Resting Stage Occurrence and Auxospore Formation of Diatoms Collected from Fjordic Sediments in Sechelt Inlet, British Columbia, Canada

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RESTING STAGE OCCURRENCE AND AUXOSPORE FORMATION OF DIATOMS COLLECTED FROM FJORDIC SEDIMENTS IN SECHELT INLET, BRITISH COLUMBIA, CANADA

By

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ABSTRACT

Sutherland, T.F., Taylor, F.J.R., and Pond, S. 2023. Resting Stage Occurrence and Auxospore Formation of Diatoms Collected from Fjordic Sediments in Sechelt Inlet, British Columbia, Canada. Can. Tech. Rep. Fish. Aquat. Sci. 3521: vi + 21 p.

Phytoplankton assemblages were determined from sediment cores collected from both the high-energy Skookumchuck Narrows (station-1) and the low-energy Narrows Inlet (station-3) located in Sechelt Inlet, British Columbia. A coarse sediment-texture with a defined water-sediment interface was observed at station 1, reflecting strong hydrodynamic sorting. Alternately, a diffuse, mobile, nepheloid-silt layer, without a defined water-sediment interface, existed at the depositional station-3. At station-3, sedimented phytoplankton concentrations were relatively higher than those at station-1, where *Chaetoceros* resting spores were absent. A 10-day, serial-dilution, sediment incubation provided phytoplankton growth. *Skeletonema costatum*, *Chaetoceros* spp., and *Thalassiosira nordenskioeldii* were dominant taxa in both 1) the incubation experiment and 2) a 1989 spring-bloom in associated overlying waters. The incubation ratio of initial to maximum concentrations, suggests that sedimented phytoplankton contributions to overlying waters should not rely solely on sediment phytoplankton estimates. *Skeletonema* auxospore formation gave rise to large diameter post-auxospore cells (19.9 μm) relative to small resting pre-auxospore cells (7.9 μm). The accumulation of small-diameter cells at deep sediment-water interfaces promotes auxosporulation upon exposure of favourable, over-lying water conditions. The synchronization of deep-water renewal, entrainment of nepheloid layers, and diatom size-restitution through auxosporulation will play an important role in the initiation and reoccurrence of spring blooms.

RÉSUMÉ

Sutherland, T.F., Taylor, F.J.R., and Pond, S. 2023. Resting Stage Occurrence and Auxospore Formation of Diatoms Collected from Fjordic Sediments in Sechelt Inlet, British Columbia, Canada. Can. Tech. Rep. Fish. Aquat. Sci. 3521: vi + 21 p.

On a déterminé les assemblages phytoplanctoniques dans des carottes de sédiments prélevées dans le passage Skookumchuck à énergie élevée (station 1) et dans le bras Narrows à énergie faible (station 3), situés dans le bras Sechelt, en Colombie-Britannique. On a observé une texture sédimentaire grossière avec une interface eau-sédiments définie à la station 1, reflétant un fort tri hydrodynamique. En contrepartie, on a observé une couche de limon néphéloïde diffuse et mobile, sans interface eau-sédiments, à la station 3. À la station 3, les concentrations de phytoplancton sédimenté étaient relativement plus élevées que celles de la station 1, où les spores dormantes de *Chaetoceros* étaient absentes. Une incubation de sédiments de 10 jours en dilution en série a permis de surveiller la croissance du phyloplancton. *Skeletonema costatum*, *Chaetoceros* spp., et *Thalassiosira nordenskioeldii* étaient les taxons dominants à la fois dans 1) l'expérience d'incubation et 2) une prolifération printanière en 1989 dans les eaux sus-jacentes associées. Le rapport entre les concentrations initiales et maximales dans l'incubation indique que les contributions du phytoplancton sédimenté aux eaux sus-jacentes ne devraient pas reposer uniquement sur les estimations du phytoplancton sédimenté. La formation d'auxospore de *Skeletonema* a donné lieu à des cellules post-auxospore de grand diamètre (19,9 μm) par rapport aux petites cellules pré-auxospore au repos (7,9 μm). L'accumulation de cellules de petit diamètre aux interfaces eau-sédiments profonds favorise l'auxosporulation au moment de l'exposition à des conditions favorables d'eau sus-jacente. La synchronisation du renouvellement des eaux profondes, l'entraînement des couches néphéloïdes et la restitution de la taille des diatomées par l'auxosporulation joueront un rôle important dans le déclenchement et la réapparition des proliférations printanières.

1.0 INTRODUCTION

Estuarine convection of phytoplankton resting stages to the highly-illuminated surface waters of favourable growth conditions has great ecological significance for the establishment diatom populations within enclosed bays and fjords. The retention of phytoplankton within fjords may be enhanced by the characteristic two-layer circulation pattern and slow flushing rates that retain both phytoplankton and sediment in the return flow. The formation and sedimentation of resting stages during adverse conditions will favour the accumulation of phytoplankton at the seabed surface, since phytoplankton resting stages exhibit faster sinking rates relative to those of vegetative cells (Davis et al. 1980; Hargraves and French, 1983; Lewis et al. 1985). Phytoplankton act like fine silt particles and settle in the low turbulent regions of fjords potentially forming “seed beds” (Dale, 1976; Lewis, 1985; Anderson and Keafer, 1985). Seed beds play an important role in the initiation and reoccurrence of phytoplankton blooms.

Comparisons of corresponding benthic and pelagic phytoplankton communities within estuaries would elucidate information regarding the "reseeding" theory (Pitcher 1990; Itakura et al. 1997; Montresor et al. 2013). For example, the repeated occurrence of the vegetative cells of *Chaetoceros radicans*, *Chaetoceros vanheurckii*, *Chaetoceros debile*, and *Chaetoceros didymus* in the upper water column of the inner region of a British Columbian fjord, Saanich Inlet, along with the occurrence of corresponding resting spores in the sediments support the idea that these spores serve to "re-seed" this region (Roeloffs, 1983; McQuoid and Hobson, 1995). *Chaetoceros* resting spores have been shown to contribute significantly to various sedimentary environments (Calvert, 1966; De Vries and Schrader, 1981; Roelofs, 1983; Sancetta, 1989). In addition, the overwintering conditions of fjordic sediments will provide phytoplankton resting stages with the mandatory dormant period of dark and cold conditions required for the germination in the spring time (Davis et al. 1980; Drebes, 1977; Von Stosch, 1979; Ellegaard and Ribeiro, 2018). Wind- or tide-induced resuspension of sediment in shallow waters will promote the exposure of resting spores to changes in temperature and light conditions necessary for the germination and vegetative growth of resting spores (Eilertsen et al. 1995; McQuoid and Hobson, 1995).

The formation of resting stages in diatoms is induced by the seasonal onset of nutrient-depleted surface waters (Davis et al. 1980; Von Stosch, 1979; French and Hargraves, 1985), cold temperatures (French and Hargraves, 1985), lower light levels, and shorter photoperiods (Von Stosch, 1979). Hargraves and French (1983) have suggested that resting spore formation may avoid potential damage caused by photo-oxidative effects and metabolic imbalance in the presence of highly irradiated, nutrient-depleted surface waters. Resting spore formation would aid in 1) the retention of a certain species in an area during adverse conditions (long-term mechanism) and 2) the passage through zooplankton guts and subsequent pellettization (short-term mechanism). The latter mechanism would promote sedimentation of diatoms to the benthos via rapid transport of fecal pellets as well as the horizontal dispersal of species to an environment of favourable growth conditions (French and Hargraves, 1980).

