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PATTERNS OF DIEL VERTICAL MIGRATION IN FRESHWATER EPIPELIC DIATOMS: AN *IN SITU* EVALUATION OF FACTORS AFFECTING MOVEMENT

by

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Für meinen Vater, Manfred.

Mein größter Wunsch – daß Du dieses und vieles Andere mit uns
noch hättest erleben können...

Abstract

It has long been recognized that epipelic (sediment-dwelling) algae exhibit patterns of diel vertical migration in response to fluctuating environmental parameters. Due to the inherent difficulties of sampling permanently submerged habitats, the vast majority of research on the subject has been performed on intertidal sediments. Those studies that addressed migration in submerged habitats largely relied on removal of material from the natural environment. For the purposes of this project, I modified an existing sampling technique to permit *in situ* analysis of vertical migration in freshwater epipelic algae. Selecting diatoms as my study organisms, I was able to determine that vertical movement does indeed occur under water and that results derived from *in vitro* investigations are not truly representative. Through the use of relatively modern sensory equipment, I was further able to ascertain that movement in epipelic diatoms is very closely linked to both cumulative insolation and immediate changes in solar intensity. By applying the newly devised *in situ* sampling technique north of the arctic circle, I was able to show that patterns of migration during 24-hour daylight remain very similar to those at more southerly latitudes. A simultaneous species-specific evaluation of migration demonstrated considerable differences in migratory patterns between species. It was proposed that this variability arose primarily as a result of differences in light tolerance.

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Chapter 1.

INTRODUCTION

The epipelon comprises the community of microorganisms typically inhabiting the top three to five millimetres of submerged and semi-submerged sediments in aquatic environments. It is a relatively ubiquitous association, occurring in the vast majority of surface waters in which sediments accumulate and at depths to which adequate light penetrates (Round 1956, 1964, 1981). Highly dynamic by nature, this particular habitat places considerable stresses on the organisms that reside within it. Due to currents, wave action, tides, bioturbation, and constant sediment deposition, the substratum is perpetually in flux (MacIntyre *et al.* 1996). Moreover, it is an environment of extreme and variable physicochemical gradients (Jönsson *et al.* 1994). In order to maintain a physiologically suitable position within the sediments, or to regain that position following disturbance, a large proportion of microbes within the epipelon are dependent on motility (Round 1956, 1964, Harper 1976, Häder & Hoiczyk 1992). This is particularly true of epipelic algae, which are generally intolerant of anoxic conditions typical of deeper sediments and which must remain reasonably close to the sediment surface in order to meet their photosynthetic needs (Palmer & Round 1965, Round 1981). Aside from a few mat- and chain-forming species, only algae capable of movement are likely to survive in the continuously reworked sediments of the epipelon (Round 1981, Happey-Wood & Jones 1988).

Vertical migration in epipelic algae is speculated to serve a broad range of purposes. It reduces the risk of burial associated with constant sediment deposition (Harper 1977). Similarly, it allows cells to regain the sediment surface following a disturbance event (Happey-Wood & Jones 1988). It likely permits avoidance of recurring disruptive influences, such as scouring and potential relocation due to tidal action (Aleem 1950, Pomeroy 1959, Hopkins 1966). It may also facilitate sexual reproduction by increasing the likelihood of contact between individuals of the same species (Häder & Hoiczyk

1992). Furthermore, it probably reduces grazing pressure by allowing algae to submerge themselves in the sediments when they are not actively photosynthesizing (Hickman 1971, Harper 1976, Admiraal *et al.* 1982, Baillie 1987, Underwood & Paterson 1993a, b, Smith & Underwood 1998). At the same time, movement into the sediments may enhance nutrient uptake (Happey-Wood & Jones 1988). Lastly, and most importantly, migration permits cells to seek an appropriate vertical position in or on the sediments relative to insolation and photosynthetic requirements (Aleem 1950, Häder & Hoiczyk 1992). By the same token, it probably facilitates avoidance of damaging levels of visible and ultraviolet radiation (Nelson & Castenholz 1982, Häder & Hoiczyk 1992, Kingston 1999). It is the role of insolation in determining patterns of vertical migration, in terms of photoperiod and intensity, that has received by far the most attention in the literature.

Since the early 20th century, a large number of studies have investigated the phenomenon of vertical migration in epipelic algae. Beginning with simple observations on diurnal changes in the intensity of green colouration on exposed intertidal sediments (Bracher 1919, Fauré-Fremiet 1951, Callame & Debyser 1954), research on migratory behaviour rapidly evolved to incorporate investigations of diel and tidally-regulated periodicity. Due to the inherent difficulties of sampling in permanently-submerged habitats, much of the early work on vertical migration in epipelic algae was performed on intertidal sediments (e.g. Palmer & Round 1965, Round & Palmer 1966, Round 1966, Admiraal *et al.* 1982). As a result, researchers had to take into account the influences of both photoperiod and tidal patterns in their evaluation of algal movements. Although results were frequently contradictory at some level, several common findings began to appear. In general, algae were found to emerge from the sediments either slightly before or shortly after sunrise, reaching a peak in surficial abundance toward mid-morning. In most instances, this was followed by a brief period of downward migration during midday and another surficial maximum during mid- to late afternoon. Numbers again declined around the time of sunset. This pattern was usually modified by a tidal influence, which, depending on the time of day, was characterised by cell movement out of the sediments at

ebb, and into the substratum at flow. In most cases, both insolation- and tidally-mediated migratory patterns were shown to persist under constant laboratory conditions (Palmer & Round 1965, Round & Palmer 1966, Happey-Wood & Jones 1988, Jönsson *et al.* 1994). Very few cells were detected on the sediment surface during the night. Maintenance of the migratory pattern under constant illumination or constant darkness (Happey-Wood & Jones 1988), albeit muted and generally deteriorating over time, led to the conclusion that vertical migration in epipelic algae is regulated by an endogenous factor and modified by environmental stimuli (Bracher 1919, 1937, Hopkins 1966, Eppley *et al.* 1968).

Later studies of vertical migration in freshwater algae in large part relied on removal of material from the natural habitat and subsequent transfer to a laboratory (Round & Happey 1965, Round & Eaton 1966, Brown *et al.* 1972), thereby introducing a suite of experimental artefacts. It has been proposed that physical shock can cause downward migration of the epipelic community (Hopkins 1966). Hence, collection and transport of sediments may have had a marked impact on observed results. Reorganization of naturally stratified sediment particles may have introduced additional bias. Moreover, sample dewatering and subsequent sediment compaction in the laboratory could also have had a substantial effect. Lastly, sediment cohesion, largely associated with extracellular polysaccharides (EPS) extruded by diatoms during locomotion (Paterson 1986, 1989, Daborn *et al.* 1993, Noh & Choi 1998, Smith & Underwood 1998, Sutherland & Grant 1998, Oxborough *et al.* 2003, Underwood & Paterson 2003), may have been compromised. Despite these various considerations, it was not until 1974 that the first attempt was made to examine *in situ* migration of a permanently-submerged algal population in a freshwater pond (Harper 1976). Although reasonably successful in showing that migration does occur under these circumstances, this research relied on relatively simplistic methods and equipment. Hence, results were reported on a fairly coarse scale.

Results published by Harper (1976) provided definitive proof that vertical migration does occur naturally in the epipelon of freshwater environments. Furthermore, the overall patterns of movement she described were reasonably consistent with the findings of previous researchers (Round & Happey 1965, Round & Eaton 1966). Unfortunately, the light meter employed by Harper was primitive, at best, and ceased to function at an early stage in the study. Hence, as was the case for most of the preceding work on vertical migration in epipelic algae, it was impossible to determine the existence of any immediate relationship between light intensity and algal movement. Nonetheless, Harper was able to show that peak periods of movement in algae, in this case the diatom *Pinnularia viridis*, occurred around 08:00 and 17:00. Furthermore, she demonstrated that maximum surficial diatom abundance occurred between 10:00 and 12:00. However, considerable doubt is cast on these results due to the fact that values from three separate, non-consecutive study dates were reported as a single series of hourly averages over a span of 16 hours and compared to an illumination curve obtained on the first day of sampling.

The purpose of this study was to augment previous findings on vertical movements in the epipelon through an investigation of *in situ* patterns of diel vertical migration in freshwater epipelic diatoms. By using a research method devised by Taylor and Palmer (1963) and specifically modified for this project to facilitate *in situ* sampling of epipelic algae, I endeavoured to obtain additional evidence for the existence of migratory behaviour in a range of freshwater systems. In addition, through the use of modern sensory equipment, I hoped to establish a clear and definitive link between diatom migration and environmental stimuli. An attempt was also made to elucidate on the endogenous component of migratory behaviour in epipelic diatoms. Lastly, it was my intention to identify interspecific differences in migratory patterns of these algae. Diatoms were selected as the study organism based on their preponderance in the epipelon (Round 1956, Moore 1974, Jönsson 1994, Smith & Underwood 1998, Underwood and Paterson 2003), their comparatively high degree of motility (Happey-Wood & Jones 1988, Häder & Hoiczyk 1992), and their relative ease of identification.

Chapter two addresses the development and efficacy of the modified sampling technique, henceforth referred to as the 'ring method'. It goes on to compare migratory curves of epipelic diatoms obtained via the ring method and those derived using the traditional 'lens tissue' approach, which requires removal of sediments from the natural habitat. As a means of explaining some of the observed dissimilarities between results of the two sampling techniques, the chapter also provides a brief comparison of sediment structure from one method to the next using low-temperature scanning electron microscopy. Lastly, as a means of more clearly identifying the causal agents of movement in freshwater epipelic diatoms, chapter two investigates the relationships between several environmental stimuli and the migratory pattern.

Numerous studies have investigated patterns of vertical migration under constant illumination (e.g. Happey-Wood & Jones 1988, Jönsson *et al.* 1994). However, none have examined the phenomenon under conditions of continuous natural daylight. Chapter three describes the results of such an experiment, designed to examine the migratory behaviour of freshwater epipelic diatoms under 24-hour natural insolation and accomplished via the aforementioned ring method of sampling. Through a study of diel vertical migration in epipelic diatoms of an arctic stream and pond during the constant light of polar noon, the chapter endeavours to address the role of an endogenous component in defining patterns of movement. Furthermore, in order to address previous comments and observations on interspecific differences in migratory behaviour (e.g. Round & Happey 1965, Round & Palmer 1966, Round 1979), chapter three discusses patterns of movement on a per species basis.

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Chapter 2.

PATTERNS OF DIURNAL VERTICAL MIGRATION IN FRESHWATER EPIPELIC DIATOMS: DEVELOPMENT, EVALUATION, AND APPLICATION OF A NOVEL *IN SITU* SAMPLING PROTOCOL

2.1. Introduction

A diverse array of organisms typically lives on and within the top three to five millimetres of submerged and semi-submerged littoral sediments. Termed the epipelon, this community occurs in most waters where sediments accumulate and at depths to which sufficient light penetrates (Round 1956, 1964, 1981). The associated habitat is inherently dynamic in nature, being subject to considerable turbation, both biotic and abiotic, as well as constant deposition of sediments (MacIntyre *et al.* 1996). Furthermore, it is an environment of steep and variable physicochemical gradients (Jönsson *et al.* 1994, Paterson 1995). In order to maintain a physiologically suitable vertical position within the microhabitat, or to regain that position following disturbance, a large proportion of microorganisms within the epipelon rely on a capacity for movement (Round 1956, 1964, Harper 1976, Häder & Hoiczyk 1992). This is particularly true of epipelic algae, which are generally intolerant of anoxic conditions typical of deeper sediments and which must reside in reasonable proximity to the sediment surface in order to meet their photosynthetic requirements (Palmer & Round 1965, Round 1981). With the exception of various mat- and chain-forming species, algae capable of positive phototaxis are most likely to survive in the constantly moving sediments of the epipelon (Round 1981, Happey-Wood & Jones 1988). Moreover, the ability to move may facilitate contact with other members of the same species (Häder & Hoiczyk 1992) and considerably reduce grazing pressure on these organisms (Hickman 1971, Harper 1976, Admiraal *et al.* 1982, Baillie 1987, Underwood & Paterson 1993a, b, Smith & Underwood 1998).

Epipelic algae play a major role in carbon fixation, particularly in shallow lakes, ponds, and streams where phytoplanktonic growth is often comparatively low (Round 1964, Round 1981, Hickman 1971, 1983, Bebout & Garcia-Pichel 1995, MacIntyre *et al.* 1996). The majority of epipelic algae undergo a series of diurnal vertical migrations into and out of the sediment in response to both physical and chemical stimuli (Häder & Hoiczyk 1992). As a result of this movement, an additional periodicity in photosynthesis may arise, which should be taken into account for the determination of daily rates of production (Brown *et al.* 1972, Noh & Choi 1998, Smith & Underwood 1998). Furthermore, an understanding of this migratory behaviour will help improve methods of estimating cell abundance in the epipelon (Round & Happey 1965). Lastly, since numerous grazing invertebrates and small fishes rely on the microphytobenthos as a primary food source (Admiraal *et al.* 1982, Baillie 1987), a relevance to trophic interactions with regard to optimum feeding times is indicated (Noh & Choi 1998, Buffan-Dubau & Carman 2000, Orvain & Sauriau 2002).

It is generally accepted that vertical migration in epipelic algae is the product of an endogenous, or physiologically-regulated, rhythm in both motility and phototactic sensitivity (Fauré-Fremiet 1951, Hopkins 1966, Round & Palmer 1966, Round 1977, Happey-Wood & Jones 1988, Suzuki & Johnson 2001). However, several environmental parameters have been proposed as potential modifiers of this rhythm. Although most of these have been effectively ruled out (Hopkins 1963), insolation has repeatedly been implicated as a major factor in algal migration (Fischer *et al.* 1977, Admiraal *et al.*, 1982, Bothwell *et al.* 1989). Its primary role appears to be that of a 'pacemaker' (Round & Eaton 1966, Müller 1973, Fisher *et al.* 1977), acting to entrain the endogenous component of the rhythm through marked changes at dawn and dusk. Moreover, sunlight serves as a directional stimulus by guiding migrating algae to the sediment surface. By the same token, it helps regulate the abundance of cells on the substratum at a given point in time. On days of low illumination, or during periods of darkness, few algae tend to be present on the sediment surface (Harper 1976, Bothwell *et al.* 1989). Conversely,

surficial algal abundance may be quite high at times of elevated light intensity (Harper 1976, Fisher *et al.* 1977). It has also been postulated that high photon flux may cause algae to burrow in order to avoid photoinhibition associated with damaging levels of solar and ultraviolet radiation (Hopkins 1966, Round 1979, Nelson & Castenholz 1982, Häder & Hoiczyk 1992, Daborn *et al.* 1993, Yallop *et al.* 1994, Barranguet *et al.* 1998, Underwood *et al.* 1998).

Pennate diatoms (Heterokontophyta, Bacillariophyceae) comprise a conspicuous constituent of the motile epipelon in most aquatic environments (Moore 1974, Smith & Underwood 1998, Underwood and Paterson 2003). They move by gliding, extruding a viscous mucilage of extracellular polysaccharide (EPS), which expands upon contact with water and pushes the cell forward or backward along its longitudinal axis (Paterson 1986, 1989, Jönsson *et al.* 1994, Noh & Choi 1998, Smith & Underwood 1998). In the absence of a directional stimulus, movement in diatoms is random with frequent pauses and reversals of direction (Nultsch 1974, Harper 1977, Häder & Hoicyk 1992). Although these erratic patterns of motility persist in the presence of a guiding stimulus, the net direction of travel will be a product of response to that stimulus.

EPS trails produced by an assortment of epipelic microbes, most notably by large pennate diatoms (Paterson 1986, Taylor & Patterson 1998), persist for a time in the sediment. If the diatom community is sufficiently populous, a complex network of EPS strands may form, contributing considerably to the cohesiveness of the sediment matrix (Paterson 1986, 1989, Daborn *et al.* 1993, Noh & Choi 1998, Smith & Underwood 1998, Sutherland & Grant 1998, Oxborough *et al.* 2003, Underwood & Paterson 2003). By forming inter-particle bonds in the sediment, this network may increase substratum stability and, therefore, resistance to turbation (Yallop *et al.* 1994). This, in turn, may reduce the impact of disturbance and enhance the ease with which algae migrate through the substratum (Admiraal *et al.* 1982). Pre-existing mucilage trails may also act as a type of 'bridge', providing algae with a means of traversing larger interstices between

sediment grains.

Numerous studies have addressed the nature of motility in epipelic algae, with particular reference to diel patterns in movement (e.g. Bracher 1919, 1929, 1937, Aleem 1950, Fauré-Fremiet 1951, Callame & Debyser 1954, Perkins 1960, Palmer & Round 1965, Round & Happey 1965, Round & Eaton 1966, Harper 1976, Happey-Wood & Jones 1988). Of these, however, very few have investigated migratory behaviour of epipelic algae on undisturbed, permanently submerged freshwater sediments (Jönsson *et al.* 1994). Aside from Harper (1976), those few workers that did attempt to interpret diel cycles in non-tidal freshwater material did so under laboratory conditions (e.g. Fischer *et al.* 1977), potentially introducing a suite of artefacts that may have led to inaccurate results. Disruptive collection methods and artificial sampling environments unrepresentative of the natural habitat undoubtedly had considerable influence on the algae under scrutiny. Happey and Round (1965) indicated that *in situ* sampling of submerged habitats was not feasible, given the techniques and materials available at the time. However, Underwood and Paterson (1993b) emphasized the need for *in situ* sampling as a means of addressing what they termed "the complex coupling between physical and biological processes" in the epipelon.

Objectives

A) Development of a New Sampling Technique

The primary objective of this project was to develop a method for *in situ* sampling of epipelic diatoms in freshwater ponds. This new protocol, hereafter referred to as the 'ring method', would incorporate and improve upon the 'lens tissue technique' developed by Taylor and Palmer (1963). This latter technique entails the placement of small lens tissue squares on sediments that are either tidally exposed (Taylor and Palmer 1963) or completely removed from the natural habitat (Eaton and Moss 1966). Algae in the sediments travel upward between the tissue fibres and can be readily 'captured' through collection of the lens tissue, thereby providing a 'snapshot' of the microphytobenthic

assemblage at any point in time. The lens tissue technique, by nature, is not conducive to sampling in submerged habitats. The newly developed ring method was designed to overcome this major limitation of the lens tissue technique by facilitating *in situ* analysis of vertical migration patterns in permanently inundated habitats.

The efficacy of the new sampling approach was to be evaluated through a series of comparative experiments. These were designed to compare patterns of diurnal vertical migration in diatoms observed via the ring method with those determined by way of the traditional lens tissue technique. It was predicted that the ring method would provide a more accurate representation of actual *in situ* migratory patterns. Due to the lack of a protective water column, algae examined using the lens tissue technique were expected to exhibit greater sensitivity to changes in light levels, being quicker to migrate back into the sediments at higher intensities that may inhibit photosynthesis and incite a negative phototactic response (Daborn *et al.* 1993, Yallop *et al.* 1994). Furthermore, as the result of a theorized increase in sediment density, a factor presumed to reduce light penetration and inhibit cell movement, a greater response time during the upward phase of migration was anticipated in algae sampled using the lens tissue approach. Lastly, the number of diatoms participating in migration was expected to be considerably less when determined using the lens tissue method. This prediction was formulated on the assumption that mixing of the sediment sample, an accepted component of the lens tissue technique, will damage some cells and irretrievably bury others. Moreover, subsequent dewatering may dehydrate existing EPS trails (Paterson 1995, Underwood & Paterson 2003) and prevent diatoms from producing new ones (Paterson 1988). The results of this may have been indirectly evidenced by Aleem (1950), who noted that diatoms in recently exposed tidal sediments appeared first in depressions, where water content is typically greater.

Sediment characteristics, including the condition of EPS trails, were to be evaluated under Low-Temperature Scanning Electron Microscopy (LTSEM) in order to better assess the source of any differences in migratory behaviour between the two sampling

techniques. Due to the water-solubility of EPS strands, as well as the disruptive nature of the *in vitro* sampling method, LTSEM analysis was expected to reveal considerably fewer and less organised EPS trails in samples subjected to the lens tissue technique. It was thought that a reduction in sediment stability arising from damage to the EPS matrix might account for differences in diatom migration. In addition, a more dense arrangement of sediment grains was anticipated in these samples as a result of water removal as well as mixing of the sample. This increased density was predicted to reduce light penetration and inhibit diatom movement, thereby increasing the time required for cells sampled *in vitro* to respond to changes in insolation.

B) Factors Regulating Vertical Migration in Epipelic Diatoms

The second objective of this study was to analyse physical and chemical data collected during the development of the ring method with the purpose of identifying the major factors regulating diatom migration in the epipelon. Based on previous findings (Bracher 1919, 1929, 1939, Aleem 1950, Pomeroy 1959, Admiraal *et al.* 1982), it was predicted that the primary environmental influence on the migratory pattern would be light intensity. Moreover, it was expected that the study of movement under naturally fluctuating daylight, along with the use of modern sensory equipment capable of detecting photon flux across a broad range of wavelengths, would more clearly characterise the role of light in migration. This prediction was based on perceived flaws in previous experimental designs, which in large part relied on artificial light environments (e.g. Round & Happey 1965) or used detection equipment that measured illumination over a narrow range of poorly-defined wavelengths in the visible spectrum (e.g. Harper 1976). It was anticipated that short-term fluctuations in surficial algal numbers described by other researchers (Round & Happey 1965, Round & Palmer 1966) would be much more closely correlated with changes in light intensity than previously recognized.

2.2. Materials and Methods

2.2.1. Development of an *in situ* sampling technique

Clear acrylic tubing with an inside diameter of 18 mm and a wall thickness of 6 mm was cut across its width into 13 mm slices. The resulting rings were placed flat side down on a small tray to which a thin coating of clear silicon sealant had been freshly applied. They were then transferred, silicon side down, to a large sheet of lens tissue (61 x 91 cm). Once all rings had been adhered to the tissue, they were left to cure overnight. The following morning, the lens tissue was gently inverted onto a flat surface such that the adhered rings were underneath the paper. A scalpel was employed to cut the tissue around the outside edge of each acrylic ring. The result of the described procedure was a tightly-drawn skin of tissue paper entirely covering one end of each segment of tubing (Figure 2.1).

Sampling

A small (~ 0.1 ha), slightly alkaline (pH 8.1) pond located within Jasper National Park (JNP), Alberta (53° 2' 0" N, 118° 5' 51" W; ~1100 m asl) was chosen as the study site based on its reasonably firm, inorganic sediments, water clarity, and accessibility. Aquatic macrophytes in the pond were predominantly *Scirpus* sp., while terrestrial flora was dominated by *Picea mariana* on the west side and small shrubs on the east side. A relatively even, uninterrupted area of sediment at a water depth of 20 cm was selected within this site. At 17:00 on July 30, 1998, thirty-nine sampling rings were placed tissue side down, within two to three centimetres of one another, on the designated substratum. Beginning at 08:00 the following morning, three randomly-chosen rings were removed from the pond every hour until 17:00 (total of 30 rings). In order to minimize the number of cells lost from the lens tissue during transport through the water column, each ring was carefully grasped with forceps, turned such that the tissue was perpendicular to the sediments, and very slowly lifted from the pond. Thereafter, the surface formerly in

contact with the substratum was given a standardised 3-second rinse from a distilled water bottle in order to remove excess sediment from the sample. The ring was then inverted, tissue side up, and placed into a well-ventilated area until the lens tissue had dried completely. Once dry, the tissue was cut from the inside rim of the acrylic ring and transferred to a 1.5 ml microcentrifuge tube for storage. The nine rings remaining in the water after completion of sampling were included as a contingency in the event that any rings were damaged or became subject to disturbance.

At each sampling time, dissolved oxygen concentration (DO), water temperature, pH, and light intensity were recorded. Water temperature and DO were measured as close to the sediment surface as possible using a YSI FT 50/55 DO meter. A Fisher 119 pH/temperature meter was used to determine the pH of a water sample collected directly adjacent to the sediment. Light intensity was measured both at the pond surface and at the sediment surface using a Li-Cor Model LI-185A meter with a submersible quantum millivolt sensor.

Sample Processing

In the laboratory, the samples were treated according to Eaton and Moss (1966). Following the addition of 20 mg of potassium dichromate crystals, 0.30 ml distilled water, and 0.20 ml 98% sulphuric acid to each microcentrifuge tube, the samples were gently shaken and allowed to settle overnight. The described chemical mixture results in the formation of chromic acid, which digests both the tissue fibres and organic cell components and leaves only sand grains and siliceous diatom valves intact.

The following day, the microcentrifuge tubes were placed in a Beckman Microfuge B centrifuge and spun for five minutes. Thereafter, 0.35 ml of supernatant were carefully removed from each tube while great care was taken not to disrupt the pellet that had formed at the bottom. The removed acid was replaced with an equal volume of distilled water and the sample was again shaken and centrifuged for another five minutes. This

process of centrifugation and resuspension was repeated four to eight times until all samples registered a neutral pH.

Once a pH approaching 7 was attained, the samples were gently shaken one more time and immediately pipetted to circular cover glasses (18 mm, #1), 30 of which had previously been placed on a warming tray. Following complete evaporation of the water, each cover slip was subjected to the following series of steps. A drop of Naphrax mounting medium (refractive index ~ 1.74) was applied to the centre of a microscope slide that had been preheating on a hotplate. This was permitted to bubble for several seconds before the cover glass was placed, sample side down, onto the hot medium. Ten seconds later, the slide was removed from the plate and allowed to cool while gentle pressure was applied to the cover glass in order to ensure complete coverage of the mounting medium and prevent the formation of air bubbles between the slide and cover. In this way, permanent mounts were made from all of the samples.

Each slide prepared in the previous manner was examined at 1000 x magnification (oil immersion) under a Leitz Laborlux S microscope until a minimum of 300 fields of view had been traversed. A comparable method has been shown to give statistically robust results (Eaton & Moss 1966). Since siliceous diatom shells (frustules), comprising two halves or 'valves', frequently become separated during preparation, the algae were counted in terms of valves rather than whole cells. If fewer than 200 diatom valves were counted within the 300 fields, additional fields, up to a maximum of 800, were examined until 200 valves had been enumerated. Complete frustules were counted as two valves and, wherever possible, valves were identified to species. Broken diatom segments were counted as estimated fractions of whole valves. The number of individuals of each species per fields counted was recalculated as the number per slide. This value was then adjusted to reflect the number of cells of each species per square centimetre of lens tissue and interpreted as the number per square centimetre of sediment surface. Diatom nomenclature was primarily determined according to Krammer and Lange-Bertalot (1986,

1988, 1991a, 1991b).

2.2.2. Comparison Between Sampling Techniques

This component of the study was conducted on a reasonably even, uninterrupted section of fairly compact, organic sediment in a small pond (the 'Grebe Water', 53° 24' 37" N, 113° 45' 7" W; ~670 m asl) at the University of Alberta Devonian Botanic Garden (DBG). Hourly sampling took place between 08:00 and 17:00 on September 23, 1998, October 28, 1998, June 4, 1999, June 11, 1999, and June 17, 1999. Experiments were set up between 16:00 and 17:00 on the afternoon of the day prior to each sampling date. Due to unforeseen circumstances, the apparatus for the October 28 experiment was put into place one hour before the onset of sampling.

Ring Method

With the exception of water depth and the number of rings used, *in situ* sampling occurred as described in section 2.2.1 above. Twenty-four sampling rings were placed on the sediment at a water depth of 10 cm and allowed to stabilize overnight.

Lens Tissue Technique

Collection of sediment samples was performed according to techniques modified from Round and Happey (1965) and Round and Eaton (1966). A glass tube (100 cm length, 1 cm outside diameter) was used to collect surficial sediments from the pond at a water depth of 10 cm. The tube was held at an angle and drawn across a designated area of the substratum while a stopper at the opposite end was slowly released, causing water and sediment to rush in. This mixture was then transferred to the base of a plastic petri dish pre-drilled with fifteen evenly-spaced holes of 0.5 cm diameter and containing a piece of glass microfibre paper (Whatman 9.0 cm GF/A). Water was permitted to drain from the holes while sediment was retained by the filter paper. This procedure was repeated several times until the dish contained evenly-distributed sediment to a depth of

one centimetre. Once sufficient water had drained to expose the sediment surface, the dish was placed inside an inverted petri dish lid in order to inhibit further water loss. Four additional petri dishes were treated in the same manner. Following the addition of 6 sampling rings to each dish, the latter were set down directly adjacent to the pond and permitted to equilibrate overnight.

