



PhD Thesis

**STUDIES ON THE LIFE CYCLE OF AKINETE
FORMING CYANOBACTERIUM *Cylindrospermopsis*
raciborskii IN THE TEMPERATE REGION**

A thesis approved by the Faculty of Environmental Sciences and Process Engineering at the Brandenburg University of Technology in Cottbus in partial fulfilment of the requirement for the award of the academic degree of PhD in Environmental and Resource management

By

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In loving memory of my father

**Peter Fon Tingwey
(10.12.1939-07.10.2001)**

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Declaration

“I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text”.

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CERTIFICATE

This is to certify that the thesis entitled STUDIES ON THE LIFE CYCLES OF AKINETE FORMING CYANOBACTERIUM *Cylindrospermopsis raciborskii* IN THE TEMPERATE REGION submitted by Emilienne Ingie Tingwey to the Brandenburg University of Technology, Cottbus, Germany for the award of the degree of Doctor of Philosophy is a bona fide record of the research work carried out by her under my supervision and guidance. The content of the thesis, in full or parts have not been submitted to any other Institute or University for the award of any other degree or diploma.

Place

Date

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ABSTRACT

In recent years *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju a potentially toxic freshwater cyanobacterium of the order Nostocales initially classified as a tropical species, has invaded the temperate region and has established in lakes in northern Germany. The formation of dormant cells (akinetes) is an important part of the life cycle which enables Nostocales species to survive harsh conditions like the winter in the temperate zone

In this thesis, my aim is to throw more light on the life cycle of the invasive cyanobacterium *Cylindrospermopsis raciborskii* and also to compare it to native Nostocales species of the genus *Aphanizomenon* using derived field and laboratory data. This would help in the understanding of the invasion of *C. raciborskii* and spread and how it can be contained.

Abiotic factors influencing the development and growth of photoautotrophic planktonic organisms in nature are examined and their variation with the years is discussed. A comprehensive look at *C. raciborskii*'s akinetes in the sediment and the filaments and akinetes in the water column is done. The timing and temperature condition of akinete germination, recruitment, formation in the pelagic and importation into the sediment is determined. An assessment of the role of abiotic factors on the dynamics and abundance of filaments is done with a statistical model used to isolate which abiotic factors are most influential on the population growth of *C. raciborskii*. Akinetes distribution on the bed of the lake and the effect of loss processes on their abundance are looked into. *Cylindrospermopsis* is compared to native species *Aphanizomenon* spp in terms of the seasonal dynamics and the germination condition of their akinetes.

The main findings in this thesis were that the seasonal dynamics of *C. raciborskii* had a similar trend but different timing of each event in the four years of the study. The variabilities were due to the different timing of the abiotic conditions. But in all temperature was identified as the most important factor influencing the timing of most events with germination of akinetes occurring at 13°C which is lower than so far reported in literature. Subsequent increment in temperature to at least 23°C was found to be essential for the development of high populations of *C. raciborskii* in the water column. With a drop of temperature in autumn the filaments are stimulated in to producing akinetes which settle out into the sediment and remain there until the next spring when favourable conditions force them to germinate. They might also be consumed before germination by non identified grazer or losses due to microbial infections are discussed. Overall, the native Nostocales species of the genus *Aphanizomenon* have a selective advantage over the invaded species *C. raciborskii* under low temperatures and temperature fluctuations in spring as observed from the field and laboratory studies.

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1. INTRODUCTION

Algal blooms that adversely affect environmental, plant, or animal health are referred to as harmful algal blooms (HABs) (Backer, 2002). They occur in freshwater, marine and estuary systems and are quickly becoming a public health issue. These blooms are primarily associated with surface scum from blue-green algae or cyanobacteria that occur in nutrient enriched freshwater lakes and reservoirs. The negative environmental impacts caused by HABs include reduced light levels and reduced dissolved oxygen concentrations that may lead to fish kills, and may even cause shifts in plankton populations (Jones and Sauter, 2005).

Algae are a diverse group of organisms once thought to belong to a single class, plants and have since the earliest times, defied precise definition (South and Whittick, 1987, Canter-Lund and Lund, 1995). Most fresh water algae are of microscopic size, that is they or their general structure cannot be seen by unaided vision. The charophytes (stoneworts) are an exception in that most are bushy plants 20 or more cm in height. The largest known algae, seaweeds, can reach about 60 metres in length. Even as algae vary greatly in size, so they do in colour. Seaweeds, the large algae of the seashore, are various shades of green, brown and red. Microscopic algae of the sea and inland waters also show almost every shade between and mixture of these colours plus forms which are blue-green and, in a few cases, blue. The colour common to many members of a group can be the basis of its name (Canter-Lund and Lund, 1995).

Biochemically and physiologically, the algae are similar in many respects to other plants. They possess the same basic biochemical pathways; all possess chlorophyll *a* and have carbohydrates and protein end-products comparable to those of higher plants. Therefore many algae are ideal experimental organisms in that, due to their small size and easy manipulation in liquid media, they can be studied under controlled conditions in the laboratory (South and Whittick, 1987).

The algae are ubiquitous, occurring in practically every habitable environment on earth, ranging from hot and cold deserts to the soil, permanent ice and snow fields, and every kind of aquatic habitat. They are the major primary producers of organic compounds, and they play a central role as the base of the food chain in aquatic systems. They are a source of human food, and chemical extracts from algae are used in the manufacture of food and many other products. They are also 'nuisance' organisms in municipal water supply systems and in aquatic resources subject to eutrophication (South and Whittick, 1987).

1.1 Systematic classification of algae

The classification and nomenclature of algae follows plant, animals or bacterial systems depending on whether you consider a given alga to be a plant or bacterium. The algae belong in the kingdom Protocista.

Table 1: classification of algae (Source: Adapted from Encyclopaedia Britannica-online; South and Whittick, 1987)

Kingdom		Division	Class	Example	
Protocista	Prokaryota	Cyanophycota	Cyanophyceae	<i>Aphanizomenon</i> <i>Cylindrospermopsis</i>	
		Prochlorophycota	Prochlorophyceae	<i>Prochlorococcus</i> ,	
		Archaeobacteria		<i>Methanococcus</i>	
	Eukaryota	Rhodophycota (red algae)	Rhodophyceae		<i>Bangia</i> , <i>Porphyra</i> .
		Chromophycota		Bacillariophyceae (diatoms)	<i>Cyclotella</i>
				Bicosoecophyceae	<i>Bicosoeca</i>
				Chrysophyceae (golden algae)	<i>Chrysocapsa</i> ,
				Dictyochophyceae	<i>Pedinella</i> .
				Eustigmatophyceae	<i>Eustigmatos</i>
				Phaeophyceae (brown algae)	<i>Macrocystis</i>
				Prymnesiophyceae (Haptophyceae)	<i>Chrysochromulina</i> ,
				Raphidophyceae (Chloromonadophyceae)	<i>Heterosigma</i> , <i>Vacuolaria</i> ,
				Synurophyceae	<i>Synura</i> ,
				Xanthophyceae (yellow-green algae)	<i>Vaucheria</i> .
			Pyrrhophycota (Dinoflagellata)	Dinophyceae;	<i>Peridinium</i> , <i>Polykrikos</i>
			Euglenophycota	Euglenophyceae	<i>Euglena</i>
		Chlorophycota (green algae)		Chlorophyceae	<i>Chlorella</i> ,
				Charophyceae	<i>Spirogyra</i> ,
				Micromonadophyceae	<i>Micromonas</i> .
				Pleurostrophyceae	<i>Tetraselmis</i>
	Ulvophyceae		<i>Ulva</i>		
	Cryptophyta	Cryptophyceae	<i>Cryptomonas</i> ,		

Adapted from Parker (1982) this scheme recognises a separation between prokaryotic forms which lack membrane-bounded organelles (e.g. Golgi, mitochondria etc) and eukaryotic forms which have membrane bound organelles (See Table 1). Prokaryota comprise the

Schizomycophyta (bacteria), the Cyanophycota (or cyanobacteria, the 'blue-green algae'), the Prochlorophycota and the Archaeobacteria while the eukaryotic form includes algae and all other plants.

1.2 Algal morphology

A great deal of variation exist in the morphology of the algal body known as thallus (Graham and Wilcox, 2000). The algae are simply constructed ranging from single-celled forms to aggregations of cells, filaments, or parenchymatous thalli. Yet the relative simplicity of the algae is misleading, because even the smallest may exhibit, at the cellular level, a high degree of complexity (South and Whittick, 1987). Many possess extensive mucilaginous sheaths.

1.2.1 Unicells and colonies

Many algae occur as solitary cells (unicells) while others may be made up of several to many individual cells held together loosely or in a highly organised fashion forming a colony¹. Some unicellular algae are non-motile while others possess one (or more) of various means of locomotion found among the algae (e.g. flagella) (Graham and Wilcox, 2000).

1.2.2 Filaments

A simple filament consists of a single row of cells firmly attached to one another, and with no branching (South and Whittick, 1987). It is a common growth form among the algae where the daughter cells remain attached to each other following cell division forming a chain of cells. Filaments may be unbranched or branched and may also to uniseriate² or multiseriate³. True filaments can be distinguished from a linear colony by the fact that cells of the adjacent cells of the former share a wall whereas cells of the later each possess their own individual walls (Graham and Wilcox, 2000). In Cyanophyceae the simple filament is the trichome. The individual cells may be extremely small (<1.0µm diameter in Lyngbya). They may be of equal width throughout its length (e.g. Oscillatoria), with only slight modification of the terminal cell, or markedly tapered from base to apex, with the terminal portion hair-like (e.g. Scytonema, Rivularia) and the basal cell a heterocyst (South and Whittick, 1987).

¹ A colony is an assemblage of individual cells or a predictable number and arrangement of cells that remain constant throughout the life of the individual. A colony of the latter type is known as coenobium (Graham & Wilcox, 2000).

² Uniseriate-a single series of cells

³ Multiseriate or pluriseriate is when a few to many individual filaments fuse together to form a larger, more complex structure.

1.2.3 Coenocytic or siphonaceous forms

This is less common in algae. The organisms basically consist of one large multinucleate cell, without cross walls (Graham and Wilcox, 2000).

1.2.4 Parenchymatous and pseudoparenchymatous algae

Pseudoparenchymatous algae have thalli that superficially resemble parenchyma⁴. But which are actually composed of appressed filaments or amorphous cell aggregates. Parenchyma and pseudoparenchymatous algae assume a wide range of shapes (sheets, tubes, stem- and leaf-like arrangements, etc) and size (microscopic to lengths of 50m or greater). Simple filamentous cyanophytes readily fragment into hormogonia. These result either from natural breaks, or from special biconcave cells known as separation discs or necridia. The hormogonia are normally liberated from the parent sheath, but may remain within it (South and Whittick, 1987).

1.3 Reproduction in algae

Reproduction in the algae involves a number of functions apart from an increase in the number of individuals. These include mechanisms to aid in dispersal and survival under adverse conditions, and mechanisms to bring about the enhancement of species fitness through genetic recombination (South and Whittick, 1987). The algae demonstrate as much variation in reproduction as they do in morphology. Vegetative, asexual, and sexual processes are involved, and in many forms an alternation of generations occurs (South and Whittick, 1987, Graham and Wilcox, 2000). Asexual reproduction predominates, involving binary fission and multiple fission. Multiple fission results in endospores⁵ and hormogonia⁶. True sexual reproduction is lacking, although there are reports of a low-frequency occurrence of genetic recombination in some species (South and Whittick, 1987).

1.3.1 Asexual reproduction by spores

Asexual reproduction is a means by which an individual organism can produce additional copies of itself without such unions of cytoplasmic and nuclear materials or meiosis (Graham and Wilcox, 2000). The event includes the formation of specialized unicellular spores which may appear singly (monospore) or in pairs (bisporangia) or in an irregular mass of

⁴ Parenchyma is a term used to describe plant or algal tissue that is composed of relatively undifferentiated, isodiametric cells generated by a meristem (A cell or group of cells capable of repeated division)

⁵ Endospores or baeocytes

⁶ Hormogonium is a short gliding trichome(s).

monospores known as paraspores. Asexual spores are functionally distinguished from gametes in that they develop without syngamy⁷. Their formation can be by abstriction of the cell contents or internally within the parent cell respectively known as exospores and endospores. There are different possible types of asexual spores that algae can produce. It might also combine two or more descriptions (South and Whittick, 1987).

1.3.1.1 Zoospores and aplanospores formation

Motile spores which possess flagella are termed zoospores (South and Whittick, 1987). They may be produced within vegetative cells or in specialised cells, depending on the organism. Zoospores contain all of the components necessary to form a new individual. Sometimes rather than forming flagella, the spores begin their development before being released from the parental cell or sporangium (Graham and Wilcox, 2000). These non-motile spores termed aplanospores possess the ontogenetic possibility of developing into zoospores (South and Whittick, 1987, Graham and Wilcox, 2000).

1.3.1.2 Autospore or monospore production

Autospores and monospores are also non-motile spores, but unlike aplanospores, lack the capacity to develop into zoospores (Graham and Wilcox, 2000). They typically look like miniature versions of the parental cells from which they are derived (Graham and Wilcox, 2000, South and Whittick, 1987). They are known as autospores in green algae where as in red algae they are known as monospores (Graham and Wilcox, 2000).

1.3.1.3 Autocolony formation

In coenobia, each cell goes through a consistent number of successive divisions giving rise to a miniature version -an autocolony- of the original coenobium. Depending on the organism, autocolonies may be formed from non-motile or motile cells that arrange themselves in a pattern identical to that of the parental cells (Graham and Wilcox, 2000).

Spores or specialised vegetative cells may enable survival under conditions adverse to vegetative growth. These usually have thickened or otherwise modified cell walls and reduced metabolic activity. They are particularly common in fresh water, where they may have to withstand complete desiccation or be exposed to wider variations in temperature and nutrient than are their marine counterparts (South and Whittick, 1987). These include:

⁷ They do not fuse to produce zygotes.

1.3.1.4 Akinetes

An akinete is a vegetative cell that develops a thickened cell wall and is enlarged, compared to typical vegetative cells (Graham and Wilcox, 2000). They are responsible for perennation in several orders of the cyanophyceae, including some unicellular forms. Apart from the thickened walls and larger size, they possess abundant reserves of stored nitrogen in cyanophycean bodies and are resistant structure that allow the alga to survive harsh environmental conditions and germinating when they improve (South and Whittick, 1987, Graham and Wilcox, 2000). Rather than a means of producing additional copies of the individual during active growth, akinetes represent a type of survival mechanism (Graham and Wilcox, 2000).

1.3.1.5 Zygo spores

Zygote in some algal groups may also remain dormant and are termed zygo spores. Many flagellates are capable of forming temporary non-motile 'palmelloid' stages with cells embedded in a gelatinous matrix. This may be a response to adverse conditions, but as both cell division and spore formation may occur in the palmellae they cannot be regarded as resting stages. One of the characteristic features of the Chrysophytes is their ability to form siliceous statospores or resting cysts. During formation of the resting cell, the cytoplasm of the parent cell migrates into this cyst, which is then sealed by a plug. On germination the plug dissolves and one or more motile cells emerge (South and Whittick, 1987).

1.3.2 Sexual reproduction

In algae sexual reproduction involves two functionally opposite processes:

1. **Syngamy**, a two-stage process involving the cytoplasmic fusion of haploid gametes⁸ (plasmogamy) followed by nuclear fusion and pairing of chromosomes (karyogamy), to form a diploid zygote (South and Whittick, 1987, Graham and Wilcox, 2000). The homologous chromosome contributed by each of the two gametes pair and at some point are partitioned into haploid cells through the process of meiosis (Graham and Wilcox, 2000).
2. **Meiosis**, the interchange of genetic information (in synapsis) and the independent assortment of chromosomes into the resultant cells.

⁸ Gametes are reproductive cells

1.3.2.1 Syngamy

Gametes may be nearly identical to vegetative cells in appearance or vastly different, depending upon the alga (Graham and Wilcox, 2000). There are four types of gametes and gamete fusion processes:

- **Isogamy**- In this type of sexual reproduction, motile gametes that are more or less identical morphologically (size and shape) fuse with each other.
- **Anisogamy**- Here the two motile gametes are of different sizes and/or behaviour. One gamete (the male) is smaller and more motile than the other (the females) gametes.
- **Oogamy**- In this case, a flagellate (sperm) or non-flagellate cell fuses with a larger immobile egg (female gamete). E.g. rhodophytes
- **Autogamy**-there is the fusion of two gametic nuclei within an individual.

In anisogamous and oogamous species, the two types of gametes may be produced by the same individual, in which case the species is termed monocious. If they are produced by separate individuals, it is said to be dioecious. When gametes from the same individual are able to fuse and produce viable offspring, the organism is termed homothallic⁹. If such gametes are incompatible, then two individuals of different genetic makeup are required for successful mating, and the organism is termed heterothallic¹⁰ (South and Whittick, 1987, Graham and Wilcox, 2000).

1.3.2.2 Meiosis

Algae exhibit an amazing diversity of life histories. The primary difference between them include the point where meiosis occurs in relation to syngamy and the type of cells it produces, and whether or not there is more than one free-living stage present in the lifecycle (Graham and Wilcox, 2000). In the algae four such meiotic sites can be distinguished but three principal life history types.

1. The major portion of the life cycle which is the vegetative phase is spent in the haploid state. Meiosis takes place during germination of the zygote, which is the only diploid phase; such meiosis is termed **zygotic**. E.g. *Chlamydomonas*.

⁹ Homothallic means self fertile

¹⁰ heterothallic means self-sterile

2. The vegetative phase is diploid with meiotic division taking place during the formation of gametes, which are the only haploid phase; such meiosis is termed **gametic**. E.g. Fucus
3. Two or three multicellular phases occur. The sporophytes typically diploid produce spores through meiosis (**sporic**). After spore formation, the spores germinate to form a haploid plant which produces gametes (gametophyte) by mitosis. The recurring sequence of sporophyte and gametophyte is termed alternation of generation. E.g. Ulva
4. During vegetative cell division and which is not immediately followed by spore or gamete formation. One part of the thallus is thus haploid the other diploid; such meiosis is termed **somatic**.

Finally in some algae, one or both gametes may develop into haploid individuals if they do not happen to fuse with another gamete to form a zygote. This phenomenon is referred to as parthenogenesis (Graham and Wilcox, 2000).

1.3.3 Vegetative reproduction

1.3.3.1 Cellular bisection

In many unicellular algae, reproduction is simply by longitudinal or traverse cell division. In multicellular algae (or colonies with indeterminate numbers of cells), this process would lead to growth of the individual, i.e. an increase in size and the number of its cells. In unicells cell division leads to population growth (Graham and Wilcox, 2000). Vegetative reproduction via simple mitotic division has been reported in all the algal divisions with unicellular representatives. In diatoms the two valves move apart in cytokinesis and new valves form in the cleavage furrow (Figure 1).

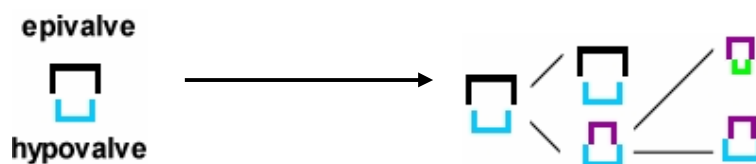


Figure 1: Diatom Frustule: Diatoms shrink with successive generations (Source: http://www.mbari.org/staff/conn/botany/phytoplankton/phytoplankton_diatom_asexual.HTM, 26.03.2007)

Both the epitheca and hypotheca of the parent become the epivalves of the offspring; and thus in actively growing diatom population there is a progressive diminution of cell size. Usually

the return to maximum size is brought about via sexual reproduction and auxospore formation though instances of size regeneration via extrusion of cytoplasm and the regeneration of larger valves have been reported.

1.3.3.2 Fragmentation

Some multicellular and non-coenobic colonial algae give rise to new individuals through fragmentation. In colonies and some filamentous species this can be a simple, more or less random process whereby an individual breaks into two or more pieces, each of which can continue to grow. In other algae the process is more specialised with, for example, predictably occurring weak links in filaments or elaborate budlike propagules in some multicellular algae.

Vegetative reproduction through fragmentation is widely reported from all algal divisions in both freshwater and marine habitats, and is especially common in filamentous species. Some algae produce specialized vegetative propagules, hormogonia, consisting of short filaments of undifferentiated cells formed by the disintegration of some filamentous cyanophytes (Graham and Wilcox, 2000).

1.4 Benthic recruitment and its importance to the phytoplankton population development particularly with regard to blue-green algae

Resting cells (e.g. akinetes) formed when conditions are unfavourable settle out of the water column onto or in the sediment. On the return of favourable conditions vegetative colonies or filaments either actively migrate into the water column through the formation of gas vesicles (Trimbee and Harris, 1984; Barbiero and Welch, 1992; Hansson, 1996) or are passively recruited when turbulence resuspends the resting cells to an environment conducive of vegetative growth and formation of colonies (Lund, 1954; Reynolds, 1972). Recruitment of photosynthetic microorganisms from surface sediments to the water column occurs frequently in freshwater lakes. Understanding the size and timing of this process is one key factor for understanding and managing phytoplankton blooms (Hansson *et al.*, 1994, Schelske, 2002, Brunberg and Blomqvist, 2003).

The ability of a species both to survive periods when environmental factors are unsuitable for growth and to maintain a potential 'inoculum' poised to exploit the return of favourable conditions, is of fundamental importance to the planktonic way of life. It is manifested in different ways among the various groups of phytoplankton (Reynolds, 1984).

In dinoflagellates (E.g *Ceratium hirundinella*) there is an alternation between the vegetative population and a distinctive resting phase. These dinoflagellates in temperate lakes build up their population through the summer. Later in the summer or autumn, an abrupt decline in number of vegetative cells takes place with a concomitant production of distinctive cysts which settle out onto the sediment (Reynolds, 1984). According to Reynolds *et al.* (1983) about 35-42% of the vegetative cells are encyst. Excystment or germination takes place late in winter with a naked flagellated cell or gymnoceratium, emerging through an exit slit and soon developing the thecal plates of the vegetative form. In a study carried out in the Esthwaite water, recruitment of these vegetative cells into the plankton occurred at a water temperature of 5°C where the population remained static or declines slightly until in April or May, that there was cell division or exponential increase (Heaney *et al.*, 1981). However in that study it was established that few cysts produced the preceding autumn were required to germinate in order to establish the basis of a dominant summer population. In another study an enhanced spring recruitment of *Ceratium* to the plankton of Crose Mere generally followed prolific cyst production the previous autumn (Reynolds, 1984).

In the case of the Nostocales, (e.g. *Aphanizomenon*, *Cylindrospermopsis*, *Anabaena*, *Gloeotrichia* sp), asexual spores, akinetes, produced when conditions become unfavourable for the vegetative cells eventually settle down to the sediment. With the return of favourable conditions they too undergo germination and recruitment. In another study by Reynolds (1972) in Crose Mere, it was observed that the akinetes of *Anabaena*, are episodically resuspended into the open water column where germination eventually occurs after a certain minimum temperature and/or insolation requirement has been satisfied (Reynolds, 1984).

Microcystis of the order Chroococcales overwinters as vegetative colonies that reside on the lake bottom where they can survive for several months or years without light or oxygen (Reynolds, 1984, Visser *et al.*, 2005). In spring the benthic colonies reinvade the water column. Various conditions have been suggested for its recruitment. Trimbee and Harris (1984) noted that low oxygen concentration, resulting from the onset of stratification, may enhance the recruitment of cyanobacteria while Oliver *et al.* (1985) suggested that some colonies regain buoyancy under anaerobic conditions, when the ferric iron compounds that weigh them down are reduced to soluble ferrous compounds. Penetration of relatively high irradiance levels to the sediment seems necessary for this process since gas vesicle formation in the dark occurs only after earlier accumulation of energy reserves (Deacon and Walsby, 1990). Cáceres and Reynolds (1984) acknowledged the role played by anoxic conditions and

light in the recruitment process but also concluded that an increase in temperature may be critical for the renewed activity of *Microcystis*.

Recruitment in *Microcystis* may be more or less a continuous process during winter and spring as a result of the ongoing decrease of the carbohydrate content in the colonies. In autumn, colonies sinking down may initially have high carbohydrate content but will metabolise it in the low irradiance at or near the sediment. At a certain time in winter or spring, dependent on the initial carbohydrate concentration, oxygen conditions and temperature, buoyancy will be regained if the gas vesicles have remained intact. In some cases recruitment of *Microcystis* may result from resuspension after wind induced mixing and/or bioturbation (Visser *et al.*, 2005).

With the diatoms, while some centric ones such as *Melosira* spp. can survive for long periods (months to years) on the sediment of lakes, other pennate diatoms do not have such resting stages though *Asterionelle* and *Fragilaria* often settle out live and may remain capable of revived vegetative growth if resuspended within 1-3 months. The vegetative protoplast of the centric diatoms contract into physiological-resting globules still within the frustule with those of *Melosira* spp readily germinating when resuspended into the water column with suitable growth conditions. In a nutshell, diatoms, together with many small planktonic species of cryptomonads and chlorellids have no means of perennation save that of being capable of attaining relatively fast rates of growth (Reynolds, 1984).

Other resting propagules produced by phytoplankton include the sexually-produced zygotes of *Eudorina* and *Volvox* and the siliceous cysts of Chrysophyceae such as *Synura* and *Uroglena*. The sexually produced zygotes survive for several months after being deposited on the bottom, still within the colonial mucilage (Reynolds, 1984).

In a nutshell, algae adapted to undertake directional migrations may be recruited from the sediment surface to the water column, where they grow and are subject to grazing by zooplankters. Eventually, the recruited algae return to the sediment surface as dead cells, as cells initiating another cycle, or as resting stages such as cysts or spores. Therefore this benthic population may function as a “seed bank” that provides insurance against catastrophic events in the planktonic part of the population (Barbiero and Welch 1992, Hansson, 1996). It may also serve as an inoculum for planktonic growth or even contribute significantly to the size of the pelagic population (Barbiero and Welch 1992, Hansson, 1996, Head *et al.*, 1999, Karlsson-Elfgren and Brunberg, 2004).

Algal recruitment from the sediment is a highly dynamic and fluctuating phenomenon, varying both spatially and temporally among lakes and along depth gradients within lakes. However, clear and repeatable patterns can be distinguished. Different taxa have their main recruitment at different depths (Hansson, 1996).

Hansson found in his study in 1996 that some species, such as *Peridinium pusillum*, were exclusively recruited from very shallow sediments; others, such as *Synura* and *Gymnodinium*, had high recruitment at depths down to 7 m, whereas *Cryptomonas* was mainly recruited from depths of 4-9 m. None of the species he investigated showed recruitment maxima at water deeper than 9 m, making him to conclude that recruitment is primarily a littoral phenomenon. Though in some cases there was the influence of sediment focussing in which cells which are normally recruited in the littoral were found in traps in the deeper parts of the lake. This was noticed in a study by Trimbee and Harris (1984) on Lake Guelph. *Aphanizomenon* in the Lake appeared in mid-May and reached its peak spring-early summer abundance on 23 May. Recruitment of *Aphanizomenon* in Guelph Lake, in 1981, occurred preferentially at lake depth of 2m and filaments were then transported towards 10m lake depth (where the sedimentation traps were suspended). This suggestion was based on the observation that the abundance of *Aphanizomenon* at 10m depth lagged that at Station 2m depth by 7 days. The fact that filaments showed up in the downward trap at 2 m about a week before dispersal to the lower traps suggests that filaments were transported from Station at 2m depth section of the lake to the 10m depth. This means for a success of seed banks in deep lakes the seeds should be located in the littoral so as to germinate. Location of seeds in the deeper would not germinate and hence not recruited.

Phytoplankton productivity is most often thought to be determined primarily by nutrient concentrations in the mixed layers of lakes. However, interactions with the sediments can play an important role in determining phytoplankton community size and structure, particularly in shallow, unstratified lakes (Barbiero and Kann, 1994). The sediment surface and the water column offer completely different environmental conditions with respect to grazing pressure and light, oxygen, and nutrient availabilities. Thus, in a temporally changing environment, there may be adaptive gains in migrating between these two habitats (Hansson, 1996).

The populations of cyanobacteria that form part of the phytoplankton communities in temperate lakes undergo periodic fluctuations in abundance too. During unfavourable environmental conditions a number of species persist as resting stages in the surface sediments of lakes (Reynolds, 1984; Reynolds, 1987, Head *et al.*, 1999) with certain species

of both spore- and non-spore forming cyanobacteria spending an initial period of development in or on the sediment (Pettersson *et al.*, 1993). The occurrence of specialized resting spores (akinetes), and the possession of gas vacuoles, provides mechanisms for both overwintering and subsequent inoculation into the water column (Barbiero and Kann, 1994). The sediments of lakes with periodically large populations of planktonic cyanobacteria will usually contain large numbers of these resting stages which represent potentially significant inocula for subsequent development of pelagic populations (Head *et al.*, 1999; Barbiero and Welch, 1992). Akinetes are very tolerant to extreme conditions, such as desiccation and freezing, and they can survive buried in the sediment for many years, although the level of tolerance may vary with species (Baker and Bellifemine 2000).

Benthic accumulations of a number of genera of blue-green algae have been found in a variety of shallow lakes (Takamura *et al.*, 1984, Barbiero and Kann, 1994) with recruitment from the sediment observed in them just as is in various other algal groups, (Barbiero and Welch 1992; Pettersson *et al.* 1993; Hansson *et al.*, 1994, Hansson, 1996). A number of studies have been carried out to assess the importance of benthic recruitment to the development of blue-green algae population in a shallow, unstratified lake, and to measure their qualitative role in the pelagic. According to Hansson (1996) studies of the links between benthic and pelagic habitats may improve the understanding of unexplained variation in population dynamics and succession patterns in algal communities.

1.4.1 Process of recruitment

Several studies have now demonstrated active recruitment of cyanobacteria from the sediments into the water column (Trimbee and Harris, 1984; Barbiero and Welch, 1992; Barbiero and Kann, 1994; Hansson, 1996, Head *et al.*, 1999). The process of recruitment can be divided into three different phases: germination, a potential growth phase on the sediment, and migration to the pelagic. Akinetes may spend a variable time on the sediment before germination, depending on a number of factors such as depth of deposition, burial in the sediment, and the amount of disturbance of the sediment surface.

Active migration from the sediment occurs after germination when the formation of gas vacuoles is completed. Migration may also be facilitated by resuspension events (Karlsson-Elfgren and Brunberg, 2004).

1.4.2 Performance of recruitment studies

Most recruitment studies have been performed with samplers modified from sedimentation traps which are turned upside down, in some cases equipped with nets to prevent grazing losses, and placed at different distances from the sediments (Karlsson-Elfgren and Brunberg, 2004).

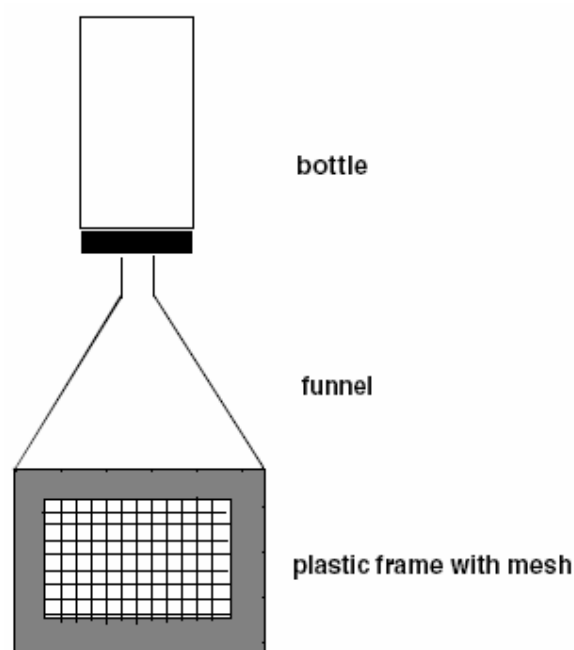


Figure 2: An illustration of a trap for recruiting algae. The trap consists of a transparent glass bottle, attached to a transparent plastic funnel. Underneath the funnel is attached a circular plastic frame with mesh covered sides (mesh size 50 μm). The plastic frame excludes any horizontally transported algae and allows the trap to stand by itself at the sediment surface without disturbing the water flow through the trap, and hence, the chemical environment in the trap will be similar to the lake conditions (STÅHL-DELBANCO *et al.*, 2003).

The sediment traps consist of plastic funnels with attached bottles, mounted on support stands and stacked into the sediment at different distances from the sediment (Figure 2). With these traps, it is always assumed that they catch only algae migrating from an area of sediment equal to the funnel area (Barbiero and Welch, 1992) and that horizontal transportation of migrating cyanobacteria is negligible (Karlsson-Elfgren and Brunberg, 2004). Barbiero and Welch (1992) likewise Hansson (1996) performed tests with trap sides shielded with netting, which indicated minor influence of lateral transportation. Others have experienced a certain amount of “contamination” of their traps by plankton from the surrounding water (Head *et al.*, 1999). In another case of improvement of the traditional trap, the collecting jars were

painted black to exclude light and minimise growth of trapped photosynthetic cyanobacteria. In this same trap the end funnels were covered with 80µm to prevent large zooplankters and mites from entering the traps and thus reducing the grazing pressure (Head *et al.*, 1999). But no comparison between light and dark traps was made.

Karlsson-Elfgrén and Brunberg (2004) used a new design of the traps, which were placed directly on the sediment to avoid problems with horizontal transportation, especially at the shallow site (1m depth) of Lake Limmaren. In their case the traps were constructed of big transparent plastic enclosures that were opened at the bottom. To allow exchange of water but not of the studied cyanobacterial species, two openings were cut on the side of the flask and covered by 40 mm mesh. The traps were attached to the bottom by long spikes penetrating into the sediment. On top of each trap, a 500-mL plastic bottle filled with filtered (40 mm mesh) lake water was attached to collect filaments moving upward (Karlsson-Elfgrén and Brunberg, 2004).

1.4.3 Conditions governing recruitment

Several abiotic factors may initiate or prevent recruitment in algae. Variables of potential importance for initiating recruitment and active migration from the sediment surface are high insolation of $\geq 150\mu\text{Em}^{-2}\text{s}^{-1}$ (Hansson, 1996, Head *et al.*, 1999), appropriate oxygen conditions (or a factor related to oxygen concentration) at the sediment surface (Trimbee and Harris, 1984; Hansson, 1993, Barbiero and Kann, 1994, Hansson, 1996, Head *et al.*, 1999), temperature and water mixing (Robarts and Zohary, 1987). A reduction in light is related to an increased recruitment rate for most genera. There exists a threshold light level below which a further deterioration in light climate initiates recruitment, above this threshold alterations in light availability are of minor importance for recruitment of algae from the sediment. However, taxonomy does not give any clue to the observed recruitment patterns (Hansson, 1993).

The results regarding the optimal oxygen conditions vary. Some studies have indicated that oxygenated (about 10mg l^{-1}) waters seem to promote the recruitment of *Aphanizomenon spp.* (Trimbee and Harris, 1984, Barbiero and Kann, 1994) and *Anabaena spp.* (Reynolds, 1972).