The resuspension of a small-diameter, senescent diatom population to the highly illuminated, nutrient-rich mixed surface waters may also provide an environmental window for synchronous auxospore formation. The coordination of the exposure to changes in environmental growth factors following a period of cell size reduction (Drebes, 1977; Perez-Martinez et al. 1992) and arrestment of mitotic growth (Ambrust et al. 1990) is necessary to induce auxospore formation. Resuspension and subsequent auxosporulation of senescent diatoms in the spring will result in an "inoculum" of large-diameter diatoms capable of rapid vegetative growth during favourable environmental conditions (Garrison 1981; Pitcher, 1990). To gain a better understanding of the

starting point or recruitment mechanism of sedimented phytoplankton, sediment samples were collected from Sechelt Inlet, British Columbia and analyzed for phytoplankton abundance and diversity. The diatom resting spores in sediment samples were incubated under favourable growth conditions and the vegetative diatom populations examined to determine the potential influence of sedimented phytoplankton on initiating spring or summer blooms in overlying waters.

2.0 STUDY SITE

Jervis Inlet connects with the Strait of Georgia, which lies between Vancouver Island and the southwestern coastline of British Columbia. The Jervis Inlet system harbours other adjoining inlets, including Sechelt Inlet (49 40 N, 123 45 W; Figure 1) that connects to the southeastern border of Jervis Inlet via a channel (Skookumchuck Narrows).

The dimensions of the main inlet include a length of 29 km, an average width of 1.2 km and a maximum depth of 300 m. An adjoining inlet, Narrows Inlet, enters Sechelt Inlet on the eastern border and has a length of 14 km, a maximum depth of 85 m and is divided in the middle by a 14-m sill (Tzoonie Narrows). The benthos of station 1, located in Skookumchuck Narrows, consists of a scoured hard-bottom seabed with coarse sediment and epifauna, while the benthos of station 3, located in the inner region of Narrows Inlet, is characterized by a soft, unconsolidated fine material, reflecting a depositional setting. For additional descriptions of water circulation patterns within Sechelt Inlet, refer to Lazier (1963).

A shallow sill (5-10m depth) that divides Skookumchuck Narrows from Sechelt Inlet, generates a high-energy, turbulent, unidirectional tidal-jet that influences hydrodynamics on both sides of the sill (Sechelt Rapids). This shallow sill also contributes to the development of a three-layer water-column in the main Sechelt inlet (station 2) and a high-energy, well-mixed water-column in Skookumchuck Narrows (station 1) (Pickard,1961; Lazier 1963). Alternately, a two-layer water-column exists in the Narrows Inlet basin (station 3; max 80 m) located beyond the sill (Tzoonie Narrows) that generates a relatively lower energetic tidal jet (station 3). Seasonal water-column profiles of temperature, salinity, sigma-t, and dissolved oxygen (January – June 1991) demonstrate the contrast between the well-mixed water column in Skookumchuck Narrows (station 1) and the well-stratified water-column (station 3) (Figure 2) (Tinis 1995). While bottom-water renewal is not an annual event in Sechelt Inlet (Lazier, 1963; Pickard, 1961), it takes place more frequently in the Narrows Inlet basin generally between the late-winter and early-summer period (Tinis and Pond, 2001). Both basins may experience a tidal-jet intrusion that can facilitate a mid-water or bottom-water renewal, that relies on the water-properties at station 2 (Figure 2). Dissolved nitrate and phosphate levels in the bottom waters of both stations typically remain above non-limiting levels for phytoplankton growth (Smethie, 1987), while oxygen levels can reach low values for both station 1 (3.5 ml L^{-1}) and station 3 (0 ml L^{-1}) prior to renewal events (Tinis & Pond, 2001). Additional information regarding previous oceanographic and planktonic studies in Sechelt Inlet are discussed in Pickard (1975), Lazier (1963), Thomson (1981), Haigh et al. (1992).

3.0 METHODS

Water profiles of physical attributes: Vertical profiles of oceanographic attributes (temperature, salinity, sigma-t, and dissolved oxygen) in the upper water-column were established at stations 1, 2, and 3 using a Guildine 8709 CTD Rosette (Tinis, 1995).

Sediment samples: Sediment samples were collected at a depth of 80 m from station 1 and station 3 on February 19 and 20, 1990. A Shippex Grab™ was used to collect sediment from station 1 due to the coarse nature of the seabed, while a Pedersen Corer™ was used to collect a core sample from station 3, due to the unconsolidated nature of the seabed. The top-two centimeters of the water-sediment interface were collected and stored temporarily in the dark at 5 °C until analyzed in the laboratory. A Canadian Standard Sieve Series™ was used to determine the relative amount of each sediment size class found in each sediment sample. The abundance of various phytoplankton groups present in the surficial sediment samples were determined from direct counts. An aliquot was taken from each suspended test tube, fixed with Lugol's solution, allowed to settle for 24 hours in a two-ml settling chamber and viewed under an inverted microscope (Utermohl method; Hasle, 1978). Phytoplankton counts were made across 6 star-shaped transects of known area and on low, medium and high power before converting the counts to cells ml^{-1} sediment.

Incubation of resting spores of phytoplankton: A serial dilution technique (Throndsen, 1978) was used to enumerate phytoplankton groups generated from the sediment during a 10 day incubation period. Three-ml of sediment were collected from sediment sample (station 1) and added to a 25 x 150 mm glass test-tube containing 27 ml of HESNW medium based on a salinity of 28 psu (Harrison et al. 1980). All glassware and equipment was soaked for 24 hours in a ten percent 1N HCl solution, rinsed with distilled water three times, and autoclaved for twenty minutes in a Standard Laboratory Castle ™ autoclave at 20 psi. Three ml of this sediment and growth medium mixture were then subsampled using a 60-cc syringe and added to a set of three replicate test-tubes, each containing 27 ml of autoclaved HESNW medium. The remaining subsample except for the last three ml was expelled from the syringe. Twenty-seven ml of HESNW medium was then drawn into the syringe to produce a 10:1 dilution. This new dilution was suspended and three ml were added to a new set of replicate test tubes containing 27 ml of HESNW medium. Three ml of this 10:1 dilution were retained in the syringe. The above procedure was repeated to produce a dilution inoculum of 100:1. The result was a serial dilution of 10^{-1}, 10^{-2}, and 10^{-3} inocula with three replicates for each dilution step. This entire procedure was repeated for the sediment sample collected from station 3.

The test tubes containing the sediment dilutions were stored in an incubation chamber at 16 °C under a 14:10 light:dark cycle at an irradiance of about 35 quanta m^{-2} s^{-1}. Culture tubes were randomized daily to reduce the effects of possible light intensity variations emitted along the length of the fluorescent lights. Direct counts were performed at day one and every subsequent three days. The experiment was terminated on day 10 when the counting procedure was rendered inaccurate due to the clumping of phytoplankton and increase in bacteria that was observed in the sample dilutions collected from station 3.

Statistics: A t-test was used to determine differences between mean cell diameter of pre-auxospore and post-auxospore cells of *Skeletonema costatum*. The differences between station, dilution, and time on the ratio of auxospore to vegetative cells were tested using a Three-Factor ANOVA.

4.0 RESULTS

Environmental characteristics, sediment size distributions, and phytoplankton assemblages: Station 1 is a relatively high-energy and well-mixed environment, while station 3 experiences a lower hydrographic-energy with a highly-stratified environment on a seasonal basis (Figure 2). Although light levels were not measured at the seabed surface (80 m), the 1% light levels in the upper water column ranged seasonally between 4 and 20 m at both stations. In

general, a deep water-renewal event (50-80 m) at station 3 appears to have occurred between April and June, 1991, where a notable increase occurred in salinity, density and oxygen values along with a concomitant decrease in temperature, suggesting strong seasonal variation in near-bottom waters.

Figure 3 shows the contrasting sediment grain size distributions between stations 1 and 3, where station 1 has a strongly-skewed distribution that was weighted towards the very coarse sand/gravel (79%) and station 3 has a more even distribution with an emphasis on very fine sand (Wentworth 1922). The remaining finer grain-size categories at station 1 fell under a 5% relative proportion, with the exception of the silt category (0%). The surface of the sediment sample had a clear sediment-water interface with angular-shaped rocks and shell fragments (one to two cm in diameter) indicative of a high-energy environment. The sediment:phytoplankton equivalent size-categories (0-250 μm) was relatively very low at station 1 when compared to that of station 3.