Sampling

Sampling proceeded at 08:00 on the following morning. Forceps were used to withdraw two randomly-selected acrylic rings from the pond in the same manner described previously. In this way, loss of diatoms during transport through the water column was minimized. An additional two rings were chosen randomly and removed from the petri dishes. All four rings were subjected to a standardised 3-second rinse of distilled water and permitted to air dry. Once completely dry, the lens tissues were cut away from the inside of the rings and transferred individually to 1.5 ml microcentrifuge tubes. The entire process was repeated hourly until 17:00.

At each sampling time, light intensity was measured both at the sediment surface and at the air/water interface using a Li-Cor Model LI-185A meter with a submersible quantum millivolt sensor. On September 23 and October 28, 1998, *in situ* sampling included additional measurements of DO and water temperature (YSI FT 50/55 DO meter). On the latter date, pH was also recorded from water samples collected at the sediment-water interface.

Sample Processing

All samples were processed and examined as described under 'sample processing' in section 2.2.1.

Data Analysis

Due to the low degree of replication inherent in a study such as this one, it was not possible to obtain statistically robust results. Autocorrelation and pseudoreplication further confounded the issue. In order to overcome these problems, data interpretation relied heavily on qualitative inferences rather than mathematical manipulation. Valve abundance, light intensity, and chemical data were communicated graphically as simple spline curves using Sigmaplot 5.0 software.

2.2.3. Low-Temperature Scanning Electron Microscopy

On June 4th, 1999, two additional sets of samples were collected in the following manner. During the *in situ* experiment, four plastic straws of 0.5 cm diameter and 20 cm length were gently pushed 3 cm into the sediment of the sampling site at locations where they would not disturb the experiment already in progress. While its top was firmly sealed, each straw was gently removed from the pond and plunged into a dewar of liquid nitrogen. Once the nitrogen had ceased to bubble, the straw was deposited in a cooler and carefully covered with dry ice. An additional four straws were employed in a similar fashion to retrieve samples from the petri dishes used for the lens tissue technique. In this case, the core length was limited to the one centimetre depth of sediment within the petri dishes.

Sample Preparation

The frozen samples were taken into the scanning electron microscope (SEM) lab on the following morning for examination according to techniques modified from Paterson (1995). In preparation for study under LTSEM, a single straw was removed from the dry ice and plunged, sample end down, into a cup of liquid nitrogen (-196 °C). While immersed, the straw was cut away from the frozen specimen, leaving only a bullet of sediment in the nitrogen. A cryo-adhesive was then added to a specially-designed microscope stub, which had an elongate depression running down its length. In

preparation for rapid manipulation, the stub was already affixed to the SEM transfer rod. The sediment bullet was removed from the liquid nitrogen with forceps, laid lengthwise into the cryo-adhesive, and submerged until half of its diameter was protruding from the stub. The stub was then plunged back into the nitrogen in order to fix the specimen in place. Once the nitrogen had almost ceased bubbling, the sediment protruding above the stub was removed. Pliers were used to apply pressure on either side of the sample, thereby fracturing the frozen sediment and exposing the internal structure with minimal physical damage. The stub was subsequently removed from the cup and quickly inserted into the transfer chamber of the SEM, which was promptly isolated and evacuated. The sample was advanced into the pre-cooled microscope (JEOL 6301F, Field Emission SEM) where it was gently heated in order to sublimate both ice crystals that may have formed on the fracture face as well as some of the frozen water between the sediment particles. During this time, it was closely monitored at a low accelerating voltage in order to ensure that sufficient ice was removed and that no damage to the specimen was incurred.

Once adequate sublimation had taken place, the specimen was removed from the microscope and inserted into a sputter-coating device. It was coated with gold and again returned to the microscope for examination at a higher accelerating voltage. Within the top 5 mm of sediment, twenty horizontal transects running parallel to the surface were analysed for structure and cohesion of particles. Ten randomly chosen fields of view were digitally captured for subsequent comparisons. All eight samples were treated in the described fashion.

2.3. Results and Discussion

A complete data set for Chapter 2 is presented in Appendices 2.1. through 2.6.

The primary objective of this project was to design a method for *in situ* analysis of

algal migration in the epipelon that would cause minimal disturbance of the substratum and the algal community. The newly-developed ring method of sampling was able to meet these criteria, clearly detecting *in situ* fluctuations in epipelic diatom abundance under conditions of natural daylight in the JNP pond (Figure 2.2). Patterns in diatom movement were similar to previously-reported findings (e.g. Round & Happey 1965, Harper 1976), suggesting that they were not the product of random chance. This conclusion is further supported by a visible relationship between diatom numbers and insolation, which will be further addressed in the following pages.

Similar work on vertical migration by Harper (1976) used a tripod-mounted microscope to evaluate the surficial abundance of a single species of diatom in a small freshwater pond. Although this system was restricted to water of less than 50 mm depth, it was sufficient to confirm that migration does occur in permanently-inundated habitats. Due, however, to its requirement for rapid on-site enumeration, its applications were limited. Unlike Harper's (1976) approach, the ring method has been shown to permit enumeration of the motile algal community on a given area of sediment at a chosen point in time, regardless of water depth. Based on previous findings, as well as sample analysis prior to acid digestion, it is anticipated that the technique could be applied to almost any motile microalgae in the epipelon. In the current study, diatoms were chosen as the study organism simply on the basis of their high degree of motility (Aleem 1950, Noh & Choi 1998), their common abundance in cohesive sediments (Moore 1974, Smith & Underwood 1998, Buffan-Dubau & Carman 2000), and their relative ease of identification following acid digestion.

Based on the JNP results, further investigation of the ring technique was deemed appropriate and an additional series of experiments was performed.

2.3.1. Comparison of Sampling Techniques

Summary

In terms of the migratory pattern of epipelic diatoms, a number of differences were noted between the *in situ* and *in vitro* sampling techniques (Figures 2.3, 2.5, 2.7, 2.8, 2.9). As predicted, algae sampled *in vitro* were generally more sensitive to light, exhibiting suppressed surficial abundance at high illumination. Moreover, they appeared to migrate downward more dramatically once light intensity had reached elevated midday values. However, contrary to expectation, the number of diatoms appearing on the sediment surface over the course of a sampling period was generally comparable between the sampling techniques (Figure 2.11), although the sum of hourly averages *in vitro* did substantially exceed *in situ* values on two occasions. Although it cannot be stated with certainty that one protocol is superior to the other, it was concluded that the ring method, due to its *in situ* application and a reduced potential for bias arising from sampling artefacts, is the preferred technique.

Background

Round & Eaton (1966) attested that their study, which used the lens tissue technique, relied on the assumption that algal migration in freshwater streams is a natural phenomenon not induced by transference to the laboratory. Disruptive sampling techniques (Round & Happey 1965, Palmer & Round 1965, Round 1966, Round & Eaton 1966), algal sensitivity to physical shock and high light intensity (Hopkins 1966), and changes in physical sediment characteristics (Admiraal *et al.* 1982, Daborn *et al.* 1993, Hay *et al.* 1993, Underwood & Paterson 1993b), led to the suggestion that past results reported from pond and stream sampling (Round & Happey 1965, Round & Eaton 1966) are suspect and must be considered with some scepticism. Designed to avoid these potential sources of error, the ring method was an attempt to incorporate the principles of the lens tissue technique into a sampling protocol that could be applied in permanently submerged littoral habitats. Although not the first study of its kind, it was, to the best of my knowledge, the first to facilitate in-depth *in situ* analysis of the species and numbers

of epipelic algae involved in migration.

Sediment-inhabiting organisms display considerable sensitivity to environmental stimuli, thereby precluding the removal and transport of sediments for study (Paterson 1989, Underwood & Paterson 1993b). Recognising the shortfalls of *in vitro* sampling, Harper (1976) developed a reasonably successful, albeit somewhat awkward, *in situ* method for analysing vertical migration with minimal disturbance of surficial sediments. However, although critical of previous sampling techniques, she failed to address any differences between results obtained using those techniques and conclusions derived from the method she ultimately applied. With this in mind, the following paragraphs will analyse diurnal migration of epipelic diatoms from three different perspectives and relate the observed differences to the applied sampling technique.

The Migratory Pattern

As anticipated, diatoms sampled using the ring method were less impacted by higher light intensities than their counterparts in petri dishes (Figures 2.3, 2.7, 2.9). This was most clearly evidenced by marked *in vitro* suppression of surficial diatom abundance during midday on days of elevated photon flux (Figures 2.3, 2.9). At these same times, diatoms in the pond either exhibited an inverse response to insolation (Figure 2.3), moving downward as illumination increased and *vice versa*, or responded with positive phototactic behaviour (Figure 2.9). On a day during which light intensity did not fluctuate appreciably, a sudden *in vitro* reduction in surficial diatom abundance was of considerably greater magnitude than a corresponding drop in the pond (Figure 2.7).

Less dramatic *in situ* responses to high insolation are likely attributable to the shading effects of the overlying water column, which may be further complicated by the ultraviolet radiation attenuating properties of Dissolved Organic Matter (DOM) in the water (Vinebrooke & Leavitt 1996). However, this presupposes an ability of microalgae to detect and respond to radiation in the ultraviolet range. The results of several researchers (Bebout and Garcia-Pichel 1995, Nadeau *et al.* 1999) strongly suggest such

an ability in cyanobacteria and its existence in the diatom *Gyrosigma balticum* has also been confirmed (Underwood *et al.* 1999). Sundbäck *et al.* (1996) noted, as did the author, that downward migration in diatoms was initiated in response to high insolation. This was interpreted as a potential strategy for avoiding increased levels of damaging ultraviolet-B (UV-B) radiation and deemed worthy of further investigation. The results of the current study clearly support those conclusions. However, since the employed photometer senses light only in wavelengths of between 400 to 700 nm, this possibility cannot be explored any further with the data available.

On a day of low average photon flux (Figure 2.5), both sampling techniques indicated very similar responses of diatoms to changes in light intensity. Aside from an early *in vitro* peak, both curves followed their respective insolation graphs very closely.

Rates of Emergence

The results of this study failed to provide clear evidence in support of the hypothesis that algae sampled via the lens tissue technique would emerge from the sediment more slowly than those sampled *in situ*. The original argument was based on the assumption that disturbed and dewatered samples would become more compact, thereby impeding algal movement, particularly for larger cells. However, it is conceivable that the increased density of particle placement in the sediment, visibly confirmed through SEM analysis (Figure 2.10), might facilitate diatom migration by increasing the likelihood of a moving cell making the necessary contact with an adjacent sediment grain.

The prediction that diatoms sampled *in situ* would exhibit upward migration earlier in the day was most strongly contradicted by a notably larger early morning *in vitro* peak in algal abundance on October 28, 1998 (Figure 2.5). This may have been the result of cell divisions, which tend to peak in frequency toward dawn and generally occur on the sediment surface (Round & Eaton 1966, Round 1978). However, it seems much more likely, based on the lack of a corresponding *in situ* rise, that the unexpected increase was an artefact arising from the brief one-hour equilibration period on this day. It might be

argued that the recent physical shock incurred as a result of sediment removal from the natural habitat may have been sufficient to induce upward movement in the algal cells as a means of avoiding burial (Hopkins 1966). It would seem reasonable that, upon reaching the sediment surface, the diatoms resumed their cycle of phototactic responsiveness and drifted downward as a result of relatively low light levels.

Two additional instances of markedly dissimilar emergence rates were noted on September 23, 1998, and June 11, 1999. In the first case, *in vitro* abundance of surficial diatoms increased more rapidly between 08:00 and 09:00 (Figure 2.3). It was concluded that this was the result of a lacking water column, which led to a somewhat higher light intensity than algae sampled *in situ* would have encountered at that time. This may have been adequate to increase the rate of upward migration in the petri dishes beyond that in the pond. On the second sampling date, however, the inverse scenario was realised (Figure 2.8). In this case, *in situ* diatom abundance rose more rapidly in response to higher light levels, which may have already been sufficiently elevated to inhibit upward movement *in vitro* due to the absence of a water column and associated DOM.

Cell Abundance

The prediction of reduced algal abundance in samples subjected to the lens tissue technique was refuted by the collected data. On two out of five sampling dates (Figure 2.11), daily *in vitro* totals markedly exceeded *in situ* numbers. This may have been a factor of increased sediment density and lower light penetration in the petri dishes, causing complete emergence in algae that might otherwise cease upward movement within a few millimetres of the sediment surface. On the other hand, this may demonstrate an additional bias of the lens tissue technique. Since the glass tube method collects only the top few millimetres of sediment – the region of highest cell abundance (Bracher 1919, Aleem 1950, Pomeroy 1959, Noh & Choi 1998) – and combines multiple samples in a petri dish, the resulting algal density per unit sediment volume will be much greater than *in situ*.

Although bias introduced through the glass tube method is the most plausible explanation for lower *in situ* diatom counts, the discrepancy may also have resulted from one of three potential flaws in the ring method. Firstly, it is possible that the acrylic rings retained a slightly positive buoyancy in water, causing them to make incomplete contact with the sediment. Similarly, it might be suggested that, although an effort was made to select a relatively level substratum, the sediment was sufficiently uneven to prevent total coverage under the lens tissue. Lastly, the extraction method may have resulted in the loss of cells to the water column.

2.3.2. Low-Temperature Scanning Electron Microscopy

Summary

LTSEM was used to examine differences in sediment structure between the two sampling techniques as a means of explaining observed dissimilarities in migratory patterns. As anticipated, EPS strands in sediments sampled via the lens tissue technique were disrupted and frequently obscured by adhering detrital matter (Figure 2.10). Similar damage was not observed in sediments subjected to the ring method. Moreover, particle placement in sediment collected directly from the pond was visibly less dense than that of sediment recovered from the petri dishes.

The EPS Matrix

A substantial proportion of sediment stability arises from EPS trails extruded by microorganisms, particularly pennate diatoms (Paterson 1986, 1988, 1989, Underwood & Paterson 1993a, b), during locomotion. These trails tend to bind sediment particles in a common matrix (Smith & Underwood 1998) and provide a cohesive influence that may be lost during mixing as the biologically-derived mucilage is displaced or dissolved (Paterson 1988, 1989). LTSEM analysis of sediments proved quite revealing with regard to the effects of sampling disturbance on sediment integrity. However, the interpretation of differences between substratum structure from one sampling technique to the other, relative to migratory patterns, remains open to discussion.

Visual examination of scanning electron micrographs demonstrated considerable differences in sediment structure between the sampling methods (Figure 2.10). Material collected *in situ* exhibited scattered networks of EPS trails which were similar to those previously described by Paterson (1989). Despite a lack of evidence for motile algae other than diatoms in the samples, EPS trails were attributed to microbial movement in general. The observed trails were reticulate and three-dimensional in structure, tending to span interstices between sediment grains. Although various minute particles were found clinging to the mucopolysaccharide, EPS matrices were clearly defined and reasonably unobstructed (Figure 2.10).

As hypothesised, EPS trails in sediments subjected to the lens tissue technique were frequently disrupted (Figure 2.10). Clearly less extensive, they were fouled with what appeared to be adhering detrital particles of a flocculent nature. Having lost their reticulate structure, multiple strands of EPS were often reorganised into amorphous clumps, forming a dense mass within the sediment matrix. The delicate, three-dimensional structure seen *in situ* was absent.

EPS trails in the sediment may act as 'bridges' (Yallop *et al.* 1994, Sutherland & Grant 1998), aiding diatoms in traversing interstices between particles. As a result, loosely arranged sediments may not represent a significant impediment to locomotion in these cells. However, since EPS trails in samples subjected to the lens tissue technique were considerably damaged, it might be predicted that the progress of cells travelling through this material would be retarded. On the other hand, the higher density of grain arrangement within sampled sediments may preclude the need for expansive polymer trails. The results, which in two instances indicate a higher *in vitro* abundance of diatoms (Figure 2.11), tend to support the latter proposal.

The reticulate organisation of EPS strands observed within undisturbed sediment is inconsistent with the findings of Paterson (1995), who claimed that the "natural structure is of a more sheet-like appearance" than the reticulate arrangement left by other

preparatory techniques. This may be attributable to a much higher diatom abundance in the marine material examined by Paterson. High cell density could lead to overlaying of trails to the point where they become wider and more expansive, thereby losing the net-like organisation they may originally have had. Low diatom density on the chosen sampling date may explain why the overall abundance of EPS trails was low. Furthermore, since Paterson's work was done in marine and estuarine environments, species composition may have had a substantial impact on the difference between my findings and his. Finally, based on the ephemeral nature of the water-soluble EPS strands (Paterson 1988, 1989), as well as their modest presence in the sampled sediments, it is conceivable that, beyond their initial propulsive capacity, they play little role at all in algal migration.

Sediment Density

As anticipated, an attempt to characterise grain placement in the sediments under LTSEM suggested that sediments become more compact in the absence of a water column (Figure 2.10). Although no definitive conclusions could be drawn from the available micrographs, it appeared that particles *in situ* were more loosely packed than those in the experimental dishes. It might be argued that the more dense alignment of sediment grains arising from the lens tissue technique could directly impede diatom mobility, thereby increasing response time during migration. Clearly, the results do not support this conclusion. Alternatively, one might suggest that the increased proximity between sediment grains might actually facilitate cell locomotion. The latter proposal arises due to the nature of movement in diatoms. Since diatom cells extrude EPS as a means of propelling themselves (Smith & Underwood 1998, Sutherland *et al.* 1998) and since they require a substratum for this mechanism to be effective, it stands to reason that a more dense arrangement of sediment particles would increase the likelihood of a moving cell making the necessary contact with an adjacent substratum and thereby directing itself toward or away from a given stimulus. Given the sampling results, the second argument is likely the stronger one. Previous comments regarding sampling bias aside, this may help explain the larger number of cells participating in migration when

sampled via the lens tissue technique.

Density of particle placement within a sampled substratum may further influence migratory behaviour through modification of the light stimulus. It might be reasoned that, although the filtering effect of the water column is lost through the lens tissue technique, the increased sediment density may at least partly counteract this problem by reducing the penetration depth of solar radiation into the substratum. This could modify certain aspects of migration, such as emergence times. Following emergence, however, diatoms are fully exposed to solar radiation and the sediment no longer fulfills a shading function. Moreover, shallower penetration depths of sunlight due to denser sediment structure may incite algae that might otherwise remain slightly below the sediment surface to emerge fully in order to photosynthesize effectively.

2.3.3. Environmental Regulation of Migration

Summary

The second purpose of this chapter was to characterise the environmental stimuli potentially influencing the migratory pattern in freshwater epipelic diatoms. In order to do this, a number of environmental parameters, including water temperature, pH, DO, and light intensity, were measured on an hourly basis during the development of the ring method. As predicted, all but illumination were ruled out as direct influences on vertical migration in epipelic diatoms.

2.3.3.1. Temperature, pH, and Dissolved Oxygen

In order to accurately regulate a circadian rhythm, an exogenous pacemaker must undergo clearly-defined changes of a significant magnitude on a diel basis. Periods of weak insolation alternating with ones of higher intensity, for example, have been implicated in the regulation of other biological cycles (Palmer & Round 1965). Aside from light, however, very few environmental parameters fluctuate sufficiently in

amplitude to entrain a pattern of vertical migration in epipelic algae. Moreover, many variables that could potentially act as a pacemaker are too unpredictable. Temperature is one of these. Several workers have suggested that an endogenous rhythm, in order to be an accurate time-giver, must be temperature-independent within reasonable ecological parameters (Bruce & Pittendrigh 1956, Palmer 1974, Suzuki & Johnson 2001). Water temperature can be a function of numerous environmental parameters and, as such, is rarely a good indicator of time. Moreover, depending on the habitat, water temperature may vary enormously with season or not show any significant fluctuations at all. Similarly, pH and DO are unlikely to fulfill the role of an effective pacemaker. Despite recognizing that they are unlikely to affect the circadian rhythm of migration, I felt it necessary to eliminate these three factors as potential stimuli behind minor oscillations in surficial diatom abundance during the period of emergence.

The results clearly support the suggestion that diurnal vertical migration in freshwater epipelic diatoms is not directly related to water temperature (Figures 2.2, 2.4, 2.6). On all three sampling dates, temperature exhibited a gradual increase from morning until early evening. No apparent link between this trend and daytime fluctuations in diatom density was determined.

Dissolved oxygen concentration and pH were ruled out by Aleem (1950) and Hopkins (1963) as factors in the vertical migration of epipelic algae. However, these results are dated and were collected on tidally-influenced sediments, suggesting that a revisitation of the indicated variables in permanently-inundated freshwater systems was warranted. Investigation of both DO and pH values measured during the JNP study (Figure 2.2) and the DBG component of the project (Figures 2.4, 2.6) did not yield any relationship with the pattern of migration in epipelic diatoms.

2.3.3.2. Light

Background

Past studies of vertical migration in epipelic algal communities were often conducted under constant light levels in the laboratory (e.g. Round & Happey 1965, Round 1966, Round 1979). Others, performed under natural light regimes, failed to address short-term fluctuations in solar intensity arising from cloud cover (e.g. Palmer & Round 1965, Round & Palmer 1966). Those few experiments that did measure light values were conducted with equipment that detected poorly defined wavelengths of radiation in the visible spectrum (e.g. Round & Eaton 1966, Harper 1976). Round and Palmer (1966) noted that algal migration curves were rarely smooth, suggesting the intervention of a process more complex than a simple up-down pattern with an intervening surface interval. However, they were unable to identify a cause for these fluctuations. Since the sensor applied for the current study detects photons specifically in the photosynthetically active range of between 400 and 700 nanometre wavelengths, a more detailed investigation than seen in previous studies was made possible.

It is generally agreed that light plays a major role in algal migration, acting as a directional stimulus and, in terms of photoperiod, adjusting the phase of an endogenous rhythm in phototactic sensitivity (Fauré-Fremiet 1951, Palmer & Round 1965, Hopkins 1966, Round & Eaton 1966, Eppley *et al.* 1968, Happey-Wood & Jones 1988). However, past studies of vertical migration in epipelic algae appear to have overlooked

the surprisingly close relationship between surficial algal abundance and short-term changes in light intensity that became evident during the course of this investigation. Brief variations in surficial abundance may be partly due to sampling artefacts arising from a heterogeneous distribution of cells in the habitat. Yet, the results strongly suggest that they are a direct consequence of at least two different factors. The first is short-term changes in solar intensity as a result of cloud cover or other exogenous factors, which indicates that diatoms may be much more responsive to momentary fluctuations in insolation than previously thought. The second is photoinhibition.

In order to better evaluate the effect of insolation on migration in epipelic diatoms, results of five sampling dates were grouped into three arbitrarily-assigned categories of low, medium, and high light intensity, based on average values for each day. Since light measurements were only made at hourly intervals, all inhibitory thresholds stated here are provided strictly as estimates.

Low Light (mean intensity <500 μmol·cm^{-2}·s^{-1})

Considerable cloud cover at the DBG on October 28, 1998 provided noteworthy insight into the migratory behaviour of epipelic diatoms under average light conditions of less than 500 μmol·cm^{-2}·s^{-1} (Figure 2.5, A). The surficial abundance curve on this day closely emulated the illumination graph, suggesting a direct relationship between diatom abundance and insolation.

Reductions in diatom abundance concurrent with drops in illumination are a somewhat surprising feature of vertical migration during low-light conditions. This phenomenon, particularly during morning upward migration, suggests that a minimum light intensity is required not only to stimulate upward movement in the algae but also to maintain their presence on the sediment surface (Bracher 1929, 1937). In the absence of such a threshold, one would expect that a decline in photon flux would simply reduce the rate of emergence from the substratum, likely expressed as a more gradual slope on the abundance curve. However, this is clearly not the case. Even slightly decreased

insolation, as seen between 11:00 and 12:00 (Figure 2.5, A), appears sufficient to cause downward migration. This raises the additional question of why a seemingly negligible drop in light intensity to values that were previously sufficient to encourage upward movement should result in downward migration. Also evidenced *in vitro* (Figure 2.5, B), this behaviour may support claims of interspecific differences in minimum light thresholds. If certain species require higher light intensities to initiate upward migration, as reported in the past (Round 1979, Paterson 1986, Hay *et al.* 1993), then those most recently emerged from the sediment as a result of increasing photon flux will likely be the first to drift downward again following a reduction in insolation. This interpretation readily explains the illustrated abundance curves.

Once solar intensity has reached a certain value, it becomes a directional stimulus, causing specific algal species to migrate upwards. As that value is surpassed, algae with higher thresholds (Paterson 1986), as well as those situated in deeper sediment, also begin their upward migration, leading to an increased abundance of cells on the sediment surface. However, if light levels are reduced below the indicated value, the directional influence is lost. As a result, gravity becomes a determining factor for the algae, resulting in a net downward migration of cells. Although this behaviour has repeatedly been referred to as 'geotaxis' or 'gravitaxis' in the literature (Palmer & Round 1965, Round & Happey 1965, Round & Eaton 1966, Round & Palmer 1966, Round 1978, Happey-Wood & Jones 1988), implying a physiological response to gravity, it seems more likely that the absence of a directional stimulus, such as light, would inevitably result in a gradual downward flux in the otherwise randomly-moving cells (Nultsch 1974, Harper 1977). This would account for the presence of a small number of cells on the sediment surface at night (Round & Eaton 1966). Moreover, the slow descent, described as downward 'drift' by Round (1978), coupled with a cycle in cell motility (Hopkins 1965, Round 1966, Round 1978, Happey-Wood & Jones 1988), would explain why the algae do not burrow to depths from which they cannot recover. Proponents of the geotaxis theory have attributed this phenomenon to a rhythmic cycle in responsiveness to gravity (Happey-Wood & Jones 1988) as well as an ability to detect chemical gradients

in the sediment (Palmer & Round 1965). Conversely, the latter have also been proposed as a directional stimulus for downward migration, as well as predawn upward movement, in lieu of a geotactic explanation (Nultsch 1974). Due, however, to their variability between habitats and sediment types, it is unlikely that chemical gradients have a significant impact on short-term trends in algal migration.

Medium Light (mean intensity 500-1000 $\mu mol \cdot cm^{-2} \cdot s^{-1}$)

Average light intensities of between 500 and 1000 $\mu mol \cdot cm^{-2} \cdot s^{-1}$ were seen on two sampling dates (Figures 2.3, 2.9). On the first day (September 23, 1998), photon flux underwent an increasing trend to 1000 $\mu mol \cdot cm^{-2} \cdot s^{-1}$ at 11:00 (Figure 2.3, A). Beyond 550 $\mu mol \cdot cm^{-2} \cdot s^{-1}$, however, the density of diatom valves on the sediment surface began a very clear oscillation opposite light intensity. This, again, reinforces the theory of light thresholds, suggesting a maximum limit for positive phototaxis. Round (1978) proposed that midday downward migration is illustrative of passive downward 'drift' resulting from a decrease in phototactic sensitivity. However, the behaviour is far more indicative of negative phototaxis, as a means of avoiding or recovering from presumed 'photo-inhibition' (Round 1979, Daborn *et al.* 1993,Yallop *et al.* 1994). High insolation and enhanced UV-B radiation (Underwood *et al.* 1999) appear to elicit an active avoidance response in diatoms, causing them to move downward.

On the second day of average medium light intensity (June 17, 1999), illumination was impeded by patchy cloud cover until sometime between 13:00 and 14:00, at which point a maximum value in excess of 1200 $\mu mol \cdot cm^{-2} \cdot s^{-1}$, concurrent with a peak in algal abundance, was seen (Figure 2.9, A). This is inconsistent with findings described in the previous paragraphs and may indicate a cumulative effect of sunlight. On the first day, light levels rose much more rapidly, leading to an inverse response in diatom migration by 11:00. On the second day, however, illumination remained below 200 $\mu mol \cdot cm^{-2} \cdot s^{-1}$ for most of the morning and did not begin to increase notably until 11:00. As a result of these low light levels, the additive effect may have been minimal, thereby explaining why the large rise in photon flux during early afternoon did not have a detrimental effect on

by 11:00. On the second day, however, illumination remained below 200 $\mu mol \cdot cm^{-2} \cdot s^{-1}$ for most of the morning and did not begin to increase notably until 11:00. As a result of these low light levels, the additive effect may have been minimal, thereby explaining why the large rise in photon flux during early afternoon did not have a detrimental effect on surficial diatom abundance. This suggestion of cumulative impacts on migration is supported by the findings of Fauré-Fremiet (1951), who noted that diatoms left in constant light on a microscope slide reversed their phototropism and become photophobic after a few hours. Furthermore, these findings are consistent with those of Admiraal *et al.* (1982), who suggest that the light requirements of cells may be met during a few hours sojourn on the sediment surface.

