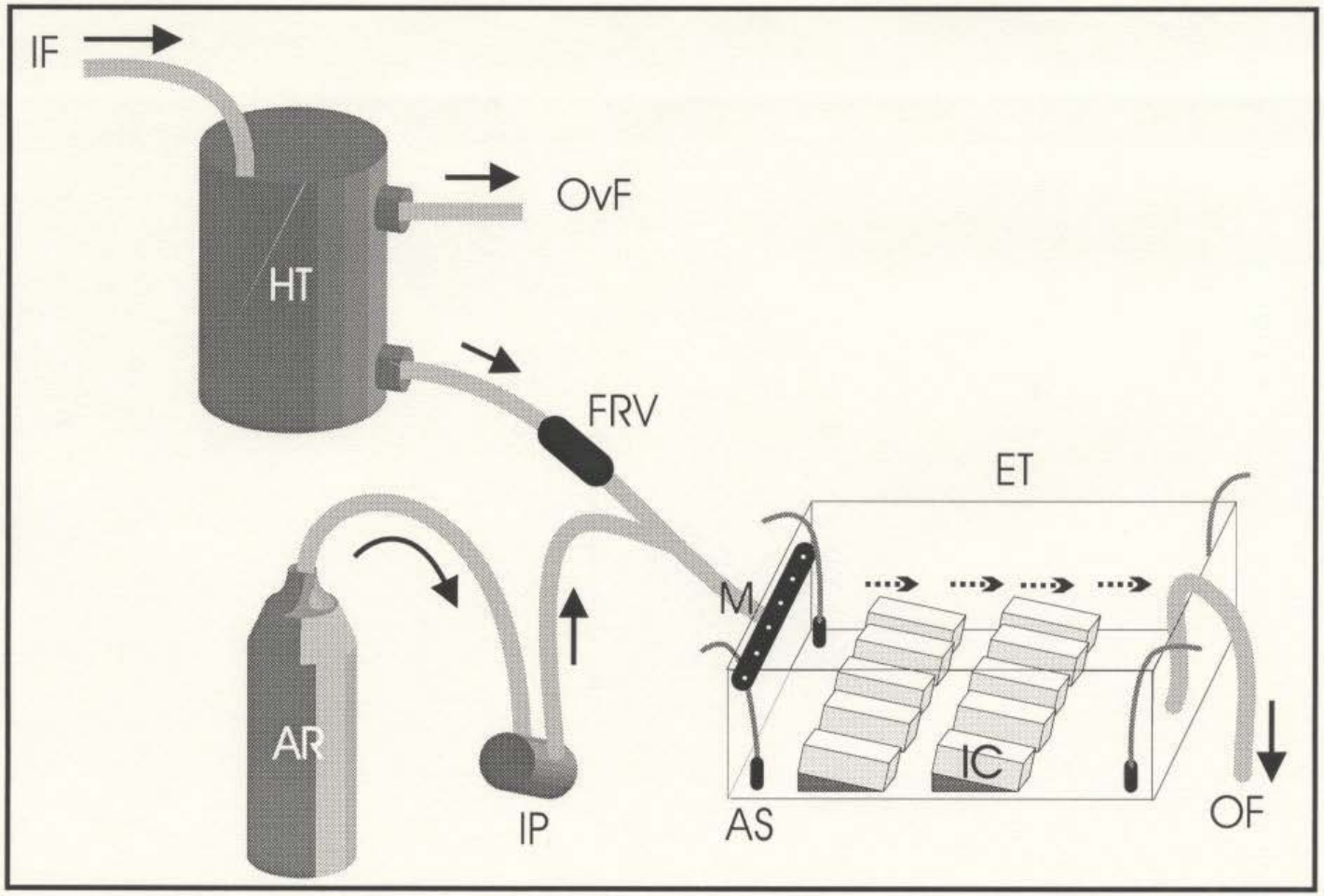

Fig. 4.1: Experimental apparatus for measuring gut retention time. AR = algae reservoir, AS = airstone, ET = experimental tray, FRV = flow restrictor valve, HT = header tank, IC = incubation chamber, IF = seawater inflow, IP = inline pump, M = manifold, OF = outflow, OvF = seawater overflow. Arrows indicate direction of seawater and algae flow.

experimental tray was maintained at 750 - 850 ml min^{-1}. Algal culture was pumped from 15 l glass carboys into the experimental tray using a peristaltic pump (Cole-Parmer Masterflex) at ca. 5.3 ml min^{-1}. The algal cells were allowed to mix with seawater in a 10 cm length of 1.5 cm dia. tubing before the suspension flowed into one end of the experimental tray through one of six openings in a PVC manifold. Aerators were placed in the four corners of the experimental tray to maintain a homogeneous algal suspension. Algal concentrations within the tray were 15 - 18 x 10^3 cells ml^{-1} throughout the experiment. A preliminary test of the system with algal cells but no animals present confirmed homogeneity: random seawater samples from the experimental tray showed similar concentrations of algae ($n = 10$, $p = 0.447$).

The ten incubation chambers were arranged in two rows, the narrow end of each chamber facing the inflow and raised at 35° to facilitate collection of mussel biodeposits. Mussels were scrubbed clean of epibionts and placed individually in the chambers with the ventral side upwards and the exhalant siphon directed downward. After delivery of the sediment slurry, the incubation chambers were covered with a 500 μm mesh screen to prevent loss of biodeposits.

4.2.4 Delivery of sediment pulse and collection of biodeposits

Mussels were placed in the experimental chambers for 3 h to allow embyssment and to establish steady-state feeding. When the shell valves were gaping widely, the sediment slurry was delivered directly into the inhalant siphon using a micropipette. This

method of direct delivery was chosen for two reasons (1) to ensure each mussel received similar concentrations of the dinoflagellate cyst - bead slurry initially, (2) to provide sufficient cysts, because the limited volume of concentrated sediment was inadequate for delivery in suspension in either a static or flow-through apparatus. Sediment was slowly delivered by hand, taking care to avoid contact with the margin of the inhalant canal. Seven 200 μl aliquots of sediment for each of five animals required 35 minutes, approximately one minute for each delivery. Rates faster than 200 μl min^{-1} disturbed the flow into the inhalant siphon, often resulting in its closure. The mussels in the row of incubation chambers near the inflow received LCS to prevent contamination by HCS, while the mussels in the row behind simultaneously received HCS.

Immediately prior to sediment delivery, all biodeposits (faeces and pseudofaeces) were collected separately for each animal with a large-bore micropipette and placed in glass vials (7 ml), each containing 1.5 ml FSW. Biodeposits were then collected every 45 min for the first 4.5 h. Subsequent samples were taken at 6, 9, 12, 16, 20, and 24 h. Faeces are expelled through the exhalant siphon, whereas pseudofaeces are rejected through the inhalant siphon at the dorsal margin. In this study, pseudofaeces were easily visually distinguished from faeces, thereby facilitating their quantitative separation and recovery. All samples were stored in the dark at 4°C prior to processing.

Biodeposit samples were examined in triplicate as described in Section 2.2.4. The total numbers of *Scrippsiella* sp. cysts, *A. fundyense* hypnozygotes, cysts resembling *A. ostenfeldii,* and beads were determined for each sample of faeces and pseudofaeces. The

ostenfeldii, and beads were determined for each sample of faeces and pseudofaeces. The coefficient of variation, estimated by five replicate counts of a single sample, was 8.2%.

4.2.5 Data analysis

The relative percentage of cysts and beads egested in faeces and pseudofaeces was calculated as a proportion of the initial concentration. The gut retention times for *A. ostenfeldii* cysts and polystyrene beads of the same size were estimated for each individual mussel and defined as the time required for egestion of 90% of the total number passed in faeces (Bayne *et al.* 1987; Hawkins *et al.* 1990). It was not possible to estimate the gut retention times for *Scrippsiella* sp. cysts and *A. fundyense* hypnozygotes as they were not found in the faecal samples.