The depth for maximum recruitment differed among lakes and among algal species. This means that the water depth itself is obviously not a factor behind the observed patterns in life-form shift drawn from results of experiments performed at one depth which generated a wide spectrum in recruitment patterns (Hansson, 1993). Head *et al.* (1999) found that the greatest

recruitment of buoyant filaments was recorded in shallower sites where insolation, temperature and dissolved oxygen levels were highest particularly for *Aphanizomenon flos-aquae*. The requirements for high insolation suggested that an initial period of growth and development might occur on the sediment surface following the germination of akinetes. A consequence of this is that large areas of the benthos in steeply sided lakes which receive rapidly diminishing levels of irradiance and where temperatures are low will represent poor environments for the development of resting stages. Conversely, a lake with a large surface area of sediments in shallow, warm water and receiving high levels of insolation will represent a much more favourable environment for such benthic stages to develop and migrate into the overlying water column. Indeed the recruitment rates of all species in Head *et al.*'s study were low when the thermal stratification was at its strongest during July. This was also a period of low insolation, when the euphotic depth was reduced due to shading by phytoplankton cells in the epilimnion (Head *et al.*, 1999). Moreover, Head *et al.* (1999) said that variations in environmental conditions observed with depth are not important factors and that no evidence was found that stability of the water column or anoxic conditions influenced such movements; he then concluded that no single over-riding environmental factor of those initiates the recruitment of cyanobacteria from the sediments in Lake Esthwaite. In contrast, a compilation of data from several different lakes indicated that recruitment of cyanobacteria in general (except possibly *Aphanizomenon*) was enhanced by anoxic conditions over lake sediments (Trimbee and Prepas, 1988, Karlsson-Elfgren and Brunberg, 2004). Growth or recruitment of this blue-green algal species is initiated under different environmental conditions. While recruitment of *Aphanizomenon* is favoured when the bottom waters are oxygenated that of the these three species of blue-green algae coincides with high surface temperatures and the development of anoxia, high NH₃ and low pH The recruitment of *Aphanizomenon* flakes is favoured when the bottom waters are oxygenated (Trimbee and Harris, 1984).

Upward migration may not always be successful because there are factors that prevent the algae from reaching water with suitable light conditions. Such a factor is the thermocline, which may function as a "lid" for algal migration to the euphotic zone (Hansson *et al.*, 1994, Hansson, 1996). During stratified conditions, the environmental conditions, including O₂, redox potential, temperature, and nutrient concentration, differ considerably between a sediment surface above and below the thermocline. Especially low oxygen concentration has been suggested as affecting recruitment of algae from the sediment surface to the water (Trimbee and Harris, 1984, Hansson, 1993). Hansson (1995) tested the hypothesis that

conditions provided by stratification affected algal recruitment by simultaneously quantifying recruitment above and below the thermocline in the deep (Maximum depth of 14m) unproductive Long Lake, U.S.A. Traps were placed at both the shallow (2m) and deep (8m) parts of the lake with considerably different environmental conditions (such as light reaching the sediment and oxic conditions at the shallow point in contrast to the deep point) reigning at each points. In his findings with different phytoplankton species, *Gymnodinium splendens* had the highest exchange of individual between the sediment surface and the water column with the number of migrating cells being 10 times lower at the deep compared to the shallow site in June/July and vice versa in August. Since this species had showed phototactic behaviour in response to a sudden increase in irradiance he expected recruitment during the day but on the contrary in his study *Gymnodinium* sp. usually migrated upward during the night, indicating that receptors sensitive to stimuli other than light may have been active.

In another study by Hansson *et al.* (1994), such dephasing in recruitment at different depths was attributed to alteration between stratified and mixed conditions. The algal groups showing dephasing in recruitment along the depth gradient have receptors sensitive to the surrounding oxygen concentration. When oxygen concentration is low, a cell at the sediment surface can expect a thermocline, which may not be possible to penetrate, and therefore the cell would stay at the sediment surface until the receptor indicates mixed conditions (Hansson *et al.*, 1994). In Long Lake, oxygen concentration were zero at the deep site until about 20th August, when oxygen-rich water penetrated the thermocline coincident with the considerable upward movement of *Gymnodinium*. Hence, the observations in the Long Lake study strengthen his notion that oxygen concentration (or a variable associated with oxygen concentration) affects migration in some algal species and that the thermocline functions as a barrier for vertical migration to the euphotic zone. This is in contrast with the observation in a Finnish lake that *Cryptomonas marssonii* was able to cross a temperature gradient of up to 10° C (Salonen *et al.*, 1984). Hansson (1995) also noted that a change from 0.0 to 0.1 mg O₂ L⁻¹ between 30 July and 23 August coincided with a threefold increase in recruitment of *Gymnodinium* whereas a simultaneous increase of almost 1.0 mg O₂.L⁻¹ at the shallow site had no detectable effect on the migratory behaviour suggesting that possible oxygen receptors (or receptors correlated with oxygen concentration) are of an “on-off” character (Hessen and van Donk, 1993); i.e. they distinguish only between presence and absence of the variable.

Hence, for the species investigated, the thermocline has a crucial impact on whether a recruitment period will be successful (i.e. whether the algae will reach the euphotic zone). If a major part of the recruitment of a species occurs below the thermocline, a large meta- or

hypolimnetic population of cells would be expected—a notion that seems to hold true for these lakes, which often show 10 times higher metalimnetic chlorophyll values than they do above the thermocline (Carpenter *et al.*, unpubl.). This should have a competitive advantage and should therefore occur more often among the dominating species than non-migratory species do. That adaptation to shift habitat is related to a higher frequency of dominance (Hansson, 1996).

In all life-form shift is an active and directional phenomenon mainly triggered by environmental factors, such as: light availability (e.g., *Gymnodinium*, *Synura* and *Dinobryon*), depth of oxycline (e.g., *Synura* and *Cryptomonas*), nutrients (*Cryptomonas*), and possibly even grazer abundance (*Peridinium pusillum*, *P. wisconsinense* and *Dinobryon*). However, depending on the environmental conditions, different algal species are recruited from the same sediment, implying that different parts of the algal "seed-bank" are activated under different conditions (Hansson, 1993).

1.4.4 Impact of recruitment

The sediment surface and the water column offer completely different environmental conditions with respect to grazing pressure and light, oxygen, and nutrient availabilities. Thus, in a temporally changing environment, there may be adaptive gains in migrating between these two habitats (Hansson, 1996). In a study by Hansson (1996) the grazing rates on the algal species investigated differed between the two lakes used probably due to differences in zooplankton size.

His results indicated that recruitment of the species *Cryptomonas* was usually high compared to sinking and grazing. Further more in both lakes, the daily recruitment of Species *Gymnodinium* never or seldom exceeded 10% of the abundance with grazing reducing the abundance by 6-50% per day. The recruitment rate of *Peridinium pusillum* was higher than the rates of the loss processes in both lakes. However, rates of recruitment of *Cryptomonas*, *Gymnodinium*, and *P. pusillum* usually exceeded loss rates. The mean abundance per zooplankter was almost two times higher in one than in the other. Differences in zooplankton community composition may also have caused different grazing pressures on different algal sizes and morphologies. In both lakes, grazing pressure on the small *Peridinium pusillum* was high relative to the other recruited species (*Cryptomonas*, *Gymnodinium*,). In one of the lakes, the abundance of *P. pusillum* was high when the abundance of herbivorous zooplankton was low and vice versa. This made him to suggest that grazing was a key factor in the population dynamics of *P. pusillum*. Hence, high abundances of grazers may also prevent recruitment

from having a large effect on pelagic abundance of algae susceptible to grazing, although indirect effects such as responses in recruitment rates to zooplankton exudates cannot be excluded.

Ståhl-Delbanco *et al.* (2003) on the contrary found from their work that recruitment of *Microcystis* did not seem to be affected by grazers. They commended that since cyanobacteria are of low nutritional value, can be toxic, and their shape may mechanically interfere with the filtering processes they are therefore not suitable as food for several zooplankton species. Also since resting stages of *Microcystis* are large, they are also difficult to graze, which can explain why they do not need an avoidance strategy.

1.4.5 Quantitative importance of algal recruitment from the sediment

Some species regularly exhibit recruitment and recruitment is quantitatively important for the planktonic part of the population (e.g. *Gymnodinium sp.*, *Cryptomonas sp.*, *P. pusillum*, and *Synura sp.*, Hansson, 1996). Planktonic population of some phytoplankton are derived largely from benthic stocks (Barbiero and Welch, 1992). The quantitative importance of these benthic stage, akinetes, in the annual life-history of planktonic cyanobacteria as has been reported is that planktonic populations can originate from 'recruitment' following an initial period of development on the sediments (Barbiero and Welch, 1992, Trimbee and Harris, 1984, Hansson *et al.*, 1994, Head *et al.*, 1999). Several authors investigated the impact of recruitment on the size of the pelagic population (Trimbee and Harris, 1984, Barbiero and Welch, 1992, Barbiero and Kann, 1994, Hansson *et al.*, 1994, Forsell and Pettersson, 1995, Hansson, 1996, Perakis *et al.*, 1996, Head *et al.*, 1999, Karlsson-Elfgren and Brunberg, 2004). The general conclusion to be drawn from these studies is that the recruitment in most cases only contributes a minor part of the pelagic biomass, although the results vary between different lakes and different species (Table 2). Still, the migrating cyanobacteria may be important as an inoculum for the pelagic growth or for nutrient cycling (Istvánovics *et al.* 1993, Pettersson *et al.* 1993, Perakis *et al.* 1996, Karlsson-Elfgren and Brunberg, 2004).

Recruitment of cyanobacterium, *Gloeotrichia echinulata*, from the benthos has been estimated to account for over 40% of the number of colonies in the planktonic populations of Green Lake in Seattle, Washington, U.S.A. (Barbiero and Welch, 1992, Barbiero, 1993) and about 50% in Lake Erken, Sweden (Forsell and Pettersson, 1995, Head *et al.*, 1999). Therefore the pelagic populations in these lakes were largely derived from benthic stocks.

In other studies recruitment has not significantly affected the population. An investigation on the Esthwaite Water indicated that the upward vertical movements of the main planktonic species of cyanobacteria (*Anabaena flos-aquae*, *Aphanizomenon flos-aquae* and *Oscillatoria agardhii*) from the sediment–water interface did not affect the magnitude of established planktonic populations.

Table 2: Contribution from recruitment of some phytoplankton from the benthos to the maximum phytoplankton population in the pelagial of some lakes

Lake	Max depth	Mean depth	Area	Trophy	Species present	Estimated contribution to max pelagic population
Lake Limmaren (Karlsson-Elfgren and Brunberg,2004)	7.8m	4.6m	5.5km ²	Eutrophic	<i>Anabaena spp</i>	0.05%
					<i>Anabaena circinalis</i>	0.003%
					<i>Aphanizomenon flos-aquae</i>	0.02%
					<i>Microcystis spp</i>	negligible
Lake Limmaren (Brunberg and Blomqvist,2003)	7.8m	4.6m	5.5km ²	Eutrophic	<i>Microcystis spp</i>	0.5%
Green lake (Barbiero and Welch 1992)	9.1 m	3.8 m	1.05 km ²	Eutrophic	<i>A. flos-aquae</i>	0.62%
					<i>Anabaena spp</i>	0.62%
					<i>Gloeotrichia echinulata</i>	40%
					<i>Microcystis spp</i>	negligible
Agency Lake (Barbiero and Kann 1994)		2.4m	35.54km ²	hypereutrophic	<i>A. flos-aquae</i>	8.2%
					<i>Microcystis aeruginosa</i>	negligible
Lake Erken (Karlsson-Elfgren,2003)	21m	9m	24km ²	Moderately eutrophic	<i>Gloeotrichia echinulata</i>	2.6%
Esthwaite Water,England (Head <i>et al.</i> , 1999)	15.5m	6.4m	1.1km ²	Eutrophic	<i>Anabaena flos-aquae</i>	Recruitment did not affect the magnitude of established planktonic populations
					<i>A. flos-aquae</i>	
					<i>Oscillatoria. agardhii</i>	
					<i>Gomphosphaerie naegeliana</i>	Recruitment played an important role
Guelph Lake (Trimbee and Harris, 1984)	12.2		4km ²	Eutrophic	<i>Lyngbya Birgei</i>	~2-4%
					<i>Microcystis aeruginosa</i>	~2-4%
					<i>Aphanizomenon</i>	
					<i>G. naegeliana</i>	~2-4%
Lake Krankesjön (Ståhl-Delbanco <i>et al.</i> ,2003)	3m	1.5m		Moderately eutrophic	<i>Microcystis</i>	17%

These species were already well established by the time the first quantitative trapping experiments were carried out. The pelagic populations was several orders of magnitude larger than the estimates attributed to vertical movements, and the level of recruitment occurring from the sediments was insignificant compared to the size of the planktonic population.

The presence of the small, long-term planktonic populations, suggested that low numbers of filaments persisting in the water column, were likely to be more important inocula than recruitment from benthic stocks at the onset population increase. Such pelagic populations are already positioned in the water column to take immediate advantage of improved conditions (Head *et al.*, 1999). Similar results for these same species of cyanobacteria were earlier recorded in recruitment studies on lake Green, U.S.A. and Guelph Lake, Southern Ontario, Canada (Barbiero and Welch, 1992, Trimbee and Harris, 1984).

Barbiero and Welch (1992) came up with the results that an average of approximately 40% of the water column population of *Gloeotrichia echinulata* was derived from sediment colonies. In contrast the development of neither *Aphanizomenon* nor *Anabaena* population was shown to be heavily subsidised by the input from benthic sources. Migration rates as a percentage of population increase accounted only for less than 2% of the planktonic population increase (Barbiero and Welch, 1992) giving a similar result to that of Trimbee and Harris, 1984 who registered a 2-4% of maximum standing crop for the cyanobacterium *Microcystis*. Contradicting the percentage contribution of recruited *Microcystis* to the pelagial, Ståhl-Delbanco *et al.* (2003) found a correlation between the abundance of *Microcystis* and the recruitment rate with an overall, the daily recruitment from the sediment surface forming approximately 17% of the standing stock in the water column suggests that recruitment was important for the development of the pelagic population.

Previous studies by Jones (1979) have shown that overwintering benthic stocks of *Aphanizomenon* are unnecessary for plankton development in Lough Neagh. But in 1994 benthic recruitment of *Aphanizomenon flos-aquae* was found to account for up to 8.2% of the summer planktonic population in Agency Lake (Barbiero and Kann, 1994). From this Barbiero and Welch (1992) suggested that benthic reserves of this alga constitute a 'seed bank' providing insurance against possible washout and concluding from their own study that population growth of *Aphanizomenon* and *Anabaena* takes place mainly in the water column, while some input provided by benthic filaments, migration from the sediment represent a minor contribution to water column population.

In Head *et al.* (1999) study, the pelagic populations were several orders of magnitude larger than the estimates attributed to vertical movements, and the level of recruitment occurring from the sediments was insignificant compared to the size of the planktonic population. From this they concluded that the presence of the small, long-term planktonic populations, suggested that low numbers of filaments persisting in the water column, are likely to be more important inocula than recruitment from benthic stocks at the onset population increase. As said before such pelagic populations are already positioned in the water column to take immediate advantage of improved conditions. This strategy of maintaining a small population in the phytoplankton until the return of conditions conducive to population growth is common amongst phytoplankton (Reynolds, 1984), including cyanobacteria even with known benthic resting stages (Rother and Fay, 1977, Jones, 1979, Head, 1999).

These studies suggests that migration from the sediment surface via recruitment as well as growth may be important factors contributing to increased abundances of different species during succession in phytoplankton communities. Hence, the traditional model for fluctuations in quantification of recruitment can thus help predict population maxima in the water column, which is of special interest for management and prevention of outbreaks of bloom-forming algae. Recruitment may also be important in explaining observed dominance and succession patterns in phytoplankton communities (Hansson, 1996)

1.4.6 Qualitative importance algae recruitment from the sediment

Besides quantitative evidence about the extent of recruitment of cyanobacteria from the sediment, large-scale recruitment could also explain internal loading of phosphorus (P) in shallow lakes, where classical iron redox mechanisms would not be operative¹¹ (Osgood, 1988, Barbiero and Kann, 1994). Phosphorus supplied from lake sediments may occur as anaerobic or aerobic release, excretion by bottom feeding fish, active macrophyte transport or as release upon their decay. Internally supplied phosphorus may also occur by a different mechanism of phosphorus translocation (Osgood, 1988).

A result of algal recruitment from the sediments to the epilimnion would be a transport of phosphorus (P) from the sediments to the water column (internal P loading). Like other algal

¹¹Phosphate is usually present in sediments in organic and inorganic forms. The inorganic forms are iron-bound and calcium-bound phosphates. These fractions are particularly sensitive to redox, O₂, P-load and pH variations of the system and their mobility depend on it. Iron hydroxides are responsible for the iron-bound-phosphate adsorption. Laboratory experiments with Fe(OOH) showed that pH increases produced liberation of P adsorbed onto the iron complexes because of competition with OH⁻ ions. The very low redox potentials induce the reduction of Fe (III) from the Fe (OOH) to Fe (II). In oxic conditions, Fe (III) is very reactive forming Fe(OOH). Fe (OOH) solubility and, as a consequence, its reactivity depends on pH and organic chelators (Gomez *et al.*, 1999).

groups, cyanobacteria have the ability to store a luxury uptake of P (Fitzgerald and Nelson, 1966, Stewart *et al.*, 1978, Pettersson *et al.*, 1993). A benthic population has a good opportunity to take up P from the pore water, which is very rich in P, followed by transport to the water column where it can use this P for a number of cell divisions. Some shallow lakes have an internal loading of P that coincides with a development of cyanobacteria in the water column, indicating algal P transport from the sediments (Pettersson *et al.*, 1993) A typical example is Spring Lake located in the Twin Cities Metropolitan Area, Minnesota, U.S.A. (44°42', 93 °28') in which Osgood (1988) found out that its internal phosphorus loading rates were greater than observed in other lakes and that internal loading mechanisms other than translocation by blue-greens are probably less important with there being an association between *Aphanizomenon* and increasing lake phosphorus when external inputs have ceased. He also found out that in years when *Aphanizomenon* flakes were not present, internal phosphorus inputs were less.

1.5. Special properties of cyanobacteria

Taking a closer look at the prokaryotic algae, all possessing chlorophyll *a* and carrying out aerobic photosynthesis are classified in the Cyanophycota; those possessing chlorophylls *a* and *b* and carrying out aerobic photosynthesis are classified in the prochlorophycota. Prochlorophytes cells also contain β -carotene, zeaxanthin and cryptoxanthin, but lack the phycobiliproteins of the cyanophycota. Also unlike the cyanophycota, their thylakoids are arranged in pairs or stacks; thylakoid architecture resembles that of green algae and higher plants (South and Whittick, 1987). As a logical consequence of the definition of a bacterium, R. Y. Stanier created the name "cyanobacteria" as a replacement for "blue-green algae"(Rippka and Cohen-Bazire, 1983).

Cyanobacteria (blue-green algae) have been among the most studied of all planktonic groups (Wetzel, 2001). While exhibiting an algal morphology, the cyanophycota possess an admixture of bacterial features (South and Whittick, 1987). These organisms are structurally and physiologically like bacteria (Figure 3) but they photosynthesize functionally like plants in the aquatic system (Wetzel, 2001). They lack a nuclear envelope and mitochondria; their photosynthetic lamellae (thylakoids) are single or unstacked and distributed peripherally in the cytoplasm and not within a membrane-bound chloroplast. They possess chlorophyll *a* and phycobiliproteins (Phycocyanin, allophycocyanin and phycoerythrin) with polyglucan granules (carbohydrates) and cyanophycin (combined nitrogen) as storage products. The cell (Figure 3) wall is four-layered, with the major, structural layer of peptidoglycan (as in

bacteria) (South and Whittick, 1987). As such, cyanobacteria entered the 8th issue of Bergey's Manual of Determinative Bacteriology 1974 as members of the Procaryotae, this kingdom being composed of two divisions, Cyanobacteria and Bacteria (Rippka and Cohen-Bazire, 1983).

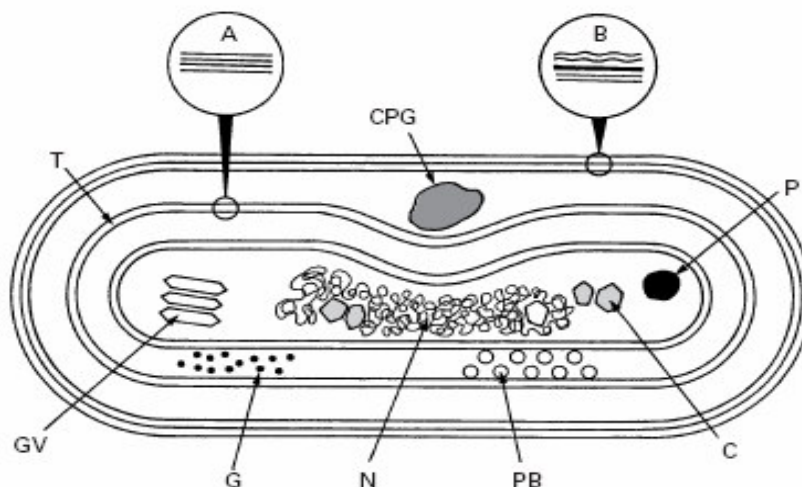


Figure 3: Schematic diagram of a thin section of a cyanobacteria cell. Abbreviation: C-carboxysome; CPG-cyanophycin granules; T-thylakoid; P-polyphosphate granules; N-nucleoplasmic region; G-glycogen granules; PB-phycobilisome; GV-gas vesicle. Inset (A) is an enlarged view of a thylakoid, showing the paired unit membranes. Inset (B) is an enlarged view of the cell envelope, showing the outer membrane (top), the peptidoglycan layer and the cytoplasmic membrane. (Source: Adams *et al.*, 1999)

A major controversy, concerning cyanobacteria is whether they should be treated taxonomically like bacteria or eukaryotic algae. Molecular biologist often tend towards the former, whereas ecologists find it easier to classify cyanobacteria on the basis of structural attributes which is commonly done for eukaryotic algae. Taxonomists have historically used morphological features to define cyanobacteria taxa including variations in cyanobacteria thallus structure, which include occurrence as unicells, colonies, unbranched filaments, or branched filaments. Two types of branches occur in the cyanobacteria: false branches, which are outgrowth of filaments that occur adjacent to a dead cell or a specialized cell, and true branches whose development involves continued division by a cell at right angles to that of the main filament axis. Cyanobacteria taxa can be further defined by the presence or absence of specialized cells such as exospores, endospores, akinetes or heterocysts and even by the presence or absence of mucilaginous sheath. The term trichome is used as a synonym for an ensheathed individual filament. Production of hormogonium¹² is also considered as well as

¹² The term hormogonium is applied to a short filament that results from break-up of longer filaments and serves as a means of vegetative reproduction. Separation disks or necridia (dead and collapsed cells), death is probably genetically programmed for the production of hormogonia or false branches.

structures visible with the light microscope such as gas vacuoles (Graham and Wilcox, 2000). Various classification models have been put forth and ongoing studies of Cyanobacteria result in frequent changes. One proposes the following principal orders of Cyanobacteria (Table 3) to be Chroococcales, Pleurocapsales, Stigonematales and Nostocales (South and Whittick, 1987, Wetzel, 2001). They occur in unicellular, filamentous and colonial forms, and are enclosed in a mucilaginous sheath either individually or in colonies (Wetzel, 2001).

Although vegetative reproduction by fragmentation is common in cyanobacteria, some cells are differentiated and specifically involved in reproduction and perennation (Wetzel, 2001). Cytokinesis, or binary fission, is a relatively simple process in the cyanobacteria and is normally a consequence of cell expansion. Three types of cytokinesis have been described in the cyanobacteria: a constrictive type, a septum-forming type, and a budding type. In the constrictive type, simultaneous invagination of walls occurs. In the septum type a septum is formed by invagination of the cytoplasmic membrane and the peptidoglycan layer, before invagination of the outer membrane commences. The thylakoids are pushed towards the centre and are cut across as the septum is completed. An unusual feature of cyanophyte cytokinesis is that septum formation is a continuous process and three or more different septa may be initiated in sequence (South and Whittick, 1987).

As shown in Table 3 specialized features include the ability to fix atmospheric nitrogen (which under aerobic condition is associated with specialized cells, the heterocysts), possession of akinetes (Figure 4) and the trichomes, a feature of some filamentous forms in which the entire row of cells is sheathed by a common outer wall layer (South and Whittick, 1987). Akinetes (non-motile, resistant cells, resting stages) form in a number of species (all species of the order Nostocales and some species of the order Stigonematales; not formed by Oscillatoriales or coccal cyanobacteria) as enlarged, thick-walled cells that accumulate proteinaceous reserves in the form of cyanophycin granules and polyphosphate in polyphosphate granules. Under favourable conditions, akinetes germinate directly into trichomes or into hormogonia. Hormogonia are short, slightly modified pieces of trichomes that fragment from the parent trichome and move away by means of gliding motions to develop into a new filament.

Unique to cyanobacteria are heterocysts. These occur in any filamentous species and are the major sites of nitrogen fixation. The heterocysts occur only in the same orders like akinetes, i.e. regularly in Nostocales and non-regularly in Stigonematales (Wetzel, 2001). Heterocysts

develop from vegetative cells which form thick envelope over the cell wall except for a pore at each pole where the heterocyst is connected to adjacent vegetative cells (Figure 5).

Table 3: Classification of Cyanobacteria (Source: Koning, 1994)

Order	Morphology	Dispersal	Genera
Chroococcales	Unicellular or Colonial	Cell division, formation of nannocytes, and by budding.	<i>Cyanothece</i> , <i>Aphanothee</i> , <i>Merismopedia</i> , <i>Chroococcus</i> , <i>Gloeocapsa</i> , <i>Microcystis</i> , <i>Chamaesiphon</i> , and <i>Eucapsis</i> .
Pleurocapsales	Unicellular or Colonial.	Cell division and endospores	<i>Cyanocystis</i> <i>Chamaesiphon</i> and <i>Pleurocapsa</i> .
Oscillatoriales	Filamentous	Formation of hormogonia. Any branching in the filament is false, and neither heterocysts nor akinetes are produced	<i>Oscillatoria</i> , <i>Lyngbya</i> , <i>Microcoleus</i> , <i>Phormidium</i> , <i>Arthrospira</i> , and <i>Spirulina</i> .
Nostocales	Filamentous	Formation of hormogonia. Any branching is false, and both heterocysts and akinetes can be produced	<i>Nostoc</i> , <i>Anabaena</i> , <i>Cylindrospermum</i> , <i>Aphanizomenon</i> , <i>Scytonema</i> , <i>Gloeotrichia</i> , and <i>Rivularia</i> .
Stigonematales	Filamentous	Formation of hormogonia. Branching is true. Heterocysts and akinetes can both be produced	<i>Stigonema</i> , <i>Hapalosiphon</i> , and <i>Fisherella</i> .

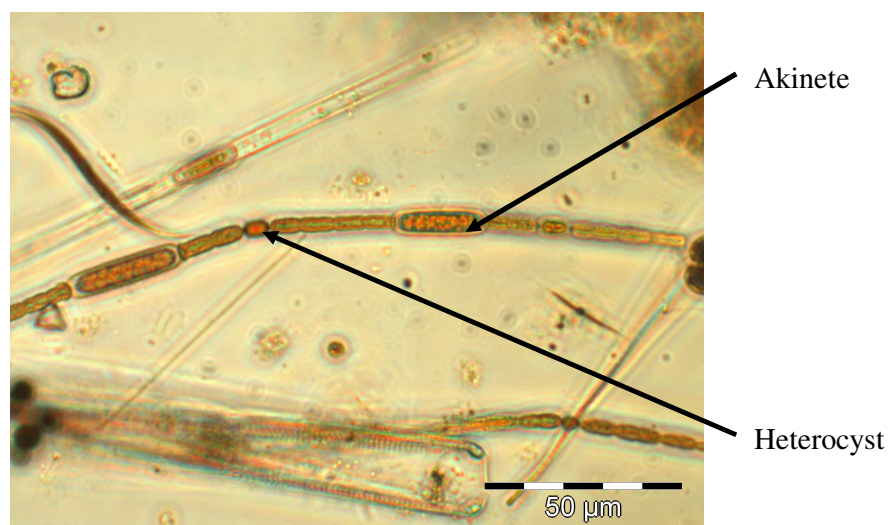


Figure 4: Photomicrographs illustrating some of the differentiated cell types of cyanobacteria. (Photo: Mbunya, A.N.)

The envelope provides a barrier to diffusion of gases into the cells therefore creating an ideal environment for nitrogen fixation since nitrogenase enzyme is inactivated by oxygen (Wetzel, 2001).

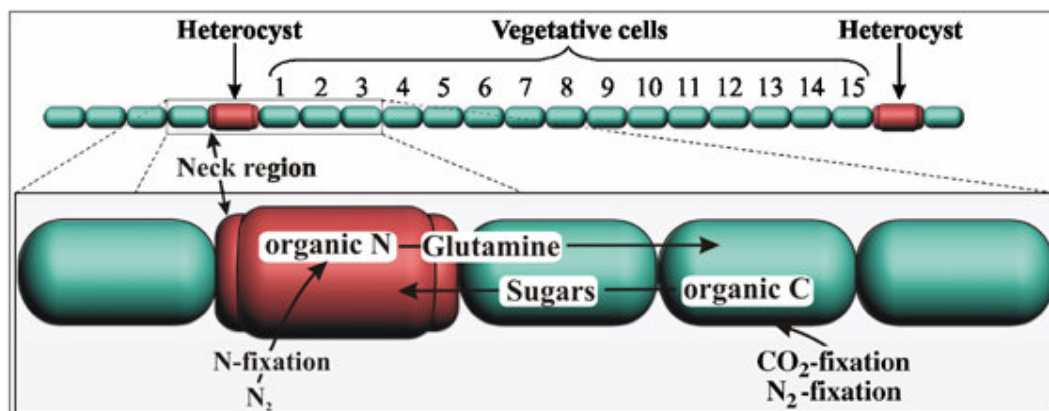


Figure 5: Model of CO_2 - and N_2 -fixation and transport in a filament of *Anabaena oscillarioides* showing two heterocysts. The numbers indicate vegetative cells at different distances from the heterocysts. (Source: Radu *et al.*, 2007)

The cyanobacteria comprise a very diverse group of organisms. They can be found all over the world, for instance in the sea, damp soil, glaciers, deserts and hot springs (Elfgren, 2003) but most of all are a common feature of many aquatic systems including tropical and temperate lakes, rivers and estuaries. In these aquatic systems, their cell densities, species composition, vertical distribution, longevity and timing of the population maxima differ. To a large degree this may be explained by climatic and meteorological conditions that influence the degree of stratification and mixing as well as light and nutrient availability. It is this physical and chemical setting that provides the stage upon which competitive interactions between species are enacted. Many studies have addressed the question of why cyanobacteria should be so successful in such a wide range of environmental conditions. Explanations proposed for cyanobacteria dominance include traits to take advantage of warmer water temperatures, to capture reduced photosynthetic photon flux densities (PPFD), competitive advantage under low concentrations of soluble reactive phosphorous (SRP) since they have high affinities for phosphorus. They can take up SRP rapidly (like other bacteria, too) and can out compete higher classes of algae to access low dissolved carbon dioxide concentration. Other advantageous conditions include buoyancy regulation, reduced zooplankton grazing and a capacity to store phosphorus (Oliver *et al.*, 2000).

In freshwater, cyanobacteria can be found in both benthic and pelagic habitats. In the latter they can become extremely dominant, forming dense blooms (Elfgren, 2003). There are about

150 known genera of cyanobacteria, 40 of which are known to be toxic (Saker *et al.*, 1999). Since first being reported in Australia (Francis, 1878), potentially toxic cyanobacterial blooms and their associated toxins have presented a serious and on going problem for the water industry (Moore, 2004). The primary toxin-producing cyanobacteria include *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis*, *Nodularia*, and *Planktothrix* (*Oscillatoria*). There are two major groups of toxins which are named for their effects on animals: neurotoxins¹³ and hepatotoxins¹⁴. Neurotoxins (anatoxin-a(s), saxitoxins, and neosaxitoxin) interfere with nerve communication and hepatotoxins (microcystins, cylindrospermopsin, and nodularins) block protein synthesis, promote chromosome breakage, and may even cause tumors (Jones and Sauter, 2005).

¹³ A substance that causes damage to nerves or nerve tissue (www.medterms.com).

¹⁴ A poisonous substance produced during the metabolism and growth of certain microorganisms and some higher plant and animal species that affect or damages the liver (www.thefreedictionary.com).

2. LITERATURE REVIEW ON *Cylindrospermopsis raciborskii* IN THE TEMPERATE REGION

Cylindrospermopsis raciborskii (Woloszynska) Seenayya *et* Subba Raju (Figure 6) is one of the potentially toxic freshwater cyanobacteria (Jones and Sauter, 2005). It was described as *Anabaena raciborskii* by Woloszynska (1912) from a plankton sample collected in a small pond in Central Java in 1899-1900. The sample was brought to Poland by Professor *Raciborskii*. Following the establishment of the genus *Anabaenopsis* for species with terminal heterocytes, *Anabaena raciborskii* was moved to *Anabaenopsis*. But then, heterocytes of this species develop in a quite different way from those of other *Anabaenopsis* species. In *Cylindrospermopsis*, heterocyst develops from terminal cells while in *Anabaenopsis* from pairs of cells in the middle of the filament thus breaking them into two. *Cylindrospermopsis*, at that time a monospecific genus, was separated from *Anabaenopsis* (Seenayya and Subba Raju 1972 in Padisák, 1997).

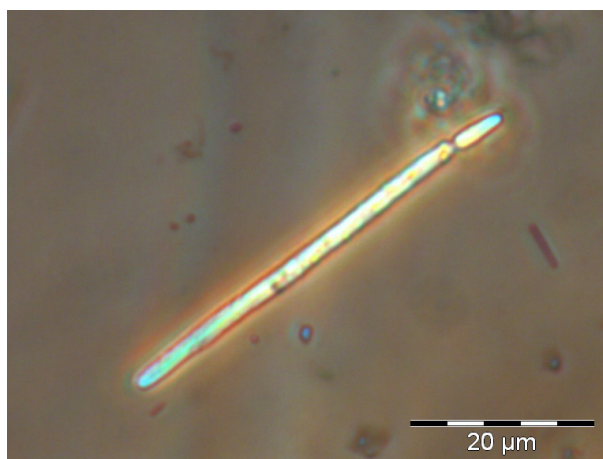


Figure 6: Mature *C. raciborskii* with heterocyst present at one ends (Photo: Mbunya, A.N)

C. raciborskii had been thought to be non-toxic for a long time. In November 1979 an outbreak of hepato-enteritis occurred on palm island, Australia involving 148 people a few days after treatment of Solomon Dam, a drinking water supply for the island, with copper sulphate to control dense algal bloom (Bourke *et al.*, 1983). Following scientific investigation, this outbreak was linked to the toxin cylindrospermopsin produced by *C. raciborskii* present in the water at that time (Bourke *et al.*, 1983, Hawkins *et al.*, 1985, Ohtani *et al.*, 1992). *C. raciborskii* does not only produce cylindrospermopsin (CYN) but also saxitoxin, anatoxin-a, and paralytic shellfish poisons (PSPs) (Jones and Sauter, 2005) which is a major health hazard

for humans (Hawkins *et al.*, 1985) and livestock (Moore *et al.*, 2005). *C. raciborskii* is unique to cyanobacteria for two reasons. First, it typically does not form surface ‘blooms’ (Jones and Sauter, 2005). Maximum densities of its cells occur at 2-3 m below the surface (Saker and Griffiths, 2001). Second, *C. raciborskii* does not produce the volatile organic compounds such as geosmin and 2-methylisoborneol (MIB) that cause taste and odour problems that are commonly associated with algal blooms (Chiswell *et al.*, 1997).

C. raciborskii populations are easy to identify, if at least some of the filaments carry heterocytes and/or akinetes, and it remains identifiable in either Lugol- or formaldehyde-preserved samples (Padisák, 1997). The trichome morphology changes during population growth (Singh, 1962). This blue-green alga characteristically occurs in lakes with pH of 8.0-8.7 (Padisák, 1997). It does not occur in acidic waters (Fogg *et al.*, 1973). Being a heterocytic blue-green alga, *C. raciborskii* dominance is often attributed to the ability to fix atmospheric nitrogen to overcome N-deficiency. The appearance of heterocyte can be used as an indicator to fix nitrogen (Padisák, 1997).

