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Differentiation of Morphology and Toxicity in Harmful Algal Blooms Caused by the Raphidophyte Alga *Heterosigma akashiwo*

By

Edward Adam Black
B.Sc. University of British Columbia, 1974
M.Sc. University of British Columbia 1978

A Dissertation Submitted in Partial Fulfilment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In the Department of Biology

We accept this dissertation as conforming
to the required standard

Dr. L.A. Hobson, Supervisor (Department of Biology)

Dr. C.W. Harwyshyn, Departmental Member (Department of Biology)

Dr. R.G.B Reid, Departmental Member (Department of Biology)

Dr. P.C. Wan , Outside Member (Chemistry Department)

Dr. R. A.. Horner, External Examiner (Department of Biology)

Supervisor: Dr. Louis A. Hobson

Abstract

The purpose of this study was to examine the sequence of changes in the gross morphology and toxicity of the raphidophyte alga *Heterosigma akashiwo* in the context of blooms of this species in the Strait of Georgia, British Columbia. Field work focused on this alga's 1989 fish-killing bloom. That bloom was extensive, with discolouration of the water extending from Jervis Inlet through Malaspina Strait into the Strait of Georgia, and south to Cypress Island in Washington State. Excystment events on Spanish Banks in Vancouver harbour preceded population development in the Strait of Georgia during July and August but toxicity was only noted in the last two days of August and through September. Fish kills occurred near Cypress Island, and in Jervis Inlet and the contiguous waters of Agamemnon channel. These locations represented the geographic extremes of the discoloured waters.

In Malaspina Strait and Jervis Inlet, information was collected on water column structure, macronutrient concentrations, and the distribution of algae and fish mortalities. Fish mortalities were coincident with the first sighting of waters discoloured with *Heterosigma akashiwo* in Agamemnon Channel and Jervis Inlet. The alga appeared to be transported to the areas by currents. Algal concentrations were higher at the Malaspina Strait end of a transect into Jervis Inlet and during flood rather than ebb tides. The water column in Malaspina Strait and Jervis Inlet was stratified and both inorganic nitrogen and phosphorus in surface waters were low enough to limit growth of *H. akashiwo*. Though this alga can migrate vertically to obtain nutrients at depth, there was no evidence of migration during this toxic event. Termination of the bloom was associated with a

weakening of the water column stratification and the reappearance of non-limiting concentrations of nitrogen in the surface waters.

Work in culture demonstrated that there was a sequence of reproducible changes in cell size and shape that improved uptake when nutrients were at the concentrations seen in the Strait at the start of the toxic bloom. These changes involved reduction in cell volume by a factor of between 2- and 4-fold and cells changing from rounded, almost football-shaped cells (oblate spheroids), to plate-like (prolate spheroids). Mathematical modeling suggests that the volume changes could improve nutrient uptake by a factor of 21 to 38%. The changes in shape could improve nutrient uptake dynamics by a further 7.5%. Depending on the nutrient history of the population, changes in cell shape could continue beyond the plate-like form with significant portions of the algal population developing surface protuberances when adequate nutrients and energy were available to the population. The elaboration of surface processes could further improve uptake dynamics. A numerical model to describe these shapes was not available so the degree of improvement could not be quantified.

As cells reach the end of their life cycle in culture, they revert to the oblate spheroid form and, if no new nutrients are added, will form resting cysts. However, cyst formation can also be triggered earlier in the life cycle by the addition of nutrients. This may benefit the species by ensuring that cells form cysts in shallow waters where spring temperatures are adequate to facilitate excystment of cells.

In addition to changes in shape, *Heterosigma akashiwo* cells produce a toxic agent which can suppress growth of nutrient competitors and kill predators, or make the algae unpalatable. Production of those toxins begins and declines immediately prior to

decline in population numbers. Toxic effects of the alga can be caused to disappear at any time by the addition of nutrients. Similar to the differentiation of cell shape, the level of toxicity expressed by the cells appears to be correlated with the nutrient history of the population. Cells grown in higher nutrient concentrations appear to be more toxic; however, the population need not have high numbers of cells with processes to be toxic.

Heterosigma akashiwo would appear to have adaptations which enhance its ability to compete and survive in the nutrient-limited waters of late summer. The adaptions include both changes in gross morphology of the cells and in the production of toxins which reduce the effects of competition and predation. Population growth and formation of concentrations of the alga capable of discolouring the water can be independent of the occurrence of toxicity. Lack of vertical migration and a protracted period of a stable, stratified water column with depleted nutrients appear to be critical to the genesis of toxicity in wild populations.

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Dr. P.C. Wan , Outside Member (Chemistry Department)

Dr. R. A.. Horner, External Examiner (Department of Biology)

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1.0 Introduction

Toxic algal blooms have been in existence for a very long time, but the study of harmful algal blooms (HABs) is a relatively recent undertaking. Toxins which are found in these blooms are produced by such phylogenetically ancient groups as blue-green algae and bacteria (Cembella 1998, Doucette *et al.* 1998). Further, genera which are associated with toxin production, such as *Alexandrium,* are known in the fossil record (Wall 1980). Historically, the Christian bible records the occurrence of what is generally believed to be a toxic algal bloom in Exodus during the time of Moses (Exodus, Chapter 7 Verse 18-19) in the 13th century B.C. (Weeks 1998). An early record of the human symptoms of exposure to algal toxins can be found in the 1794 diary of Captain George Vancouver (Quayle 1969). The linkage of algal blooms and toxicity, based on scientific evidence, occurred in the 1930s (Sommer *et al.* 1937, Sommer and Meyer 1937). Purification and characterization of a phytotoxin, saxitoxin, occured in the late 1950s and 60s (Schantz *et al.* 1957, 1966) and the first international conference on toxic algae occurred in 1975 (LoCicero 1980).

Cembella (1998) best summed up the present state of our knowledge of toxic algal blooms at the 1996 NATO workshop when he stated " For several decades, the scientific community working on HABs has attempted the transition from a purely descriptive approach to HAB dynamics (magnitude, species composition, range extension etc.) to an interpretation of the causative processes and mechanisms underlying bloom initiation and development. ... it is fair to state we remain far from this goal." Knowledge of bloom

causation requires an understanding of the alga's response to its natural environment, not simply a knowledge of the tolerances of the cell's physiology or its responses to static algal culture conditions. What is required is a knowledge of the cell's responses to the kind of environmental dynamics it would experience in nature.

The problem can be stated in broad generalities as a need to define processes (progression of changes) and mechanisms (factors which enable the changes to occur). It is however, more difficult to formulate an approach that will provide an understanding of why toxic algal blooms develop from the perspective of the cell. The difficulty arises, in part, because definition of the phenomenon is assumed to imply that toxicity is a result of the occurrence of a bloom. However, not all blooms contain toxin-producing organisms. The type of algal abundance under consideration herein is both a bloom and is toxic however, for most toxic species high nutrient conditions promoting algal growth are associated with the lowest level of toxin production (Cembella 1998, Bates 1998, Wright and Cembella 1998). Phrased in this fashion it becomes clear that what is under study is a special subset of blooms. Their derivation involves a sequence of events that must first create a bloom then make it toxic. To understand the processes and mechanisms of bloom development we must therefore analyze blooms from their initiation as a growing population of algal cells through to the development of toxicity.

Another problem in the study of toxic blooms has been incomplete documentation of environmental conditions that give rise to and support a toxic bloom. Because we cannot predict when or where a bloom will occur, studies of algal blooms are generally initiated once the bloom has formed and has developed toxicity. Thus, most of our data on individual blooms does not include the period of bloom derivation, only its progression and demise. With this limitation our best hope for understanding the development of toxic

blooms is to describe blooms from their beginning through to their demise in nature and then to examine the mechanism of toxin production in the controlled environment of the laboratory.

In British Columbia the largest algal-mediated fisheries losses are caused by a raphidophyte alga, *Heterosigma akashiwo* (Hada) Sournia (hereafter also referred to as *Heterosigma*). Most losses are experienced by the salmon farming industry, though some are also believed to occur in the shellfish culture industries (Pers. Comm. Delia Becker, Cortez Island Shellfish Growers Co-operative, pers. comm.). The scale of the salmon farming loses is illustrated in Table I.

As described by Smayda (1998) the vegetative *Heterosigma* cell that forms blooms is small and slightly laterally compressed (10-25 μm long and 8-15 μm in breadth) varying in shape between spheroid to oblong. It has two flagella inserted in a shallow lateral grove: a rapid beating anterior flagellum provides the motile force; an shorter rigid flagellum trails posteriorly. It can have between 4 and 95 small yellow to brown discoid chloroplasts and has no cell wall.

Heterosigma akashiwo taxonomy has been confused by its close resemblance to *Olisthodiscus luteus* which was first described from brackish waters(Carter 1937). Some authors consider the strong flattening of *O. luteus* an adaptation to interstitial microhabitats and that pelagic blooms are caused by *Heterosigma* (Larsen and Moestrup 1989, Smayda 1998). Reviews of the taxomony of the species can be found

Table I. Major financial losses in British Columbia salmon farming caused by harmful algal blooms between 1986 and 1997. (J.N.C. Whyte, pers. comm.)

Year	Estimated Loss ($000,000)	Algal Species Responsible
1986	2.5	*Heterosigma*
1987	3.0	*Chaetoceros sp.*
1988	4.0	*Heterosigma* & *Chaetoceros sp.*
1989	4.0	*Heterosigma*
1990	4.0	*Heterosigma*
1991	1.5	*Heterosigma*
1997	10-20	*Heterosigma*

in Hulburt (1965), Loeblich and Fine (1977), Hara and Chihara (1987), Taylor (1992) and Throndsen (1996). *Heterosigma akashiwo* has also been known as: *Entomosigma akashiwo* Hada; *Heterosigma inlandica* (Hada) (Hada 1968); *Heterosigma carterae* (Taylor 1992) and Microflagellate X (Gowen 1987). The designation of *Heterosigma akashiwo* made by Hara and Chihara in 1987 will be used throughout this document.

Some authors *Heterosigma* is considered to be one of the most representative of toxic flagellate bloom species (Khan *et al.* 1997). This species and associated fisheries losses are not limited to British Columbia. The species has been reported in the waters of New Zealand (Boustead *et al.* 1989,Taylor 1990), Australia (Smayda 1998), Thailand (Lirdwitayaprasit *et al.* 1996), Singapore (Taylor 1990), Taiwan (Shen and Chaing 1971), China (Tseng *et al.* 1993), Korea (Park 1991), Japan (Hara and Chaing 1987, Honjo 1978), Kamchatka Islands (Konovalova 1995), British Columbia (Black 1991), Washington (Rensel *et al.* 1989), California (Lackey and Clendenning 1965), Peru (Rojas de Mendiola 1979), Chile (Taylor 1990), Florida (Tomas 1998),Massachussetts (Tomas 1980), New York (Mahoney and McLaughlin 1977), Bermuda (Tomas 1980), Faroe Islands (ICES 1991, Larsen and Moestrup 1989), Ireland (ICES 1991), Scotland (Gowen 1987), Norway(Throndsen 1969), Belgium (Conrad and Kufferath 1954, Honjo 1993), England (Careter 1937, Lackey and Lackey 1963), France (IFREMER 1994), Spain (Bravo et al. 1995), Portugal (IFREMER 1994, Sampayo and Moita 1984), The Adriatic Sea (Marasovic and Pucher-Petkovic 1985), and Namibia (Smayda 1998). Most of these records are of the presence of *Heterosigma akashiwo* as algal blooms.

Blooms of this species along with fisheries losses are common in B.C. (Table I) and the literature is extensive, amounting to approximately 350 papers (Smayda 1998).

Even with such a large information base, prediction of the occurrence of toxic *Heterosigma* blooms is difficult (J.N.C. Whyte, Fisheries and Oceans Canada, pers. comm. and F.J.R Taylor, University of British Columbia, pers. comm.) and there is a lack of information on conditions both in nature and in culture which lead to the production of toxins.

The life cycle of *Heterosigma akashiwo* has two benthic resting stages : a non-motile resting stage agglutinated into plasmodial masses of large numbers encapsulated by mucilage (Tomas 1978b) and resting cysts (Imai *et al.* 1993). The cysts tolerate low sediment oxygen concentrations and high sulfide levels (Montani *et al.* 1995). They are capable of dark survival for at least eight months at 11 °C. Cysts attain their highest germination rates at temperatures above 14 °C. Vegetative cells range from spheroid to ovoid to oblong (Hara and Chihara 1987) with measured cellular division rates varying between .88 and 5 day^{-1} (Langdon 1986, Honjo and Tabatta 1985). Vegetative cells can undergo diel vertical migration characterized by daytime ascent and nighttime descent (Hatano *et al.* 1983, Wada *et al.* 1985, 1987, Watanabe *et al.* 1988, MacKenzie 1991). Use of this feature to access nutrients below a pycnocline is aided by the ability of cells to swim through significant changes in temperature and salinity; cells can traverse changes of 6.5 °C and 5.7 ppt encountered in pycnoclines. The nutrient status of cells influences vertical migration. For example, nitrogen depleted cells cease vertical migration (Hatano *et al.* 1983, Takahashi and Fukazawa 1982, Takahashi and Ikawa 1987, Yamochi and Abe 1984).

Studies of how blooms of this species terminate are inadequate. There have been studies on the possible role of predation and viruses in ending *H. akashiwo* blooms. These studies however, have been done without reference to the potential toxicity of the alga. The formation of cysts might also play a role in ending blooms. Though cysts have been described both in nature and in cultured populations little has been done to examine environmental signals which initiate cyst formation. The underlying and untested assumption is that the primary environmental signal for cyst formation is lack of nutrients.

Many of the blooms recorded in Table I occurred within or in waters adjacent to the Strait of Georgia (J.N.C. Whyte pers. comm.). The biological and physical oceanography of this water body has been the subject of numerous studies, which have been compiled in a number of reviews (Harrison *et al.* 1983, LeBlond 1983, Thomson 1981, Waldichuck 1957). In summary, the strait can be viewed as a coastal basin with tributary inlets and restricted flow from the Pacific Ocean over shallow narrow sills at its northern and southern extremes. There are six well-developed inlets contiguous with the strait: In the southern part these are Saanich, Burrard, and Jervis Inlets, and Howe Sound; and in the northern part, Desolation and Pendrell Sounds, and Bute Inlet. These vary in the amount of runoff they receive but all are positive estuaries with seaward flow at the surface and inflow at depth.

The structure and circulation of the southern part of the basin is strongly affected by the discharge of the Fraser River, particularly during the summer when it receives the

snow and glacier melt from the interior of British Columbia. The discharge from the Fraser can contribute up to 80% of the freshwater input to the strait. Most years the central body of the strait is strongly stratified with seasonal low concentrations of nitrate and phosphate, as well as the highest surface water temperatures occurring during the summer. A frontal zone often forms in front of the river and some of the highest phytoplankton biomass and productivity in the strait are found there.

Currents in the strait are affected by mixed semidiurnal tides entering from the north and south (Thomson 1981). This results in a generally counter-clockwise circulation pattern with the strongest currents along the mainland shore. There is also a minor southern counter-clockwise gyre occupying most of the basin south of Texada Island.

Much of the impact of Georgia Strait blooms of *Heterosigma akashiwo* is witnessed by salmon farmers in the mouth of Jervis Inlet and Agamemnon Channel in the summer and early fall months (Black 1986, 1987, 1988, 1990a, 1990b, 1991, 1994). This area is at the north-eastern edge of the southern gyre and near the southern end of Texada Island.

Little work has been done on the physical oceanography of this sub-area of the strait. Jervis Inlet extends 85 km inland, is deep (approximately 600 meters) and has a deep sill (280 meters) at its mouth. The mouth of the inlet starts at Malaspina Strait behind Texada Island and runs approximately 28 km east before the main body of the inlet turns north. The oceanography of the mouth of Jervis inlet is complicated by three

tributary waters that empty into it. On the north side, 18 km from the strait, is Hotham Sound a broad, unsilled basin 5 km wide at its mouth extending 15 km miles to the north. On the south side, 20 km from the strait, two water bodies empty into Jervis Inlet. From the southeast waters flowing over a very shallow (14 m) sill are from the Sechelt Inlet system. This system is composed of approximately 68 km of waterways which include Sechelt inlet (30 km) and two tributary inlets, Salmon (23 km long) and Narrows Inlet (15 km long). Entering Jervis Inlet from the southwest are the waters of Aggamemnon Channel. The channel's southern terminus is in Malaspina Strait at the south end of Texada Island approximately 15 km south of the entrance to Jervis Inlet.

Lazier (1963) documented the structure and flow of waters in Jervis Inlet. The structure of the water column in the mouth of the inlet does not appear to be significantly affected by waters entering from any of these tributary systems. The Inlet has an estuarine circulation pattern with a thin surface layer of water moving to the strait driven by freshwater from land drainage and precipitation. As this low salinity surface layer flows seaward it entrains saltwater from below it causing a subsurface inflow of waters. An estuarine circulation is caused and controlled by the amount of surface runoff. Because the surface runoff in Jervis Inlet is relatively small, other factors such as changes in meteorological and oceanographic conditions will produce flows that overwhelm the forces driving the estuarine circulation pattern.

Lazier's data suggest that the estuarine circulation persists in Jervis Inlet from May to at least July. Between July and October, the strength of that circulation pattern

decreases dramatically as the amount of freshwater entering the system decreases. Surface salinities increase at all sampling stations up to the head of the inlet. However, of particular interest is that the low salinity surface layer is deeper and lower in salt content at the head and mouth of the inlet than at mid-inlet. Further, the salinity at the mouth of the inlet is lower than that at the head of the inlet. This would appear to be the result of the low salinity plume from the Fraser River spreading over the surface of the Strait of Georgia and entering the mouth of Jervis Inlet. Lazier (1963) hypothesizes that the waters leaving the inlet do so at depths below 50 m at this time of year.

His analysis suggests that during July, outflow of waters from Jervis Inlet occur below a surface layer. This is because the cooler, low salinity of the glacier-fed waters of the inlet are denser than the warm, low density surface waters of the strait. The depth of the summer outflow layer may be as much as 50 meters in July. By October the density of the surface waters has risen sufficiently that there is a net outflow of surface water from Jervis Inlet.

As many of the toxic blooms of this species occur in the late summer (Black 1987, 1988, 90b, 91, and Rensel 1989) the relation of the physical and biological oceanography of the Strait of Georgia to the occurrence of toxic algal blooms in the Jervis Inlet/Agamemnon Channel area is central to our understanding of the development of toxic *Heterosigma akashiwo* blooms in this area. To understand the linkage between the oceanography of the area that engenders the blooms and the development of toxicity which is so commonly witnessed by salmon farmers we must understand, in the context of

the environment in which a bloom develops, what benefits the individual cells might derive. Our best hope for achieving this is to describe the occurrence of toxic blooms in their natural environment from their beginning to their demise, then to examine the mechanism of toxin production in the controlled environment of the laboratory. E.B. Wilson's assertion that "The key to every biological problem must finally be sought in the cell" (Cembella 1998) does, for toxic blooms, clearly show where we will find the "keystone" knowledge for understanding this phenomenon.

2.0 Methods For Studies of Wild Populations

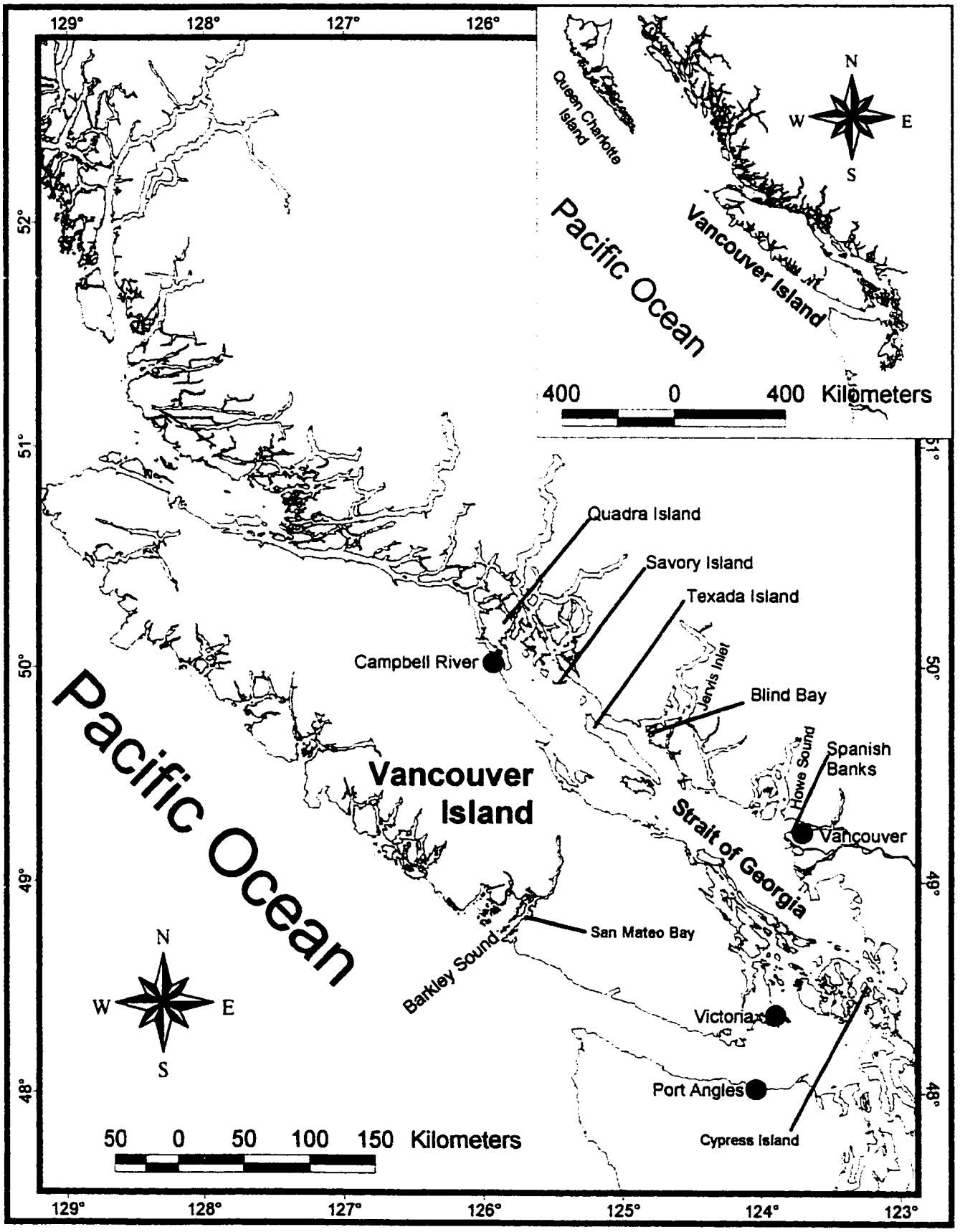
2.1 Sampling Stations

Location of areas sampled or discussed in the text are shown in Figure 1. Samples used to document temporal and spatial occurrences of *Heterosigma akashiwo* were obtained from fifteen sites along the coast of British Columbia during 1989 (Figure 2). These samples were from several depths between 0 and 5 m and are used only to indicate presence or absence of *H. akashiwo* at certain times of the year (Table II).

The visual extent of discoloured waters associated with the September 1989 bloom (Figure 3) was derived from two sources: observations from a low level (under 300 m) aircraft reconnaissance of the lower Strait of Georgia flown September 9; and reports from salmon farmers in the United States for the period of September 7 to 15.

Samples for interpreting the progression of the bloom on the Sunshine Coast (August 28 to October 5) were taken from three sets of stations: five stations in a transect from Malaspina Strait up Agamemnon Channel to Jervis Inlet (Figure 4) were sampled once at the beginning of the bloom and were used to determine whether or not there was a pattern in the changes in surface concentration of *H. akashiwo* along the transect: two stations in Agamemnon Channel and Jervis Inlet, sampled daily, were used to determine temporal changes in algal concentrations (Figure 5); and, three stations in Malaspina Strait, Sechelt Inlet and Jervis Inlet were sampled four times (September 8, 12, 19 and

Figure 1. Locations mentioned in the text. .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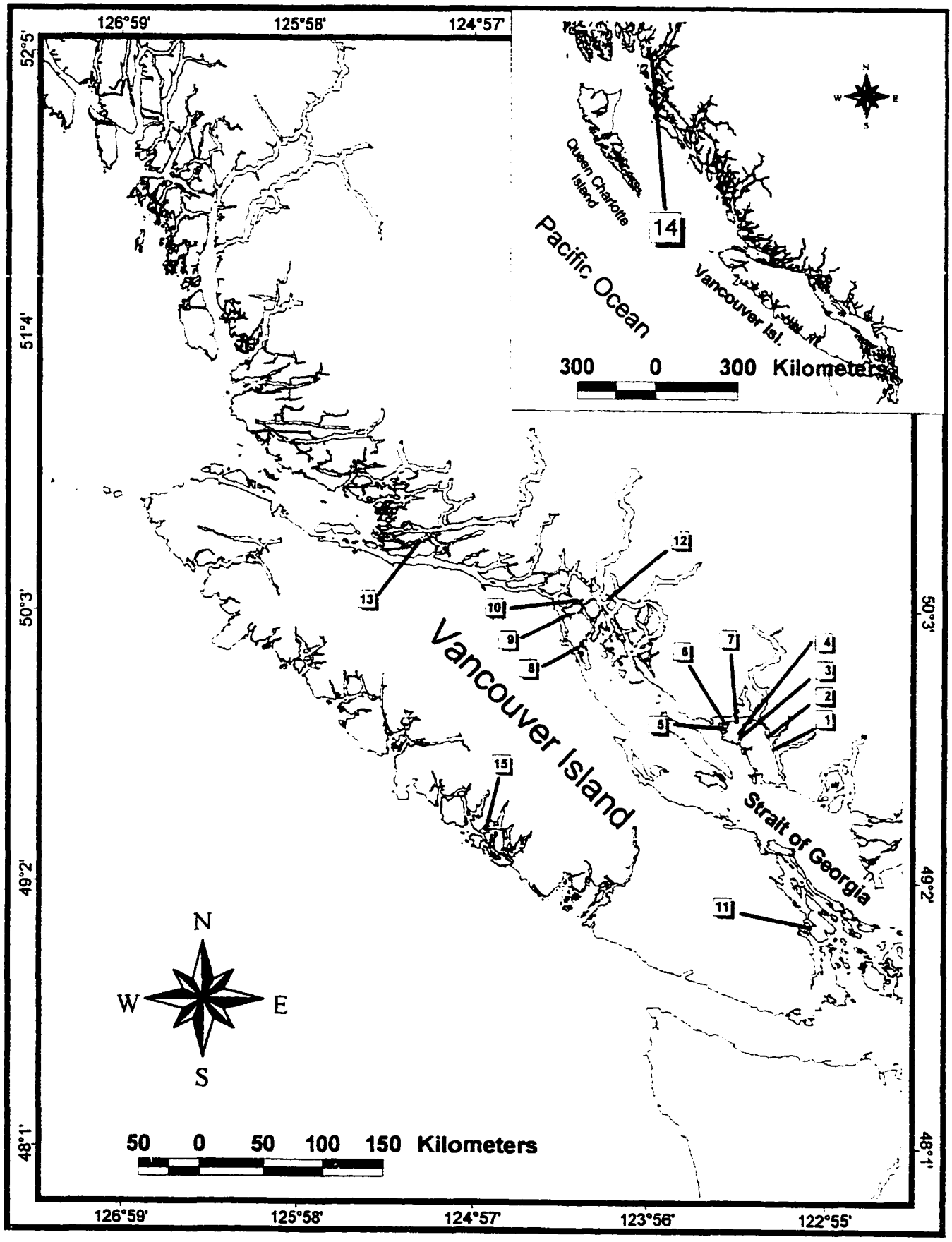
S
400 0 400 Kilometers
Quadra Island
Savory Island
Texada Island
Campbell River
Jervis Inlet
Blind Bay
Howe Sound
Spanish Banks
Vancouver
Strait of Georgia
San Mateo Bay
Barkley Sound
Victoria
Port Angles
Cypress Island
50 0 50 100 150 Kilometers

Figure 2. Location of sites used in the monthly sampling documented in data in Table II.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51°4'
50°3'
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Pacific Ocean
Queen Charlotte Island
Vancouver Isl.
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Vancouver Island
Strait of Georgia
13
12
10
9
8
7
6
5
4
3
2
1
15
11
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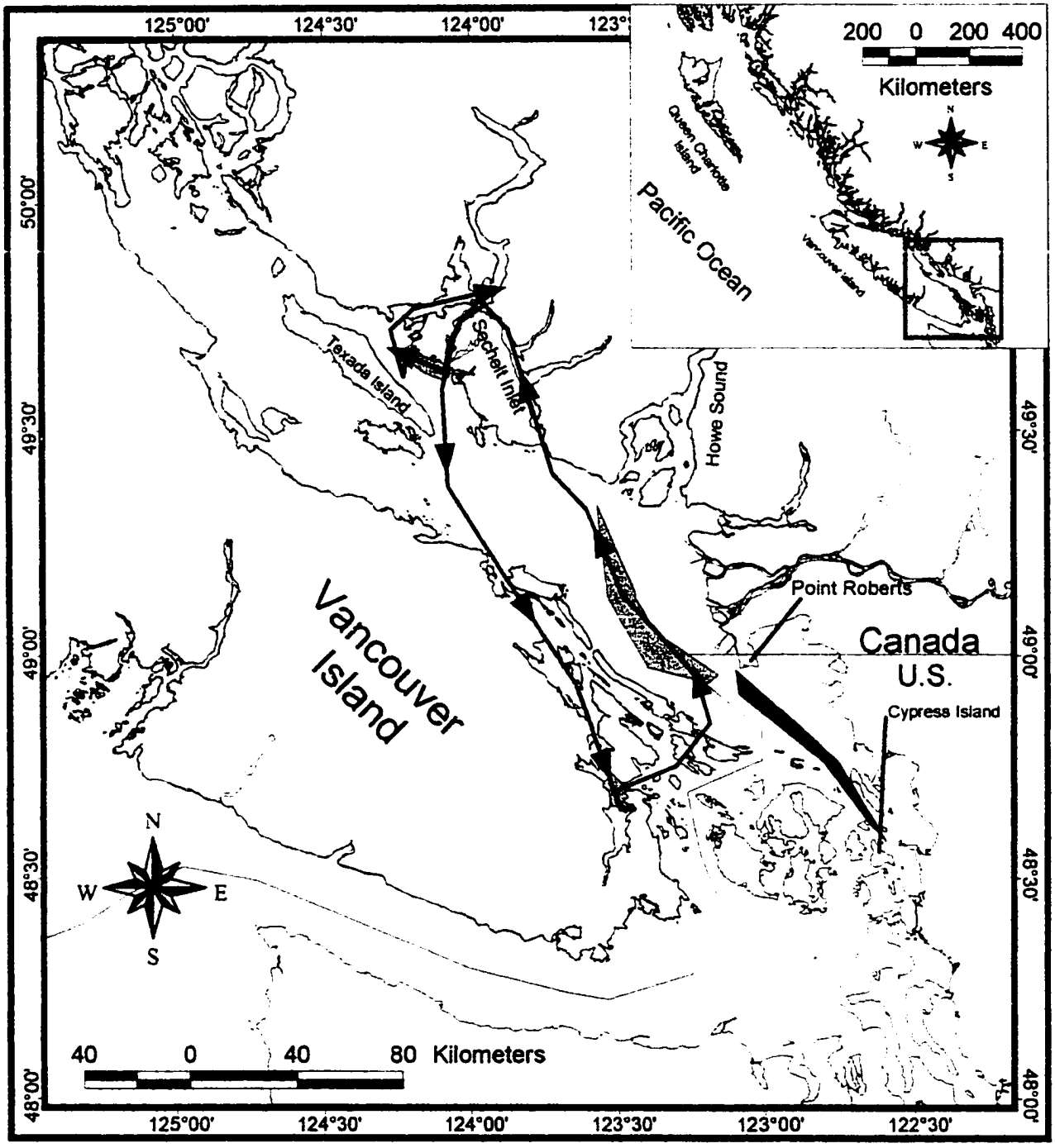
E
S
50 0 50 100 150 Kilometers

Table II. Occurrence of *Heterosigma akashiwo* in 1989 plankton samples from the coast of British Columbia. (The designation 4/8 indicates that of 8 samples taken, 4 contained *H. akashiwo*)

SITE #	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.
Strait of Georgia							
1	0/2	0/3	0/4		0/3		0/5
2	0/4	0/4	0/3	2/4	1/3	7/7	2/3
3	1/3	0/5	0/4	1/4	3/5	6/6	0/1
4	0/4	0/3	0/3	1/3	5/6	4/4	4/4
5	0/3	0/4	0/4	0/2	3/4	1/1	0/1
6	0/2	0/3	0/4	0/4	3/4	5/5	1/3
7			0/2	2/5	3/5	2/4	2/4
8	2/3	0/5	0/4				
9	0/4	0/3	0/2	0/3	1/1	0/5	
10	1/4	0/3	0/3	0/1	3/3	0/4	
11		0/4	0/3	0/1	4/5	2/3	
12	0/3	0/4	0/4	0/3	0/3	0/4	0/4
Total	4/32	0/40	0/40	6/30	26/42	27/43	9/25
Outside the Strait of Georgia							
13		0/3	0/5	0/5	1/3	1/5	0/3
14	0/4	0/3	0/4	0/5	0/5	1/5	0/5
15					0/1	3/5	0/4
Total	0/4	0/6	0/9	0/10	1/8	4/15	0/12

Figure 3. The spatial extent of discoloured waters associated with the bloom. Arrows indicate the aircraft's flight path. Shaded areas under the flight path indicate where the bloom was observed September 9, 1989. The shaded area extending from Point Roberts to Cypress Island shows is where salmon farmers from the United States reported seeing the bloom between September 7 and September 15, 1989.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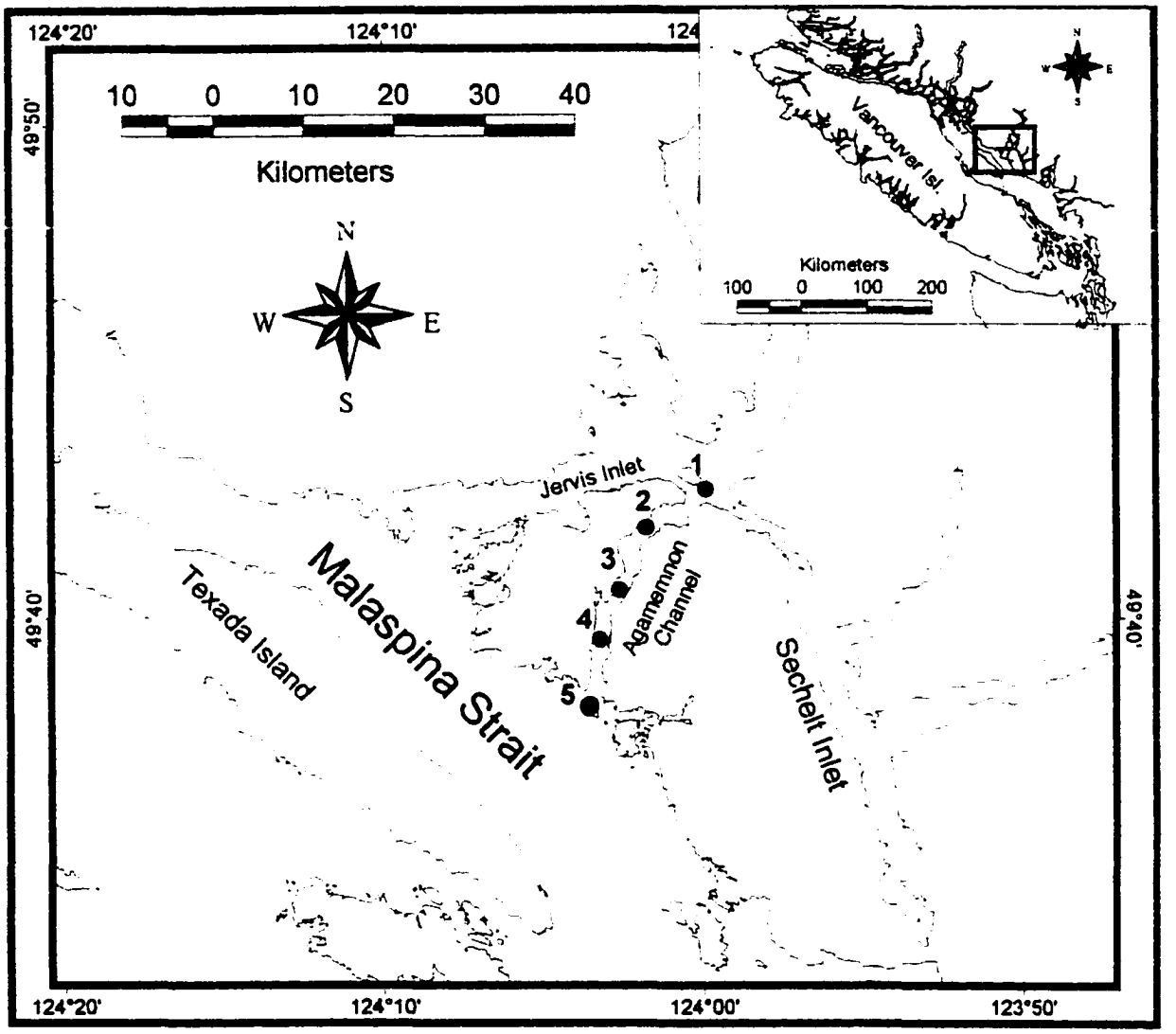
Pacific Ocean
Queen Charlotte Island
Vancouver Island
Texada Island
Sechelt Inlet
Howe Sound
Point Roberts
Canada
U.S.
Cypress Island
Vancouver Island
40 0 40 80 Kilometers

Figure 4. Locations used to determine the changing pattern of surface concentrations of *H. akashiwo* in Malaspina Strait up Agamemnon Channel to Jervis Inlet.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Jervis Inlet
1
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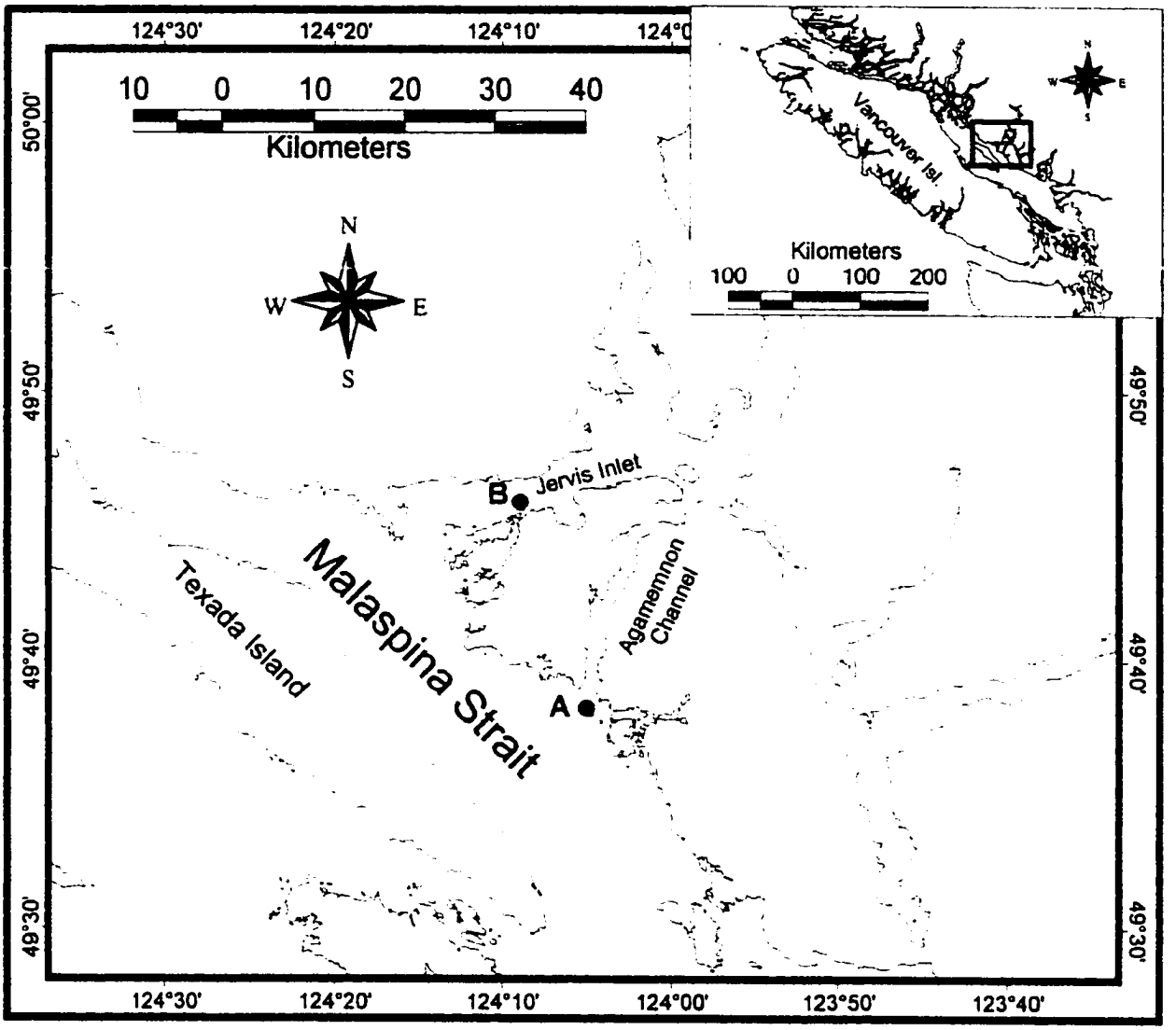
4
5
Malaspina Strait
Texada Island
Agamemnon Channel
Sechelt Inlet
Vancouver Isl.
Kilometers
100 0 100 200

Figure 5. Locations in Agamemnon Channel and Jervis Inlet used to determine temporal changes in concentrations of *Heterosigma akashiwo*.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124°00'
123°50'
123°40'
50°00'
49°50'
49°40'
49°30'
10 0 10 20 30 40
Kilometers
N
W
E
S
Jervis Inlet
B
A
Malaspina Strait
Texada Island
Agamemnon Channel
Vancouver Isl.
Kilometers
100 0 100 200

October 5) and were used to determine the vertical distribution of *H. akashiwo* and the physical and chemical characteristics of the water column (Figure 6).