The apparent lack of photoinhibition on the second day may also have been the result of one of two seasonal influences. Firstly, since the epipelic standing crop varies considerably with time of year (Hickman & Round 1970), the species composition of the diatom population in September was notably different from that in June (Table 2.1). It is predictable, based on the range of morphologies and physiologies between them, that at least a proportion of the species comprising the indicated assemblage, sampled in mid-June, may have had an innate tolerance to higher insolation. Secondly, the early summer community may simply have been adapted to longer periods of daylight and higher peak insolation values, a seasonal phenomenon previously evidenced by Barranguet *et al.* (1998). Lastly, it is conceivable that the exhibited response may have resulted from a combination of both species composition and seasonal adaptation.

High Light (mean intensity >1000 $\mu mol \cdot cm^{-2} \cdot s^{-1}$)

Average incident radiation in excess of 1000 $\mu mol \cdot cm^{-2} \cdot s^{-1}$ was recorded on three separate sampling dates. Based on data collected on these days, light clearly plays an additional role in migration at higher intensities. In most instances, diatom abundance decreased as light intensity exceeded between 550 and 750 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This range coincides with the photosynthetic saturation level of marine phytoplankton (Lee 1989),

suggesting that an upper tolerance limit (Round & Eaton 1966, Round 1979, Daborn *et al.* 1993, Yallop *et al.* 1994) may lead to a negative phototactic response by the algae as visible light and UVB levels exceed a certain value. This avoidance of photoinhibitory light levels is expressed in the form of downward migration (Blanchard 1994, Blanchard *et al.* 1994, Barranguet *et al.* 1998, Kingston 1999).

High average insolation values were first recorded during the JNP component of the project. Results from this day strongly suggest that fluctuations in surficial diatom abundance are a direct result of short-term variability in solar intensity. Aside from an early morning peak, surficial diatom abundance was clearly related to illumination (Figure 2.2, A). Cell numbers increased until 11:00, at which point they began to oscillate opposite light intensity. This inverse relationship beyond 1500 $\mu mol \cdot cm^{-2} \cdot s^{-1}$ strongly supports the light threshold theory presented previously. Interestingly, the indicated threshold, interpolated from the graph, is double the threshold interpreted from the DBG data. This difference may have been a function of water clarity. The DBG site was visibly higher in DOM and was therefore subject to considerably lower light penetration. This is consistent with the findings of other researchers, which indicate that algae grown under full sunlight are more resistant to photoinhibition than cells of the same species grown under shaded conditions (Round 1967, Vonshak & Guy 1992). Since light-incubated algae are much more tolerant of high photon flux, it is reasonable to expect a greater threshold in diatoms of the clear JNP waters.

A second incidence of high average light conditions was recorded at the DBG site on June 4, 1999 (Figure 2.7, A). Aside from minor fluctuations around midday, light intensity followed a typical curve, increasing steadily until early afternoon and dropping off thereafter. Diatom abundance appeared to reach a peak at an insolation value of close to 750 $\mu mol \cdot cm^{-2} \cdot s^{-1}$ during mid-morning, again suggesting a much lower maximum threshold than was seen in the JNP pond. A slight increase in surficial diatom density during midday, despite continuing high insolation, was attributed to the preceding

downward migration. The described submergence may have acted as a "self-imposed dark period" (Palmer & Round 1965), providing cells with an opportunity to recover from previous exposure to solar radiation. A subsequent mid-afternoon peak in cell abundance coincided with declining illumination and may also have been facilitated by a period of recovery associated with a preceding downward movement.

The final day of high light intensity occurred on June 11, 1999 (Figure 2.8, A). Aside from a fairly sharp decline and rapid recovery around noon, insolation again described a typical curve, peaking at midday and decreasing thereafter. As in the previous paragraph, the initial maximum in surficial diatom abundance was noted at an illumination value of approximately 750 $\mu mol \cdot cm^{-2} \cdot s^{-1}$. Thereafter, diatom density began to fluctuate opposite light intensity, almost mirroring the latter curve for the remainder of the sampling period. A late-afternoon drop in insolation, which again approached the 750 $\mu mol \cdot cm^{-2} \cdot s^{-1}$ level, appeared to draw a proportion of the cells back to the surface.

Although large photon fluxes seemed to cause a negative response in the algae, it is interesting to note that, for the most part, the surficial population displayed a rapid recovery as soon as light levels began to drop, despite the fact that they remained well above what had been identified as the presumed inhibitory value. Since light measurements were made on an hourly basis, it is quite conceivable that the impetus behind certain migrations was missed. However, it is also possible, particularly based on its frequency of occurrence, that the upward movement of algae in bright sunlight during midday arises at least partly from a rapid recovery mechanism within the photosystem (Hay *et al.* 1993). Once a diatom moves into the sediment, it may begin regenerating its photosystems in the partial shade afforded by the sediment particles. As soon as light levels begin to drop, the alga can re-emerge and resume photosynthesising. The regeneration period may help explain why the algae move upward despite levels of insolation that previously caused downward migration.

Additional Artefacts

Eaton and Moss (1966) questioned the extent to which light transmits through lens tissue, suggesting that some degree of experimental bias may arise as a direct result of attenuation. This is undoubtedly the case, as a brief test performed during the course of the experiments herein described revealed. However, it was felt that the twenty percent reduction in solar intensity determined from this test was quite acceptable in the absence of a less disruptive sampling method. Furthermore, one might argue that the tissue paper may do little more than emulate the light filtration characteristics of an additional layer of sediment. Eaton and Moss (1966) also addressed the issue of dead and dividing cells as a potential source of error during sample analysis. The former point was not considered of concern, since the sampling method clearly relies on the ability of a cell to move. Dividing cells, on the other hand, although probably not of major consequence overall, may have played a considerable role on days during which very large morning peaks in *in situ* diatom abundance were detected. Surprisingly few cells were found in the active stages of division during sample analysis, however. This may have been due to the considerably disruptive preparation method. Conversely, the apparent lack of dividing cells may reflect the findings of Saburova and Polikarpov (2003), which suggest that the proportion of diatom cells undergoing mitosis increases with increasing depth in the sediment.

2.4. Conclusion

The main objective of this project, namely to develop a method for *in situ* sampling of the epipelon that would incorporate the advantages of the lens tissue technique, was met. Results obtained through the ring method strongly suggest that vertical migration does occur in permanently inundated non-tidal habitats and seem to indicate that it is an effective and viable sampling protocol. Moreover, similarities in results obtained through the ring method and through the traditional lens tissue technique, particularly during low light conditions, indicate a considerable degree of accuracy in identifying the migratory

pattern of epipelic diatoms. Based on examination of lens tissues prior to acid digestion, it is predicted that the ring method is not restricted to sampling of diatoms alone. Rather, it could be used to analyse a wide range of motile microalgae in the epipelon.

Based primarily on a considerably reduced potential for the introduction of sampling artefacts, the ring method was deemed superior to the lens tissue technique. However, a number of additional factors were considered in making this determination. As anticipated, appreciable differences were recorded between migratory patterns identified using the ring method and those observed using the lens tissue technique. Due to the lack of a protective water column, algae sampled *in vitro* were generally more sensitive to high levels of insolation, tending to exhibit a greater degree of suppression and more pronounced avoidance behaviour. Contrary to expectation, there was little difference in morning emergence times between the sampling protocols. However, overall cell abundance on the sediment surface was occasionally greater in the petri dishes than it was in the pond. This was mainly attributed to sampling artefacts of the lens tissue technique, which may have resulted in an artificially elevated concentration of diatoms in the sediment.

LTSEM analysis of sediment matrices revealed considerable differences between sampling techniques. EPS trails were, as expected, noticeably disrupted in the petri dishes. The reticulate structure seen *in situ* was nearly absent *in vitro* and EPS strands were frequently clumped together and fouled with detrital matter. Although this may have hampered diatom migration, the EPS matrix was not sufficiently extensive to permit a definitive statement on its role in algal movement.

In samples subjected to the lens tissue technique, LTSEM analysis showed visible sediment compaction. Contrary to expectation, this did not have an apparent impact on *in vitro* emergence times. A more dense arrangement of particles may have facilitated, rather than impaired, diatom movement due to an increased availability of substrata

across which to travel. In addition, decreased light penetration arising from closer placement of sediment grains may help explain the discrepancy in observed cell abundance between sampling techniques. Whereas cells located *in situ* may receive sufficient insolation slightly below the sediment surface, those in the petri dishes may have been forced to emerge completely in order to photosynthesize at optimum capacity.

Insolation was implicated as the primary environmental factor regulating migration in epipelic diatoms. Moreover, the possibility of a surprisingly close and, to the best of my knowledge, previously unrecognised relationship between short-term fluctuations in light intensity and surficial diatom abundance was identified. This may help explain minor diurnal variations in surficial cell abundance previously described by Round & Happey (1965) and Round & Palmer (1966).

During low-light conditions, cell density closely emulated the insolation curve. Downward movement of cells concurrent with short-term reductions in solar radiation supports previous suggestions that a minimum light intensity is required to initiate upward migration as well as maintain an appreciable algal presence on the sediment surface. Interspecific differences in this presumed light threshold are thought to explain decreases in surficial abundance, despite light levels that remained higher than those which previously appeared to stimulate upward migration. Those algae with the highest minimum threshold were expected to be the first to resubmerge following a decrease in photon flux. Conversely, those with lower thresholds were predicted to tolerate a larger reduction in light intensity and remain on the sediment surface after the previous group had drifted downward.

Medium and high light conditions served to illustrate several additional characteristics of vertical migration in epipelic diatoms. The existence of a maximum light threshold was clearly indicated. Light in excess of the upper tolerance limit appeared to incite negative phototaxis, causing an avoidance response in algal cells and leading to active

downward migration. This threshold may also be a function of cumulative effects. A very rapid increase in insolation, and therefore a reduced additive impact, may permit cells to endure much higher radiation values before being forced to burrow. Time of year, in terms of solar intensity, day length, and light acclimatization, and species-specific tolerances are also thought to play a role in defining light thresholds.

In addition to supporting the proposed existence of light thresholds, high insolation values revealed an interesting function of downward migration during midday. Not only does it serve as an avoidance tactic, protecting the cell from damaging radiation, but it also appears to serve in a regenerative capacity, allowing the alga to repair or recharge its photosystems prior to returning to the sediment surface. A marked impact of ultraviolet radiation on migration was also implicated as a factor in downward movement during peak photon flux; an opportunity for additional research is indicated.

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Table 2.1. Top ten most abundant diatom species by sampling location and date, based on sum of hourly counts for each day. Species dominance refers to relative abundance, with the most abundant species occurring at level one and the least abundant at level ten.

Species Dominance	Site and Date					
	Jasper August 01, 1998	Devon September 23, 1998	Devon October 28, 1998	Devon June 04, 1999	Devon June 11, 1999	Devon June 17, 1999
1	*Achnanthes minutissima*	*Navicula cryptocephala*	*Navicula cryptocephala*	*Nitzschia spp.*	*Gomphonema augur*	*Nitzschia spp.*
2	*Navicula bryophila*	*Navicula veneta*	*Nitzschia spp.*	*Navicula cincta*	*Nitzschia spp.*	*Navicula cryptocephala*
3	*Navicula cryptocephala*	*Navicula capitatoradiata*	*Navicula radiosafallax*	*Gomphonema truncatum*	*Navicula cincta*	*Navicula cincta*
4	*Encyonema norvegica*	*Nitzschia spp.*	*Navicula capitata*	*Gomphonema parvulum*	*Navicula cryptocephala*	*Navicula capitata*
5	*Nitzschia spp.*	*Navicula trivialis*	*Navicula cuspidata*	*Navicula veneta*	*Gomphonema truncatum*	*Navicula veneta*
6	*Navicula cf. halophila*	*Navicula seibigii*	*Navicula cincta*	*Gomphonema acuminatum*	*Navicula capitata*	*Navicula pseudolanceolata*
7	*Brachysira brebissonii*	*Gomphonema parvulum*	*Cocconeis placentula*	*Navicula cryptocephala*	*Gomphonema parvulum*	*Navicula seibigii*
8	*Diploneis sp.*	*Navicula accomoda*	*Navicula pseudolanceolata*	*Navicula seibigii*	*Navicula veneta*	*Gomphonema parvulum*
9	*Amphora sp.*	*Cocconeis placentula*	*Navicula capitatoradiata*	*Navicula capitata*	*Gomphonema gracile*	*Navicula radiosafallax*
10	*Encyonema silesiaca*	*Navicula wildii*	*Navicula pupula*	*Cocconeis placentula*	*Navicula seibigii*	*Gomphonema truncatum*

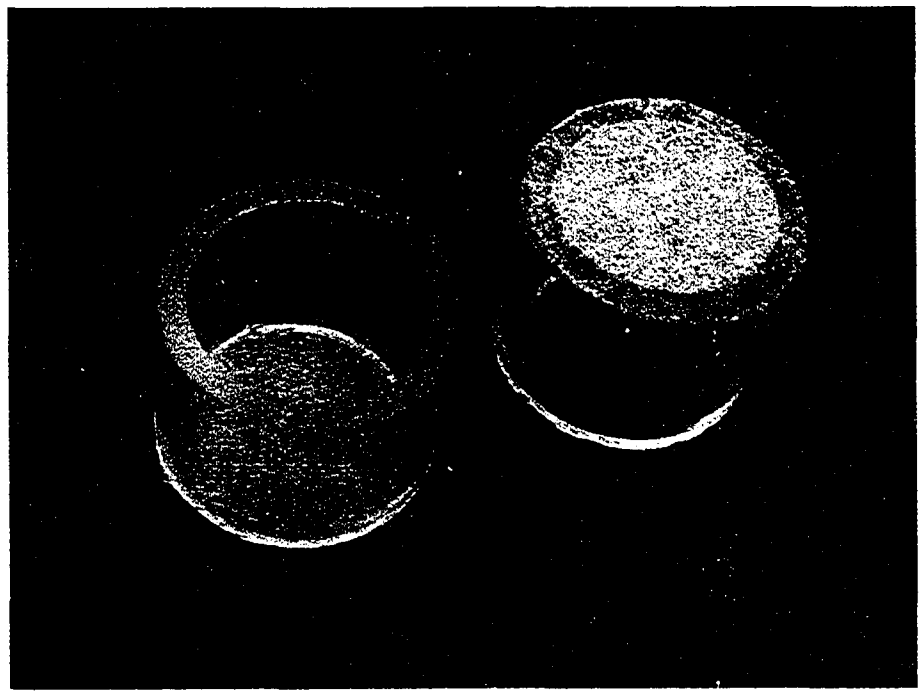


Figure 2.1. Acrylic rings employed for the 'ring method' of sampling (A = upright, B = inverted). Vertically migrating algae in the epipelon travel between the tissue fibres and are 'captured' through removal of the ring from the sediment.

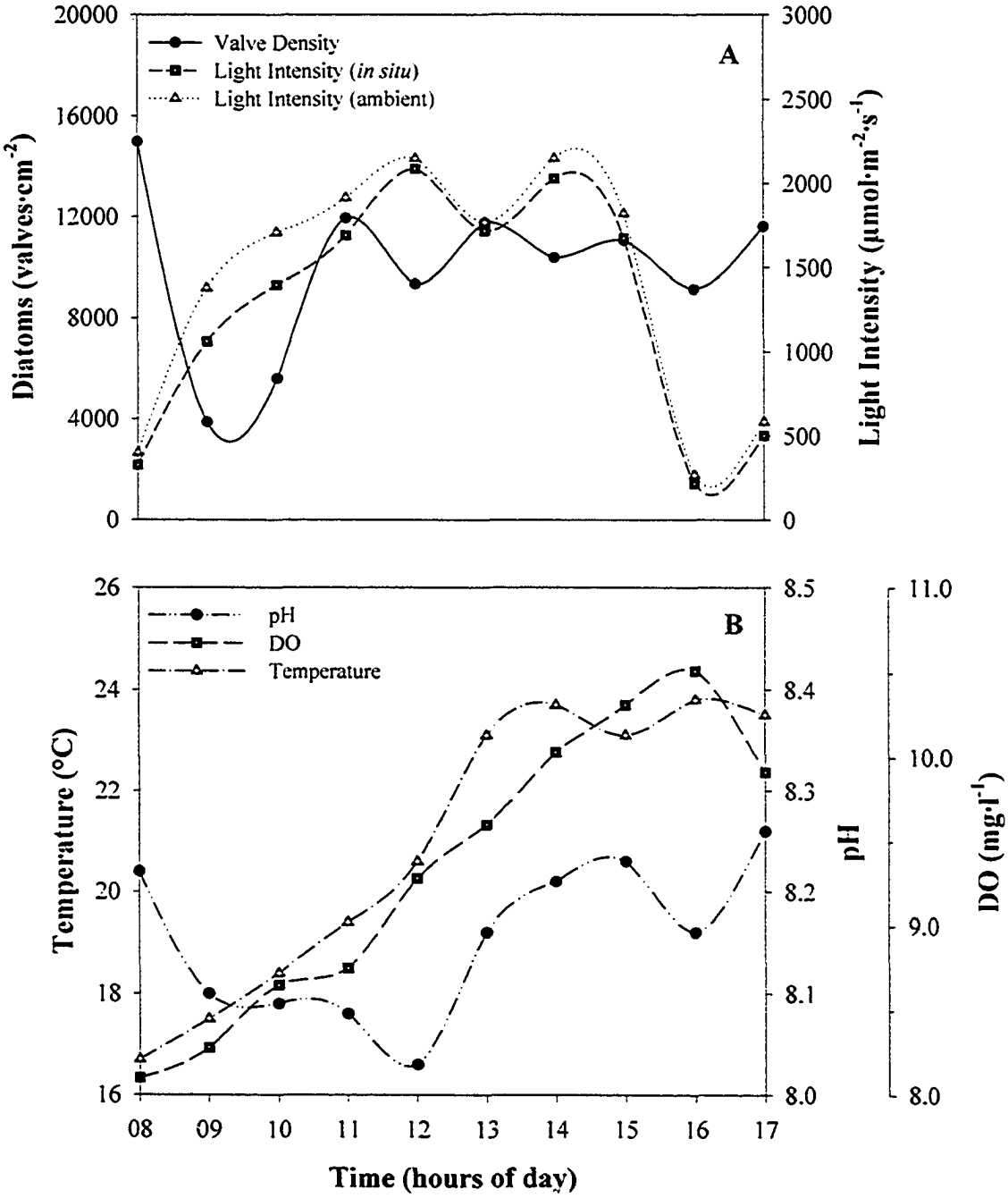


Figure 2.2. Changes in total diatom valve abundance and light intensity (A) and pH, dissolved oxygen concentration, and temperature (B) on the sediment surface of a pond in Jasper National Park on August 29, 1998. Ambient light intensity refers to insolation at the pond surface.

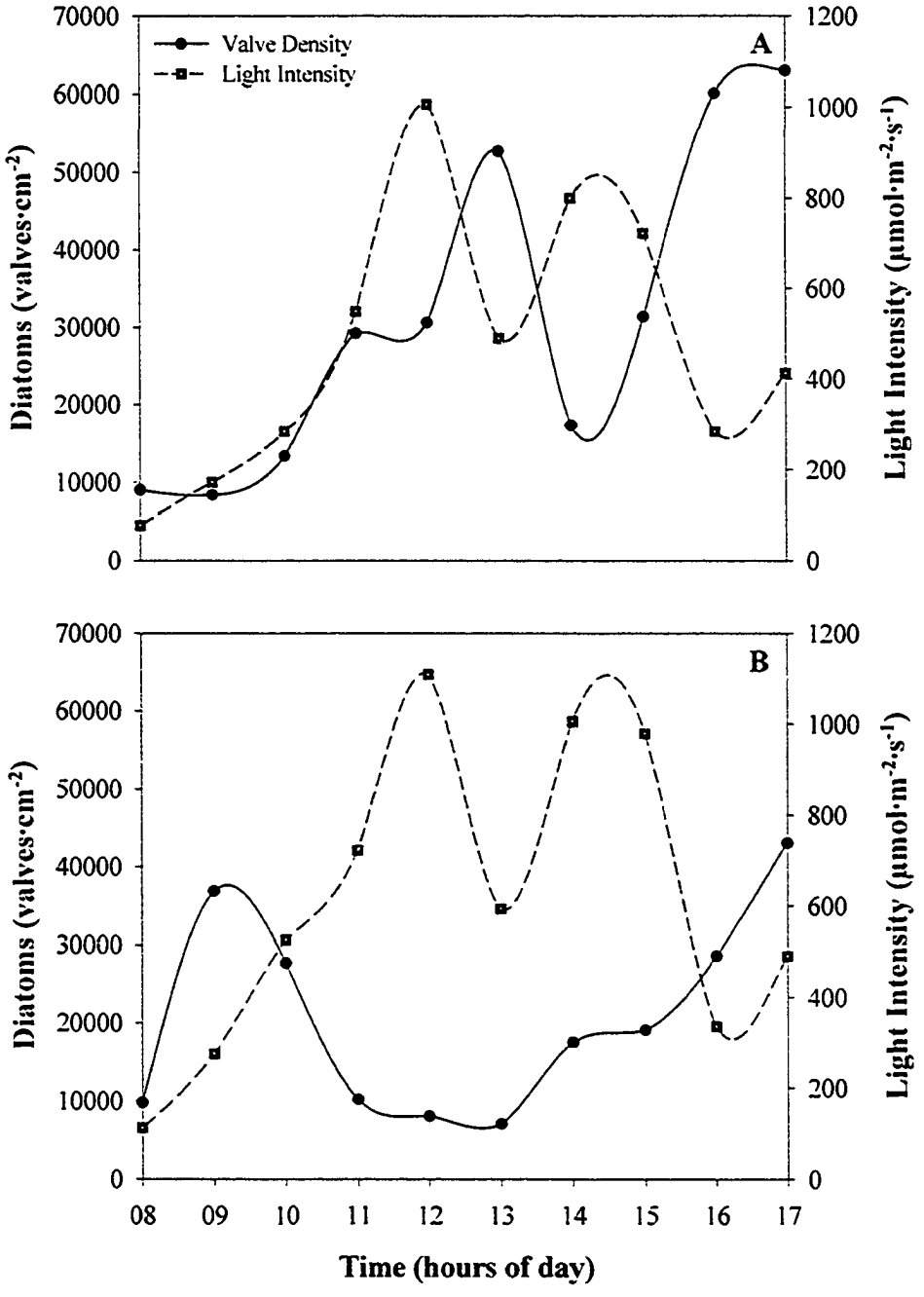


Figure 2.3. Changes in light intensity and mean diatom valve abundance on the sediment surface in the Grebe Water on September 23, 1998 (A = *In situ*, B = *In vitro*).

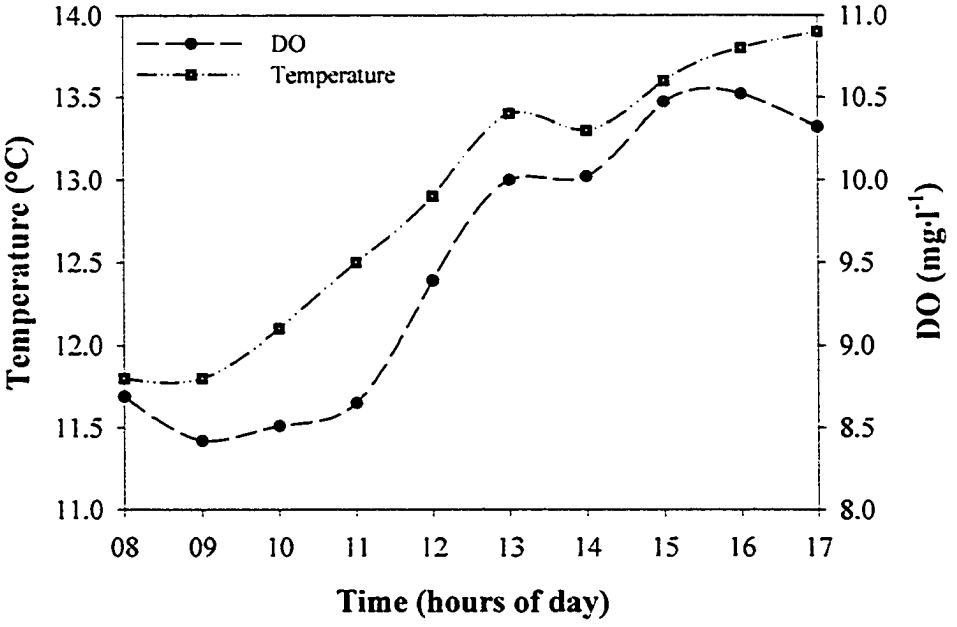


Figure 2.4. Changes in dissolved oxygen concentration (DO) and water temperature at the sediment surface in the Grebe Water on September 23, 1998.

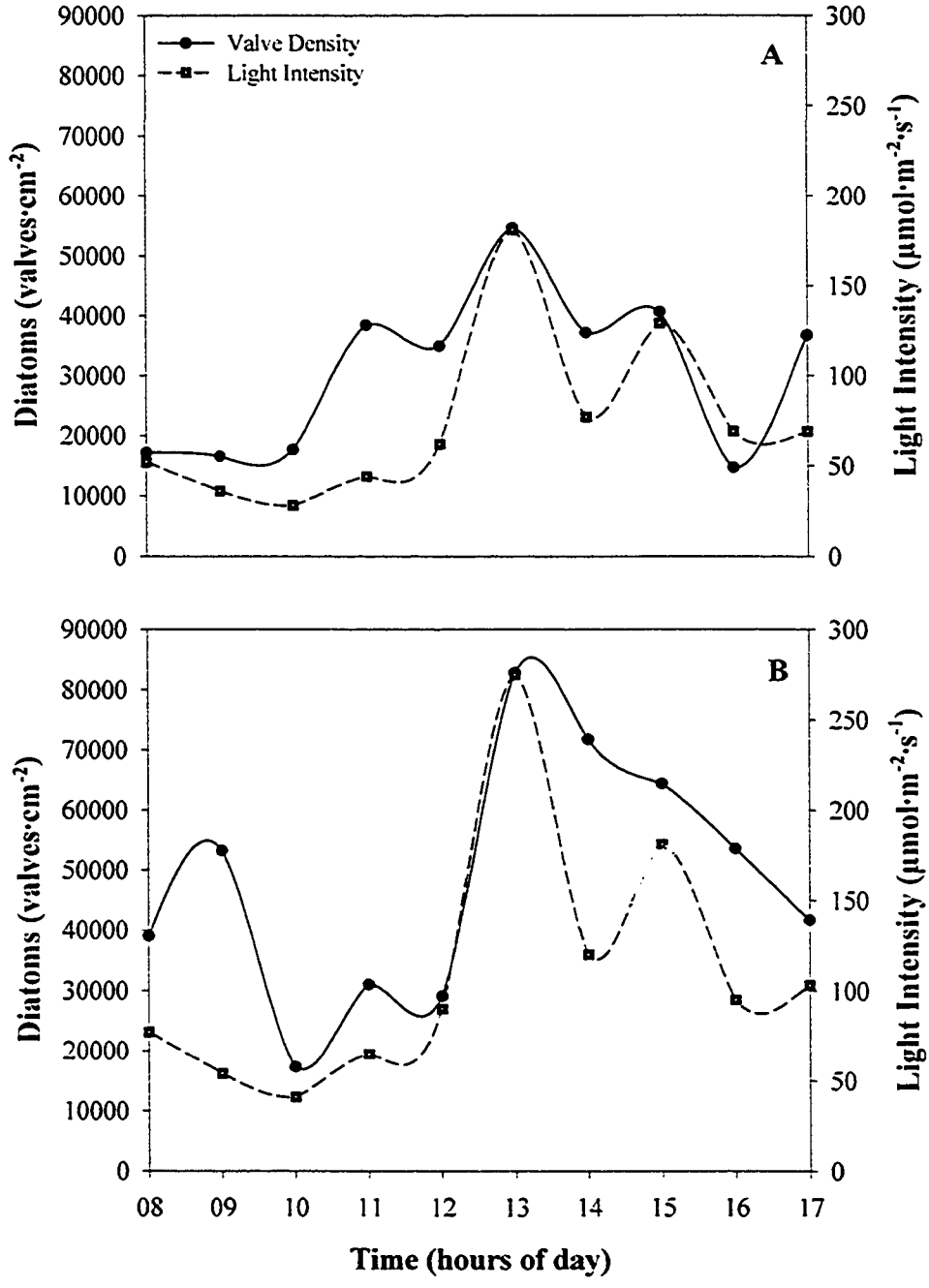


Figure 2.5. Changes in light intensity and mean diatom valve abundance on the sediment surface in the Grebe Water on October 28, 1998 (A = *In situ*, B = *In vitro*).