The fact that *C. raciborskii* can persist in very dense populations without sudden collapses implies that it tolerates low light due to self shading. Secchi-disc readings of 30cm are characteristic during *C. raciborskii* blooms. Shafik *et al.* (1997) experiment with ACT-9502 *C. raciborskii* strain from Lake Balton have shown that maximal growth occurred at $120 \mu\text{Em}^{-2}\text{s}^{-1}$. Photon fluxes higher than $200 \mu\text{Em}^{-2}\text{s}^{-1}$ inhibited growth. This supports the fact that they grow at very low light intensities (Padisák, 1997).

Initially classified as a tropical species, *Cylindrospermopsis raciborskii* has been described as expanding with species found in the tropical-subtropical regions of each continent and data are accumulating from temperate regions of North America and more prominently of Europe. The cyanobacterium that was confined to a tropical environment (Hawkins *et al.*, 1985) due to its affinity for warm water temperatures (25-30°C) (Jones and Sauter, 2005), has since been found in sub-tropical and temperate climatic regions on all continents except Antarctica, making its presence a global water quality issue (Moore, 2004). *C. raciborskii* has become an invading species in the 20th century reaching the northernmost margin of its distribution in Germany at latitudes of 53-54°N (Krienitz and Hegewald, 1996). In 1990, it was found in Lake Lieps, northern Germany and so far this has been the northernmost record of the species (Krienitz and Hegewald, 1996).

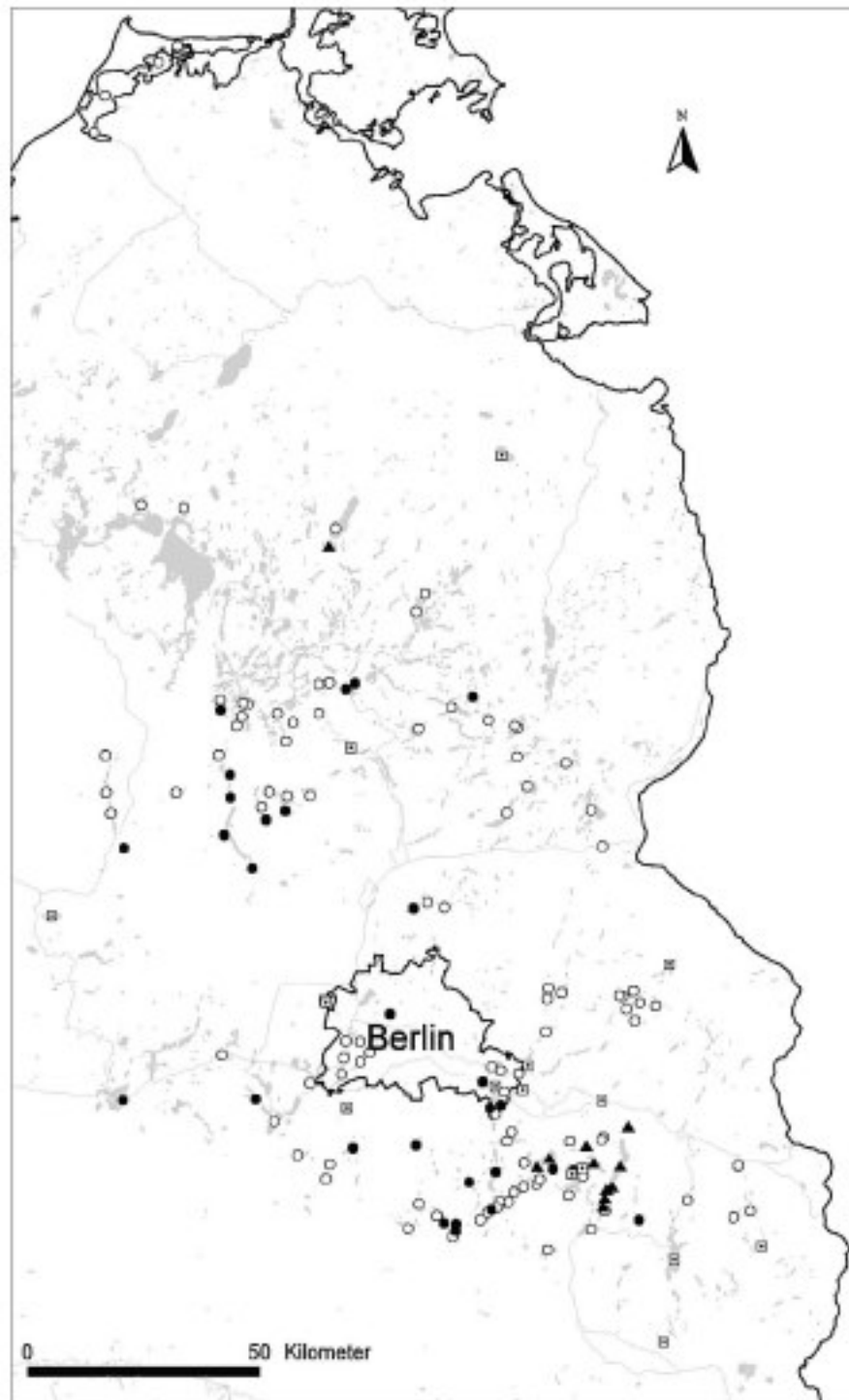


Figure 7: Distribution of *C. raciborskii* in lakes in the Berlin- Brandenburg area before 2004 and the increase of lakes inhabited by *C. raciborskii* discovered by the screening program conducted in the *Cylindrospermopsis*-project in 2004. Legend: ▲ *C. raciborskii* report prior to 2004 ○ Lake sampled in 2004 but *C. raciborskii* not detected; ● lake sampled in 2004 and *C. raciborskii* detected □ Report of *C. raciborskii* in non-peer-reviewed literature. (Source: Stüken *et al.*, 2006)

The only significant data on the occurrence of *C. raciborskii* in Germany resulted from a study on the phytoplankton dynamics in some lakes of the Scharmützelsee region (south east of Berlin) where *C. raciborskii* was identified in 1994 (Rücker *et al.*, 1997, Wiedner *et al.*, 2007). Now, it is known to be widely distributed in Northern Germany (Figure 7, Stüken *et al.*, 2006). These data demonstrated that the occurrence of *C. raciborskii* was not a temporary event. *C. raciborskii* populations occurred frequently since 1994. The species is most abundant in shallow eutrophic lakes with relative proportions on the total phytoplankton biovolume being up to 20% during its population maximum which occurs mostly during August though not forming blooms (Wiedner *et al.*, 2007).

They are also highly ecophysiolegically adaptive with data ranging from subtropical oligotrophic Kariba reservoir in the southern hemisphere, small, shallow, hypertrophic, temperate ponds in the northern one to rivers or other intensively flushed environments and from newly built reservoirs (Padisák, 1997).

Independently from latitude, *C. raciborskii* populations grow and increase only in warm waters (>25°C) water. Consequently, it can maintain large populations in permanently warm waters all over the year while its appearance is restricted to shorter summer periods towards higher latitudes. At the northernmost limits of its presently known geographic distribution, *C. raciborskii* builds up large populations only in exceptionally warm years while in others only single filaments appear, if at all, late summers. This interannual variability can make *C. raciborskii* a good indicator species of global climate change for temperate regions (Padisák, 1997).

Shallow lakes have higher mean water temperature compared to deep lakes in the temperate zone, and therefore fulfil the high temperature requirement of *C. raciborskii* (Wiedner *et al.*, 2007). The high temperature requirement of *C. raciborskii* is partly related to the high temperature optimum of akinete germination. Laboratory experiment with akinetes deriving from lake Balaton showed that high percent of germination occurs only in the temperature range of 22-23.5°C (Padisák, 1997). Tropical populations of the specie require temperatures above 22°C for germination and above 25°C for optimum growth (Wiedner *et al.*, 2007). But *C. raciborskii* is not restricted only to shallow lakes in the Scharmützelsee region, and can be explain by differences in temperature requirement with those in the dimictic Scharmützelsee (maximum depth-29m) growing at temperatures as low as 15°C (Wiedner *et al.*, 2007). Mischke (2003) stated that *C. raciborskii* has a temperature requirement of about 17°C.

Although *C. raciborskii* is one of the species that can dominate yearlong or almost yearlong in the tropical regions, continuous dominance is more the exception than the rule. In deep tropical lakes *C. raciborskii* filaments with akinetes are not or only very rarely reported. In the temperate region, the species occurs exclusively in the warmest periods of the year. In these lakes *C. raciborskii* is sensitive to meteorological conditions. This is due to at least two factors. One is that cold summer may prevent the germination of akinetes, and if so, the alga either does not occur at all or only in small amounts. The second is that it seems to be sensitive to wind-induced physical disturbance. Which is an important factor since all these lakes are shallow and polymictic (Padisák, 1997). Thus *C. raciborskii* could be prevented by artificial destratification (aeration) as described by Hawkins and Griffiths (1993). In subtropical and temperate regions, they exhibit characteristic morphological series during annual population dynamics (Singh, 1962). Akinetes appear late in the population cycle; usually after the population peaked (Padisák, 1997).

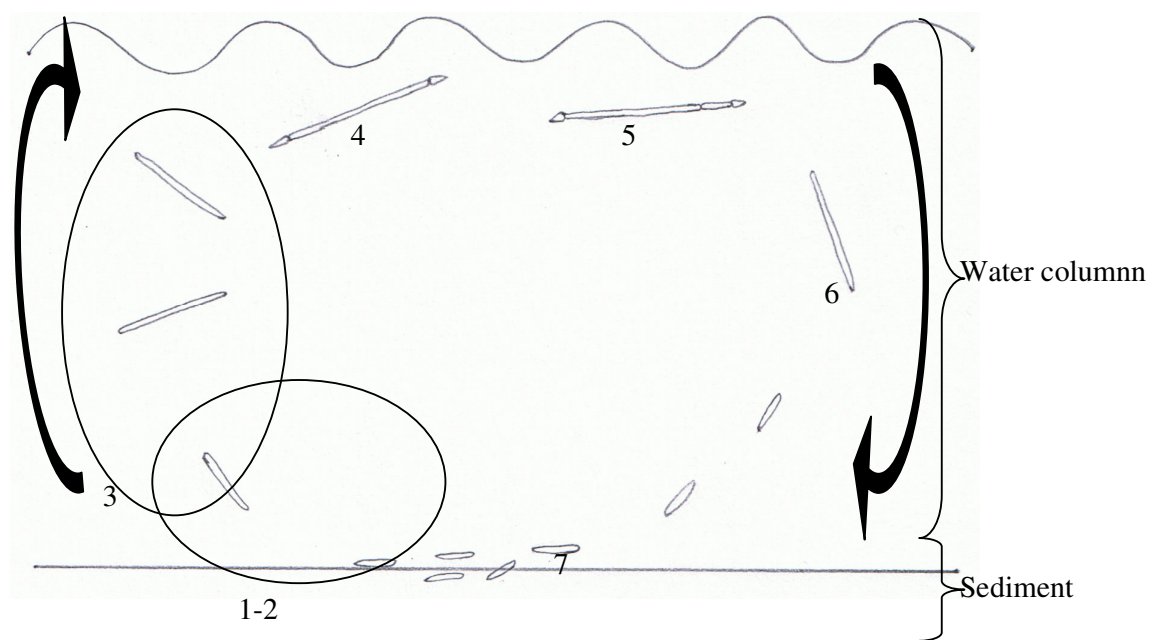


Figure 8: A sketch of the life cycle of *C. raciborskii* with numbers corresponding to the development stages sited above (Adapted from Elfgren, 2003).

The life cycle of *C. raciborskii* consists of several different phases (Figure 8):

- 1) Germination on the sediment.
- 2) Growth period in/on the sediment (hormogonium/young filament).
- 3) Formation of gas vesicles and migration up into the water.

- 4) Growth and division in the water column, heterocyst formation at one or both ends of the filament and N-fixation is possible.
- 5) Formation of akinetes adjacent to the heterocysts or somewhere in the middle of the filaments.
- 6) Loss of the outer parts of the vegetative filaments and sinking out of the water (It might also sink with the filament).
- 7) Period of resting and maturation of the akinetes.

Because of its increasing global colonisation, toxicity and ability to form dense blooms that hamper the multiple use of freshwater bodies, this cyanobacterium continues to be of interest to water quality managers. Until now, no field study of germination for *C. raciborskii* has been carried out in the northern hemisphere despite its acknowledged presence and possible toxicity. Only one study is mentioned by Padisák (1997) from Lake Balaton in Hungary which is in South-East Europe. As a function of its primary distribution in tropical ecotypes, most of what is known about conditions favouring *C. raciborskii* growth is based upon tropical environments. However, with its expanding distribution into temperate climates, different trends seem to be emerging. For example, while *C. raciborskii* typically occurs in deep (>50m), stratified lakes in tropical regions, it seems that it prefers shallow waters in temperate zones (i.e. <10m) (Padisák, 1997). Additionally, annual *C. raciborskii* populations in temperate systems appear to be highly variable and extremely dependent on temperature and climate. Little is known of the early developmental phases of its life cycle, despite the relative importance of akinetes as a source of sedimentary inoculums for subsequent seasonal recruitment (Moore *et al.*, 2004). Compared to experiments, field studies are much more difficult to carry out and to interpret due to the high number and variability of abiotic and biotic factors, but they are close to nature. Knowledge on life cycle of Nostocales is important to understand the annual course of population development and to understand the northwards spread of *C. raciborskii* and the establishment of stable populations in the Northern hemisphere. Collected data are necessary to calibrate mathematical models which are one of the tools for understanding of northwards spread and developing management strategies for *C. raciborskii* and other Nostocales. Understanding the life cycle of *C. raciborskii* by identifying the mechanism that regulates its seasonal dynamics the course of future development of this cyanobacterium can be predicted thereby measures could be developed for the prevention of *C. raciborskii* mass invasion of other fresh water bodies in this region.

3. OBJECTIVES AND HYPOTHESES

The occurrence, abundance and proliferation of *C. raciborskii* are of major concern, due to its toxicity, in water bodies used as drinking water sources or for recreation. Some investigators who did not detect any ecophysiological difference between the tropical and temperate strains, proposed climate change to have been as the potential causes of the invasive spread of *C. raciborskii* (Briand *et al.*, 2004). To others ecotype selection has been the main cause of the spread. While others found strains that can survive at low temperatures, which is more suggestive of adaptation and selection mechanisms (Chonudomkul *et al.*, 2004). Strains isolated from Lake Scharmützelsee region were found to be toxic in several bioassays. All isolates showed toxic effects to freshly isolated rat hepatocytes, human hepatoblastoma and human colon adenocarcinoma cells. Some isolates showed liver and spleen damage and inflammation of the intestine in the mouse bioassay. *C. raciborskii* could not be implicated as the cause of the first occurrence of cylindrospermopsin at two German lakes (Melangsee and Langer See) because none of the strains of *C. raciborskii* isolated from these lakes to date produce CYN (Fastner *et al.*, 2003). These results give strong evidence that the German isolate of *C. raciborskii* contain unidentified or unknown toxins (Fastner *et al.*, 2003, Wiedner *et al.*, 2007). *C. raciborskii* has established in lakes in the Berlin area. Too little is known about its ecology to assess the potential colonisation and health hazards it may pose in water used for drinking and recreation. A better understanding of the life cycle of this cyanobacterium would help throw more light on the cause of its invasion and spread and how it can be contained.

The life cycle of the akinete forming cyanobacterium, *C. raciborskii*, is the main subject of this thesis and the following questions are addressed:

1. How are the akinetes distributed horizontally on the bed of a lake (horizontal inhomogeneity)?

Hypothesis: The akinetes are not evenly distributed on the lake bed. There should be more akinetes in the central and/ or deepest parts of the lake. This is the so-called sediment focusing effect.

2. When and under which abiotic conditions (light and temperature) does germination of *C. raciborskii* occur in the temperate zone (i.e. at the northern limit of its geographical distribution)?

According to Moore (2004) germination occurs at temperatures between 15 and 30°C in tropical Australian strains. But Mischke (2003) also found that *C. raciborskii* has a temperature requirement of about 17°C in temperate lakes. Wiedner *et al.* (2007) also stated this cyanobacterium in the Scharmützelsee region grows at temperatures as low as 15°C.

Hypothesis 1: The minimum temperature for the germination of *C. raciborskii* is below 15°C and the light requirement depends on the temperature.

Hypothesis 2: Germination occurs in the later part of spring depending on the temperature at the time.

3. How is germination related to the developing population in the pelagial of the respective year?

Hypothesis: The growth conditions after hatching of the akinetes influence the success of *C. raciborskii*'s further development. The freshly emerged (naked) germlings are more sensitive to temperature and light stress than adult trichomes. Changing (bad) weather conditions after germination can reduce population growth for *C. raciborskii*.

4. When and under which conditions do the filaments of *C. raciborskii* form akinetes in the pelagial?

Hypothesis: Trichomes start developing akinetes in the autumn when temperatures start dropping and no further development of akinetes is realised after a certain temperature mark.

5. How is the annual import of akinetes reflected in the sediment?

Hypothesis: There are more akinetes in the sediment following a season of high biovolume of the *C. raciborskii* trichome in the pelagial and vice versa.

6. Is there a loss of akinetes in the sediment until the next spring?

Hypothesis: With the presence of other organisms in the lake, possible loss processes reducing the akinetes may be grazing and direct ingestion by consumers or infection by fungi, bacteria or viruses or mechanical displacement or coverage in the sediment.

7. What are the differences between the invasive species, *C. raciborskii* and the native species, *Aphanizomenon* spp concerning germination temperature and light need?

Hypothesis: there is not so much difference between these species since they belong to the same order Nostocales and they coexist in many lakes.

4. STUDY SITE AND METHODOLOGY

4.1 Study area

Investigations were performed on Lake Melangsee (52° 10.00' N, 013° 59.00' E) in Germany (Figure 9). This lake was chosen because *C. raciborskii* had been detected there in 1995. It is a shallow (2.4m maximum depth) eutrophic and Polymictic Lake situated in Brandenburg, northeast Germany (54°35'E). This lake which belongs to the catchment area of the River Dahme originates from the Weichsel glacier and is located at the border of the south plateau of the Berlin glacial valley (Schmitt and Nixdorf, 1999).

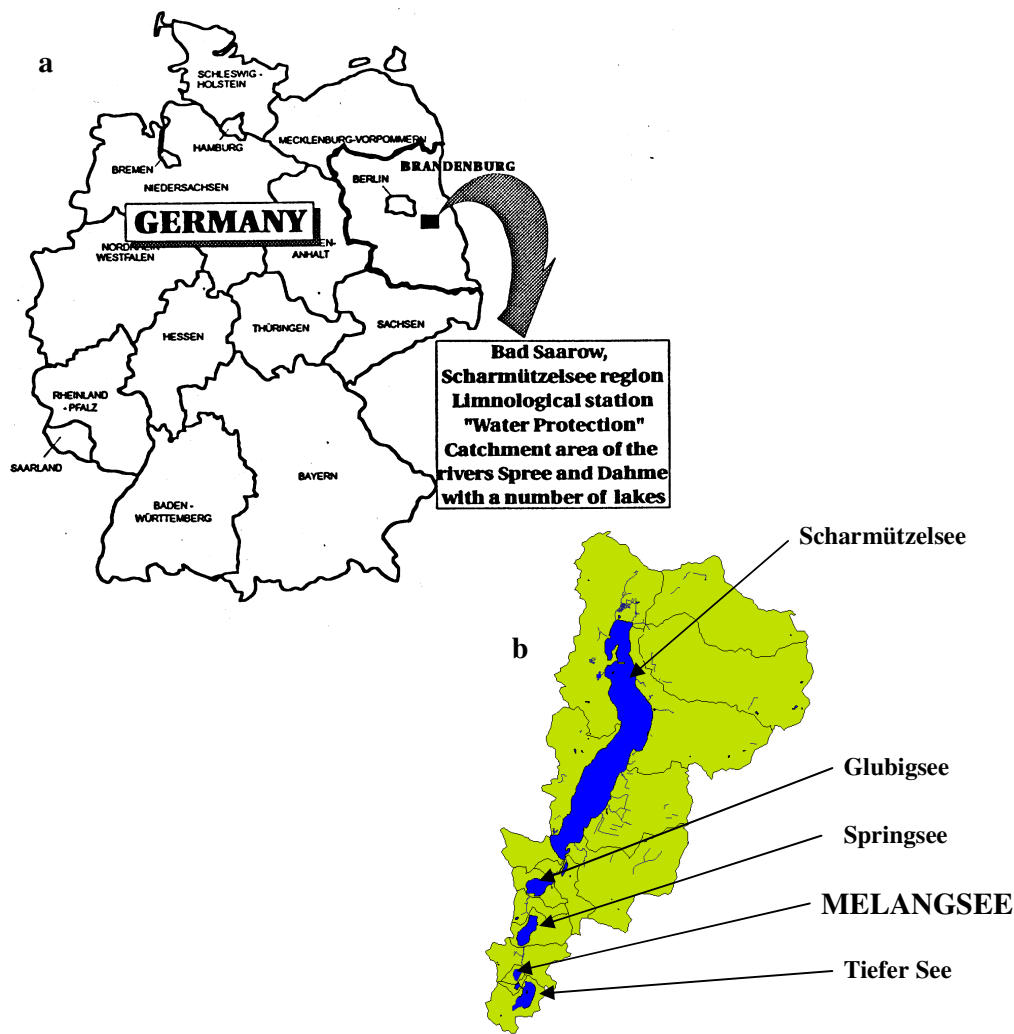


Figure 9: (a)The map of Germany and the study area (Source: Nixdorf and Deneke, 1997) (b)The chain of lakes in to which the Melangsee belongs(Source: Hämmerling and Nixdorf, 2004)

Lake Melangsee receives inflow from groundwater and from mesotrophic Lake Tiefer See. A part of the inflow is regulated by the activities of the fish management of Lake Tiefer See and runs through a wetland before reaching Lake Melangsee. Therefore, discharge and water level fluctuations are controlled artificially. Particulate inputs are very low. Lake Melangsee was used for intensive fishery until 1990, resulting in its eutrophic character (Schmitt and Nixdorf, 1999).

The lake is surrounded by hills, forests (Figure 10) and settlements and is less affected by wind (Rücker *et al.*, 1997). The average residence time of the water is 22 days. The main characteristics of the lake are given in Table 4.

The phytoplankton communities of the lake are recurrently dominated by cyanobacteria from early spring until late autumn (Nixdorf and Deneke, 1997). Taxa of the order Oscillatoriales-*Limnothrix* spp. and *Pseudanabaena* spp. form mass developments in late summer (Rücker *et al.*, 1997). Before Oscillatoriales become dominant, taxa of the order Nostocales, including *C. raciborskii*, and several taxa of the genera *Aphanizomenon* and *Anabaena* are more abundant (Wiedner *et al.*, 2002).

Table 4: Morphometric and hydrologic parameters of Lake Melangsee (left column). Trophic characteristics are given as ranges of annual means and vegetation (April-October) means for the years 2004 – 2006. (Chl *a* – chlorophyll *a*, TP - total phosphorus, DIP – dissolved inorganic phosphorus, TN – total nitrogen, NO_{tot} – sum of nitrate plus nitrite, NH₄ - ammonia)

			Annual mean	Vegetation mean
Maximum depth	2.4 m	Secchi depth (m)	0.84 – 1.00	0.62– 0.75
Mean depth	1.6 m	Chl <i>a</i> (µg l ⁻¹)	41.76 – 67.75	50.53–78.59
Area	0.11 km ²	TP (µg l ⁻¹)	39.97 – 43.67	44.45–52.70
Volume	0.17x10 ⁶ m ³	DIP (µg l ⁻¹)	4.31 – 8.49	4.86–10.17
Catchment area	5 km ²	TN (mg l ⁻¹)	0.68 – 0.93	0.70– 1.07
Residence time	22 days	NO _{tot} (µg l ⁻¹)	10.60 – 21.26	8.26– 9.28
		NH ₄ (µg l ⁻¹)	37.41 –149.22	11.11–39.99

4.2 Field measurement

On each sampling date, the Secchi depth was measured using a 25cm Secchi disc. This data permitted a rough determination of the trophic state of the lake. Along side the transparency

depth, vertical depth profiles for water temperature, pH and oxygen saturation and oxygen concentration at half-meter intervals were obtained using a multiparameter probe (H20, Hydrolab, Austin, TX, USA). Based on these depth profiles, the depth of the mixed water column from which the mixed sample was to be collected was determined on site.



Figure 10: Lake Melangsee with the data logger situated at the regular sampling point (Photo: Rucker)

Light intensity used for photosynthesis by these cyanobacteria was measured in the form of photosynthetically active radiation¹⁵ (PAR). Using two spherical quantum sensors (SA 193, Li-Cor, Lincoln, NE, USA) fixed at half-meter intervals, PAR was measured at variable depths along the water column. The vertical attenuation coefficient (K_d) or fraction of solar energy lost due to depth-related attenuation and scattering, was calculated as the difference between the upper and lower readings using Kirk's equation 1 below (Kirk, 1994 in Wiedner *et al.*, 2007).

$$K_d = \frac{\ln I_1 - \ln I_2}{Z_1 - Z_2} \quad \text{Equation 1}$$

Whereby I_1 is the PAR at depth z_1 and I_2 is the PAR at depth z_2 .

The underwater light available for absorption at mean and maximum depth in Lake Melangsee were calculated using the Beer-Lambert Law (equation 2).

¹⁵ **Photosynthetically Active Radiation (PAR)**-It is the spectral range of solar light from 400 to 700 nanometers that is useful to photosynthetic organisms in the process of photosynthesis. This spectral region corresponds more or less with the range of light visible to the human eye (Gates, 1980).

$$I_z = I_0 e^{-Kz} \quad \text{Equation 2}$$

Where I_z is irradiance at a given depth; I_0 is irradiance at the surface; k is the vertical attenuation coefficient (K_d) or fraction of solar energy lost due to depth-related attenuation and scattering; and z is depth in metres.

Mean PAR in the mixed water column (I_{mix}) was calculated as the product of underwater light attenuation and total incident radiation (I_0) using Riley's equation 3 below (Riley 1957 in Wiedner et al. (2007))

$$I_{mix} = 0.45 \cdot I_0 \cdot \left(\frac{1 - e^{-K_d \cdot Z_{mix}}}{K_d \cdot Z_{mix}} \right) \quad \text{Equation 3}$$

Where z_{mix} is the depth of the mixed water column and was assumed to be equal to the mean depth of the lake (ratio of lake volume to lake area). I_0 was defined as the fortnightly mean of total incident radiation prior to the sampling date. Total incident radiation data was kindly provided by the meteorological observatory of the German Weather Service in Lindenberg. The ratio of photosynthetically active radiation to total incident radiation and losses due to reflection on the water surface were considered using a factor of 0.45 according to Behrendt and Nixdorf (1993).

4.3 Sampling and analysis of water samples

4.3.1 Chemical analysis

The lake water was sampled biweekly at the deepest point from January 2004 to 2007. Mixed samples from the whole water column were derived by taking samples at half-meter intervals with a 2.3 l Limnos (Turku, Finland) sampler and stored in a single vat. They were then stored by appropriate means until the time for chemical and microscopical analysis.

In the laboratory aliquots of the mixed samples were analyzed to determine concentrations of ammonia ($\text{NH}_4\text{-N}$), nitrate plus nitrite ($\text{NO}_{\text{total}}\text{-N}$), dissolved inorganic phosphorus (DIP), total phosphorus (TP), and total nitrogen (TN), according to standard methods (DEV 1976–1998). Total phosphorus and total nitrogen concentrations were determined from 100ml aliquots of mixed water sample acidified with sulphuric acid using the FIA-LAB II system (MLE GmbH, Radebeul). Part of the mixed sample was filtered through a membrane filter (0.45 μm pore size) and analysed to determine dissolved inorganic phosphorus (DIP), ammonium ($\text{NH}_4\text{-N}$) and nitrite plus nitrate ($\text{NO}_{\text{total}}\text{-N}$) concentrations. Detection limits of the

dissolved nutrients were $1.7\mu\text{g l}^{-1}$ for DIP, $64\mu\text{g l}^{-1}$ for $\text{NH}_4\text{-N}$ and $3.2\mu\text{g l}^{-1}$ for $\text{NO}_{\text{total-N}}$. Dissolved inorganic nitrogen (DIN) represents the sum of $\text{NH}_4\text{-N}$ plus $\text{NO}_{\text{total-N}}$.

4.3.2 Phytoplankton analysis

Phytoplankton composition and biovolume were estimated using an aliquot fixed with Lugol's solution studied under an inverse microscope according to Utermöhl (1958) and Rott (1981). A quantitative analysis of the biovolume of the different stages of *C. raciborskii* was performed using 100ml aliquots of the mixed water sample fixed in Lugol's solution. When analysis was not done on the day of sampling, the fixed solutions were stored in the refrigerator. On the day of analysis, they were removed at least two hours prior to analyses for acclimatization. The sample and all equipment used should be of a similar temperature to promote a random distribution of plankton in the sedimentation chamber. Following acclimatization, the samples were manually homogenised by alternately and gently turning the sample bottles upside down for a minute. The filling of the sedimentation chamber followed suit. The 10ml plankton chambers (Hydrobios) placed on a horizontal surface, were filled directly with the homogenised sample and the chamber closed with a cover glass avoiding air bubbles. In some cases the samples were diluted with water to ensure that the concentration of the organism was sufficiently low to prevent clogging and to optimise the counting process. The prepared chambers to be examined by microscopy were then allowed for at least eight hours for sedimentation to take place in a dark, vibration free and moist air environment.

4.4 Sediment sampling and analysis

The sediment of Lake Melangsee was sampled for akinetes monthly to bi-weekly from March 2005 to December 2007. A Kajak-Brinkhurst Sediment Core Sampler was used to obtain the sediment cores (Figure 11). Ensuring that the rope was securely fastened to the corer and the other end of the rope attach to the boat, the valve was opened and the trigger mechanism set. The corer was lowered and then allowed to penetrate the sediments with its own weight avoiding any disturbance by impact. The messenger sent down to release the trigger mechanism. The sampler was then carefully retrieved and a stopper placed into the bottom opening before removing from the water to prevent loss of the sample. Most of the water overlying the sediments in the core tube was carefully siphon off. A rubber stopper of a size sufficient to fit inside the liner tube tightly to form a watertight seal on an extruder was then used to gently and slowly force the core upward to the top of the tube. As the sediment core

was extruded, 2cm slice was carefully cut. With a clean spatula, the sediment was carefully stirred to homogenize, then scoop into a pre-labelled sediment sample bottle and place in a cooler with ice packs as soon as they are transferred to the labelled bottles. In the laboratory the samples were immediately preserved with 10% formalin¹⁶ by adding 3ml of formalin to 25ml of sediment for an end concentration of 10.71%v/v then kept in the refrigerator awaiting analysis. On the day of analysis, the samples were removed from the refrigerator and left on the bench to acclimatise for at least two hours. Half a millilitre of the preserved sediment sample was put into 50ml glass vial and 49,5ml of cold boiled pipe water and 8 droplets of Lugol solution added to it. The mixture was gently homogenised and some poured into a 2.973ml sedimentation chamber. For each sample two different sedimentation chambers were prepared. The closed chamber was then left in a moist dark environment for at least four hours for sedimentation proceeded by microscopy.

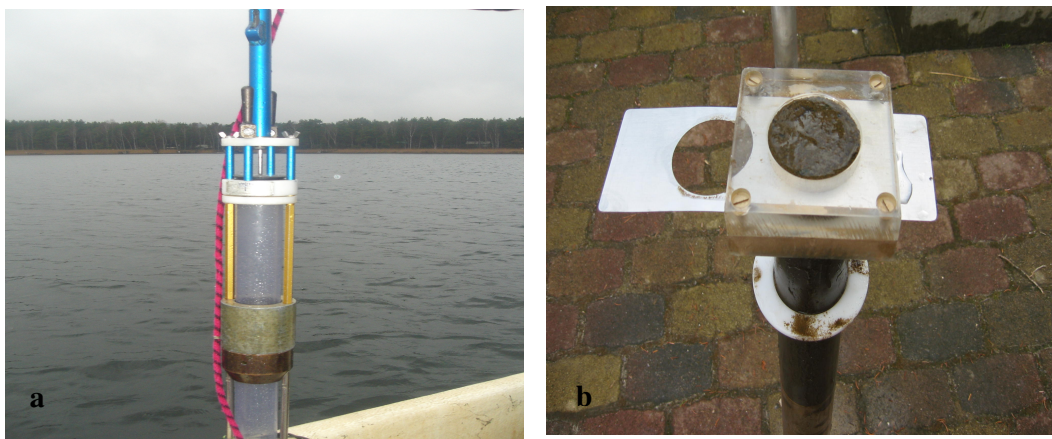


Figure 11: (a) Kajak-Brinkhurst Sediment Core Sampler (b) Upper 2cm of sediment being cut off (Photo: Tingwey, E. I.)

4.4.1 Annual variation of *C. raciborskii* akinetes

Sediment samples at the point where it had always been sampled in the previous years were used for the enumeration of seasonal course of akinete number. Three sediment cores were collected from the sampling station at each sampling event. The uppermost 2 cm of the sediment cores were mixed and treated as above.

¹⁶ 10% formaline or Formol = 9 parts of water + 1part of 37%formaldehyde
Formaldehyde is produced as a gas by oxidation of methyl alcohol. Always sold 37-40% aqueous formaldehyde

4.4.2 Determination of akinete horizontal patchiness

In order to determine the horizontal patchiness of the bed of the lake, four other sampling points in addition to the regular sampling point were sampled on the 24th of November 2005. The regular sampling point was point number 1 (Figure 12). To each of these sampling points, five different sediment sub samples were collected as shown in Figure 12 represented by the arms of the stars. The sub samples were treated and analysed independently as above.

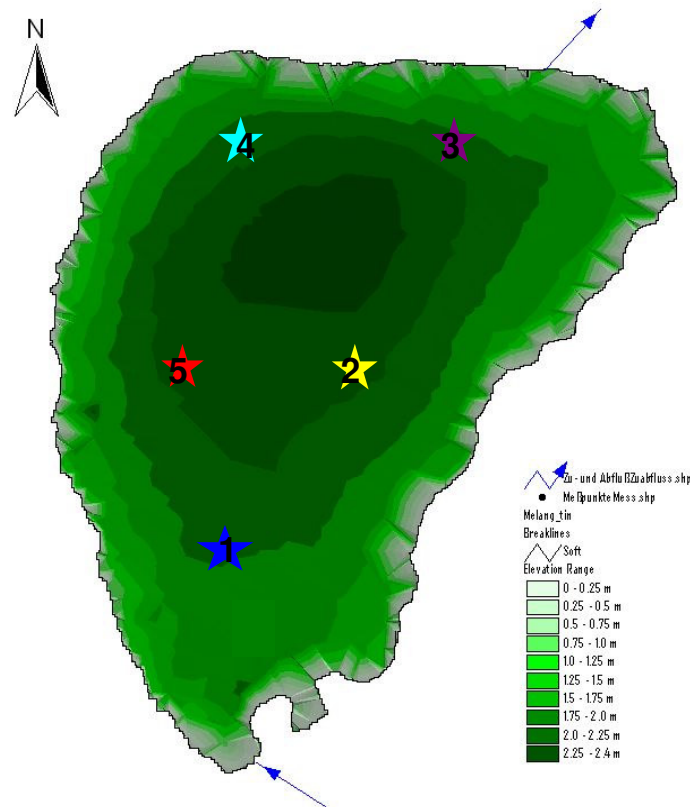


Figure 12: Bathymetric map of Lake Melangsee with sampling points and depth lines. Point number one is the regular sampling point. The blue arrows show the direction of water flow (Map designer: M. Hemm).