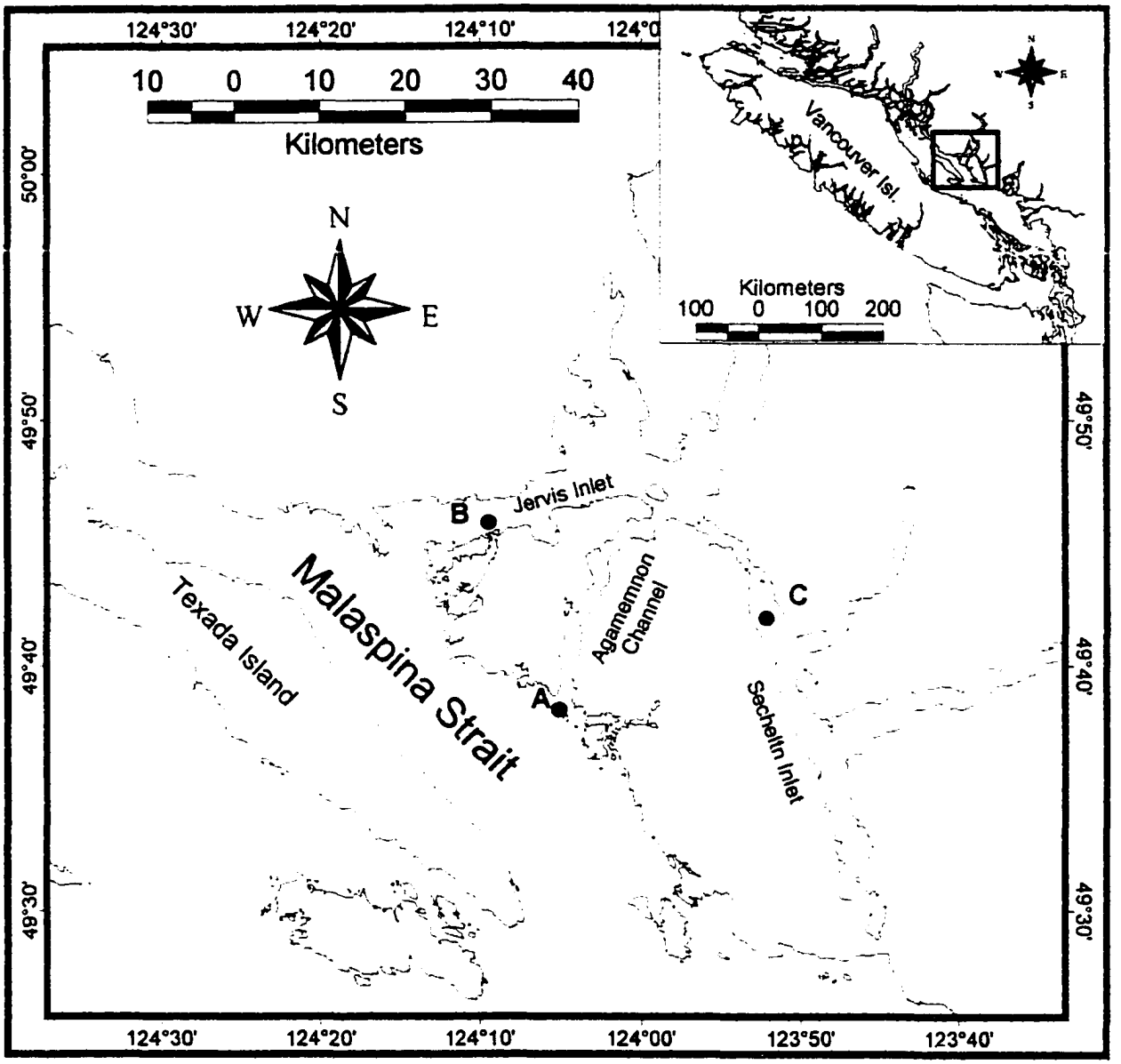
2.2 Phytoplankton Analysis

At each site Niskin sampling bottles were used to collect 500 ml of water which was preserved with 1% Lugol's acid-iodine solution (Steedman 1978, Tregouboff and Rose 1957). From each sample a subsample of 10 ml was settled for 6-12 hours and analysed by the Utermöhl method (Steedman 1978, Utermöhl 1958). The lesser of the entire contents of the settled column or 200 cells were enumerated.

2.3 Water Quality Analysis

A HydroLab Ltd., Surveyor II depth, temperature, salinity and oxygen meter was used to determine temperature, salinity and oxygen concentrations in the upper water column on September 7, 8, 12, 19 and October 5. Readings were initially take at 0, 3, 10, 30 and 50 m. After establishing the depth of the pycnocline on September 7 and 8, a reading was thereafter taken from 15 meters rather than 50 meters. Phytoplankton samples were also taken at these depth.

Figure 6. Locations in Agamemnon Channel, Jervis Inlet, and Sechelt Inlet used to determine vertical distribution of *Heterosigma akashiwo* and water column properties.


124°30'
124°20'
124°10'
124°0
10 0 10 20 30 40
Kilometers
N
W
E
S
50°00'
49°50'
49°40'
49°30'
Vancouver Isl.
Kilometers
100 0 100 200
Jervis Inlet
B
Malaspina Strait
Texada Island
Agamemnon Channel
C
A
Sechelt Inlet
124°30'
124°20'
124°10'
124°00'
123°50'
123°40'

Analytical methodologies described by McQuaker (1989) were used to determine concentrations of orthophosphate, ammonia, nitrite and nitrate. Orthophosphate was determined by ascorbic acid reduction to create a blue colour whose colour intensity was then measured in a spectrophotometer at 885 nm. Ammonia concentrations were determined using the Berthelot reaction followed by colorimetry at 630 nm. Nitrite analysis was performed using the diazontization technique followed by spectrophotometry at 520 nm. Nitrate analysis was performed by reducing all nitrate to nitrite then analyzing nitrate and correcting for pre-formed nitrate.

2.4 Fish Mortalities

During visits to sampling sites on September 7, 8, 12, 19 and October 5 notes on penned fish behavior were made. After bloom termination farmers were canvassed by phone to determine whether or not a pattern could be discerned from the reported mortalities. They were asked when they first saw the bloom on their site, when they thought they first experienced losses, what portion of their stock was lost and did they notice any change in their fishes behaviour during the progression of the bloom . Not all farmers were willing to discuss their losses but those that were provided an intriguing and generally consistent pattern.

2.5 The 1996 Bloom

A bloom of *H. akashiwo* was seen July 27 1996 during a flight from Port Hardy on the northern end of Vancouver Island to Vancouver International Airport (Figure 7). Four 100 ml samples of the water were obtained from a depth of 1 meter at the Department of Fisheries and Oceans dock in North Vancouver. One of the samples was preserved in 1% Lugol's solution and the others were untreated to obtain live cells for later culture work. Cells in the fixed samples were enumerated using a model TA II Coulter Counter. Bioassays were carried out as in Black *et al.* (1991). Filtered sea water for controls was obtained from the sea water system of the Department of Fisheries and Ocean's laboratory in North Vancouver.

3.0 Results from Studies of Wild Populations

3.1 The Bloom of 1989

3.1.1 Seasonal and geographic distribution of *Heterosigma akashiwo*

This algal species is found throughout the waters of coastal British Columbia. *H. akashiwo* occurs both within and outside the Strait of Georgia mainly in August and September, though it does occur in some samples as early as April. It also appears to be a

more frequent constituent of waters of the Strait of Georgia and contiguous waterways than of the rest of the coastal waters.

Observation of the discoloured surface waters of and contiguous to the Strait of Georgia, indicated that the 1989 bloom extended in a continuous band from off Point Roberts, across in front of Vancouver to Howe Sound (Figure 7). Discoloured water was also evident in Malaspina Strait, Agamemnon Channel, and Jervis Inlet on the east side of the Strait of Georgia and in the mouth of Saanich Inlet on the west side of the strait.

Puget Sound fish farmers reported a streak of the bloom extending from the southern tip of Point Roberts to Cypress Island near the entrance to Puget Sound (Figure 3). Mortalities of cultured salmon were reported at Cypress Island, and *H. akashiwo* was reported to have formed a monospecific bloom at that location.

3.1.2 Occurrence of *Heterosigma akashiwo* on the Sunshine Coast

In Agamemnon Channel there was a marked increase in abundance of *H. akashiwo* on August 30, 1998 (Figure 8) and cell concentrations was generally high over the next month varying between 100 and 10,000 cell/ml. Three distinct increases were discernible starting August 30, September 1, and September 15. Cell

Figure 7. Spatial extent of discoloured waters associated with the 1996 bloom. The arrows represents the flight path of the plane. The area that had discoloured water associated with the Fraser River plume is shaded with horizontal bars. The discoloured waters associated with the bloom are stippled.

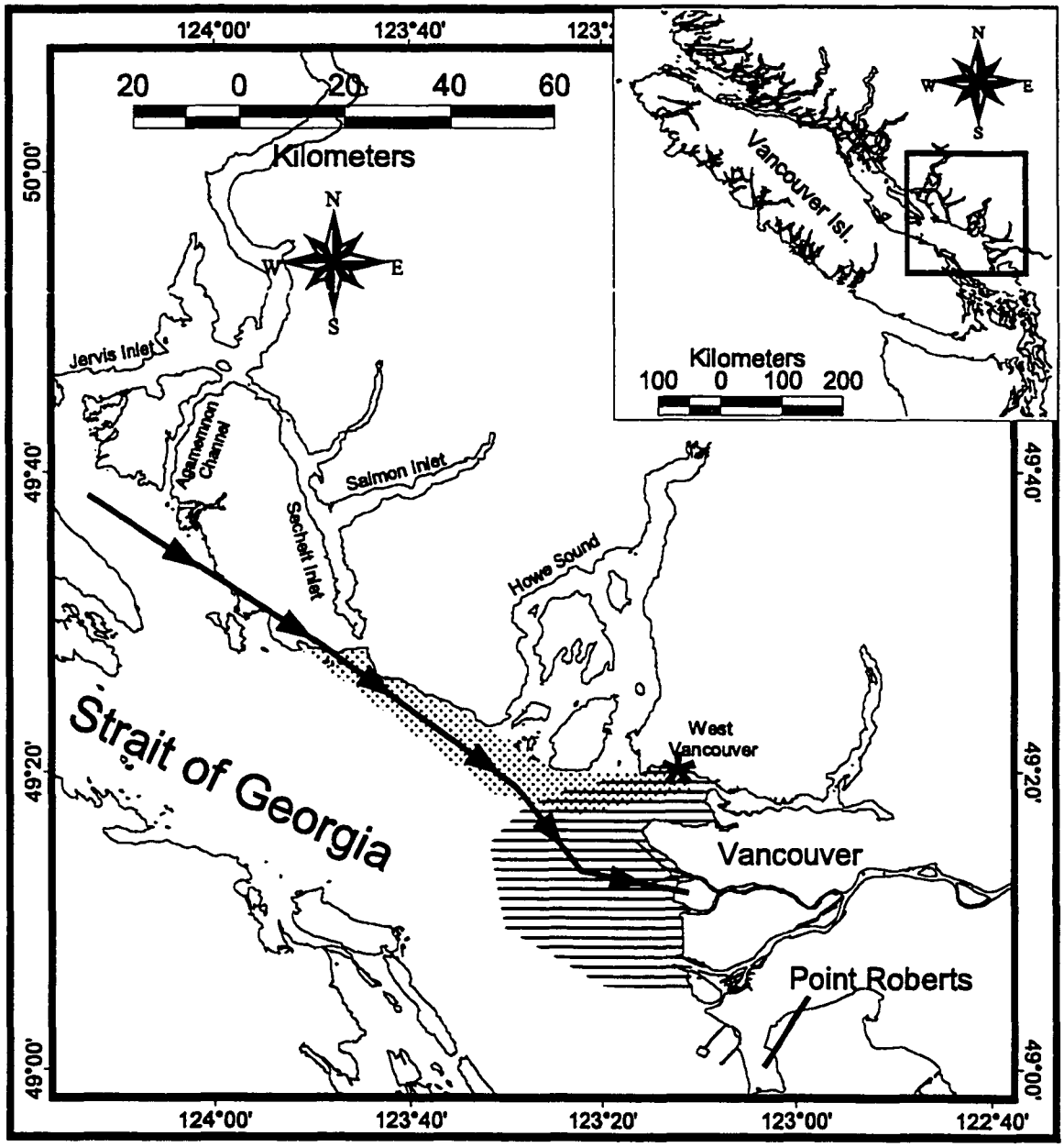

124°00'
123°40'
123°
20 0 20 40 60
Kilometers
N
W
E
S
50°00'
49°40'
49°20'
49°00'
Jervis Inlet
Agamemnon Channel
Sechelt Inlet
Salmon Inlet
Howe Sound
Strait of Georgia
West Vancouver
Vancouver
Point Roberts
123°20'
123°00'
122°40'
Vancouver Isl.
Kilometers
100 0 100 200

Figure 8. Concentration of *H. akashiwo* cells at 1 m depth in Agamemnon Channel (solid line) and Jervis Inlet (dashed line). Sampling locations are as shown in Figure 5.


10000
1000
100
10
1
Cell Conentration (Cells/ml)
28/08/99
29/08/99
30/08/99
31/08/99
01/09/99
02/09/99
03/09/99
04/09/99
05/09/99
06/09/99
07/09/99
08/09/99
09/09/99
10/09/99
11/09/99
12/09/99
13/09/99
14/09/99
15/09/99
16/09/99
17/09/99
18/09/99
19/09/99
20/09/99
21/09/99
22/09/99
23/09/99
24/09/99
25/09/99
26/09/99
27/09/99
28/09/99
29/09/99
30/09/99
01/10/99
02/10/99
03/10/99
04/10/99
05/10/99
Date

concentrations on ebb and flood tides were examined between September 4 and 27 (Figure 9). Concentrations in flooding tidal waters were as high or higher than those on ebb tides on all days except Sept 6, 12, 13 and 25. Cell concentrations decreased approaching Jervis Inlet from Malaspina Strait on September 7 (Figure 10). In contrast to fluctuating concentrations in Agamemnon channel, cell concentrations in Jervis Inlet increased on September 7 and remained relatively constant until September 25, when concentrations in Agamemnon Channel started their final decline (Figure 8).

Vertical profiles of *H. akashiwo* concentrations show strong surface orientation at all stations during the blooms (Figure 11). Gradually there is a decrease in surface concentrations until October 5, when concentrations are low at all depths. Profiles of cell concentrations during the night of September 11 and the day of September 12 did not show any evidence of vertical migration of cells (Figure 12).

In 1989 phytoplankton sampling for this study stopped on October 5. A little over a week later, October 14, salmon farms at the confluence of Agamemnon Channel and Malaspina Strait reported surface waters to be again discoloured by algae. The bloom only lasted three or four days. While no quantification was available, samples taken by the farmers confirmed that it was another bloom of *H. akashiwo*.

Figure 9. Concentration of *H. akashiwo* at 1 m depth in Agamemnon Channel on the ebb (dashed line) and flood tide (solid line). Sampling locations are as shown on Figure 5.

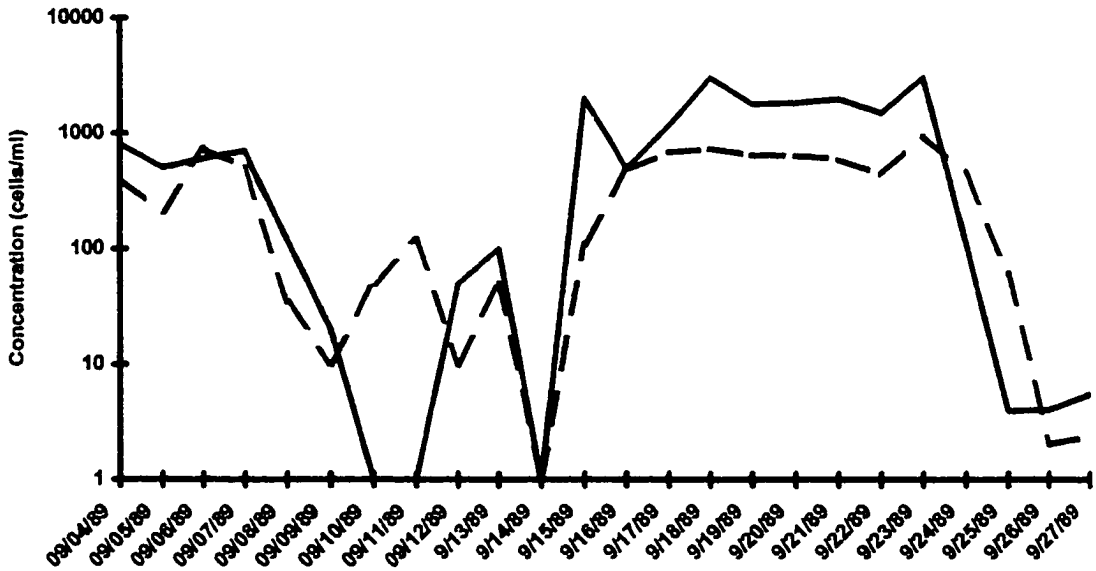

10000
1000
100
10
1
Concentration (cells/ml)
09/04/89
09/05/89
09/06/89
09/07/89
09/08/89
09/09/89
09/10/89
09/11/89
09/12/89
9/13/89
9/14/89
9/15/89
9/16/89
9/17/89
9/18/89
9/19/89
9/20/89
9/21/89
9/22/89
9/23/89
9/24/89
9/25/89
9/26/89
9/27/89

Figure 10. Concentrations of *H. akashiwo* in samples taken at 1 m depth in transect from Malaspina Strait, up Agamemnon Channel to Jervis Inlet on Sept. 7. Sampling locations are illustrated in Figure 4.

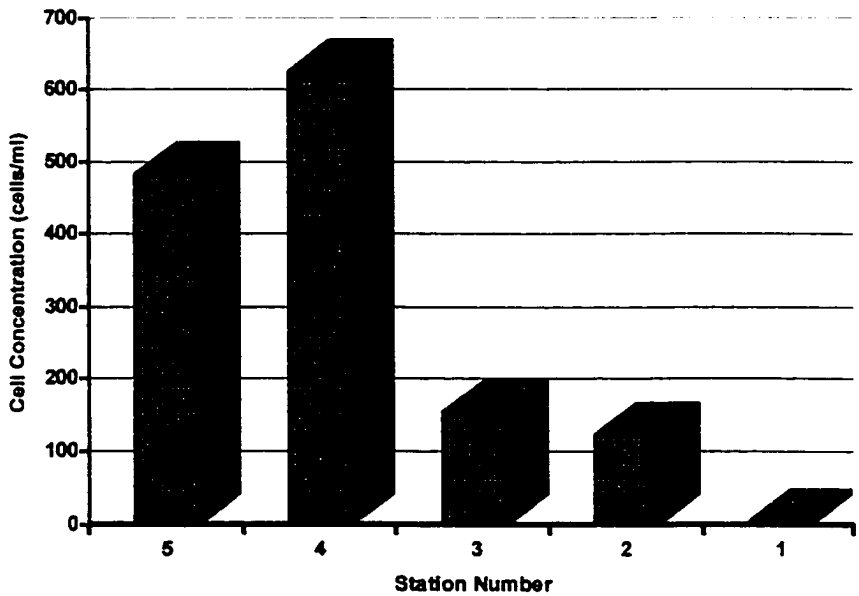

700
600
500
400
300
200
100
0
Cell Concentration (cells/ml)
5
4
3
2
1
Station Number
Malaspina Strait
Jervis Inlet

Figure 11. Depth profile of *H. akashiwo* concentrations in: Malaspina Strait (top); Jervis Inlet (middle); and Sechelt Inlet (bottom).

Closed circles = September 7,
Open circles = September 8,
Asterisk = September 12,
Open triangles = September 19,
Closed triangles = October 5.

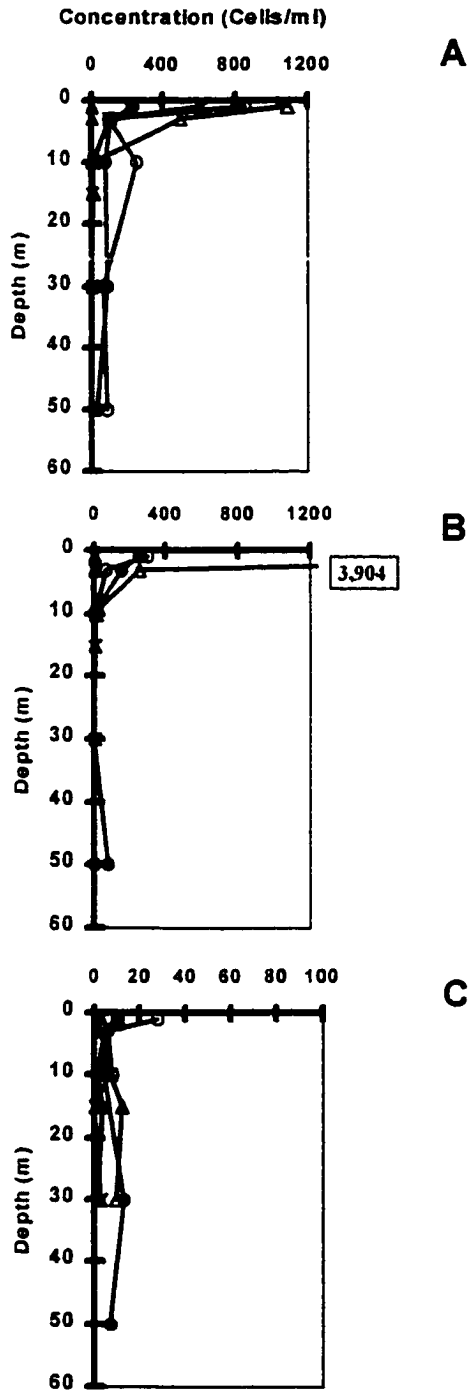
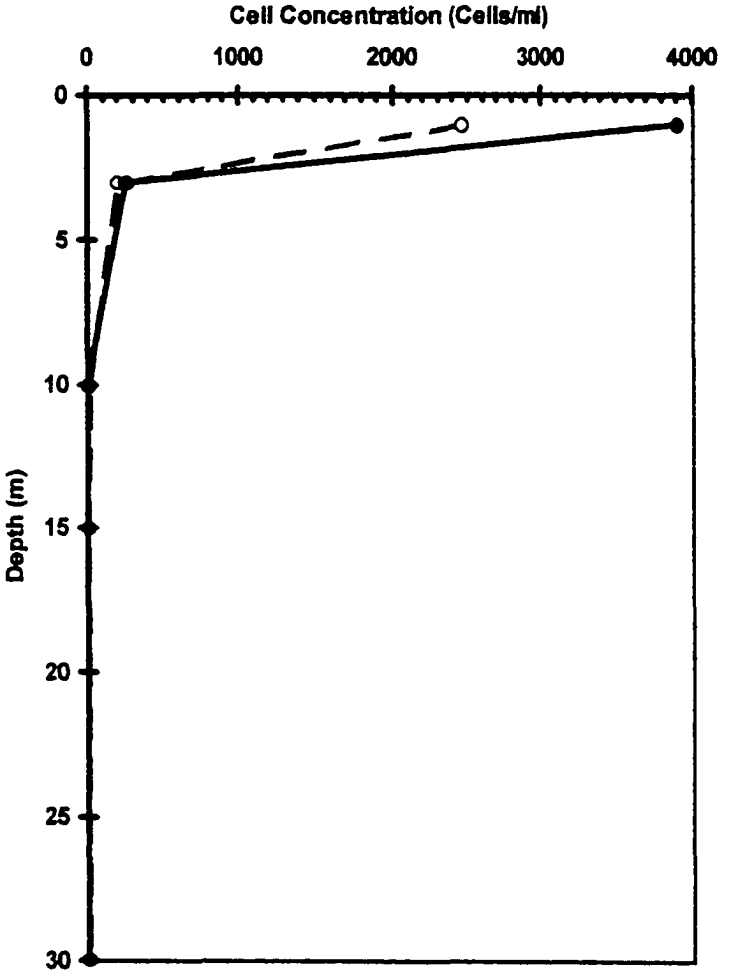

Concentration (Cells/ml)
A
0
400
800
1200
Depth (m)
0
10
20
30
40
50
60
B
3,904
C
20
40
60
80
100

Figure 12. September 11-12, day and night depth profiles of *H. akashiwo* concentrations in Jervis Inlet. Open circles represents samples taken during the night of September 11. Closed circles are samples from the day of September 12.


Cell Concentration (Cells/ml)
0
1000
2000
3000
4000
0
5
10
15
20
25
30
Depth (m)

3.1.3 Structure of the Water Column

The relative strength of stratification is illustrated in figure 13 and also in Table III as buoyancy frequencies of waters at 30 m depth. Water columns (between the surface and 15 m) in Malaspina Strait, Jervis Inlet and Sechelt Inlet were strongly stratified on September 7, 8 and 12. Stratification weakened between September 19 and October 5. During September stratification was stronger in the waters of Malaspina Strait and Jervis Inlet. At the end of the bloom on October 5, the surface waters of Jervis inlet were more strongly stratified than either the waters of Malaspina Strait or Sechelt Inlet. Over all in this period the decline in the strength of stratification was most pronounced in the waters of Malaspina Strait and Sechelt Inlet, and least in Jervis Inlet. Salinities (Figure 14) at all stations ranged from approximately 25 to 26 ppt on September 7-8 when blooms began. Surface waters at the end of the bloom, October 5, were more saline (approximately 28 to 29.5 ppt).

Sea surface temperatures at all locations were above 16 °C as blooms started (Figure 14). Jervis Inlet water was approximately one degree warmer than in those of Sechelt Inlet, and almost two degrees warmer than in those of Malaspina Strait. At the end of the bloom, surface temperatures in Malaspina Strait had dropped to approximately 12 °C, while those in Sechelt Inlet were approximately one degree warmer. Water temperatures decresaed most rapidly in Jervis Inlet, and slowest in Sechelt Inlet.

Figure 13. Depth profiles of water density in: Malaspina Strait (top); Jervis Inlet (middle); and Sechelt Inlet (bottom).

Closed circles = September 7,
Open circles = September 8,
Asterisk = September 12,
Open triangles = September 19,
Closed triangles = October 5.

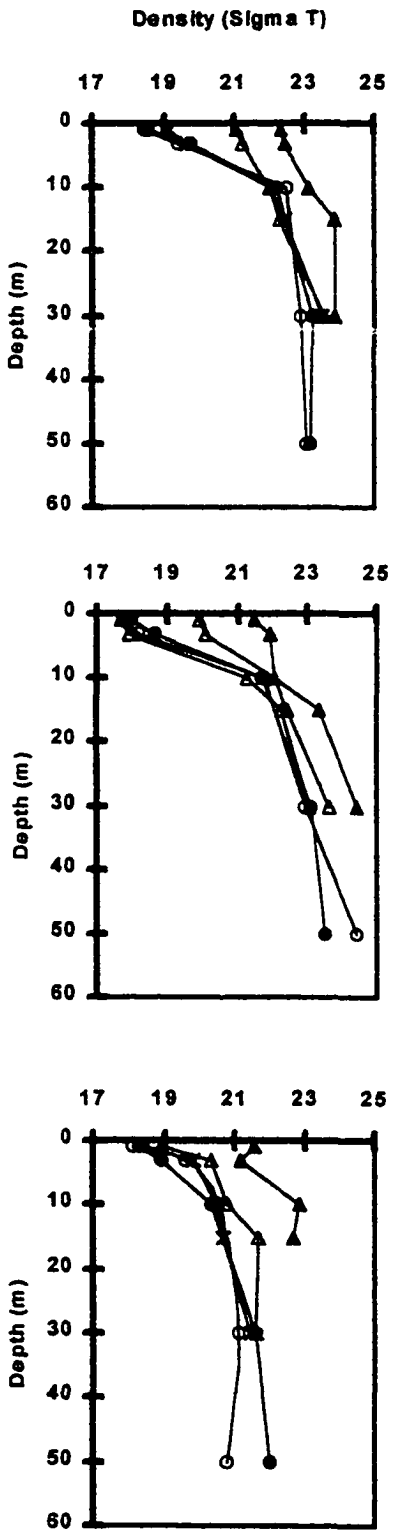
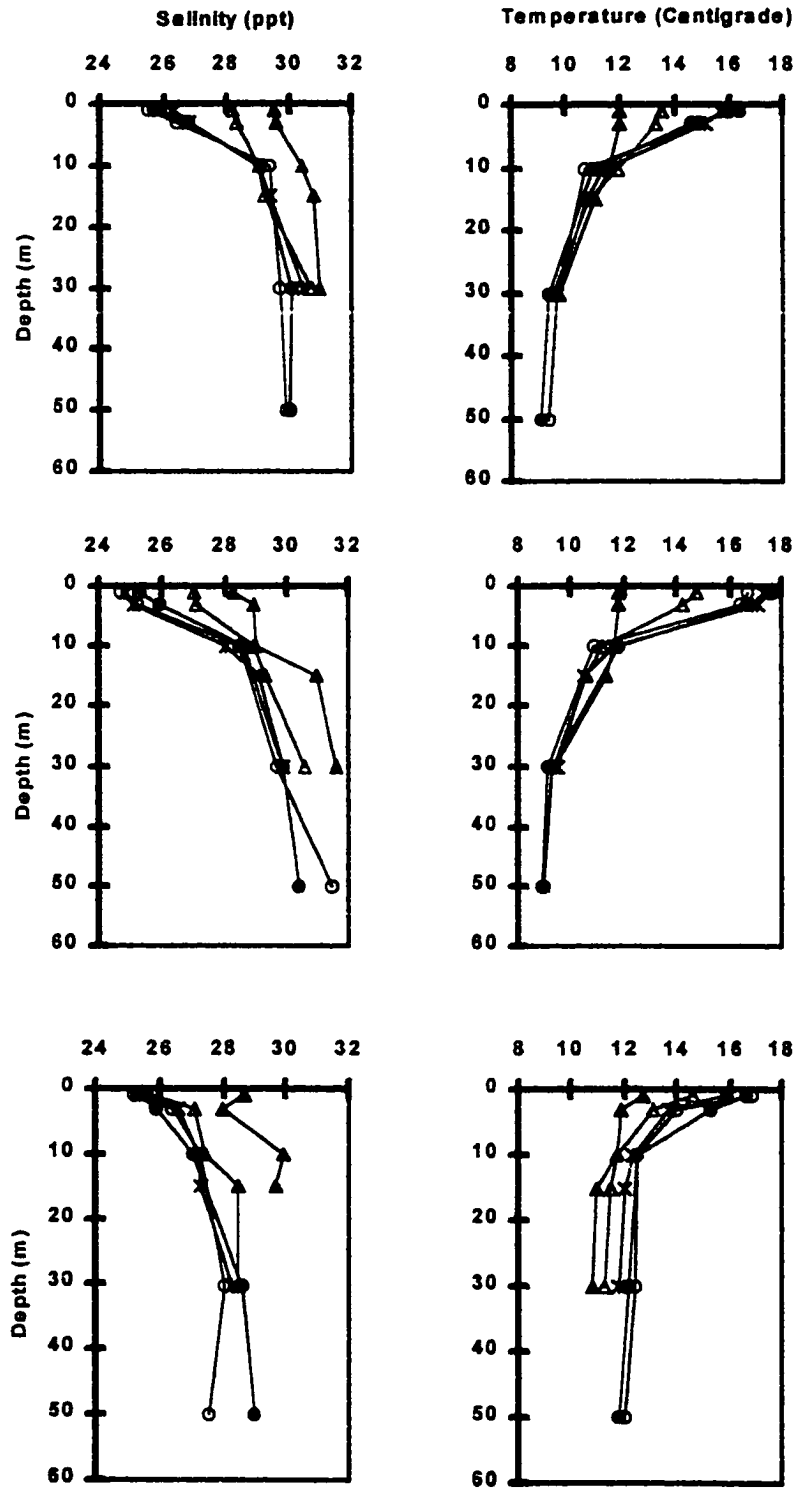

Density (Sigma T)
17
19
21
23
25
0
10
20
30
40
50
60
Depth (m)

Table III. Water column buoyancy

Buoyancy Fequency of Water Between 0 and 30 m (cm/sec * 10^{-3})			
	Malaspina St.	Jervis Inlet	Sechelt Inlet
September 6	2.862	3.053	2.443
September 7	2.788	3.110	2.312
September 11	2.730	3.157	2.304
September 18	1.980	2.477	2.159
October 4	1.500	2.122	1.291

Figure 14. Salinity and temperature profiles of the water column during the bloom. Malaspina Strait (top); Jervis Inlet (middle); and Sechelt Inlet (bottom).

Closed circles = September 7,
Open circles = September 8,
Asterisk = September 12,
Open triangles = September 19,
Closed triangles = October 5.


Salinity (ppt)
Temperature (Centigrade)
Depth (m)
24 26 28 30 32
8 10 12 14 16 18
0 10 20 30 40 50 60

3.1.4 Dissolved Inorganic Nitrogen (DIN)

Dissolved inorganic nitrogen is often separated into a new component (NO_2^-:NO_3^-) and a recycled component (NH_4^+). Generally the new DIN component was less abundant than NH_4^+ in Jervis and Sechelt Inlets at depths between the sea surface and 50 m (Figure 15). New nitrogen was undetectable in surface waters of all three water bodies on September 8 and the nutricline was between 1 and 3 m in Malaspina Strait. In contrast, the nutricline began at greater depth, between 3 and 30 m, in Jervis and Sechelt Inlets. Over the life of the bloom the position of the nutricline gradually shoaled such that by the end of the bloom significant levels of new N were found in the surface waters of all tested areas.

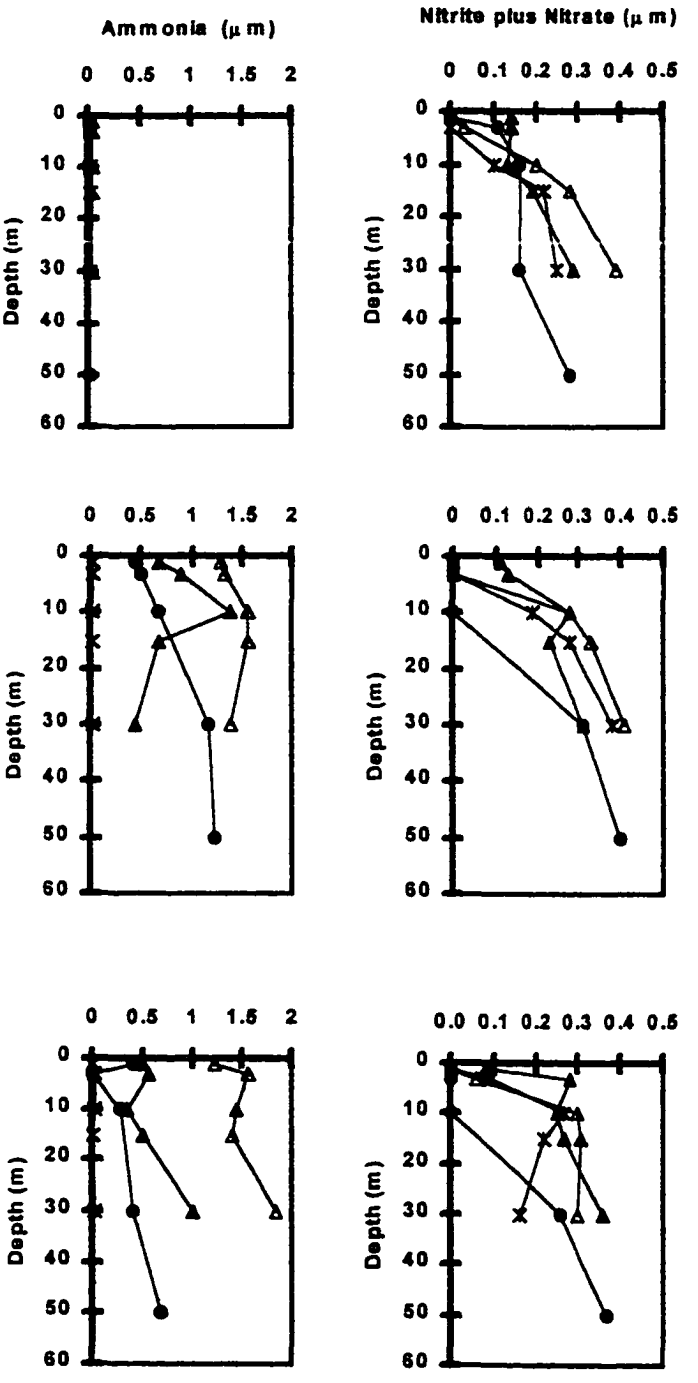
In contrast, re-cycled NH_4^+ was generally much more abundant in Jervis and Sechelt Inlets but not in Mapaspina Strait (Figure 15). However, on September 12, concentrations of NH_4^+-N in all samples taken in the two inlets was low, ranging between 0.005 and .0026 μM. Malaspina Strait values ranged between 0.01 and 0.03 μM at all depths and times. No pronounced nutricline was apparent for this nutrient.

3.1.5 Orthophospate Levels

The distribution of orthophosphate levels at all sites on September 8 demonstrate the presence of a marked nutricline. In Malaspina Strait the nutricline began at depths between 1 and 3 m, whereas in Jervis and Sechelt Inlets it began at 10 m (Figure 16).

Figure 15. Depth profiles of the concentration of ammonia, and combined nitrite-nitrate in: Malaspina Strait (top); Jervis Inlet (middle); and Sechelt Inlet (bottom).

Closed circles = September 7,
Open circles = September 8,
Asterisk = September 12,
Open triangles = September 19,
Closed triangles = October 5.


Ammonia (µm)
Nitrite plus Nitrate (µm)
Depth (m)
0 0.5 1 1.5 2
0 0.1 0.2 0.3 0.4 0.5
0.0 0.1 0.2 0.3 0.4 0.5
0 10 20 30 40 50 60

Lowest surface values occurred on September 19 and in all three water bodies. These varied between .003 to .008 μM. Generally the depth and strength of this nutricline decreased through the bloom.

