Morphological and biochemical variability within eastern North American populations of *Asterionella* Hassall: Possible taxonomic implications

by
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Abstract

Morphological plasticity is an inherent property of most diatom taxa which complicates taxonomic identification and the use of diatoms as ecological indicators. Consequently, physiological, biochemical, cytological and ecological traits, in combination with phenetic characterization, are now all employed to delineate species. The objective of this study was to explore taxonomic relationships and morphological variability within the *Asterionella formosa / ralfsii* complex. Populations of *Asterionella* were examined from clear and coloured lakes in eastern North America, taxonomic characters were measured using light and scanning electron microscopy, and isolates were grown in culture for pigment extraction and analysis.

Cell length data and shifts in population distributions were followed within and between lakes over several seasons. Length class distributions were seen to follow one of three patterns: 1) an increase in length from the spring 1990 sample to the spring 1991 sample, 2) an increase in length from the spring of 1991 to the fall of 1991, or 3) an increase in length from the fall of 1992 to the spring of 1993. Mean population cell length increases occurred when cells declined to one-third to one-half the maximum, suggesting that auxospore formation had taken place. A few of the lakes followed no obvious cycle but always had a wide distribution of length classes. Average cell length of populations were 1) less than 60 μm, 2) approximately 70 μm, or 3) greater than 80 μm. In addition, the number of valves per colony were counted to determine if there were differences in colony size between clear and coloured lakes. Although differences were seen in populations from a few lakes, most colonies had between five and eight cells, typical for this genus. Within-lake variability was similar to between-lake variability, with clear and coloured lakes overlapping in most cases.

Five morphological character states which proved to be stable and conservative and therefore useful for taxonomic identification, were measured on cleaned *Asterionella* valves. Populations were compared seasonally as well as within and between lakes. In all cases, differences were seen in one or more characters as well as for values reported in the literature for *A. formosa* and *A. ralfsii* and its varieties. Using principal components analysis, variability in morphological characters could not be connected with any measured environmental variable.

Pigment analysis revealed distinct differences in ratios of xanthophylls and chlorophylls between *A. formosa* and *A. ralfsii* var. *americana* cells grown in culture. The dominant pigment in the *A. formosa* clones was fucoxanthin and the combined xanthophyll component was twice that which was seen for chlorophyll a. *Asterionella ralfsii* var. *americana* clones had higher ratios of chlorophyll a to any other single or combined pigments.

In conclusion, valve and colony morphology did not differentiate between any of the *Asterionella* populations sampled from the eastern North American sites investigated in this study. Intermediate morphological forms were seen that possessed traits common to *A. formosa* and *A. ralfsii* and its varieties. However, pigment complement as well as pH preference were different in clones clearly identifiable in the classical sense, as *A. formosa* and *A. ralfsii* var. *americana*. Thus the *Asterionella* populations in this study form a morphological continuum with *A. formosa* at one extreme and *A. ralfsii* at the other extreme. Clearly, the *Asterionella formosa / ralfsii* complex is an example of adaptive radiation in diatoms. Therefore, the use of these widespread and abundant taxa as bioindicators and as environmental proxies in paleoecological reconstructions should be with great caution. If this taxon is evolving and changing morphologically as the data from this study indicate, then present methods used for taxonomic identification are inadequate and misleading. Greater emphasis needs to be placed on variability across and within populations in all aspects of the biology of the taxon under investigation. This work strongly suggests that diatomists should be more inclusive and less exclusive when erecting taxa. Local or geographic populations of a taxon should be considered ecotypes, with variability a function of differing ecological conditions, possible founder effects or genetic mutations.

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I. INTRODUCTION

I.1 An historical perspective of diatom taxonomy

Diatom identification and classification has undergone many changes since the first documented occurrence of diatoms in the early 1700's. By the latter half of that century diatoms were being described and given Latin binomials. The interpretation of the nature of diatoms was much in dispute and classification was divided between the animal and the plant kingdoms. The publication of Kützing's monograph in 1844 treated all diatoms as plants, effectively ending the confusion and uncertainty.

The study of diatoms kept pace with the development of the microscope in the latter part of the 19th century. This was a great time for world-wide expeditions and collections by many enthusiastic amateurs. Many of the earlier diatomists, notably Grunow and Cleve, were encouraged to produce their own monographs of diatom genera. It is from this time and to these workers that we owe a great debt, for their persistence, careful observations, and superb type collections that have allowed future generations of diatomists to continue exploring the world of the diatom.

In the 20th century, interest in diatoms expanded from the fascination of the siliceous frustule and its structure, to the biology of these organisms. The occurrence of different diatoms in different habitats had always been a puzzle, the physiology and biochemistry were poorly understood in most diatoms, and the role of diatoms in the ecosystem was beginning to be appreciated. Great advances in these areas have been made in the last 90 years and information regarding the genetics, internal structures and sexuality have begun to be investigated.

The biological species concept was first introduced by Mayr (1940) and Dobzhansky (1937) and was designed to apply to sexually reproducing organisms living sympatrically. Because this concept is limiting and does not include asexual or parthenogenetic organisms, many biologists have found it wanting. Considerations about the amount of diversity in mechanisms of speciation and the relationship between speciation and macroevolutionary patterns have also been areas of conflict for many evolutionary biologists (Templeton 1987). The purpose of classification is not only to group organisms into convenient clusters for identification and phylogenetic comparison but also to indicate subtleties in biochemical information, ecological preference, and physiological response.

In most diatoms, the sexual reproductive system is unknown or at best poorly understood. Consequently, it is difficult to apply to these organisms a concept based on reproductive isolation within populations such as that proposed by Mayr (1940) and Dobzhansky (1937). The classification and organization of taxa within the Bacillariophyceae has been, for the greater part, based on the structure of the siliceous cell wall. In the past, these observations were collected using light

microscopy and did not discern the finer features we now know the diatom frustule to possess. Taxa erected before the common usage of the electron microscope are often found to contain a greater diversity of genera and species than previously thought (Round *et al.* 1990).

Physiological and biochemical differences are known to occur within different diatom taxa, but the extent of within-group variation is unknown for all but a few isolated cases. Diatom communities occurring in natural conditions are subject to interactions between other organisms, whether it be competition, predation, parasitism, changing environmental conditions unique to each individual system, biochemical variations within each water body or watershed and, in some cases, the influence of anthropogenic impacts. The extent to which these factors contribute toward the astonishing plasticity evident in the diatom frustule is poorly understood.

Morphological variability within diatoms has been a source of concern for investigators since the earliest attempts at classification. This is quite valid, as taxonomic identification and classification is still, for the greatest part, based on the appearance of the siliceous frustule under microscopic examination. There are two schools of thought on how this variability should be treated. One group (Lange-Bertalot 1976, 1980; Lange-Bertalot & Simonsen 1978; Simonsen 1979) takes a broad view and combines all morphological variants together, while the other (Round 1978a, 1978b; Round *et al.* 1990) strictly divides variants into species, varieties or forms. The problem is one of defining boundaries between different morphotypes which are adequate to delimit species.

Taxonomic unit descriptions are based on conservative and, hopefully, stable morphological features. The major problem at lower levels of organization in diatoms is the lack of definitive boundaries between morphotypes. Very little is known concerning the ability of morphotypes to interbreed, or how much clonal variability exists within a population. Phenetic information, not reproductive features, are used as characters to delimit taxa. It is not known to what extent this reflects the genetic make-up of a population. If, during reproduction, something affects the valve formation, morphotypes may occur that differ phenotypically but not genotypically. The difficulty lies in defining the range of morphotypes that should be included in a species and in determining where natural breaks occur between species. If valve outline and size change with successive cell divisions in natural populations, a range of morphological types should be seen. Sampling frequency could determine whether the various forms are stages in a continuum or discrete entities (Cox 1984).

Generally, comparative anatomy and morphology are the basis for erecting taxa from family level down to form. In natural collections of marine centric diatoms, cells have been found that have two very different valves, each with characteristics of different genera (Wood 1959). In clonal

cultures, cells of one species have been shown to produce valves of other "species" (Holmes and Reimann 1966; Holmes 1967; Drebes 1967). Taxonomic 'forms' have been established in some species based on morphotypes, but often cells have one valve of one form and the other valve of a different form. Very little is known about hybridization between morphotypes: if it can occur or to what extent it happens in natural populations. Isolated observations on four diatom species have shown that selective interbreeding or hybridization may occur between different forms or varieties of these diatoms (von Stosch 1962; Geitler 1958,1969; Mann 1984).

Very little attention has been given to using other features of the cell to distinguish differences or similarities in species. *Skeletonema costatum* and *S. tropicum* are distinguished solely by the number of chloroplasts each cell contains, but it is not known how these populations are separated genetically (Hasle 1973). A re-evaluation of several naviculoid diatoms that were originally classified by the chloroplast type has resulted in a number of species being removed to other genera (Cox 1987b). Strains of diatoms similar in morphology but found in different localities have been shown to be very different physiologically (Guillard & Ryther 1962; Guillard 1968; Guillard *et al.* 1974; Hutchinson 1967). Genotypic variability in morphologically identical clones has been shown from their electrophoretic banding patterns and has been coupled with differences in growth rates, chlorophyll content and carbon uptake (Gallagher 1980,1982). Sequence differences in the small subunit of rRNA were seen in different geographical races of *Skeletonema* (Medlin *et al.* 1988).

In this study, the freshwater genus *Asterionella* was the diatom under investigation, for several reasons. The amount of morphological variability and the different biochemical, physiological, and ecological attributes found in this genus have led past investigators to consider it as containing at least two, and perhaps three, distinct species. If diatom taxonomy is to be based on phenetic characters, then definite differences in these characters should delimit species. If, on the other hand, diatom taxonomy is to encompass everything known about the organism in question, then a more precise classification must be developed which includes all known traits; ecological, physiological, and biochemical as well as morphological.

Future studies on extant diatoms will have to include morphological, cytological, physiological, and biochemical data if classification and identification of diatoms at the generic level and lower is to be phylogenetically informative. It cannot be assumed that a character which is stable in one genus is necessarily stable in a different genus. Each character will have to be examined independently to decide which can be used as diagnostic for that genus. As Mann (1984)

postulated: "Data is usable for taxonomy only if it exhibits variation between the taxa in question; it will be used only if it is economic to gather."

I.2 Background on the Taxonomy of *Asterionella*

Asterionella is a small genus of 11 species, only 3 of which are freshwater species: *A. formosa*, *A. glacialis*, and *A. ralfsii*. Among these, *A. formosa* is the best known and the most widely studied. *Asterionella glacialis* is a relatively rare species but has been recorded in northern Ontario. *A. ralfsii* is as ubiquitous as *A. formosa* but is found in small dystrophic water bodies with low pH values.

There has been a great deal of uncertainty in the past over the taxonomic treatment of *Asterionella*. In the mid-to late-1800's, the genus was redescribed under other names and combinations, in part because of the variability of frustule morphology and also because there are so few characters that separate it from other closely related araphid pennate taxa. Genus-specific characteristics occur either singly or in combination in many closely affiliated taxa.

The genus *Asterionella* was first validly published by Hassall in a pamphlet entitled "Microscopic Examination of the Water Supplied to the Inhabitants of London and the Suburban Districts" in 1850. According to the International Code of Botanical Nomenclature (Article 44), the good illustration and diagnostic annotations are considered as a valid publication even though a description is missing. The genus at that time was considered monotypic so, according to Article 42 of the code, *Asterionella formosa* Hassall was a valid publication of that genus.

Asterionella ralfsii was first validly published by Smith in 1865 as a name but with no accompanying illustration. The type location was Dolgelly in North Wales. Later published illustrations from the original material were very uninformative, so the species remained obscure and subsequent discoveries were not identified as *A. ralfsii*. They were either misidentified as *Peronia*, a raphid diatom or as a variety of *A. formosa* or *A. gracillima*. *Asterionella ralfsii* has been recorded in Africa, Madagascar, Europe, and the eastern seaboard of North America. Walker and Paterson (1986) found this species abundant in sediments of deep, strongly acidified coloured lakes in Atlantic Canada, while Patrick and Reimer (1966) have described it as favouring shallow dystrophic lakes. Foged (1962) found *A. ralfsii* in acidic oligotrophic conditions and provisionally assigned the pH category as acidobiontic. Besides the nominate variety, the varieties *hustedtiana* and *americana* have been described by Körner (1970).

Patrick and Reimer (1966) recognised three freshwater species of *Asterionella*: *A. bleakeleyi* W. Smith, an estuarine species similar in appearance to *A. formosa*; *A. ralfsii* W. Smith, a species found in shallow dystrophic water with low pH and mineral content; and *A. formosa* Hass. which is

most often found in mesotrophic to eutrophic waters. For this last species they list two varieties: *A. formosa* var. *formosa*, Hass. and *A. formosa* var. *gracillima* (Hantz.) Grun.

In the most recent taxonomic treatment of *Asterionella*, two species groups are recognised (Körner 1970). The freshwater species are represented by *A. formosa* and *A. ralfsii* and are linked to the marine group by the morphologically intergrading *A. notata*. *Asterionella bleakeleyi* is considered one of 36 synonyms of *A. formosa* as is *A. gracillima* and *A. formosa* var. *gracillima (sensu* Patrick and Reimer). Revised descriptions were given for these species and two new varieties of *A. ralfsii* were described. *Asterionella ralfsii* var. *americana* Körner corresponds to those that were found in the New England and Mid-Atlantic states of North America in waters that were 'weakly' acidic. *Asterionella ralfsii* var. *hustedtiana* Körner is the German variety and is found in waters with pH values between 6 and 7. Körner considered *Asterionella ralfsii* var. *ralfsii* to be a mid, western and northern European species found in the plankton of still, fresh waters of pH between 5 and 6.

Asterionella ralfsii var. *americana* is one of the few planktonic diatoms that occurs in lakes of pH less than 5.5. There have been problems in the past fitting this diatom to one of Hustedt's pH preference categories, and thus its status as an indicator species is still uncertain. Charles *et al.* (1990) described two 'forms' of *A. ralfsii* var. *americana*: a short form (<45 - 60 μm) and a longer form (> 60 μm). They classed the short form of *A. ralfsii* var. *americana* as acidobiontic and the long form as acidophilous. Only long forms were seen in a study of 35 Quebec lakes and these were considered acidophilous (Dixit *et al.* 1988). Many authors have stated that the lakes where *A. ralfsii* var. *americana* is found are acidic, but have not indicated a pH preference group or whether short or long forms were present (Taylor *et al.* 1986; Walker and Paterson 1986; Anderson *et al.* 1993). It has been suggested that the pH classification within the *Asterionella* species complex may be dependent on water chemistry and changes in DOC-mediated trace metal avaliability (Gensemer *et al.* 1993). In controlled culture studies of *A. ralfsii*, var. *americana* a decrease in mean cell length was shown to occur with additions of Al (Gensemer 1990). Its response to acidification and dissolved organic matter (DOM), especially fulvic and humic materials, is unknown. An incomplete understanding of the taxonomic and ecologic relationships between *A. ralfsii* var. *americana* and closely related forms has also contributed to the confusion surrounding this taxon.

The freshwater *Asterionella* are ubiquitous, planktonic, star-shaped colonies. The morphology of the two freshwater species, *sensu* Körner, is very similar, both in the individual cells and the colony form. The high degree of morphological plasticity seen, especially in *A. ralfsii* var. *americana*, and the relationship this plasticity may have to ecological conditions is still unknown.

The two forms of *A. ralfsii* var. *americana* described by Charles *et al.* (1990) from the Adirondacks have been seen in different areas in North America and have been linked with a number of variables: depth of the lake, pH or alkalinity of the water, solute concentrations of Ca, Mg, SiO_2, trace metals, and humic content (Anderson *et al.* 1993; Riseng *et al.* 1991; Gensemer 1991a, 1991b; Walker and Paterson 1986; Charles *et al.* 1990). It may be that the natural distribution of the freshwater *Asterionella* is linked to environmental conditions that, over time, may have altered the physiology and biochemistry of what we now regard as two separate species.

I.3 Previous studies of *Asterionella*

The ecology, physiology and, to a lesser extent, the morphology of *Asterionella*, both under natural conditions and in culture, have been the focus of many studies since the mid 1940's. Some of the earliest work on *A. formosa* was undertaken in the English Lake District by Hilda Canter and John Lund (Canter & Lund 1948; Lund 1949,1950). They examined population fluctuations in relation to fungal epidemics and the effects of nutrient depletion, especially phosphates, silicates and nitrates, on the spring bloom of *Asterionella*. As well, Lund looked into the possible origin and nature of the cells producing the seasonal maxima in the plankton. The data covered a period of twelve years and culture experiments were done, as well as observations on naturally occurring populations. No reproductive stages or auxospores were ever seen in the sediments or lake water, although Lund did later consider the possibility that the variety *acaroides* may be the auxospore of *A. formosa*. It always seemed to occur after the maxima in the plankton and in conditions of nutrient depletion (Lund 1961).

In 1953, a follow-up study to Lund's in the English Lake District was done by Mackereth on phosphorus utilization by *Asterionella*. He found that a minimum amount of phosphorus was required by the cells and that they could retain a reserve of this nutrient which could be utilized at the onset of cell division. The cells seemed to have an excellent ability to accumulate phosphorus even from very low external concentrations. The growth rate of *A. formosa* and the relationship between phosphorus, silica, water temperature and intensity and duration of illumination was clarified by Hughes and Lund (1962). A comprehensive report on the standing crop of *A. formosa* in relation to changes in depth, chemical, and physical conditions in the north basin of Lake Windermere subsequently added to the knowledge of that genus (Lund *et al.* 1963).

In a study of two alpine lakes in Colorado, the populations of *A. formosa* were found to be parasitised by the same chytrid fungi that Canter and Lund had seen in English lakes (Koob 1966). The length class distribution of the *Asterionella* cells was linked with the occurrence of the parasite.

Koob did not find any correlations between the growth phase of the parasitic population and the concentration or growth phase of the host population. Temperature was found to be an important factor in the mortality of *A. formosa* infected with parasitic fungi both in culture experiments and in field populations (Kudoh & Takahashi 1990). It was suggested that this type of host-parasite interaction was a significant factor in seasonal changes of the population density of *A. formosa* in some eutrophic lakes.

It has often been presumed that the transport of algae between lakes is a function of water birds, animals, or the wind. Many diatoms, especially soil diatoms, can withstand drying for short periods of time, but the same has not been shown for planktonic species. As *Asterionella* is one of the most widely distributed of all planktonic algae, its ability to remain alive once dried would explain, to some extent, the distribution of this diatom. It was found that once 87% of the water was lost, *Asterionella* cells began to die (Jaworski and Lund 1970). They could remain alive for up to 19 days under very water reduced conditions but could not withstand desiccation, making it very unlikely for wind to be the main distribution vector between lakes. It may be reasonable to suggest that damp mud carried by water birds or animals, if transported rapidly between lakes, is the main dispersal vector of *Asterionella*, but there is no evidence to substantiate this idea.

Diatoms require silica and when it is at reduced or low levels it is more limiting to growth than phosphorus or nitrogen. The patterns of seasonal periodicity and the dominance of different planktonic diatoms were examined by Kilham (1971) with relation to the ambient silica concentrations in eutrophic lakes. This study suggested a strong correlation between the dominance of specific diatoms and the concentration of silica in the water. The relationship of *A. formosa* to other planktonic diatom species was complicated by the fact that it is found in both eutrophic and oligotrophic waters. It was suggested that the trophic status of the lake influenced the success of *A. formosa* and that silica demand might be used as an index of increasing eutrophication. Kilham concluded that more information was required on the ecology of this species before anything definitive about silica requirements and a eutrophication index could be established.

A subsequent study was done with clones of *A. formosa* from lakes of different trophic status to examine the kinetics of silicon-limited growth and intraspecific response differences (Kilham 1975). The level of Si at which growth ceased in the clone from the English Lake District (Lake Windermere) was in agreement with that which had been considered limiting in natural populations from that lake. The difference in the Si growth kinetics seen in the second clone was difficult to interpret as no data were available for that lake, (Lake Ohrid, Yugoslavia), but adaptation to different

environmental conditions was suggested for the variance. Nutrient kinetics, the influence of trace metals, pH, and Al have all been studied in relation to silica and phosphorus limited growth rates in *Asterionella* (Tilman & Kilham 1976; Gensemer 1991; Riseng *et al.* 1991). The effects that additions of Al had on Si-limited cultures of *A. ralfsii* var. *americana* influenced cell size as a function of growth rate (Gensemer 1990). This physiological information has clarified many differences between the two freshwater *Asterionella*, especially when comparing this information to natural populations.

Variation in diatom morphology is well known, but the factors that influence these changes are poorly understood. Considering the many physiological studies on *Asterionella*, very little work has been done on the morphology of the two freshwater species. Size variation has been related to the cell growth cycle and reproduction and, although auxospore formation has not been seen in *Asterionella*, it has been seen in other closely related araphid genera (Geitler 1939). Clones of *A. formosa* which were derived from a single parent colony and studied over a four month period in culture were shown to differ both morphologically and physiologically (Happey-Wood & Hughes 1980). A decrease in cell length was always seen. A study of the *A. formosa* population in Lake Taupo, New Zealand examined both extant and palaeo-populations (Rawlence 1986). The data suggested a series of irregular changes in cell length rather than cyclical changes. There was also an inverse relationship seen between increasing frustule length and the abundance of the *Asterionella* population relative to other diatoms. Electrophoretic analysis on 101 clones of *A. formosa* showed that multiple isolates from the same population were highly homogeneous genetically, at least in small or morphometrically simple lakes (Soudek & Robinson 1983). Isolates from different populations clearly showed significant genetic differences, and in large lakes more than one genotype was suspected.

1.4 Research Background and Challenge

Similarities between species of the freshwater diatom, *Asterionella*, have been a source of problems in many studies (Stoneburner and Smock 1980; Delorme *et al.* 1984; Blouin 1989). Although their ecological and physiological preferences have been shown to be quite different, both *A. formosa* and *A. ralfsii* have very similar single cell and colony morphology. This makes the two taxa very difficult to differentiate using light microscopy. In addition, there is very little information in the literature about *A. ralfsii*, but a great deal about *A. formosa* (Foged 1962). *Asterionella formosa* has been found in lakes with nutrient status varying from oligotrophic to eutrophic, but the earliest report of *A. ralfsii* claimed it favours shallow dystrophic lakes (Patrick & Reimer 1966). More recently, Charles *et al.* (1990) found different forms of *A. ralfsii* var. *americana* occurring in lakes of different

sizes and humic content and considered them to be different ecotypes. Anderson *et al.* (1993) showed that the ecology of the two forms as defined by Charles *et al.* is very different in small, oligotrophic, low conductance lakes in northern New England.

The initial objective of this study was to determine morphological variation in the *Asterionella ralfsii / formosa* complex and to clarify taxonomic relationships within the complex. This project was based on *Asterionella ralfsii* and any related forms found in the sampled sites, primarily Kejimkujik National Park (KNP), Nova Scotia. Increased industrialization has had an enormous effect on freshwater systems both in North America and in Europe, and long range transport of atmospheric pollutants has had an impact in areas far removed from the point source of those pollutants. In North America, the Atlantic provinces and the New England states have been the recipients of acidic deposition from the mid-western United States and Canada. The lack of buffering capacity in the soils and bedrock in these areas has resulted in rapid acidification of the freshwater lakes and streams. This, in turn, has influenced the chemical characteristics and biological communities found in these areas (Kerekes 1989). The growing concern about possible harmful effects and the impact of acid precipitation led to a continuation of the Kejimkujik Watershed Study initiated by the Canadian Wildlife Service and Canadian Parks Service in 1970. The Atmospheric Environment Service, Canadian Wildlife Service, Inland Waters Directorate, Canadian Parks Service of Environment Canada, Forestry Canada, Department of Fisheries and Oceans and contracted scientists from universities and the private sector have all contributed to this study since then. Kejimkujik National Park (KNP), Nova Scotia, was chosen as one of the primary study sites because of many special features not seen in other watersheds. As well as being one of the most sensitive aquatic receptor systems in North America, KNP contains clear and coloured lakes in close proximity that have almost identical inorganic water chemistry but widely divergent pH values. The watershed is for the greater part within the confines of a national park and can, therefore, be protected from disturbances caused by logging or settlement. The *Asterionella* in these lakes has previously been described as *A. formosa* (Delorme et al. 1984) and as a 'form' of *A. ralfsii* (Duthie 1989).

Delorme and co-workers examined the pH trends in a sediment core from Kejimkujik Lake, the largest lake in the Kejimkujik watershed, and described one of the dominant diatoms as *Asterionella formosa* (Delorme *et al.* 1984). This diatom, according to Hustedt's pH spectrum, is alkaliphilic. Of the ten most common diatoms reported, only one other fell into this category (*Melosira islandica*). All others were reported as either acidophilic or acidobiontic. The discrepancies seen in the measured lake pH and the diatom inferred (DI) pH of the surface sediment

sample led one of the authors to re-examine the core (Duthie 1989). Using additional taxonomic references and an index calibrated for lakes in Atlantic Canada (Walker and Paterson 1986), as well as newer ecological information on diatom species, the core was re-analysed. This reinterpretation resulted in the identification of an *Asterionella* with a morphology similar to that seen in *Asterionella ralfsii* var. *ralfsii*, *Asterionella ralfsii* var. *americana*, and to *Asterionella formosa* (Duthie 1989).

Nygaard (1956) proposed an index for inferring lake water pH from diatoms. He based his system on a pH spectrum for diatoms devised by Hustedt (1937-39), who grouped diatoms according to the hydrogen-ion concentration of the water in which their occurrence was optimal. Five categories were established by Nygaard:

alkalibiontic: occurring at pH values above 7

alkaliphilous: occurring at pH values about 7 and with widest distribution at pH above 7

circumneutral: occurring around pH 7

acidophilous : occurring at about pH 7 and with widest distribution at pH values below 7

acidobiontic : occurring at pH values below 7; optimum distribution at pH 5.5 or less

Nygaard (1956) used these categories to develop a quantitative treatment which weighed the extreme groups, the acidobiontics and alkalibiontics, and stressed the ecological significance of these groups over the more commonly occurring alkaliphilous, acidophilous and circumneutral taxa. Merilãinen (1967) later applied the resulting indices, (alpha, omega and epsilon), to the diatom floras in 14 lakes and ponds in Finland and found them to be a useful method for computing the functional pH of lake water. The limitations of this model were addressed by Renberg and Hellberg (1982), who calculated the coefficients by the least square regression method rather than assigning an arbitrary value as Nygaard had done. Since then, other workers have modified the linear function in accordance with varying ecological parameters found in lakes from different geographical localities (Charles 1985; Davis & Anderson 1985; Dixit *et al.* 1988; Taylor *et al.* 1986; Walker & Paterson 1986).

Recent studies (Charles *et al.* 1990; Anderson *et al.* 1993) have indicated that much of the variation seen in the *Asterionella* complex may be related to environmental gradients, but the actual causal factors remain unknown. Morphological variation was examined in this study from populations occurring in lakes in KNP, as well as occasional collections obtained from lakes in Ontario, Canada and New Jersey, U.S.A. These covered a range of pH values, temperatures and humic content in lakes sampled in both the spring and fall bloom periods. The question of whether morphological differences could be used as taxonomic characters to assure correct identification of

the different species and, if morphological variability could then be related to either the measured pH or the humic content of a lake, a broader understanding of this genus might ensue. The accuracy of using *Asterionella* species as a biological indicator for inferring past or present pH was also considered. Whether morphological variability occurred in populations of *Asterionella* from the spring and fall blooms periods in these lakes might indicate a possible physiological dependency on temperature. It was noted that when grown in culture, *A. formosa* and the form of *A. ralfsii* form KNP were different colours, so it was hypothesised that the pigment ratios might be useful as a biochemical marker to separate the two possible species. Population studies of diatoms have been conducted over time from a single location (Cox 1987a; Jewson 1992; Lund 1949,1950) but very few studies have examined a single diatom genus from multiple sites over time (Mann 1988). The results of this study will contribute to a broader understanding of the autecology and taxonomy of this cosmopolitan diatom genus.

I.5 Hypotheses and Research Design

The following groups of questions will be addressed in this thesis:

What is the taxonomic relationship between *A. ralfsii* and *A. formosa*? Is *A. ralfsii* var. *americana* a real taxon or is it a variant of *A. formosa*? If *A. ralfsii* var. *americana* is to be considered a separate taxon, then is it the only *Asterionella* to be found in Kejimkujik National Park and the other sites today? Are there others in the *A. ralfsii* complex at these sites and, if so, what is the taxonomic relationship between them?

Is *Asterionella ralfsii* var. *americana* an ecotype of *A. formosa* adapted to different lake water conditions and, are these adaptations evident in morphological variability? The *Asterionella* seen in Kejimkujik National Park have characteristics that have been described for *A. ralfsii* var. *ralfsii*, *A. ralfsii* var. *americana* and *A. formosa* (Duthie 1989). How extensive is the morphological variability in the *Asterionella* cells from the collection sites in Kejimkujik National Park? Are there differences in the morphology of the *Asterionella* found in the clear lakes as opposed to those found in coloured lakes? If there are differences, what are they and do they distinguish different taxa or ecotypes? Can these differences be related to the humic content or to the pH of the lake in which a population was found? Are there variations in the morphology of the *Asterionella* populations within a lake over different seasons? If so, is it the same taxon or are there different ecotypes occurring at different times of the year? Are the characteristics of the colonies similar between clear and coloured lakes? Is there any seasonal change in the size or number of cells per colony? Does this reflect temperature-dependent ecotypes found in a single lake?

Can the pigment composition of *A. ralfsii* var. *americana* be used to differentiate it from *A. formosa*? Is this a biochemical marker which could be useful in the identification of these two species?

HYPOTHESIS ONE

There are no clear morphological differences between the *Asterionella* taxa described as *A. formosa* and *A. ralfsii* var. *americana*.

HYPOTHESIS TWO

Morphological variability in *Asterionella* populations can be attributed to environmental factors such as humic content, changes in temperature or the pH of the lakes in which these populations are found.

HYPOTHESIS THREE

Differences in pigmentation between clones of *A. formosa* and *A. ralfsii* can be used in extant populations to differentiate these two taxa.

To test these hypotheses, planktonic net collections, pH, conductivity, and temperature measurements were obtained from lakes and streams over the course of four years (1990 - 1993) and two seasons (spring and fall). Clear and coloured lakes and streams were sampled in Nova Scotia (Kejimkujik National Park and McIntyre Lake, Cape Breton) in the spring of 1990, 1991, 1993 and in the fall of 1991 and 1992. Clear lakes in Ontario were sampled in the spring of 1991 and 1993. Birchwood Lake, New Jersey and Thirty Acre Pond, Rhode Island were sampled in the fall of 1993. Using the light microscope, valve length of at least 100 cells were measured and the number of cells per colony counted. Subsamples were prepared for electron microscopy and different valve characters were measured (valve length, mid cell width, head pole width, foot pole width, number of spines in 6 μm). Average values from these measurements were compared with reported measurements found in the literature (Rabenhorst 1864, Van Heurck 1896, Boyer 1927, Foged 1962, Patrick and Reimer 1966, Körner 1970). The morphological measurements obtained from the scanning electron microscope, (SEM), were compared by plotting the measured characters against the lake water variables (pH, conductivity, temperature) to determine factors which may influence morphological variability. Principle components were analyzed on some samples to verify the results obtained from the plots. Factor analysis, anovas and multiple regressions were also generated using morphological characters and environmental variables. A statistical comparison was done using the honestly significant interval (HSI) analysis method which gives a more accurate representation of the uncertainty interval than the confidence interval for the mean or the standard error of the mean (Andrews *et al.* 1980).

In addition, slides from the Natural History Museum (B.M.#24422), the Botanisches Museum Berlin-Dahlem (Berlin #25636,#25635) and the National Museum of Natural Sciences (Ottawa #30236,#30235), containing *Asterionella* were examined with the light microscope and 100 cells were scored. A portion of the specimen *Diatoma stellare* (isotype of *A. ralfsii* var. *ralfsii*) from the British Museum was prepared and examined with the scanning microscope. Valve characters were measured on those cells which displayed the entire suite of characters.

Clonal cultures were established from colonies identified as *A. formosa*, isolated from Georgian Bay, Ontario and from colonies identified as *A. ralfsii* var. *americana* isolated from Birchwood Lake, New Jersey (type location). All cultures were grown under identical conditions until they contained enough pigment for analyses by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Silica and phosphorus assays were done to determine the amounts of these nutrients left in the medium at the time of cell harvest.

II. METHODS AND MATERIALS FOR COLLECTION AND MICROSCOPY

II.1 Study Sites

The primary study site for this project was Kejimkujik National Park, located in south-central Nova Scotia, approximately 50 - 60 km from both the Atlantic Ocean and the Bay of Fundy. It lies on Devonian granite and is considered to be one of the freshwater systems most sensitive to long range acidic precipitation in North America. It is 94% forested, surrounded predominantly by mixed conifer-angiosperm stands typical of the Acadian Forest Region (Rowe 1972). There are extensive fens, bogs and heath-dominated wetlands throughout the park. The lakes are mainly shallow and range in pH from 4.2 to 6.0. Some of the lakes in the park are brown-coloured due to dissolved humic substances leaching in from boggy organic substrata. The lakes are polymictic, oligotrophic, weakly buffered and either acidic or susceptible to acidification (Freedman *et al.* 1989).

Twenty-six sites in Kejimkujik National Park were sampled (Figure 1). Eight of them were clear lakes and nine were coloured. Two outflows from the larger lakes were sampled; Mersey River out of Kejimkujik Lake and Peskowesk Brook out of Peskowesk Lake. The Mersey River at Jakes Landing, an inflow into Kejimkujik Lake, and a connecting stream between Peskowesk and Peskawa Lakes, Poison Ivy Falls, were also sampled.

Other sites included in this study were one clear lake, McIntyre Lake, from lower Cape Breton Island. It is isolated from Kejimkujik National Park and lies in a different watershed but, like the lakes within the park boundaries, is shallow. Periodic samples were taken from four lakes in the Haliburton-Muskoka area of central Ontario (Plastic Lake, Leonard Lake, Joseph Lake, McKay Lake). This area has also been classed as environmentally sensitive due to the poor buffering capacity offered by the granitic Canadian Shield. Sampling was done for *A. formosa* in Georgian Bay at Wiarton, Ontario with varying success. The type location, Birchwood Lake in New Jersey, U.S.A. was sampled for *A. ralfsii* var. *americana,* as was 30 Acre Pond, Rhode Island, U.S.A.

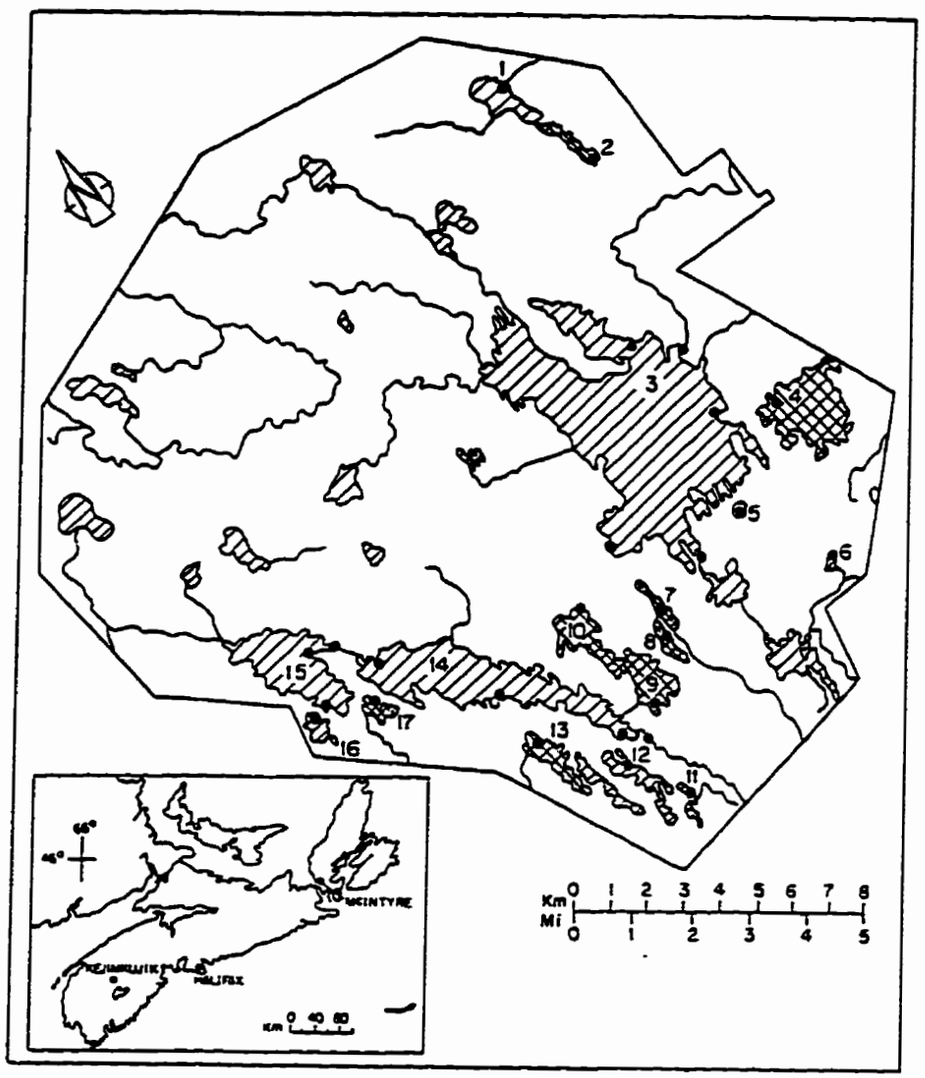

Figure 1.Sampled sites from Kejimkujik National Park. 1) Big Dam West 2) Big Dam East 3) Kejimkujik Lake (from north to south - Jeremys Bay, Kedgemakooge, Jakes Landing, Merrymakedge, Mersey River outflow, Minards Bay) 4) Grafton 5) Snake 6) McGinty 7) North Cranberry 8) Puzzle 9) Cobrielle 10) Mountain 11) Little Peskowesk 12) Hilchmakaar 13) Back 14) Peskowesk (from east to west- Peskowesk Brook, lower, mid and upper sites) 15) Peskawa (Poison Ivy Falls, upper and lower sites) 16)Pebbleloggitch 17) Beaverskin

To clarify the identity of the *Asterionella* in this study, samples of this diatom, as well as measured lake water variables such as colour, pH, temperature and conductivity, were collected over a period of four years at most sites. A representative subsample of the *Asterionella* population from each site was measured with both the light microscope (at least 100 valves) and the scanning electron microscope (from 3 to 14 valves) and included all possible morphological characters which could be used to delimit the species. These measurements were used, in conjunction with the lake water variables, to determine if there was a relationship between environmental factors and changes in morphology. Single colonies from the acidic Birchwood Lake, New Jersey, U.S.A. and the alkaline Georgian Bay, Ontario were isolated and grown in culture for pigment separation and analyses. These two sites are believed to have representative populations of *A. ralfsii* var. *americana* and *A. formosa* respectively.

II.2 Field Methods

All lakes examined in this study were sampled in the same way. An attempt was made to match the sampling times with the spring and fall bloom periods of the *Asterionella* populations in as many lakes as possible. Nova Scotian sites were sampled in early June of 1990, 1991, 1993 and late October 1991 and 1992. Sites in Ontario were sampled in late May or early June of 1991 and 1993. The New Jersey and Rhode Island sites were sampled once in September 1993.

Planktonic net drags (50 μm mesh) were taken from the upper 1.5 m of the lake. The sample was split into two plastic containers. If samples of live material were required, one of the containers was set in a cool place with access to sunlight until transported back to the laboratory. The other part of the sample was preserved with glutaraldehyde or alcohol. Conductivity, temperature and pH readings were taken at this time with a Corning Checkmate field sensor. Water was checked visually for colour (transparent or brown).

II.3 Diatom Preparation

The preserved material was cleaned in hot concentrated nitric acid (HNO_3) or hydrogen peroxide (H_2O_2) (just below boiling point). After rinsing and centrifuging repeatedly until samples were free of acid, strewn slides were prepared and examined under 1000x with a Nikon Orthophot microscope (n.a. = 1.3). The lengths of at least 100 cells were measured and when the frequency distribution indicated a bimodal pattern another 100 cells were measured for clarification. Slides of *Asterionella* from the Berlin Museum, the Ottawa Museum and the British Museum were also measured at this time.

An aliquot of cleaned material was placed on SEM stubs and left to dry in a desiccator. The

sample of *Diatoma stellare* from the British Museum was attached to a stub with double sided tape. All stubs were later splutter coated with approximately 300 nm of gold and examined at an accelerating voltage of 15 Kv in a Hitachi S 570 scanning electron microscope (SEM). Valves showing the complete complement of valve length, head pole width, foot pole width, mid-cell width, and the number of spines in a 6 μm length along the mid portion of the valve were measured.

For colony size counts, a sample of uncleaned material was placed on a slide and examined by light microscope using 400x magnification. One hundred intact colonies were counted for the number of cells in each colony.

II.4 Statistical Methods

For light microscopy the mean cell length value for each sample was determined along with the uncertainty interval. These analyses were done using the honestly significant interval (HSI) rather than the conventional standard error of the mean or confidence interval of the mean. With the latter two methods, it is not possible to use these intervals to determine whether two means are significantly different, especially in a graphical display of the data (Andrews *et al.* 1980). With HSI, a graphical representation shows the mean value and a pooled value which measures the uncertainty of the means. The statistics are represented by the honestly significant difference (HSD) where $HSD = qS(1/n)^{1/2}$ where S = the experimental error standard deviation, n = the number of observations in each mean and q = the studentized range statistic. q is dependent on the degrees of freedom associated with the pooled error S and the selected significance level. The uncertainty interval when there is equal sample size is represented by the honestly significant interval: $HSI = y_i \pm HSD/2$ where y_i is the mean value of the observations. This results in a single interval for all comparisons. The histograms representing the mean valve length measured under the light microscope clarify many of the population trends found in the lakes.

For unequal sample sizes such as were seen in the SEM data, the formula used was slightly modified and the HSI is represented by $HSI = y_i \pm qS1/\sqrt{2}(1/2n_i)^{1/2}$ where n_i= # observations in the mean y_i. This was calculated for each individual mean value. Therefore, a comparison of several different means results in several different uncertainty intervals. When the sample size is small the result is a large uncertainty interval, and when it is large it is reciprocally small. In a graphical display using the HSI as the uncertainty interval, the means are shown to be significantly different if the associated HSI's do not overlap. Thus, this method allows for direct comparison of significant differences seen between mean values. This procedure was used in the belief that it would indicate true biological differences rather than strictly statistical differences which may not be useful in this

type of study. Plots of the valve characters versus the lake variables (pH, temperature, conductivity) were used to determine any relationship between the different parameters.

Factor analysis was performed on the average values of the character measurements obtained from the scanning electron microscope. The first factor scores were then plotted against the measured environmental variables (pH, temperature, conductivity). Regression and Anova statistics were done to determine any co-relation between morphological characters and enviromental variables.

III. MICROSCOPY

III.1.1 Introduction to results for light microscopy

In the past, valve length has been a defining character in distinguishing *A. formosa* (30 - 120 μm) from *A. ralfsii* var. *ralfsii* (20 - 60 μm) and *A. ralfsii* var. *americana* (20 - 90 μm). If these are separate taxa then the majority of valves measured should be within the size range specified for each taxon. *A. formosa* is reported to be found in clear, oligotrophic to eutrophic systems and could be expected to be found in the clear lakes in KNP. *Asterionella ralfsii* var. *ralfsii* is described as inhabiting small, boggy or swampy dystrophic water bodies with pH values between five and six. *Asterionella ralfsii* var. *americana* is reported from lakes, ponds and streams that are humic and "weakly" acidic. Both varieties of *A. ralfsii* would be expected to occur in the coloured lakes of KNP. The sampled lakes were divided into these two categories based on whether the water sample was clear or whether it was brownish, with the expectation that the longer cell length classes would predominate in the clear lakes. A third division of the sampled sites includes inflowing, outflowing or connecting streams. This was to determine if the *Asterionella* population going into or out of connecting systems reflected the population structure seen in the adjoining lakes.

The other division used in these analyses was one based on seasonality. Several previous investigators reported seasonal dimorphism in valves of *Asterionella*, possibly due to differences in water temperature. There was a marked temperature difference in the lakes of KNP from the spring sampling to the fall sampling period. Valve length classes were compared from populations occurring within lakes, within seasons (three spring samplings or two fall samplings from the same lake), and populations from within lakes, between seasons (spring to fall the same year or fall to spring of the following year). This was done to ascertain whether valve length classes and the average valve length within these populations were consistent in colder or warmer temperatures. Populations from single lakes were also followed across all five sampling periods. If cell length classes showed a gradual decrease or a large increase in size, then this result might indicate cycles of sexual reproduction. As well, clear lakes were compared with other clear lakes and coloured lakes with other coloured lakes to determine possible variability within each subset of lakes.

The number of valves per colony seen in populations of *Asterionella* was also divided according to the water colour and the season in which the sample was obtained. The average colony size was compared for all sites sampled, for clear lakes, and for coloured lakes. Colony size has been correlated to differences in Si and P in lakes so if one of these nutrients is limiting, the number

of valves in a colony may reflect this (Lund *et al.* 1963; Tilman *et al.* 1976). The mean values ± HSI for within lake variability is given for populations from two clear and two coloured lakes. Twenty seven sites in Nova Scotia were sampled in the spring and/or fall of 1990, 1991, 1992 and 1993. Nine of these sites were clear lakes and the rest were coloured lakes or inflows/outflows from coloured lakes. To facilitate discussion of the light microscope results, the sampling times are divided into (1) sites that display *Asterionella* in all three spring sampling periods, (2) sites that display *Asterionella* in both fall sampling periods, (3) sites that display *Asterionella* in both the spring and fall of 1991 i.e. colonies of this diatom within a year, (4) sites that display *Asterionella* in the fall 1992 and spring 1993 samples, i.e., sites which have populations that may have overwintered a season. Sampling sites are divided into (A) clear lakes, (B) coloured lakes and (C) connecting streams and inflow/outflow from coloured lakes. Herbarium slides and samples from sites other than Nova Scotia are presented together.

III.1.2 Results and discussion for light microscopy

III.1.2.1 Valve Length

1. All Spring Samplings

1A.**Clear Lakes**: Only four of the clear lakes contained *Asterionella* in all three spring samplings (Mountain Lake, Grafton Lake, Back Lake, McIntyre Lake). Puzzle Lake and Big Dam East Lake showed populations of *Asterionella* in two of the sampling periods while Beaverskin Lake and Cobrielle Lake exhibited *Asterionella* populations in one spring sampling period only.

Mountain, Grafton, and McIntyre Lakes showed the lowest average valve lengths in the spring of 1991 and the highest average values in the spring of 1993. Back Lake showed a gradual increase in average valve length of slightly greater than 10 μm with each successive spring sample (Figure 2).

Minimum and maximum cell length values remained fairly constant in the spring 1990 and 1991 samplings within lakes and between lakes. An exception to this was seen in Back Lake which displayed closer minimum length values in the 1991 and 1993 seasons. As well, the maximum valve length in Mountain Lake was approximately 10 μm longer than the other lakes in 1990/1991.

The greatest size range difference was seen in Back Lake in the spring of 1993 (41μm). At this time the mean valve length was greater than in any other spring sample from this lake (73.65 μm) and a slight bimodality in the length class distribution was seen. Only 8% of the valves were less than 60 μm in length as compared with 99% and 19% in 1990 and 1991 respectively (Table 1).

Mountain Lake also displayed a slight bimodality in length class distribution in the spring of 1991. The size range in cell lengths for this season was 30 μm, and 27% of the valves measured were less than 60 μm in length. Although the range in valve length was almost as high in 1990 (29 μm), only 3% of the valves were under 60 μm. All valves measured in 1993 were greater than 70 μm with the mean value (82.09 μm) the highest of any of the four clear lakes which showed *Asterionella* in all three spring samples.

Grafton and McIntyre Lakes displayed normally distributed cell length classes with the mean valve lengths of around 62μm, 60 μm, and 70 μm in 1990, 1991, and 1993 respectively. Cells that measured less than 60 μm in length were not seen in either lake in the spring 1993 samples. Forty-two percent and 61% of measured valves in Grafton Lake in 1990 and 1991 were less than 60 μm while 25% and 38% of the measured cells in McIntyre Lake in those same sampling times were less than 60 μm in length.

Two spring samples, 1990 and 1993, from Puzzle Lake which contained *Asterionella* displayed the same trend as was seen in the four clear lakes previously mentioned. Mean valve length increased in 1993 and the valve length classes shifted up. The longest cell measured for spring in any of the clear lakes was seen in this lake in 1993 (102 μm). Thirty-four percent of the measured valves were less than 60 μm in length in 1990, while there were no cells under 60 μm in 1993.

Big Dam East Lake also displayed a shift upward in cell length values. In 1990, 40% of the valves measured were in the 55 μm length class while in 1991, 47% of the valves were in the 65 μm length class. Forty one percent and 9% of the measured valves were less than 60 μm in 1990 and 1991 respectively.

1B. **Coloured Lakes**: The coloured lakes, Peskowesk and Peskawa which were sampled at three and two sites respectively, always showed *Asterionella* in some part of the lake, as did Kejimkujik Lake which was sampled at four sites. Snake Lake showed *Asterionella* in all three spring samplings, while *Asterionella* was seen in the rest of the coloured lakes in two of the spring samplings.

