Vertikalwanderung von Ciliaten: Untersuchungen zum Vorkommen, zur Kontrolle und als eine Strategie um Fraßdruck zu vermindern.

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Vorgegelgt von
Claudio Marcelo Rossberg
aus Cochabamba, Bolivien

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Berichterstatter:	Prof. Dr. Hartmut Arndt
	Prof. Dr. Helmut Hillebrandt

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Introduction.-

Heterotrophic ciliates play a very important role in aquatic foodwebs, as consumers of bacteria (Fenchel, 1980), algae and heterotrophic flagellates. They are also prey for higher consumers. Furthermore, ciliates have high growth rates and if food supply is enough they can increase in abundance in short time (Müller *et al.,* 1991). But in spite of this high growth rate, ciliate mortality through predation can be higher (Skogstad, *et al.* 1987; Sanders & Wickham, 1993; Wickham, 1995; Montagnes, 1996). Among the strategies that prey organisms have evolved to evade predation are modifications in their morphology, (Kusch, 1993a; 1993b; 1995) as well as modifications in their behaviour known as diel vertical migration (DVM).

The phenomenon of diel vertical migration has been described as the process in which pelagic organisms tend to migrate to the deep refuge in the hypolimnion during the day to then migrate to the food rich surface waters in epilimnion during the night (De Robertis, 2000). This phenomenon is widespread among the zooplankton and the reasons that induce this behaviour are not entirely understood. Most migrants are negatively photo tactic, so light appears to be the primary stimulation to induce migrations. Light intensity (Strom, 2001) and temperature (Massana *et al*., 1994; Atkinson, 2003: Sun, 2004) play an important and positive role especially over ciliate migration. The urge to evade predation could be considered as a fundamental reason, but the need to avoid unfavourable environmental conditions is also important.

A large variety of hypotheses have been offered to explain the adaptive value of vertical migration. The majority of them can be grouped in two categories according to the different components of themes they emphasize (Lampert, 1998). The first suggests that vertical migration provides a metabolic or demographic advantage and the other suggests that vertical migration leads to the avoidance of surface waters during the day in order to reduce the light dependent mortality risk. The adaptive importance of DVM as an alternative to evade predators was reported by Gilbert & Hampton, (2001); De Robertis, (2000); Laas, (2000); Pérez *et al.,* (2000); Neill, (1990) and Crawford & Lindholm (1987).

Although there are some reports concerning DVM activity on ciliates particularly on autotrophic species like *Mesodinium rubrum* (Pérez *et al.,* 2000; Crawford & Lindholm, 1987) little is known about the migratory behaviour of heterotrophic ciliates in eutrophic conditions and if this behaviour is induced by the presence of predators or adverse environmental conditions, if the migration pattern exhibited by the ciliates follow a similar or opposite pattern to the migration performed by the higher zooplankton and if this behaviour is a common or a restricted phenomenon among certain species.

It remains however a debate if vertical migration really is an evolutionary survival advantage, since from the metabolic point of view migrating animals do not gain any metabolic advantage over non-migrating ones (Guisande & Duncan*,* 1991). This would mean that the ciliates that tend to migrate towards richer surface waters do not always have higher survival chances compared to those which do not migrate. If it is a positive adaptation, then the species that do perform vertical migration are less vulnerable to predation pressure and are less subjected to remain in hazardous environments. In consequence, this activity should lead to a higher ciliate reproduction rate and hence higher abundance. On the contrary, if vertical migration is not an evolutionary advantage, then the ciliates will be vulnerable to predation risk by remaining at close contact with the predators in the epilimnion and the risk of dealing with unfavourable conditions in the hypolimnion.

This migratory behaviour observed in the zooplankton can also lead to a trade-off situation, where they are either openly exposed to predators in the epilimnion or parasites (Decaestocker *et al*., 2002) and anoxic levels (Laas *et al*., 2000) in the hypolimnion. If vertical migration behaviour is a consequence of the conflicting requirements of feeding and predator avoidance the time at which organisms migrate between a deep refuge and food rich surface waters should reflect this trade off (De Robertis, 2000).

In order to understand and to describe the causes for this migration phenomenon of a ciliate community in natural conditions, I have separated my research in three main chapters: the first, concerning primary field observations, the second field experiments and the third the annual cycle of an heterotrophic ciliate community in relation to food availability and predatory pressure in a eutrophic lake.

Chapter 1 - Ciliate vertical migration on a eutrophic lake. Field observations. In this chapter I focus mainly on the description of the vertical migration behaviour of a natural ciliate community under natural conditions during the course of a year. Results indicate that diel vertical migration behaviour does take place for the ciliates but not for all and not in every test appointment. Day and night (short term) differences in distribution could be attributed to predator avoidance, and the long term migration activity could be attributed to the changing physic-chemical conditions of the lake. My results show that after stratification two ciliate communities developed, where the epilimnion was dominated by the litostome *Askenasia* and the oligotrich *Rimostrombidium lacustris* and the hypolimnion was dominated by the prostome *Coleps*. The causes for this behaviour remain unclear, although the mechanisms driving the migration in both communities are unlikely to be identical.

Chapter 2 - Ciliate vertical migration in a eutrophic lake. Field experiments. In this chapter, I focus on the causes that would induce vertical migration on the field. It remains unclear if migration really is an advantage that ultimately leads to survival in natural conditions, and if the external influences such as predation and environmental conditions do really have an important impact over the ciliates´ community structure and potential vertical migration. To test this hypothesis field experiments were conducted to separate the effects of predator abundance and abiotic conditions at different depths. I conclude by stating that each factor is important, but it is the interaction of both which plays a fundamental role in determining ciliate abundance and survival possibilities.

Chapter 3 - Annual cycle of protozooplanktonic ciliates in relation to food availability and predatory pressure in a eutrophic lake. This chapter deals with the annual cycle and structuration of the ciliate community and its relations with other major components of the zooplankton and environmental factors. To evaluate this, I determined abundance and biovolume values during the course of a year sampling. I conclude by stating that heterotrophic ciliate assemblages are clearly influenced by the annual variation of biotic and abiotic factors, and that during prestratification ciliate abundance and biovolume were mainly controlled by a top down effect from predators and that during stratification high chlorophyll *a* concentrations in the hypolimnion would have induced a bottom up effect.

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Ciliate vertical migration in a eutrophic lake. Peliminary field observations.

Introduction.-

Ciliates play a very important role in the aquatic food-web, as consumers from bacteria (Fenchel, 1980), algae and heterotrophic flagellates they are also prey for higher consumers. Among the strategies that prey organisms have evolved to evade predation, are modifications in their morphology, (Kusch, 1993a; 1993b; 1995) as well as modifications in their behaviour, known as diel vertical migration (DVM) in which pelagic organisms migrate away from food rich surface waters during the day to avoid spatio-temporal overlap with visual predators (De Robertis, 2000).

The adaptive importance of DVM as an alternative to evade predators was reported by Lampert (1989). The presence of predators induced vertical migrations in copepods (Neill, 1990) and rotifers (Gilbert & Hampton, 2001). This migratory behaviour observed in the zooplankton can also lead to a trade-off situation, where they are either openly exposed to predators in the epilimnion or parasites (Decaestocker *et al*., 2002) and anoxic levels (Laas *et al*., 2000) in the hypolimnion. If vertical migration behaviour is a consequence of the conflicting requirements of feeding and predator avoidance the time at which organisms migrate between a deep refuge and food rich surface waters should reflect this trade off (De Robertis, 2000).

Although there are some reports concerning DVM on ciliates particularly on autotrophic species like *Mesodinium rubrum* (Pérez *et al*., 2000; Crawford & Lindholm, 1987) little is known about the migratory behaviour in heterotrophic ciliates and if this behaviour is mainly due to predator avoidance, food search or to reach more suitable habitats. Even if this behaviour remains not fully understood in heterotrophic ciliates, there are some results from recent investigations that took place in lake Speldrop that suggest that DVM takes place in eutrpophic lake conditions and that this behaviour is inversely proportional to the migratory behaviour observed in their predators.

The major goal of the first part of this project was to describe the migratory behaviour of a ciliate community under field conditions during the course of a year.

Materials and Methods.

Description of the study site.-

Lake Speldrop, a eutrophic gravel pit lake with monomictic characteristics is located between Emmerich and Rees at the lower Rhine in Germany. It is approximately 7 hectares in size and has a maximum depth of 16 meters, and presents a notorious stratification from the months of May until October. During the sampling year, the weather ranged from relatively clear, sunny windless and relatively calm waters to cloudy and through the wind swept waters. Due to the fact that the lake is surrounded by the presence of a protective forest layer, the wind influence was reduced and not so strong. Still, the wind did play an important role over wind-force triggered windmill generators placed around the lake. The lake also gives place to diverse activities, ranging from fishing, hunting, diving to didactic activities directed mostly to school and university students. Agricultural activities such as cattle ranching are also practiced in farms around the lake, which can lead to eutropphization by the constant input of nutrients (organic fertilizers) into the lake. Prior to the transport of the samples to the laboratory in the university, a pre-analysis was run in the Grietherbusch ecological investigations center (fig. 1)

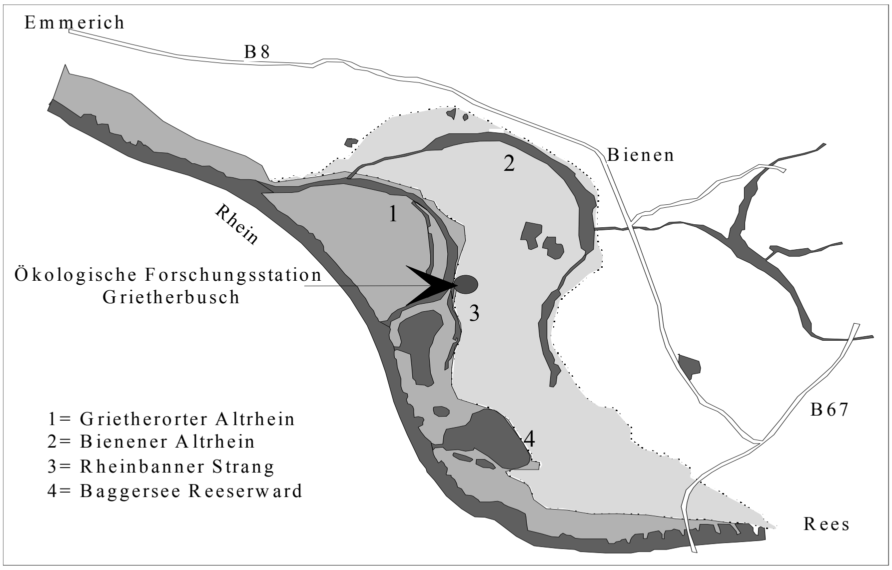


Fig. 1.- Location of the ecological field station in Grietherbusch,.

Sampling methods.-

Day and night samples were taken once a month from the months of March to October. Each sample consists of two parallel sub-samples for Ciliates at 1 meter intervals (0-14 m). Results from previous research have shown that samples taken at larger intervals are not fine-scaled enough to describe the vertical distribution of the ciliates. If there is deep chlorophyll maximum, then the depth distribution of the ciliates would be more fine scaled, due to this reason samples were taken every 50 cm within the deep chlorophyll maximum.

Samples for the ciliates were taken every meter with a 2 liter water sampler (diameter 10 cm) which can sample the exact depth. Due to the low abundance of Cladocera (*Daphnia sp, Bosmina longirostris*), Copepoda (*Thermocyclops oithonoides, Eudiaptomus gracilis*) and Diptera (*Chaoborus flavicans*) samples were taken with a 20 liter Schindler sampling unit. Ciliates were fixed with a Bouins´ solution and the Mesozooplankton with a sucrose formalin solution. At the same time vertical physico-chemical profiles for the oxygen, temperature, conductivity, pH and chlorophyll maxima were measured every meter with a YSI 6820 multisonde. The obtained physico-chemical data was immediately analyzed in-situ to determine the presence of stratification and its position within the water column.

In laboratory, ciliates were left to settle in 50 ml settling chambers, identified up to the genus level using the QPS silver staining method (Montagnes & Lynn 1987) and counted in Utermöhl counting chambers under a Zeiss Axiovert S100 microscope. The zooplankton samples were re-sampled from the original 20 liter sample to 10 ml and counted under an Olympus Optical CO 170 stereomicroscope and also identified to the closest possible level.

In order to determine significant differences among the mean day and night depth distributions a t-test analysis was used among calculated mean depths. Because 12-t test per test appointment were done, these were corrected to the significance level according to the rule from Dunn-Sidak.

Results.-

Physico-chemical parameters.-

Temperature (°C).-

The monomictic characteristic of lake Speldrop give place to only one complete circulation during the months of November to May, and allows a clearly established stratification to build up during the months of June to October. The thermocline begins to build up by mid May at a depth of 2 – 3 meters and begins to stabilize by June to July at a depth of 4 meters remaining constant during three months from July to September at a depth of 5 meters. By October however, the thermocline begins to fade (fig. 2).

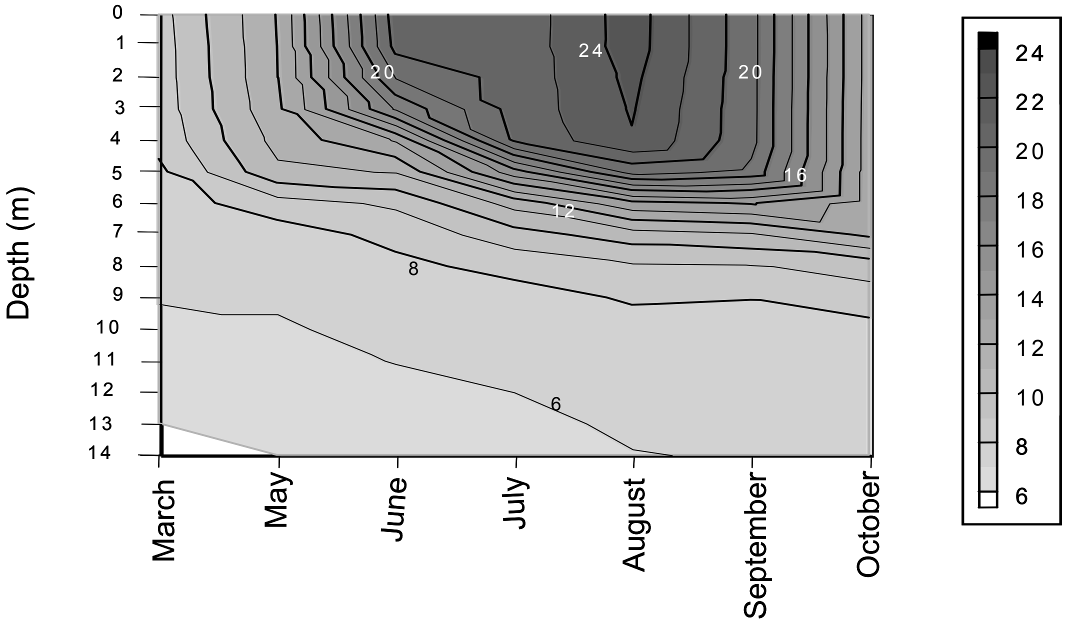


Fig. 2.- Seasonal development of the temperature (°C) in Lake Speldrop in 2002.

Oxygen (% Sat.).-

The oxygen presents the same characteristics as the temperature, but during the months of stagnation (November to May) the mixing is not complete, and a very feeble stratification is present. During stratification, the epilimnion is oxygen saturated, but on the other hand the hypolimnion reaches almost anoxic levels. These high levels could be attributed to a higher photosynthetic activity during that period of the year. In lake Speldrop, by the beginning of May the oxycline begins to form within an interval of 8 – 9 meters, which stabilizes during mid July within a depth interval of 5 – 6 meters. This allowed the formation of a notorious anoxic layer, which covered the hypolimnion (7–14 meters) during the rest of the year. The hypolimnion was also characterized by the formation of high hydrogen sulphide (H_2S) concentrations. The epilimnion though remained oxygen saturated until the end of the year (fig. 3).

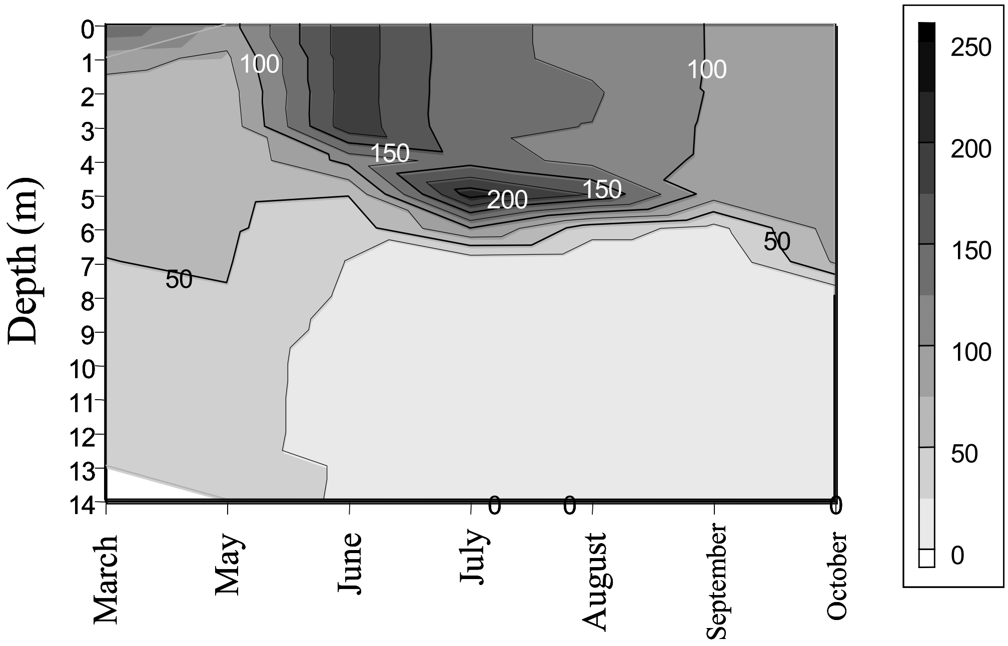


Fig. 3.- Seasonal development of the % saturation of dissolved oxygen in Lake Speldrop in 2002.

Chlorophyll a (µg/l).-

The chlorophyll *a* concentration presents an irregular pattern comparing to the temperature and the oxygen, and was not notoriously determined by stratification, due possibly to higher and lower grazing activities of the zooplankton during determined moments and places. High chlorophyll *a* concentrations were found within a depth interval of 0 to 4.5 meters in March, then remaining relatively clear until May, reaching its maximum levels within a depth interval of 9 – 11 meters from June until October. During that period the epilimnion (0 – 5 meters) was however relatively clear of algae, (fig. 4). The principal algae which would be considered as potential food recourse for the ciliates had a size < 20 µm and among these the principal ones were mainly *Coelastrum*, *Chlorella* and *Oocystes* .

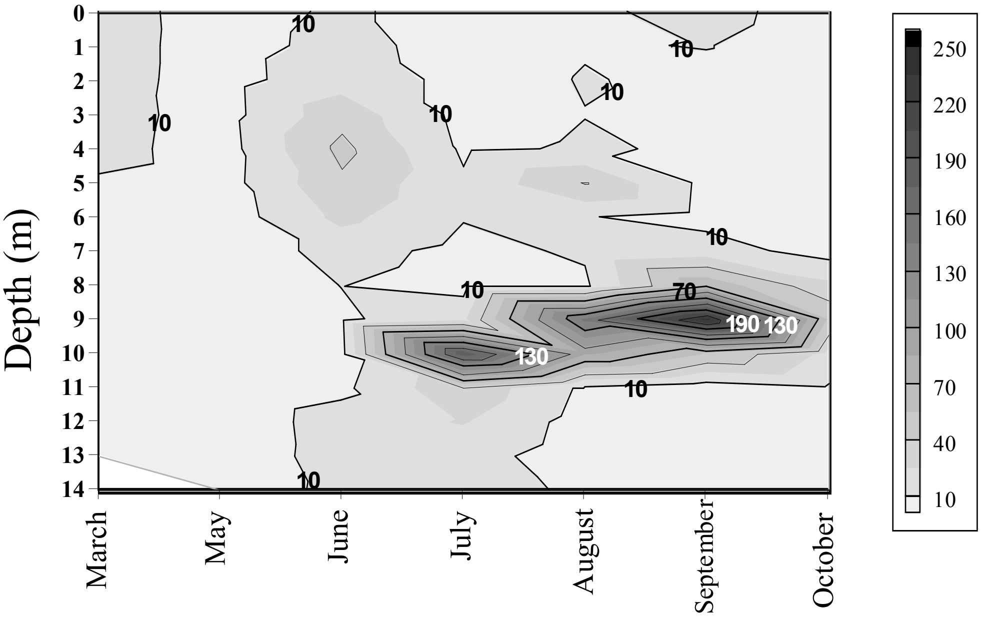


Fig. 4.- Seasonal development of chlorophyll a concentration (µg/l) in Lake Speldrop in 2002.

Biological parameters.-

Phytoplankton.-

The dominating algae during the sampling year 2002 were *Anabaena*, which comprised 43% of the total abundance, followed by *Oscillatoria* (20%). Another un-edible algae, *Fragillaria,* composed 10%. The edible algae *Chlorella* and *Coelastrum* comprised together almost 19% of the total abundance. Other species of algae (in the rectangle) were present in reduced numbers, such as *Pediastrum* and *Staurastrum* which, because of their size and shape, would be considered as “none edible” by the small migrating ciliates. The fraction that would be considered as “edible” (*Oocystes*, rectangle in figure) was much more reduced in abundance, reaching a total of 3% (fig. 5).