3.1.6 The Balance of Nitrogen and Phosphorus

Interpretation of N:P ratios is complex. It requires not merely the N:P ratios in the water but some knowledge of the range of N:P ratios in the alga in question. Data from Lirdwitayaprasit *et al.* (1996) demonstrate the most extreme value (3.14) of the ratio of cellular N and P in *H. akashiwo* available in the literature. Based on that value, the surface waters of Malaspina Strait on September 8 and 12 lack adequate N (Figure 17). In contrast, deep waters on those dates and all depths on September 19 and October 5 lack adequate P. Surface waters of Jervis Inlet and Sechelt Inlet had sufficient N on September 8 but by September 12 it was reduced to insufficient levels. It then recovered to a surplus N status for the remainder of the bloom.

3.1.7 Oxygen Profiles

Oxygen saturation under conditions found in the inlet were generally between 9 and 10 mg/l. Concentrations in surface waters were high at all stations (figure 18), varying between 9.8 and 13.5 mg. In deep waters, oxygen levels varied between 5.1 and 6.7 mg/l with oxygen at 10 m (the depth of the bottom of fish cages in the area was 10-15 m) varying between 6.1 and 8.75 mg/l.

Figure 16. Depth profiles of the concentration of orthophosphate during the bloom in: Malaspina Strait (top); Jervis Inlet (middle); Sechelt Inlet (bottom).

Closed circles = September 7,
Open circles = September 8,
Asterisk =September 12,
Open triangles = September 19,
Closed triangles = October 5.

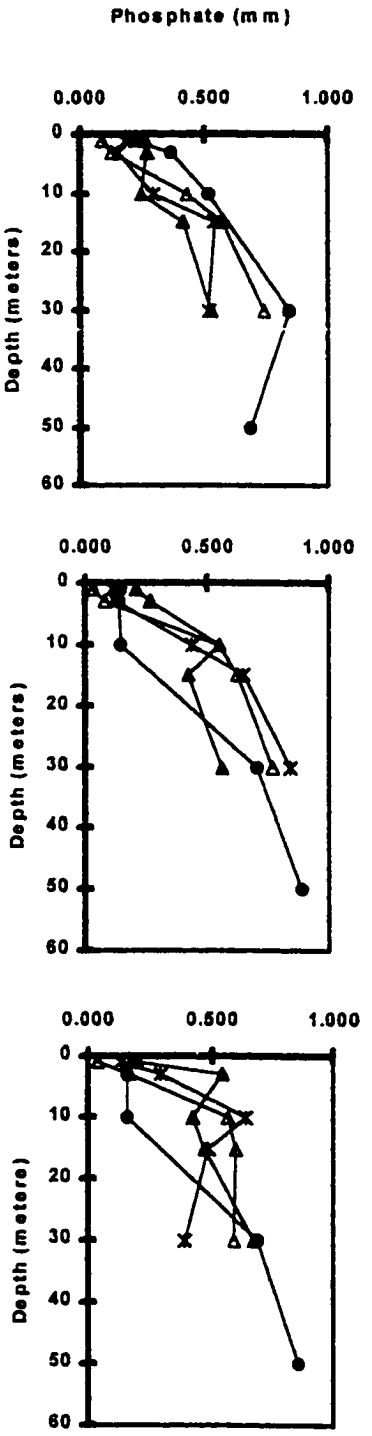

Phosphate (mm)
0.000
0.500
1.000
Depth (meters)
0
10
20
30
40
50
60

Figure 17. Depth profiles of the ratio of molar concentrations of dissolved inorganic nitrogen (N) to orthophosphate (P) in: Malaspina Strait(top); Jervis Inlet(middle); and Sechelt Inlet (bottom).

Solid circles = September 7,
Open circles = September 8,
Asterisk = September 12,
Open triangles = September 19,
Closed triangles = October 5.

The dashed vertical line represents the extreme of N:P ratios in *H. akashiwo* found in the literature.

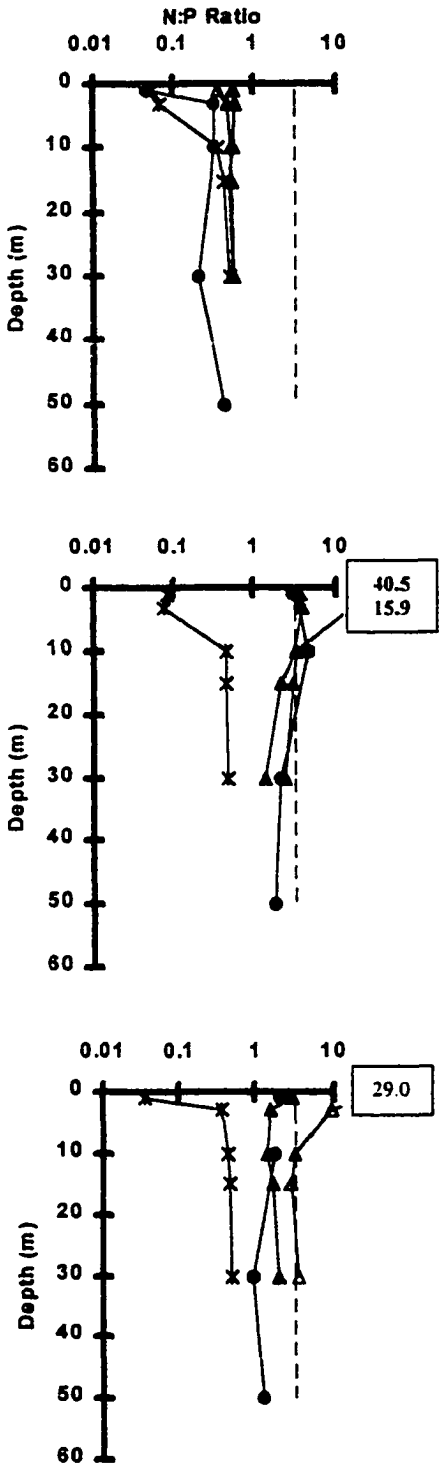
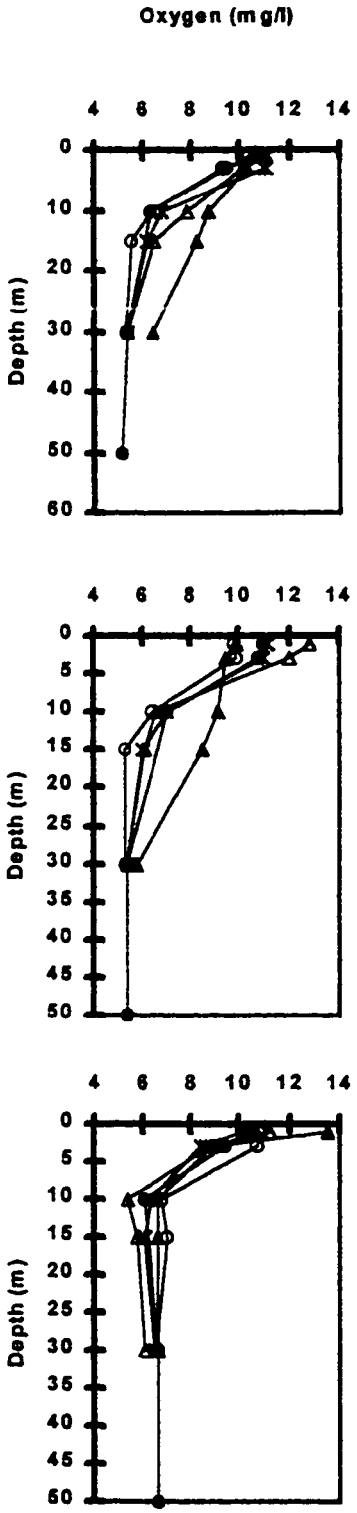

N:P Ratio
0.01
0.1
1
10
0
10
20
30
40
50
60
Depth (m)
40.5
15.9
29.0

Figure 18. Depth profiles of oxygen concentrations during the bloom in: Malaspina Strait (top); Jervis Inlet (middle); and Sechelt Inlet (bottom).

Closed circles = September 7,
Open circles = September 8,
Asterisk = September 12,
Open triangles = September 19,
Closed Triangles = October 5.


Oxygen (mg/l)
4 6 8 10 12 14
Depth (m)
0 10 20 30 40 50 60
4 6 8 10 12 14
Depth (m)
0 5 10 15 20 25 30 35 40 45 50
4 6 8 10 12 14
Depth (m)
0 5 10 15 20 25 30 35 40 45 50

3.1.8 Observations on cultured salmonids

Fish farmers in Agamemnon Channel and Jervis Inlet first noticed a change in colour of the local waters on August 28. The first fish mortalities were observed September 1 with losses being noted for approximately two weeks thereafter at various farms in Sechelt and Jervis Inlets.

Under normal operating conditions there is always some surface activity of fish in cages, which can be heard and seen at a distance of 10's of meters from the cage. Once the bloom entered the cage, surface activity ceased. Cage-cultured fish also tend to rise to the surface in anticipation of feeding when they detect activity on the floats supporting their cages. This occurred even in the presence of the red water. Farmers realizing that the bloom was densest near the surface minimized such behavior by ceasing to feed the fish and staying off the floats as much as possible. Some attempted to harvest stock before significant mortalities occurred.

Mortalities were detected in two ways. Some farmers had a net stretched tight across a stiff circular frame placed on the bottom of the cage. This "mort-ring" was then occasionally brought to the surface to determine if mortalities had occurred. Other farmers sent divers down once or twice per week to visually estimate the level of mortalities.

During the last week of the bloom and in spite of continued discolouration of the water, a number of farmers reported seeing their fish swimming at the surface of the cage throughout most of the daylight hours. The author observed the same phenomenon at sites in Agamemnon Channel. This occurred even when there was no activity on the cage systems. The fish did not appear to be in any distress despite the continuing presence of *H. akashiwo* in high densities.

The depth and perhaps the size of fish appeared to affect mortality rates experienced by the salmon farmers. Most sites had net cages that were 10 m deep. However, two sites in Agamemnon Channel had chinook salmon of comparable size (approximately 1200 gm per fish) maintained in cages that were 10 m and 12 m deep. At one site two 12 m nets containing 1200 gm chinook had mortality rates of 73.8 and 75.7 percent. Seven 10 m cages of the same sized fish had mortalities of 1.2, 1.1, 1.8. 1.5, 2.4, 2.1 and 1.6 percent. At a second site six 10 m deep cages had fish mortality rates of 64, 21, 19, 35, 40, and 38 percent (mean of 36%) while in another six 12 m deep nets fish had mortalities of 2.1, 2.4, 3.9, 2.2, 1.9, and 1.1 percent (mean of 2.3%). These cages contained chinook salmon approximately 1200 gm in size. Two other deep cages at this site contained chinook broodstock generally greater than 7.0 kg in size. These two deep nets had fish with mortality rates of 6.6, and 5.8 percent. The size dependent mortality appeared to be confirmed at two other sites in Agamemnon Channel. One farmer stated that most of his pens (number and depth not revealed) lost approximately 10% of the fish but broodstock pens lost 30%. Another farmer claimed 1+ year old fish suffered 30% mortality while 100% of brood stock was lost. Other farmers qualitatively reported that

large fish appeared to succumb more rapidly to the bloom. One farm located near a sill in Agamemnon Channel had no apparent relationship between fish size and mortality rates.

3.2 Bloom of July 1996

The flight path of the airplane from which the 1996 bloom was spotted, ran along the eastern coast of the Strait of Georgia, over Malaspina Strait across the mouth of Howe Sound into Vancouver. There were no clouds under the aircraft and no evidence of a bloom was seen in the northern portion of Georgia Strait or in Malaspina Strait. However, from the southern end of the Sunshine Coast across the mouth of Howe Sound and into Vancouver there was a broad band of brick red discolouration typical of a *H. akashiwo* bloom (a deep brown-red color) which grew more intense closer to shore (Figure 7). It was possible to see the seaward edge clearly. The northern extent of the bloom was in the clear waters of the Georgia Strait. As the extent of the bloom crossed the mouth of Howe Sound, the bloom appeared to overlay the light tan coloured plume waters of the Fraser River, and continue into the northern boundary of Vancouver Harbour. Observations from the shore suggested the bloom extended along the shore of North Vancouver to within a couple of kilometers of the Lions Gate Bridge.

Water temperature at a depth of 1 m was 19 °C and salinity was 24 ppt. Examination of sea water samples by microscopy confirmed that vegetative *H. akashiwo* cells were alive and motile at the start of the bioassay. Within two hours all cells in the sample had formed resting stages. Another examination of cells sampled directly from the

bloom determined these continued to be active vegetative cells. Counts of preserved cells gave concentrations of 432,100, 379,900, and 512,300 (mean of 431,300) cells per ml..

Mollies (*Pocelus mexicana*) and rainbow trout (*Oncorhynchus mykiss*) were used as bioassay agents to test the cells for toxicity (Black *et al.* 1991). The mollies used in the control container were 1.123, 0.646, 0.597, 0.440 and 0.435 gm (mean of .649 gm) in size. The sizes of the fish in the treatment were 1.006, 0.888, 0.669, 0.483, and 0.339 gm (mean 0.677 gm). The rainbow trout control fish were 61.9, 59.7, 51.8, 51.1, and 48.3 gm (mean of 54.5 gm) in size while the rainbow trout in the treatment container were 70.4, 65.4, 60.3, 57.0, and 45.2 gm (mean 59.7 gm) in size. None of the control or treatment fish died during the three hours of the bioassay. Wild fish of the species *Gasterosteus aculeatus* (three-spined stickleback) and *Cymatogaster agregatta* (shiner sea perch) were seen swimming in the surface waters near the experimental containers during the bioassay

4.0 Discussion of the Dynamics of Wild Populations

Population growth and toxicity in an algal bloom are not necessarily linked phenomena. Population growth can be constrained by any one of a number of factors including: light, temperature, predation, nutrients, hydrographic processes (turbulent mixing), and other factors. It is therefore not surprising that populations of potentially toxic algal species reach maximum density without detectable toxicity. When growth is

linked to toxicity, toxin expression appears to be mediated by the nutritional condition of the alga.

Any linkage between changes in available nutrients and the expression of toxicity must include a time lag. Uptake of nutrients, their incorporation into structural components of the cell, and the cells subsequent division occur sequentially, each requiring some time for completion. Watanabe *et al.* 1982 showed that it can take as much as four days for *H. akashiwo* to sequester its cellular quota of nitrogen.

Transformation of nutrients into cellular components is not a continuous process. In species which use vertical migration as part of their life history strategy, such as *H. akashiwo*, protein and carbohydrate synthesis are temporally separated. Protein synthesis occurs during the night when the cell is at depth while carbohydrate synthesis occurs during the day in the light-rich surface waters (Figueras and Fraga 1990, Figueras and Rios 1993, Watanabe *et al.* 1990).

Many algae, including *H. akashiwo* (Satoh and Fujii 1989, Takahashi and Hara 1989), engage in synchronous division, which occurs during the dark period subsequent to nutrient uptake. As a result, numerical expression of the nutritional experience of cells necessarily has a lag of about 24 hours before it can be expressed as an increase in cell numbers. Confirmation of the lag phenomenon is presented by Watanabe *et al.* (1982) who noted that cell division continues for more than a day after minimum concentrations of nitrogen and phosphorus occur in the culture media.

During the lag between nutrient uptake and numerical expression, flagellated cells can experience changes in water quality as their motility permits them to remain at the sea surface in spite of the replacement of their natal waters by down or upwelling currents. Therefore to understand the role of nutrition to a toxic bloom in nature, the nutritional experience of the alga before the expression of toxicity must be understood.

4.1 Origins of the Seed Population

First mortalities of caged fish on the Sunshine Coast (Agamemnon Channel, Jervis and Sechelt Inlets) occurred at or about the same time the abundance of *H. akashiwo* increased in that area. To understand the derivation of toxicity, it is necessary to examine the life of the cells prior to their arrival on the Sunshine Coast.

Yamochi (1984) showed the life cycle of *H. akashiwo* beginning in the winter with most of the cells dormant as resting stages in bottom sediments. In the spring, when waters warmed the sediments to 10 °C or more, the resting stages begin excyst and the newly-produced vegetative cells migrate to the sea surface. Tomas (1980b), examining *H. akashiwo* in Narragansett Bay found that temperatures below 10 °C discouraged effective excystment. Yamochi (1984) showed that Japanese populations reached maximum excystment rates when sediment temperatures reached approximately 16 °C.

In British Columbia, only shallow sediments are likely to reach these temperatures as most deep waters of the open coast and the Strait of Georgia never reach 10 °C (IOS 1989, Waldichuck 1957). Even in shallow areas, the overlying waters heat the sediments to maximum excystment rates for only a short period each year. Coastal surface waters of the Pacific coast of British Columbia generally vary between 10 and 15 °C in July or August (IOS 1989). The exception to this is the surface waters in the Strait of Georgia which are much warmer and can reach temperatures as high as 20 °C in the summer. Surface temperatures of 10 °C are observed in surface waters of the Strait in April or May, and temperatures of 16 °C are sometimes measured between June and August (IOS 1989).

The appearance of *H. akashiwo* in British Columbia waters generally conform to the pattern described by Yamochi (1984) where *H. akashiwo* was found in the water column as early as April (Table II). Haigh (1988) found *H. akashiwo* in the northern Strait of Georgia as early as March. However, in neither study was the species common or abundant at this time of year. In Narragansett Bay, populations start to increase in May when surface waters are about 10 °C, and increase to maximum abundance in June when water temperatures are approximately 15 °C (Tomas 1980b).

At Spanish Banks in Vancouver Harbour, Taylor and Haigh (1993) showed that in 1989, 1990 and 1991, *Heterosigma* increased its abundance dramatically at the end of May or beginning of June, just after the surface water temperature rose above 15 °C. They also showed that subsequent to excystment in Vancouver Harbour, concentrations of

H. akashiwo spread up the east side of the Strait of Georgia before spreading to the western side of the Strait.

Broad geographic sampling (Figure 2) indicated that *H. akashiwo* occurred in the Strait of Georgia in April 1989 (Table II). The excystment documented by Taylor and Haigh (1993) in the same year occurred in June , and could not account for that April pulse. The data in Haigh (1988) suggest another possible source of *H. akashiwo* in the Strait of Georgia. In April of 1989 the only large concentration of this species in the northern Strait occurred northeast of Savory Island. This beaches of this island may be the source of an excystment event that was responsible the early record of *H. akashiwo* in the strait in 1989. Savory Island (Figure 1) has a shallow, wide and 7 km long, muddy beach would be well suited to act as a source bed for *H. akashiwo*.

However, it appears likely that the source of cells for the toxic bloom on the Sunshine Coast in September 1989 originated in the area of Vancouver Harbour. Surface currents generally supply water to the area in front of Agamemnon Channel and Jervis Inlet from the waters off Vancouver Habour (Waldichuck 1957). The bloom first occurred in Agamemnon Channel to the south of Hardy Island and during less than one week, it appeared in Jervis Inlet to the north of the Island (Figure 3). Cell concentration decreased moving from the Strait of Georgia up Agamemnon Channel (Figure 4 & 5). Throughout the bloom event, flood tides in Agamemnon Channel tended to have higher cell concentrations than ebb tides (Figure 6). The bloom on the Sunshine Coast

terminated in early October when concentrations of *H. akashiwo* declined in Vancouver Harbour (Taylor and Haigh 1993).

4.2 Population Growth and Maintenance.

Waldichuck (1957) described many of the features of the oceanography of the Strait of Georgia salient to a discussion of the environment in which the 1989 bloom of *H. akashiwo* developed. Surface waters of the Strait south of Texada Island are dominated by the Fraser River plume, which is a major source of nutrients and adds buoyancy to the water column in this part of the Strait. Stratification inhibits mixing and convection between surface waters and deeper, nutrient-rich waters. During the bloom on the Sunshine Coast, the distribution of *H. akashiwo* was heavily focused in the upper part of the top layer of water. Most of the algae were in the top three meters of the water column with the highest density of algae at the surface (Figure 11).

Before discussing population growth of this species in the Strait of Georgia, the nutrient resources of the Strait and the alga's ability to access those nutrients needs to be addressed.

4.2.1 Nutrients

Associated with the isolation of the surface waters is a gradual decline in nutrients as the summer progresses. Harrison *et al.* (1983), in reviewing data nutrient availability in the Strait of Georgia, showed that nitrate levels in the winter surface waters were similar to those found in the deep waters of the Strait (about 25 μM). For most of the rest of the year nitrogenous nutrients were above levels limiting to algal growth, except for a short period at the end of summer when nitrogen may be limiting.

Ammonium ion is the preferred source of nitrogen for most phytoplankton because it is the form of inorganic nitrogen which has the least energetic cost for take-up and assimilation . It is generated as a product of the protein catabolism by zooplankton and excreted in the surface waters.

At the onset of the bloom on the Sunshine Coast at the site of the surface station in Malaspina Strait (Figure 3), and presumably in the southern Strait of Georgia, concentrations of NH_4^+ could limit population growth rates (Figure 15) with total dissolved inorganic nitrogen concentrations below 0.2 μM from a depth of 30 m to the sea surface. Most mid-latitude neritic phytoplankton are growth limited when sources of inorganic nitrogen or phosphorus drop below 0.4 to 2.0 μM (Eppley *et al.* 1969) and Tomas (1979c) demonstrated that *H. akashiwo* had a K_s (the half-saturation constant for nutrient uptake) for NH_4^+ of 2 μM.

In contrast to the generation of NH_4^+, NO_3^- is created by bacterial nitrification of NO_2^-. For phytoplankton to use NO_3^- (here after used to indicate the combined

concentration of NO_2^- and NO_3^-) they face not only slower uptake but also higher energetic costs. Tomas (1979) also demonstrated that the K_s for NO_3^- for *H. akashiwo* was also approximately 2 μM. Thus both NH_4^+ and NO_3^- may have limited the growth of this species (Figure 15) in the Strait of Georgia prior to the transport of the bloom to Agamemnon Channel and Jervis Inlet.

The bloom originated in waters in which NH_4^+ concentrations were very low, and the dominant source of inorganic nitrogen was NO_3^-, in contrast to Jervis and Sechelt Inlets in which the dominant supply of nitrogen was in the form of NH_4^+ at the beginning of the bloom (Figure 15).

Changes in nutrient levels indicated that as the algae entered Jervis Inlet (Figure 15), and moved from there into Sechelt Inlet (Figure 15), they initially drew down the concentration of NH_4^+ (September 8 to 12) to levels comparable to those in Malaspina Strait. At the same time, with the supply of NH_4^+ depleted, levels of NO_3^- continued to decline in the surface waters of the Strait.

As the bloom progressed, the relative amounts of NH_4^+ and NO_3^- in Jervis and Sechelt Inlets again shifted to a dominance of NH_4^+. Concentrations of NO_3^- in surface waters did not change but the concentration of NH_4^+ in surface waters increased (Figure 15) to levels closer (between 0.5 and 1.0 μM) to the value of K_s.

The cause of the increase in NH_4^+ between September 12 and 19[t] is problematic. The underlying waters were not responsible for the increased level of NH_4^+. Neither orthophosphate nor NO_3^- levels evidenced large changes until October 5 (Figure 15 & 16). Thus, changes in NH_4^+ cannot be the result of upwelling events resupplying NH_4^+ from the underlying sediments in the inlets. A reduction in the uptake by phytoplankton is unlikely to be the cause because the bloom is largely *H. akashiwo*, the concentration of which did not decrease to reduce the demand on the supply of NH_4^+.

The most likely origin of the increased NH_4^+ was a sudden increase in the abundance of the heterotroph community following a decline in that community caused by the advent of the *Heterosigma* bloom. This alga is known to produce bioactive substances that kill fish (Black *et al.* 1990, Kahn *et al.* 1997) and suppresses growth of bacteria (Tomas 1980b). There is evidence that the toxic bloom entering the Sunshine Coast was producing bioactive compounds, in that the appearance of the bloom coincided with mass fish mortalities. The toxin also may have initially had an antibacterial effect on the heterotrophic bacterial community in the waters of the Sunshine Coast. Antibacterial activity of phytoplankton is well known for diatoms (Aubert et al. 1968, Aubert and Gambarrotta 1972, Cooper and Battat 1983, Duff and Bruce 1966, Gauthier 1980, Gauthier et al. 1978, Kogure et al 1979, Oda *et al.* 1992, Viso et al. 1987), and flagellated phytoplankton (Austin and Day 1990, Austin et al. 1992, Burkholder et al. 1960, Cooper and Battat 1983, Viso et al. 1987). The combination of reduced bacterial activity plus the increased demand for ammonia by concentrations of *Haterosigma* would then account for the initial decline in ammonia concentration in the surface waters.

The antibacterial activity may have been transitory. When fish first experienced the bloom, few fish remained near the surface and any fish which did was in obvious distress, having difficulty in maintaining equilibrium and making only sporadic swimming efforts. In the latter half of the bloom, fish behavior returned to normal with fish swimming continuously at the surface without any obvious signs of distress. This would imply that the effectiveness of bioactive substances produced by *Heterosigma* had declined or disappeared later in the progression of the bloom event. If, at the same time antimicrobial effect disappeared, new heterotrophic activity on organisms killed by *Heterosigma* would result in a sudden increase in production of ammonia. This would increase concentrations of ammonia until the *Nitrosomas/Nitrobacter* community and simular NO_3^- producing bacteria increased in abundance in response to the elevated availability of ammonia. The consequence of this shift would be a repeated, though less dramatic, reduction in the abundance of ammonia while the abundance of nitrate would increase.

The dynamics of NO_3^- would be further affected by changes in the effectiveness of stratification in isolating surface waters from the waters under them. When NH_4^- concentrations declined, the primary source of any increase in NO_3^- in the surface waters would be from diffusion and localized mixing of deeper water with surface waters. As the pycnocline decreased in intensity after September 12 such mixing events were more likely to occur, and with increasing frequency. This would also have added NO_3^- to the surface waters.

While nitrogen is generally presumed to be a limiting nutrient in marine waters, orthophosphate also appears to be growth limiting in the waters examined. Tomas (1979) demonstrated that orthophosphate became a growth limiting factor at concentrations below 1-2 μM. All water samples taken during this study had orthophosphate concentrations less than 1.0 μM. Samples from 30 m or deeper had concentrations between 0.5 and 1.0 μM, surface samples ranged from 0.0 to 0.25 μM.

Orthophosphate concentrations in the surface waters of Malaspina Strait declined from September 8 to September 19, then rebounded by October 5, as the pycnocline broke down and nutrient-rich deep waters move towards the surface. The same general pattern occurred in the waters of Jervis and Sechelt Inlets.

With both N and P in concentrations low enough to be growth limiting, it may be important to try to determine which controls growth. The relative amounts of nutrients are believed to have a role in both biotoxin production (Boyer *et al.* 1987) and the selection of the dominant toxic algal species (Smayda 1990).

The classic way of evaluating the relative abundance of nutrients is to compare the ratio of the cell quota of various nutrients in the phytoplankton to the ratio of those nutrients in the environment. The Redfield ratio in part describes the ratio of nitrogen and phosphorus found generally in particulate matter in oceanic waters as 16:1 (Goldman *et al.* 1979). This is commonly used as the set point from which to evaluate the relative

abundance of these nutrients. Those same authors suggest that values for phytoplankton from 10:1 to 20:1 should be considered to conform to this ratio. There is data on the chemical composition of phytoplankton in both productive and unproductive waters to support this approach (Goldman *et al.* 1979, Strickland *et al.* 1969 and Rhee and Gothman 1981).

The rational for translating these cellular nutrient ratios into an indicator of aqueous N or P limitation does have some weaknesses. Goldman *et al.* (1979) point out that it can be deceptive to evaluate nutrient levels in natural systems based on this criterion. In natural systems phosphorus is being recycled much faster than nitrogen. This implies that at values much higher than 15:1 nitrogen would still be the limiting nutrient. Goldman *et al.* (1979) used nutrient ratios of 50:1 or greater to indicate phosphorus limitation and ratios of 15:1 or less to indicate nitrogen limitation.

A further weakness of this approach however is that the original Redfield ratio appears to be based on nutrients stored in healthy cells (exponential growth phase) (Goldman 1979). Such an assumption may have value for some phytoplankton species even under conditions of nutrient limitation. However, luxury consumption of nutrients by species which vertically migrate could confound such analyses. It becomes very difficult to determine what is a cell with a balanced proportion of nutrients. Cells in exponential growth could easily have accumulated much higher levels of phosphate than are required for "balanced" nutrient reserves. Thus there is a potential bias in the opposite

direction to that implicit in the analysis of nutrient composition based solely on the Redfield ratio.

For these reasons, rather than using the value of less than 15:1 to represent a nitrogen-limited environment, it is perhaps best to determine the most extreme N:P ratio in growth-limited N starved cells. Thus, N:P ratios markedly below this level may be indicative of growth limitation by nitrogen.

A number of authors have presented data on the N:P ratio within nutrient-limited *H. akashiwo*. Watanabe *et al.* (1982) found a ratio of about 15.2:1, which is close to the Redfield ratio. Others have determined relatively higher amounts of phosphorus in nutrient-limited cells. Data presented in Lirdwitayaprasit *et al.* (1996) suggests that the ratio may be as low as 3.15:1 in nutrient-limited cells.

Examination of nitrogen to phosphorus ratios in the waters of the study area suggest that nutrient imbalance in the Strait of Georgia where the bloom originated, were more extreme (with nitrogen limiting) than in Jervis and Sechelt Inlets, though the advent of the bloom in these areas caused a temporary move to similar conditions. The N:P ratios in the waters of the Strait of Georgia were at all times below 1:1 (Figure 17). At the start of the bloom on the Sunshine Coast the ratio was below 0.1:1 and remained so until after September 12. In contrast, ratios in the waters of Jervis and Sechelt Inlets were above 1:1 on September 8 and only dropped below 0.1:1 on September 12 and thereafter were in excess of 1:1.

In summary, while both nitrogen and phosphorus could limit growth of this species, nitrogen appears to be the more likely limiting factor based on its low concentration and the low N:P ratios in near surface waters. Further, toxic cells may affect ambient concentrations of nitrogen not only through consumption but also through negative effects of toxins on the bacterial component of the plankton community.

4.2.2 Vertical Migration

To survive and grow in a nutrient-depleted environment, phytoplankton have evolved strategies which either improve nutrient uptake rates at low concentrations, or permit access to new sources of nutrients. One strategy is evidenced in the seasonal shift in species composition. During the spring transition from an unstratified to a stratified water column structure, there is also a shift from large non-motile diatoms to flagellated cells. Many flagellate phytoplankton have the advantage of luxury consumption and storage of nutrients plus the ability to migrate between nutrient-rich but light-limited waters beneath the pycnocline where they can obtain scarce nutrients and the light-rich but nutrient-depleted surface waters where they process stored nutrients into useable cellular products.

H. akashiwo can migrate vertically (Yamochi and Abe 1984, Watanabe *et al.* 1988, MacKenzie 1991) and accumulate nutrients at depth in the dark and return to the surface to photosynthetically utilize those nutrients during the day (Watanabe *et al.* 1988). The

question then is, can this species migrate all the way to the depth at which nutrients become available?

Hershberger *et al.* (1997) suggested that the presence of a layer of freshwater overlaying more saline waters triggered cessation of vertical migration. In June, when the resting stages of *H. akashiwo* excyst, the Strait of Georgia is well-stratified and the Fraser River is approaching its maximum freshet (Harrison *et al.* 1983). The probability that *Heterosigma* would encounter a freshwater layer in the Strait of Georgia would seem greatest at this time. No data was available to comment on the relevance of this to the 1989 toxic bloom however, it was not till almost September that the toxic bloom was noted. As the Strait is well populated and had fish farms operating all around its' periphery at that time (B.C. Ministry of Fisheries, Pers. comm.), it is unlikely a toxic bloom would have gone unnoticed for several months. If, as will be discussed later, lack of nutrients is integral to the development of a toxic bloom it must be said that it does not seem likely that it was critical to the development of this bloom.

An alternate explanation might reside in the development of stratification of the water column during the summer months. By the beginning of September the freshet is almost finished and the out-flow volume of the Fraser is nearing its winter low. Between June and September surface water temperatures generally increase and the depth of the pycnocline gradually increases to approximately 10 m depth (Waldichuck 1957). Data in this study show that at the time of the bloom on the Sunshine Coast in 1989 the pycnocline depth was between 5 and 10 m deep.

Laboratory studies have shown that *Heterosigma akashiwo can* migrate in the water column at speeds of up to approximately 1 m/hr (Gaines and Taylor 1989, Satoh and Fujii 1989, Takahashi and Hara 1989). As migration is linked to light conditions experienced by cells (Takahashi and Hara 1989) the maximum depth it can achieve is limited by the length of dark hours. If we generously allow that there may be 10 hours of darkness during which the cells have to migrate down to a maximum depth and return to the euphotic zone, at the extreme the cells would be able to migrate through approximately 5 m of depth. Toxic blooms of this species occur in the Seto Inland Sea of Japan where stratification is typically between 5 and 10 m (Watanabe et al. 1989).

There is however, reason to believe that the 5 m depth limit is not an absolute limit to migration by this species. Maximum migration depths as deep as 10 m have been documented by MacKenzie (1991) during a bloom of *H. akashiwo* in New Zealand. In that study, sunset was at 20:42 hr. and sunrise was at 05:10 hr. The migration to the surface took 6.5 hours between 04:00 hr. and 10:30 hr. Migration rates documented in the laboratory can not account for such distances being covered in such a limited time.

Micro-currents within the stratified layer can potentially augment migration rates. As the sun sets, warm surface waters cool faster than the waters underlying them due to evaporation and back radiation. With decreased temperature and consequent increase in density the water sinks and creates convection currents which might increase the rate of descent of *H. akashiwo* through the water column.

To create such a current, the difference between the temperature in the cooled surface and warmer underlying water must be great enough to overcome any density gradient engendered by differences in salinity between the surface and deeper waters. MacKenzie's (1991) data suggest that salinity differences were no more than 0.6 ppt , which would only require a cooling of just over 3 °C to overcome the effect of salinity on density. Unfortunately, coincident temperature data were not obtained in that study. However, it is noteworthy that their data show that the salinity gradient present during daylight hours all but disappears during the night, suggesting microcurrent mixing occurred.

If the rate of descent is augmented, the rate of ascent may also require an increase for the migration to be completed within a comparable period. During the dark period, *H. akashiwo* is known to accumulate fatty particles (Wada *et al.* 1985, Hara *et al.* 1985, Wada *et al.* 1987) composed principally of triacylglycerol (Wada *et al.* 1987). These fatty particles are assimilated during daylight. The development of these neutral fats in the cell would reduce its density and, as other authors have commented (Wada *et al.* 1985, 1987), can be expected to facilitate upward vertical migration.

Migration however, is not a constant feature of this species. Lack of vertical migration was apparent in the 1989 bloom (figure 12) and its lack has previously been reported in the literature. Nagasaki *et al.* (1996) noted that vertical migration ceases as

cells become smaller and Aizdaicher (1993) found migration ceased before cells formed resting cysts.

Some mechanisms which result in cessation of migration have been documented. Experiments have also shown that continuous dark will disrupt the migration pattern resulting in an even distribution of the cells throughout the water column (Hatano *et al.* 1983, Takahashi and Hara 1989). Takahashi and Hara (1989) demonstrated that light levels required for initiation of the migratory pattern are approximately the same as those required for cell division (i.e. minimum compensatory light levels).

At the very high cell concentrations seen in blooms associated with fish mortality, there is considerable opportunity for self-shading by cells. Secchi disc readings during a number of these blooms have been less than 1 m (Mackenzie 1991, Black *et al.* 1991) suggesting a light compensation depth of approximately 2 m. However as the majority of the cells in the bloom under discussion are concentrated in the top 3 m, it seems unlikely that cells are light limited.

The effect of nutrients on migration is equally dramatic. Hatano *et al.* (1983) demonstrated that before cessation of population growth in nitrogen-limited cultures *Heterosigma akashiwo* stopped migrating. They also demonstrated that the time to re-initiation of migration depends on how long the cells had endured nutrient limitation. Cells that were nitrogen limited for 10 days could recover vertical migration the same day new nutrients were supplied. Cells that had been starved for 28 days required one day and

cells that had been starved for 42 days required 4 days to reinitiate vertical migration. For the entire 27 days of this study (Figure 15) and presumably for some period before that bloom cells from the Strait of Georgia were nitrogen starved. Nitrogen limitation is therefore the likely cause of suppression of migratory behaviour of the bloom organism when it was in the Jervis Inlet/Agamemnon Channel area.

4.2.3 Population Growth in the Strait of Georgia

H. akashiwo may have been present for as much as 5 months prior to the appearance of the toxic form on the Sunshine Coast in 1989. It was first observed in April when it occurred in three widely separated locations in the Strait of Georgia. In May and June, the alga was absent from samples despite the fact that data from Taylor and Haigh (1993) clearly demonstrated that there was a supply of further excysting cells to enhance the populations in the Strait of Georgia during that period.

Cells reappeared in samples during July, and occurred in more than half the samples in August and September, and approximately a third of the samples in October. The toxicity of the population became apparent in the last few days of August.

This seasonal bimodal occurrence of *H. akashiwo* is a common feature of other populations of this alga. For example Tomas (1980b) found that *H. akashiwo* had a bimodal annual distribution of abundance in Narragansett Bay for most years of 17

years. The initial peak of abundance was in May-June and the other in September-October.

In Narragansett Bay however, the initial peak of *H. akashiwo* can also occur in April-May. Tomas(1980b) suggested that the initial peak in abundance is driven by timing of excystment when nitrogen levels in surface waters are limiting and phosphate is in the range of 0.5-1.0 μm. No information was given on the depth of the pycnocline, though surface water temperature increased from this time until August, which suggests that the stratification remained, and the pycnocline deepened over this period.

Tomas (1980b) however, had difficulty in explaining the reason for the summer decline in abundance of *H. akashiwo*. In contrast to the situation in British Columbia, the summer decline in abundance of *H. akashiwo* in Narragansett Bay occurred during a period of increasing nutrient abundance (nitrate levels during this period reached approximately 6 μM and phosphorus attained its yearly maximum of 2-3 μM.). This co-occurred with declining abundance of herbivorous zooplankton and increasing abundance of diatoms. During the summer decline of *H. akashiwo* in the Strait of Georgia, nutrient levels in the surface waters declined and the pycnocline became progressively deeper. The fall peak in abundance of *H. akashiwo* may represent the net outcome of continual cell division and release from predation associated with the development of toxicity.

The bioactivity/toxicity of *H. akashiwo* is one of the broadest seen among phytoplankton. It has been shown in laboratory studies to be effective against a wide

spectrum of bacteria, fungi, and invertebrate species which might either compete for nutrients in the nutrient-depleted surface waters or prey upon cells (Table IV). Field studies confirm many of these observations. The late summer (October-November) peak of *H. akashiwo* is associated with the decline of *Skeletonema costatum* cell numbers and the decline of zooplankton biomass in Narragansett Bay (Tomas 1980b, Pratt 1966). Other field studies have shown that there is an inverse relation between the abundance of tintinnid species (*Tintinnopsis tubulosoides* and *Favella* sp.) and the abundance of *H. akashiwo* (Verity and Stoecker 1982).

In addition, *H. akashiwo* has been shown to be responsible for the mortality of a broad spectrum of fish species in culture. The only bioassay work done on naturally occurring blooms has demonstrated that cell counts of 200,000 to 800,000 cells/ml will kill all fish in a bioassay in time between 40 and 200 minutes (Black *et al.* 1991). But not all blooms of *H. akashiwo* are necessarily toxic as demonstrated by the bioassay work done in the 1996 bloom in Vancouver (Figure 7). There, cell concentrations in excess of 400,000 cells/ml failed to kill any fish in over 210 minutes.

4.3 Bloom Termination

There are potentially both exogenous and endogenous causes of bloom termination. Exogenous mechanisms include a shift in physical or chemical environments

Table IV. Organisms against which *Heterosigma akashiwo* has demonstrated toxicity.