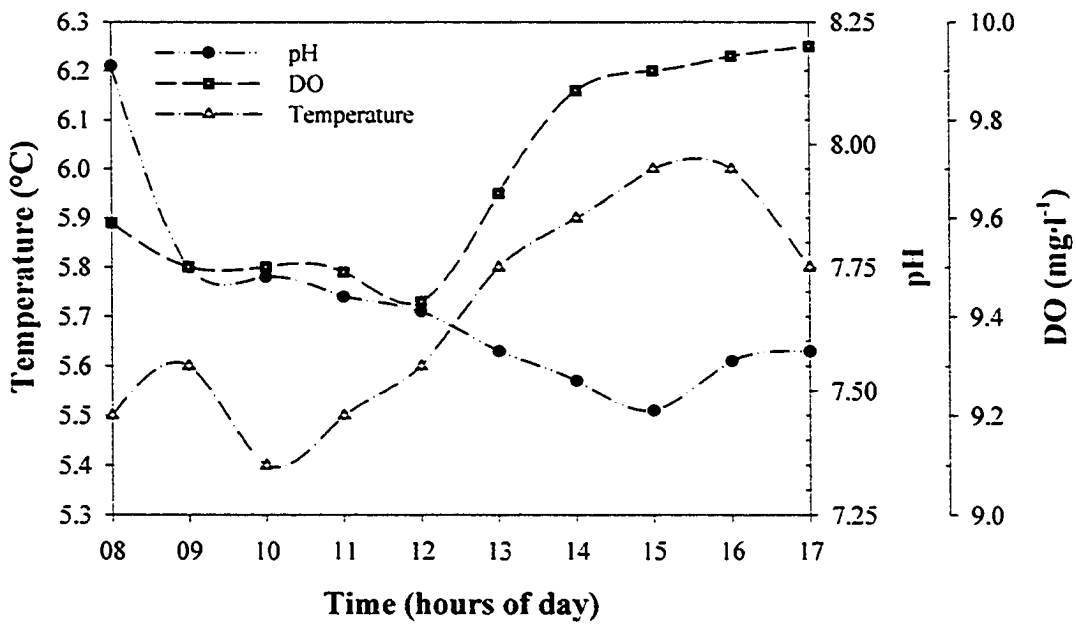


Figure 2.6. Changes in pH, dissolved oxygen concentration (DO), and water temperature at the sediment surface in the Grebe Water on October 28, 1998.

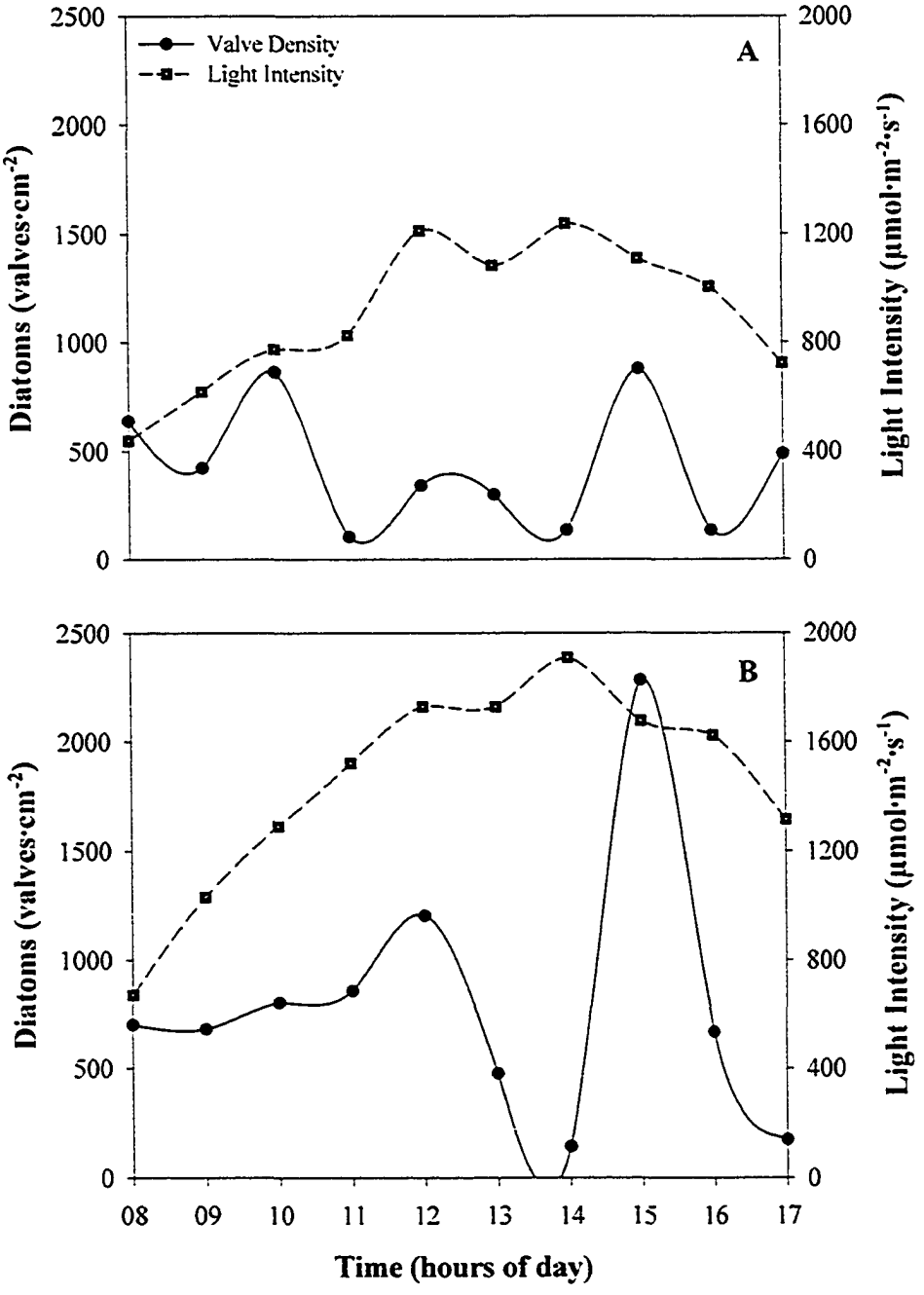


Figure 2.7. Changes in light intensity and mean diatom valve abundance on the sediment surface in the Grebe Water on June 4, 1999 (A = *In situ*, B = *In vitro*).

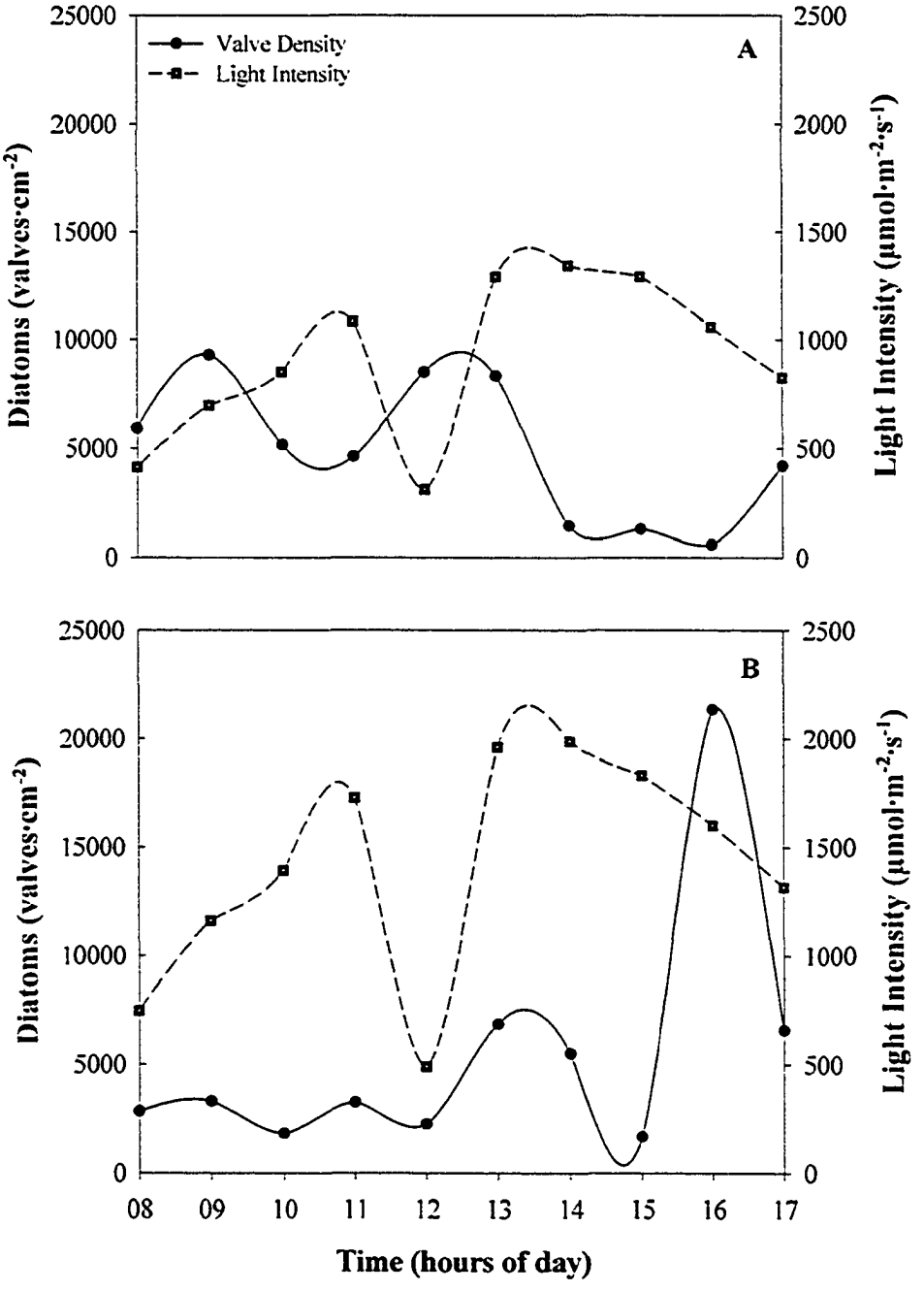


Figure 2.8. Changes in light intensity and mean diatom valve abundance on the sediment surface in the Grebe Water on June 11, 1999 (A = *In situ*, B = *In vitro*).

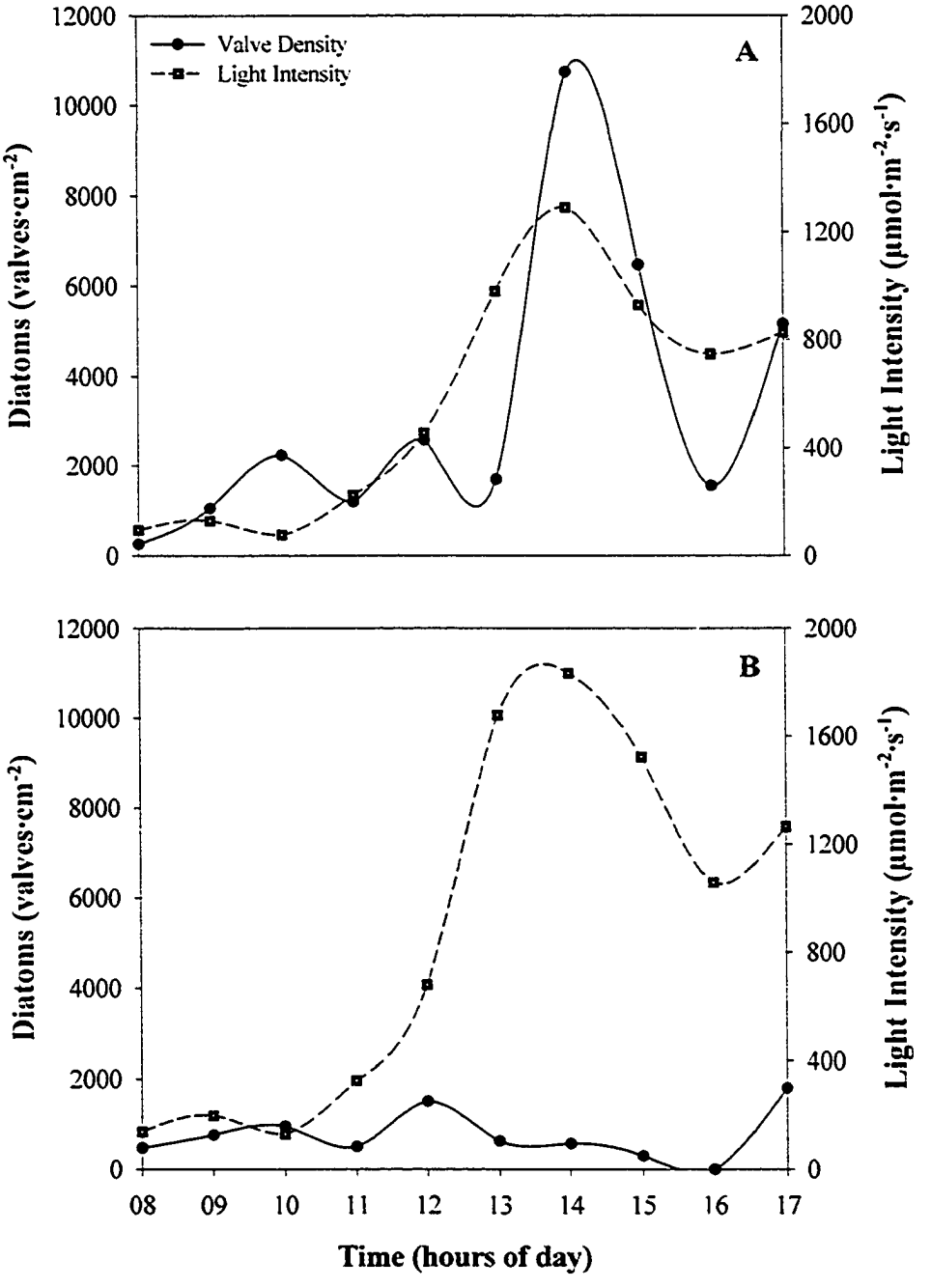
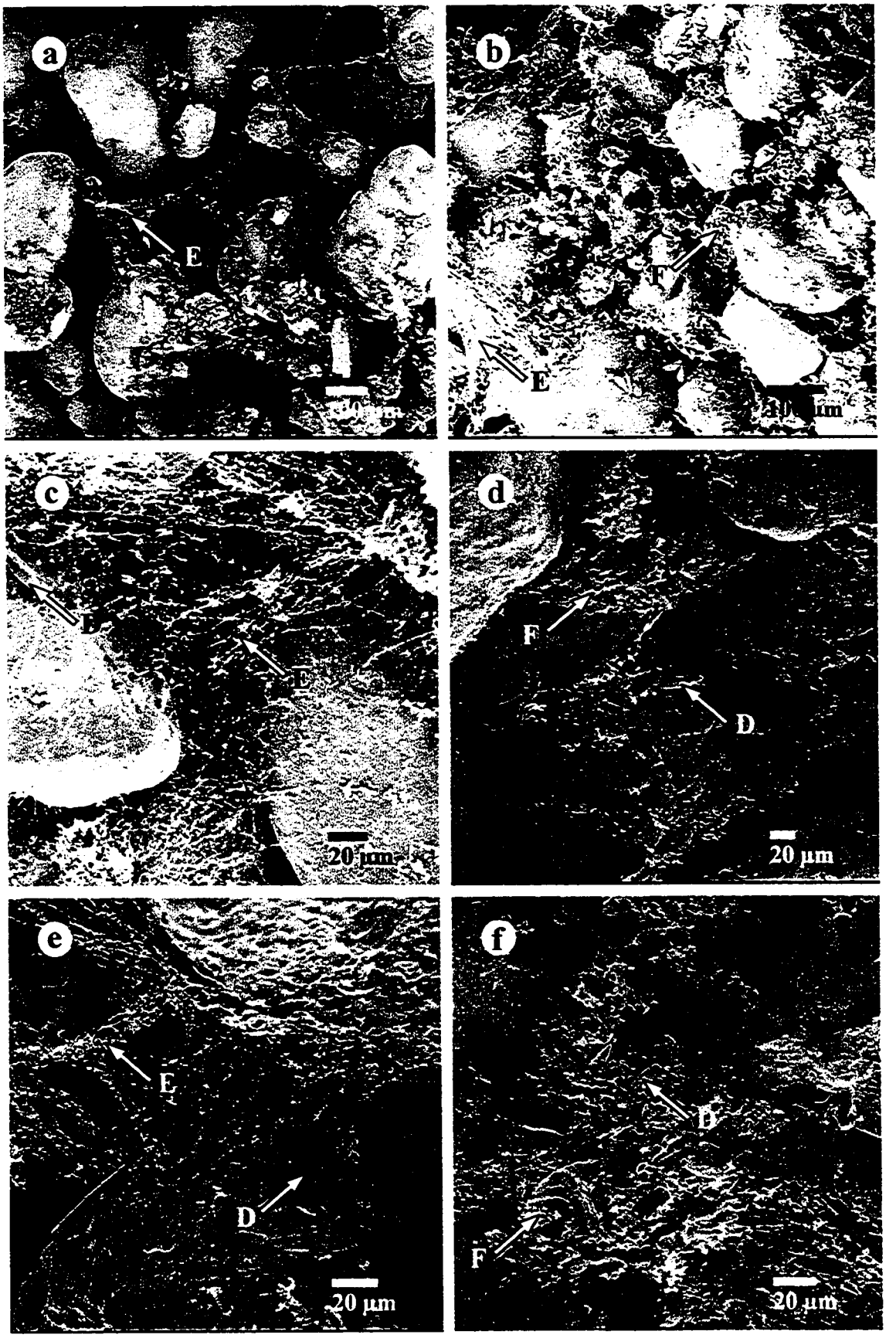


Figure 2.9. Changes in light intensity and mean diatom valve abundance on the sediment surface in the Grebe Water on June 17, 1999 (A = *In situ*, B = *In vitro*).

Figure 2.10. Low-temperature scanning electron micrographs of sediments sampled via the ring method (a, c, e) and the lens tissue technique (b, d, e). Images are paired horizontally according to similar magnifications (E = EPS trails, F = flocculent debris, D = diatom).



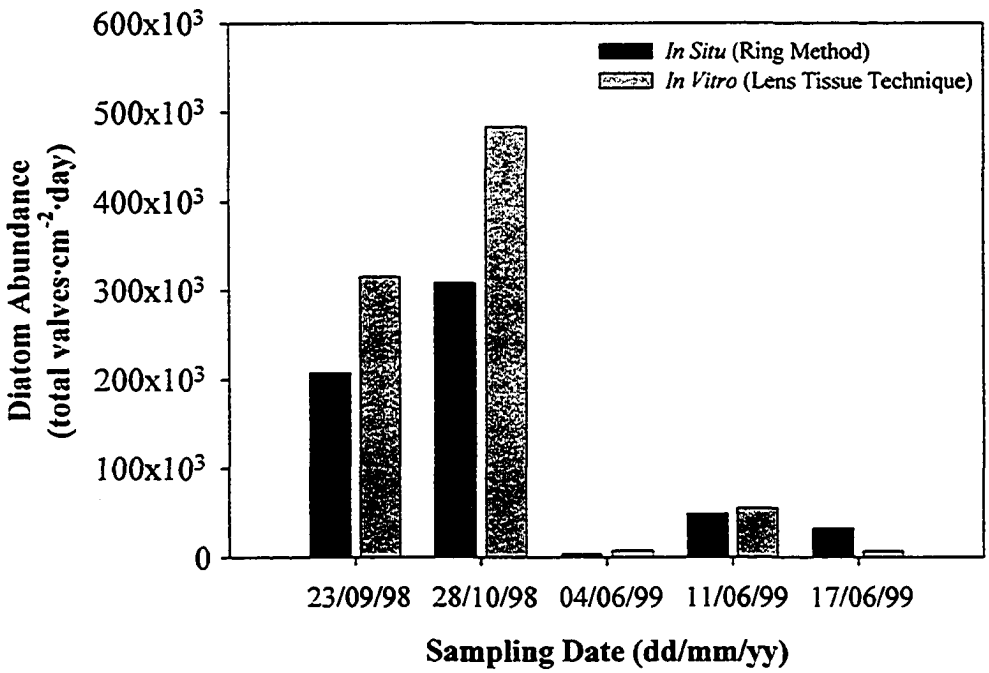


Figure 2.11. Sum of mean hourly surficial valve densities in the epipelon of the Grebe Water based on sampling technique and date.

Chapter 3.

PATTERNS OF DIEL VERTICAL MIGRATION IN FRESHWATER EPIPELIC DIATOMS: MOVEMENT UNDER THE CONTINUOUS ILLUMINATION OF POLAR NOON

3.1. Introduction

It has long been recognized that the majority of motile algal species in the epipelon undergo a series of diel vertical migrations in response to changing environmental parameters. Of the stimuli thus far implicated in patterns of algal movement, the most influential appears to be light intensity. However, past results have repeatedly suggested the involvement of an additional endogenous factor (Fauré-Fremiet 1951, Hastings & Sweeney 1957, Palmer & Round 1965, Hopkins 1966). It is generally agreed that this mechanism, commonly termed a 'biological clock' (Round & Happey 1965, Round & Eaton 1966, Round & Palmer 1966), is responsible for regulating the period, if not the amplitude, of migration. The nature of this internal clock has been a point of some contention and, through an investigation of algal migration under conditions of natural 24-hour daylight, will be addressed in the ensuing chapter.

Endogenous rhythms are the result of innate physiological processes and, as such, are generally independent of environmental changes (Jarosch 1962, Müller 1978). However, the latter do play a vital role in modifying cyclic behaviour (Palmer & Round 1965, Enright & Hamner 1967). Numerous researchers have attempted to verify the presence of an endogenous component to migration in epipelic algae (e.g. Eppley *et al.* 1968, Happey-Wood & Jones 1988, Jönsson *et al.* 1994). Through artificial manipulation of daily light patterns, they hoped to establish whether or not algae exhibit a rhythm of sensitivity to photon flux. The results of such experiments suggest that epipelic algae undergo daily cycles in both rates of movement and responsiveness to light (Happey-

Wood & Jones 1988). In most cases, it was found that a typical migratory cycle persisted for several days under constant illumination while the pattern was either muted (Round & Happey 1965, Round & Eaton 1966,) or lost (Palmer & Round 1965, Round & Palmer 1966, Jönsson *et al.* 1994) under continuous darkness. In the case of *Euglena gracilis*, a single light signal was sufficient to adjust the phase of the migratory rhythm (Bruce & Pittendrigh 1958). Resumption of migration following reintroduction of the algae into a natural light-dark cycle suggests that the endogenous clock continues to run despite the lack of an overt display (Round & Palmer 1966).

Loss of the migratory pattern over time, whether under constant illumination or continuous darkness, has been attributed to the lack of a 'pacemaker' (Round & Eaton 1966) or 'Zeitgeber' (Müller 1973). The pacemaker, a predictable and marked change in a specific environmental parameter, serves to entrain a biological rhythm. In the case of algal migration, as with many other biological processes (e.g. Buchanan & Haney 1980), the pacemaker is most likely a significant change in insolation characteristic of sunrise and sunset. Although a number of studies have investigated algal movement in the absence of a pacemaker (Palmer & Round 1965, Round & Happey 1965, Round & Eaton 1966, Round & Palmer 1966, Jönsson *et al.* 1994), only a few appear to have done so under conditions of natural 24-hour insolation (Müller-Haeckel 1973, Müller-Haeckel & Solem 1976). However, rather than addressing migration in the epipelon, the latter studies investigated diurnal patterns of drift in epilithic algae of streams. Müller-Haeckel (1973) discovered that the daily rhythmicity in the number of cells of certain species breaking loose from the substrate was considerably less synchronized with the 24-hour period near the summer solstice than it was at other times of the year. Interestingly, the pattern became more precise under cloudy conditions.

Objectives

A) The Effects of 24-Hour Daylight on Migration

The primary purpose of this chapter was to examine diel patterns of vertical migration in epipelic diatoms subjected to continuous natural daylight. In order to do so, the ring method of *in situ* sampling was applied in subarctic aquatic environments near the time of the summer solstice, a period frequently referred to as 'polar noon'. Characterized by extreme lighting conditions (Müller 1973, Head *et. al* 1985) and high diatom standing crop (Moore 1974), this choice of region and season was expected to give the best possible representation of algal movement regimes in the presumed absence of a pacemaker.

Due to the lack of a pacemaker during the 24-hour illumination of polar noon, it was hypothesized that the biological clock would no longer be synchronised, leading to less distinct patterns of migration in epipelic diatoms and to considerable movement throughout the entire day. This was further based on the assumption that the most prevalent regulatory influences on migration, aside from small fluctuations in illumination, would be the photosynthetic thresholds of individual cells as well as the cumulative impact of constant exposure to solar radiation. Since the behaviour of individual diatoms would no longer be entrained to the same rhythm, it was anticipated that some degree of equilibrium would be reached in terms of the number of algae travelling upward and those migrating down. Therefore, fluctuations in surficial diatom density were expected to be generally muted. However, as a result of diel changes in the angular height of the sun, which at lower values reduces the penetration of light into water, it was predicted that the most distinct periods of algal migration would still occur during 'typical' daylight hours. On days of minimal cloud cover, a suppression of surficial algal abundance was anticipated around solar noon (14:00; Herzberg Institute of Astrophysics 2003) due to the high intensity of sunlight above the arctic circle. Based on findings in the previous chapter as well as those of Müller-Haeckel (1973), it was also predicted that, on days of lower photon flux due to cloud cover, the vertical migration

graph would closely approximate the illumination curve.

B) Interspecific Differences in Migration

The second objective of this chapter was a species-specific analysis of diatom migration in the epipelon. Previous research established that patterns of migration in epipelic algae may vary between species as a result of differences in light tolerance and velocity of movement (Round & Happey 1965, Round & Palmer 1966, Round 1979). In order to assess whether fluctuations in surficial algal abundance during 24-hour insolation are related to such interspecific differences or merely attributable to the behaviour of individual cells, diatom counts were performed on a species-specific basis. Due to the aforementioned absence of a pacemaker, it was expected that algal movement would rely primarily on the relationship between illumination and the light thresholds of individual cells and species. As a result of differing velocities of travel and dissimilar minimum light requirements for the initiation of a phototactic response, interspecific variability in migratory patterns was predicted to emerge on days of fluctuating, low intensity insolation. On days of consistently high light intensity, however, migratory behaviour was expected to rely on the light tolerance, vertical distribution, and previous exposure of individual cells, thereby resulting in asynchronous movements of diatoms in the epipelon.

3.2. Materials and Methods

Sampling rings were prepared in advance as described in section 2.1.1. A total of 210 slices of clear acrylic tubing (height, 13 mm; inside diameter, 18 mm; outside diameter, 24 mm) were adhered to large sheets of lens tissue using clear silicon sealant. Once the silicon had dried, a scalpel was used to cut around the outer edge of each ring, thereby freeing it from the lens tissue. The result was a series of cylindrical sampling 'chambers', each with one open end and one end completely covered by tissue.

Sampling

At 18:00 on July 9, 1999, sixty sampling rings were placed tissue side down on a reasonably even, uninterrupted section of organic sediment in a small, slow-flowing stream (68° 19' 9" N, 133° 27' 35" W; ~170 m asl) located in a willow-dominated peatland southwest of Inuvik, Northwest Territories. An additional 60 rings were placed in a small wetland pond (68° 19' 6" N, 133° 25' 21" W; ~170m asl) situated in a *Sphagnum* bog roughly a kilometre away from the first site. In both cases, rings were placed at a water depth of four centimetres. In order to minimize the effects of recent disturbance on the migratory pattern, the rings were given an equilibration period of seven hours. Sampling began at 01:00 with the hourly removal of two randomly-selected rings from each of the two sites until 00:00 the next evening. At each sampling time, light intensity was measured at the sediment surface using a Li-Cor Model LI-185A meter with a submersible quantum millivolt sensor.