4.5 *In situ* determination of time and conditions of akinete germination (Beaker experiment)

The sediment was sampled accordingly on the 10th of April 2007. It was examined microscopically to make sure that no germlings but intact akinetes were present. Following this assurance, 15 urine beakers (ca. 130ml) were each filled with 1.5g of the fresh sediment. They were then topped with cooled (4°C) lake water filtered through 0.45µm membrane filters. Each beaker was then covered with a semi permeable membrane (8.5 x 8.5; Pall

Corporation Supor® 200; 0.2µm) and closed with a lid into which a hole of about 4cm diameter had been cut. The prepared beakers were then put into the lake at a depth of 1.5m. One beaker was harvested every second or third day and after homogenization, 50ml of its content was fixed with formalin and the rest with Lugol's solution. Phytoplankton analysis was carried out on the beaker lugol fixed samples. On each day that a beaker was removed, both light and temperature profile was measured. In addition to that the sediment was also sampled, fixed and analysed as above.

4.6 *In vitro* determination of akinete germination temperature

For this germination experiment, fresh sediment collected from the lake was suspended in N-free culture medium (modified after Nicklisch, 1992). The flasks were placed in a culture chamber and cultivated at temperatures of 13, 15, and 17, respectively. All the flasks were placed in light intensity of $130\mu\text{E m}^{-2}\text{s}^{-1}$ which corresponds to the mean light in the mixed layer in Lake Melangsee in May. This was done in a light: dark cycle of 12:12 h. Subsamples of 20 ml were taken from the flask daily over a two weeks period, fixed with Lugols' solution and stored into the refrigerator until further analysis. When it was the day for further analysis, these samples were treated and allowed to sediment just like the water column samples

4.7 Microscopical analysis of sedimented samples

Enumeration of akinetes and estimation of biovolume of filaments employed the Utermöhl technique (Utermöhl, 1958). Counting was done under a Nikon Diaphot inverse microscope at 400x magnification. Chambers were gently moved to the microscope to avoid resuspension. A total of 120 fields were counted per chamber and this was done in the direction of transect indicated in Figure 13.

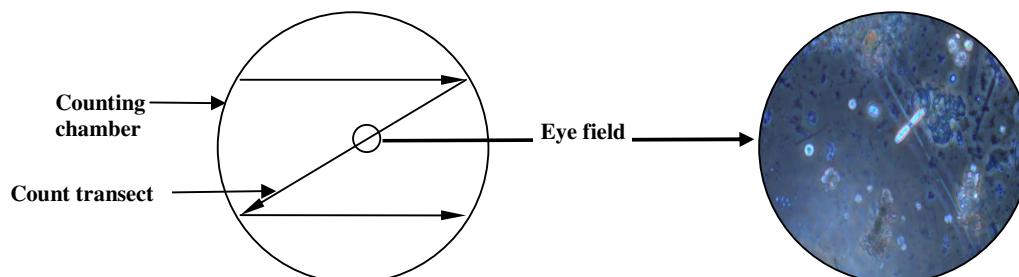



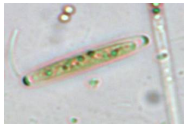



Figure 13: Counting techniques used to enumerate cyanobacteria in water and sediment samples

For the sediment samples, simple enumeration of the akinetes was done. The observed akinetes were characterize into different states as intact, half filled and empty envelopes to get an idea of their germination potency. Intact akinetes were those assumed to be able to

germinate; half filled were assumed to be no more able to germinate and empty shells have been germinated earlier or died for some unknown reasons.

For the samples of the water column, growth changes were measured by determining the biovolume of filaments and or the different stages. In all cases the length and the width of each filament or germling or akinete were measured in all the 120 eye fields examined. Akinetes on the mature filaments in the pelagic samples were also measured just like the filaments themselves.

Table 5: Different growth phases of *C. raciborskii* (photos: J. Rucker)

	Type	Description	Picture
Phase 1	Akinete	Full and intact akinete	
Phase 2	Growing akinete	Elongated with endospore separated from the exospore	
Phase 3	Growing akinete	Hatched akinete with germling partially in the shell	
Phase 4	Germling	Young germling of less than 30µm length without heterocyst	
Phase 5	Growing filament	Filament of less than 30µm with heterocyst or filament of more than 30 µm with or without heterocyst	

Measurements were made with an ocular micrometer calibrated against a standard scale-bar slide to 0.1µm precision at 400x and 200x magnification under the inverted microscope. The growth phases were separated according to Moore *et al.* (2004). With phase separated as shown in the Table 5.

In the case of the *in vitro* akinete germination, some of the different growing phases groups according to Moore *et al.* (2004) were enumerated as one. The free mature akinetes together with the elongated akinetes having their endospore separated from the exospore classified as two groups by Moore *et al.* (2004) were considered as the group akinetes in this case. The

growing filament emerging from the akinete shell (phase 3) were taken as growing akinetes while the phase 3, germlings without envelope and in the size of up to 30 μm were the germlings group. Lastly the phase 5 (growing filament above 30 μm) were considered as growing filaments. The following phases (Figure 14) of development were differentiated in the germination experiment.

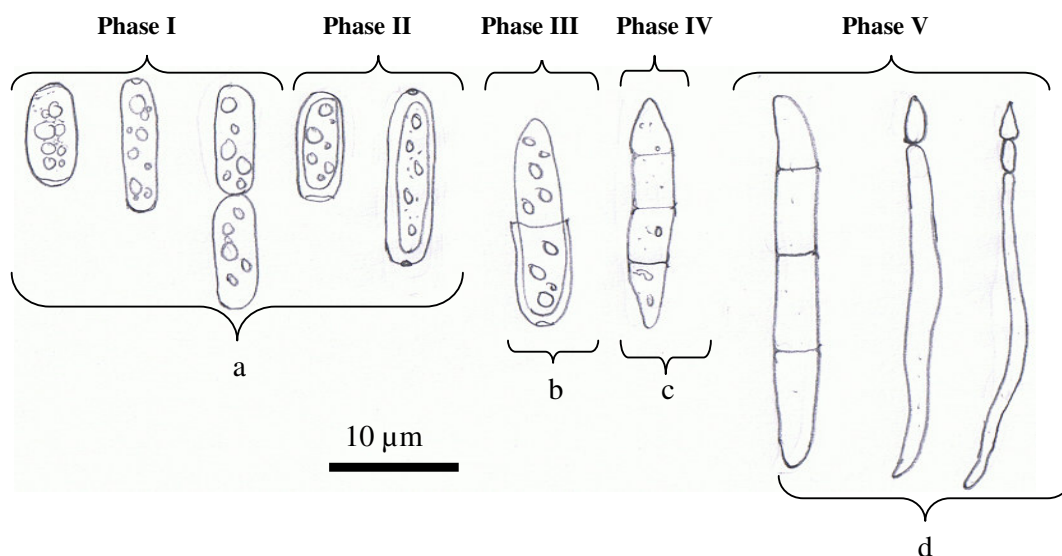


Figure 14: Drawings showing the different groups into which growing *C. raciborskii* was separated. Those above the diagram are according to Moore *et al.* (2004) refer to Table 5. And the groups specifically for the *in vitro* germination experiment a-Akinetes, b-growing akinetes, c-germlings, d-growing filaments (Adapted from Moore, 2004). The scale bar is only valid for Phases I – IV. Filaments of Phase V have a width of 0.5 μm to 2.5 μm and a length of at least 30 μm .

Along side the study of *C. raciborskii* considered to be an invasive cyanobacterium of tropical origin, work was also done and data collected on the native Nostocales species of *Aphanizomenon*. *Aphanizomenon gracile* was the most abundant species of that genus in Melangsee.

4.8 Data analysis

All results were routinely recorded on standard Microsoft Excel spreadsheets for data processing, with the following entries: sample identification, species names, numbers of cells counted, cell length and width (where necessary) and number (Appendix 1 and 2). The data was later stored in electronic database and checked to eliminate unexpected errors. All variables in the data were transformed either into biovolume or number of cells per litre and saved with the count and identification data. To get the biovolume of the organisms,

calculation was done using the geometric formulas given by Rott (1981) in which this specie is taken to be cylindrical (Willén, 1976). Hence its volume derived from the equation 4 below:

$$Volume = \frac{1}{4} \pi w^2 l \quad \text{Equation 4}$$

Where w is the width and l is the length of the cylinder.

4.8.1 Net change rate of akinete and phytoplankton biovolume

The net change rate (v) between sampling dates t_1 and t_2 ($t_2 > t_1$) is defined by equation 5 below.

$$v = \frac{\ln C_{bioV_2} - \ln C_{bioV_1}}{t_2 - t_1} \quad \text{Equation 5}$$

Where C_{bioV_1} is the biovolume at time t_1 and C_{bioV_2} is the biovolume at time t_2 .

4.8.2 Statistical analysis

The horizontal homogeneity determination counts were analysed statistically using the software package SPSS 9.0 for windows. The non-parametric test Kruskal-Wallis was ran on the data to see if there was any significant difference of the other points from the normal sampling point. In a case where there was a significant difference we again ran the data through the Mann-Whitney U test to know exactly which points data differ from the normal sampling point. Graphs were created using Origin Pro 6.1 (Origin Lab Corporation, Northampton, MA, USA). In order to evaluate the main factors controlling akinete germination a correlation analysis was performed using SPSS too.

4.8.3 Influence of abiotic factors on population growth of *C. raciborskii* studied by Partial Least Square (PLS) Statistical analysis.

In order to pick out the most important physical conditions in determining *C. raciborskii* filament abundance several factors were considered. Those considered were average water and air temperature, mean PAR, hours of sunshine, global radiation, wind direction, maximum wind and average wind. To identify the factors that correlated with the population abundance and biomass, and to build a predictive model for this variable, a partial least square (PLS) multiple regression model analysis¹⁷ was run with these conditions using the

¹⁷ Partial least squares analysis is a multivariate statistical technique that allows comparison between multiple response variables and multiple explanatory variables. It was designed to deal with multiple regression

programme Statistica 6.1 from StatSoft, Inc. The conditions were average water and air temperature, mean PAR, hours of sunshine, global radiation, wind direction, maximum wind and average wind. To each condition, seven different cases were taken into consideration which included from one day average to seven days average. Thus filament biovolume in the pelagial was treated as a dependent (response) variable, and the other water environmental conditions named above were treated as continuous predictor variables. Using a stepwise method, alternate group of predictors were eliminated to determine which group gave the highest percentage of explaining the variation in abundance. Elimination involved starting with all predictors variables and deleting any that are not significant, a sequential elimination of predictors with coefficient of determination (R^2) nearer zero.

when data has small sample, missing values, or multicollinearity. The goal of partial least squares is to predict Y (the dependents) from X (the predictors) and to describe the common structure underlying the two variables (Abdi, 2003). It allows for the identification of underlying factors, which are a linear combination of the explanatory variables or X which best model the response or Y variables (Talbot, 1997). Partial least squares optimal linear relationships are computed between latent variables and can be interpreted as the best set of predictions available for a study given all the limitations (Falk & Miller, 1992). As an extension of multiple linear regression, partial least squares regression has many of the same assumptions. For example, one should be concerned with outliers and nonlinear data relationships when using partial least squares. Although PLS can be used for theory confirmation, it can also be used to suggest where relationships might or might not exist and to suggest propositions for later testing. PLS is a predictive technique and not an interpretive technique, except for exploratory analysis as a prelude to an interpretive technique such as multiple linear regression or structural equation modeling. When the number of predictors is large compared to the number of observations one of the many approaches is to eliminate some predictors (e.g., using stepwise methods) (Abdi, 2003).

5. RESULTS

5.1 Influence of temperature and light on germination of *Cylindrospermopsis raciborskii* akinetes

5.1.1 Results from field studies

Field studies which constituted a larger part of this work took place between January 2004 and December 2007. But the study of the akinetes of *C. raciborskii* in the sediment only commenced in March 2005. Abiotic factors have been noted to have some influence on the behaviour of organisms in nature.

5.1.1.1 Characterisation of abiotic conditions

5.1.1.1.1 Meteorological factors

Patterns of phytoplankton species occurrence and distributions are determined by multiple abiotic factors, including temperature and light. Air temperature and solar radiation income plays a role in influencing the temperature of the lake. Data collected by the meteorological observatory of the German Weather Service in Lindenberg indicated the following seasonal air temperature change through out the study period (Figure 15).

With summer months running from June to August, the year 2004 had the coldest of summers during the study period. The mean daily air temperature during that period remained below 20°C in the first half of the summer period and above during the second half. The highest mean daily air temperature in the course of that year was 24.9°C on the 12th of August 2004. On this day too a maximum air temperature of 31.1°C was recorded. Unlike 2004, in 2005 we saw a fluctuation in the daily temperatures in summer. Towards the end of spring (March to May) of 2005, mean daily air temperature had risen to a 25.3°C on the 28th of May 2005 only for it to drop again at the beginning of summer to a low 9.8°C four days after the peak. Two day after the 9.8°C drop the mean daily air temperature peaked to 19.3°C followed again by another drop to a mean of 9.2°C. Such alternation patterns were noticed through out the summer period of 2005. The 2006 summer was different from that of 2005 and 2004 in that it had the greatest warming of all the studied years and it was more stable. Once the mean daily air temperature had risen after spring and at the beginning of summer, it remained more or less above 20°C. Decrease in temperature during the summer of this year was just to a

minimum of 16°C for the mean daily air temperatures. For 2007, the mean daily air temperature was below 20°C during most of the summer months.

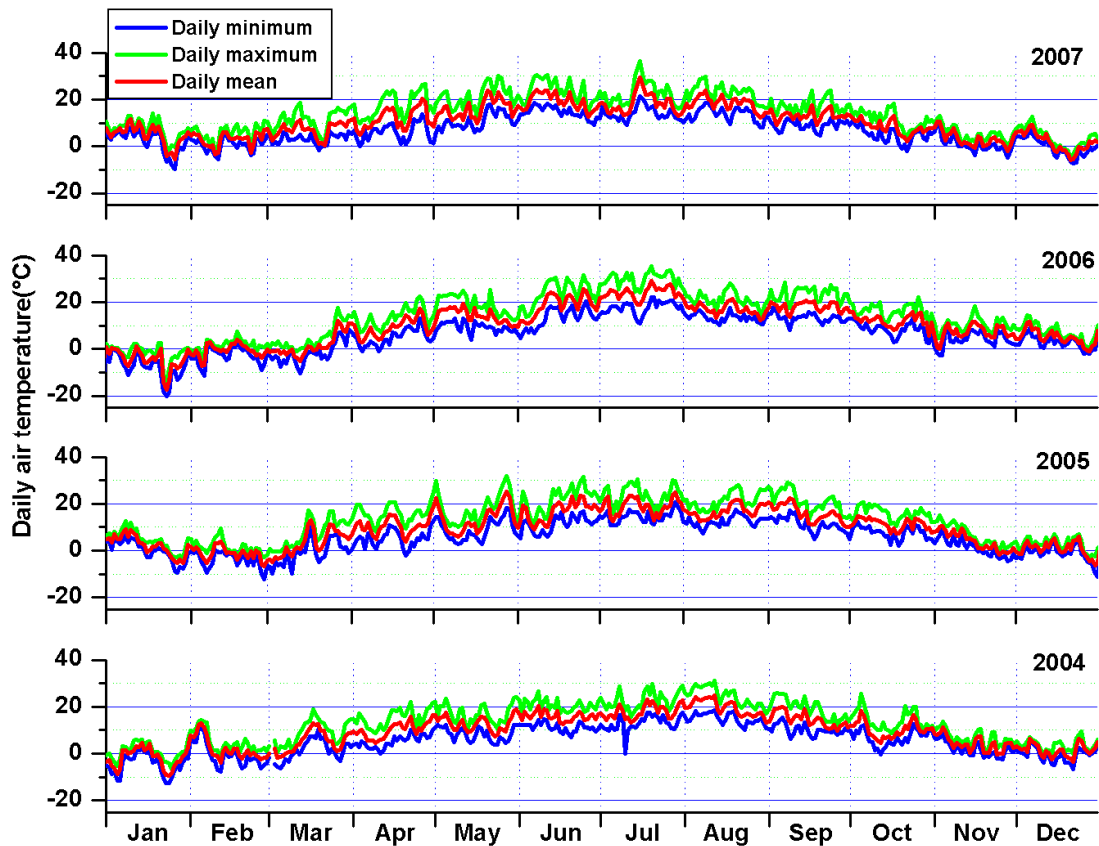


Figure 15: Daily air temperature at the study area from 2004 to 2007. Min = minimum, max= maximum and Mean= Mean air temperature. Data from the meteorological observatory of the German Weather Service, Lindenberg, Germany.

The monthly air temperature anomaly relative to the long term average from 1961 to 1990 period gotten from the above air temperature changes in the course of the years gave the following (Figure 16). The graph shows air temperature anomalies (departures from normal) with each bar representing mean air temperature anomaly of one month and the points the actual monthly average temperature of the respective years. The bars are either above or below point zero which represents the monthly average of the air temperature from 1961 to 1990 considered as the normal average temperature (Müller-Westermeier, 1996). The year 2004 started of with a negative monthly mean air temperature anomaly in the middle of winter and move to positive one for the next three months (February, March and April) of up to +2°C. The last month of spring of that same year (May) was negative to the normal value, same with the month of June and July whose values were very close to the normal average temperature value from 1961 to 1990. The rest of the year of 2004 as from the month of

August had a positive mean air temperature anomaly right into the end of the winter in January of 2005. The fluctuations of the daily air temperatures we noticed in 2005 (Figure 15) can clearly be seen here with the air temperature anomalies in Figure 16. The air temperature anomaly varied from above normal in one month to below normal in the next within seasons. These fluctuations in temperature did not occur in the years 2006 and 2007. In the summer of 2006 a temperature of 6°C above normal was recorded in July making it the year with the highest temperature above normal in summers of the study period. These positive air temperature anomalies continued into autumn 2006, winter of 2006/2007 right into spring 2007 with a temperature anomaly of 6°C above normal measured in January of 2007 too. Towards the end of the year 2007 temperature anomalies were then below normal.

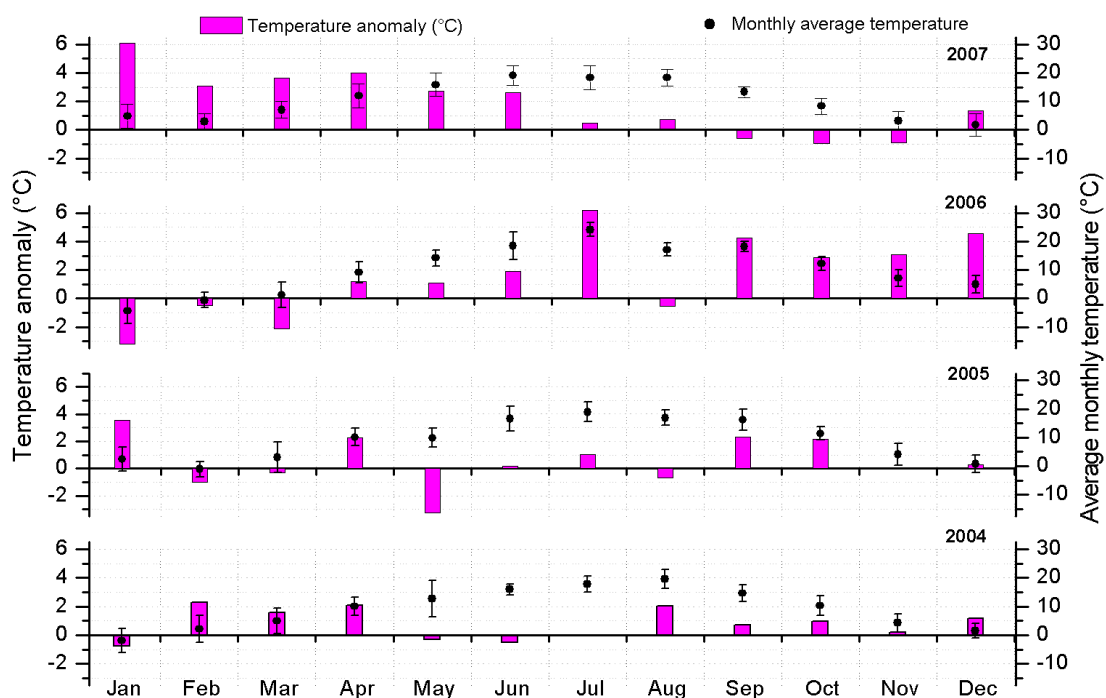


Figure 16: Monthly mean air temperature anomaly (Pink Column) for the years 2004-2007 compared to the 1961-90 average (Müller-Westermeier, 1996). The dots indicate the monthly mean with the error bars being the standard deviation of the daily mean values. The deviation is given in absolute values.

Just like the air temperatures in the summer months (June to August) of 2004 daily sum of global radiations within this period were below 2500 Jcm⁻² most of the time except for three peaks. The exceptions were one in June and the other two in July. The highest daily sum of global radiation in the course of that year was 2956 Jcm⁻² on the 07th July 2004 which was just 8 Jcm⁻² above what was registered during the last three day of May 2004. On the contrary in 2005 we saw more peaks above 2500 Jcm⁻² particularly in the month of June. Towards the end of spring (March to May) of 2005, as from may 13th the daily sum of global radiation had

increased to 2845 Jcm^{-2} again to drop below 2500 Jcm^{-2} at the beginning of summer. The same trend of up and down alternations in values occurring with the air temperature of 2005 was also noticed with the global radiations in the study area this same year. The peaks went as far as 458 Jcm^{-2} on the 21st of July 2005 in summer. In terms of the daily sum of global radiation, the summer of 2006 was different from that of 2005 and 2004 in that it remained almost throughout June and July above 1500 Jcm^{-2} with more and larger peaks as compared to the other years above 2500 Jcm^{-2} . Drop during the summer of this year were just ones to a minimum of 1185 Jcm^{-2} on the 30th of June 2006. For 2007, there were also some fluctuations during the summer months. For the first two weeks of June 2007 the radiations were above mostly 2000 Jcm^{-2} followed by four weeks in which it was more often less than the 2000 Jcm^{-2} . In the last two weeks of July 2007 right into the first week of August sum of daily radiations were more often above 2000 Jcm^{-2} on the study area.

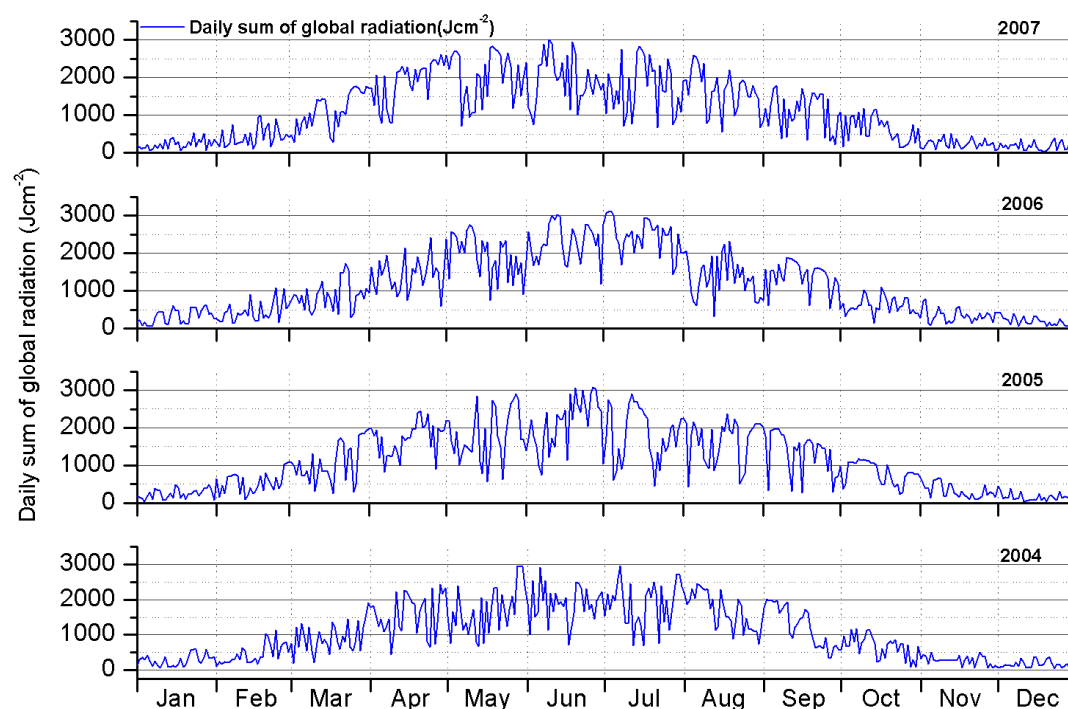


Figure 17: Daily sum of global radiation on the study area from 2004 to 2007. Data from the German Weather Service in Lindenberg. J=Joules

From the above global radiation changes on the sampling site in the course of the year, the mean monthly global radiation was also calculated from all of the available observations within each month to determine the global radiation anomaly relative to the long term average from 1993 to 2007 period . The following was gotten (Figure 18).

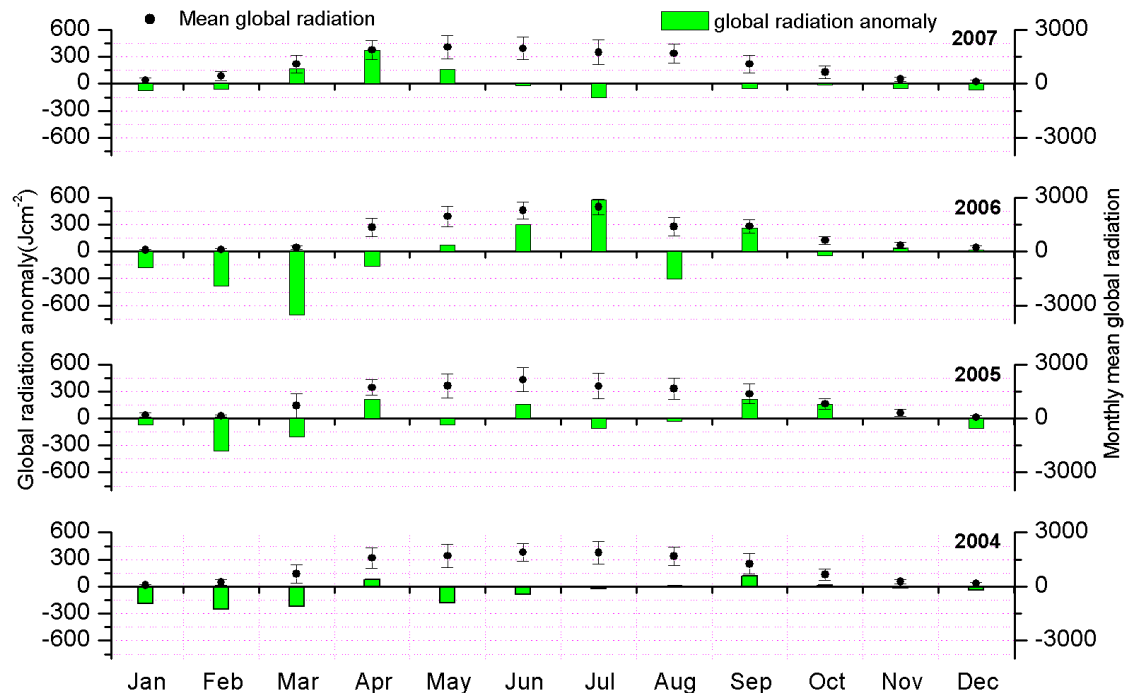


Figure 18: Monthly mean global radiation anomaly for the years 2004-2007 compared to the 1993-2007 average (1993-2007 average calculated by Jacqueline Rucker). The dots indicate the monthly mean with the error bars being the standard deviation of the daily mean values. The deviation is given in absolute values.

The graph shows global radiation anomalies (departures from normal) with each bar representing mean global radiation of one month. Both the highest and the lowest deviation from the normal occurred in 2006. While the negative global radiation anomaly value of -706 Jcm^{-2} was recorded for the month of March 2006, a value of 573 above normal was recorded for the month of July 2006. The normal in this case is the monthly average of global radiation from 1993-2007. For the first three months of 2004, there was a negative deviation from the normal while in the last two months of spring there was some fluctuation from the normal starting off with a positive deviation and ending with a negative one. This negative deviation from the normal continued into the first month of summer 2004. But then for the rest of the summer of 2004 there was little anomaly recorded. Just like we had alternation in temperature anomaly for the year 2005, same was observed for the monthly mean global radiation anomaly with one month being above normal and the very next one below. 2006 and 2007 were more stable compared to 2005 with 2007 having little or no deviation from the normal through out the year but for the spring months.

The air temperature was reflected in the water temperature. The average pelagic temperature showed a clear annual cycle, varying from the lowest 1.7°C on February 13th, 2006 to the

highest of 25.7°C on the 1st of August 2006 (Figure 25a). The fluctuation in the 2005 air temperatures was also seen in the average pelagic temperature of the same year (Figure 25a). In the summer of 2005, there was a pelagic temperature peak of 25°C on the 28th of June. Two weeks after (7th July 2005) this peak, the pelagic temperature had dropped to 19°C. Another peak of 23°C occurred on the 18th of July followed by drop of the pelagic temperature to 19°C on the 21st of July. The warm nature of the year 2006 was reflected by the pelagic water temperature getting to a maximum of 30 °C on the 26th of July above the 24.4°C, 26.6°C, and 27.7°C of 2004, 2005 and 2007 respectively. The pelagic temperature remain above 20°C in 2006 the 11th of June to the 13th of August while for the other years within this period on summer temperature drops below 20°C were recorded.

In contrast to air and water temperature the decoupling of global radiation¹⁸ and under water light was obvious because of the dependence of underwater light on attenuation. Under water light values in Figure 19 indicate their variability with depth. Through out the study period there was little or no light getting to the sediment at a depth of 2m. The highest value of 24.8 $\mu\text{Em}^{-2}\text{s}^{-1}$ recorded at this sediment depth of 2m was on the 14th of April 2007 and the least of no light at all at the bottom was on the 13th of August 2006. At the beginning of 2004 there was less than 50 $\mu\text{Em}^{-2}\text{s}^{-1}$ at the depth of 0.5m but by the first week of February we already noticed in the availability of light to the depth of 1m. Much more light was disposable during the spring months getting to a maximum of 402 $\mu\text{Em}^{-2}\text{s}^{-1}$ at a depth of 2m on 14th April 2004. From the month of June to the end of the year there was then less than 150 $\mu\text{Em}^{-2}\text{s}^{-1}$ at the 1m depth of the lake. A slight difference was noticed with 2005 in that about 150 $\mu\text{Em}^{-2}\text{s}^{-1}$ light was available at the 1m depth in January with less in February. The spring months of 2005

¹⁸ Solar radiation reaching the Earth's surface comprises a mixture of ultraviolet light, visible light, and near-visible infra-red radiation. All this radiation conveys heat and its energy content measured in joules is referred to as radiant energy. A Joule is the energy expended, or work done, when a force of 1 Newton moves through 1 metre. E.g. holding a 100 gram weight against the Earth's gravitational attraction requires a force of 1 N; lifting the weight through 1 vertical metre represents 1 J of work done or energy expended. If converted to heat, 4.2 KiloJoule (kJ) of energy will increase the temperature of a kilogram of water by 1°C. The rate of flow of the radiant energy is called the radiant flux (units: watts; 1W = 1 J/s) and irradiance is the radiant flux incident on a receiving surface from all directions per unit area of surface (units: watts/m² or J/m²/s). All the properties of radiant flux depend on the wavelength of the radiation. Photosynthetic Available Radiation (PAR) is the radiation in the 400 nm to 700 nm waveband (a continuous range of wavelengths between two limits), which can be captured and used by photo-autotrophs. The units for PAR can be specified in energy terms (watts/m²) or in photon terms which is a measure of the number of photons in the 400 nm to 700 nm waveband that are incident per unit time on a unit surface. A photon is a quantum of radiant energy embodying only a very small amount of energy whose amount depends on the wavelength of the photon. The quantity of photons is measured in moles of photons (1 mole of photons =, 6.022 x 10²³ photons = 1 Einstein (E)). PAR is measured in $\mu\text{E}/\text{m}^2/\text{s}$ or $\mu\text{E}/\text{s}/\text{m}^2$. A microEinstein (μE) is a convenient amount, being 6.022 x 10¹⁷ photons. For approximate conversion, 1 J of PAR energy = 4.15 μE of PAR photons (Source: <http://www.lifesciences.napier.ac.uk/teaching/Eu/muI.htm#PAR>).

were more or less the same like in 2004 but for the summer months of June and July 2005 which had more light available in the water column compared to 2004. This continues right to the end of 2005. Light availability above $150\mu\text{Em}^{-2}\text{s}^{-1}$ at a depth of 1m only started off later in the middle of spring of 2006. Worth noting is the fact that the difference between light available at the 0.5m depth and 1m depth was wide through out the months of June and July 2006 unlike the other sampling years with less light available in the water column from August 2006 to the rest of the year. The year 2007 had the most underwater light through out the year as compared to the other sampling years.

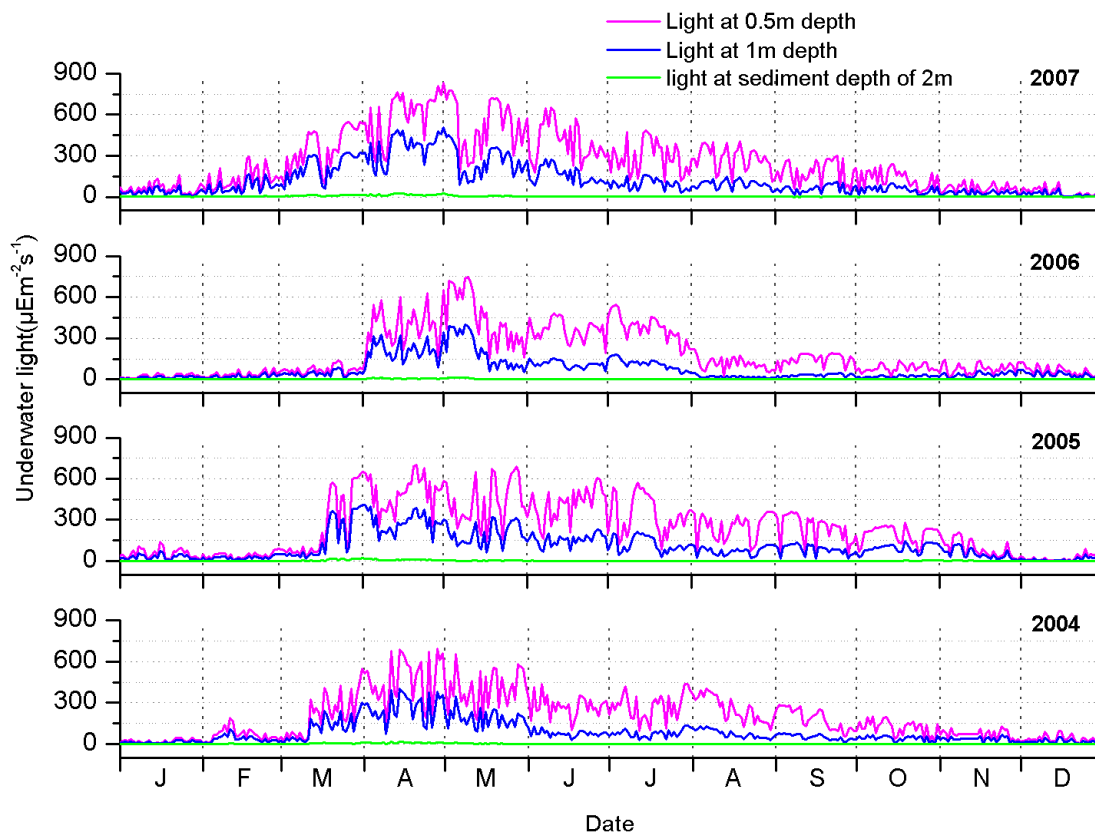


Figure 19: Underwater light in Lake Melangsee at different depth in the course of the study period.