Minimum and maximum valve length values were relatively constant in the spring of 1990 in Peskawa and Peskowesk Lakes. Average valve length was around 80 μm for all sites. An increase in the average valve length of approximately 13 μm was seen in the spring of 1991 at both the upper and lower Peskawa sites and at the upper and lower Peskowesk sites. The mid Peskowesk site decreased slightly (5 μm) in 1991. Cell length averages remained at the 1991 values in Peskawa Lake and at the upper Peskowesk site in the spring of 1993. A decrease of 24 μm was seen at the

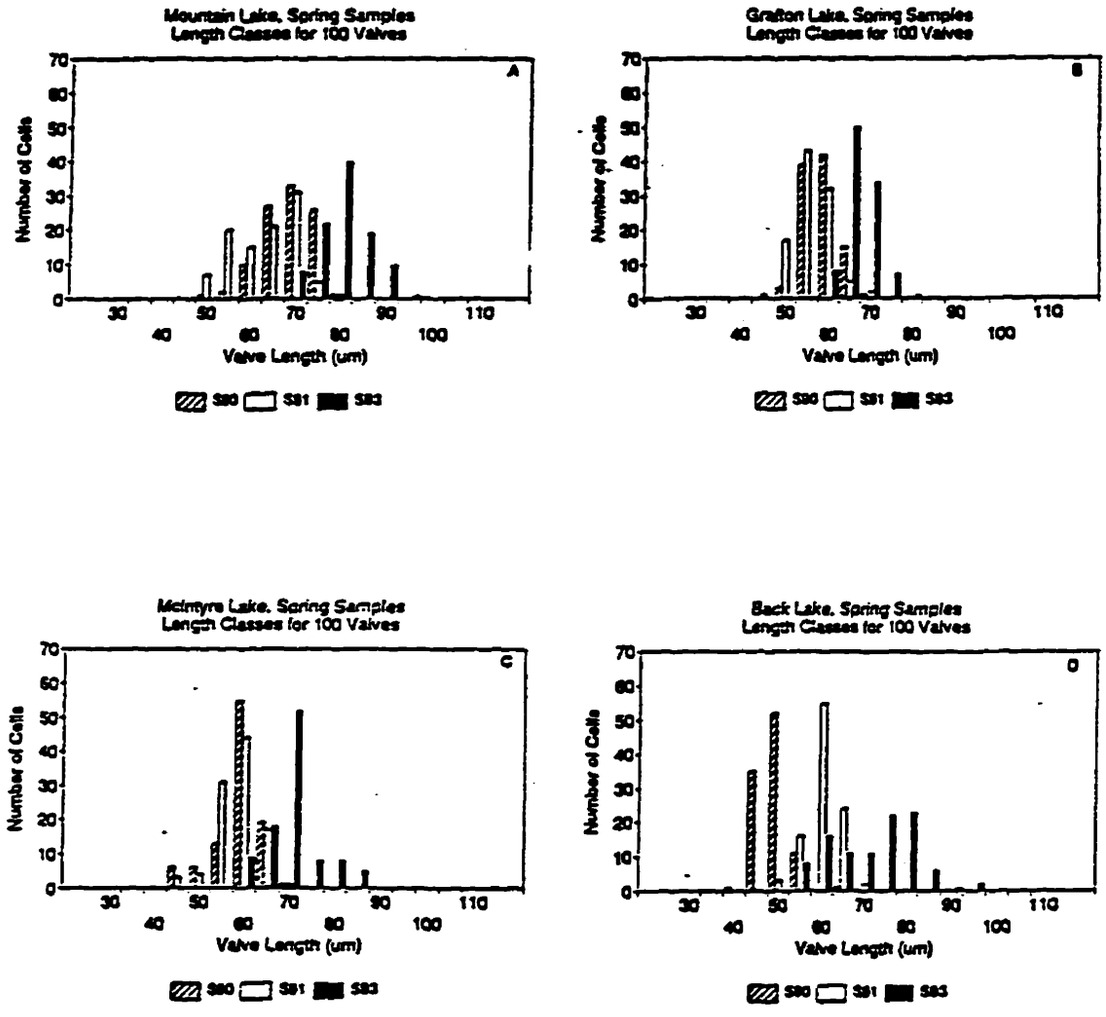

Figure 2. Histograms showing valve length classes in four clear lakes for the spring samplings 1990, 1991, and 1993. A) Mountain Lake B) Grafton Lake C) McIntyre Lake D) Back Lake

Table 1. Minimum, maximum and average cell length values for four clear lakes in the spring 1990, 1991 and 1993. (N = 100 t=0.025)

Lake & Season	Min (μm)	Max (μm)	Mean ± SEx2 (μm)
Back S90	44	70	52.12 ± 0.61
Grafton S90	53	72	61.89 ± 0.71
McIntyre S90	48	72	62.02 ± 1.00
Mountain S90	52	81	71.75 ± 1.08
Back S91	52	72	63.25 ± 0.69
Grafton S91	50	74	59.54 ± 0.84
McIntyre S91	49	72	61.72 ± 0.84
Mountain S91	52	82	66.38 ± 1.42
Back S93	55	96	73.65 ± 1.91
Grafton S93	60	80	69.32 ± 0.73
McIntyre S93	62	88	71.99 ± 1.14
Mountain S93	71	96	82.09 ± 1.06

lower Peskowesk site at this time. The average values for the mid Peskowesk 1991 and lower Peskowesk 1993 sites (74.22 μm and 70.26 μm respectively) were the lowest values seen in any part of these lakes at time when *Asterionella* occurred. No *Asterionella* was found in the sring 1993 sampling at the mid Peskowesk site.

From the five sites in Peskowesk and Peskawa Lakes over the three spring sampling periods, only 4% of the cells measured were less than 60 μm. The smallest cell measured was obtained from the mid Peskowesk site (46μm) in the spring of 1991. Cells as long as 110 μm were present in the upper site of Peskowesk Lake in the spring of 1993 (Table 2).

Three sites on the east side, Jeremys Bay, Kedgemakooge, and Merrymakedge and one on

the west side, Minards Bay were sampled from Kejimkujik Lake. *Asterionella* was seen in all spring samples except for Kedgemakooge in 1990. Minimum and maximum cell length values were relatively constant over the three sites with *Asterionella* in the spring of 1990. Minimum values for cell length were as low as 37 μm and 38 μm in Jeremys Bay in 1991 and 1993 respectively. Maximum cell length was seen in populations from Kedgemakooge and Minards Bay in 1991 (94 μm) and at Merrymakedge in 1993 (109 μm).

Shorter cells, that is cells of less than 60 μm, were most prevalent in the spring of 1990. Forty four percent of cells measured from Merrymakedge, 20 % from Minards Bay and 19 % from Jeremys Bay were in this category. Shorter cell lengths were never seen in the Kedgemakooge samples but were always found at the other three sites with the exception of Minards Bay in 1993. The average cell length values varied little in 1990 and 1993, 5 μm and 1 μm, respectively. The greatest variability in the different populations was found in 1991. The lowest average value was found in Jeremys Bay (64.88 μm) and the highest at Kedgemakooge (81.05 μm) (Table 3).

Snake Lake was the only other coloured lake to show *Asterionella* in all three spring sampling periods. The population in this lake was typically much shorter than those seen in other lakes with mean values from 56.1 μm to 58.64 μm. Thirty eight percent, 80% and 65% of the valves counted were less than 60 μm in length in the spring samples from 1990, 1991, and 1993 respectively. A bimodal distribution was seen in the spring 1993 sample so another 100 cells were measured. The combined measurements from the 200 cells exhibited the same bimodal pattern as was seen in the smaller measurement.

Of the other coloured lakes, McGinty Lake was the only one to have populations of *Asterionella* in the spring of 1990 and 1991. The average cell length values were 62.13 μm ± 0.84 and 60.88 μm ± 1.58 for 1990 and 1991, respectively. Eighteen percent of the cells were shorter than 60 μm in 1990, while 39% were in this range in 1991. Cell length classes were normally distributed in both samplings.

In the spring of 1990 and 1993, *Asterionella* was present in Pebbleloggitch Lake. There was a 13 μm increase in cell length over that time period. The average cell length in 1990 was 77.81 μm ± 1.77 with 2% of the measured population shorter than 60 μm. In 1993, all cells were 70 μm or greater in length with an average length of 90.87 ± 1.17 μm. The longest cell was 104 μm in length.

Asterionella colonies were seen in Hilchmaakar, and Big Dam West Lakes in the spring of 1990 and 1993. Both lakes showed a high percentage of short cells in 1990, 56% in Hilchmaakar

Table 2. Minimum, maximum, and average cell length values for five sites in Peskowesk and Peskawa Lakes in the spring of 1990, 1991, and 1993. (N = 100 t= 0.025)

Lake and Season	Min (μm)	Max (μm)	Mean ± SEx2 (μm)
Peskawa (U) S90	66	88	80.07 ± 0.81
Peskawa (L) S90	68	92	79.81 ± 0.82
Peskowesk (U) S90	70	90	79.57 ± 0.86
Peskowesk (M) S90	70	90	79.51 ± 0.79
Peskowesk (L) S90	58	91	81.12 ± 1.09
Peskawa (U) S91	52	106	94.19 ± 1.51
Peskawa (L) S91	74	108	93.99 ± 1.40
Peskowesk (U) S91	72	106	93.06 ± 1.40
Peskowesk (M) S91	46	86	74.22 ± 1.29
Peskowesk (L) S91	78	110	94.80 ± 1.42
Peskawa (U) S93	68	104	93.58 ± 1.36
Peskawa (L) S93	52	108	94.38 ± 1.61
Peskowesk (U) S93	62	110	93.28 ± 1.59
Peskowesk (M) S93	---	---	---
Peskowesk (L) S93	60	94	70.26 ± 1.29

and 45% in Big Dam West as compared to 1% and 7% in 1993. This is reflected in the average cell length values which increased 10 μm and 16 μm in 1993. Cell length classes were seen to be normally distributed.

1C. **Connecting stream and inflow/outflow from coloured lakes:** The Mersey River Outflow from Kejimkujik Lake and Poison Ivy Falls, the connecting rapids between Peskowesk and Peskawa Lakes, also showed a good population of *Asterionella* in all three spring sampling seasons. Peskowesk

Table 3. Minimum, maximum and average cell length values for four sites in Kejimkujik Lake in the spring of 1990,1991, and 1993.(N = 100 t = 0.025)

Site and Season	Min (μm)	Max (μm)	Mean ± SEx2 (μm)
Jeremys Bay S90	38	80	66.20 ± 1.24
Kedgemakooge S90	---	---	----------
Merrymakedge S90	36	76	61.10 ± 0.97
Minards Bay S90	35	78	64.95 ± 1.47
Jeremys Bay S91	37	79	64.88 ± 0.96
Kedgemakooge S91	66	94	81.05 ± 1.00
Merrymakedge S91	54	78	65.16 ± 0.84
Minards Bay S91	52	94	79.10 ± 1.24
Jeremys Bay S93	38	98	73.65 ± 1.32
Kedgemakooge S93	63	88	73.31 ± 0.98
Merrymakedge S93	48	109	74.29 ± 1.43
Minards Bay S93	63	88	73.31 ± 1.09

Brook coming out of Lake Peskowesk and the Mersey River at Jakes Landing going into Kejimkujik Lake showed *Asterionella* populations in two of the three spring samplings.

Minimum and maximum cell length values at Poison Ivy Falls were found to be in close agreement with the lower Peskawa and the upper Peskowesk sites. Average cell length values followed the same trend as was seen at these sites with an increase in the 1991 and 1993 samples of greater than 10 μm. In all cells measured, only one cell was less than 60 μm long and that was seen in the 1993 spring sample.

The two samples (1990 and 1991) from Peskowesk Brook were also in agreement with the upper and lower Peskowesk sites. Again, average values for cell length were very similar and the

cell length classes were found to be normally distributed. There were never any cells less than 60 μm long, the shortest being 63 μm seen in 1990 and the longest 110 μm seen in the spring of 1991. Cell measurements in 1990 and 1993 at Jakes Landing were in close agreement to the three sites along the east side of Kejimkujik Lake. Average valve lengths differed only by 4 μm between spring 1990 and spring 1993. Short cell lengths were most prevalent in the spring of 1990 and were 23% of the measured population. Eight percent of the cells were shorter than 60 μm in the spring of 1993. This is in agreement with what was seen at Merrymakedge and Jeremys Bay.

The Mersey River outflow from Kejimkujik Lake showed little variablilty within the minimum and maximum cell length values and displayed a close similarity to the values seen at the Minards Bay site. Average cell length values ranged from a low of 71.15 ± 1.3 μm in the spring of 1990 to a high of 79.91 ± 1.28 μm in 1991. Cells less than 60 μm long were found in all three years but cells longer than 95 μm were never seen. Cell length classes were normally distributed in all spring sampling times.

2. Both Fall Samplings 2A. Clear Lakes: Mountain, Back and Grafton Lakes all showed populations of *Asterionella* in both fall samplings. This diatom was seen in Big Dam East and McIntyre Lakes in the fall of 1991 but was not seen in Beaverskin or Puzzle Lakes at these times. Average cell length values remained constant in Back Lake over the two fall sampling periods with cells measuring less than 60 μm in length abundant in both years (42% and 44% in 1991 and 1992, respectively). Cell length classes were normally distributed in both samples with the shortest cell measured in the fall of 1992 (47 μm). Average cell length in Grafton Lake declined 5 μm from the fall of 1991 to fall 1992 (73.72 ±0.86 μm to 68.58 ± 0.97 μm). Cell length classes were normally distributed and only 5% of cells measured in 1992 were less than 60 μm long. The longest cell lengths were present in Mountain Lake (98 μm) in the fall of 1991. Mountain Lake also displayed bimodal cell class length distribution with peaks around 65 μm and 85 μm at this time. An additional 100 cells were measured to verify bimodality in this sample. Average cell length values in 1991 were 81.5 ± 2.04 μm and declined approximately 6 μm in the following year.

2B. **Coloured Lakes:** *Asterionella* colonies were evident at three sites on Kejimkujik Lake, Merrymakedge and Jeremys Bay on the east side and Minards Bay on the west side, in the fall samplings. As well, Snake, Big Dam West and Little Peskowesk Lakes had populations of *Asterionella* in both years. McGinty and Hilchmaakar Lakes had fall populations of *Asterionella* in 1991 only. The mid Peskowesk site was the only place in the Peskawa/Peskowesk series to show

Asterionella and this occurred in 1992.

Cells measured from the three sites in Kejimkujik Lake were very consistent in length between the sites. A slight decrease of approximately 5 μm in the average valve length was noticed from 1991 to 1992. None of the measured cells were less than 60 μm in length in any of the samples, and all cell length classes were seen to be normally distributed (Table 4).

Of the three coloured lakes which showed populations of *Asterionella* in both fall samplings, Big Dam West was the most consistent in average cell length. Only one measured cell was less than 60 μm in length in 1991 and all were greater than 64 μm in length in 1992. Length classes in both samples were normally distributed. This was not the case for Little Peskowesk and Snake Lakes. Bimodal cell length distributions were found in both lakes in the fall 1991 samples so an additional 100 cells were measured.

In both lakes, a high proportion of measured cells were found to be less than 60 μm in length; 56% and 24% in Snake Lake and 68% and 53% in Little Peskowesk Lake. The resulting average cell length values were some of the shortest recorded from all years and seasons.

2C. **Connecting streams and inflow/outflows from coloured lakes**: Jakes Landing on the Mersey River and the Mersey River outflow from Kejimkujik Lake were found to have *Asterionella* in both fall samplings. Like Peskawa and Peskowesk Lakes, neither Poison Ivy Falls connecting these lakes or Peskowesk Brook showed *Asterionella* at these times. A slight decline of 5 μm in average valve length was seen at Jakes Landing from 1991 to 1992. The Mersey River outflow from Kejimkujik Lake remained relatively constant over the two fall sampling seasons. Both sites were reflective of the populations seen in the lake itself. Cell length classes were seen to be normally distributed in all cases and all measured cells were greater than 60 μm in length.

3. Sites which contained Asterionella in both spring and fall 1991

3A. **Clear Lakes:** Five clear lakes showed populations of *Asterionella* in the spring and again in the fall of 1991. Grafton, Back, Big Dam East and McIntyre Lakes had normally distributed cell length classes but Mountain Lake showed bimodality in both the spring and fall samples. Back Lake showed very little variation in the average cell length and was approximately 1.7 μm shorter in the fall than in the spring. Nineteen percent of cells measured in Back Lake in the spring were less than 60 μm long, while 42% were short cells in the fall. Cells measuring less than 60 μm long were present in the other four lakes in the spring only (Grafton 61%, McIntyre 38%, Mountain 27%, Big Dam East 9%) and all lakes showed an increase in average cell length of between 14.2 μm - 15.1

Table 4. Minimum, maximum and average cell length values for three sites in Kejimkujik Lake and three coloured lakes in the fall of 1991 and 1992. (N = 100, t = 0.025)

Site and Season	Min (μm)	Max (μm)	Mean ± SEx2 (μm)
Merrymakedge F91	68	88	78.80 ± 0.89
Jeremys Bay F91	70	96	79.66 ± 0.94
Minards Bay F91	65	89	79.01 ± 0.88
Big Dam West F91	58	96	74.05 ± 1.32
Snake F91	41	88	59.95 ± 2.60
Little Peskowesk F91	38	79	54.46 ± 2.18
Merrymakedge F92	60	88	73.17 ± 1.00
	62	93	74.87 ± 1.19
Jeremys Bay F92	66	100	75.92 ± 1.21
Minards Bay F92	62	96	75.46 ± 1.08
	62	108	77.13 ± 1.53
Big Dam West F92	64	90	74.17 ± 0.97
Snake F92	32	85	64.46 ± 2.39
Little Peskowesk F92	38	74	55.32 ± 2.10

μm in the fall samples. Mountain Lake showed peaks in cell length classes at 55 and 70 μm in the spring and 65 and 85 μm in the fall. When another 100 cells were measured, the bimodality remained, but the spring peaks shifted to 70 and 90 μm and the average valve length increased 8 μm. The range of cell length averages was greatest in Mountain Lake which also had maximum cell length averages in both seasons.

3B. **Coloured Lakes:** Three sites in Kejimkujik Lake and three other coloured lakes showed populations of *Asterionella* in both the spring and fall 1991 samples. Cell length classes were always seen to be normally distributed in Kejimkujik Lake but bimodality was seen in McGinty Lake in the

spring and Snake and Little Peskowesk Lakes in the fall.

Average cell length in the fall remained constant at the west side of Kejimkujik Lake in Minards Bay in 1991 but decreased in Jeremys Bay by 15 μm and increased by 13 μm at Merrymakedge, both on the east shore of Kejimkujik Lake. Only 2% of measured cells from Minards Bay were less than 60 μm in length in the spring while 12% and 16% of the cells in Jeremys Bay and Merrymakedge, respectively, were less than 60 μm long. Cells from Jeremys Bay were both the longest (96 μm) and the shortest (37 μm) of the three sites in Kejimkujik Lake.

Average cell lengths varied little in McGinty and Snake Lakes over 1991 but decreased 16 μm from the spring to the fall in Little Peskowesk Lake. Average cell lengths were approximately 61 μm in McGinty, 57 μm in Snake and 70 μm in Little Peskowesk in the spring samples with 39%, 80% and 12% of the cells less than 60 μm long. Shorter cells were in the majority in all fall samplings which is reflected in the average cell lengths, which were less than 60 μm in all samples. Bimodality in cell length classes was seen in Snake and Little Peskowesk Lakes in the fall samples and McGinty Lake in the spring sample. All three lakes showed peaks at 45 μm and at 65 μm. After a second one hundred cells were measured the lower peak in Little Peskowesk shifted to 40 μm. Three peaks were evident in Snake Lake; 45μm, 65 μm and 85 μm. Although a slight peak was still seen at 45 μm after 200 cells were measured the largest peaks were at 60 μm and 80 μm in McGinty Lake. The longest (106 μm)and shortest (32 μm) cells were measured in Little Peskowesk Lake in the spring.

3C. **Connecting streams and inflow/outflow from coloured lakes :** The Mersey River outflow from Kejimkujik Lake was the only site to show *Asterionella* in both the spring and fall of 1991. Average cell length values were 79.91 ±1.28 μm in the spring and 77.95 ± 0.92 μm in the fall. Maximum and minimum cell length values were both seen in the spring sampling period and only one cell measured less than 60 μm in length. Cell length classes were normally distributed with average values similar to those seen at Minards Bay in both seasons and Jeremys Bay and Merrymakedge in the spring and fall, respectively.

4. Fall 1992 and Spring 1993

4A. **Clear Lakes:** Three clear lakes (Mountain, Back and Grafton) showed populations of *Asterionella* in the fall of 1992 and then again in the spring of 1993. In all cases the average cell length in the spring was longer than that measured in the fall with the greatest increase seen in Back Lake (+13 μm). Grafton Lake showed the least amount of variability with average cell length increasing less than 1 μm. The shortest cells measured were in Back Lake (47 μm) in the fall of

1992 while the longest were in Back and Mountain Lakes (96 μm) in the spring of 1993. Forty-four percent of the measured cells in the fall Back Lake sample were less than 60 μm long. Only 8% were in this size range the following spring and a slight bimodality was seen in the cell length classes. There were no measured cells which were less than 60 μm in length in Mountain Lake and only 5% of the measured cells in the fall Grafton Lake sample were in this size range.

4B. **Coloured Lakes:** Three sites from Kejimkujik Lake, Minards Bay on the west side and Merrymakedge and Jeremys Bay on the east side, contained *Asterionella* in both the fall 1992 and spring 1993 samples. As well, Snake, Little Peskowesk and Big Dam West Lakes showed populations of *Asterionella* at these times.

Measured cells from all sites around Kejimkujik Lake were normally distributed in both sampling periods and showed very little change in average cell length. Minimum cell length values ranged from 60 μm to 66 μm with the exception of a single cell in the spring sample taken from Merrymakedge (48 μm). Maximum measured cell length ranged from 88 μm to 109 μm with the longest cell seen at Merrymakedge in the spring sample. Jeremys Bay and Merrymakedge had 4% and 1% of the measured cells less than 60 μm in length in the spring sample respectively.

Cell length classes were normally distributed in both seasons in the three coloured lakes except for Little Peskowesk Lake in the fall 1992. Slight bimodality was evident with peaks at 40 and 60 μm, and, after an additional 100 cells were measured, these peaks remained. Minimum cell length values remained constant in Little Peskowesk but the maximum cell length increased 37 μm from the fall to the spring. Average values in the fall sample were 55.32 $\pm$ 2.10 μm and an increase of 12 μm was seen in the spring sample. Fifty three percent of the cells measured were less than 60 μm in length in the fall sample and only 5% were in this size range the following spring.

Big Dam West also showed an increase in average cell length of approximately 5 μm from the fall of 1992 to the spring of 1993. Cell length ranged from 64 - 90 μm in the fall sample but ranged from 36 - 112 μm in the spring sample, 7% of which were shorter than 60 μm. Because of this wide range in valve length and the higher standard error (SE x2 = 3.00) another 100 cells were measured. The resulting average valve length differed by only 0.2% and was still normally distributed.

Average length values were seen to decrease approximately 8 μm in Snake Lake from the fall to the spring. The shortest measured cell from any of the coloured systems was present in the fall 1992 sample (32 μm). Length classes were normally distributed and the range in cell size constant. The average cell length value in the spring sample was less than 60 μm and 65% of all cell measured were less than 60 μm in length. Twenty four percent were in this size range in the fall sample.

4C. **Connecting streams and inflow/outflow from coloured lakes:** The Mersey River coming into Kejimkujik Lake at Jakes Landing and the Mersey River outflow were the only two sites to show populations of *Asterionella* in both the fall 1992 and the spring 1993. Average valve length was constant at the outflow but decreased slightly from the fall to the spring at Jakes Landing. Both sites were normally distributed in both seasons. Cells measuring less than 60 μm in length were seen in the spring only (8% at Jakes Landing and 2% at the Mersey River outflow). The range in cell length sizes was greatest at Jakes Landing in the spring and was similar to that seen at Jeremys Bay and Merrymakedge.

Within and Between Lakes

Clear Lakes. 1. Mountain Lake: Cell length classes were normally distributed in the spring of 1990, 1993, and the fall of 1992 (Figure 3A). A bimodal distribution of cell length classes was found in both 1991 samples. In the spring of 1990 the majority of the cells were between the 65 - 75 μm classes (86%) but the following spring a shift downward was evident. Slight bimodality was seen with a downward shift in peaks to 70 μm and 55 μm. The fall 1991 sample displayed distinct bimodality with a shift upward in the cell length classes to 65 and 85 μm. All cell length classes under 60 μm had disappeared at that time but 85, 90 and 95 μm classes were added. Distribution of cell length classes returned to a normal distribution in the fall of 1992 with the majority of the cell seen between the 65 and 80 μm (92%). The 90 and 85 μm length classes were greatly reduced but the 70, 75, and 80 μm classes increased. The following spring, 1993, showed the cells normally distributed around 80 μm with 91% of all measured cells falling between the 75 - 90 μm length classes. No cells measured less than 70 μm long and only a few were longer than 94 μm. The greatest average cell lengths within this lake were seen in the spring 1993 (82.09 μm) and the shortest in the spring 1990 (71.75 μm). In three sampling periods, spring 1990, spring 1993 and fall 1992, the average length of the cells in Mountain Lake was the greatest seen in any of the clear lakes sampled. There were no significant differences seen between the mean cell length values in the fall 1991 and spring 1993 samples (Figure 5A). All other seasonal averages were seen to be statistically different. When comparing the mean values to other clear lakes no statistical differences were seen between this lake in the spring and fall of 1991 and Big Dam East or Hilchmaakar in the spring of 1990.

2. Grafton Lake: All measured cells from the five seasons were normally distributed (Figure 3B). In the spring of 1990, the majority of the cells fell into the 55 and 60 μm length classes. Average cell

length values in the spring of 1991 were the lowest values for this lake and all the clear lakes sampled (59.54 μm). A decrease in the 65 μm length class and an increase in the 50 μm length class occurred. The greatest average cell length within the lake was found in the fall of 1991 (73.72 μm), and 92% of all measured cells fell within the 65 - 70 μm length classes. All cells less than 60 μm disappeared while cells greater than 65 μm were dominant. The following fall, a slight shift downward was seen with the majority of cells in the 65 or 70 μm classes. A few cells in the 50 and 55 μm classes were found. Grafton Lake had the lowest average values of any of the clear lakes sampled in 1993 (69.32 μm). The major peak remained at 65 μm. There was no significant difference in the average cell length values in the spring of 1990 and 1991 or between the fall 1992 and spring 1993 (Figure 5B). The mean value for the fall 1991 was statistically different from the other two groups. McIntyre Lake and Grafton Lake showed no significant difference in the mean cell length. This was also the case in Big Dam East in the spring of 1990.

3. Back Lake: Normally distributed cell length classes were present in all sampling times in Back Lake except for the spring of 1993 when slight bimodality was noticed (Figure 4A). The lowest average cell length values for all clear lakes sampled occurred in this lake in the spring of 1990 and in both fall samples. As well, Back Lake had the lowest average values of any lake, clear or coloured in the spring of 1990 (52.12 μm). Within the lake, the lowest average length measurement occurred in 1990 and the highest in 1993 (73.65 μm). In the spring 1990 the majority of the cells fell in the 45 and 50 μm cell length classes. Small groups occurred at 40 and 65 μm. Cell length classes shifted upward the following spring. Cells measuring 40 - 50 μm dissappeared and those between 50 and 60 μm were greatly reduced. The majority of cells were now in the 60 μm length class with the addition of a 65 μm class and an increase in the 70 μm class. The majority of cells were still in the 60 μm length class in the fall of 1991 with an increase seen in the 55 μm class. Cells in the 70 μm class had dissappeared but there was an addition of a small 45 μm class. In the fall of 1992 the major cell length classes were still at 55 and 60 μm with a small increase in the 50 μm class. The 65 μm group decreased but there was the addition of 70 and 75 μm groups. In the spring of 1993 a shift upward was seen along with a much broader cell length distribution. The shortest cells were now in the 55 μm length class with the length classes extending to 95 μm. Slight bimodality was present at this time. There were no statistically significant differences in the mean cell values for the spring of 1991, fall 1991 and fall 1992 (Figure 5C). The other two samples were different. No significant difference was found between Back Lake and McIntyre Lake in the spring of 1991.

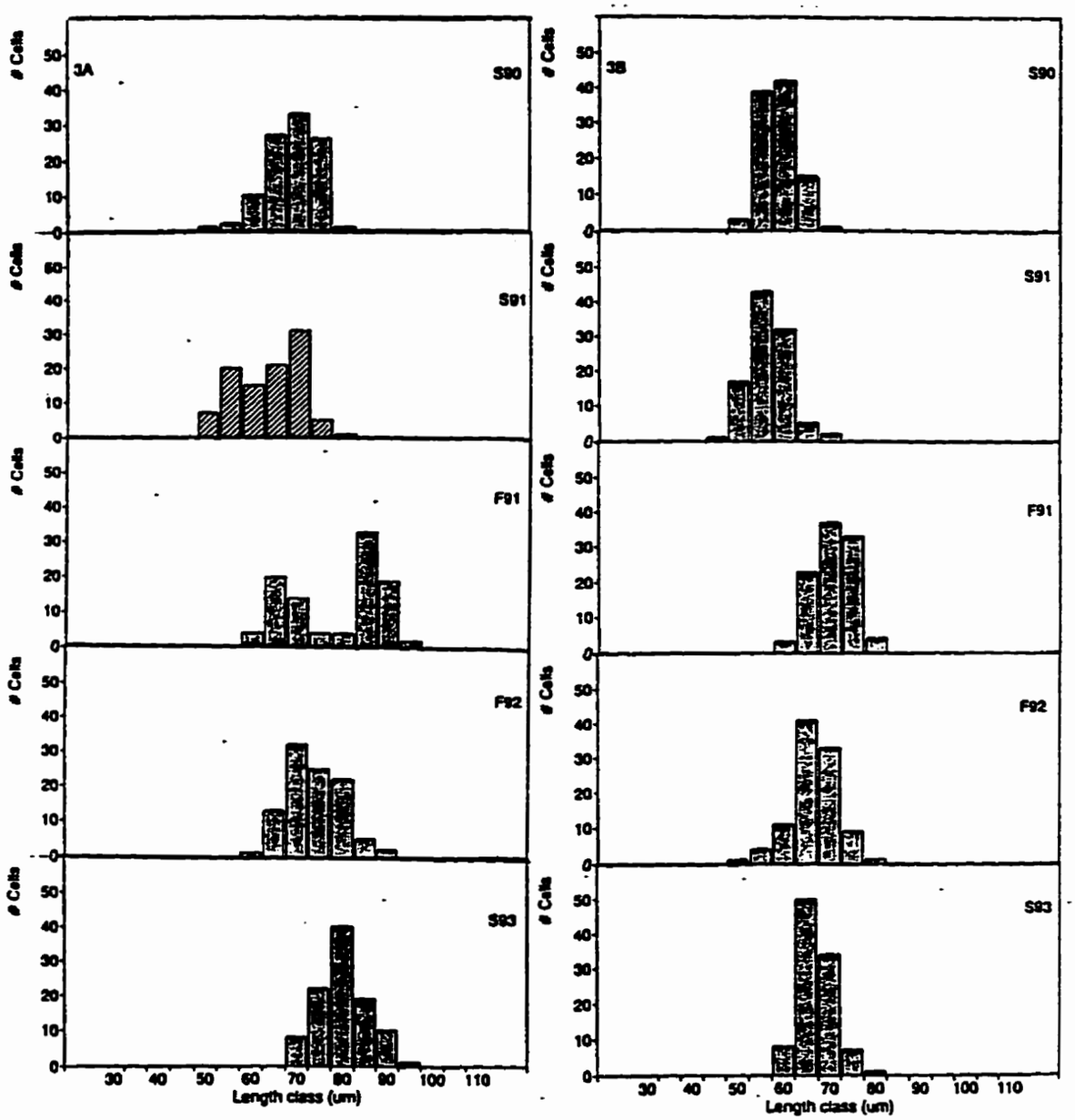

Figure 3. Histograms showing the change in valve length classes over time. 3A) Mountain Lake 3B) Grafton Lake

4. McIntyre Lake: *Asterionella* was found in McIntyre Lake in all spring samples and in the fall of 1991 (Figure 4B). All cell length classes were normally distributed. Within the lake the lowest average cell length value occurred in the spring of 1991 (61.72 μm) and the highest in the fall of 1991 (75.90 μm). The majority of the cells measured in the spring of 1990 and 1991 fell within the 55 - 65 μm length classes with a shift upward to 65 - 70 μm in the fall of 1991 and to 65 -80 in the spring of 1993. There were no significant differences in the mean cell lengths in the spring of 1990 and 1991 (Figure 5D). The fall 1991 and spring 1993 samples were different from each other as well as different from the spring 1990-1991 samples. Similarities in this lake and Grafton and Back Lakes have already been mentioned.

5. Big Dam East Lake: Samples containing *Asterionella* were found in Big Dam East in the spring of 1990 and in the two sampling times in 1991. All cell length classes were normally distributed. Average cell length was the greatest for any of the clear lakes in both 1991 samples (67.62 μm and 82.16 μm) with the fall sample the longest average value measured an any of the clear lakes in any season. The lowest average cell length value within this lake was found in the spring of 1990 (64.11 μm). Mean cell length values were statistically different in all three samples, although in the spring of 1990 cell lengths in this lake were statistically similar to Grafton Lake (Figure 6).

Coloured Lakes: 1. Peskawa/Peskowesk Series: This grouping includes Peskawa and Peskowesk Lakes, the connecting rapids, Poison Ivy Falls, and the outflow from Peskowesk Lake, Peskowesk Brook. *Asterionella* was present at these sites in the spring samplings only, with the exception of the mid Peskowesk site and Peskowesk Brook, which showed no *Asterionella* in the spring of 1993. The mid Peskowesk site did have *Asterionella* present in the fall of 1992, unlike any of the other Peskowesk sites. All cell length classes were normally distributed. Average length values from all sites in 1990 were approximately 80 μm with an increase in 1991 to 94 μm. The exception to this was the mid Peskowesk site in 1991, where the average cell length values were 20 μm shorter that those at the other sites. In 1993 the average cell length was approximately 93 μm, except at the lower Peskowesk site which was 23 μm shorter than at any of the other sites. The average length values within this system were greater than any other lake, clear or coloured, for the spring samples, with the lower Peskowesk site having the highest value in the spring of 1990 (80.62 μm), Poison Ivy Falls in 1991 (94.97 μm) and lower Peskawa in 1993 (94.38 μm). There were no significant differences between the mean valve length in any of the seven sites in 1990. All sites in 1991 and

1993 except the mid Peskowesk site showed no significant differences in average cell length. The fall 1991 and spring 1993 samples from the mid Peskowesk site showed no significant differences from each other but were significantly different from the other sites in the spring of 1990, 1991 and 1993 (Figures 7a, 7b).

2. Kejimkujik Lake Series: This grouping includes the four sites on the lake as well as the Mersey River inflow at Jakes Landing and the Mersey River outflow from the south east portion of Kejimkujik Lake. *Asterionella* was found at three sites on Kejimkujik Lake and in the Mersey River outflow in all sampling seasons. No *Asterionella* was found at Jakes Landing in the spring of 1991 or at Kedgemakooge in the spring of 1990 or at either fall sampling times. Cell length classes were normally distributed in all samples. In the spring of 1990 average cell length ranged between a low of 61.10 μm at Merrymakedge to a high of 71.15 μm at the Mersey River outflow. The following spring displayed no set pattern of cell length averages and ranged between 64.88 μm in Jeremys Bay to 81.05 at Kedgemakooge. All sites in the fall of 1991 showed a 2 μm range with the greatest average cell length value of any lake, clear or coloured occurring at Jakes Landing (80.75 μm). In the fall of 1992, the average cell length was again consistent and ranged between 74 - 76 μm. The average cell length from Minards Bay was the greatest seen in any of the lakes, clear or coloured, and was 76.29 μm. A decrease in average cell length was again seen in the spring of 1993 and average cell length ranged from 70 - 75 μm.

In the spring of 1990, the cell length classes in Minards Bay were normally distributed around the 65 μm length class (Figure 8A). Small groups occurred at 30, 45, 50 and 75 μm. The following spring the cell length classes shifted upward and were distributed around 75 μm with small groups occurring at 50 and 55 μm and the disappearance of the 60 μm class. Length classes at 80, 85, and 90 μm appeared at this time. The fall 1991 sample was similar to the spring sample but the 50, 55, and 90 μm groups had dissappeared, narrowing the distribution of the length classes. Fall 1992 showed a slight downward shift with the majority of the cells occurring in the 70 μm class. The 85 μm class dissappeared and was replaced by 90 and 95 μm length classes. Spring of 1993 was similar to the previous fall except the small classes at 90 and 95 μm were replaced with one at 85 μm and another at 100 μm. There were no significant differences in average cell length between the spring 1991, fall 1991, and fall 1992 samples or between the fall 1992 and the spring 1993 samples from Minards Bay (Figure 10A).

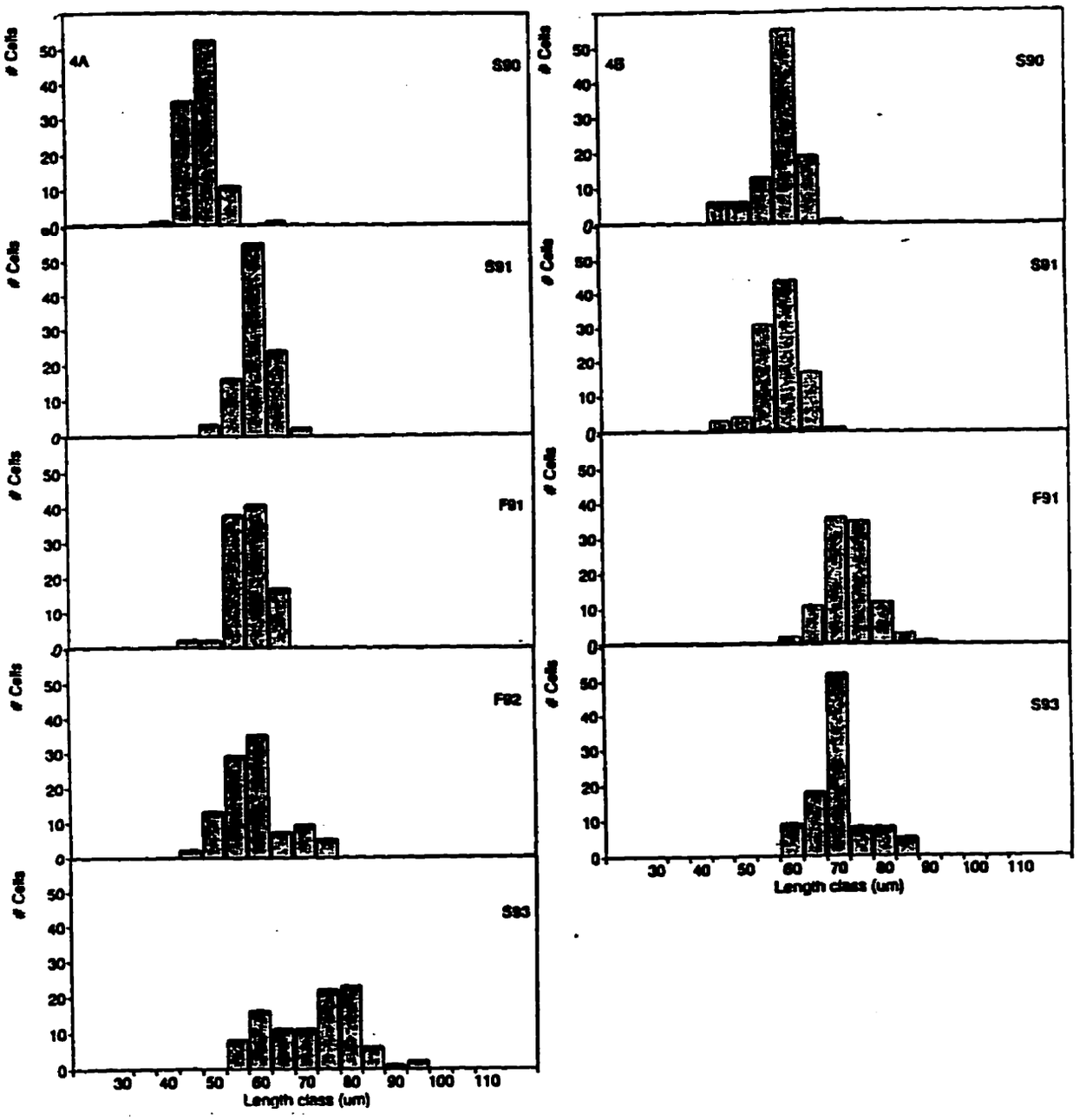

Figure 4. Histograms showing the change in valve length classes over time. 4A) Back Lake 4B) McIntyre Lake

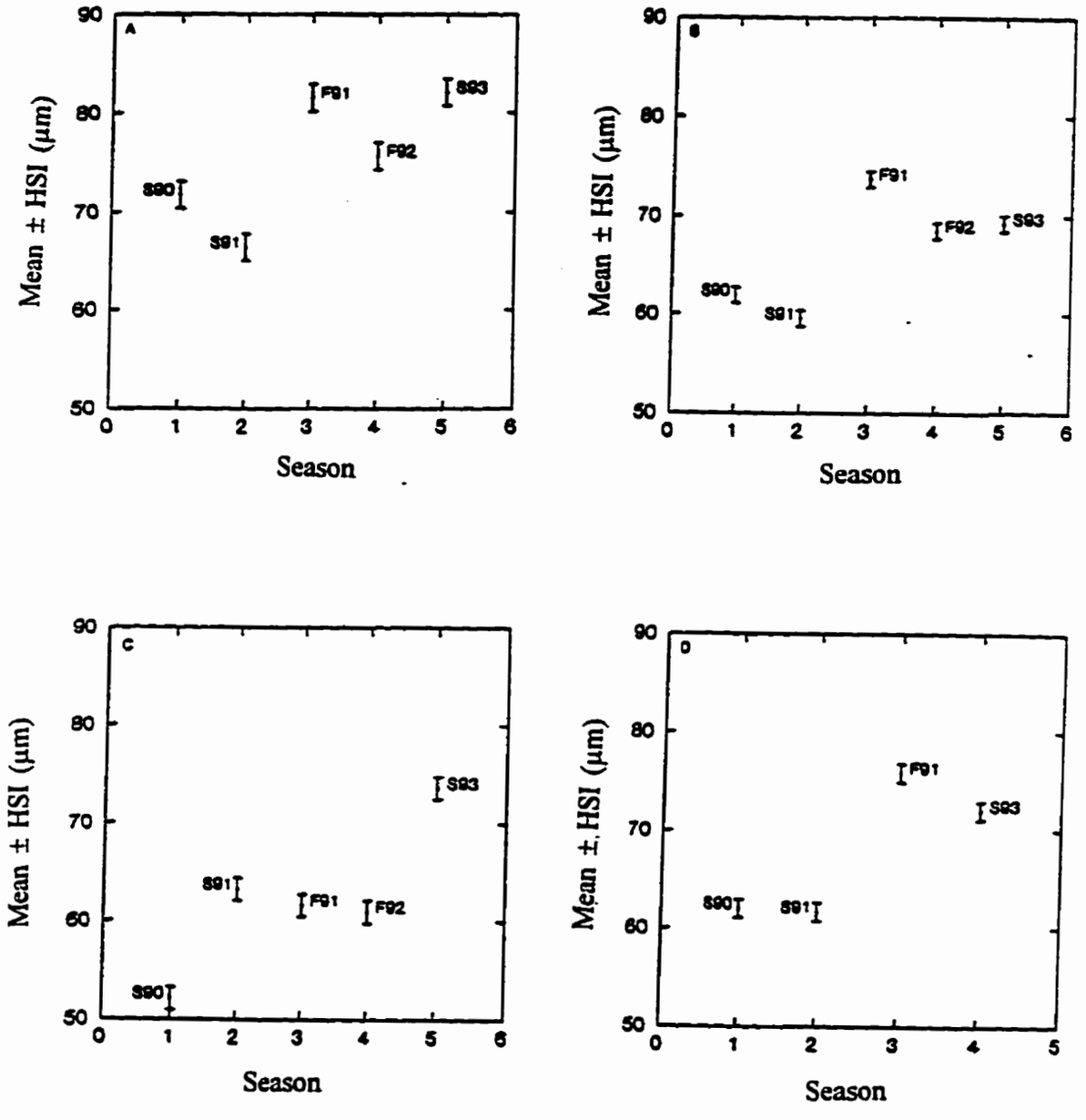

Figure 5. Mean valve length values ± HSI for A) Mountain Lake B) Grafton Lake C) Back Lake D) McIntyre Lake

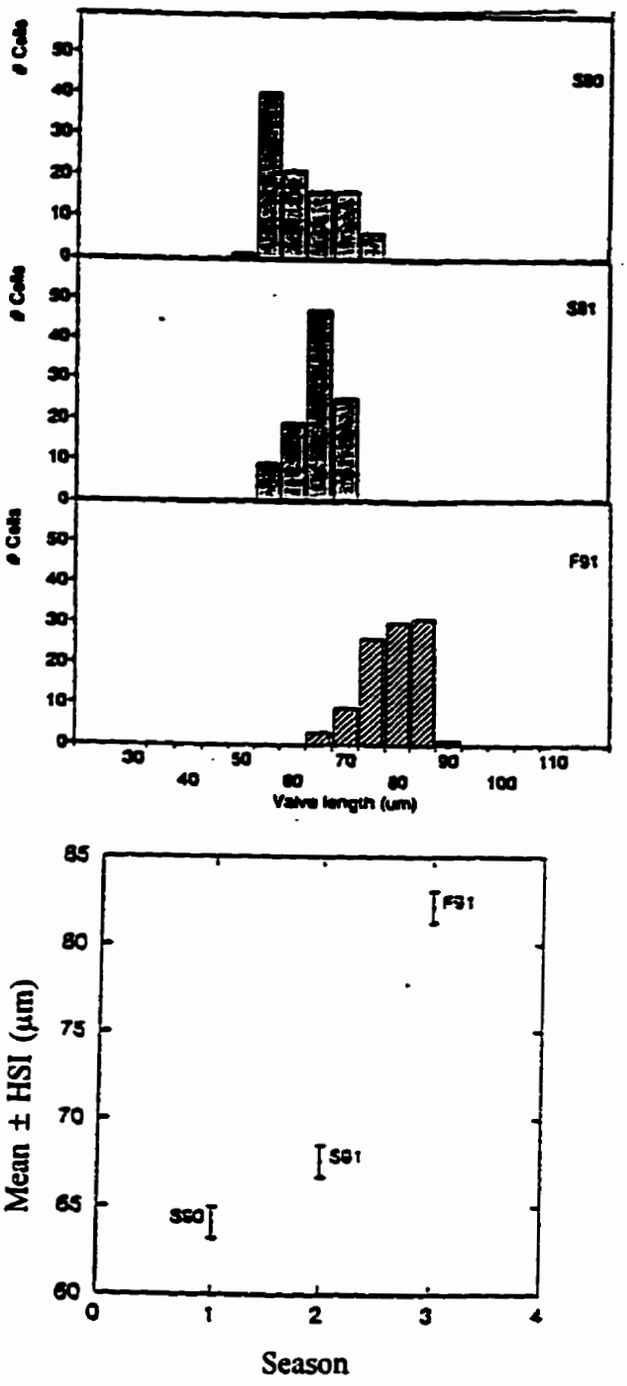

Figure 6. Histograms showing the change in valve length classes over time and a plot of the mean values ± HSI for Big Dam East Lake.

Cell length classes in Jeremys Bay in the spring of 1990 were normally distributed around the 65 μm cell length class. Small groups occurred at 35, 50, and 75 μm (Figure 8B). The following spring showed a slight shift downward to the 60 μm length class. A large shift upward occurred in the fall of 1991 with the majority of cells occurring in the 75 μm length class. All groups below 65 μm disappeared and new length classes were seen at 80,85, and 95 μm. Cell length classes shifted again in the fall of 1992 downward to 70 μm. Small groups were seen in the 85, 90, 95, and 100 μm classes. A shift downward in the spring of 1993 resulted in the loss of the 85, 95, and 100 μm classes. Instead cells were seen in the 30,50,55, and 60 μm length classes. The average cell length in the spring of 1990 and 1991 were seen to have no significant differences. The average cell lengths in the other three seasons were all statistically different from each other and from the spring 90/91 averages (Figure 10B).

Cell length classes in Merrymakedge were normally distributed and the majority of cells were in the 55 - 60 μm length classes in the spring of 1990 (Figure 9A). Small groups occurred at 35, 40, and 75 μm. The following spring there was a slight shift upward with the majority of cells falling in the 60 - 65 μm length classes. The 35 and 40 μm classes dissappeared and the 50 and 55 μm groups were much reduced. The shift to longer cell length continued in the fall of 1991 with the majority of cells now in the 75 μm length class. All cells less than 65 μm had disappeared at this time but 80 and 85 μm groups had appeared. A slight shift downward occurred in the fall of 1992 and the majority of cells were in the 70 μm length class. A few cells were in the 60 μm class. The following spring showed the cells still centered around the 70 μm class but new groups at 105,90, and 45 μm had appeared. There were no significant differences seen in the average cell length values in the populations from the fall 1992 and spring 1993 samples (Figure 10C).

Cell length classes in the Mersey River outflow were normally distributed in the spring of 1990 with the majority of cells occurring in the 65 μm length class (Figure 9B). Small groups were in the 55 and 85 μm length classes. The following spring there was a shift upward with the cells centered around 75 μm. There was an increase in the 85 μm length class and 40 and 90 μm classes appeared. The small 55 μm class has disappeared at this time. The following fall showed a very similar cell length distribution but at that time all cells are at least 65 μm in length. A slight shift downward occurred in the fall of 1992 with the majority of cells in the 70 μm class and all cell lenghts falling between 60 and 84 μm. In the spring of 1993, the cell length distribution was similar to the previous fall but had the addition of small groups at 90, 55, and 50 μm. No significant differences were found between the fall of 1992 and the spring of 1993 or between the spring and fall 1991 samples. The average cell length was significantly different in 1990 (Figure 10D).