Species	Effect	Reference
Bacteria		
- Undefined sp.	Growth suppression	Tomas 1980b
Fungus		
- *Aspergillus niger*	Growth suppression	Nagari et al. 1990
Algae		
- *Skeletonema costatum*	Growth suppression	Tomas 1980b, Pratt 1966 Honjo 1986, Stuart 1972
- *Chaetoceros* sp.	Growth suppression	Honjo 1978
- *Thalassiosira* sp.	Growth suppression	Honjo 1978
Tintinids		
- *Tintinnopsis tubulosoides*	Feeding suppression	Verity and Stoecker 1982
- *Favella* sp	Feeding suppression	Verity and Stoecker 1982
Rotifers		
- *Brachionus plicatilis*	Feeding suppression	Chotiyaputta and Hirayama 1978
Copepods		
- *Pseudodiaptomus marinus*	Feeding suppression	Uye and Takamatsu 1998
- *Arcatia omorii*	Feeding suppression	Uye and Takamatsu 1998
- *Acartia hudsonica*	Feeding suppression	Tomas and Deason 1981
- *Acartia tonsa*	Feeding suppression	Tomas and Deason 1981
Crustacea		
- *Artemia salina*	Mortality	This Study
Bivalves		
- *Mytilus edulis*	Feeding suppression	Ward and Targett 1989
- *Crassostrea gigas*	Feeding suppression	Welling 1982
Fish		
- *Oncorhynchus nerka*	Mortality	Yang et al. 1993
- *Oncorhyncus tshawytscha*	Mortality	Boustead et al. 1989, Black 1990, 1991
- *Oncorhyncus kisutch*	Mortality	Black 1990, 1991
- *Salmo salar*	Mortality	Black 1990, 1991
- *Oncorhyncus mykiss*	Mortality	Black et al. 1991
- *Seriola quinqueradiata*	Mortality	Khan et al. 1997, Honjo 1993
- *Centropristis striata*	Mortality	Khan et al. 1997
- *Cantharus cantharus*	Mortality	Kahn et al. 1997
- *Pagrus major*	Mortality	Kahn et al. 1997
- *Pocelus mexicana*	Mortality	This study

beyond the tolerances of the alga or viral infection, and predation. In contrast, cellular senescence or encystment brought on by an internal cellular mechanism such as endogenous rhythms may be responsible for the termination of some blooms.

The bloom that was observed on the Sunshine Coast ended between 26 and 28 September (Figure 8). Between September 19 and October 5, surface water temperatures dropped from around 14 to 12 °C in Malaspina Strait, and from approximately 15 to 12 °C in Agamemnon Channel. These temperatures are well within the range at which natural populations of this species occur. Populations of motile vegetative cells have been found at temperatures as low as 3-4 °C in Narragansett Bay (Tomas1980b) and Norwegian coastal waters (Throndsen 1969). Also significant growth is seen at temperatures above 10 °C in culture (Tomas1978a) with optimal growth typically occurring at temperatures between 15 and 25 °C (Watanabe *et al.* 1982). Further, the subsequent re-occurrence of the species in bloom concentrations approximately two weeks later in the middle of October suggested that neither declining temperature nor light were the proximal cause of the bloom's termination.

Viral infection (Nagasaki *et al.* 1995, Itakura *et al.* 1996b) and predation (Tomas1980b) have been responsible for the demise of some blooms. However, it is clear that these mechanisms are not universal or reliable predictors of bloom termination (Nagasaki *et al.* 1996, Tomas1980b). In the case of the bloom on the Sunshine Coast, while no quantitative data were taken, surface water samples taken throughout this study were almost continuously monospecific for *H. akashiwo* except prior to the occurrence of

the bloom in Agamemnon Channel. The few zooplankton seen at the end of the study did not occur until the collapse of the bloom was already well initiated. No direct comment can be made on the role of viruses in the termination of the 1989 bloom, yet it is difficult to see why a viral infection would appear and disappear from the parent population in the Strait of Georgia within two weeks.

Among other causes for bloom termination are nutrient depletion and co-occurring cyst formation. *H. akashiwo* is known to produce cysts or resting stages (Imai *et al.* 1993, Tomas1980b) at the end of its life cycle. Reviews of resting cyst formation in dinoflagellates (Dale 1983, Pfiester and Anderson 1987) and diatoms (McQuoid and Hobson 1996) consider lack of nutrients to be a primary environmental trigger for cyst (resting stage) formation.

From an evolutionary point of view, it would seem a reasonable strategy for a species to use encystment to escape periods of nutrient starvation. In a species like *H. akashiwo*, which requires a shallow area to successfully perform excystment (as discussed earlier), this would make particular sense if the resting or encysted cells were nearly neutrally buoyant, and could subsequently float to a littoral area where future light and nutrient conditions might prove favorable to excystment and regrowth.

The resting stages of *H. akashiwo* however are negatively buoyant (Tomas1978b). As such, formation of resting stages would in most instances result in the cells sedimenting in deep waters where it would be unlikely that water temperatures would reach the critical

level required for excystment. The explanation of bloom termination through the formation of *H. akashiwo* cysts due to a lack of nutrients also does not fit the pattern seen in recorded blooms. During the termination of natural blooms of *H. akashiwo* in Naragansett Bay (Tomas1980b), Nagasaki Bay (Nagasaki *et al.* 1996) and Hiroshima Bay (Itakura 1996a), nutrient levels were rising well above levels at which nutrients would be limiting. In this study, nutrient levels appeared to be adequate to support continued population growth while nutrient levels were increasing. In all these instances water temperatures were well above those which are associated with forced encystment.

Observation of the 1995 bloom in Vancouver Harbour suggests a possible explanation for bloom termination, which will be discussed further in a later section. In he confluence of marine and riverine waters. which presumably had some nutrients after their exit from the mouth of the Fraser River, *H. akashiwo* cells appeared to be rapidly encysting. Cells went from their vegetative form to fully formed resting cysts in a matter of an hour or two. Thus, I suggest that an increase in nutrient concentration after a period of nutrient starvation might trigger resting cysts formation. Such a mechanism would enhance the survival potential of encysted vegetative cells.

As seen earlier in this section, *H. akashiwo* is most abundant in the stratified and nutrient depleted surface waters of late summer. Rivers emptying in to this environment are likely to result in a localized increase of nutrients in the surface waters of the estuary. Under these conditions were *Heterosigma* to use the sudden increase in nutrients as an environmental trigger for encystment, it would have a far greater chance of sedimenting

out in an estuarine environment. Encystment in an estuary would increase the likelihood of the cysts being deposited in shallow environment. While much of the waters overlying deep waters in the Strait of Georgia are unlikely to experience temperatures that would encourage excystment, shallow areas such as those in river deltas are more likely to reliably reach optimal excystment temperatures.

Having examined the natural environment in which toxic and non-toxic blooms of *Heterosigma akashiwo* occur the test of our knowledge is to see if similar conditions (depletion and addition of nutrients) create the same effects on development of toxicity and encystment of cells in laboratory cultures.

5.0 Methods for Studies of Cultured Populations

5.1 Culture Environment

Cultures were grown in sea water after it had been passed through a .45 μm Millipore ™ filter. Nutrients used were as published in Harrison et al. (1980). Cultures were conducted in 6-l round bottom flasks and 6-l plastic bags held in a rectangular chicken wire mesh frame 40 cm wide by 3 cm deep by 45 cm high. The algae were illuminated with Cool-White fluorescent ™ bulbs supplying $2.5 * 10^{16}$ quanta/sec/cm^2 as measured with a Biospherical Instrument Inc. Model QSL-100. Cultures were maintained at a constant temperature of 20 ± 1 °C on a 16/8 light/dark cycle.

5.2 Enumeration

Cell concentrations and sizes were determined using a Coulter TA II particle counter. In this machine, size measurement is based on the varying degree to which cells of different sizes obstruct electrolyte passing through an aperture. With a single electrode on each side of the aperture, the electrolyte acts as an electrical conduit and changes in resistance between the electrodes occur in proportion to the degree to which the cross sectional area of the aperture is reduced as the cells pass through it. The cyclonic flow of medium passing through the aperture tends to align particles with their longest dimension perpendicular to the aperture. In effect the measurement made is of total cross-sectional area obstructing the orifice and is less sensitive to changes in shape than to changes in

total cross-sectional area. Each increase in size discussed in this study (small-medium and medium-large) represents a doubling of the cross sectional area of the cell.

Cell shape was determined by light microscopy. Under 500 to 1000 X magnification, using bright-field or phase-contrast illumination, over 100 cells were examined, enumerated and placed in each of 4 cell-shape categories or morphs. Cell shape was classified as smooth, bumpy, 1-2 protuberances or 2+ protuberances. These categories were defined as:

Smooth - cells whose surfaces lacked either bumps or protuberances. This category included both the classic bean-shaped vegetative cell and the spherical resting stage (Tomas1978b).

Bumpy - cells which have surface protrusions which are shorter than they are wide at the base.

1-2 Protrusions - cells which have 1 or 2 surface protrusions whose length is greater than the width of the protrusion at its base.

2+ Protrusions - cells which have more than 2 surface protrusions whose length is greater than the width of the protrusion at its base.

5.3 Culture Manipulation

To determine if cell concentrations at stationary phase were nutrient limited or light limited by self shading, four 5-l containers were inoculated with approximately $5*10^6$ cells. Cultures were then grown to stationary phase in sea water plus nutrients (Harrison *et al.* 1980). On day 16, while the cultures were in stationary phase, two of the four cultures were again fertilized as on day 0. All cultures were documented through a further 12 days of growth. Changes in cell size were enumerated. Changes in cell shape were noted.

Three 5-l cultures were used to document changes in cell shape and size under different conditions of light and agitation. All cultures were supplied with a gentle stream of filtered air and inoculated with $1.3 * 10^7$ cells. Two of the cultures were in round (spherical) 6-l flasks. One of these cultures had the air stream flow over the surface of the culture (a culture without agitation), while the other had the air released near the bottom of the culture flask which rose as a continuous stream of bubbles to the culture's surface (the agitated culture). These were used to determine the effect of agitation on cultures. The third culture was contained in a clear plastic bag held laterally compressed in a rectangular wire basket. The bag culture was mounted upright and illuminated from the side so light penetrated the minimum amount of culture. The air supply was delivered above the surface of the culture as in the unagitated culture. The effect of light on culture development was determined by comparing the round unagitated flask culture (24 cm

diameter) with the culture in a rectangular, wire basket-mounted bag (3 cm thick). During sampling of the unagitated round flask cultures, length measurements were made by microscopy to determine mean cell length of each of the morphological forms of the species.

The effect of nutrient dose on size and shape differentiation was examined by sequential dosing triplicate cultures. Three 5 l bag cultures were each inoculated with $3.5*10^7$ cells and half the nutrient dosage recommended by Harrison *et al.*(1980). In this and all subsequent cultures air was release above the surface of the culture as before. The cell concentrations in the cultures were allowed to grow and then decline until cell concentrations were approximately the same concentration as that when cultures were originally inoculated. At that time the cultures were re-fertilized with the full dose of nutrients recommended by Harrison *et al.*(1980) and the cultures were again allowed to grow until cell concentrations began to decline.

5.4 Bioassays

Trout and brine shrimp (*Artemia salina*) bioassays were done in cultures taken from round bottom flasks. Most molly bioassays were done in algal cultures from laterally compressed bags. The exception was the use of mollies in conjunction with trout bioassays. All cultures were allowed to cool to room temperature (18 °C) prior to beginning the bioassay.

The first bioassays used trout, brine shrimp and mollies that had been maintained in room temperature sea water for at least 4 hours prior to bioassay. Brine shrimp were raised under incandescent lamps in 21 °C filtered sea water and were used within 48 hours of hatching to avoid any complications which might occur through the initiation of feeding by the shrimp. The complete brine shrimp bioassay methodology is in Vadnais (1995).

Trout bioassays were done to determine the effect of the addition of new nutrients on toxin expression. The inoculum of $1.47*10^8$ cells was put in each of three 5-l bag cultures and grown until protuberant forms were common in cultures. All three cultures were then assayed for toxicity, and then one of the cultures was re-inoculated with nutrients. Four days later the re-inoculated culture and one of the other cultures were again assayed. Twenty-six days later the remaining culture which had not been re-fertilized was assayed. Filtered sea water was used as a control medium in all bioassays.

When re-fertilization occurred the unused portion of the toxic culture used for the bioassay was divided into three new assay containers and diluted with filtered sea water by factors of 10, 100 and 1000 respectively. Trout bioassays were performed on each of these dilutions. For subsequent molly bioassays, bag cultures of *H. akashiwo* were grown until all described cell forms were apparent in culture.

The effect of culture volume on the assay was examined using 250, 350, and 500 ml of culture placed in three 500 ml beakers. Two 1000 ml beakers received 750 ml and 1000 ml of culture. A fourth 500 ml beaker received 250 ml of filtered sea water as a control for the bioassay. Five mollies of approximately the same size were place in each beaker and time to death was recorded for each fish. Death was defined by a lack of movement by the gill operculum and subsequent failure to show any response when the fish were prodded with a glass rod.

To examine the effect of nutrient dose on toxicity, 5-l bag cultures were started in: filtered sea water; sea water plus half the nutrient dose recommended by Harrison *et al.* (1980); and, sea water plus the full recommended dose of nutrients. The donor culture for the inoculum had declining cell concentrations suggesting that nutrients might be limiting cell growth. On day 8 one liter of the inoculum ($1.19 * 10^8$ cells) was placed in each of 3 new bags along with 4 liters of filtered sea water. Each bag was then given either none, half or a full dose of the nutrients recommended by Harrison *et al.* (1980). Subsequently at day 12, 20 and 28, 500 ml beakers were filled from each of the cultures and molly assays were done each using 10 fish. Due to a mishap only the bag with full nutrient dose was available for assay on day 35. The controls for all assays were filtered sea water.

6.0 Results From Studies of Cultured Populations

6.1 Cell size and shape

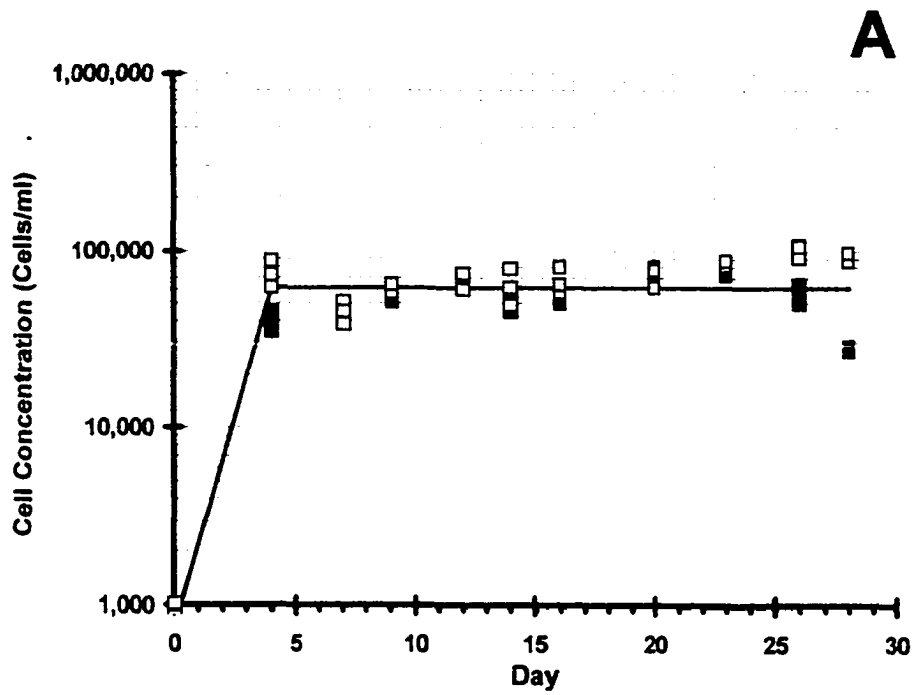
6.1.1 Light and nutrient limitation of culture growth

To determine whether culture growth was limited by nutrients or light, two of four identical cultures were supplied additional nutrients on day 16 during stationary phase of the culture cycle. Stationary phase was attained in all of the cultures by day 4. All four cultures attained concentrations of approximately 4-10*10^4 cells ml^{-1} (Figure 19). In cultures that were not re-fertilized, concentrations during the period day 4 to 16 (mean = 5.56 * 10^4, sd = +- 1.49 * 10^4 cell ml^{-1}) and the period day 22 to 27 (mean = 6.58 * 10^4, sd = +- 1.71 * 10^4 cell ml^{-1}) were not significantly different. In cultures which were re-inoculation with nutrients, the average cell concentration prior (day 4 to 16) to re-fertilization (mean = 5.69 * 10^4, sd = +- 1.30 * 10^4 cell ml^{-1}) and from day 22-27 after re-fertilization (mean = 7.31 * 10^4, sd = +- 2.23 * 10^4 cell ml^{-1}) also did not differ significantly.

6.1.2 Differentiation of gross cell size and shape

The same cultures were used to determine if cell size was influenced by re-fertilization. Cell size varied systematically throughout the culture cycle. All cultures showed the same pattern from day 1 to 16 (Figure 20). Upon inoculation the proportion of large and medium cells increased as cell concentration increased. The proportion of the three cell sizes remained relatively constant from day 4 to day 12 and

Figure 19. Effect of nutrient addition on cell concentration during stationary phase of population growth. The response of two replicate cultures (open and closed symbols) not re-inoculated with nutrients are represented in A.; The response of two replicate cultures that were re-inoculated on day 16 are shown in B.


A
1,000,000
100,000
10,000
1,000
Cell Concentration (Cells/ml)
0
5
10
15
20
25
30
Day

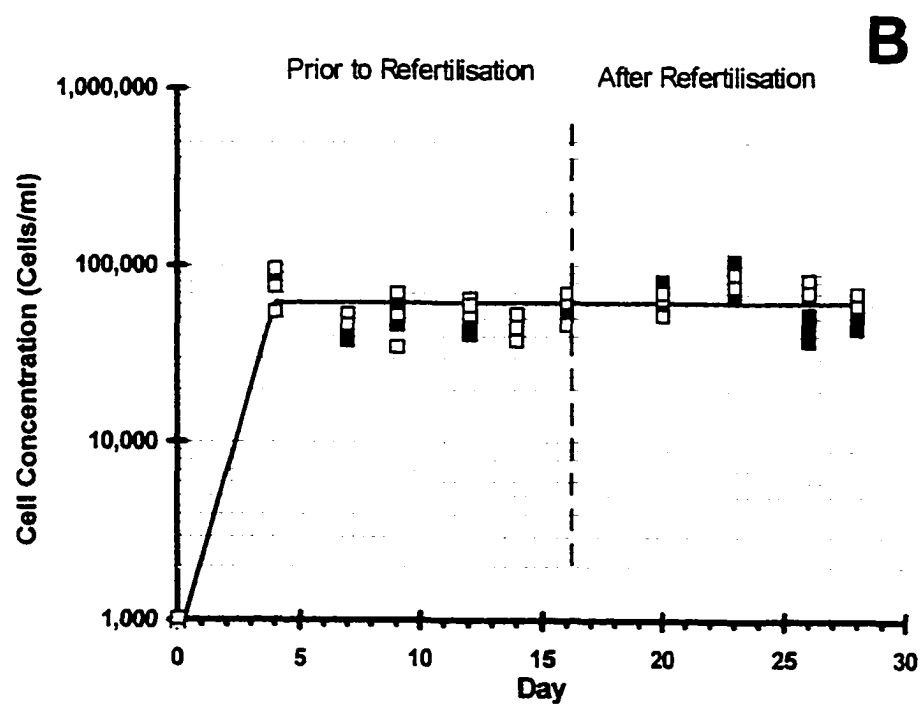
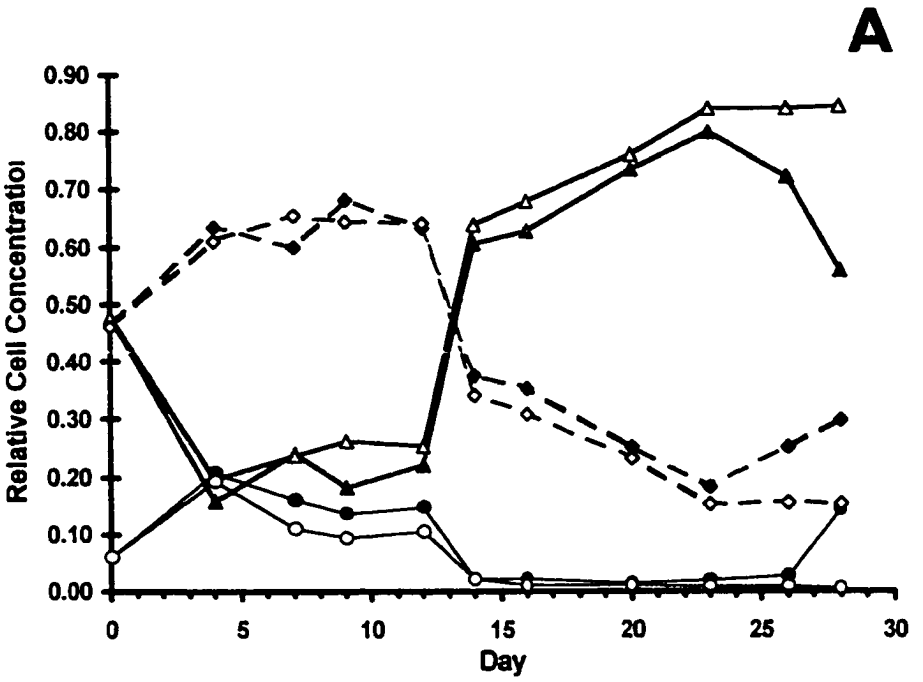
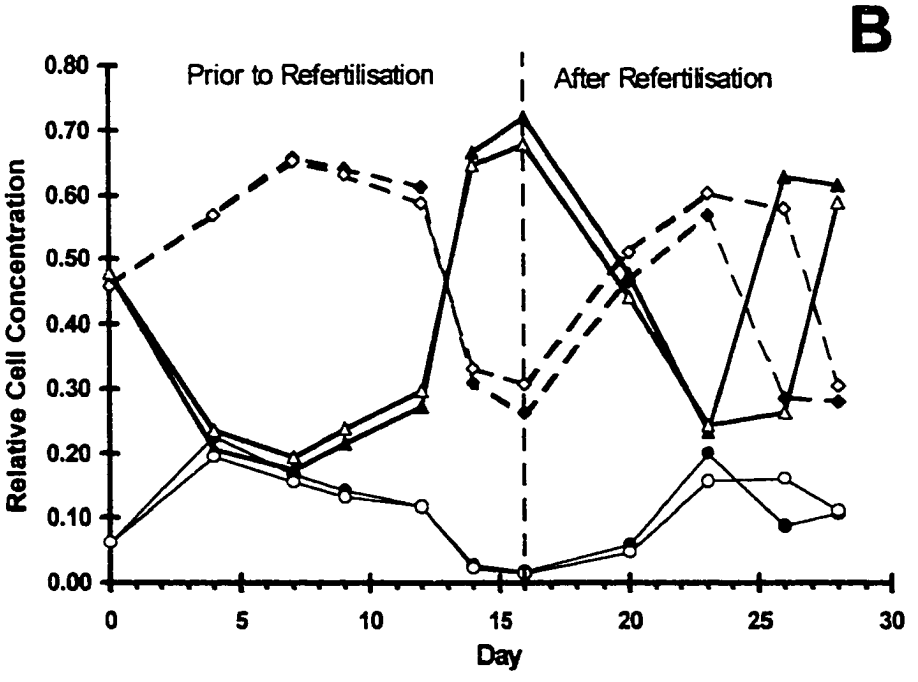

B
Prior to Refertilisation
After Refertilisation
1,000,000
100,000
10,000
1,000
Cell Concentration (Cells/ml)
0
5
10
15
20
25
30
Day

Figure 20. Effect of nutrient addition on cell size during stationary phase of population growth. These are the cultures documented in Figure 19. Cultures not re-inoculated with nutrients are shown in A. Cultures that were re-inoculated on day 16 are shown in culture B. Round symbols represent large cells, diamonds represent medium sized cells and triangles represent small cells. Each data point represents the mean of three samples from a culture. Each Line represents data from a single algal culture.


A
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Relative Cell Concentratio
0
5
10
15
20
25
30
Day


B
Prior to Refertilisation
After Refertilisation
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Relative Cell Concentration
0
5
10
15
20
25
30
Day

then between day 12 and 14 there was an increase in the proportion of small cells. In the two cultures not re-fertilized (Figure 20A) the proportion of small cells in the culture continued to increase until day 23. Beyond day 23 the culture with stable cell concentrations had a constant proportion of small cells while the culture with decreasing cell concentrations experienced a general decline in the portion of small cells and an increase in the proportion of large cells. In contrast, in the two cultures which had been re-fertilized (Figure 20B) there was a decline in the proportion of small cells after nutrients were added followed 3 or 5 days later by an increase in the proportion of small cells. These changes mimic the changes in the proportions of each size class seen at the start of all cultures.

A previously unreported progressive change in the shape of *H. akashiwo* cells was noted during this experiment. The range of variation in cell shape is illustrated in figure 21 and Plate 1. Cultures derived from samples of *H. akashiwo* collected in San Mateo Bay (Barkely Sound), Dixie Cove (between Hardy and Nelson Islands in the Strait of Georgia) and Narrows Inlet (in the Sechelt Inlet System) were examined. All were found to contain the full range of cell shapes. Vegetatively growing cells are variable in length (10 - 27 μm), breadth (7 - 14 μm) and depth (14 - 4 μm). The shape of vegetatively dividing cells replete with nutrients and light (during exponential growth phase) is bean-shaped with a roughly circular cross-sectional profile and mid-lateral insertion of the flagella. The lateral insertion of the flagella remains constant for the vegetative cells throughout the culture cycle. As cultures age the shape of the cell

Figure 21. Variation in gross external morphology of *Heterosigma akashiwo* cells seen in culture.

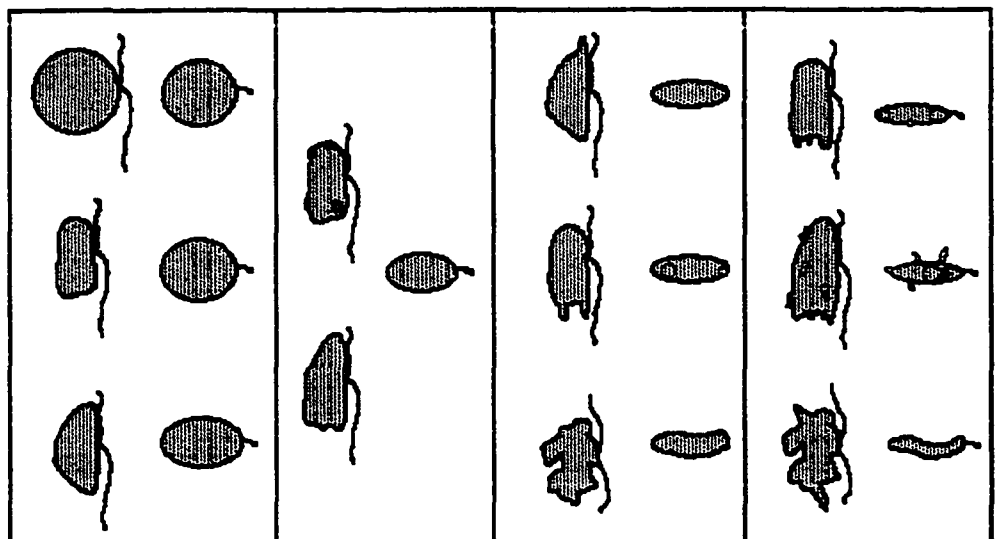

Smooth
Bumpy
1-2 Protuberances
>2 Protuberances

Plate 1. Observed variation in gross cell morphology. The 10 μm scale bar applies to all photographs.
A – traditional smooth form. **B** – Cysts. **C** and **D** – bumpy form.
E and **F** – 1-2 protuberances form, **G** and **H** – 2+ protuberances form.

B

becomes more flattened and it may develop surficial bumps. Following the initial occurrence of the bumps, cells can develop protrusions usually on the trailing edge of the cell. As cultures continue to age the protrusions become larger and more numerous; Protrusions can then be found on the lateral, dorsal, and ventral surfaces of the cells. Chloroplasts, visible during the formation of the protuberances, remain in the body of the cell and do not appear to be part of the protrusions. With time, cellular pigmentation becomes more dense and chloroplasts can not be seen.

Protuberances were variable in length and diameter. The smallest protuberances detected were approximately 0.5 μm in diameter, and reached 3 μm in length. At this size light diffraction around the cell hampered precise measurement. The very small diameter protuberances were among the first to occur on cells. The majority of protuberances were 2-4 μm in diameter and 3-6 μm in length. Small tubular protuberances with diameters less than the depth of the cell body, and a rounded distal tip (See the cell drawn in the upper right hand corner of figure 20 for an example of how a cell with both these types of protuberances might look.) were more common early in culture. Later another protuberance shape was noted . These protuberances were as deep in cross-section as the cell, laterally compressed and exhibited angular rather than curved bending at some point along their length.

The two cultures which were re-fertilized (Figure19B and 20B) had a different pattern of change in shape than those in cultures without re-fertilization (Figure 19A and

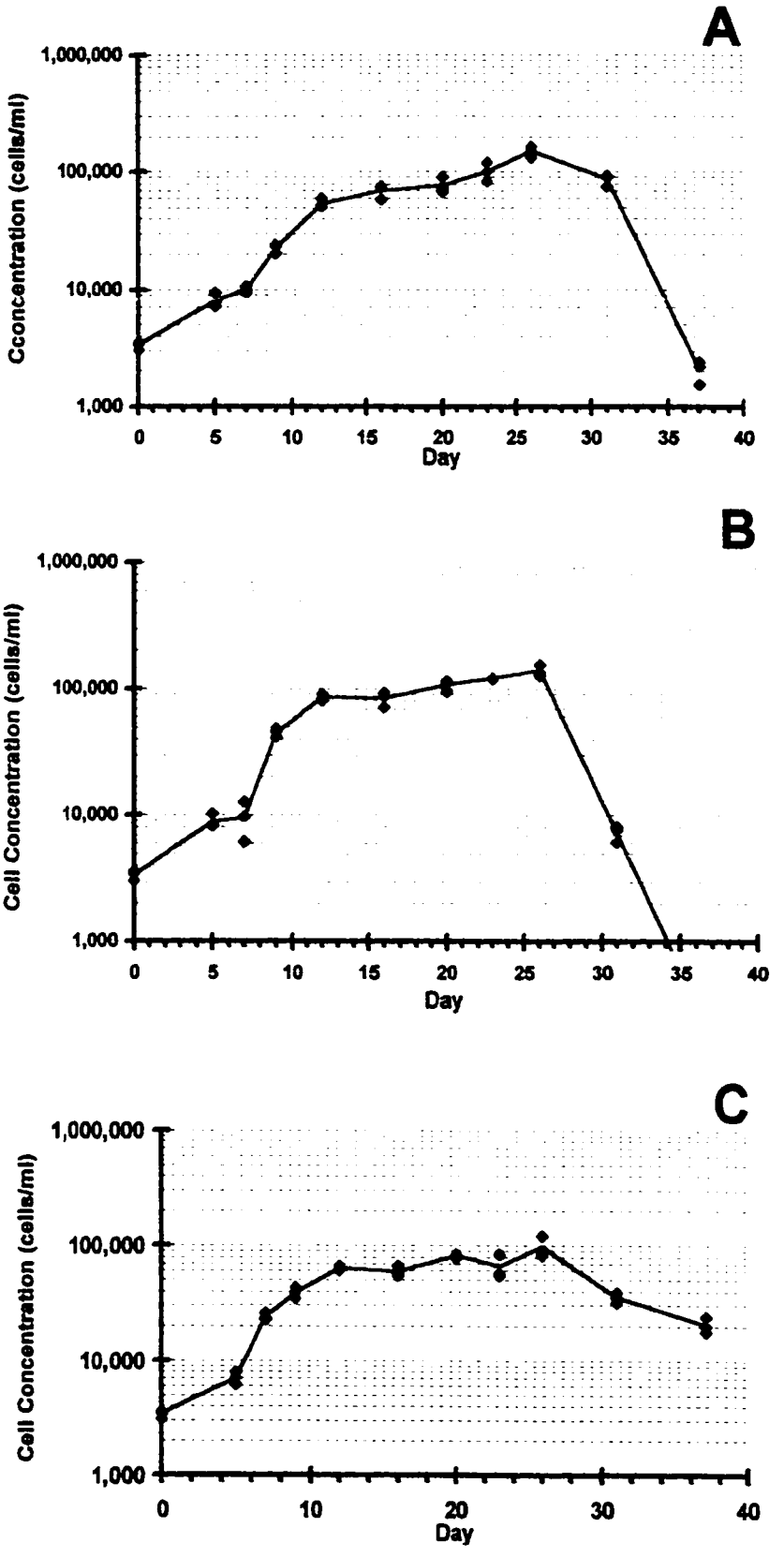
20A) described on the previous page. At re-fertilization all cultures were dominated by laterally compressed, bean-shaped, bumpy cells. Upon addition of the nutrients the culture again became dominated by smooth bean-shaped cells almost circular in cross-section. In both re-fertilized cultures immediately after nutrients were added spherical resting cells appeared. Spherical resting stage cells are generally 16 -26 μm in diameter though, occasionally a second smaller (4 - 12 μm) group of spherical cells was seen in culture. These resting cells did not appear in the two cultures that were not re-fertilized at this time. However the resting stages did occur much later in one of those populations when cell densities started to decline.

Changes in the cell's swimming behavior occurred as the gross morphology of the cell changed. During the smooth phase the cells swam in a slow helical spiral through the medium with occasional changes in direction. When the cultures were dominated by laterally compressed cells (both those with and those without protuberances), the helical motion was more rapid and changes in direction were much more frequent and abrupt.

6.1.3 Effects of agitation and light on growth and changes in cell size and shape

The effects of agitation and light level can be seen in figures 22, 23 and 24. In terms of population growth (figure 22) all treatments appear to reach stationary phase

Figure 22. Effect of light and agitation on cell population growth: spherical culture without agitation (A); spherical culture with agitation (B); laterally compressed culture without agitation (C)

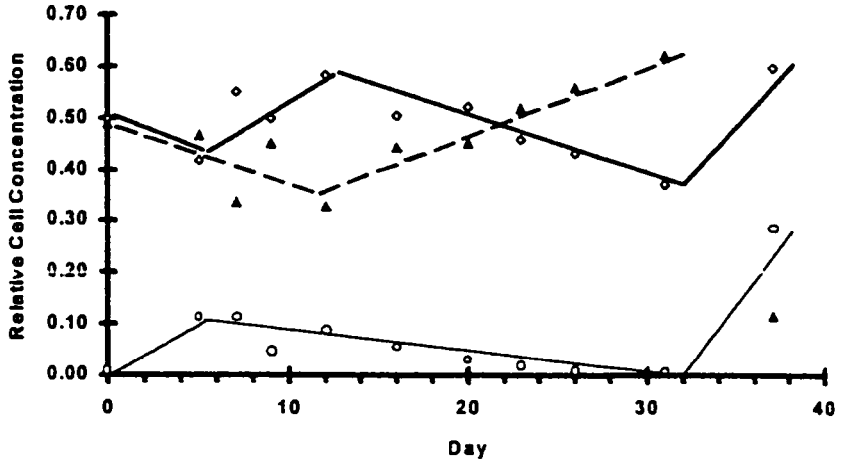

A
1,000,000
100,000
10,000
1,000
Cconcentration (cells/ml)
0
5
10
15
20
25
30
35
40
Day
B
1,000,000
100,000
10,000
1,000
Cell Concentration (cells/ml)
0
5
10
15
20
25
30
35
40
Day
C
1,000,000
100,000
10,000
1,000
Cell Concentration (cells/ml)
0
10
20
30
40
Day

between day 12 and 26. The principal difference in the growth pattern of the three cultures is that the thin culture (bottom figure) declined at a rate that is markedly slower than those in either of the thicker round flask cultures. Carbon dioxide did not appear to be limiting in these cultures as the culture with air bubbled directly into the medium (Figure 22b) did not show additional growth over the other cultures (Figure 22a and c).

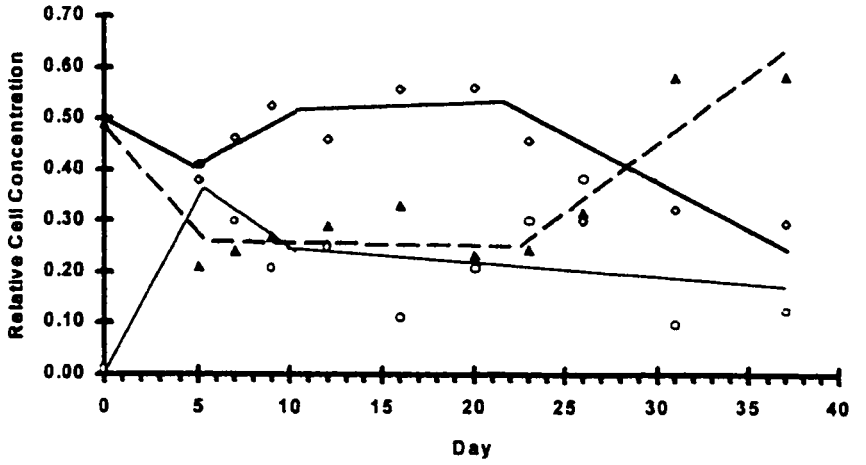
Differentiation of cell size (Figure 23) followed a common pattern. While the sequence is consistent, the timing and proportion of the population attained in each of the forms varies. In all three cultures there were sequential increases in the proportion of large, medium, and small cells. All cultures started with less than 5% of the population composed of large cells. Initially, the fraction of large cells increased in all cultures. In the agitated and thin-bag cultures, that portion of the population became much larger than that seen in the control (un-agitated) culture. After the initial increase, the proportion of large cells in the control culture decreased from around 11% to 0% on day 31, then increased to almost 30% by day 37. In the agitated culture the proportion of large cells increased to 40% then decreased after day 7 but maintained more than 11% large cells until day 37 (Figure 23b). In the thin-bag culture the percentage of large cells decreased from 40% on day 7 to less than 5% by day 16 and remained at this low level till day 26 when it increased to approximately 18% by day 37 (Figure 23c). When cell concentrations began to decline in control and thin bag cultures, the percentage of large cells increased. This terminal increase in the proportion of large cells did not occur in the agitated culture though cell concentrations decreased.

Figure 23. Effect of light and agitation on cell size during culture: spherical culture without agitation (A); spherical culture with agitation (B); laterally compressed culture (C). Circles = large cells, Diamonds = medium cells, Triangles = small cells

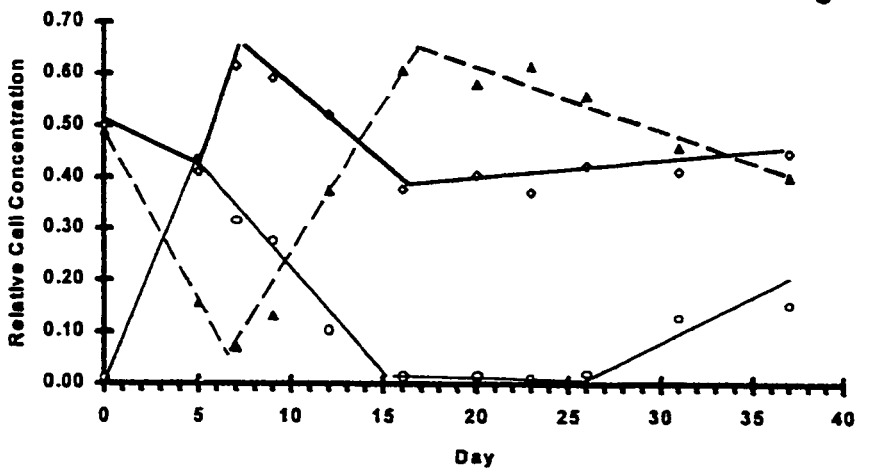
A


Relative Cell Concentration
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
10
20
30
40
Day

B


Relative Cell Concentration
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
5
10
15
20
25
30
35
40
Day

C


Relative Cell Concentration
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
5
10
15
20
25
30
35
40
Day

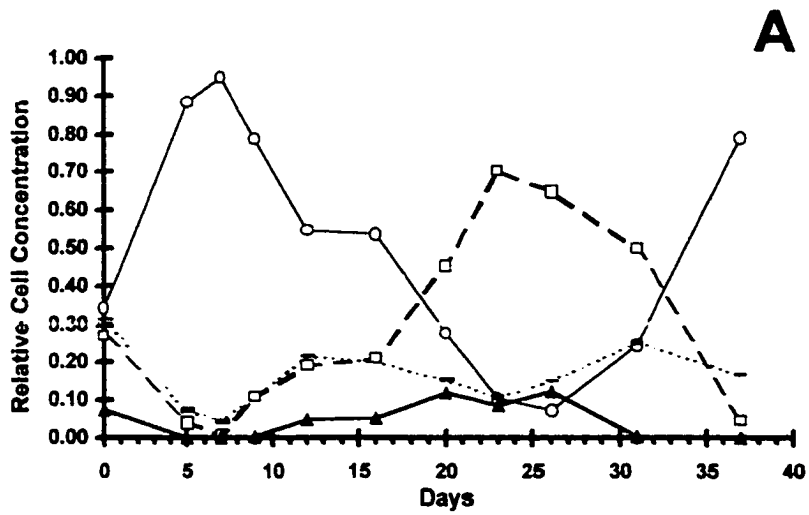
Cell shape also appeared to follow a basic pattern. After the culture was fertilized, smooth forms became more common until they constituted almost the entire cell population (Figure 24). The bumpy cells and cells with one or two protuberances(1-2s) increased in numbers about day 10. The number of cells with more than two protuberances (2+s) increased between day 15 and 20. After the peak in abundance of the protuberant forms, and before any decline in population size, the smooth forms increased again.