The fluctuation in the 2005 global radiations were also seen in the mean PAR at the mixed zone (I_{mix}) of the same year (Figure 25a). In the summer of 2005, there were two more major peaks registering above $170\mu\text{Em}^{-2}\text{s}^{-1}$ after the major peak on spring. On the contrary in 2004, 2006, 2007 other peaks after the major spring peak got only to a maximum of $106\mu\text{Em}^{-2}\text{s}^{-1}$. Mean PAR at the mixed zone (I_{mix}) in the water column was least in the winter of 2004 measuring $4.3\mu\text{Em}^{-2}\text{s}^{-1}$ on the 21st of January and the maximum was recorded as $238\mu\text{Em}^{-2}\text{s}^{-1}$ in spring of 2007 specifically on the 6th of May (Figure 25a). In all the years, I_{mix} rose in

spring had the highest peak between early May and mid-May. These peaks were between 45 to sixty days before the maximum temperature had been reached (Figure 25a). By the time temperature peaks, I_{mix} drops to three quarters its peak value in all the years. These relevant growth factors therefore had a similar pattern of recurrence in the years studied.

5.1.1.1.2 Concentration of dissolved inorganic nutrients and total nutrients

All physical and chemical data for the different sampling times could not be reported in this work but some are shown in Figure 20. Dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) showed a distinct pattern of recurrence in all years studied. In addition to temperature and light, dissolved inorganic nitrogen concentrations ($\text{DIN} = \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$) were generally low during summer, but during the autumn and winter there was a high amount about five times that registered during the summer season. In 2005 a decrease in nutrient concentrations (DIN) was observed from a peak of $497.6\mu\text{g l}^{-1}$ on the 4th of January to another of $280.6\mu\text{g l}^{-1}$ on the 3rd of March and finally to a low value of 2.2 on the 2nd of May. Subsequently there was some slight increases (did not go above $75\mu\text{g l}^{-1}$) and decreases (got to zero or below detection limits on the 22nd of August) through out the summer months.

As from the beginning of the last month of autumn 2005 (2nd November), dissolved inorganic nitrogen was already above the maximum summer level of $71.1\mu\text{g l}^{-1}$ and kept increasing into the year 2006. Unlike 2005, the amount of DIN giving a peak value in January 2006 was lower ($501.4\mu\text{g l}^{-1}$) than the second peak in April ($587.8\mu\text{g l}^{-1}$). After this second peak there was a sharp drop two weeks after to $30.0\mu\text{g l}^{-1}$ on the 10th of April. Following this decrease the amount of DIN remain low (below $75\mu\text{g l}^{-1}$) in the water through out the rest of spring, summer and part of autumn 2006. Just like in 2005, increase above the maximum summer value was recorded on the 1st of November 2006 ($130.7\mu\text{g l}^{-1}$). The maximum value was recorded in this winter (2006/2007) on the 12th December 2006. Maximum summer values were quite high compared to the other years, reaching $129.4\mu\text{g l}^{-1}$ at the end of August 2007. Ammonium had a greater contribution to the fluctuation in DIN values than NO_g . In the case of nitrogen deficiency, *C. raciborskii* and other Nostocales have a competitive advantage, namely N_2 fixation. Dissolved inorganic phosphorus (DIP) was low during winters and started increasing in concentration in the water as from June of each year. The maximum amounts were registered towards the end of September with a variation in concentration between the years.

In the summer 2006 the highest amount of $28\mu\text{g l}^{-1}$ was recorded in the water column of the Melangsee. DIN: SRP ratio by mass ranged from 1:1 to 142:1 and was higher than the

Redfield ratio (N:P= 7.2:1 by mass and 16:1 by atomic ration), suggesting potential nitrogen limitation occurred at some point while at its other there was an excess. Chlorophyll *a* concentrations (Figure 20) ranged from 4.3 μg Chlorophyll *a* l^{-1} in December of 2005 to 182 μg Chl *a* l^{-1} in August of 2006. The seasonal cycle of phytoplankton Chl *a* biomass coincided with a decline in nutrient concentrations that occurred in April.

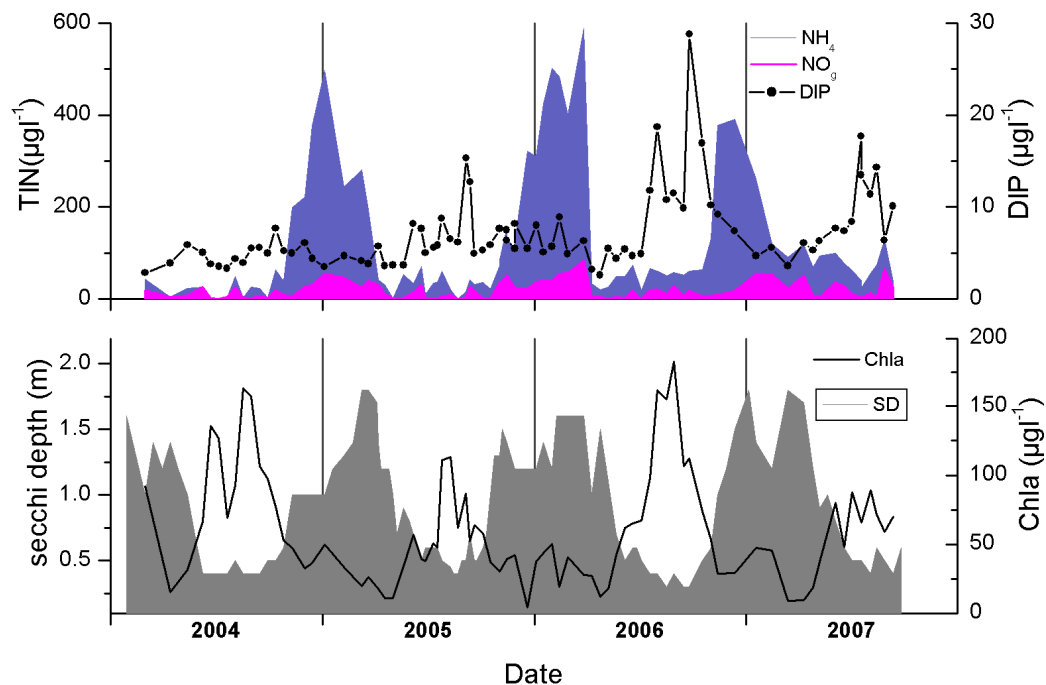


Figure 20: Some of the lake physical and chemical values during the study period. TIN=Total inorganic nitrogen, NH_4 = ammonium $\text{NO}_g = \text{NO}_3^- + \text{NO}_2$, DIP=Dissolved Inorganic Phosphorus, Chla= Chlorophyll a, SD= Secchi Depth.

5.1.1.1.3. Occurrence of temporal thermal stratification in the lake

The air temperature and global radiation warm up the water of the lake (from top to bottom). During the summer and winter times of the year the difference between the surface temperature and the bottom temperature can lead to thermal stratification of the lake caused by the different temperature dependent density of the water. The temperature difference between surface and bottom is an indicator of the strength of stratification, respectively the density gradient in a lake. Seasonal stratification is defined as the condition when the temperature difference between surface and deep water is $> 1^\circ\text{C}$ (Stefan *et al.*, 1996). Temporal stratification events in shallow lakes are not unusual. A look at this temperature difference in the summers of our study period gave us the different times at which some temporal stratification event might have taken place despite the fact lake Melangsee is polymictic (Figure 21).

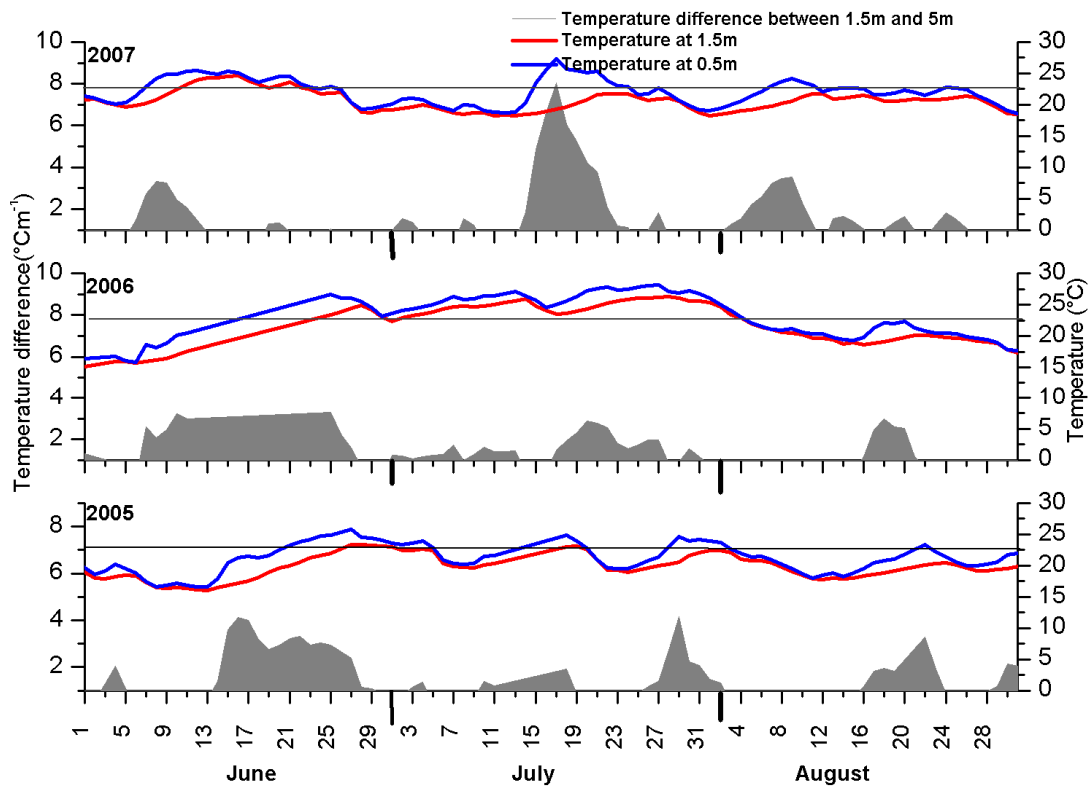


Figure 21: Periods in the summers of the sampling period at which temperature difference between the water in the depth of 0.5m and 1.5m was at or above 1°C and which a temporal stratification was assumed to have taken place

The highest temperature difference of about 8°C per meter was observed during the month of July 2007. Taking the temperature difference $>1^{\circ}\text{Cm}^{-1}$ to be an indication of stratification we can see that at the beginning of the summer of 2005, there was short two day stratification. This was followed by an eight day period of no stratification. From the middle of June 2005 to two days before the end of the month there was a long period of stratification. July and August of 2005 also had some short periods of some stratification. Compared to 2005, the month of June 2006 had a longer period of stratification almost through out the month of June, compared to 2005. This was again followed by more within the months of July and August. Though 2007 registered the highest temperature difference per meter, its months of June had the shortest period of stratification (7 days) in June of all the three years. Another major stratification occurred in the middle of July. It should be noted that during this periods of stratification the bottom temperature for the years 2005 and 2007 remained at or below 22.5°C while that of the year 2006 was mostly above this mark

5.1.1.2 Long-term study on akinetes abundance in the surface sediment of Melangsee (With focus on germination periods in spring)

The quantification of the *C. raciborskii* akinetes in the sediment started on the 11th of March 2005 (Figure 22). Akinete abundance at this time of the year was 123×10^3 akinetes per ml of fresh sediment. It should be noted that they were found in the sediment of the lake all year round through out the study period. As the temperature kept increasing in this spring of 2005, the number of akinetes in the sediment reached a maximum of 151×10^3 akinetes per ml of sediment on the 18th of April 2005 after five weeks of the initial sampling with the average sediment temperature on this day reading 13.3 °C. This was followed by sharp decrease also reflected in the negative net changing rate ($-0.1d^{-1}$), fourteen days after. With further increment in temperature there was still some decrease in the akinete number but not as much as before. There was stabilisation through out the summer months with little or no changes.

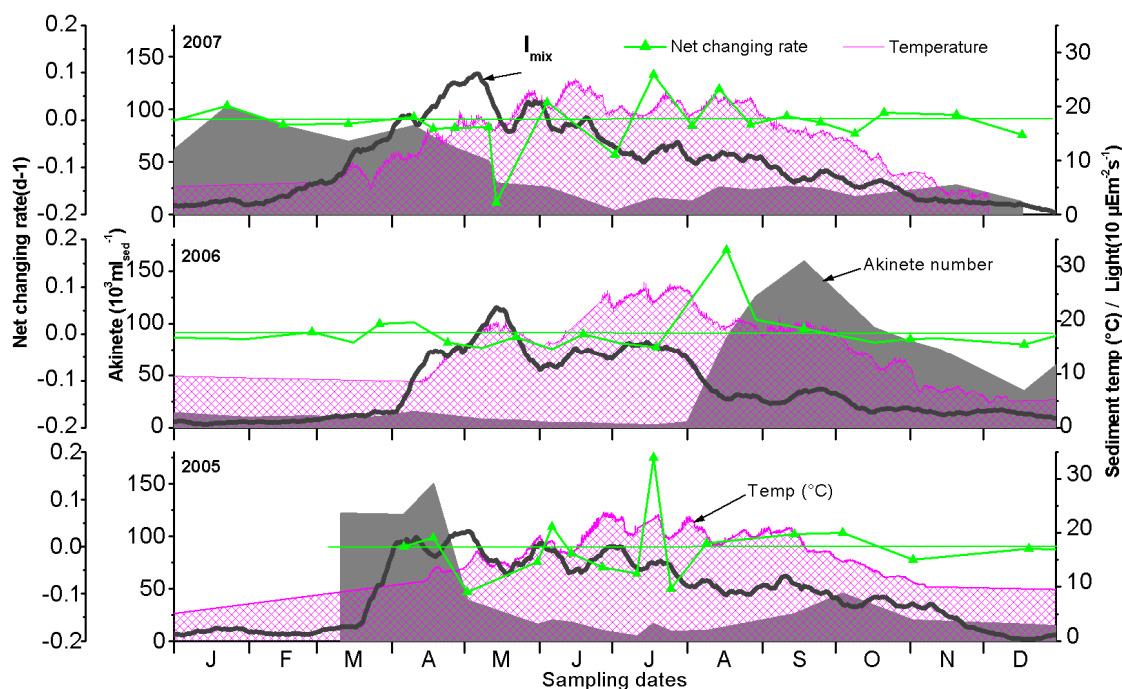


Figure 22: Variation of akinete number with temperature at the sediment and light in the mixed zone for the different years from 2005-2007.

As from the beginning of August 2005, there was the onset of a gradual increase in the number of akinetes in the sediment but the number was not as high as they were at the beginning on the year. With not so many akinetes in the sediment at the beginning of 2006, there was still some decrease in akinete number from the peak on the 24th of April to the 8th of May 2006. The average temperature of the sediment on this 24th of April was 13.3 °C.

Contrary to 2005, there was a higher amount of akinetes at the end of 2006 during the cold seasons. In 2007, there was a difference between the count on the 10th of April which was 84.3×10^3 and that of the 18th of April which was 73.3×10^3 reflected by a negative growth rate of $-0.01d^{-1}$. The sediment temperature between these two dates changed from 11°C on the 10th getting to 13°C by the 13th and up to 16°C by the 18th of April 2007. Nevertheless there was a more sharp decrease in number later in the month of May. At the approach of the cold winter of 2005, 2006 and 2007 there was a noticeable increase in abundance of the akinetes in the sediment as they sank from the filaments in the water column but the amount was variable between the years. Akinetes in the sediment population peaks occurred in autumn between mid September and mid October with maximum abundance values of 45×10^3 akinetes per ml registered on the 4th of October 2005, 171×10^3 akinetes per ml on September 18th 2006 and 26.3×10^3 on September 11th 2007. The akinete number in the sediment varied greatly between the years with winter of 2006/2007 registering the highest during the sampling period.

5.1.2 *In vitro* temperature conditions for *Cylindrospermopsis raciborskii* akinete germination (Germination experiment)

In our annual study of the life cycle, the akinetes showed a sharp decrease in 2005 when temperatures were just about 13°C (Figure 22). The laboratory experiment was run to determine the temperature at which akinetes germinate despite literature on the fact that it takes place at a temperature higher than 15°C, down to 13°C (Figure 23). Germination was examined at three temperatures (13, 15 and 17°C) under $130\mu Em^{-2}s^{-1}$ (which corresponds to the mean light in the mixed layer in Melangsee in May) for 11 days. Akinete germination was evaluated by morphological changes i.e. whether the endospore (germlings) had elongated and separated from the exospore (akinete shell) (growing akinetes), already exiting the akinete shell, endospore less than 30µm completely out of the shell (germlings) or filaments above 30µm (growing filament). In all the Erlenmeyer flasks incubated at the different temperatures and at the same light condition of $130\mu Em^{-2}s^{-1}$, were the different growth stages at some point in time. The classification of phases in this section is according to Figure 14. In the first (day zero) sample gotten from the beaker incubated at 17°C there were only intact akinetes. After a day of incubation there was the presence of growing akinetes and germlings from the samples drawn from the incubated flasks (17°C) in addition to the intact akinetes. In the second days extraction, small amount of growing filaments were already present and germlings relative to the growing filaments and akinetes. Growing filaments then constituted the majority of the extractions of the third day onward with little akinetes present. Samples extracted from the

flask incubated at 15°C had some similarities in constitution and timing to that incubated at 17°C.

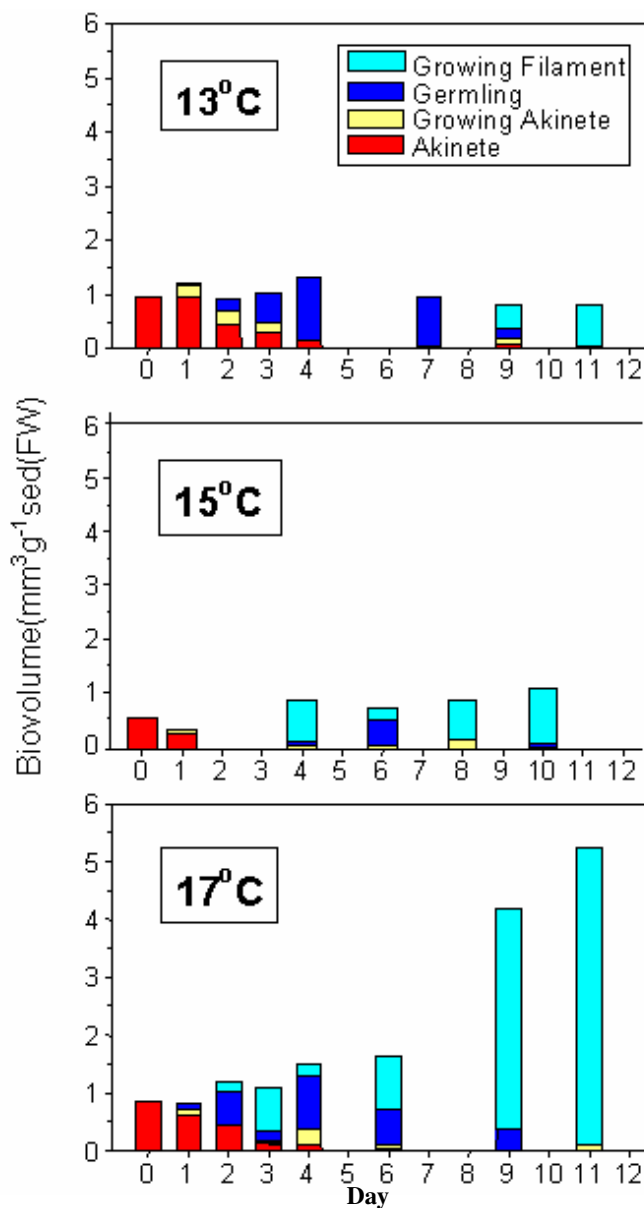


Figure 23: Biovolume of different phases of development of *Cylindrospermopsis raciborskii* in the course of the germination experiments at different temperatures with sediment of Lake Melangsee. For the description of the different groups referred to Figure 14

Just like the 17°C flask, samples extracted from the 15°C flask on day zero had only intact akinetes in them with growing akinetes appearing after a day of incubation. Furthermore the germling and the growing filaments appeared only after four days of incubation at that temperature. It should be noted that unlike the samples drawn from the flask incubated at 17°C, we did not notice any increase in the biovolume of the filaments with subsequent extraction. Over the eleven days period the biovolume in the 17°C flask multiplied about five

times, starting off at less than $1 \text{ mm}^3 \text{ g}^{-1} \text{ sed}$ and ending with over $5 \text{ mm}^3 \text{ g}^{-1} \text{ sediment}$ after 11 days of incubation. At 13°C not only did the growing filament appear after 9 days of incubation, the biovolume was decreasing as the days went by. The growing akinetes were also present after a day of incubation and germling after a day.

5.1.3 Condition for akinete germination and estimation of the recruitment period for *C. raciborskii* in Lake Melangsee (Beaker experiment 2007)

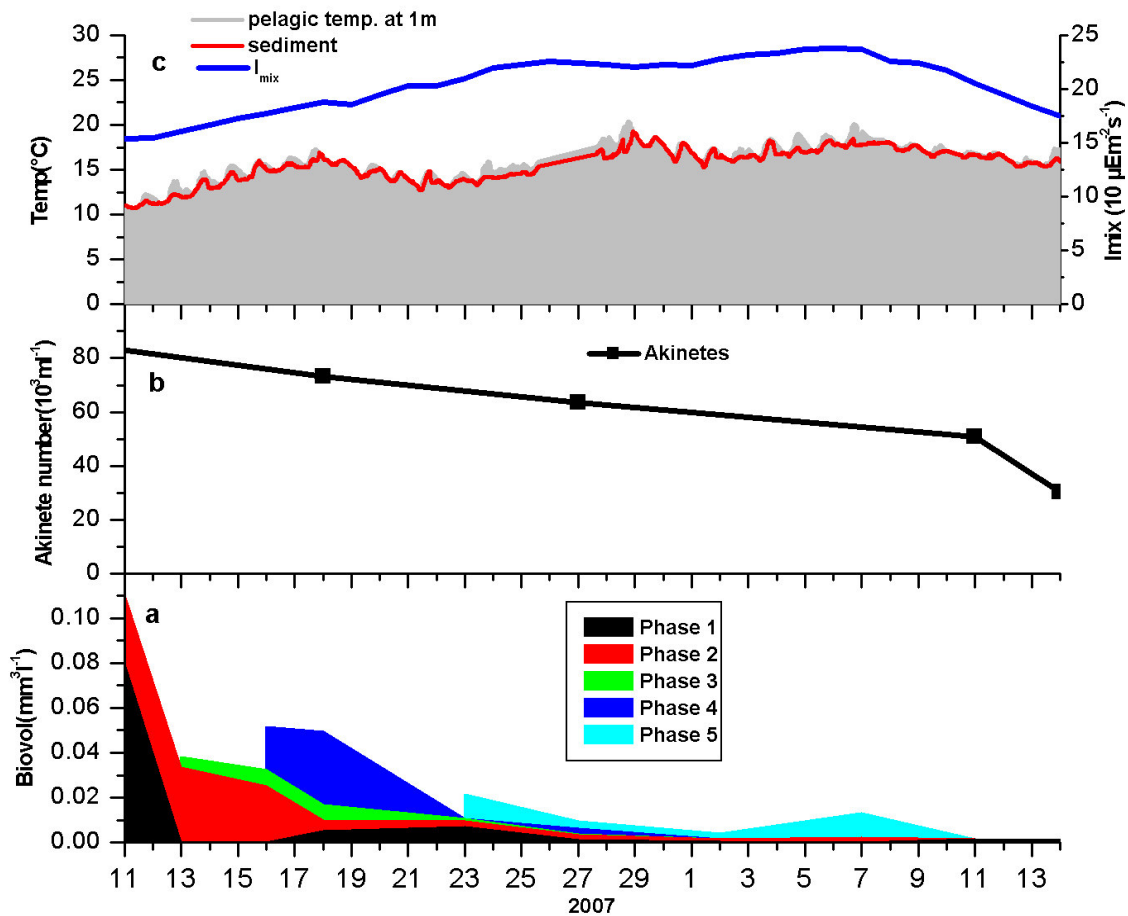


Figure 24: Dynamics of *C. raciborskii* in the short term experiment (a) the suspension of filtrated lake water and sediment within the beakers (b) Akinetes in sediment (c) Temperature and light condition within the period

Due to the fact that akinete numbers in the lake sediment dropped sharply between two sampling dates in 2005 and 2006 (Figure 22), with sampling interval of two week, and none or very few growing akinetes (phases 2 and 3 of .

Table 1) were observed in the sediment samples in these years, the short term experiment was carried out in spring of 2007 to further investigate the germination in the field. The investigation was to find out when akinetes germinate, how long the germlings stay in the

sediment until they form gas vesicles and become buoyant and disappear in the water column (recruitment) and when filaments appear in the water column. The results given in Figure 24 indicate that at the start of the experiment on the 11th of April, both intact akinetes (Phase 1) and growing akinetes with separated endospore and exospore (phase 2) were found in the samples. None of the other stages (according to Table 5) were seen. But just two days after the start of this exercise, growing akinetes emerging from the endospore (phase 3) appeared. Germlings (phases 4) and growing filaments (phase 5) followed suit 5 and 12 days respectively after. The appearance of the germlings occurred at a water temperature of 12.5 to 13°C and light in the mixed layer of around 175 $\mu\text{Em}^{-2}\text{s}^{-1}$.

5.2 Influence of light and temperature on the population dynamics of *C. Raciborskii* in the water column

5.2.1 Results of long-term study

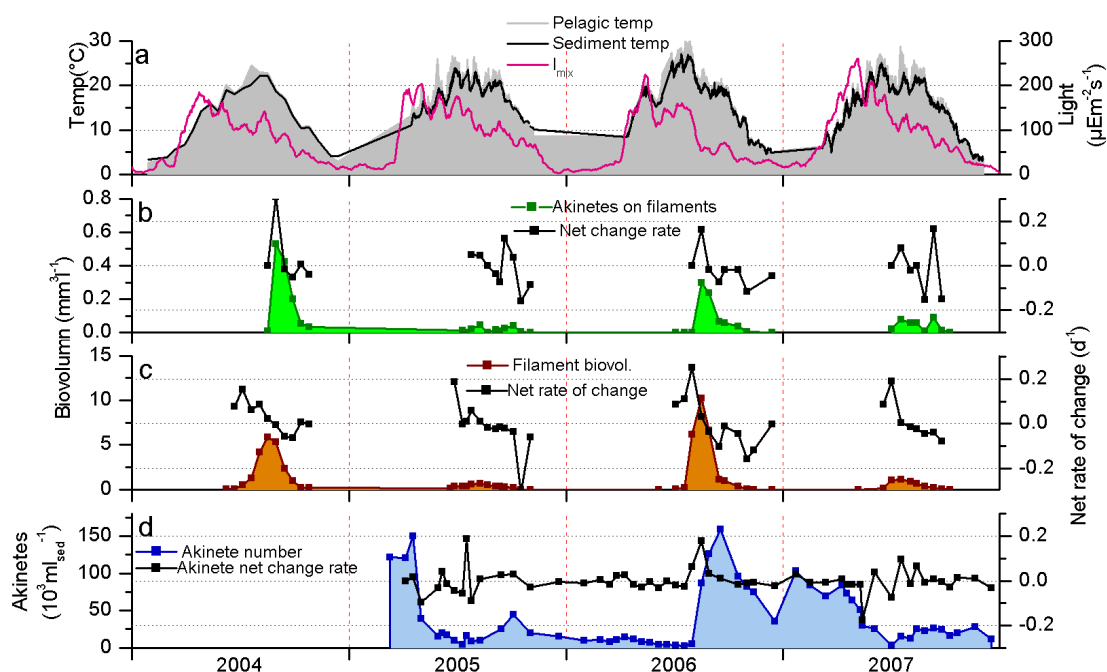


Figure 25: The yearly trend of *C. raciborskii* in Lake Melangsee. (a) Average pelagic temperature and light in the mixed zone (b) Akinete biovolume in the water column and its net rate of change (c) Filament biovolume and net rate of change in the water column (d) akinete number and net rate of change in the sediment (Ave= average, Temp= Temperature, I_{mix} =mean PAR at the mixed zone, sed= Sediment, biovol.= biovolume)

The seasonal dynamics of *C. raciborskii* exhibited a strong similar pattern of recurrence just like the akinetes in the sediment in all the investigated years. The development of *C. raciborskii* (filament biovolume and akinete number) in relation to the pelagial and sediment

temperature and light conditions thriving in the mixed zone in the lake as from 2004 to 2007 is shown in Figure 25. This seasonal cycle of filaments was characterized by a summer peak. The highest population of *C. raciborskii* filaments biovolume in the water column was registered in 2006 (Figure 25c) corresponding to $10.23\text{mm}^3\text{l}^{-1}$ on the 17th of August. Filament blooms that were numerically high in July and August were generally low during the autumn and nil in winter seasons. The trend of the akinetes numbers on the filaments in the water matched those of the filament themselves and this was reflected later in the sediment during the resting period by either a high or low counts of akinetes.

A closer look at just the filaments in the water column is shown in Figure 26

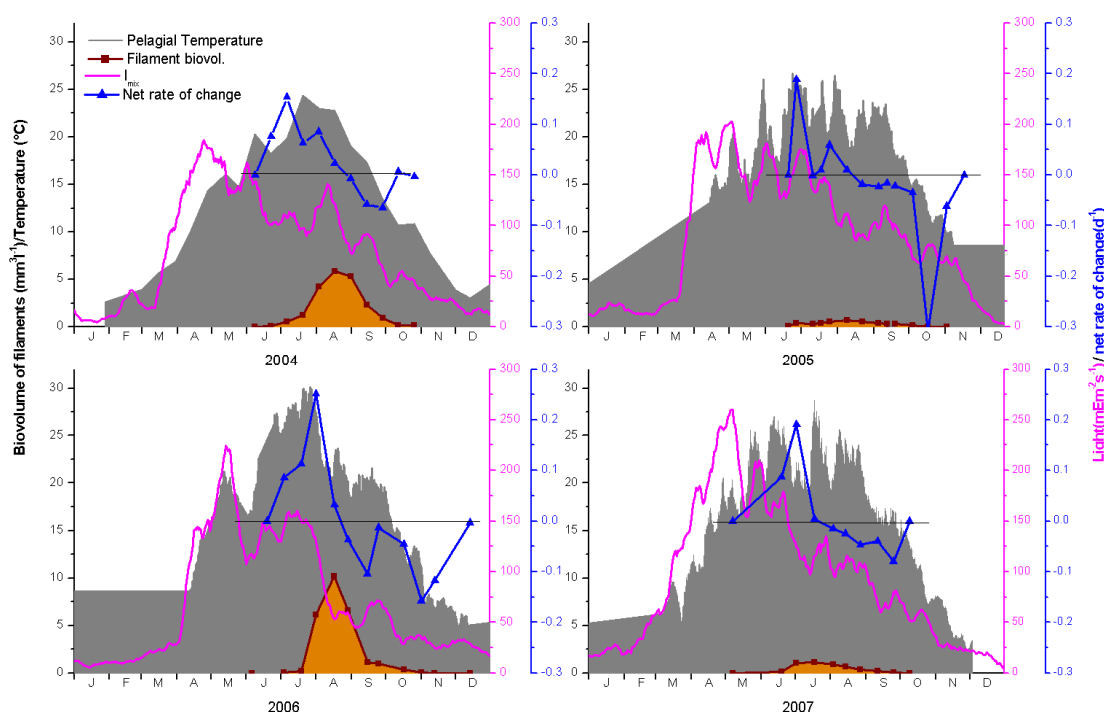


Figure 26: Annual trend of the biovolume and net change rate of *C. raciborskii* filaments in the water column, temperature and mean PAR in the mixed layer from 2004-2007 (2004, 2005 and 2007 phytoplankton data from Anette Tworeck -LBH Freiburg)

Results of the direct counting technique indicated that in all the years mature *C. raciborskii* filaments appeared in the water in June. The appearance of the filament was towards the end of June in 2005 and 2007, while that for 2004 and 2006 was at the beginning of the same month. The first *C. raciborskii* trichomes were identified in the pelagial habitat around the 8th June 2004, 20th June 2005, 06th June 2006, 19th June 2007 each corresponding to 160, 171, 157, 170 Julian days respectively. This summer appearances of the filaments coincided with an increase above 16°C in the water column temperature and a decline in nutrient

concentrations (DIN) that occurred between April and May. A noticeable increase only occurs at the beginning of July. The seasonal cycle of *C. raciborskii* filaments abundances was characterized by a peak in August and a decline in September. 2004 showed as an increase from $0.02\text{mm}^3\text{l}^{-1}$ to a peak of 5.83 on the 17th of August 2004. The summer of 2005 had a biovolume range of between $0.0012\text{mm}^3\text{l}^{-1}$ as minimum and $0.67\text{mm}^3\text{l}^{-1}$ as maximum count on the 9th of August 2005. In 2006 abundance of the mature filament population was higher than the previous years of 2005 during the summer period. This ranged between $0.06\text{mm}^3\text{l}^{-1}$ and $10.23\text{mm}^3\text{l}^{-1}$ with the highest biovolume registered on the 17th of August 2006. The 3rd of August registered the highest value of $0.88\text{mm}^3\text{l}^{-1}$ for the year of 2007.

Biovolume based estimates of filaments net change rate indicated in Figure 26, exhibited similar patterns, with the highest values occurring between July and August (Figure 26) and the lowest between September and October. Maximum net change rates ranged from -0.31d^{-1} on the 17th of October 2005 to 0.25d^{-1} in August 2006. For net change rate variations were similar to the variation observed in the filament community.

5.2.2 Influence of abiotic factors on population growth of *C. Raciborskii* studied by Partial Least Square (PLS) statistical analysis.

Using the measured values of average water and air temperature, mean PAR in the mixed water column, hours of sunshine, global radiation, wind direction, maximum wind and average wind, result from a PLS multiple regression analysis indicated that several models (combination of different predictors) could be used to explain this variability in filament biovolume with dependence on all the physical conditions considered. For each model different coefficient of determination¹⁹ were gotten. The model that gave the highest coefficient of determination (Figure 27) indicated that water temperature, air temperature and global radiation were the most influential of all the predictors. Further analysis to determine the strength of each predictor indicated that air temperature and global radiation had the highest strength of influence on the model (Figure 28).

¹⁹ The coefficient of determination (R^2 value) is an indicator of how well the model fits the data (e.g., an *R-square* close to 1.0 indicates that we have accounted for almost all of the variability with the variables specified in the model) or how much of the total variation in the dependent variable can be accounted for by the Regression Function. If we have an *R-square* of 0.4 then we know that we have explained 40% of the original variability, and are left with 60% residual variability. Ideally, we would like to explain most if not all of the original variability. It is therefore the proportion of variability in a data set that is accounted for by a statistical model.