4.3 Results

4.3.1 Selective rejection of dinoflagellate cysts

Copious amounts of loosely-aggregated pseudofaeces were produced within 3 minutes of the initial delivery of the dinoflagellate cyst and bead sediment slurry into the inhalant siphon of the mussel. Pseudofaeces production ceased after 2.25 h. Examination of the contents of this material revealed that a large proportion of the initial particles delivered in both LCS and HCS (*Scrippsiella* sp. cysts, *Alexandrium fundyense* hypnozygotes, cysts resembling *A. ostenfeldii*, polystyrene beads) were rejected in

pseudofaeces (Fig. 4.2). With the exception of *A. fundyense* cysts in LCS, the mean percent rejection of particles ranged from 53 - 89%.

Dinoflagellate cysts and beads were not egested for the first 1.5 h. Subsequent faecal samples contained detritus, polystyrene beads and *A. ostenfeldii* cysts. No *Scrippsiella* sp., or *A. fundyense* cysts were egested by the mussels. Less than 20% of the initial amounts of *A. ostenfeldii* cysts and polystyrene beads were recovered in faecal material.

The total numbers of particles in biodeposits (both pseudofaeces and faeces) accounted for between 60 and 90% of the initial particle composition of LCS and HCS.

4.3.2 Estimates of gut retention time

Plots of the percentages of *A. ostenfeldii* cysts in mussel faeces, relative to the total number egested, revealed peaks at two sampling periods (Fig. 4.3). The first peak of egestion occurred at 3 h in faeces from both groups of mussels, the second at 6 and 9 h for LCS treated mussels and at 6 h for HCS treated mussels.

Peaks of egestion were also recorded for relative percentages of polystyrene beads in mussel faeces, although these were not as marked as in *A. ostenfeldii* cysts (Fig. 4.4). The first peak occurred at 3.75 h in faeces from all mussels. The second peaks were not clearly defined, partially due to data variability. Following the first peak, egestion of beads was gradual in faeces from LCS contaminated mussels. The second peak of bead

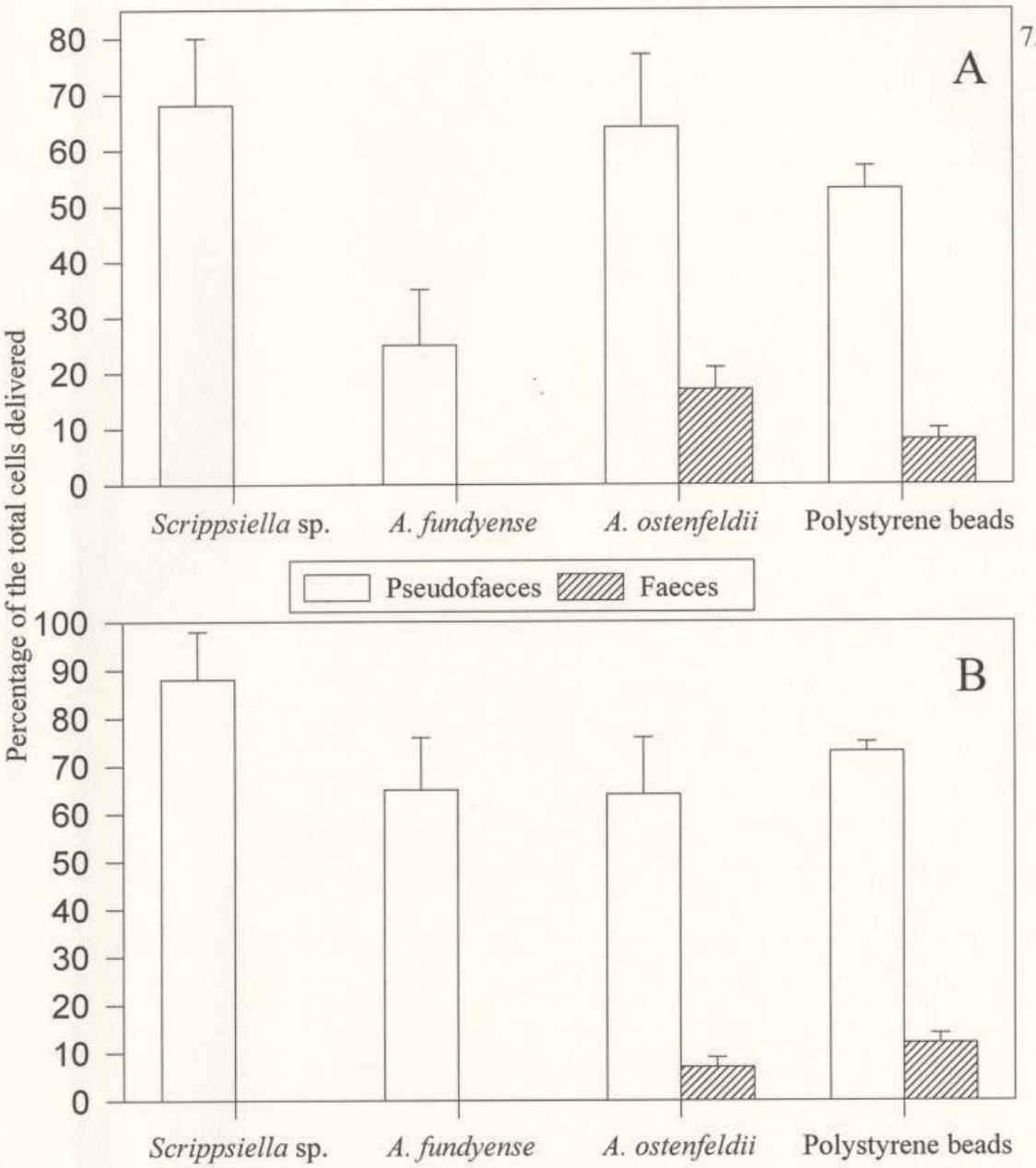

Fig. 4.2: Mean percentages of dinoflagellate cyst species and beads in mussel biodeposits, relative to the initial concentration delivered (n = 5). A = Low concentration sediment; B = High concentration sediment. Error bars represent the standard error of the mean.