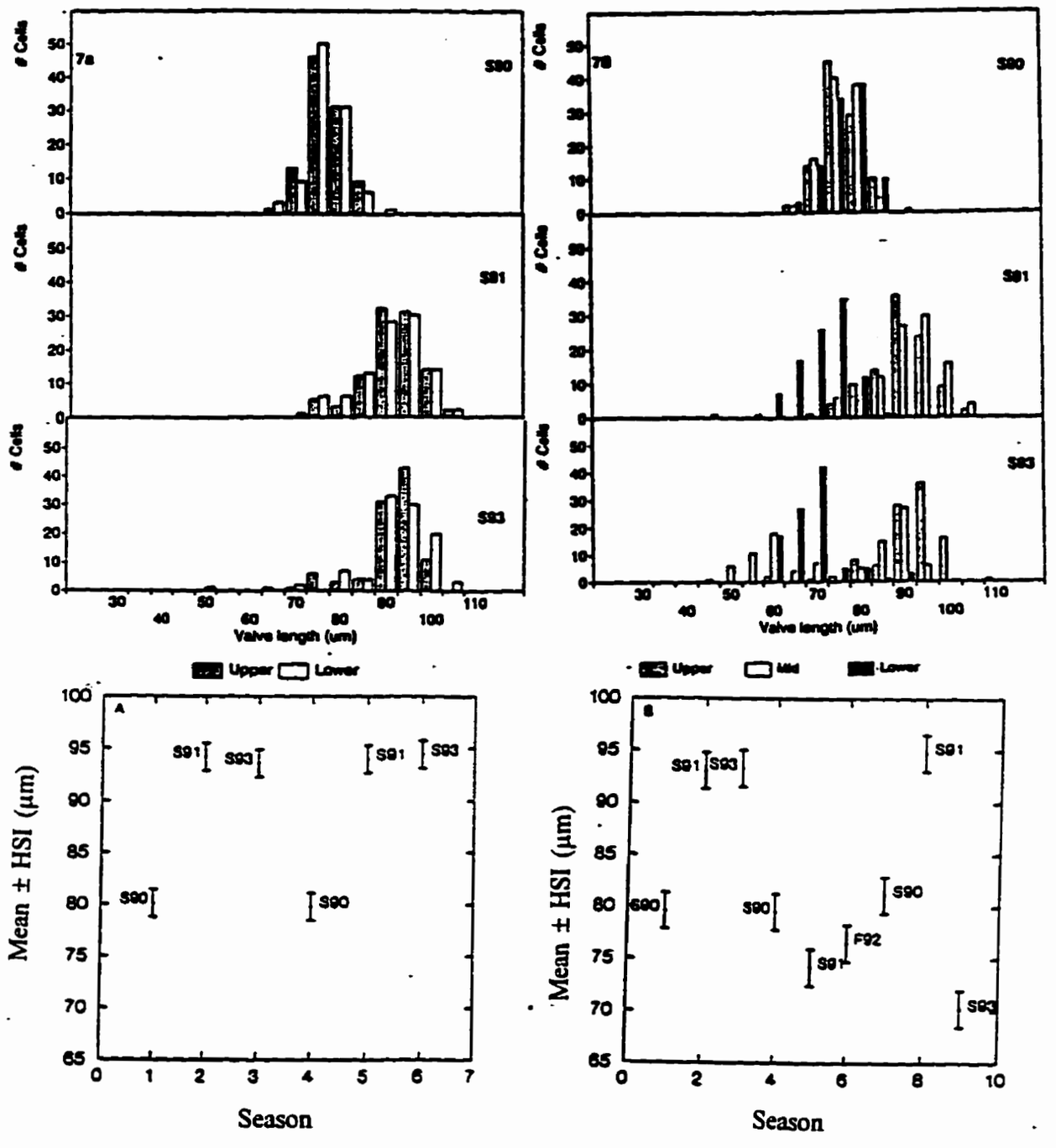

Figure 7. Histograms showing changes in valve length classes over time and a plot of mean values ± HSI. 7A) Peskawa Lake 7B) Peskowesk Lake

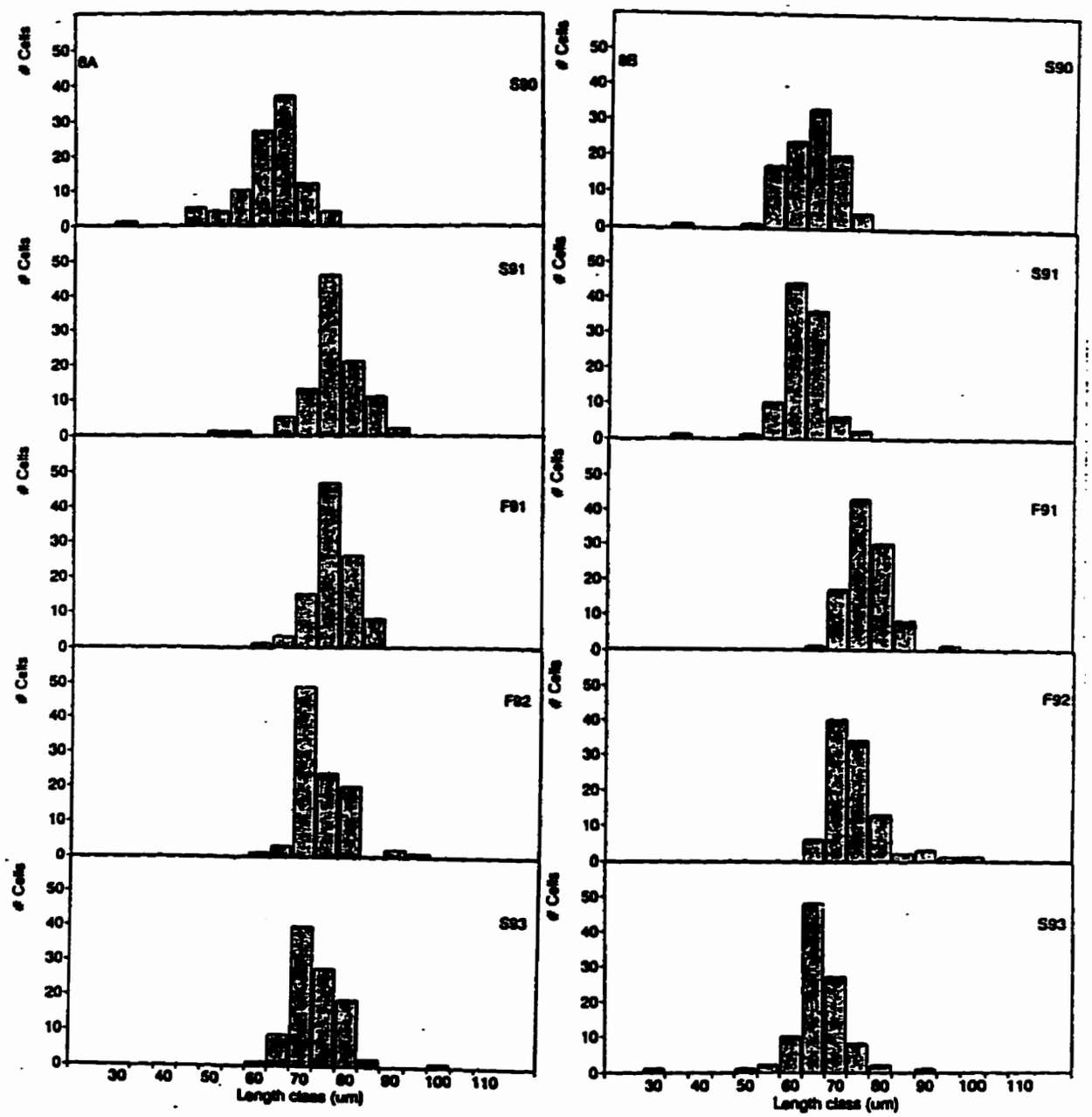

Figure 8. Histograms showing the change in valve length classes over time. 8A) Minards Bay 8B) Jeremys Bay

In the spring of 1990, cell length classes at Jakes Landing were centered around 65 μm with small groups seen at 30, 40, 45, and 50 μm (Figure 11). The longest cells were in the 75 μm length class. In the fall of 1991 cell length classes had shifted up and were centered around 75 μm. The shortest cells were in the 65 μm group and the longest in the 95 μm group. There was a slight shift downward the following fall but the distribution of the length classes was very similar to that of the previous fall. In the spring of 1993, the majority of the cells were still centered around 70 μm, but the distribution of cell length classes was much wider. Small groups occurred in the 30 to 60 μm length classes while the longest cells were in the 90 μm length class. All average cell length values were statistically different.

A statistical comparison of the average cell length values in this series of sites from Kejimkujik Lake showed many similarities. Table 5 shows the relatedness of average cell length values. At three sites (Merrymakedge,S90; Mersey River outflow S90; Jakes Landing S91), the average cell length values are unique in that they are statistically different from any other site in that season. In most other cases there is little statistical difference between the average cell length in one or all sites.

3. Snake Lake: This was the only coloured lake, with the exception of three sites on Kejimkujik Lake, which contained *Asterionella* in all five sampling seasons (Figure 12A). Average cell length values were relatively constant and always less than 60 μm, except in the fall of 1992 (64.46 μm). Average cell measurements from the three spring samples were the lowest of any of the coloured lakes (58.64 μm, 56.53 μm, and 56.10 μm in 1990, 1991, and 1993, respectively) and in the spring of 1992 and 1993 were the shortest of any lake, clear or coloured. Cell length class distribution in the spring of 1990 was negatively skewed with the majority of cells around 60 μm. Small groups were seen in the 30 μm to 50 μm length classes as well as the 75 μm length class. In the spring of 1991 length class distribution had shifted down and was positively skewed with the majority of cells found between 40 and 59 μm. Small groups were evident in the length classes from 60 μm to 100 μm. That fall all length classes above 85 μm or below 40 μm had disappeared and length class distribution was weakly trimodal with peaks at 45, 65, and 75 μm. In the fall of 1992 cell length classes shifted upward but the length class distribution showed a negative skewedness. The majority of cells were seen between the 60 and 70 μm length classes with small groups appearing in the 30 and 35 μm classes. Length classes in the spring of 1993 had shifted downward with most cells falling in the 40 - 64 μm range. There were no significant differences among the average cell length values in all three spring samples and the fall of 1991 (Figure 13A). The fall 1992 sample was

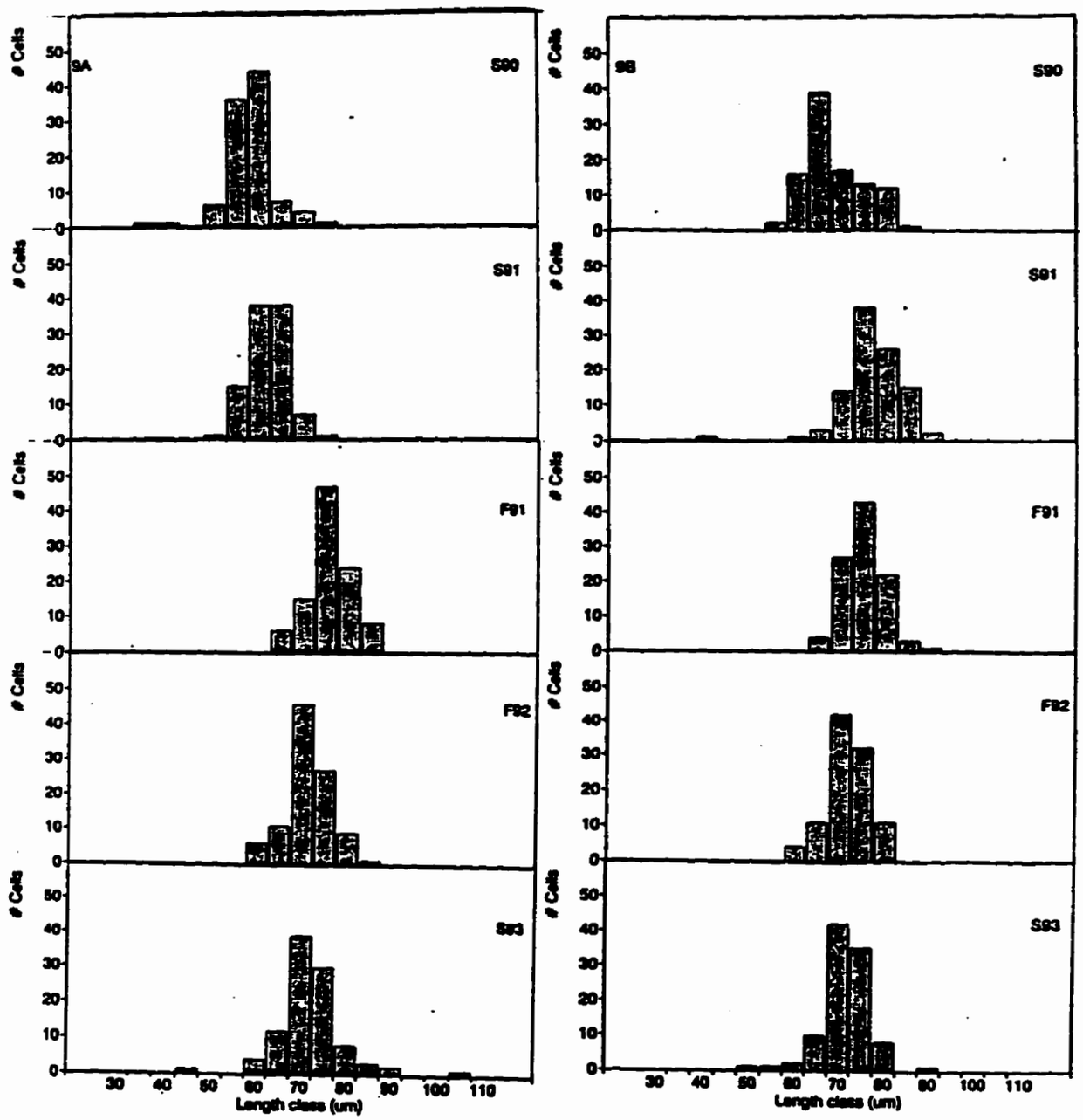

Figure 9. Histograms showing the change in valve length classes over time. 9A) Merrymakedge 9B) Mersey River outflow.

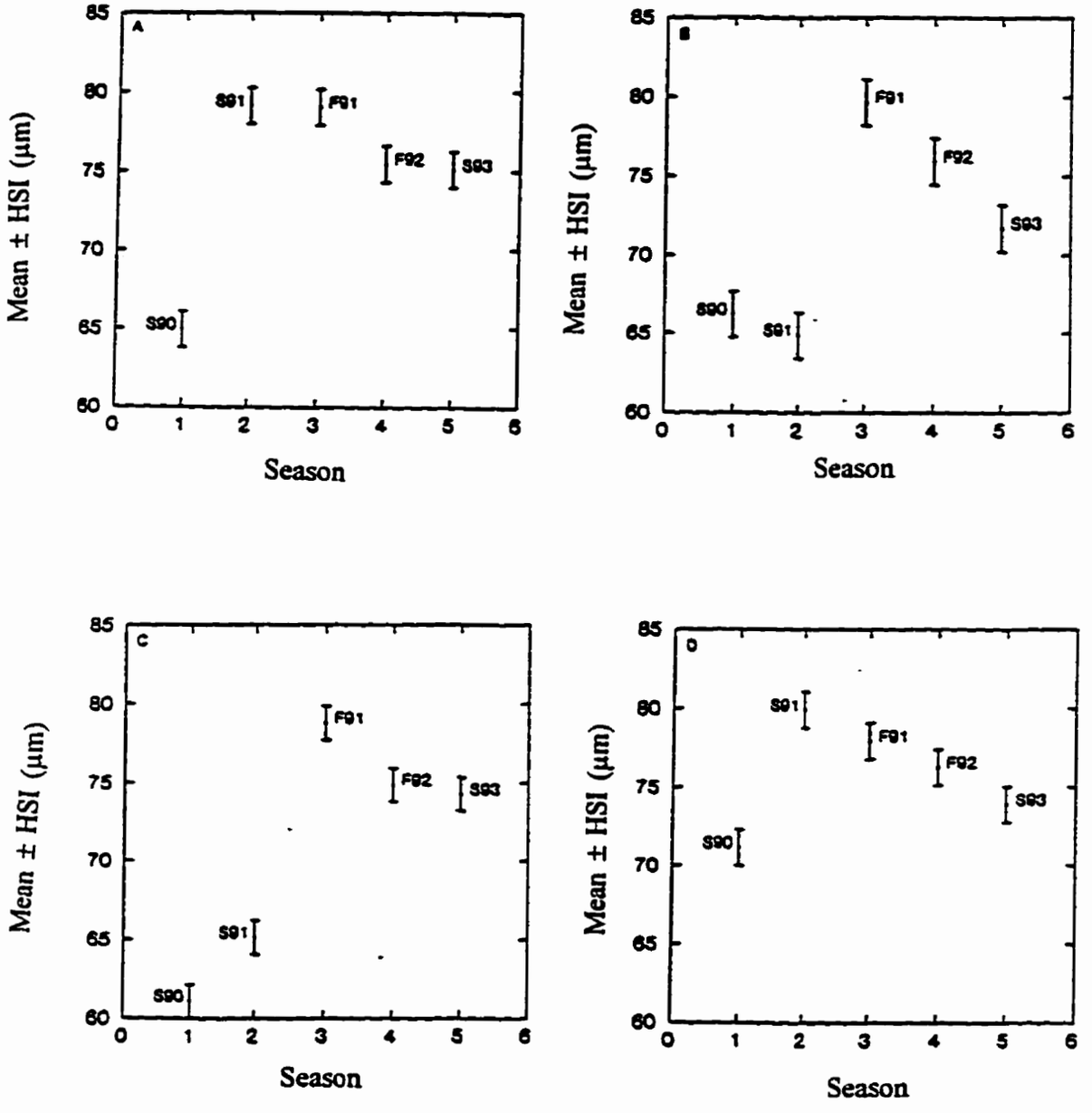

Figure 10. Mean cell length values ± HSI for A) Minards Bay B) Jeremys Bay C) Merrymakedge D) Mersey River outflow

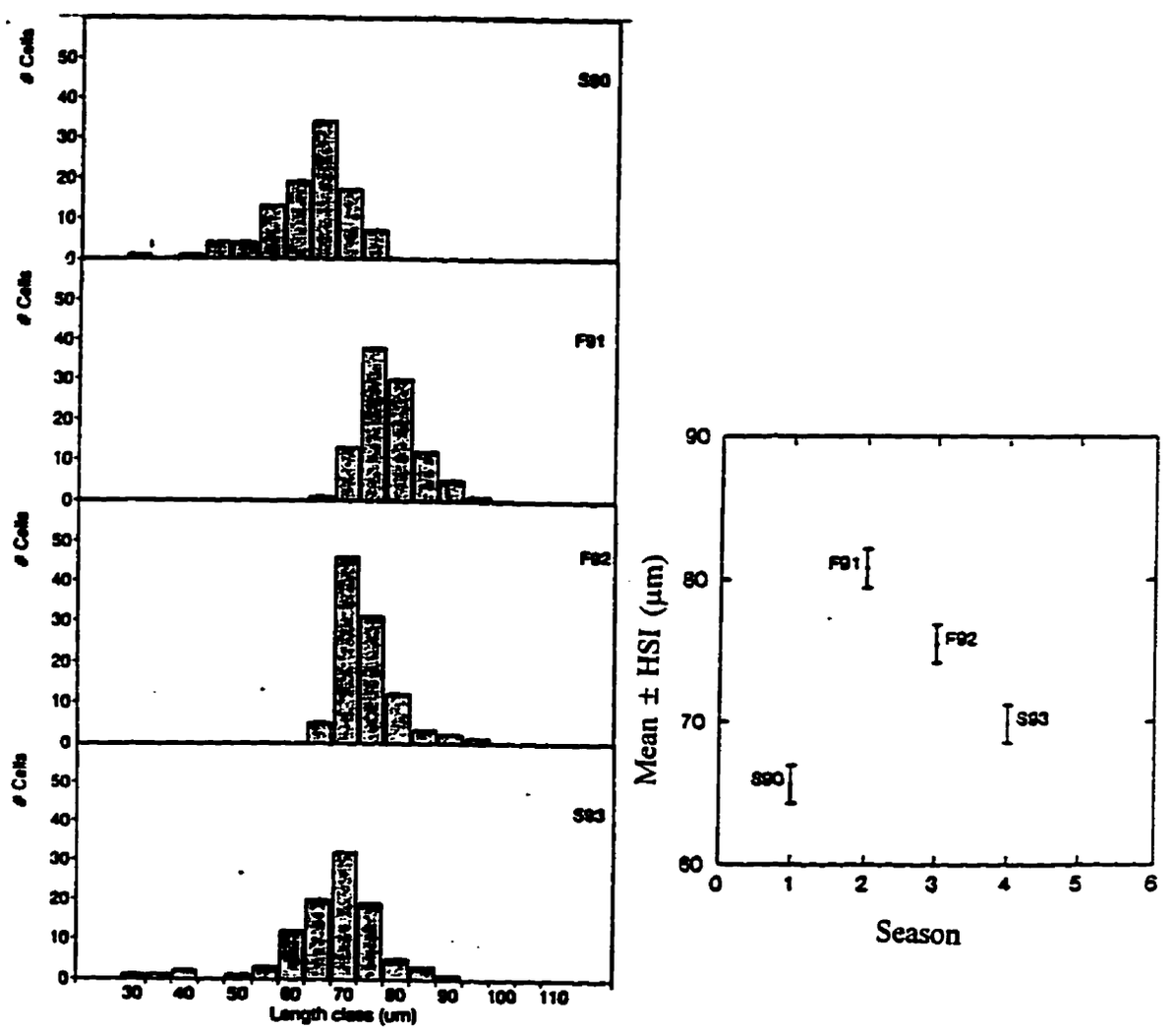

Figure 11. Histogram showing changes in valve length classes over time and a plot of the mean values ± HSI for Jakes Landing

Table 5. Cell length averages which are not significantly different for sites in the Kejimkujik Lake system. Sites are identified by reference numbers (e.g. Minards Bay: Ref.# 1)

Season Site and Ref. #	Spring 1990	Spring 1991	Fall 1991	Fall 1992	Spring 1993
1. Minards Bay	2,5	4	2,3,4,5	2,5	3,4
2. Jeremys Bay	1,5	3	1,3,4,5	1,3,4,5	4,5
3. Merrymakedge		2	1,2,4,5	2,4,5	1,4
4. Mersey River Outflow		1	1,2,3	2,3,5	1,2,3
5. Jakes Landing	1,2		1,2,3	1,2,3,4	2

different from all other seasons except from the fall of 1991.

4. Little Peskowesk Lake: *Asterionella* was not present in the spring 1990 sample but was found at all other sampling times (Figure 12B). Cell length classes were normally distributed in both the spring 1991 and 1993. At both these times the average cell length was 14 - 16 μm longer than those measured in the two fall samples (68 - 70 μm in the spring and 54 - 55 μm in the fall). In the fall samples, bimodal distributions were seen in the cell length classes. In 1991, peaks were at 45 and 65 μm and after a second hundred cells were measured the peak at 45 μm shifted to 40 μm. Average cell length values were reduced by 2 μm. In the fall of 1992 only a slight bimodality was noticed with the peak occurrence of cells at 40 and 60 μm. When a second hundred cells were measured, the curve became almost normal with the major peak occurring at 60 μm and a very minor peak at 40 μm. The average cell length increased by 2 μm. The average cell length values in both fall samples were the lowest in any of the lakes that were sampled, clear and coloured. There were no significant differences between the two spring samples or between the two fall samples but these two groups were different from each other (Figure 13B).

5. Big Dam West Lake: *Asterionella* occurred in this lake in all but the spring of 1991 sample. In all these samples, cell length classes were normally distributed. The shortest average cell size occurred in 1990 with increases of 11 μm in the fall of 1991 and a further increase of 6 μm in the spring of 1993. The greatest average length value for coloured lakes in the fall of 1991 found in this lake (74.05 μm). In the spring of 1993 cell size ranged from 36 μm to 112 μm, a span of 76 μm. Because this was a larger range in cell size than had been seen before, another 100 cells were

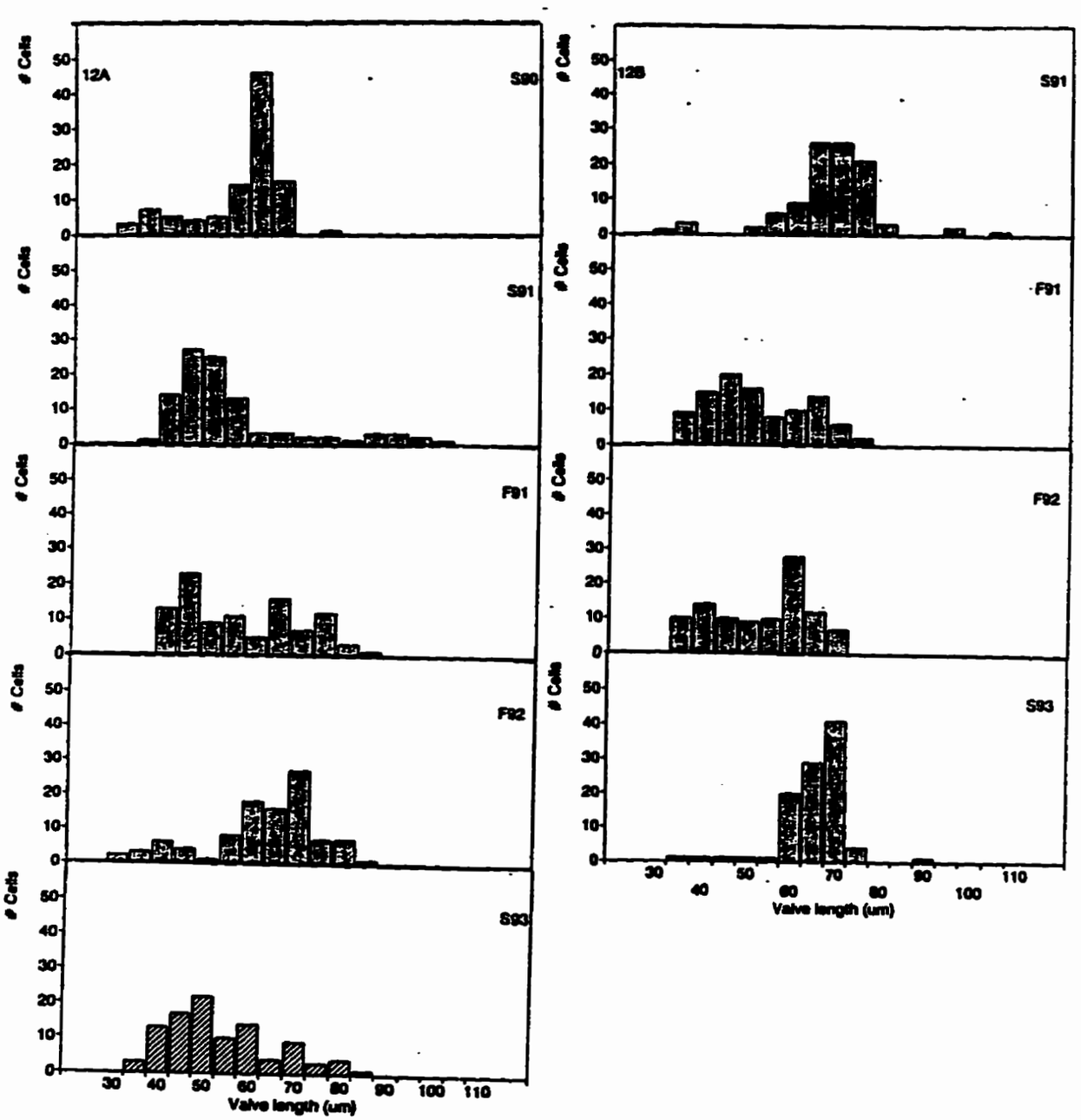

Figure 12. Histograms showing the change in valve length classes over time. 12A) Snake Lake 12B) Little Peskowesk Lake

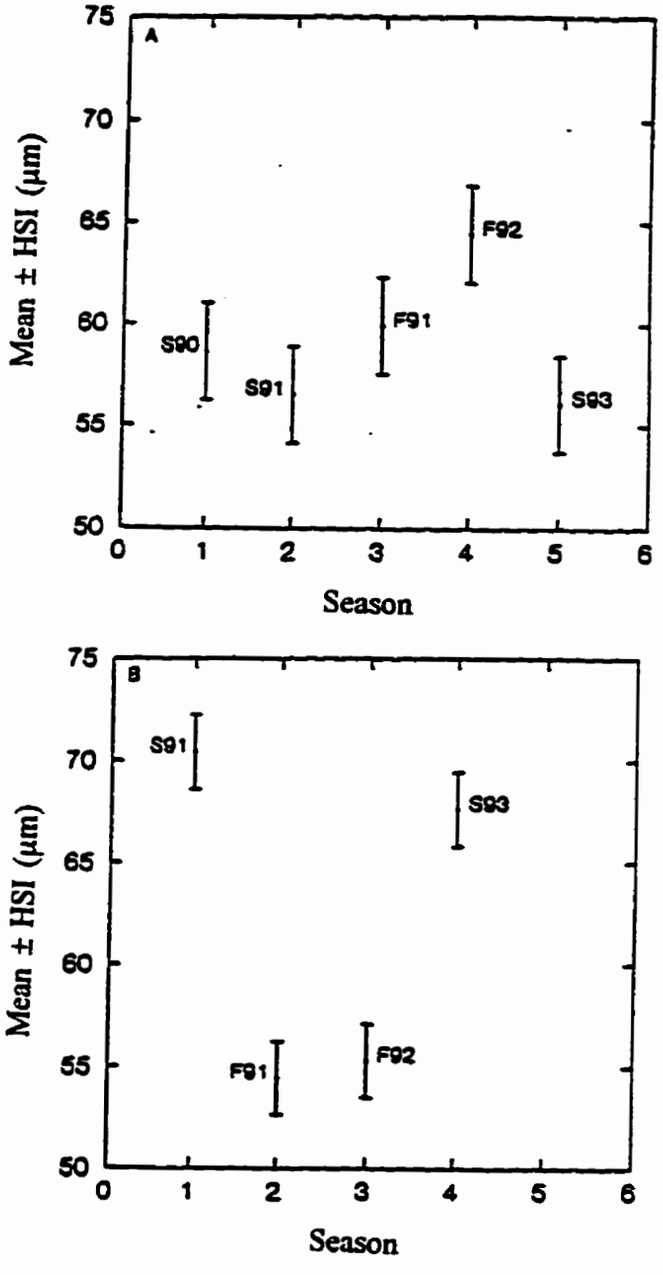

Figure 13. Mean cell length values ± HSI A) Snake Lake B) Little Peskowesk Lake

measured. The average value for 100 cells was 79.52 μm and for 200 cells 79.33 μm, indicating that this was a valid representation for this population. There was no significant difference between the two fallsamples but both spring samples were different from each other and from the fall samples.

6. McGinty Lake: *Asterionella* was present in the first three sampling times, spring 1990, 1991 and fall 1991. At these times cell length classes was normally distributed Average cell lengths were quite consistent with a slight decrease of 1 or 2 μm each sampling time (62.13μm, S90; 60.88μm, S91; 59.16μm, F91). However, statistical analyses showed no differences between spring 1990 and spring 1991 or between spring 1991 and fall 1991 but did indicate a difference between the spring 1990 and the fall 1991 samples.

Other lakes and Herbarium slides

1. ***A. ralfsii* var. *americana*, (type slide) Birchwood Lake, New Jersey, Berlin # 25636:** Cell length classes ranged from 30 μm to 90 μm with 64% falling in the 55 - 70 μm length classes. Length classes were normally distributed and the average cell length value for 100 cells was 61.5 ± 2.52 μm.

2. ***A. ralfsii* var. *americana*, Pine Rock Lake, New Brunswick, Ottawa # 30236:** Cell length classes ranged from 30 - 55 μm with 98% of measured cells falling in the 40 - 50 μm length classes. Length classes were normally distributed and the average cell length value for 100 cells was 48.70 ± 1.12 μm.

3. ***A. ralfsii* var. *americana*, Folly Lake, New Brunswick, Ottawa # 30235:** Cell length classes ranged from 30 - 70 μm with 94% of measured cells falling in the 30 -50 μm length classes. Length classes were normally distributed and the average cell length value for 100 cells was 45.73 ± 1.74 μm.

4. ***A. ralfsii* var. *hustedtiana*, (type slide) Berlin # 25635:** Cell length classes ranged from 30 - 50 μm with 91% of measured cells falling in the 30 - 40 μm length classes. Length classes were normally distributed and the average cell length value for 100 cells was 39.51 ± 0.92 μm.

5. ***A. ralfsii* var. *ralfsii* (type slide) Dolgelly, Wales British Museum # 24422:** All measured cells fell in the 35 - 40 μm cell length classes. Length classes were normally distributed and the average cell length for 100 measured cells was 39.72 μm.

6. **Leonard Lake, Ontario Spring 1991, # 99:** Slight bimodality was found in the Leonard Lake sample with 55% of cells falling in the 50 - 60 μm classes and 41% of measured cells falling in the 65 - 75 μm length classes. The average cell length value for 100 cells was 64.48 ± 1.67 μm.

7. **Joseph Lake, Ontario Spring 1991, #96:** Cell length classes were normally distributed with 95%

of measured cells falling in the 45 - 60 μm length classes. Average cell length for 100 measured cells was 54.44 ± 1.13 μm.

8. McKay Lake, Ontario Spring 1991, #100: Cell length classes were normally distributed with 94% of cells falling in th 50 - 65 μm cell length classes. Average length for 100 measured cells was 59.77 ± 1.02 μm.

9. Birchwood Lake, New Jersey, Spring 1993, #201: Cell length classes were normally distributed with 81% of the cells falling in the 45 - 50 μm length classes. Average length for 100 measured cells was 51.61 μm ± 0.76.

10. Thirty Acre Pond, Rhode Island, Spring 1993, #203: Cell length classes were normally distributed with 58% of the cells occurring between 75 μm and 89 μm. Average length for 100 measured cells was 77.33 ± 2.08 μm.

11. Plastic Lake, Ontario, Spring 1993, #200: Cell length classes had a bimodal distribution with peaks occurring at 65 μm and 100 μm. Average length for 100 measured cells was 88.98 ± 3.18 μm.

Length class distribution followed one of three patterns. In the first group, the length class distribution shifted upward from the spring of 1990 over the year to the spring of 1991 (Back, Big Dam East, Peskowesk Brook, Poison Ivy Falls, Peskawa, McGinty, Minards Bay and the upper and lower sites in Peskowesk). The class sizes that disappear differ from lake to lake. In the Peskawa\Peskowesk series the 60 - 70 μm groups become nonexistent or insignificant and length classes from 90 μm to 110 μm appear and dominate the distribution. This would suggest that when cells reach 1/3 to 1/2 of the maximum size, auxospore formation is triggered in the smaller cells. From the only fall collection of *Asterionella* (mid Peskowesk site fall 1992), it is evident that some cells attain a much smaller size, a result of continued asexual division over the summer months. If the populations in the other parts of this system follow this pattern, then the actual size at which auxosporulation is triggered is much less than what is suggested by considering the spring samples alone. All sites from this series showed a statistical difference in the mean cell length values from the spring of 1990 to the following spring. This is not only statistically significant but biologically significant as well, because the occurrence of a second different population of *Asterionella* 15 - 20 μm longer than that seen the previous year is highly unlikely. Previous studies have shown that the average rate of cell size reduction is approximately 2.0 - 2.5 μm per year (Lozeron 1902; Mann 1988; Wesenberg-Lund 1908), so an increase in size would suggest that restitution of cell size had occurred at some time between collection dates. The other lakes in this group all showed unique patterns in length classes. Cell classes in Big Dam East increased 10 μm from the spring of 1990

over the winter, to the spring of 1991, and then a further 20 μm from the spring of 1991 to the fall of that same year suggesting the 50 and 55 μm length classes to be the trigger size for cell size restitution in this lake. Back Lake showed a similar trend, except the second increase in cell size occurred between the fall of 1992 and the spring of 1993.

The situation in McGinty Lake was slightly different. Although there appears to be a shift upward of 5 μm in the spring of 1991, the mean cell length was actually less than in 1990 (62.13 μm in 1990, 60.88 μm in 1991). The number of cells in each length class were more evenly distributed in the 1991 sample and did not show the major peak in the 60 μm class. It is difficult to conclude, from these data, anything about the *Asterionella* in this lake. A 3 μm difference in cell length between the spring of 1990 and the fall of 1991 is statistically significant but is within the range of measurement error with light microscopy. The statistical method used, (HSI, honestly significant interval) gave the greatest uncertainty interval of any test of this type (confidence intervals, standard error, least significant difference) yet, if these organisms decrease at an approximate rate of 2.5 μm per year (Lozeron 1902; Mann 1988; Wesenberg-Lund 1908), the differences in the three populations from McGinty Lake are not biologically important.

In the second group, a similar increase in valve length occurred between the spring 1991 sampling and the fall of that same year. Sites in this group include Grafton, Merrymakedge, McIntyre, Mountain, Mersey River outflow, Jeremys Bay, and Big Dam East. The dominant classes in this group shifted upward 10 - 20 μm over the summer period. The sites in the Kejimkujik series (Merrymakedge, Jeremys Bay, Mersey River outflow) were all very similar in cell length distribution and the majority of cells were generally seen in 4 or 5 length classes. All of these sites are on the east side of Kejimkujik Lake and are, therefore, probably subject to very similar environmental influences. The shift in length classes at the other Kejimkujik site, Minards Bay, occurred between the spring of 1990 and 1991. Of all the sites in the Kejimkujik series, this is the most isolated from the others and may, therefore, be influenced by different factors or by the same factors at different times than the other sites. All classes shorter than 60 μm disappeared with the upward shift, and the greatest increase was seen in the 75 μm and above length classes. This would suggest that in this system the trigger size for restitution of cell size is 50 - 55 μm, again about 1/2 of the length of the largest cells. McIntyre and Grafton Lakes showed similar trends to what was seen in Kejimkujik Lake, both in the distribution of length classes and in the disappearance of cells shorter than 60 μm over the summer 1991. There were no significant differences between the mean cell lengths in these two lakes. The change in length classes in Big Dam East over the summer of 1991 was 20 μm and

removed all classes less than 65 μm while substituting classes from 75 μm to 90 μm in the fall. Although it is evident that there had been a shift in the distribution of cell sizes, *Asterionella* was only present in this lake the first three sampling periods, so no long trends were observed. There were no similarities seen in the mean valve length between this lake and Big Dam West, from which it is separated by only a 100 m rapids. Unlike the Peskawa/Peskowesk series, the influences of physical, chemical and environmental factors on these two adjoined lakes is different when considering the morphology of the *Asterionella* populations from these two sites. Mountain Lake not only displayed an increase in length classes between the spring and fall of 1991, but also again between the fall of 1992 and the spring of 1993. In this lake, however, bimodal distributions of the length classes in 1991 somewhat obscured what was happening in the lake. The one fact that does stand out is that the mean cell length in the fall of 1991 and the spring of 1993 are not statistically different, although these values are always different among the other three seasons and among any one season and these two. There is a loss of cells in the 50 - 60 μm length classes in the spring of 1991 and the subsequent appearance of 85 μm, 90 μm, and 95 μm length classes the following fall. Similarly, the 60 μm and 65 μm length classes disappeared in the spring of 1993 but large increases were seen in all groups greater than 80 μm. Although it is unclear what had triggered the increase in cell size, it is evident that it occurred twice, involving cells ranging from 50 μm to 65 μm.

The third group of sites showed an upward shift from the fall of 1992 to the following spring. These sites included Back and Mountain lakes, previously mentioned, and Little Peskowesk Lake. The histogram (Figure 12B) for the four seasons from the spring of 1991 to the spring of 1993 shows the population in Little Peskowesk Lake with a very broad initial distribution. By the fall of 1992 most of the cells were evenly distributed across eight length classes with a peak in cell length at 60 μm. The following spring very few cells remained in the 35 - 55 μm length classes but the peak in cell length has shifted to 70 μm with a few seen at 75 and 90 μm. This closely reflects the initial cell length distribution seen in the spring of 1991 and statistically there are no differences between both fall populations or between both spring populations.

Snake Lake did not fit into any particular group. The distribution of length classes was always very broad and sometimes spanned as many as 14 classes. Mean cell length in this lake was always quite short and exceeded 60 μm only once. This population is closer to what Charles *et al.* (1990) described for the Adirondack lakes, short cells occurring in a shallow coloured lake with a depressed pH.

III.1.2.2 Colony size

The number of cells per colony was counted from twenty six sites in the spring of 1990, twenty one sites in the spring of 1991, fifteen sites in the fall of 1991 and twenty two sites in the spring of 1993. The fall 1992 sites were preserved in Lugols solution which disrupted the colony formation and disintegrated the bonds between cells. There is, therefore, no colony data available for this season. Colony size in the combined sites for each season were statistically compared. Clear lakes were analyzed together as were coloured sites for each season. Examples are also shown for within lake variation for two clear lakes and two coloured lakes over all four seasons. Abbreviations used to identify the different sites seen on the mean ± HSI plots in Figures 14 through 17 are given in Table 6.

Spring 1990: All sites: Mean colony size in the spring of 1990 varied from a low of 4.77 valves per colony at Back Lake to a high of 8.27 at the mid-Peskowesk site. Four sites had fewer than six cells per colony: Puzzle, Back, Beaverskin and the lower Peskawa site. The mid Peskowesk site was the only site to exceed eight cells per colony in this sampling season. All other sites fell between 6.1 cells to 7.9 cells per colony (Figure 14A). Five groups were seen with no significant differences between the means (Table 7).

Clear sites: Colony size was compared in seven clear lakes in the spring of 1990 and at all sites the mean colony size was less than eight cells per colony (Figure 15A). The mean size ± HSI distinguished three groups which showed no significant differences: Puzzle, Back and Beaverskin Lakes, all with fewer than six cells per colony; Mountain, Grafton and Big Dam East Lakes (6.9-7.5 cells/colony); and Mountain and McIntyre Lakes.

Coloured sites: When the coloured sites were compared in the spring of 1990, colony size ranged from a low of 6.1 cells per colony at the Mersey River outflow to a high of 8.27 at the mid Peskowesk site. The mid-Peskowesk site was the only site to exceed 8.0 cells per colony in this sampling period and at no site was the mean colony size less than 6.0 cells per colony. Four groupings were found to be significantly different from each other (Figure 16A). The Mersey River outflow and the lower Peskawa site formed the smallest group. Two of the groups overlapped at the majority of the sites and the fourth group consisted of six sites, three of which were found in one of the larger groups (upper Peskawa and Peskowesk sites, and Minards Bay).

Table 6. Abbreviations used to indicate sites in the plots of mean valve length ± HSI for Figures 14,15,16 and Tables 7,8,9.

Site Clear		Site Coloured		Site Coloured	
Mountain	MN	Peskowesk Upper	PU	Minards Bay	MB
Puzzle	PZ	Peskowesk Mid	PM	Jeremys Bay	JB
Back	BA	Peskowesk Lower	PL	Merrymakedge	MK
Beaver-skin	BS	Peskawa Upper	WU	Kedgemakooge	KK
Grafton	GR	Peskawa Lower	WL	Jakes Landing	JA
Big Dam East	BE	Poison Ivy Falls	PIF	Mersey River outflow	MR
McIntyre	MC	Peskowesk Brook	PB	McGinty	MG
Cobrielle	CO	Little Peskowesk	LP	Hilchmaakar	HI
		Pebbleloggitch	PE	Big Dam West	BW
		Snake	SN	Birchwood	BD

Spring 1991: <u>All sites</u>: Mean colony size in the spring of 1991 varied from a low of 4.03 valves per colony at Back Lake to a high of 8.47 at Mountain Lake. These are both clear water lakes. Snake Lake was the only other site which had the mean colony size less than six valves per colony. Peskowesk Brook and the mid-Peskowesk site were the only other sites that had mean colony size greater than eight valves per colony. The three sites with greater than eight cells per colony were all coloured water sites. The remaining sixteen sites showed colony sizes which varied from 6.68 cells per colony to 7.98 cells per colony (Figure 14B). Both Back and Snake Lakes were significantly different from each other and all other sites. Three other major groups were seen (Table 8).

<u>Clear Sites</u>: Only two of the five sites in the 1991 sampling showed no significant difference from each other (Big Dam East and McIntyre). Back, Grafton and Mountain Lakes were significantly different from each other and from the other two sites (Figure 15B).

<u>Coloured Sites</u>: Sixteen coloured sites were analyzed in the spring of 1991 (Figure 16B). Colony size ranged from a low of 5.22 cells per colony at Snake Lake to a high of 8.03 at the mid-

Table 7. Sites which showed no significant difference in the mean colony size for the spring 1990 sampling period. Refer to Table 6 for site abbreviations.

Group 1	Group 2	Group 3	Group 4	Group 5
BA	SN	SN	PM	MG
PZ	BE	BE	JA	MC
BS	MB	WL	PE	BE
WL	GR	GR	BW	MK
	WU	WU	PL	MN
	PIF	PIF	MK	HI
	PU	PU	HI	JB
	MR	MR	MN	JA
		PZ	MC	MB
				BW
				GR
				PL

Peskowesk site. These were the only two sites which had either fewer than 6 cells or greater than 8 cells per colony. The fourteen other sites all showed mean colony size to be between 6.74 and 7.98 cells. Snake Lake was significantly different from all other coloured sites. Four other groups can be separated but overlap each other at most of the sites.

Fall 1991: All Sites: A much broader range in colony size was seen in the fall 1991 samples although only fifteen sites contained *Asterionella* in this sampling period. The only site where the mean colony size was less than six cells was Minards Bay (5.59 cells per colony) and the highest value was in Hilchmaakar Lake (12.18 cells per colony). Colony size in seven sites ranged from 6.94 cells per colony to 7.91 cells per colony, and in seven other sites ranged from 8.03 to 12.18 cells per colony (Figure 14C). Hilchmaakar in the upper range and Minards Bay on the lower range were significantly different from all other sites. Two other groupings were seen: Grafton, McIntyre

Table 8. Sites which showed no significant differences in mean colony size in the spring of 1991 sampling period. Refer to Table 6 for site abbreviations.

Group 1	Group 2	Group 3
MG	PU,PM,PL	MN
WL	WU,WL	PE
PU	PIF	WU
PIF	BM	PM
BE	MR	PL
MC	PE	PB
	LP	GR
	GR	
	KK	
	MK	
	JB	
	BE	

and McGinty Lakes, and all sites except Grafton, Hilchmaakar and Minards Bay.

Clear Sites: Colony size was compared in five clear lakes in the fall of 1991. Three groupings were significantly different from each other: 1. McIntyre, Big Dam East and Back Lakes; 2. Mountain, Back, and Big Dam East Lakes; and 3. Grafton and Mountain Lakes (Figure 15C).

Coloured Sites: Ten coloured sites were compared in the fall of 1991. Colony size in the coloured sites were both the highest and lowest seen in this season (Figure 16C). The highest number of cells per colony were seen in Hilchmaakar Lake (12.18) and the lowest was at Minards Bay (5.59). Two other sites had colonies with greater than 8 cells per colony and only one other site had colonies of fewer than 7 cells. Both Hilchmaakar and Minards Bay were significantly different from all other sites. Two other groups were distinguished from each other on the basis of one lake (McGinty Lake).

Spring 1993: All sites: Mean colony size varied in the KNP sites in the spring of 1993 from a low of 4.40 at Back Lake to a high of 8.57 at the upper Peskawa site (Figure 14D). Colony size was also counted at the same time for Birchwood Lake, New Jersey (9.25 cells per colony). Two other sites as well as Back Lake had colonies with fewer than six cells: Mountain (5.34) and Puzzle (5.83).Five other sites as well as the upper Peskawa and Birchwood sites showed colony size greater than eight cells: Grafton (8.05), lower Peskawa (8.16), upper Peskowesk (8.70), Pebbleloggitch (8.04), and Big Dam West (8.36). Twelve sites ranged from 6.84 cells / colony to 7.99 cells / colony. Colony size in Back Lake was significantly different from all other sites. Mountain and Puzzle Lakes were similar to each other but significantly different from all other sites. Birchwood Lake was not seen to be significantly different from the upper Peskawa and Peskowesk sites. Three other groupings were seen (Table 9).

Clear Sites: Six clear lakes were compared for differences in colony size in the spring of 1993 (Figure 15D). Back and Grafton Lakes were significantly different from all other sites. Mountain and Puzzle Lakes, and Cobrielle and McIntyre Lakes, were similar to each other but these two groups were significantly different from the other four sites.

Coloured sites: All colonies measured in the spring 1993 sampling from coloured sites had a mean colony size greater than 7 cells with all but two sites, falling between 7.5 and 8.5 cells per colony (Figure 16D). The mean colony size at Birchwood Lake and the upper Peskowesk site was greater than 8.5 cells. These two sites along with the upper Peskawa site formed a significantly different group. Two other large groups were seen with many of the sites overlapping into both groups.