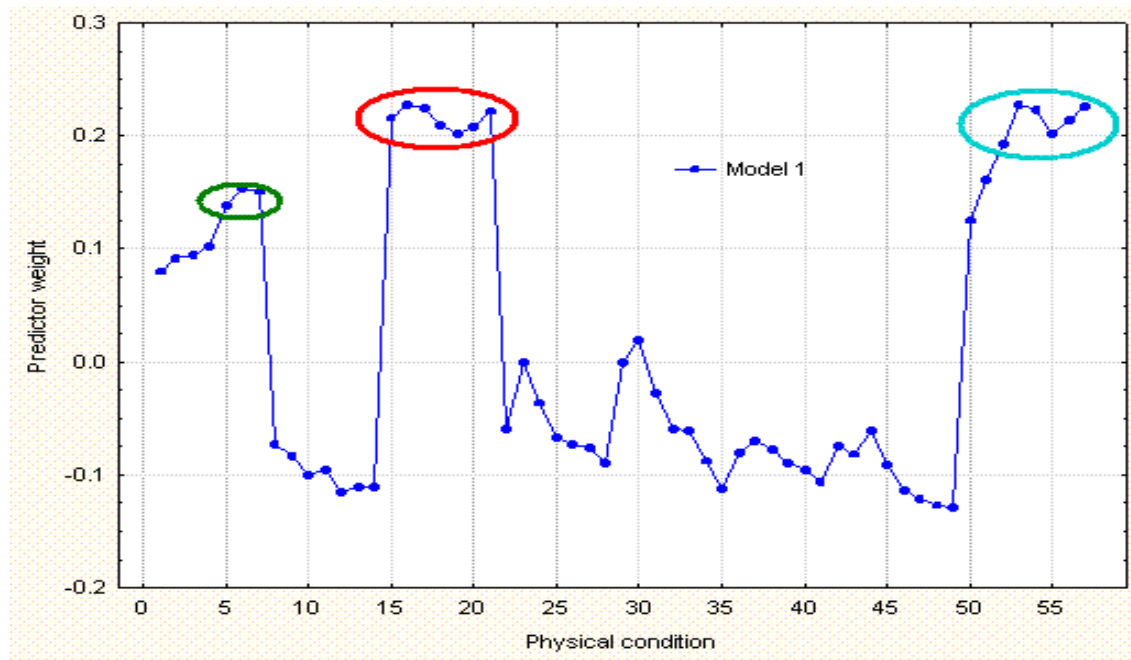


Figure 27: Weight of the predictor variables to the model explaining 44% of the biovolume variability. Those circled in red are the best physical condition (refer to Appendix II for which physical condition is represented by the numbers on the X-axis) Green= water temperature, Red= air temperature, blue= global radiation.

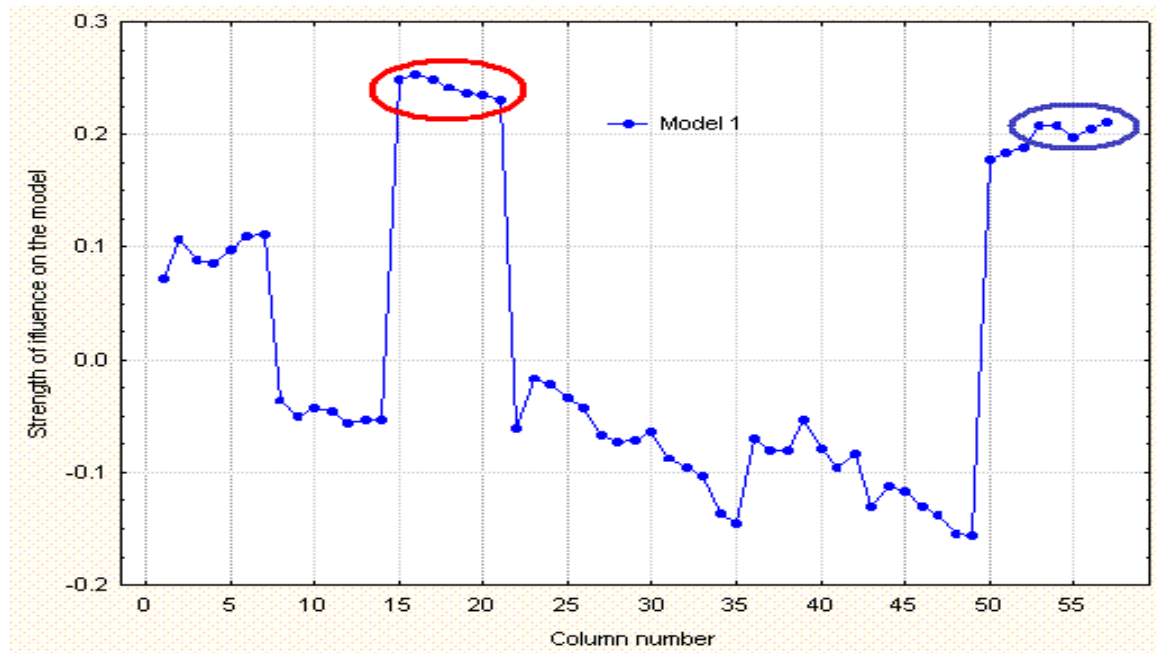


Figure 28: Strength of influence of the physical condition on the model (refer to Appendix II for which physical condition is represented by the numbers on the X-axis) Red= air temperature, blue= global radiation.

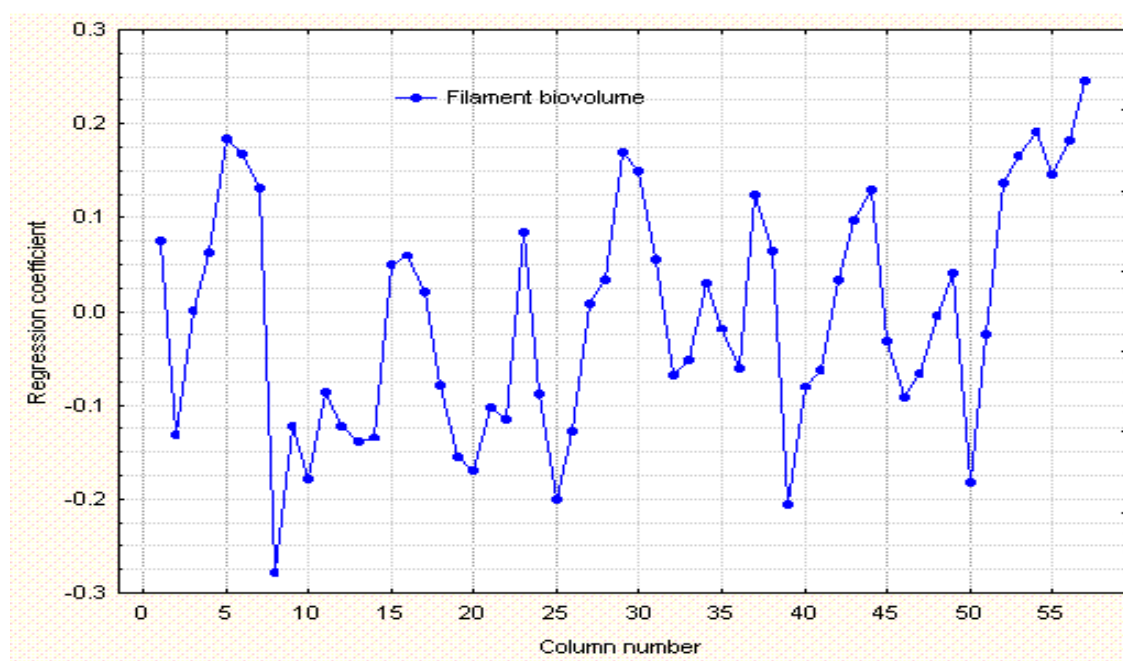


Figure 29: Regression coefficient of each individual physical condition. The physical conditions the column number represent are in Appendix III..

Table 6: Different coefficient of determination gotten from running the PLS in the indicated group of predictors

Predictors	R ² value
5D water temp 7D global radiation	0.25
5D water temp 7D PAR	0.24
5D water temp 7D global radiation 7D PAR	0.42
5D water temp 7D global radiation 7D PAR 5D air temperature	0.40

Each individual physical condition had scaled regression coefficient value²⁰ (or B coefficient) values indicated in Figure 29. The backward and sequential elimination of predictors with coefficient of determination (R²) nearer zero got us the most important predictors of

²⁰ Regression coefficient or B coefficient represent the independent contribution of each independent variable to the prediction of the dependent variable. It is also known as partial correlation. When the regression line is linear ($y = ax + b$) the regression coefficient is the constant (a) that represents the rate of change of one variable (y) as a function of changes in the other (x); it is the slope of the regression line and can take any value to infinity.

Cylindrospermopsis raciborskii filaments biovolume which were five days water temperature average, 7 days global radiation. Next to these were seven days average of I_{mix} and five days average of air temperatures but not hours of sunshine. The different R^2 gotten when different groups of predictors were considered is given in Table 6.

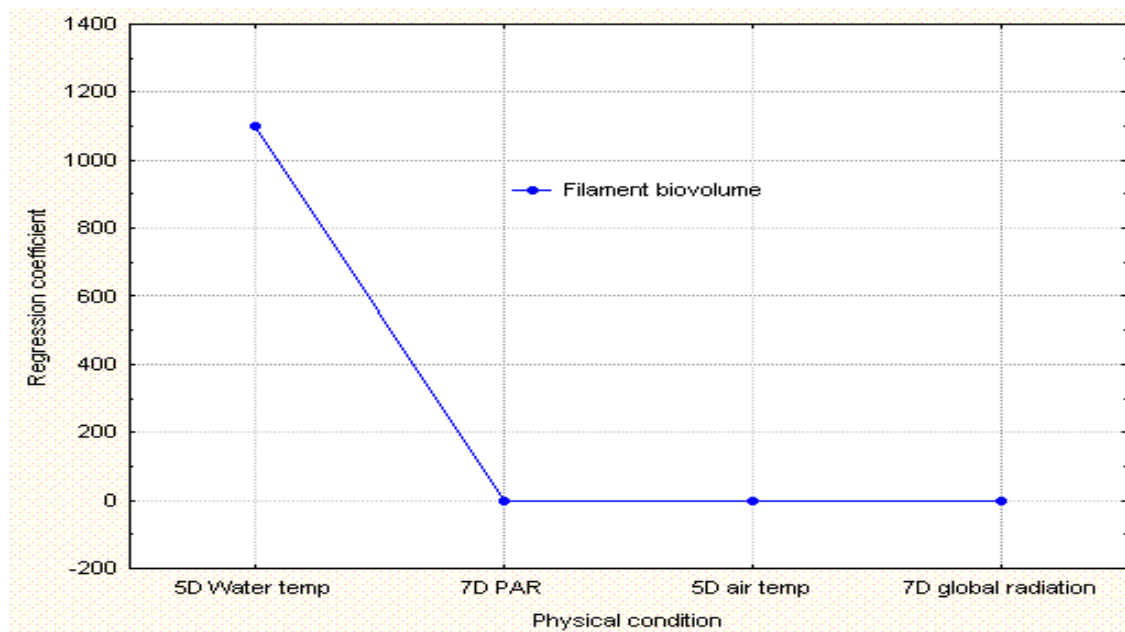


Figure 30: Regression coefficient of the most important predictors which are five days average temperature (5D water Temp), seven day average PAR in the mixed water column (7D PAR), five days air temperature (5D temp air) and seven days global radiation (7D global radiation).

5.3 Development of germlings and young filaments-a detailed quantification during the field study 2006

Data of LBH for the years 2004, 2005 and 2007 were used to show seasonal development in the pelagic. But I did a detailed counting in 2006 and differentiated the phases of developments (Figure 31). In addition to the different phases, there was also a quantification of the akinete present within the filament during this period. Grouping was also according to Table 5.

It can be noticed that the intact akinetes (phase 1) were not found in the water column in the early part of the cycle (end of May to almost the end of June) until around the middle of August when the number of mature filaments greater the 30 μ m in length (phase 5) started reducing. Neither the elongated akinetes with endospores separated from the exospore (phase 2) nor emerging germlings (phase 3) were seen in any of the water samples at all in the course of this study. Germlings of less than 30 μ m (phase 4) and filaments above 30 μ m (phase 5)

appeared almost at the same time at the beginning of June but with the filaments above 30µm registering a higher count than those less than 30µm in length. While phase 4 germlings peaked earlier by the end of July, the ones with length of at least 30µm (phase 5) peaked by the middle of August. A few akinetes appeared on the filament at the beginning of July but increment in biovolume only became noticeable at the end of that same month of July. A lot more were present on the filament after the peaking of the matured phase 5 filaments.

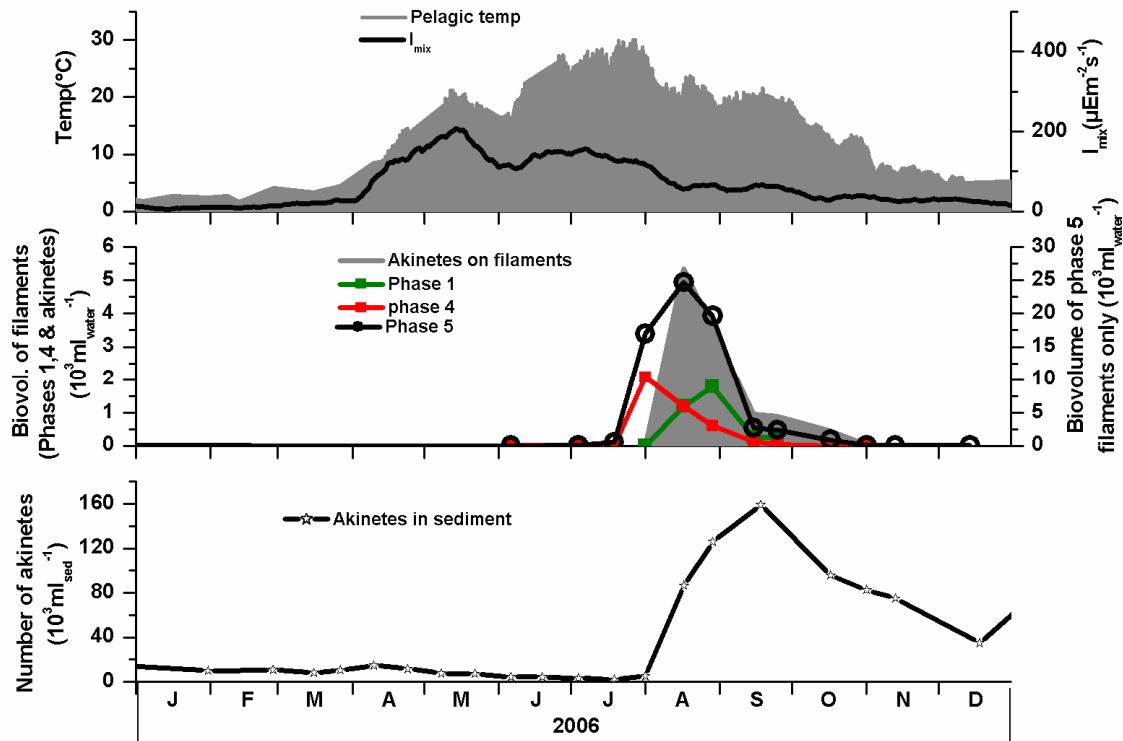


Figure 31: Different phases of growth of *C. raciborskii* in the water column in 2006 and temperature and Mean PAR within this period.

5.4 Akinete formation in the pelagial and import to the sediment

5.4.1 Akinete formation in the pelagial

In the course of our four years study, the akinetes started to grow on the filaments in the pelagic when the population maximum was exceeded (Figure 32). The year 2004 had the highest biovolume of akinetes on the filaments registering $0.53 \text{ mm}^3 \text{ l}^{-1}$ on the 31st of August. The timing of the onset of akinete formation varied with the years. Compared to the other sampling years in which the akinetes started appearing on the filament in July, 2004 was different in that the onset came right in the middle of the month of August. Though in 2006 the first akinetes developed on the filaments at the beginning of July, a sharp increase only

occurred one month after. Not only was there variation with the onset of akinete formation but also with the timing and the number of peaks in the different years. The years 2004 and 2006 both had a single peak for the abundance of akinetes in the pelagial occurring at the end and in the middle of August respectively. Meanwhile, 2005 and 2007 both had two peaks. The first occurring in August and July respectively and the second taking place in at the beginning of October and mid September respectively. With all the above difference, by the end of October of each year no akinetes were seen in samples from the pelagial.

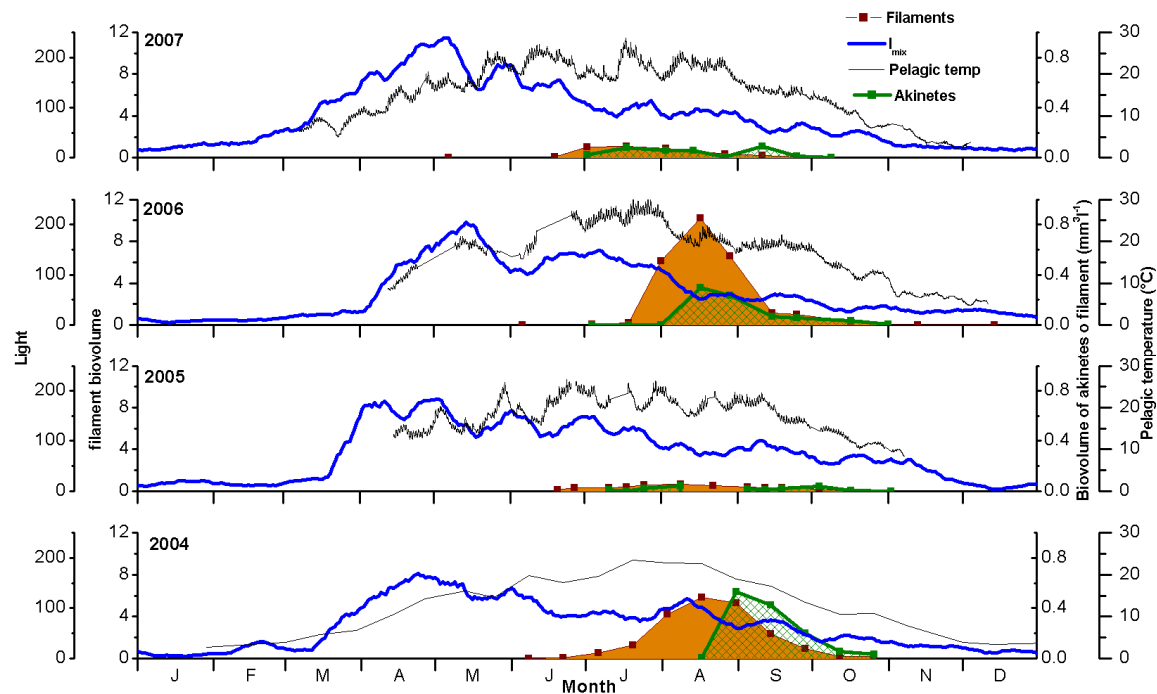


Figure 32: Timing of akinete production in Lake Melangsee from 2004 to 2007

5.4.2 Akinetes horizontal patchiness

C. raciborskii akinetes were found in all samples from the five different sampling points. The average numbers of filled akinetes ranged from 16.6×10^3 to 2.8×10^3 per ml of fresh sediment. Variability in average akinete numbers measured over the five horizontal spatial scales is shown in Figure 33. Differences in maximum and minimum values of akinetes number in all the akinete groups varied within the sampling point. The largest differences were in the numbers of empty envelopes in sampling point five indicated by the standard error. The bars are the averages of the five subsamples counted at each sampling point along side the standard error. The average number of akinetes in samples from point 1 and 2 were almost the same for both the filled and half filled groups but differed within the group of empty envelopes. Point 2 had more than twice the average amount of empty akinetes found at sampling point

1(37.4×10^3 in point 2 to 13.6×10^3 in point 1). Within the three akinete groups, samples from point 3 had less amount of akinetes compared to points 1 and 2 but more akinetes than those in points 4 and 5 in the filled and half filled groups. Sampling point 4 and 5 were the same (16.6×10^3 akinetes) in the case of the filled akinetes but not in the half filled and empty envelope groups.

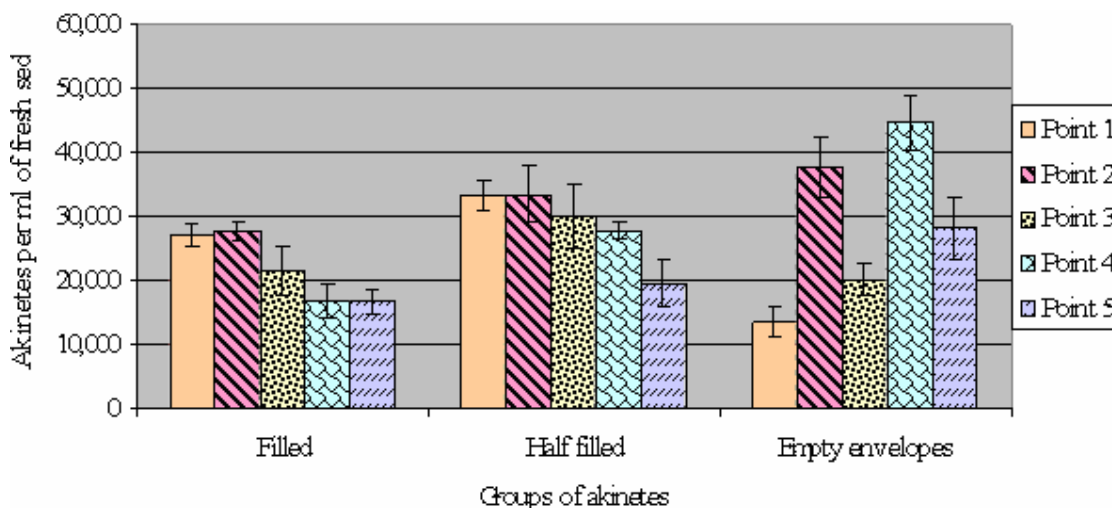


Figure 33: Average number of akinetes counted at the different sampling points. The bars are standard errors.

In all the three groups of akinetes samples from point 4 and 5 appeared quite different from the regular sampling point 1. While the average number of filled and half filled akinetes were less in points 4 and 5 than point 1, the number of empty envelopes were instead more in points 4 and 5 compared to points 1. The Kruskal-Wallis non-parametric test ($P < 0.05$) run on all the groups and the ‘sum of all akinetes’ of the counts gave the results in Table 7.

Table 7: Kruskal-Wallis test results of the different akinete groups and sum of all akinete counts.

	Filled akinetes	Half filled	Empty envelopes	Sum of all akinetes
Chi-square	13.477			8.635
Degree of freedom (df)	4	4	4	4
Asymptotic Significance²¹	.009	.115	0.002	.071

The Kruskal-Wallis gave a p-value of 0.009 and 0.071 for the filled akinete groups and the sum of all the groups respectively while the test results of the Mann-Whitney U test ran on the filled akinete group and empty envelopes is seen in Table 8 and Table 9 respectively. The

²¹ Asymptotic significance (p-value) is a measure of how much evidence we have against the null hypothesis.

highest p-value was recorded in the analysis between points 4 and 5 and 1 and 2 whereas the least other than zero was gotten in the analysis between points 4 or 5 with points 3.

Table 8: Mann-Whitney test result (p-value) on the filled akinete counts

Sampling point	1	2	3	4	5
1	0	0.833	0.175	0.016	0.012
2			0.142	0.009	0.009
3				0.402	0.248
4					0.834
5					0

Table 9: Mann-Whitney test result (p-value) on the empty envelopes counts

Sampling point	1	2	3	4	5
1	0	0.009	0.09	0.009	0.06
2			0.016	0.21	0.30
3				0.009	0.25
4					0.047
5					0

5.4.3 Dynamics of akinetes in the surface sediment and determination of its import from the water column

5.4.3.1 How the annual import of akinetes from the pelagial is reflected in the sediment.

In the year 2006, the water column had more filaments as compared to the years 2005 and 2007. This high biovolume was equally reflected in the number of akinetes in the water column and the sediments in the respective years. There were more akinetes in the sediment of 2006 followed by 2007 and then 2005, following the same pattern of abundance as the filament in the water column. An analysis of the mean akinetes per ml of water in the water column compared to the rate of akinete increment in the sediment gives the results in Figure 35. In these figures which are for the years 2005, 2006 and 2007, we can see that the rate at which akinetes increase in the sediment increases with an increase in the amount of akinetes in the water column. Given that scattered plot is not so clear, a linear trend line through the points for the various years.

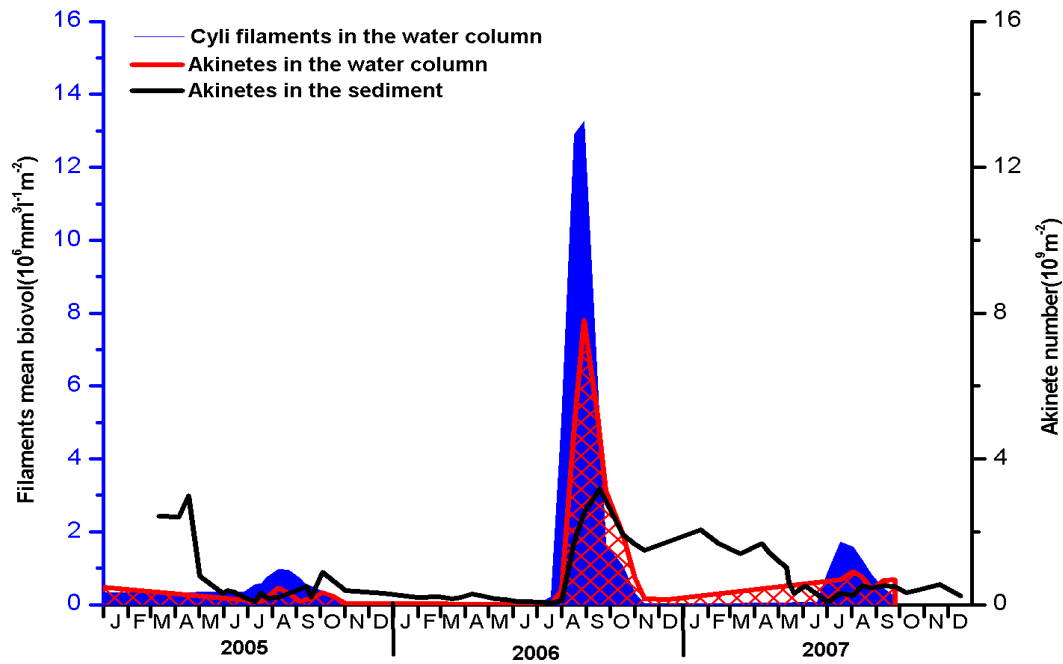


Figure 34: Mean biovolume of *C. raciborskii* filaments and akinetes in the pelagial and akinetes in the sediment between sampling dates

These same results were reflected when the akinete increment rates were plotted against the mean number of akinetes per square meter of the lake (Figure 36).

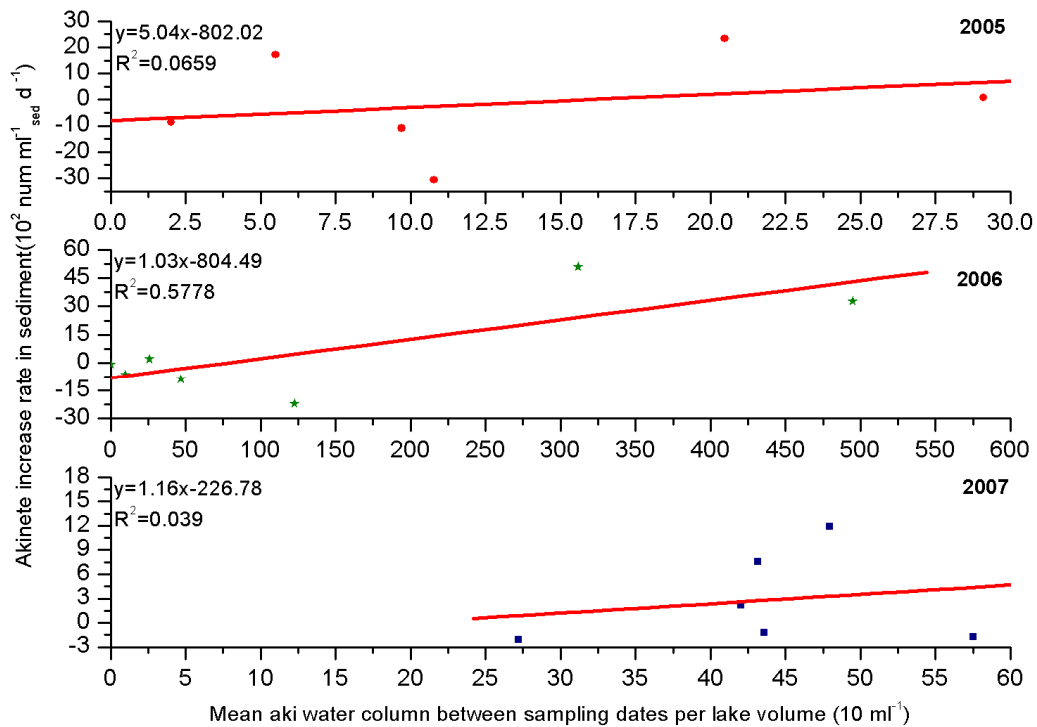


Figure 35: Akinete input from a ml of water column into a ml of sediment for the years 2005 to 2007

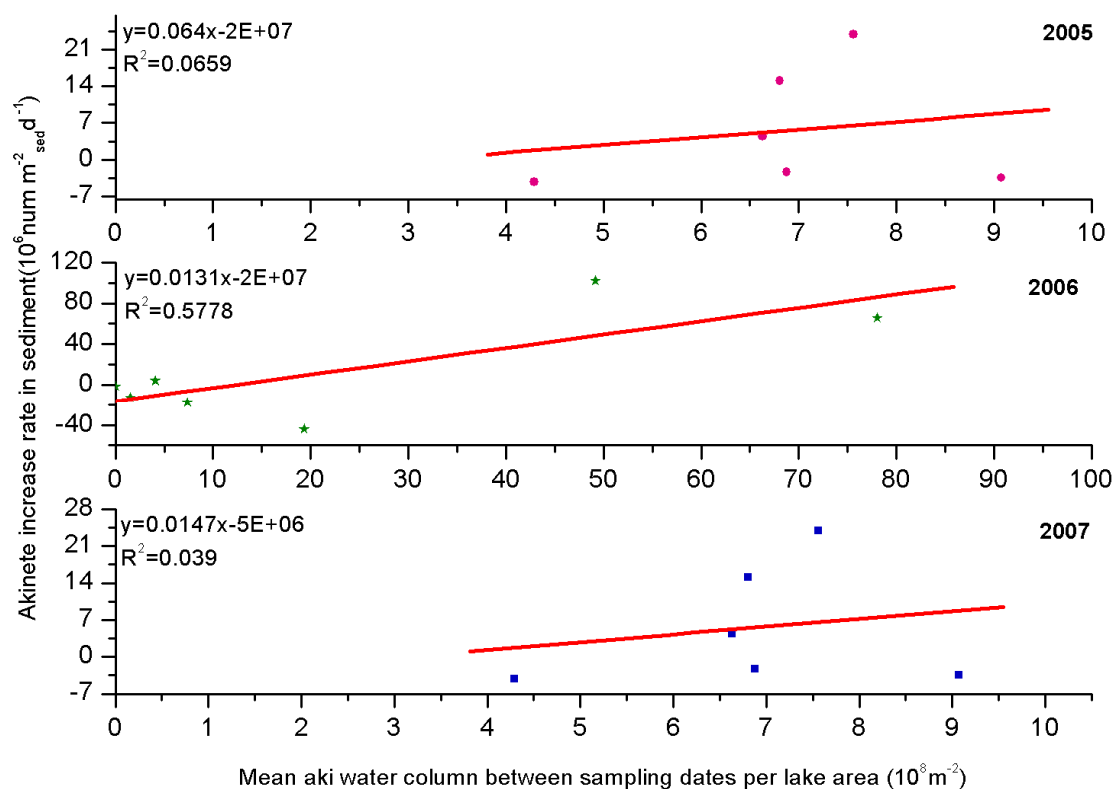


Figure 36: Akinete input per surface of sediment (m²) for the years 2005, 2006 and 2007.

5.4.3.2 Qualifying the role of loss processes on akinetes in the sediment

The fluctuations of akinete abundance in the sediment (Figure 22) over the sampling period indicate that in autumn the akinete numbers are increasing as they settle out of the sediment. They then peak and then start decreasing again within the same winter. We took a closer look at this period between maximum counts of akinetes and decrease due to germination in spring. To prevent redundant data and in an attempt to correct the systematic bias in the data and also to remove the impact of non-biological influences on it (Boer 2004), we normalise²² the data by dividing each value by the maximum value which is the value at the start of the considered period. Two winters which were considered were that of 2005/2006 and that of 2006/2007. The normalised values were plotted (Figure 37) against the number of days from which the maximum number of akinetes were recorded for each winter. To find the parameter values that most closely match the data curve fitting was done. The non-linear lorentzian fit curve to the normalised values gave the results (Table 10) with very low chi square and a goodness of fit (R²) very close to one indicating a very good fit.

²² Normalisation allows the recognition of biological information in data, comparison of data from one array to another and comparison of one microarray platform to another (Boer, 2004).

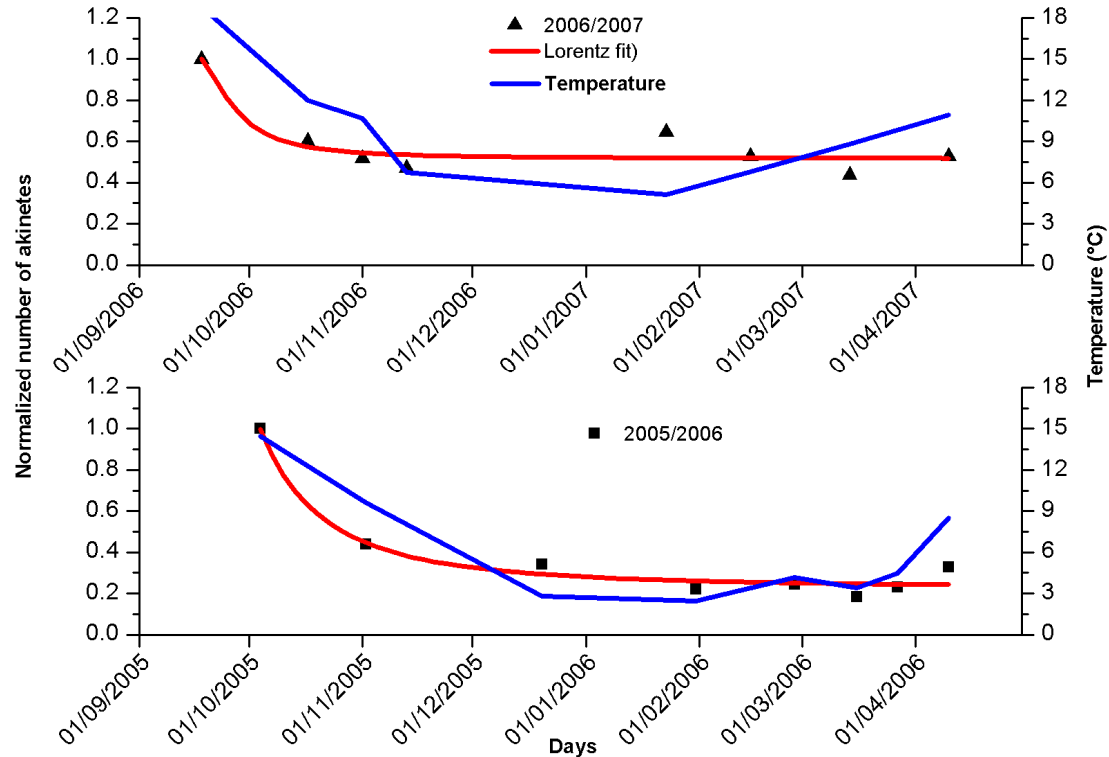


Figure 37: Decrease in the number of akinetes in the sediment with time (days) as from when the maximum number was registered in the lake sediment.