The largest sediment grain size categories observed at station 3 consisted of very fine sand (31%, 63-150 μm) and fine sand (32%, 150-250 μm). The most notable observation of this sediment core sample was the lack of a clear sediment-water interface. For example, the sediment texture gradually changed along a core-barrel vertical-continuum from a consolidated, dense-sediment base to a highly-porous flocculent framework at the core-barrel top. This porous surface layer (height: 15-cm) consisted of a cloudy, buoyant, nepheloid layer with a diffuse sediment-water interface. While storing the core sample in a cold dark area, the clear water at the top of the core barrel was carefully removed, while the nepheloid layer settled to an unconsolidated, fragile, gel-mud interface. The sediment grain shape in every size classification at this site consisted of well-rounded, spherical grains.

The concentrations and species richness of phytoplankton taxa in the surficial sediment samples of station 3, prior to the incubation experiment, tended to be higher than those observed at station 1 (Figure 3). During the incubation period, *Skeletonema costatum*, *Thalassiosira nordenskioeldii*, and *Chaetoceros* spp. were the dominant species observed at both stations (Figure 4), even though the abundance of *T. nordenskioeldii* was not sufficient to be detected on day 1 of the incubation trial. These diatom species were observed to dominate the spring bloom succession in the overlying waters at these stations in 1989 and other surrounding BC waters (Sutherland, 1991; Haigh et al. 1992; Taylor et al. 1994). Resting spores of several *Chaetoceros* species (*Chaetoceros debile*, *Chaetoceros didymus*, *Chaetoceros laciniosum*, *Chaetoceros radicans*, and unidentified *Chaetoceros* spp.) that form under nutrient-limited conditions were observed at station 3 only. The potentially harmful diatom species, *Chaetoceros concavicorne,* was also observed at station 3 only.

Incubation experiment: The phytoplankton succession observed during the incubation experiment consisted of diatoms during the initial stages followed by nanoflagellates and flagellates in the final stages. However, the results reported in this study will focus solely on the growth of the dominant phytoplankton group (diatoms) that occurred during the sediment incubations. Figure 5 shows the maximum concentration of the dominant species of diatoms, *Skeletonema costatum*, *Chaetoceros* spp., and *Thalassiosira nordenskioeldii* reached during the 10 day incubation period. The disappearance of *Chaetoceros* resting spores after day 4 of the incubation period (station 3) suggests that germination of vegetative cells took place. Chain-forming diatoms were observed to gain buoyancy at this time point of the incubation period and ascend vertically within the test tubes.

Initially, a higher number of diatom species (species richness) was present in station 3 samples at the onset of the incubation experiment relative to those present in station 1 samples (Figure 4). However, in the final stages of the incubation experiment, no difference between species richness was observed between stations (Figure 5). Note that the absence or low abundance of the "rare" diatom species observed at station 1 initially fell below the counting limit of accuracy and therefore should be analyzed with skepticism. The diatom species observed at station 3 tended to fall above the 200 number counting limit required to achieve an accepted degree of accuracy of 15% (Utermohl Method; Lund et al. 1958). Thus, the incubation experiments provided a better estimate of both "rare" and viable diatom species present in the sediment samples.

Although the concentrations of most diatom species were generally higher at station 3 on day 1 of the incubation, the early onset of stationary growth exhibited by many diatom species may have contributed to lower maximum concentrations achieved by station 3 diatoms relative to those of station 1 diatoms (Figure 6). The onset of stationary phase by certain diatoms in station 3 sediment dilutions may be due to 1) the exhaustion of a specific nutrient following a sharp increase in growth; 2) low-quality pre-conditioning of sediment porewater characterized by low oxygen and dissolved-nutrients (Smethie, 1987) and/or 3) an inhibitory effect produced by high concentrations of a chain-forming microbe observed after day 4. Alternately, stationary growth was not reached by *Chaetoceros laciniosum* and *Chaetoceros didymus* present in station 3 samples. These diatom species showed steady growth over time and sometimes reached higher maximum concentrations than those of corresponding station 1 species. Figure 7 shows time-series plots of the relative concentrations of *Skeletonema costatum* for each dilution. Higher maximum concentrations of *Skeletonema costatum* occurred at a greater dilution at both stations.

Auxospore formation of *Skeletonema costatum:* Auxospores of *Skeletonema costatum* formed between day one and day four of the incubation experiment (Figure 8). The mean diameter of the pre-auxospore cells (7.9 microns) was significantly different from that of the post-auxospore cells (19.9 microns; $P = < 0.001$). A Three-Factor Anova comparison revealed that day ($P = 0.014$) and dilution ($P = 0.026$) both had a significant effect on the ratios of auxospore to vegetative cells, while station did not ($P = 0.123$). Several large vegetative cells were attached to the hemispherical auxospore cells on day 4, suggesting that asexual cell division of the large cells had taken place after the formation of auxospores. The highest ratios of auxospore to vegetative cells were observed on day four at both stations, and was subsequently diluted by the vegetative growth of large-diameter cells. Although the initial mean concentration of vegetative cells of *S. costatum* at station 1 (4306 cells ml^{-1}) was significantly different from that of *S. costatum* observed at station 3 (851,396 cells ml^{-1}, $P = 0.02$), the ratio of auxospore to vegetative cells did not differ between stations.

5.0 DISCUSSION

Phytoplankton assemblage: Three diatom groups, *Skeletonema sp.*, *Chaetoceros* spp., and *Thalassiosira sp.*, were observed to occur in high concentrations within the incubation experiment (Figure 3 and 5) as well as predominate in the associated natural overlying waters during a spring bloom (Sutherland, 1991; Haigh et al. 1992; Taylor et al. 1994). These three diatom groups also predominate spring blooms in contiguous waters (Strait of Georgia) connected to Sechelt Inlet via Jervis Inlet (Harrison et al. 1983; Haigh et al. 1990, 1991), suggesting a benthic-pelagic coupling of these diatoms in this region. However, the incubation experiment shows that the concentrations and ratios of diatoms generated during the grow-out period do not reflect the initial concentrations and ratios of diatoms observed in the sediment sample. The succession of diatoms taking place throughout the incubation experiment appeared to depend on a combination of factors: 1) the initial sediment concentration of each species (Figure 4); 2) the variations in the growth rates

exhibited by each species (Figure 6); and 3) the dilution conditions of the experiment (Figure 7). Estimates of diatom species present at the seabed alone may underestimate the diatom abundance and composition in surface waters following germination and growth. Thus, incubation experiments are essential in determining the contribution of both "rare" and buoyant viable cells to primary production in overlying waters.

The different hydrographic conditions observed at stations 1 and 3 resulted in the different concentrations of varying sediment grain size and phytoplankton between stations (Figure 3 and 4). The high accumulation of phytoplankton and silt at the seabed at station 3 may be due to 1) the strong estuarine return flow; 2) the parallel retention of grazers, pelletization and rapid transport to the benthos, or 3) the flocculation and consequent sedimentation at the fresh/salt water interface that occurs in this region. The source of lateral transport in the form of a strong tidal jet present at station 1 probably kept silt and diatom cells in suspension longer and transported them to a region outside the influence of the tidal jet. The presence of larger-diameter sediment grains at station 1 supports this observation. If vertical migration patterns of herbivores exhibit an avoidance of the outgoing surface layer of the two-layer estuarine system, they will be retained in the region of station 3. The incorporation of resting spores into fecal pellets of herbivores may provide a rapid transport to the sediments (Hargraves and French, 1983). Davis et al. (1980) also found that *Leptocylindrus danicus* appeared to sink unmodified via transportation through grazers in the CEPEX controlled experiment in Saanich Inlet, British Columbia.