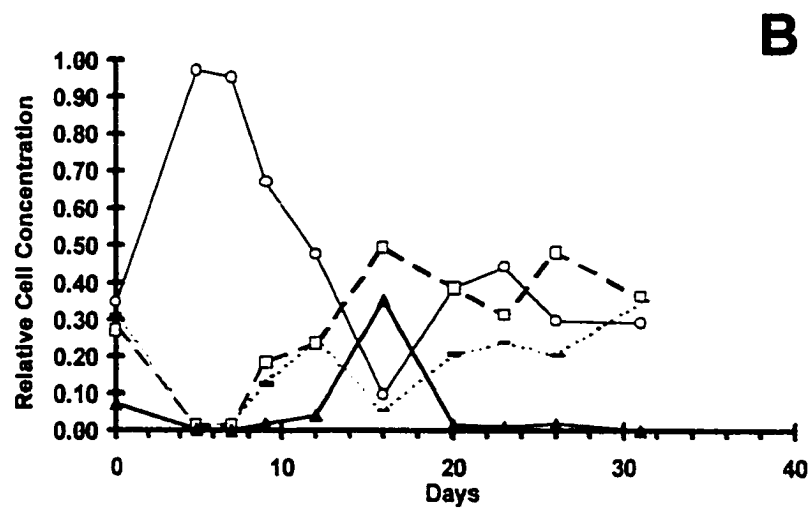
Table V demonstrates that the evolution of the various shapes during the cell cycle generally did not involve a significant change in cell length. The exception may be that spherical cells appear to be smaller than the 2+ protuberances cells. However for cells in progression from smooth to 2+ protuberances the changes observed in cell size appear to be primarily due to changes in cross-sectional area of the cells.

6.1.4 The effect of nutrient abundance on cell size and shape

Responses of *H. akashiwo* to varying levels of nutrient concentration are seen in figures 25 and 26. In response to half the nutrient dose suggested by Harrison *et al.* figure 25a (days 0-20), cell concentrations underwent approximately a three-fold increase before the culture started to decline (day 10). This occurred with little change in cell size (day 0-20 in Figure 25b) or cell shape (day 0-20 in figure 26). In contrast, when the same cultures where given twice the concentration of nutrients (Figure 26, days 20-120)

Figure 24. Effect of light and agitation on differentiation of cell shape: spherical culture without agitation (A); spherical culture with agitation (B); laterally compressed culture (C). Circle = smooth, Bar = bumpy, Squares = 1-2s, Triangles = 2+s


A
Relative Cell Concentration
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
5
10
15
20
25
30
35
40
Days


B
Relative Cell Concentration
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
10
20
30
40
Days

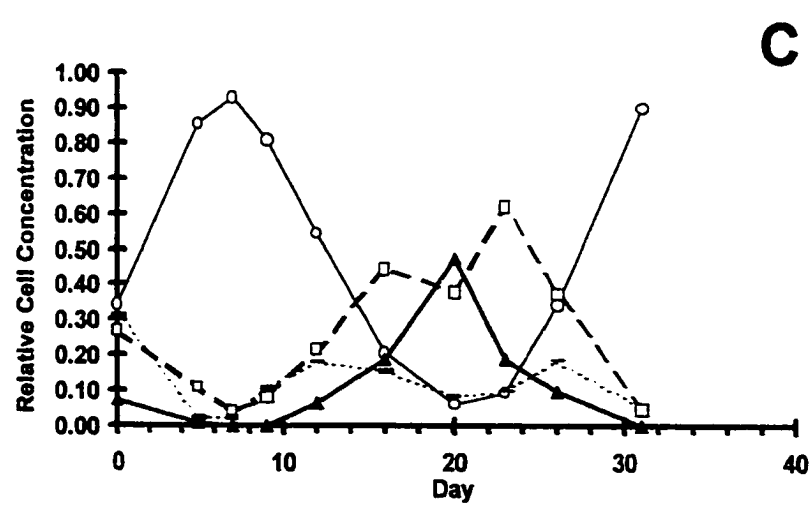
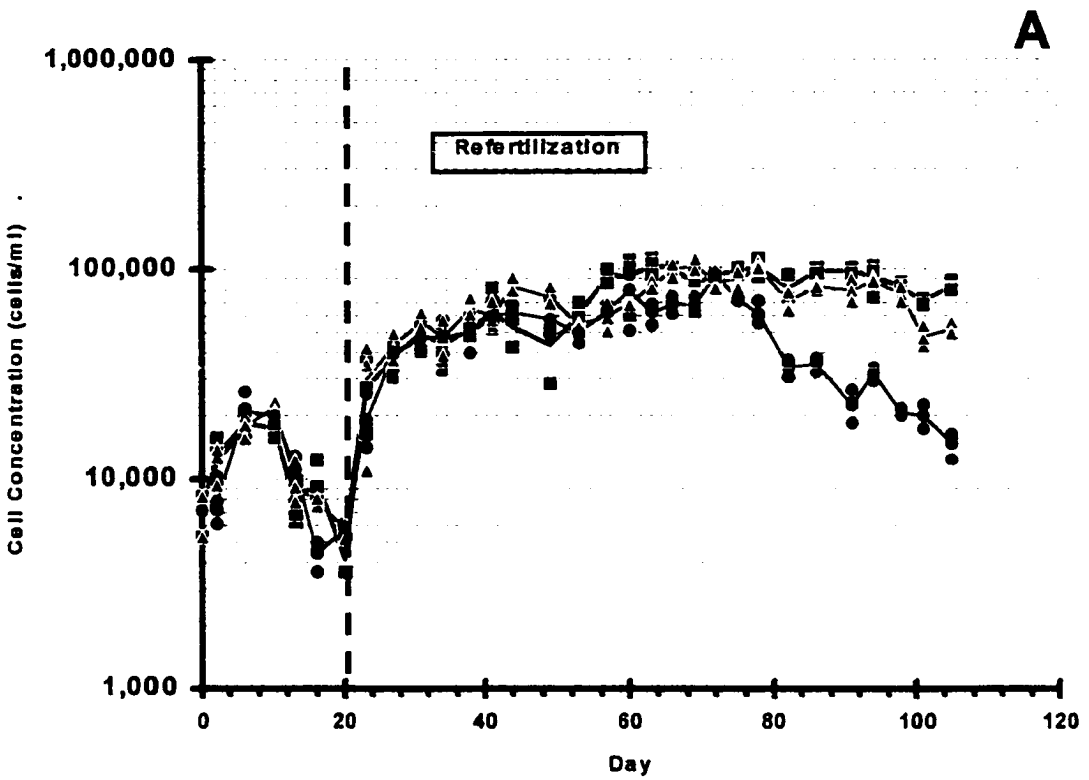
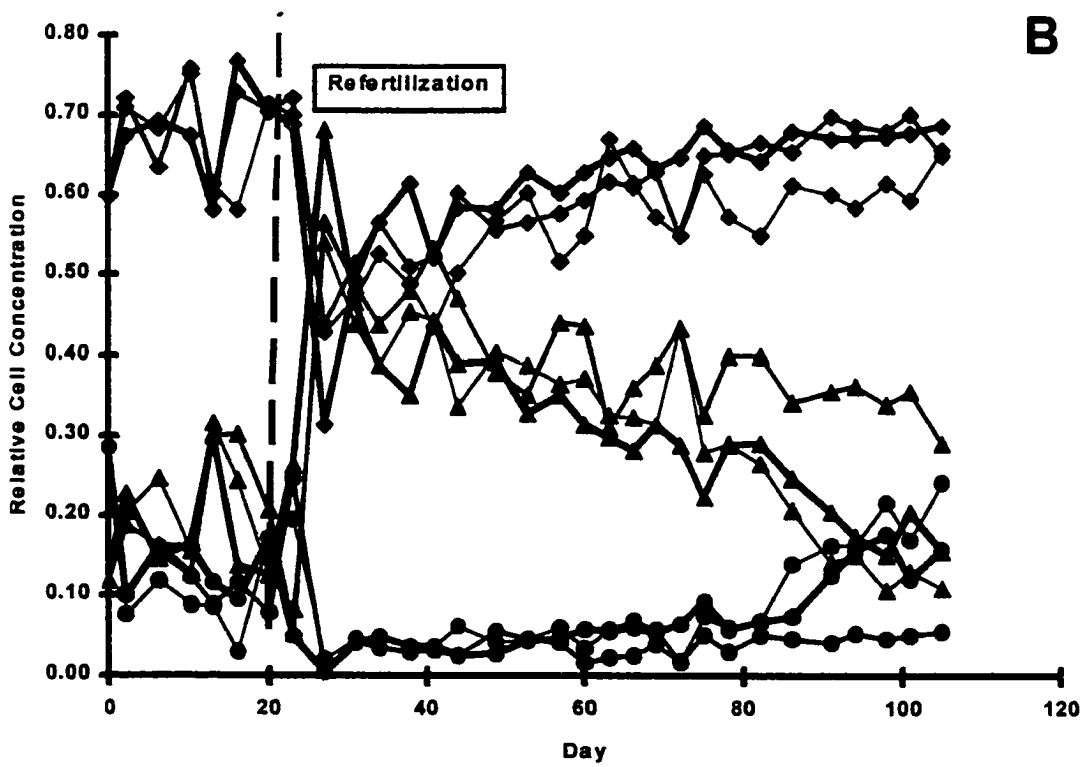

C
Relative Cell Concentration
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
10
20
30
40
Day

Table V The relation between cell shape and length.

	Cell Length (μm)		
	Average	Sd	n
Spheres	16.62	2.35	23
Smooth	18.58	4.26	28
Bumpy	18.25	3.96	21
1-2 Protuberance	18.49	2.44	31
2+ Protuberances	24.42	4.68	8

Figure 25. Changes in cell concentration and size resulting from different levels of nutrient addition. During days 0 to 20 cultures were grown on half the concentration of nutrients recommended by Harrison *et al.*(1980). The amount of nutrients added is doubled at re-fertilization (indicated by the dashed vertical line at day 20). Each line represents a different flat culture container. A - Numerical population growth. Lines connect mean values for each culture. B - Changes in cell size. Triangles = small cells, Diamonds = medium cells, Circles = large cells.

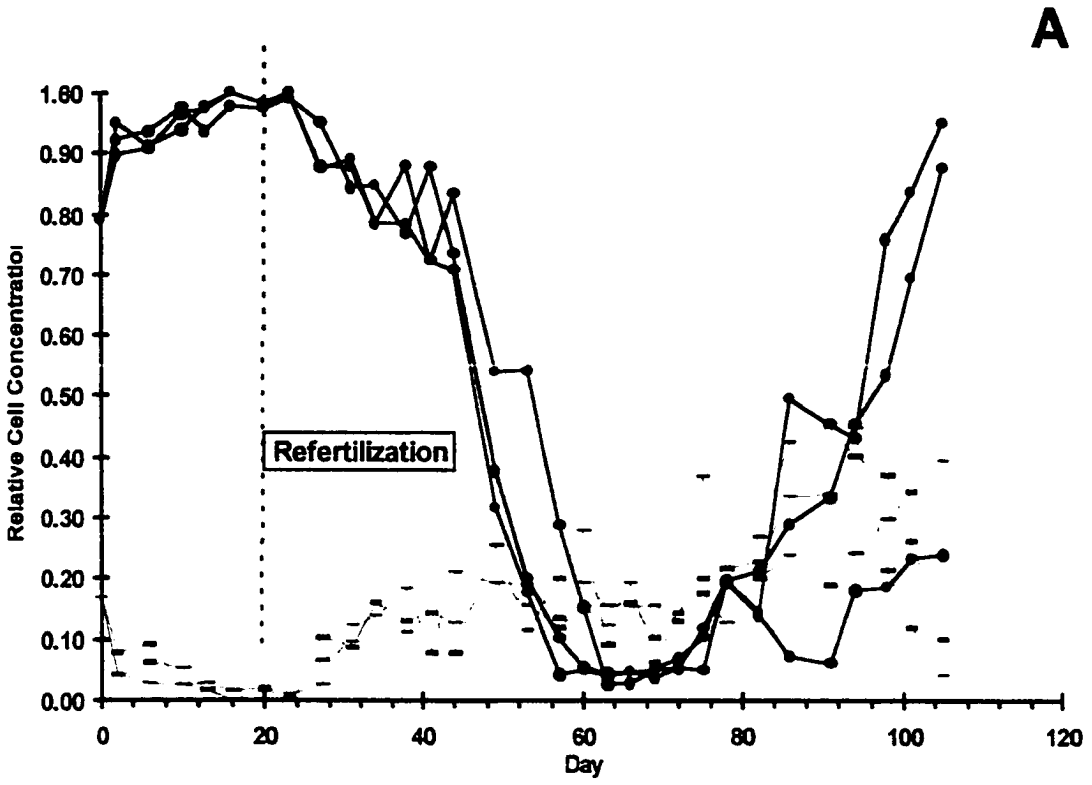

A
1,000,000
100,000
10,000
1,000
Cell Concentration (cells/ml)
Refertilization
0
20
40
60
80
100
120
Day

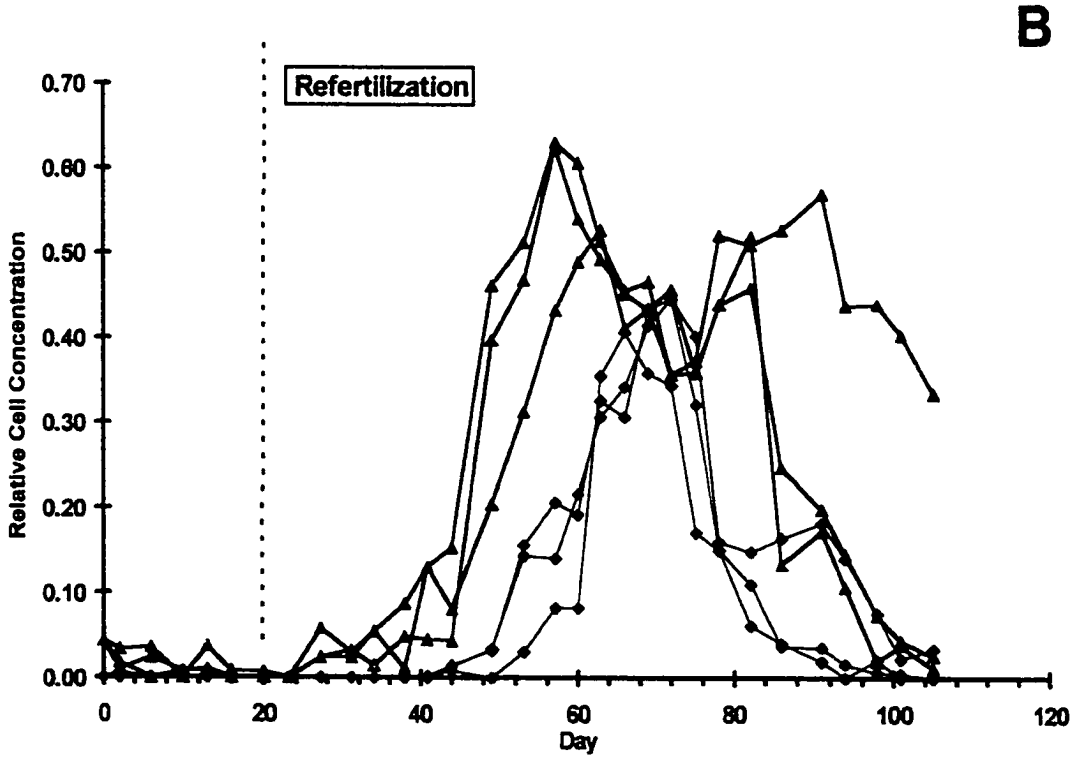

B
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Relative Cell Concentration
Refertilization
0
20
40
60
80
100
120
Day

showed dramatic changes in cell concentration, duration, size and shape. Cell concentrations increased close to 10 fold (Figure 25a). The growth cycle also lasted over 100 days as opposed to the 20 days when cells were given half the nutrient concentrations on day 0. The initial supply of half the recommended nutrient supply (Harrison *et al.* 1980) only slightly modified the proportions of various cell sizes (Figure 25b days 0-20) and cell shapes (Figure 26, days 0-20). No clear pattern could be seen in these changes. When the larger supply of nutrients was supplied (day 20) clear and marked changes in the proportions of each cell size and shape were seen. Changes in cell size (Figure 25b) did not follow the pattern seen earlier (Figures 20 and 23). In figure 25b for a couple of days after re-fertilization (days 20 – 22) two of the three cultures had an increased abundance of large cells. Where an increase in the proportion of large cells was not detected it is impossible to say whether an increase in the proportion of large cells occurred in the period between the samples on days 20 and 22. Large cells did increase in relative abundance towards the end of the culture period. The most striking difference between the pattern in figures 20 and 23 and that seen in figure 25b was that where the abundance of small cells increased and medium sized cells decreased in figures 20 and 23, small cells decreased and medium cells increased in abundance in figure 25b.

The three flat-bag unagitated cultures shown in Figure 26 did follow the same general pattern of changes in cell shape previously described for unagitated cultures (Figure 24a and 24c). Initially, smooth cells dominated the cultures. Then the proportion

Figure 26. Changes in cell shape under different levels of nutrient addition. The amount of nutrients added (see Figure 25) is doubled at re-fertilization (indicated by the dashed vertical line). In Graph A - Circles = smooth cells and Bars = bumpy cells. In Graph B - Triangles = cells with 1 or 2 protuberances and Diamonds = cells with more than 2 protuberances. The there are three cultures represented in graphs A and B. The values from A and B for any one culture sum to a value of 1.0.


A
Relative Cell Concentration
1.60
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Refertilization
0
20
40
60
80
100
120
Day


B
Refertilization
Relative Cell Concentration
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
20
40
60
80
100
120
Day

of bumpy cells increased. This was followed by and increase in the proportion of cells with one or two protuberances which was in turn followed by an increase in the proportion of cells with more than two protuberances. After day 72 the proportion of cells with more than 2 protuberances declined. Shortly thereafter the abundance of cells with one or two protuberances declined. At this time the contribution of smooth cells to population numbers started to increase and continued to do so till the end of the experiment. The pattern shown for the agitated culture in figure 24b is very similar through to the point at which the cells with more than 2 protuberances decline in abundance. The period after this does see some increase in the contribution of smooth cells to the population however, the contribution of cells with one or two protuberances does not decline as precipitously as it does in unagitated cultures.

6.2 Toxicity

Initial bioassay work was carried out with brine shrimp (*Artemia salina*). While shrimp are useful in a laboratory setting (Vadnais 1995) most field observations have focused on fish. Figure 27 shows temporal variation in cell concentration (Figure 27a) and size (Figure 27b) in three 5-l flat-bag cultures. On day 43 a bioassay was done on each of the cultures exposing rainbow trout (*Oncorhynchus mykiss*) (Table VI) and brine shrimp to the cultures. At the end of 130 minutes all trout exposed to the cells were dead and all control trout were alive. Shrimp were not killed by exposure to the same algal culture. As an after-thought two mollies (*Pocelus mexicana*) were placed in a small amount (approximately 350 ml) of leftover culture B (Table VI). At the end of the bioassay on day 43, and after examining the trout and brine shrimp for mortalities, it was noted that the mollies had also expired.

Eight days later cells from cultures with and without re-fertilization were again assayed using trout. The culture without re-fertilization (Table VI, Culture A) remained toxic, while the re-fertilized culture (Culture B) was not toxic. On day 69 culture C, which had been re-fertilized with three times the standard dose of nutrients, was again bioassayed. No toxicity was detected.

Figure 27. Population and size differentiation seen in cultures used to evaluate the effect of nutrients on toxin production (Table VI). Three 5-l bag cultures were used. Long dashed lines represent culture A. This culture was not re-fertilized. Short dashed lines represent culture B. This culture was re-fertilized on day 43. Solid lines represent culture C. This culture was re-fertilized on day 43 with three times the amount of nutrient used in re-fertilization of culture B. **Graph A** - numerical population growth. Lines connect mean values of three samples from each culture. **Graph B** - changes in cell size. In graph B Triangles = small cells, Diamonds = medium cells, Circles = large cells. The vertical dashed line at day 43 represents the re-fertilization of cultures B and C. Arrows indicate when bioassays were performed.

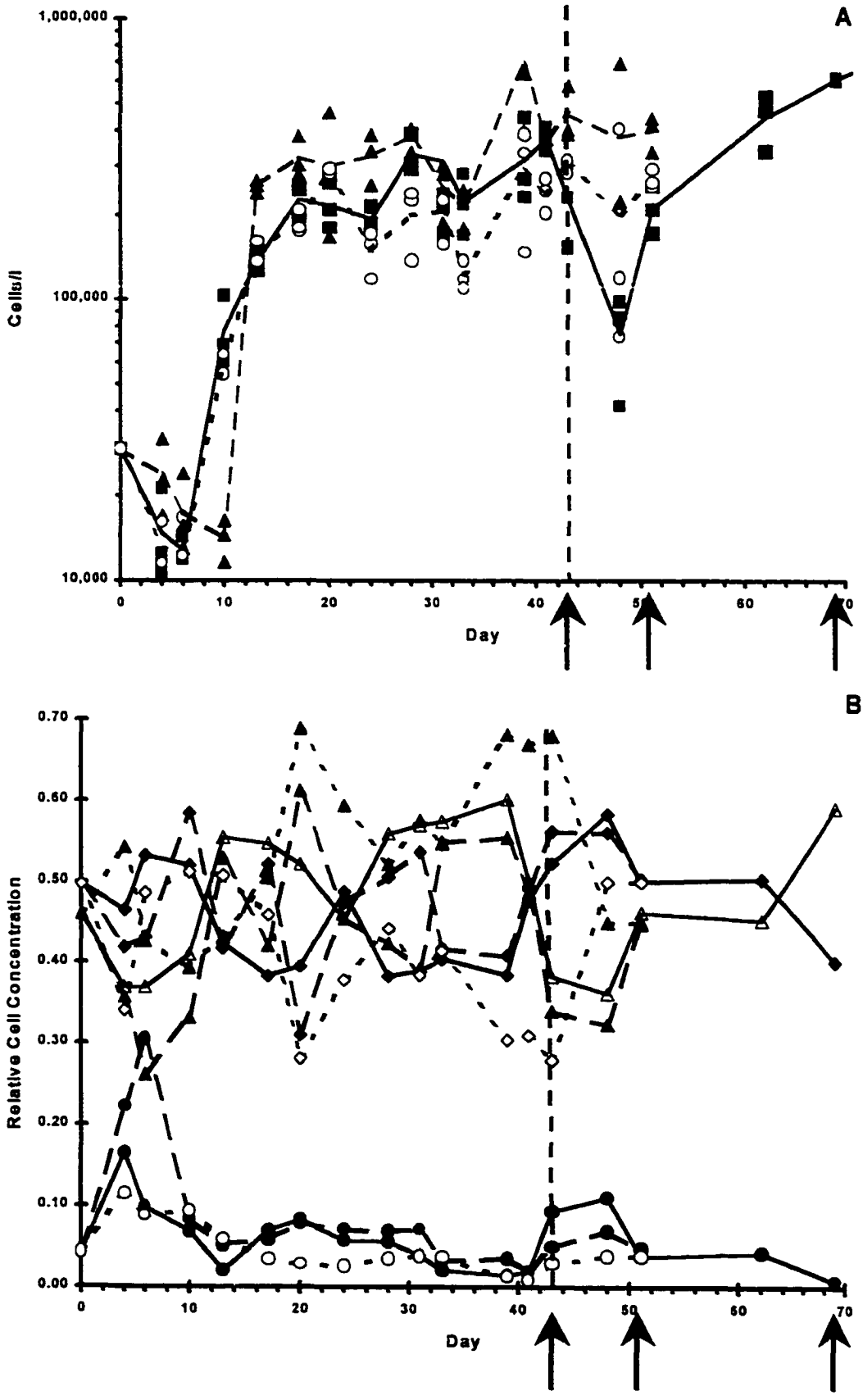

A
1,000,000
100,000
10,000
Cells/l
0
10
20
30
40
50
60
70
Day
B
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Relative Cell Concentration
0
10
20
30
40
50
60
70
Day

Table VI. Toxic effect of algae on trout in cultures with and without re-fertilization. The cultures used are those represented in figures 27 and 28 . Culture A was not re-fertilized. Culture B was re-fertilized with a standard dose of nutrients while culture C received three times the standard dose of nutrients. The presence of a bar as an entry on the time to death column means the fish did not die during the bioassay. Time to death is measured in minutes and the weight is that of the fish in grams.

Day 43

	Culture A		Culture B		Culture C		Control	
	Time to Death	Weight	Time to Death	Weight	Time to Death	Weight	Time to Death	Weight
	70	7.3	50	9.4	75	7.9	-	8.9
	95	11.1	95	8.3	80	12.1	-	9.9
	120	8.2	100	8.7	115	13.3	-	10.3
	125	7.5	125	13	120	9	-	11.8
	130	12.3	125	11	130	10.4	-	12.4
Mean	108	9.28	99	9.68	104	10.54		10.26
Stn. Dev.	25.15	2.27	30.70	2.51	24.85	2.20		2.14

Day 51

	Culture A		Culture B		Culture C		Control	
	Time to Death	Weight	Time to Death	Weight	Time to Death	Weight	Time to Death	Weight
	65	6.7	-	5.2			-	4.3
	68	8.4	-	7.9			-	6.8
	92	7.5	-	10			-	6.8
	120	4.4	-	10.4			-	8.7
	135	12.7	-	11.3			-	14.9
Mean	98	8.34		8.96				8.30
Stn. Dev.	31.06	2.97		2.44				4.01

Day 69

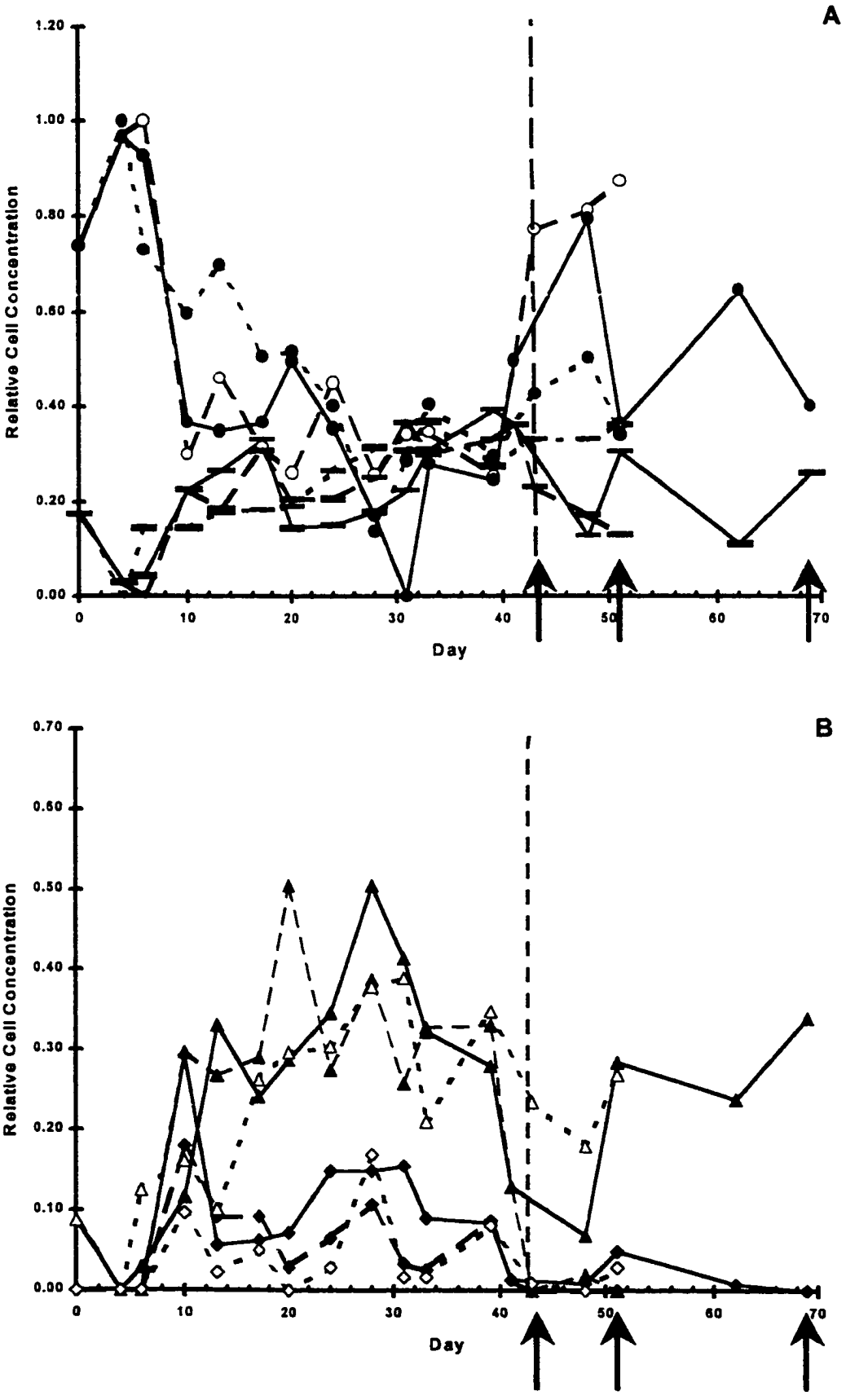
	Culture A		Culture B		Culture C		Control	
	Time to Death	Weight	Time to Death	Weight	Time to Death	Weight	Time to Death	Weight
					-	23.6	-	27.4
					-	31.1	-	22.1
					-	16.3	-	12.3
					-	8.9	-	8.1
					-	5.7	-	7.4
Mean						17.12		15.46
Stn. Dev.						10.44		8.88

Toxicity is not wholly dependent on cell concentrations. During bioassays on day 43 cultures A, B and C were all toxic and had mean cell concentrations of 4.54(± 0.185), 3.04(± 0.701) and 2.25(± 1.45) $*10^5$ cells/ml (Figure 27a). On day 51 cultures A (toxic) and B (non-toxic) had mean cell concentrations of 3.97 (± 0.559) and 2.11 (± 0.042) $*10^5$ cells/ml. The cell concentration in culture C (non-toxic) on day 69 was 6.09(± 0.004) $*10^5$ cells/ml. While there were significant differences in algal concentrations between the cultures, the highest concentration was not toxic even though its cell concentration was almost three times that of the toxic culture with the lowest cell concentration.

Toxicity also does not appear to be directly dependent on the distribution of cell sizes in a culture. On day 43 the proportion of small, medium and large cells (Figure 27b) in cultures A, B and C (toxic) were ..34/.53/.04, .68/.28/.01 and .38/.52/.01 respectively. On day 51 in cultures A (toxic) and B (non-toxic), for both cultures those values were .45/.50/.05. On day 69 the proportions in culture C (non-toxic) were .49/.50/.01. The ratios in the nontoxic cultures of day 69 and day 51 lay between the ratios for the toxic cultures.

Care must be taken when evaluating the relation between cell shape and toxicity of a culture. On day 43 the proportion of smooth, bumpy, 1-2 protuberances and 2+ protuberances cells in toxic cultures A, B and C was .77/.23/.0/.0, .43/.33/.23/.01 and .50/.36/.13/.01 respectively. On day 51 in cultures A (toxic) and B (non-toxic) those values were .87/.13/.00/.00 and .34/.36/.21/.03. On day 69 the proportions in non-toxic culture C were .40/.26/.34/.00. To control for potential systematic changes in toxicity

Figure 28. Shape differentiation in cultures used to evaluate the effect of nutrients on toxin production (Table VII). Three 5 liter bag cultures were used. **Graph A** - The relative abundance of smooth (Circles) and bumpy cells (Bars). **Graph B** - The relative abundance of 1-2 protuberances cells (Triangles) and 2+ protuberances cells (Diamonds). Short dashed lines represent culture A. Long dashed lines represent culture B. Solid lines represent culture C. The vertical dashed line at day 43 represents the re-fertilization of cultures B and C. Arrows indicate when bioassays were performed.


A
Relative Cell Concentration
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0
10
20
30
40
50
60
70
Day
B
Relative Cell Concentration
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
10
20
30
40
50
60
70
Day

during the culture cycle only cultures at the same point in their differentiation of shape may be compared. In this experiment only the cultures on day 43 are appropriate and at that point there were no detectable differences in toxicity between the cultures (TableVI).

While the precise value for the proportion of each cell shape does not appear to be directly related to toxicity of the culture examined in another manner there does appear to be a relation between cell shape and toxicity. Toxicity appears to be present late in the progressive elaboration of cell shapes that occurs in a cultured population. In the case of culture C on day 69 the proportion of cells with 1or 2 protuberances was 34%. This was the highest of any of the cultures when they were tested for toxicity. However, there the pattern of cell shape changes appeared to be in its early stages as no peak for the abundance of this cell type was reached nor was a minimum evidenced for the smooth or bumpy cells since the refertilization of the culture.

The question of the effect of culture dilution on toxicity became important during the trout bioassay work. Trout grow rapidly and no local supplier hatches trout throughout the year. As a consequence, the same size fish are only available for a short time each year. This can be seen in the difference in size between the fish used in the bioassays on day 43 and day 69. On day 43, fish samples had means between 9.5 and 10.5 g. By day 69, the mean fish size had increased to 15.5 to 17.1 g. The larger fish required more culture medium to keep the fish fully immersed during the assay. The smaller fish could be assayed with as little as 1500 ml of algal culture but the larger fish required a minimum of 3000 ml. Under those conditions and only being able to grow a maximum of three 5-l

cultures at a time, either large volumes of toxic material were required to run numerous simultaneous tests on the same-sized fish or smaller bioassay animals were required.

An attempt was made to determine whether the cultures could be diluted to allow a number of bioassays to be completed at the same time. The trout in the earlier bioassay were juvenile fish. Over the period of an experiment fish might triple in size. The available culture facilities could not produce adequate volumes of culture in individual containers to accommodate multiple assays of the larger fish. To allow for fish growth during the experiment it was thought that as much as a 10-fold dilution of the culture might be required to have adequate bioassay material. However, data in Table VII demonstrated that dilution of a culture by a factor of 10 or more resulted in a loss of detectable toxicity. For these reasons the decision was made to use mollies for the bioassay work. These fish are seldom larger than 2 g in size. They are also bred throughout the year and are readily available in a variety of sizes at any time.

The amount of medium required to conduct a bioassay using mollies was determined by conducting simultaneous bioassays on the same culture in 5 different volumes taken from one container of algae. In table VIII the cell concentration in each of the five volumes of algae are recorded. Concentrations of cells in four of the volumes, 250, 375, 750 and 1000 ml, were not significantly different. Though the concentration of algae in the 500 ml aliquot was higher than those in the other containers, mean time to death of mollies in the algal aliquots containers did not vary (Figure 29). One of the mollies in the 250 ml of sea water used as a control died after 300 minutes (Figure 29).

Table VII. The effect of various dilutions of an algal culture on its toxicity to mollies. The presence of a "-" in the table indicates that the fish survived the bioassay period. Time to death is measured in minutes. The weight of the fish is in grams.

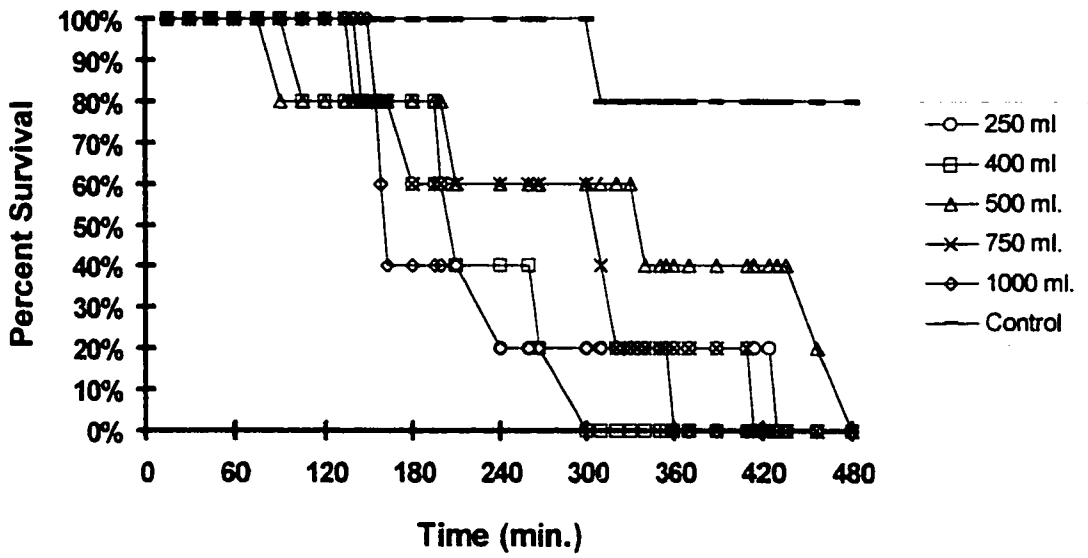
Dilution	Full Strength		1/10		1/100	
	Time to Death	Weight	Time to Death	Weight	Time to Death	Weight
	65	8.7	-	14.3	-	12.8
	68	8.4	-	10.3	-	15.2
	92	7.5	-	6.8	-	8.0
	120	4.4	-	9.7	-	8.0
	135	12.7	-	4.2	-	8.7
Mean	96	8.34		9.06		10.54
Stn. Dev.	31.06	2.97		3.81		3.28

Dilution	1/1000		Control	
	Time to Death	Weight	Time to Death	Weight
	-	16.9	-	14.9
	-	4.1	-	4.3
	-	4.5	-	6.8
	-	7.1	-	5.2
	-	5.2	-	8.7
Mean		7.56		7.98
Stn. Dev.		5.35		4.22

Table VIII. Volumes of media and concentrations of *Heterosigma akashiwo* cultures used in bioassays to determine the effect of assay volume on mean time to death illustrated in figure 29. Replicates 1 through 3 represent three separate enumerations of the concentration of cells at the start of the assay.

Volume	Replicate 1	Replicate 2	Replicate 3	Mean	Stnd. Dev.
1000 ml	38,954	28,354	47,054	38,121	9,378
750ml	34,254	27,854	35,254	32,454	4,015
500 ml	47,654	48,754	42,854	46,421	3,137
375 ml	34,354	20,754	36,154	30,421	8,420
250 ml	22,854	33,754	34,354	30,321	6,473
Grand Mean				35,548	8,525

Figure 29. Mean time to death in containers of various sizes as documented in Table VIII.