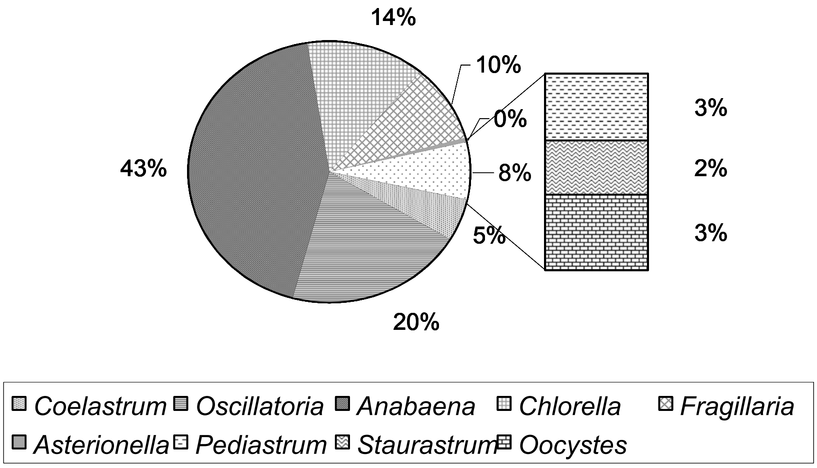


Fig. 5.- Percentual composition of the phytoplankton community in Lake Speldrop in 2002.

The depth distribution of the edible algae during the months of May, September and October can be appreciated on figure 6. In May, prior to stratification, the algal abundances were reduced and evenly distributed, possibly leading the ciliates to perform migrations in the search for food. There are also no marked differences between their day and night distributions, only the day abundances are lower than the night, possibly due to higher grazing activities during the day. By September and the consolidation of the stratification, the biggest part of the edible algal abundance was concentrated in the hypolimnion, but anyway the epilimnion had enough disposable algae as food resource. October presents a similar distribution pattern, but its abundances are lower, and the overall abundance of *Chlorella* gives way to other important edible algae.

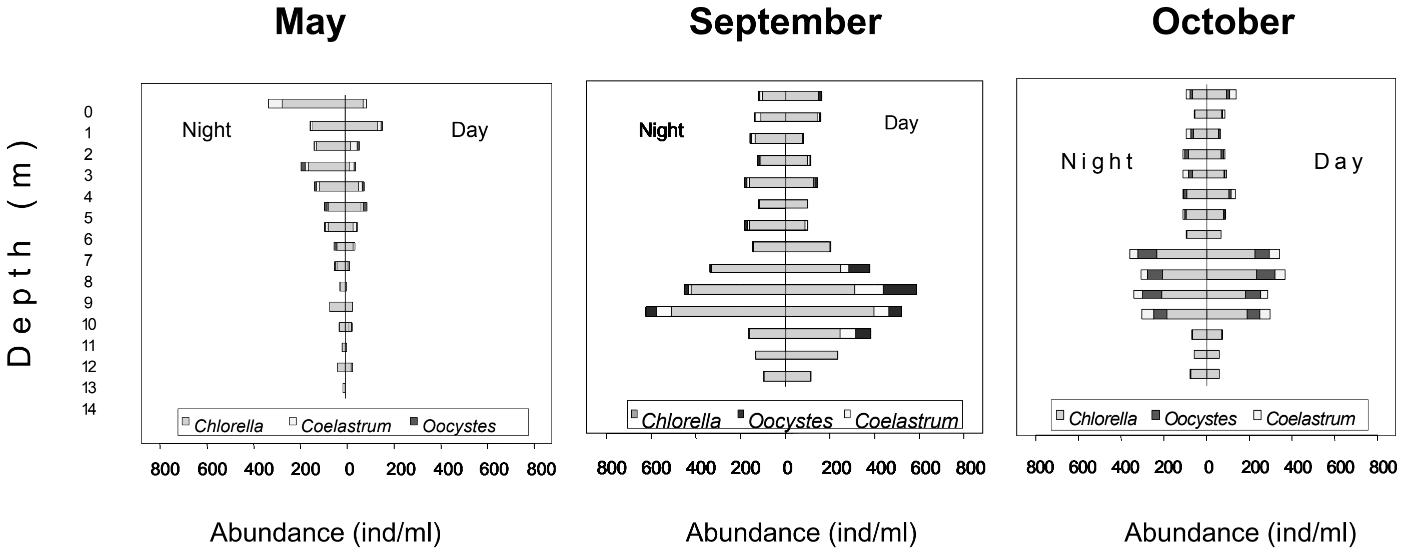


Fig. 6.- Vertical distribution profiles for the principal “edible” algae *(Chlorella, Oocystes* and *Coelastrum)* in Lake Speldrop in 2002.

Zooplankton.-

Rotifers.-

Rotifers present a similar temporal distribution pattern to that exhibited by the ciliates, except for September where abundance and composition were drastically reduced, recovering by the month of October. A total of 10 morph-species were identified. The dominant species during 5 of the sampling months was *Keratella cochlearis* comprising up to 77.58 % of the total abundance during the month of August. During the month of March the dominating rotifer was *Polyarthra remata* (50.2%) and in June *Conochilus* (48.75%). The graphical representation of the rotifers´ community structure and temporal variation can be appreciated on figure 7. *Polyarthra remata* was present in all sampling months as well as *Keratella cochlearis,* which reached the highest rotifer abundance ever reached during the sampling. Other species were rare, found only sporadically even if they got to be the dominating species in one month, such as *Conochilus* in June. Other rotifer species were extremely rare and had very low abundances such as *Synchaeta, Asplanchna, Ascomorpha, Brachiouns* and *Filinia. Keratella quadrata* was also very important during the month of May but in the following months eventually lost importance.

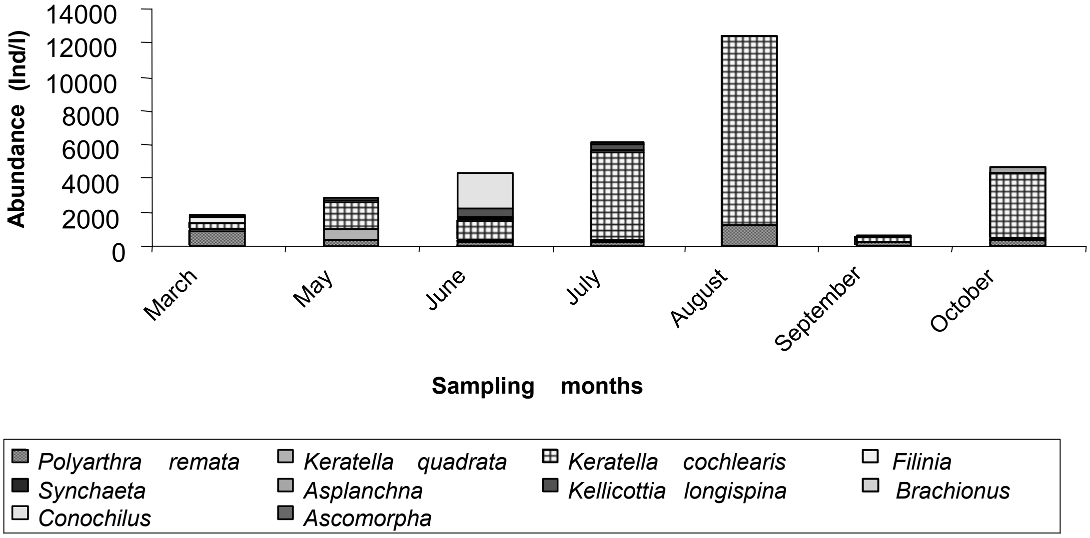


Fig. 7,- Rotifer taxonomic composition in lake Speldrop in 2002.

Copepods and Cladocerans.-

During the first three months, the Copepoda and the Cladocera present different community structures. From March to June, the Copepoda are relatively constant, with little variation. During these three months the Nauplii were the dominant group, followed by *Thermocyclops oithonoides* and the copepodites. *Eudiaptomus gracilis* was also present in all samples, but it remained in very small numbers. On the contrary, the Cladocera present a different community structure pattern from March until May, where their abundance grows progressively. The dominant species was *Daphnia sp.*, followed by *Bosmina longirostris* which by June gained an important place. From July to October, both groups present a similar distribution pattern, with a low abundance in July, to recover by August and to a rapid decrease by September and October.

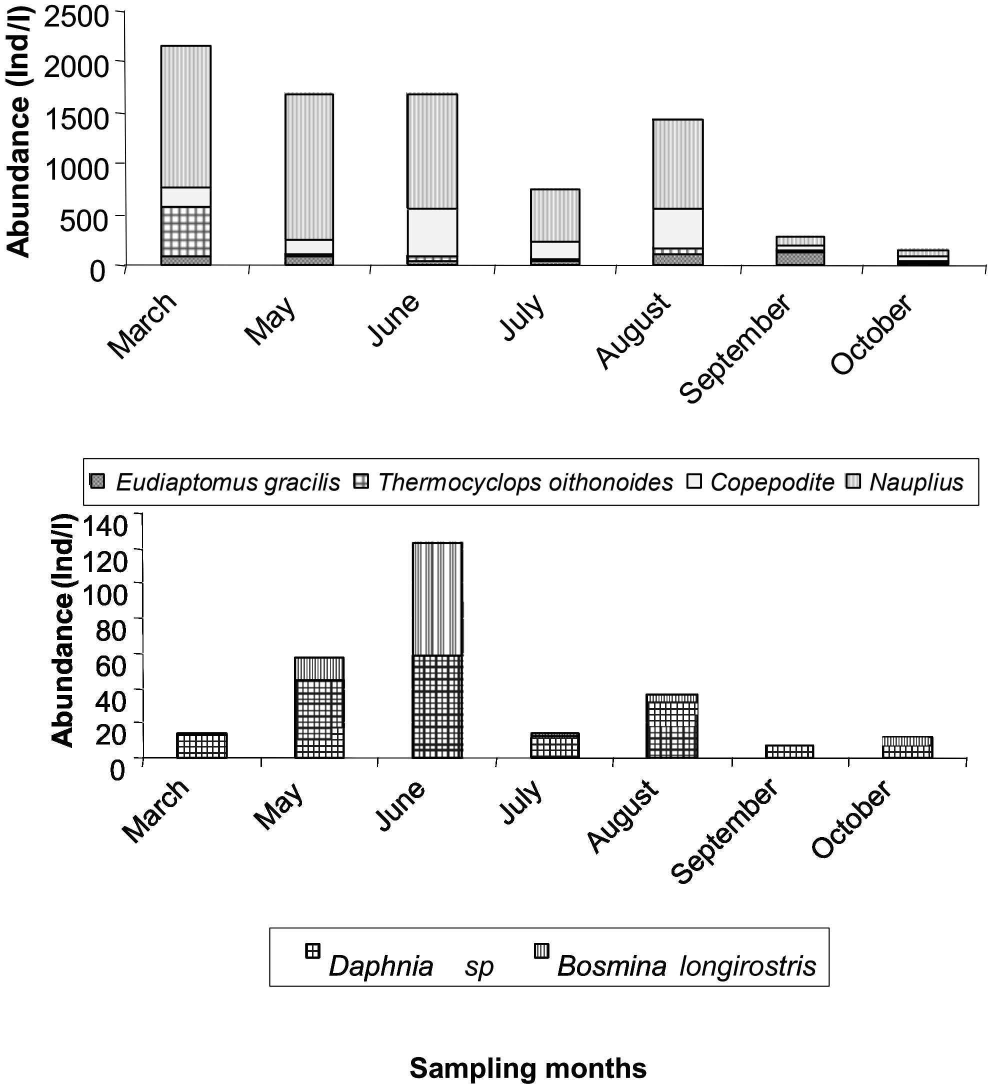


Fig.9,- Copepod and cladoceran composition in lake Speldrop in 2002.

Chaoborus flavicans.-

During the first 3 months the abundances are rather low in contrast to the high abundance found for the cladocerans and the copepods. This inverse relation can also be seen during the month of August where *Chaoborus flavicans* reached the highest abundance and the abundance for Cladocera was very low. The abundance for *Chaoborus flavicans* found in September decreased one half compared to the abundance found in August, and decreased much more during the month of October.

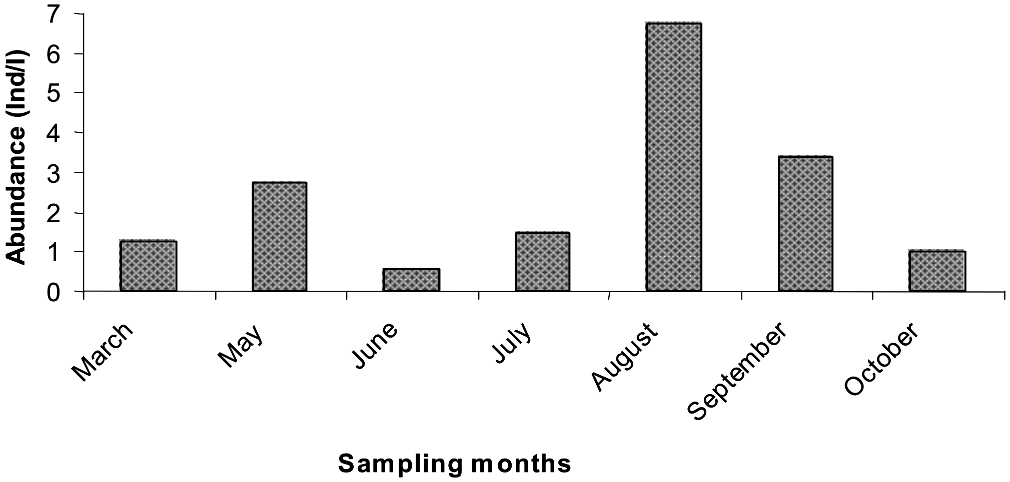


Fig. 11.- Temporal abundance for *Chaoborus flavicans* in lake Speldrop in 2002.

Ciliates.-

A total of 22 morph-species were identified during the year 2002. Total density (%) and species richness increased throughput the sampling year as well as variations within the ciliate communities. The minimal richness and density were found during the month of March. The dominant group found during that month were the Tintinnids (37.30%), whereas the maximal richness and density were found during the month of September having *Coleps* (39.65%) as the dominant group. Diversity remained constantly low, averaging 0.74 according to the Simpson´s diversity index. The lowest diversity was found in July (0.84) and the highest in October (0.56). The increase of species richness and total density were possibly due to the increase of available food resources (Chlorophyll *a* concentration in June 17.56 µg/l to a maximal concentration in September 33.94 µg/l). The increase of the algal abundances did not only provide a sufficient food resource, it also provided possible shelter against predators but can also represent a physical barrier to deviate or block migration in the ciliates. (Table 1).

Table 1.- Richness, diversity total density and principal dominant ciliate species in lake Speldrop in 2002.

	Total density (ind/ml)	**Richness**	**Dominant species %**	**Diversity (Simpson diversity index)**
March	19	9	Tintinnid (37.30)	0.76
May	39	17	*Halteria* (46.29)	0.73
June	35	16	*Coleps* (34.60)	0.74
July	58	20	*Halteria* (25.86)	0.84
August	89	21	*Holophrya* (20.80)	0.81
September	118	22	*Coleps* (39.65)	0.75
October	98	22	*Coleps* (63.78)	0.56

The graphical representation of the ciliates´ community structure and its variations along the sampling months is shown in fig. 13. The communities grew exponentially and gradually changed their biological structure. These changes within the ciliates´ communities will indeed influence their migratory behavior, either by direct interspecific or intraspecific pressure like competition or predation and indirectly by forcing the more sensitive ciliates to migrate to better sectors. As the system changes, the more resistant species will remain in one determined sector of the lake, while the more sensitive species will be forced to migrate to more adequate sectors.

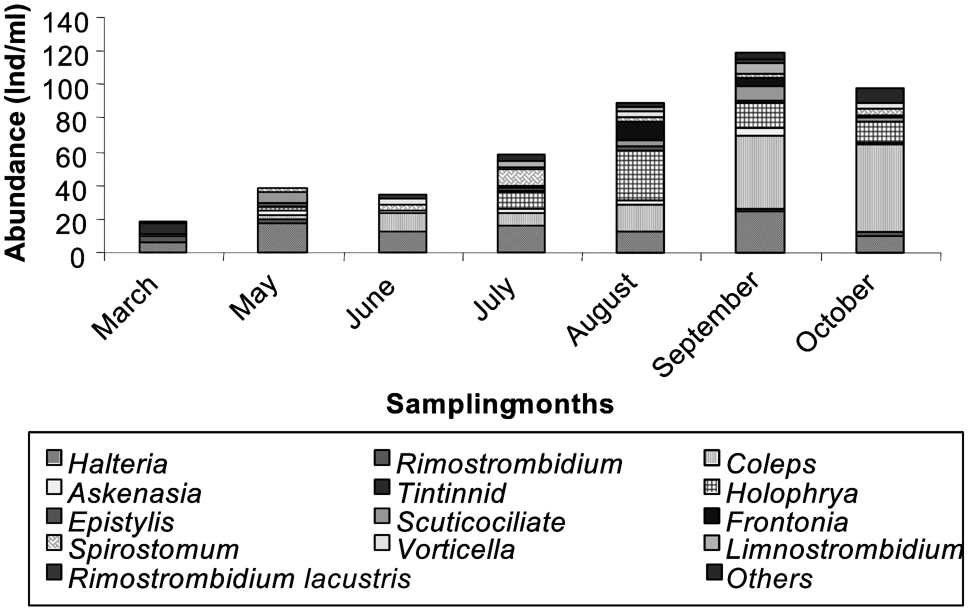


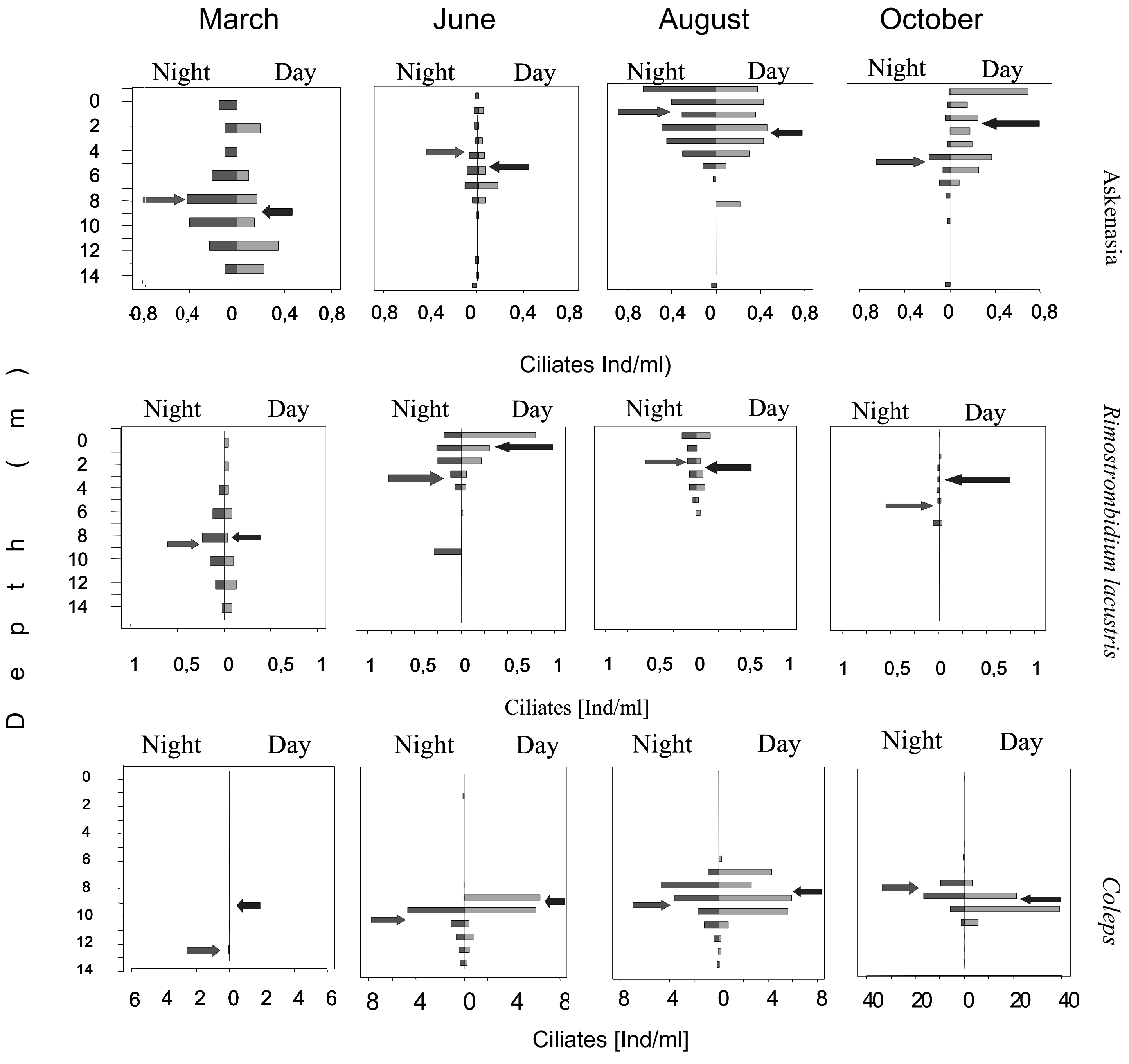
Fig. 13,- Ciliate taxonomic composition in lake Speldrop in 2002.