Following collection, lens tissues were placed in a well-ventilated area and permitted to air dry. Once completely dried, tissues were cut away from the inside edge of their respective cylinders and placed individually into 1.5 ml microcentrifuge tubes for storage and subsequent analysis in the laboratory.

At 15:00 on July 10, 1999, ninety more rings were placed on the stream sediments directly adjacent to the previous sampling site. Beginning at 01:00, three randomly-chosen rings were collected at hourly intervals until 00:00 the following night. As before, each tissue was dried, gently cut from the acrylic ring, and placed in a microcentrifuge tube for storage and transport.

Sample Processing

Lens tissues were acid-digested according to Eaton and Moss (1966). As described in section 2.1.1, 20 mg of potassium dichromate crystals, 0.30 ml of distilled water, and 0.20 ml of 98% sulphuric acid were added to each microcentrifuge tube. The samples

were shaken and allowed to settle overnight. The following morning, eighteen of the tubes were transferred to a Beckman Microfuge B centrifuge and spun for five minutes. Thereafter, 0.35 ml of supernatant were carefully pipetted from each tube and replaced with an equal volume of distilled water. The samples were again agitated and centrifuged for another five minutes. This process of centrifugation and resuspension was repeated four to eight times for each set of 18 vials until all samples registered a neutral pH (7).

The processed samples were again agitated and immediately pipetted in their entirety to individual circular cover glasses (18 mm, #1), which had previously been placed on a warming tray. Once all water had evaporated, each cover slip underwent the following sequence of treatments. A small amount of Naphrax® mounting medium (refractive index ~1.74) was applied to the centre of a microscope slide that had been preheating on a hotplate. This was allowed to bubble for several seconds before the cover glass was placed, sample side down, onto the hot medium. Ten seconds later, the slide was removed from the plate and permitted to cool while gentle pressure was applied to the cover glass, thereby ensuring complete coverage of the mounting medium and preventing the formation of air bubbles between the slide and cover. In this way, permanent mounts were made from all of the samples.

Following preparation, each permanent slide was examined at 1000 x magnification (oil immersion) under a Leitz Laborlux S microscope until a minimum of 300 fields of view had been traversed. If less than 200 diatom valves were counted within the 300 fields, additional fields, up to a maximum of 800, were appraised until at least 200 valves had been enumerated. Whole frustules were counted as two valves and, wherever possible, valves were identified to species using Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b) as the primary taxonomic reference. In order to expedite the process, members of the genus *Nitzschia*, which are frequently difficult to differentiate, were not resolved to the species level. Diatom segments were scored as estimated fractions of whole valves. The number of valves of each species per fields counted was

recalculated as the total per slide and adjusted to reflect the number for each species per square centimetre of lens tissue. The resulting value was interpreted as the number of valves of each species per square centimetre of sediment surface.

Statistical Analyses

Due to considerations of low replication, autocorrelation, and pseudoreplication, indepth statistical analyses were largely deemed inappropriate for this study. However, some species-specific comparisons were possible. In order to ensure meaningful results and reasonably continuous data, species-specific analyses were restricted to those species appearing in substantial numbers and at most sampling intervals. In the stream habitat, this translated into the top nine species, by abundance, during high insolation sampling and the top eleven species during low insolation sampling. In the wetland pond, this limited analyses to the top six species. Similarities in migratory patterns between these species were determined through Pearson correlation matrices while significance of the calculated coefficients was ascertained through Bonferroni probabilities. Statistical work was performed with Systat 10 software, while valve abundance and light intensity were communicated as simple spline curves using Sigmaplot 5.0. Closely-correlated species abundance curves were depicted graphically as a single line with standard error bars.

3.3. Results and Discussion

A complete data set for Chapter 3 is presented in Appendices 3.1. through 3.3.

3.3.1. Patterns of Migration under 24-Hour Daylight

The Pacemaker

Contrary to expectation, patterns of insolation during polar noon appear sufficient to function in a pacemaker capacity. Examination of the light intensity curve (Figure 3-1) clearly reveals periods of distinct change around 11:00 and 17:00. However, a potential

artefact must be considered when making this determination. The submersible quantum sensor used to measure illumination functions most accurately when placed such that the detection surface is directly facing the light source. For the sake of consistency, however, the sensor was always placed flat on the submerged substrate. As a result, it is likely that light levels did not drop quite as low as indicated. A similar statement was made by Kruell (1976), who, after making all measurements with a photocell consistently directed at the zenith, emphasized that the sensor should always be directed toward the sun. Nonetheless, it is known that the amount of light reflected from the surface of a water body increases as the angular height of the sun decreases. Consequently, what appears as relatively bright conditions to a terrestrial organism may be considerably darker under water, depending on the time of day. Although this phenomenon was to some degree anticipated, its full extent was not realised until the results had been tabulated. Therefore, the presumption that a pacemaker would be lacking during polar noon was, at least in terms of aquatic environments, likely erroneous. Dramatic and predictable changes in light intensity were quite evident, thereby necessitating a slight reevaluation of the original hypotheses.

High Average Light Intensity

During a day of high light intensity (July 9, 1999), slightly more than half of total daily diatom migration in both the stream (51%) and wetland (54%) sites occurred between 11:00 and 21:00 (Figure 3-1), a time frame clearly delineated by marked changes in photon flux. However, the sum of hourly means for surficial diatom valve abundance in the stream exceeded that of the wetland by close to 800,000 valves. Interestingly, despite the different species compositions, abundance curves for both sampling sites were reasonably well correlated after 02:00 (r^2=0.55, $p<0.01$) and remarkably similar after 09:00 (r^2=0.78, $p<0.01$). Although the cause for a large peak in surficial valve density in the stream site between 01:00 and 04:00 is unclear, it has previously been noted that the final stages of diatom cell division tend to occur on the sediment surface during early morning (Round & Eaton 1966, Round 1978), thereby resulting in a brief increase in cell

abundance. This explanation, however, fails to address the lack of a corresponding peak in the wetland pond. Moreover, it is inconsistent with the findings of Saburova and Polikarpov (2003), which indicate that the proportion of diatom cells undergoing mitosis increases with increasing depth in the sediment.

Prior to 10:00, surficial diatom abundance in both the stream (Figure 3-1, A) and the pond (Figure 3-1, B) exhibited fairly regular, small magnitude up-down fluctuations. Based on the striking correlation between the migratory curves of all the dominant species in the pond (Table 3-3), it is conceivable that perceived changes in diatom abundance during this time of relatively constant illumination may have been the result of sampling error, such as incomplete contact between the tissue paper and sediment, or a heterogeneous distribution of cells across the sampling area. Much weaker correlation between several species in the stream (Table 3-2) does not support this suggestion. Alternatively, the reasonably consistent up-down pattern of the fluctuations indicates that movement during this time of relatively stable illumination may have been regulated by a cumulative effect of fairly constant light intensity on algal physiology. The observed cyclic behaviour was probably the result of differences in photosynthetic thresholds between individual diatoms, as well as variable depth distribution of cells within the sediment. Since a combination of these factors would leave some cells moving up while others moved down, surficial cell density would be in a state of constant, although small amplitude, oscillation. The actual timing of these fluctuations, in terms of cusps and troughs, would likely have been predetermined by the previous solar maximum.

As anticipated, the most distinct period of algal movement, aside from early morning activity in the stream, occurred in association with the time of highest light intensity between 08:00 and 21:00 (Figure 3-1). An initial suppression of surficial algal abundance, presumably the result of rapidly increasing insolation, was noted shortly after 11:00 in both the stream and pond habitats. Although consistent with hypothesised behaviour, this clearly refutes the statements of Stanley and Daly (1976), who claimed

that algae of northern latitudes are not inhibited by elevated light levels. Despite high illumination, however, a brief period of upward movement followed in both sampling sites at 15:00. Coinciding with a maximum illumination event, these were likely facilitated by preceding downward migrations, which probably provide cells with an opportunity to regenerate their photosystems (Palmer & Round 1965, Hay *et al.* 1993). In both ecosystems, surficial valve maxima occurred simultaneously with the onset of decreasing light levels during late afternoon. This is consistent with previous findings (Stanley & Daly 1976) suggesting that the daily photosynthetic peak in arctic ponds occurs during the latter half the day.

Low Average Light Intensity

As hypothesised, surficial diatom abundance in the stream during a day of low average light intensity was very closely synchronised with illumination (Figure 3-2). This agrees with the findings of Müller-Haeckel (1973), who noted that patterns of drift in epilithic stream algae became increasingly synchronised with light levels during overcast days. Prior to 15:00, I observed a fairly strong correlation between valve density and insolation (r^2= 0.74, $p<0.01$). Despite only a very small increase in light intensity between 16:00 and 17:00, combined cell abundance began to oscillate opposite photon flux until 21:00. This supports earlier statements suggesting a cumulative effect of exposure to solar radiation on algal physiology leading to a negative phototactic response. As was discovered during the high light intensity experiment, somewhat more than half (57%) the total number of diatom valves counted on this day were enumerated during the 10-hour period between 11:00 and 21:00 – the interval of greatest insolation. The sum of hourly mean abundances for this day fell short of that calculated for the same site during high insolation sampling by more than 250,000 valves. This suggests that light penetration into the sediment on a day of low illumination may have been insufficient to promote upward movement in some, presumably deeper-situated, cells. Moreover, as suggested in the following section, this may have been the product of differences between species in terms of the minimum light intensity required for the initiation of upward

movement.

3.3.2. Species-Specific Analysis of Migration

High Average Light Intensity

Species-specific analysis of hourly changes in the surficial algal assemblage of the stream ecosystem during polar noon revealed some unexpected characteristics of diatom migration. The most notable of these occurred on day one of sampling, during which average light intensities were relatively high as a result of negligible cloud cover. Changes in surficial abundance of several selected species representative of different migratory responses were plotted and compared over a 24-hour period (Figures 3-3, 3-4). Although a number of strong correlations were noted between species (Table 3-1), one prevalent exception was apparent. While combined *Nitzschia* species, representing the bulk of diatom abundance in the stream, reached maximum surficial density near 19:00 (Figure 3-3, A), the majority of diatom species exhibited abundance peaks at the time of highest insolation, around 15:00 (Figure 3-3, B).

Density maxima during peak illumination are inconsistent with hypothesised behaviour and the results of the previous chapter, both of which suggest that photosynthetic limitations cause epipelic diatoms to seek shelter from elevated photon flux. However, examination of a single species, *Eunotia bilunaris*, represented in both the stream and wetland pond, revealed a remarkable phenomenon (Figure 3-5). In the stream, *E. bilunaris* abundance peaked together with light intensity (~15:00). In the pond, on the other hand, where *Nitzschia* species comprised a comparatively small proportion of the diatom community, *E. bilunaris* reached its maximum concurrently with *Nitzschia* species in the stream (Figure 3.3, A) at approximately 19:00. Moreover, up-down fluctuations in surficial cell density of the two *E. bilunaris* populations prior to 11:00 were staggered (Figure 3-5), suggesting that the previous day's abundance maxima may also have been offset. Not only did the dominant species in the wetland (Figure 3-6)

attain peak surficial density at the same time as *Nitzschia* in the stream (Figure 3.3, A), but they also exhibited similar smaller peaks at both 16:00 and 21:00. As opposed to the stream diatoms, the migratory patterns of the dominant pond species were all strongly correlated with one another (Table 3-3) throughout the day.

In the absence of the wetland pond data, it might be suggested that the majority of the stream species are adapted to high light and preferentially photosynthesize under these conditions. Based on the entire data set, however, it seems reasonable to suggest the influence of a previously unconsidered factor. The most parsimonious explanation, given the data at hand, is that *Nitzschia* species in the epipelic community exert what can best be described as inhibitory, or possibly allelopathic, effects on other diatoms in the sediment. This precludes the emergence of other species during what appears, from a photosynthetic perspective, to be the most desirable time, forcing these diatoms to alter their patterns of movement and appear on the sediment surface concurrently with highest insolation values. Due to the inherent difficulty of identifying *Nitzschia* cells to the species level, as well as the considerable number of samples that had to be analysed, members of this group were simply assigned to the genus. Unfortunately, this means that the actual species responsible for the proposed inhibitory influence remain unknown. However, the impact on other members of the epipelic community is evident.

Inderjit (1994) lamented the current paucity of *in situ* studies regarding algal allelopathy. Although data presented here may provide a good starting point for additional research, it must be emphasized that various physical or chemical differences between the sampling habitats could have contributed to observed migratory behaviour. Since illumination curves for the stream and pond environments were essentially identical during sampling, and mean insolation at the sediment-water interface was quite comparable between the sites (stream, $\bar{x}$ = 484 $\mu mol \cdot m^{-2} \cdot s^{-1}$; pond, $\bar{x}$ = 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$), it is unlikely that water colour or turbidity (i.e. light penetration) played a role in observed migratory differences. However, it is also possible that an unmeasured parameter, such as

ion concentrations, pH, carbon dioxide, or oxygen, may have played a role. Allelopathic effects of other species, algal or otherwise, may also have been responsible. For instance, two stream diatoms, *Navicula molestiformis* and *Gomphonema parvulum* (Figure 3-4, A), exhibited abundance curves that, although statistically unrelated to each other, were reasonably well-correlated with that of *Nitzschia* (Table 3-1). Yet, based on the relatively small numbers of the former two, it seems logical to suggest that the dominant suppressive impact, if indeed algal, was exerted by *Nitzschia* species. This being the case, it would appear that *N. molestiformis* and *G. parvulum* were, to some degree, unaffected by the inhibitory influence.

It might also be suggested that the relatively high density of *Nitzschia* cells in the stream site was sufficient to cause an adjustment in the migratory patterns of other species. Interspecific differences in emergence times and speed of diatom locomotion have repeatedly been noted (Paterson 1986, Hay *et al.* 1993). *Nitzschia* species are typically long and narrow, which likely makes them better able to negotiate tight sediment interstices, allowing them to attain and populate the sediment surface faster than other, bulkier species. This, along with their vastly superior abundance, may give them a competitive advantage, suppressing other species through sheer numbers and associated shading effects. Regular occurrence of such behaviour might be sufficient to act in a "pacemaker" capacity, causing a shift in the migratory phase of other diatoms, a phenomenon accomplished by various researchers in the past through simple *in vitro* manipulation of light stimuli (Bruce & Pittendrigh 1956, 1958, Jarosch 1962). Moreover, this mass movement of *Nitzschia* cells would produce copious quantities of extracellular polysaccharide (EPS; Paterson 1986, 1988, 1989), which might further inhibit the upward migration of other algae in the epipelon. Since EPS in sediments is water-soluble and relatively short-lived (Paterson 1989), it is conceivable that the constraints imparted by it would be removed fairly rapidly through a simple process of dissolution. Hay *et al.* (1993) commented on a relevant phenomenon in an intertidal salt marsh where, due to rare tidal coverage, EPS was presumed to have accumulated to the point where diatoms

were immobilized and no longer capable of migration.

Additional evidence for interspecific variability in light tolerance is presented in Figure 3-4. A comparison between the migratory patterns of *Gomphonema parvulum* and *Navicula molestiformis* (Figure 3-4, A) in the stream habitat revealed reasonably similar behaviour between 12:00 and 20:00 – the time of highest photon flux. However, after 20:00, the two patterns became distinctly inverse, suggesting that cells of *G. parvulum*, which began migrating upward again after a brief decline between 19:00 and 20:00, recovered more quickly from extended exposure to solar radiation. A similar inverse relationship earlier in the day, between 7:00 and 12:00 (Figure 3-4, A), suggests that cells of *N. molestiformis* may be considerably more sensitive to insolation than those of *G. parvulum*. During this time, the surficial density of *G. parvulum* mimicked the insolation curve quite closely. Density of *N. molestiformis*, on the other hand, decreased as light intensity increased and *vice versa*.

A similar comparison between *G. parvulum* and *Navicula seibigii* on the first sampling date revealed abundance curves that mirrored each other almost perfectly after 14:00 (Figure 3-4, B). In this case, a slightly higher light threshold in *N. seibigii*, observed between 14:00 and 15:00, appears to have been responsible for this phenomenon. At this point in time, *G. parvulum* abundance dropped, whereas *N. seibigii* continued to rise. After 15:00, the time of maximum insolation, *N. seibigii*, apparently having exceeded its light tolerance, migrated downward, while cells of *G. parvulum* began to increase in surficial density. Through their previous downward migration, the latter likely had an opportunity to recover from exposure to high light intensity and could resume photosythesising. This inverse relationship, apparently driven by photosynthetic thresholds, was evident for the remainder of the day. During low average light intensity on the second sampling date, however, the previously observed behaviour was no longer in evidence. In fact, a reasonable correlation was noted between the indicated *Navicula* species and *G. parvulum* on day two (r^2=0.455, $p<0.01$).

Low Average Light Intensity

Subsequent species-specific analysis of vertical migration in the stream site during low average light levels ($\bar{x}$ = 222 $\mu mol \cdot m^{-2} \cdot s^{-1}$) revealed considerable deviation from high light results (Figures 3-7, 3-8, 3-9). As on the previous sampling date, the total surficial abundance curve (Figure 3-2) was determined almost exclusively by a proliferation of *Nitzschia* species (Figure 3-7, A). Consequently, the graph of combined diatom density is not entirely representative of the migratory pattern displayed by other, less abundant species (3-7, B; 3-8, 3-9). Furthermore, a change in species dominance was noted on this day. During low light conditions, each of ten species, plus *Nitzschia* spp., were classified as abundant, occurring in the majority of samples collected at the stream site. During high insolation, only eight species, plus *Nitzschia* spp., were recorded with sufficient frequency to be categorised as dominant. Of these eight species, only *Eunotia bilunaris* and *Eunotia subarcuatoides* were not dominant in the low light conditions of day two. Since the total abundances of both *E. bilunaris* and *E. subarcuatoides* were markedly greater during high average light intensity (3654 and 4378 valves$\cdot cm^{-2}$ *versus* 1969 and 1556 valves$\cdot cm^{-2}$, on days one (high light) and two (low light), respectively), while other species became more abundant on the lower insolation day, it was concluded that the *Eunotia* species are better adapted to conditions of high photon flux. Their reduced abundance during low average light intensity may reflect a minimum threshold to initiate a positive phototactic response and/or to maintain cells of the two *Eunotia* species at the sediment surface.

In general, migratory patterns of the various diatom species were closely related to photon flux during day two of sampling, with the majority of cells achieving surficial abundance maxima during late afternoon. The surficial density of *Nitzschia* species, for example, exhibited a positive relationship with insolation until about 15:00, at which point an inverse response became evident (Figure 3-7, A). *Nitzschia* cell density reached a large peak at 16:00, followed by a smaller maximum at 18:00. Despite an overall lack of statistically significant correlation with that of *Nitzschia* species, the migration of

Neidium affine, *Pinnularia gibba*, and *Stauroneis phoenicenteron* (Figure 3-7, B) exhibited a very similar afternoon pattern, with an inverse response beginning at 15:00. The previously described oscillating up-down pattern was seen in these species during the morning. *Encyonema silesiaca*, *Gomphonema parvulum*, *Navicula molestiformis* , and *Navicula seibigii*, all with reasonably well correlated surficial abundances (Table 3-2), also achieved maxima at 18:00 (Figure 3-8, A). *Gomphonema lagerheimii*, on the other hand, peaked at 09:00 and 20:00 (Figure 3-8, B), the latter maximum occurring just after the time of highest insolation and as photon flux dropped abruptly. An inverse response to light was evidenced by this species after 17:00. This behaviour implies that photosynthetic requirements of *G. lagerheimii* are likely met at relatively low light intensity and that inhibition occurs considerably sooner than it does in some other species. Even lower tolerances are suggested by the movements of *Neidium bisulcatum* and *Navicula pupula*, which exhibited distinct negative phototactic responses as early as 12:00 and 05:00, respectively (Figure 3-9). Interestingly, both of these species displayed positive phototaxis for a brief period around 15:00 but resumed an inverse response shortly thereafter.

The previously proposed inhibitory influence of *Nitzschia* species, manifested during high light conditions on day one, was not in evidence during reduced insolation on day two. The reason for this is unclear. However, it is noteworthy that the major *Nitzschia* peak during high average insolation on day one (~122,000 valves·cm^{-2}; Figure 3-3, A) was almost twice as large as the afternoon maximum during day two (~68,000 valves·cm^{-2}; Figure 3-7, A). It is conceivable that this smaller surficial density of *Nitzschia* frustules during the afternoon of day two was insufficient to act as a substantial hindrance to the movements of other species. On the other hand, assuming the existence of an allelopathic interaction, it might be suggested that the production of the relevant chemicals is dependent on adequate sunlight. According to Inderjit (1994), field research on allelopathic interactions among algae is severely lacking, particularly with regard to the physiological and ecological effects of algal toxins. He further indicates that chemicals

released by certain algal species can alter nutrient accumulation and availability, thereby affecting the abundance and distribution of other algae, microorganisms, and hydrophytes in the aquatic ecosystem. Lastly, Inderjit (1994) suggests that a demonstration of algal allelopathy under field conditions is essential, placing a high priority on, among other things, the variability of allelopathic influences under a range of environmental parameters. In light of these comments, the interspecific effects seen here are clearly worthy of more detailed investigation.

3.4. Conclusion

The loss of synchrony in characteristically diel behaviours under the 24-hour natural daylight of polar noon has been observed for a fairly broad range of organisms (e.g. Müller 1973, 1978, Buchanan & Haney 1980). In general, this disruption of behavioural patterns has been attributed to the lack of a pacemaker, which, in most cases, has been identified as either the daily light-dark cycle (Müller 1973, 1978) or the marked changes in insolation associated with sunrise and sunset (Buchanan & Haney 1980). It was originally predicted that this presumed lack of a pacemaker would lead to a loss of entrainment in the migratory behaviour of epipelic diatoms and that vertical movement would occur unchecked throughout the 24-hour period. However, it was subsequently discovered that, despite constant illumination, the pacemaker may still be a viable stimulus in aquatic environments. Since light reflection from the water's surface increases with the angle of incidence, marked morning and evening changes in insolation, deemed necessary for entrainment of many biological rhythms, still take place on submerged sediments during polar noon. Hence, for the experiments herein described, most diatom movement occurred in association with the time of greatest fluctuations in light intensity, between approximately 08:00 and 21:00. Despite the erroneous assumption of a lacking pacemaker, this latter discovery was consistent with hypothesised results.

As predicted, surficial diatom abundance on a day of elevated photon flux was substantially depressed around the time of solar noon (14:00 in Inuvik, on July 9, 1999; Herzberg Institute of Astrophysics 2003). This coincides surprisingly well with a daytime surficial minimum at 15:00 for an epipelic *Amphora ovalis* population in Lake Kinneret, Israel (Round 1978). However, noteworthy deviation from this tendency toward a midday minimum in diatom density during maximum insolation was seen in the stream site, where a very large *Nitzschia* population is thought to have forced other species in the epipelon to emerge from the sediments during undesirably high light intensity. The mechanism for competitive exclusion in this instance is hypothesised to be primarily mechanical, via sheer density of *Nitzschia* cells and associated EPS production. However, the potential for allelopathic interactions is not ruled out. Interestingly, the inhibitory effect was not observed at the same site during a day of low average insolation. A need for further study is indicated.

Hypothesised small magnitude up-down fluctuations in surficial diatom abundance were variously evident during consistently low photon flux between 21:00 and 08:00. Although not as prevalent as originally expected, these fluctuations are thought to be the product of photosynthetic thresholds – in response to cumulative impacts of exposure to light – and vertical distribution of diatoms in the sediment. The timing of cusps and troughs is proposed to be regulated by preceding surficial abundance maxima. Previous research has shown that algal motility persists to some degree outside of bounds imposed by the pacemaker and that cells can be made to migrate upward at night (Round & Eaton 1966). The results of this study not only confirm those findings but provide reasonably solid evidence that the phenomenon may occur in nature.

A considerable degree of interspecific variability seen in the migratory pattern was consistent with previous findings (Round & Happey 1965, Round & Palmer 1966, Round 1979). Contrary to expectation, these differences were observed on both high and low insolation days. I predicted that diatoms migrating under constant, relatively intense

photon flux would begin to move based on individual circumstances, rather than species-specific limitations, eventually exhibiting a complete lack of synchrony with one another. Since this hypothesis relied on the absence of both a pacemaker and dark (or low insolation) period, it is not surprising that the theory was disproven. However, the prediction that migratory curves would follow insolation quite closely during low photon flux was, aside from inverse responses typically noted between mid-afternoon and early evening, validated. On a day of relatively low average light intensity, movement patterns in the majority of diatoms, depending on what appeared to be species-specific light tolerances, exhibited very distinct direct or inverse relationships with insolation for most of the day.

This study of vertical migration in epipelic diatoms of northerly latitudes during polar noon was, in large part, motivated by a desire to assess the persistence of a biological clock mechanism in the presumed absence of a pacemaker. However, since the pacemaker appears to be maintained in submerged aquatic habitats, despite 24-hour daylight, no definitive conclusions can be drawn with regard to this question. Indeed, but for the results of previous work, which indicate that certain species of algae exhibit 'anticipation of dawn' (Round & Happey 1965, Round & Eaton 1966), emerging from sediments prior to sunrise, while others maintain a migratory period, albeit muted, in constant darkness (Round & Happey 1965, Round 1966), the perceived migration of diatoms during nighttime hours might lead one to suggest that the pacemaker has little, if any, effect on movement. In short, migration in epipelic diatoms, at least during polar noon, may be solely a response to immediate light conditions and may not rely on an endogenous component, as such. It is proposed that a similar study of a diatom community in exposed sediments at a comparable latitude and time of year would remove the perceived shading influence of an overlying water column and yield some more definitive answers.

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Table 3.1. Pearson matrix depicting correlation (r^2 values) between surficial abundance of epipelic diatom species over time in an arctic stream during a 23-hour period of high average light conditions (*0.05>p>0.01, **p<0.01).

	E. bilunaris	*E. subarcuatoides*	*G. parvulum*	*N. molestiformis*	*N. pupula.*	*N. seibigii*	*Nitzschia spp.*	*P. gibba*	*S. phoenicenteron*
Eunotia bilunaris	1.000	-	-	-	-	-	-	-	-
Eunotia subarcuatoides	0.885 **	1.000	-	-	-	-	-	-	-
Gomphonema parvulum	0.228	-0.004	1.000	-	-	-	-	-	-
Navicula molestiformis	0.239	0.225	0.361	1.000	-	-	-	-	-
Navicula pupula	0.669 **	0.775 **	0.012	0.265	1.000	-	-	-	-
Navicula seibigii	0.456	0.515	0.159	0.316	0.894 **	1.000	-	-	-
Nitzschia spp.	0.068	0.094	0.614 *	0.588 *	0.074	0.192	1.000	-	-
Pinularia gibba	0.662 **	0.749 **	0.134	0.413	0.747 **	0.664 **	0.434	1.000	-
Stauroneis phoenicenteron	0.619 **	0.624 **	0.372	0.335	0.841 **	0.864 **	0.324	0.724 **	1.000

Table 3.2. Pearson matrix depicting correlation (r^2 values) between surficial abundance of epipelic diatom species over time in an arctic stream during a 22-hour period of low average light conditions (*0.05>p>0.01, **p<0.01).

	E. silesiaca	*G. lagerheimii*	*G. parvulum*	*N. affine*	*N. bisulcatum*	*N. molestiformis*	*N. pupula*	*N. seibigii*	*Nitzschia spp.*	*P. gibba*	*S. phoenicenteron*
Encyonema silesiaca	1.000	-	-	-	-	-	-	-	-	-	-
Gomphonema lagerheimii	0.045	1.000	-	-	-	-	-	-	-	-	-
Gomphonema parvulum	0.616**	-0.224	1.000	-	-	-	-	-	-	-	-
Neidium affine	0.247	-0.207	0.386	1.000	-	-	-	-	-	-	-
Neidium bisulcatum	0.247	-0.125	0.222	0.333	1.000	-	-	-	-	-	-
Navicula molestiformis	0.775**	-0.047	0.544**	0.149*	0.014	1.000	-	-	-	-	-
Navicula pupula	0.414*	0.078	0.319	0.013	0.060	0.085	1.000	-	-	-	-
Navicula seibigii	0.560**	0.110	0.455**	0.417*	0.382	0.275	0.149	1.000	-	-	-
Nitzschia spp.	0.634**	-0.298	0.673**	0.390	0.155	0.781**	0.273	0.248	1.000	-	-
Pinularia gibba	0.439*	0.186	0.376	0.510**	0.020	0.152	0.407	0.567**	0.277	1.000	-
Stauroneis phoenicenteron	0.384	-0.005	0.454*	0.566**	0.300	0.288	0.116	0.356	0.418*	0.060	1.000

Table 3.3. Pearson matrix depicting correlation (r^2 values) between surficial abundance of epipelic diatom species over time in an arctic wetland pond during a 23-hour period of high average light conditions (**p<0.01).