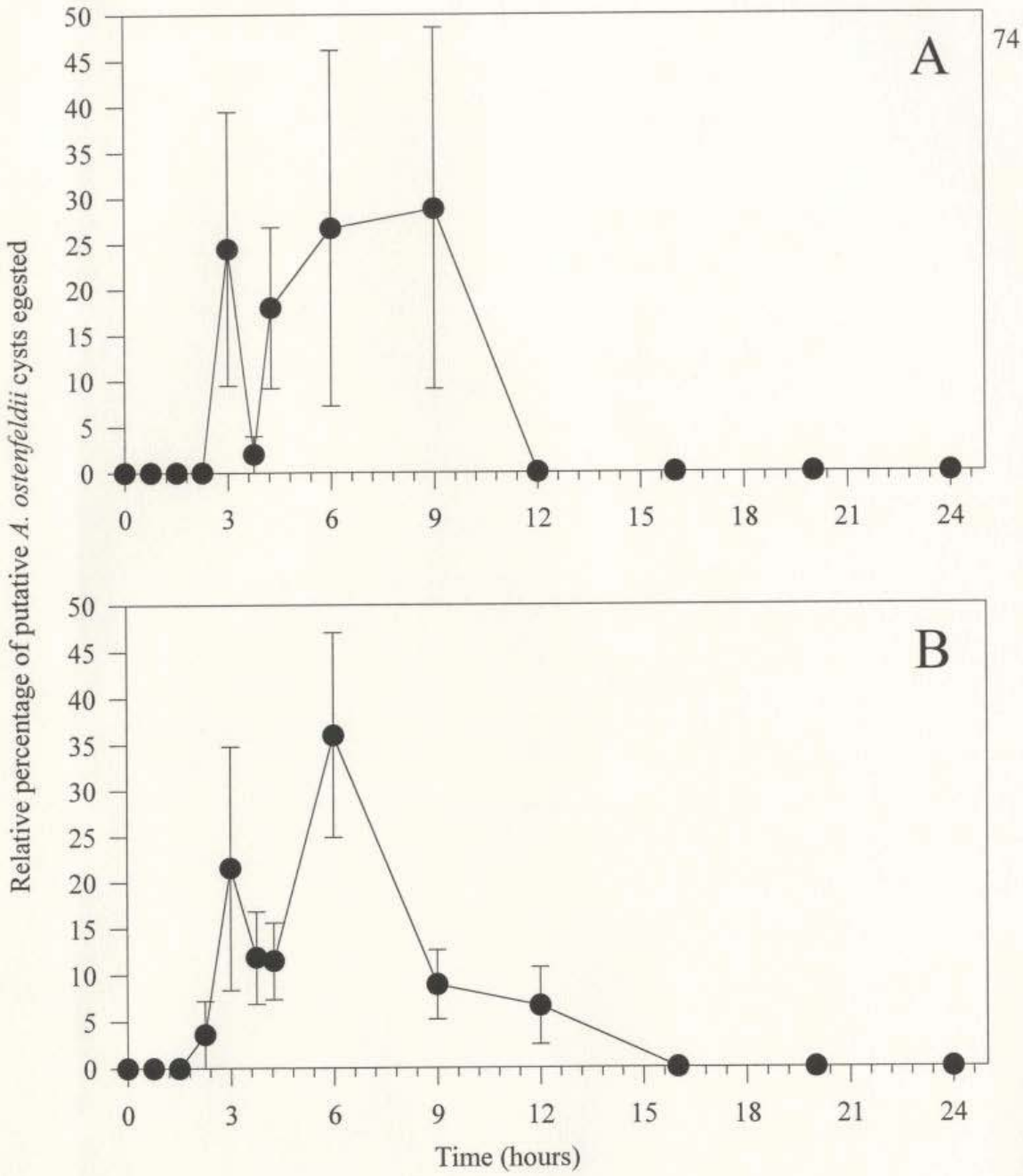

Fig. 4.3: Mean percentages of putative *A. ostenfeldii* cysts egested in 24 h, relative to the total number in mussel faeces (n = 5).
A = Low concentration sediment; B = High concentration sediment.
Error bars represent the standard error of the mean.

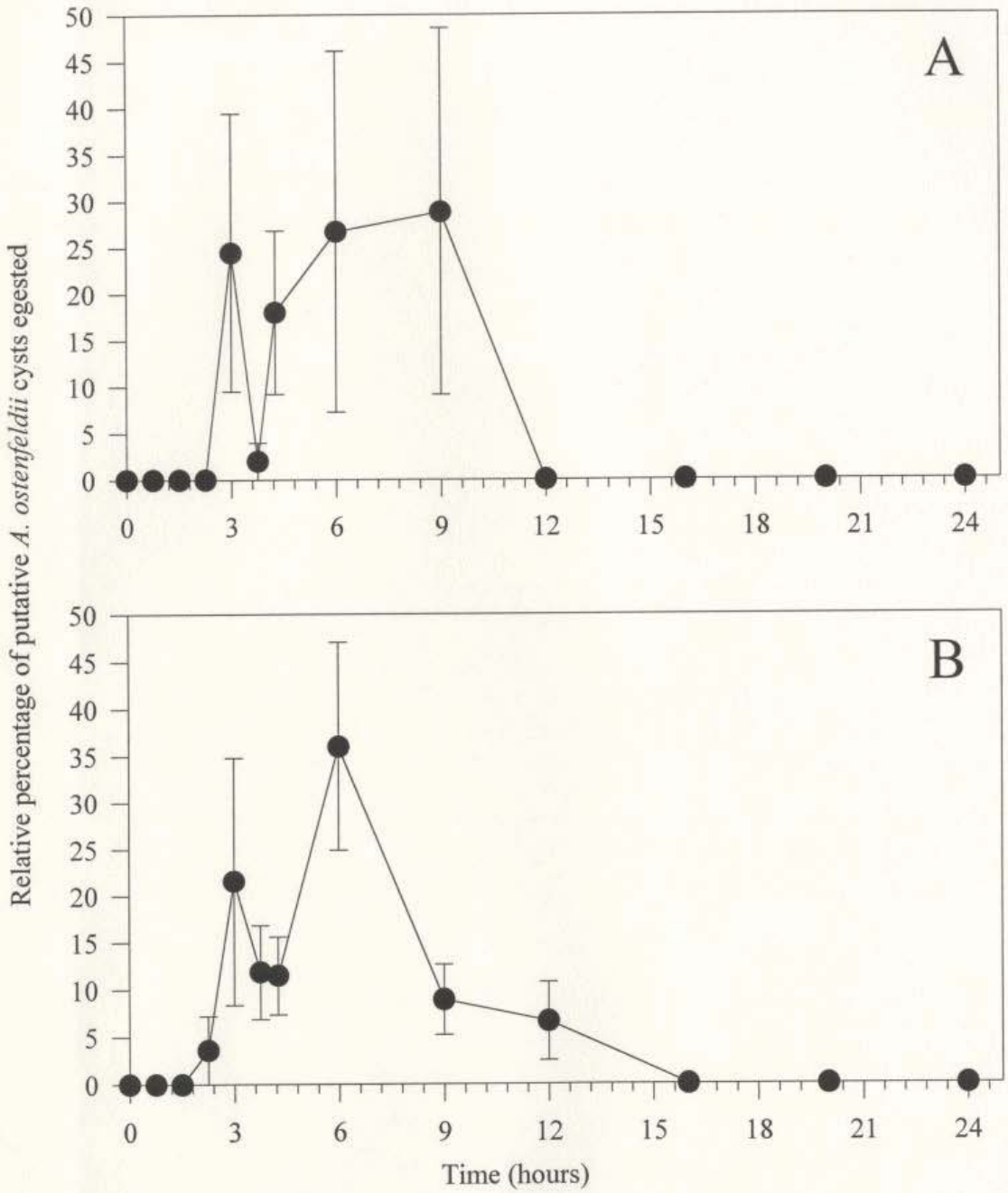

Fig. 4.3: Mean percentages of putative *A. ostenfeldii* cysts egested in 24 h, relative to the total number in mussel faeces (n = 5).
A = Low concentration sediment; B = High concentration sediment.
Error bars represent the standard error of the mean.

egestion from HCS treated mussels occurred at 6 h. The first peak probably corresponds to egestion of intestinal faeces, the second to the egestion of glandular faeces.

Polystyrene beads were egested over a longer period of time than dinoflagellate cysts resembling *A. ostenfeldii*. The estimated gut retention time of the cysts, calculated as the passage of 90% of the total amount egested, was between 6 and 9 h. By 9 h, 93 - 100% of the cysts had been passed in faeces from mussels. The estimated gut retention time of the 40 μm polystyrene beads was between 12 and 16 h. Approximately 94 - 98% of the beads passed in faeces of mussels had been egested by 16 h.