The presence of *Chaetoceros* resting spores at the seabed at station 3 may be related to the prolonged nutrient-depleted condition of the surface waters observed between June and August, 1989 (Sutherland, 1991). The increased density of the heavily armoured, double-theca frustule will increase sinking rates of resting spores and provide rapid transport to the benthos compared to that of vegetative cells (Davis et al. 1980; Hargraves and French, 1983). The weaker lateral transport present at station 3 would allow a fast vertical separation of resting spores from planktonic vegetative cells. This differential sinking rate and low frequency of sampling may explain why resting spores were not observed in the intermittent plankton samples collected at station 3. Alternately, the absence of *Chaetoceros* resting spores at the seabed of station 1 may be due to either the non-limiting nutrient conditions in the upper water column or the strong lateral transport provided by a tidal jet. These continuous current conditions would lengthen the suspension time of fast-sinking resting spores and potentially prevent sedimentation.

A large number of chlorophyll-containing cells present at the seabed at station 3 survived the dark, anoxic, and high H_2S conditions in the Narrows Inlet basin. Resting spores generally have double theca and restricted contact between the spore interior and external environment, and therefore differ morphologically from their corresponding vegetative cells (Stockwell and Hargraves, 1984), as opposed to resting cells which are structurally similar to vegetative cells (Hargraves, 1979). Although a double theca was not observed on diatoms such as, *Skeletonema costatum* and *Thalassiosira nordenskioeldii*, it is possible that these species form a "physiological" resting spore with thickened frustules (Hargraves, 1976). The cellular contents of *S. costatum* in the core samples of both regions were observed to be compact and withdrawn around the chloroplasts and away from the frustule similar to those diatoms observed in Narragansett Bay and Lough Neagh sediments (Hargraves and French, 1975, Jewson, 1992). *S. costatum* present in the sediments of Narragansett Bay, USA, were found to be physiologically similar to most diatom resting spores of other species (Hargraves and French, 1975). In addition, the diatom, *Aulacoseira subarctica*, which lacked a morphologically distinct resting stage was observed to survive 18 months in sediments of Lough Neagh, Northern Ireland (Jewson, 1992).

Auxospore Formation: The success of auxospore formation of a dispersed planktonic diatom population depends on the concentration of diatom cells in time and space (Brawley and Johnson, 1992). Accumulation of a sufficient number of potentially inducible cells within the seabed will influence the synchronization and success of auxospore production in a population. The small-diameter, resting cells of *Skeletonema costatum* observed in the sediment samples indicate that 1) the size of the source population in the upper water column was small prior to sedimentation and 2) the sedimentation of small cells is favoured over large cells in this region over time. Older populations of chain-forming diatoms or resting stages have been observed to have higher sinking rates relative to vegetative cells (Smayda, 1974). These small-diameter resting cells of *S. costatum* may represent a population that experienced decreases in cell diameters associated with successive vegetative division during optimal rapid growth conditions in the overlying water column.

The formation of auxospores of *Skeletonema costatum* gave rise to new vegetative cells that were significantly larger than the pre-auxospore resting cells. Auxospore formation is triggered in diatom populations that have reached a critical size of 30 to 40 percent of maximum valve diameter through asexual division (Drebes, 1977; Perez-Martinez et al. 1992). Cell size restitution through sexual reproduction is necessary if normal physiological processes are affected by shifts in the surface area to volume ratio, the number of chloroplasts, or by changes in the nucleus/plasma/vacuole ratio resulting from progressive size reduction (reviewed by Drebes, 1977).

The formation of auxospores may also result if diatom populations arrest mitotic division in cell cycle phases of either G1 or G2 during periods of low irradiance (Armbrust et al. 1990). Upon illumination of a G1 or G2 phase population, the induction of sexual reproduction may help to synchronize gametogenesis, thereby, increasing the success of fertilization. The limitation of auxosporulation to a cell cycle phase may reduce the "cost" of interrupting asexual reproduction to undergo sexual reproduction (Lewis, 1983; 1984). The low-light levels at depth experienced by the sedimented cells in this study would allow for the progression into the appropriate mitotic phase for synchronous auxosporulation. The synchronization of sexual reproduction in *Skeletonema costatum* has been shown to be influenced by the length of the senescent period of the batch inoculum (Harrison, 1973).

Although the production of auxospores in centric diatoms is limited to a population of a specific size threshold (Drebes, 1977; Perez-Martinez et al. 1992) or of a specific mitotic phase (Armbrust et al. 1990), sharp changes in environmental factors, such as, light intensity, photoperiod, temperature (Holmes, 1967; Smith, 1966; Harrison, 1973; Vaulot and Chisholm, 1987; Ambrust et al. 1990; Eilertsen et al. 1995), nutrients (Davis et al. 1973), and salinity (Schultz and Trainer, 1968) have been shown to induce sexual reproduction and therefore auxosporylation. Harrison (1973) found that auxospores of *Skeletonema costatum* were formed after a senescent batch inoculum was exposed to limiting levels (< 2 uM) and subsequent increases of silicate concentrations. Ambrust et al. (1990) found that *Thalassiosira weissflogii* could be induced to undergo gametogenesis by transferring cultures from low light or darkness to continuous light conditions. Comparing the bottom water conditions of both regions to the experimental incubation conditions in this study, it appears that increases in light, temperature, and oxygen could act as controlling environmental factors in the induction of auxospore formation of *S. costatum*.

Entrainment of an over-wintering, phytoplankton-based, nepheloid layer to surface waters could take the form as a mid- or bottom-water renewal event that slowly entrains and carries a 1) mid-water phytoplankton flocculent layer supported by a mid-water pycnocline; or a 2) tall, diffuse nepheloid layer rafting above the water-sediment interface, respectively. In Narrows Inlet basin,

water renewal takes place slowly as either mid- or bottom-water exchanges with fairly consistent annual or seasonal events (Pickard, 1961; Lazier, 1963; Tinis & Pond, 2001). For example, in 1991, a deep-water renewal event replaced a relatively warmer, low-oxygen, and low salinity resident bottom-water mass with dense colder intruding waters with higher salinity, density, and oxygen levels (Figure 2) (Tinis & Pond, 2001). Other research has found that phytoplankton resting spores and vegetative cells can cycle between the benthos and pelagic settings in coastal upwelling-dominated systems (Garrison, 1981; Pitcher et al. 1990). Lewis et al. (1985) found that a very small proportion of resting cells are required to seed a phytoplankton bloom.

The upward entrainment of auxosporulation of small-diameter cells of *Skeletonema costatum* may play a major role in the initiation of phytoplankton blooms and influence the succession phytoplankton species in fjords. The increase in cell size following auxospore formation, triggered by experimental conditions, may have ecological significance with respect to the seasonal size changes and subsequent growth of diatoms. The resuspension of small-sized benthic cells into overlying waters of favourable growth conditions during the spring may trigger auxospore formation. A population undergoing rapid increases in cell numbers during a spring bloom would benefit from the formation of auxospores and consequent restitution of a large-sized buoyant population. Harrison (1973) found that the wide-diameter post-auxospore cells had higher growth rates than the thin-diameter pre-auxospore cells. Bellinger (1977) found that size restitution of the planktonic population of *Stephanodiscus astraea* in a reservoir in England took place in autumn and was maintained through the winter. The cell diameter of *S. astraea* decreased quickly in the spring due to rapid growth and then decreased slowly in the summer as a consequence of slower cell division.