The middle depth for the 10 most frequently found ciliates plus *Daphnia sp.* and larvae of *Chaoborus flavicans*, was determined during their day and night distributions in the lake Speldrop in 2002. *R. lacustris* is *Rimostrombidium lacustris*, Scuticociliate is a ciliate from the subclass Scuticociliatia which could not be determined any further. The day tests were taken between 12:00 and 15:00, the night tests at least 2 hours after sundown. A starlet shows a significance level of P = 0.005, 2 starlets a significance level of P = 0.0005 and 3 starlets a significance level of P = 0.00005. Empty spaces show that no species were found in the lake.

Table 2.- Calculated mean depths for the 10 most frequent ciliate species, *Daphnia sp.* and *Chaoborus flavicans*.

Species	Month													
	March		May		June		July		August		Sept		Oct	
	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night
Rimostombidium	7,5	6,5	5,3	8,6***	1,5	4,5***	3,0	4,9***	1,6	1,7	3,9	4,2	3,1	5,7***
R. lacustris	8,3	8,5	-	-	0,9	3,4***	1,9	4,0***	2,5	1,9***	2,8	3,7*	4,7	6,5***
Limnostrombidium	-	-	-	-	0,9	2,0***	2,1	3,8***	2,5	2,2	2,4	3,4*	3,5	4,0***
Halteria	6,1	6,5	4,6	4,9	2,3	2,8	2,8	3,3	5,6	5,3	4,8	3,8	4,0	5,7
Askenasia	9,4	7,8	6,9	5,1***	7,0	6,4**	2,3	3,2**	3,1	2,4*	2,5	2,8	2,3	5,3***
Scuticociliat	-	-	9,1	6,6*	8,3	10,2***	7,6	8,1	8,4	8,4	8,4	7,8*	9,0	11,0***
Coleps	9,5	14,0***	13,0	13,4	9,8	10,6	9,2	9,4	8,8	9,0	8,9	8,7	9,7	8,9
Spirostomum	-	-	13,1	14,0***	10,3	10,4	8,8	8,2	8,8	8,0***	8,4	8,2	9,1	8,0***
Holophyra	-	-	9,9	9,2	-	-	7,5	7,9	6,5	7,9	7,5	7,0	8,0	7,9
Caenomorpha	-	-	-	-	12,4	12,1***	10,3	10,5	10,6	9,8***	9,5	10,1**	7,3	10,1***
Daphnia sp	5,1	5,0	6,8	3,3	7,1	7,0	4,9	5,7	6,5	5,9	3,3	5,3	7,1	5,8
Chaoborus flavicans	10,7	4,8***	12,0	4,5***	9,5	5,9***	11,4	5,1*	11,8	6,0***	12,3	5,5	12,0	5,6***

The depth distributions for the ciliates *Askenasia*, *Rimostrombidium lacustris* and *Coleps* as well for *Daphnia sp* and *Chaoborus flavicans* can be appreciated on figure 14. The ciliates *Askenasia* and *Rimostrombidium lacustris* performed their migrations mainly in the epilimnon. Prior to stratification, the litostome *Askenasia* is evenly distributed along the depth profile, and progressively begins to move towards the epilimnion as the stratification begins to form. *Rimostrombidium lacustris* presents the same pattern, but opposing to *Askenasia*, its abundance gradually decreases along with the stratification. This reduction in abundance is possibly not only due to changing environmental conditions, but to possible predation activities. The high intolerance of both species against anoxic conditions would restrict their main abundance to the epilimnion. The prostome *Coleps* on the other hand, is mainly distributed in the hypolimnion where it also presents in some degree, migration activities. The reason for the residence of *Coleps* on the hypoimnion could be explained by the following reasons, during the sampling year the hypolimnion was densely populated with purple sulphur bacteria, which meant an abundant food supply. Another explanation would be the high tolerance of *Coleps* against anoxic conditions in the hyplimnion. There was therefore no urge for *Coleps* to perform migration over the stratification borders. The cladoceran *Daphnia sp* also did perform vertical migration activities, and its abundances were inversely related to the abundances of the equally migrating *Chaoborus flavicans*. Both species did perform vertical migrations in an upward direction, but only *Chaoboris flavicans* did migrate across the stratification boundaries, whereas *Daphnia sp* mostly migrated within the metalimnetic area.



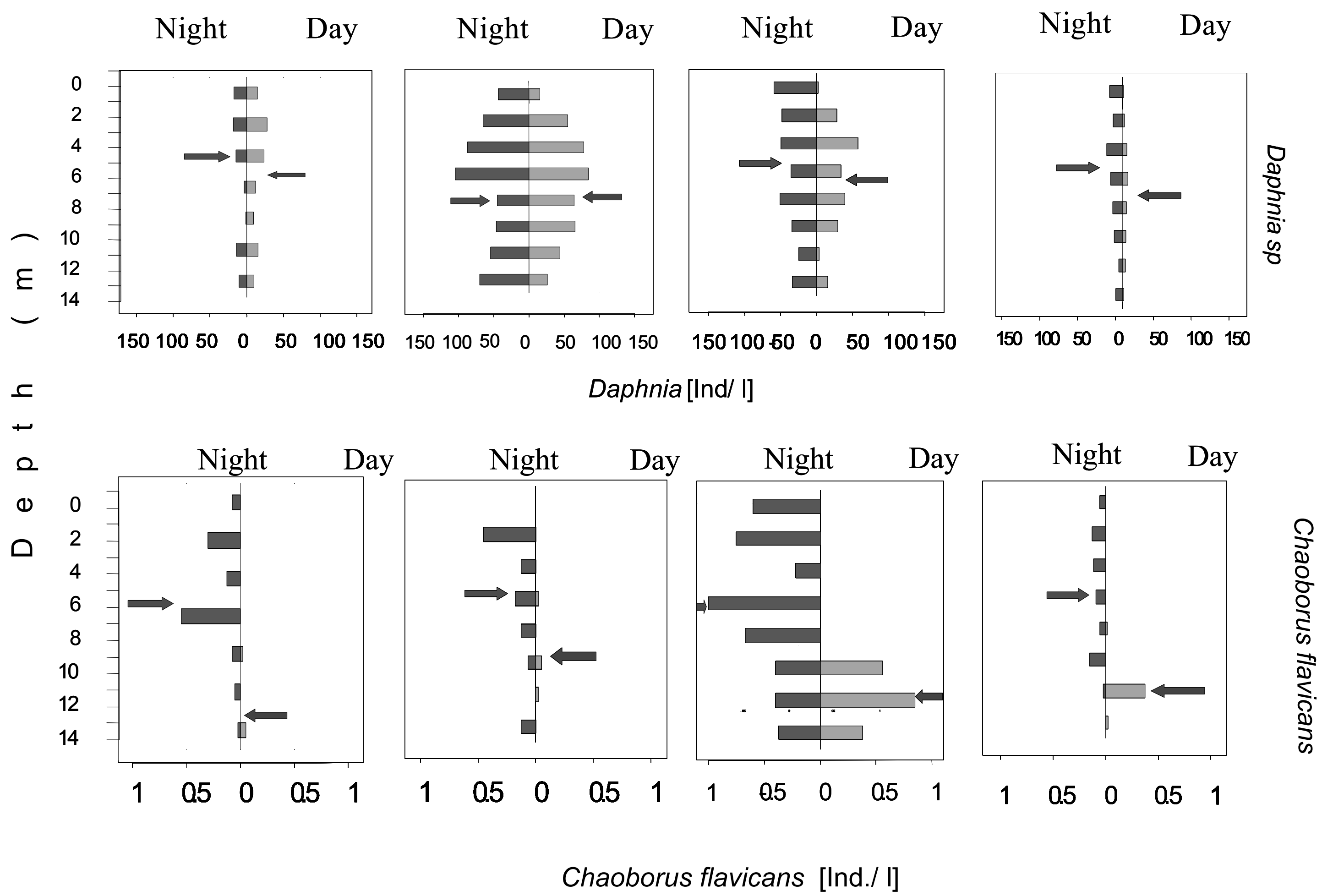


Fig. 14.- Day and night depth vertical distributions for *Askenasia, Rimostrombidium lacustris*, *Coleps*, *Daphnia sp* and *Chaobous flavicans.* The arrows indicate the main depth found for each organism. The right bars indicate day distribution, and the left night distribution. The abundance units are individuals per mililiter for the ciliates and individuals per liter for the zooplankton.

Discussion.-

During the sampling campaign in 2002, diel vertical migration (DVM) activity was observed for the ciliates, but not for all, and not within every test appointment. While the day and night (short term) differences in distribution could be attributed to predator avoidance, the long term migration activity could be attributed to the changing physic-chemical conditions of the lake. The gradual stratification of the lake and variation within the physicochemical characteristics would eventually set boundaries on the ciliate distribution patterns, according to their auto-ecological requirements. Species adapted to survive in aerobic environments would be restricted to the epilimnion and those capable of surviving under anoxic conditions would be restricted to the hypolimnion. Water column interleaving during stratification would have played an important role in determining the structure and distribution of the ciliate community and ultimately their migratory behaviour (Lovejoy *et a*l., 2002).

Edible algae (mainly *Chlorella*, *Coelastrum* and *Oocystes*) were low in abundance but evenly distributed throughout the depth profile. This would exclude migration activity strictly related to food search. Algal concentration would also play an important role by physically regulating vertical migration in the ciliates. During stratification, the main algal concentration occurred within the metalimnion. This would lead to the formation of ciliate aggregations (patches) due to changes in the locomotory pattern of the ciliates when they encounter food (Stocker *et al.,* 1984). Furthermore, during stratification the hypolimnion was populated by a dense population of purple sulphur bacteria, which meant an important food resource for the ciliates and other zooplankton adapted to live in hypolimnetic conditions (Overmann, *et al*., 1989).

Vertical migration was evidently influenced by the presence of zooplankton. Constant predation pressure by the rotifers does have a negative impact over ciliate abundance (Weisse & Frahm, 2002). This pressure would induce predator avoidance behaviour (Kusch, 1993), eventually leading to perform migration activities. Not only did the rotifers play a negative role over the ciliates, but also the copepods, which are able to feed efficiently on the ciliates, thus allowing the dissolved organic carbon transfer to higher trophic levels from the bacterial producers (Overmann *et al.,* 1989). As grazers of the phytoplankton, ciliates would simply follow the grazing activities of higher zooplankton groups (Fenchel, 1980).

In addition, the higher feeding activity of the crustaceans during the night might explain the upwards directed migration of the ciliates as a defensive mechanism against predation (Arndt & Heerkloss, 1989). The presence of the higher predator *Chaoborus flavicans* would provoke migration activities in the copepods (Neill, 1990) and eventually provoke migration in the ciliates. The presence of *Eudiaptomus gracilis* at the bottom of the epilimnion and the top of the metalimnion would also induce migrating activities in the ciliates (Benoit, *et al.,,* 2002).

The copepods and cladocerans did perform migration activities as a result from fish predation in the system (Gliwicz, 1986), followed by the rotifers avoiding the copepods (Dieguez & Gilbert, 2004). These migration activities would eventually cascade down to the ciliates, either by predator avoidance or by simply following as casual scavengers. The reasons for this behavioural cascade remain unclear, if predator induced kairomones are gradually transferred from one species to the next (species specific) or if a “general” kairomone would eventually induce migration behaviour on all components of the zooplankton.

Real migrations would be considered as those which are longer than 1 m, which is the distance between the sampling depths and longer than the size of the sampling unit, which is 50 cm. This would not be the case for all the ciliates, even though significant differences among depths were found. This could be attributed to the instability of the boat during sampling, having as a result possible “sampling errors”.

I considered migration activity within a whole population; however this population response may be misleading in terms of the behaviour of constituent individuals. Individuals may vary speed and direction of their movements, so that at any time some animals move upwards while the others rest or move downwards (Lampert, 1989). Furthermore, the long migration observed for *Coleps* during the month of March could be scarcely due to real migration, but possibly also due to an error during the sampling activity. Nevertheless, even given the adverse conditions, the idea that the ciliates are capable of migrating distances longer than the established distance is realistic. Small ciliates are capable of reaching an average speed of 1,2 mm per second which would cost only 8% of their metabolic rate (Crawford, 1992) and can reach vertical speeds from 1 to 2,5 meters per hour (Jonnson, 1989).

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Ciliate vertical migration in a eutrophic lake. Field experiments.

Introduction.-

Diel vertical migration (DVM) is common in the zooplankton and the main causes that can lead to this activity on the field can be either attributed to predator evasion, food search as well as the need to evade unfavourable habitats and search for better and more suitable sectors in the lake during stressful periods (Dekaestecker *et al*., 2002; Lass, 2000; Neill, 1990; Lampert, 1989). Although this migration activity was proven for autotrophic ciliates such *as Mesodinium rubrum* (Pérez *et al.,* 2000; Crawford & Lindholm, 1987) recent results have shown that heterotrophic ciliates do perform DVM activity in eutropphic conditions and that this activity was species specific and was not always consistent with predator activity.

It remains however unclear if migration really is an advantage that ultimately leads to survival in natural conditions, and if the external influences such as predation and environmental conditions do really have an important impact over the ciliates´ community structure and potential vertical migration. If vertical migration really is an evolutionary survival advantage, then the species that do perform vertical migration are less vulnerable to predation pressure and are less subjected to remain in hazardous environments. In consequence, this activity should lead to a higher ciliate reproduction rate and hence higher abundance. On the contrary, if vertical migration is not an evolutionary advantage, then the ciliates will be vulnerable to predation risk by remaining at close contact with the predators in the epilimnion and the risk of dealing with unfavourable conditions in the hypolimnion.

In order to test this hypothesis, field experiments were conducted to separate the effects of predator abundance and abiotic conditions at different depths, in which a ciliate community from the epilimnion was transplanted to the hypolimnion and a ciliate community was transplanted from the hypolimnion to the epilimnion, either with or without zooplankton.

Materials and methods.-

Sampling methods.-

The experiments were run during the months of April, August and October of the year 2003 in Lake Speldrop. The same spot in the center of the lake was chosen as the sampling sector, and anchored the boat to this same spot. In order to separate the ciliates from the external environmental conditions during the experiment transparent dialyse bags (7.64 * 30 cm) were used, which were attached to 2 transparent plexiglas boards 30 * 70 cm with lateral incisions. The 24 bags were estimated as the following: for the 2 meter set, 6 replicates for the control (2 meters), of which 3 were with zooplankton and 3 without zooplankton, 6 replicates for the transplanted (2 in 6 meters), having 3 with zooplankton and 3 without. The same treatment was applied for the 6 meter set, totalling 24 plastic bags that were randomly placed in the plexiglas boards. These boards were then fastened to ropes, to which a floating buoy was attached to the top and a weight on the bottom to keep the whole set in a vertical position. Then they were left to settle for 24 hours (fig. 1). At the same time ciliate and zooplankton depth profiles were taken to determine the composition and abundance of the total community.

After 24 hours samples were taken from the lake. Ciliate samples were then fixed with a Bouins´ solution and the zooplankton samples were fixed with a sucrose formalin solution. Samples were then taken to the laboratory where they were left to settle in 50 ml settling chambers, identified up to the genus level using the QPS silver staining method (Montagnes & Lynn, 1987) and counted in Utermöhl counting chambers under a Zeiss Axiovert S100 microscope. The zooplankton samples were re-sampled from the original 20 liter sample to 10 ml and counted under an Olympus Optical CO 170 stereomicroscope and also identified to the closest possible level. The influence of the external factors can be evaluated by the growth difference between the control and transplanted samples. The influence of predation can be evaluated by the difference between the with and without zooplankton samples In order to statistically analyze the interaction among the original depth, transplanted depth either with or with out zooplankton (independent or grouping variables) and the ciliate abundance (dependent factor) a 3 way Analysis of Variance was used.

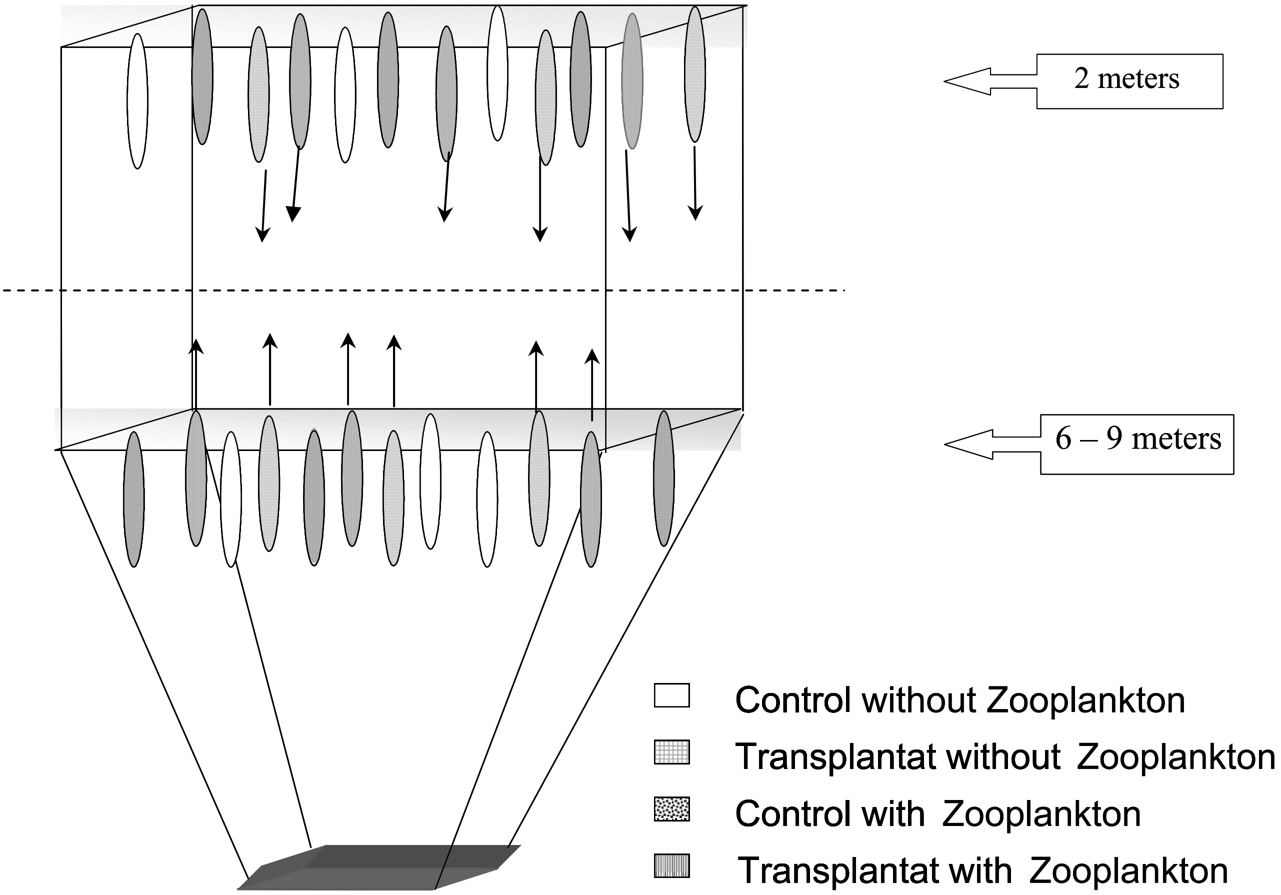


Fig 1.- Experimental design and placement of the dialyse bags in lake Speldrop in 2003.

Results.-

Experiment 1 April 2003

Physico-chemical parameters- Temperature (°C) and % saturation oxygen

The temperature (°C) presents very little variation within the first 4 meters, then descending abruptly within the next 2 meters from 12 °C to 6°C, then remaining constant (5-6°C) down to the hypolimnion. The oxygen presented 90% saturation in the surface, then increased in value within the first 4 meters to reach a maximum value in the 4 – 5 meter depth interval (120% saturation), to descend abruptly to 60% saturation in only 1 meter, then remaining relatively constant for the next 2 meters then descending very steeply to reach anoxic levels in the hypolimnion. Water transparency reached to a depth of 8 meters allowing photosynthetic activity.

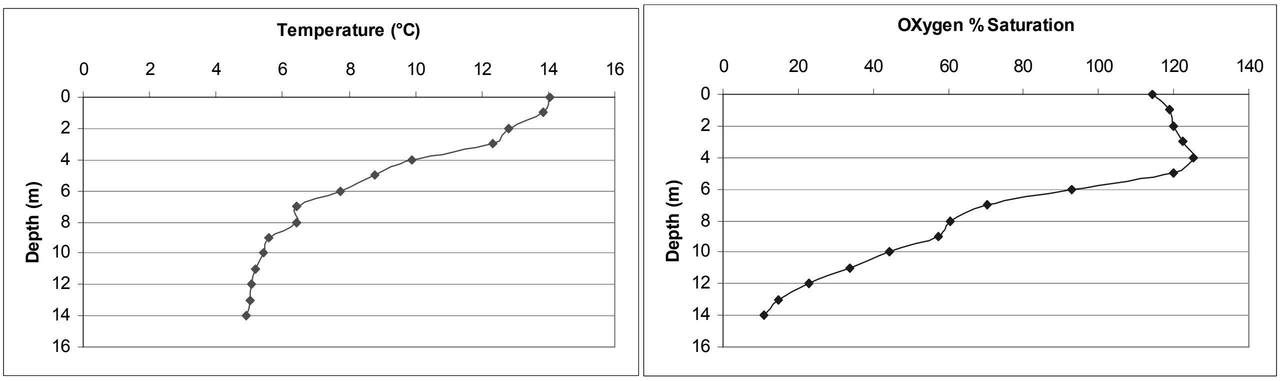


Fig. 2.- Temperature (°C) and oxygen concentration (%O_2 sat.) during the month of April 2003.