4.4 Discussion

4.4.1 Pre-ingestive selection

Pseudofaeces contain material remove from suspension, but rejected within the mantle cavity and not ingested. Above a threshold concentration of particulate matter, an increasing amount of material retained by the gill is rejected as pseudofaeces. In this study, between 53 and 89% of the particles were rejected as pseudofaeces. This may represent selection against the cysts and beads, or the result of overloading the gills with sediment. The sediment slurry of dinoflagellate cysts and beads, delivered into the inhalant siphon in discrete pulses of 0.62 mg min^{-1} LCS and 0.86 mg min^{-1} HCS exceeded the normal capacity of the gills. In conditions of high particle concentration and increased frequency of clogging of the gill, the functional state of the gill has been described by Jørgensen (1976) as a "cleaning" state, characterized by copious mucus and

corresponding pseudofaeces production. Mussels reject a greater proportion of filtered material as pseudofaeces with increased seston concentration (Foster-Smith 1975; Widdows *et al.* 1979; Bayne *et al.* 1993). Bayne *et al.* (1993) report 40% rejection of filtered material at seston concentrations of 6 - 10 g l^{-1}. Below a threshold for feeding selectivity, mussels lose their ability to selectively filter out phytoplankton from mixed particle assemblages (Newell *et al.* 1989).

Pre-ingestive rejection of particles as pseudofaeces may be affected by particle size, shape, electrical charge and chemical cues such as algal ectocrines (Newell & Jordan 1983; Newell *et al.* 1989; Ward & Targett 1989; Kemp *et al.* 1990). Selection can occur between different algal species (Shumway *et al.* 1985b), and between algal cells and inorganic particles (Kiørboe *et al.* 1980; Kiørboe & Möhlenberg 1981; Newell & Jordan 1983). In this study, with the exception of *A. fundyense* hypnozygotes in low concentration, cysts of *Scrippsiella* sp., *A. fundyense* and putative *A. ostenfeldii* were rejected as pseudofaeces in similar proportions as polystyrene beads. The concentration of *A. fundyense* cysts in LCS was very low (10 cysts ml^{-1}) in comparison to other particles, making it difficult to detect a signal. Under the conditions in this study, the mussel gill did not differentiate between potentially toxic dinoflagellate cysts (*A. fundyense* and *A. ostenfeldii*), non-toxic *Scrippsiella* sp. cysts and polystyrene beads of similar size and shape.

4.4.2 Post-ingestive selection

In mixed cell suspension, mussels are capable of differential processing of specific algae within the gut, possibly as a result of different routes of passage through the digestive system. When food is abundant, some of the ingested material can be transported through the gut undigested, by-passing the digestive gland (Thompson & Bayne 1972) and voided as intestinal faeces (van Weel 1961). Following assimilation of digested material in the digestive gland, the remaining unassimilated matter is voided as glandular faeces (Thompson & Bayne 1972, 1974).

The high proportion of both dinoflagellate cysts and beads recovered in biodeposits suggests that these particles are relatively indigestible. The wall of the *Alexandrium* cyst may contain a sporopollenin-like polymer (Anderson & Wall 1978) which is refractory. Bricelj *et al.* (1984) report that algal cells containing sporopollenin bypass the digestive gland and are eliminated faster than more digestible species.

There was no egestion of either *Scrippsiella* sp. cysts or *A. fundyense* hypnozygotes. The absence of these cells in the faeces may be due to (1) selective rejection during pre-ingestion or (2) digestion and utilisation of the cells. *Scrippsiella* sp. hypnozygotes and putative *A. ostenfeldii* cysts were rejected as pseudofaeces in similar proportions, the remaining proportions of cysts presumably being ingested. However, only *A. ostenfeldii* cysts were egested, together with the polystyrene beads.

4.4.3 Gut retention times

Although the capacity of mussels to produce both intestinal and glandular faeces has been recognized in the literature, the two peaks which often result have not been reported in gut retention time studies, in which curves are normally fitted to the data. Figure 3b in Bayne *et al.* (1987) clearly shows two peaks at 2 h and 5 h in the relative activity of ^{14}C. Furthermore, Figure 2 in Hawkins *et al.* (1990) shows two peaks, the first at about 1 h, the second at about 3 h. In the present study, Figures 4.3 and 4.4 demonstrate peaks of particle egestion in intestinal and glandular faeces. Egestion of cysts resembling *A. ostenfeldii* peaked in intestinal faeces at 3 h and in glandular faeces at about 6 h. Polystyrene beads in intestinal faeces were most concentrated at 3.75 h and in glandular faeces at about 6 h. Although both *A. ostenfeldii* cysts and beads were egested intact in glandular faeces and were not assimilated in the digestive gland - stomach mass, it is possible that some cells may have been digested.

Polystyrene beads were egested over a longer period of time than *A. ostenfeldii* cysts. The estimated gut retention time for the dinoflagellate cysts was 9 h, compared with 16 h for the beads. Gut retention times are highly variable. Gut retention times of adult mussels, determined with radio-labelled food, range from about 2 to 13 h (Bayne *et al.* 1987, 1989; Hawkins *et al.* 1990). Mean retention times of bovine red blood cells in mussels range between 2.4 and 8.3 h (Bayne *et al.* 1984). The gut retention time of dinoflagellate cysts has not been previously determined, although Scarratt *et al.* (1993) proposed 12 h minimum for purging *A. tamarense* cysts.

The polystyrene beads used in this study were similar in size and shape to the putative *A. ostenfeldii* cysts. Both particles were rejected in similar proportions in pseudofaeces and faeces. However, the dinoflagellate cysts were egested more rapidly than the beads. The mechanisms effecting selective rejection after ingestion are poorly understood, although if the unidentified cysts are indeed *A. ostenfeldii*, exudates (possibly PSP toxins) may be a factor. Although direct measurements of the specific toxicity of *A. ostenfeldii* cultured isolates suggest there is a low risk of PSP associated with this species (Hansen *et al.* 1992), Hansen (1989) found that exudates of this algal species in culture reversed ciliary movements in the tintinnid ciliate *Favella ehrenbergii*, as well as swelling and cell lysis. Therefore, it is important that the cyst resembling *A. ostenfeldii* be identified and the relative toxicities of both the benthic resting stage and the motile vegetative stage be determined, as these could have serious implications for aquaculture operations in Notre Dame Bay, Newfoundland.

CHAPTER V

Summary and avenues for further investigation

The research presented in this thesis examined the role mussels may play in recirculating cysts within an aquaculture site, the impact of passage through the bivalve gut on the viability of cysts, and the retention time of the cysts within the bivalve digestive tract.

The dinoflagellate cyst composition of faecal material from contaminated mussels in a closed aquaculture site was compared with the composition of the underlying sediment. Mussels from the top and bottom of mussel socks were similarly contaminated with dinoflagellate cysts as resuspension effects carried cysts throughout the site. *Scrippsiella* sp. cysts dominated the dinoflagellate cyst population of the sediment, however fewer cysts of this species were egested in faecal material. Cysts resembling *A. ostenfeldii* were more abundant in mussel faeces. The comparison of the relative dinoflagellate cyst composition of the sediment analysed with the composition of faecal material collected from suspended mussel cultures suggests *A. ostenfeldii* cysts are not digested in the mussel gut.

To compare pre- and post-ingestive selection in the laboratory, mussels were treated with sediment contaminated with cysts of several dinoflagellate species (*Scrippsiella* sp., *A. fundyense*, putative *A. ostenfeldii*). Non-toxic *Scrippsiella* sp.

hypnozygotes and potentially toxic *A. ostenfeldii* cysts were rejected as pseudofaeces in similar proportions, the remaining cysts presumably ingested. However, only *A. ostenfeldii* cysts were egested in faeces. This suggests there may not be digestion of *A. ostenfeldii* hypnozygotes in the bivalve gut and digestion of *Scrippsiella* sp. cysts. Although this finding is consistent with the results of field contaminated mussels, it is not unequivocal evidence.

The egestion of *A. ostenfeldii* hypnozygotes in *Mytilus edulis* faeces requires further investigation in the laboratory. Sediment contaminated with dinoflagellate cysts should be delivered in more dilute concentrations to prevent overloading the gill. *Scrippsiella* sp. cysts could be labelled, if possible, with a surface particle or charge to trace the route of passage through the bivalve gut. Although *Scrippsiella* sp. and putative *A. ostenfeldii* cysts are similar in size, shape and density, they are apparently differentiated within the mussel digestive tract.