Within Site Comparisons

Four sites were chosen to compare colony size over the four sampling periods; two clear lakes, Back and Grafton, and two coloured sites, Snake and Merrymakedge. There was no recognizable pattern at any site (Figure 17). In all but the Merrymakedge site, the mean colony size was greater in the fall sample and was seen to be significantly different in the two clear lakes. Back Lake showed no significant differences between the three spring samples and Grafton Lake showed no significant difference between the spring 1991 and 1993 samples. The spring 1990 and the fall 1992 mean colony size in Grafton Lake were different from each other and from the other two seasons. In Snake Lake, the fall 1991 and the spring 1993 mean colony sizes showed no significant differences while both the spring 1990 and 1991 were different from each other and from the other two sampling periods. At Merrymakedge the spring 1990 and both 1991 mean colony sizes showed

Table 9. Sites which showed no significant differences in mean colony size in the spring 1993 sampling period. Refer to Table 6 for site abbreviations.

Group 1	Group 2	Group 3
CO	BW	MC
PL	MB	MB
SN	PE	PE
MC	PIF	PIF
MB	WL	WL
	HI	HI
	PL	PL
	LP	LP
	MR	MR
	SN	SN
	GR	GR
	MK	MK
	KK	KK
	JA	JA

no significant difference nor did the three spring sampled measurements from this site.

The genus *Asterionella* is well known for its star shaped colonial formation yet this character receives little attention by most authors. In the past, attempts to erect different species based on colony size or formation has not been accepted, generally because of the similarities of valve morphology. More recently, variations in colony size have been considered as useful indicators of phytoplankton nutrient status (Tilman *et al.* 1976). Previous work has indicated that when phosphorus or silica is limiting, colony morphology is affected. An increase in colony size is seen when Si is limiting (from 6-8 up to 20 or more) and a decrease is seen when P is limiting (down to

2-4 cells per colony).

The histograms of mean colony size for each season show an overlap in many of the sites (Figure 14). An attempt was made to distinguish different groupings but many of the lakes fell into more than one category. Based on colony size, there was no distinction between clear or coloured lakes. The majority of *Asterionella* colonies averaged between five and nine cells per colony, indicating that phosphorus and silicate were not limiting factors. This is generally considered to be normal colony size for all *Asterionella* species.

Hilchmaakar Lake was the only site which displayed *Asterionella* colonies greater than ten cells (fall 1991). This might indicate that silicate was limiting in this lake at this time. Small colony size, that is colonies of less than six cells, were seen in a few lakes. In all spring samples, the average colony size in Back Lake was less than six cells per colony, yet was within the normal range (6-8 cells) in the fall sample. Puzzle, Beaverskin, Snake and Mountain Lakes all showed small colony size in one sampling time or another. This may indicate phosphorus limitation but, as water chemistry was not done, it remains speculative. A poor understanding in this genus of colony morphology and its function does not allow for a definitive explanation of the variation seen over the different seasons. It is not known how long the newly formed valves take to separate after undergoing division, or what effect massive bloom conditions have on neighbouring colonies.

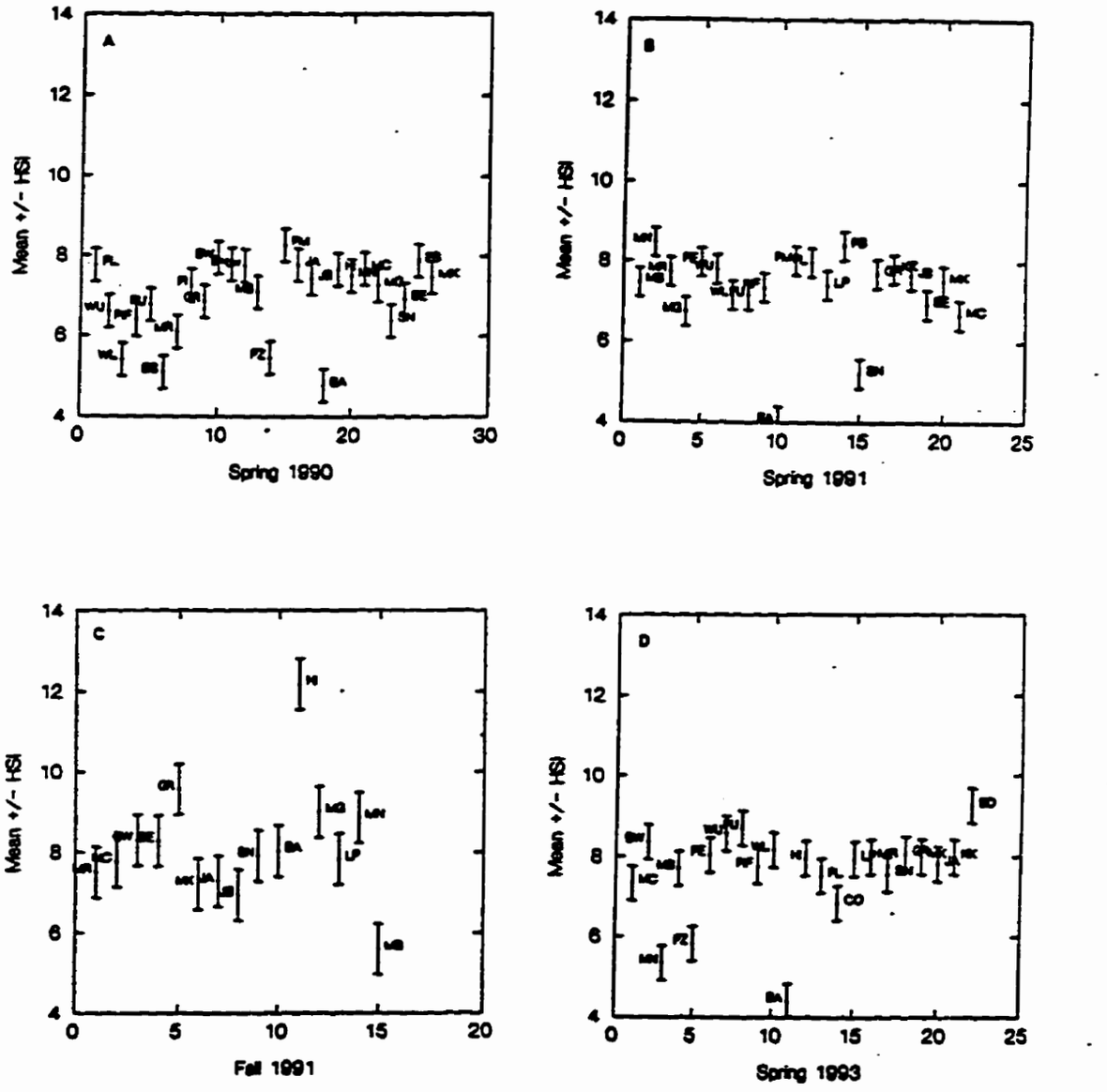

Figure 14. Mean ± HSI for colony size counts in all sites in A) Spring 1990 B) Spring 1991 C) Fall 1991 D) Spring 1993

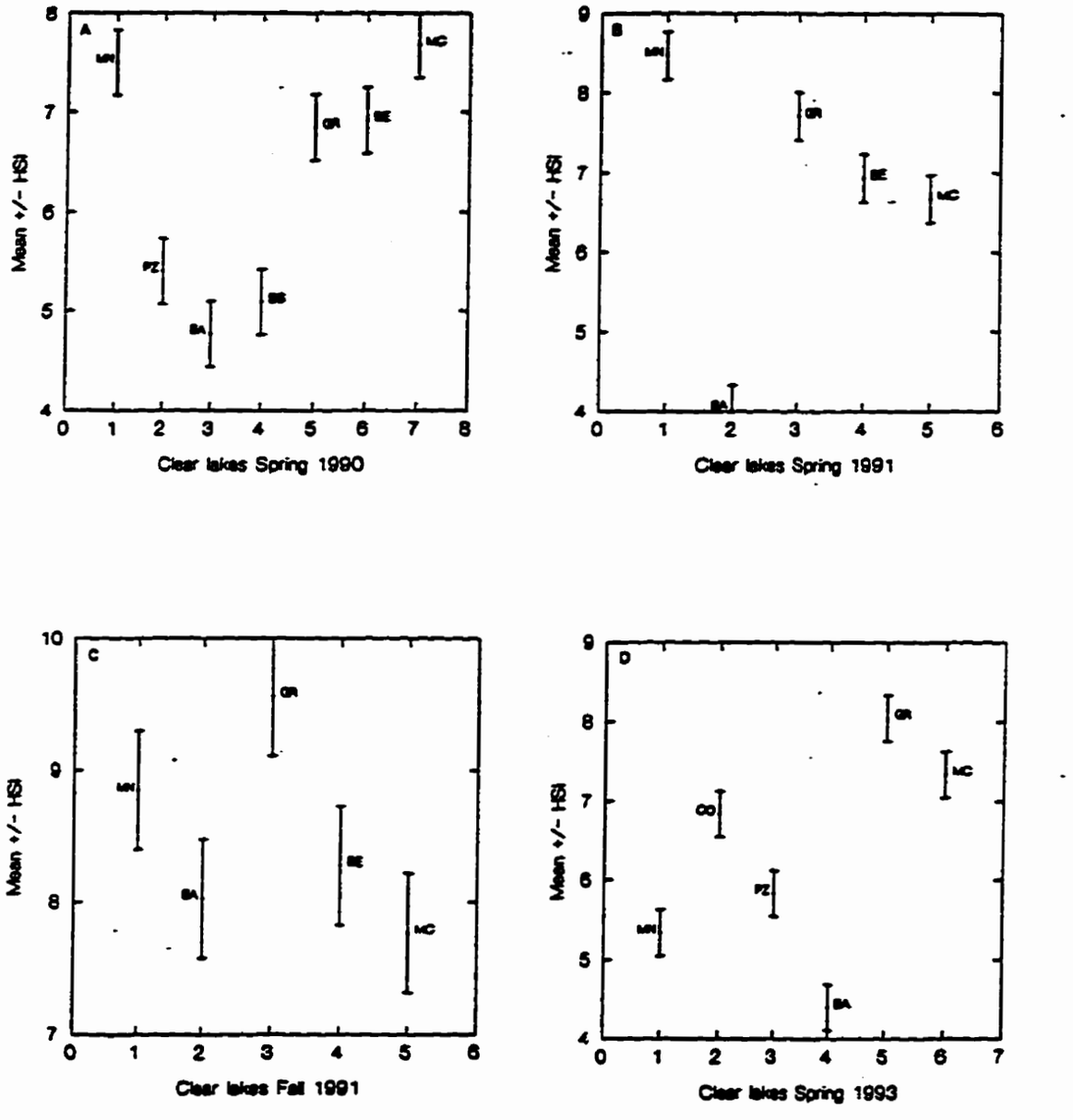

Figure 15. Mean ± HSI for colony size in all clear lakes in A) Spring 1990 B) Spring 1991 C) Fall 1991 D) Spring 1993

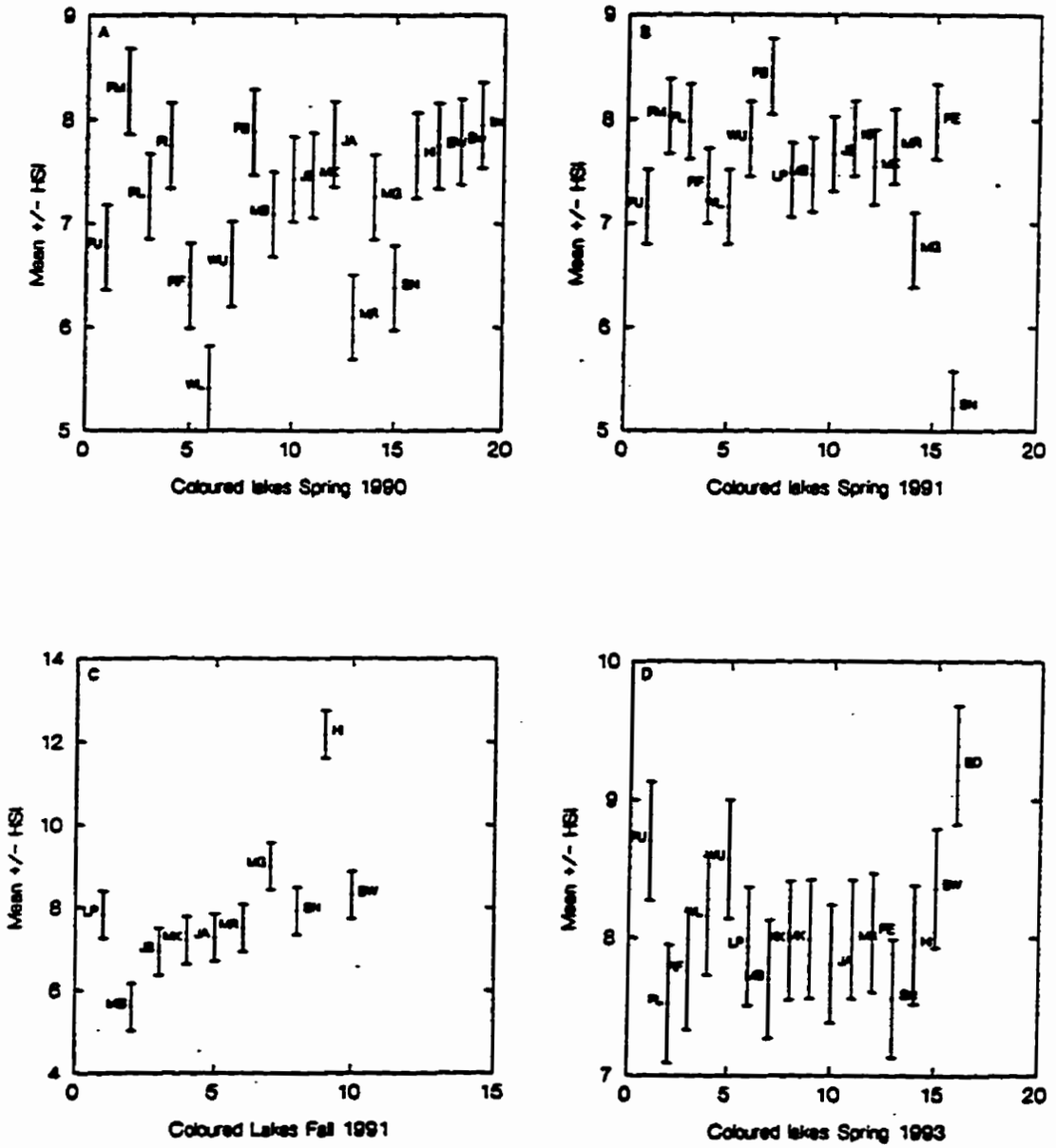

Figure 16. Mean ± HSI for colony size for all coloured lakes in A) Spring 1990 B) Spring 1991 C) Fall 1991 D) Spring 1993

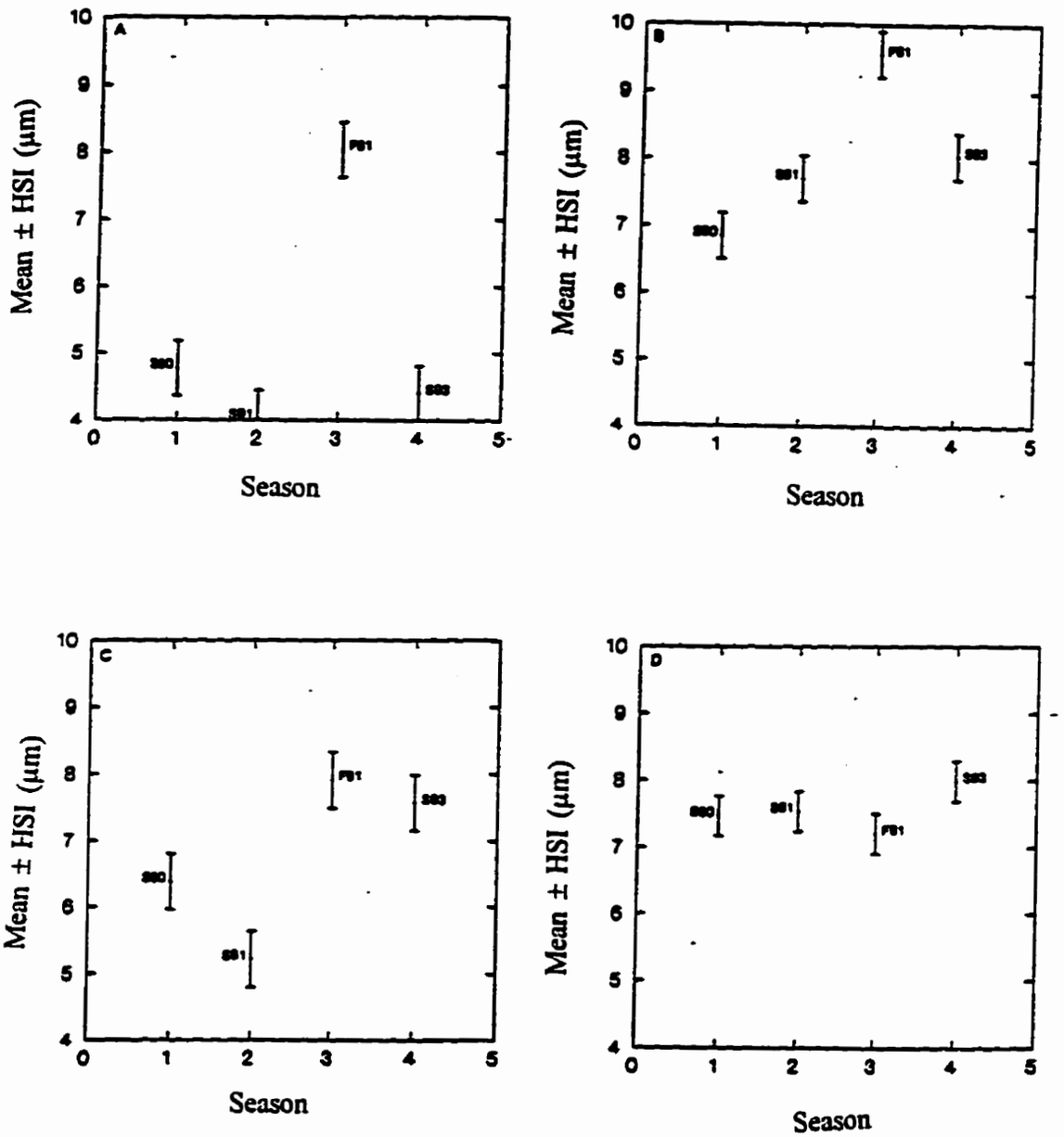

Figure 17. Within lake variability of the mean ± HSI for colony size in A) Back Lake B) Grafton Lake C) Snake Lake D) Merrymakedge

III.2 Scanning Microscopy

III.2.I Introduction for Scanning electron microscopy results

Asterionella, like many other araphid pennate diatoms, has very few morphological characters which can be measured. The transapical size of the poles and mid cell, the number of spines or spinules in a given length along the valve margin and the actual length of the valve were the only characters that could be measured with any accuracy. These are all found on the outside of the valve and their measurement depends on the correct orientation of the valve on the SEM stub. The outside of the valve was measured because in living forms a light microscope would be used and the measurements would be of the external structure. Internal features such as the labiate process and the pole pore fields (which were obscure on most valves externally) were seen and will be discussed as they could not be measured on the same valve that the external measurements were taken.

The scanning electron microscope allows a more detailed view of the fine structure of the valve and a greater accuracy for measurement. The only values available from the literature for comparison are valve length, and transapical width of the mid-cell region, head and foot poles (Körner 1970). Again, as was seen with the valve length, most of these measurements overlap. Descriptions given by different authorities on the appearance or aspect of *A. formosa* and *A. ralfsii* and its varieties are subjective and often confusing. The discussion of the SEM measurements and the general appearance of the valves will compare what was seen by different investigators to what was seen in this study.

Fifteen sites from Nova Scotia and five sites from Ontario and the United States were statistically analyzed for similarities in the five characters measured on the *Asterionella* populations using the SEM. The characters were considered within lakes over the different sampling times as well as the similarities within characters among lakes and seasons. The number of observations from each sampling period were different so the formula for HSI was modified to take this into consideration. Two of the clear lakes, Back and Grafton, had measurements for all five sampling periods and two, McIntyre and Mountain, had measurements for only three of the sampling times. Sites with coloured water included two from Kejimkujik Lake, Jeremys Bay and Minards Bay, the inflow into Kejimkujik Lake, Jakes Landing, and the outflow from Kejimkujik Lake, Mersey River. All of these sites had measurements for all five sampling times, as did Snake Lake. Upper and lower sites from Peskowesk and Peskawa as well as the joining rapids, Poison Ivy Falls, and Little Peskowesk Lake were also considered, individually and in combination. The five sites from outside Nova Scotia were compared as one group, as well as in different combinations with the Nova Scotian

sites.

III.2.2 Results and discussion for scanning electron microscopy

Within lakes, between seasons

Clear Lakes: Grafton Lake: (N = 7,5,7,3,7 for S90,S91,F91,F92,S93, respectively) Foot pole size was different in the spring 1990 sample and the two other spring samples. Measurements at the mid-cell were different from spring 1990 to the following spring, which was statistically similar to all other sampling times. There was no significant difference seen in the valve length, head pole size or number of spines in 6 μm over any sampling period.

Back Lake: (N = 6,4,5,7,6 for S90,S91,F91,F92,S93, respectively) Differences in head pole size were found between spring 1990 measurements and fall of 1992 measurements. Mid cell width was also different from the spring 1990 and 1993 samples and the fall 1992 measurements. Valve length, foot pole, and the number of spines in 6 μm was consistent between sampling times and showed no statistical difference.

Mountain Lake: (N = 9,3,6 for S91,F92,S93, respectively) Measurements were taken from Mountain Lake samples of *Asterionella* in the spring of 1991 and 1993 and the fall of 1992. The only statistical difference was seen in the mid-cell measurements between the spring of 1991 and the fall of 1992. All other characters measured showed no statistical difference between sampling times.

McIntyre Lake: (N = 6,6,8 for S90,F92,S93, respectively) Measurements were taken from McIntyre Lake *Asterionella* in the spring of 1990 and 1993 and the fall of 1992. All valve length measurements were statistically different from each other. Foot pole measurements were different in the two spring samples and the mid-cell measurement showed statistical differences between the spring 1990 sample and the fall 1992 sample. The number of spines in 6 μm and the head pole showed no differences between seasons.

Coloured Sites: Snake Lake: (N = 6,4,5,8,5 for S90,S91,F91,F92,S93, respectively) All measurements of characters on Snake Lake *Asterionella* showed no significant difference between the sampling times except for the number of spines seen in 6 μm in the spring 1991 and fall 1992 samples.

Little Peskowesk Lake: (N = 5,8,6 for F91,F92,S93, respectively) Measurements of *Asterionella* characters were taken in the two fall sampling times as well as the spring 1993 sampling time. Mid cell width was significantly different at all three times and the number of spines differed between the two fall *Asterionella* samples. No statistical differences were found in the valve length, head pole or

foot pole measurements.

Jeremys Bay: (N = 3,6,6,6,6 for S90,S91,F91,F92,S93, respectively) The only statistical difference in characters measured on the Jeremys Bay *Asterionella* was between the number of spines in 6 μm in the spring 1991 and fall 1992 sampling times. All other measured characters were not found to be statistically different.

Minards Bay: (N = 4,8,4,6,7 for S90,S91,F91,F92,S93, respectively) Mid cell width was the only character measured on the Minards Bay *Asterionella* which showed any statistical difference. This was seen between the spring 1991 and 1993 samples. All other characters measured were statistically similar over all seasons.

Jakes Landing: (N = 3,5,7,6,4 for S90,S91,F91,F92,S93, respectively) Statistical differences in valve length of the *Asterionella* from Jakes Landing were found between the spring 1990 and 1991 samples. Head pole width was similar in the spring 1990 and 1993 samples but were statistically different at the other three sampling times. Foot pole size, mid cell width and the number of spines in 6 μm was statistically similar in all cases.

Mersey River Outflow: (N = 4,6,8,7,7 for S90,S91,F91,F92,S93, respectively) Valve length in the spring of 1990 was significantly different in the *Asterionella* from this site than in all other sampling periods. Statistical differences were also present in the foot pole measurements from spring of 1990 and 1993. Head pole width, mid cell width and the number of spines in 6 μm was similar in all five sampling times.

Peskawa Upper Site: (N = 4,6,7 for S90,S91,S93, respectively) Measurements were taken from the three spring blooms of *Asterionella* at this site. Valve length was statistically different between the spring 1990 and the other two samples. Differences were found in the head pole measurement in the spring 1990 and 1991 and in the mid cell measurements from spring 1991 and 1993. The number of spines in 6 μm and the foot pole width were statistically similar in all three spring samples.

Peskawa Lower Site: (N = 5,9,7,6 for S90,S91,F92,S93, respectively) Measurements were taken on *Asterionella* in all three spring samples as well as the fall 1992 sample. Valve length was similar in the spring 1990 and 1991 samples and in the fall 1992 and spring 1993 samples. There was no difference between spring 1990 and 1993 but there was between spring 1991 and 1993. Foot pole size was statistically different in the fall 1992 sample from all three spring samples. The measurement taken at the mid cell point of these populations differed in the spring of 1990 from all other times, as did the fall 1992 measurements. Spring 1991 and 1993 measurements for this character were similar to each other. The number of spines in 6 μm differed in the fall 1992 sample

from the spring 1991 and 1993 samples but was similar to the spring 1990 measurements. Measurements from the head pole were consistently the same across all seasons.

Poison Ivy Falls: (N = 4,7,3,7 for S90,S91,F92,S93, respectively) Character measurements were taken from the *Asterionella* at this site in all three spring sampling times as well as the fall 1992. Valve length measurements differed in the spring 1990 from the other two spring measurements but were similar to those seen in the fall 1992 sample. Mid cell measurements were different in both the spring 1991 and 1993 from each other as well as from the fall sample. The number of spines in 6 μm was statistically different between the spring 1991 and fall 1992 samples. Head pole and foot pole measurements were statistically similar in all four sampling periods.

Peskowesk Lake, Upper site: (N = 8,7,7 for S90,S91,S93, respectively) Measurements for characters on the *Asterionella* populations were taken in all three spring sampling periods. All five characters showed no statistical difference at any of these times.

Peskowesk Lake, Lower site: (N = 5,8,5 for S90,S91,S93, respectively) Character measurements were taken in all three spring sampling periods for the *Asterionella* populations at this site. The valve length in the spring 1993 sample was statistically different than in the other two spring samples. Differences were also noted between measurements taken on the foot pole and mid cell width in the spring 1990 and 1993 samples. The number of spines in 6 μm and the head pole size showed no statistical difference among the three spring seasons.

Other Lakes: (N = 6,14,11,8,10 for Pl200, Bw201, TAP203, Pl169, GB168, respectively) This group of lakes includes Plastic Lake, Dorset, Ontario in the spring of 1993 (Pl169) and 1994 (Pl200), Georgian Bay at Wiarton, Ontario (GB168) in the spring of 1993, Birchwood Lake, New Jersey, U.S.A. (Bw201) in the spring 1994 and Thirty Acre Pond, Rhode Island U.S.A. (TAP203) in the spring of 1994.

Valve length was statistically different between Pl200 and all other sites and between Bw201 and all other sites. Head pole size was similar at all sites except for Pl200 which was statistically different from the other four sites. The size of the foot pole was statistically similar between Bw201, Pl169, and GB168, between Pl200 TAP203 and Pl169, and between Bw201, Pl169, and GB168. Two groupings were seen in the mid cell measurement which were statistically similar within the group but different between the groups. One group includes Pl200, Bw201 and TAP203 and the other group consists of Pl169 and GB168. The number of spines in 6 μm was the only measured character which showed statistical similarities between all five sites.

Between lakes

Five morphological characters were measured on samples of *Asterionella* from different sites

and then compared statistically for differences. Nine sites from the Nova Scotian data set as well as five from other areas were variously combined. Pl200, Pl169, GB168, Bw201, and TAP203 were compared as a group, "Other", against Snake, Back, Grafton, Mcintyre, and Mountain lakes over the different sampling periods that *Asterionella* was found in these Nova Scotian lakes. As well, combinations of lakes were compared to establish which were the most similar or dissimilar in morphological characters across the seasons.

Snake Lake: Measurements of all five characters from *Asterionella* valves in all five sampling periods were statistically analysed in combination with Back Lake (all five sampling times), Plastic Lake (Pl169 and Pl200), Georgian Bay (GB168), Birchwood Lake (Bw201), and Thirty Acre Pond (TAP203).

Table 10 shows that the combined error in the group "Other" is not as discriminating as comparing each lake individually with Snake Lake. The length of the valve and the size of the foot pole show the greatest amount of variation in all seasons. Differences in head pole measurements were seen only in the spring of 1990 between Birchwood Lake and Snake Lake in the grouped Other comparison, but with individual lake comparison the length of the valve and the number of spines were different. Differences in the width of the valve at mid-cell and the number of spines in 6 μm were found in the individual lake comparisons of Thirty Acre Pond and the Plastic Lake sample of 1993.

In the combined "Other" group, seasonal differences were not seen in any of the measured characters on the *Asterionella* in Snake Lake and Plastic 169 or Snake Lake and Georgian Bay in the spring of 1990, spring of 1991, or fall of 1992. The same is true for Birchwood Lake in all seasons but spring of 1990. There was no season when *Asterionella* from Snake Lake and Thirty Acre Pond had statistically similar foot pole measurements. Valve length and foot pole measurements were the major differences in the Plastic Lake 200 and Snake Lake *Asterionella.*

The greatest number of differences between measurements of characters in Back and Snake Lakes was in the size of the head pole. There was no statistical difference in measurements of the foot pole and the number of spines on the *Asterionella* in these two lakes in any season (Table 11). Differences appear to be greatest between seasons rather than within seasons and between lakes.

Back Lake: Back Lake was compared with the "Other " group as well as individually with TAP203, BW201, Pl200, Grafton, McIntyre, Mountain and as previously mentioned Snake Lakes. Major differences were seen in the head pole, foot pole and valve length measurements. Differences in mid cell measurements and spines in 6 μm were seen in the individual measurements of Thirty Acre Pond

Table 10. Statistical differences in measured characters of *Asterionella* from Snake Lake and five other lakes, individually and in combination. Column 1 indicates the individual lake being compared in column 3 to Snake Lake. Column 2 is the combination of all 5 sites compared against Snake Lake. (VL:valve length, HP:head pole, FP:foot pole, MC:mid cell, SP:spines in 6 μm, S90:spring 1990, S91:spring 1991, F91:fall 1991, F92:fall 1992, S93:spring 1993) Boldface shows significant results whether comparing an individual lake or the combination of lakes.

Snake	Other	Individual
Thrity Acre Pond (TAP203)	**FP,all seasons**	**FP,all seasons** MC S93 SP S91
Birchwood Lake (BW201)	HP S90	VL S90 SP S91,S93
Plastic Lake (Pl200)	**VL,all seasons** **FP S90,F91,F92,S93**	**VL,all seasons** **FP S90,F91,F92,S93**
Georgian Bay (GB168)	**VL F91** **FP S93**	VL S91,**F91**,S93 **FP S93**
Plastic Lake (Pl169)	**VL F91** **FP F91,S93** **MC S93**	VL S91,**F91**,S93 FP S90,**F91**,F92,**S93** **MC S93** SP F92

and Birchwood Lake but all other statistical differences were the same.

The only seasonal similarities in all measurements in the "Other" group were those in the fall of 1991 among Back Lake, the Plastic Lake 169 sample and the Birchwood (Bw201) sample and again between Back Lake in the fall of 1992 and Birchwood Lake. In the spring of 1990 there were no head pole measurements which were statistically similar to those from Back Lake (Table 12).

Back Lake was also compared to three other clear lakes, Grafton, McIntyre, and Mountain. Measurements were obtained from *Asterionella* in Back and Grafton Lakes in all five sampling times but from McIntyre Lake only in the spring of 1990 and 1993 and the fall of 1992. *Asterionella* in Mountain Lake were measured in the spring of 1991 and 1992 as well as the fall of 1992 (Table 13).

Table 11. Statistical differences between Snake and Back Lakes in all five sampling seasons. (S90:spring 1990, S91:spring 1991, F91:fall 1991, F92:fall 1992, S93:spring 1993)

Character	Snake Lake	Back Lake
Valve Length	F91	S93
Head pole	S90	S90,S91,F92,S93
	S91	S90
	F92	S90
Foot pole	None	None
Mid cell	S93	F91
Spines in 6μm	None (Snake S91,F92 different)	None

Table 12. Statistical differences between *Asterionella* characters from Back Lake samples and the combined "Other" group as well as Back Lake and individual lakes from the "Other" group. (VL: valve length, HP: head pole, FP: foot pole, MC: mid cell, SP: spines in 6 μm.)

Back	Other	Individual
Thirty Acre Pond	HP S90,S91,F92,S93	HP S90,S91,F92,S93
(TAP203)	FP all seasons	FP all seasons
		MC S93
Birchwood Lake	VL S90,S91,S93	VL all seasons
(Bw201)	HP S90	HP S90
		SP F91
Plastic Lake (S94)	VL all seasons	VL all seasons
(Pl200)	HP S90,S91,F92,S93	HP S90,S91,F92,S93
	FP S90,S91,F92,S93	FP S90,S91,F92,S93

No statistical differences were found in any season in the valve lengths of the *Asterionella* in Back and Grafton Lakes, the head pole and spine measurements in McIntyre and Back Lakes or the foot pole and spine measurements in Mountain and Back Lakes. The head pole measurements in Grafton and Mountain lakes showed few similarities with the Back Lake head pole measurements in most seasons. Foot pole measurements were seen to be most different between Back and McIntyre lakes especially in the spring of 1990.

Grafton Lake: As well as the comparisons already mentioned, Grafton Lake was also compared with the "Other" group (Pl169, Pl200, GB168, Bw201, TAP203) and to Mountain and McIntyre Lakes. Table 14 shows the differences between Grafton Lake when analysed against the "Other" group. The greatest differences are in the spring of 1990, at which time the foot pole was found to be most dissimilar. *Asterionella* from Birchwood Lake were the most different from all of the lakes compared and, unlike any of the others, the foot pole was statistically similar to those seen in Grafton Lake. The *Asterionella* in the spring 1991 sample from Grafton Lake were statistically the most similar and differences were seen in Birchwood Lake and Thirty Acre Pond only.

When comparing all lakes across all seasons, the *Asterionella* in Birchwood Lake and Thirty Acre Pond were different from Grafton Lake in some character in all seasons. The size of the foot pole in TAP203 was consistently different from that in Grafton Lake in all seasons while, Bw201 was most different in valve length and head pole measurements.

The most similar were the *Asterionella* from Georgian Bay, which showed no statistical differences in either 1991 sample or in the spring 1993 sample from Grafton Lake. Only three measurements were statistically different between the *Asterionella* in Georgian Bay and those in Grafton Lake: headpole measurements in the spring of 1990 and fall 1992 and foot pole measurement in 1990.

The Plastic Lake samples from the spring of 1993 (Pl169) and 1994 (Pl200) showed no significant statistical differences from the *Asterionella* in Grafton Lake in the spring of 1991. Differences were seen in valve length and foot pole measurements in the Pl200 *Asterionella* and in foot pole and mid cell measurements in the Pl169 *Asterionella* when compared with Grafton Lake *Asterionella*.

In the statistical comparison of *Asterionella* characters between Grafton and McIntyre Lakes, many differences were seen (Table 15). The only character which was statistically similar in the

Table 13. Differences in statistical comparison of *Asterionella* characters from Back and Grafton Lakes in all five sampling periods, Back and McIntyre Lakes in spring 1990, 1993 and fall 1992, and Back and Mountain Lakes in spring 1991, 1993 and fall 1992.

Character	Back Lake	Grafton Lake
Valve length	NONE	NONE
Head pole	S90,S91,F92,S93	All seasons
Foot pole	S93	S91,S93
Mid cell	F91	S90,F91,S93
Spines in 6 μm	S90,F92	S90
	Back Lake	**McIntyre Lake**
Valve length	F92	S90,S93
Head pole	NONE	NONE
Foot pole	S90	S90,F92
	S91,F91	S90
	F92,S93	S90,F92
Mid cell	F91	S90,S93
Spines in 6 μm	NONE	NONE
	Back Lake	**Mountain Lake**
Valve length	All seasons	S91
Head pole	S90,F92	S91,S93
	S91,S93	S91
Foot pole	NONE	NONE
Mid cell	F91	S91
Spines in 6 μm	NONE	NONE

Table 14. Statistical comparison of characters measured from all five seasons *Asterionella* appeared in Grafton Lake with the combined group "Other". (VL:valve length, HP: head pole, FP: foot pole, MC: mid cell, SP: spines in 6 μm) * indicates statistical differences (t=0.95)

Season	Char.	Pl 200	Pl 169	GB 168	Bw 201	TAP 203
S90	VL				*	
	HP			*	*	
	FP	*	*	*		*
	MC		*		*	*
	SP				*	
S91	VL				*	
	HP				*	
	FP					*
F91	VL				*	
	HP				*	
	FP	*	*			*
	MC		*			*
F92	VL	*				
	HP			*	*	*
	FP					*
S93	VL	*			*	
	FP	*				*
	MC		*			

Asterionella of both these lakes was the valve length. The measurement at the mid point of the valve was different only between the spring 1990 Grafton and fall 1992 McIntyre *Asterionella*. Head pole

and foot pole measurements were very dissimilar between these two sites.

The size ranges not only of the length of the valve but also of other measured features of the *Asterionella* examined in this study from Nova Scotia, were found to overlap *A. formosa* on the upper end and *A. ralfsii* on the lower end according to Patrick and Reimer (1966) or, following Körner (1970), within the range of both *A. formosa* and *A. ralfsii*. The frustule of *A. formosa* is variously described in the literature as being: long, linear, and rod-like with capitately widened ends (Körner 1970), linear and a little narrower toward the end of the valves (Patrick and Reimer 1966), linear and unequally inflated at the ends (Boyer 1926) and narrowly linear with apices unequally capitate (Van Heurck 1896). Valve length has been considered very variable by most authors but some have tried to erect varieties of *A. formosa* based on this criterion (Heiberg 1863; Lozeron 1902). Körner noticed a reduction in the apical axis in both natural and clonal populations of *A. formosa* and considered valve length too variable a character with which to differentiate possible varieties of *A. formosa*. A large amount of variability was noticed in the transapical width of the head and foot pole as well as the amount of constriction between the poles and the main body of the valve. The overlap of these characters was so complete that Körner (1970) did not feel that separation into different forms based on these criteria was justified. Marginal spinules were mentioned as occurring along the valve edges.

The nominate variety of *A. ralfsii*, as well as var. *americana* and var. *hustedtiana*, have been described as appearing heteropolar, shorter than *A. formosa* and with a bulging cell center (Körner 1970; Patrick and Reimer 1966; Boyer 1926; Van Heurck 1896). The upper length ranges of all varieties of *A. ralfsii* are well within the *A. formosa* upper limit but the lower range is 10 - 20 μm shorter than the lower limit for *A. formosa* (Table 16). Delimitation of the different varieties appears to be based on the shape of the head and foot poles and by the position of the pseudoraphe and transapical striae since there is little difference in the valve length parameters. Marginal spinules in var. *hustedtiana* are described as being "robust" while in the other two varieties they are described as small.

The appearance of the frustules from all sites in this study was generally linear, but when shorter cells were encountered, the transapical valve width (mid cell width) did tend to be broader than that toward the apices (Plate I). Most cells exhibited heteropolarity between the apices with the foot pole usually larger than the head pole. Occasionally cells were seen where the transapical width of the head and foot poles was approximately equal but more commonly little differentiation was seen between the head pole and the main body of the valve. The frustule shapes

Table 15. Statistical differences in *Asterionella* valve characters between Grafton and McIntyre Lakes and Grafton and Mountain Lakes over all five seasons *Asterionella* was seen in Grafton Lake and in the spring of 1990, 1993 and fall 1992 in McIntyre Lake and the spring 1991, 1993 and fall 1992 in Mountain Lake. ($p = 0.95$)

Character	Grafton Lake	McIntyre Lake
Valve length	NONE	NONE
Head pole	S90	S90,F92,S93
	S91	S90
	F91	S90,F92
	F92	S90,F92,S93
Foot pole	S90	S90,F92,S93
	S91,F92,S93	S90
	F91	S90,F92
Mid cell	S90	F92
Spines in 6 μm	S90,S91	F92,S93
	Grafton Lake	**Mountain Lake**
Valve length	F92,S93	S91
Head pole	NONE	NONE
Foot pole	NONE	NONE
Mid cell	S90	F92
	S91	S91
Spines in 6 μm	NONE	NONE

of the longer cells were similar to the gradation described for *A. formosa* by Körner (1970) while the shorter cells (≤ 60 μm) appeared similar to what was described for *A. ralfsii* and its varieties. The amount of inflation in the mid cell area is a subjective observation when made under the light microscope and, in most studies, this is the case. It is, therefore, difficult to describe the aspect or

visual appearance of a valve with any consistency between different observers.

SEM measurements in this study from all but the Nova Scotian sites showed that the upper and lower size limits for the transapical mid cell width were within the range given in the literature for *A. formosa*. Valves from Birchwood Lake, New Jersey and Plastic Lake, Ontario were wide enough to agree with the transapical mid cell width for *A. ralfsii* var. *americana* as described by Körner, but populations from the other sites did not fit this criteria. The lower size limit for mid cell width in the literature for any of the described *Asterionella* species was larger than that which was measured in some valves from populations in Nova Scotian lakes. However, when all measurements were averaged from a single site this value was always within the measured values for *A. formosa* and often within the measured values for *A. ralfsii* var. *americana*. Marginal spinules were seen on the full valve length and the foot pole, and larger spines were seen on all head poles (Plates II,V).

The shape and size of the valve poles was used by Körner (1970) to differentiate the different species of *Asterionella*. The foot poles of neighbouring cells abut one another in the center of a colony, while the head poles are the ends away from the colony center. Transapical measurements of these two poles overlap in *A. formosa* and the shape is described as being variable; sometimes pear-shaped, rounded, compressed or constricted. The poles may be the same size, the foot pole larger than the head pole, or any intermediate shapes between the two extremes. Both poles show pore fields with a labiate process present in a depression in the foot pole, and often in the head pole as well (Körner 1970). Patrick and Reimer (1966) described the valve as being capitate, with the foot pole much larger and strongly capitate and the head pole much smaller and not as distinctly capitate. They made no mention of pore fields or labiate processes in either pole. Boyer (1926) describes *A. formosa* as being unequally inflated at the ends, with one end attenuated and the other capitate. The description given by Van Heurck (1896) mentions the base (foot pole) being very strongly capitate and the superior apex (head pole) much smaller. No mention is given by either Boyer or Van Heurck of pore fields or labiate processes, but these features are almost impossible to discern with a light microscope.

Körner (1970) described three varieties of *A. ralfsii*. The nominate variety was seen as having a ± spherically inflated foot pole and a small spherically elongated head pole, both poles being clearly constricted from the middle of the valve. Pore fields were seen at both poles but that of the head pole was not well developed. A labiate process was usually seen in close proximity to the pseudoraphe. The poles of the nominate variety have been described by others as being capitate on one end and smaller and rounded on the other (Patrick and Reimer 1966) or attenuate at one end

and rounded capitate at the other (Boyer 1927). Körner (1970) described two varieties of *A. ralfsii*. The variety *hustedtiana* Körner was described as having an elongated-roundish inflated foot pole and a slightly widened elongate head pole, both of which are separated from the middle of the cell by an elongate length of valve. Pore fields are located in both poles, a small one in the head pole and a larger asymmetrical one in the foot pole. A labiate process, found in close proximity to the pseudoraphe was usually present at both poles. Variety *americana* Körner was described as having a considerably inflated triangular foot pole with the head pole weakly widening into an elongate-roundish shape. Short pore fields were present at both poles and, in the foot pole, the pore field continued across the valve in a transapical direction. Labiate processes, situated near the valvar margins were present in both poles. Again, as with the aspect of the whole cell, descriptive terms can be ambiguous or misleading when applied by different investigators.

The size of the head poles from sites in this study varied on both the upper and lower range limits. Some head poles were as small as 0.6 μm when measured transapically and all sites except Birchwood Lake, New Jersey exceeded the *A. ralfsii* var. *americana* range for this character. The shape was also variable and included rounded, slightly inflated head poles with a definite constriction between the pole and the rest of the cell, elongately rounded head poles with little constriction between the pole and the body of the cell, and head poles which showed little difference in width from the main body of the cell but were almost pointed at the apex (Plate II). Apical pore fields were seen in all head poles and usually consisted of five rows of pores. A labiate process was sometimes present and sometimes absent on the interior surface of the pole. When present, it was located in the center of the valve below the pore field, oriented either horizontally or vertically, on the left side of the pole, oriented either toward the center of the valve apex or toward the outer valve margin, or on the right side of the pole oriented toward the cell apex or the outer valve margin (head pole positioned to the top). Large spines (3 - 12) were evident on all head poles (Plates III,IV).

The transapical width of the foot pole was also very variable. The size range (1.8 - 6.0 μm) in the Nova Scotian lakes and from Georgian Bay, Ontario is a much broader range than that given by Körner for any of the *A. ralfsii* varieties, but does fall within the limits given for *A. formosa*. The foot pole measurements from the two U.S. sites and from Plastic Lake, Ontario were within the range given for *A. formosa* and *A. ralfsii* var. *ralfsii* and var. *americana*. The overall shape of the foot pole was not as variable as was seen in the head pole but ranged from rounded, to slightly flattened, to having a definite cleft at the apex. Foot poles were always set off from the rest of the valve by a constriction (Plate 5). A pole pore field was always present, varying in size from five

rows of pores to as many as twelve rows of pores. Sometimes the pore field was restricted to the immediate apex of the cell and in other valves it was seen to be continuous across the upper quarter of the foot pole. Spinules around the valve margin varied from small and delicate to larger and robust. A single labiate process was sometimes present in the foot pole. When present, it was found on the left or right side of the valve below the pore field, or in the centre of the cell just above the transapical striae (foot pole positioned to the top). The orientation of the labiate process slit was sometimes vertical, sometimes horizontal, and sometimes pointed toward the outer valve wall or the centre of the valve apex (Plates VI,VII).

In all species the transapical striae are described as being fine and occurring close together (Körner 1970; Patrick and Reimer 1966; Boyer 1926; Van Heurck 1896). In *A. formosa* a narrow, straight pseudoraphe was evident and ended in front of the pore fields. A narrow pseudoraphe is mentioned in the nominate variety of *A. ralfsii*, while a narrow crooked pseudoraphe is mentioned for var. *hustedtiana* (Körner 1970). In *A. ralfsii* var. *americana* the transapical striae were restricted to the margins and were variable in length, demarking an irregular pseudoraphe which broadens at the foot pole (Körner 1970). In the samples from the study sites no definite pattern was seen. In some valves the striae continued across the valve mantle and were seen immediately adjacent to the pore fields. In other valves the striae were shorter and delimited a narrow pseudoraphe in the valvar axis. They were never seen to occur on the margins only.

III.2.3 Character Measurements and Environmental Variables

Temperature, pH, and conductivity measurements were taken at all sites at the time of sampling. These environmental variables were plotted against the character measurements taken from valves examined with the SEM (length of valve, size of head pole, size of foot pole, mid cell width, number of spines in 6 μm). Box plots were used to assess whether discrete groupings occurred based on the valve character, the measured environmental variable or the colour of the water ie. humic content. Figure 18 shows a selection of those results. No single environmental variable influenced the morphological characters in any season, whether it be spring or fall, or in any lake, whether it be clear or coloured water. Principal components analysis on the morphological variables determined the component loadings for each variable and showed that the longer the cell and the larger both the head and foot poles, the wider the spines were placed along the valve margin. Almost 55% of the variance was explained by the first factor. The scores for factor 1 were then plotted against the environmental variables. No discrete groupings were seen here, and so anova and regression analysis

were done to determine any relatedness which might not be apparent in the graphs. For R (multiple correlation coefficient) to be important, p (probability) should be < 0.05. P values for the regressions were 0.113, 0.717, and 0.653 for conductivity, pH, and temperature respectively. These values were even higher for the analysis of variance: 0.908, 0.355, and 0.332 for conductivity, pH, and temperature. This shows that for this study, morphological variability can not be attributed to the water temperature, pH, or the conductivity measured in these lakes.