Ciliate and zooplankton composition and vertical distribution in Lake Speldrop

During the month of April the most abundant ciliate was *Halteria*, which was present over all the depth profile and exhibited an upward migration from 2 meters to the surface. Following in importance with a much lower abundance and no clear evidence for migration were *Askenasia*, *Limnostrombidium*, *Rimostrombidium* and *Frontonia.* The zooplankton community was dominated mainly by the cladoceran *Daphnia sp.* and the copepod *Thermocyclops oithonoides*. Following in importance were the Nauplii and *Eudiaptomus gracilis*. The rotifers were also present but in lower abundance. The principal rotifers were *Keratella quadrata* and *Asplanchna*. General main abundance was much lower during the day than during the night, and the highest abundance was found within the first 2 meters.

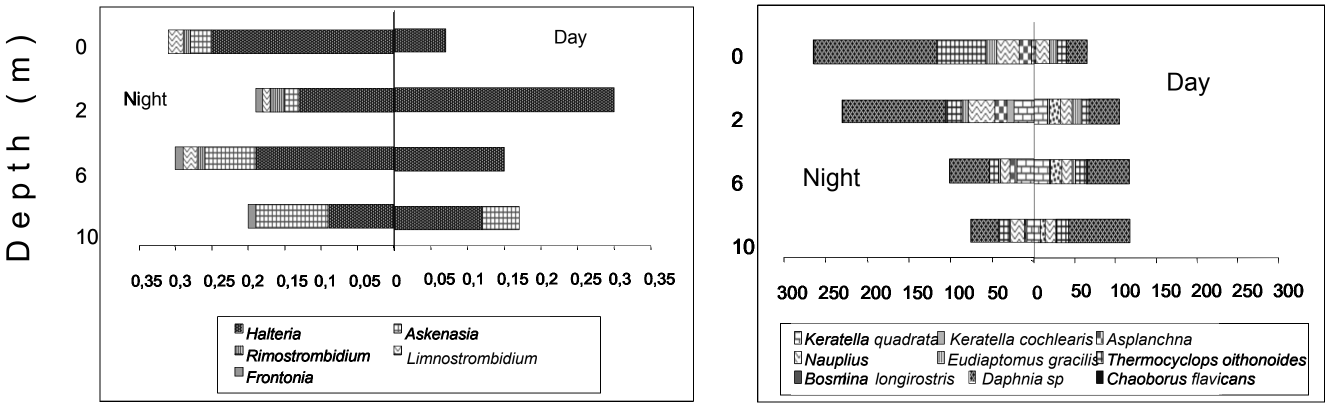

a) b)

Fig 3.- Ciliate (a) and zooplankton (b) composition and depth distribution in April 2003

2 meter set. Algae, ciliates and zooplankton.

During the month of April a total of 6 algal morph-species were found, the absolute dominating algae in all samples was the chlorophyte *Chlorella*. *Ceratium* and *Staurastrum* were also present, but with lower abundance. The maximal algal abundance was found in the 2 meter control with zooplankton sample. All other samples had a slightly less abundance, and the least abundance was found in the transplanted 2 in 6 transplanted with zooplankton sample. Among the ciliates, *Halteria* was the only representative ciliate morph-species present, with other individuals present in very low abundances. The greatest abundance was found in the initial sample, not differing very much from the experimental treatments. The 2 in 6 transplanted sample had the highest zooplankton abundance, whereas the lowest was found in the 2 meter control sample. The dominating species in the experimental samples was *Daphnia sp.,* together with the copepods *Eudiaptomus gracilis* and *Thermocyclops oithonoides*. An increase in abundance and number of species was observed in the 2 in 6 transplanted sample, where *Daphnia sp.* was still very important, together with other cladoceran species such as *Bosmina longirostris* and rotifer species such as *Keratella quadrata, Keratella cochlearis* and *Kellicottia longispina* which also gained in importance. The higher zooplankton abundance in the transplanted sample would explain the low algal and ciliate abundance in the same sample. The grazing and predatory activity of the zooplankton was more intense when transplanted to a lower depth, which would explain the lower abundance of the algae and the ciliates. The adverse environmental conditions would also play an important role. On the 2 meter control with zooplankton sample the zooplankton had a rather low abundance, the ciliates and especially the algae hence had a much higher abundance. The 2 meter set had better environmental conditions, which induced a higher reproductive rate for the algae and the ciliates, which was not affected by the low zooplankton abundance.

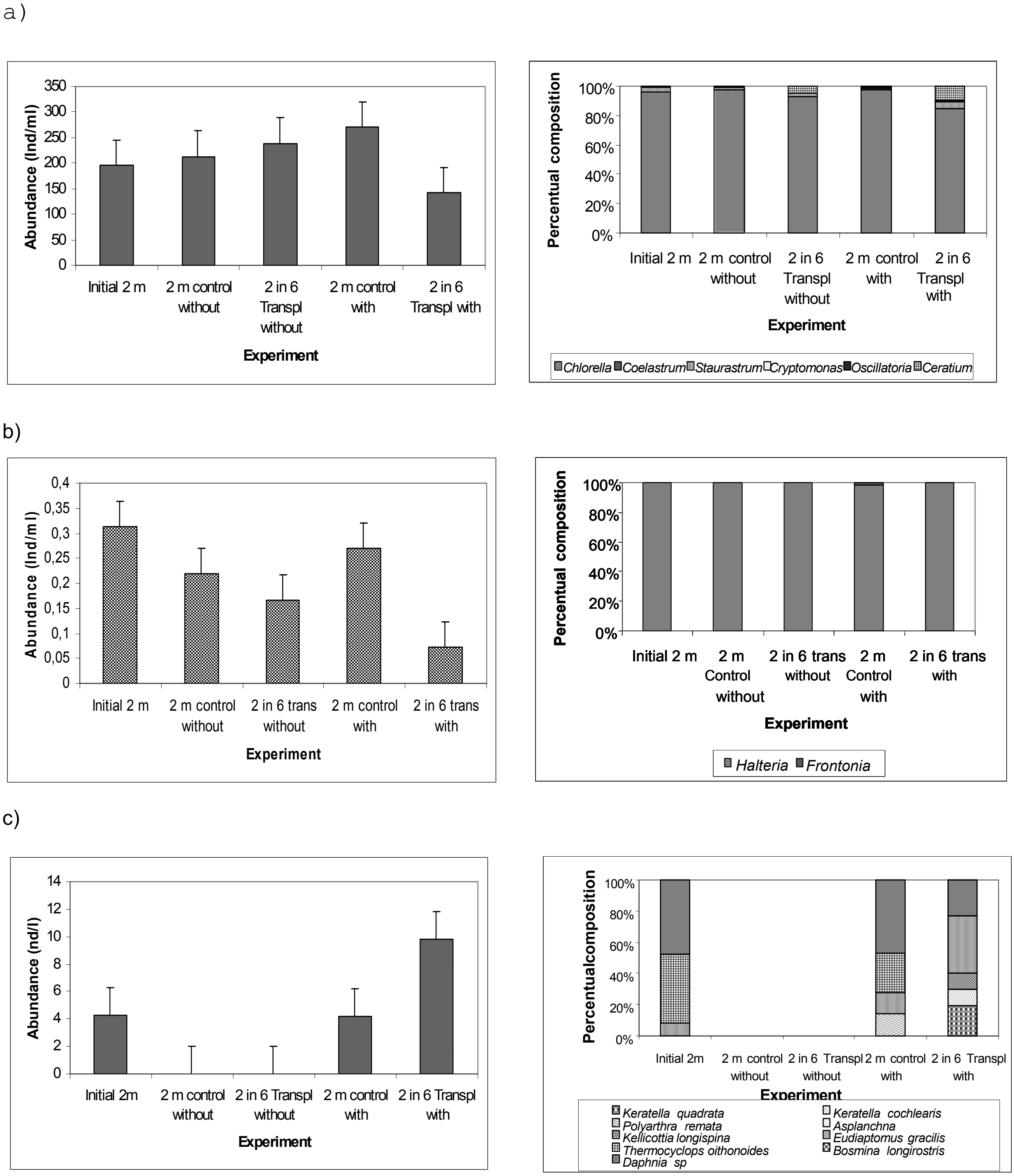
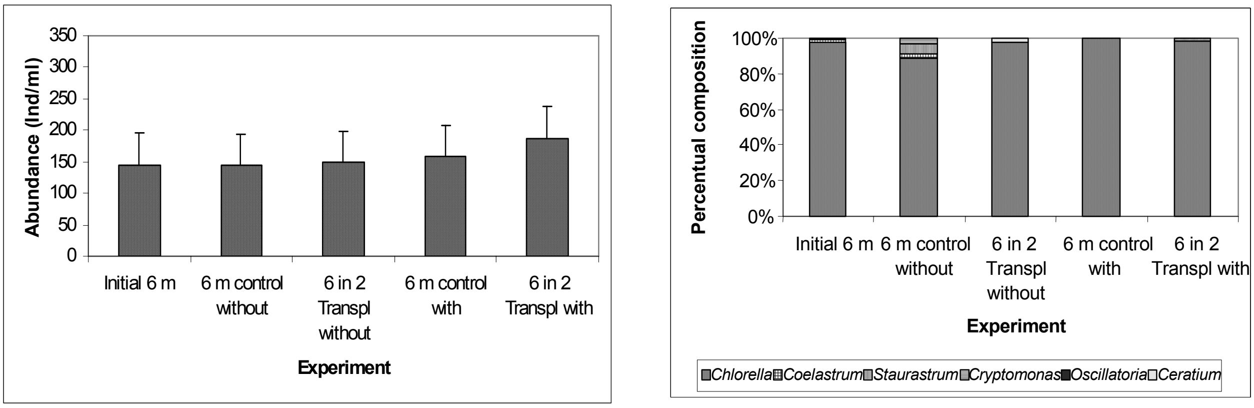


Fig 4.- Total and percentual algal (a), ciliate (b) and zooplankton (c) abundance and percentage composition in the 2 meter set for April 2003.

6 meter set. Algae, ciliates and zooplankton.

In the 6 meter set the dominant algae was also *Chlorella.* Other algae such as *Staurastrum, Ceratium*, and *Coelastrum* were also present, but in very low abundances. In general, the algae had lower abundances when compared to the 2 meter experimental set. When transplanted to a higher depth, algal abundance increased, even with the presence of zooplankton. The maximal abundance for the ciliates was found in the 6 in 2 transplanted without zooplankton sample and the minimal abundance was found in the 6 in 2 transplanted sample with zooplankton. The dominating ciliate was also *Halteria*, and the other ciliates presented very low abundances. In contrast to the initial 2 meter sample, as with the algae, the initial 6 meter sample was much more reduced in ciliate abundance, as well as the experimental samples. The zooplankton abundances were generally more reduced than in the 2 meter sample. Together with the dominating *Daphnia sp.* and *Eudiaptomus gracilis* were other species which were also important such as *Thermocyclops oithonoides* and the rotifers *Asplanchna* and *Polyarthra remata.* The effect of transplanting to a higher depth had different causes, for instance the algae had a higher reproductive due to the lower grazing activity from the ciliates and the zooplankton, which on the other hand presented much lower abundances possibly due to higher predatory activity amongst the zooplankton and the ciliates themselves.

a)



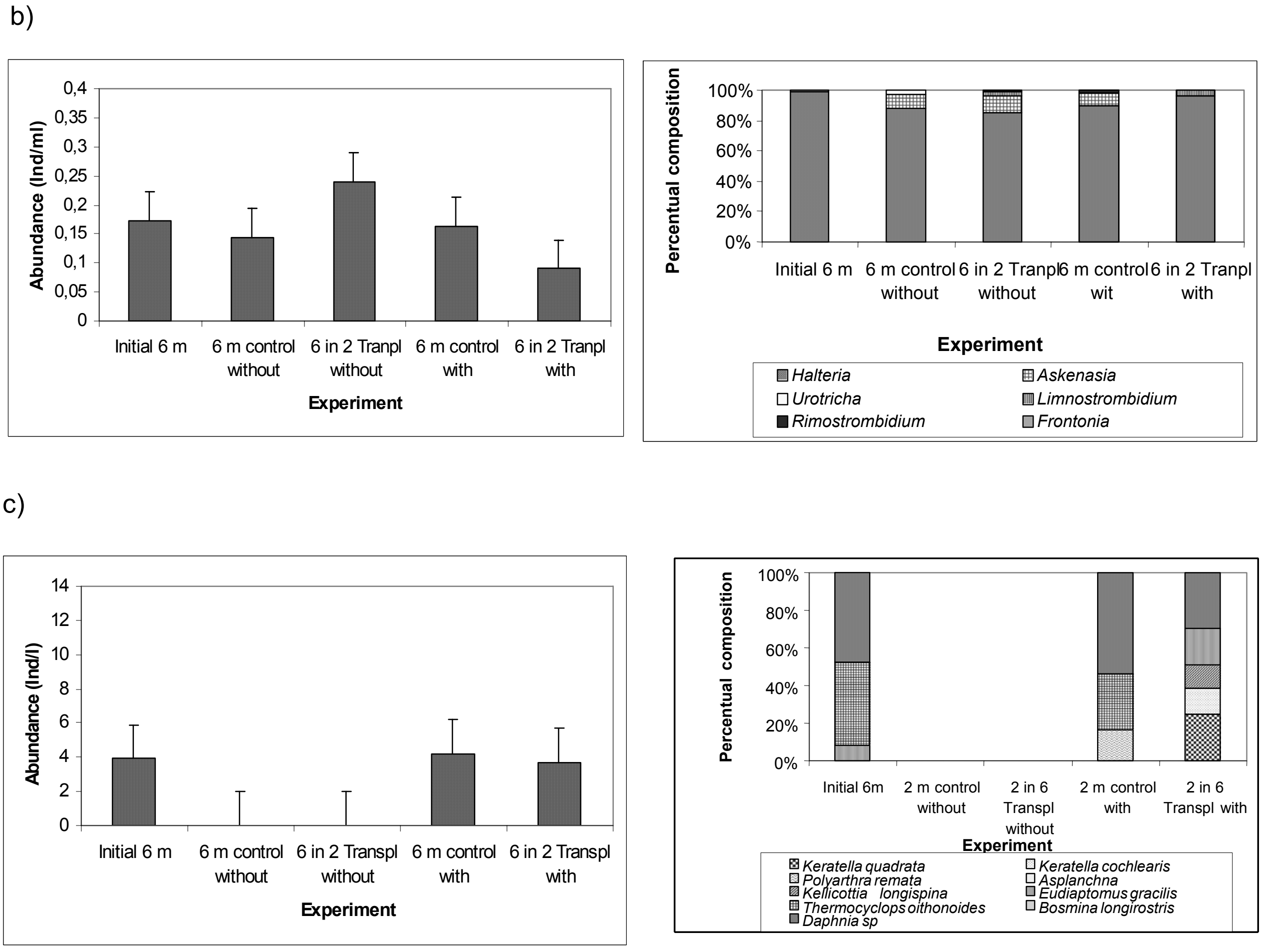


Fig 5.- Total algal (a), ciliate (b) and zooplankton (c) abundance and percentual composition in the 6 meter set for April 2003.

The results for the 3 way analysis of variance for the ciliates indicate significant differences among treatments. The original depth (control) and the transplanted depth (incubated depth) for *Halteria* without zooplankton were statistically different (p = 0.037*), where the total ciliate abundance was either reduced (0.25 ind/ml to 0,18 ind/ml when transplanted to a lower depth) or increased (0.14 ind/ml to 0.24 ind/ml when transplanted to a higher depth). When transplanted with zooplankton, these statistical differences were much more evident (p = 0.014*), where *Halteria* abundance decreased from 0.27 ind/ml to 0.07 ind/ml when transplanted to a lower depth. The effect of predation over the ciliates was more reduced when transplanted to a higher depth, due in part to lower zooplankton abundance and the relatively higher ciliate reproductive rates.

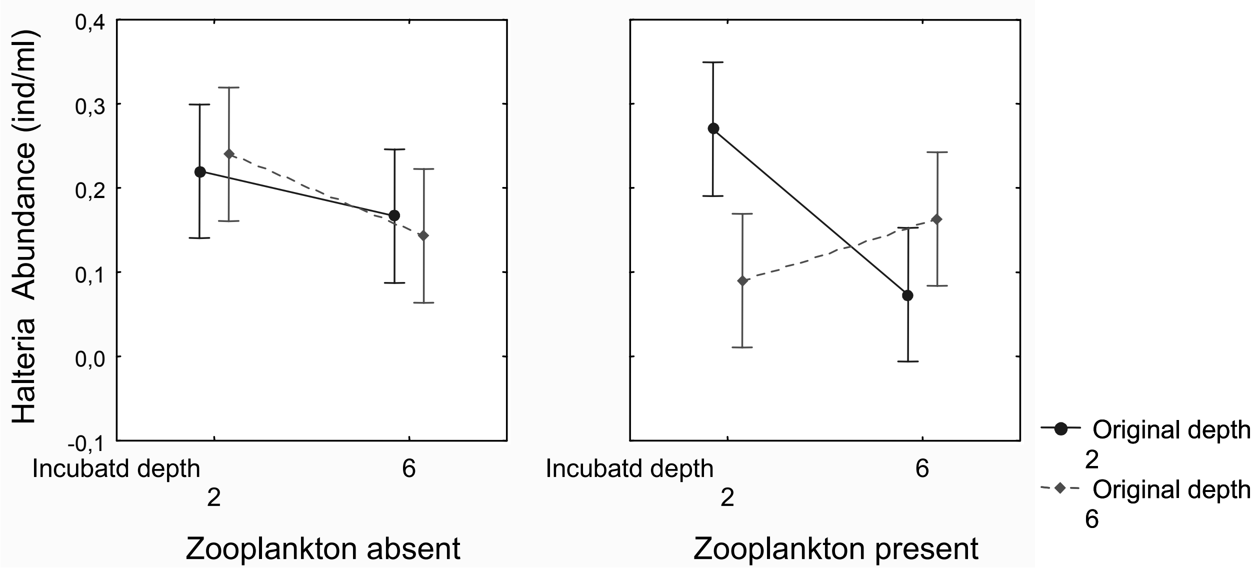


Fig. 6.- 3 way ANONA test results for the April 2003 experiment

Experiment 2 : August 2003.

Physico-chemical parameters: Temperature (°C) and % oxygen saturation.-

The temperature remains constant for the first 3 meters (25°C), then descends abruptly to 10°C in the following 3 meters and continues to descend to 5°C from 8 meters and remains constant from 9 meters to 14 meters. The oxygen profile presented a different pattern, with minimal variations from surface to 4 meters. Saturation peak was found in 5 meters to descend very rapidly to less than 50% saturation in 6 meters and then reaching anoxic levels from 7 meters to 14 meters in the hypolimnion. Water transparency reached from surface to a depth of 2.87 meters, therefore allowing enough light penetration to enhance photosynthetic activity and in consequence to produce high oxygen levels.

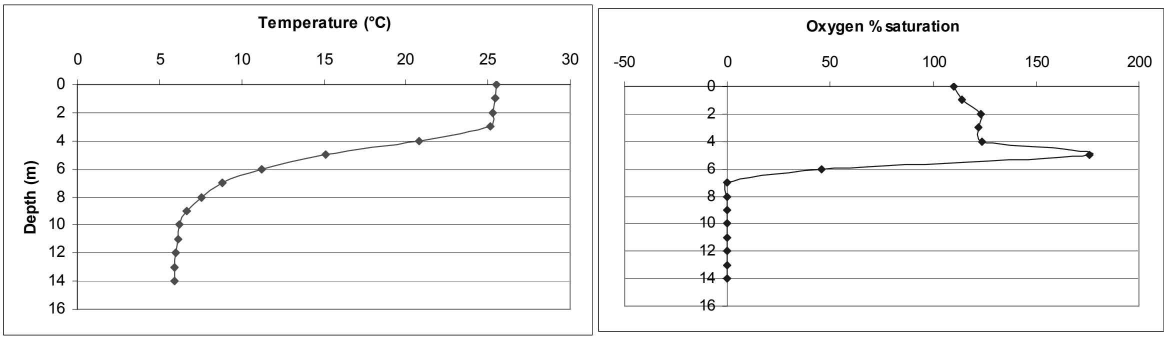


Fig. 7.- Temperature (°C) and oxygen concentration (%O_2 sat.) during the month of August 2003.

Ciliate and zooplankton composition and vertical distribution in Lake Speldrop

The ciliates were irregularly distributed along the depth profile. High abundances were found during the day in the depth interval from 6 to 10 meters. The dominant ciliates during the day were *Halteria*, *Holophrya* and *Spirostomum*. Less important were *Rimostrombidium*, and *Plagiopyla*. During the night however, general abundance was greatly reduced to one third of the original when compared with the abundance found during the day. During the night, minimal abundance were found in the 6 meters level, represented by the ciliates *Halteria* and *Rimostrombidium lacustris.* Other ciliates, such as *Rimostrombidium,* gained in importance in the 2 meter level. *Keratella cochlearis* was the dominating species among the zooplankton, followed by *Bosmina longirostris* and *Daphnia sp*. which in turn presented much lower abundances when compared to the April experiment.