Auxospore cells can be used as indicators for the recruitment of new populations of diatoms. Establishment of a new large-sized population of *Stephanodiscus* through the formation of auxospores, followed by a decay of the old small-sized (pre-auxospore) population was recorded in an English reservoir (Round, 1982). A similar trend of old (small pre-auxospore) and new (post-auxospore) populations of *Skeletonema costatum* was observed in the incubation of water-sediment interface samples. For example, by day ten of the experiment small cells of *S. costatum* were not observed. Jewson (1992) reported that maximum number of auxospores of the freshwater diatom, *Aulacoseira subarctica*, represented only 0.16 percent of the total field population at one time. The fraction of auxospores to total population numbers (4%) is higher in our study probably due to the accumulation of viable cells at the seabed (Figure 8). Other investigators have observed the disappearance of small-diameter diatoms from the upper water column associated with a decline in silica concentrations in late spring followed by an increase in large-diameter cells during resuspension events during the winter or early spring (Jewson, 1992; Perez-Martinez et al. 1992). This observed shift in the size distribution due to upward entrainment of auxospores cells has been observed to occur biannually prior to the spring and fall blooms (Jewson, 1992).

Synchronization of the appropriate size and mitotic phase of a population, along with the presence of sufficient numbers of cells (through sedimentation) and the timing of exposure to environmental induction cues is important in determining the success of sexual reproduction (Jewson, 1992). The impact of auxospore production of a sedimented population on overlying waters relies on 1) the accumulation of senescent cells (through sedimentation and retention in fjords due to estuarine return flow), 2) the length of the senescent period, 3) the viability of certain phytoplankton species in adverse bottom waters (e.g. ammonium and H_2S accumulation in low-oxygen conditions), and 4) the timing and frequency of entrainment of cells due to seasonal increases in the depth of mixing.

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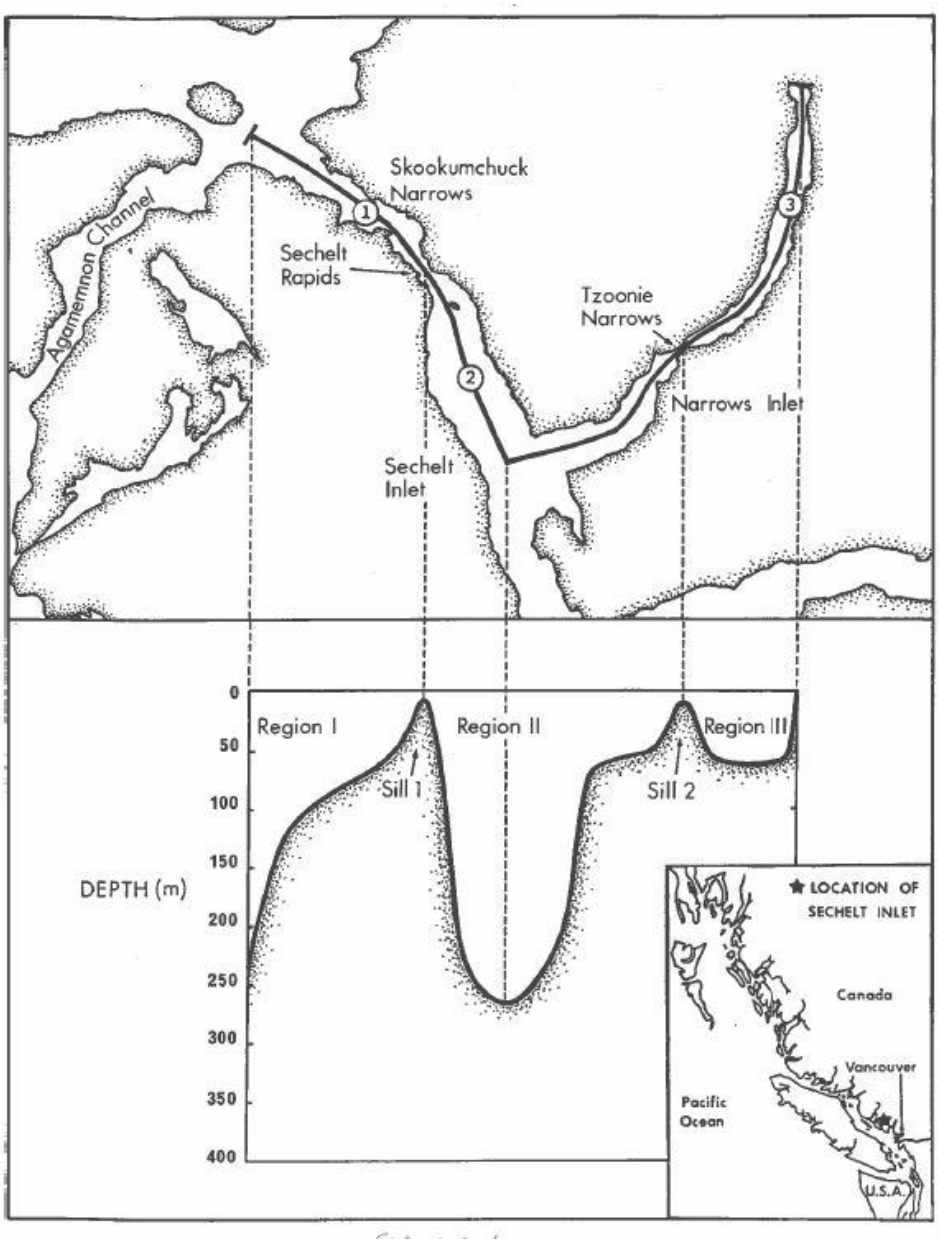

Figure 1: Station locations in Sechelt Inlet, British Columbia, Canada. Vertical profiles of oceanographic attributes (temperature, salinity, sigma-t, and dissolved oxygen) were collected at stations 1, 2, and 3, while sediment samples were collected at stations 1 and 3.