Table 10: Non- linear test results for the akinete loss in the sediment

	Chi square	R ²
2005/2006	0.00398	0.96794
2006/2007	0.00706	0.87247

5.5 *Cylindrospermopsis raciborskii* in relation to native species

Aphanizomenon

Results of the dynamics of *Aphanizomenon* spp. as seen in Figure 38 exhibited the same seasonal pattern like *C. raciborskii* in both the akinetes in the sediment and the filaments in the water column.

Just like *C. raciborskii*, enumeration of the akinetes in the sediment started only in March of 2005 with akinete abundance at that time of the year being 33.9×10^3 akinetes per ml of fresh sediment. Similarly, they were found in the sediment of the lake all year round through out the study period.

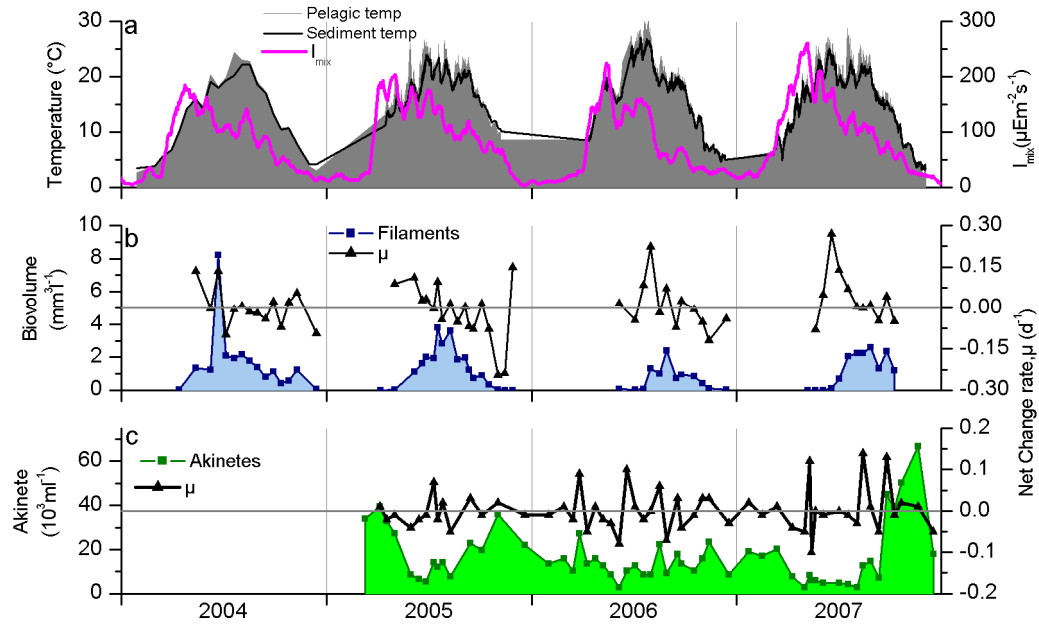


Figure 38: Seasonal dynamics of *Aphanizomenon* spp in Lake Melangsee. (a) Average pelagic temperature and light in the mixed zone (b) Filament biovolume and net rate of change in the water column (c) akinete number and net rate of change in the sediment (Temp= Temperature, I_{mix} =mean PAR at the mixed zone, μ = net change rate)

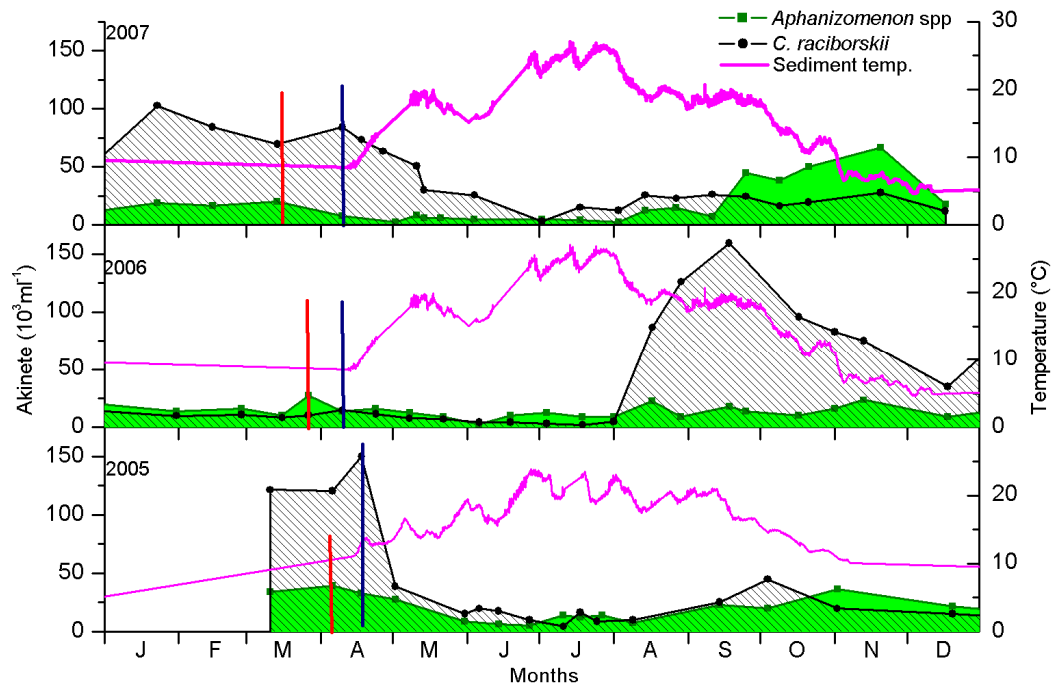


Figure 39: A comparison of the akinete abundance of *C. raciborskii* and *Aphanizomenon* spp. The red and blue vertical line indicates the point of *Aphanizomenon* spp and *C. raciborskii* respectively.

Akinetes of *Aphanizomenon* spp. in the sediment recorded the highest abundance during the study period on the 20th of November 2007 with a value of 66.8×10^3 akinetes per ml of fresh sediment. The year 2004 had the highest biovolume of filament in the water column of $8.21 \text{ mm}^3 \text{ l}^{-1}$ on the 22nd of June 2004. Through out the study period, there was no similarity in the abundance trend between the akinetes in the sediment and the filaments in the water columns for the *Aphanizomenon* spp. But again we can not see so much difference in the population size of *Aphanizomenon* spp. akinete as we see with *C. raciborskii* in the course of the years both within and between the cold seasons. In comparing the invading *C. raciborskii* to this native species of *Aphanizomenon* (Figure 39), we observed that *C. raciborskii* akinetes were more abundant than those of *Aphanizomenon*. During the spring months of March and April of each year, with increasing sediment temperature we also noticed a sharp (indicated by a negative net change rate) decrease in the number of akinetes of *Aphanizomenon* spp in the sediment.

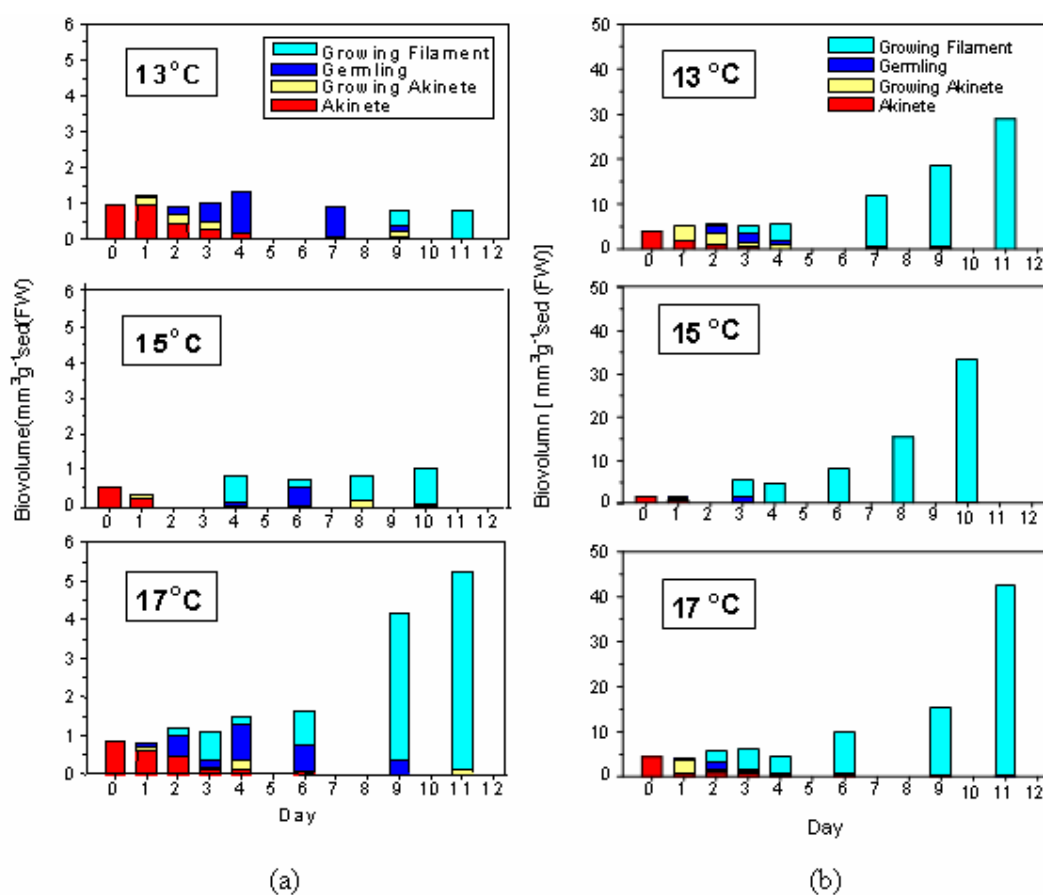


Figure 40: Biovolume of different phases of development (a) *Cylindrospermopsis raciborskii* (b) *Aphanizomenon* species in the course of the germination experiments at different temperatures with sediment of Lake Melangsee.

But it comes earlier in time than that of *C. raciborskii*. For *Aphanizomenon* this decrease took place at the point where the sediment temperature reads about $\leq 12^{\circ}\text{C}$ for all the years.

The laboratory germination experiment with the sediment indicated that just like the *C. raciborskii*, on day zero (Figure 40b) there were only akinetes found in the flasks to be cultured at all three temperatures. After a day of culture at all three temperatures there was already the presence of growing akinetes in all the flasks. But again at the culture temperature of 15 and 17°C we had germlings in addition present in the one day culture samples. By the third day there was already the presence of growing germlings in all the culture flasks.

The difference in abundance was not only with the akinetes in the sediment but also with the filaments in the water column. The biovolume of *C. raciborskii* filaments were more than that of *Aphanizomenon* spp. the summer of 2006 but not for 2004, 2005 and 2007. Filaments of *Aphanizomenon* spp. appear in the water column as early as April but with the time of a greater increase varying with the years. In 2004 and 2005, this sharp increase occurred early in May but for the years 2006 and 2007 these increase was noticed only in July and June respectively. By the beginning of September of each year the filament population in the water column starts decreasing.

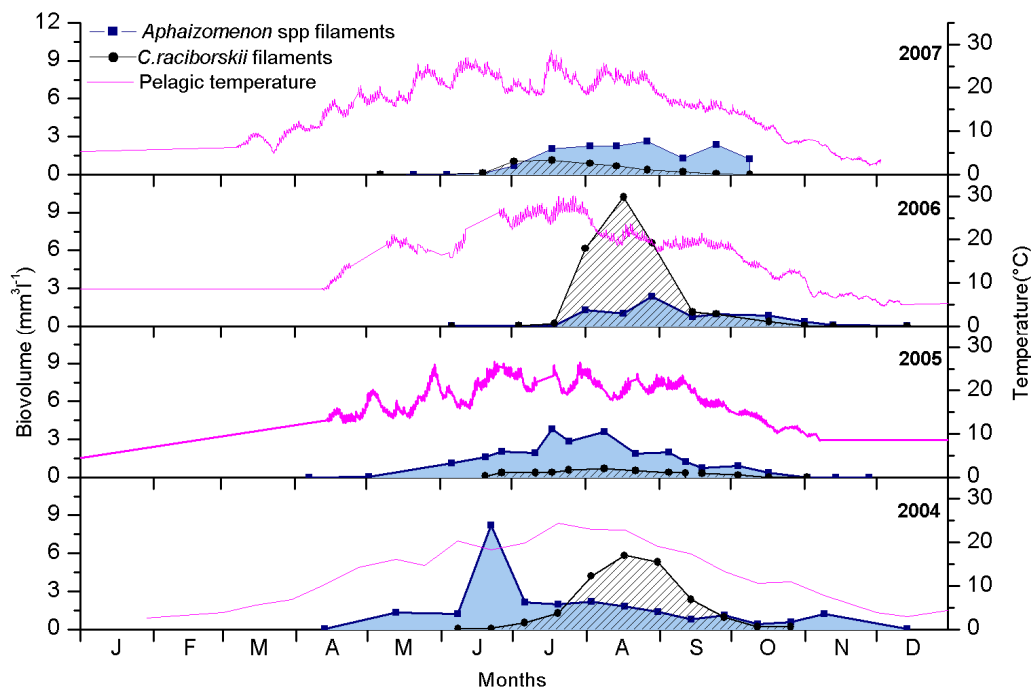


Figure 41: A comparison of the filament abundance of *C. raciborskii* and *Aphanizomenon* spp.

6. DISCUSSION

6.1 Influence of temperature and light on germination of *C. Raciborskii* akinetes

6.1.1 Interpretation of field study results

6.1.1.1 Characterisation of abiotic conditions

6.1.1.1.1 Meteorological factors

Surface air temperature change is a primary measure of global climate change (Hansen *et al.* 1996). According to the mean air temperature values, the summer of 2006 which had the greatest and more stable values could easily be assumed to be the warmest in our sampling period record. But to get a clearer picture it is necessary to translate these values into air temperature anomalies. The reason to work with anomalies, rather than absolute temperature is that absolute temperature varies markedly in short distances, while monthly or annual temperature anomalies are representative of a much larger region. If we hear a temperature (say 15°C), we instinctively translate it into hot or cold, but our translation key depends on the season and region, the same temperature may be 'hot' in winter and 'cold' in July, since by 'hot' we always mean 'hotter than normal', i.e. we translate all absolute temperatures automatically into anomalies whether we are aware of it or not (Schmunk, 2005). The positive air temperature anomaly values represented by the bars above zero imply that a particular month was warmer than normal and vice versa (Figure 16). The year 2004 started off with a colder winter of less than 1°C lower than the normal 1961-1990 value. The spring of that same year was warmer by about 2°C moving on to a summer close to the reference range but which ended up warmer by plus 2°C and then into a warmer autumn. Therefore from all indications, the year 2004 had a warmer spring and autumn but a normal summer. The alternation in the position of the bars the year 2005 indicate that in one month the air temperature was above normal followed by the proceeding month being colder than normal indicate by the bars below zero. This alternation occurring through out the year 2005 had the months April and May temperature anomalies furthest apart. Meanwhile, the alternations with the summer months were not so great. This therefore implies the instability of the weather temperature conditions of 2005. The summer of 2006 was the warmest of all the four years of the study period reaching a peak of 6°C above normal. Autumn and winter of that same year were also warmer than normal to the point of 6°C anomaly recorded for the month of January 2007. The

autumn of 2007 was the coldest of the studied years. The data therefore show warming for the first six months of 2007 and a weak cooling trend for the last six months. These variations in temperature with the years would have some impact on the life cycle *C. raciborskii* which depends on temperature among other things therefore the consequences of these patterns are discussed in section 6.2 below.

6.1.1.1.2. Concentration of Dissolved Inorganic Nutrients and Total Nutrients

Nitrogen is one of the major constituents of living cells (the others being carbon as the main constituent, hydrogen, and phosphorous) and is also a major nutrient necessary for primary productivity. Ammonium (NH_4^+), a major nitrogen species in fresh waters is most easily assimilated by photosynthetic organisms. The sharp decrease in the quantity of DIN (which is mostly made of NH_4) in the lake can be associated with an increase in primary production and growth of photosynthetic organisms. Primary production increase indicates the excystment and rejuvenation of organisms which were dormant during the winter period. For the year 2005 and 2006 this decrease in DIN took place within the last week of March and the first week of April. A corresponding increase in chlorophyll *a* and lowering of the secchi depth (an indication of primary production) followed the decrease in DIN. On the contrary, we cannot tie the decreases in DIN at the end of 2006 to an increase in biomass as temperatures were too low at this point to support life. There might have been primary production taking place or this might have been a methodological error. We suggest that in 2007, rejuvenation of primary producers actually started at the beginning of April when there was a decrease of DIN from $117.85\mu\text{gl}^{-1}$ to $69.8\mu\text{gl}^{-1}$ in the course of two weeks. Ammonia (NH_3) is generated by the heterotrophic (decomposer) bacteria from the decomposition of proteins and other N-containing organic molecules. With decomposition there is the increase in the amount NH_4 in the water as we noticed through out the year at the end of autumn. With encystment and formation of resting cells taking place in autumn, the rest of the vegetative part of organisms die off and are then decomposed by bacteria thereby releasing NH_4 . Moreover there are no organisms at this time to consume the ammonium by chemical oxidation nor is there flushing of the lake.

The low amount of DIP in the lake water can be attributed to the rapid uptake by algae and bacteria. *C. raciborskii* is also a strong competitor for phosphorus (Wiedner *et al.*, 2007). Strains isolated from Lake Balaton had a high uptake affinity and a high storage capacity for phosphorus (Istánovics *et al.*, 2000). *C. raciborskii* was also found to exploit phosphorus very efficiently in Florida lakes, where increases in phytoplankton biomass yield per unit

phosphorus occurred when the species was dominant (Dobberfuhl 2003, Wiedner *et al.*, 2007). In our lake, with the increase in biomass there is just a constant amount DIP. But after the peaks of filament in the water column which indicated the end of biomass increase, we notice the onset and subsequent increase in the level of DIP. *C. raciborskii* needs high phosphorus concentrations for akinete formation (Moore *et al.* 2003, 2005, Wiedner *et al.*, 2007). With the onset of the formation of akinetes on the filament which begins in the middle of August, there is the peaking of DIP and subsequent decrease in the water concluding the fact that our own species of *C. raciborskii* has a high phosphorus demand for akinete formation.

6.1.1.2 Identification of *C. raciborskii*'s germination period in spring from the Long-term study on akinetes abundance in the surface sediment of Lake Melangsee

The akinetes in the sediment exhibited a strong seasonal pattern, reaching a maximum on September 18th 2006 with a count of 159.2×10^3 akinetes per ml of sediment. In all three years, the number of akinetes decreased in spring dropping to about three quarters its peak value within two weeks in accordance with increasing light intensity in the mixed zone and sediment temperature. This decrease was clearly reflected in the net change rate of the akinete number in the sediment drastically decreasing from 0.018 d^{-1} to -0.096 d^{-1} and from 0.007 d^{-1} to -0.174 d^{-1} for the springs of the years 2005 and 2007 respectively in which the akinetes were more abundant in the sediment as oppose to the spring of 2006. Though with fewer akinetes during the 2005/2006 winter, there was still some noticeable decrease in net change rate in the spring of 2006. We therefore attribute this decrease in the number of intact akinete numbers in the sediment in spring and a negative net change rate to germination of akinetes. From the long term result, it can be noticed that at some points in the course of the year the number of akinetes decrease other than in spring. From this we say that not in all cases would a decrease in the number of akinetes be as a result of germination. This because other factors such as environmental come into play to initiate germination (see section 6.1.1.3 below) and a decrease in biomass in an environment can be attributed to other factors which we would look into in section 6.4.3 below.

6.1.1.3 Some abiotic conditions which trigger akinete to germinate

Temperature

In spring of the year 2005, (Figure 22), the rapid decrease in akinetes numbers from 1.5×10^5 akinetes/ml to 0.4×10^5 akinetes/ml in the samples from the 18th of April to that of 2nd of May

which we have attributed to germination took place when the sediment temperature increased from 12.5°C to 14.5°C. This same pattern was observed in 2006. Though with not so many akinetes in the sediment, there was still a decrease in akinete number from 1.2×10^4 to 6.5×10^3 akinetes/ml in samples from the 24th of April (sediment temperature of 13°C) to the 8th of May (sediment temperature of 17°C) respectively. As for the year 2007, there was a decrease (from 8.4×10^4 to 7.5×10^4) in the number of akinetes in the sediment from the 10th to 18th of April. This decrease corresponded to a sediment temperature increase from 11°C to 16°C. According to Moore (2004), germination occurred in all temperature (15-30 °C). But in our case this decrease which we suggest to be as a result of germination was noticed when sediment temperatures got to around 13°C for all the years. Though there was a noticeable decrease in the number of akinetes in the sediment from within the winter period of the sampling years, we cannot link it to germination since the corresponding temperature at this time are between 3°C and 9 °C, temperatures at which germination cannot occur. We can therefore say that akinete germination occurs between 13°C and 20°C sediment temperature in the temperate Lake Melangsee.

Light intensity

Increasing temperature in the temperate region is linked to light intensity but dependent on turbidity of the water. The light first increases followed by an increase in temperature. As mentioned above light plays a role second to temperature. During the periods of akinete germination (April and May) in 2005, 2006 and 2007, the light in the mixed layer measures between 129 and $190 \mu\text{Em}^{-2}\text{s}^{-1}$ (Figure 22). The range of 129 to $190 \mu\text{Em}^{-2}\text{s}^{-1}$ correspond to the temperature of 13 to 15°C which implies that at this light intensity after winter, germination is expected to start but moreover at an appropriate temperature. Other investigators have suggested that light might be more important in determining the timing and magnitude of recruitment, provided that temperature is above some threshold. The importance of an increase in light in initiating recruitment has been suggested by previous authors for *Gloeoetrichia echinulata* (Barbiero, 1993) and *Anabaena circinalis* (Reynolds, 1972). In addition to its probable role in initiating akinete germination, the dependence of recruitment on a period of high light also suggests an initial period of growth on the sediments, as appears to be the case with *G. echinulata* (Barbiero, 1993). Our study gives something of the contrary in that the filaments start appearing in the water column only after the highest peak of light conditions in the pelagic. Once the amount of light starts decreasing after the peak and getting to about $150 \mu\text{Em}^{-2}\text{s}^{-1}$ we then notice their presence. We can therefore say after germination on the sediment the filaments starts migrating only when the amount of light starts decreasing.

An initial period of growth on the sediments prior to recruitment into the water column would lead to two potential adaptive advantages. First, attaining larger size prior to entering the plankton could reduce grazing losses due to herbivory, as hypothesized by Lynch (1980). Second, it would allow for uptake and storage of P while the cells are in contact with the P reserve in interstitial pore water (Barbiero and Kann, 1994). This shows that germination starts only when the temperature is above a threshold level with the aid of a particular level of light intensity after the winter period. But after the water body has gotten the required temperature, a decrease in light intensity in the mixing zone triggers upward movement. Baker and Bellifemine (2004) stated that in akinetes there is a link between germination and photosynthesis; however the initial response by akinetes to light exposure is respiratory oxygen uptake. And that while photosynthetic activity provides the energy for akinete germination, the rate of germination is determined by the respiratory activity of the akinetes, which is dependent largely on the optimum temperature. This generally corresponds to the optimum temperature for cell growth. This confirms the fact that seasonal increases in water and sediment temperature could therefore provide the trigger for the recruitment of populations from the benthic resting stages, more so than increased light availability (Baker and Bellifemine, 2000).

According to Moore (2004), germination occurred at all temperatures (15-30 °C), light intensities (50–400 $\mu\text{Em}^{-2}\text{s}^{-1}$) and varying phosphorus and nitrogen concentrations investigated, except in the absence of light. But in our case this decrease which we suggest to be a result of germination was noticed when sediment temperatures got to around 13°C for all the years and light in the mixed zone of about 120 $\mu\text{Em}^{-2}\text{s}^{-1}$. Through out the study period light at the sediment only got a maximum of 24.8 $\mu\text{Em}^{-2}\text{s}^{-1}$ making us to dismiss the effect of light on germination in the field and only focus on the temperature at the sediment. Furthermore Moore (2004) stated that from his laboratory experiment results that, under conditions of higher temperature and light intensities germination increased. From our results in 2007 we saw a sharp decrease in akinete number in the sediment between the 10th and the 18th of April when the sediment temperature had risen to 18°C on May 9th. But interestingly to Moore (2004) from his laboratory experiments, a greater reduction of the akinete concentrations in culture was observed with increasing light intensities. In our field experiment we cannot say with certainty that light intensity had an effect on the timing of germination as light at the sediment was 20 $\mu\text{Em}^{-2}\text{s}^{-1}$ way less than the interval of 50–400 $\mu\text{Em}^{-2}\text{s}^{-1}$ suggested by Moore (2004) in his work. Never the less light in the mixed zone was in this range having an indirect effect on the sediment in heating up the water column eventually heats up the sediment.

6.1.2 The use of laboratory experiments to further identify the temperature range in which akinetes germinate

The different groups based on morphology into which *C. raciborskii* was separated indicated some stage of development. Those that fell into the akinete group were just the normal mature akinetes. Growing akinetes were attributed to those showing early signs of germination, in being longer with their endospore separated from the exospore. Germlings and growing filaments were trichomes less than 30µm and above 30µm in length respectively. The presence of only the mature akinetes on day zero served as control to this experiment that no germination was taking place prior to the incubation. At all the temperatures there was an indication of germination within the first days of incubation by the presence of growing akinetes. At all three temperatures there was complete growth by the end of the incubation period but the point was how successful was the germling growth after germination. One important aspect of the germination observed was that germlings of *Cylindrospermopsis* hatched out of the envelope of the akinetes at the 13°C temperature but did not show any significant further growth. Filaments appeared in the 13 °C sample only on the 9th day of incubation. On the contrary at 17°C following germination, we witness an increase in the biovolume by the 6th day. We therefore suggest that *Cylindrospermopsis raciborskii* germinates at temperatures as low as 13°C but if maintained at this temperature the population would die out. Further increase in temperature to at least 17°C is important for this species to survive.

6.1.3 Estimated duration of the recruitment period of *C. raciborskii* from the sediment into the water column

Germination indeed took place within the two weeks of running the experiment indicated by the appearance of the different phases according to Table 5 within this period. The endospores emerged from the exospores just within two day after the start. There is quite some time between the start of germination and the appearance of germlings in the water column of up to 6 weeks. Germination starts in the middle of April and according to the result of 2006 (Figure 31), the germlings less than 30µm in length and those greater than 30µm in length only appear at the beginning of June. But in our beaker every akinete /filament is arrested there and cannot leave to the open water. At the start of the experiment there was already indication of initiation of germination with the phase 2 akinetes (Figure 24) being present. But again it took just 12 days (23rd of April) for the mature filament (phase 5) to be present in the beaker. Since there was the absence of phase two in the water column, we can say that this stage of

development takes place in/on the sediment. A good number of previous studies have indicated that germination is followed by a potential growth phase on the sediment before migration to the pelagic (Osgood, 1988, Barbiero and Kann, 1994; Karlsson, 2003; Karlsson-Elfgren and Brunberg, 2004). A good example is what happens in the life cycle of another cyanobacterium *Gloeotrichia echinulata* where the switch between the benthic and pelagic life stage is preceded by germination of the akinetes, followed by a period of growth on the sediment before gas vesicles are formed and the new colonies migrate up into the water (Barbiero, 1993, Karlsson-Elfgren and Brunberg, 2004). In this light, we can suggest the absence of gas vesicles in the phase 2 and phase 3 akinetes but present in phase 4 (germlings <30µm in length) and 5 as they were identified in the water samples from the water column. In our beaker, these young germling appear after five days of initiation of germination. By estimation, since the time between initiation of germination and the filaments appearance in the water column is approximately 50 days and it took five days from onset of germination for phase 4 to be present in the beaker, we can say that recruitment of these filaments into the water column through formation of gas vesicles takes about 45 days. But more work in measuring direct recruitment with traps in the lake is needed to throw more light on the timing and duration of this process.

6.1.4 Timing of the initiation of akinete germination

The time for the onset of akinete germination should depend on the conditions reigning in the lake at a particular time. Since we have seen above that temperature is the most important factor, it means that the point at which germination is initiated is the point at which the required temperature is attained. In our study area temperatures start increasing in spring and the temperature of 13°C which we observed initiates germination from the laboratory experiment is usually attend towards in the middle of April to the end of May. Therefore, it can be said that the time at which germination occurs is towards the end of April and the month of May but all these depending on how warm the year is or at what time the sediment attains this temperature of 13°C. A look at Figure 42 (which is an out look of the germination periods of Figure 22) it can be seen that the required temperature of 13°C for germination is reached in the sediment on different days in the different years. This was reached on Julian day number 108, 114, 104 which correspond respectively to the years 2005, 2006 and 2007. A difference of timing of 10 days occurred between 2007 and 2006 while between 2006 and 2005 it was 6 days.

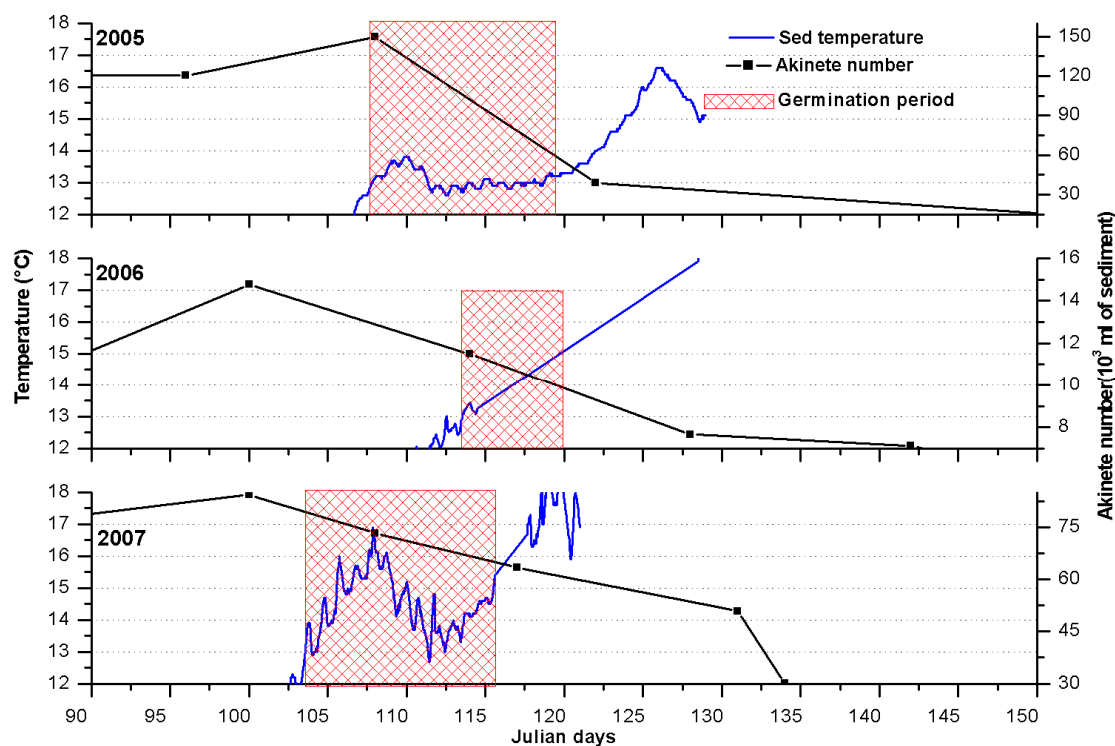


Figure 42: Timing of akinete germination during the different sampling years and sediment temperature (An outlook of Figure 22)

Even though there is some decrease in the number of akinetes prior to attending the 13°C mark in all the years we cannot attribute this to germination since it has already been proven in the laboratory and nowhere in literature, that germination cannot take place at such low temperatures (below 13°C). The sharp decrease in the number of akinetes after the required temperature has been attained indicates germination and the levelling off after this decrease indicates the end of germination. We can see in Figure 42 that the onset of the germination period varies with the year. In 2007, in which the sediment got the required temperature (13°C) earlier as compared to 2006, had the onset of germination just two days earlier than the year 2005. Interestingly the year 2006 which was a very warm year from April had its onset of germination eight days later than the year 2005. This indicates the variation in the timing of germination which mostly depends on temperature as indicated by the partial least square analysis. Should we have warmer springs this timing would also shift to become earlier in the year. It is therefore a fact that the temperature of the lake sediment is a great indicator of the time when akinetes would germinate. Once the point of 13°C is attained and should the temperature continue to increase one should expect to find filaments in the water column within 50 days there.

6.2 Influence of light and temperature on population dynamics of *C. raciborskii* in the water column

6.2.1 Characterization of this influence from the long-term field study

Just like the environmental variables, *C. raciborskii* displayed strong seasonal and interannual temporal variations. During the four years-long sampling period described in the present study, the abundance of *C. raciborskii* filaments was out of the range of values previously reported for the Melangsee (Wiedner *et al.*, 2007). But, the seasonal pattern, which was characterized by a maximum occurring during the warm season and lower values in the autumn, was similar to those observed during a sampling period from 1995 to 2000 (Wiedner *et al.*, 2007). However, the maximum biovolume value ($10.23\text{mm}^3\text{l}^{-1}$) observed in the current study in 2006 was 10% higher than in 1999 ($9.2\text{mm}^3\text{l}^{-1}$) (Wiedner *et al.*, 2007). Our results suggest that the abundance of this species in the lake varies with years which can be linked to the environmental conditions in which it is found. The maximum net change rate recorded during this study period was 0.26d^{-1} . Saker and Griffiths (2000) found that *C. raciborskii* from isolates in culture had a maximum growth rate of $0.9\text{--}1.2\text{d}^{-1}$ at its optimum temperature ($>25^\circ\text{C}$) and a maximum growth rate of only $0.3\text{--}0.5\text{d}^{-1}$ at 20°C . A fact supported by Ryan and Hamilton (2003). In our lake the net change rate highest value registered at a temperature $>25^\circ\text{C}$ was 0.26d^{-1} which fell out of range of that stated by Saker and Griffiths (2000) in his culture experiment (where they have almost no loss processes as we have in the field) and Ryan and Hamilton (2003) in his *in situ* study on this species in subtropical, shallow lakes. This suggests that *C. raciborskii* populations did not grow as rapidly following their first detection in summer in temperate lakes as did in the subtropics under the same temperature conditions.

In temperate climates according to Grover and Chrzanowski (2006), seasonal variations of temperature and other environmental variables are apparently large enough to produce predictable changes in phytoplankton composition against a background of hydrological variability. A direct effect of temperature on algal growth might explain summer dominance by cyanobacteria. But in our study we realized that even though through out the four years the temperature in the mixed zone at some point were at or above 25°C during the summer season there was annual temporal variability in filaments abundance. It was not the same with all the summers. Some summers had higher filaments abundance than others. We therefore infer that the stability of this required temperature for a period of time is required for the success and

multiplication of the vegetative filaments and that they have other adaptations permitting abundance despite nutrient depletion. The nutrient supply had evidently little or nothing to do with the dynamics of the cyanobacteria. In this wise we realize that the year 2004 and 2006 which had a very stable water temperature during the summer realized a higher abundance of filaments in the lake than the 2005 and 2007 which had fluctuating water temperatures. The summer abundances found in 2004 and 2006 were 3 or 4 orders of magnitude higher than the 2003 and 2007 values.