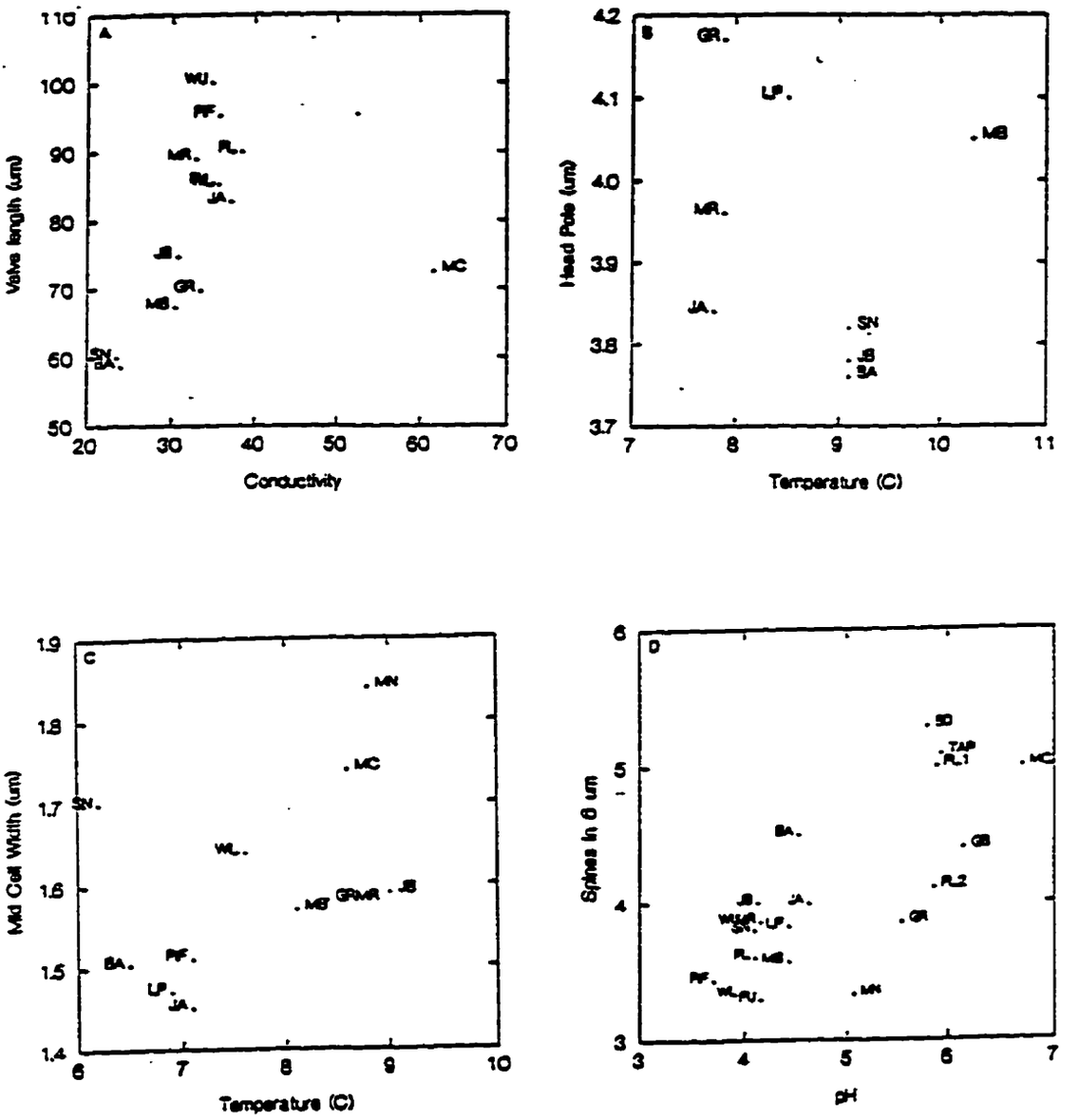

Figure 18. Examples of environmental variables plotted against character variables. Refer to Table 6 for abbreviations of the sites. A) Spring 1990 B) Fall 1991 C) Fall 1992 D) Spring 1993

III.3 SYNOPSIS OF MICROSCOPY RESULTS

This study considered populations of the diatom genus *Asterionella* from twenty-six sites in Nova Scotia as well as populations from the eastern United States and central Ontario, Canada. Some of these lakes were humic and therefore the water was a tea colour, while the water in other lakes was clear and the humic content minimal. Past investigations of *Asterionella* have suggested that different species have adapted to different ecological conditions, one of those being humic content. The occurrence of *A. ralfsii* var. *ralfsii* and var. *americana* has always been reported in coloured/humic lakes while *A. formosa* has been reported from clear water lakes. In this study the lakes were separated according to whether they were clear or humic in an attempt to match the morphological data with the humic content of the lake.

One of the most commonly used characters to distinguish one species of diatom from another, in addition to the general morphology of the valve, is the length of the valve. Although the size ranges in many species may overlap there are generally upper and lower limits which most authorities agree on. *Asterionella formosa* is not only the most commonly occurring of the *Asterionella* species but is also the largest of the two recognised freshwater species (*sensu* Körner 1970). Cells range in size from 30 μm to 120 μm and, in most populations a range of cell size is evident. *Asterionella ralfsii* var. *ralfsii*, a much rarer taxon, has valve limits of 20 μm to 60 μm. The cell length range for *A. ralfsii* var. *hustedtiana*, the European variety, is within the nominate variety range (25 - 50 μm) while the valve length of the North American var. *americana* extends to 90 μm. Valve length alone can not be used to delimit a species but is generally considered to be a stable character in the determination of the different species. Tables 16 and 17 present measurements of *A. formosa* and *A. ralfsii* and its varieties from the literature as well as measurements obtained from the *Asterionella* in this study.

Cells as short as 32 μm were seen (Snake Lake) as well as those as long as 110 μm (Peskowesk Lake). Although the lengths of the majority of cells were generally found within 3 or 4 class ranges (a class range being 5 μm), those in some populations were distributed from 35 to 100 μm (Snake Lake, spring 1991). In most lakes however, the populations of *Asterionella* fell into one of three categories, with few exceptions: those with the majority of cells in and around the 50 μm class (Snake), those with the majority of cells in and around the 70 μm class (Kejimkujik) and those with the majority of cells greater than 80 μm (Peskawa, Peskowesk). Three patterns in length class distribution over time were seen: an increase in cell length from one spring sampling to the following spring, an increase from spring to the fall of the same year, and an increase from the fall sampling to

the following spring.

The *Asterionella* populations examined in this study showed no pattern in the relationship between cell length and the colour of the lake water. Longer cell lengths were seen consistently in some of the more coloured lakes and, in the three spring samplings of the Peskawa/Peskowesk Lake series, all but six cells were greater than 60 μm. Cells less than 60 μm long were seen in six of eight clear lakes and nine of thirteen coloured lakes and, in Snake Lake (coloured) and Back Lake (clear), were seen in all five seasons. Although general patterns in cell length were seen, the *Asterionella* populations in each lake or lake system were unique. There were no trends which could be attributed to the humic content of the lake water as both short and long celled populations were seen in clear and coloured lakes.

Based on valve length, Charles *et al.* (1990) suggested the possible occurrence of two forms or ecotypes of *A. ralfsii* var. *americana* in the lakes he studied in the Adirondacks. Twelve lakes, eight of which had acidified recently, were compared against a calibration set of 37 lakes. A shorter form of *A. ralfsii* var. *americana* (<45-60 μm) occurred most commonly in lakes with lower pH (<5.0) and coloured water while the longer form (>60 μm) was more common in lakes with higher pH and clearer water. It is unclear from the literature how these findings on valve length have been used. Although Charles *et al.* (1990) first described the short form as being < 45 - 60 μm, most subsequent authors have used the lower limit (45 μm) as their criterion for short cells.

The 63 lakes studied by Anderson *et al.* (1993) from 1978 to 1984 were small, deep, humic lakes with pH ranging from 4.4 to 7.9 and either oligotrophic or slightly mesotrophic. Both *A. formosa* and the short and long forms of *A. ralfsii* var. *americana* were reported in these lakes. *Asterionella formosa* was almost completely restricted to lakes with pH ≥ 6.2 although it was common in lakes with low alkalinity. The distribution of this taxon was seen to be correlated to lake morphometry and landscape variables and occurred most commonly in lakes that were large, deep and more mesotrophic than other lakes in the data set. The short form of *A. ralfsii* var. *americana* (<45 μm) predominated in some lakes (8) while only the long form (≥ 45 μm) occurred in others (12 lakes). The short form occurred most commonly in lakes that were shallow relative to transparency and no statistical relationship to water colour was found. The long form was found in lakes with reduced transparency and its presence was positively correlated with water colour. This is contrary to the distribution of these two forms in the Adirondack study (Charles *et al.* 1990) but in agreement with what was seen in this study.

The other major division used in this study was that of seasonality. Spring temperatures in

the KNP lakes was found to be much lower than that seen in the fall sampling periods. Ruttner (1937) proposed two races of *A. formosa* based on water temperature: a cold stenothermal race he called var. *hypolimnetica* and a eurythermal race, var. *epilimnetica* which was supposed to represent the nominate race. Variety *hypolimnetica* was always small celled (36 μm - 54 μm) and showed no cycle of size reduction where var. *epilimnetica* was larger (39 μm - 90 μm) and showed cycles of decreasing size with assumed auxospore formation. Körner (1970) proposed a similar idea after examining seasonal collections of populations of *Asterionella* from Lake Michigan. He described three possible forms based on the differences in head and foot pole width which were then labelled A, B, and C. The type B cells were not found to be distinguishable from the other forms upon closer examination, and so were abandoned. Certain characteristics were shared by the populations occurring in the winter and spring and others by populations occurring in the summer and fall. Cold water forms were predominantly of the C type, found mainly in the hypolimnion, while type A cells were considered warm water forms, mainly in the epilimnion. He did not see a corresponding seasonal dimorphism in the European collections.

Size variability from the populations in this study followed no set pattern although the measured temperatures were as much as half a magnitude different between the warmer spring and the cold fall ranges. Only one and two clear lakes and five and four coloured lakes in the fall 1991 and 1992, respectively, had cells in the shorter length classes. The spring 1990 samples showed an equal number of clear and coloured lakes (six) with cells in the shorter length classes. The spring 1991 samples showed six coloured and five clear lakes with cells in the shorter length classes. Nine lakes with cells in the smaller length classes were seen in the spring of 1993, six of those being clear lakes and three being coloured lakes. This study shows that although a few populations are predominantly short celled, most display a range of cell lengths and variability in morphological features which could change with the season. In most of the lakes, increases occurred over winter, although populations in some lakes increased in length over the summer months. In all cases, length increases occurred after a bloom period.

Dissolved Si concentrations have a direct effect on cell length with a decrease occurring when Si levels are low (Anderson *et al.* 1993). Again this seems to be contrary to what Charles *et al.* (1990) reported from Bear Pond and also to what was seen in ELA Lake 223 (Findlay 1984). In Bear Pond, a site with pH values < 5.0, the Si levels were very low (0.2 mg L^{-1}) and the abundance of most other diatom species was declining, yet populations of the long form of *A. ralfsii* var. *americana* showed an increase in abundance beginning around 1950. A similar observation was

made when the pH of ELA Lake 223 was artificially lowered to 5.2. The appearance of the long form of *A. ralfsii* var. *americana* coincided with the drop in dissolved SiO_2 levels (< 1.0 mg L^{-1}) (Findlay 1984). Anderson *et al.* (1993) proposed the idea that the short form was benthic and could survive in lakes with low Si (≤ 0.1 mg L^{-1}) because of contact with Si-rich substrates. They suggested that the longer form was predominantly planktonic in deeper lakes with higher Si content. Gensemer (1990), using *A. ralfsii* var. *americana* in culture studies, found a decrease in valve length to be related to additions of Al, especially at high growth rates. However, Anderson *et al.* (1993) found no relationship between valve length and total Al in natural conditions.

Interactions between environmental and biological factors have been shown to influence cell size (Jewson 1992). An increase or decrease in temperature, nutrients, parasitism, and light are just a few of the factors which may stimulate or retard cell size. Although sexual reproduction is considered an integral part of the diatom life cycle, little is known about the sexual cycle in most diatom species. It has been suggested that this stage has been lost in some genera, but a more likely reason for the lack of detection is that it is of such short duration and sporadic occurrence when compared to the predominantly asexual phase, that it largely goes unnoticed. When cell size is reduced below 30 - 40 % of the maximum, this factor, along with internal cell mechanisms and environmental conditions, may trigger cells to undergo sexual differentiation, leading to restitution of maximum cell size. This suggests that in any given population of diatoms a multimodal distribution of length classes could be evident (Mann 1988). Each class should represent the time since auxosporulation and selective pressures occurring over time. These age classes are affected differentially by selection and, depending on whether selection favours smaller or larger cells, the modality of the length classes may change. The absence of a length class does not necessarily mean that it is not present in that population, but that selective pressures are being exerted on that particular size of cell. This may explain the distribution of size classes seen in the *Asterionella* populations in some of the Kejimkujik National Park lakes. The change in cell length classes from one season to another could indicate that a proportion of that population has obtained optimum conditions to induce sexuality.

Another possibility which would account for the changes in length classes and the bimodality seen in some lakes, is the presence of more than one clonal population. These clones would share the same ecological preferences but be genetically separate for the most part. If different clonal varieties make up a population in any given lake, then it is possible that these lines could remain reproductively isolated from each other for many generations, (the asexual generation time of diatoms

being as high as one division per day under optimum conditions). Most individuals in a population would be the result of clonal reproduction and only a few individuals would, at any given time, be direct offspring of a mating event or have the correct combination of conditions for sexual initiation. The cyclical nature of the populations in this study, especially in the cases where bimodal length distributions were seen would suggest that more than one mutational variant was present in these lakes.

If *Asterionella* does undergo some form of sexual reproduction and genetic recombination does occur, then the question should be how much actual gene exchange has happened. Is the recombined genome affected differently than the parental population? This diatom, like most others, undergoes periodic blooms, usually in the spring and/or fall seasons, during which asexual reproduction occurs rapidly. This suggests that cells in close proximity to one another are clonal siblings that have come from the same biparental stock. Whether sexual reproduction occurs in the water column or on the sediments, the likelihood of the recombining pair having vastly different genetic material is small. Only those clonal members that are out of phase with the majority of the population would be likely to find a mate of different clonal stock and successfully reorganise the genome of a new clonal population. This might be the type of event that led to the combination of character states seen in this study and may be the answer to why Charles *et al.* (1990) and Anderson *et al.* (1993) had such different findings in their study lakes.

Differences in morphology which were seen in the natural populations of *Asterionella* from the lakes studied, encompassed a greater range of variability than that which has been reported in the literature to this time. The type location for *A. ralfsii* var. *americana*, (Birchwood Lake, New Jersey), was sampled for this study and the only discrepancy seen between measurements, as reported by Körner, was in the size of the foot pole. This measurement was smaller in this study but was within the range reported for *A. ralfsii* var. *ralfsii*. The populations studied from the Nova Scotian lakes encompassed a much broader range of sizes, for all measured characters, than what has been reported. They did not fit the *A. formosa* description or measurements, nor did they agree with any of the *A. ralfsii* varieties. Intermediate forms were seen with combined attributes of all reported *Asterionella*. This would suggest that these particular populations are possibly hybrid forms of *A. formosa* and the varieties of *A. ralfsii* as described by Körner, that is, clonal variants which have combined the genome of the most suitable parental stock. Populations examined from Georgian Bay and Plastic Lake, Ontario, were in closer agreement to the *Asterionella* described as *A. formosa* with the exception of the transapical width of the head pole.

As can be seen by the cell length values and morphometric measurements from this study, a much broader range in the morphology of *Asterionella* is evident. These findings are not confined to either clear or humic lakes, nor to temperature differences which were evident in the different sampling seasons. If sexuality in this organism can, in part, be triggered by the smaller size of the valve, then the maximum size for most of the *Asterionella* populations in the study lakes, clear and coloured, is well within the range of that reported for *A. formosa*. Although auxospores were not seen, cyclical changes in the length of valves were evident, indicating the possibility of sexual reproduction occurring in these populations.

The two freshwater *Asterionella* species (*sensu* Körner) have been shown to be physiologically and biochemically different (Riseng 1991; present study), and this may be a result of different evolutionary pathways. Many phytoplankton studies have used clonal isolates in an attempt to explain diversity in such attributes as silicon metabolism (Eppley *et al.* 1969; Kilham 1975; Nelson *et al.* 1976; Gensemer *et al.* 1993), effects of temperature on growth rates (Ruttner 1937; Lewin 1955; Hulburt & Guillard 1968), toxicity (Maranda *et al.* 1985; Cembella *et al.* 1986; Gensemer 1991a, 1991b), pH dependent growth rates (Wee *et al.* 1991), vitamin requirements (Lewin & Lewin 1960; Guillard 1968; Hargraves & Guillard 1974) and other physiological or biochemical traits in populations. These studies perhaps emphasize the diversity within populations rather than explain actual physiological and morphological responses under natural conditions, simply because in most cases few clones were used, and those that were used were the ones capable of being cultured and maintained under laboratory conditions. Changing environmental conditions in natural situations may exert pressure to select a particular genotype or phenotype which, when cultured, will show a particular trait not necessarily present in the entire population. This is often used to explain interspecies differences rather than within species variation. It is necessary to understand changes in diatom morphology which occur in response to environmental conditions, if diatom taxonomy is to be useful. Paleoecological studies often use diatoms for inferring changes in pH, fluctuations in water levels and heavy metal contamination. It is necessary therefore, to recognise natural phenotypic variability within the species and variability which occurs in response to changing environmental conditions. Physiological and biochemical changes are important and integral parts of the organism, but care should be exercised when extrapolating data to natural populations. The strains or races of the experimental organism should have the collection conditions and location clearly specified.

Table 16. Measured parameters of the freshwater species of *Asterionella* as reported in the literature.

Species	Authority	Valve Length (μm)	Mid-cell Width (μm)	Head Pole (μm)	Foot Pole (μm)
A. formosa	Patrick & Reimer 1966	40 - 130	1.0-3.0	NA	NA
	Boyer 1927	to 100	NA	NA	NA
	Van Heurck 1896	70 - 100	NA	NA	NA
	Rabenhorst 1864	40 - 130	1.0-2.0	NA	NA
	Körner 1970	30 - 120	1.3-6.0	1.5-4.0	3.0-12.0
A. ralfsii var. ralfsii	Patrick & Reimer 1966	20 - 50	3.0	NA	NA
	Boyer 1927	30	NA	NA	NA
	Van Heurck 1896	35 - 45	NA	NA	NA
	Körner 1970	20 - 60	2.0-3.5	1.0-2.0	3.0-5.0
A. ralfsii var. hustedtiana	Körner 1970	25 - 50	2.0-3.2	1.6-2.4	2.4-3.5
A. ralfsii var. americana	Körner 1970	20 - 90	1.5-2.4	1.0-2.0	3.5-5.0

Table 17. Measurements of valve parameters from the freshwater *Asterionella* sampled from lakes in Nova Scotia and Ontario, Canada, and Rhode Island and New Jersey, U.S.A. Length measurements of slides identified as *A. ralfsii* var. *americana* (Ottawa #30236, 30235, Berlin #25636), *A. ralfsii* var. *hustedtiana* (Berlin #25635) and the nominate variety *A. ralfsii* var. *ralfsii* (B.M. #24422) are also included.

Site	Valve length (μm)	Mid-cell Width (μm)	Head Pole (μm)	Foot Pole (μm)
Nova Scotia	32 - 112	0.6-3.0	0.6-3.6	1.8-6.0
Thirty Acre Pond, Rhode Island	52 - 96	1.4-2.3	1.5-3.0	3.6-4.1
Birchwood Lake, New Jersey	42 - 64	1.5-2.0	1.0-1.4	3.2-3.8
Georgian Bay, Ontario	60 - 120	1.4-2.0	1.0-3.0	3.8-5.5
Plastic Lake, Ontario	61 - 112	1.8-2.2	1.0-3.0	3.8-5.0
Ontario lakes (Joseph, McKay, Leonard)	34 - 88	NA	NA	NA
Slides Ottawa # 30236,30235	32 - 70	NA	NA	NA
Slide Berlin # 25636	32 - 90	NA	NA	NA
Slide Berlin # 25635	28 - 54	NA	NA	NA
Slide B.M. # 24422	36 - 45	NA	NA	NA

IV. PIGMENT DETERMINATION

IV.1.1 Culture and Extraction Methods

Single colonies were isolated from material collected from Birchwood Lake, New Jersey (*A. ralfsii* var. *americana*), and Georgian Bay, Ontario (*A. formosa*), and grown in a modified FRAQUIL medium (Morel *et al.* 1975). Collections from the Nova Scotian lakes became contaminated and could therefore not be used. The FRAQUIL medium was further modified for *A. ralfsii* by buffering with 250 mg L^{-1} MES to about pH 5.4, lowering Na_2 EDTA to 0.5 µmol L^{-1}, and adding 400 µmol L^{-1} boron (Na salt) and 400 µmol L^{-1} Si to the medium (Gensemer 1990). Double-distilled deionized water (DDW) and reagent grade chemicals were used to prepare media stock solutions. All culture tubes and media were autoclaved and cooled to 18°C before use. Approximately 1 mL of stock cultures were transferred into 150 mL culture tubes containing the appropriate medium. Six to twelve replicates were used for each clone. All replicates were incubated in a constant environment chamber (Percival Mfg. Co., Boone, IA) at 18°C and lighted with cool white fluorescent bulbs on a 14:10 h light:dark cycle. In vivo fluorescence was checked at the same time daily using a Sequoia-Turner model 450 fluorometer after allowing the culture tubes to acclimate to darkness for 10 -15 minutes (Brand *et al.* 1981). Growth rates were determined as the slope of the linear portion of the relationship between days and the Ln-transformed fluorescence unit. Cells were harvested for pigment analysis toward the end of the logarithmic growth phase and before entering the stationary phase.

All replicates of a single clone were combined and the total volume measured. A 4 mL subsample was removed and preserved in Lugols solution for later cell counts and to check for any anomalies in cell morphology. The remaining sample was filtered using a GF/C Whatman glass microfibre filter of 1.2 µm pore size, which trapped the diatom cells on the filter. The growth medium was filtered directly into tubes used for phosphorus analyses only, or into a polycarbonate beaker for later Si analyses. Five mL of 100% acetone were then added to the filter in a 20 mL vial, agitated, and placed in the freezer for approximately 20 min. The pigment extract was then removed and the filter was repeatedly rinsed with 100% acetone until the solution coming from the filter was clear. The resulting acetone extract was then filtered using a 0.2 µm pore size polycarbonate filter to remove any filter fibres or frustule debris. The acetone extracts were read at specific wavelengths which corresponded to the pigments known to occur in diatoms, using a Perkin-Elmer Lambda 3 spectrophotometer (Chl a:429-430, Chl c:441-443, ß-carotene:449-450, fucoxanthin:446-447, diatoxanthin:451, diadinoxanthin:446). This was done to determine if all expected pigments were

present. All extractions and readings were done under subdued light to reduce the possibility of degradation of the pigments.

For chromatography, an equal quantity of peroxide-free diethyl ether was added to the acetone extracts and placed in the freezer for 5 min. Cold 10% NaCl solution was added to the diethyl ether - acetone extract mixture (approximately 15% of the total volume) and the solution was inverted gently several times to precipitate any particulate matter that may have remained. The pigments migrated into the ether hyperphase, and the acetone and water insoluble impurities in the aqueous hypophase were discarded. The ether extract was then concentrated into a smaller volume under a stream of nitrogen gas. Any remaining water was removed by either repeated centrifugation or by the addititon of solid NaCl. The pigment extract was used immediately for chromatography or was stored at -30C until used.

IV.1.2 Methods for cell counts

One mL of the preserved sample was placed in a Sedgewick-Rafter chamber and allowed to settle for 15 min. The number of cells in 1 to 4 strips of the chamber were counted under low magnification using a Whipple grid to measure the width of the strip. The number of cells per mL was calculated using a standard method (Greenberg et al. 1992)

$$\#\ \text{cell/ ml} = \frac{(C)(V)}{(L)(D)(W)(S)}$$

where V= volume of 1 Whipple field = (L)(D)(W)= 1000mm

C= # organisms counted

L= length of each strip (S-R cell length) mm

D= depth of strip (S-R cell depth) mm

W= width of strip (Whipple grid image width) mm

S= # strips counted

The number of cells per mL was then converted into cells harvested from representative samples (Appendix 2).

IV.1.3 Methods for silicate assay

Soluble silicate was measured colorimetrically by the silicomolybdate method modified for a sample size of 10 mL (Strickland and Parsons 1972). Calibration standards were 1,3,5,10,20,50,80, and 100 µM sodium silicoflouride solutions (Na_2SiF_6). One and 10 cm path lengths were used at a wavelength of 810 nm on a LKB Ultrospec II spectrophotometer to measure reactive silicate. The

extinction of the solution was corrected by subtracting the reagent blank. The reactive silicate was calculated from the expression: $y = mx+b$ where

y = the measured absorbance of the solution

m = X coefficient calculated from the calibration standards for 1 or 10 cm cells

b = y intercept calculated from the calibration standards for 1 or 10 cm cells

This calculation gives the concentration of reactive silicate left in the growth medium at the time of harvest (μg-at Si /L)(Appendix 4).

IV.1.4 Methods for phosphorus assay

Three replicates of 35 mL each were analysed for the amount of phosphorus remaining in the growth medium after harvesting each culture. Only analytical grade reagents were used. The basic colorimetric method of Strickland and Parsons (1972) was followed, which involved mixing 4 parts of pre-mix (double distilled water, ammonium molybdate, antimony potassium tartrate and concentrated sulphuric acid) with 1 part ascorbic acid, then adding 10% of this reagent (V/V) to the sample. Absorbance was measured at 895 nm on a LKB Ultrospec II spectrophotometer using a 10 cm cell. Final concentration was calculated by averaging the three replicates and subtracting the blank then multiplying the absorbance by 142.86. (Appendix 3).

IV.1.5 Methods for thin layer chromatography

Whatman 100 μm layer cellulose plates were used for thin layer chromatography. A micropipette was used to spot the plates 1.5 cm from the bottom of the plate. Two solvent systems were used initially : for first dimension, n-propanol in light petroleum (BP 60-80 C fraction) = 2.5:97.5 (V\V); for second dimension, chloroform: light petroleum (BP 60-80 C fraction): acetone = 30:70:0.5 (V\V\V) (Stauber & Jeffrey 1988). Solvents were added to chromatography tanks lined with filter paper and left to equlibrate until saturation. Changes in the quantities of the various solvents were subsequently used in an attempt to resolve the different pigments in one or two dimensions. Running time of the pigments varied from 10 - 20 min in both dimensions. Upon removing the plates from the first dimension tank, spots were measured for Rf values and the colour was noted under subdued light and/or UV light before being place in the second tank. The procedure was the same after the plates were run in the second dimension. After some of the runs in the first dimension, the spots were scraped off the plate with a metal spatula and eluted in a small quantity of solvent (acetone or diethyl ether for chlorophylls, ethanol for xanthophylls) and measured on a Perkin-Elmer Lambda 3 UV / Vis spectrophotometer.

IV.1.6 Methods for high performance liquid chromatography

Separation of algal pigments was done using a Shimadzu HPLC 10A System, equipped with 2 LC-10AD pumps, SCL-10A system controller, SIL-10A autoinjector, FCV-11AL solvent switching system and a SPD-M10A diode array detector. A 5 μm C-18 column (Supelcosil 25 cm x 4.6 mm I.D.) was used. A three solvent system was found to be necessary, as the pigments needed to be loaded on the column in a buffered solution (Gieskes & Kraay 1986; Roy 1987) and eluted with a strong organic solvent. Solvents used were: Solvent A - 40:40:20 acetonitrile:methanol:0.1M ammonium acetate; Solvent B - 60:35:5 acetonitrile:methanol:water; Solvent C - 75:25 methanol:hexane. All solvents were degassed with helium prior to use. 200 μL of extracted pigments were injected on the column. The elution solvent profile used was 100% A at 1 min, switch to 100% B at 3 min, hold at 100% B until 12 min, linear gradient to 80% C at 20 min, hold until 30 min. The flow rate was maintained at 1.5 mL / min.

Eluted pigments were detected by diode array at 300 to 600 nm, and identified by comparison with standards (Sigma Chemical Co. Type IV ß-carotene from carrots; Chlorophyll a from *Anacystis nidulans*) or by published absorbance spectra (Tanada 1951; Parsons 1961; Jeffrey 1972; Healey *et al.* 1967; Eskins *et al.* 1977; Abaychi & Riley 1979; Lichtenthaler 1987). Absorbance of pigments were quantified at 430 nm (40 nm band width) and the absorbance maximum for each of the major peaks was recorded. Retention time was used to identify similar pigments in all samples, then the ratio of pigments was calculated by comparing the areas under the absorbance curve for each identified peak. Twenty different *A. ralfsii* clones and six different *A. formosa* clones were processed.

IV.2 RESULTS FOR PIGMENT DETERMINATION

IV.2.1 Results for cell counts, dissolved reactive silicate and phosphorus

Table 18 shows a representative sample for the number of cells harvested for pigment extraction and the remaining dissolved reactive silicate and phosphorus left in the growth medium at the time of harvest. Sample calculations for these assays can be seen in appendices 2, 3, and 4.

The number of cells in culture at the time of harvest were counted for random clones in the samples representing *Asterionella formosa* (T-7 and T-8) and *Asterionella ralfsii* var. *americana* (BW5-, BW6-, BW10-, BW12-). In all cases, the cells were in exponential growth and showed no signs of morphological abnormalities. The number of cells / mL from the combined replicates for

each clone varied in both the *A. formosa* samples and the *A. ralfsii* var. *americana* samples. A decline was seen in the number of cells / mL in the latter clones (BW5-9 = 2.3 x 10^6 vs. BW5-10 = 0.99 x 10^6) in most cases.

Table 18. Number of cells / mL, dissolved reactive silicate and phosphorus remaining in the growth medium for clones of *Asterionella formosa* (T-7-, T-8-) and *Asterionella ralfsii* var. *americana* (BW5-,BW6-,BW10-,BW12-) at the time of harvest for pigment extraction.

Sample #mL	# cells/mL (x 10^6)	Si (µg-at/L)	P (µg/L)
BW5-9	2.30	11.31	246.86
BW5-10	0.99	9.67	248.58
BW6-10	1.76	8.10	248.58
BW6-11	1.12	9.90	243.15
BW10-11	2.80	2.01	236.29
BW10-14	0.73	2.97	255.29
BW12-12	1.33	10.35	244.58
BW12-13	1.29	15.05	234.58
BW12-15	1.40	9.11	228.86
BW12-16	1.65	8.94	236.15
T-7-5	6.00	11.06	169.57
T-7-7	2.11	15.05	224.00
T-8-8	4.63	4.72	158.29
T-8-9	2.41	9.43	188.00
T-8-11	2.43	3.39	184.43

The amount of dissolved reactive silicate and phosphorus left in the growth medium showed no evidence of limiting the growth of any of the cultures used for pigment extraction. Levels of total phosphorus were generally higher in the *A. ralfsii* clones than in the *A. formosa* clones. Soluble silicate showed no definite pattern of uptake with regard to cells / mL in culture.

IV.2.2 Results for absorbance spectra and thin layer chromatography

Separation of pigments by thin layer chromatography was not very successful, regardless of the solvent system used and regardless of whether the pigment extract was from an *A. ralfsii* var. *americana* or an *A. formosa* clone. From the origin to the solvent front, four spots occurred. The first three were quite distinct pigment separations, while the fourth was a mixture of more than one pigment. A very minor spot always remained at the origin whether development was in the first or second dimension. This was tentatively identified as chlorophyll c, both by its Rf (retardation factor) (Rf = 0) and by its colour (pale green) (Jeffrey 1968,1974). It never occurred in sufficient quantities to permit scraping off the plates and elution with a solvent to confirm its identification by its absorption maximum. The second separation was an orange pigment. By comparing Rf values, colour, and absorption maxima, this was identified as fucoxanthin (Vesk & Jeffrey 1987; Stauber & Jeffrey 1988). The third distinct component was yellow in colour and, again by comparison to reported values in the literature, was identified as diadinoxanthin (Jeffrey 1968; Stauber & Jeffrey 1988). A large spot occurred near the solvent front which contained the remainder of the expected pigments; chlorophyll a, ß-carotene, and diatoxanthin. Under UV light the chlorophyll fluoresced while the carotenoids remained almost black. These three pigments did not separate well regardless of the solvent system and regardless of whether the plate was run in one or two dimensions. The fluorescent portion of this last spot was scraped off and eluted with either diethyl ether or acetone for absorbance determination. Table 19 shows maximum absorbance and Rf values found in this study for the yellow and orange pigments and the fluorescent pigment in the fourth spot for three *A. ralfsii* clones and three *A. formosa* clones.

Absorption maxima were recorded at wavelengths from 700 nm to 400 nm. All of the pigments absorbed to some extent between 400 nm and 500 nm, so by referring to the literature, peaks for the different pigments were identified. A comparison of absorption maxima from this study and from results in the literature can be seen in Appendix 5. Most values from this study are in good agreement with reported values and any variation might be considered a function of the solvent in which the pigment was eluted. The extraction method was adequate enough to recover all of the

Table 19. Rf values for first dimension and maximum absorbance values seen in three *A. ralfsii* var. *americana* clones (BW5-9,BW6-10,BW10-10) and three *A. formosa* clones (T-7-1,T-8-6, F-6-5). Tentative identification based on Rf values and maximum absorbance values are: orange -fucoxanthin; yellow -diadinoxanthin; green - chlorophyll a. (N = 3)

Clone #	Colour	Rf	Max. Abs.
BW5-9	orange	0.49	444-452
BW6-10	orange	0.49	448
BW10-10	orange	0.37	443-445
T-7-1	orange	0.51	447-449
T-8-6	orange	0.45	448
F-6-5	orange	0.38	447-449
BW5-9	yellow	0.65	448
BW6-10	yellow	0.65	433-438
BW10-10	yellow	0.53	443-447
T-7-1	yellow	0.61	446-449
T-8-6	yellow	0.59	444-448
F-6-5	yellow	0.55	444-447
BW5-9	green	0.84	428
BW6-10	green	0.86	424-430
BW10-10	green	0.77	428-430
T-7-1	green	0.71	428-430
T-8-6	green	0.83	428-430
F-6-5	green	0.75	428-431

expected pigments from the diatoms even if the solvent systems used were not accurate enough to separate all of the pigments.

IV.2.3 Results for high performance liquid chromatography (HPLC)

Chromatograms from the HPLC showed good resolution of all major pigments. Six major peaks or groups of peaks corresponded to the pigment array expected in diatoms. Degradation products were seen for all pigments in most cases, but a comparison of the spectra could identify a degradation product of a particular pigment. The area under the array of peaks for both diatoxanthin and diadinoxanthin were combined and represent the other xanthophylls present in the ratio calculations (from approximately RT=10 to RT=13 where RT= retention time) (Stransky & Hager 1970). Chlorophyll a was calculated from the areas under the retention times corresponding to this pigment (RT = 19.58) and its degradation product (RT = 21.50). Figures 19 and 20 show spectra corresponding to the identified pigments. Absorbance maxima for pigment peaks were as follows: Chl a - 430 nm, Chl c - 441 nm, ß-carotene - 450 nm with minor peak at 476 nm, fucoxanthin - 446 nm, diatoxanthin - 442 nm with minor peak at 464 nm, diadinoxanthin - 446 nm with minor peaks at 423 and 475 nm, chlorophyll degradation - 408 nm. Representative chromatograms showing the major pigment peaks are presented in Figure 21 for *A. formosa* and *A. ralfsii* var. *americana*. Table 20 lists the pigments and the corresponding peak numbers and retention times seen on these chromatograms.

Ratios were calculated for the amounts of Chl a : Chl c, ß-carotene, and fucoxanthin as well as for fucoxanthin and the other xanthophylls. Comparisons were also made between the pooled xanthophylls (fucoxanthin, diatoxanthin, diadinoxanthin) and chlorophyll a. Ratios for all samples analysed are given in Appendix 6.

Chlorophyll a was always present in the most abundant amounts in all samples while chlorophyll c was often present in such small quantities as to be barely detectable. The retention times and the spectra did not vary significantly for each of these pigments but results from calculations for this ratio were very variable. The chl a : c ratio was generally lower in the *A. formosa* samples than in the *A. ralfsii* var. *americana* samples, with *A. ralfsii* var. *americana* showing, on average, thirty times more chlorophyll a than chlorophyll c.

Fucoxanthin was also a very abundant pigment, with an average of 0.89 chl a : 1 fucoxanthin in the *A. formosa* clones. Generally there was 2 - 4 times the amount of chlorophyll a relative to fucoxanthin in the *A. ralfsii* var. *americana* clones (average: 3.03 : 1, chlorophyll a : fucoxanthin).

The chlorophyll a : ß-carotene ratio showed a lot of fluctuation among the samples. Retention time was again relatively consistent, but in some cases ß-carotene was not eluted. Average

values for this ratio were lower in the *A. formosa* samples than in the *A. ralfsii* var. *americana* samples, but again variablilty was so great as to place these values in question.

When all the xanthophylls were combined and then compared with the chlorophyll a portion, the *A. formosa* samples showed twice the amount of xanthophylls to chlorophyll a than did the *A. ralfsii* var. *americana* samples. This seemed to be a better measure of pigment composition than using either the chl a : c or chl a : ß-carotene ratios.

The ratios of fucoxanthin to the other combined xanthophylls was also a relatively consistent measurement. In the *A. formosa* samples this ratio was always greater than 1:1 and averaged 1.31 fucoxanthin : 1 other combined xanthophylls. The *A. ralfsii* var. *americana* samples showed an opposite trend with all values less than 1:1 and averaging 0.68:1 fucoxanthin to other xanthophylls.

Table 20. Pigment identified and corresponding peak number and retention time seen in Figure 21. (BW12-14 *A. ralfsii* var. americana ; T-8-10 - *A. formosa*)

Pigment	BW12-14		T-8-10	
	RT	#	RT	#
Chl a	19.63	21	19.65	20
Chl c	5.87	3	5.89	3
ß-carotene	23.19	26	23.21	28
fucoxanthin	9.11	6	9.11	4
diatoxanthin	11.03	11	10.41	7
diadinoxanthin	11.39	12	11.03	8
Chl degradation	21.61	25	21.65	25

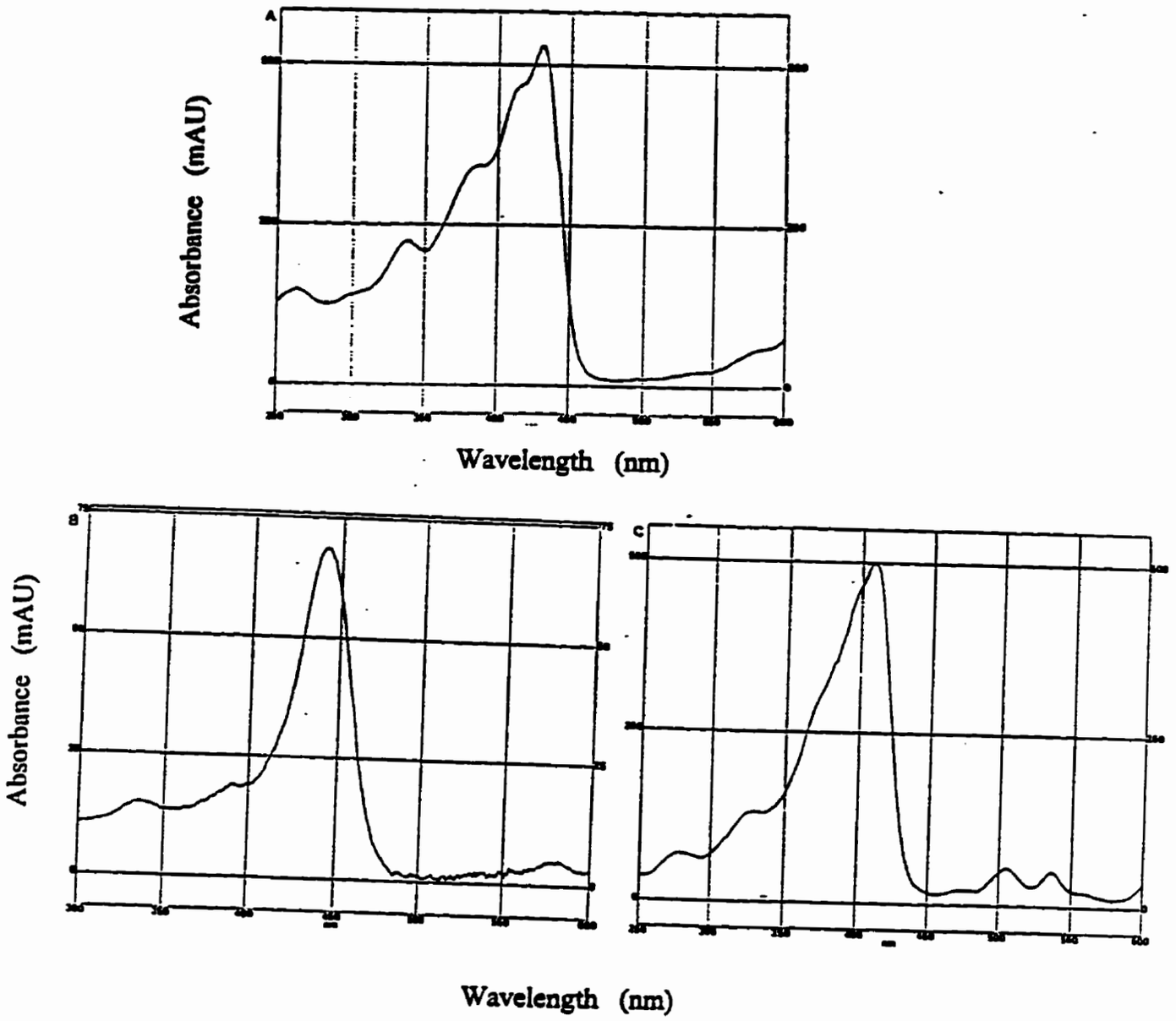

Figure 19. Absorption spectra for (A) chlorophyll a (RT = 19.58, N = 26), (B) chlorophyll c (RT = 5.69, N = 19), and (C) a chlorophyll degredation product (RT = 21.50, N = 23).

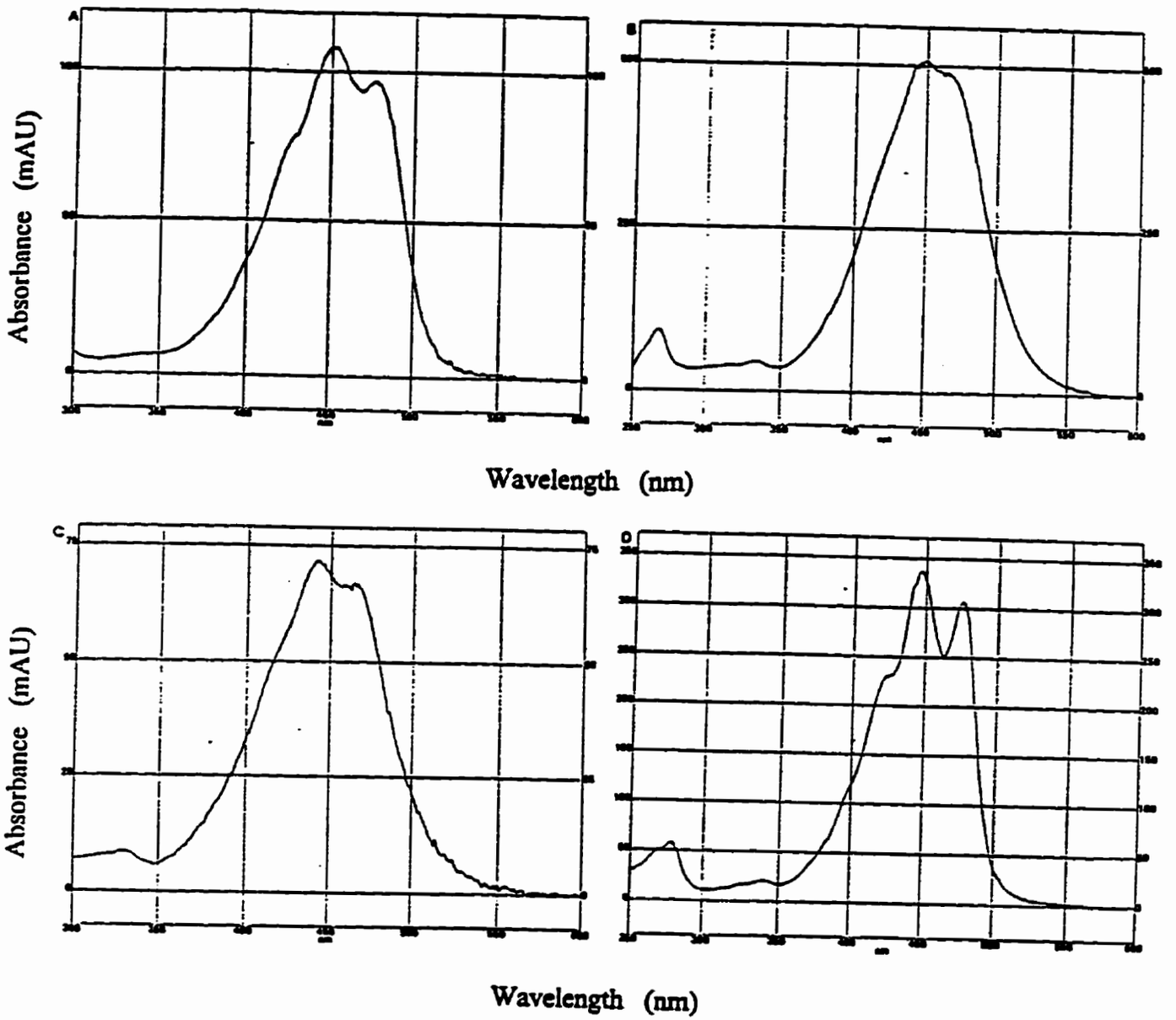

Figure 20. Absorption spectra for (A) ß-carotene (RT = 23.16, N = 21), (B) fucoxanthin (RT = 9.14, N = 26), (C) diatoxanthin (RT = 10.11 lower limit, N = 26), (D) diadinoxanthin (RT = 12.57 upper limit, N = 26)

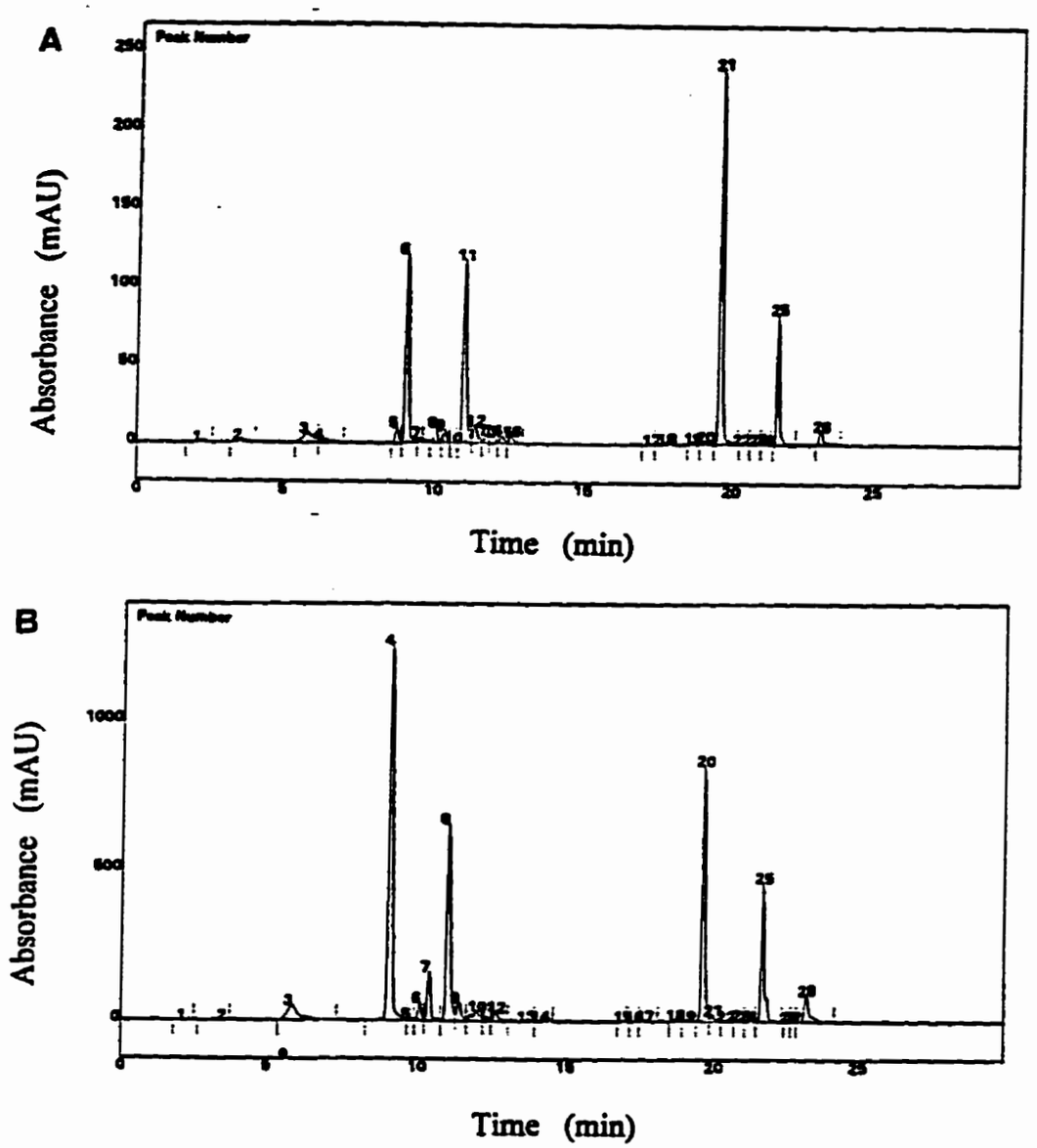

Figure 21. HPLC chromatograms of (A) *A. ralfsii* var *americana* and (B) *A. formosa* pigments. Refer to Table 20 for peak identification

IV.3 Discussion of results for culture conditions and pigment determination

The effects of Si and P on the growth of *Asterionella* have been studied extensively both in culture and in natural conditions (Lund 1950; Lund *et al.* 1963; Kilham 1971; Kilham 1975; Bailey-Watts 1976; Tilman & Kilham 1976; Tilman *et al.* 1976; Gensemer 1990, 1991a, 1991b; Gensemer *et al.* 1993; Hughes & Lund 1962). Limitation of either one of these nutrients may change the colony or cell morphology and consequently photosynthetic capabilities. In natural conditions, phosphorus may range from 0.0 to 20.0 µg P/L and occasionally slightly higher, while silicate ranges from 0.0 to 30 µg/L (Werner 1977; Mackereth 1953). *Asterionella* has been shown to take up and store phosphorus from very low concentrations and then use these reserves when phosphorus becomes limiting. Colony size is influenced by limitation of both phosphorus and silicate. In the former case, colony size decreases while in the latter it increases (Lund *et al.* 1963; Tilman *et al.* 1976). Limitation of silicate also affects frustule morphology and metabolic processes. Decreases in cell length occur, protein, DNA, chlorophyll and carotenoid synthesis are inhibited and photosynthesis is reduced (Darley & Volcani 1969; Werner 1978). It is for these reasons that these two nutrients were monitored in the cultures used for pigment determination in this study. The silicate and phosphorus remaining in the culture medium was always in adequate supply, although occasionally the silicate was near the lower limit (Table 18).