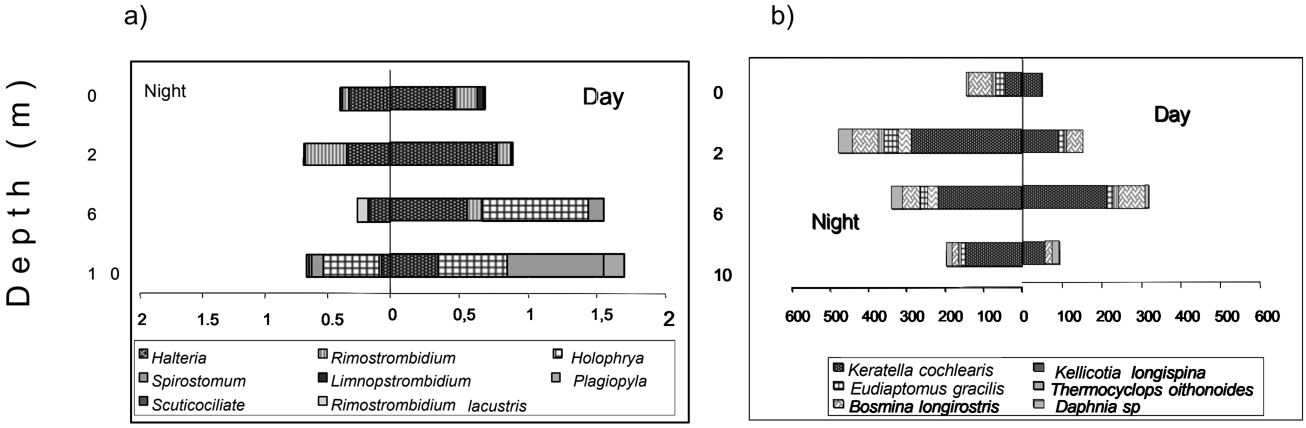


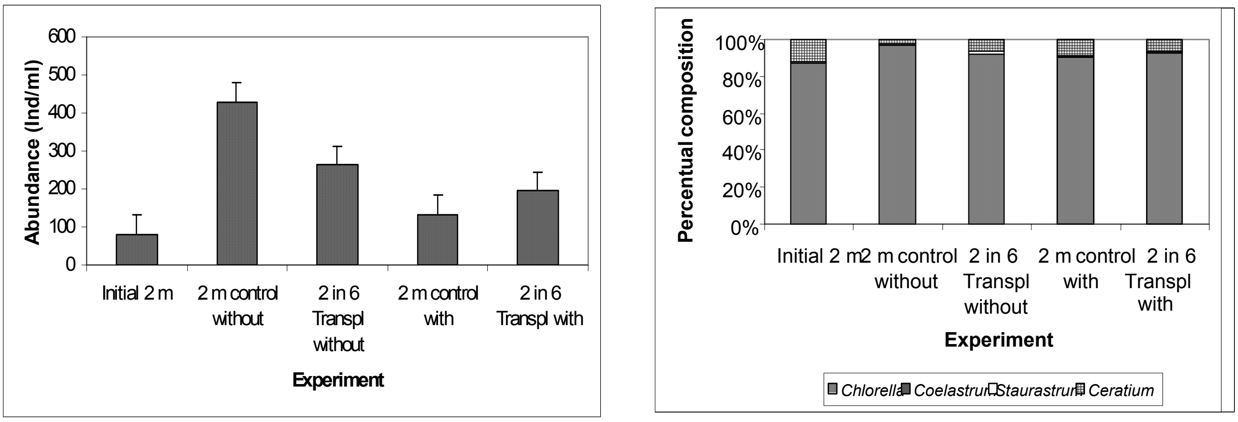
Fig 8.- Ciliate (a) and zooplancton (b)depth distribution in August 2003

2 meter set. Algae, ciliates and zooplankton.

The dominating alga on the 2 meter set was *Chlorella*, comprising more than 80% of the total abundance, followed by *Ceratium*. The lowest abundance was found in the initial sample. During the experiment, *Chlorella* abundance reached its maximum in the 2 meter without zooplankton sample, due to the absence of grazing zooplankton. But when transplanted to a deeper level, abundance was reduced. When faced with zooplankton at the control depth, abundances were much lower. Grazing activity was thus much greater in the 2 meter control with zooplankton sample than the transplanted sample, which would explain the slight difference in abundance. The initial sample was dominated by *Halteria* which presented the highest abundance, together with less important species such as *Rimostrombidium* and

Frontonia. The control sample without zooplankton had only *Halteria* as the most important species, whereas the transplanted without zooplankton had a smaller abundance but the presence of entirely different species among them: *Limnostrombidium* and *Spirostomum*. The highest abundance for the zooplankton was found in the initial sample, and the experimental samples had extremely low abundances. The dominating species in all samples was *Bosmina longirostris*, followed by *Thermocyclops oithonoides* and *Eudiaptomus gracilis*. *Daphnia sp* and the rotifers were also present, but in much lower abundances.

a)



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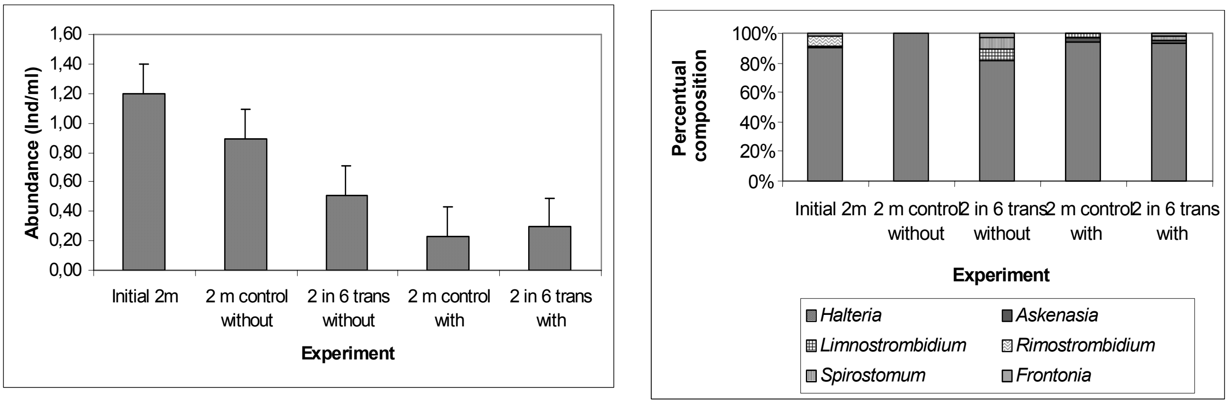


c)

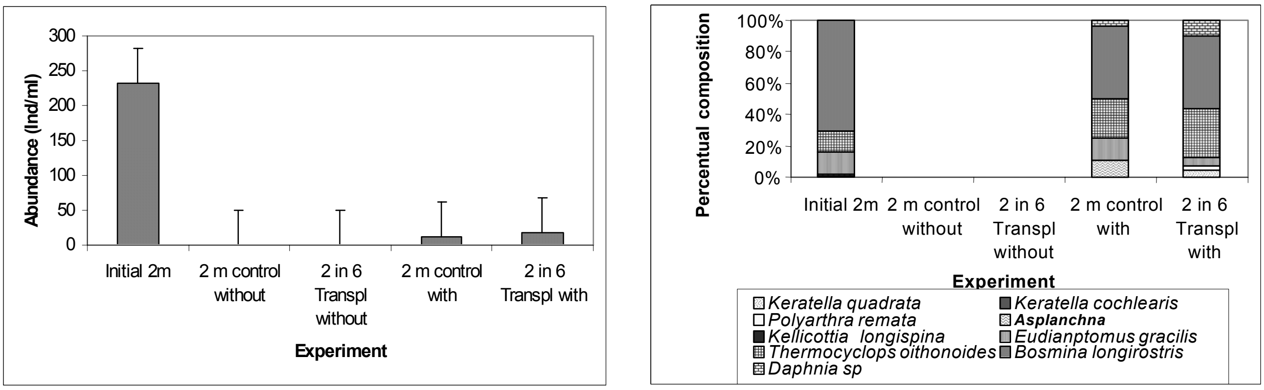
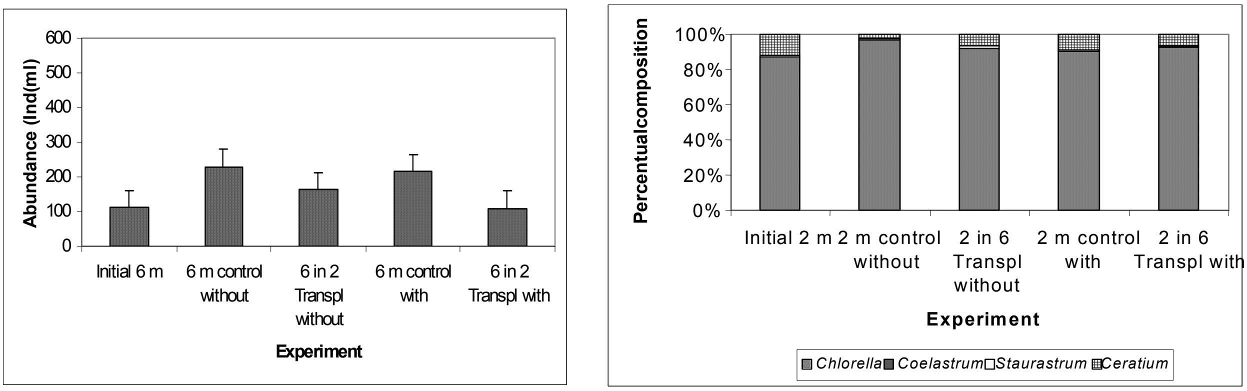


Fig 9.- Total algal (a), ciliate (b) and zooplankton (c) abundance and percentual

6 meter set. Algae, Ciliates and Zooplankton

In the 6 meter experimental set, general abundances for the algae were much lower. As in the 2 meter set, very low abundance was found in the initial sample. The maximal abundance was found in the 6 meter control without zooplankton sample, which decreased when transplanted to a higher depth. Grazing activity was rather low in the 6 meter level, which increased when transplanted to a higher depth, which would explain the reduction in algal abundance. The initial 6 meter sample was dominated by *Halteria* together with *Askenasia*, *Limnostrombidium* and *Rimostrombidium*. Total abundance was also fairly high. The 6 meter control without zooplankton sample presented together with the dominating *Halteria* and *Spirostomum*, and was not very different from the transplanted sample, where *Halteria* reached the highest abundance. The control sample had also *Spirostomum* but *Frontonia* was present instead of *Limnostrombidium*. The transplanted sample as the previous 2 meter sample, *Bosmina longirostris* was the dominating species, together with *Eudiaptomus gracilis* and *Thermocyclops oithonoides*. The control and transplanted samples were reduced in numbers and presented mostly *Bosmina longirostris* and *Thermocyclops oithonoides.*

a)



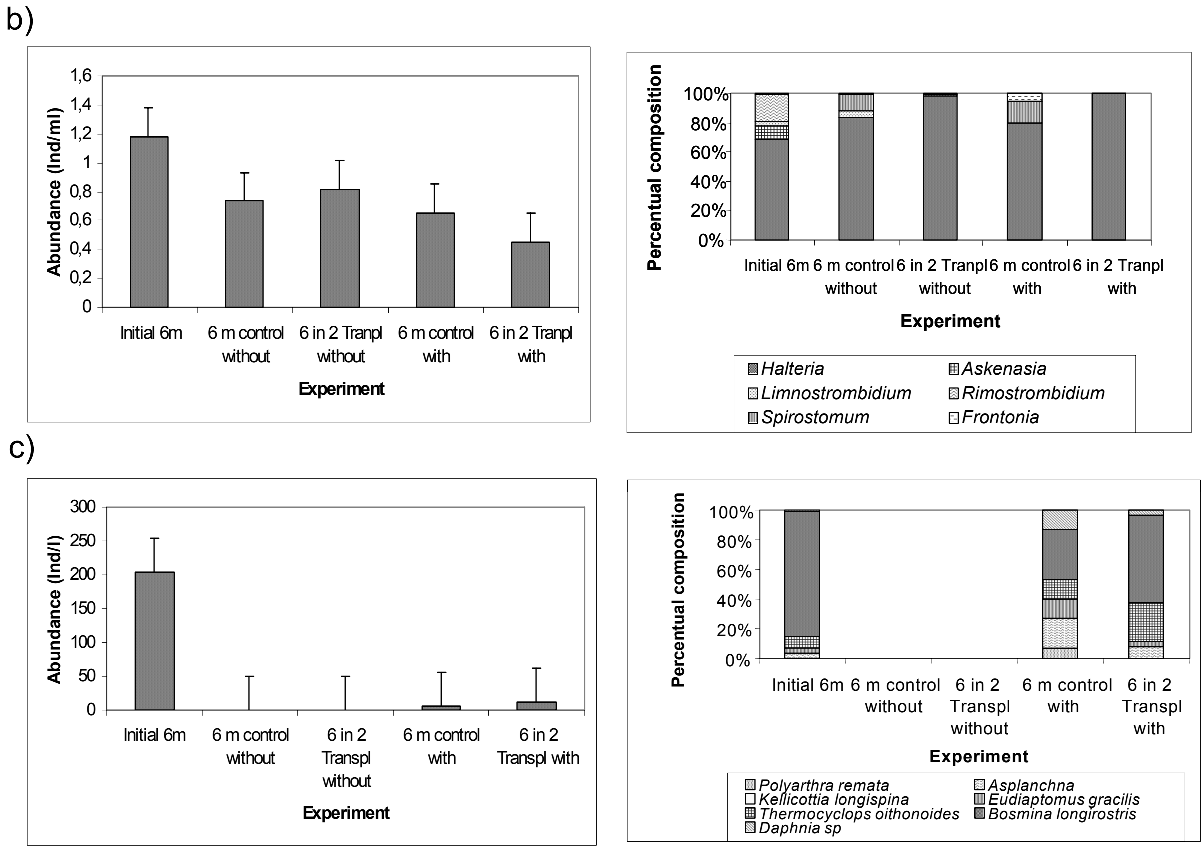


Fig 10.- Total algal (a), ciliate (b) and zooplankton (c) abundance and percentual composition in the 6 meter set for August 2003.

The results for the 3 way analysis of variance did not show any significant differences among the treatments for any of the ciliates , except for *Spirostomum* (p = 0.026*), which increased in abundance when transplanted to a lower depth, and decreased when transplanted to a higher depth, regardless to the presence of zooplankton.

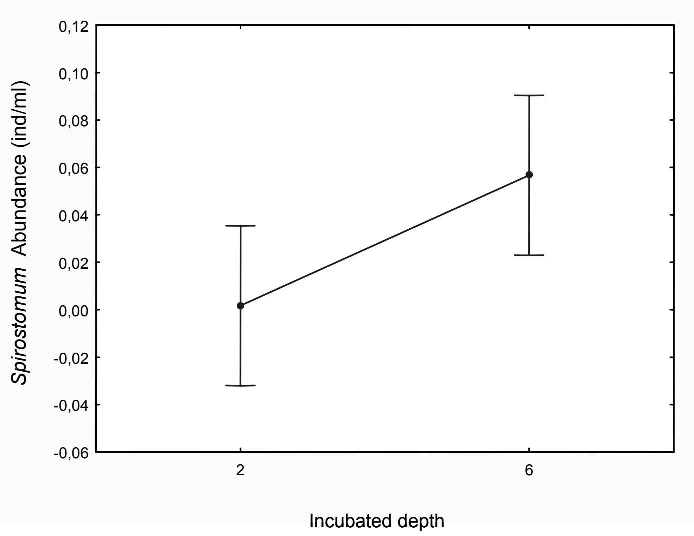


Fig. 11.- 3 way ANOVA results for the August 2003 experiment

Experiment 3 : October 2003.

Physico-chemical parameters.Temperature (°C) and % saturation oxygen .-

The temperature profile presented a constant value of 8 °C for the first 7 meters, then descending to 7° C at 8 meters to then continue descending to 6° C for the following 4 meters. The oxygen profile presented a similar pattern, with a constant value of 90% saturation for 6 meters. Then descending to 75% in the 7 meter depth level to then descend very rapidly to reach practically anoxic levels from 8 meters downwards. The stratification border was thus present at a different layer than the previous months, so the placing of the experimental samples had to be different. The weather during that month was quiet cold and windy, which would explain the thorough mix-up in the upper layers

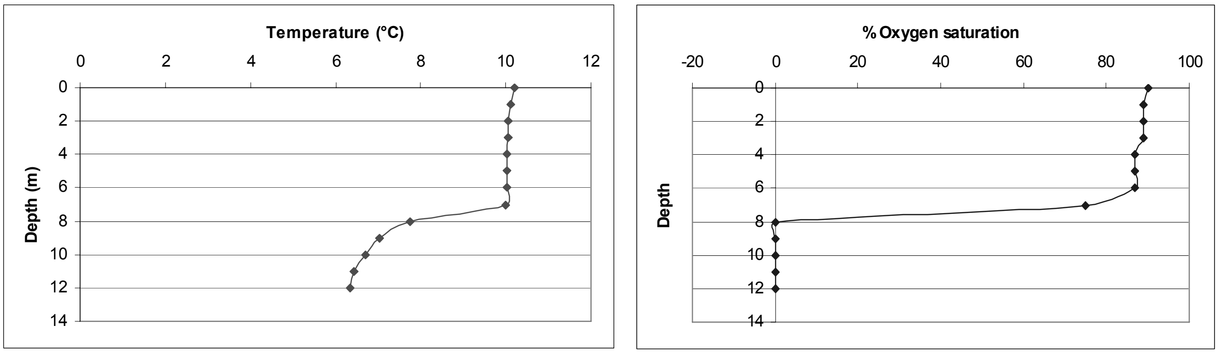


Fig. 12.- Temperature (°C) and oxygen concentration (%O_2 sat.) during the month of October 2003.

Ciliate and zooplankton vertical distribution in Lake Speldrop in October 2003

During the month of October the ciliates presented a regular depth distribution with nearly equal day and night abundances. The dominating ciliates were clearly *Askenasia* and *Halteria*, which were present throughout the depth profile. Following in importance was *Rimostrombidium*. Other ciliates, such as *Spirostomum*, *Caenomorpha*, *Coleps* and others, were also present but in much lower abundances. Vertical migration can also be appreciated, where day time distribution patterns were different from the night time distributions. The preferred depth for the ciliates during the day was 2 meters, then during the night either migrating to the surface or to a deeper layer. The zooplankton depth distribution was irregular, with a great difference among the day and night distributions. The greatest abundance was found during the night, and during the day abundance was reduced. The dominating organism was the rotifer *Keratella cochlearis*, which presents a vertical migration pattern, along with other organisms, among those the copepods *Eudiaptomus gracilis*, *Thermocyclops oithonoides* as well as the cladocerans *Daphnia sp.* and *Bosmina lomnirostris*. The rotifers *Asplanchna* and *Polyarthra remata* did not perform migrations, especially *Asplanchna,* which presented equal day and night distributions along the depth profile (fig. 19).

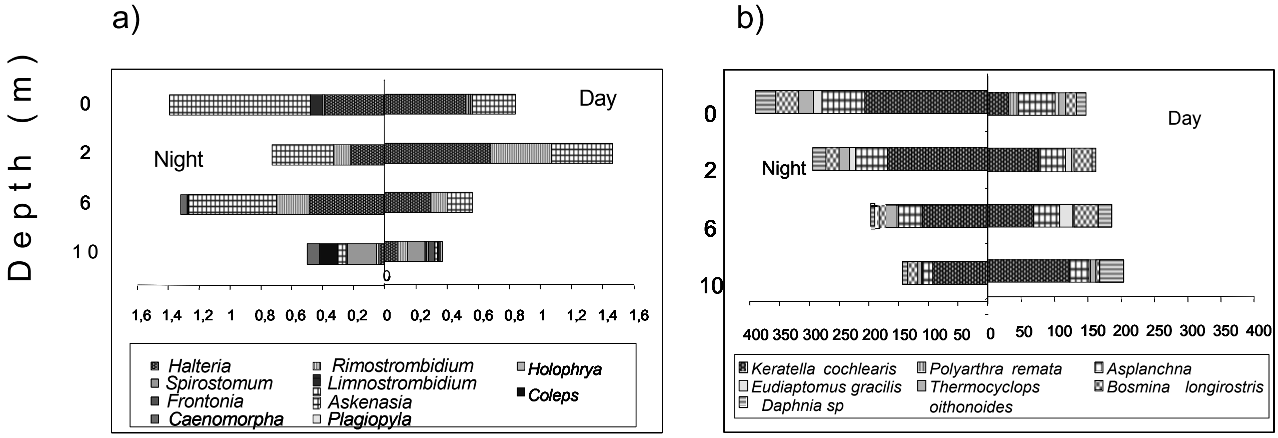


Fig 13.- Ciliate (a) and zooplankton (b) depth distribution in October 2003

2 meter set. Algae, ciliates and zooplankton.

The dominating algae on the 2 meter set was *Choleralla*, comprising more then 90 % of the total, followed by *Staurastrum* which was present in much smaller abundances. The highest abundance was found both in the 2 meter control without zooplankton sample and the 2 in 9 transplanted without zooplankton. The lowest abundances were found in the initial sample and the control with zooplankton sample and the transplanted 2 in 9 with zooplankton sample. Among the ciliates the dominant species were *Halteria* and *Askenasia* followed by *Rimostrombidium* and *Limnostrombidium*. Other ciliates such as *Frontonia* and *Urotricha* were also present, but in smaller abundances. The dominant zooplankton species in the 2 meter control sample were the rotifers *Keratella quadrata*, *Asplanchna* and *Brachionus*. Also present was the copepod *Thermocyclops oithonoides*. The 2 meter control with zooplankton had the presence of the cladocerans *Bosmina longirostris* and *Daphnia sp.* together with the rotifers *Asplanchna* and *Brachionus.* The 2 in 9 transplanted with zooplankton sample had two main representatives: *Bosmina longirostris* and *Thermocyclops oithonoides*. All samples were relatively low in abundance (less than 8 individuals per liter) when compared to the 9 meter set.