	250 ml	375 ml	500 ml.	750 ml.	1000 ml.
Mean Time to Death	241	216	312	273	213
Standard Deviation	111	75	158	112	90

The possibility that variations in fish size (weight) might affect the mean time to death in the bioassays was investigated. In this experiment 39 fish were assayed in 1.0 l of culture and a similar number were placed in the same volume of sea water. As no controls died even after 300 minutes it was decided to let the experiment continue until all experimental fish had expired. Over the approximately 500 minutes of this assay none of the control fish died. This assay involved maintaining the control fish in less water per fish than those reported in the controls for figure 29. It thus seems likely that the mortality reported in figure 29 after 300 minutes was the result of a single fish which had been damage during transfer between holding and experimental vessels or was otherwise compromised before the bioassay. Even so, to avoid potential problems with mortalities in controls, it was decided that in comparing toxicity only fish mortalities which occur in the first 4 hours(240 minutes) would be considered valid data.

There appears to be a trend of increasing time to death with increasing size of molly used in the bioassay. There is however considerable scatter around the trend line in figure 30. Regression analysis of the data yielded an F value of 2.26. The probability of the relationship existing between time to death and the size of the assay animal is considerably greater than 75% but less than 90% (Rolf and Sokal 1969). While the analysis does not demonstate a significant regression (α = 95%) caution suggested that sizes of fish used in comparison of bioassays be kept as similar as possible.

Figure 30. Relation of molly size to time to death.

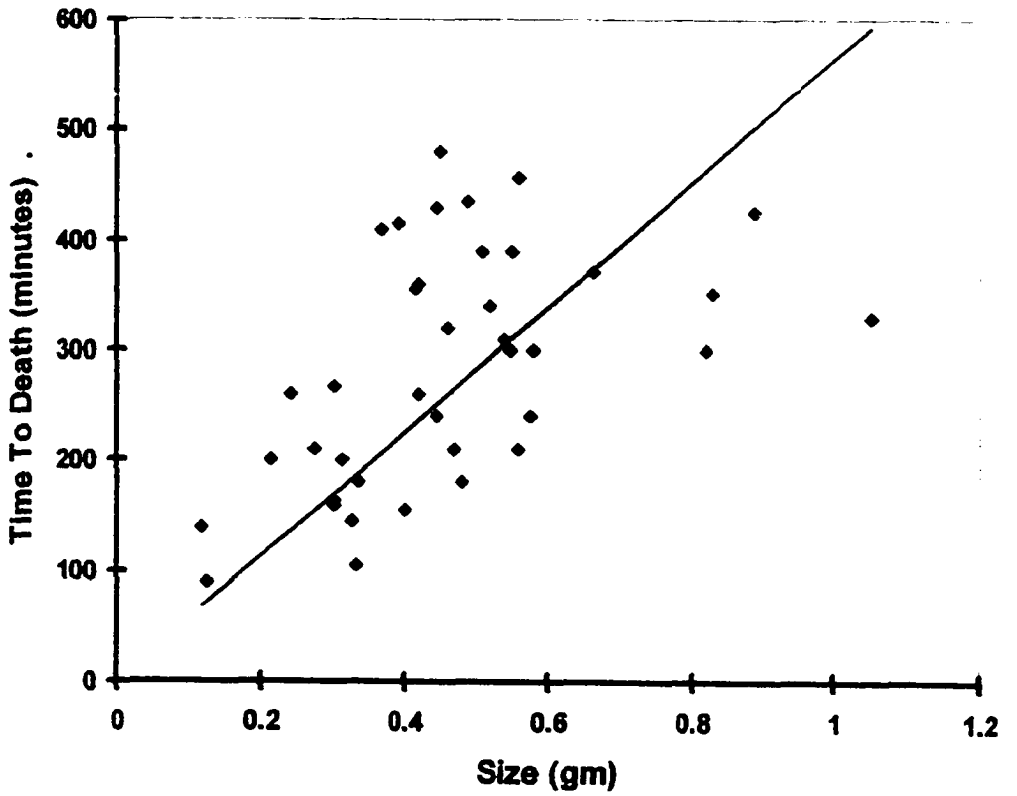

600
500
400
300
200
100
0
Time To Death (minutes)
0
0.2
0.4
0.6
0.8
1
1.2
Size (gm)

Table IX. Regression statistics for the relationship between molly size and time to death illustrated in Figure 30

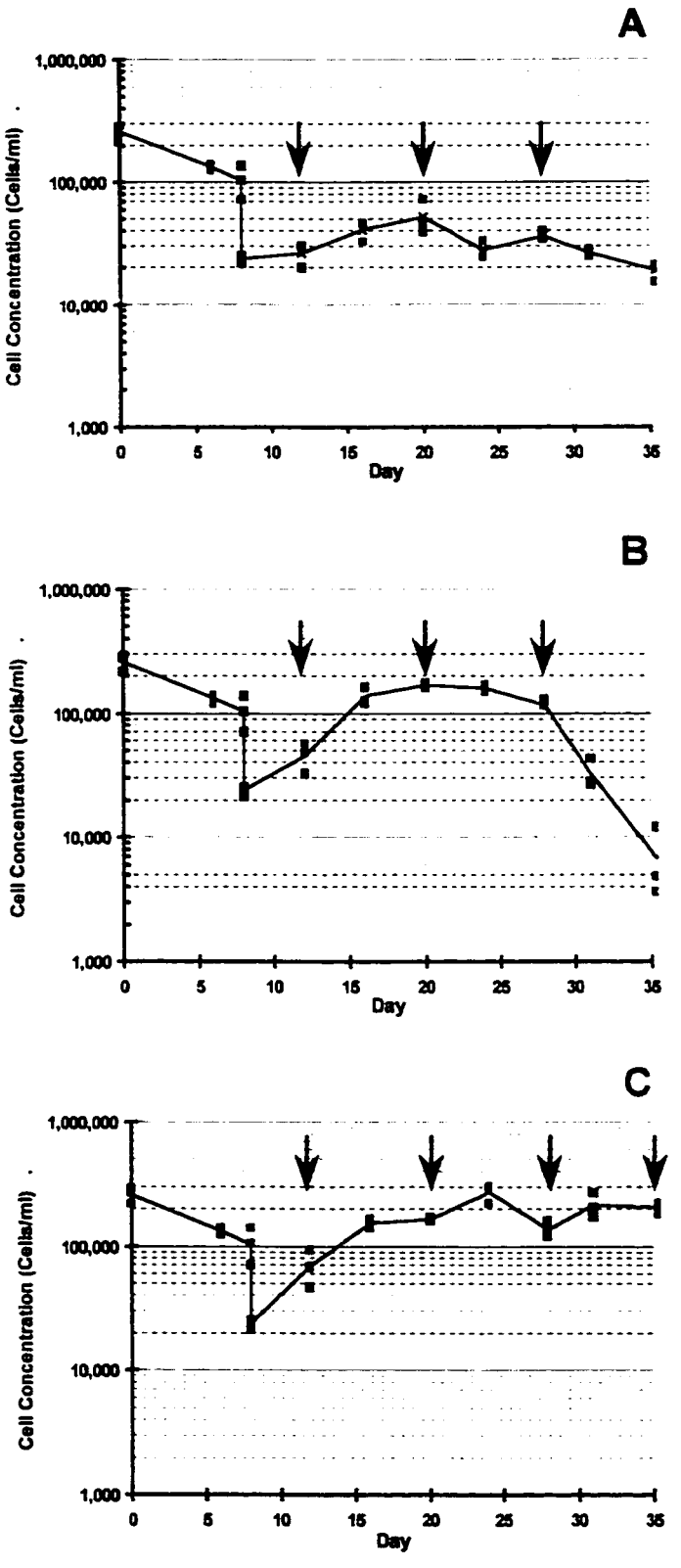
ANOVA

	df	*SS*	*MS*	*F*
Regression	1	-27297.1	-27297.1	-2.25598
Residual	38	459796.2	12099.9	
Total	39	432499.1		

The final analysis was to determine the effect that nutrient dosage had on the toxicity of the alga and whether that toxicity varied during culture. The three levels of nutrient enrichment (sea water, sea water plus half the nutrient dose recommended by Harrison *et al.* 1990, and sea water plus the full recommended dose) of an algal culture resulted in a graduated response in population size, cell size and cell shape.

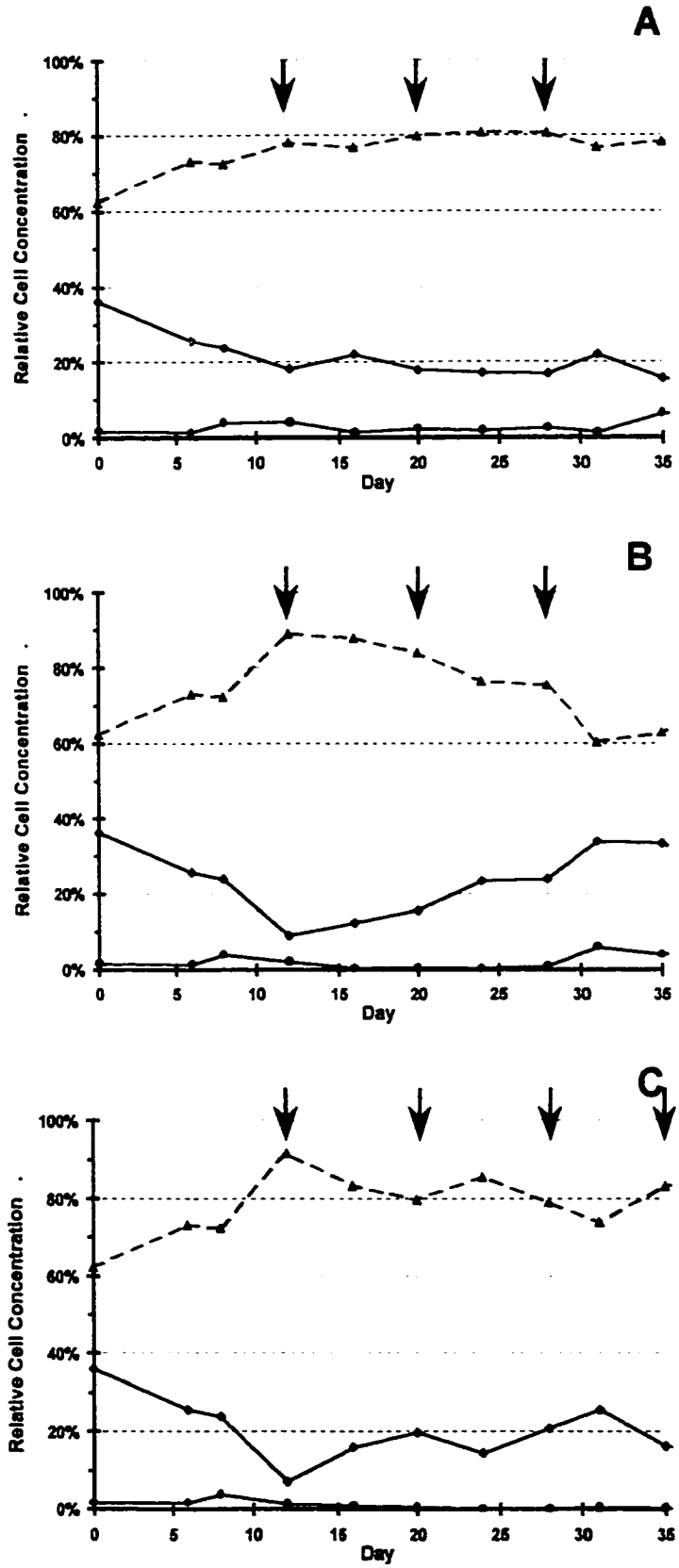
The numerical response of an algal population to three concentrations of nutrients is depicted in Figure 31. Cell concentrations in the parent culture were tracked from day 0 to day 8 to ensure the donor population was in decline. This suggests that the cells were close to exhausting their internal nutrient stores and were either encysting or dying. Addition of filtered sea water elicited a slight (approximately 2-fold) increase in cell concentration with the concentration again declining after day 19 (Figure 31a). Addition of sea water plus half the nutrient dose recommended by Harrison *et al.* (1980) resulted in a larger increase in population size (approximately 9-fold) and concentrations again declined after day 19 (Figure 31b). The numerical response to the full recommended dose of nutrients was still stronger (Figure 31c). The population of algae increased approximately 10-fold and did not decline before the end of the experiment on day 35.

Figure 31. Population development at three different levels of nutrient addition. The parent population was tracked till day 8 when it was split into three separate cultures. At that time water and nutrients were added to make up 5-l cultures. Three counts of cell concentration were taken and a line connecting the mean value is plotted. A - only filtered sea water added; B - sea water and half the nutrient dose recommended by Harrison *et al.* (1980); C - sea water and the full dose of recommended nutrients. Arrows show when bioassays were preformed on each culture.


A
1,000,000
100,000
10,000
1,000
Cell Concentration (Cells/ml)
0
5
10
15
20
25
30
35
Day
B
1,000,000
100,000
10,000
1,000
Cell Concentration (Cells/ml)
0
5
10
15
20
25
30
35
Day
C
1,000,000
100,000
10,000
1,000
Cell Concentration (Cells/ml)
0
5
10
15
20
25
30
35
Day

The response in cell shape also increased with an increased supply of nutrients (Figure 32). In the parent culture between day 0 and day 8 small cells were increasing in abundance in the culture while medium cells declined. With the addition of sea water this trend was terminated by day 12 and the proportion of small and medium cells became more or less constant, at approximately 80 and 20 percent of the population respectively, till almost the end of the experiment. Large cells in the parent population increased only slightly between days 6 and 8. After addition of sea water the concentration of large cells fell and remained constant until day 35 when there was a slight increase in their number. Upon addition of sea water plus half of Harrison *et al.* 's (1980) recommended dose of nutrient there was a more pronounced response in cell size. After day 8 the proportion of small cells in the population increased to 90% on day 12 after which their contribution to the population decreased till day 31 when they were about 60% of the population. Medium cells declined in abundance to approximately 10% on day 12 after which they increased to around 35% on day 35. After addition of sea water the concentration of large cells fell and remained constant until day 35 when there was a slight increase in their number. With the addition of sea water plus the full recommended dose of nutrient changes in the proportion of cells mimicked those seen in the half nutrient dose vessel until day 12. Thereafter the declines and increases in the proportion of small and medium sized cells were again noted however, the degree to which these changes occurred was less pronounced and there was no increase in large cells towards the end of the experiment.

Figure 32. Cell size differentiation at three levels of nutrient addition. The parent population was tracked till day 8 when it was split into three separate cultures. At that time water and nutrients were added to make up 5-l cultures. Circles = large cells, Diamonds = medium cells, Triangles = small cells. A - only filtered sea water added; B - sea water and half the nutrient dose recommended by Harrison *et al.* (1980); C - sea water and the full dose of recommended nutrients. Arrows show when bioassays were preformed on each culture.


A
B
C
Relative Cell Concentration
100%
80%
60%
40%
20%
0%
0
5
10
15
20
25
30
35
Day

In addition to numbers and size of cells the proportion of various shapes of cells also varies with nutrient dose (Figure 33). In the parent culture (day 0 to 8) the proportion of smooth cells declined, bumpy cells increased and both cells with 1-2 and 2+ protuberences remained unchanged. In the vessel with only sea water added (Figure 33a), the smooth cells declined to 0% by day 20 then rose to 7% of the population. Bumpy cells also dropped to 0% on day 20 before rising to 4% on day 35. In the same vessels the portion of 1-2 protrusions and 2+ protrusions all dropped to zero on day 20 then recovered to 3 and 1% of the population respectively. In the vessel with sea water plus half the recommended amount of nutrients (Figure 33b) bumpy cells and 1-2 protuberance cells rose to 26% and 13 % of the population on day 28. Cells with 2+ protuberances never rose above 2% percent of the population. In the container with sea water plus the full dose of recommended nutrients (Figure 33c) bumpy cells and 1-2 protuberance cells rose to 38% and 17 % of the population on day 20. Cells with 2+ protuberances never rose to 3% percent of the population.

Toxicity responded in relation to the amount of nutrients supplied. Cultures receiving more nutrients killed mollies more rapidly at the peak of measured toxicity (Day 20) in each of the cultures. Toxicity in all cultures varied over the culture cycle. It was undetectable on day 12, by day 20 it peaked and at day 28 and, in culture c, day 35 it declined (Figure 34).

Figure 33. Cell shape differentiation at three levels of nutrient addition. The parent population was tracked till day 8 when it was split into three separate cultures. At that time water and nutrients were added to make up 5-l cultures. Circles = smooth cells, Bars = bumpy cells, Triangles = cells with 1 or 2 protuberances, Diamonds = cells with more than 2 protuberances. A - only filtered sea water added; B - sea water and half the nutrient dose recommended by Harrison *et al.* (1990); C - sea water and the full dose of recommended nutrients. Arrows show when bioassays were preformed on each culture.

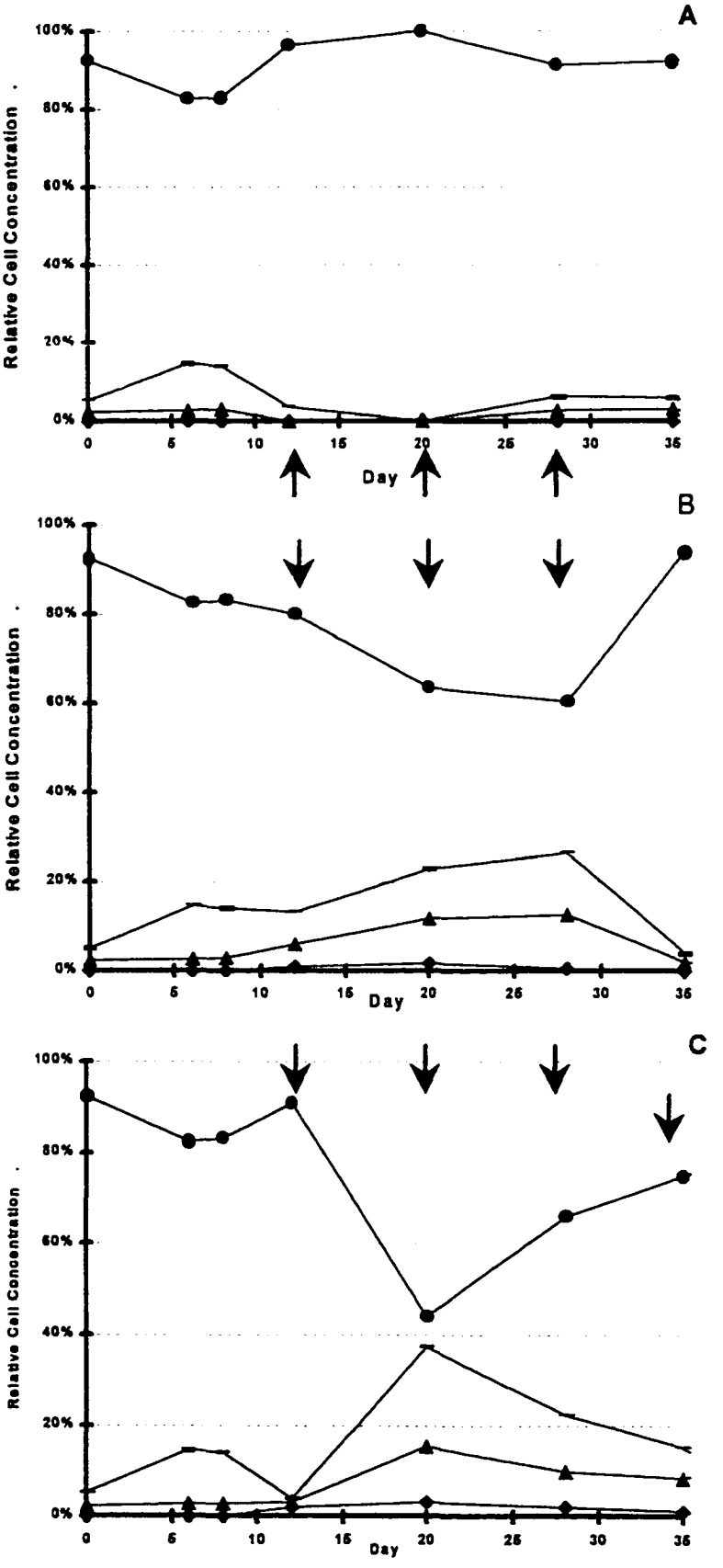
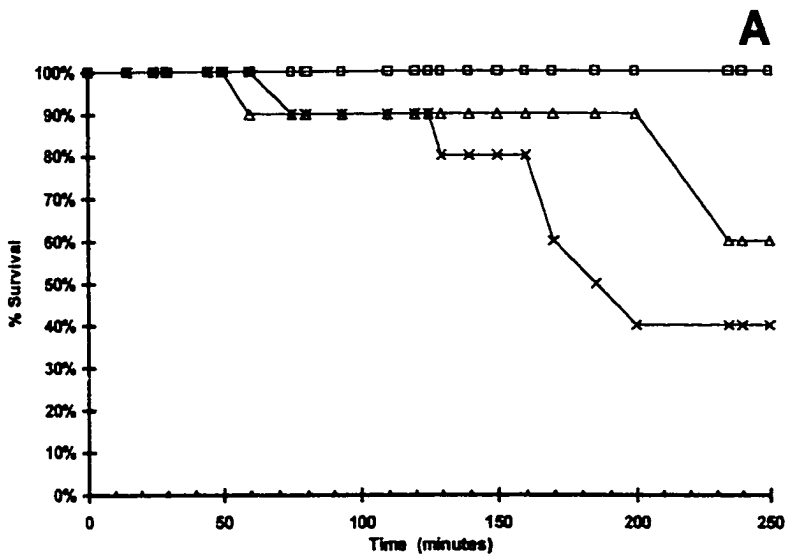
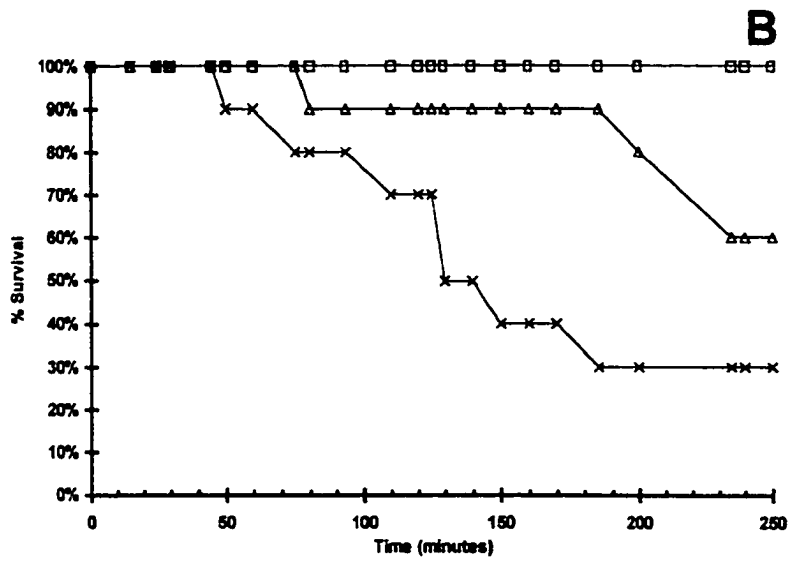
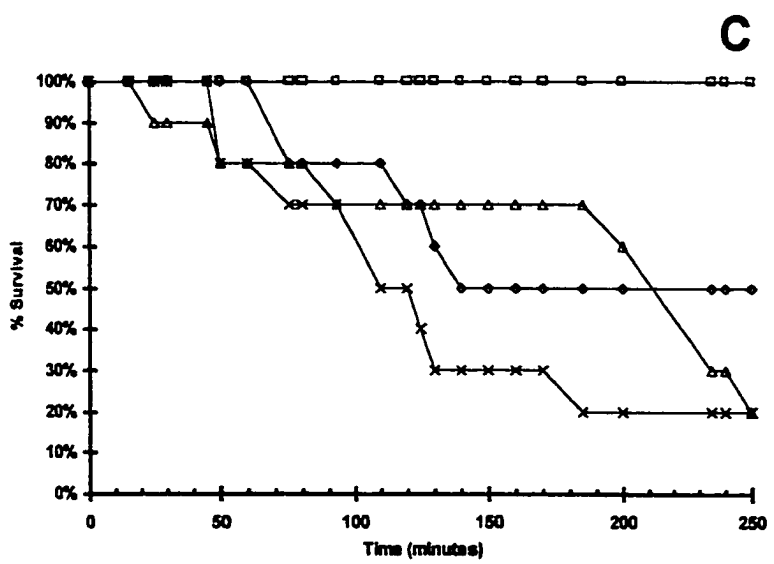

A
B
C
Relative Cell Concentration
100%
80%
60%
40%
20%
0%
0
5
10
15
20
25
30
35
Day

Figure 34. Culture toxicity at three levels of nutrient addition. Top - only filtered sea water added; Middle - sea water and half the nutrient dose recommended by Harrison *et al.* (1980); Bottom -sea water and the full nutrient dose. Circles = controls, Squares = day 12, Crosses = day 20, Triangles = day 28, Diamonds = day 35.


A
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Survival
0
50
100
150
200
250
Time (minutes)


B
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Survival
0
50
100
150
200
250
Time (minutes)


C
100%
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0%
% Survival
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50
100
150
200
250
Time (minutes)

7.0 Discussion of Studies of Cultured Populations

7.1 Nutrient Uptake

The importance of cell morphology generally, and in particular cell size and shape, has been central to many discussions of phytoplankton ecology . So pervasive is the topic of cell size that writing about phytoplankton ecology Chisholm (1992) stated "....plankton ecologists fall out into two groups: Those who delight in finding the patterns in nature that can be explained by size, and those who delight in finding exceptions to the size-dependent rules."

Comparisons of size differences among species have focused on how those size differences affect the cell's role in ecosystems (e.g. Chisholm 1992, Kiørboe 1993, Longhurst 1989, Warwick and Joint 1987). Research has demonstrated that, during summer months, differences in cell size of even a few μm may be significant in inter-species competition (Chisholm 1992, Morel et al. 1991). For example, Pasciak and Gavis (1974) demonstrated for *Cyclotella nana* that a doubling of the cell size diameter from 2 to 4 μm was associated with a 6 fold increase in competitive ability to take up nitrate.

These comparisons between species often specifically examined the effect of size on the uptake of inorganic nitrogen in marine waters. Results of nitrate and ammonium uptake measurements taken in the field suggest larger plankton are more dependent on

nitrate, and the smaller pico- and nanoplankton prefer ammonium (Probyn 1985, Owens *et al.* 1990).

Uptake and storage of those nitrogen species appear to differ in large and small cells. Larger plankton, with their greater cellular volume, have more room for vacuoles in which they can store inorganic nitrogen (Dortch 1982). This gives the larger cells not only a greater capacity to store nitrogen but also an enhanced ability to supply nitrogen to its metabolism over periods when inorganic nitrogen is absent or limited in the cell's external environment. Consequently, large cells tend to dominate in environments with fluctuating nitrate levels.

At the other extreme, small cells are more limited in the volume available to store nitrogenous nutrients, therefore they specialize in rapid uptake and incorporation of nitrogen into the metabolism. This becomes an advantage for cells which utilize the ammonium that dominates in the surface stratified waters of late summer when nutrient levels in surface waters are low and vary slowly. Ammonia is generally not stored in large quantities in phytoplankton cells (Stolte and Riegmen 1996). In addition it is subject to more leakage from the cell, as a portion of cytosolic ammonium de-ionizes into ammonia, which can then passively diffuse down a concentration gradient across the cell membrane and back into the environment. Thus, unlike nitrate that once in a cell is trapped there and can be utilized over longer periods of time, ammonium molecules must be moved as rapidly as possible from the environment and incorporated into non-diffusible metabolites. To prevent creation of a nutrient gradient encouraging leakage of ammonia out of the cell,

the ammonium nitrogen must rapidly cross the plasmalemma, move through the cytoplasm, across the chloroplast wall where it can be captured by the glutamine/glutamate synthetase enzyme system and incorporated into amino acids. To make this transition time as short as possible it is necessary to keep the distances traveled as short as possible hence the advantage of having a smaller cell volume.

Heterosigma is small but not one of the smallest phytoplankters. The range of phytoplankton cell volume is from 1 to 10^6 μm^3 (Stolte and Riegman 1996) with the lower limit for oxygenic phototrophes being 1 μm^3 (Raven 1994) and the lower limit for eukaryotic cells being 2 μm^3 (Courties *et al.* 1994). Given the variable morphology of *Heterosigma*, precise estimation of cell volume of this species is not possible, however, Cattolico (1978), and Thompson *et al.* (1991) approximated it to be between 4.9 $*10^2$ and 1.8 $*10^3$ μm^3.

Some authors have noted changes in the size of *Heterosigma* cells (Cattolico, 1978, Thompson *et al.* 1991, Langdon 1986, Kohata and Watanabe 1986). However, it is difficult to compare much of that work directly to this study. Some authors report cell volume based on measurements under a microscope and an assumption of constant cell shape. A few authors also conform to the approach taken herein and report shifts in volume across Coulter Counter channels. (A shift of one channel in a Coulter Counter represents a nominal doubling of cell volume.) Within the literature reporting size ranges based on measeurments with a microscope, there are considerable differences in volumes

seen. Cattolico (1978) and Thompson *et al.* (1991) suggest a size range in excess of 3-fold. That is significantly greater than the 1.5-fold increase noted by Langdon (1986). The Coulter Counter based work of Kohata and Watanabe (1986) suggests the range in volume spans three channels (a 4-fold shift in size) which is similar to the shift (small-medium, and medium-large) observed in this study (Figures 23 and 25). Shifts in the size of *Heterosigma* cells in natural populations, while not formally and quantitatively documented, have been seen by myself and by other individuals examining wild populations at fish farms (L. Sams pers. comm.).

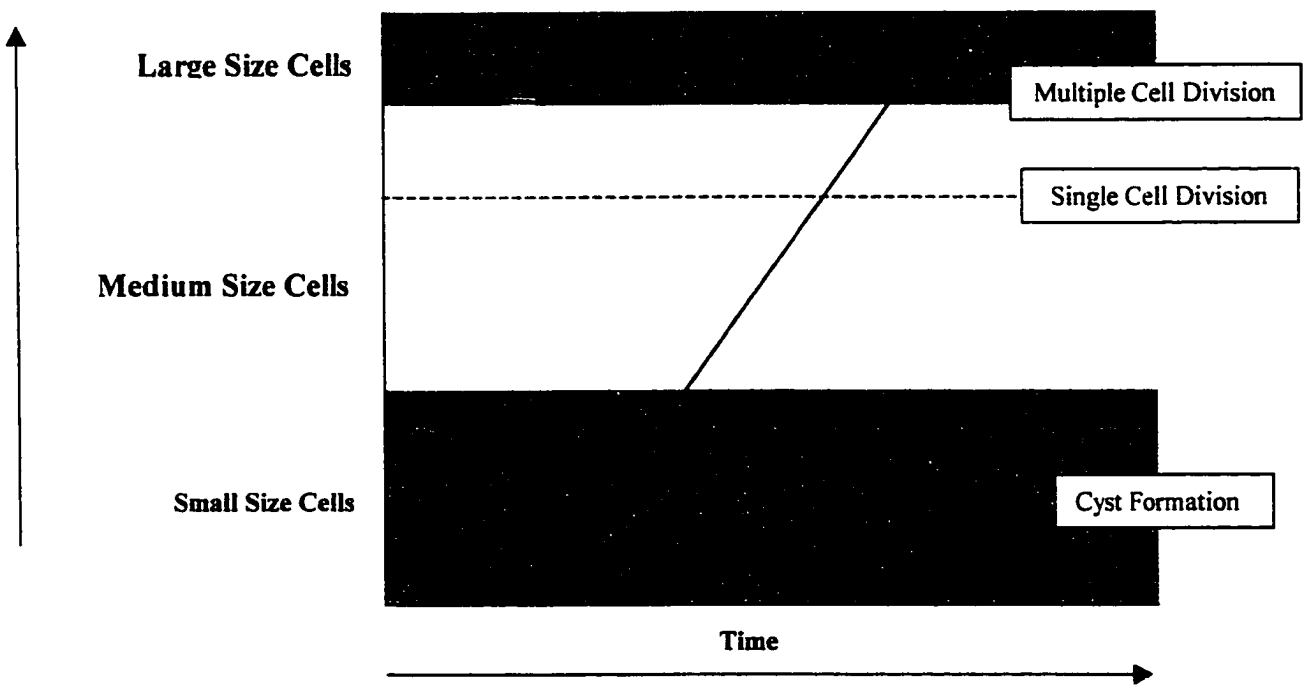
The significance of variation in cell size within a species has been discussed by a number of authors (Dor 1985, Grover 1989, Gavis 1976, Swift and Meunier 1976, Thompson et al. 1991). Interpretation of the adaptive significance of changes in cell size within a species however, can be difficult. Changes can be driven by either physical or biological variables. For example, volume changes can occur due to conservation of mass during cell division or changes can be initiated by changes in the cell's external osmotic environment. Changes based on physical requirements alone are unlikely to be subject to natural selection. It is easy to imagine however, that changes under metabolic control could be selected for. To separate these types of factors, controlled experimental conditions are required that would be difficult to achieve under field conditions. Laboratory work has supplied some information in this area.

While Coulter Counter data is useful in interpreting long-term gradual changes in cell numbers and sizes in an algal population, it is extremely difficult to interpret changes in cell size as seen by the Coulter Coulter in samples separated by 48 hrs or less or the difference seen between two consecutive samples.. To illustrate this point consider the following possible model of growth and consequence data as it might be detected by a Coulter Counter (Figures 35 - 37). Events in each diagram represent potential cellular events that can occur in 48 hours following a cell's exposure to a nutrient source. (A day and night for nutrient uptake; a day for metabolic incorporation of the nutrients into metabolic products; and, a night for cellular division).

Figure 35 is a generalized diagram that illustrates several features of *Heterosigma* cell growth. The cell's potential growth is represented by the gray arrow. There are several size thresholds along this trajectory. One of these is for the formation of cysts. Below this size there are inadequate cellular reserves to form viable cysts. Similarly, there are minimum size thresholds for a cell to undergo single or multiple division(s) in a dark period. The precise cell sizes for these thresholds have not been quantified and the relation of the cell sizes to Coulter Counter cell size is hypothetical.

It is also important to note that these thresholds do not imply an obligatory cell response. It seems likely that some assessment of the cells external as well as its internal environment (cellular reserves) would be involved in the decision for cell division or cyst formation. For example if a cell achieved the threshold for encystment but nutrients were still very abundant some or all of the cells may opt for continued growth rather than

Figure 35. A conceptual diagram of the dynamics and problems of Coulter Counter detection of *Heterosigma* cell division. Cell sizes detectable by the Coulter Counter (small, medium and large) are shown as wide gray or white bands on the graph. Horizontal dashed lines indicate minimum threshold cell size for the formation of either cysts or cell division. The gray line with an arrow-head represents potential cellular growth.

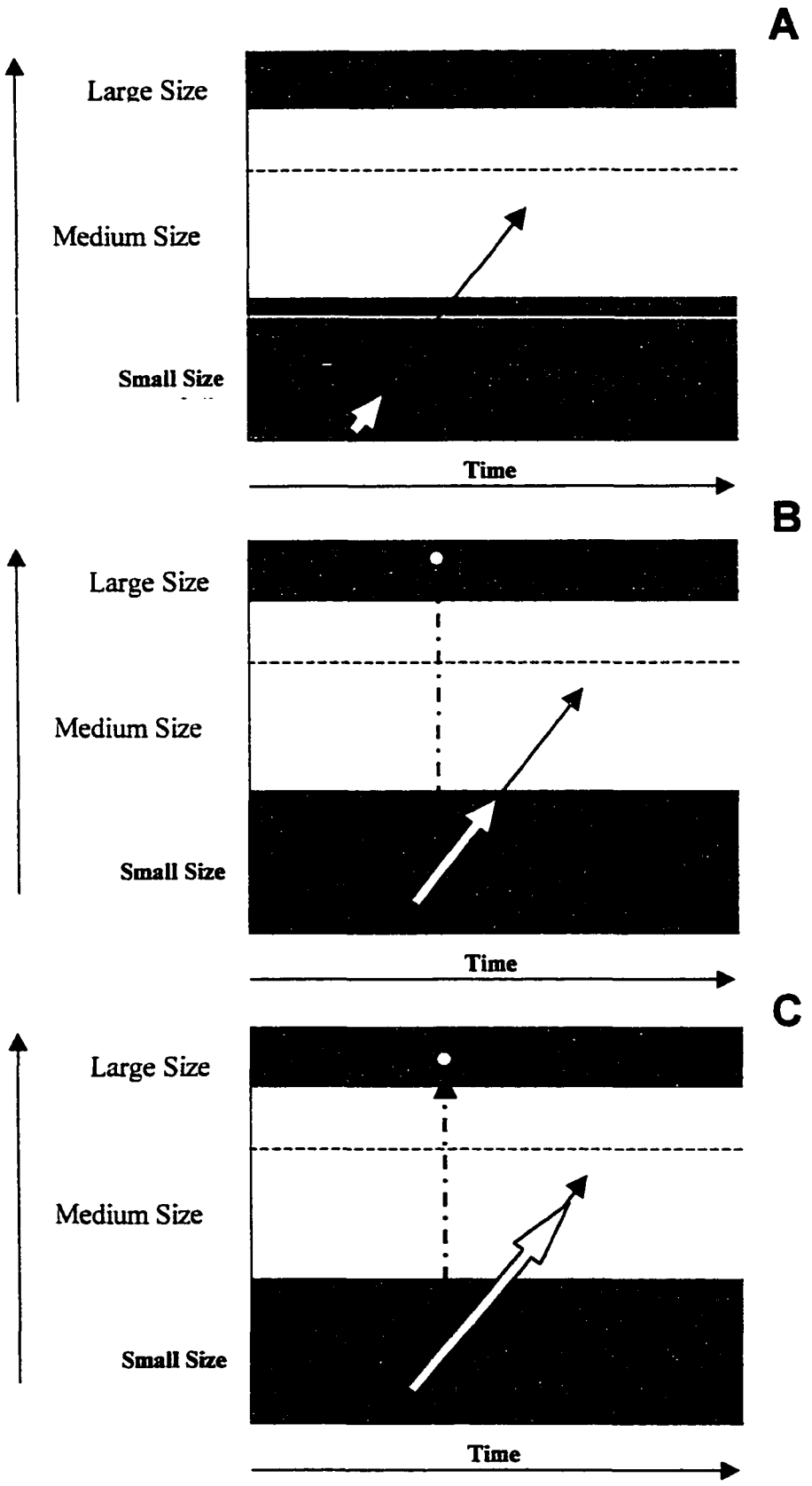

Large Size Cells
Medium Size Cells
Small Size Cells
Multiple Cell Division
Single Cell Division
Cyst Formation
Time

encystment. If, in contrast, the cell had experienced a period of declining nutrient availability and then experienced a pulse of nutrients the cells may opt for encystment. Similarly, a cell has the option for continued cell growth after the threshold for a single division is achieved. The key to exercising that option may hinge on whether the cell is in an environment of continuing nutrient abundance or on whether nutrient abundance has declined. In an environment of continuing nutrient abundance the cell might delay division to enable further accumulation of nutrient that would enable multiple division. Nutrients in the above are used as one possible trigger in the decisions to excyst or divide. There are others including light and temperature. Further, one or more of these and other environmental parameters might be involved in triggering these decisions. In spite of these limitations and uncertainties, the above model is useful to illustrate difficulties in discussion of cell sizes based only on Coulter Counter data.

In populations after nutrients are added it may not always be possible to detect a change that has occurred in the population. Figure 36a illustrates that cell growth may occur without detectable changes in the numbers in the population or the distribution of cell sizes shown by the Coulter Counter.

Even where numbers in a population decline over 48 hrs it would be difficult to understand the implications of changes in cell numbers and size. Figure 36b shows that nutrient addition may result in non-detectable cellular growth that is however, adequate to stimulate cyst formation. Consequent Coulter Counter readings would show a shift in cell size that might include a decline in small cells and increase in large cells. Without

Figure 36. Effect of three levels of nutrient addition on Coulter Counter data. **A.** Addition of nutrients adequate to cause undetectable cell growth but inadequate to cause cyst formation or cell division. **B.** Addition of nutrients to cause undetectable cell growth but adequate for cyst formation. **C** Addition of enough nutrients to cause detectable cell growth, and cyst formation but inadequate to attain enough cell growth to allow cell division. The base diagram is as in Figure 35 with addition of the broad white arrow to represent growth realized as a result of the amount of nutrients accumulated, and the line of dots and dashes to represent generation of cysts.