Cells were examined from the cultures to ensure that healthy populations were used for pigment extraction. Six to twelve replicates were combined to obtain sufficient pigment extract and it was essential that each replicate be in the exponential stage of growth. The number of cells/mL harvested from the cultures could be considered as healthy, growing populations although not heavy cultures (Eskins *et al.* 1977). The morphology of individual cells was also checked at this time. Cells were seen to vary in length in some populations but there was no evidence of "Janus" cells. However, when examining old cultures which had not been supplied with new nutrients, definite morphological changes were seen. Initially, a reduction in length occurred when new cell walls were laid down at approximately 2/3 of the initial valve length. The head pole disappeared and that end of the cell took on linear proportions that are typically seen in the body of the valve. The foot pole gradually became asymmetric and curved to one side. Cells continued to decrease in length and eventually took on a reniform shape. Reduction in length continued until the cells were only twice as long as wide. All reduction appeared to occur along the length of the valve with very little occurring across the breadth of the cell. The valves eventually assumed an oval shape and had no resemblance to the initial "*Asterionella*" shape. These oval cells persisted for a few weeks but eventually died

out. Progression through these stages is shown in Plates VIII and IX.

Morphological differences in *Asterionella* populations have been seen in natural populations and in culture by other investigators (Körner 1970; Rawlence 1986; Gensemer 1990; Kling 1993). Usually a decrease was seen in the cell length, but Körner (1970) and Rawlence (1986) both observed oval or irregularly shaped cells. Rawlence speculated that they might be auxospores about to undergo gametogenesis. Kling (1993) observed colonies containing cells of different lengths as did Gensemer (1990), but both attributed these observations to nutrient limitation or stress. The clonal populations in this study when the Janus cells were seen, were very likely under nutrient stress (the growth medium was not assayed), and as senescence and death occurred shortly after the cells assumed an oval shape, it is not very likely that these were auxospores.

The purpose of thin layer chromatography (TLC) is to characterize and identify unknown compounds and TLC techniques for the determination of pigments have altered greatly in the past few decades. Much of this is due to the persistence of Jeffery and co-workers in developing better solvent systems and adsorbents for phytopigments (Jeffrey 1968, 1972, 1974, 1976, 1981; Vesk & Jeffrey 1987; Stauber & Jeffrey 1988). An attempt was made in this study to follow the methodology of these former studies to try and determine the pigment composition of two taxa of *Asterionella*. Extraction was carried out using 100% acetone rather than 90% acetone normally recommended, in order to inactivate any chlorophyllase which may have been present (Jeffrey 1974; Wright & Shearer 1984). Cellulose plates were used because they are considered the most efficient at separating the carotenoids. The initial solvent systems used followed Stauber and Jeffrey (1988) because they found that it separated all the major diatom pigments.

The TLC results from this study were very poor. Although some separation did occur in the more polar pigments, chlorophyll a, ß-crotene, and diatoxanthin all ran together to the solvent front. Using the second solvent system and running the plate in the second dimension did not separate these pigments or clarify their identity. There are a number of possible explanations for the lack of separation. The role that temperature and humidity play in TLC determinations is, as yet, poorly understood. The ability of the adsorbent to uptake water vapour and the ambient air temperature have been seen to cause fluxuations in Rf values and changes in spot compactness (de Zeeuw 1972). Unfortunately temperature and humidity are not possible to control in an open laboratory situation. Solvent demixing can also be a problem when a multicomponent solvent is used. This is due to the different affinities of the components to the adsorbent and will cause changes in running times and Rf values of the different compounds being separated. The role of the solvent vapour in the TLC

chamber and the question of saturated vs. unsaturated atmospheres is still unresolved. The solvent front appeared to be at the top of the filter paper used to equlibrate the chamber but if demixing occurred, different components might have been in different areas of the tank. This would cause streaking of the compounds and incomplete separation. Differences in adsorbent properties such as pore size and volume, particle size and surface area, thickness of the adsorbent on the plate, and the number of active sites are closely related to adsorption capacity, vapour adsorption rate, solvent flow rate and position of the demixing fronts. In this study cellulose plates were used, and although each manufacturer attempts to maintain standards, it is difficult to compare results between different studies even when the TLC plates are acquired from the same manufacturer (de Zeeuw 1972). The pigment load is also important. Efforts were made to apply the same amount of pigment in each determination but it was impossible to quantify this, as dilution of the pigments changed between samples. The size of the chamber, atmospheric conditions, partitioning of the solvent systems and particularly, the nonuniformity of different techniques among users will all influence TLC results in different ways.

The separation of chlorophyll c, diadinoxanthin and fucoxanthin was very clear. Rf values, colour of the spots and absorbance maxima were in close enough agreement to those in the literature that a positive identification was made. Quantitative recovery of pigments is apparently possible (Jeffrey 1968,1981) but was not attempted in this study as there was no way to measure the degradation, loss or potential loss of pigments through the different stages of preparation. When the pigment extract was examined using the spectrophotometer the expected absorbance maxima for each pigment were seen although peak absorbance was often overlapping.

Results for high performance liquid chromatography (HPLC) were generally much better than for the TLC. This method is highly sensitive and reproducible for the separation, quantitative and qualitative determination of photosynthetic pigments. Because photosynthetic pigments are readily oxidizable and are photolabile, the identification of pigments spectrophotometrically during analysis has the advantage of reducing the amount of pigment degradation which might occur using other methods. A step-wise gradient elution was used in this study rather than isocratic elution as it is preferable when the separation of compounds involves a range of polarities, as in pigment analysis (Roy 1987). Absorption spectra, a function of the structure of the compound, are quite characteristic for each compound and were seen to be consistent over all of the samples examined in this study. Retention times may differ but the spectral quality of pigments remains quite consistent (Mantoura & Llewellyn 1983).

Separation of all pigment components was good, but quantitative problems arose with the

chlorophylls and ß-carotene. Only very minute amounts of chlorophyll c and ß-carotene were found in the culture extracts and the ratios of these to chlorophyll a were inconsistent throughout the determinations. This could have been a result of light conditions in the culture chambers or a function of the solvent system used to elute the pigments.

Variation in light intensity and periodicity have been shown to change the quantity of pigments by influencing the number and size of chloroplasts, the number of thylakoids in each chloroplast and the amount of photosynthetically active enzymes (Jorgensen 1977). The light-dark cycle used in this study is considered optimal for growth but the effects on the photosynthetic apparatus have not been studied. It is well known however that a lower light intensity or a shorter period of light inversely affects the rate of photosynthesis (Talling 1957; Jorgensen 1964,1969; Paasche 1968).

Many different solvent systems were considered for the elution of the algal pigments (Eskins *et al.* 1977; Abayachi & Riley 1979; Braumann & Grimme 1979; Wright & Shearer 1984; Sartory 1985; Gieskes & Kraay 1986; Roy 1987; Wright *et al.* 1991). Resolution of pigments, especially chlorophyll c, the time required to run a single sample, the use of more than one solvent system and the overestimation of some pigments were potential problems with these systems.

The principle pigments in diatoms are considered to be chlorophylls a and c, and fucoxanthin with the latter two considered accessory pigments (Jorgensen 1977). Chlorophyll a is generally the major photosynthetic pigment and is known to occur in at least four different forms. Chlorophyll c is found in at least two forms (Jeffrey 1969,1972,1976) and functions by transferring absorbed energy to chlorophyll a. Fucoxanthin absorbs light at longer wavelengths than most other carotenoids and has been shown to be as effective in this capacity as chlorophyll a (Dutton & Manning 1941). In quantitative terms, ß-carotene does not seem to be as important as the xanthophylls and did not occur in any great quantity in this study. The other two xanthophylls, diatoxanthin and diadinoxanthin have been shown to be interconvertible (Stransky & Hager 1970) and are generally believed to work in a protective role when light intensities threaten the photosystems.

When grown in culture the *A. ralfsii* var. *americana* cells appeared "greener" than the "brownish" *A. formosa* cells. Differences in the ratios of chlorophylls and carotenoids were suspected. HPLC determinations showed that with regard to fucoxanthin, diadinoxanthin, diatoxanthin and chlorophyll a, these differences were definable. Comparison of the chlorophyll a : fucoxanthin ratios showed that the quantity of chlorophyll a was three times greater in the *A. ralfsii* var. *americana* clones compared with the *A. formosa* clones. Chlorophyll a dominated the pigment

composition in the *A. ralfsii* var. *americana* clones when compared with fucoxanthin by itself or when fucoxanthin was grouped with diatoxanthin and diadinoxanthinin. In the *A. formosa* clones, fucoxanthin predominated and the combined xanthophyll component was almost twice that of what was seen for chlorophyll a.

Comparisons of fucoxanthin to the other combined xanthophylls showed that the *A. formosa* clones contained twice as much fucoxanthin as the *A. ralfsii* var. *americana* clones. Chlorophyll a to fucoxanthin ratio was three times greater in the *A. ralfsii* var. *americana* clones than that which was seen in the *A. formosa* clones. The *A. formosa* clones had more than twice the combined xanthophyll to chlorophyll a content than was seen in the *A. ralfsii* var. *americana* clones. Previous work has shown that the chlorophyll c content should account for between 11 - 37% of the total chlorophyll in a diatom (Jeffrey 1972; Bunt 1968). The estimates of chlorophyll c in this study were very unreliable and varied so much that they could not be used with any confidence. Only trace quantities were seen on the TLC's, so it can be assumed that the chlorophyll c in these clones was at such low levels as to be unimportant in these growth conditions. When clonal type populations of *A. formosa* and *A. ralfsii* var. *americana* are grown in culture under the same conditions, they exhibit a different complememt of pigments. In *A. formosa*, fucoxanthin predominates, giving the populations a brownish colour. In *A. ralfsii* var. *americana*, the relative proportion of chl a is higher, giving the populations a greenish colour. Because environmental conditions and the stage of development was similar, the change in pigment complement is probably genetic in origin and may be a valuable characteristic to use in distinguishing *A. formosa* and *A. ralfsii* var *americana* populations.

V. Synopsis and Conclusions

The primary focus of this study was morphological variability in natural populations of the diatom *Asterionella*. Clear and coloured lakes in Kejimkujik National Park, Nova Scotia were sampled seasonally over a four year period in an attempt to ascertain which freshwater taxon or taxa of this diatom genus occurred in these systems. A secondary study on the pigment ratios was undertaken after noticing visual colour differences in clonal populations from different geographical locations.

Three hypotheses were addressed in this study. The first questioned the taxonomic relationship between *A. formosa* and *A. ralfsii* var. *americana*. The second hypothesis investigated the morphological variability in populations of *Asterionella* from Kejimkujik National Park lakes. The third hypothesis considered the possibility of using pigments as biochemical markers to aid in taxonomic identification. To clarify any relationship between *A. formosa* and *A. ralfsii* var. *americana*, morphology and pigmentation were examined first to ascertain the variability, then to determine if environmental factors may be a contributing agent.

Morphological variation in the siliceous frustules of *Asterionella* from natural populations was examined. The initial step was to measure the length of the valves from populations found in a wide range of lakes, both clear and coloured, and encompassing a range of pH values. These were compared to the values reported by other investigators, because valve length has traditionally been one of the main criteria for separation at the specific level. The number of valves per colony was also counted to determine whether this differed between clear and coloured lakes.

In the past, diatom taxa have been erected on the basis of morphological characters observed by light microscopy and the overall aspect of the valve as perceived by the investigator. The generic description uses pooled information derived from common basic features, that is, shared derived traits, seen in all the species included in the genus. This can present difficulties when the frustule has very few distinctive components. Like most other araphid diatoms, the valve wall of *Asterionella* is very simple. Thus, separation at the specific level based on phenetic differences is reduced to a few quantitative measurements and subjective observations. Measurements of characters as reported in the literature for *Asterionella*, with the exception of the work done by Körner (1970), were all obtained by light microscopy. This has severely limited the circumscription of this genus and has emphasized subjective observations to a greater degree than quantitative measures of the few characters available. The determination of morphologically conservative and stable character states was a primary concern. Then, the question of whether phenetic criteria are enough to delimit species

needed to be addressed.

Scanning electron microscopy was used to see the finer details of the frustules and to measure characters which could be used for identification and delimitation of possibly different taxa. Six character states were initially measured, five of which were found to be independent of each other. These five character states were considered to be independent, stable and conservative and could be used to express possible differences between taxa.

Diatom taxonomy has recently undergone a great deal of revision, in part, because of the technological advances in microscopy. As a consequence of this, type material has been reexamined, the taxonomic position of many taxa has changed, and new genera and species have been erected (Round *et al.* 1990). In some cases, overlap of morphological characters has shown more similarities than differences in some species, leading to the combining of several taxa together. With the more frequent use of electron microscopy in diatom studies, the terminology for describing different structures on the diatom valve has increased to the point where there is the risk of confusing or misusing these new terms. Attempts have been made to standardize the terminology, (Anonymous 1975; Ross *et al.* 1979), but continued investigations inevitably produce new structures which require new names.

The progressive size reduction of the diatom valve and the restitution of cell size have been subjects of interest to diatomists since the mid nineteenth century (Wallich 1860; Macdonald 1869; Pfitzer 1869,1871; Tomaschek 1873). It was suggested that reduction in valve dimensions follows a binomial progression and is predictable in terms of the girdle band thickness. Since this idea was first proposed many exceptions have been noted, especially in the pennate species. Size reduction, if it occurs at all, is often confined to the apical axis and is not accompanied to the same extent in the transapical axis. In some cases the smaller of the two daughter cells resulting from a division shows a lag and divides a generation later than its larger sister cell. It is now generally believed that not only the thickness of the girdle bands but also the relationship between the epivalve and hypovalve exerts control over cell size reduction. In many pennate species, thickenings occur at the apices of cells, resulting in a lack of flexibility and restricting the membrane limited vesicles within which the new valves form (Round 1972; Crawford 1981).

Longer cells are thought to be "new" cells, that is, cells formed after sexual reproduction. They undergo repeated asexual divisions with each generation of cells becoming progressively smaller. When a certain size is reached, the smaller cells either continue to decrease in size until they are no longer viable, or are triggered by various environmental and physiological factors to

undergo sexual reproduction. Because of the restrictions placed on the cell by the siliceous cell wall, sexual reproduction in most species is believed to be the primary mode of restitution of cell size. The size of the initial cell is dependent on the size of the auxospore, which is dependent on the environmental conditions present at the time of auxospore formation (Jewson 1992).

Like many one-celled eukaryotes, increases in population size in diatoms are generally related to asexual reproduction and a rapid generation time. Unlike many protistans, the genetic material of diatoms is gathered into discrete chromosomes which segregate in a highly regular manner during cell division. The only haploid cells in the diatom life cycle are the gametes, which must fuse before cell division. There is no distinction between somatic cells and reproductive generative cells as there is in multicellular organisms (with the exception of oogamous sex in centric diatoms). Instead, whether mitotically or meiotically dividing, a new individual is produced. Consequently, populations are generally clonal lines which may remain reproductively isolated from each other for several generations (Richardson 1994).

Asterionella is a planktonic diatom which may develop bloom conditions in the spring and/or fall. This is usually the result of rapid asexual reproduction, the outcome of which is large clonal populations best suited to survive in the environmental conditions present at that time. When conditions become limiting for *Asterionella* and they cease to be abundant in the water column, then the colonies fall to the sediment surface. Factors which are known to induce sexual reproduction in diatoms would be present: internal factors in cells of a smaller size and external environmental factors (Mann 1988; Jewson 1992). However, if all cells in a colony undergo size reduction at the same rate then the most likely pairing would occur between cells from the same clonal population. Continuous directional selection in predominantly asexually reproducing populations could increase hidden genetic variability in an initially rare genotype, making it possible to become the dominant form (Wood & Leatham 1992). Occasional recombination would reestablish the greatest range of heritable phenotypic variability most suited for the environmental conditions present at that time. Mutations, if non-detrimental, would persist throughout the asexual phase of the life cycle and be transmitted into what might often be millions of clonal descendants. If recessive, the mutation would not be expressed given the diploid nature of diatoms, but if dominant, the new mutated line may be morphologically or physiologically different from other clonal strains (Richardson 1994). The resulting diatom population would be a combination of clones generated both asexually and sexually, with considerable adaptive capabilities.

Although sexual conjugation was not seen in any *Asterionella* in this study, cell length data

suggest that different portions of the populations undergo sex on a rotational basis when cell size and environmental conditions are favourable. Mann (1988) thought that the length classes represented annual periods of auxosporulation and that these year classes were affected differentially by selection. He found that in some *Asterionella* populations, a constant size seemed to be maintained. In others, he saw what appeared to be size reduction cycles with the rate of decline from 2.0 μm to 2.5 μm per year in cells measuring 100 μm or more. Mann suggested that this diatom may have a life cycle as long as forty years but Jewson (1992) did not feel that there was sufficient evidence for life cycles longer than eight years. Regardless, the natural diatom populations would be predominantly clonal, with very few individuals in the population the direct offspring of a mating event.

Cell size itself can be altered by changes in environmental conditions which may alter cell metabolism and hence valve structure (Schmid 1979). Changing environmental conditions may select for a particular phenotype, one which is either larger or smaller than the majority of the population, but also one which is capable of utilizing the present environmental, chemical and biological conditions most efficiently. Smaller cells have a greater surface area to volume ratio than do larger cells, increasing the ability to absorb nutrients, often when competition for nutrients is the greatest (Bellinger 1977). Increases in water temperature, which generally occur in the spring, raise the general metabolic rate and increase colony size (Hayakawa *et al.* 1994). The sinking rate of cells, the physiology and nutrient absorption are all influenced by changing environmental conditions acting on the phytoplankton populations.

The length of the *Asterionella* valves was measured from populations in the lakes from Kejimkujik National Park, Nova Scotia, and from other sites in Ontario and the United States. Herbaria slides identified as containing the different species and varieties of *Asterionella* were measured as well. The type slide of *A. ralfsii* var. *americana* and the sample population obtained for this study from the type location were in very close agreement with respect to valve length and other morphological measurements. Morphological similarities were also seen in the *A. formosa* populations acquired in Georgian Bay, Ontario and those measured from Lake Michigan by Körner in 1970. This would suggest that some populations of *Asterionella* are quite stable morphologically, at least for the twenty year period seen between these two studies.

A range of valve lengths and shapes were seen in the Kejimkujik lakes, encompassing those recorded for all of the fresh water *Asterionella* taxa. The descriptions given in the literature for the overall form of the valve are very ambiguous and subjective. It is unclear what different investigators are seeing when modifiers such as "slightly" or "strongly" or "highly" are used to

describe the aspect of the valve (i.e. slightly capitate, strongly widened valve ends). Assuming that a range of ecotypical strains were seen in the natural populations in this study, such descriptive terms could be applied to some valves in every system. This does not define differences, rather it promotes the inclusion of these different forms into a large overlapping group, making it impossible to delimit taxa on a phenetic basis. A great deal of descriptive licence has been used in the past which has added to the confusion in demarking diatom species. Without material from the Kejimjujik lakes obtained in the past or future, it is difficult to say how the morphology of these populations may or may not change over time. It is probable that the variability seen is typical for these lakes, and that these populations are clonal ecotypes intermediate in form and physiology to *A. formosa* and *A. ralfsii* var. *americana*.

The morphological characters measured in the *Asterionella* from the study lakes did not show any correlation to pH, lake colour, conductivity or temperature. Regardless of the statistical analyses, the morphological character states were independent of these factors in this study. Other investigators have done more rigorous testing of lake water chemistry and environmental variables on lakes from the north eastern United States (Charles *et al.* 1990; Anderson *et al.* 1993; Dixit *et al.* 1993). The results of these studies have not been consistent, leading one to the conclusion that no single factor, or combination of factors found so far, is responsible for the variability seen in this genus. It is more likely that ecotypical strains have been selected by the conditions present in particular geographical areas and arose by means of mutations or genetic recombination.

The six to eight-celled stellate colony formation is considered a defining feature of the genus *Asterionella*. I hypothesized that colony size was related to the humic content of the lake; that is, could I tell whether an *Asterionella* colony came from a clear or a coloured lake by the number of individuals in a colony. Physiological studies have linked both colony size and valve length to the availability of silica, phosphorus or both (Lund 1963; Tilman *et al.* 1976; Gensemer 1990). Under Si limitation, the number of cells per colony increases, but when P is limiting, a decrease in cells per colony occurs. The availability of nutrients has been linked directly to chemical interactions with dissolved organic carbon (DOC) (Francko 1986; Jones 1990) and indirectly by complexing with inorganic Al, affecting P bioavailability (Nalewajko & Paul 1985; Jansson *et al.* 1986). If DOC were in some way affecting the nutrient availability in the lakes in KNP, fewer valves per colony should have been evident in the coloured lakes. If silica were a limiting factor, then colony size should be greater than expected and cell length should be in the lower class ranges.

Colony size could not be related to humic content and, overall, more clear lakes showed

smaller colony size than coloured lakes. This may simply be a function of the ability *Asterionella* has shown to utilize phosphorus at very low levels or may indicate that P was never limiting in these systems. Small cell size was not related to larger colony size but rather was more a function of the cyclical nature of the individual lake population. This would indicate that Si was not an important influence or that the smaller valve size seen in some of the lakes was possibly a function of genotypic variability in these populations.

If the delimitation of a diatom species is not strictly phenetic, then other possible factors should be considered. Studies in the past have examined the biochemistry, genetics and physiology of diatoms considered to be the same species (Gallagher 1980; Brand *et al.* 1981; Gallagher *et al.* 1984; Theriot 1987). Often microalgal species were characterized by a single clonal isolate with little knowledge of the within species variability (Wood and Leatham 1992). Actual interclonal variation is for the greater part unknown, but studies on two or three clonal isolates of the same species have shown enormous diversity within natural populations (Hulburt & Guillard 1968; Hargraves and Guillard 1974; Murphy & Belastock 1980). It is not known if interclonal variation is being truly represented or simply that the clones used in these studies were those that could be maintained in culture.

Asterionella has been used in many physiological studies on pH, nutrients, and metals (Lund *et al.* 1963; Kilham 1971, 1975; Gensemer 1990, 1991a, 1991b). It is commonly found in temperate regions and is often the major component of the phytoplankton in the spring and fall. Colonies are relatively large, making them easy to isolate for culture experiments. A great deal of work has been done, beginning with Lund in the 1950's, on the biology of this organism. It is therefore an attractive diatom to use in this type of work.

Previous studies have shown that physiologically, what have been described as *A. formosa* and *A. ralfsii* var. *americana* are very different. For example, pH, Al, and EDTA have been shown to affect growth rates in clones of *A. formosa* and *A. ralfsii* var. *americana* in a species-specific manner (Riseng *et al.* 1991). Within *A. formosa*, it has been shown that the silicon uptake and growth rates of two strains were different (Kilham 1975). Electrophoretic analysis of populations of *A. formosa* has also been shown to have different banding patterns for proteins and some enzymes (Soudek & Robinson 1983). Morphologically similar strains of *A. formosa* isolated on different occasions showed contrasting physiological behaviour and two morphologically distinct clonal types from the same original isolate differed physiologically (Happey-Wood & Hughes 1980). While growing *Asterionella* isolates in culture for this study, it was observed that the cultures designated as

A. formosa were a different colour from those which were isolated from Kejikujik National Park. Due to contamination of the clones from KNP, colonies were isolated from the type location of *A. ralfsii* var. *americana* and were similar in colour to those isolated from KNP. These, along with the *A. formosa* clones from Georgian Bay, Ontario, were used for pigment analysis.

The clones of both *A. formosa* and *A. ralfsii* var. *americana* used for pigment analysis were monitored closely for available P and Si and checked for changes in morphology before harvest. Initially, thin layer chromatography was used to separate the pigments. The results were unsatisfactory and were impossible to quantify, so high performance liquid chromatography was then used. Resolution of the different compounds was much better and, although quantitative estimates were not undertaken, comparative ratios did show substantial differences in the pigments between these two freshwater *Asterionella*, especially for the xanthophyll complement. A number of clones were used to represent the two populations, and within those populations findings were consistent. Pigment ratios may prove to be good biochemical markers for separating these two taxa of *Asterionella*, but a larger sample size is necessary to draw a definite conclusion.

In this study several problems were encountered which indicate that *Asterionella* is not a suitable organism for experimental studies. Only fully intact, healthy colonies were isolated for use in the pigment analysis and although conditions were the same, some grew and some died. This suggests that there was an initial tolerance difference between these isolates with respect to the adaptability to culture conditions. For any given successfully growing clone, twelve culture tubes containing the same growth medium were inoculated with an aliquot of the parent material. These were placed randomly in a growth chamber under an identical temperature and light regime. It was very seldom that all twelve tubes survived and often, only one half of the replicates grew. As all conditions were identical, it is not clear why some died and some grew, but a viral infection is one possibility (R. Smith pers. comm.). The study of algal viruses is in its infancy, but may provide the answers to many problems inherent in the successful growth and maintenance of algae in culture.

If the clones were subjected to any kind of stress at all or had been in culture for any amount of time, "Janus" cells would begin to appear and after a few weeks the entire culture would consist of these abnormal cells. Transfer to fresh medium did not alter this. Clones sent to culture collections did the same thing, making it impossible to do follow-up work on the same clones (R. Gensemer pers. comm.). For these reasons, it becomes very important to use fresh isolates of *Asterionella* for any type of physiological or biochemical study and to ensure, before the beginning of the experiment, that the colony formation and valve integrity are intact. Care should also be

exercised in extrapolating data obtained under culture conditions to natural populations when intrapopulation and interpopulation variability are poorly understood.

This study has shown that the morphology of populations of *Asterionella* in eastern Canada does not conform to the descriptions or parameters reported for *A. formosa* or *A. ralfsii* var. *americana*. A variety of morphotypes exist and a range of measurements and descriptions for each character could be found in populations from all the sites in Kejimkujik National Park at one time or another. Whether the variability is genetic or in response to environmental conditions is as yet, unknown. Some populations of this genus have shown similar morphological plasticity in eastern North America (D. Charles pers. comm) while others seem to maintain morphological integrity (populations from the type location of *A. ralfsii* var. *americana* and Georgian Bay, Ontario). It is possible that these eastern populations have adapted to changing environmental conditions due to anthropogenic input which has impacted this area of North America quite intensely. The perceived similarities in the pigments from the type location of *A. ralfsii* var. *americana* and the Kejimkujik lakes would suggest that the parental populations originated or developed in the eastern part of North America. The successful growth of some clones from KNP at lower pH levels than were used for the Georgian Bay *Asterionella* would also suggest an inherent physiological and biochemical difference.

The genus *Asterionella* was defined more than a century ago based on morphological characteristics as observed by light microscopy. Since then, there have been numerous problems in this genus at the specific and varietal levels. A major challenge of this work was to decide what species or variety of *Asterionella* occurred in lakes in eastern Canada and what the relationship was to the accepted classification of this genus.

Classification of biological organisms assumes a relatedness of every entity in that group. This is generally a simple matter at the generic level where homologous traits indicate shared ancestral lines. Classification at the specific level or lower has been a controversial issue since the time of Darwin. The question of whether species are real units in nature or simply man-made arbitrary units erected for the convenience of interpreting variability, has been an ongoing argument in evolutionary study and systematics. Various concepts have been proposed in attempts to define and delimit what a species is. The biological species concept (BSC) is perhaps the most widely known and recognized attempt to accommodate these ideas.

The biological species concept is based on the recognition of groups of morphologically

similar individuals that differ from other such groups. It is largely a phenetic concept which deals with populations that are distributed spatially and temporally, are interrelated through mutual interbreeding and are separated from other groups by reproductive barriers. Three basic aspects went into the development of this idea: 1.) variation of characteristics over large geographical areas 2.) changes in these characteristics as populations adapt to environmental challenges or interact with other populations, and 3.) integration of individuals into populations to form common genetic pools through direct processes as well as indirectly through ecological interactions (Sokal & Crovello 1970). This is perhaps not such a bad definition for organisms that reproduce sexually, but does not consider clonal populations reproducing asexually or predominantly parthenogenic populations where genetic exchange does not occur or occurs infrequently.

Sexual reproduction is known in a few diatoms but is implied in many by restitution of cell size. Like most pennate diatoms, *Asterionella* has never been shown to undergo sexual reproduction, yet restitution of cell size does occur. This would suggest that although primarily asexual, genetic exchange and recombination does occur at some time in the life history. If *Asterionella formosa* and *A. ralfsii* are to be considered "true" species, that is, if they do undergo genetic recombination and fit the other criteria suggested in the biological species concept, then what is the status of the *Asterionella* populations seen in eastern Canada? Are the two varieties of *A. ralfsii* suggested by Körner different enough from the nominate variety to justify separation or is the biological species concept unsuited for predominantly asexual populations where sexual reproduction is implied but not proven to occur?

Asexual reproduction has been considered an evolutionary dead end (Muller 1964; Mayr 1970; White 1973; Maynard Smith 1978). In the absence of genetic recombination, a population is considered to have lost the only means of ridding itself of deleterious mutations and to have restricted the potential for phenotypic evolution in response to variable environmental conditions. This assumes that obligate asexual or parthenogenic organisms have not evolved a higher level of sensitivity to environmental effects. There is no direct evidence to date that the gradual accumulation of deleterious mutations leads to the extinction of diploid unisexuals or that the fitness is reduced (Lynch & Gabriel 1983). The costs of sex are quite high for any biological organism, making asexual reproduction with occasional sexual recombination a very attractive strategy from an evolutionary point of view (Lynch & Gabriel 1983; Lynch 1984; Wood & Leatham 1992; Richardson 1994).

It cannot be disputed that diatoms are very successful organisms. They are a dominant

component of the earth's present biota and contribute up to 25% of the total global photosynthetic production (Werner 1977). Rapid reproductive rates under favourable conditions, either meiotically or mitotically, have ensured successful colonization and maintenance of populations in most water bodies as well as in the soil. Although sexuality has been observed in very few diatoms, the possibility of potentially several thousand genetically distinct clonal lines would suggest selective differences that would ensure success under most conditions. If sexual reproduction is as infrequent as most diatomists suspect, and physiological, biochemical and morphological differences are inherent in this group of organisms, then the standard biological species concept (BSC) may be inappropriate for diatoms. One of the central tenants of the BSC is phenetic similarity, but this study has shown that the genus *Asterionella* in eastern Canada is a combination of morphological intermediates. Cell length data from this study suggests sexual reproduction occurred but, as yet, this is unproven. The lack of successful growth of many of the colonies isolated for pigment analysis suggests that the clonal lines have different tolerances. Without knowledge of a common gene pool in this organism, another basic aspect of the BSC is not fulfilled. Geographically, what have been described as variable "forms" of *A. ralfsii* var. *americana* occur in the eastern United States (Charles *et al.* 1990), and in this study, in eastern Canada. Körner's type location for the variety he described as *americana* is the eastern seaboard of the U.S., and is therefore not discretely isolated from these other intermediate forms, the third aspect of the BSC.

If the biological species concept is not a workable idea for predominantly asexually reproducing or clonal populations what should it be replaced with? In this study we have what appear to be morphologically dissimilar populations in a series of lakes in close proximity to each other. None of these populations could be separated as being distinct, or as possessing all necessary characters to include them into a known classification. Grouping taxa into complexes may facilitate recognition, but could also lead to the loss of vital ecological information. It would perhaps be more useful to separate "ecotypes" or "strains" based on morphology, physiology, biochemistry, DNA analysis, ecological preferences, and any other known trait. Unless there are very evident visual differences, the species, varietal and form levels of nomenclature in diatoms are confusing and seldom sufficient to describe the multitudinal differences in predominantly clonal populations.

The use of *Asterionella* as an indicator genus is in need of re-evaluation. This diatom has been widely used to infer pH from sediment cores and from cleaned phytoplankton samples. If *A. formosa* and *A. ralfsii* are to be retained as separate species then the ecological conditions and the floristic composition must be known in order to assign a specific epithet. This genus has shown

integration in morphology so that differentiating cleaned material is difficult if not impossible at the best, as evidenced by Delorme *et al.* (1984). Both *Asterionella* species appear to have pH preferences (*A. formosa* optimum pH 7; *A. ralfsii* pH 6) but both have been shown to grow under similar pH levels (Riseng *et al.* 1991; Gensemer *et al.* 1993). Taxa with wide ecological amplitudes are of little use as indicators, and this makes it necessary to know the conditions under which growth occurred and what valid diatom indicators were present.

This study has shown that morphologically, the *Asterionella* in Kejimkujik National Park fit neither the description for *A. formosa* nor for *A. ralfsii* var. *americana*. Physiologically, growth of the KNP populations occurs at pH values not tolerated by *A. formosa* but within the range described for *A. ralfsii* and its varieties. This would mean that the *Asterionella* in Kejimkujik National Park are an ecotype of *A. ralfsii* because of their ability to grow in low pH lakes rather than on any phenetic basis. It also suggests that using this organism as an indicator species in eastern North America could be misleading if the floristic composition is unknown and good indicator species are not present. Although pigments were untested in the KNP collections, the colour was visuallly similar to that seen from clones taken from the type location of *A. ralfsii* var. *americana* as described by Körner (1970). Consequently, biochemically, the *Asterionella* from KNP appear to have a closer relationship with *A. ralfsii* than with *A. formosa*. Körner's separation of *A. ralfsii* into an American variety and a European variety is unnecessary and without foundation morphologically because of lack of consistency in the North American forms. This has led to confusion in delimiting this taxon without elucidating any basic differences.

I would recommend that *Asterionella* not be used in the future for physiological or biochemical studies unless stringent monitoring for nutrient limitation and morphological abnormalities is adhered to. Then, care must be exercised in extrapolating these data to natural populations. The extreme morphological plasticity seen in clones subjected to any amount of stress was not only seen in this study but has occurred in culture collections and in previous work on this organism by other investigators (F. Round pers. comm.). Different genetic strains are probable, and until extensive work using multiple isolates is undertaken, the danger of assuming all individuals in a population are reacting similarly to external influences, remains.

I propose that the two freshwater *Asterionella* taxa, *A.formosa* and *A. ralfsii* be retained as separate species. There does appear to be populations which conform to the classical descriptions found in the literature but these did not occur in the eastern North American populations seen in this study or in many other populations previously investigated (Delorme *et al.* 1983, Charles 1985,

Charles *et al.* 1990, Anderson et al. 1993). Morphological overlap observed in this study, suggests that future investigators should perform other, more stringent methods to determine which of the *Asterionella* taxa the population is more closely alligned with physiologically, biochemically and ecologically. Microscopy, whether it be light or electron, failed to define clear separation in this taxon based on morphology alone.

Other diatom taxa have been examined using SEM and have been shown to possess characters which are useful for defining separate taxa. This might be a useful tool when beginning a study but then the assumption must be that conditions have been static over time, and that all of that taxon seen at a particular site are the same and no integration has occurred with other genotypes or phenotypes. In a stable system under bloom conditions it may be possible to delimit taxa at the specific level but unless there are obvious morphological differences, the use of light microscopy will not differentiate integradation of possible ecotypes or morphological forms seen in most diatom populations. One need only to examine the classical diatom texts to see the range of morphotypes included under a single taxonomic name to realize that adaptive radiation in the Bacillariophyceae is an integral component of these organisms. This work has shown that what has been considered defining characteristics of the freshwater *Asterionella* taxa are not consistent, at least in North American populations. With a group of organisms that are constantly subjected to changing conditions and which have the ability to readily alter their phenotype, as many ditoms do, then taxonomy at the lower levels i.e. species and lower, should remain flexible and inclusive.

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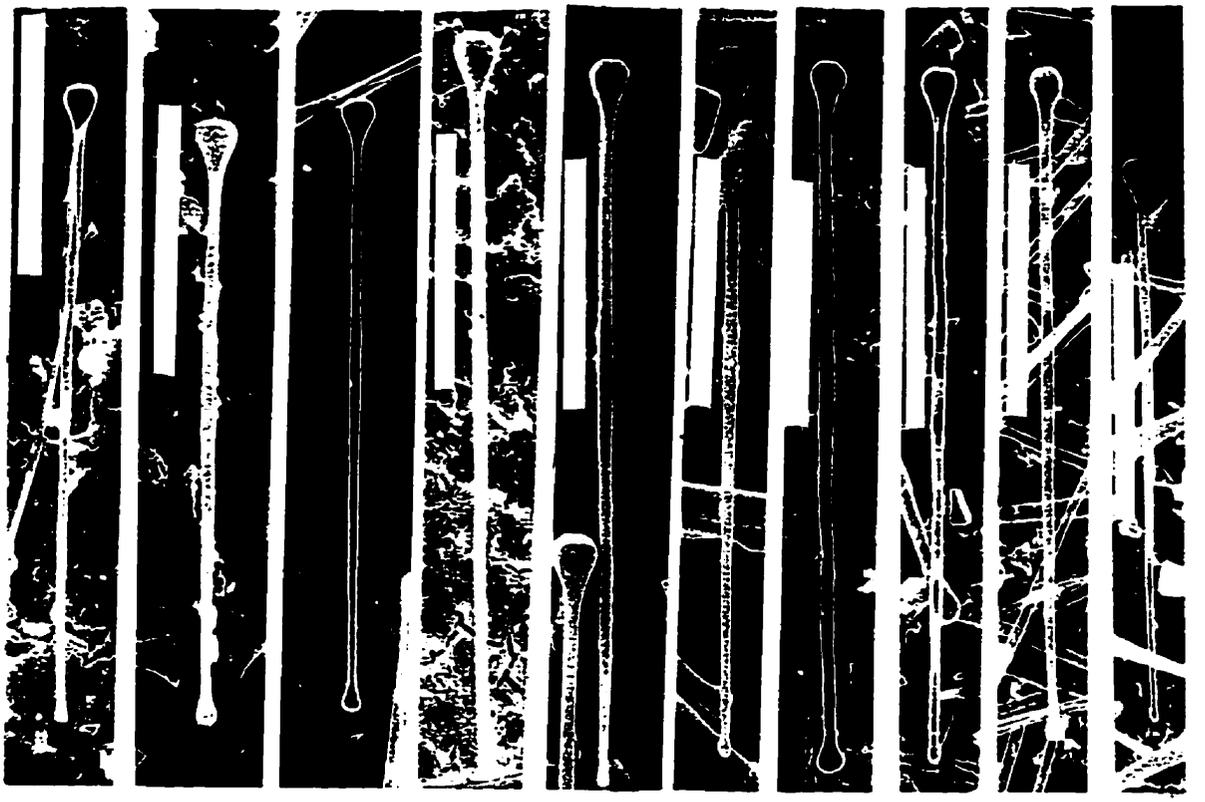
Plate I.

Variation in size and shape of *Asterionella* valves examined using S.E.M. A - E: cells ≥90 μm; F - N: cells < 90 μm > 65 μm; O - S: cells ≤65 μm; Valves are selected from all five sampling periods and 16 lakes

A) Mountain Lake S91 bar = 38 μm, cell 102 μm long
B) Upper Peskawa Lake S93 bar = 38 μm, cell 95 μm long
C) Upper Peskowesk Lake S90 bar = 38 μm, cell 95 μm long
D) Big Dam East Lake S90 bar = 30 μm, cell 92 μm long
E) Lower Peskowesk Lake S91 bar = 30 μm, cell 90 μm long
F) Big Dam West Lake F92 bar = 30 μm, cell 88 μm long
G) Plastic Lake bar = 30 μm, cell 88 μm long
H) Grafton Lake S90 bar = 30 μm, cell 82 μm long
I) McIntyre Lake S90 bar = 30 μm, cell 80 μm long
J) Mersey River Outflow F91 bar = 38 μm, cell 80 μm long
K) Jeremys Bay S93 bar = 27 μm, cell 80 μm long
L) Snake Lake F92 bar = 25 μm, cell 77 μm long
M) Little Peskowesk Lake F92 bar = 23 μm, cell 75 μm long
N) Plastic Lake bar = 27 μm, cell 74 μm long
O) Back Lake S90 bar = 23 μm, cell 65 μm long
P) Little Peskowesk Lake F91 bar = 27 μm, cell 65 μm long
Q) Birchwood Lake bar = 17.5 μm, cell 54 μm long
R) Little Peskowesk Lake F92 bar = 15 μm, cell 45 μm long
S) Little Peskowesk Lake F92 bar = 13.5 μm, cell 40 μm long



A B C D E F G H I J

K L M N O P Q R S

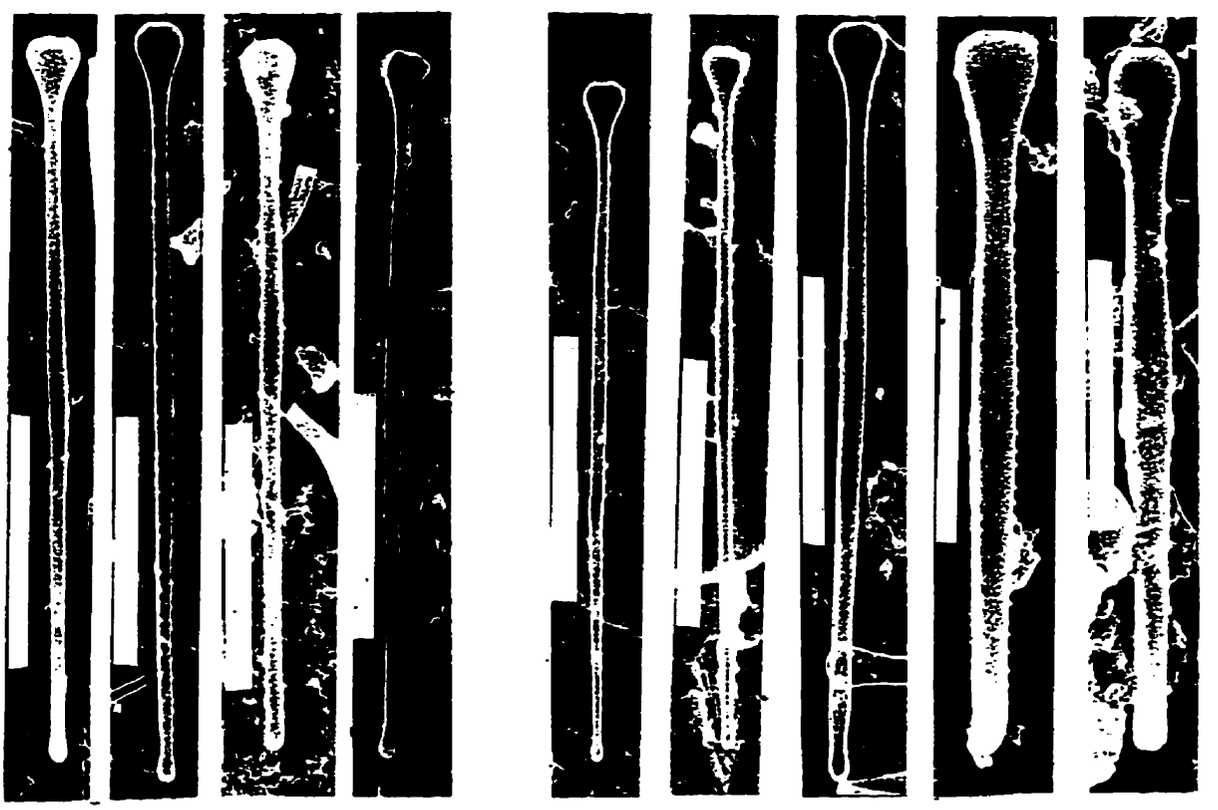


Plate II

Scanning electron micrographs of *Asterionella*. Exterior view of head poles. Note variation in shape and number of spines around the apex of the head pole. All valves oriented with the head pole to the top.

A) Head pole attenuate, not differentiated from the body of the valve, spines very reduced. bar = 2.0 μm

B) Head pole rounded, well differentiated from the body of the valve, twelve spines. bar = 2.5 μm

C) Head pole attenuate, only slightly differentiated from the body of the valve, eight spines. bar = 1.0 μm

D) Head pole rounded, well differentiated from the body of the valve, eight spines. bar = 2.0 μm

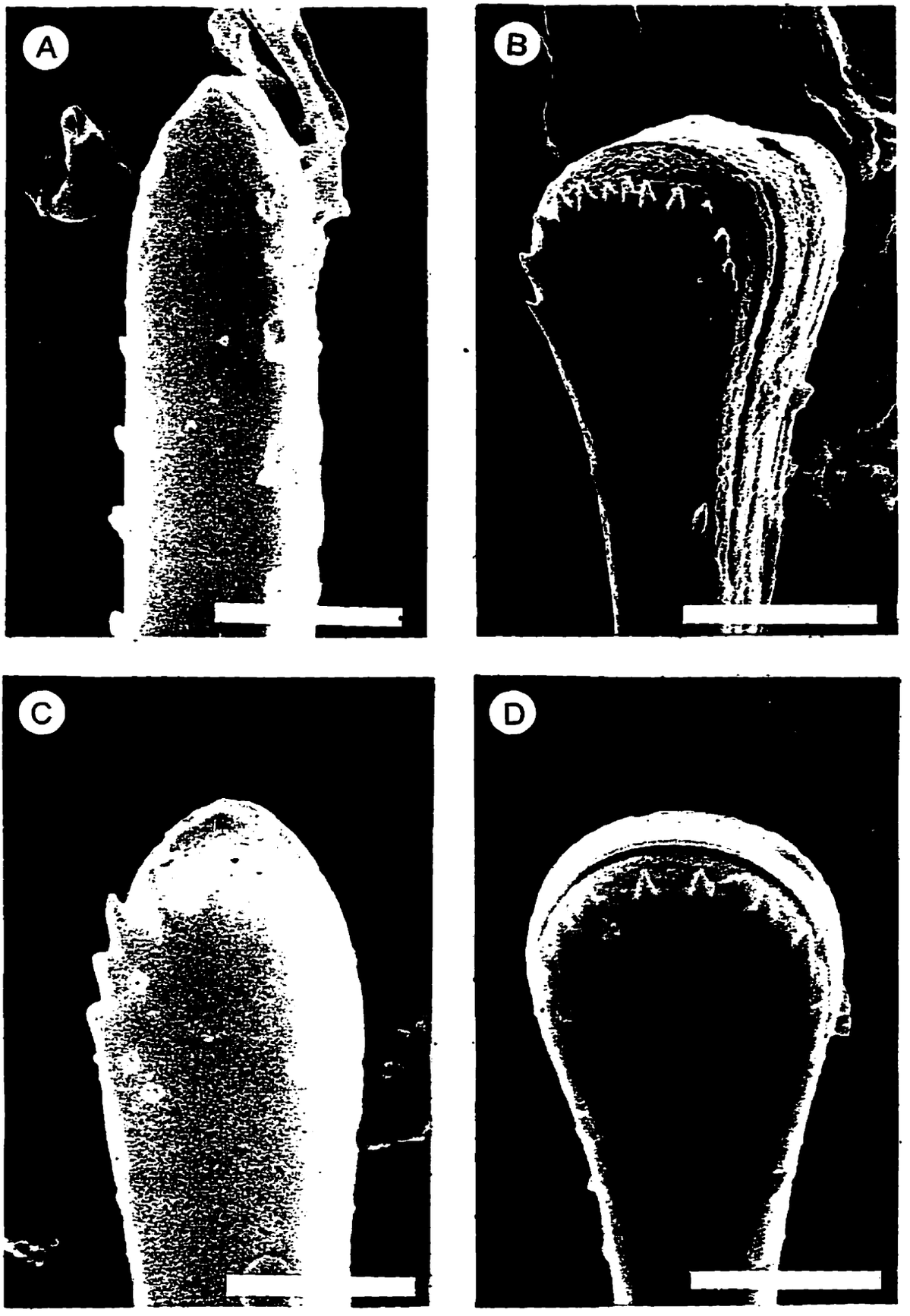

A
B
C
D

Plate III

Scanning electron micrographs of *Asterionella*. Interior view of head pole. Note the variation in the shape, location and orientation of the labiate process, if present, and the apical pore field. All valves oriented with the head pole to the top.