The egestion of *A. ostenfeldii* hypnozygotes by *M. edulis* in faeces provides a mechanism for the recycling of dinoflagellate cysts to the sediment floor. The estimated 8% daily replenishment rate of the bottom sediment *A. ostenfeldii* hypnozygotes by faecal pellet deposition from the overlying mussel culture is a considerable contribution to the maintenance of *A. ostenfeldii* cyst populations. The volume and vertical displacement of resuspended sediment under different environmental conditions require study to provide a better understanding of contamination of mussels in shallow aquaculture sites and the role mussels may play in recirculating dinoflagellate cysts.

Intact *A. ostenfeldii* cysts in mussel faeces remained viable after passage through the bivalve gut. The estimated gut retention time was 9 h. The thick-walled dinoflagellate cysts egested in faeces were of similar viability as cysts found in the sediment. Putative *A. ostenfeldii* cysts were similar in appearance to the description given by MacKenzie *et al.* (1996), however identification of vegetative cells from these cysts was not possible and requires further study. Cysts resembling *A. ostenfeldii* germinated in about 10 - 14 days under the culture conditions described, compared with the 20 h germination time previously reported (MacKenzie *et al.* 1996), possibly due to the different culture media. The egestion of viable cysts from mussels could have serious implications for current aquaculture practice. The transport of contaminated bivalves among sites may result in the transfer of viable cysts, capable of germinating and seeding a potentially toxic dinoflagellate bloom in a previously uncontaminated area.

References

Anderson, D. M. 1980. Effects of temperature conditioning on development and germination of *Gonyaulax tamarensis* (Dinophyceae) hypnozygotes. *J. Phycol.* 16:166-172.

Anderson, D. M. & Keafer, B. A. 1987. An endogenous annual clock in the toxic marine dinoflagellate *Gonyaulax tamarensis*. *Nature* 325: 616-617.

Anderson, D. M. & Morel, F. M. M. 1979. The seeding of two red tide blooms by the germination of benthic *Gonyaulax tamarensis* hypnocysts. *Estuar. Coast. Mar. Sci.* 8:279-293.

Anderson, D. M. & Wall, D. 1978. Potential importance of benthic cysts of *Gonyaulax tamarensis* and *G. excavata* in initiating toxic dinoflagellate blooms. *J. Phycol.* 14(2):224-234.

Anderson, D. M., Fukuyo, Y. & Matsuoka, K. 1995. Cyst methodologies. *In* Hallegraeff. G. M., Anderson, D. M. & Cembella, A. D. [Eds.] *Manual on Harmful Marine Microalgae*. IOC Manuals and Guides No. 33, UNESCO, pp. 229-250.

Anderson, D. M., Kulis, D. M. & Binder, B. J. 1984. Sexuality and cyst formation in the dinoflagellate *Gonyaulax tamarensis*: cyst yield in batch cultures. *J. Phycol.* 20:418-425.

Anderson, D. M., Kulis, D. M., Doucette, G. J., Gallagher, J. C. & Balech, E. 1994. Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeastern United States and Canada. *Mar. Biol.* 120:467-478.

Anderson, D. M., Lively, J. J., Reardon, E. M. & Price, C. A. 1985. Sinking characteristics of dinoflagellate cysts. *Limnol. Oceanogr.* 30(5):1000-1009.

Anderson, D. M., Taylor, C. D. & Armbrust, E. V. 1987. The effects of darkness and anaerobiosis on dinoflagellate cyst germination. *Limnol. Oceanogr.* 32(2):340-351.

Balech, E. 1985. The genus *Alexandrium* or *Gonyaulax* of the Tamarensis group. *In* Anderson, D. M., White, A. W. & Baden, D. G. [Eds.] *Toxic Dinoflagellates*. Elsevier Science Publishing Co., Inc., New York, pp. 33-38.

Balech, E. & Tangen, K. 1985. Morphology and taxonomy of toxic species in the *tamarensis* group (Dinophyceae): *Alexandrium excavatum* (Braarud) comb. nov. and *Alexandrium ostenfeldii* (Paulsen) comb. nov. *Sarsia* 70:333-343.

Bayne, B. L., Hawkins, A. J. S. & Navarro, E. 1987. Feeding and digestion by the mussel *Mytilus edulis* L. (Bivalvia: Mollusca) in mixtures of silt and algal cells at low concentrations. *J. Exp. Mar. Biol. Ecol.* 111:1-22.

Bayne, B. L., Hawkins, A. J. S., Navarro, E. & Iglesias, I. P. 1989. Effects of seston concentrations on feeding, digestion and growth in the mussel *Mytilus edulis*. *Mar. Ecol. Prog. Ser.* 55:47-54.

Bayne, B. L., Iglesias, J. I. P., Hawkins, A. J. S., Navarro, E., Heral, M. & Deslous-Paoli, J. M. 1993. Feeding behaviour of the mussel, *Mytilus edulis*: responses to variations in quantity and organic content of the seston. *J. Mar. Biol. Assoc. U.K.* 73:813-829.

Bayne, B. L., Klumpp, D. W. & Clarke, K. R. 1984. Aspects of feeding, including estimates of gut residence time, in three mytilid species (Bivalvia, Mollusca) at two contrasting sites in the Cape Peninsula, South Africa. *Oecologia* 64:26-33.

Binder, B. J. & Anderson, D. M. 1986. Green light-mediated photomorphogenesis in a dinoflagellate resting cyst. *Nature* 322: 659-661.

Binder, B. J. & Anderson, D. M. 1987. Physiological and environmental control of germination in *Scrippsiella trochoidea* (Dinophyceae) resting cysts. *J. Phycol.* 23: 99-107.

Blanco, J. 1995. Cyst production in four species of neritic dinoflagellates. *J. Plankton Res.* 17(1):165-182.

Blanco, J. & Campos, M. J. 1988. The effect of water conditioned by a PSP-producing dinoflagellate on the growth of four algal species used as food for vertebrates. *Aquaculture* 68: 289-298.

Bourne, N. 1965. Paralytic shellfish poison in sea scallops (*Placopecten magellanicus*, Gmelin). *J. Fish. Res. Bd. Can.* 22(5):1137-1149.

Bricelj, V. M., Bass, A. E. & Lopez, G. R. 1984. Absorption and gut passage time of microalgae in a suspension feeder: An evaluation of the ^{51}Cr:^{14}C twin tracer technique. *Mar. Ecol. Prog. Ser.* 17:57-63.

Bricelj, V. M., Greene, M. & Cembella, A. D. 1993. Growth of the blue mussel *Mytilus edulis* on toxic *Alexandrium fundyense* and effects of gut passage on dinoflagellate cells.

In Smayda, T. J. & Shimizu, Y. [Eds.] *Toxic Phytoplankton Blooms in the Sea.* Elsevier Science Publishers B.V., Amsterdam, pp. 371-376.

Bricelj, V. M., Lee, J. H., Cembella, A. D. & Anderson, D. M. 1990. Uptake of *Alexandrium fundyense* by *Mytilus edulis* and *Mercenaria mercenaria* under controlled conditions. *In* Granéli, E., Sundstrom, B., Edler, L. & Anderson, D. M. [Eds.] *Toxic Marine Phytoplankton.* Elsevier Science Publishing Co., Inc., New York, pp. 269-274.

Brooks, J. 1971. Some chemical and geochemical studies on sporopollenin. *In* Brooks, J., Grant, P. R., Muir, M., van Gijzel, P. & Shaw, G. [Eds.] *Sporopollenin.* Academic Press, London, pp. 351-407.

Cannon, J. A. 1993. Germination of the toxic dinoflagellate, *Alexandrium minutum*, from sediments in the Port River, South Australia. *In* Smayda, T. J. & Shimizu, Y. [Eds.] *Toxic Phytoplankton Blooms in the Sea.* Elsevier Science Publishers B.V., Amsterdam, pp. 103-107.