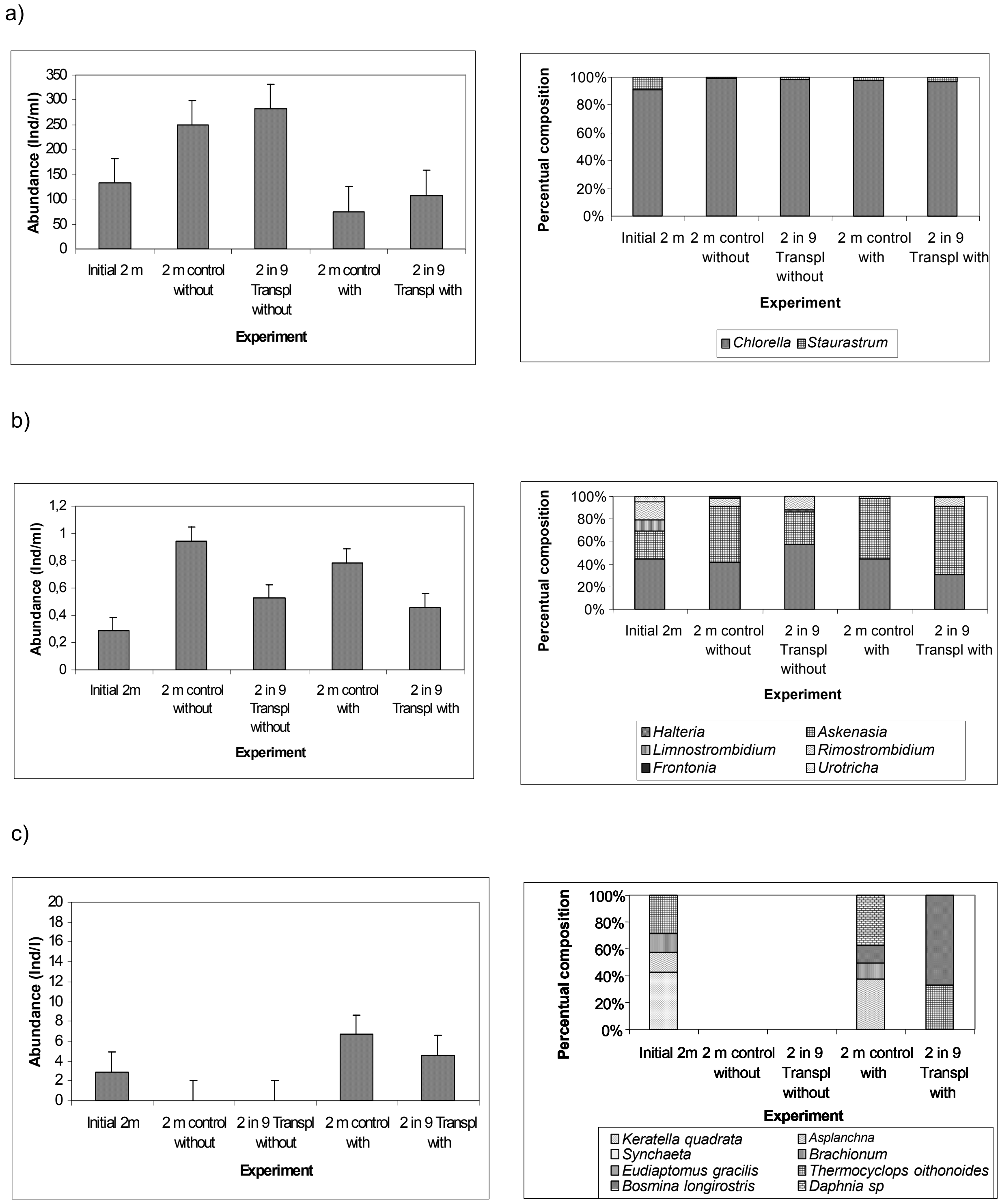
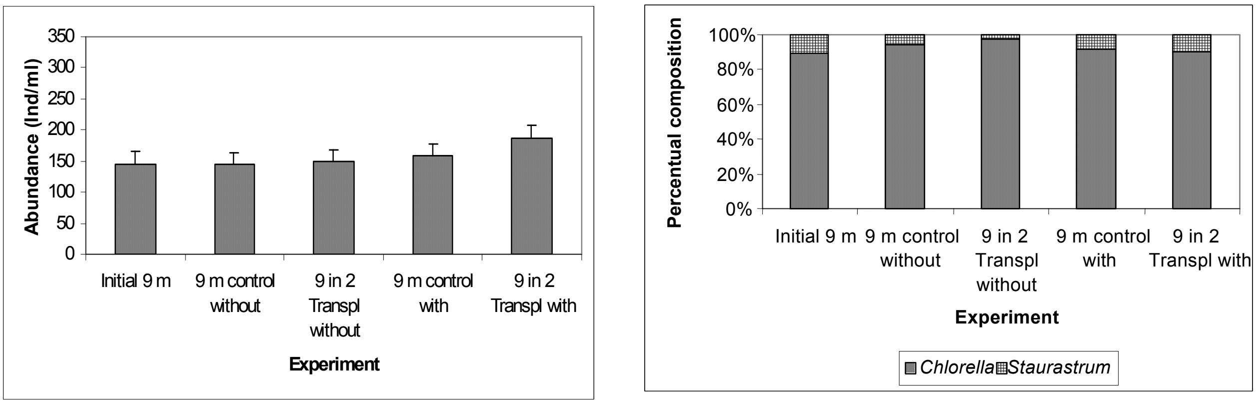


Fig. 14.- Total algal (a), ciliate (b) and (c) zooplancton composition and percentual composition n the 2 meter set for October 2003.

9 meter set. Algae, ciliates and zooplankton.

The dominating algae in the 9 meter sample was *Chlorella*, which comprised more then 80% of the total abundance, followed by *Staurastrum*, which presented very low abundances. The maximal abundance was found in the 9 in 2 transplanted sample without zooplankton. The minimal abundance was found in the 9 meter control sample. *Halteria* and *Askenasia* were the principal ciliates in the 9 meter sample, followed by *Rimostrombidium* and *Epistylis*. As well as with the algae, maximal abundance was found in the 9 in 2 transplanted sample without zooplankton and the minimal abundance, contrary to the algae, in the 9 in 2 transplanted sample with zooplankton. Among the zooplankton the dominating species was *Keratella cochlearis* in the initial and the transplanted 9 in 2 with zooplankton samples. The 9 meter control sample had the largest number of species, with *Thermocyclops oithonoides* as the dominant species, followed by *Keratella cochlearis* and *Eudiaptomus gracilis*. *Daphnia sp.* was also present, but in lower abundances and absent in the initial sample. The highest abundance was found in the 9 in 2 transplanted sample with zooplankton, and the lowest in the initial 9 meter sample.

a



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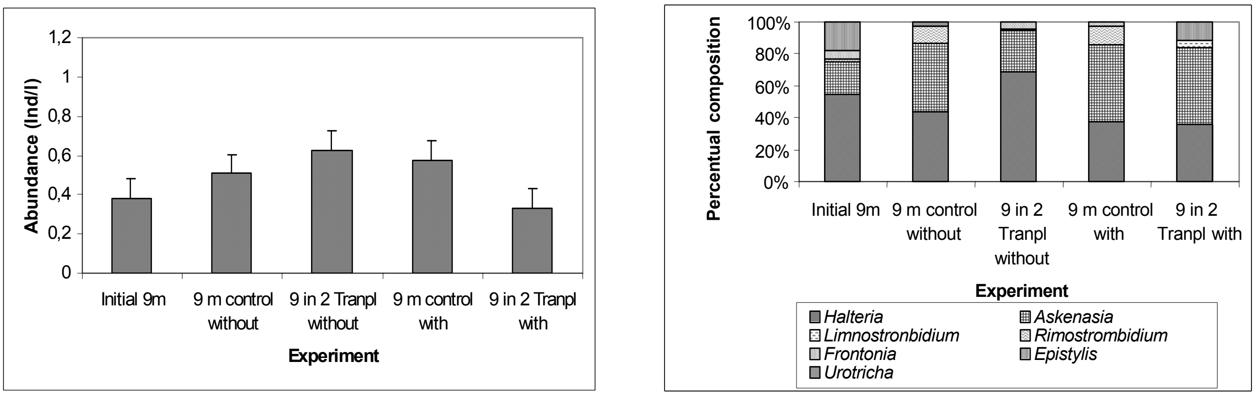


c)

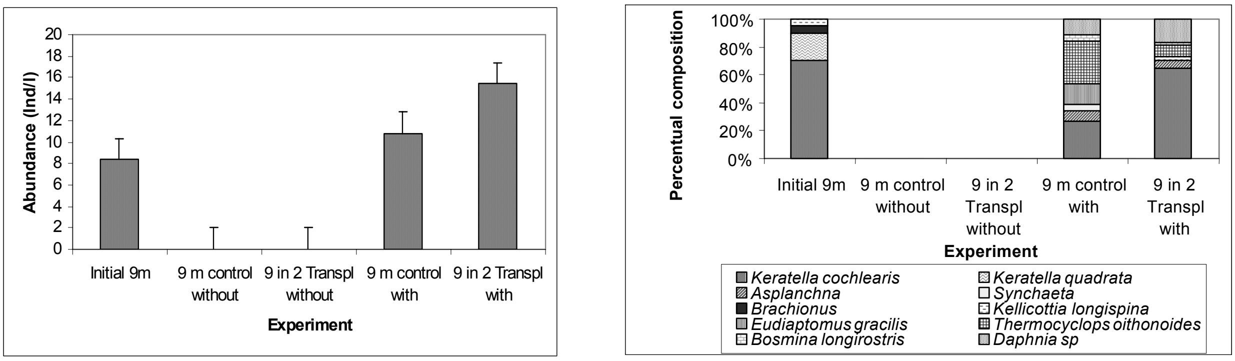
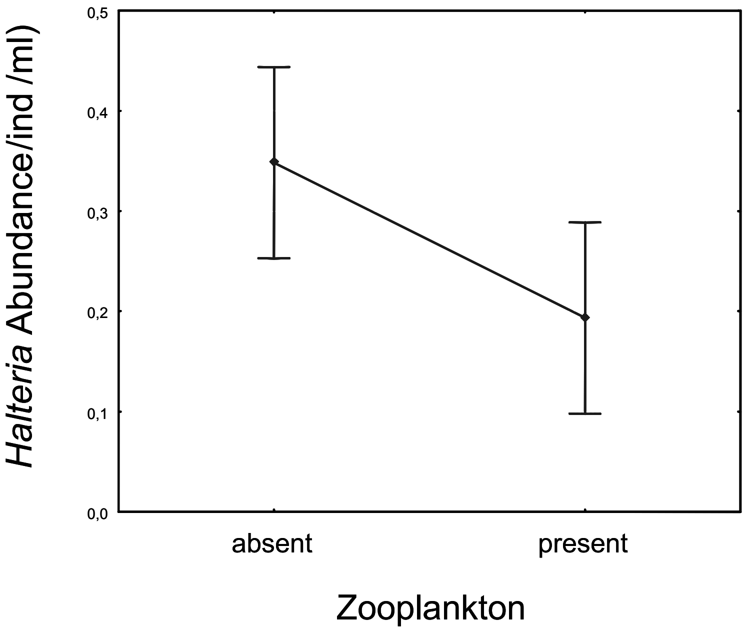
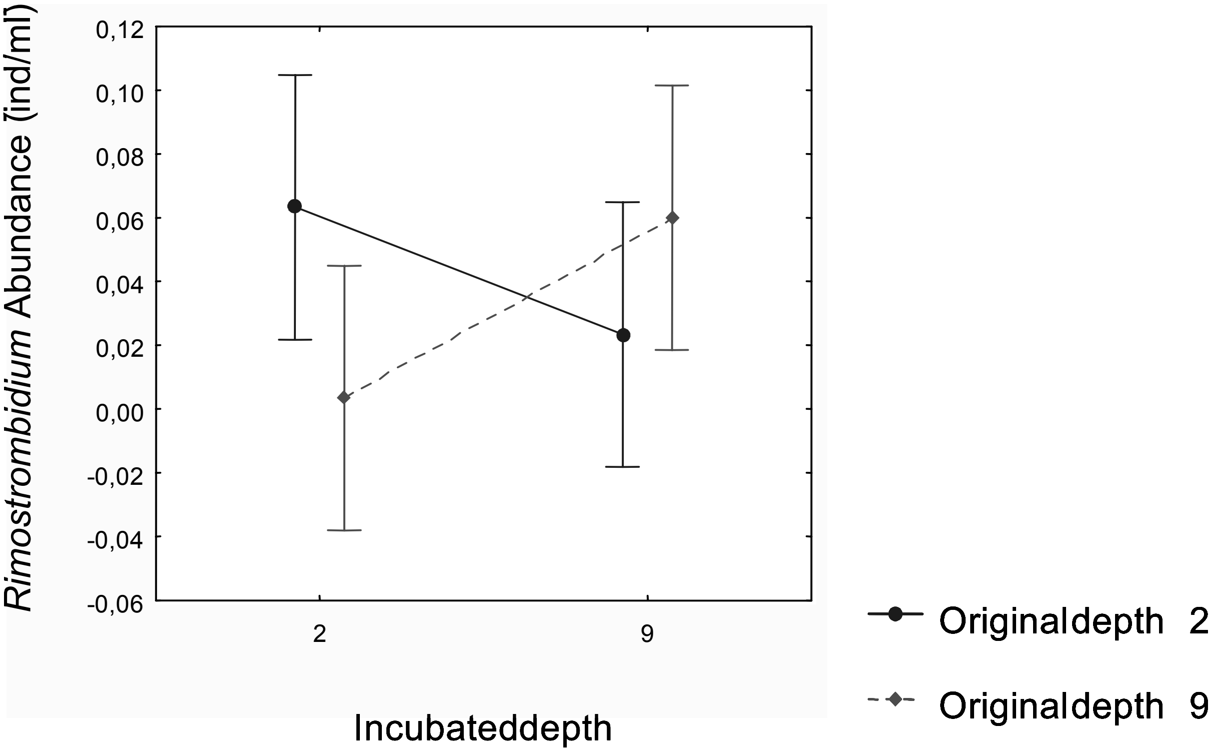
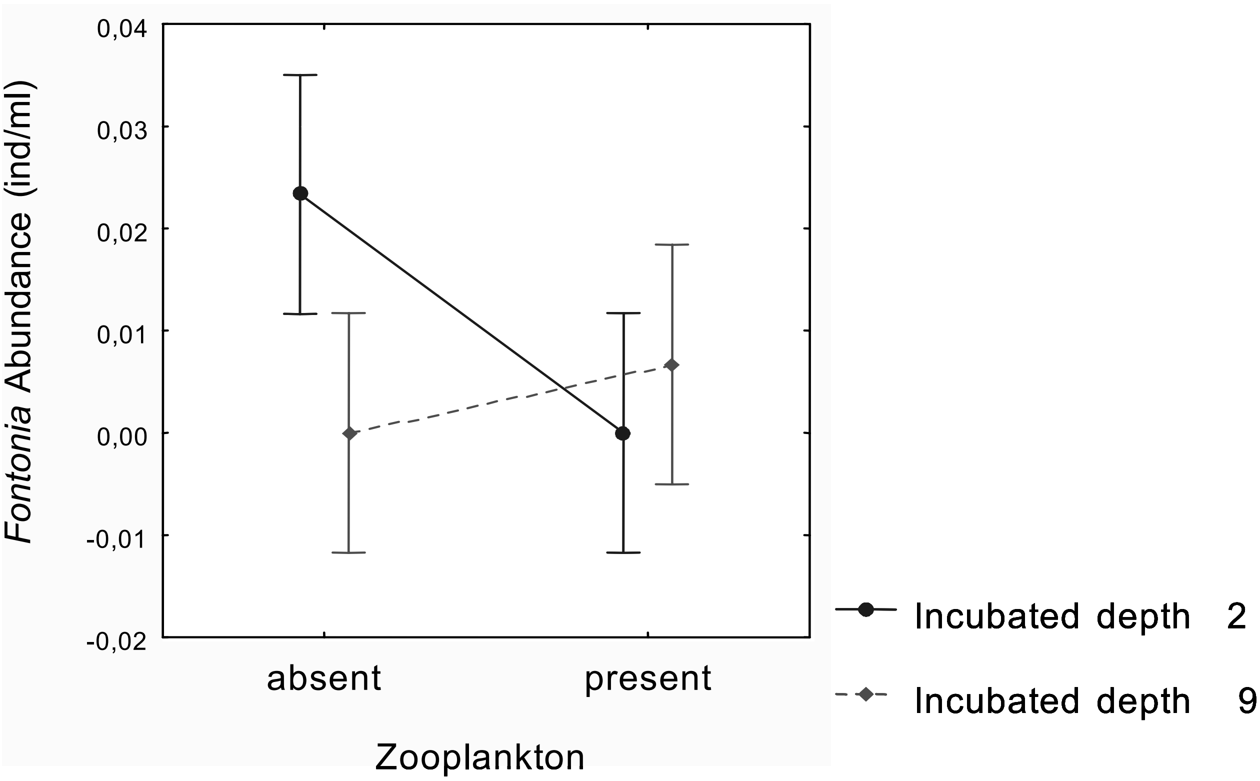


Fig 15.- Total algal (a), ciliate (b) and zooplankton (c) abundance and percentual composition in the 9 meter set for October 2003

During the month of October, statistical differences were found for *Halteria* (p = 0.027*) when faced to the zooplankton, regardless to be transplanted or not, to a different depth. Statistical differences were also found for *Rimostrombidium* (p = 0.025*), which decreased in abundance when transplanted to a higher depth (from 9 meters to 2 meters) and in a much lower degree, when transplanted to a lower depth without the presence of the zooplankton. The presence of zooplankton was more evident for *Frontonia* (p = 0.01**), which decreased in abundance when incubated in the 2 meter set with the presence of zooplankton. When incubated in the 9 meter set, predator influence was not evident.







Fig, 16.- 3 way ANOVA results for the October 2003 experiment.

Discussion

My results demonstrate that vertical migration does have an important effect, either positive or negative over the ciliates` survival and mortality rates. The advantages or disadvantages of vertical migration lie close together, and often it is difficult to differentiate among them. The common migratory trend for the biggest part of the zooplankton organisms suggests that migrations are advantageous to those organisms, that migrate up during the night to feed in the rich surface waters and then migrate down to remain in the deep water refuges (Lampert, 1989; Neill, 1990; Gilbert & Hampton, 2001; De Robertis, 2000; Pérez *et al.,* 2000; Crawford & Lindholm, 1987).

Ciliates that migrate to a favourable habitat (mostly the epilimnion) would have higher survival possibilities and higher reproductive rates than those which remain in the unfavourable habitat (hypolimnion). This would explain the increase in the abundance of *Halteria* when incubated at a higher depth, where the effects of higher light intensities do have a positive effect over ciliate growth rate, which can be explained by the light aided photo-oxidative break down of ingested organic matter (Strom, 2001) as well as higher temperature values, which are positively correlated with the ciliate growth rates (Massana *et al*., 1994; Atkinson, 2003; Sun, 2004). However, the deleterious effect of direct UV radiation cannot be excluded. In most cases, low abundances for the ciliates and zooplankton were found directly at the surface especially during the day.

Ciliate growth rates would be even greater in the absence of zooplankton (Wickham, 1998). The low rates of predation, and the favourable environmental conditions would lead to a rapid increase in ciliate abundance. Eventually, this increase would then be regulated by a higher predatory activity from the zooplankton in the favourable habitat (Broglio *et al.,,* 2001; 2004; Modenutti *et al*., 2003; Marchesault & Mazumder, 1997) especially from the copepods which are more effective consumers of protozoa than cladocerans, particularly in eutrophic conditions (Burns & Schallenberg, 2001).

The environmental conditions during the month of October would have played an important role in determining the ciliates´ migration activity. During this month, the hypolimnion had reached total anoxic conditions, which would have been an unfavourable strategy for *Rimostrombidium* to perform migrations to this region, even with the risk of predation in the epilimnion. However, when transplanted to a higher depth level where conditions were better abundances were also reduced, even without the presence of zooplankton, which could be a result of the relatively low edible algal abundances, inter-specific competition and predation among ciliates. During this month *Halteria* and *Askenasia* were particularly abundant. Another factor could be that during sampling some zooplankton predators slipped through the mesh, and their predatory activity was enhanced in the epilimnion.

Zooplankton predation was more evident for *Frontonia* when left at the 2 meter set where predatory activity was higher, and food resources were lower (bacterioplankton). On the other hand, predatory activity was much more reduced when left at the 9 meter set although zooplankton abundances were not low in fact they were much higher than in the 2 meter set. *Frontonia* was found mainly concentrated in the hypolimnetic area (initial sample, 9 meter set), which had a rich abundance in purple sulphur bacteria, which meant available food resource. This would also explain the increase in abundance even with the presence of zooplankton.

This migratory activity would also lead to a trade off situation (Decaestecker *et al*., 2002), where ciliates, which migrate up in order to evade unfavourable habitats in the hypolimnion are faced to a higher predatory threat in the epilimnion. On the other side, ciliates, that migrate down in order to evade predatory pressure in the epilimnion are ultimately faced with the risk of dealing with unfavourable environmental conditions in the hypolimnion. From the metabolic point of view, migrating animals do not gain any metabolic advantage over non-migrating ones (Guisande & Duncan, 1991). This would mean that the ciliates that tend to migrate towards richer surface waters do not always have higher survival chances compared to those which do not migrate, since this migrating activity would also “overlap” with the parallel migration movement of their potential predators, unless correct “migration timing” is performed (De Robertis, 2002).