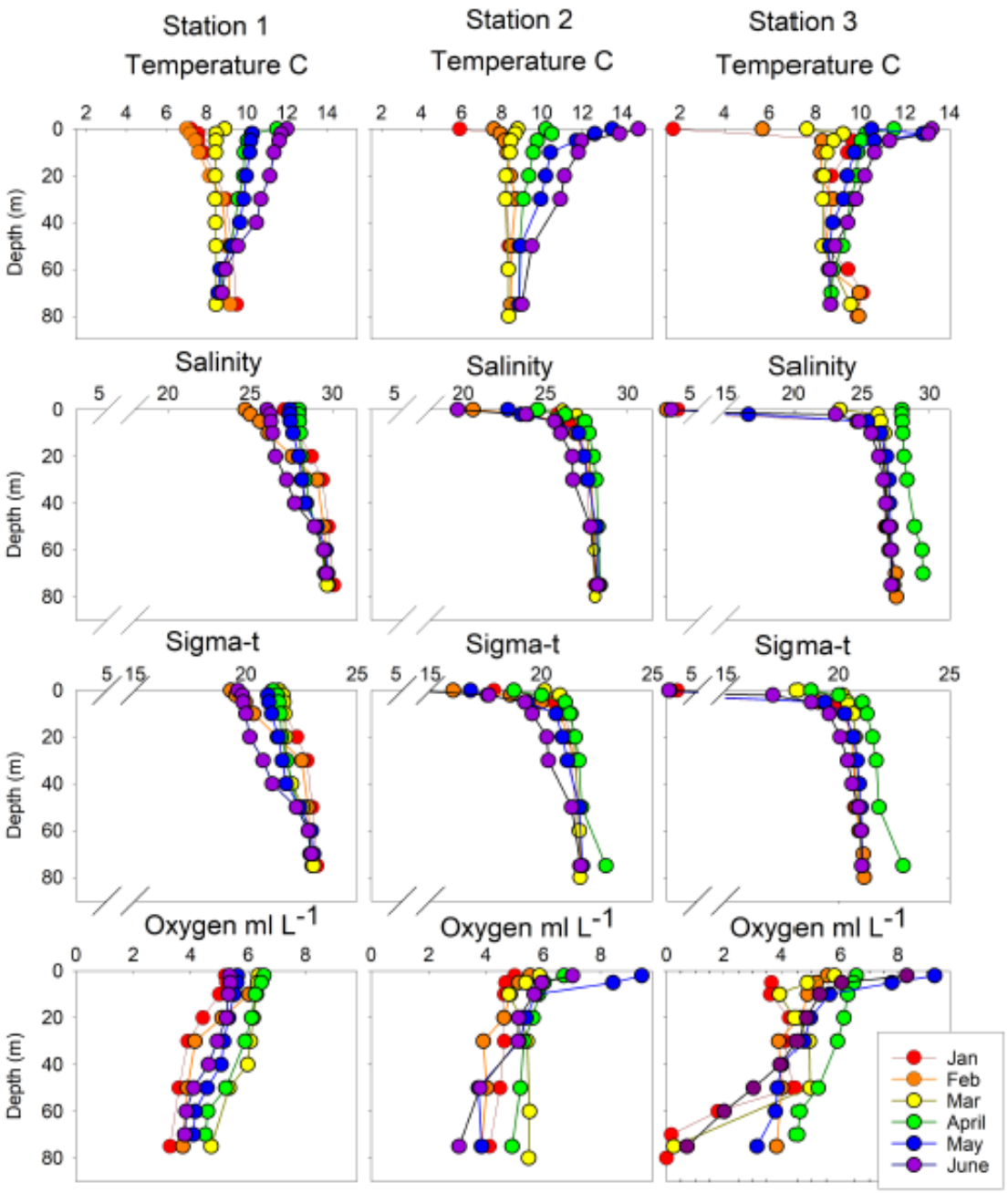

Figure 2: Vertical profiles of oceanographic variables (temperature, salinity, sigma-t, and dissolved oxygen) at stations 1, 2, and 3. Station 2 was surveyed between stations 1 and 3 to provide water-column conditions to serve as a common hydrodynamic exchange point between these stations.

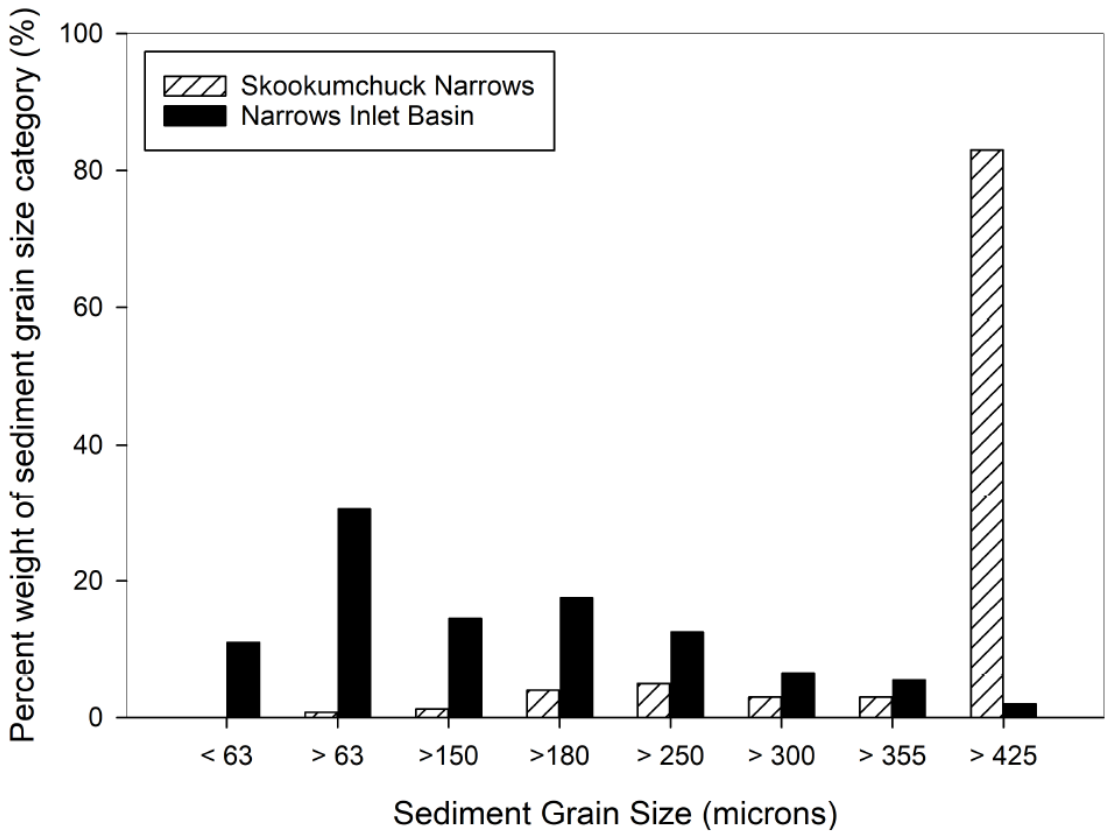

Figure 3: Sediment grain size distribution of core samples collected from stations 1 and 3.

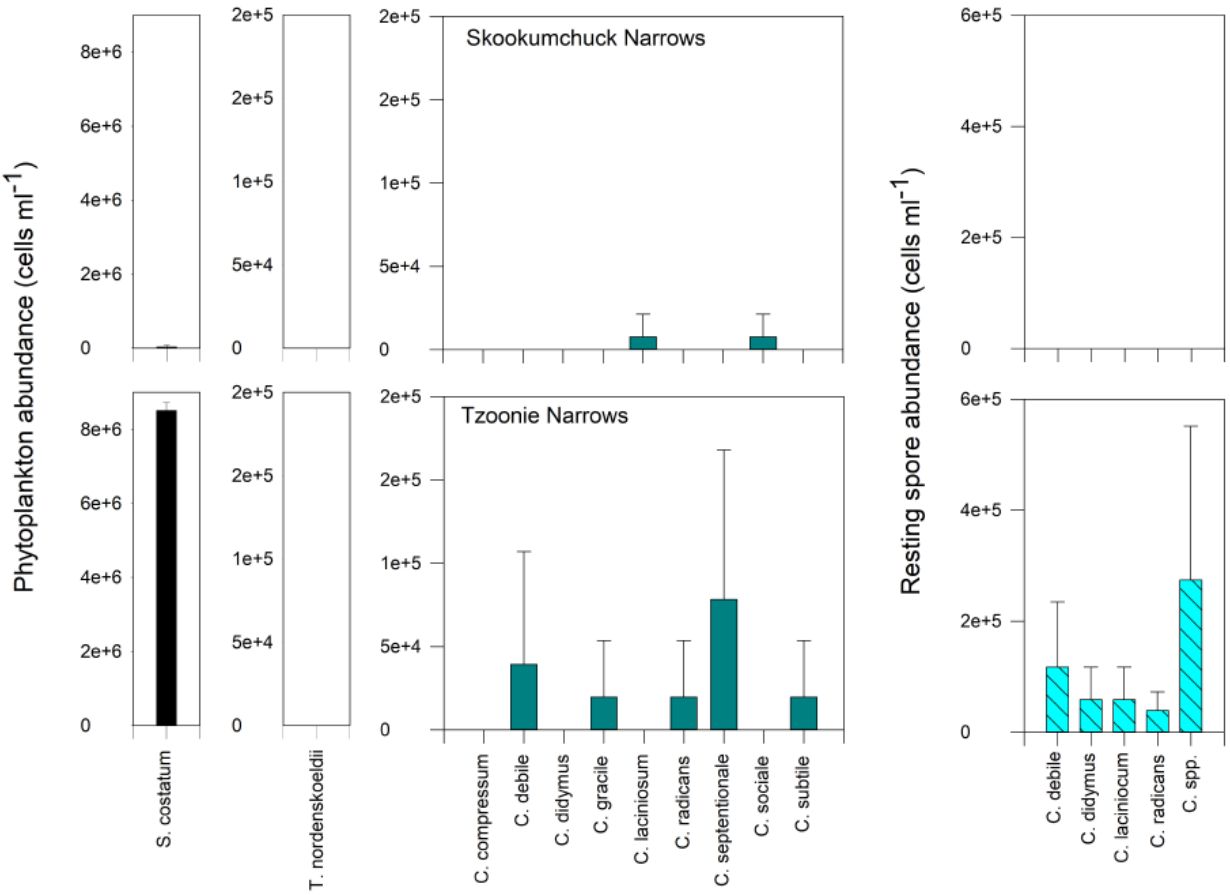

Figure 4: Diatom and resting spore concentrations in sediment samples collected from stations 1 and 3.