A) Head pole rounded, labiate process vertically oriented, left hand side. bar = 2.0 μm

B) Head pole with little differentiation from the body of the cell, labiate process vertically oriented, left hand side. bar = 1.2 μm

C) Head pole rounded, labiate process vertically oriented, right hand side. bar = 1.5 μm

D) Head pole with little differentiation from the body of the cell, labiate process vertically oriented, right hand side. bar = 1.36 μm

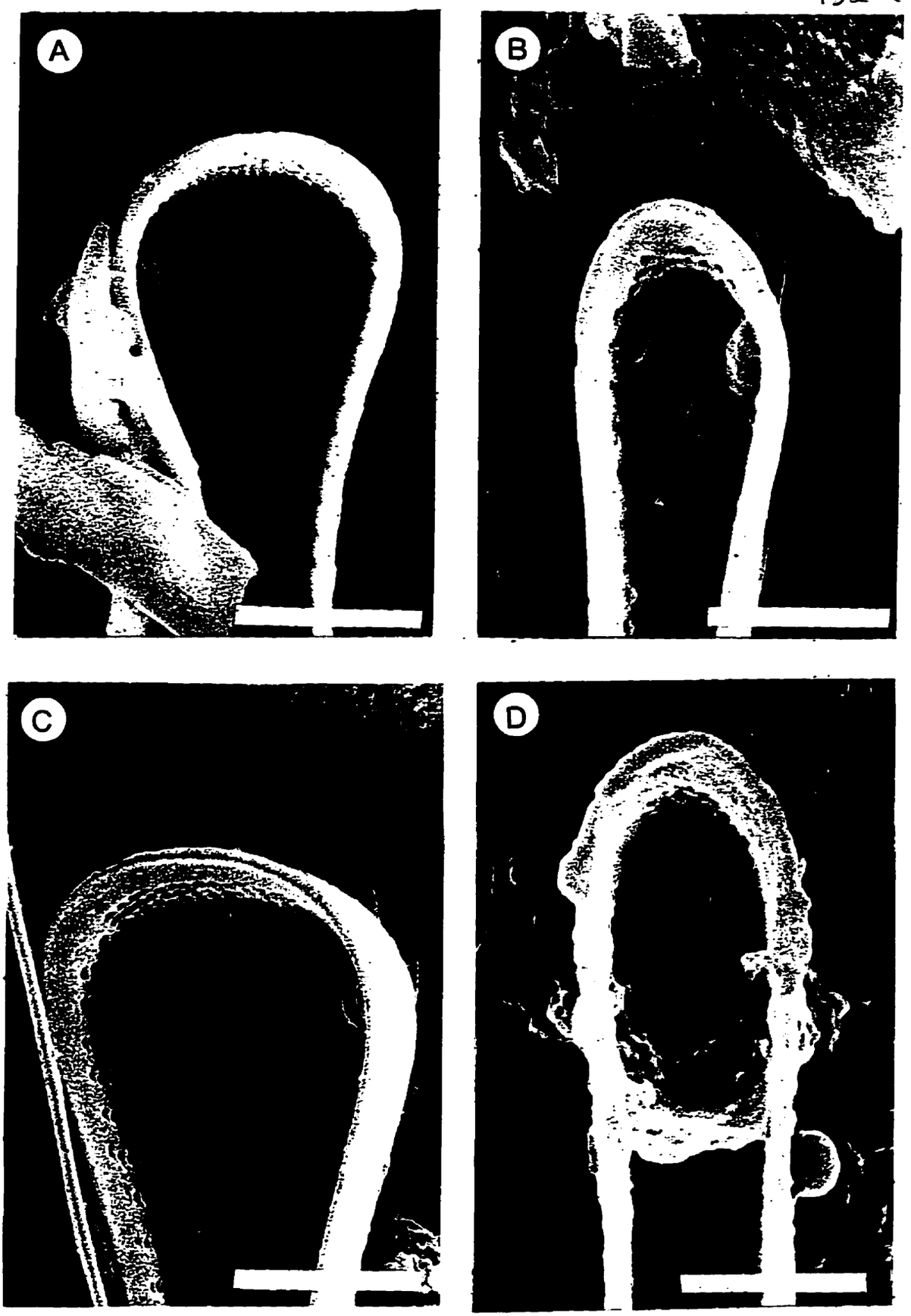

A
B
C
D

Plate IV

Scanning electron micrographs of *Asterionella*. Interior view of head pole. Note the variation in the shape, location and orientation of the labiate process, if present, and the apical pore field. All valves oriented with the head pole to the top.

A) Labiate process centrally located, vertically oriented. bar = 1.0 μm

B) Labiate process centrally located, horizontally oriented. bar = 1.5 μm

C) Head pole with little differentiation from the body of the cell, labiate process absent. bar = 1.0 μ

D) Head pole rounded, labiate process absent. bar = 2.7 μm

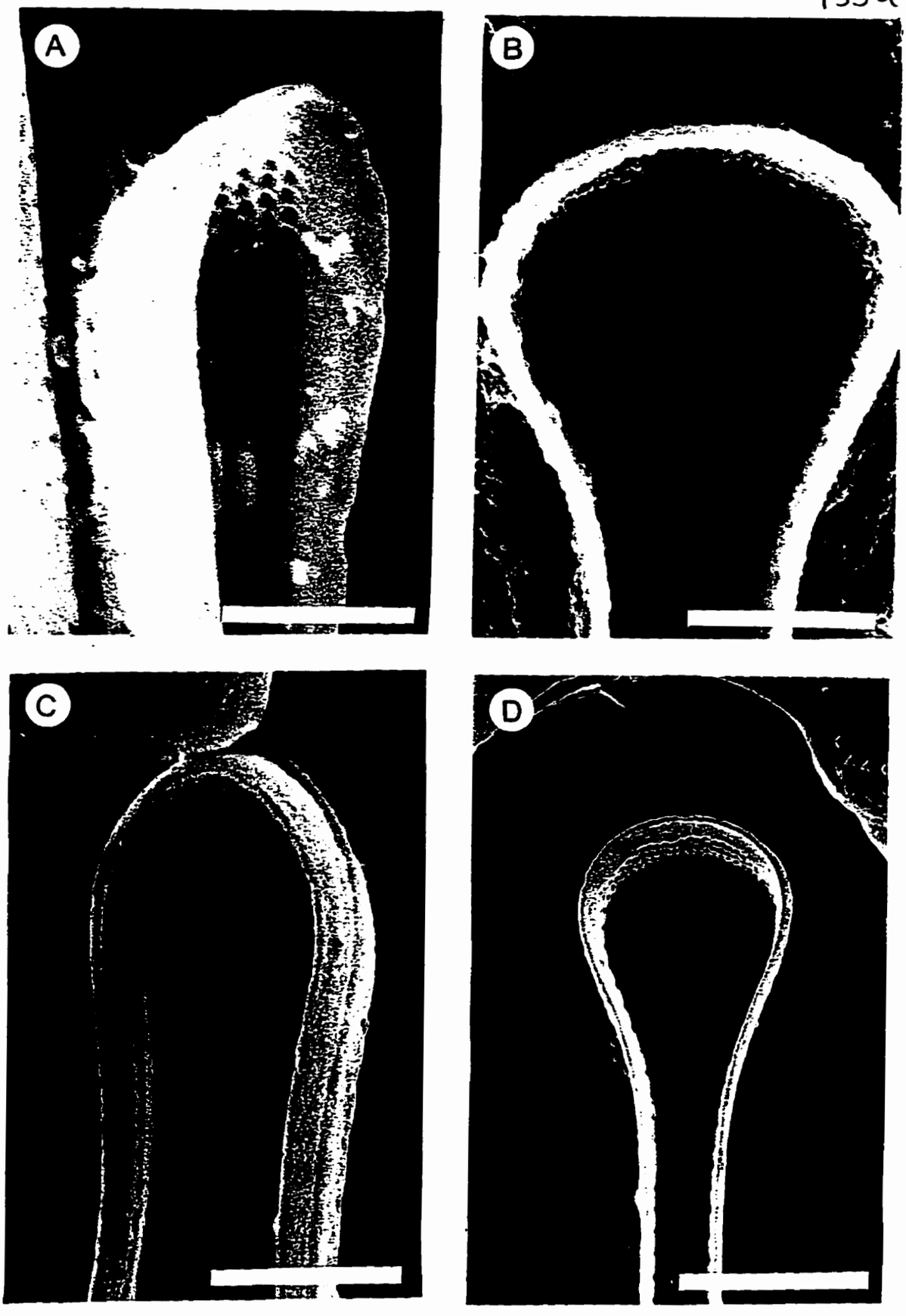

A
B
C
D

Plate V

Scanning electron micrographs of *Asterionella*. Exterior view of valves at foot poles. Note the variation in the shape and number of spines around the apex of the pole. All valves oriented with the foot pole to the top.

A) Foot pole rounded with indentation at the apex of the pole. Spinules many and reduced in size. bar = 3.0 μm

B) Foot pole rounded with cingulum indented at the apex of the pole. Spines many and sharply pointed. bar = 3.0 μm

C) Foot pole rounded. Spinules small around the valve mantle graduating to small spines along the body of the valve, pore field distinct. bar = 3.0 μm

D) Foot pole slightly flattened and less distinctly constricted from the body of the valve. Spinules small and irregularly placed. bar = 2.7 μm

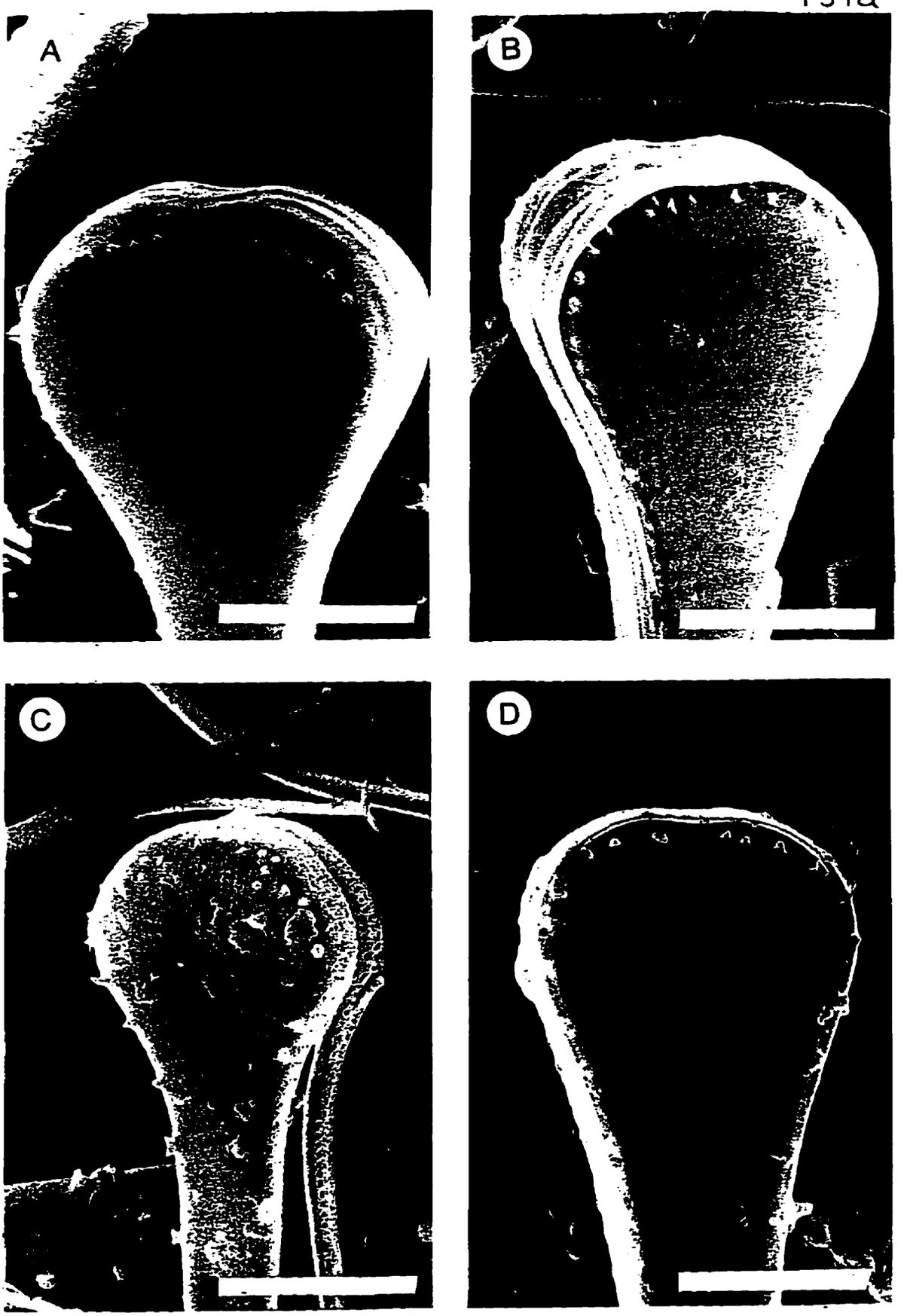

A
B
C
D

Plate VI

Scanning electron micrographs of *Asterionella*. Interior view of valves at foot poles. Note the apical pore field, shape of the pole, and location and orientation of the labiate process. All valves oriented with the foot pole to the top.

A) Foot pole with labiate process located centrally, adjacent to the pore field, transapical orientation. bar = 2.0 μm

B) Foot pole with labiate process located centrally, below the pore field, transapical orientation. bar = 2.7 μm

C) Foot pole with labiate process located below the pore field on the right hand side, parallel to longitudinal axis. bar = 2.7 μm

D) Foot pole with labiate process located below the pore field on the left hand side, transapical orientation. bar = 2.3 μm

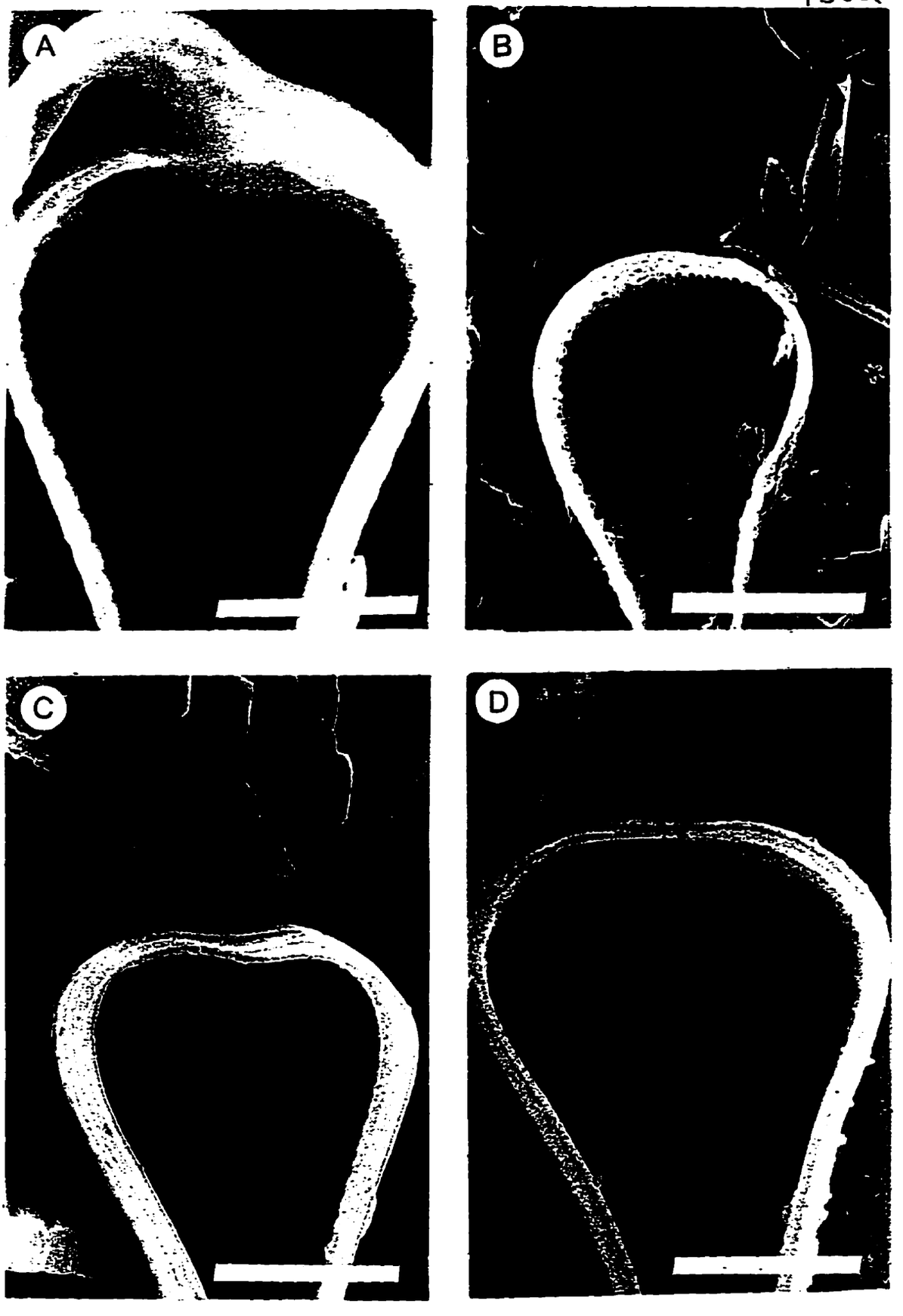

A
B
C
D

Plate VII

Scanning electron micrographs of *Asterionella*. Interior view of valve at foot poles. Note the apical pore field, cleft at the apex of the pole, and location and orientation of the labiate process. All valves oriented with the foot pole to the top.

A) Foot pole with labiate process on the right hand side below the pore field, line of lip oriented toward the top center of the valve. bar = 3.0 μm

B) Foot pole with labiate process on the left hand side below the pore field, line of lip oriented toward the upper, outer side of the valve. bar = 3.0 μm

C) Foot pole with labiate process on the left hand side below the pore field, line of lip oriented toward the top center of the valve. bar = 2.0 μm

D) Foot pole with labiate process on the right hand side below the pore field, line of lip oriented toward the upper outer side of the valve. bar = 2.0 μm

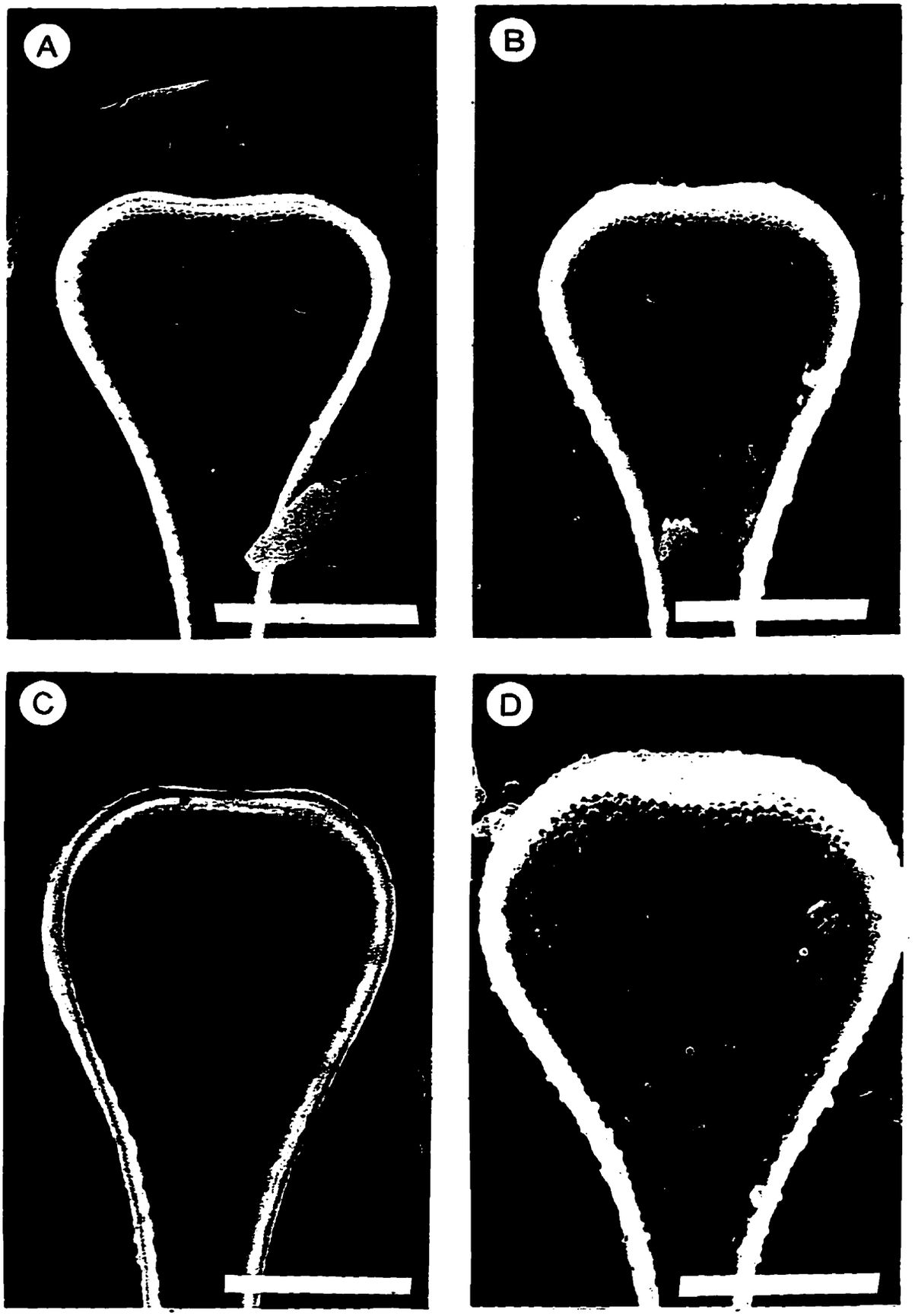

A
B
C
D

Plate VIII

Scanning electron micrographs of *Asterionella*. Morphological changes seen in cultured cells of *Asterionella*

A) "Normal" cells in culture. Head pole and foot pole are well defined. Note the reniform shaped cells in the background. bar = 38.0 μm

B) Head pole and foot pole are no longer distinct from the body of the cell. Spines on valve margin persist. bar = 10.0 μm

C) Reduction in valve length and an increase in the number of girdle bands. bar = 5.0 μm

D) One of several intermediate shapes. Note that the spines along the valve margin stil persist. bar = 4.3 μm

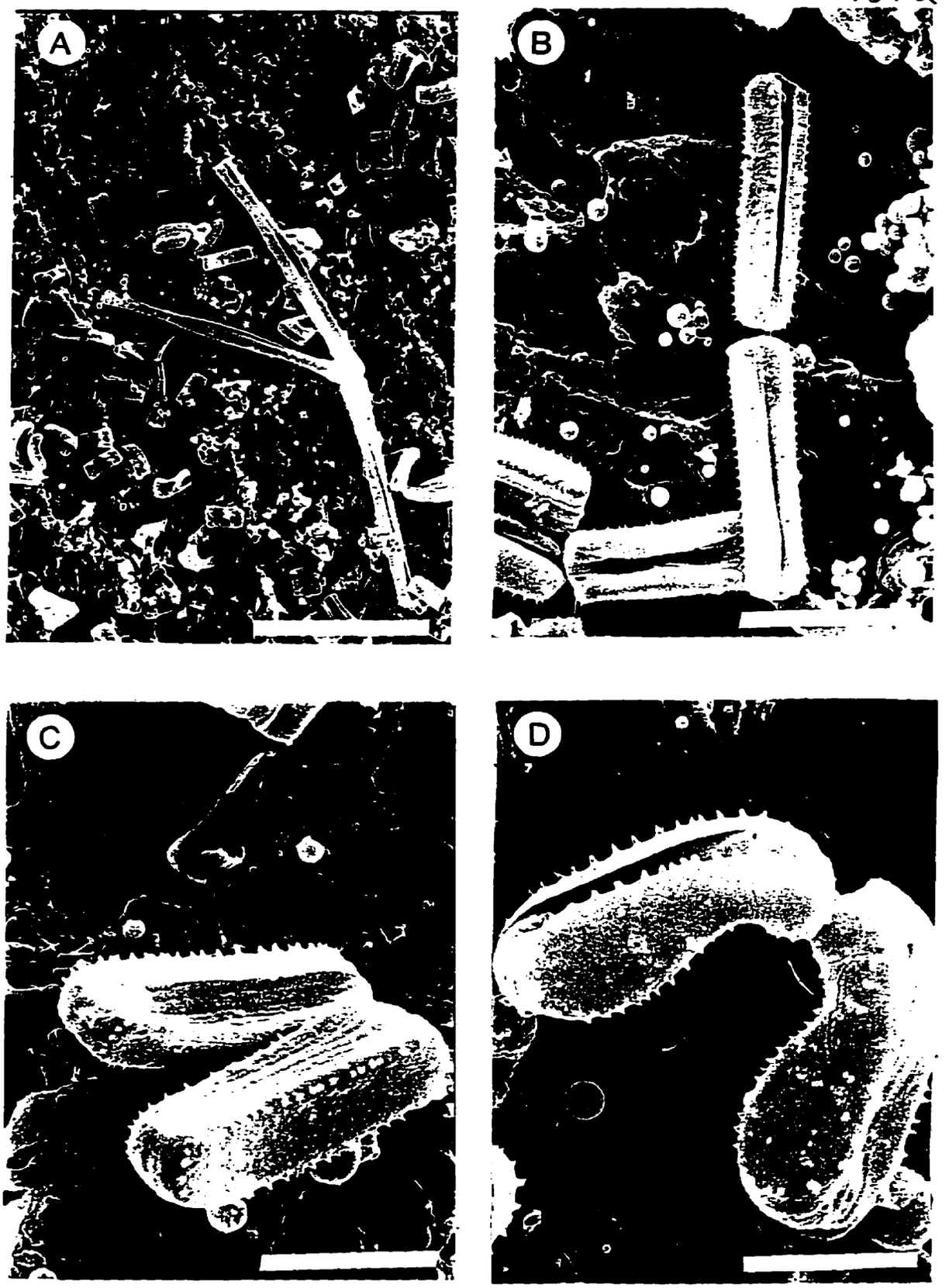

A
B
C
D

Plate IX

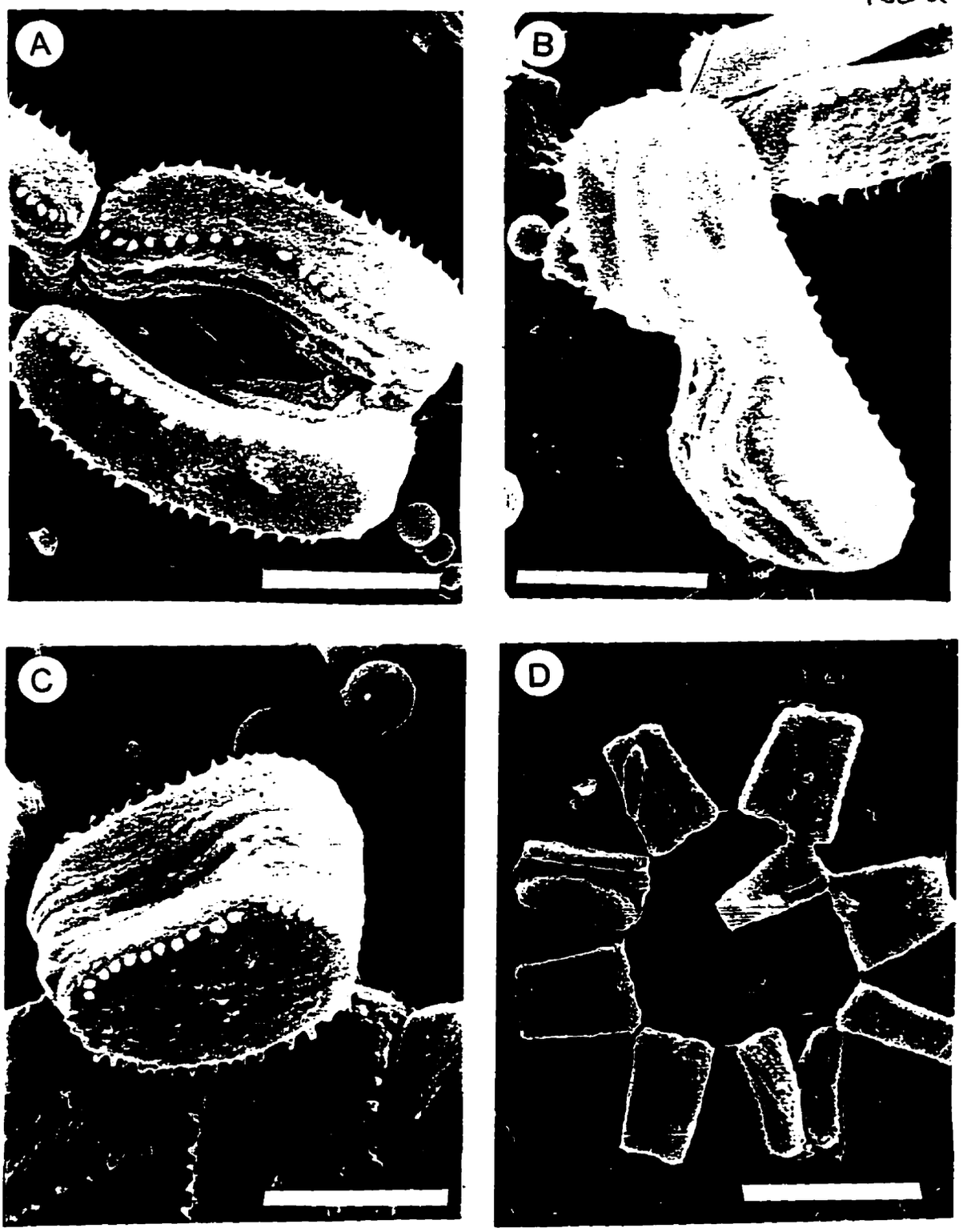
Scanning electron micrographs of *Asterionella*. Morphological variation in cultured cells of *Asterionella*

A) Reniform cells were one of the most commonly occurring forms. Note multiple girdle bands. bar = 4.3 μm

B) Intermediate form which will become two oblong to rounded cells upon the next division. Spines along the valve margin persist. bar = 3.0 μm

C) The final form seen before death of culture. Note the multiple girdle bands and persistent spines. bar = 3.0 μm

D) Although most cells of reduced size were seen either singly or in pairs, some multicellular colonies were evident. bar = 13.6 μm


A
B
C
D

Appendix 1. pH, conductivity and temperature measurements for *Asterionella* populations used in scanning electron microscopy

	pH	Cond	Temp
Back45	5.12	24	22.4
Back77	5.00	22.4	15.8
Back120	4.91	26.6	9.1
Back152	5.00	23.6	6.5
Back183	4.52	22.3	16.4
Graf31	5.66	33.4	19.1
Graf83	5.34	29.7	19
Graf115	5.93	33.8	7.9
Graf140	6.40	40	8.4
Graf194	5.55	35.1	18.5
Mnt66	4.75	21.8	17
Mnt144	5.00	28.2	8.8
Mnt173	5.07	23	18.8
McI53	6.45	61.6	19.2
McI132	6.60	65	8.6
McI170	6.70	62.3	13.4
Sna57	4.65	23.5	23
Sna82	4.32	21.2	16.9
Sna119	4.55	37.3	9.1
Sna159	4.80	28	6.2
Sna193	4.10	25.2	18.3
Jbay41	4.73	30.7	17.6
Jbay89	4.20	27.6	21
Jbay118	4.81	29.9	9.1
Jbay134	5.20	31	9
Jbay198	4.13	34.6	20.3
MBay36	4.76	30.4	22.7
Mbay65	4.62	26.3	18.6
Mbay131	5.54	29.7	10.3

Mbay143	5.00	43	8.1
Mbay174	4.42	29.3	19.6
Jakes87	4.67	27.5	17.9
Jakes40	4.90	37.1	16.2
Jakes117	5.02	32.8	7.8
Jakes167	4.20	49.8	7.1
Jakes196	4.62	33.3	18.3
MRO29	4.72	32.8	17.4
MRO68	4.70	26.5	17.7
MRO111	5.04	30.5	7.9
MRO139	4.50	25.5	8.6
MRO190	4.16	29.6	16.5
LPesk125	5.25	31	8.5
LPesk155	4.40	27.8	6.9
LPesk189	4.42	26.2	19.3
PwesL1	4.82	38.4	17.2
PwesL79	4.35	26.2	14.4
PwesL186	4.11	27.4	18
PwesU25	4.40	34.9	18.8
PwesU74	4.27	28.5	15.6
PwesU179	4.16	29.1	15.4
PwaU19	4.67	34.8	18.4
PwaU72	4.27	28.2	14.9
PwaU178	4.01	29.1	15.2
PwaL21	4.95	35.6	19.4
PwaL73	4.33	29.6	15.6
PwaL148	3.90	26.6	7.6
PwaL181	3.99	33.3	15.2
PIF23	4.43	35.7	16.4
PIF75	4.26	28.4	15.6
PIF149	3.8	27.2	7.1
PIF180	3.71	29.3	15.4
PI200	5.89	22	11.2

Bw201	5.8	61.2	11.9
TAP203	5.93	99.2	11.7
Pl169	5.86	21.1	13.6
GB168	6.14	192	7.6

Appendix 2

Sample calculation for # cells / ml

Volume of S-R chamber = $1000mm^3$

cells/ml = C x 1000mm / L x D x W x S

= cells counted x 1000mm /

50mm x 1mm x 0.008mm x # strips

for BW12-10 $2082 \times 1000mm^3 / 0.40 \times 2 = 2.6 \times 10^6$

Cell counts made on *Asterionella ralfsii* clones (BW5-, BW6-, BW10-, BW12-) and *Asterionella formosa* clones.

Clone #	# Cells	Transect #	Cells / ml ($x10^6$)	Final Vol. (ml)	# Cells harvest ($x10^8$)
BW5-4	685	2	0.9	106	0.91
BW5-5	1362	2	1.7	130	2.21
BW5-6	1330	2	1.7	202	3.36
BW5-7	1152	1	2.9	234	6.74
BW5-8	1676	2	2.1	384	8.04
BW5-9	1868	2	2.3	333	7.78
BW5-10	1188	3	1.0	344	3.41
BW5-14	1038	2	1.3	310	4.02
BW6-2	1603	2	2.0	182	3.65
BW6-3	928	2	1.2	248	2.88
BW6-5	664	2	0.8	215	1.78
BW6-10	1411	2	1.8	354	6.24

BW6-11	898	2	1.1	280	3.14
BW6-15	929	2	1.2	132	2.28
BW10-2	2172	2	2.7	176	4.78
BW10-3	1160	2	1.5	251	3.64
BW10-4	909	2	1.1	171	1.94
BW10-5	1437	2	1.8	217	3.90
BW10-6	1816	2	2.3	238	5.40
BW10-9	1491	2	1.8	429	8.00
BW10-10	1505	2	1.9	236	4.44
BW10-11	2261	2	2.8	384	10.90
BW10-13	1041	2	1.3	132	1.72
BW10-14	1175	4	0.7	303	2.23
BW10-16	1546	2	1.8	261	4.65
BW10-17	1481	2	1.9	121	2.24
BW12-1	601	2	0.8	225	1.69
BW12-2	2946	2	3.7	108	4.00
BW12-3	1478	2	1.8	204	3.77
BW12-4	1161	2	1.5	200	2.90
BW12-5	1331	2	1.7	239	3.98
BW12-6	1715	2	2.1	234	5.02
BW12-8	2719	2	3.4	272	9.24
BW12-9	1320	4	0.8	375	0.31
BW12-10	2082	2	2.6	318	8.28

BW12-12	1061	2	1.3	288	3.82
BW12-13	1547	3	1.3	330	4.25
BW12-14	1168	3	1.0	193	1.88
BW12-15	1117	2	1.4	228	3.18
BW12-16	1321	2	1.8	261	4.84
T-7-1	1568	1	3.9	209	8.19
T-7-2	1578	1	3.9	237	9.35
T-7-3	2433	1	6.1	241	14.70
T-7-4	1727	2	2.2	439	9.39
T-7-5	2383	1	6.0	262	15.60
T-7-7	1687	2	2.1	400	8.44
T-8-1	1881	1	4.7	231	10.90
T-8-2	1858	1	4.6	236	11.00
T-8-3	2040	1	5.1	225	11.50
T-8-5	1005	1	2.5	408	10.00
T-8-8	1852	1	4.6	133	6.16
T-8-9	1162	1	2.4	393	11.40
T-8-10	1157	1	2.9	267	7.72
T-8-11	1940	2	2.4	265	6.43
T-8-12	1084	1	2.7	232	6.29
T-8-15	1280	1	3.2	226	7.23
F-1-1	1353	1	3.4	256	8.66
F-6-1	875	1	2.2	205	4.48

F-6-4	4151	2	5.2	261	13.50
F-6-5	3114	2	3.9	346	13.50

Appendix 3

Values for dissolved reactive phosphorus left in the media after harvesting clones of *Asterionella formosa* (T-7, T-8) and *Asterionella ralfsii* (BW5-, BW6-, BW10-, BW12-). Final absorbance was calculated by averaging three replicates of the same clone and subtracting the blank (0.037).
final absorbance x 142.86 = µg P / L
Media = 2.062 x 142.86 = 294.57 µg / L

BW5-9	246.86	BW12-14	247.00
BW5-10	248.58	BW12-15	228.86
BW5-11	264.58	BW12-16	236.15
BW5-13	235.29	T-7-5	169.57
BW5-14	187.15	T-7-7	224.00
BW6-10	248.58	T-8-6	147.86
BW6-11	243.15	T-8-8	158.29
BW6-12	261.72	T-8-9	188.00
BW6-13	258.43	T-8-10	159.43
BW6-14	221.43	T-8-11	184.43
BW6-15	194.43	T-8-12	230.43
BW10-11	236.29	T-8-13	199.43
BW10-13	231.43	T-8-14	151.86
BW10-14	255.29	T-8-15	151.86
BW10-15	242.00		
BW10-16	215.58		
BW10-17	225.72		
BW12-10	238.15		
BW12-12	244.58		
BW12-13	234.58		

Appendix 4

Sample calculation for dissolved reactive silicate remaining in the growth media after harvesting clones of *Asterionella formosa* and *Asterionella ralfsii*.

Si standard

0.96g/188.05546 mol wt. = 0.005 mol or 5104.88 μmol

For dilution: 2 ml standard/100ml H_2O X 5104.88 =

102.10 μmol

For Si calibration standards

Conc	Vol	Si Std.	
100	100ml	2.0ml	= 102.10μmol
80	100ml	1.6ml	= 81.68μmol
50	100ml	1.0ml	= 51.05μmol
20	100m	0.4ml	= 20.42μmol
10	100m	0.2ml	= 10.21μmol
5	100m	0.1ml	= 5.10μmol
3	100m	0.06ml	= 3.06μmol
1	100m	0.02ml	= 1.02μmol

Corrected absorbance of Si standards used to calculate the concentration of Si remaining in the growth media at the time of harvesting *Asterionella formosa* (T-7-, T-8-) and *Asterionella ralfsii* (BW5-, BW6-, BW10-, BW12-)

Conc	Corrected Absorbance	
1	0.110	Using a 10 cm cell
3	0.269	Constant = 0.042106
5	0.449	X coefficient = 0.076978
10	0.803	R^2 = 0.9975
20	0.234	Using a 1 cm cell
50	0.544	Constant = 0.025184
80	0.872	X coefficient = 0.01045
100	1.064	R^2 = 0.9996

For 10 cm cell: Corrected absorbance - 0.042106 / 0.076978 = [Si] µg-at / L

For 1 cm cell: Corrected absorbance - 0.025184 / 0.01045 = [Si] µg-at / L

Concentrations of Si remaining in the growth media after harvesting *Asterionella formosa* (T-7-, T-8-) and *Asterionella ralfsii* (BW5-, BW6-, BW10-, BW12-) for pigment analysis.

Clone #	Corrected Absorbance	[Si] µg-at/L
BW5-9	0.913	11.31
BW5-10	0.787	9.67
BW5-13	0.742	9.09
BW5-14	0.147	1.36
BW6-10	0.666	8.10
BW6-11	0.805	9.90
BW6-13	0.403	4.69
BW6-14	0.818	10.08
BW6-15	0.245	2.64
BW10-11	0.197	2.01
BW10-13	0.274	3.01
BW10-14	0.271	2.97
BW10-15	0.264	2.88
BW12-10	0.351	4.01
BW12-12	0.839	10.35
BW12-13	1.201	15.05
BW12-15	0.743	9.11
BW12-16	0.737	8.94
T-7-5	0.894	11.06
T-7-7	1.200	15.05
T-8-5	0.949	11.78
T-8-6	1.208	15.15
T-8-11	0.303	3.39
T-8-12	0.618	7.48
T-8-13	0.230	2.44

Appendix. 5

Comparison of absorbance maxima in this study to values seen in other studies.(DEE= diethylether; V&J Vesk & Jeffrey 1987; M&L Mantoura & Llewellyn 1983; S&J Stauber and Jeffrey 1988).

Pigment	Solvent	Abs. max	Ref.	Organism
Chl a	DEE	428	V&J 1987	Chryso
Chl a	90% acetone	432	M&L 1983	assorted
Chl a	DEE	429	S&J 1988	diatoms
Chl a	100% acetone	430	this study	diatoms
ß-carotene	DEE	449	V&J 1987	Chryso
ß-carotene	ethanol	450	M&L 1983	assorted
ß-carotene	ethanol	449	S&J 1988	diatoms
ß-carotene	100% acetone	450	this study	diatoms
Chl c	methanol	443	M&L 1983	assorted
Chl c	Acetone	442	Jeffrey 1969	Phaeo
Chl c	methanol	443	M&L 1983	assorted
Chl c	100% acetone	441	this study	diatoms
diatoxanthin	ethanol	451	S&J 1988	diatoms
diatoxanthin	100% acetone	442	this study	diatoms
diadinoxanthin	ethanol	446	S&J 1988	diatoms
diadinoxanthin	ethanol	446	V&J 1987	Chryso
diadinoxanthin	100% acetone	446	this study	diatoms
fucoxanthin	acetone	447	S&J 1988	diatoms
fucoxanthin	DEE	446	S&J 1988	diatoms
fucoxanthin	100% acetone	446	this study	diatoms

Appendix 6.

Pigment ratios measured from *A. formosa* samples (T-) and *A. ralfsii* samples (BW-) using HPLC

SAMPLE	CHLa:c	CHLa:ßCAROTENE	CHLa:FUCOXANTHIN
T-7-7	31:1	84:1	1:1
T-8-9	5.4:1	88:1	1:1
T-8-10	9:1	11:1	1:1
T-8-11	10.4:1	15.7:1	0.97:1
T-8-14	14.9:1	10.4:1	0.67:1
T-8-15	127:1	NA	0.69:1
AVERAGE	32.88	41.72	0.89
BW5-10	950:1	163:1	3.3:1
BW5-11	NA	27.7:1	3.2:1
BW5-13	332:1	24:1	2.5:1
BW5-14	1103:1	NA	3.3:1
BW6-11	NA	15:1	3.3:1
BW6-12	1727:1	27:1	2.1:1
BW6-14	NA	NA	1.7:1
BW6-1501	450:1	NA	2.7:1
BW6-1502	NA	185:1	2.8:1
BW10-12	338:1	114:1	2.8:1
BW10-13	NA	163:1	3.4:1
BW10-14	260:1	26:1	4.9:1
BW10-16	NA	21.3:1	2.0:1
BW10-17	4749:1	23.4:1	4.2:1
BW10-1701	1347:1	NA	3.0:1
BW12-12	NA	13.5:1	3.1:1
BW12-13	4.5:1	30.4:1	3.4:1
BW12-14	18:1	24.4:1	3.0:1
BW12-15	127:1	20.4:1	3.1:1
BW12-16	1011:1	46:1	2.8:1
AVERAGE	988.2	57.77	3.03

SAMPLE	FUCOXANTHIN:OTHER XANTHOPHYLLS	XANTHOPHYLLS:CHL a
T-07-7	1.2:1	1.8:1
T-8-9	1.1:1	1.8:1
T-8-10	1.2:1	1.7:1
T-8-11	1.5:1	1.7:1
T-8-14	1.68:1	2.4:1
T-8-15	1.8:1	2.4:1
AVERAGE	1.3:1	1.97:1
BW5-10	0.62:1	0.80:1
BW5-11	0.57:1	0.86:1
BW5-13	0.92:1	0.82:1
BW5-14	0.58:1	0.84:1
BW6-11	0.65:1	0.76:1
BW6-12	0.98:1	0.94:1
BW6-14	0.60:1	1.59:1
BW6-1501	0.59:1	0.99:1
BW6-1502	0.63:1	0.93:1
BW10-12	0.66:1	0.90:1
BW10-13	0.61:1	0.77:1
BW10-14	0.83:1	0.45:1
BW10-16	0.55:1	1.42:1
BW10-17	0.78:1	0.55:1
BW10-1701	0.53:1	0.95:1
BW12-12	0.66:1	0.83:1
BW12-13	0.79:1	0.66:1
BW12-14	0.63:1	0.87:1
BW12-15	0.76:1	0.74:1
BW12-16	0.62:1	0.93:1
AVERAGE	0.68:1	0.88:1

Appendix 7. Cell length data for spring 1990,1991,1993 and fall 1991 and 1992

Spring 1990		Size (um)									
		32	33	34	35	36	37	38	39	40	41
42	43	44	45	46	47	48	49	50	51	52	53
54	55	56	57	58	59	60	61	62	63	64	65
66	67	68	69	70	71	72	73	74	75	76	77
78	79	80	81	82	83	84	85	86	87	88	89
90	91	92	93	94							
McI #53		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	4	1	1	0	3	0
3	0	1	0	5	1	6	2	12	11	24	6
11	1	6	0	1	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0							
BDE #59		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0
0	0	7	5	13	4	11	3	7	2	8	1
4	0	7	1	4	2	10	1	3	0	3	0
1	0	2	0	0	0	0	0	0	0	0	0
0	0	0	0	0							
MMK #61		0	0	0	0	1	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0	2	0
3	1	4	3	10	5	14	6	17	5	14	2
5	0	2	0	0	1	2	0	1	0	1	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0							
Hil #47		0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	1	0	0	2	6	0
7	3	10	3	8	3	12	4	12	2	12	4

5 1 3 0 0 1 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0

Mnt #51 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 1
0 0 0 0 0 1 0 1 1 3 1 4
1 5 2 8 2 10 2 9 1 13 8 12
2 9 2 1 1 0 0 0 0 0 0 0
0 0 0 0 0 0

J Bay #41 0 0 0 0 0 0 1 0 0 0
0 0 0 0 0 0 0 0 0 0 1 0
0 0 2 0 5 4 6 3 7 1 9 4
7 1 8 3 14 4 12 0 3 1 1 1
1 0 1 0 0 0 0 0 0 0 0 0
0 0 0 0 0

Pwesk L #3 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 1 0 0 1 1 3 1 1 7 2 8
3 11 4 8 6 16 3 9 4 6 0 2
0 2 0 0 0 1

Pwesk M #4 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 2 0 3 1 7 5 6
4 12 5 13 4 15 9 7 3 2 0 1
0 1 0 0 0 0

Pwesk L #1			0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	1	0	0
0	2	0	0	1	0	0	0	0	6	0	4
2	5	2	12	5	17	9	10	3	12	1	5
0	1	1	0	0	0						
Pesk Br #6		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	1	0
0	0	0	0	2	0	0	0	3	1	4	4
13	2	21	6	14	3	13	4	5	0	1	0
0	1	0	0	1							
McG #55		0	0	0	0	0	0	0	0	0	0
0	0	0	0	2	0	1	0	2	0	0	0
2	2	1	0	2	0	6	0	25	12	29	5
6	0	5	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0							
Pwa@PIF #21		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	2	0	2	0	5	2	8	1
17	4	20	2	17	2	7	3	3	1	0	0
2	0	1	0	0							
Snake #57		0	0	3	0	3	0	0	0	4	0
0	0	4	1	0	0	1	0	3	1	0	1

3	0	5	0	3	0	6	2	13	3	27	1
8	2	1	1	3	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0							

Back #45		0	0	0	0	0	0	0	0	0	0
0	0	1	0	1	0	5	0	29	0	34	0
18	0	8	0	3	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0							

Puz #38		0	0	0	0	0	0	0	0	0	0
0	0	1	1	2	1	4	0	1	2	8	0
1	0	3	0	2	1	7	2	4	3	8	3
7	4	12	0	9	2	3	1	3	1	2	0
2	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0							

M Bay #36		0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	2	2	0	1	2	1	0
1	0	1	0	5	2	2	0	4	4	13	6
6	7	12	1	11	1	6	4	1	0	2	1
1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0							

BDW #35		0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	1	0	0	1	0	3	0
5	1	3	0	4	5	11	1	8	3	12	1

10	1	6	1	6	3	6	2	1	1	2	0
1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0							

BDW #34		0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	3	0	0	0
5	0	6	5	10	4	11	3	5	3	10	3
3	3	0	0	6	2	4	2	3	2	2	0
4	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0							

BDW #33		1	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	1	0	1	0	0	0
3	2	4	2	8	4	15	4	8	3	7	5
10	2	1	3	8	1	2	0	2	0	2	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0							

Graf #31		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	1
2	0	1	2	10	6	20	8	13	3	11	7
5	3	5	1	1	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0							

Jakes #40		0	0	1	0	0	0	0	0	0	0
0	0	1	0	0	0	1	0	3	1	2	0
1	0	2	4	2	0	5	2	3	1	9	4
7	0	8	5	14	4	5	1	5	2	2	1

1	2	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0							

MRO #29		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	1	0	4	0	6	6
8	7	9	3	12	3	6	4	4	0	5	1
5	2	0	0	5	2	4	1	1	0	0	0
0	0	0	0	0							

Bskin #27		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	4	0	4	0	8	2
7	6	22	3	11	1	15	1	9	1	2	2
1	0	0	0	0							

Pwesk@PIF #25			0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0		0	0	0
0	0	0	0	0	2	0	3	2	5	4	11
4	13	4	13	5	11	2	9	2	6	0	2
0	2	0	0	0	0						

PIF #23		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	3	1	5	0	10	3
9	6	15	5	15	4	7	5	4	3	3	0

1 0 0 0 0

Pwa L #19 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
1 0 0 0 0 0 1 1 5 6 9 3
9 3 22 3 9 4 11 4 6 0 3 0
0 0 0 0 0

Spring 1991 32 33 34 35 36 37 38 39 40 41
42 43 44 45 46 47 48 49 50 51 52 53
54 55 56 57 58 59 60 61 62 63 64 65
66 67 68 69 70 71 72 73 74 75 76 77
78 79 80 81 82 83 84 85 86 87 88 89
90 91 92 93 94 95 96 97 98 99 100 101
102 103 104 105 106 107 108 109 110

Graf #83 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 1 0 3 3
6 5 6 6 10 5 16 7 15 2 7 1
3 0 1 0 1 1 0 0 1 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0

Pwesk M #78 0 0 0 0 0 0 0 0 0 0
0 0 0 0 1 0 0 0 0 0 0 0
0 0 0 0 0 0 1 1 1 2 2 1
4 1 4 2 6 3 5 2 12 4 10 6
3 4 12 3 5 1 3 0 1 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0