A
Large Size
Medium Size
Small Size
Time
B
Large Size
Medium Size
Small Size
Time
C
Large Size
Medium Size
Small Size
Time

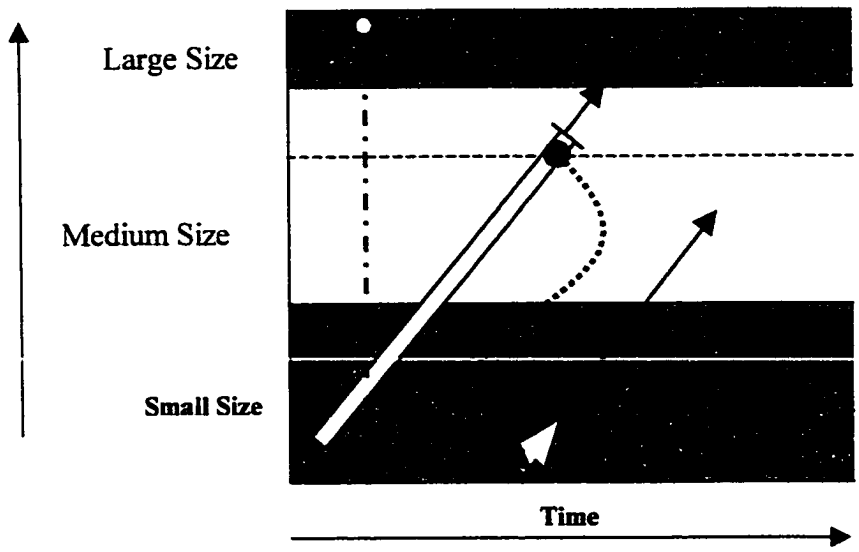
supplementary information (Is there a decline in cell concentration? What portion of the cells became cysts?) it is impossible to determine if the shift in 36b represents growth to large cells some of which have divided to produce more small cells or if only formation of cysts was involved, or if both formation of large cells and cysts were involved. Figure 36c illustrates detectable cell growth and cyst formation that may appear in the readings as an increase in medium and large cells. Even with knowledge of a decline in cell concentrations the Coulter Counter Data in figure 36c could represent growth of cells to the medium and large sizes as well as it could represent growth only to medium sized cells and formation of cysts.

Even where the population numbers are increasing it is difficult to accurately evaluate what is occurring with cell size. For example, without some knowledge of the relative proportion of cells encysting the Coulter Couter data from two different levels of nutrient addition might look similar. In figure 37a just enough nutrients were added to permit the cells to just pass the threshold for a cell division. The resultant daughter cells would show up as small cells. In figure 37c enough nutrients were supplied to permit the cell to grow very large and divide more than once. This would also show up as a proliferation of small cells in the population. If the later scenario also resulted in an increased proportion of the cells turning to cysts the resultant Coulter Counter data in figures 37a and 37c might be indistinguishable.

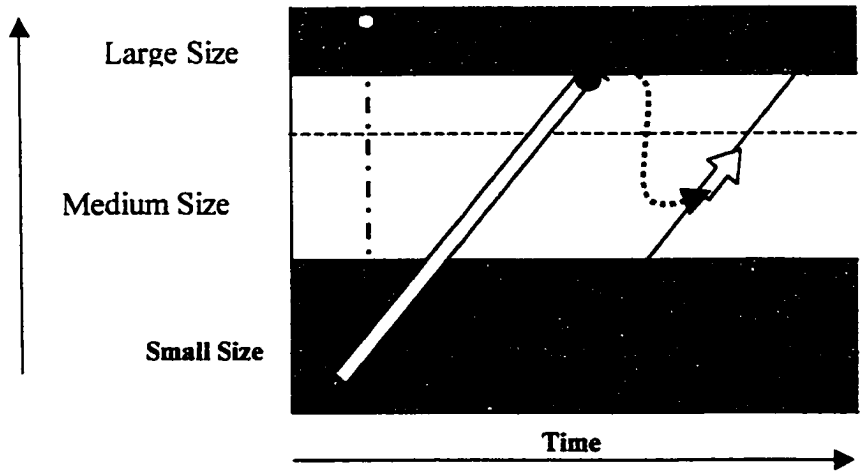
While division of a cell results in the creation of smaller cells it need not result in the creation of small-sized cells as seen by the Coulter Counter. Figure 37b illustrates

Figure 37 Effect of three higher levels of nutrient addition on Coulter Counter data. **A.** Addition of nutrients adequate to cause cyst formation detectable and a single division into small daughter cells. **B.** Addition of enough nutrients to cause cyst formation and a single division into medium sized daughter cells. **C.** Addition of enough nutrients to cause cyst formation and division of cells into daughter cells of a size adequate to immediately undergo another division into small cells. The base diagram is as in Figure 35 and 36 with addition of the curved dashed line indicating cell division and daughter cells the size of which depends on the size of the dividing parent cell.

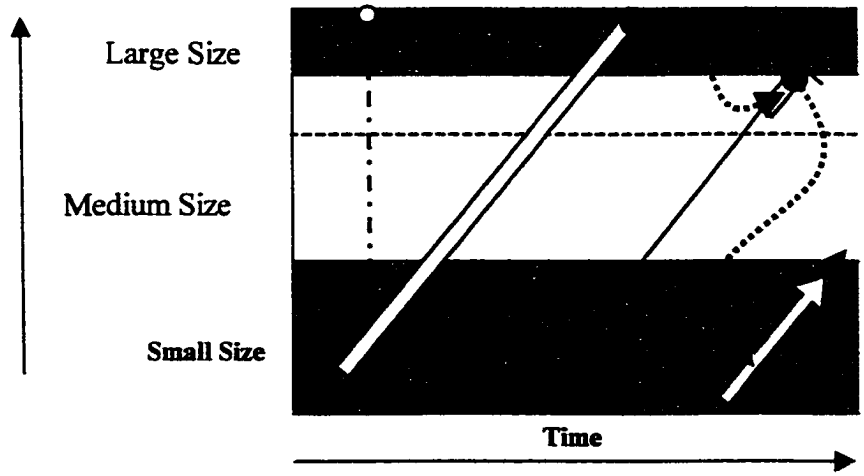
A


Large Size
Medium Size
Small Size
Time

B


Large Size
Medium Size
Small Size
Time

C


Large Size
Medium Size
Small Size
Time

cells being given more than enough nutrient to attain a single division but not enough to attain multiple divisions. Consequent shifts in Coulter Counter data would show an increase in numbers of medium cells rather than small cells.

In spite of these severe limitations some information can be deduced from the cell-size changes seen in this study. Changes in the size of cultured *H. akashiwo* in this study do not appear to be entirely dependent on cell division (Figure 19 and 20). When re-fertilized cultures make a second increase in the proportion of small cells there was no increase in population size. The possiblity exists that these changes could be brought about by changes in the external osmotic environment associated with addition of nutrients to the culture. This seems unlikely as addition of the nutrients to sea water has little effect on osmolartity (Harrison *et al.* 1980), and if the changes in cell size were a passive response of the cells brought on by changes in the external osmotic environment associated with the addition of new nutrients, the changes would occur within minutes after exposure to a new osmotic regime. In Figures 20, 23, 25 and 32 the changes in cell sizes require days to complete and thus may be under biological control.

Some factors driving changes of cell size in wild and cultured populations have been documented. Studies outlined below suggest these changes are not a passive response to the external environment but may be the result of the metabolic activity of the cell. For example increases in size follow active uptake of dissolved inorganic nitrogen (DIN). Thompson *et al.* (1991) found that DIN, supplied to *Heterosigma* only in the form

of ammonium, was responsible for increases in cell size and that neither changes in other HESW (Harrison *et al.* 1980) nutrients or light levels caused a comparable change in cell volume to those seen in this work. The authors concluded that while photon flux density had a minor effect, increases in cell size were primarily effected through transport of ammonium across the cell membrane. In this study cell expansion occurred when all ammonium was replaced by nitrate (this is the original HESW formulation of Harrison *et al.* 1980). Thus, it appears that uptake of nitrate as well as ammonium can initiate changes in cell size.

Light appears to have a major effect on cell size. In the two un-agitated cultures of Figure 23 (a and c) the same illumination was applied. The culture represented by Figure 23c is in a flat (3 cm thick) container. The culture represented by Figures 23a was in a thicker (24 cm) spherical container thus cells in this container would, on average, be exposed to lower light levels due to attenuation of the light passing a greater distance through the medium. The algae experiencing better light conditions (Figure 23c) showed an increase in cell size that was more pronounced (large-sized cells increased from almost 0% to 40% of the population) than was evident in the spherical culture container (Figure 23a where large-sized cells increased from almost 0% to approximately 11% of the population).

The cause of the difference in cell size response between this study and those of Thompson *et al.* (1991) may have its origins in the chemical structure of nitrogen species

being moved across the plasmalemma. Nitrate uptake is known to utilize energy to move the nutrient into the cell. Nitrate uptake is mediated by membrane-bound molecules which require energy to move nitrate across the plasmalemma (Huppe *et al.* 1994, Farr *et al.* 1994). When nitrate is present it activates the oxidative pentose phosphate (OPP) pathway which can supply energy from starch reserves. In the presence of light, the OPP pathway is suppressed and the energy for nitrate uptake comes directly from photosynthesis (Huppe *et al.* 1994, Farr *et al.* 1994). Ammonium uptake is also believed to require energy (Loban and Harrison 1994) and is associated with carrier molecules (Kleiner 1981). The source or sources of energy are not known. It is clear however, that the presence of ammonium does not stimulate activation of the OPP pathway (Huppe *et al.* 1994). Thus, with different mechanisms effecting delivery of energy for nitrogen uptake, it is not unreasonable to expect that the uptake dynamics of the two types of nitrogen sources (ammonium and nitrate) would differ.

The importance of nitrogen uptake to changes in cell size is that changes in cytosolic nitrogen may cause water to flow across the cell membrane to balance the change in osmotic pressure created by the uptake of DIN. These changes in cell size would not therefore, be passive responses to changes in the external environment but would be caused by changes in the internal cell environment, which in turn are generated by metabolic processes upon which natural selection can be effective.

The lack of rigid silica or cellulose components in the cellular structure of *Heterosigma* cells pre-adapt it for changes in cell volume that may be of adaptive significance for its particular life history. Unlike diatoms and dinoflagellates, *Heterosigma* has no rigid external casing to the cell that might limit cellular expansion. Lacking a cell wall, armor plates or other external rigid structures allows *Heterosigma* to more effectively accumulate internal DIN stores. This would be a particularly useful feature in a cell, like *Heterosigma*, that may have limited time for nutrient uptake because of limitation on nitrogen uptake associated with diel vertical migration. In nitrogen uptake associated with diel vertical migration the cell has to migrate from surface waters to the nutricline and back to the surface in the dark. In the short summer nights this limits the time that the cell can spend at depth taking up nitrogen. A mechanism which prevents the build up of internal pressure caused by swelling of the cell against a rigid encasing structure would enhance uptake of the nutrient by reducing pressure which might create a reverse osmotic pressure forcing free cytosolic ammonia from the cell.

In addition to changes in cell size, changes in shape can have an important effect on the ability to take up nutrients. In their seminal paper "Absorption of nutrients by aquatic plants" Munk and Riley (1952) examined the potential effect of changes in shape on nutrient uptake. They examined three generalized cell shapes: spheres, plate-like volumes (oblate spheroids) and long tubular or football like volumes (prolate spheroids). Of these a sphere is the least beneficial shape for nutrient uptake. For the same cell

volume a plate-like shape was better and a long tubular shape was the best shape for nutrient up-take.

Gavis (1976) mathematically defined the environmental conditions under which shape could be expected to play an important role in the cell's competitive ability for nutrients. He further explored the effect of cell shape on nutrient uptake using the Monod type Equation (1) to describe nutrient uptake kinetics (Table X).

$$V = V_m C/(K+C) \qquad (1)$$

Choice of this mathematical description for nutrient uptake would not be appropriate for all algal species or for all environments. The description is based on the assumption that the nutrient in question is limiting and that the environmental levels of that nutrient are stable for extended periods. This description also implies that the effect of internal stores of the nutrient is negligible on V and K. These assumptions would be seriously violated most of the year (fall, winter, spring, and much of summer) when nutrient levels are high. They would also be violated at the end of the summer as a shallow weak pycnocline is repeatedly formed and broken down by wind and other forces causing nutrient concentrations to fluctuate markedly. The assumption that internal nutrient stores have negligible effects on V and K is also likely to be violated in a nutrient replete environment. If cells did not have a way of limiting V they would accumulate nitrogen in the cytosol until the consequent uptake of water caused the cell to lyse.

Table X. Definition of model parameters and values derived from the literature.

Parameter	Definitions from Gavis (1976) and (measurement units)
V	Rate of nutrient uptake (μmol/cell/hr)
V_m	Maximum rate of nutrient uptake (μmol/cell/hr)
C	Ambient concentration of nutrient in the environment (μM)
K	Half saturation concentration (μM)
C_o	Concentration of nutrient at cell surface (μM)
P	influence of diffusion transport on nutrient uptake rate (non-dimensional scaling factor)
R	linear dimension characteristic of organism's semi-major axis or radius (cm)
A	semi-minor axis length (cm)
α	shape factor (non-dimensional scaling factor)
D	Diffusivity of nutrient (cm^2 sec^{-1})

Parameter	Value	Reference
V_m for NH_4^+	0.022 μmol $cell^{-1}$ hr^{-1}	Tomas1979
K for NH_4^+	2.00 μM	Tomas1979
D	1.5 X 10^{-5} cm sec^{-1}	Pasciak and Gavis 1974

The situation with toxic *Heterosigma* blooms may however, represent one instance where the application of this description is appropriate. Toxic *Heterosigma* blooms often occur in the late summer, at a time when freshwater input to the ambient environment is at the yearly minimum. During this period warming of surface waters over a protracted period results in a deep pycnocline. Under these conditions *Heterosigma* would not be able to regularly access nutrients by vertical migration, the cell's internal stores of nutrients would become depleted, and thus the cell's nutrient uptake kinetics would be adequately described by the Monod equation.

Events at the plasmalemma / environment boundary define conditions under which cell shape can modify nutrient uptake rates. Where the rate of nutrient uptake (V) exceeds the rate of diffusion from the ambient environment to the cell surface, nutrients in the area bordering the cell surface (C_o) become depleted (i.e. $C > C_o$), and nutrient uptake is limited by diffusion rate to the cell surface.

The Monod equation for diffusion-limited nutrient transport was reformulated by Gavis (1976, equation 2) as:

$$V = V_m C_o /(K+ C_o), \text{ where } C_o < C \qquad (2)$$

Because both V_m and K are fixed physiological-based processes, C_o is the only parameter which can be influenced by shape. Under these conditions C_o is the effective

concentration of the nutrient at the cell surface. Gavis (1976, equation 3) developed a quadratic description of the dynamics of nutrient diffusion:

$$(C_o/K)^2 + (1/P + 1 - C/K)\,(C_o/K) - (C/K) = 0 \qquad (3)$$

Pasciak and Gavis' (1974) defined rules for application of this formula by examining two limiting cases for the value of 1/P. They noted that when 1/P is much larger than the absolute value of (1-C) then $C_o = PC$. Thus as P decreases, cellular uptake of nutrients is more effective at reducing C_o without any change in C. When 1/P is much smaller than 1-C then $C_o = C$ because the rate of uptake of nutrients is equal to or less than the rate at which the nutrient can diffuse from the environment to the cell surface.

Within equation 3, P is a unit-less factor quantifying the influence of diffusion on nutrient uptake. P is defined as:

$$P \equiv 14.4\,\pi\,R\,\alpha\,D\,K/V_m \qquad (4)$$

Where K and V_m are physiological parameters, D is a physical constant, R describes the size of the cell and α describes the shape of the cell. The mathematical descriptions of simple forms such as spheres, tubes and plate used to approximate cell shapes were derived by Gavis (1976) using the "uniformly accessible surface" approximation wherein:

- for spheres $\alpha = 1$
- for prolate spheroids (tube-like shapes), $\alpha \equiv 2\,e/\ln[(1 + e)/(1 - e)]$, and $e = 1$ for purely tubular shapes

- for oblate spheroids (plate-like shapes), $\alpha \equiv e / \tan^{-1} (e/\sqrt{(1-e^2)}) = e/\sin^{-1} e$, and $e = 1$ for purely plate shaped objects

In the cases of prolate and oblate spheroids $e = (1 - a^2/R^2)^{-1/2}$.

The effect of changes in shape of an algal species on the uptake dynamics of a specified nutrient can be approximated using the above formulae. Ammonium has been chosen as the nutrient of interest because when *Heterosigma* blooms, that nutrient is the most abundant source of nitrogen, yet it is at the same time at concentrations that limit the alga's growth.

Parameters used for these calculations in tables XI and XII are listed in table X. Values for cell dimensions and environmental concentrations of ammonium are taken from data reported earlier in this work (Section 6.1 and Figure 15).

Changes in cell shape have the greatest potential to impact nutrient uptake dynamics when nutrient uptake is limited by diffusion from the environment to the cell surface. The potential for diffusion-limited nutrient uptake (DLU) can be evaluated from calculated values of the form describing parameter P. Pasciak and Gavis (1974) suggest values less than 2 represent the occurrence of DLU. As can be seen in table XII, values of P for the various shapes of *Heterosigma* are all an order of magnitude less than 2 for both

ammonium and nitrate concentrations found during the advent of the bloom on the sunshine coast.

As noted earlier *Heterosigma* first necessarily undergoes changes in size associated with cell division associated with population growth then it changes cell shape (here we are referring to both the flattening of the cell and the development of protuberances.). The reason for this may laie in a hierarchy of benefits to changing shape and size. Table XI demonstrates the effect on competitive ability (1/P) for ammonium of reducing *Heterosigma* cell volume without changing cell shape. That effect is marked. Reducing cell volume by half reduces P by 21%. A further reduction by half brings P down another 17%. Thus in a nutrient-limited environment reduction of cell volume through cell division could significantly improve nutrient uptake.

Elaboration of cell shape provides further competitive advantage to the cell. The move from a prolate sphere to an oblate sphere could reduce P a further 7.5%. Presumably the elaboration of protuberances further improves the cell's competitive ability. Mathematical description of this form is difficult because the number and shape of the protuberances is variable (Plate 1).

Table XI. The effect of a reduction in volume of a spherical *Heterosigma* cell on diffusion-limited nutrient uptake. A lower value of P indicates improved diffusion-limited nutrient uptake.

	Full volume	1/2 volume	1/4 volume
R (μ)	9	7.15	5.65
V_{max} (μmole cell^{-1} hr^{-1})	3.70E-07	3.70E-07	3.70E-07
K (μM)	2.00	2.00	2.00
D (cm sec^{-1})	0.000015	0.000015	0.000015
P	3.30E-01	2.62E-01	2.07E-01

Table XII. The effect of changing *Heterosigma akashiwo* cell shape and motility on diffusion-limited nutrient uptake. A lower value of P indicates improved diffusion-limited nutrient uptake.

	For NH_4^+ Limitation			For NO_3^- Limitation		
	H. akashiwo	*H. akashiwo*	*H. akashiwo*	*H. akashiwo*	*H. akashiwo*	*H. akashiwo*
	Prolate	Oblate	Sphere	Prolate	Oblate	Sphere
α	0.85	0.79	1.00	0.85	0.79	1.00
R (μm)	9	9	9	9	9	9
a (μm)	7	3.5		7	3.5	
e	0.629	0.921		0.629	0.921	
V_{max} (μmole ce	3.70E-07	3.70E-07	3.70E-07	5.80E-07	5.80E-07	5.80E-07
K (μM)	2.08	2.08	2.08	2.23	2.23	2.23
D (cm sec^{-1})	0.000015	0.000015	0.000015	0.000015	0.000015	0.000015
P (Non-motile)	2.92E-01	2.70E-01	3.43E-01	2.00E-01	1.85E-01	2.35E-01
u (μm sec^{-1})	145.000	145.000	145.000	145.000	145.000	145.000
p' (Motile)	4.19E-01	3.88E-01	4.93E-01	2.87E-01	2.65E-01	3.37E-01

Not all shape changes however, are adaptive to overcoming DLU. When *Heterosigma* moves towards the resting stage morphology or swells up into the smooth bean-shaped morphology in response to increased nutrients, the advantage for uptake of low concentration of nutrients is lost. The increase in P between prolate and spherical forms is approximately 17%. This is not surprising as resting stage cells should not require nutrients so a decreased ability to compete for DLU is not a competitive disadvantage to the cell during this stage of its life cycle. Similarly, if nutrients are abundant the shift to smooth cells is also not a competitive disadvatage.

Comparison of the ability of different species to compete for nutrients based on the shape factor is difficult, because values of P has been calculated for few algal species. A starting point would be to examine *Heterosigma* relative to a number of non-motile cells. Prasciak and Gavis (1974) examined 8 species of diatoms for their nitrate competitive value using the above approach. Table XIIIa is a subset of those data selected for a range of cell sizes and extreme values of P. Of the species they examined only *Coscinodiscus lineatus* is a wide spread oceanic species and only this species has a P value low enough to suggest that DLU might be a significant consideration in its life history. As can be seen in Tables XI and XII *Heterosigma* is within the cell size range of the species in table XIIIa, but has a P value lower than any of the diatom species in table XIIIa.

Table XIII. Comparative values of P and P' for motile and non-motile phytoplankton. (A) non-motile species from Pasciak and Gavis 1974. (B) Motile species from Prasciak and Gavis 1974, and from Gavis 1976.

(A) Non-motile Species

	D. brightwelli	*N. closterium*	*C. lineatus*	*C. nana* 3H
α	1.00	1.00	1.00	1.00
R (μm)	75	15	25	2
a (μm)				
e				
V (μmole cell^{-1} hr^{-1}	1.25E-06	1.30E-08	9.00E-06	7.40E-09
K (μM)	0.6	2.8	2.8	1.87
D (cm sec^{-1})	0.000015	0.000015	0.000015	0.000015
P	2.44E+00	2.19E+02	5.28E-01	3.43E+01

(B) Motile species

	G.splendens	*P. minimum*	*D. tertiolecta*
α	1.00	1.00	1.00
R (μm)	20	10	4
a (μm)			
e			
V_{max} (μmole cell^{-1} hr^{-1}	4.00E-05	4.50E-08	1.07E-08
K (μM)	15	8.5	1.4
D (cm sec^{-1})	0.000015	0.000015	0.000015
P	5.09E-01	1.28E+02	3.55E+01
Re=	0.008	0.001	0.0016
Pe=	5.33	0.67	1.07
u (μ sec^{-1})	2.00E+02	5.00E+01	2.00E+02
P'	9.0E-01	1.50E+02	4.4E+01

Motion of the cell through the water increases the rate of transport of nutrient from the environment to the cell (effectively increasing the value of P). Pasciak and Gavis (1974) described the effect of movement on nutrient uptake dynamics on DLU. In their model the shape factor P for a cell in motion at speed u, is designated as P' and is defined as:

$$P' = P (1 + (uR / 2D)) \qquad (5)$$

Estimates of the rates at which *Heterosigma* moves through the water are variable. The upper limit to the velocity of planktonic cells appears to be about 300 $\mu m\ sec^{-1}$ (Lewin 1962). Hatano *et al.* (1983) calculated *Heterosigma*'s rate of decent at 139 $\mu m\ sec^{-1}$ and Throndsen (1973) gave similar values of between 130 to 160 $\mu m\ sec^{-1}$. The lower extreme of estimated velocities for this alga is Hatano *et al.*'s (1983) suggested rate of ascent of 10 $\mu m\ sec^{-1}$ which corresponds well to the estimate of Bauerfeind *et al.* (1986) of 20 $\mu m\ sec^{-1}$. These values however, are derived from estimates based on the rate of migration of a population in a water column. As has been mentioned earlier *Heterosigma* changes direction of its swimming motion abruptly and radically. In culture a subjective judgment of cell motility as seen through a microscope would suggest that the rate of movement of individual cells is fairly consistent throughout the culture cycle as well as within the diel cycle. The lower values probably represent a lack of a consistent

net direction of movement rather than a 10-fold reduction in the speed at which the cells move through the water column.

Using a nominal value of 145 μm sec^{-1} for the rate of movement of *Heterosigma* through the water it is possible to estimate the impact cell motility will have on DLU, and rank the effect of motility relative to the effects changes in size and shape might have on DLU. Using the values of 2Ru/v and 2Ru/D a multiplier can be selected and taken from the work of Gavis (1976) to estimate the effect of motility in reducing DLU. The value of 2Ru/v for *Heterosigma* is 0.0026 and 2Ru/D is 1.74. Based on these values and Gavis' 1976 calculations the ratio of P'/P is approximately 1.2 or a 20% increase in P due to the movement of the cell. In other words movement through the water column reduces the impact of DLU about the same amount that reducing the cell volume by half does, and more than that achieved by a change in shape from a prolate to an oblate spheroid.

There is little published in the literature to allow comparison of the impact of motility on potential DLU among phytoplankton species. Pasciak and Gavis (1974) and Gavis (1976) calculated P' for three species in the same size range as *Heterosigma.* Those species were: *Gymnodinium splendens, Prorocentrum minimum* and *Dunaliella tertiolecta.* Table XIIIb is an abridged version of their data. The % increases in P attained by movement of the cells of each of these species are 177, 118 and 124% respectively. *Heterosigma* is within this range however, where motility all but eliminates the potential

for DLU (P'>2) in the dinoflagellate species, the P' achieved by *Heterosigma* suggests that even with the effects of motility it is likely to experience DLU.

The discussion to this point has largely ignored the effect of the development of protuberances on DLU other than to state that P or P' is likely increased by their appearance. The reason for this is a lack of appropriate mathematical models to describe the surface areas of cell shapes having protuberences. Given the scale of the impact that motility, size and simple changes in cell shape have been shown to affect P, it would seem unlikely that the protuberances would have enough of an effect to eliminate the potential DLU.

Other questions about these protuberances however, can be addressed. The development of protuberances in this species is remarkable for its all but complete absence as a subject for publications on this species. *Heterosigma* is an extensively studied organism that is frequently used in laboratory studies. The literature on this species includes over 300 papers (Smayda 1998). Over 170 papers were examined in this study. They dealt with a wide variety of aspects of this species, yet only two scant references were made to any development of protuberances.

It must be asked why such an obvious phenomenon has received so little notice in the literature. Most field samples are collected and preserved for subsequent examination. When preserved the plasmalamma of this species contracts around the numerous chloroplasts. All resemblance to the live specimen's external morphology is lost, including any evidence of surface protrusions. For samples examined in the field, the published literature would not support a supposition that cells with protuberances were a *Heterosigma* species. Only Carter's original 1937 description of the closely related species notes the occurrences of protuberances in samples taken directly from the ocean. Generally the literature does not identify these surface protuberances as characteristic of *Heterosigma* sp. If cells with protuberances were seen, it is likely they would be considered diagnostic of a separate species. If protuberant cells were seen in samples of wild populations, they might be observed in some but not all samples containing *Heterosigma*, further reinforcing the impression that these cells represent an aberrant form or separate species. As figures 25 and 26 (days 0-20) demonstrate, populations can complete a cycle of growth and decline with very few of the cells developing surface protuberances, and unless examined at magnifications in excess of 800X it would be easy to overlook this feature.

The study of cultured populations would be more likely to lead to recognition of the protuberant form as a form of *Heterosigma*. However, even this is unlikely to occur because most culture collections are propagated by keeping the culture in logarithmic growth. As figures 22, 24, 25 and 26 show, the frequency of protuberant forms during this stage would be low and the person culturing the cells may well attribute any such

forms to unstable aberrations in cell morphology which can occur in culture which have been re-propagated many times.

Interpretation of the adaptive significance of size and shape characteristics would be more difficult if the two characteristics appeared to be linked. If protuberences only occurred on small cells it would be necessary to inquire if the changes in shape are adaptive or merely a consequence of aging and the cell's loss of ability to maintain its shape. The changes in shape in *Heterosigma* however, do not appear to be linked to cell size. For example in figures 22 and 24 the proportion of protuberant forms in the population generally increased while the proportion of small cells in the population increased. In contrast in Figures 25 and 26 protuberant forms increased while the proportion of small cells decreased.

That these protuberant forms are not spontaneous morphological aberrations but are rather part of an adaptive repertoire of cell morphologies would seem to flow from the observations: that they occur across a number of strains from widely separated locations; that they can be induced; that they are an intermediate rather than terminal form in the life cycle; and that the potential adaptive significance of the forms is in accord with environmental conditions in which the species are found to occur.

The development of surface protuberances on *Heterosigma* is also not a strain or area specific phenomenon. Strains from the Strait of Georgia (Dixie Cove on Hardy

Island), from Narrows Inlet (a small fjordic inlet which flows into Sechelt Inlet which in turn empties out through Jervis inlet into the Strait of Georgia), and from San Mateo Bay at the mouth of Barkely Sound on the west coast of Vancouver Island all develop the protuberances. Tomas (1978b) working with samples collected from Narragansett Bay on the west coast of the Atlantic Ocean also noted occasionally seeing protuberances in his cultures, as did Carter in her 1937 description of the species from a lagoon on the eastern shore of the Atlantic.

Protuberances appear to develop in cells with nutrient or energy reserves. The increase in protuberances only occurs after uptake of nutrients and expression of that uptake as an increase in cell size (Figures 22, 23, 25 and 26). The rise and fall in the proportion of protuberant forms occurs in most cultures prior to the decline of the cell population (Figure 22, 24, 25 and 26), presumably as a result of declining internal and external nutrient stores. The exception to this may occur in agitated cultures (Figure 22b and 24b). The reason for the exception is not clear.

At the other end of the growth cycle, decline of the abundance of protuberant forms precedes the decline of population numbers and a return to a preponderance of smooth forms(Figures 22 and 23, and figures 25 and 26, days 20-120). The re-occurrence of smooth forms in the population at the end of the growth cycle is characterized by the formation of spherical resting stages that show up in size class profiles as an increased proportion of large-sized cells. Formation of those resting stages is energetically costly

and requires the presence of energy stores within the cell (McQuoid and Hobson 1996, Von Stosch and Fecher 1979).

Further evidence that development of protuberances requires cellular energy reserves is the observation that the extent to which protuberances are expressed depends on the level of nutrient uptake (Figure 25, 26, 31 and 33) and can be enhanced in the presence of a fixed amount of nutrients by increased illumination (Figures 24a and 24c).

7.2 Toxicology

One of the features which excites interest in *Heterosigma akashiwo* is its toxicity to other marine organisms. This effect is due to a toxic agent, that is water borne and of low molecular weight (Black *et al.* 1991, Stuart 1972, J.N.C. Whyte Pers. Comm.). Changes in cell shape that increase the transfer rate of the toxin to the environment will enhance the adaptive advantage the cell obtains by producing a toxin that can affect its external environment. Thus, we have a parallel to the nutrient uptake phenomenon discussed earlier, except it involves mass transfer out of the cell rather than into the cell.

To quantitatively model the potential effect cell shape may have on toxin transfer to the environment we need to know: the chemical formula of the toxin; whether the toxic agent is actively secreted from the cell or passively leaks out; what factors control the rate

of toxin production; and what that rate is. Much of this information is unknown though some progress towards answering those questions was made in this study. Regardless of the answers to these questions, cell shape will influence the cell's ability to affect its environment through the use of a toxin because the rate of transfer to the environment will be influenced by the concentration of the toxin in the boundary layer around the cell. Further, the rate of transfer from that boundary layer to the surrounding environment will be increased by the shape and movement of the cell through the water column.

Two types of chemicals have been suggested as the basis for *Heterosigma*'s toxicity. Yang *et al.* (1995) suggested that toxicity was derived from production of superoxide radicals, hydrogen peroxide and hydroxyl radicals. This was based on the observation that when added to cultures, the enzymes catalase and superoxide dismutase reduced toxicity. This suggestion is given some support by the previous discovery that the closely related Raphidophycean species, *Chattonella marina* and *C. antiqua* produce potentially toxic concentrations of superoxide (Tanaka *et al.* 1994 and Oda *et al.* 1992). The other suggested toxin is brevetoxin. In HPLC analysis, substances that co-elute with the brevetoxin components PbTx-2, PbTx-3, PbTx-4 and an oxidized form of PbTx-2 have been detected in cultures isolated from a toxic *Heterosigma* bloom (Kahn *et al.* 1997). Brevetoxins are usually found to be produced by the dinoflagellate *Gymnodinium breve* (Wright and Cembella 1998) though there have also been reports of these toxins being associated withother Rhaphidiophyte species including: *Chattonella antiqua, C. marina* and *Fibrocapsa japonica* (Ahmed *et al.* 1995, Kahn *et al.* 1996a, 1996b).

These suggestions however, are not supported by either the methodology used or by observations on blooms in nature. Superoxide occurs as a dissociation by-product of oxygen production and is a commonly used plant defense mechanism against pathogens. Yang *et al.* (1995) demonstrated the presence of superoxide radicals in toxic cultures and that the concentration of superoxide was dependent on cell concentrations. However they did not demonstrate that the trout mortalities were related to the concentrations of superoxides. A key component of their presumptive proof was that the addition of catalase and superoxide effectively reduced toxicity of the culture. What was not shown was that the reaction that reduced toxicity, used these substances as enzymes that lowered the energy requirements for thew reaction to occur rather than as substrates. Further, superoxide, hydrogen peroxide, and hydroxyl radicals damage cells, leaving easily detected tissue damage. Oda *et al.* (1992), working with *Chattonella marina* noted that when yellowtail tuna were exposed, such damage was apparent in gill tissue, as did Onoue and Nozawa (1989) when red sea bream were killed by exposure to *Chattonella* sp. None of this gill damage was seen when fish died during a natural bloom of *Heterosigma* in B.C. (Black *et al.* 1991). Fish used by Yang *et al.*(1995) were not examined for such damage though the discussion assumes its presence. Yang *et al.* (1995) and Kahn *et al.* (1997) both observed a violent behavioral reaction when fish were exposed to toxic *Heterosigma* yet this reaction was not apparent in fish exposed to either natural blooms or the toxic cultures reported in this study nor by others (Black *et al.*1991).

While the precise chemical formulation of the toxic agent remains uncertain progress has been made towards elucidating a number of other aspects, chief among these is some of the factors affecting toxin production.

Techniques for measuring toxicity are constrained by a lack of knowledge of the chemical formulation of the toxic agent or agents and by the size of algal culture that can be prepared. In this study, a fish bioassay was chosen because much of the concern about the occurrence of this alga in nature has focused on the effect this species has on cultured fish (Boustead et al. 1989, Black 1990, 1991, Honjo 1993, Khan et al. 1997). Salmonids were initially chosen because they were available and of concern to the mariculture industry (Boustead et al. 1989, Black 1990, 1991). Unfortunately, the volume of available algal cultures was limiting as the growth of salmonids over the course of an experiment required dilution of cultures to perform the bioassays and as demonstrated, dilution of the medium removes toxicity (Table VII). For these reasons bioassays were modified to use mollies (*Pocila mexicana)* instead of salmonids as the bioassay subject.

Time to death for mollies exposed to *Heterosigma* is sufficiently short to make them an adequate test animal. Depending on the size of the fish, mean time to death is likely to be approximately 2 to 6 hours (Figure 30). While not as rapid as the trout mortalities (50-135 minutes) (Table VI), it is adequate for assay purposes. Further, the bioassay can be performed with small aliquots of culture material (Figure 29) thereby

reducing the limitation set on experimental procedures by the volume of the culture vessels.

Toxicity appears to be initiated and terminated by nutrient conditions. Toxicity is not apparent during early growth of the algal culture and declines before senescence occurs (Figures 31 and 34). Peak toxicity is expressed during stationary phase but not during the exponential or senescence phases of the culture cycle (Figures 27, 29, 31 and 34). Exponential phase of population growth indicates the likely presence of nutrients. Stationary phase may or may not indicate that nutrients are limiting cell division but nutrients can be assumed to be limiting when the population declines in the senescent phase of the culture cycle. In cultures in this study it appears that nutrients were not limiting during stationary phase. Light limitation caused by self-shading of the cells appears to determine the limit to population growth (figure 19). It is during this period and close to the decline in population size that toxicity is expressed suggesting that the maximum toxin production is in part a response to declining concentrations of nutrients. This seems to be supported by the observation that addition of Harrison *et al.*'s (1980) nutrient broth caused the cessation of toxicity (Figure 27 and Table VI).

In *Heterosigma* the strength of the toxic reaction is dependent on the nutrient history of the cells rather than on the numerical abundance of cells. The rate of fish mortality was related to the level of nutrients supplied to the culture (Figure 34). All cultures were toxic on day 20 in that experiment. However, on days 28 and 35 before

cell concentrations declined in the culture supplied with the full concentration of nutrients recommended by Harrison *et al.*, toxicity declined.

In a closely related species changes in cell shape appear to be linked to changes in toxicity. *Heterosigma* cell shape does not necessarily coincide with changes in toxicity however, the closely related species *Chattonella marina* also produces toxins, the timing of which correlates to changes in cell shape that would alter the rate of toxin transfer to the environment (Ahmed *et al.* 1995). These changes though, are not those that would be expected if cell shape was important in moving the toxins into the surrounding water body. As cells of *C. marina* change from spindle-shaped in exponential and early stationary phase, to more rounded cells in late stationary and senescence phases, toxicity develops. The spindle shape would be a more efficient shape for transfer of toxin to the environment. Yet during growth of the culture population when spindle cells are most abundant, toxin levels within the cell increase and levels in the media are below detection. Then, in late stationary phase and senescence the cells take on the less efficient rounded-shape for mass transfer to the environment, toxin levels in the environment increase and the toxin level of the cells remains constant.

In *Heterosigma* culture the protuberant forms, which would be more effective at moving a toxin into the environment, can be present to a greater or lesser degree when

toxicity is expressed (Figure 27, 28 and Table VI, and, Figure 33 and 34, day 20). However, the maximum proportion of protuberant cells appears to indicate the level of toxicity of a culture. In Figure 33a protuberant cells were absent from the culture when it was toxic (Figure 34a). As the maximum level of protuberant cells increased in the cultures (Figures 33a, 33b and 33c) the level of toxicity of those cultures also increased (Figures 34a, 34b and 34c). The increase in toxicity of the cultures coincides with an increase in the proportion of the cell population that has a shaped that is better suited for transfer of toxins to the environment.

The reason for increasing toxicity coinciding with increasing maximum frequency of protuberant forms expressed during a culture cycle is not clear. As toxicity is present in non-protuberant forms the increased toxicity could be due to either increased rate of transfer out of the cell associated with the change in shape of the cell or to increased production of toxins within the cell. To differentiate between these options it would be necessary to bioassay a series of cultures at peak toxicity. Each of the cultures would have to be light-limited (achieve the same cell concentrations) while having received different concentrations of nutrient and as in Figure 33a none of those nutrient concentrations would be adequate to elicit the development of the protuberant forms. That experiment remains to be done.

Evidence of the identity of the nutrient or nutrients controlling toxicity in natural populations is absent and unlikely to be identifiable in the context of a single study like this one. Most experimental studies manipulate specific nutrients in algal cultures to determine effects on toxin production. However there is also evidence that the proportion of the various nutrients available to the alga affects toxin production (Boyer *et al.* 1987, Johanson and Graneli 1998). As the nutritional range of phytoplanton involves both inorganic and organic nutrients the number of individual and combination of nutrients which might be involved is large. Not only is the number of nutrients and the number of their combinations daunting but, if total toxicity is to be explained it must be demonstrated in those studies that any identified toxins account for the total toxicity seen in the bioassay. Then the nutritional triggers for production of each of the toxins must be isolated.

8.0 Conclusions

The record of the 1989 toxic *Heterosigma akashiwo* bloom in the Strait of Georgia is the most complete of any harmful algal bloom. It covers the bloom cycle from excystment of the algal population to bloom collapse and sets a good basis for interpreting the results of laboratory studies on the development of populations and toxicity of *Heterosigma akashiwo*.