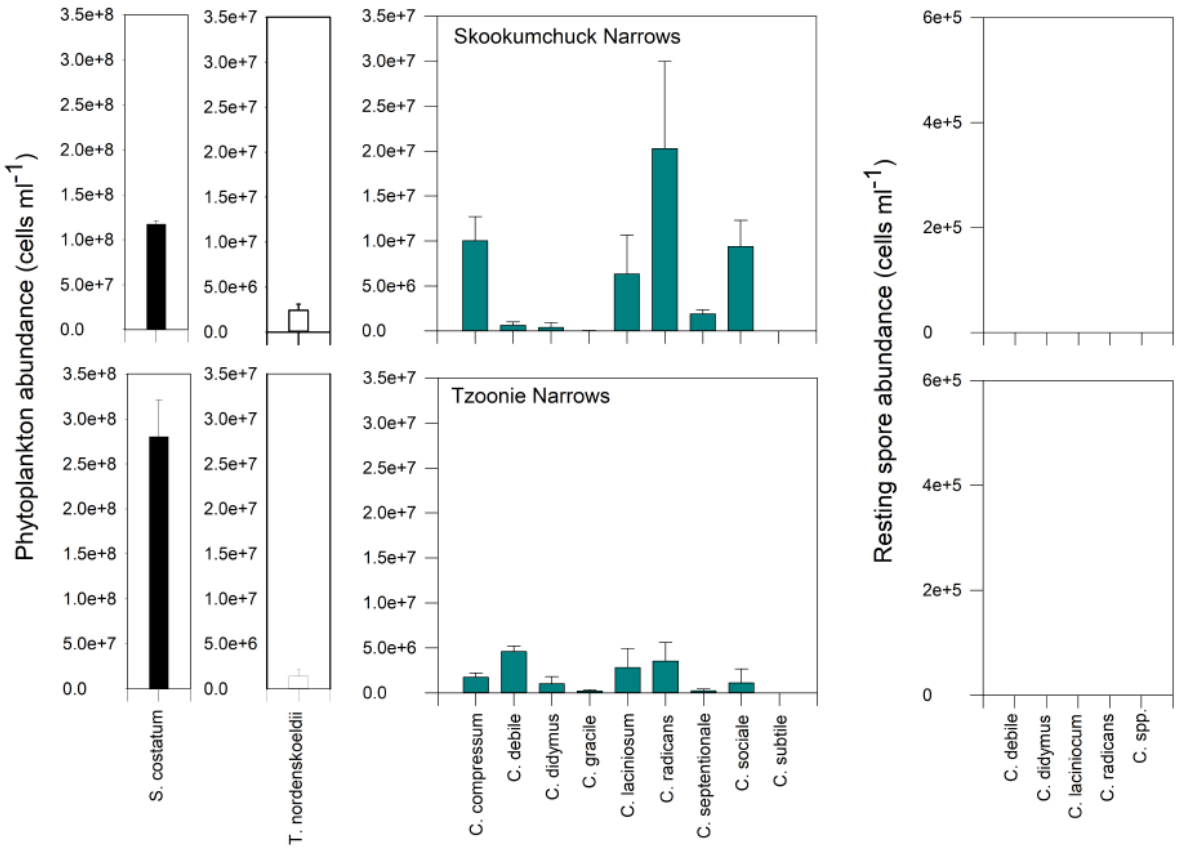

Figure 5: Maximum diatom and resting spore concentrations generated during an incubation trial of sediment samples collected from stations 1 and 3.

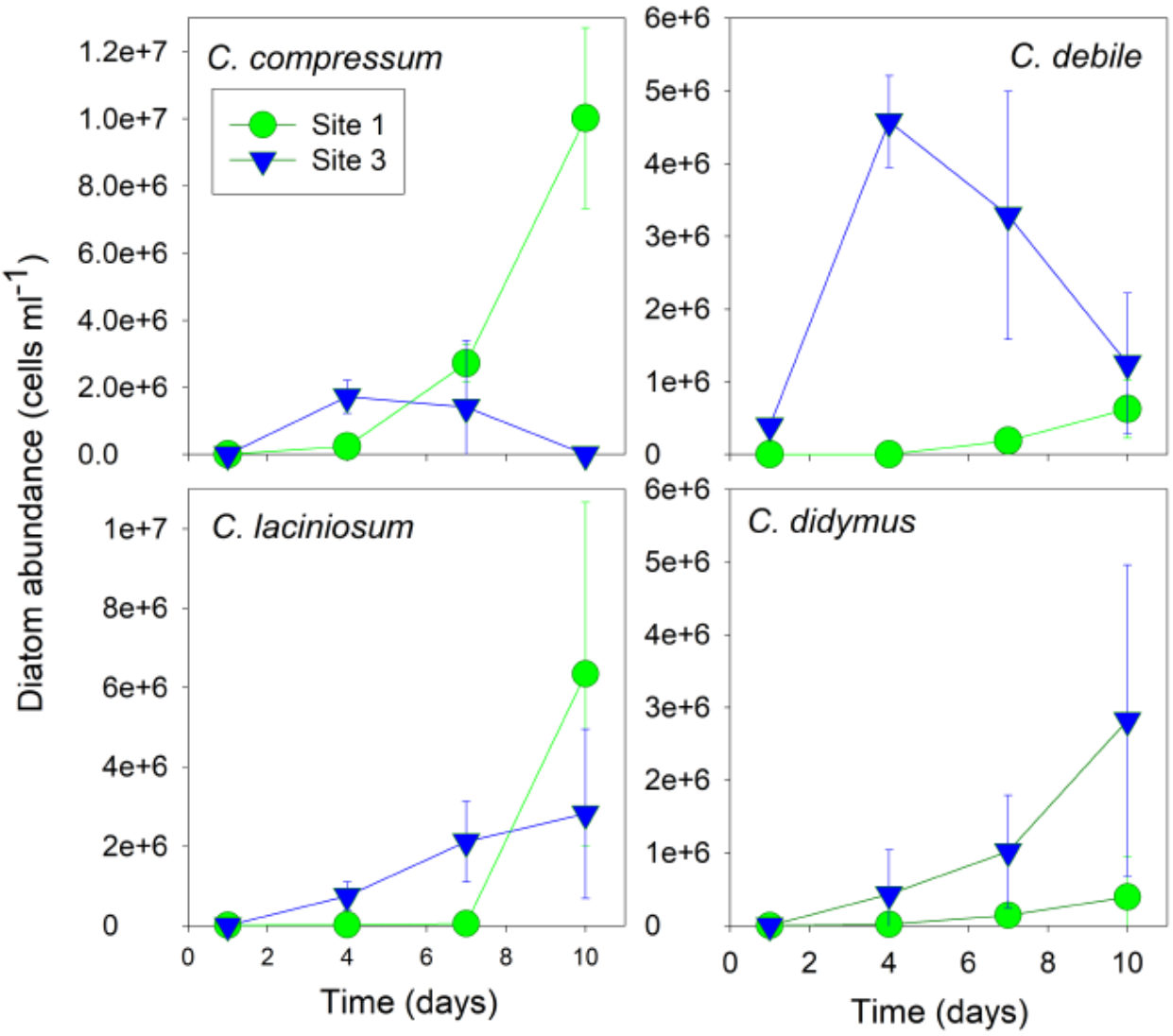

Figure 6: Growth curves of the diatoms, *Chaetoceros compressum*, *Chaetoceros debile*, *Chaetoceros laciniosum*, and *Chaetoceros didymus* generated during an incubation trial of sediment samples collected from station 1 and 3.