Cembella, A. D. & Shumway, S. E. 1995. Anatomical and spatio-temporal variation in PSP toxin composition in natural populations of the surfclam *Spisula solidissima* in the Gulf of Maine. *In* Lassus, P., Arzul, G., Erard, E., Gentien, P. & Marcaillou, C. [Eds.] *Harmful Marine Algal Blooms.* Lavoisier, Intercept Ltd., pp. 421-426.

Cembella, A.D. & Therriault, C. 1987. Population dynamics and toxin composition of *Protogonyaulax tamarensis* from the St. Lawrence estuary. *In* Okaichi, T., Anderson, D.M. & Nemoto, T. [Eds.] *Red Tides: Biology, Environmental Science and Toxicology.* Elsevier Science Publishing Co., Inc., New York, pp. 81-84.

Cembella, A.D. & Todd, E. 1993. Seafood toxins of algal origin and their control in Canada. *In* Falconer, I.R. [Ed.] *Algal Toxins in Seafood and Drinking Water.* Academic Press, London, pp. 129-144.

Cembella, A. D., Destombe, C. & Turgeon, J. 1990. Toxin composition of alternative life history stages of *Alexandrium*, as determined by high-performance liquid chromatography. *In* Granéli, E., Sundström, B., Edler, L. & Anderson, D. M. [Eds.] *Toxic Marine Phytoplankton.* Elsevier Science Publishing Co., Inc., New York, pp. 333-338.

Cembella, A. D., Quilliam, M. A., Lewis, N. I., Bauder, A. G. & Wright, J. L. C. 1998. Identifying the planktonic origin and distribution of spirolides in coastal Nova Scotian waters. *In* Reguera, B., Blanco, J., Fernandez, M. L. & Wyatt, T. [Eds.] *Harmful Algae.* IOC Press, in press.

Cembella, A. D., Turgeon, J., Therriault, C. & Beland, P. 1988. Spatial distribution of *Protogonyaulax tamarensis* resting cysts in nearshore sediments along the north coast of the lower St. Lawrence estuary. *J. Shellfish Res.* 7(4):597-609.

Dale, B. 1976. Cyst formation, sedimentation, and preservation: factors affecting dinoflagellate assemblages in recent sediments from Trondheimsfjord, Norway. *Rev. Paleobot. Palynol.* 22:39-60.

Dale, B. 1977. Cysts of the toxic red-tide dinoflagellate *Gonyaulax excavata* (Braarud) Balech from Oslofjorden, Norway. *Sarsia* 63:29-34.

Dale, B. 1983. Dinoflagellate resting cysts: benthic phytoplankton. *In* Fryxell, G. A. [Ed.] *Survival strategies of the algae*. Cambridge University Press, New York, pp. 69-136.

Dale, B., Yentsch, C. & Hurst, H. W. 1978. Toxicity in resting cysts of the red-tide dinoflagellate *Gonyaulax excavata* from deeper water coastal sediments. *Science* 201:1223-1225.

Dale, B., Baden, D.G., Bary, B.M., Edler, L., Fraga, S., Jenkinson, I.R., Hallegraeff, G.M., Okaichi, T., Tangen, K., Taylor, F.J.R., White, A.W., Yentsch, C.M. & Yentsch, C.S. 1987. The problems of toxic dinoflagellate blooms in aquaculture. *In* Proceedings: International Conference and Workshop, Sherkin Island Marine Station.

Desbiens, M. & Cembella, A. D. 1993. Minimization of PSP toxin accumulation in cultured blue mussels (*Mytilus edulis*) by vertical displacement in the water column. *In* Smayda, T. J. & Shimizu, Y. [Eds.] *Toxic Phytoplankton Blooms in the Sea*. Elsevier Science Publishers, New York, pp. 395-399.

Desbiens, M., Coulombe, F., Gaudreault, J., Cembella, A.D. & Laroque, R. 1990. PSP toxicity of wild and cultured blue mussels induced by *Alexandrium* excavatum in Gaspé Bay (Canada): implications for aquaculture. *In* Granéli, E., Sundstrom, B., Edler, L. & Anderson, D.M. [Eds.] *Toxic Marine Phytoplankton*. Elsevier Science Publishers, New York, pp. 459-462.

Foster-Smith, R. L. 1975. The effect of concentration of suspension on the filtration rates and pseudofaecal production for *Mytilus edulis* L., *Cerastoderma edule* (L.) and *Venerupis pullastra*. *J. Exp. Mar. Biol. Ecol.* 17:1-22.

Fox, D. L. & Coe, W. R. 1943. Biology of the California sea-mussel (*Mytilus californianus*), II. Nutrition, metabolism, growth and calcium deposition. *J. Exp. Zool.* 93:205-249.

Fritz, L. & Triemer, R. E. 1985. A rapid simple technique utilizing calcofluor white M2R for the visualization of dinoflagellate thecal plates. *J. Phycol.* 21:662-664.

Fritz, L., Anderson, D. M. & Triemer, R. E. 1989. Ultrastructural aspects of sexual reproduction in the red tide dinoflagellate *Gonyaulax tamarensis*. *J. Phycol.* 25:95-107.

Gainey, L. F. J. & Shumway, S. E. 1988. A compendium of the responses of bivalve molluscs to toxic dinoflagellates. *J. Shellfish Res.* 7(4):623-628.

Guillard, R. R. L. & Hargraves, P. E. 1993. *Stichochrysis immobilis* is a diatom, not a chrysophyte. *Phycologia* 32(3):234-236.

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

Hall, S. & Reichardt, P.B. 1984. Cryptic paralytic shellfish toxins. *In* Ragelis, E.P. [Ed.] *Seafood Toxins*. ACS Symposium Series 262, American Chemical Society, Washington, D.C., pp. 113-123.

Hallegraeff, G. M. & Bolch, C. J. 1992. Transport of diatom and dinoflagellate resting spores in ships' ballast water: implications for plankton biogeography and aquaculture. *J. Plankton Res.* 14(8):1067-1084.

Hallegraeff, G. M., Bolch, C. J., Bryan, J. & Koerbin, B. 1990. Microalgal spores in ships' ballast water: a danger to aquaculture. *In* Graneli, E. et al. [Eds.] *Toxic Marine Phytoplankton*. Elsevier Publishing Co., Inc., New York, pp. 475-480.

Hansen, P. J. 1989. The red tide dinoflagellate *Alexandrium tamarense*: effects on behaviour and growth of a tintinnid ciliate. *Mar. Ecol. Prog. Ser.* 53:105-116.

Hansen, P. J., Cembella, A. D. & Moestrup, O. 1992. The marine dinoflagellate *Alexandrium ostenfeldii*: paralytic shellfish toxin concentration, composition and toxicity to a tintinnid ciliate. *J. Phycol.* 28:597-603.

Haven, D. S. & Morales-Alamo, R. 1966. Aspects of biodeposition by oysters and other invertebrate filter feeders. *Limn. Oceanogr.* 11:487-498.

Haven, D. S. & Morales-Alamo, R. 1972. Biodeposition as a factor in sedimentation of fine suspended solids in estuaries. *Geol. Soc. Amer.*, Memoir 133, pp. 121-130.

Hawkins, A. J. S. & Bayne, B. L. 1992. Physiological interrelations, and the regulation of production. *Devo. Aqua. Fish. Sci.* 25:171-222.

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

Heaney, S. I., Chapman, D. V. & Morrison, H. R. 1983. The role of the cyst stage in the seasonal growth on the dinoflagellate *Ceratium hirundinella* within a small productive lake. *Br. Phycol. J.* 18:47-59.