The 1989 bloom was derived from an excystment event that occurred on Spanish Banks in the Fraser River estuary. The cells algae then multiplied in the Strait of Georgia. The distribution of subsequent discolouration of waters occurred in a halo in front of the Fraser River plume. Cultured populations become light-limited at concentrations below those seen in the discoulored waters of the bloom suggesting that oceanographic concentrating mechanisms may be an important factor producing the concentrations seen in the 1998 bloom. Currents then transported the toxic bloom into the area of the Sunshine Coast where fish mortalities occurred.

That toxicity is not necessarily directly related to concentration of cells. This was demonstrated in the laboratory studies and by the fact that the greater concentrations of *Heterosigma* in the 1996 bloom were non-toxic while the lower concetrations in the 1989 bloom were toxic. It was shown that the level of toxicity during a bloom is dependent not only on the concentration of cells, but on the nutrient history of the cells. These studies also show that in wild and cultured populations *H. akashiwo* becomes less toxic during senescence.

Toxicity developed in waters extremely low in inorganic nitrogenous nutrients. In culture, nutrients are usually depleted prior to senescence of cells. Laboratory studies also indicated that toxicity in culture occurs immediately prior to cell senescence. In nature nutrients might normally be available to the algae through its diel migration to deeper

waters. However, field data suggest that at this time of the bloom in 1989 the algae no longer migrated vertically.

For the first time it has been shown that *H. akashiwo* can alter its shape in a manner that may constitute an adaptation to increase both its ability to take up nutrients at low ambient concentrations, and to permit more rapid dispersion of an unknown toxic agent or agents from the cell.

A sudden increase in the abundance of nutrients appears to cause formation of cysts in both populations which have access to nutrients (light-limited) and those with out nutrient access. This is unusual among phytoplankton which are generally thought to form cysts in response to deteriorating environmental factors such as low ambient nutrient levels. For *Heterosigma akashiwo* however, formation of cysts in response to increasing nutrients may be adaptive. It may help the algae to encyst in estuarine waters and reside in the shallow muds of an estuary, which the following year are likely to experience temperatures high enough to stimulate excystment. If the species were to encyst due to a lack of nutrients, many of the negatively buoyant cells would settle in deep waters that are very unlikely to experience temperatures high enough to stimulate excystment.

Literature cited

Ahmed, M.S., O. Arakawa and Y. Onoue 1995. Toxicity of cultured *Chattonella marina.* In: *Harmful marine algal blooms.* P. Lassus, G. Arzul, E. Erard-Le Denn, P. Gentien and C. Marcaillou-Le Baut (eds.) Lavoisier, Paris 499-504.

Aizdaicher, N.A. 1993. Morphological investigations of flagellate alga *Heterosigma akashiwo* in culture. *Russian Journal of Marine Biology.* 18(5-6): 163-166

Aubert, M., D. Pesando, and H. Thouvenot 1968. Action antibotique d'extraits planctoniques vis a vis de germes anerobies. *Reviews International de Oceanography de Mediteranean*, X: 259-265

Aubert, J., and J.P. Gambarotta 1972. Etude de l'action antibacterienne d'especes phytoplanktoniques marine vis a vis de germes anerobies. *Reviews International de Oceanography de Mediteranean*, XXV: 39-47

Austin, B., and J.G. Day 1990. Inhibition of prawn pathogenic *Vibrio spp.* By a commercial spray dried preparation of *Tetraselmis suecica. Aquaculture,* 90: 389-392.

Austin, B., E. Baudet, and M. Stobie 1992. Inhibition of bacterial fish pathogens by *Tetraselmis suecica. Journal of Fish Disease* 15: 55-61

Bates, S.S., 1998. Ecophysiology and metabolism of ASP Toxin Production. In: *Physiological Ecology of Harmful Algal Blooms.* D.M. Anderson, A.D. Cembella and G.M. Hallegraeff (Eds.) NATO ASI Series, G41:405-427.

Bauerfeind, M., M. Elbr□chter, R. Steiner and J. Trondsen 1986. Application of laser doppler spectroscopy (LDS) in determining swimming velocities of motile phytoplankton. *Marine Biology* 93: 323-327.

Boyer, G.L., J.J. Sullivan, R.J. Anderson, P.J. Harrison and F.J.R. Taylor 1987. Effects of nutrient limitation on toxin production and composition in the marine dinoflagellate *Protogonyaulax tamarensis. Marine Biology* 96: 123 - 128

Black, E.A. 1986. Plankton Kills Fish. British Columbia Mariculture Newsletter Vol. 6(3): 23-26.

Black, E.A. 1987. Minimizing the Effect of Plankton Blooms on Cage Cultured Fish. B.C. Ministry of Agriculture and Fisheries, Aquaculture Information Bulletin No.13, 3 pages.

Black, E.A. 1988. Integrated Management of Plankton Problems. In: Proceedings of the Workshop on Exceptional Marine blooms: Their Impact on Fisheries and Aquaculture. W.E.L. Clayton (ED.), Bamfield Marine Station. Bamefield, Canada, June 5-7, 1988, pp. 1-6.

Black, E.A. 1990a. Algal Blooms in British Columbia. Red Tide Newsletter, 3(2): 11-12.

Black, E.A. 1990b. The impact of harmful phytoplankton on Atlantic salmon. In: The Atlantic salmon workshop. E.W. Britton (ed.) B.C. Ministry of Agriculture Fisheries and Food. Aquaculture Industry Development Report. No. 90-4: 45-48

Black, E. A. 1991. Studies on the management of salmon farming algal problems. In: Pacific coast research on toxic marine Algae. J.R. Forbes ed. *Canadian Technical Report of Hydrography and Ocean Science* 135: 29-31

Black, E.A. 1994. Controlling fish farm losses to algal events. Fourth Canadian Workshop on Harmful Marine Algae. *Canadian Journal of Fisheries and Aquatic Science.* Tech. Report 2016: 3.

Black, E. A., J. N. C. Whyte, J.W. Bagshaw and N.G. Ginther 1991. The effects of *Heterosigma akashiwo* on juvenile *Oncorhynchus tshawytcha* and its implications for fish culture. *Journal of Applied Ichthyology.* 7: 168-175

Boustead, N., H. Chang, R. Pridmore, P. Todd 1989. Big Glory Bay algal bloom identified. *Freshwater Catch* 39:3-4.

Bravo, I., B. Reguera and S. Fraga 1995. Description of different morpohotypes of *Dinophysis acuminata* complex in the Galacian Rias Baixas in 1991. In: *Harmful Marine Algal Blooms.* P. Lasus, G. Arzul, E. Erard, P Getien and C. Marcaillou [eds.] Lavoisier, New York. pp 21-26.

Burkholder, P.R., L.M. Burkholder and L.R. Almodovar 1960. Antibiotic activity of some marine algae of Puerto Rico. *Botony Marine* 2: 149-156.

Carter, N. 1937. New or interesting algae from backish water. *Archives Protistenk* 90: 1-68.

Cattolico, R.A. 1978. Variation in plastid number. Effect on chloroplast and nuclear deoxyribonucleic acid compliment in the unicellular alga *Olithodiscus luteus. Plant Physiology.* 62: 558-562.

Cembella, A. 1998. Ecophysiology and metabolism of paralytic shellfish toxins in marine microalagae. In: *Physiological Ecology of Harmful Algal Blooms.* D.M. Anderson, A.D. Cembella and G.M. Hallegraeff (Eds.) NATO ASI Series, G41: 381-403

Chisholm, S.W. 1992. Phytoplankton size. In: *Primary Productivity and Biogeochemical Cycle in the Sea.* Falkowski, P.G. and A.D. Woodhead (eds.) Plenum Press, New York. pp. 213-237.

Chotiyaputta, C., and K. Hirayama 1978. Food selectivity of the rotifer *Brachionus plicatilis* feeding on phytoplankton. *Marine Biology* 45: 105-111.

Conrad, W., and H. Kufferath.1954. Recherches sur les eau saumatres des environs de Lilloo. perti II. Descrictive. *Inst. R. Sci. Nat. Belg. Mem.* 127:1-346

Cooper, S. and D.L. Battat 1983. Production of antibacterial activities by two bacillariophyceae growth in dialysis culture. *Canadian Journal of Microbiology* 29: 338-341.

Courties, C., A. Vaquer, M. Troussellier, J.Lautier, M.J. Chretiennot-Dinet, J. Neveux, C. Machado and H. Claustre 1994. Smallest eukaryotic organism. *Nature* 370: 255.

Dale, B. 1983. Dinoflagellate resting cysts: "benthic plankton". In: *Survival Strategies of the algae.* G.A. Fryxell [ed.] Cambridge University Press, New York. pp. 69-137.

Doucette, G.J., Kodama, M., Fraga, S., and Gallacher, S. 1998. Bacterial Interactions with harmful algal: bloom ecology, toxigenesis and cytology In: *Physiological Ecology of Harmful Algal Blooms* D.M. Anderson, A.D. Cembella and G.M. Hallegraeff (Eds.) NATO ASI Series, G41: 619-647

Dor, I. 1985. Long-lasting effect of dilution on cell volume, motility, division rate and vertical distribution of Dunaliella parva Lerche. *Journal of Experimental Marine Biology and Ecology* 91: 183-197.

Dortch, Q. 1982. Effect of growth conditions on accumulation of internal nitrate, ammonium, amino acids and protein in three marine diatoms. *Journal of Experimental Marine Biology and Ecology* 61: 243-264

Duff, D.C.B. and D.L. Bruce 1966 The antibacterial activity of marine planktonic algae. *Canadian Journal of Microbiology.* 12: 877-884.

Eppley, R.W., N.J. Rogers, and J.J. McCarthy 1969. Half-saturation constants for uptake

of nitrate and ammonium by marine phytoplankton. *Limnology and Oceanography* 14: 912 - 920

Farr, T.J., H.C. Huppe, and D.H. Turpin 1994. Coordination of Chloroplastic metabolism in N-limited *Chlamydomonas reinhardtii* by redox modulation: I The activation of phosphoribulosekinase and glucose-6-phosphate dehydrogenase is relative to the photosynthetic supply of electrons. *Plant Physiology* 105: 1037-1042

Figueiras, F.G and F. Fraga 1990. Vertical nutrient transport during proliferation of *gynmodinium catenatum* Graham in Ria de Vigo, northwest Spain. In: *Toxic Marine Phytpolankton.* E. Graneli, Bo Sundstrom, L. Edler and D.M. Anderson.(eds.) Elsevier, New York. pp. 144-148.

Figueiras, F.G. and A.F. Rios 1993. Phytoplankton succession, red tides and the hydrographic regiem in the Rias Bajas of Galacia. In: *Toxic Phytoplankton Blooms in the Sea.* T.J. Smayda and Y.Shimizu (Eds.) Elsevier, New York. pp. 239-244.

Gaines, G., F.J.R. Taylor 1989. A mariculturist's guide to potentially harmful marine phytoplankton of the Pacific coast of North America. British Columbia's Ministry of Agriculture and Fisheries, Information Report No. 10, 53 pages.

Gauthier, M.J. 1980. Note sur la frequence de la production d'antibiotiques liqidiques chez les algues planctoniques. *Review du International Oceanography de Mediteranean*, Vol. LVIII, pp. 41 - 44.

Gauthier, M.J., P. Bernard, and M. Aubert 1978. Production d'un antibiotique lipidique photosensible par la diatomee marine *Chaetoceros lauderi* (RALFS). *Annals of Microbiology* 129B: 63-70.

Gavis, J. 1976. Munk and Riley revisited: Nutrient diffusion transport and rates of phytoplankton growth. *Journal of Marine Research* 34: 161-179.

Goldman, J.C., J.J. McCarthy and D.G. Peavey 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature* 279: 210-215

Gowen, R.J. 1987.Toxic Phytoplankton in Scottish Waters. *International Council for the Exploration of the Seas Rapport et Proces-Verbaux des Reunions*1 87:89-93

Grover, J.P. 1989. Influence of cell shape and size on algal competitive ability. *Journal of Phycology* 25: 402-405.

Hada, Y. 1968 Protozoan plankton of the Inland Sea, Setonaikai II Mastigophora and Sarcodina. *Bulletin Suzugamine Women's College of Natural Sciences* 14: 1-28

Haigh, R. 1988. The effect of stratification on microplankton communities in the northern Strait of Georgia. Master of Science Thesis. University of British Columbia. 287 pages.

Hanto, S., Y. Hara, M. Takahashi 1983. Preliminary study on the effects of photoperiod and nutrients on the vertical migratory behavior of a red tide flagellate *Heterosigma akashiwo*. *Japanese Journal of Phycology* 31: 263-269.

Hara, Y., and M. Chihara 1987. Morphology, ultrastructure and taxonomy of the raphidophycean alga *Heterosigma akashiwo*. *Botanical Magazine of Tokyo* 100: 151-163

Hara, Y., I. Inouye, M. Chihara 1985. Morphology and ultrastructure of *Olisthodiscus luteus* (Raphidophyceae) with special reference to the taxonomy. *Botonical Magazine of Tokyo* 98: 251-262.

Harrison, P.J., J.D. Fulton, F.J.R. Taylor and T.R. Parsons 1983. Review of the biological oceanography of the Strait of Georgia: pelagic environment. . *Canadian Journal of Fisheries and Aquatic Sciences* 40: 1064-1094.

Harrison, P.J., R.E.Waters and F.J.R. Taylor 1980. A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. *Journal of Phycology* 16: 28-35.

Hatano, S., Y. Hara, and M. takahashi 1983. Preliminary study on the effects of photoperiod and nutrients on the vertical migration behavior of a red tide flagellate. *Heterosigma akashiwo*. *Japanese Journal of Phycology* 31: 263-269.

Hershberger, P..K., J.E. Rensel, A.L. Matter and F.B Taub 1997. Vertical migration of the chloromonad flagellate *Heterosigma carterae* in columns: implications for bloom development. *Canadian Journal of Fisheries and Aquatic Sciences* 2228-2234.

Honjo, T. 1978. Changes of phytoplankton composition and its characteristics during red tide season. *Bulletin of the Plankton Society of Japan* 25: 13-19.

Honjo, T. 1993. Overview on bloom dymanics and physiological ecology of Heterosigma akashiwo. In: *Toxic Phytoplankton Blooms in the Sea*. T. J. Smayda and Y. Shimizu eds. Elsevier, New York, pp. 33-41

Honjo, T. and K. Tabata 1985. Growth dynamics of *Olithodiscus luteus* in outdoor with coastal waterand in small vessels. *Limnology and Oceanography* 30: 653-664

Hulburt, E.M. 1965. Flagellates from brackish watersin the vicinity of Woods Hole, Massachusetts. *Journal of Phycology* 1: 87-94

Huppe, H.C., T.J. Farr and D.H. Turpin 1994. Coordination of Chloroplastic metabolism in N-limited *Chlamydomonas reinhardtii* by redox modulation: II Redox modulation activates the oxidative pentose phosphate pathway during photosynthetic Nitrate assimilation. *Plant Physiology* 105: 1043-1048

ICES 1991. Effects of harmful algal blooms on mariculture and marine fisheries. International *Council for the Exploration of the Seas. Cooperative Research Report* 181. 38 pages

IFREMER 1994. Mortalities des poissons d'levage par phytoplankton toxique, Camaret 27-28 Sept. 1994 IFREMER DEL/AA/94 - MK Le 30 Septembre 1994.

Imai, I., S. Itakura, and K. Itoh 1993. Cysts of the red tide flagellate *Heterosigma akashiwo*, Raphidophyceae, found in bottom sediments of northern Hiroshima Bay, Japan. *Nippon Suisan Gakkaishi* 59: 1669-1673.

Itakura, S., K. Nagasaki, M. Yamaguchi and I. Imai 1996a Species succession between Skeletonema costatum and Heterosigma akashiwo in Hiroshima Bay, Japan, with special reference to the resting stage cells in the bottom sediments. . In: *Harmful and Toxic Algal Blooms*. T. Yasumoto, Y. Oshima and Y. Fukuyo (eds.) Intergovernmental Oceanographic Commision of UNESCO. pp. 373-376.

Itakura, S., K. Nagasaki, M. Yamaguchi and I. Imai 1996b. Cyst formation in the red tide flagellate *Heterosigma akashiwo*(Raphidophyceae). *Journal of Plankton Research* 18: 1975-1979.

IOS, 1989. Monthly summaries of marine conditions: Coast of British Columbia. No 14. Manuscript of the Data Assessment Division, Institute of Ocean Sciences. October 1989. 15 pages.

Johanson, N. and E. Graneli 1998. Toxicity of *Chrysochromulina polylepis* cells grown in different N:P ratios. In: *Harmful Algae*. B. Reguera, J. Blanco, M.L. Fernandez, and T. Waytt (eds.) Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO pp. 329-330.

Khan, S., O. Arakawa and Y.Onoune 1996a. A toxicological study of the marine phytoflagellate *Chattonella antiqua* (Raphidophyceae). *Phycologia* 35: 239-244.

Khan, S., O. Arakawa and Y.Onoune 1996b. Neurotoxin production by a chloromonad *Fibrocapsa japonica*(Raphidophyceae). *Journal of the World Aquaculture Society* 27: 254-263

Khan, S., O. Arakawa and Y.Onoune 1997. Neurotoxins in a toxic red tide of *Heterosigma akashiwo* (Raphidophyceae) in Kagoshima Bay, Japan. *Aquaculture Research* 28: 9-14

Kleiner, D. 1981. The transport of NH3 and NH4+ across biological membranes. *Biochima et Biophysica Acta* 639: 41-52

Kiørboe, T. 1993. Turbulence, phytoplankton cell size, and the structure of pelagic food webs. In: *Advances in Marine Biology*, Blaxter, J.H.S, and A.J. Southward (eds.). Academic Press, London. 29: 1-72

Kogure, K., U. Shimida and N. Taga 1979. Effect of *Skeletonema costatum* (Grev.) Cleve on the growth of marine bacteria. *Journal of Experimental Biology and Ecology*, 36: 201 - 215

Kohata, K. and M. Watanabe 1978. Synchronous division and the pattern of diel vertical migration of *Heterosigma akashiwo* (Hada) Hada (Raphidophyceae) in laboratory culture. *Journal of Experimental Marine Biology and Ecology* 100: 209-224.

Konovalova, G.V. 1995. The dominant and potentially dangerous species of phytoplankton in the coastal waters of east Kamchatka. In: *Harmful Marine Algal Blooms*. P. Lasus, G. Arzul, E. Erard, P Getien and C. Marcaillou (eds.). Lavoisier, New York. pp 169-174.

Lackey, J. B., and E. Lackey 1963. Microscopic algae and protozoa in the waters near Plymouth in August. 1962. *Journal of the Marine Biological Association of the UK* 43: 797-805.

Lackey, J.B., and K. A. Clendenning 1965. Ecology of the microbiota of San Diego Bay, California. *Transactions of the San Diego Society of Natural Hististory* 14: 9-40

Langdon C. 1986. On the causes of interspecific differences in the growth-irradiance for phytoplankton. Part I. A comparative study of the growth-irradiance relationship of three marine phytoplankton species: *Skeletonmena costatum, Olisthodiscus luteus* and *Gonyaulax tamarensis. Journal of Plankton Research* 9: 459-482.

Larsen, J. amd Ø. Moestrup 1989. *Guide to toxic and potentially toxic marine algae*. Fish Inspection Service, Ministry of Fisheries, Copenhagen. 61 pages.

Lazier, J.R.N. 1963. Some aspects of the oceanographic structure in the Jervis Inlet system. Masters of Science Thesis. University of British Columbia. 55 pages.

LeBlond, P.H. 1983. The Strait of Georgia: functional anatomy of an inland sea. *Cananadian Journal of Fisheries Aquatic Sciences* 40: 1033-1063.

Lewin, R.A. 1962. *Physiology and biochemistry of algae*. New York, Academic Press, 929 pages.

Lirdwitayaprasit, T , T. Ochi, and S. Montani 1996. Changes in cell chemical composition during the growth cycle of *Chattonella* sp. and *Heterosigma* sp, found in the shrimp ponds at Chantaburi, Thialand. In: *Harmful and Toxic Algal Blooms*. T. Yasumoto, Y. Oshima and Y. Fukuyo (eds.) Intergovernmental Oceanographic Commision of UNESCO. pp. 251-254.

Loban, C.S. and P.J. Harrison 1994. *Seaweed ecology and physiology*. Cambridge University Press, New York. 366 pages.

Longhurst, A.R. 1989. Pelagic ecology: definition of pathways for material and energy flux. In: *Océanologie: Actualité et Prospective*. Denis, M.M.(ed.) Centre d'Océanologie de Marseille. pp. 263-288.

Loeblich III, A.R. and K.E. Fine 1977. Marine Chloromonads: More widely distributed in neritic environments than previously thought. *Proceedings of the Biological Society of Washington*. 90: 388-399.

LoCicero, V.R. 1980. *The first international conference on toxic dinoflagellates*. Massachusetts Science and Technology Foundation. Wakefield, Massachusetts, 541 pages.

MacKenzie, L. 1991. Toxic and noxious phytoplankton in Big Glory Bay, Stewart Island, New Zealand. *Journal of Applied Phycology* 3: 19-34

McQuaker, N.R. 1989. *A Laboratory Manaual for the Chemical Analysis of Waters, Wastewaters, Sediments and Biological Materials*. Part I. B.C. Ministry of Environment, Data Standards Group, Victoria, B.C. 354 pages

McQuoid, M.R. and L.A. Hobson 1996. Diatom resting stages. *Journal of Phycology* 32: 889-902

Mahoney, J.B. and J.J.A. McLaughlin 1977. The association of phytoflagellate blooms in lower New York Bay with hypertrophication. *Journal of Experimental Marine Biology and Ecology* 28: 53-65

Marasovic, I. and T. Pucher-Petkovic 1985. Effects of eutrophication on the structure of the coastal phytoplankton community. *Rapport et Communicatio de International Mer de Mediteranian 29:137-139*.

Marsot, P. and L. Houle 1989. Excretion cellulaire et morphogenese de *Phaeodactlum tricornutum* (Bacillariophyceae). *Botanica marina* 32: 355-367.

Montani, S., K. Ichimi, S. Meksumpun and T. Okaichi 1995 The effects of dissolved oxygen and sulphide on germination of the cysts of some different phytoflagellates. In: *Harmful Marine Algal Blooms*. P. Lassus, G. Arzul, E. Erard-Le Denn, P. Getien and C. Marcaillou-Le Baut (eds.) Lavoisier, Paris. pp. 627-632

Morel, F.M.M., R.J.M. Hudson and N.M. Price 1991. Limitation of productivity by trace metals in the sea. *Limnology and Oceanography* 36: 1742-1755.

Munk, W.H. and G.A. Riley 1952. Absorption of nutrients by aquatic Plants. *Journal of Marine Research* 11: 215-240.

Nagai, H, M. Satake, M. Murata and T Ysumoto 1990. Screening of marine phytoplankton for antifungal substances. In: *Toxic Marine Phytpolankton*. E. Graneli, Bo Sundstrom, L. Edler and D.M. Anderson.(eds.) Elsevier, New York. pp. 385-390.

Nagasaki, K., S. Itakura, I. Imai, S. Nakagiri and M. Yamaguchi 1996.The disintegration process of a Heterosigma akashiwo (Raphidophyceae) red tide in northern Hiroshima Bay, Japan, during the summer of 1994. In: *Harmful and Toxic Algal Blooms*. T. Yasumoto, Y. Oshima and Y. Fukuyo (eds.) Intergovernmental Oceanographic Commision of UNESCO. pp. 251-254.

Nagasaki, K., I. Imai, S. Itakura, M. Ando and Y. Ishida 1995. Viral infection in *Heterosigam akashiwo* (Raphidophyceae); A possible termination mechanism of the noxious red tide. In: *Harmful Marine Algal Blooms*. P. Lassus, G. Arzul, E. Erard-Le Denn, P. Getien and C. Marcaillou-Le Baut (eds.) Lavoisier, Paris. pp. 639-644.

Oda, T., A. Ishimatsu, M. Shimada, S. Takashita and T. Muramatsu 1992. Oxygen-radical-mediated toxic effects of the red tide flagellate *Chattonella marina* on *Vibrio alginolyticus*. *Marine Biology* 112: 505-509.

Owens N.J.P., E.M.S. Woodward, J. Aiken, I.E. Bellan and A.P. Rees 1990. Primary production and nitrogen assimilation in the North Sea during July 1987. *Netherland Journal of Sea Research* 25: 143-154.

Onoue, Y. and K Nozawa 1989. Seperation of toxins from harmful red tides occuring along the coast of Kagoshima Prefecture. In: *Red Tides: Biology, Environmental Science and Toxicology*. Okaichi, T., D.M. Anderson and T. Nemoto (eds.). Elsevier, New York. pp. 371-374

Park, J.S. 1991. Red Tide Occurrence and countermeasure in Korea. In: *Recent Approaches on Red Tides*. Korean National Fisheries Research and Development Agency Report 1991 24 pages.

Parsons, T and M. Takahashi 1973. *Biological Oceanographic Processes*. Pergamon Press. New York. 186 pages.

Pasciak, W.J and J. Gavis 1974. Transport limitation of nutrient uptake in phytoplankton. *Limnology and Oceanography* 19: 881-888.

Pfiester, L.A. and D.M. Anderson 1987. Dionoflagellate reproduction. In: *Biology of Dinoflagellates*. F.J.R. Taylor (ed.). Blackwell Scientific Publications, london. pp. 611-648.

Pratt, D. M. 1966. Competition between Skeletonema costatum and Olisthodiscus luteus in Narragansett Bay and in culture. *Limnology and Oceanography* 11: 447-455.

Probyn, T.A. 1985. Nitrogen uptake by size fractionated phytoplankton in the southern Benguela upwelling system. *Marine Ecology Progress Series* 22: 259-258.

Quayle, D.B. 1969. Paralytic shellfish poisoning in British Columbia. *Bulletin of the Fisheries Research Board of Canada* No. 168: 1-68.

Rao, D.V.S. and G. Wohlgeschaffen 1990. Morphological variants of *Nitzchia pungens* Grunow f. *multiseries* Hasle. *Botanica Marina* 33: 545-550.

Raven, J.A. 1994. Why are there no planktonic O_2 evolvers with volumes less than 10^{-19} m^3? *Journal of Plankton Research* 16: 565-580.

Reed, R.H. and W.D.P. Stewart 1985. Osmotic adjustment and organic solute accumulation in unicellular cyanobacter from freshwater and marine habitats. *Marine Biology* 88: 1-9.

Rensel, J., R.A. Horner and J.E. Postel 1989. Effects of phtoplankton blooms on salmon aquaculture in Puget Sound, Washington: Initial research. *Northwest Environmental Journal* 5: 53-69.

Rhee, G.Y. and I.J. Gotham 1981. Optimum N:P ratios and coexistence of planktonic algae. *Archive of Microbiology* 25: 486-489

Rohlf, F.J. and R.R. Sokal 1969. *Statistical Tables*. W.H. Freeman and Company, San Francisco. 253 pages.

Rojas de Mendiola B. 1979. Red tide along the Peruvian coast. In: *Toxic Dinoflagellate Blooms*. Taylor, D.L., and H.H. Seliger (eds.). Elsevier, Amsterdam. pp. 183-190.

Sampayo, M. and M.T. Moita 1984. An *Olisthodiscus luteus* red water: Its dynamics during 24 hours. ICES CM 1984/B:12 11p.

Satoh, E., T. Fujii 1989. Photoperiod regu;lation of cell division and chloroplast replication in *Heterosigma akashiwo*. In: *Red Tides: Biology, Environmental Science and Toxicology*. Okaichi,T., D. Anderson and Nemoto (Eds.) Elsevier, New York. pp. 217-220

Schantz, E.J., Lynch, J.M., Vayvada, G., Matsumoto, K., and Rapoport, H. 1966. The purification and characterization of the poison produced by *Gonyaulax catenella* in axenic culture. *Biochemistry* 5: 1191-1195.

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

Shen, S.-I., and Y.-M. Chiang 1971. Notes on organisms causing brown water in milkfish ponds. *Fishery Series: China-American Joint Communication on Rural Reconstruction.* 11:84-84.

Smayda, T.J. 1990. Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. In: *Toxic marine Phytoplankton*. E. Graneli, Bo Sundstrom, L. Edler and D.M. Anderson. (eds.). Elsevier, New York. pp. 29-40

Smayda, T.J. 1998. Ecophysiology and bloom dynamics of *Heterosigma akashiwo*. In: *Physiological Ecology of Harmful Algal Blooms*. D.M. Anderson, A.D. Cembella and G.M. Hallegraeff. (eds.). Springer-Verlag, Berlin, NATO ASI series Vol. G 41: 113-131.

Sommer, U. 1985. Comparison between steady state and non-steady state competition: experiments with natural phytoplankton. *Limnology and Oceanography* 34: 1109-1112.

Sommer, H and Meyer, K.F. 1937. Paralytic shell fish poisoning. *Archives of Parasitology* 24:560-598.

Sommer, H., Whedon, W.H., Kofoid, C.A. and Stohler, R. 1937. Relation of paralytic shellfish poison to plankton of the genus *Gonyaulax*. *Archieves of Pathology* 24:537-559.

Steedman, H.F. 1976, *Zooplankton fixation and preservation*. Paris, Unesco Press, Monographs on oceanographic methodology, 350 pages.

Stolt, W. and R. Riegman 1996. A model approach for size-selective competition of marine phytoplankton for fluctuating nitrate and ammonium. *Journal of Phycology* 32: 732-740.

Strickland, J.D.H., O. Holm-Hansen, R.W. Eppley and R.J. Linn. 1969. The use of a deep tank in plankton ecology. I Studies of the growth and composition of phytoplankton crops at low nutrient levels. *Limnology and Oceanography* 14: 23-34.

Stuart, M.C. 1972. The effect of *Olisthodiscus luteus* upon the growth of *Skeletonema costatum* (Grev.) Cleve. Masters Thesis, University of Rhode Island. 83 pages.

Swift, E. and V. Meunier 1976. Effects of light intensity on division rate, stimulatable bioluminescence and cell size of the oceanic dinoflagellates *Dissodinium lunula, Pyrocystis fusiformis* and *P. noctiluca. Journal of Phycology* 12: 14-22.

Takahashi, K., and N. Fukukawa 1982. A mechanism of red tide formation. IIEffect of selective nutrient stimulation on the growth of different phytoplankton species in natural water. *Mar. Biol.* 70:267-273.

Takahashi, M., and Y. Hara 1989. Control of diel migration and cell division rhythm of *Heterosigma akashiwo* by day and night cycles. In: *Red Tides: Biology, Environmental Science and Toxicology.* Okaichi,T., D. Anderson and Nemoto (eds.) Elsevier, New York. pp. 265-268

Takahashi, M., and T. Ikawa 1987. Effect of nutrient starvation on photosynthetic carbon mechanism in the marine Raphidophycean flagellate *Heterosigma akashiwo. Plant Cell Physiol.* 28:840-852.

Tanaka, K., Y. Muto and M. Shimada 1994. Generation of superoxide anion radicals by the marine phytoplankton organism, *Chattonella antiqua. Journal of Plankton Research* 16:161-169.

Taylor, F.J.R. 1990. Red tides, brown tides and other harmful algal blooms: the view into the 1990's. . In: *Toxic marine Phytoplankton.* E. Graneli, Bo Sundstrom, L. Edler and D.M. Anderson. Elsevier, New York. pp. 527-533.

Taylor, F.J.R. 1992. The Taxonomy of harmful Marine Phytoplankton. *Giornale Botanico Italiano* 126(2): 209-219.

Taylor, F.J.R. and R. Haigh 1993. The ecology of fish killing blooms of the Chloromonad flagellate *Heterosigma* in the Strait of Georgia and adjacent waters. In: *Toxic Phytoplankton Blooms in the Sea.* T.J. Smayda and Y. Shimizu (Eds.) Elsevier, New York. pp. 705-710.

Thompson, P.A., P.J. Harrison, and J.S. Parslow 1991. Influence of irradiance on cell volume and carbon quota for ten species of marine phytoplankton. *Journal of Phycology* 27: 351-360.

Thomson, R.E. 1981. *Oceanography of the British Columbia coast.* Canadian Special Publication of Fisheries and Aquatic Sciences 56: 291 pages.

Throndsen, J. 1969. Flagellates of Norwegian Coastal Waters. *Nytt Magasin for Botanikk* 16: 161-216.

Throndsen, J. 1973 Motility in some marine nanoplankton flagellates. *Norwegian Journal of Zoology* 21:193-200

Throndsen, J. 1996. Note on the taxonomy of *Heterosigma akashiwo* (Raphidophyceae) *Phycologia* 35:367

Tomas, C.R. 1978a. *Olisthodiscus luteus*(Chrysophyceae) I. Effects of salinity and temperature on growth motility and survival. *Journal of Phycology*, 14: 309-313.

Tomas, C.R. 1978b. *Olisthodiscus luteus* (Chrysophyceae) II. Formation and survival of a benthic stage. *Journal of Phycology*, 14: 314-319.

Tomas, C.R. 1979. *Olisthodiscus luteus* (Chrysophyceae) III. Uptake and utilization of nitrogen and phosphorus. *Journal of Phycology*, 15: 5-12

Tomas, C.R. 1980a. *Olisthodiscus luteus*(Chrysophyceae) IV. Effects of light intensity and temperature on photosynthesis and cellular composition. *Journal of Phycology* 16: 140-156

Tomas, C.R. 1980b. *Olisthodiscus luteus*(Chrysophyceae) V. Its occurrence, abundance and dynamics in Narragansett Bay, Rhode Island. *Journal of Phycology*, 16: 157-166.

Tomas, C. 1998. Blooms of potentially harmful Raphidophyte flagellates in Florida coastal waters. In: *Harmful Algae* B. Reguera, J.Blanco, M.L. Fernandez and T. Wyatt. (eds.) Xunta de Galacia and International Oceanographic Commission of UNESCO. Santiago de Compostela. pp. 101-103

Tomas, C.R., E.E. Deason 1981. The influence of grazing by two *Acartia* species on *Olithodiscus luteus* Carter. *Marine Ecology*, 2(3): 215-223

Tregoubouff, G. and M. Rose 1957. *Manuel de planctonologie mediterraneenne.* Vol. 1. Paris, Centre National de la Recherche Scientifique. 587 pages.

Trondsen, J. 1973. Motility in some marine nanoplankton flagellates. *Norwegian Journal of Zoology* 21:193-200

Tseng, C.K., M.J. Zhou and J.Z.Zou 1993. Toxic phytoplankton studies in China. In: *Toxic Phtoplankton Blooms in the Sea* T.J. Smayda and Y Shimizu (eds.). Elsevier, New York. pp. 347-352.

Turpin, D.H. and P.J. Harrison 1980. Cell size manipulation in natural marine, planktonic, diatom communities. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 1193-1195.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt. d. Int. Vereinig. f. Limnologie*, 9: 39 .

Uye, S. and K. Takamatsu 1990. Feeding interactions between planktonic copepods and red-tide flagellates from Japanese coastal waters. *Marine Ecology Progress Series*, 59: 97-107.

Vadnais, D. A. 1995. The role of carbonic anhydrase and a model to predict toxicity in *Hetereosigma carterae* Taylor. Masters of Science Thesis, Biology Department, University of Victoria. 63 pages

Verity, P.G. and D. Stoecker 1982. Effects of *Olisthodiscus luteus* on the growth and abundance of tintinnids. *Marine Biology*, 72: 79-87.

Viso, A.C., D. Pesando and C. Baby 1987. Antibacterial and antifungical properties of some marine diatoms in culture. *Botanica Marine* 30: 41 - 45

Von Stosch, H. A. and K. Fecher 1979. "Internal thecae" of *Eunotia soleirolii* (Bacillariophyceae): Development, structure and function as resting spores. *Journal of Phycology* 15: 223-243

Wada, M., Y. Hara, M. Kato, and T. Fujii 1987. Diurnal appearance, fine structure, and chemical composition of fatty particles in *Heterosigma akashiwo* (Raphidophyceae). *Protoplasma* 137: 134-139.

Wada, M., A. Miyazaki, and T. Fujii 1985. On the mechanism of diurnal vertical migration and dark uptake of nitrate and phosphate of the red tide flagellates, *Heterosigma akashiwo* Hada and *Chattonella antiqua* (Hada) Ono (Raphidophyceae). *Japanese Journal of Phycology* 31: 161-166.

Waldichuck, M. 1957. Physical Oceanography of the Strait of Georgia, British Columbia. *Journal of the Fisheries Research Board of Canada* 14: 321-486.

Wall, D. 1980. Taxonomy and cysts of red-tide dinoflagellates. In: V.R. LoCicero (Ed.). *The first international conference on toxic dinoflagellates*. Massachusetts Science and Technology Foundation. 249-256

Ward, J.E. and N.M. Targett 1989. Influence of marine microalgal metabolites on the feeding behavior of the blue mussel *Mytilus edulis*. *Marine Biology* 101: 313-321.

Warwick, R.M. and I.R. Joint 1987. The size distribution of organisms in the Celtic Sea: from bacteria to metazoa. *Oecologia* 73: 185-191.

Watanabe, M., K. Kohata, and M. Kunugi 1988. Phosphate accumulation and metabolism by Heterosigma akashiwo (Raphidophyceae) during diel migration in a startified microcosm. *Journal of Phycology* 24: 22-28.

Watanabe, M., K. Kohata, and M. Kunugi 1989. 31p-NMR study of polyphosphate metabolism associated with diel vertical migration by *Heterosigma akashiwo* under salinity and phosphate stratification. In: *Red Tides: Biology, environmental science and toxicology*. Okalchi, T., D. Anderson and Namoto (eds.). Elsevier, New York. pp.321-323.

Watanabe, M., K. Kohata, and M. Kunugi 1990. Nitrogen and phosphate accumulation by *Chattonella antiqua* during diel vertical migration in a stratified microcosm. In: *Toxic marine Phytoplankton*. E. Graneli, Bo Sundstrom, L. Edler and D.M. Anderson (eds.). Elsevier, New York. pp. 244-248

Watanabe, M.M., Y. Nakamura, S. Mori, and S. Yamochi 1982. Effects of physico-chemical factors and nutrients on the growth of *Heterosigma akashiwo* Hada from Osaka Bay, Japan. *Japanese Journal of Phycology* 30: 279-288

Weeks, K.R. 1998. *The lost tomb*. William Morrow and Co., New York, 332 pages.

Welling, K. 1982. The effects of extracellular products of *Olisthodiscus luteus* Carter on the survival of oyster larvae (*Crassostrea gigas* Thunberg) and on the growth of larval-associated bacteria. Master of Science Thesis, University of Washington. 60 pages.

Wright, J.L.C., and A.D. Cembella 1998. Ecophysiology and biosynthesis of polyether marine biotoxins. In: *Physiological Ecology of Harmful Algal Blooms*. D.M. Anderson, A.D. Cembella and G.M. Hallegraeff (eds.). Springer-Verlag, Berlin, NATO ASI series Vol. G 41: 427-452.

Yamochi, S. and T. Abe. 1984. Mechanisms to initiate a Heterosigma akashiwo red tide on Osaka Bay. *Marine Biology* 83: 255-261.

Yamochi, S., T. Abe and H. Jon 1982. Study on the mechanisms of red water bloomsby *Olithodiscus luteus* at Tanigawa Fishing Port, Osaka Bay – Characteristics in occurrence of *O. luteus* and its diurnal vertical migration. *Research, Per. National. Institute for Environmental Study of Japan*. 30: 191-214

Yamochi, S. 1984. Mechanisms for outbreak of *Heterosigma akashiwo* red tide in Osaka Bay, Japan. *Journal of the Oceanographic Society of Japan*. 40: 343-348.

Yang, C. Z., A. M. Yousif, T. Perkins and L. J. Albright 1993. The mode of toxic phytoplankter, Heterosigma akashiwo on juvenile sockeye salmon (Oncorhyncus nerka) In: *Harmful Marine Algal Blooms*. P. Lassus, G. Arzul, E. Erard-Le Denn, P. Getien and C. Marcaillou-Le Baut (eds.) Lavoisier, Paris. pp. 333-338.

Yang, C.Z., L.J. Albright and A.N. Yousif 1995. Oxygen-radical-mediated effects of the toxic phytoplankter *Heterosigma carterae* on juvenile rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 23(2): 101-108