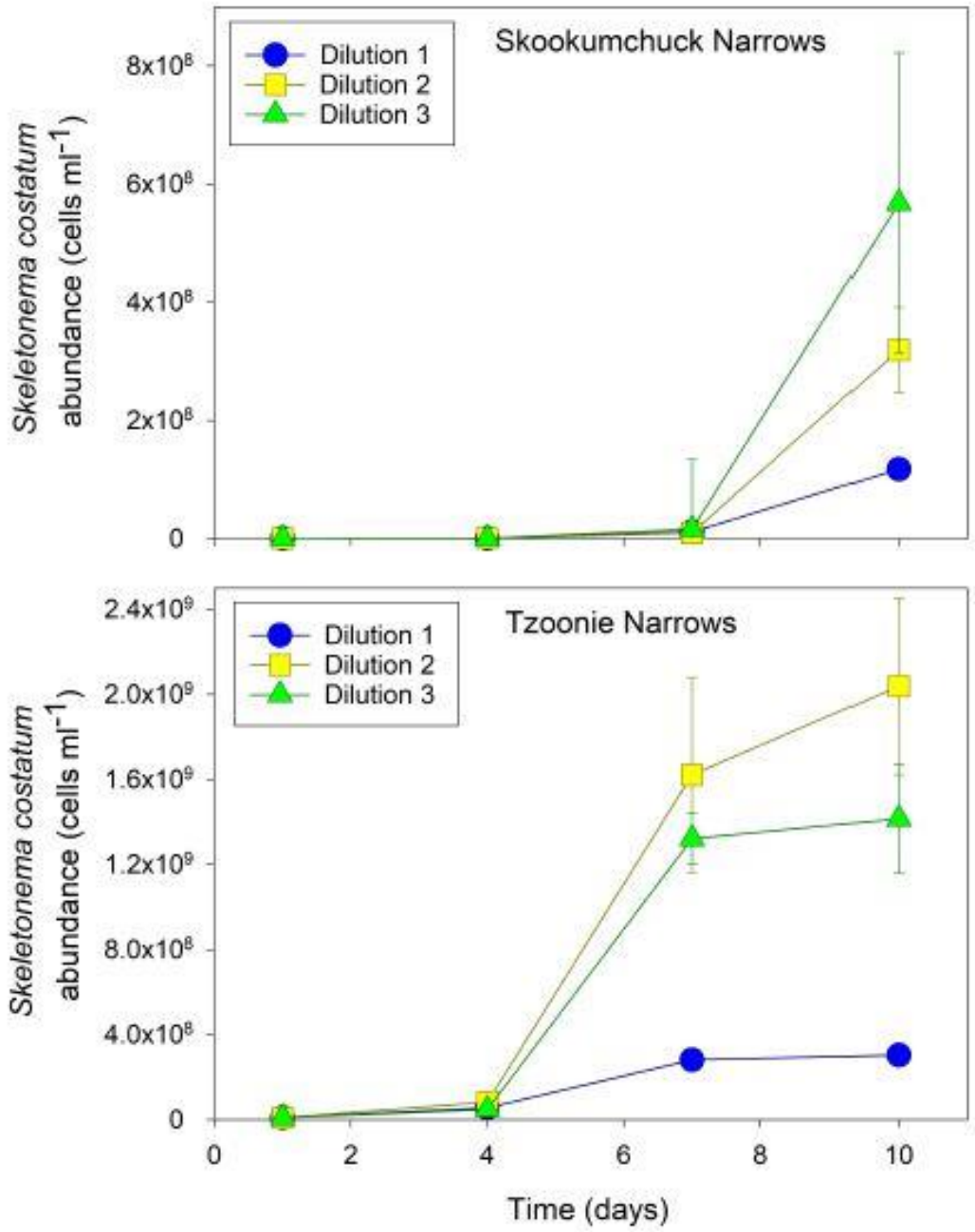

Figure 7: Growth curves of *Skeletonema costatum* generated during an incubation experiment consisting of 3 sediment dilutions.

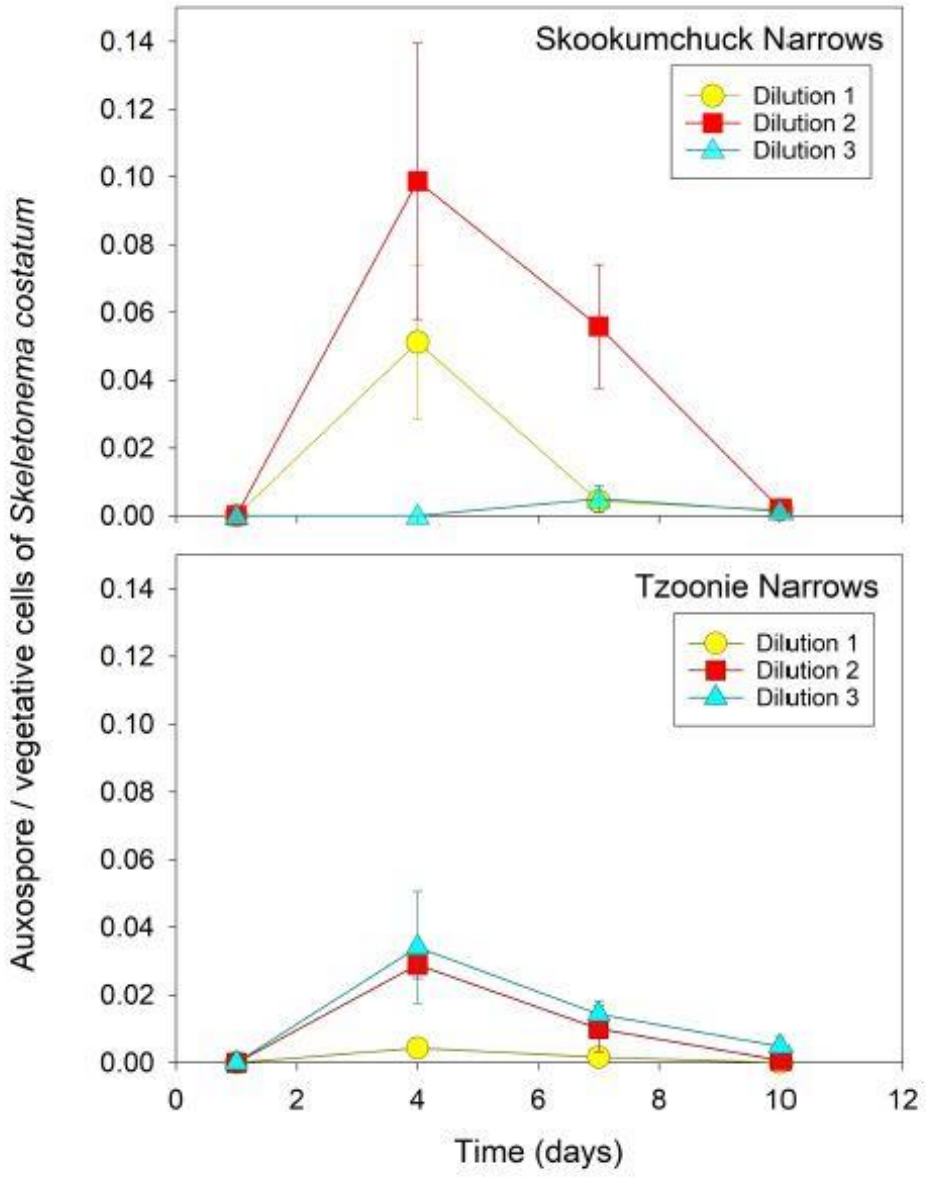

Figure 8: The ratio of auxospore to vegetative cells of *Skeletonema costatum* generated from an incubation experiment consisting of 3 sediment dilutions.