Ishikawa, A. & Taniguchi, A. 1994. The role of cysts on population dynamics of *Scrippsiella* spp. (Dinophyceae) in Onagawa Bay, northeast Japan. *Mar. Biol.* 119:39-44.

Jaramillo, E., Bertran, C. & Bravo, A. 1992. Mussel biodeposition in an estuary in southern Chile. *Mar. Ecol. Prog. Ser.* 82:85-94.

Jørgensen, C. B. 1976. Comparative studies on the functions of gills in suspension feeding bivalves, with special reference to effects of serotonin. *Biol. Bull.* 151: 331-343.

Kemp, P. F., Newell, S. Y. & Krambeck, C. 1990. Effects of filter-feeding by the ribbed mussel *Geukenia demissa* on the water-column microbiota of a *Spartina alterniflora* saltmarsh. *Mar. Ecol. Prog. Ser.* 59:119-131.

Kiørboe, T. & Möhlenberg, F. 1981. Particle selection in suspension-feeding bivalves. *Mar. Ecol. Prog. Ser.* 5:291-296.

Kiørboe, T., Möhlenberg, F. & Nohr, O. 1980. Feeding, particle selection and carbon absorption in *Mytilus edulis* in different mixtures of algae and resuspended bottom material. *Ophelia* 19(2):193-205.

Kiørboe, T., Möhlenberg, F. & Nohr, O. 1981. Effect of suspended bottom material on growth and energetics in *Mytilus edulis*. *Mar. Biol.* 61:283-288.

Levasseur, M., Berard-Therriault, L. & Bonneau, E. 1996. Distribution of the toxic dinoflagellate *Alexandrium ostenfeldii* in the Gulf of St. Lawrence, Canada. *In* Penney, R. W. [Ed.] *Proceedings of the Fifth Canadian Workshop on Harmful Marine Algae*. Can. Tech. Rep. Fish. Aquat. Sci. 2138, p.119.

Lewis, C. M., Yentsch, C. M. & Dale, B. 1979. Distribution of *Gonyaulax excavata* in the sediments of Gulf of Maine. *In* Taylor, D. L. & Seliger, H. H. [Eds.] *Toxic Dinoflagellate Blooms*. Elsevier North Holland, Inc., Amsterdam, pp. 235-238.

Loeblich, A. R. I. & Smith, V. E. 1968. Chloroplast pigments of the marine dinoflagellate *Gyrodinium resplendens*. *Lipids* 3(1):5-13.

Lund, J. W. G., Kipling, C. & Cren, E. D. 1958. The inverted microscope method of estimating algal numbers, and the statistical basis of estimation by counting. *Hydrobiologia* 11:143-170.

MacKenzie, L., White, D., Oshima, Y. & Kapa, J. 1996. The resting cyst and toxicity of *Alexandrium ostenfeldii* (Dinophyceae) in New Zealand. *Phycologia* 35(2):148-155.

Maranda, L., Anderson, D.M. & Shimizu, Y. 1985. Comparison of toxicity between populations of *Gonyaulax tamarensis* of eastern North American waters. *Est. Coast. Shelf Sci.* 21:401-410.

Marsden, I. D. & Shumway, S. E. 1992. Effects of the toxic dinoflagellate *Alexandrium tamarense* on the greenshell mussel *Perna canaliculus*. *N.Z. J. Mar. Fresh. Res.* 26:371-378.

Marsden, I. D. & Shumway, S. E. 1993. The effect of a toxic dinoflagellate (*Alexandrium tamarense*) on the oxygen uptake of juvenile filter feeding bivalve molluscs. *Comp. Biochem. Physiol.* 106A(4):769-773.

Martin, B. B. & Martin, D. F. 1987. Enhanced activity of a red tide (*Ptychodiscus brevis*) cytolytic fraction (aponin) from the green alga, *Nannochloris* sp. *J. Environ. Sci. Health* A22: 457-462.

Matsuoka, K., Fukuyu, Y. & Anderson, D. M. 1989. Methods for modern dinoflagellate cyst studies. *In* Okaichi, T., Anderson, D. M. & Nemoto, T. [Eds.] *Red Tides: Biology Environmental Sciences and Toxicology*. Elsevier Science Publishing Co., Inc., New York, pp. 461-480.

McKenzie, C. H. 1993. Report on the occurrence and abundance of *Alexandrium fundyense* cysts in marine sediments at seven mussel aquaculture sites in Newfoundland. Ocean Sciences Centre, Memorial University of Newfoundland, 10 p.

McKenzie, C. H. 1994. Report on the occurrence and abundance of *Alexandrium fundyense* cysts in marine sediments Part II. Six aquaculture sites in Newfoundland. Ocean Sciences Centre, Memorial University of Newfoundland, 9 p.

McKenzie, C. H., Thompson, R. J., Parrish, C. C., Harper, F. M. & Hatfield, E. A. 1997. Bloom dynamics of the toxic dinoflagellate *Alexandrium fundyense*. *In* Proceedings of the VIII International Conference on Harmful Algae, 25-29 June, Vigo, Spain. In press.

Medcof, J.C., Leim, A.H., Needler, A.B. & Needler, A.W.H. 1947. Paralytic shellfish poisoning on the Canadian Atlantic coast. *Bull. Fish Res. Bd Can.* 75, 32 p.

Navarro, J. M. 1983. Oferta alimenticia natural y su efecto sobre los procesos fisiológicos del bivalvo *Mytilus chilensis* en la bahía de Yaldad, Chiloé (Sur de Chile). *Mems. Asoc. Latinoam. Acuicult.*, 5: 175-187.

Navarro, J. M. & Thompson, R. J. 1997. Biodeposition by the horse mussel *Modiolus modiolus* (Dillwyn) during the spring diatom bloom. *J. Exp. Mar. Biol. Ecol.* 209:1-13.

Newell, R. I. E. & Jordan, S. J. 1983. Preferential ingestion of organic material by the American oyster *Crassostrea virginica*. *Mar. Ecol. Prog. Ser.* 13:47-53.

Newell, C. R., Shumway, S. E., Cucci, T. L. & Selvin, R. 1989. The effects of natural seston particle size and type on feeding rates, feeding selectivity and food resource availability for the mussel *Mytilus edulis* Linnaeus, 1758 at bottom culture sites in Maine. *J. Shellfish Res.* 8(1):187-196.

Oshima, Y., Sugino, K., Itakura, H., Hirota, M. & Yasumoto, T. 1990. Comparative studies on paralytic shellfish toxin profile of dinoflagellates and bivalves. *In* Granéli, E., Sundström, B., Edler, L. & Anderson, D.M. [Eds.] *Toxic Marine Phytoplankton*. Elsevier Science Publishing Co., Inc. New York, pp. 391-396.

Oshima, Y., Itakura, H., Lee, K.C., Yasumoto, T., Blackburn, S. & Hallegraeff, G. 1993. Toxin production by the dinoflagellate *Gymnodinium catenatum*. *In* Smayda, T., & Shimizu, T. [Eds.] *Toxic Phytoplankton Blooms in the Sea*. Elsevier Science Publishing Co., Inc. New York, pp. 907-912.

Pfiester, L. A. & Anderson, D. M. 1987. Dinoflagellate reproduction. *In* Taylor, F. J. R. [Ed.] *The Biology of Dinoflagellates*. Blackwell Scientific Publications, London, pp. 611-648.

Prakash, A. 1967. Growth and toxicity of a marine dinoflagellate, *Gonyaulax tamarensis*. *J. Fish. Res. Bd. Can.* 24:1589-1606.

Purchon, R. D. 1977. *The Biology of the Mollusca*, 2nd Ed. Pergamon Press, Oxford. 560 pp.

Reid, R. G. B. 1965. The structure and function of the stomach in bivalve molluscs. *J. Zool.* 147: 156-184.