6.2.2 Influence of abiotic factors on population growth of *C. raciborskii* studied by Partial Least Square (PLS) Statistical analysis

According to Padisák (1997) and Briand *et al.* (2002) the occurrence of *C. raciborskii* blooms depends mainly on climatic parameters: water and air temperature and hours of sunshine. In addition to the above three, mean PAR, global radiation, wind direction, maximum wind and average wind which were considered for the PLS analysis, 26 different models could be derived when all the predictors were considered. For each models, different percentage of explanation were gotten with the highest having a coefficient of determination, $R^2=0.44$. This best model which we would consider further is the one which could explain the highest, 44% of the variation in the biovolume with dependence on all the physical factors considered. Of all the factors considered the most important contributors which bore high weight to this model were the variables water temperature, air temperature and global radiation (Figure 27) with air temperature and global radiation carrying more weight than even water temperature. Further analysis to depict which condition could most influence the model still indicated that the variables air temperature and global radiation are the most influential variables (Figure 28) that really define the model considered.

Following the backward elimination, the four most important predictors of *C. raciborskii* identified from the coefficient of determination values, R^2 , were identified as average of seven days I_{mix} , seven days global radiation, five days air temperature and five days water temperature (Table 6). All could explain 40 % of the variability given an R^2 value of 0.40. But when the 5 days average in air temperature was taken out the R^2 value increased to 0.43. All the remaining three were in this light responsible for 43% of the *C. raciborskii* biovolume variation. Meanwhile, 5D water temperatures / 7D global radiations and the 5D water temperature / 7D I_{mix} were responsible for 25% and 24 % respectively of the variation. These were almost equal but lower than what the three together gave as R^2 . Nevertheless it was the five days water temperature which had the greatest influence on the biovolume variability

according to Figure 30 indicated by a high regression coefficient of about 1100. Should all other factors be at a normal level knowing the water temperature of a lake, we can use to predict the population changes on the sixth day following the measurement.

This confirms observations by Padisak (1997) and Briand *et al* (2002) that water temperature is a key factor to cell multiplication and to add to this is the fact that visible changes can only be observed within five days of the temperature change.

This key observation can be confirmed by the observation that the year 2006 which had a high average air and water temperature had a corresponding high population of *C. raciborskii* just as the year 2004 (Figure 26). But in the years 2005 and 2007 with a lower average daily and water temperature as compared to 2006 and 2004 also had a lower population of in the lake compared to 2006 and 2004. It is important to note that global radiation and light intensity in the mixed zone (PAR) comes second in place to water temperature according to our analysis. This fact was observed in 2007 which had the highest maximum PAR during the sampling period but still the population was not so high as compared to the year 2006 because of its lower reigning summer temperatures.

6.3 Development of germlings and young filaments.

6.3.1 Detailed quantification during the field study 2006

The annual seasonal dynamics of *C. raciborskii* in Lake Melangsee can be divided into two stages (Figure 25). In the first stage (May, June and July of 2005 and 2006) mass development of *C. raciborskii* filaments and a second stage in which filaments with akinetes occur in the water column (2006-Figure 31). During the first stage, the number of akinetes in the sediment does not increase. The first stage can be linked to the germination of akinetes in the sediment as seen clearly in 2006 of Figure 31 at the beginning of June. In a study by Reynolds (1974), he observed that the akinetes of *Anabaena*, are episodically resuspended into the open water column where germination eventually occurs after a certain minimum temperature and/or insolation requirement has been satisfied. Despite the fact that in this study carried out in 2006, the temperature in the mixed zone at the beginning of June was already a 15°C, two degrees above the temperature of 13°C at which we found germination to take place, we never found any growing akinete with elongating envelope (Phase 2) nor growing filament emerging from the akinete shell (phase 3) in our samples. Phase 2 and 3 is an indication of akinetes germination. We therefore say that just like the cyanobacterium *Aphanizomenon spp* (Barbiero and Kann, 1994) and *Gloeotrichia echinulata* (Karlsson-

Elfgren and Brunberg, 2004), *C. raciborskii* germination takes place in the sediment and then the phase 4 stages are recruited into the water column. The absence of the phase 2 and 3 in the 2006 water samples confirm the fact that this stage of akinetes germination takes place in the sediment and there are no gas vacuoles present at this stage of development to move them into the water column. Though the phase 3 germlings eventually grow into the growing filaments of less than 30 μ m (phase 4), these short filaments are supposed to appear first in the water column before the longer ones (phase 5) given the fact they are first formed. But for the fact that they all appear in the water column at the same time implies that in the course of phase 4 being recruited from the sediment to the water column they also grow in length. Otherwise it might also imply that the two weeks interval between sampling might have been too long to conclude that they appear at the same time therefore leaving room for further investigations on a daily basis following germination which we carried out in the field in 2007. Nevertheless, the longer filaments greater than 30 μ m peak after those less than 30 μ m given the fact that in addition these longer ones developing from the shorter ones, these longer filaments also undergo fragmentation leading to the increase in biovolume and a later peaking. It is worth noting that fragmentation is only possible when the conditions in the water column are favourable for its multiplication as the investigator Singh (1962) said that the chemical nature and the high temperatures (28-30°C) of water are responsible for the development of blooms.

6.3.2 Determining the time and the place of transformation between the different growth phases.

The 2007 experiment carried out with beakers had one harvested every two to three days for examination. As noted before all the akinetes and filaments were arrested in the beakers and could not leave to the open water. In it the germlings less than 30 μ m and those above 30 μ m were present in the beakers harvested five and twelve days following the onset of germination respectively. This implies that it would take at least a week for the young germlings emerging out of the shell to grow and reach at least 30 μ m in length. But again in the field, in 2006 the time between the onset of germination and the appearance of the filaments in the water column is at least 45 days. Within two weeks from the onset of germination in the sediment no filaments were noticed in the water column. This leads us to conclude that in the course of movement of the germlings less than 30 μ m from the sediment to the water column, they also increase in length too thus we find both appearing in the water at the same time. As such a fortnightly sampling of the column of a water body is not too long but again certain stages following germination are missed out. These early stages we suggest could be more

vulnerable for target in case of trying to kill the cyanobacterium. More studies at the level of vertical recruitment are necessary to know at which depth this transformation from phase 4 to 5 takes place. Knowing the time and point of transformation could be of great help to control these cyanobacteria should they become a nuisance.

6.4 Relationship of akinete germination to the developing population in the pelagial

With temperature being a crucial factor in the regulation of the onset of the pelagic population (Wiedner *et al.*, 2007), the germination of akinetes plays a role in the population in the pelagic. At the start of the sediment sampling towards the end of the winter 2004/2005 the akinete maximum was almost as high (150×10^3 akinetes per ml) as that of the winter of 2006/2007 (160×10^3 akinetes per ml). On the other hand in the winter of 2005/2006 (44×10^3 akinetes per ml) the akinete maximum in the sediment was less than the previously mentioned winters. But this relatively small amount of akinetes in 2005/2006 gave rise to a highest amount of filament biovolume during the sampling period in the summer of 2006. Unlike the previous case of 2005/2006, the great amount of akinetes in the sediment in the winter of 2004/2005 and 2006/2007 did not give a corresponding high pelagic filament biovolume in the following summer. Therefore the *C. raciborskii* population in the pelagial is not only dependent on the amount of akinetes in the previous season but on germination which is also dependent on the condition reigning in the lake. Implying that, the reigning temperature following germination of akinetes determines the population in the pelagial. The year 2006 was a warm year and stable (Figure 15) as compared to the that of 2005 and 2007 with temperatures during the most part of summer staying above 20°C and we can see that during this warm year there was a corresponding high pelagic population. But in 2005 with the alteration in temperature around the 20°C this lead to the *C. raciborskii* population not doing well likewise with the year 2007 which had the average temperature below 20°C resulting in a low population of *C. raciborskii*. Definitely the growth conditions after hatching of the akinetes influences on the success of *C. raciborskii*'s further development.

Some investigators concluded from culture experiments that, conditions favouring *C. raciborskii* growth include warm surface waters of over 25°C, seasonal warming of hypolimnetic waters (>23°C), low light conditions (Briand *et al.* 2004), highly stable thermally-stratified water columns (McGregor and Fabbro 2000, Dokulil and Mayer 1996), and environmental consistency (McGregor and Fabbro 2000). The different periods (Figure 21) following germination in the sampling years were the summer months of June, July and

August. Given the fact that during these months in 2005 to 2007 had periods of temporal stratification we would have expected *C. raciborskii* to grow very well in all the summers but that was not the case. This could have been one of the reasons why the population did not do well during these years compared to 2006. This we say is as a result of the fact that in the summer of 2006 the bottom water temperature stayed constantly at least 23°C, a temperature at which Briand *et al.* (2004) noted to be one of the requirements favouring *C. raciborskii* growth and that the duration of temporal stratification periods in June and July was longer compared to 2005 and 2007. We therefore suggest the higher water temperature in 2006 and high thermal stability favoured *C. raciborskii* growth.

We can rightly say that the fluctuating temperatures of 2005 and the low temperatures of 2007 early in spring may have destroyed the emerging or naked germlings which are supposed to be more sensitive to temperature stress than the adult or the growth rate was lower than loss rate. Moreover the temperature and stability of the water column following the filaments appearance in it is very important as it would determine the population growth of *C. raciborskii* population. This ties to what we saw in the germination experiment that for naked germling develop to full and healthy adults, a constant rise in temperature and temperatures above a certain limit is necessary. Therefore the daily mean temperature in the water column should be at least 23°C in order to have a high population of *C. raciborskii* in the water column.

6.5 Akinetes formation in the pelagial and their import into the sediment

6.5.1 Factors that trigger akinete formation in the pelagial

The precise factors that induce akinete formation remain obscure. In some species akinete formation seems to be triggered by phosphate deficiency, whereas in others a lack of energy (as light) seems to be responsible. In yet another species the differentiation of akinetes is induced by particular organic compounds that are themselves secreted by akinetes, so that akinete formation is an autocatalytic process (Hoek *et al.*, 1995). So far no autocatalytic process has been reported in the case of *C. raciborskii*. In *Anabaena* sp. factors including phosphorus limitation (van Dok and Hart, 1996), temperature (Li *et al.*, 1997) and light limitation (Fay *et al.*, 1984) have been associated with this process of akinete differentiation.

Fluctuations in temperature remain an important trigger for akinete differentiation in all isolates of *C. raciborskii* (Moore, 2004). In his laboratory experiment with an Australian

strain of *C. raciborskii* (AWT 205/1), Moore *et al.* (2003) found that temperature fluctuations in magnitude and frequency are involved in triggering akinete differentiation. In magnitude, Moore *et al.* (2003) in his laboratory work saw the formation of *C. raciborskii* akinetes when temperatures were dropped from an initial 25°C to 15°C and 20°C. The differentiation occurred approximately 10 days after this temperature shocks. A similar time frame observed by van Dok and Hart (1996) in *Anabaena circinalis*. From our field results, if we look back 10 days in time from the first appearance of akinetes on the filament we note that, our lake temperature was around 22°C and peaked at around 19°C. Though through out the years the akinetes appear ones temperatures in the mixed zone get to 22°C, a sharp increase only occurs when it has dropped below 20°C a temperature at which Li *et al.* (1997) reported the development of akinetes in *Anabaena* strains. We therefore suggest that, 22°C trigger the differentiation of akinetes on the filaments in our lake.

But again it is suggested that if isolates were to experience a single temperature fluctuation, then a small number of akinetes would differentiate and that however, more akinetes would differentiate should the organism detect frequent temperature fluctuations of increasing magnitude (Moore *et al.*, 2005). The greatest akinete differentiation occurring when *C. raciborskii* experience multiple nightly temperature shocks (<5°C) (Moore, 2004). During our study there were several temperature fluctuations in the summer of 2005 and 2007 and a single one in 2004 and 2006. We had water column temperatures decreasing from 23°C to 18°C within 12 days and back to 23°C in another ten days. Contrary to Moore *et al.* (2005), not as many akinetes were produced in 2005 and 2007 with its many fluctuations as compared to 2004 and 2006 a single decrease in water column temperatures. From this we say that though the fluctuation frequencies might play a role in the amount of akinetes produced, again the length of this fluctuation frequencies matter. If these fluctuations were within a single day as suggested by Moore *et al.* (2005) then more akinetes would be produced but if it is within a season with we would find less akinetes being produced. With the 2005 and 2007 fluctuating temperature around the 20°C mark we also noticed a fluctuation in the akinete differentiation identified by the filaments peaking twice between August and September of each year.

Light was also noted to be of extreme importance for akinete differentiation in *C. raciborskii*, with the quantity of light determining the success of the process (Moore *et al.*, 2005). Moore (2004) found the akinete differentiation occurring when *C. raciborskii* was provided with light intensities of $100\mu\text{Em}^{-2}\text{s}^{-1}$. He later reported that at this light intensity the akinetes produced were degraded and suggests the light being insufficient to support its full

development. Just like we did for the temperature, when we look 9 days before the appearance of the akinetes, on such days the light conditions at one meter depth vary with the years between $115\mu\text{Em}^{-2}\text{s}^{-1}$ in 2004 and $185\mu\text{Em}^{-2}\text{s}^{-1}$ in 2005. Again we can suggest that temperature is more important than light when it comes to the amount of akinetes produced. This becomes more evident in that even though 2005 and 2007 had almost the same amount of light prior to the onset of akinete productions as 2004 and 2006, a lower biovolume of akinetes were produced in the previous years as compared to the later (Figure 32).

Moore (2004) also found the greatest akinete differentiation at phosphorus concentrations of $> 70 \mu\text{g L}^{-1}$. Meanwhile laboratory experiment with the Australian strain of *C. raciborskii* (AWT 205/1), Moore (2003) found that phosphorus limitation of 0 and $2\mu\text{gl}^{-1}$ did not trigger akinete differentiation but in a concentration of 22 and $55\mu\text{gl}^{-1}$ there was its production when temperatures were constant at 25°C . At that low phosphorus concentration (0 and $2\mu\text{gl}^{-1}$), akinetes were observed at some point and was attributed error occurrence of nightly temperature shocks. However, in our lake Melangsee field study we observe that through out spring and beginning of summer of each year, the amount of DIP remained below $10\mu\text{gl}^{-1}$ but an increase in DIP in the months of July always coincided with the onset of akinete differentiation. The least we had during the four years was $2.60\mu\text{gl}^{-1}$ at the end of April 2006 which is germination period. Again in each year within a month prior to the first identification of akinetes in pelagic, the DIP was at least $5\mu\text{gl}^{-1}$ agreeing to what Moore *et al.* (2005) noted in his laboratory work as $5 \mu\text{gl}^{-1}$ being the threshold. We can therefore rule out phosphorus deficiency to be the cause since through out the study period dissolved inorganic phosphorus concentrations were never below $2\mu\text{gl}^{-1}$. Fay *et al.* (1984) noted availability of phosphorus being an important factor required for akinete differentiation but again Li *et al.* (1997) found in *Anabaena* sp. that temperature was the most important environmental factor causing this.

These conditions supported akinete development, maturation and cell growth. The identified triggers supporting maximum akinete differentiation in the AWT 205/1 (Sydney) temperate isolate did not support the formation of similar akinete concentrations when applied to a tropical (Rockhampton) and subtropical (Brisbane) isolate of *C. raciborskii* (Moore, 2004). And so we also see some difference in the condition of formation of akinetes in our lake. But again more laboratory work with isolates from our lake would give a definite picture.

Based on our findings, the success of akinete differentiation in natural populations is controlled by multiple water temperature changes coupled with suitable light intensities and phosphorous concentrations. Because the time at which these condition occur the lakes vary

with the years, we see a difference in the time at which the akinetes first appear in the pelagial. As noted before the akinetes starts being formed when the population maximum is exceeded. With the vegetative population maximum being reached ones temperatures starts decreasing implies the akinetes too start formed at such points.

6.5.2 Akinetes horizontal patchiness on the lake sediment

From the graph, the standard error bars of point 2 and 3 in the intact akinete group overlapped with that of point one. Those of point 4 and 5 do not overlap with that of point 1. The error bars (Figure 33) are not so wide for the 'filled' and 'half filled' but for the 'empty envelopes', we can therefore be confident our values representing the true value. Though upper error bar for the different sampling points overlap each other in a not so similar pattern for the different groups of akinetes, we cannot say with certainty that they do not differ significantly. However, we are confident that there is a significant difference in some cases.

With the statistical test ran on the different groups into which the various akinetes were separated into we got p-values of 0.009, 0.115, 0.002 for the filled, half filled and empty akinetes respectively (Table 7). This implies that there is some significant difference (p-value is less than significant level of 0.05) among the filled akinetes and the empty envelopes counts from the various points and not among the half-filled. But when we ran this same Kruskal-Wallis test on the sum of all the akinetes, it indicated no significant difference among the points ($p=0.071$).

The Mann-Whitney U statistical test results run on the filled akinetes count indicated (Table 8) there being a significant difference between point 1 and points 4 and 5 but not between points 1 and points 2 and 3. Points 2 and 3 are along the line of flow of water in the lake with point 1 being nearer the inflow and point 3 nearer the outflow. Sampling of the lake for this study was done at the end of November and it is assumed that the filled akinetes came from that year's summer bloom period. Though the maximum depths and areas of the studied lakes are not very different in depth the bathymetric map of the lake indicates that, sample point 4 and 5 have a steeper gradient to the deepest part as compared to the other three point (1,2 and 3). According Likens and Davis (1975) and Davis and Ford (1982) the movement of sediment from shallower areas towards deeper ones expressed as focusing is caused by water currents, especially during overturn. In addition focusing is more intensive in lake depressions with steeper slopes but it also occurs in lakes with gently sloping bottoms (Blais and Klaff, 1995). We therefore suggest that there was some movement of the filled akinetes if not by gravitation down the steep slope then during mixing resulting in the movement of filled

akinetes from points 4 and 5 to the deepest point of the lake even though the deepest point of the lake was not sampled. This resulted in the uneven accumulation of filled akinetes on the lake sediment as we observed. Therefore, providing a good basis for distinguishing areas with different sedimentation regimes as also observed by Yang *et al.*, 2002.

Even though we are more interested in the filled akinetes which can germinate to give rise to another bloom when conditions become favourable, we had a look too at the empty akinetes. Analysis of the spatial distribution of the empty envelopes in surface sediment of Lake Melangsee revealed quite high variations abundance, predominating mostly in the calm north western part of the lake. Empty envelopes accumulate over the years with germination of the filled intact ones. Point 2 and 4 had significantly more of the empty envelopes compared to point 1 unlike points 3 and 5. Some might decay others might be buried or washed to the calmer water shore. We therefore, suggest this as a reason for their non-uniform quantities. It might also be as results of counting error as they were more difficult to identify.

So far, akinetes are found all over the bed of Lake Melangsee with some part having a higher number than others. In this light, we can rightly conclude our sampling point represents the lake and akinetes are more or less equally distributed along the line of flow of the lake which has a lower gradient. The akinetes are therefore not evenly distributed on the lake bed making us to suggest that there is some little sediment focusing effect on one side of the lake. Though Hilton's (1985) results of on a good number of lakes concluded that polymictic lakes with a maximum depth of $\leq 3\text{m}$ do not have sediment focusing taking place in them we contradict this by claiming from our study that Lake Melangsee a polymictic lake with a maximum depth of 2.4 and which therefore falls in this category of lakes has some slight sediment focusing taking place in it.

6.5.3 Dynamics of akinetes in the surface sediment /calculation of import

6.5.3.1 How the annual import of akinetes from the pelagial is reflected in the sediment

The akinetes enumerated in the sediment are produced by filaments in the water column. They later break off from these dead filaments and settled down into the sediment. Therefore, it is but logical that should there be more filaments in the water column during the blooming season there should be more akinetes in the sediment in the resting season that follows. Figure 35 and Figure 36 clearly indicates that the rate at which akinetes increase in the sediment increases with increase in both mean akinetes per water column and also akinetes per lake area. The gradient of the trend line throws more light on the strength of this relationship. The

strongest gradient is noticed in the relationship of the year 2006 which had a very high abundance of both akinetes in the water column and in the sediment. This was followed by 2007 and then 2005 whose trend line was almost horizontal but again still had this positive correlation. The goodness of fit value R^2 is not so good for the years 2005 and 2006 which had very little small amount of akinetes during their summer. But the year 2006 which had a comparable larger amount of akinetes had a good fit value²³ of 0.6. Again from these results we can rightly say that the annual import is reflected in the sediment in that there are more akinetes in the sediment following a season of high biovolume of the *C. raciborskii* trichomes in the pelagial and vice versa. A high biovolume of trichome in the pelagial gives a corresponding high number and biovolume of akinetes in the pelagial.

6.5.3.2 Qualifying the role of loss processes on akinetes in the sediment

The loss processes that could play a role in controlling the akinetes number in the sediment are consumption by animals (grazing) or death and decomposition. When all have left the pelagic waters and there is no more production by filaments, we then have a peak, with the net change rate decreasing and getting to zero. Under conditions of which there are no loss processes there should have been maintenance of the peak level until the following spring in which there is germination with resulting decrease in akinete abundance. In the course of quantifying the akinetes in the sediment we notice a decrease in the abundance from the peak value over the winter period, with a negative net change rate value within this same period. When germination takes place in spring we saw a negative rate of change in numbers of akinetes of $-0.1d^{-1}$, $-0.3d^{-1}$, $-0.17d^{-1}$ for the years 2005, 2006 and 2007 respectively. In the course of the winter period during the three years we had a maximum negative net change rate of $-0.03d^{-1}$ which is very minimal compared to that during the germination period. During hibernation the net change rate is more or less around zero. But it should be noted that once a maximum number akinetes are enumerated around the end of September to the beginning of October there is the plunging of the net change rate value below the zero mark.

As the temperature keeps dropping (within the first 25 days after the peak of akinetes in the sediment of each year) in the water column from about 15°C to about 9°C we see a drastic decrease in the number of akinetes in the sediment. With further decrease in temperature there is less loss of akinetes which eventually gives way to a horizontal fitness line indicating no loss at all (Figure 37). We can say that at this point in time that the decrease from day zero to

²³ The goodness of fit of a statistical model describes how well it fits a set of observations. Measures of goodness of fit typically summarize the discrepancy between observed values and the values expected under the model in question.

the day 25 was as a result of consumption of the akinetes by zooplankton which are also preparing for the winter leading to a decrease in the abundance.

At a temperature of around 9°C the zooplankton must have also gone into hibernation hence we notice no further decrease. Following hibernation of the zooplankton we then have a stable population with no grazing losses in the course of the winter. After day 25 following the peak of akinetes there temperatures starts increasing to beyond the 9°C (2006/2007) but we do not notice any corresponding decrease in the number of akinetes which we can also attribute to grazing. It should be noted that 9°C is too low for any decrease to be attributed to germination. The grazers might be out but the akinetes are not available on the sediment surface as they might have been buried in the sediment in the course of time. We can therefore say that there is a loss of akinetes in the sediment until the next spring as a result of a higher risk of being consumed by animal organisms at the onset of winter.

6.6 Some comparison of the invasive *C. raciborskii* to native species *Aphanizomenon*

Aphanizomenon also belongs to the order Nostocales, with trichomes that are either solitary or gathered in small or large fascicles which are arranged in layers that can be macroscopic in water (Cronberg and Annadotter, 2006). They have more or less straight trichomes ranging from at least 15µm in length and 2.5-3.75µm wide that are slightly constricted at the cross-walls with elongated, slightly narrowing to tapered ends. Their heterocysts are solitary, spherical or slightly cylindrical and are located along the filament. Akinetes of *Aphanizomenon* species are cylindrical with blunt ends, located a distance from the heterocyst and not at the end of the trichome.

Just like *C. raciborskii*, *Aphanizomenon* spp. undergoes a life cycle. The akinete population did not vary much with the winters of the different years as was the case with *C. raciborskii*. More over as they were formed in autumn we did not notice a big drop in the population in the course of the cold winter period as was the case with *C. raciborskii*. We suggest that loss processes in the lake which affects the invasive cyanobacterium does not have a noticeable toll on the native species. The akinetes of *C. raciborskii* might be better food for grazers than *Aphanizomenon* spp. This could be related to the fact that the *C. raciborskii* in Lake Melangsee does not produce the toxin cylindrospermopsin whereas some species of *Aphanizomenon* do (Wiedner *et al.*, 2007). Another difference we noticed in the life cycle of both species is that unlike *C. raciborskii*, the amount of settling akinetes is not dependent on

current population size. Implying a high biovolume of the filaments in the water column does not necessarily guarantee a higher number of akinetes in the sediment in the following cold season. The summer of 2005 had a higher filament population than that of 2007, but we can see (Figure 38) the winter of 2005/2006 had a lower amount of akinetes as compared to the winter of 2007.

Akinetes which are formed when conditions become unfavourable such as lower temperature in autumn and winter, germinate again when the temperature increases to and above a certain point in spring. This point of germination as said before is identified by the decreasing number of intact akinetes in the surface sediment. We noticed that germination of *Aphanizomenon* started two weeks earlier than in *Cylindrospermopsis* in 2005 and 2007, resulting in a longer lag phase until appearance of the species in the water column (14 - 54 days for *Aphanizomenon*, and 74 - 99 days for *Cylindrospermopsis*). This earlier germination of *Aphanizomenon* spp. implies it takes place at a lower temperature compared to *C. raciborskii*. The germination point corresponded to a sediment temperature of about 12°C of each year. With the laboratory culture experiment we found that germination took place at the lowest of the temperatures chosen indicated by the presence of the growing filaments in all the culture flask. But unlike the *C. raciborskii* in which we saw a decrease in the population size in the 13°C flask with time, we instead saw a doubling of the population size in *Aphanizomenon* spp. which indicates that at temperatures of 13°C germination and growth of *Aphanizomenon* spp takes place. Also leaving us to suggest that in connection with field sampling results, germination can take place at even lower temperatures than 13°C.

The population size of invaded *Cylindrospermopsis* differs much more from year to year compared to the native *Aphanizomenon* species (Figure 41). While *Aphanizomenon* averagely maintains almost the same population in the summers of the study period we saw that *C. raciborskii* having a higher population in 2004 and 2006 when there were stable temperature condition. This intensifies the notion that *Aphanizomenon* spp. is more established in this lake and can be there for a long time. But should climatic conditions not favour the growth of *C. raciborskii*, less and less akinetes would be form at the end of each season and eventually this species may die out from the lake.

7. CONCLUSION

This thesis investigated the life cycle of the nostocales *Cylindrospermopsis raciborskii* and *Aphanizomenon* spp using field data acquisition and processing methods to provide information in temperate shallow lakes. Laboratory data were also generated and used in the perspective of proposing an integrated approach that exploits optimally the already available data from the lake. I provide in the following sections a summary of its main results, general conclusions and some directions for future research.

7.1 General conclusions

Field data have played an important role in documenting the conditions under which cyanobacteria thrive and how they react to these conditions. Historically, laboratory derived data was the primary source for characterizing these conditions and it is still used today. Because we used field studies which are much more difficult to carry out and to interpret due to the high number and variability but which are closer to nature, we were able to use a detailed biological approach for population dynamics coupled with physical and biological variations in the water column during the years. But again we did do some laboratory experiments. There are substantial seasonal as well as interannual variations in the size and composition of this cyanobacterium's filaments and akinetes in the water column and akinetes in the sediment, and also in physical and chemical parameters.

First of all a field study on looking at the horizontal patchiness of akinetes on the lake sediment showed that even though the akinetes are not evenly distributed on the lake bed as we hypothesized, the main sampling point though not the deepest point of the lake is highly representative of the lake. We conclude based on our results and in line with other investigations on other lakes such as ours that some sediment focusing does take place in Lake Melangsee on the steep side but not along the slope with a lower gradient. This therefore gives the discrepancy in the akinetes distribution on the sediment.

We also showed that the seasonal dynamics of *C. raciborskii* in Lake Melangsee had a similar trend but distinctly different timing in the four years during which they were studied. Though the physical, chemical and meteorological environment of the lake appeared to be complex and inter-related we showed that the main reasons for the variabilities in this cyanobacteria abundance are the different meteorological conditions. The timing of onset of spring and autumn are most relevant to the development of some of the stages of this species:

Field data indicated germination of akinetes results in decreasing akinete abundance in the sediment in spring and occurred at 13°C. From the laboratory experiment in addition to the field data we proved our hypothesis that the minimum temperature for the germination of *C. raciborskii* is actually below 15°. The temperature at which germination occurred was 13°C and it is an important parameter to look out in the lake for the initiation of germination.

From the long term study we realized that the time at which the sediment attained this 13°C in spring varies with the year. This makes us to say that a fixed time cannot be given for the start of akinete germination. When akinetes germinate in the temperate zone is a matter of when the warm seasons come. This leads us to say that should we have earlier springs this timing would also shift to become earlier in the year. It is therefore a fact that the temperature of the lake sediment is a great indicator of the time when akinetes would germinate.

In our work we did not see any direct impact of light to the initiation of germination since there was no light at the sediment throughout the study period. An indirect effect of light was suggested as a heating factor. Light heats the water column and there by heats the sediment too.

From our results attaining this 13°C mark initiated germination but again we found that subsequent increase in temperature is essential for the thriving and blooming at the beginning, during and at the end of summer.

We showed that the onset of germination was only when the temperature was at or above 13°C and with the aid of a particular level of light intensity after the winter period. But after the water body has gotten the required temperature, a decrease in light intensity in the mixing zone triggers upward movement. Increase in water and sediment temperature provide the trigger for the recruitment of populations from the benthic resting stages, more so than increased light availability meaning in the advent of higher spring temperatures as a consequence of global warming it would promote the flourishing of this species and why not its colonisation of other freshwater bodies.

Following germination of the akinetes, the germlings are supposed to establish a population in the pelagial in the respective years. From our study we found that *C. raciborskii* abundance in the pelagic depends not only on the physical processes in the water column such as light and temperature but also most importantly on the conditions following germination when the freshly emerged and naked germlings are out. An increase in temperature from the 13°C that initiated germination and away from light is needed for further growth and maturity of these

germlings since the freshly emerged germlings are more sensitive to temperature and light stress than adult trichomes. This period of maturity we identified to take place in the benthic zone of the lake

Our study gave us the possibility to estimate a time frame between germination of akinetes and appearance of filaments in the pelagial and considering that the germling spend some time of the sediment. This time frame we suggest to be 50 days. Within the first five days of this 50 days period the germlings emerge from the exospore and it takes about 45 day for them to mature and move into the pelagial. For this reason we suggest that this time frame of about 50 days following the attainment of the germination threshold temperature of 13°C is a good period to target these species in the case of trying to eliminate them from the water body. Claiming the newly emerged germlings are more susceptible to adverse conditions than the adult filaments. Moreover for the mature filament to continue living and multiplying asexually a stable water column in addition to the temperature above 23°C is necessary to have a high population of *C. raciborskii* in the water column.

Just like we hypothesised, we concluded after our study that trichomes start developing akinetes in autumn when temperatures in the water column drop to 22°C. But again we noted that more akinetes are formed when this temperatures decrease gradually than when there is a sharp drop to say 20°C. The timing of the akinetes production in the pelagial comes in as a function of the reigning environmental conditions which are decreasing temperatures to 22°C.

The production of akinetes in the pelagial is reflected in the sediment in that there are more akinetes in the sediment following a season of high biovolume of the *C. raciborskii* trichomes in the pelagial and vice versa. A high biovolume of trichomes in the pelagial gives a corresponding high number and biovolume of akinetes in the sediment. But it is still not a guarantee that the number would remain the same until the following spring. We found in the course of our study that there was a loss of akinetes in the sediment until the next spring. This loss of akinetes we attributed to grazing as they have a high risk of being consumed by grazers at the onset of winter and onset of spring.

In a selective comparison of *C. raciborskii* and *Aphanizomenon* spp it came to our notice that though their akinetes can germinate at temperatures as low as 13°C, only *Aphanizomenon* spp germling can grow further and thrive well at this temperature This native species has a selective advantage over the invaded species under low temperatures and temperature fluctuations as observed in the field when weather is frequently changing in that their population did not change much over the years.

The links between abundance and population dynamics of *C. raciborskii* filaments and akinetes allowed us to form a relationship between physical conditions and changes in biomass. In our study, the development of *C. raciborskii* adjusts itself to the dynamics of temperature and light conditions in the lake which was also a reflection of conditions out of the lake. Water temperature was a key factor to cell multiplication and differentiation

7.2 Directions for future research

An open window which is still left for investigation in future is to find

- Whether this germination can be initiated below 12 C and its dependence on light.
- Determine which processes are actually responsible to the loss of akinetes in the sediment.
- Could there be a loss of the filaments as a result of grazing too?
- Are depth of deposition and time of the year important for the germination frequency of *C. raciborskii*?
- What conditions determine the size of the recruited inoculum of *C. raciborskii*?
- How much does recruitment contribute to the pelagic population of *C. raciborskii*?
- How large is the recruitment from sediments at different depths during the season for *C. raciborskii* and *Aphanizomenon* spp?

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Contributions

Parameter	Task	Name	Laboratory of analyses
Determination of light intensity and temperature anomaly	Global radiation and air temperature		Meteorological Observatory in Lindenberg, Germany.
	Calculation of I_{mix}	Jacqueline Rücker	
	Calculation of temperature anomaly	Emilienne Tingwey	
Seasonal dynamics	Sampling and field measurements and fixation	Ingo Henschke, Jacqueline Rücker Emilienne Tingwey(One sampling date)	Chair of fresh water conservation, Bad saarow
	Chemical analysis of water sample	Gudrun Lippert Ute Abel	Chair of fresh water conservation, Bad Saarow
	Filament biovolume	Anette Tworeck Emilienne Tingwey	LBH, Freiburg BTU Cottbus
	Akinete quantification of all experiments and champagnes	Emilienne Tingwey	BTU Cottbus
In vitro experiment	Culturing	Andrea Laundart	Bad saarow
	Quantification	Emilienne Tingwey	BTU Cottbus
In situ experiment	Beaker preparation	J. Rücker	
	Beaker harvesting	Ingo Henschke	Lake Melangsee
	Akinetes and filament quantification	Emilienne Tingwey	BTU Cottbus
Determination of akinete patchiness	Sampling	Ingo Henschke	Lake Melangsee
	Quantification and analysis	Emilienne Tingwey	BTU Cottbus
Data analysis		Emilienne Tingwey	BTU Cottbus

Date / sample	Number of fields counted	<i>Cylindrospermopsis</i> akinetes			<i>Aphanizomenon gracile</i> akinetes			other <i>Aphanizomenon</i> akinetes		
		Intact	half filled	empty	full	half filled	empty	full	half filled	empty

Appendix II

Germination Experiment								
	Cylindrospermopsis				Aphanisomenon			
	Width(40x)	Length(40x)	Phase	Heterocyst No	Width(40x)	Length(40x)	Phase	Heterocyst No
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Appendix III

Column	Condition
1	1 day temperature average
2	2 days temperature average
3	3 days temperature average
4	4 days temperature average
5	5 days temperature average
6	6 days temperature average
7	7 days temperature average
8	1 day PAR
9	2 days PAR
10	3 days PAR
11	4 days PAR
12	5 days PAR
13	6 days PAR
14	7 days PAR
15	1 day air temperature
16	2 days air temperature
17	3 days air temperature
18	4 days air temperature
19	5 days air temperature
20	6 days air temperature
21	7 days air temperature
22	1 day wind
23	2 days wind
24	3 days wind
25	4 days wind
26	5 days wind
27	6 days wind
28	7 days wind
29	1 days wind direction
30	2 day wind direction
31	3 day wind direction
32	4 day wind direction
33	5 day wind direction
34	6 day wind direction
35	7 day wind direction
36	1 days precipitation
37	2 day precipitation
38	3 day precipitation
39	4 day precipitation
40	5 day precipitation
41	6 day precipitation
42	7 day precipitation
43	1 day maximum wind
44	2 days maximum wind
45	3 days maximum wind
46	4 days maximum wind
47	5 days maximum wind
48	6 days maximum wind
49	7 days maximum wind
50	Daily hours of sunshine
51	1 day global radiation

52	2 days global radiation
53	3 days global radiation
54	4 days global radiation
55	5 days global radiation
56	6 days global radiation
57	7 days global radiation