	B. brebissonii	*E. sinuata*	*E. bilunaris*	*F. rhomboides*	*N. soehrensis*	*N. sublissima*
Brachysira brebissonii	1.000	-	-	-	-	-
Reimeria sinuata	0.720**	1.000	-	-	-	-
Eunotia bilunaris	0.852**	0.633**	1.000	-	-	-
Frustulia rhomboides	0.796**	0.820**	0.696**	1.000	-	-
Navicula soehrensis	0.899**	0.798**	0.757**	0.731**	1.000	-
Navicula sublissima	0.750**	0.585**	0.840**	0.797**	0.628**	1.000

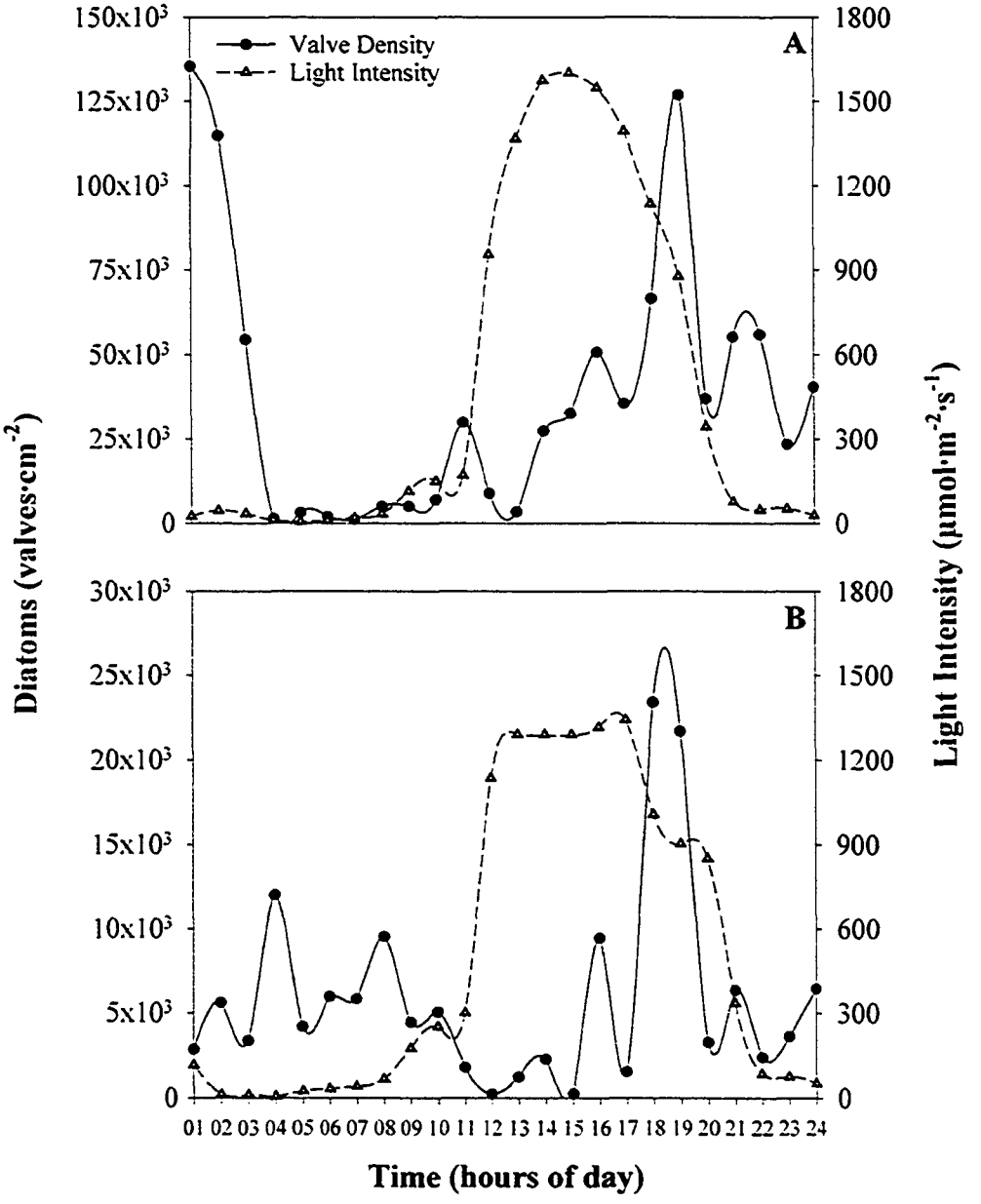

Figure 3.1. Changes in light intensity and surficial diatom valve density on submerged sediments of a small stream (A) and small wetland pool (B) near Inuvik, NWT, during a 23-hour period of high average insolation on July 9, 1999.

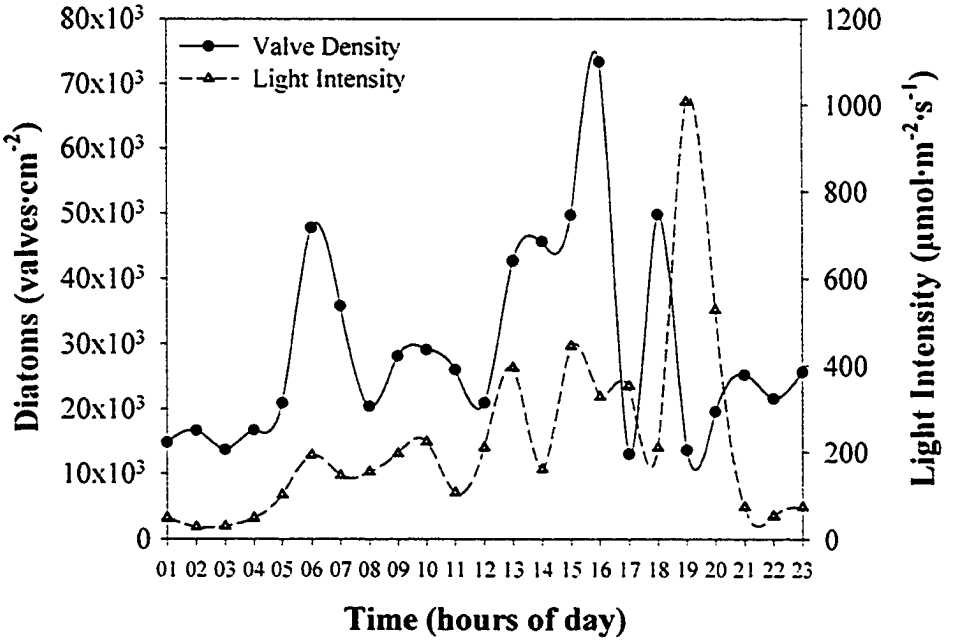

Figure 3.2. Changes in light intensity and surficial diatom valve density on submerged sediments of a small stream near Inuvik, NWT, during a 22-hour period of low average insolation on July 10, 1999.

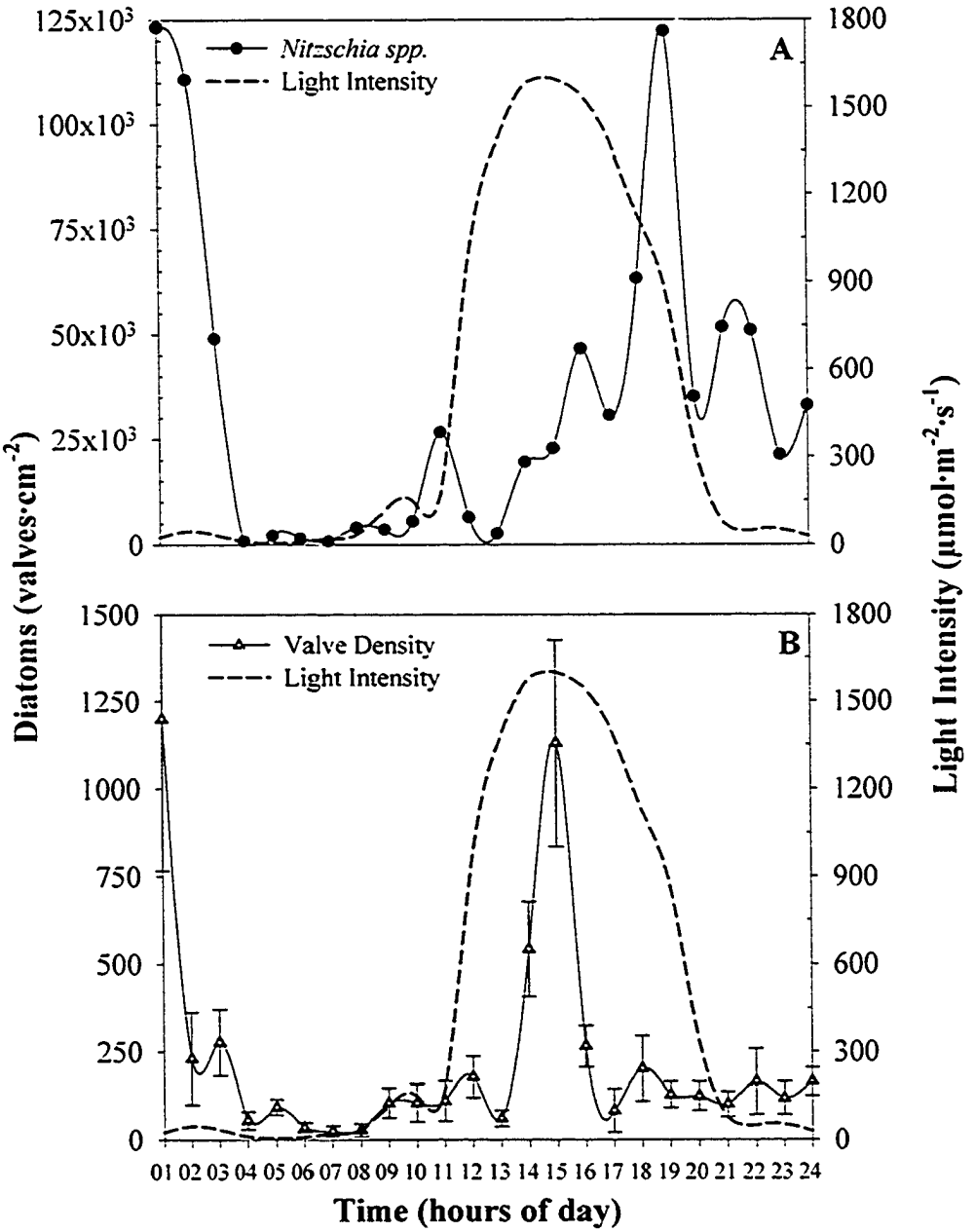

Figure 3.3. Changes in light intensity and surficial diatom valve density on submerged sediments of a small stream near Inuvik, NWT, during a 23-hour period of high average insolation. (A) Pooled *Nitzschia* species; (B) mean (± SE) of six correlated species (*Eunotia bilunaris*, *E. subarcuatoides*, *Navicula pupula*, *N. seibigii*, *Pinularia gibba*, *Stauroneis phoenicenteron*).

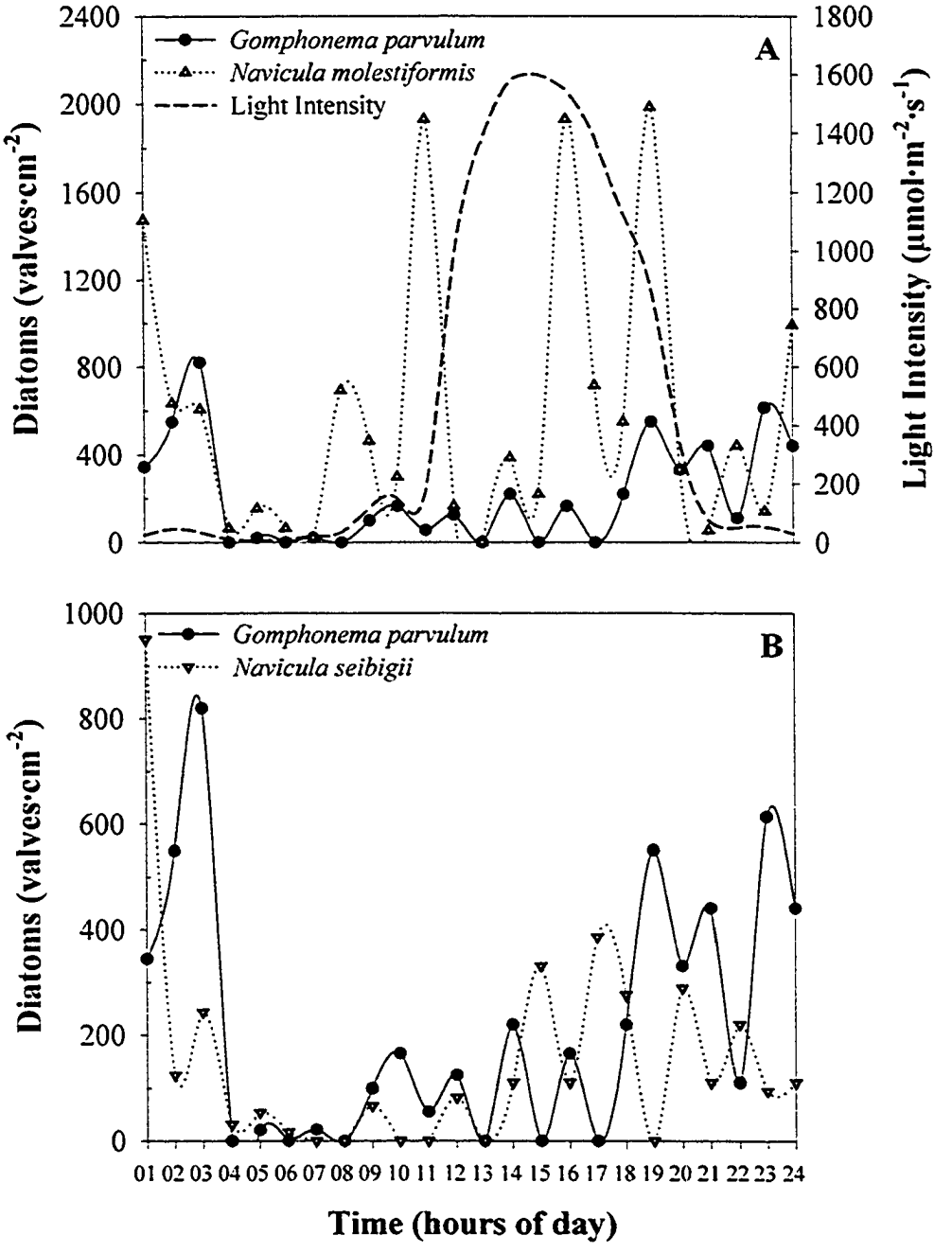

Figure 3.4. Changes in light intensity and surficial diatom valve density on submerged sediments of a small stream near Inuvik, NWT, during a 23-hour period of high average insolation. A comparison between the migratory patterns of *G. parvulum* and (A) *N. molestiformis* and (B) *N. seibigii*.

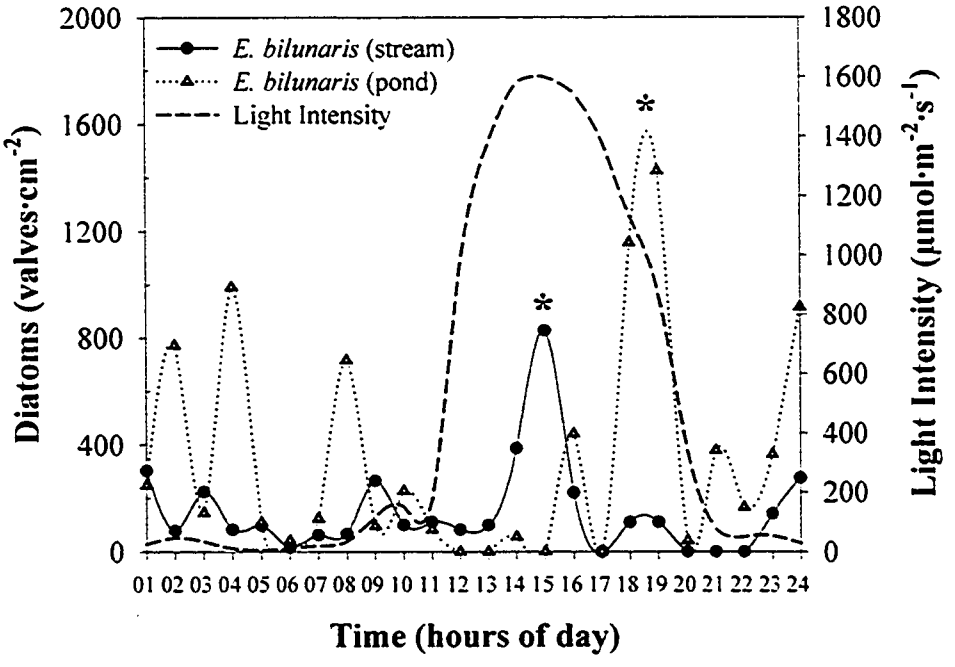

Figure 3.5. Changes in light intensity and surficial density of the diatom *Eunotia bilunaris* on submerged sediments of a small stream and wetland pond near Inuvik, NWT, during a 23-hour period of high average insolation. Asterisks denote surficial density peaks for each population.

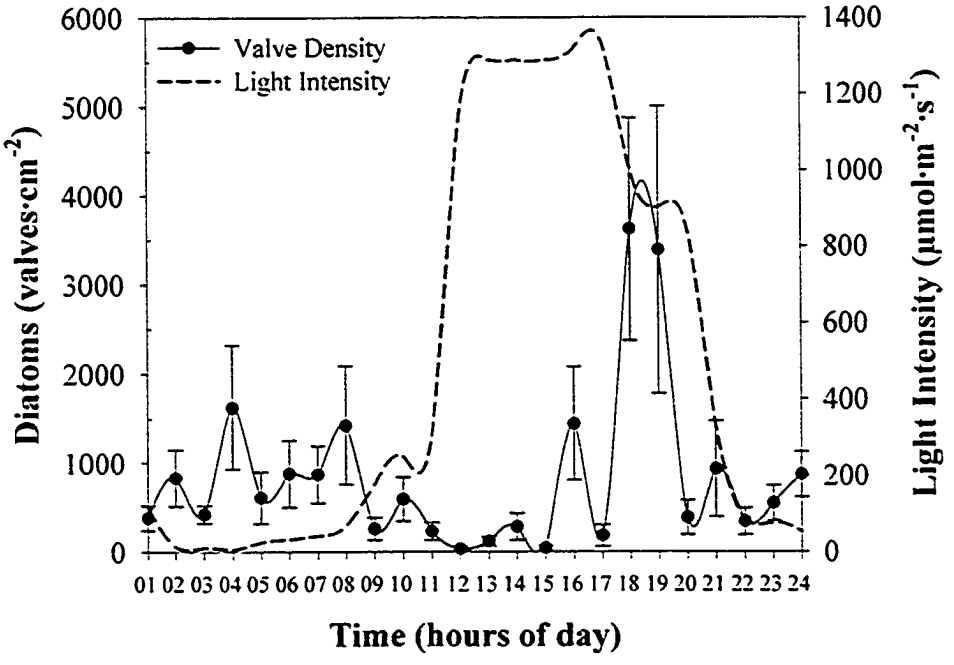

Figure 3.6. Changes in light intensity and surficial diatom valve density on submerged sediments of a small wetland pond near Inuvik, NWT, during a 22-hour period of high average insolation. Hourly means (± SE) of 6 correlated species (*Brachysira brebissonii*, *Reimeria sinuata*, *Eunotia bilunaris*, *Frustulia rhomboides*, *Navicula soehrensis*, *N. sublissima*) are depicted.

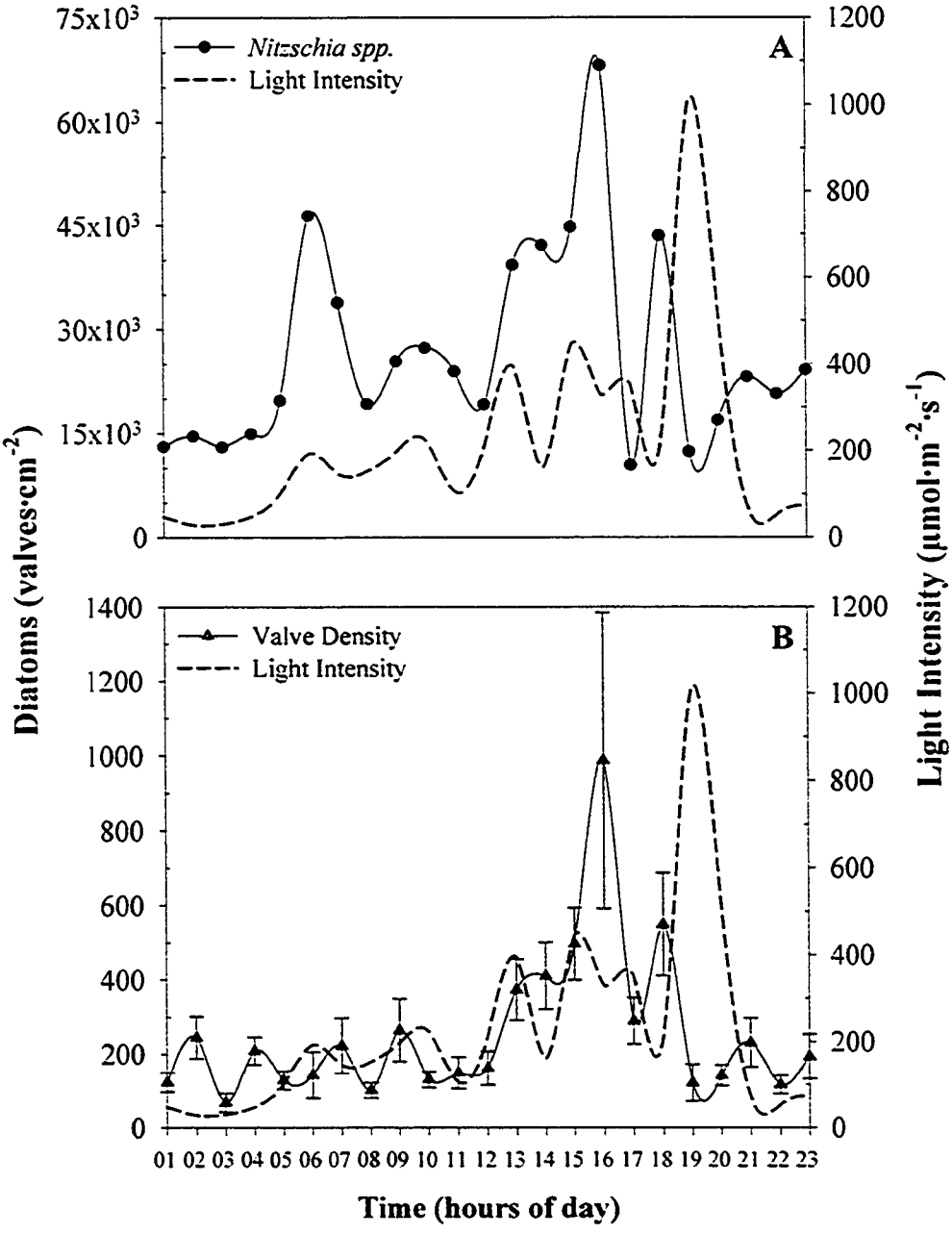

Figure 3.7. Changes in light intensity and surficial diatom valve density on submerged sediments of a small stream near Inuvik, NWT, during a 22-hour period of low average insolation. (A) Pooled *Nitzschia* species; (B) mean (± SE) of 3 correlated species (*Neidium affine*, *Pinnularia gibba*, and *Stauroneis phoenicenteron*).

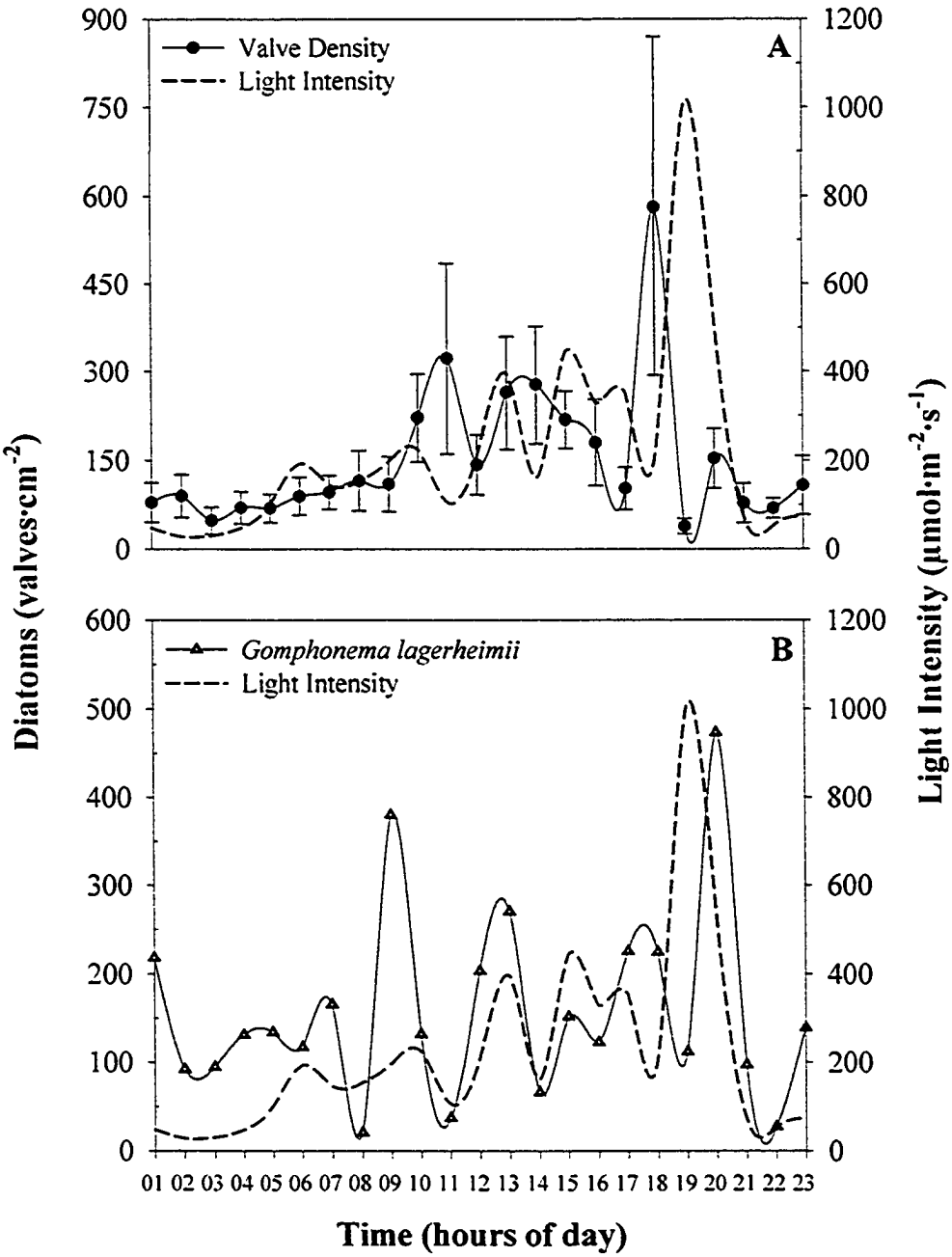

Figure 3.8. Changes in light intensity and surficial diatom valve density on submerged sediments of a small stream near Inuvik, NWT, during a 22-hour period of low average insolation. (A) Mean (± SE) of 4 correlated species (*Encyonema silesiaca*, *Gomphonema parvulum*, *Navicula molestiformis*, and *Navicula seibigii*); (B) *Gomphonema lagerheimii*.