Sakshaug, E. 1990. Problems in the methodology of studying phytoplankton. *In* Morris, I. [Ed.] *The Physiological Ecology of Phytoplankton*. Blackwell Scientific, pp. 57-91.

Scarratt, A. M., Scarratt, D. J. & Scarratt, M. G. 1993. Survival of live *Alexandrium tamarense* cells in mussel and scallop spat under simulated transfer conditions. *J. Shellfish Res.* 12(2):383-388.

Schwinghamer, P., Anderson, D. M. & Kulis, D. M. 1991. Separation and concentration of living dinoflagellate resting cysts from marine sediments via density-gradient centrifugation. *Limnol. Oceanogr.* 36(3):588-592.

Schwinghamer, P., Hawryluk, M., Powell, C. & MacKenzie, C. H. 1994. Resuspended hypnozygotes of *Alexandrium fundyense* associated with winter occurrence of PSP in inshore Newfoundland waters. *Aquaculture* 122:171-179.

Shimizu, Y. 1987. Dinoflagellate toxins. *In* Taylor, F.J.R. [Ed.] *The biology of dinoflagellates*. Blackwell Scientific Press, Oxford, UK, pp. 282-315.

Shumway, S. E. 1990. A review of the effects of algal blooms on shellfish and aquaculture. *J. World Aquacul. Soc.* 21(2):65-104.

Shumway, S. E. & Cucci, T. L. 1987. The effects of the toxic dinoflagellate *Protogonyaulax tamarensis* on the feeding and behaviour of bivalve molluscs. *Aquat. Tox.* 10:9-27.

Shumway, S. E., Cucci, T. L., Gainey, L. & Yentsch, C. M. 1985a. A preliminary study of the behavioral and physiological effects of *Gonyaulax tamarensis* on bivalve molluscs. *In* Anderson, D. M., White, A. W. & Baden, D. G. [Eds.] *Toxic Dinoflagellates*. Elsevier Science Publishing Co., Inc., New York, pp. 389-394.

Shumway, S. E., Cucci, T. L., Newell, R. C. & Yentsch, C. M. 1985b. Particle selection, ingestion, and absorption in filter-feeding bivalves. *J. Exp. Mar. Biol. Ecol.* 91:77-92.

Shumway, S. E., Selvin, R. & Schick, D. F. 1987. Food resources related to habitat in the scallop, *Placopecten magellanicus* (Gmelin, 1791): a qualitative study. *J. Shellfish Res.* 6(2):89-95.

Steidinger, K. A. 1975. Basic factors influencing red tides. *In* LoCicero, V. R. [Ed.] *Proceedings of the First International Conference on Toxic Dinoflagellate Blooms*, November 1974, Boston, Massachusetts. Massachusetts Science and Technology Foundation, Wakefield, MA, pp. 153-162.

Steidinger, K. A. & Baden, D. G. 1984. Toxic marine dinoflagellates. *In* Spector, D.L.[Ed.] *Dinoflagellates*. Academic Press Inc., Toronto, pp. 201-261.

Taghon, G. L. 1981. Beyond selection: optimal ingestion rate as a funciton of food value. *Am. Nat.* 118: 202-214.

Taylor, F.J.R. 1984. Toxic dinoflagellates: taxonomic and biogeographic aspects with emphasis on *Protogonyaulax*. *In* Ragelis, E.P. [Ed.] *Seafood Toxins*. ACS Symposium Series 262, American Chemical Society, Washington, D.C., pp. 77-97.

Thompson, R. J. & Bayne, B. L. 1972. Active metabolism associated with feeding in the mussel *Mytilus edulis* L. *J. Exp. Mar. Biol. Ecol.* 9:111-124.

Thompson, R. J. & Bayne, B. L. 1974. Some relationships between growth, metabolism and food in the mussel *Mytilus edulis*. *Mar. Biol.* 27:317-326.

Todd, E. C. D. 1995. Estimated costs of paralytic shellfish, diarrhetic shellfish and ciguatera poisoning in Canada. *In* Lassus, P., Arzul, G., Erard, E., Gentien, P. & Marcaillou, C. [Eds.] *Harmful Marine Algal Blooms*. Lavoisier, Intercept Ltd., pp. 831-834.

Tsuchiya, M. 1980. Biodeposit production by the mussel *Mytilus edulis* L. on rocky shores. *J. Exp. Mar. Biol. Ecol.* 47:203-222.

Turpin, D. H., Dobell, P. E. R. & Taylor, F. J. R. 1978. Sexuality and cyst formation in Pacific strains of the toxic dinoflagellate *Gonyaulax tamarensis*. *J. Phycol.* 14(2):235-238.

Twarog, B.M. & Yamaguchi, H. 1974. Resistance to paralytic shellfish toxins in bivalve molluscs. *In* LoCicero, V.R. [Ed.] *Proceedings of the First International Conference on*

Toxic Dinoflagellate Blooms, November 1974, Boston, Massachusetts. Massachusetts Science and Technology Foundation, Wakefield, MA, pp. 381-393.

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

Tyler, M. A., Coat, D. W. & Anderson, D. M. 1982. Encystment in a dynamic environment: deposition of dinoflagellate cysts by a frontal convergence. *Mar. Ecol. Prog. Ser.* 7:163-178.

van Weel, P. B. 1961. The comparative physiology of digestion in molluscs. *Am. Zool.* 1: 245-252.

Venrick, E. L. 1978. How many cells to count? *In* Sournia, A. [Ed.] *Phytoplankton Manual.* UNESCO Monographs on Oceanographic Methodology, pp. 167-180.

von Stosch, H. A. 1973. Observations on vegetative reproduction and sexual life cycles of two freshwater dinoflagellates, *Gymnodinium pseudopalustre* Schiller and *Woloszynskia apiculata* sp. nov. *Br. Phycol. J.* 8:105-134.

Wall, D. 1971. Biological problems concerning fossilizable dinoflagellates. *Geo. Sci. Man.* 3:1-15.

Wall, D. 1975. Taxonomy of cysts of red tide dinoflagellates. *In* LoCicero, V.R. [Ed.] *Proceedings of the First International Conference on Toxic Dinoflagellate Blooms,* November 1974, Boston, Massachusetts. Massachusetts Science and Technology Foundation, Wakefield, MA, pp. 249-255.

Wall, D. & Dale, B. 1968. Modern dinoflagellate cysts and evolution of the Peridiniales. *Micropaleo.* 14:265-304.

Ward, J. E. & Targett, N. M. 1989. Influence of marine microalgal metabolites on the feeding behaviour of the blue mussel *Mytilus edulis*. *Mar. Biol.* 101:313-321.

White, A. W. & Lewis, C. M. 1982. Resting cysts of the toxic, red tide dinoflagellate *Gonyaulax excavata* in Bay of Fundy sediments. *Can. J. Fish. Aquat. Sci.* 39:1185-1194.

White, D. R. L. & White, A. W. 1985. First report of paralytic shellfish poisoning in Newfoundland. *In* Anderson, D. M., White, A. W. & Baden, D. G. [Eds.] *Toxic Dinoflagellates*. Elsevier Science Publishing Co., Inc., New York, pp. 511-516.

Widdows, J. & Bayne, B. L. 1971. Temperature acclimation of *Mytilus edulis* with reference of its energy budget. *J. Mar. Biol. Asso. U.K.* 51:827-843.

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

Yamaguchi, M., Itakura, S., Imai, I. & Ishida, Y. 1995. A rapid and precise technique for enumeration of resting cysts of *Alexandrium* spp. (Dinophyceae) in natural sediments. *Phycologia* 34(3):207-214.

Yentsch, C. M. & Mague, F. C. 1979. Motile cells and cysts: two probable mechanisms of intoxication of shellfish in New England waters. *In* Taylor, D. L. & Seliger, H. H. [Eds.] *Toxic Dinoflagellate Blooms*. Elsevier North Holland, Inc., New York, pp. 127-130.