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Annual cycle of protozooplanktonic ciliates in relation to food availability and predatory pressure in a eutrophic lake.

Introduction.-

It has been long since recognized that the biological community that inhabits a given sector is composed by species adapted to live under the specific conditions that prevail there. The temporal modification of the lake's physical and chemical conditions during the stratification period will ultimately affect the structure and functioning of the biological community. The temporal and spatial variations for zooplankton communities as a whole have been well documented (Mathes & Arndt, 1995; Bojanic, 2001; Modigh, 2001; D´Álcala *et a*l., 2004; Bojanic *et al*., 2005).

The annual cycle of the ciliate community and its role within the pelagic food web can be clearly influenced by bottom up (resource induced) and top down (predator induced) factors (Rothhaupt, 2000; Adrian & Schneider, 1999; McQueen & Post, 1996; Wickham 1995, 1998; Sarnelle, 1997; Jack & Gilbert, 1997; Burns & Gilbert, 1993).as well as environmental conditions (Gong *et al*., 2005; Rodrigo *et al*., 2001).

The present study was carried out in order to get more information about the seasonal changes in the quantitative and qualitative composition of a heterotrophic ciliate community during the course of a year taking in account not only the variations of abundance and biovolume, but its relation with other major components of the zooplankton and environmental factors.

Material and Methods.-

Sampling methods.- Day and night samples were taken once a month from the months of March to October during the year 2002 in lake Speldrop. Each sample consisted of two parallel sub-samples for ciliates at 1 meter intervals (0-14 m) which were taken every meter with a 2 liter water sampler (diameter 10 cm) which can sample the exact depth. Due to the low abundance of Cladocera (*Daphnia sp, Bosmina longirostris*), Copepoda (*Thermocyclops oithonoides, Eudiaptomus gracilis*) and Diptera (*Chaoborus flavicans*) samples for the zooplankton were taken with a 20 liter Schindler sampling unit. Ciliates were fixed with Bouins´ solution and the Mesozooplankton in a sucrose formalin solution. At the same time vertical physico-chemical profiles for the oxygen, temperature, conductivity, pH and chlorophyll maxima were measured every meter with an YSI 6820 multisonde. The obtained physico-chemical data was immediately analyzed in-situ to determine the presence of stratification and its position within the water column.

In laboratory, ciliates were left to settle in 50 ml Utermöhl chambers,, identified up to the genus level using the QPS silver staining method (Montagnes & Lynn, 1987) and counted under a Zeiss Axiovert S100 microscope. The zooplankton samples were re-sampled from the original 20 liter sample to 10 ml and counted under a Olympus Optical CO 170 stereomicroscope and also identified to the closest possible level.

For the biovolume, dimensions (length and width) were measured and volume calculated, assuming the organism approximates a cube, sphere cylinder or some other known geometrical form, and compare with reference data (Wetzel & Likens, 1991).

In order to simplify analysis, ciliates were grouped according to their corresponding higher groups, Oligotrichida (*Halteria, Rimostrombidium, Limnostrombidium,, Rimostrombidium lacustris*), Prostomatidae (*Coleps, Holophrya, Urotricha*), Litostomatea (*Askenasia, Phyalina, Lacrymaria*), Peritrichia (*Epistylis, Vorticella*), Heterotrichida (*Spirostomum, Caenomorpha*), Scuticociliatida (scuticociliates) and Hymnostomata *(Frontonia, Sarthrophilus muscorum*).

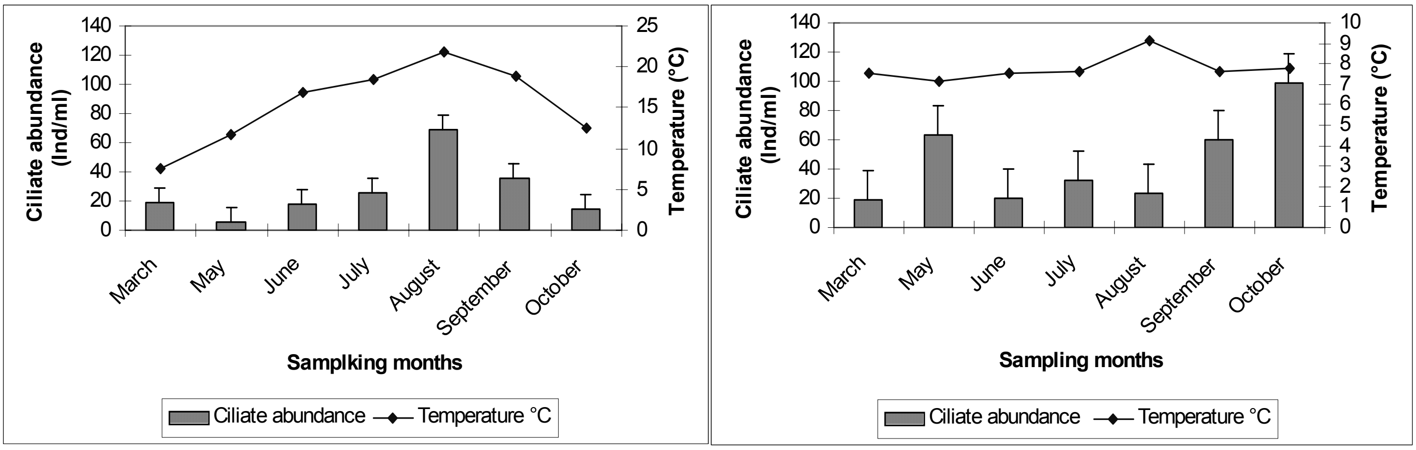
To analyze results, I used two multivariate techniques, a cluster analysis (according to Ward´s method, Euclidean distances) was used to classify taxonomic groups and months according to their abundances which were previously transformed and standardized by the procedure Log (X +1). These classified groups were then ordered and plotted in two main axes by a factorial analysis.

Results.-

Environmental factors .-

Temperature.-

The temperature did play an important role determining the annual cycle of the ciliates´ abundance. Ciliate abundances were more reduced in the epilimnion than in the hypolimnion. The highest ciliate abundances were found during the month of August, which coincides with the highest temperature value (21,7°C), and low abundances were found during the months of March, May, June, July and October where temperature (°C) averaged 13° C. Ciliate abundances were much higher in the hypolimnion than the epilimnion. . Maximal ciliate abundances were found during the months of June, September and especially during October. The temperature did not present major fluctuations, averaging 7,76° C during the sampling year. The highest temperature value was found during the month of August, (13° C), which in turn, presented very low ciliate abundance (fig 1).



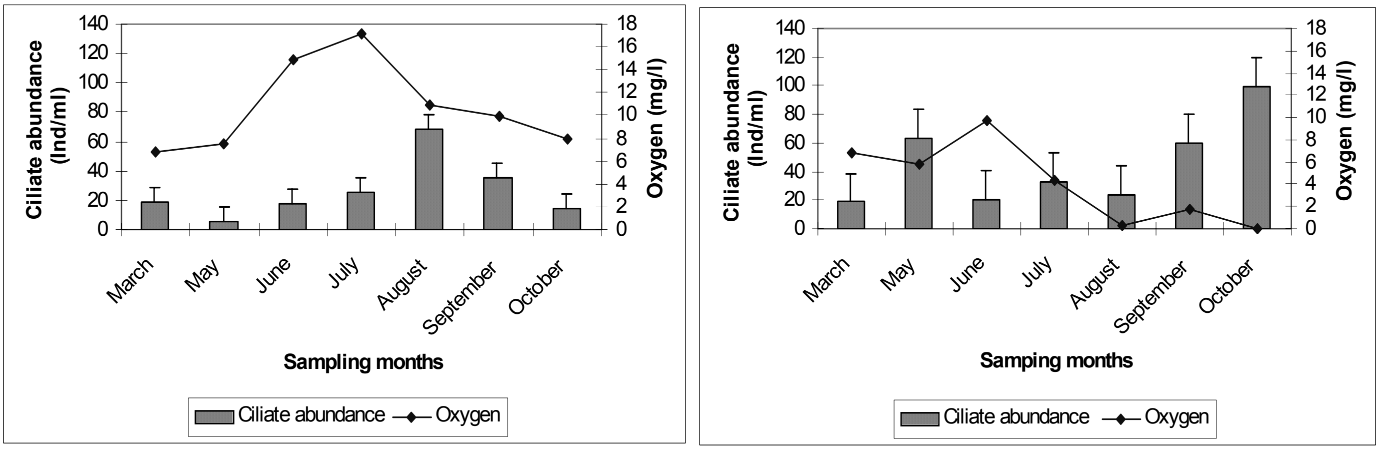
a) Epilimnion

b) Hypolimnion

Fig. 1.- Annual temperature (°C) variation with ciliate abundance in the epilimnion (a) and hypolimnion (b)

Oxygen.-

In the epilimnion, the highest oxygen concentrations were found during the months of June and July. The lowest during the months of March, May, August September and October. In general, the epilimnion remained well oxygenated throughout the year. On the contrary, ciliate abundances were low in the epilimnion, even at high oxygen levels. The highest ciliate abundance was found during the month of August, were oxygen concentration reached a value of 10,8 mg/l. The oxygen concentration in the hypolimnion was much more reduced. During the months of March until July, oxygen levels averaged 6,7 mg/l, The hypolimnion during the months of August until October had very low oxygen concentrations, averaging 0,6 mg/l. High ciliate abundances were found during this period, as well as during the month of July. Even at similar oxygen concentrations in the epilimnion and hypolimnion during the month of May, (7,4 mg/l and 5,8 mg/l respectively) ciliate abundances were different during this month (fig. 2)


a) Epilimnion

b) Hypolimnion

Fig. 2.- Annual oxygen (mg/l) variation with ciliate abundance in the epilimnion (a) and hypolimnion (b)

Species composition

The percentual composition for the abundance and biovolume considering the main ciliate groups can be appreciated on figure 1. During the month of March, the dominating group were the oligotrichs, comprising almost 90% of the total abundance, followed by the Litostomatea. There is a progressive decrease in oligotrich abundance from the months of May towards August, during the stratification period of the lake, with a slight increase during the month of September, but to be reduced again during the month of October. On the other hand, the prostomes experienced a progressive increase in abundance from the months of May towards October, where it reached its maximal abundance. Other groups, among them Hymenostomata, Heterotrichida, Peritrichida, and Scuticociliata, were also present throughout the sampling year, but in much lesser abundance, and experienced very little variation among their abundances during the sampling year. The least number of taxonomic groups was found during the month of March, which gradually increases along stratification.

According to the biovolume, the month of March was dominated by Oligotrica and Litostomatea. The Prostomatida were present during the whole sampling year, with maximal values found during the period of stratification, comprising the months of July towards September. Another very important group during pre-stratification and stratification were the Hymenostomata. The months of June and July were characterized by the presence of Heterotrichida. The groups Peritricha and the Scuticociliates presented very low biovolume values during the sampling year.

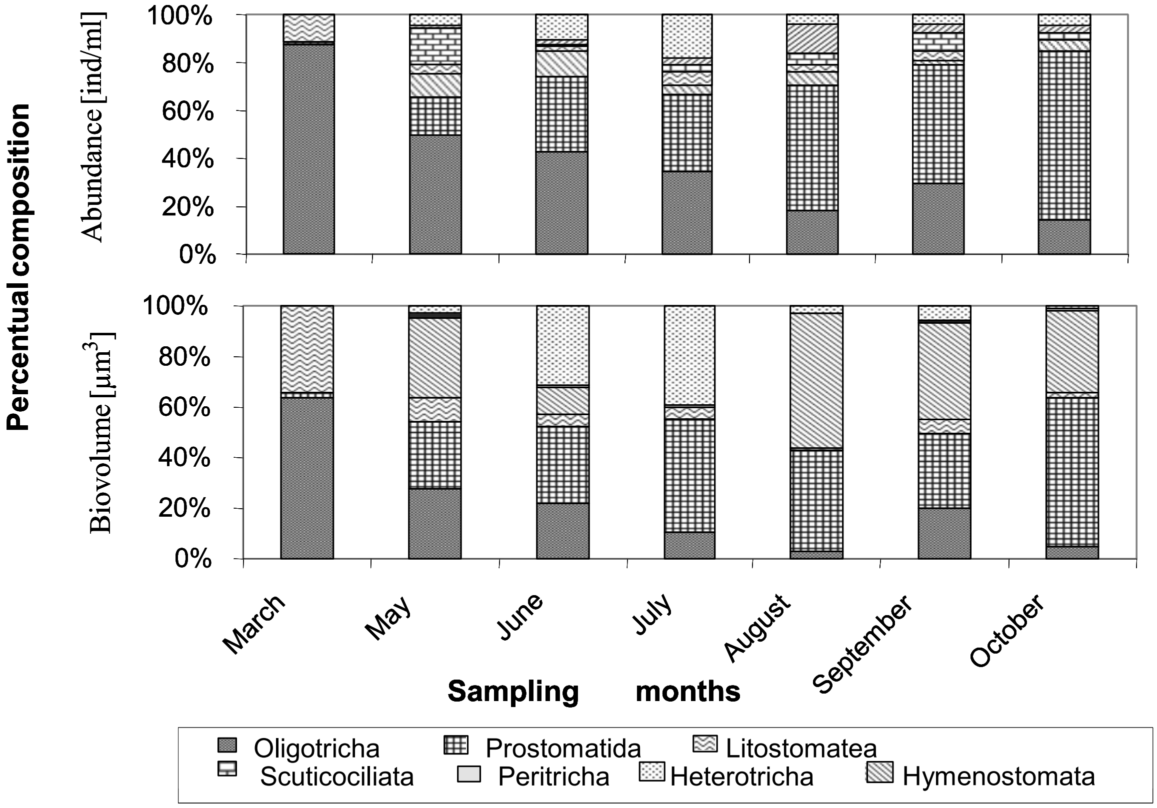


Fig.3.- Percentual composition for the ciliates´ abundance (a) and biovolume (b) during the sampling year

At a Euclidean distance of 1,5, the results for the cluster analysis shows the formation of 3 main distinctive groups among the sampling months and for the ciliates´ abundances; The first, March previous to stratification, which is independent to the other groups, the second

May, June and July marking beginning of stratification and the third, from August towards October during stratification.

For the ciliate abundances at a Euclidean distance of 1,0 there is a formation of 4 main groups, the first two groups consisting primarily of Oligotricha and Prostomatida,, which presented the highest abundance values throughout the sampling year. The Oligotricha were most abundant during the period of pre-stratification and the Prostomatida was more abundant during stratification. The group conformed by Heterotricha and Peritricha had very low abundances and were present mainly during the period of pre-stratification. The last group, comprising Litostomatea, Hymenostomata and Scuticociliata were present mainly during the period of stratification. Litostomatea is separated from the other two due to the fact that it was also present during pre-stratification.

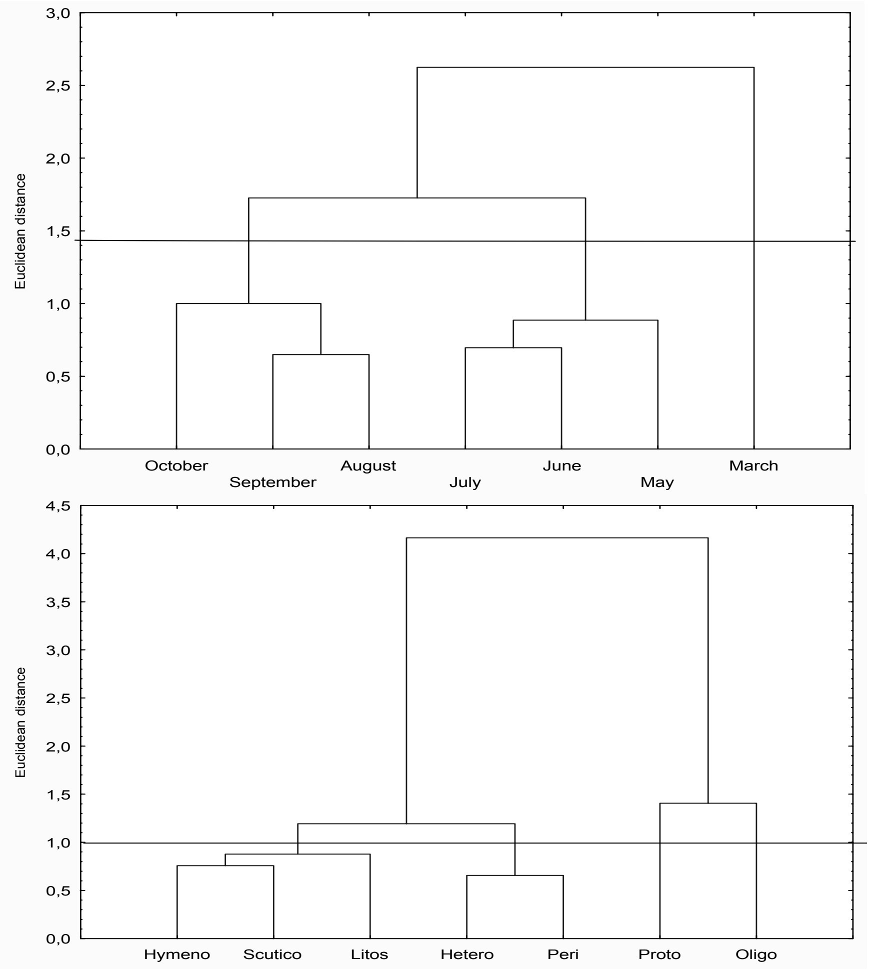


Fig. 4.- Cluster analysis results for the months (a) and groups (b)

During the sampling year, the ciliates dominated the total zooplankton abundance (ind/l), followed by the rotifers, Copepoda and Cladocera. Larger predators (*Chaoborus flavicans*) were also present, but in reduced numbers. The lowest abundance for the ciliates was found during the months of March and June, which is consistent with the presence of higher predatory groups. During the month of March, both the rotifers and the copepods represented the principal predatory pressure against the ciliates. During the month of June, copepod abundance decreased, but rotifer abundance increased. The highest ciliate abundance was found during the months of September and October. During these months, predatory pressure was very reduced, especially during the month of September. Rotifer abundance was also very reduced during the month of October. The main fluctuations of ciliate abundance were found during the period of pre-stratification, whereas a stabilization follows during the period of stratification.

Total biovolume (µm^3/ml) presented a different pattern, were the ciliates represented less than 20% of the total. Very low ciliate biovolume values were found during the months of March towards July. Furthermore, rotifer, copepod, cladoceran and *Chaoborus flavicans* biovolume values were relatively high during these months. It is important to note, that during the month of June, *Chaoborus flavicans´* biovolume was reduced, and rotifer and cladoceran biovolumes were high. By the beginning of stratification, there is a slight increase of ciliate biovolume especially during the months of August and September, which is consistent with very low rotifer, copepod and cladoceran biovolume values. This could be the consequence of a very high *Chaoborus flavicans* biovolume. There is a slight reduction for the ciliate biovolume during the month of October, were rotifer biovolume increased and *Chaoborus flavicans* biovolume decreased but copepod and cladoceran biovolume values were extremely low. The high ciliate and *Chaoborus flavicans* biovolume values during the months of August and September could be explained by the fact that *Chaoborus flavicans* did not represent a direct predatory threat for the ciliates as it did fort he other groups. The 5 main groups were clearly represented in their total abundance and biovolume values during the period of pre-stratification, which comprises the months of March towards July, This changes however by the beginning of stratification from the months of July towards October, where only 2 – 3 groups are present,