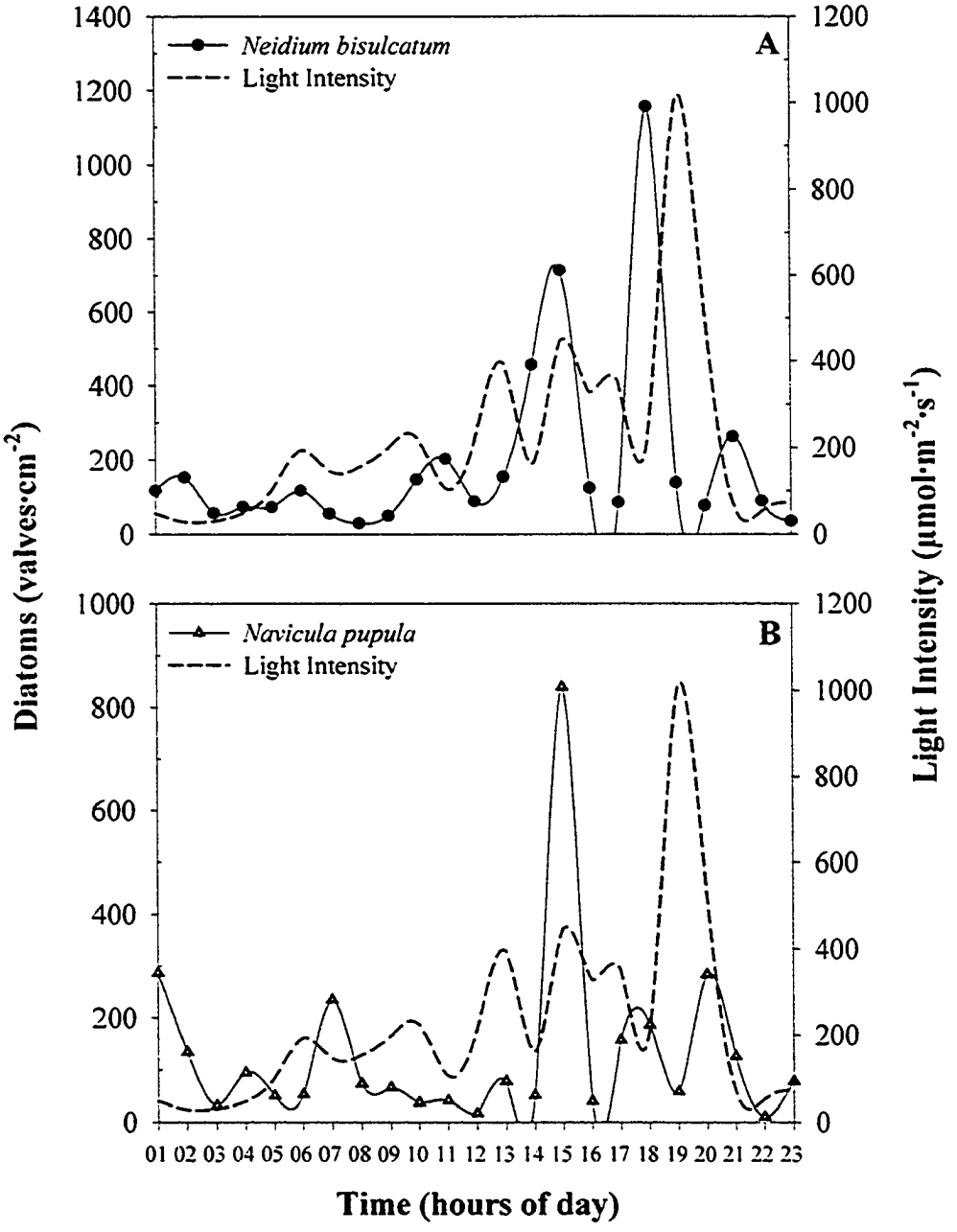

Figure 3.9. Fluctuations in light intensity and valve density of (A) *Neidium bisulcatum* and (B) *Navicula pupula* on submerged sediments of a small stream near Inuvik, NWT, during a 22-hour period of low average insolation.

Chapter 4.

CONCLUSION

Jönsson *et al.* (1994) noted a distinct paucity of studies detailing migratory behaviour in epipelic algae of submerged, undisturbed sediments. To date, this lack of *in situ* work has largely been attributable to the absence of an appropriate sampling protocol. Harper (1976) was able to circumvent this problem through the use of a fairly simple device comprising a tripod and microscope tube. This apparatus permitted her to execute small-scale algal counts on the sediments of a small, shallow pond. Unfortunately, shallow depth limitations, the requirement for real-time observations, and primitive light measurement equipment placed severe restrictions on the usefulness of both acquired data and the method itself. Jönsson *et al.* (1994) chose to avoid the inherent difficulties of *in situ* sampling through removal of an intact sediment core from the natural environment. Collected from a permanently submerged marine habitat, this core was transported to the laboratory where, via an elaborate experimental setup, it was maintained under conditions designed to mimic the natural state. However, it is highly unlikely that the laboratory design was able to perfectly replicate the natural habitat.

Through modification of a sampling approach devised by Taylor and Palmer (1963), I set out to elucidate on various aspects of migratory behaviour in epipelic diatoms of permanently submerged freshwater habitats. The revised sampling technique, dubbed the 'ring method', was intended to facilitate *in situ* sampling of motile algae in the epipelon with minimal disturbance of the sediments. Results indicate not only that the ring method functions very well for investigations of diatom movement in the epipelon but also that vertical migration does indeed occur in freshwater habitats. Incidental observations also suggest that the ring method would likely prove useful for similar examinations of several other algal taxa.

A perceived shortcoming of previous investigations into diel vertical migration of freshwater epipelic algae was the considerable extent of disturbance that accompanied removal of sediments from the natural habitat (e.g. Round & Happey 1965, Round & Eaton 1966). In order to evaluate the impacts of translocation on diatom movement, I executed a series of simultaneous *in situ* and *in vitro* experiments, the latter of which employed a technique typically used in the past (e.g. Round & Happey 1965, Round & Eaton 1966, Brown *et al.* 1972). As hypothesized, diatoms sampled *in vitro* appeared to be substantially more sensitive to insolation, exhibiting more pronounced avoidance behaviour at times of elevated light intensity. This was largely attributed to the lack of a protective water column. A marked difference in diatom abundance between sampling techniques was thought to be an artefact of sediment collection, which may have led to artificially elevated *in vitro* diatom densities. As anticipated, low-temperature scanning electron microscopy revealed that sediments removed from the aquatic system were more compact than those left undisturbed. Moreover, LTSEM revealed that extracellular polysaccharide trails, produced by diatoms during gliding movement, were fouled and disturbed in sediments sampled *in vitro*. Contrary to expectation, neither sediment compaction nor disruption of EPS trails was shown to have any immediate impact on the migratory pattern.

An additional goal of this project was to evaluate the influence of various environmental parameters on diatom migration. As predicted, the only variable with an apparent impact on patterns of movement was light intensity. Moreover, the use of relatively modern sensory equipment revealed a surprisingly close relationship between surficial diatom abundance and photon flux. During morning hours and lower light intensities, a positive relationship was evidenced. Later on in the day and during periods of high insolation, diatoms began to exhibit a negative response to sunlight, suggesting not only a maximum threshold in terms of immediate intensity but also an additive effect of illumination on the algae. Diurnal downward migration is concluded to serve in both an avoidance and regenerative capacity. Reaction times of diatoms to short-term changes

in photon flux were remarkably fast and deemed worthy of a more detailed investigation.

Various studies have investigated the role of an endogenous component in the vertical migration of epipelic algae (e.g. Happey-Wood & Jones 1988, Jönsson *et al.* 1994). They generally did so via the examination of rhythms of movement under constant illumination. Since all of these studies were performed under artificial light in a laboratory setting, the likelihood of experimental bias in derived results is substantial. Hence, I chose to execute a similar *in situ* project under the 24-hour daylight of polar noon, using the newly-devised ring method. Although slight changes in light intensity during morning and evening were certainly anticipated, I felt that these would be insufficient to act in the 'pacemaker' capacity typically deemed necessary for entrainment of an endogenous biological rhythm. Unfortunately, the full impact of changes in angular height of the sun, which can drastically affect the amount of light penetrating water, was not realized beforehand. Changes in photon flux at the substratum level appeared adequate to act in a pacemaker capacity. As a result, the bulk of vertical migration still took place during 'traditional' daylight hours.

In accordance with hypothesized results, migratory behaviour of epipelic diatoms during the constant illumination of polar noon was very much a factor of light intensity. During a day of low average insolation, surficial algal density tended to follow the illumination curve quite closely from morning until mid- to late-afternoon, at which point an inverse response, presumed to be the result of a cumulative effect of exposure to sunlight, was exhibited. On a day of high average light intensity, a more pronounced suppression of surficial diatom abundance was evidenced during midday. Contrary to the hypothesis, which predicted erratic patterns of migration based on the presumed absence of a pacemaker, diatom movement on the high insolation day also appeared to have a distinct relationship with light intensity. As on the low illumination day, cell density began to increase during early morning and decreased again toward mid-evening. Movement at night during both days generally appeared limited to a muted, low-

magnitude, oscillating up-down pattern, as might be expected for slow-moving cells travelling in the absence of a directional stimulus.

A species-specific analysis of patterns in movement north of the arctic circle yielded considerable interspecific variability. Consistent with both hypothesized and previously-reported results (Round & Happey 1965, Round & Palmer 1966, Round 1979), this appeared to be the product of different minimum light requirements for the initiation of upward migration and dissimilar maximum thresholds of light tolerance. For instance, while some species appeared on the sediment surface in greater numbers during a day of high average light intensity, the majority seemed to prefer lower illumination and were more abundant on the sediment under conditions of reduced diurnal photon flux. Similarly, some species began to exhibit an apparent sensitivity and negative response toward sunlight during mid- to late morning while the surficial density of others demonstrated a positive relationship with solar intensity until early- or late afternoon.

Species-specific evaluation of vertical migration in diatoms of an arctic stream and pond revealed an additional and unexpected aspect of movement in diatoms. During a day of high average insolation, the diatom community in both of these habitats reached surficial abundance maxima at roughly 19:00. However, closer investigation revealed that, in the stream, this peak was driven primarily by *Nitzschia* species. The majority of other species in the stream appeared to attain maximum surficial densities around 16:00 – the time of most intense solar irradiation. This finding was inconsistent with both hypothesized behaviour and previous findings, which suggest that diatoms tend to migrate into the sediment during times of midday maximum insolation. In the pond, however, most species, including a less dominant *Nitzschia* component, reached maxima at 19:00. Interestingly, *Eunotia bilunaris*, which was documented in both ecosystems, occurred in greatest numbers at 19:00 and 16:00 in the pond and stream, respectively. This observation suggests one of two potential explanations. Firstly, it is conceivable that *Nitzschia* species, by virtue of their sheer abundance in the stream and presumed level of

motility (over bulkier species), either shaded out other diatoms at 19:00 or inhibited their movement through extensive production of EPS trails throughout the sediment. It is proposed that regular recurrence of such behaviour may act in a pacemaker capacity for other epipelic diatoms, causing them to remain on the sediment surface during high insolation at 16:00. Secondly, it is suggested that *Nitzschia* spp. may exert an allelopathic influence on other algae in the epipelon. The potential for further study is noted.

4.1. Literature Cited

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Appendix 2.1. Surficial density (valves·cm^{-2}) of epipelic diatom species on sediments of a small pond in Jasper National Park, August 1, 1998. Species data are reported as calculated densities derived from hourly counts.

Time (hours)	Replicate	Light Intensity (µmol·m^{-2}·s^{-1})	pH	Dissolved Oxygen (mg·l)$^{-1}$	Temperature (°C)	*Achnanthes minutissima*	*Amphora* sp.1	*Brachysira brebissonii*	*Encyonema affinis*	*Encyonema norvegica*	*Encyonema silesiaca*	*Diploneis* sp.1	*Navicula angustata*	*Navicula bryophila*	*Navicula cryptocephala*	*Navicula halophila*	*Nitzschia* spp.
0800	1	350	8.17	7.94	17.1	3480	0	160	0	120	0	0	0	3120	280	40	1120
	2	320	8.20	8.00	16.9	2982	43	86	0	259	130	778	303	389	3328	216	6958
	3	460	8.29	8.36	16.7	3628	827	1102	92	4959	184	138	138	3628	3903	2434	138
0900	1	1170	8.05	8.08	17.8	122	0	0	0	143	0	0	0	225	102	61	163
	2	1350	8.08	8.20	17.2	623	0	131	0	33	33	262	0	131	328	0	787
	3	1170	8.17	8.55	17.5	1173	310	414	35	310	69	69	310	1897	3208	621	69
1000	1	1650	8.05	8.46	18.5	470	29	59	0	118	0	0	0	2116	118	0	29
	2	1320	8.08	8.75	17.9	1176	0	122	0	25	74	367	122	196	906	74	1420
	3	1890	8.15	8.74	18.4	1234	716	247	25	2148	173	49	74	1679	1654	1136	247
1100	1	1800	7.99	8.64	19.5	2469	705	647	59	5173	235	59	294	2263	3057	2263	470
	2	1890	8.07	8.57	18.7	1117	265	940	0	2145	235	29	176	1058	2674	1058	386
	3	2190	8.17	9.04	19.4	1552	0	276	0	35	35	35	10	4725	414	0	1000
1200	1	2490	8.00	9.00	20.9	1458	228	472	0	215	47	43	47	1586	2060	644	855
	2	2340	8.04	9.55	20.1	1998	705	940	88	2145	617	118	176	1117	2939	2674	588
	3	2430	8.04	9.29	20.6	1163	41	429	0	225	61	82	102	2735	694	265	490
1300	1	1950	8.10	9.70	21.8	2242	0	1311	35	345	138	0	173	4587	448	276	724
	2	1920	8.15	9.62	21.0	3168	0	138	0	92	92	3214	1148	597	2434	138	6767
	3	2100	8.22	9.47	23.1	966	310	310	69	1414	35	0	35	931	2069	724	345
1400	1	2190	8.20	10.21	22.6	283	0	0	40	80	40	80	80	1040	160	200	0
	2	2370	8.20	9.75	21.9	765	0	77	0	77	38	1837	230	115	842	0	842
	3	2490	8.23	10.14	23.7	4638	1561	1424	321	6061	413	0	0	4959	2847	1791	276

Time (hours)	Replicate	Light Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	pH	Dissolved Oxygen ($mg \cdot l)^{-1}$	Temperature (°C)	*Achnanthes minutissima*	*Amphora* sp.1	*Brachysira brebissonii*	*Encyonema affinis*	*Encyonema norvegica*	*Encyonema silesiaca*	*Diploneis* sp.1	*Navicula angustata*	*Navicula bryophila*	*Navicula cryptocephala*	*Navicula halophila*	*Nitzschia* spp.
1500	1	1920	8.21	10.17	23.8	2663	230	46	0	367	92	46	0	5970	735	92	1240
	2	1920	8.25	10.44	23.1	1381	793	470	88	1851	529	0	118	852	1793	2645	118
1600	1	210	8.01	10.55	24.2	1449	225	551	62	1408	82	61	143	1959	2429	919	307
	2	270	8.23	10.26	23.7	2490	1408	633	82	2081	551	413	0	1000	2367	3714	347
	3	270	8.24	10.72	23.8	694	116	0	0	0	0	50	0	1488	198	83	116
1700	1	570	8.27	10.39	23.6	245	20	0	0	20	0	20	0	245	41	0	0
	2	570	8.25	9.47	23.5	8486	257	0	74	147	147	3674	147	294	1396	330	4849
	3	600	8.25	9.88	23.5	1791	716	386	0	5235	193	83	28	1322	1901	2590	221

Appendix 2.2. Surficial density (valves·cm^{-2}) of epipelic diatom species on sediments of a small pond in the Devonian Botanic Garden, September 23, 1998. Species data are reported as calculated densities derived from hourly counts. Method 0 = *in situ* ring method; Method 1 = *in vitro* lens technique.

Time (hours)	Method	Light Intensity (µmol·m^{-2}·s^{-1})	Dissolved Oxygen (mg·l)$^{-1}$	Temperature (°C)	Replicate	*Achnanthes lanceolata*	*Cocconeis placentula*	*Gomphonema parvulum*	*Navicula accomoda*	*Navicula capitatoradiata*	*Navicula cryptocephala*	*Navicula seibigii*	*Navicula trivialis*	*Navicula veneta*	*Navicula wildii*	*Nitzschia* spp.
0800	0	90	8.69	11.8	1	138	358	55	799	3555	2700	772	1433	1598	0	1392
					2	0	248	165	0	1075	413	496	331	1322	165	1074
0900	0	200	8.42	11.8	1	83	248	0	744	1653	3141	1075	909	992	165	1653
					2	165	165	0	83	1157	579	579	579	1653	83	1157
1000	0	330	8.51	12.1	1	0	0	0	83	1901	496	413	413	496	0	331
					2	556	139	417	0	4306	5278	695	2361	7223	278	1389
1100	0	640	8.65	12.5	1	138	276	413	689	3582	6199	2480	3857	2066	413	1791
					2	2425	220	882	0	11020	1984	1323	3527	11902	441	2866
1200	0	1170	9.39	12.9	1	661	1488	1322	1818	13720	9588	4298	8431	5290	1322	4464
					2	165	248	0	0	1653	1736	661	909	2562	83	827
1300	0	570	10.00	13.4	1	1984	0	992	661	18184	8927	6612	9588	3967	992	1984
					2	1875	313	938	0	10938	9375	3438	2188	15938	0	6563
1400	0	930	10.02	13.3	1	0	165	0	248	3224	1488	496	1240	413	0	661
					2	261	522	1176	392	4963	6792	1176	2220	6661	131	2482
1500	0	840	10.47	13.6	1	0	0	833	3056	11946	10557	4723	5834	5001	278	1389
					2	230	230	344	115	3214	2985	2181	2526	6199	0	1148
1600	0	330	10.52	13.8	1	1653	661	2976	2645	15869	19506	4629	11902	6943	1984	12233
					2	1250	313	781	156	4219	7500	1250	4688	12813	781	5469
1700	0	480	10.32	13.9	1	661	1322	2645	3967	27771	16531	14547	9918	11241	1984	5951
					2	992	661	992	0	2314	4959	992	2645	9918	661	5290

Time (hours)	Method	Light Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Dissolved Oxygen $(mg \cdot l)^{-1}$	Temperature (°C)	Replicate	*Achnanthes lanceolata*	*Cocconeis placentula*	*Gomphonema parvulum*	*Navicula accomoda*	*Navicula capitatoradiata*	*Navicula cryptocephala*	*Navicula seibigii*	*Navicula trivialis*	*Navicula veneta*	*Navicula wildii*	*Nitzschia* spp.
0800	1	130	-	-	1	156	316	156	316	313	7500	469	1719	2656	0	1094
					2	165	110	0	55	441	1763	606	441	716	0	551
0900	1	320	-	-	1	661	2645	1322	1984	1973	26449	1984	3967	17853	0	6633
					2	331	165	165	83	1818	1322	331	827	1240	0	1984
1000	1	610	-	-	1	0	248	83	83	331	3058	413	1075	1157	0	909
					2	331	331	165	496	2810	17027	2314	5125	12729	0	6613
1100	1	840	-	-	1	165	0	248	0	496	5455	413	1075	1901	0	1572
					2	661	331	331	165	579	3141	0	744	2314	0	744
1200	1	1290	-	-	1	0	0	66	99	298	2678	198	529	2281	0	1587
					2	397	66	0	66	331	3174	198	1124	1984	0	992
1300	1	690	-	-	1	0	0	83	248	827	2976	1158	413	3472	0	910
					2	0	110	55	55	386	992	0	551	1378	0	441
1400	1	1170	-	-	1	100	200	200	0	5200	11400	1000	1900	5300	0	5800
					2	0	0	110	0	276	1047	165	771	937	0	606
1500	1	1140	-	-	1	469	156	0	0	3594	7969	938	2656	4063	0	2813
					2	165	165	331	83	1570	5125	413	1322	4959	0	1406
1600	1	390	-	-	1	165	0	661	1157	5620	7108	1157	2149	16200	0	8099
					2	331	248	248	0	1405	2314	83	744	8637	0	826
1700	1	570	-	-	1	9257	331	992	0	12563	16861	3306	5290	0	0	14547
					2	110	110	551	220	992	6502	1543	1763	7714	0	3527

Appendix 2.3. Surficial density (valves·cm-2) of epipelic diatom species on sediments of a small pond in the Devonian Botanic Garden, October 28, 1998. Species data are reported as calculated densities derived from hourly counts. Method 0 = *in situ* ring method; Method 1 = *in vitro* lens technique.

Time (hours)	Method	Light Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	pH	Dissolved Oxygen ($mg \cdot l)^{-1}$	Temperature (°C)	Replicate	*Cocconeis placentula*	*Gomphonema parvulum*	*Navicula capitatoradiata*	*Navicula capitata*	*Navicula Cincta*	*Navicula cryptocephala*	*Navicula cuspidata*	*Navicula Pseudolanceolata*	*Navicula pupula*	*Navicula radiosafallax*	*Navicula seibigii*	*Nitzschia* spp.
0800	0	60	8.16	9.59	5.5	1	230	115	1435	1779	1607	5969	115	574	172	3273	287	9413
						2	124	63	31	310	155	3533	155	0	63	558	63	4246
0900	0	42	7.75	9.50	5.6	1	631	115	459	402	2411	6888	0	344	1378	1722	115	8036
						2	98	197	197	442	639	3932	0	0	442	393	98	4178
1000	0	33	7.73	9.50	5.4	1	185	0	62	926	432	7100	247	154	0	525	0	8026
1100	0	51	7.69	9.49	5.5	1	230	0	345	805	0	2845	230	345	172	1265	0	2184
						2	661	496	827	3967	0	22481	2314	1818	579	1653	0	33640
1200	0	72	7.66	9.43	5.6	1	392	0	327	1959	65	8490	1176	0	131	28278	0	4376
						2	131	0	392	1502	718	9927	980	849	65	849	131	9078
1300	0	210	7.58	9.65	5.8	1	1654	0	2786	3309	3135	10971	2264	697	871	8969	1045	10101
						2	572	0	0	13075	1144	20756	3922	82	82	3596	163	20021
1400	0	90	7.52	9.86	5.9	1	1050	150	750	2200	1350	4650	350	150	800	8500	50	2050
						2	327	131	457	3069	392	25143	2025	131	196	4114	131	16131
1500	0	150	7.46	9.90	6.0	1	1150	100	600	4150	800	13500	650	350	700	8550	100	5300
						2	579	413	248	2480	992	22234	909	248	165	2397	165	14464
1600	0	80	7.56	9.93	6.0	1	278	0	278	729	104	1528	313	69	35	4514	0	382
						2	46	0	232	883	140	9438	558	372	47	837	93	8555
1700	0	80	7.58	9.95	5.8	1	958	295	295	2802	1180	10713	2359	295	295	2433	442	14671
0800	1	90	-	-	-	1	306	306	0	765	459	11939	689	306	306	2066	383	21505
0900	1	63	-	-	-	1	661	0	0	992	165	12563	1984	331	0	10084	0	26449
1000	1	48	-	-	-	1	200	200	200	850	0	6200	700	300	200	450	200	7800

Time (hours)	Method	Light Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	pH	Dissolved Oxygen $(mg \cdot l)^{-1}$	Temperature (°C)	Replicate	*Cocconeis placentula*	*Gomphonema parvulum*	*Navicula capitatoradiata*	*Navicula capitata*	*Navicula Cincta*	*Navicula cryptocephala*	*Navicula cuspidata*	*Navicula Pseudolanceolata*	*Navicula pupula*	*Navicula radiosafallax*	*Navicula seibigii*	*Nitzschia* spp.
1100	1	75	-	-	-	1	3125	156	0	3984	313	9375	625	469	78	1641	78	11172
1200	1	105	-	-	-	1	248	331	83	2976	465	9175	992	496	0	909	413	12977
1300	1	320	-	-	-	1	1563	104	1667	6263	1667	28958	4479	1042	1354	2604	1458	31667
1400	1	140	-	-	-	1	320	160	320	4000	400	39440	1360	1040	240	10080	480	13920
1500	1	210	-	-	-	1	909	331	0	1736	661	26697	827	496	83	1984	1240	29424
1600	1	110	-	-	-	1	402	115	0	3100	115	31454	1894	689	0	5855	574	9413
1700	1	120	-	-	-	1	547	0	156	4063	1016	16484	2422	781	469	2266	156	13359

Appendix 2.4. Surficial density (valves·cm^{-2}) of epipelic diatom species on sediments of a small pond in the Devonian Botanic Garden, June 4, 1999. Data are reported as calculated values derived from hourly counts. Method 0 = *in situ* ring method; Method 1 = *in vitro* lens technique.

Time (hours)	Method	Light Intensity (µmol·m^{-2}·s^{-1})	Replicate	*Cocconeis placentula*	*Gomphonema acuminatum*	*Gomphonema augur*	*Gomphonema gracile*	*Gomphonema parvulum*	*Gomphonema truncatum*	*Navicula capitatoradiata*	*Navicula capitata*	*Navicula Cincta*	*Navicula cryptocephala*	*Navicula Pseudolanceolata*	*Navicula pupula*	*Navicula radiosafallax*	*Navicula seibigii*	*Navicula veneta*	*Nitzschia* spp.
0800	0	510	1	71	24	0	71	0	236	0	47	24	24	0	0	47	95	165	24
			2	0	24	0	71	0	0	0	71	95	0	0	0	0	47	118	24
0900	0	720	1	0	0	21	0	0	0	0	21	0	0	0	0	0	0	21	0
			2	0	24	0	0	47	47	0	47	260	71	0	24	0	118	24	118
1000	0	900	1	21	0	0	0	41	0	0	0	62	21	0	0	0	0	0	0
			2	47	24	0	95	47	95	47	47	354	47	0	0	47	236	283	213
1100	0	960	1	0	0	0	0	0	0	0	21	21	0	0	0	0	0	0	0
			2	0	0	0	0	21	21	0	41	0	0	0	0	0	21	21	41
1200	0	1410	1	21	0	0	0	41	0	0	21	62	0	0	0	0	0	21	41
			2	0	21	21	41	21	21	0	21	145	41	21	0	41	0	0	83
1300	0	1260	1	0	0	0	28	55	0	0	0	0	0	0	0	0	28	0	55
			2	62	0	0	41	0	0	21	62	103	41	0	0	41	21	41	0
1400	0	1440	1	0	0	0	21	0	0	0	0	21	0	0	0	0	41	0	0
			2	21	21	0	21	41	0	0	0	0	0	21	0	0	21	0	41
1500	0	1290	1	0	0	0	21	0	0	0	0	62	41	0	0	0	0	21	0
			2	118	24	24	47	95	378	0	71	260	71	0	0	24	24	142	331
1600	0	1170	1	62	0	0	21	0	0	0	0	62	21	0	0	0	0	21	41
			2	0	0	0	0	0	0	0	0	41	0	0	0	0	0	0	0
1700	0	840	1	0	41	0	21	21	0	0	21	106	62	62	21	0	21	41	124
			2	0	0	0	0	62	0	0	41	165	0	21	0	62	83	0	0

Time (hours)	Method	Light Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Replicate	*Cocconeis placentula*	*Gomphonema acuminatum*	*Gomphonema augur*	*Gomphonema gracile*	*Gomphonema parvulum*	*Gomphonema truncatum*	*Navicula capitatoradiata*	*Navicula capitata*	*Navicula Cincta*	*Navicula cryptocephala*	*Navicula Pseudolanceolata*	*Navicula pupula*	*Navicula radiosafallax*	*Navicula seibigii*	*Navicula veneta*	*Nitzschia* spp.
0800	I	780	1	0	0	0	0	21	21	0	0	62	21	0	0	0	21	21	124
			2	0	71	0	71	118	24	0	24	354	118	0	0	0	47	118	165
0900	I	1200	1	0	0	0	62	0	0	0	0	41	41	0	0	0	0	21	83
			2	0	0	0	47	71	71	0	24	236	71	0	0	0	24	213	354
1000	I	1500	1	0	0	47	0	71	118	24	0	95	0	0	0	47	47	47	95
			2	0	71	47	0	118	47	0	0	47	0	0	0	0	0	71	614
1100	I	1770	1	24	24	24	118	95	165	0	0	118	47	0	0	0	0	47	827
			2	0	0	0	0	21	0	0	0	0	41	0	0	0	0	0	165
1200	I	2010	1	0	24	0	47	47	47	0	0	449	71	24	0	0	0	24	472
1300	I	2010	1	0	0	0	0	0	21	0	0	0	0	0	0	0	0	21	186
			2	0	0	0	0	83	145	21	0	63	0	0	0	0	0	41	372
1400	I	2220	1	0	0	0	0	0	21	0	0	0	0	0	0	0	0	0	165
			2	0	41	0	0	0	0	0	0	21	0	0	0	0	0	0	41
1500	I	1950	1	37	92	37	37	129	74	0	37	0	0	0	0	0	18	18	367
			2	28	606	110	165	496	496	0	0	276	248	110	0	83	28	331	744
1600	I	1890	1	0	0	0	83	186	103	0	0	103	0	0	0	0	0	0	269
			2	0	18	0	75	74	129	0	0	74	0	0	0	0	37	0	184
1700	I	1530	1	0	0	0	0	21	21	0	0	0	0	0	0	0	0	0	0
			2	0	0	41	0	83	0	0	41	41	0	0	0	0	0	0	103

Appendix 2.5. Surficial density (valves·cm^{-2}) of epipelic diatom species on sediments of a small pond in the Devonian Botanic Garden, June 11, 1999. Species data are reported as calculated densities derived from hourly counts. Method 0 = *in situ* ring method; Method 1 = *in vitro* lens technique.