BDE #90		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	2	0	1	0	6	1	6	2	5	5
7	4	13	8	15	5	10	3	7	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0			
MMK #92		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
1	0	1	1	1	2	10	3	10	4	13	8
10	4	10	5	9	2	3	1	1	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0			
McI #93		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	2	0	0	1
1	2	1	2	6	8	14	9	15	5	13	2
5	3	6	1	2	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0			
Mnt #66		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	2	1
3	1	5	2	1	3	9	2	3	5	4	1
8	2	4	1	6	5	8	2	13	3	4	0
1	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0			

J bay #89		0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	1	2	7	3	14	4	15	8
15	4	8	1	8	2	3	1	0	0	1	0
0	1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0			
McG #69		0	0	0	0	0	0	0	0	0	0
3	1	0	0	4	0	3	2	6	2	0	0
1	0	0	0	3	1	13	3	6	4	12	3
8	4	9	2	7	1	0	1	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0			
Snake #82		0	0	0	0	0	0	0	0	1	1
1	2	9	1	5	1	9	2	10	2	11	1
10	1	3	3	3	1	3	1	1	0	0	1
0	0	1	0	2	0	2	0	0	0	0	0
2	0	0	1	0	0	0	0	0	0	1	0
2	1	0	0	1	1	1	0	1	0	0	0
0	1	0	0	0	0	0	0	0			
Pwa L #72		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
2	0	3	1	0	0	1	1	3	1	2	1
5	4	8	4	12	4	11	4	7	2	7	2
5	1	4	2	2	0	0	0	0			
L Pesk #80		1	0	0	0	0	0	1	0	2	0

0	0	0	0	0	0	0	0	0	1	1	0
0	0	1	0	1	0	4	1	4	1	1	2
2	2	7	4	11	3	8	5	5	5	7	4
4	1	5	0	3	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	1	0
0	0	0	0	1	0	0	0	0			

M Bay #65		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	1	0	0	0	0	0
1	0	2	0	2	2	2	0	5	4	11	1
10	8	16	2	6	2	10	1	5	0	3	0
3	0	1	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0			

Back #77		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	2	0
1	0	1	0	2	0	13	6	19	4	22	4
14	2	4	2	2	1	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0			

KKG #88		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	1	1	4	1	3	0	3	3
12	2	19	6	10	5	7	1	10	0	4	2
2	0	2	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0			

Pwesk@PIF #74		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0

0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0	2
1	1	0	0	2	1	2	4	1	4	0	4
0	6	0	10	9	11	6	3	3	8	4	6
0	4	1	3	1	2	0	0	0	0		

Pebb #71		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	1	0	1	0	3	0
3	3	6	2	5	2	7	4	4	3	7	2
2	0	1	1	2	2	8	1	10	2	5	1
7	2	0	0	1	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0			

Pwa@PIF #73		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	1	0
1	2	2	1	2	0	2	1	3	0	2	1
7	2	6	4	9	7	7	4	9	2	8	4
6	2	1	1	1	0	1	0	0			

PIF #75		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0
0	0	1	1	0	0	0	0	1	1	3	0
10	6	10	4	8	5	10	5	9	3	10	3
4	0	2	1	2	0	0	0	0			

Pwcsk L #79		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0

0	0	0	0	0	0	0	0	0	0	0	0
2	1	3	2	1	0	2	0	3	0	5	1
3	3	4	5	11	4	5	3	12	3	7	4
5	2	5	0	2	0	0	0	2			
Pesk Br #81		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0	1
2	1	0	1	3	0	3	0	3	0	6	2
5	1	9	8	9	5	10	5	8	0	6	2
3	1	1	1	1	0	1	0	1			
MRO #68		0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	3	1	1	3	7	2	4	2
9	3	20	4	9	7	4	2	7	0	3	2
3	1	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0			
Fall											
1991											
		32	33	34	35	36	37	38	39	40	41
42	43	44	45	46	47	48	49	50	51	52	53
54	55	56	57	58	59	60	61	62	63	64	65
66	67	68	69	70	71	72	73	74	75	76	77
78	79	80	81	82	83	84	85	86	87	88	89
90	91	92	93	94	95	96	97	98	99		
MRO #111		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0

0	0	2	0	2	1	9	5	8	4	7	4
11	5	16	5	8	5	4	0	0	0	2	0
1	0	1	0	0	0	0	0	0			

McI #112		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	1
1	1	3	1	5	2	10	6	14	4	9	6
11	1	8	4	2	1	4	1	2	0	1	0
0	0	0	0	1	0	0	0	0			

BDW #113		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	3	1	1	2
0	0	7	3	15	6	11	3	7	4	6	2
8	2	2	3	1	2	2	3	1	0	2	0
0	0	1	0	0	0	1	0	0			

BDE #114		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	3	0	2	2	3	2	4	2
6	3	11	1	10	5	12	2	13	1	8	4
5	0	1	0	0	0	0	0	0			

Graf #115		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	1	1
3	2	2	3	13	6	8	5	13	5	8	5

13	4	3	1	2	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0			
MMK #116		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	2	1	3	1	5	2	5	2	4	6
15	5	17	6	10	4	4	0	3	1	4	0
0	0	0	0	0	0	0	0	0			
Jakes #117		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	1	1	3	6	2	7	4
12	4	11	4	14	2	8	2	8	1	1	0
2	1	1	3	0	0	1	0	0			
J Bay #118		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	1	5	4	4	3	8	5
10	4	16	5	10	3	9	3	4	0	1	0
3	0	0	0	0	0	1	0	0			
Snake #119		0	0	0	0	0	0	0	0	0	2
4	1	5	1	4	1	7	2	9	0	3	2
3	1	4	2	2	0	3	0	3	1	1	0
4	1	2	0	9	0	0	2	3	2	2	0
3	2	5	0	2	0	0	1	0	0	1	0

0 0 0 0 0 0 0 0 0

Back #120 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 2 0 0 0
1 1 5 2 5 4 22 8 12 6 12 3
8 3 3 3 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0

Hil #122 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 1 0 0 1 5 0 7 2
6 0 8 2 11 6 5 3 6 5 5 2
10 5 4 0 2 0 2 0 1 1 0 0
0 0 0 0 0 0 0 0 0

McG #123 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 3 1 4 4
10 2 11 6 17 3 13 4 4 2 2 2
3 0 0 0 2 1 1 1 0 0 2 0
0 0 1 0 1 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0

L Pesk #125 0 0 0 0 0 0 2 1 6 3
5 1 3 3 6 1 5 3 5 3 6 2
3 2 2 1 2 0 3 0 3 1 5 1
3 2 5 0 4 1 2 0 3 0 0 0
1 1 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0

Mnt #130		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	1	0	2	0
5	1	5	2	7	0	6	1	4	3	0	1
1	1	1	1	2	0	0	1	5	3	9	2
14	6	9	2	1	1	1	0	1			

M Bay #131		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	3	1	3	3	5	3	8	4
14	3	18	5	11	3	5	2	5	1	1	1
0	0	0	0	0	0	0	0	0			

Fall 1992

		32	33	34	35	36	37	38	39	40	41
42	43	44	45	46	47	48	49	50	51	52	53
54	55	56	57	58	59	60	61	62	63	64	65
66	67	68	69	70	71	72	73	74	75	76	77
78	79	80	81	82	83	84	85	86	87	88	89
90	91	92	93	94	95	96	97	98	99	100	101
102	103	104	105	106	107	108					

J Bay #134		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
1	1	3	1	15	0	12	4	8	11	7	5
7	4	4	2	5	0	2	2	1	0	0	0
2	0	1	0	0	0	1	0	0	0	1	0
0	0	0	0	0	0	0					
Graf #140		0	0	0	0	0	0	0	0	0	0

0	0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	3	0	0	1	4	2	4	4
11	5	16	5	15	3	7	2	6	1	4	0
3	1	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0					
BDW #133		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0
4	0	6	2	8	5	16	7	12	6	7	2
5	4	8	1	0	2	2	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0					
MRO #164		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	3	1
2	1	6	1	12	2	15	3	10	5	12	3
9	3	8	1	1	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0					
Back #152		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	1	0	2	0	2	1
8	3	7	5	10	4	18	1	9	2	5	0
5	0	1	1	0	1	2	2	4	0	1	2
2	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0					
Jakes #167		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	1	2	2	14	4	10	3	15	4	10	4
11	2	5	3	2	0	2	0	1	0	1	1
2	0	0	0	0	0	1	0	0	0	0	0

0	0	0	0	0	0	0					
M Bay #143		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0	0
0	0	2	1	14	1	17	7	10	4	9	2
7	2	9	3	5	0	3	0	0	0	0	0
0	0	2	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0					
Snake #159		1	0	1	0	0	0	3	0	2	0
0	0	4	1	3	0	0	0	1	0	0	0
0	0	2	0	5	1	6	2	4	2	4	2
2	2	10	0	8	2	7	2	8	3	2	1
1	0	4	1	1	0	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0					
MMK #136		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	5	1
2	1	5	2	14	7	13	5	7	4	8	4
9	2	3	1	3	0	2	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0					
MRO #139		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0	0
3	2	1	2	9	3	10	4	10	5	8	2
5	2	10	4	3	1	8	0	1	2	1	0
1	0	1	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0					
Mnt #144		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0	0
5	3	2	3	10	1	8	2	11	1	10	4

8 2 11 3 4 2 2 1 1 0 3 0
2 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0
L Pesk #155 0 0 0 0 0 0 11 1 2 0
3 3 7 3 5 0 4 0 2 1 6 0
3 0 3 0 6 2 14 3 4 2 10 1
5 1 10 0 3 1 3 0 1 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0
Pwesk M #153 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 1 0 2 0 1 1
2 1 2 2 4 2 5 2 7 1 3 0
0 1 2 1 5 1 0 1 0 0 0 0
0 0 0 0 1 0 4 0 5 4 6 0
7 3 7 1 9 3 3 0 0 0 0 0
0 0 0 0 0 0 0
MMK #166 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 2 0 1 1
2 1 5 2 5 1 17 6 14 3 8 4
11 1 6 1 0 0 1 0 1 0 2 0
4 0 0 1 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0
M Bay #163 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 2 0 0 0
2 1 6 3 4 3 7 6 6 1 10 4
13 3 9 1 2 0 1 0 3 0 4 0
3 0 2 1 2 0 0 0 0 0 0 0
0 0 0 0 0 0 1

Spring 1993		32	33	34	35	36	37	38	39	40	41
42	43	44	45	46	47	48	49	50	51	52	53
54	55	56	57	58	59	60	61	62	63	64	65
66	67	68	69	70	71	72	73	74	75	76	77
78	79	80	81	82	83	84	85	86	87	88	89
90	91	92	93	94	95	96	97	98	99	100	101
102	103	104	105	106	107	108	109	110	111	112	
Mnt #173		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	2	1	4	2	7	2
10	1	11	3	15	2	9	3	8	1	5	2
4	1	5	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	
Cob #187		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0
1	0	1	0	6	0	7	2	2	1	9	2
9	4	13	6	12	1	7	1	2	2	7	1
1	0	1	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	
Puz #175		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	2	0	0	0	2	1
2	0	3	1	12	4	5	4	11	3	9	5
12	3	8	1	4	2	0	0	1	0	2	0
1	1	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	
Back #183		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	2	4	1	3	3	5	1	4	2
2	1	4	2	4	0	3	1	3	1	5	2
11	3	8	5	6	1	3	0	3	1	2	0

0 0 0 0 1 0 2 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0

Graf #194 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 1 0 1 1 5 9

6 26 6 11 3 11 2 7 1 4 0 2

0 1 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0

McI #170 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 1 3 5 1

3 1 11 2 23 3 16 4 6 1 3 1

2 1 4 1 0 1 2 0 3 0 2 0

0 0 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0

Pwesk@PIF #179 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 1 0 1

0 0 0 0 0 0 0 1 0 0 0 1

0 1 0 6 0 1 0 1 0 0 1 5

0 6 1 10 6 5 4 11 3 12 6 9

2 1 3 1 0 0 0 0 0 1 0 0

Pwesk L #186 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 2 2 6 1 6 3

6 2 13 3 18 5 13 4 2 2 0 0

2 1 1 1 1 0 2 0 1 0 0 0

1 0 1 0 1 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0

PIF #180 0 0 0 0 0 0 0 0 0 0

1 0 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0 0

0	0	0	0	0	0	2	1	1	0	1	0
0	0	1	0	1	0	1	0	1	1	5	0
6	1	12	3	12	0	11	4	14	1	10	1
4	3	1	0	0	0	1	0	0	0	0	
Pwa@PIF #181		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	2	0	0	0	0	0	0	0
0	0	3	0	2	1	1	0	2	0	2	0
5	2	8	3	15	4	6	3	14	3	6	4
5	2	3	0	1	0	2	0	0	0	0	
Pwa L #178		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	1	0	1	1
1	3	2	0	1	0	0	0	0	1	1	2
6	5	7	3	10	3	14	5	13	8	4	1
5	0	1	0	0	0	0	0	0	0	0	
L Pesk #189		0	0	0	0	0	0	1	0	1	0
0	0	0	0	0	0	1	0	0	0	1	0
0	0	1	0	0	0	1	2	7	3	7	2
5	5	12	5	18	7	11	1	4	0	0	1
3	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	
M Bay #174		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0	0
3	1	4	1	6	5	11	8	10	4	8	3
11	2	8	2	5	1	3	0	2	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	
J Bay #198		0	0	0	0	0	0	1	0	0	0

0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	2	1
2	1	5	1	13	3	10	5	17	3	8	2
11	3	5	0	1	0	2	0	0	0	2	0
0	0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0	
KKG #197		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	3	0
6	0	7	4	11	4	10	4	13	3	10	3
7	3	4	2	2	1	1	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	
MMK #195		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	1	2	1	1
1	4	4	2	10	2	13	6	8	6	7	7
7	3	1	2	3	0	2	1	2	0	0	0
0	0	1	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0	
Jakes #196		1	0	0	0	0	0	1	0	0	0
1	0	1	0	0	0	0	0	0	0	1	0
0	0	0	0	2	1	0	1	3	1	7	5
5	2	4	4	12	5	7	5	3	3	6	1
8	1	2	0	1	0	2	0	1	0	2	0
0	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	
MRO #190		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0	0
0	0	1	0	0	0	0	0	0	1	1	1
2	0	5	2	7	4	14	4	13	4	6	5
15	5	4	1	2	1	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0

0	0	0	0	0	0	0	0	0	0	0	
Pebb #177		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	1	0	0	0	0	0
0	0	0	2	3	1	6	0	9	0	9	0
17	1	14	5	10	4	0	3	7	0	2	0
3	1	1	0	0	0	0	0	0	0	0	
Snake #193		0	0	0	0	0	0	3	0	2	2
3	1	5	1	4	2	8	2	6	3	6	3
4	1	3	1	4	1	0	3	7	1	3	0
0	0	4	0	4	0	4	0	1	0	2	0
1	0	2	1	0	0	1	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	
Hil #185		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	5	3	7	1
7	3	14	5	16	7	16	1	4	1	5	0
1	1	2	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	
BDW #171		0	0	0	0	1	0	0	0	1	1
1	0	0	0	0	0	0	0	1	0	0	0
1	0	0	0	1	0	0	0	2	0	2	2
1	0	5	1	7	2	3	0	8	2	2	1
3	0	7	0	2	1	7	1	3	0	2	0
7	1	1	1	4	1	1	0	1	0	6	0
1	1	0	0	1	0	2	0	0	0	1	

Ontario 1991

		32	33	34	35	36	37	38	39	40	41
42	43	44	45	46	47	48	49	50	51	52	53

54	55	56	57	58	59	60	61	62	63	64	65
66	67	68	69	70	71	72	73	74	75	76	77
78	79	80	81	82	83	84	85	86	87	88	89
90	91	92	93	94	95	96	97	98	99	100	101
102	103	104	105	106	107	108	109	110	111	112	
Leonard		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	1	0	1	1
6	0	14	2	5	3	11	1	5	0	5	0
1	0	6	2	7	0	7	2	7	1	6	0
2	0	2	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	
Joseph	0	0	1	0	0	0	0	0	0	0	0
0	0	2	1	1	3	4	8	4	13	7	17
6	6	5	5	2	5	1	2	2	0	1	0
0	3	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0		
McKay	0	0	0	0	0	0	0	0	0	0	0
0	1	1	2	0	0	1	0	1	3	0	5
1	11	3	4	3	13	7	18	4	11	2	2
1	4	1	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0		
Plastic #200		0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	4	0	2	0
6	0	8	0	5	0	8	0	1	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0
3	0	2	1	6	0	5	3	5	1	13	2

7	0	5	1	4	0	3	0	2	0	2	
Thirty #203		0	0	0	0	0	0	0	0	1	0
4	0	1	0	5	1	3	0	2	0	2	1
2	1	2	1	5	3	7	2	5	3	8	4
12	3	6	2	7	1	4	1	1	1	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	
Bwood #201		0	0	0	0	0	0	0	0	0	0
1	0	0	1	6	3	9	7	19	7	12	4
13	4	3	1	5	2	2	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	

Appendix 8. Scanning electron measurements for valves showing all five characters. All measurements are in μm. (vl: valve length, hp: head pole, fp: foot pole, mc: mid cell width, spi: spines in 6 μm)

Lake #	vl	hp	fp	mc	spi
53	73	3	2.31	1.36	4
53	73	3.8	2	1.2	6
53	70	3.8	1.76	1.2	4
53	73	3.4	2	1.36	4
53	70	2.7	2	1.36	4
53	76	3.4	2.31	1.5	4
132	59	3.4	1.76	1.76	6
132	62	3.4	1.6	1.76	4
132	60	2.8	1.76	1.76	4
132	60	3.4	2	1.6	5
132	59	3.8	1.9	2	4
132	58	3.8	1.76	1.55	6
170	65	3.7	1.2	2	7
170	75	3.6	1.8	1	5
170	62	3.2	1.45	1.5	4
170	61	3.6	1.5	1.36	4
170	62	3.4	1.1	1.5	6
170	74	3.4	2	1.5	4
170	65	3.8	1.36	1.36	5
170	61	3.6	1.5	1.4	5
31	75	4.3	1.36	1	3
31	65	4.3	1.2	1.2	3
31	68	4.3	1	1.5	3
31	70	4.3	1.5	1.36	3
31	60	4	1	1	3
31	70	4.3	0.86	1.2	4
31	80	4	0.6	1.5	4
83	65	3.8	1.76	1.5	3
83	70	4.3	1.36	1.76	4

83	60	3.8	1.5	1.7	4
83	70	4.5	1.36	1.76	3
83	65	4.3	1.5	1.76	3
115	70	4.3	1	1.2	4
115	68	4	1.6	1.36	4
115	60	4.3	1.2	1.5	4
115	72	4.3	1	1.36	4
115	72	4.3	1.2	1.2	4
115	70	4	1.5	1.76	4
115	70	4	1.5	1.5	4
140	61	4.5	1.36	1.76	4
140	58	4.3	1.2	1.38	3
140	63	4.6	1.76	1.6	4
194	62	4	1.5	1.1	4
194	70	4.3	1.36	1.2	3
194	60	4.7	1.5	1.76	3
194	68	4	1.76	1.5	4
194	63	3.8	1.36	1.76	4
194	53	3.4	1.5	1.2	4
194	58	3.4	1.36	1.76	5
34	73	2.31	1.5	1.76	4
34	86	5	1.38	1.2	4
34	80	5	1.76	1.21	4
34	70	4.3	1.2	1.5	4
34	70	4.3	1.5	1.5	4
34	75	4.3	1	1	5
34	83	4.5	1.36	1.5	4
34	80	4.5	1.5	1.36	3
34	75	4.3	1.76	1.6	3
34	70	4.3	1	1.76	4
34	80	5	1	1.3	3
34	86	5	1.36	1.36	3
34	70	4.3	1	1.5	4

34	65	4.3	1	1.1	3
185	65	3.7	1.2	1.36	4
185	60	4	1.2	1.36	4
185	55	4.3	1	1.36	4
185	60	4.5	1.36	1.36	3
185	63	4	1.36	1.2	4
185	60	4.3	1.2	1.2	4
185	60	4.3	1.2	1.1	4
61	62	4.3	1.1	1.6	4
61	65	4.5	1.76	1.76	4
61	65	4.3	1.5	1.36	5
61	63	4.1	1.5	1.8	5
61	61	4.5	1.5	2	3
92	76	4.8	1.36	1.5	4
92	76	4.6	1.5	1.76	4
92	70	3.8	1.5	1.36	4
92	70	4.3	1.76	1.5	5
92	77	4.6	1.6	1.76	5
92	70	4.3	1.5	1.76	4
116	72	3.3	1.5	1.5	5
116	70	3.4	1.36	1.3	4
116	70	3.8	1.5	1.2	3
116	70	4	1.76	1.76	3
116	65	3.6	1.36	1.5	4
116	70	3.8	1.5	1.5	4
116	70	3.8	1.6	1.36	4
136	73	3.8	1.36	1.2	5
136	74	3.8	1.5	1.2	5
136	72	3.8	1.2	1.5	4
136	65	3.8	1.36	1.2	4
136	65	3.6	1.36	1.1	3
136	65	4	1.2	1.2	5

136	73	4.5	1.6	1.1	3
166	84	5.5	1.36	1.5	3
166	64	3.8	1.76	1.5	3
166	86	4.3	1.5	1.36	3
166	73	3.8	1.5	1.5	5
166	65	4	1.5	1.2	3
195	60	4.7	1.76	1.5	3
195	70	4.3	1.4	1.76	3
195	74	4.8	1.76	1.76	3
195	63	5	1.7	1.5	3
195	68	5	1.36	2.5	3
195	72	4.8	2	2.3	3
36	63	1.76	1	1.2	6
36	70	3.8	1	1.36	4
36	61	4.3	1	1.5	4
36	75	3.8	1.5	1.2	4
65	75	2	1	1.2	5
65	70	2.5	1.36	1.76	4
65	75	5	1	1.36	4
65	75	3.8	1.2	1.2	4
65	75	3	1.36	1.5	4
65	75	4.3	1	1.36	4
65	65	1.76	0.86	1	4
65	86	5	1.2	1.2	3
131	70	4.3	1.36	2	5
131	75	3.8	1.5	1.2	3
131	75	4.3	1.36	1.36	4
131	75	3.8	1.2	1.2	4
143	65	3.7	1.36	1.45	4
143	70	4.5	1.36	1.76	5
143	70	4.1	1.36	1.2	4
143	68	4.3	1.2	1.76	4
143	65	3.8	1.5	1.5	4

143	73	4.3	1.5	1.76	4
174	68	4.3	1.2	1.5	3
174	76	4.3	1.5	1.5	5
174	62	4.3	1.36	1.76	4
174	68	4.3	1.36	1.36	3
174	86	4.3	1.5	2.31	4
174	63	3.8	1.2	1.5	3
174	57	4.3	1.2	2	3
29	86	5	2.3	3	3
29	84	5	1.76	1.5	3
29	100	4.3	1.76	1.76	3
29	86	4.7	1.76	1.5	4
68	70	4.3	1.2	1.36	5
68	70	4.7	1.36	1.36	4
68	73	4.3	1.76	1.76	3
68	70	3.8	1.76	1.65	4
68	76	4.3	1.36	1.5	4
68	75	4.3	1.36	1.36	4
111	70	3.8	1.5	1.76	4
111	70	3.8	1.5	1.5	4
111	72	3.8	1.5	1.5	3
111	86	5.5	2.31	1.5	3
111	70	3.8	2	1.5	3
111	65	3.4	1.76	1.5	3
111	70	3.8	1.36	1.1	3
111	75	3.8	1.76	2	3
139	70	4	1.36	1.36	4
139	65	3.4	1.5	1.5	4
139	70	4.6	1.5	2	4
139	75	4.6	1.76	1.76	4
139	70	3.8	1.5	1.5	4
139	68	4.5	1.6	1.6	3
139	65	4	1.5	1.36	4

190	70	3.8	1.2	1.36	4
190	62	4.3	1.2	1.6	4
190	70	4.5	1.6	1.5	4
190	65	4.1	1.5	1.45	4
190	75	4.5	1.36	1.76	4
190	65	4.3	1.5	1.5	3
190	86	5	1.36	1.2	4
73	86	3	2.7	1.5	4
73	86	2.7	2.7	1.76	3
73	88	4.5	2.5	1.76	3
73	95	5	2.31	1.5	4
73	82	4.3	2.5	1.55	3
73	82	3.8	2.5	1.25	3
73	93	5	2.5	1.76	4
73	93	5	2.7	2	3
73	86	4.3	2.31	1.5	4
148	68	3.4	1	1.6	6
148	68	4.3	1.5	1.65	4
148	70	3.6	0.9	2	6
148	61	3.6	1.36	1.36	4
148	72	3.8	1	1.76	7
148	84	4.5	2.7	1.76	7
148	63	4.3	1.2	1.36	3
181	76	4.3	2.5	1.5	3
181	76	4.5	2.5	2	4
181	87	5.3	3.4	2.2	4
181	77	5	2.7	1.9	3
181	65	4.3	1.2	1.5	3
181	80	5	2.7	1.76	3
1	86	5	2.5	1.7	2
1	96	3.8	2.7	1.5	2
1	90	4.3	2.3	1.5	3
1	90	3.8	3	2.5	4

1	88	5	3	1.76	4
25	75	4.3	2.31	2.1	2
25	86	5	2.5	1.5	4
25	95	4.3	2.31	1.6	3
25	86	5	2.7	1.6	4
25	86	5	2.5	1.5	3
25	86	4	1.2	1.8	4
25	80	4.3	1.36	1.76	4
25	90	5	2.7	1.7	4
74	90	5	3.4	1.9	2
74	88	5	3	1.85	3
74	87	4.3	2.7	1.6	4
74	90	4.3	2.52	1.36	4
74	90	5	2.7	1.5	4
74	88	4.3	2.7	1.76	3
74	80	4.3	2.5	1.76	3
78	86	4.3	2.15	1.76	2
78	75	5.1	2.7	2	3
78	88	5.1	3	1.76	4
78	86	4.3	2.15	1.76	4
78	94	3.4	2.7	2.15	3
78	74	3.6	1	1.5	3
79	75	4.3	1.2	1.5	4
79	88	5	2.7	1.76	3
79	90	4.3	2.5	1.76	3
79	86	2.31	2.31	1.5	3
79	86	5	2.7	1.6	3
79	94	5	2.31	1.85	4
79	77	3.8	1.2	1.5	3
79	75	3.8	1	1.2	3
153	50	3.4	1	1.76	4
153	57	3.4	1.2	1.5	4
153	59	3.4	1.2	1.76	3

153	57	3.4	1	1.76	6
153	72	4	1.36	1.36	6
153	63	4.3	1.76	1.76	4
153	60	3.4	1.36	2	5
179	82	4.7	2.5	1.76	3
179	90	4.8	2	1.76	4
179	87	4.3	2.7	2.2	3
179	88	4.8	3.4	2	3
179	72	3.8	1	1.76	4
179	90	5	3	1.5	3
179	86	5	2.7	2	3
186	86	3.8	2.5	1.5	4
186	60	4	1.5	1.5	4
186	60	2.7	1.2	1.36	4
186	62	4.5	1.76	1.5	3
186	64	4.3	1.5	1.2	3
125	60	4.3	1.36	1.36	3
125	43	3.8	1.36	2	2
125	65	4.3	1.36	1.5	3
125	45	3.8	1.36	1.76	2
125	60	4.3	1.76	1.76	4
155	60	4.1	1.3	1.25	4
155	45	4.3	1.36	2	5
155	55	4	1.2	1.5	4
155	63	3.8	1.36	1.36	5
155	60	4.3	1.78	1.2	5
155	45	3.8	1	1.5	8
155	50	4	1.5	1.6	4
155	50	3.8	1.2	1.36	5
189	65	4.3	1.5	1	4
189	61	3.8	1.2	1.76	3
189	61	4	1.2	1.76	4
189	55	4.3	1.2	1.2	4

189	60	4.3	1.15	1.76	4
189	62	4.3	1.2	1.5	4
69	70	4.3	1.2	2.31	4
69	73	4.3	1	3	4
69	60	4.3	1.36	1.76	5
69	80	3.8	1.2	1.2	5
69	77	4.5	1.5	1.5	4
69	74	4.3	1.5	1.76	3
69	58	3.8	1.2	1.76	5
69	73	4.3	1.2	1.36	5
168	60	4	1	1.5	7
168	75	3.4	2	1.76	7
168	60	3.8	1.5	1.76	4
168	65	3.8	1.5	1.76	5
168	70	3.8	1.5	1.2	5
168	60	3.8	1.36	1.5	2
168	60	3.8	1.5	1.5	4
168	70	3.8	1.76	1.5	4
168	70	3.8	1.76	1.25	3
168	70	3.8	1.5	1.76	3
169	65	3.8	3	1.5	4
169	73	3.3	0.75	1.5	5
169	86	4	2	1.5	3
169	65	4.8	1.76	2.31	6
169	67	4.3	1.76	2.45	4
169	65	4.3	1.5	2	3
169	63	4.3	1.37	1.76	5
169	63	3.8	2	2	4
169	67	4.3	1.76	2	3
66	80	4.3	1.2	1.65	2
66	80	3.5	1.36	1.36	4
66	70	2.7	1.5	1.2	3
66	63	4.3	0.86	1.5	4

66	84	4.6	1.36	1	4
66	75	4.4	1.2	1.2	5
66	84	4.8	1.5	1	3
66	68	4.2	1.2	1.2	3
66	86	4.3	1.2	1.36	2
144	62	3.4	1.2	1.76	4
144	61	3.6	1	1.76	4
144	70	4	1.76	2	5
173	75	4.3	1.36	1.76	3
173	65	4.3	1.2	1.36	4
173	68	3.8	1.5	1.36	4
173	74	4.3	1	1	3
173	73	4	1.1	1.5	3
173	60	3.8	1.5	1.76	3
43	75	3.8	1.76	1.5	5
43	75	5.1	1.36	1	3
43	70	3.5	1.76	1.6	3
43	73	5	1.5	1.36	3
43	70	3	1.65	1.36	4
43	75	3.8	2	2	3
43	70	3.8	1.36	1.6	4
187	72	3.8	1.2	1.5	4
187	75	4.3	1.2	1.5	3
187	63	4.3	1.2	1.5	3
187	72	4.5	1.2	1.5	2
187	75	4	1.2	1.2	3
187	76	5	1.2	1.36	3
45	60	3	1	1.2	6
45	52	2.7	1.5	1.76	5
45	60	2.7	1	1.5	4
45	55	3	1.36	1.2	5
45	65	3.8	1.36	1.5	5
45	60	2.7	0.86	1.36	3

77	63	3	1.5	1.76	3
77	60	3.8	1.36	1.36	4
77	60	3	0.86	1.76	5
77	62	3	1.2	1.36	5
120	60	4.3	1.36	2	4
120	60	3.8	2.5	3	2
120	60	3.5	1	1.6	4
120	60	3.4	1	1.5	5
120	55	3.8	1	2	4
152	52	3.4	1.2	1.5	6
152	52	3.4	1	1.36	5
152	52	3.2	1.2	1.5	5
152	55	3.6	1.2	1.8	4
152	55	2.7	1.1	1.5	4
152	75	3	1.2	1.36	5
152	48	3.2	1.2	1.5	4
183	55	3	1	1	5
183	70	3.4	1.1	1.2	5
183	65	3.4	1	1.36	4
183	57	3	1.36	1.36	4
183	65	3.8	1.1	1.5	5
183	65	3	0.95	1.5	4
175	65	3.8	2	1.5	4
175	65	3.8	1.36	1.36	3
175	65	4	1.5	1.5	4
175	65	4.3	1.6	1.6	3
175	60	3.8	1.2	1.36	4
177	80	5	2.5	1.76	2
177	77	4.6	2.7	1.5	4
177	80	5	2.5	1.5	3
177	72	5	2.33	1.76	4
177	83	5	2.5	2.31	4
177	77	4.8	2	1.5	4

177	84	4.5	2.7	1.5	4
41	76	4.3	1	1.5	4
41	75	4.5	1.1	1.36	5
41	73	4.3	1.36	1.5	4
89	70	3.8	1.2	1.5	4
89	72	2.5	1.2	1.2	4
89	77	4.3	1.36	1.5	3
89	73	4.3	1.2	1.36	4
89	90	3.8	1	1.76	3
89	73	4.3	1.5	1.25	3
118	73	4.3	1.76	1.78	4
118	60	4	1.5	1.76	4
118	68	3.8	1.5	1.36	5
118	60	3.8	1.43	1.5	4
118	70	3.8	1.5	1.2	4
118	73	3	1	1.2	4
134	73	4.3	1.36	1.76	5
134	80	4.3	1.2	1.76	5
134	76	4.6	1.76	2	5
134	75	4.3	1.2	1.76	4
134	70	3.8	1.5	1.25	5
134	70	3.8	1.5	1	5
198	64	4.3	1.76	2	4
198	62	4.7	1.36	2	4
198	88	4.3	1.36	1.5	4
198	65	4.1	1.5	1.5	4
198	65	4.3	1.36	1.45	4
198	68	4.1	1.36	1.76	4
88	74	4.6	1.2	1.2	4
88	70	4.3	1.5	1.2	4
88	74	4.3	1.5	1.76	3
88	75	4.3	1.36	1.5	4
88	68	4.5	1.5	1.65	3

88	74	4.5	1.76	1.55	3
197	67	4.5	1.2	1.36	4
197	72	5	1.2	1.76	4
197	65	4.5	1.2	1.5	3
197	65	4.3	1.36	1.76	4
197	60	4.3	1.5	1.76	5
197	62	5	1.6	2	3
10	55	3.8	0.8	1.36	4
10	80	4.3	1.36	1.2	4
10	61	4	1.2	1.36	4
10	86	4.1	1.1	1.1	5
10	78	4	0.86	1.5	4
40	83	4.3	1.5	1.76	3
40	88	4.3	1.36	1.36	4
40	77	4.3	1.5	1.36	5
87	68	3.4	1.85	1.76	5
87	63	4.4	1	1.65	3
87	65	2.8	1.1	1.55	3
87	60	3.6	1.3	1.76	4
87	33	3.8	0.95	1.45	4
117	65	3.8	1.5	1.36	4
117	42	3.4	1.36	1.2	5
117	70	3.8	1.3	1.36	5
117	72	3.8	1.36	1.3	4
117	70	4.3	2	2	5
117	72	3.8	1.5	1.52	5
117	70	4	1.5	1.5	4
167	72	4	1.36	1.36	5
167	72	4.5	1.7	1.76	5
167	68	3	1.5	1.36	5
167	65	3.8	1.5	1.36	4
167	75	4.3	1.5	1.76	5
167	70	3.4	1.55	1.1	4

196	68	4.7	1.1	1.76	4
196	70	5	1.36	1.5	4
196	63	5	1.2	1.5	4
196	55	4.7	1.36	1.76	4
23	100	5	3	2	4
23	86	5	5	2	3
23	100	5	2.7	1.65	4
23	95	2.7	1.5	1.76	4
75	93	4.8	2.31	1.76	3
75	85	5	2.5	1.76	4
75	86	4.5	2.5	1.76	3
75	88	3.8	2.5	1.5	3
75	75	4.3	1.2	2	2
75	76	3	1	1.76	3
75	87	5.1	2.7	1.5	3
149	90	5	3	1.36	4
149	86	4.7	2.5	1.76	4
149	90	5	3	1.4	4
180	80	5	2.9	1.55	4
180	80	4.7	3	1.76	4
180	80	5	2.5	1.76	4
180	88	5	2.7	1.7	3
180	85	5	3	1.76	3
180	85	5	2.7	2	3
180	80	5	2.7	1.76	3
7	86	4.7	2	1.5	3
7	90	2.5	1.76	1.2	2
7	100	4	2.5	1.55	3
7	90	5.1	2	1.76	3
7	90	5	2.8	1.5	3
81	83	5	2.31	1.76	3
81	75	4	1	1.5	3
81	86	4.3	2.7	1.36	3

81	80	4.3	2.5	1.5	3
188	74	4.3	1.27	1.5	4
188	75	4	1.2	1.25	3
188	78	4.1	1.36	1.36	4
188	70	4.3	1	1.5	4
188	68	3.8	1	1.36	4
188	74	4.3	1.5	1.2	4
19	100	6	3	1.76	4
19	100	6	2.7	1.2	4
19	100	5.5	3.6	1.5	3
19	100	4.3	2.3	1.2	3
21	86	4.2	1.5	0.75	5
21	84	4.7	3	1.5	4
21	84	4.3	2.4	1.5	4
21	86	4.5	2	0.6	4
21	86	3.4	2.6	0.7	4
72	86	4.5	2.5	1.4	3
72	84	5	2.7	1.5	3
72	86	3.8	2.5	1.6	4
72	86	4.3	2.5	1.36	3
72	85	4.3	2.31	2	3
72	86	4.3	2.5	1.5	4
178	82	5	2.31	1.5	4
178	86	5.5	2.7	1.36	4
178	86	5	2.7	2	4
178	68	4	1.2	1.76	4
178	68	4.5	1	2	4
178	86	3.8	3	1.76	3
178	80	4.7	2.31	1.76	4
57	58	4.3	1.1	1.5	4
57	63	3.4	1.36	1.76	4
57	58	4.3	1.2	1.5	4
57	63	4.3	1.2	1.6	4

57	58	4.3	1.2	1.76	4
57	60	4.3	1.2	1.36	4
82	55	3.8	1.5	1.36	3
82	50	3.8	1.5	1.76	2
82	43	3.8	1.2	1.5	2
82	55	4.3	1	2	5
119	39	3.5	0.86	1.2	5
119	45	3.8	1.1	1.76	5
119	76	5	1.36	1.76	4
119	34	3.2	1	1.76	4
119	40	3.6	1	1.76	4
159	52	3.8	1.2	1.78	5
159	70	4.5	1.5	1.76	4
159	52	3.4	1	1.76	5
159	45	3.6	1	1.5	8
159	53	3.8	0.86	1.5	6
159	65	4.3	1.5	1.76	4
159	47	3.4	1.36	1.76	4
159	62	4.3	1.5	1.76	3
193	55	3.6	1	1.5	3
193	51	3.8	1.2	1.76	4
193	51	3.6	0.9	1.5	4
193	52	3.9	0.86	1.2	4
193	53	3.9	0.86	1.2	4
35	80	5	1.1	1.5	4
35	73	4.3	1.2	1.36	2
35	75	4.3	1.5	1.2	3
35	70	4.3	1	1.5	3
35	70	4.3	1.76	1.6	3
113	63	4.3	1.36	1.76	3
113	72	4	1.2	1.5	4
113	65	4.5	1	1.5	5
113	62	4.3	1.2	1.5	5

113	65	4	1.2	1.76	3
113	65	4	1.36	1.9	5
133	76	5	1.1	1.76	5
133	78	4.7	1.2	1.36	4
133	76	4	1.36	0.9	4
133	63	3.8	1	1.5	5
133	65	4.3	1.36	1.5	4
133	76	4.8	1.2	1.76	3
171	57	3.4	0.86	1.2	5
171	70	4.3	1.2	1.5	4
171	55	3.6	1	1.5	5
171	75	5.2	1.36	1.9	4
171	90	4.3	1.36	1.5	4
171	65	4.6	1.2	1.36	3
200	65	4.1	1.5	1.76	8
200	87	3.8	1.5	1.5	4
200	86	4.5	2.5	1.2	3
200	61	4	1.5	1.76	7
200	93	4.5	2.5	1.1	4
200	96	4.9	2.3	1.76	4
203	60	3.8	2.7	2	8
203	75	3.8	2.7	1.7	7
203	38	3.8	1.5	1.76	5
203	59	4.1	2.31	1.8	6
203	75	3.8	3	1.5	3
203	76	3.6	2.5	1.36	4
203	76	4	2.7	1.76	4
203	56	3.8	2.31	1.7	4
203	58	3.6	2.6	2	7
203	53	3.8	2.31	2.31	5
203	55	4	2	2	3
201	45	3.4	1.2	1.76	5
201	46	3.8	1.1	1.5	4

201	43	3.8	1	1.76	6
201	44	3.8	1	1.76	6
201	44	3.8	1	1.76	6
201	45	3.6	1.37	1.76	5
201	45	3.2	1.2	1.76	5
201	45	3.8	1.1	1.55	5
201	48	3.4	1	1.5	7
201	45	3.4	1.2	1.5	5
201	47	3.8	1.2	2	5
201	43	3.6	1.2	1.5	4
201	45	3.2	1	1.5	5
201	62	3.8	1.2	1.5	6

Appendix 9. Colony size for spring 1990, 1991, 1993 and fall 1991

Spring 1990	4	5	6	7	8	9	10	11	12	13
14 15	16	17		Ave						
Mnt #51	1	8	13	25	39	7	3	1	2	1
0 0	0	0		7.5						
Puzz #38	43	16	17	10	12	0	0	1	0	0
1 0	0	0		5.45						
Back #45	78	2	1	4	14	1	0	0	0	0
0 0	0	0		4.77						
Bskin #27	56	9	16	8	11	0	0	0	0	0
0 0	0	0		5.09						
Graf #31	15	5	18	24	33	2	0	0	1	0
0 2	0	0		6.85						
BDE #59	8	5	25	24	31	3	3	0	1	0
0 0	0	0		6.92						
McI #53	0	7	6	19	64	5	4	1	1	0
0 0	0	0		8.24						
Pwesk@PIF #25		18	5	16	16	40	4	0	0	0
0 0	0	1	0		6.77					
Pwesk M #4	0	0	8	14	59	2	9	2	3	1
0 2	0	0		8.27						
Pwesk L #3	3	6	12	25	51	2	0	1	0	0
0 0	0	0		7.26						
Pwesk L #1	2	2	5	16	64	6	1	3	1	0
0 0	0	0		7.81						
PIF #23	18	10	21	21	26	3	1	0	0	0
0 0	0	0		6.4						
Pwa@PIF #21	50	11	11	11	14	1	1	0	1	0
0 0	0	0		5.41						
Pwa L #19	19	7	11	22	40	0	1	0	0	0
0 0	0	0		6.61						
Pesk Br. #6	1	0	9	13	64	5	3	4	0	0

0	0	0	0		7.8						
M Bay #36		5	14	26	11	27	9	4	1	2	0
1	0	0	0		7.09						
J Bay #41		2	14	26	26	10	6	0	8	3	4
0	0	0	1		7.43						
MMK #61		3	4	15	24	42	5	3	2	2	0
0	0	0	0		7.47						
Jakes #40		1	3	6	21	61	3	3	0	0	0
0	2	0	0		7.77						
MRO #29		13	27	25	16	14	2	2	1	0	0
0	0	0	0		6.1						
McG #55		0	3	20	29	44	4	0	0	0	0
0	0	0	0		5.86						
Snake #57		18	15	17	17	31	1	0	0	0	1
0	0	0	0		6.38						
Hil #47 7		0	4	4	82	1	1	1	0	0	0
0	0	0		7.66							
BDW #35		1	9	15	25	31	8	2	1	1	2
3	2	0	0		7.75						
BDW #34		1	3	15	27	35	8	3	3	2	2
0	1	0	0		7.78						
BDW #33		0	2	13	27	41	5	3	1	3	3
1	1	0	0		7.95						

Spring 1991

	4	5	6	7	8	9	10	11	12	13	14
15	16	17									

Mnt #66		0	4	11	12	42	8	9	4	2	4
2	1	1	0		8.47						
Back #77		99	0	0	1	0	0	0	0	0	0
0	0	0	0		4.03						

Graf #83	0	0	6	22	68	3	1	0	0	0		
0	0	0	0		7.71							
BDE #90	7	5	22	24	37	5	0	0	0	0		
0	0	0	0		6.94							
McI #93	24	8	9	19	33	2	1	1	0	1		
1	1	0	0		6.68							
Pwesk@PIF #74		0	6	29	29	29	0	5	0	0		
1	1	0	0	0		7.16						
Pwesk M #78	0	0	1	14	78	3	0	1	2	1		
0	0	0	0		8.03							
Pwesk L #79	1	1	2	8	80	3	3	1	0	1		
0	0	0	0		7.98							
PIF #75	0	2	22	17	56	3	0	0	0	0		
0	0	0	0		7.36							
Pwa@PIF #73	7	1	20	22	46	2	0	1	1	0		
0	0	0	0		7.16							
Pwa L #72	2	7	20	13	31	11	7	2	5	1		
0	1	0	0		7.81							
Pesk Br. #81	0	0	5	8	73	2	1	3	1	5		
0	0	2	0		8.4							
L.Pesk #80	2	10	14	34	21	8	4	3	3	1		
0	0	0	0		7.42							
M Bay #65	6	1	11	18	60	2	0	1	0	0		
0	0	0	1		7.47							
J Bay #89	1	1	11	25	54	2	3	1	1	0		
1	0	0	0		7.67							
KKG #88	1	0	4	25	58	7	4	0	1	0		
0	0	0	0		7.82							
MMK #92	0	3	6	26	64	1	0	0	0	0		
0	0	0	0		7.54							
MRO #68	1	0	7	17	70	1	4	0	0	0		
0	0	0	0		7.74							
McG #69	23	7	9	17	33	5	4	1	0	0		

1	0	0	0		6.74						
Pebb #71		1	2	5	19	65	2	0	1	0	2
1	0	2	0		7.98						
Snake #82		65	3	1	8	22	1	0	0	0	0
0	0	0	0		5.22						

Fall 1991

	4	5	6	7	8	9	10	11	12	13	14
15	16	17	18	19	20	21	22	23	24	25	Ave
Mnt #130		0	0	4	11	57	5	7	2	4	3
4	0	3	0	0	0	0	0	0	0	0	0
8.85											
Back #120		13	3	7	13	46	1	2	3	2	4
3	2	0	0	0	0	0	0	0	0	0	1
8.03											
Graf #115		0	0	2	7	61	3	1	3	2	5
2	4	10	0	0	0	0	0	0	0	0	0
9.56											
BDE #114		0	0	2	5	82	4	1	1	2	1
0	1	1	0	0	0	0	0	0	0	0	0
8.28											
Mcl #112		4	3	3	10	72	3	2	1	2	0
0	0	0	0	0	0	0	0	0	0	0	0
7.77											
L Pesk #125		13	2	3	14	54	3	1	1	3	1
1	2	2	0	0	0	0	0	0	0	0	0
7.83											
M Bay #131		52	4	6	10	19	1	4	0	0	0
2	1	1	0	0	0	0	0	0	0	0	0
5.94											
J Bay #118		7	6	19	28	38	0	1	0	1	0

0 0 0 0 0 0 0 0 0 0 0 0
6.94
MMK #116 17 8 12 19 28 6 2 1 2 0
2 1 2 0 0 0 0 0 0 0 0 0
7.21
Jakes #117 6 5 22 31 26 2 0 0 4 1
2 1 0 0 0 0 0 0 0 0 0 0
7.28
MRO #111 14 4 13 20 36 1 3 1 1 2
1 0 2 2 0 0 0 0 0 0 0 0
7.5
McG #123 0 2 4 12 55 3 6 1 5 1
6 1 1 2 0 0 0 0 1 0 0 0
9
Snake #119 17 8 8 7 35 5 6 1 3 2
3 1 2 0 1 0 0 1 0 0 0 0
7.91
Hll #122 0 0 0 1 24 4 4 6 9 7
13 16 16 0 0 0 0 0 0 0 0 0
12.18
BDW #113 0 0 4 6 75 6 2 2 2 1
0 1 1 0 0 0 0 0 0 0 0 0
8.3

Spring 1993

4 5 6 7 8 9 10 11 12 13 14
15 16 17 18 Ave

Mnt #173 44 12 20 15 6 3 0 0 0 0
0 0 0 0 0 5.36
Cob #187 8 7 15 39 28 2 0 0 1 0
0 0 0 0 0 6.84

Puzz #175	45	1	9	16	29	0	0	0	0	0
0	0	0	0	0	5.83					
Back #183	84	6	2	4	3	0	1	0	0	0
0	0	0	0	0	4.4					
Graf #194	0	0	2	4	86	5	2	0	1	0
0	0	0	0	0	8.05					
McI #170	3	5	22	29	27	7	2	2	1	0
1	1	0	0	0	7.34					
Pwesk@PIF #179		1	0	3	7	66	4	6	3	2
1	3	3	0	0	1	8.7				
Pwesk L #186	4	3	10	31	40	5	2	3	1	0
1	0	0	0	0	7.52					
PIF #180	4	0	2	11	79	2	1	1	0	0
0	0	0	0	0	7.76					
Pwa@PIF #181	2	0	3	10	76	3	0	0	2	1
0	1	1	1	0	8.16					
Pwa L #178	0	0	1	16	63	5	4	1	3	1
2	2	2	0	0	8.57					
L Pesk #189	3	0	9	12	62	5	4	0	2	3
0	0	0	0	0	7.94					
M Bay #174	6	1	3	15	71	0	2	0	0	0
0	2	0	0	0	7.7					
KKG #197	0	0	0	10	86	1	2	1	0	0
0	0	0	0	0	7.98					
MMK #195	1	0	3	8	82	1	2	1	1	1
0	0	0	0	0	7.99					
Jakes #196	4	7	21	15	37	5	5	3	2	1
0	0	0	0	0	7.43					
MRO #190	1	1	3	7	78	6	0	3	1	0
0	0	0	0	0	7.99					
Pebb #177	1	2	1	15	70	3	2	1	4	1
0	0	0	0	0	8.04					
Snake #193	11	3	5	16	53	5	1	2	2	0

1	0	1	0	0	7.56						
Hil #185		0	0	4	20	65	4	4	1	2	0
0	0	0	0	0	7.95						
BDW #171		2	0	7	23	48	4	3	2	3	2
2	2	1	1	0	8.36						
Bwood #201		14	1	6	9	8	37	7	3	3	4
1	4	4	6	0	9.28						