Time (hours)	Method	Light Intensity (μmol·m^{-2}·s^{-1})	Replicate	*Cocconeis placentula*	*Gomphonema augur*	*Gomphonema gracile*	*Gomphonema parvulum*	*Gomphonema truncatum*	*Navicula capitatoradiata*	*Navicula capitata*	*Navicula Cincta*	*Navicula cryptocephala*	*Navicula Pseudolanceolata*	*Navicula pupula*	*Navicula radiosafallax*	*Navicula seibigii*	*Navicula veneta*	*Nitzschia* spp.
0800	0	480	1	0	0	0	132	463	33	694	463	628	0	33	66	0	99	1752
			2	33	0	0	132	66	0	1025	1256	893	99	33	66	0	231	3637
0900	0	810	1	165	0	430	132	331	33	331	1223	1984	132	0	99	165	231	4893
			2	99	0	66	165	265	66	661	1322	1190	132	66	0	0	463	3934
1000	0	990	1	248	28	28	83	413	0	28	386	1047	55	0	83	28	110	3444
			2	55	28	193	83	28	55	303	386	689	55	83	28	110	276	1984
1100	0	1260	1	55	0	0	55	110	0	110	551	386	83	0	0	110	358	1350
			2	28	83	138	83	83	0	303	827	689	28	0	83	0	193	3554
1200	0	360	1	132	66	66	132	265	0	1322	694	1620	231	132	231	298	628	2678
1300	0	1500	1	83	124	0	331	207	124	1157	1570	2066	248	41	124	248	455	4381
			2	33	0	198	132	132	0	463	1091	926	66	0	99	198	430	1719
1400	0	1560	1	24	0	47	142	24	0	331	449	331	47	24	24	47	0	803
			2	0	24	0	24	0	0	142	71	47	24	0	0	0	47	213
1500	0	1500	1	24	0	0	0	0	24	0	0	24	24	0	95	0	0	47
			2	71	0	95	142	0	0	71	142	590	24	24	47	118	260	756
1600	0	1230	1	103	0	0	0	0	0	21	21	62	0	0	21	21	0	227
			2	62	0	0	0	0	0	0	62	103	0	0	0	62	0	393
1700	0	960	1	110	0	55	110	110	0	496	771	1598	331	0	0	386	331	3196
			2	0	0	71	24	71	0	0	189	189	0	0	0	24	24	283

Time (hours)	Method	Light Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Replicate	*Cocconeis placentula*	*Gomphonema augur*	*Gomphonema gracile*	*Gomphonema parvulum*	*Gomphonema truncatum*	*Navicula capitatoradiata*	*Navicula capitata*	*Navicula Cincta*	*Navicula cryptocephala*	*Navicula Pseudolanceolata*	*Navicula pupula*	*Navicula radiosafallax*	*Navicula seibigii*	*Navicula veneta*	*Nitzschia* spp.
0800	1	870	1	0	1019	55	331	248	0	83	661	83	28	0	83	0	110	138
0900	1	1350	1	71	1157	213	95	142	0	47	165	95	0	0	0	0	24	47
			2	28	1598	110	110	551	0	193	1075	496	83	0	0	28	138	138
1000	1	1620	1	0	1157	0	24	213	0	0	165	24	0	0	0	0	0	260
			2	24	1181	0	47	95	0	0	142	24	47	0	0	0	0	260
1100	1	2010	1	0	2834	71	213	165	0	0	260	0	0	0	0	71	118	213
			2	28	1488	138	193	358	0	0	110	55	0	0	0	0	0	193
1200	1	570	1	21	1074	103	83	393	0	41	124	41	0	0	0	21	21	331
1300	1	2280	1	0	351	0	62	186	0	0	0	165	21	0	0	83	41	41
			2	0	8629	198	860	893	0	99	529	33	0	0	0	66	99	1389
1400	1	2310	1	0	3664	165	276	579	0	0	220	110	0	0	55	0	28	413
1500	1	2130	1	0	614	47	213	354	0	0	71	189	0	0	0	24	0	71
			2	0	732	95	118	283	0	0	118	95	0	24	0	0	95	213
1600	1	1860	1	220	32841	0	992	771	0	0	2976	992	220	0	0	441	441	1763
			2	0	567	0	95	165	0	0	95	24	47	0	0	0	0	47
1700	1	1530	1	99	3306	165	397	760	0	66	331	430	331	0	33	0	231	165
			2	66	3141	66	165	165	33	331	893	1223	132	66	165	99	198	132

Appendix 2.6. Surficial density (valves·cm^{-2}) of epipelic diatom species on sediments of a small pond in the Devonian Botanic Garden, June 17, 1999. Species data are reported as calculated densities derived from hourly counts. Method 0 = *in situ* ring method; Method 1 = *in vitro* lens technique.

Time (hours)	Method	Light Intensity (µmol·m^{-2}·s^{-1})	Replicate	*Cocconeis placentula*	*Gomphonema acuminatum*	*Gomphonema augur*	*Gomphonema gracile*	*Gomphonema parvulum*	*Gomphonema truncatum*	*Navicula capitatoradiata*	*Navicula capitata*	*Navicula Cincta*	*Navicula cryptocephala*	*Navicula Pseudolanceolata*	*Navicula pupula*	*Navicula radiosafallax*	*Navicula seibigii*	*Navicula veneta*	*Nitzschia* spp.
0800	0	111	1	0	24	0	0	95	0	0	0	142	47	0	47	47	0	47	47
			2	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0
0900	0	150	1	55	0	0	0	0	55	0	28	358	358	28	0	0	28	220	331
			2	0	0	0	0	28	0	0	28	83	193	110	0	55	28	28	83
1000	0	90	1	193	0	0	0	0	28	0	55	83	138	0	0	28	0	83	0
			2	28	27	0	0	138	0	0	220	441	1295	83	0	55	55	193	1322
1100	0	260	1	0	0	0	0	0	0	0	95	142	213	0	0	0	0	142	47
			2	0	24	0	47	24	95	24	165	307	331	95	0	0	71	189	378
1200	0	530	1	0	0	0	0	132	66	0	463	165	1190	231	66	33	265	364	496
			2	0	0	0	0	220	55	0	110	220	386	55	0	0	28	28	579
1300	0	1140	1	21	0	0	21	41	21	0	165	310	310	165	0	21	41	62	517
1400	0	1500	1	83	83	0	0	331	41	83	1033	1612	1364	289	207	289	661	951	1653
			2	0	0	0	41	289	0	207	3182	496	1860	413	413	165	413	1240	4091
1500	0	1080	1	0	41	0	41	83	0	83	1240	579	2356	455	0	83	248	744	2893
			2	0	0	0	0	33	132	0	265	31	1124	430	33	99	165	198	1587
1600	0	870	1	0	47	0	24	47	0	24	236	47	236	47	71	47	47	165	520
1700	0	960	1	33	0	0	0	99	66	66	628	529	827	132	66	66	496	165	529
			2	33	132	0	198	165	0	33	595	628	1488	165	165	33	165	595	2215
0800	1	160	1	0	0	0	0	0	0	0	28	28	83	0	0	55	0	55	83
			2	0	28	0	0	110	138	0	0	83	55	0	0	28	0	0	165

Time (hours)	Method	Light Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Replicate	*Cocconeis placentula*	*Gomphonema acuminatum*	*Gomphonema augur*	*Gomphonema gracile*	*Gomphonema parvulum*	*Gomphonema truncatum*	*Navicula capitatoradiata*	*Navicula capitata*	*Navicula Cincta*	*Navicula cryptocephala*	*Navicula Pseudolanceolata*	*Navicula pupula*	*Navicula radiosafallax*	*Navicula seibigii*	*Navicula veneta*	*Nitzschia* spp.
0900	I	230	1	55	28	0	28	0	28	0	55	358	386	28	0	110	28	28	165
			2	0	0	0	0	28	0	0	0	55	28	0	0	0	0	28	83
1000	I	150	1	0	0	0	0	0	55	0	0	138	165	28	55	83	55	28	0
			2	28	28	28	55	55	110	248	83	0	28	28	110	165	0	303	28
1100	I	380	1	0	41	0	21	21	0	0	0	62	21	21	0	41	21	41	21
			2	22	41	0	22	22	41	0	22	145	83	22	0	41	0	41	186
1200	I	790	1	95	0	0	0	71	0	0	24	118	95	71	0	95	24	47	0
			2	0	0	0	83	0	0	0	276	1460	193	28	0	165	83	28	55
1300	I	1950	1	0	0	0	0	145	0	0	83	103	62	41	0	0	0	0	186
1400	I	2130	1	0	0	0	0	21	83	0	41	41	0	21	0	21	0	0	41
			2	21	41	0	21	124	83	41	0	165	0	0	0	0	0	21	351
1500	I	1770	1	0	0	0	0	21	0	0	0	41	21	21	0	0	0	41	145
1600	I	1230	1	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	0	0	0	0	0	0	0
1700	I	1470	1	0	28	0	55	468	165	0	55	524	193	28	0	28	55	193	1047
			2	0	0	0	24	95	24	0	0	118	71	47	0	0	71	47	260

Appendix 3.1. Surficial density (valves·cm^{-2}) of epipelic diatom species on sediments of a small arctic stream, July 9, 1999. Data are reported as calculated values derived from hourly counts. Missing replicates are due to laboratory mishaps.

Time (hours)	Light Intensity (µmol·m^{-2}·s^{-1})	Replicate	*Encynema angustata*	*Encyonema silesiaca*	*Encyonema subcuspidata*	*Encyonema tynii*	*Eunotia* sp. 1	*Eunotia bilunaris*	*Eunotia fallax*	*Eunotia subarcuatoides*	*Gomphonema lagerheimii*	*Gomphonema parvulum*	*Navicula molestiformis*	*Navicula pupula*	*Navicula seibigii*	*Neidium affine*	*Neidium bisulcatum*	*Nitzschia* spp.	*Pinnularia gibba*	*Stauroneis anceps*	*Stauroneis phoenicenteron*
0100	25	1	0	606	165	276	110	110	0	276	0	441	1378	165	165	55	110	190929	827	0	441
		2	0	0	248	2562	744	496	0	827	909	248	1570	5042	1736	0	165	55543	1075	248	3224
0200	46	1	0	99	0	132	33	33	0	99	0	66	364	165	0	0	66	83865	66	0	99
		2	0	83	41	0	41	124	0	83	0	1033	909	124	248	909	1612	137645	83	0	1653
0300	34	1	0	331	55	220	276	441	0	220	220	1543	1157	496	331	220	3031	90092	165	55	1157
		2	39	97	58	39	39	7	0	0	0	97	58	20	156	39	58	7779	0	0	331
0400	11	1	0	0	0	0	0	0	0	0	0	0	66	0	0	33	0	860	132	0	265
		2	0	62	21	0	62	165	0	0	0	0	62	0	62	0	0	909	0	0	41
0500	6	1	0	0	66	0	165	132	0	33	0	0	165	231	66	33	132	3438	132	0	231
		2	41	41	41	0	0	62	0	83	0	41	145	41	41	0	0	827	0	0	62
0600	9	1	0	0	66	33	0	9	0	33	0	0	66	132	33	0	66	2480	0	0	132
		2	0	0	0	0	41	21	0	41	62	0	62	0	0	0	0	558	0	0	21
0700	20	1	0	21	0	0	0	62	0	0	0	21	21	0	0	0	0	909	0	0	83
0800	34	1	0	0	0	0	0	66	0	0	0	0	694	0	0	33	0	4100	0	0	99
0900	114	1	0	33	33	0	0	265	0	132	66	99	463	0	66	0	66	3637	0	0	165
1000	150	1	0	66	0	0	0	99	0	231	0	165	298	0	0	165	66	5587	0	0	298
1100	172	1	0	386	110	0	0	110	0	220	55	55	1929	331	0	0	0	26669	0	0	0
1200	955	1	0	82	0	0	248	82	0	248	124	124	165	289	82	248	248	6571	0	0	372
1300	1367	1	66	99	198	0	0	99	0	132	0	0	0	33	0	0	33	2678	0	0	99
1400	1574	1	0	110	110	0	0	386	0	496	0	220	386	496	110	1212	2480	19561	661	0	1102

Time (hours)	Light Intensity (μmol·m^{-2}·s^{-1})	Replicate	*Encyonema angustata*	*Encyonema silesiaca*	*Encyonema subcuspidata*	*Encyonema tynii*	*Eunotia* sp. 1	*Eunotia bilunaris*	*Eunotia fallax*	*Eunotia subarcuatoides*	*Gomphonema lagerheimii*	*Gomphonema parvulum*	*Navicula molestiformis*	*Navicula pupula*	*Navicula seibigii*	*Neidium affine*	*Neidium bisulcatum*	*Nitzschia* spp.	*Pinnularia gibba*	*Stauroneis anceps*	*Stauroneis phoenicenteron*
1500	1600	1	110	110	0	0	331	827	0	1323	110	0	220	2425	331	165	1929	22812	716	0	1157
1600	1548	1	0	220	55	0	165	220	0	220	0	165	1929	165	110	0	0	46561	386	0	496
1700	1393	1	0	0	55	0	0	0	0	55	0	0	716	0	386	0	3582	30637	0	0	55
1800	1135	1	0	55	0	0	0	110	0	0	0	220	551	0	276	55	1102	63257	220	0	606
1900	877	1	0	827	0	0	110	110	0	165	55	551	1984	55	0	0	220	122271	220	0	220
2000	344	1	0	248	0	0	0	0	0	41	0	331	331	124	289	0	165	35128	124	0	165
2100	77	1	0	0	0	55	55	0	0	0	0	441	55	220	110	55	2204	51686	165	0	110
2200	49	1	0	0	55	0	0	0	0	55	0	110	441	0	220	276	3086	50969	110	0	606
2300	52	1	0	0	47	47	0	142	0	47	95	614	142	0	95	0	472	21254	95	0	331
2400	29	1	0	0	55	0	0	276	110	165	276	441	992	0	110	220	4243	33061	220	0	220

Appendix 3.2. Surficial density (valves·cm^{-2}) of epipelic diatom species on sediments of a small arctic stream, July 10, 1999. Data are reported as calculated values derived from hourly counts. Missing replicates are due to laboratory mishaps.

Time (hours)	Light Intensity (µmol·m^{-2}·s^{-1})	Replicate	*Encyonema silesiaca*	*Encyonema subcuspidata*	*Encyonema tynii*	*Eunotia* sp. 1	*Eunotia bilunaris*	*Eunotia subarcuatoides*	*Gomphonema* sp. 1	*Gomphonema lagerheimii*	*Gomphonema parvulum*	*Navicula molestiformis*	*Navicula pupula*	*Navicula seibigii*	*Neidium affine*	*Neidium bisulcatum*	*Nitzschia* spp.	*Pinnularia gibba*	*Stauroneis anceps*	*Stauroneis phoenicenteron*
0100	48	1	0	33	0	33	0	0	33	33	66	99	0	0	165	231	17423	0	0	0
		2	55	28	28	110	0	28	28	28	138	83	0	138	28	55	10883	28	0	28
		3	265	132	165	265	165	66	132	595	132	265	860	231	33	66	10844	132	33	298
0200	28	1	231	132	165	0	66	33	0	0	66	331	99	0	0	265	20994	0	33	231
		2	110	28	110	0	28	28	83	138	600	276	110	28	28	165	13225	0	0	138
		3	331	83	138	165	55	83	83	138	55	413	193	496	0	28	9478	138	110	276
0300	30	1	0	0	33	0	33	0	33	0	265	0	0	0	0	99	24697	33	0	165
		2	0	0	0	0	47	71	0	0	118	24	0	24	0	71	6282	0	24	0
		3	47	0	0	71	71	24	24	283	47	95	95	189	0	0	7935	95	24	142
0400	48	1	132	132	66	0	99	66	99	0	364	132	0	198	33	0	16299	0	0	66
		2	441	110	28	83	28	165	0	110	220	413	165	138	0	220	18873	28	28	220
		3	95	5	71	118	189	47	24	283	213	41	118	118	0	0	9540	95	95	189
0500	101	1	198	33	33	33	198	66	0	0	198	66	33	66	0	99	33524	66	0	132
		2	47	0	47	0	0	24	0	24	236	71	0	47	24	118	16295	0	0	47
		3	189	24	24	95	24	71	0	378	0	165	118	260	24	0	9281	213	24	118
0600	193	1	0	0	33	0	0	0	0	0	298	794	0	66	165	165	112518	0	0	231
		2	47	24	95	0	0	0	24	0	95	142	118	24	24	165	24938	47	0	236
		3	83	21	21	103	145	165	0	351	21	62	41	83	0	21	1756	21	21	83
0700	146	1	33	0	0	33	33	0	0	0	132	0	66	165	66	0	11241	0	0	99
		2	165	41	207	124	124	41	41	0	744	785	537	83	207	124	87502	83	0	248
		3	145	0	21	21	207	41	62	496	83	124	103	207	0	41	2790	41	103	124

Time (hours)	Light Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Replicate	*Encyonema silesiaca*	*Encyonema subcuspidata*	*Encyonema tynii*	*Eunotia* sp. 1	*Eunotia bilunaris*	*Eunotia subarcuatoides*	*Gomphonema* sp. 1	*Gomphonema lagerheimii*	*Gomphonema parvulum*	*Navicula molestiformis*	*Navicula pupula*	*Navicula seibigii*	*Neidium affine*	*Neidium bisulcatum*	*Nitzschia* spp.	*Pinnularia gibba*	*Stauroneis anceps*	*Stauroneis phoenicenteron*
0800	154	1	198	66	430	66	132	66	0	0	198	99	198	198	33	66	12960	99	0	496
		2	55	55	0	0	0	55	0	0	110	55	0	110	165	0	43476	55	0	110
		3	0	41	41	41	21	0	0	62	21	21	21	145	0	21	1178	0	21	83
0900	197	1	0	0	66	99	0	33	0	33	165	165	66	66	33	33	31970	66	0	66
		2	265	132	99	595	231	165	265	727	231	496	66	727	0	66	18812	198	33	298
1000	224	1	99	33	0	66	99	66	0	99	132	0	66	99	198	265	34780	66	0	760
		2	51	0	0	76	0	0	0	76	203	280	25	127	178	153	35706	153	25	331
		3	198	0	44	132	88	110	66	220	176	110	22	88	0	22	11417	110	88	198
1100	106	1	0	0	0	0	0	0	0	0	83	0	83	0	0	0	12811	0	0	248
		2	207	0	0	83	83	0	83	41	83	165	41	207	1529	537	44385	331	41	537
		3	189	24	165	71	24	95	95	71	118	543	0	189	0	71	14594	95	0	165
1200	210	1	66	0	0	0	33	0	0	0	331	66	0	0	99	165	15440	0	0	198
		2	0	24	0	0	95	24	0	0	142	118	0	0	95	24	31904	0	0	412
		3	254	76	127	229	127	153	153	610	127	483	51	356	0	76	10122	356	102	127
1300	395	1	496	41	83	124	165	124	83	0	868	909	207	455	455	165	88935	496	41	868
		2	463	99	165	66	0	33	132	231	165	364	0	231	66	298	23407	99	66	231
		3	165	0	28	358	110	83	28	579	55	55	28	248	0	0	5483	55	55	110
1400	160	1	298	33	66	66	132	99	66	33	727	992	99	231	265	760	37401	33	33	165
		2	193	0	28	28	28	28	0	55	468	909	0	496	606	579	78052	276	0	909
		3	110	55	248	110	138	83	0	110	138	138	55	220	0	28	10828	193	220	55
1500	445	1	868	41	0	372	83	496	41	83	744	703	2231	576	165	124	90918	455	83	413
		2	83	83	83	0	0	0	41	41	83	83	124	827	207	1984	22978	83	41	248
		3	331	132	132	0	132	132	66	331	331	1025	165	298	0	33	20267	231	0	165
1600	328	1	620	124	41	83	0	0	0	0	785	413	0	2149	661	372	31367	372	83	165
		2	1359	37	0	0	0	147	294	37	698	4902	72	514	37	0	169439	0	0	220
		3	118	47	142	24	47	95	24	331	0	165	47	118	0	0	3519	47	189	118

Time (hours)	Light Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Replicate	*Encyonema silesiaca*	*Encyonema subcuspidata*	*Encyonema tynii*	*Eunotia* sp. 1	*Eunotia bilunaris*	*Eunotia subarcuatoides*	*Gomphonema* sp. 1	*Gomphonema lagerheimii*	*Gomphonema parvulum*	*Navicula molesiformis*	*Navicula pupula*	*Navicula seibigii*	*Neidium affine*	*Neidium bisulcatum*	*Nitzschia* spp.	*Pinnularia gibba*	*Staurosira ancaps*	*Staurosira phoenicenteron*
1700	353	1	66	33	66	165	66	231	0	397	99	265	66	562	0	33	9191	99	0	132
		2	413	138	110	165	276	55	110	55	138	413	248	358	0	138	11516	193	55	193
1800	210	1	661	55	220	0	220	55	0	220	827	1653	110	882	827	386	79237	331	55	2700
		2	661	0	703	207	83	165	207	124	496	289	331	827	331	3058	47443	41	0	909
		3	71	71	0	47	0	118	24	331	71	142	118	0	24	24	3873	0	142	71
1900	1008	1	0	0	28	0	55	28	0	0	28	110	0	0	28	28	13417	55	0	28
		2	231	165	99	33	231	0	463	265	331	562	33	33	33	364	20399	0	0	132
		3	47	24	0	24	71	24	0	71	118	0	142	0	0	24	3070	24	24	47
2000	529	1	193	83	28	276	165	55	0	716	165	28	138	220	0	55	10690	138	55	220
		2	231	265	364	66	165	198	0	231	99	33	430	165	33	99	23044	198	0	331
2100	73	1	231	99	33	33	331	99	0	132	595	331	231	132	198	463	38549	0	0	165
		2	124	0	83	41	103	21	83	62	331	103	21	0	41	62	7749	41	0	21
2200	53	1	138	0	83	0	0	0	28	55	220	110	0	28	83	55	21159	28	0	55
		2	145	21	21	21	62	0	41	0	186	83	21	21	21	124	20167	124	21	103
2300	73	1	165	99	33	99	66	33	0	231	364	529	132	132	0	0	26515	66	33	298
		2	71	0	0	0	0	24	0	47	189	71	24	24	71	71	21726	0	0	213

Appendix 3.3. Surficial density (valves·cm^{-2}) of epipelic diatom species on sediments of a small arctic pond, July 9, 1999. Species data are reported as calculated densities derived from hourly counts. Missing replicates are due to laboratory mishaps.

Time (hours)	Light Intensity (µmol·m^{-2}·s^{-1})	Replicate	*Brachysira brebissonii*	*Eunotia bilunaris*	*Eunotia nymanniana*	*Frustulia rhomboides*	*Navicula accomoda*	*Navicula capitata*	*Navicula cryptocephala*	*Navicula cryptotenella*	*Navicula festiva*	*Navicula maceria*	*Navicula seibigii*	*Navicula soehrensis*	*Navicula sublissima*	*Nitzschia* spp.	*Pinnularia streptoraphe*	*Reimeria sinuata*
0100	116	1	992	248	83	537	0	0	0	0	0	207	83	0	248	207	0	248
0200	13	1	3802	1543	110	1267	165	110	110	110	0	165	0	0	882	331	0	0
		2	1405	0	83	661	41	0	0	0	0	41	0	0	248	0	0	165
0300	10	1	744	83	83	992	0	0	165	83	0	0	0	0	537	0	0	0
		2	868	207	248	620	0	0	0	83	248	0	455	331	413	331	0	248
0400	5	1	1157	331	0	1598	661	0	110	110	0	0	0	0	661	0	0	551
		2	9092	1653	992	1488	0	0	331	331	0	661	0	661	992	1322	0	1322
0500	24	1	386	0	0	220	0	0	0	110	0	0	0	0	0	0	0	0
		2	3251	220	110	2094	0	220	0	0	0	276	55	110	551	331	0	441
0600	32	1	4629	0	0	1598	0	0	0	0	0	220	0	331	110	110	276	441
		2	1322	83	124	1364	0	0	0	0	0	124	413	248	331	124	0	83
0700	41	1	3747	0	0	1598	0	0	0	0	0	110	0	331	331	110	220	441
		2	2232	248	83	620	0	83	0	0	0	165	0	248	289	372	83	331
0800	67	1	4574	716	0	1433	0	110	110	220	110	110	0	441	1322	110	220	55
0900	175	1	110	110	0	331	3196	110	1322	661	0	0	0	0	276	0	0	55
		2	1570	83	0	0	0	83	83	0	0	83	124	83	455	83	83	0
1000	252	1	413	124	0	248	0	165	0	331	0	0	0	0	41	0	41	0
		2	827	331	110	1543	0	0	0	381	0	276	0	441	165	1708	55	2920
1100	301	1	248	0	0	165	165	0	55	165	0	0	0	0	0	0	0	0
		2	1199	165	0	248	0	0	0	0	0	207	0	165	41	165	83	496

Time (hours)	Light Intensity (μmol·m^{-2}·s^{-1})	Replicate	*Brachysira brebissonii*	*Eunotia bilunaris*	*Eunotia nymanniana*	*Frustulia rhomboides*	*Navicula accomoda*	*Navicula capitata*	*Navicula cryptocephala*	*Navicula cryptoinerella*	*Navicula festiva*	*Navicula maceria*	*Navicula seibigii*	*Navicula soehrensis*	*Navicula subtissima*	*Nitzschia* spp.	*Pinnularia streptoraphe*	*Reimeria sinuata*
1200	135	1	9	0	0	0	0	0	0	0	0	2	0	0	2	0	0	0
		2	289	0	0	0	0	83	0	0	0	0	0	0	0	0	0	83
1300	1290	1	165	0	0	331	0	83	0	207	83	0	165	0	41	0	0	165
1400	1290	1	1708	110	0	937	441	0	276	441	0	0	0	0	110	0	0	331
		2	0	0	0	99	0	0	0	0	0	0	0	0	0	33	0	66
1500	1290	1	165	0	0	83	0	0	0	0	0	0	0	0	0	0	0	0
1600	1316	1	5290	220	110	882	220	0	0	0	0	110	0	0	220	0	110	331
		2	6833	661	165	1047	220	0	0	55	110	276	0	771	496	55	165	551
1700	1342	1	703	0	0	372	0	83	0	207	0	0	0	0	0	165	0	0
1800	1006	1	9092	1157	165	4877	331	0	0	0	496	496	165	1488	1405	0	0	3719
1900	903	1	1889	95	0	992	0	0	95	0	0	0	0	0	189	0	0	0
		2	20278	2755	0	4298	110	0	220	0	882	1212	220	4629	1543	0	0	3967
2000	851	1	744	83	0	83	83	455	620	579	0	0	0	0	0	0	0	0
		2	2167	0	0	1176	0	0	0	0	74	0	147	147	37	0	0	147
2100	335	1	827	0	41	207	124	83	0	0	0	0	868	0	207	0	0	83
		2	6754	756	0	1323	95	95	0	47	0	47	0	520	425	0	95	95
2200	85	1	1860	331	165	413	165	0	0	83	0	0	0	331	0	0	83	165
		2	703	0	83	124	0	0	0	0	0	41	124	124	0	0	0	0
2300	75	1	2362	520	236	897	0	0	0	0	0	0	0	0	425	0	283	189
		2	1240	207	0	331	0	0	83	0	0	0	83	41	124	0	0	207
2400	52	1	3086	1378	0	1598	165	0	0	0	220	551	0	0	992	882	551	110
		2	1488	455	0	496	83	0	0	0	0	0	0	248	331	0	0	248