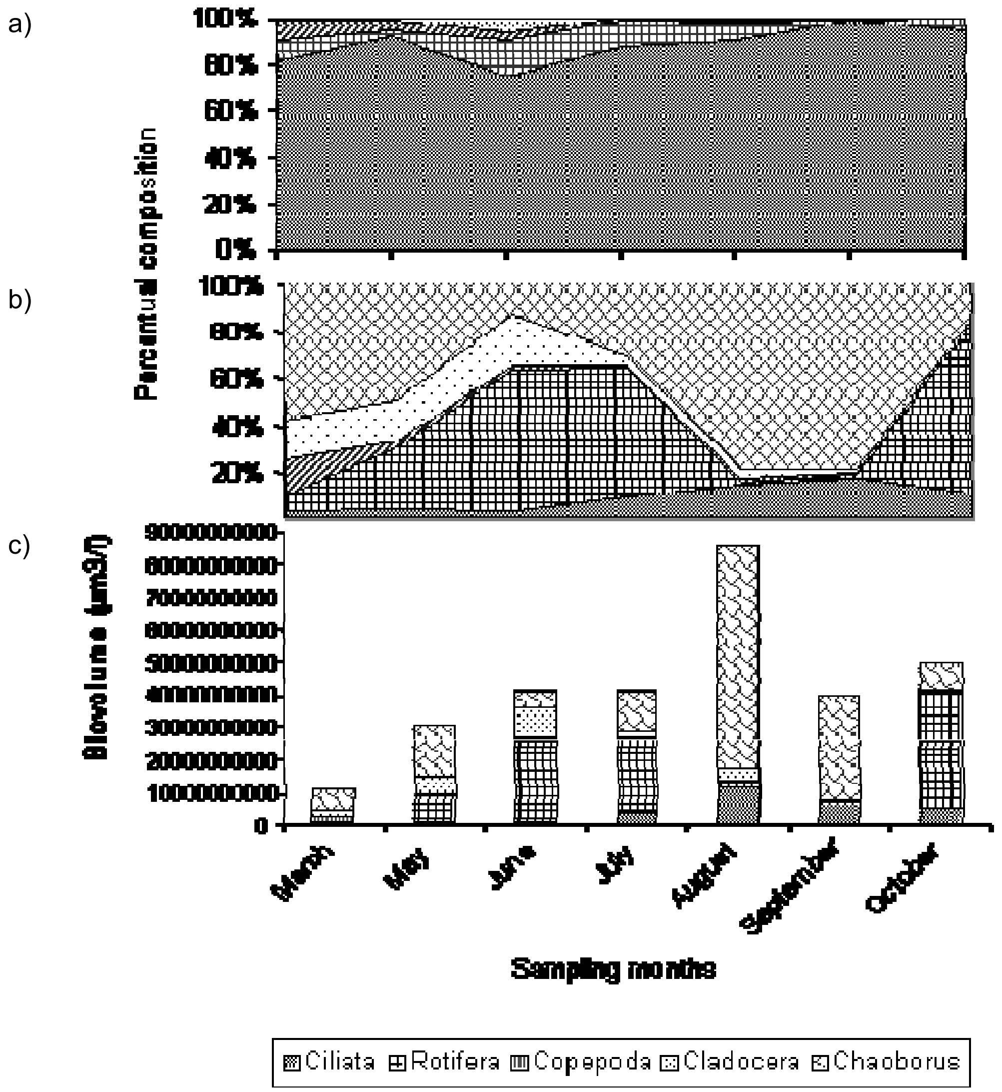
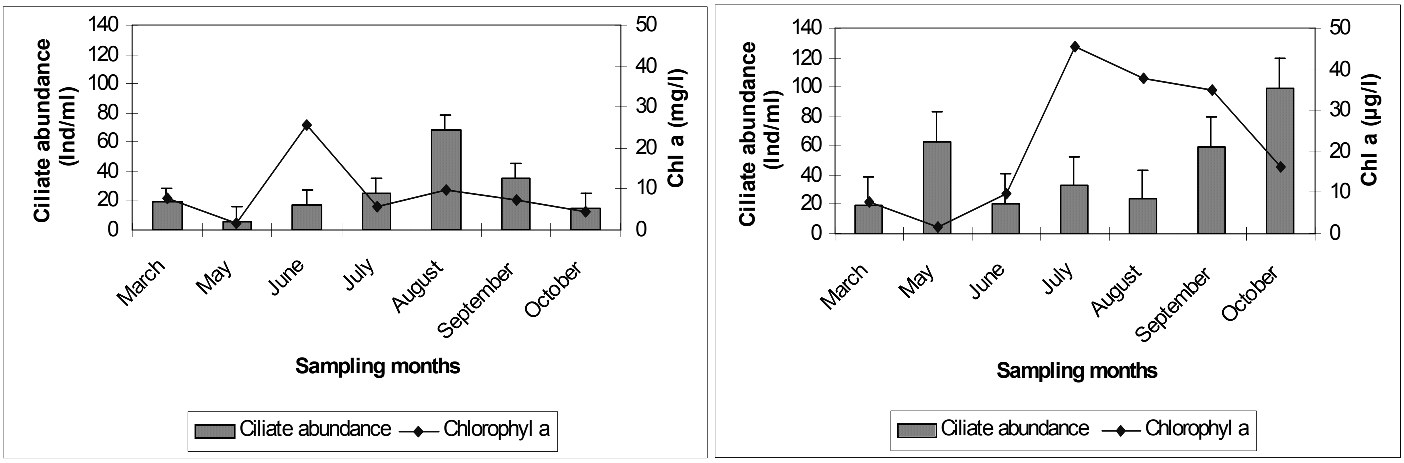


Fig. 5.- Annual percentual composition for the abundance (a) and bio volume (b,c) including the ciliates, rotifers, copepods, cladocerans and *Chaoborus flavicans*

In general, the epilimnion presented low Chl *a* concentrations, the highest concentration was found during the month of June with 25,7 µg/l. During this month, ciliate abundance was very low. The rest of he year averaged 5,7 µg/l. The high ciliate abundance found during the month of August did not match the low Chl *a* concentration (9,7 µg/l). The hypolimnion presented low Chl *a* values during the months of March, May and June (6,3 µg/l). The highest Chl *a* concentrations were found during he months of July, August and September (39.3 µg/l). This high concentration in chlorophyll *a* was due to a bloom in purple sulphur bacteria in the hypolimnion, which during the period of stratification, was saturated with H_2S. Despite high Chl *a* concentrations, ciliate abundances were rather low. The highest ciliate abundance was found during the month of October, which presented a low relation with the Chl a concentration (fig. 6).

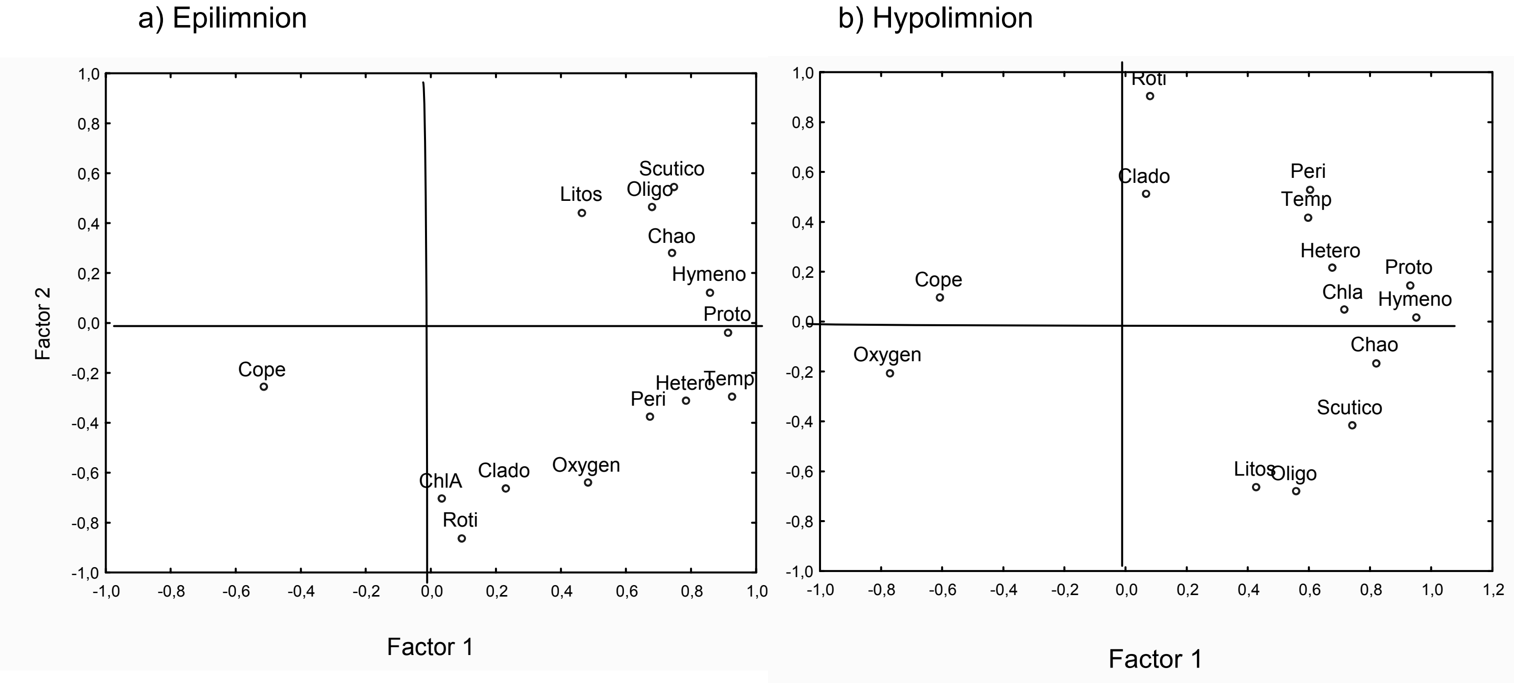


a) Epilimnion

b) Hypolimnion

Fig. 6.- Ciliate abundance and chlorophyll *a* concentration (µg/l) during the sampling year.

The factorial analysis for the epilimnion shows the projection of 15 variables in 2 main axes which explain 65,2 % of the total variation within the system. Factor 1 has a value of 41,8 % and has as main factors temperature (°C), Prostomatidae, Scuticociliata, Hymenostomata, Heterostomata and *Chaoborus flavicans*. Factor 2 has a value of 23,4 % and has as main factors Chlorophyll *a* (µg/l) and Rotifera. The analysis for the hypolimnion shows the projection of 15 variables in 2 main axes, which explain 63,5 % of the total variation. Factor 1 has a value of 43,8 % and the factors are oxygen (mg/l), Chlorophyll *a* (µg/l), Prostomatida, Scuticociliata, Hymenostomata and *Chaoborus flavicans*. Factor 2 has as value 29,7 % nad has as main factor Rotifera.


a) Epilimnion

b) Hypolimnion

Fig. 7.- Factorial analysis results for biotic and abiotic factors in the epi- and hypolimnion. .

Discussion.-

Heterotrophic ciliate assemblages are clearly influenced by the annual variation of biotic and abiotic factors. In the Speldroper Baggersee, ciliate peaks in abundance and biovolume were found during the months of August, September and October, corresponding to mid summer and beginning of autumn, but no peaks were found during spring. However, Bojanic *et al* (2005) found ciliate peaks in spring and autumn. After winter stratification (March) Oligotrich ciliate peaks were found (Macek *et al.*, 2001) although total ciliate abundance and biovolume were low. These peaks were mainly due to a bloom in *Halteria* and *Rimostrombidium* abundance and bio-volume values Ciliate biovolume peaks occurred in August (summer), and mesozoplankton biovolume peaked in June (end of spring, beginning of summer) thus, predation pressure on the ciliates was low (Johannson, *et al.* 2004).

The reduction in Oligotrich abundance and biovolume during the months of May towards August can be a result of predation by the copepods while the Prostomatida were apparently not affected which is confirned by Zollner, *et al.* (2003) who stated that selective grazing by copepods caused a clear trophic cascade via ciliates to nanoplankton. Medium-sized (20-40 mum) ciliates (mainly Oligotrichida) were particularly negatively affected by copepods whereas nanociliates (mainly Prostomatida) became more abundant. The presence of cladocerans may also play an important role in determining ciliate density during this period (Arvola & Salonen, 2001). Ciliates with low biovolume values (Scuticociliata, Peritricha and Pleurostomatida) were more abundant during spring, whereas larger biovolumes (Gymnostomata, Prostomatida, Hymenostomata and Heterotrichida) were found mostly during summer (Bojanic, 2001). According to Chiang *et al.* (2003) a 3 – 5 fold increase in Oligotrich abundance was found in summer, which contradicts with my results, since I registered a decrease in oligotrich abundance during the summer period.

Multivariate analysis results (factor analysis) show the importance of biotic and abiotic factors within the system which may best explain the changes in community structure of the ciliates (Gong *et al.,* 2005). In the epilimnion, the factors temperature (°C), and the Chlorophyll a concentration (µg/l) did influence ciliate and zooplankton community structure. The Chlorophyll a did have an important role in the epilimnion, not only as a major food resource (algae) but also by inducing photosynthesis, which would explain its close relation to the oxygen concentration (mg/l) and the presence of Cladocera and Rotifera.. In the hypolimnion, the limiting factor was the oxygen concentration (mg/l) during stratification, During this period, the hypolimnion was populated by high densities of purple sulphur bacteria and high levels of toxic H_2S, which meant an abundant food resource and additional protection against predators (aerobic) for the ciliates adapted to live in this conditions The presence of Copepoda opposing the ciliates in the epilimnion as well as in he hypolimnion might suggest high predatory activity especially in the epilimnion. The high temperatures registered during August and September, low rotifer, copepod and cladoceran abundance and biovolume values may have supported a ciliate peak in summer, which was reduced possibly due to a increase in rotifer abundance and biovolume (Mathes & Arndt, 1995).

The increase of Prostomatida observed from the months of August towards October could a result of a purple sulphur bacteria bloom in the hypolimnion. This would mean an important food resource for the ciliates and other zooplankton adapted to live in hypolimnetic conditions (Overmann *et al.,* 1989).

It is interesting to note, that the ciliates did perform migrations depending on the annual physico-chemical changes of the lake during stratification and also on the presence/absence of predators. During prestratification, the stability and homogeneity of the lake allowed the presence of the main zooplankton groups, which meant predatory threat, hence migration activity as predator evasion was induced. During stratification the abundance of the main predatory zooplankton groups decreased, and the ciliates´ migration activity was directed mainly to food search.

The results may also suggest that during the months of March until July, ciliate abundance and biovolume were mainly controlled by a top down effect from predators in the system (Rothhaupt, 2000; Adrian & Schneider, 1999; McQueen & Post, 1996; Wickham 1995, 1998; Sarnelle, 1997; Jack & Gilbert, 1997; Burns & Gilbert, 1993). High chlorophyll a concentrations from August until October as a result of purple sulphur bacteria blooms n the hypolimnion would have induced a bottom up effect that would have had as a consequence an increase in ciliate abundance and biovolume (Overmann *et al.*, 1989; Mathes & Arndt, 1995).

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General conclusions.-

In conclusion, my results for the field observations show that the ciliates do perform vertical migrations under natural conditions and that these migrations are strongly influenced by the ciliates´ own auto ecological requirements coupled with the physico-chemical variations of the lake during stratification and also by the biological interaction among individuals mainly by predation. During the months of June and October the driving force was mainly predator evasion, causing the ciliates to migrate long distances to evade predators. During the month of August the migration activity was directed to food search and was shorter ranged, due in part to a larger concentration of un-edible algae that physically hindered migration. Predator abundances were also low, so there vas no need for evasion activity. The boundaries set by stratification would have also acted as a barrier. The causes for this behaviour remain unclear, although the mechanisms driving migration in both cases are unlikely to be identical.

Concerning the field experiments, I can conclude by stating that predation and environmental conditions do play an important role in determining and regulating ciliate abundance and survival. Each factor is important, but it is the interaction of both factors that would ultimately be of fundamental importance. Ciliates that do not migrate and tend to remain at their control depths, have better and higher survival chances, partly due to the fact of adaptation of the ciliates to those environments, even with he presence of zooplankton. Survival chances would also depend if migration is directed towards better environmental conditions and the presence/absence of predatory zooplankton In general, there is no evidence that diel vertical migration itself would be an evolutionary advantage to the ciliates` survival possibilities.

The annual variation of the lake´s physico-chemcal conditions did have an important effect over the ciliate´s abundance and community structure. High abundance and biovolume values were found during the stratification period of the lake, which coincides with low zooplankton (predator) abundance and biovolume. During prestratification, the dominating ciliate group were the Oligotricha, which were mostly found in the epilimnion, which was mainly controlled by variations of the temperature (°C) and algal (photosynthetic) activity, Another important aspect is that ciliate community structure during prestratification was mainly controlled by a top-down effect from predators (Copepoda) in the system. The dominating group during stratification were the Prostomatidae, which were found mainly in the hypolimnion. The low oxygen concentration and high H_2S levels would have induced a rapid development of purple sulphur bacteria which in turn meant a sufficient food resource for the ciliates adapted to live under anoxic and toxic conditions. The high food availability and the low predator abundance might suggest that the ciliate community during stratification was mainly controlled by a bottom up effect.

Zusammenfassung

Heterotrophe Ciliaten spielen eine sehr wichtige Rolle in aquatischen Nahrungsnetzen. Als Konsumenten von Bakterien, Algen und heterotrophen Flagellaten sind sie auch gleichzeitig Beute für höhere Konsumenten. Unter den Strategien, die Beutetiere entwickelt haben, um dem konstanten Räuberdruck auszuweichen, sind Änderungen ihrer Körpermorphologie wie Dornen, Helme usw. und ihres Verhaltens, bekannt als Diel Vertical Migration (DVM) oder Vertikalwanderung während eines Tageszyklus.

Es gibt viele Arbeiten, die sich mit dem Phänomen der Vertikalenwanderung befasst haben. Jedoch berücksichtigten viele dieser Arbeiten meistens die höheren Komponenten des Zooplanktons oder autotrophe Arten wie *Mesodinium rubrum*. Über das Wanderverhalten einer heterotrophen Ciliatengemeinschaft unter natürlichen Bedingungen ist sehr wenig bekannt, insbesondere ob diese Strategie einen Vorteil für das Überleben der Ciliaten bedeutet.

Ziel der Arbeit war, die Gründe und Vorteile dieses Verhaltens in einer natürlichen Ciliatengemeinschaft zu beobachten und zu beschreiben. Um dieses Verhalten zu verstehen, habe ich meine Forschung in drei Hauptkapitel eingeteilt; das erste bezüglich primäre Feldbeobachtungen während eines Jahreszyklus, das zweite befasste sich mit Feldexperimente und der dritte mit der jährlichen Zyklus einer heterotrophen Ciliatengemeinschaft in Bezug auf die Resourcenverfügbarkeit und Prädationsdruck in einem eutrophen See.

Die Arbeit wurde am Speldroper Baggersee durchgeführt, einem eutropher Baggersee mit monomiktischen Eigenschaften, der zwischen Emmerich und Rees am Niederrhein liegt. Er ist etwa 7 Hektar groß und hat eine maximale Tiefe von 16 Metern. Während der Monate Mai - Juni und Oktober bildet sich eine stabile Schichtung mit einem klar definierten Epilimnion und Hypolimnion.

Meine Ergebnisse aus den Freilandbeobachtungen zeigten, dass die Ciliaten vertikale Wanderung unter natürlichen Feldbedingungen durchführen können, und dass diese

Wanderung unter dem Einfluss der jährlichen hydrologischen Änderungen innerhalb des Sees sowie aufgrund der biologischen Interaktion unter Individuen stattfindet.

Bezüglich der Freilandexperimente konnte ich zeigen, dass Prädation und Umweltbedingungen eine wichtige Rolle in der Bestimmung und Regulierung der Überlebenschancen der Ciliaten spielen. Dabei ist es letztendlich die Wechselwirkung von beiden Faktoren, die von grundlegender Bedeutung ist.

Über den jährlichen Zyklus wurde die Ciliatengemeinschaftsstruktur während der Vorschichtungsperiode hauptsächlich von einer Top-Down-Wirkung von Prädatoren kontrolliert. Die hohe Nahrungsverfügbarkeit und die niedrige Räuberabundanz könnten bedeuten, dass die Ciliatengemeinschaft während der Schichtung hauptsächlich von einer Bottom-Up-Wirkung kontrolliert wurde.

Abstract

Heterotrophic ciliates play a very important role in the aquatic food web. As consumers of bacteria, algae and heterotrophic flagellates they are also at the same time prey for higher consumers. Under the strategies which prey animals have developed to reduce predatory pressure are changes of their body morphology like thorns, helmets etc. and their behaviour, known as diel vertical migration (DVM).

The purpose of this study was to observe and to describe the advantages and disadvantages of the vertical migration behaviour in a natural ciliate community in the field. To better understand this behaviour, I have separated my research in three main chapters, the first concerning primary field observations during an annual cycle, the second with field experiments and the third with the annual cycle of a natural ciliate community in relation to food availability and predatory pressure.

My results for the field observations show that the ciliates do perform vertical migrations under natural conditions and that these migrations are under the influence of the annual hydrologic changes within the lake and the biological interaction among individuals.

Regarding the field experiments I conclude by stating that predation and environmental conditions do play an important role in the regulation of the survival chances of the ciliates. Each factor is important, but it is the interaction of both factors which is of basic importance.

Concerning the annual cycle the ciliate community structure was controlled by a top down effect from predation during the prestratification period. The high food availability and the low predator abundance could suggest that the ciliate community during stratification was primarily controlled by a bottom up effect.

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Erklärung

Ich versichere, daß ich die von mir vorgelegte Dissertation selbstständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit - einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; daß diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; daß sie abgesehen von unten angegebenen Teilpublikationen noch nicht veröffentlicht worden ist sowie, daß ich eine solche Veröffentlichung vor Abschluß des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. H. Arndt betreut worden.

Claudio Marcelo Rossberg

Lebenslauf

Persönliche Daten	Name: Claudio Marcelo Rossberg geboren am 06.07.1971 in Cochabamba, Bolivien verheiratet Staatsangehörigkeit: Deutsch
Eltern:	Heinz Dieter Rossberg und Teresa Rossberg geb. Vargas

Studium

12 / 2004 – 07/2005	Teilnahme am Seminar „Klinische Forschung“ am mibeg Institut Medizin in Köln.
01/2002 – 02 / 2006	Promotionstudium an der Mathematisch-Naturwissenschaftlichen Fakultät der Universität zu Köln
09 / 2000 - 10 / 2001	Umwelt - Wissenschaften an der Universität von Franche-Comté Besancon, Frankreich
01 / 1991 - 07 / 1999	Biologie Studium an der Universität von San Simon, Cochabamba, Bolivien
01 / 1990 - 12 / 1990	Vorbereitungsjahr für die Universität von San Simon, Cochabamba, Bolivien

Berufliche Tätigkeit

02 / 2005 – 07 / 2005	Praktikum am Institut für Medizinische Statistik, Informatik und Epidemiologie der Universität zu Köln.
01 / 2002 - 12 / 2004	Wissenschaftlicher Mitarbeiter am Institut für Allgemeine Ökologie und Limnologie der Universität zu Köln.
03 / 2001 - 10 / 2001	Wissenschaftlicher Mitarbeiter am Institut für Umwelt Wissenschaften und Techniken in Besancon, Frankreich
08 / 1999 - 08 / 2000	Freiberufliche Aktivitäten (Dolmetscher) und Vorbereitung für das Studium in der Universität von Besancon Frankreich (Intensiver Sprachunterricht)
08 / 1996 - 07 / 1999	Wissenschaftlicher Mitarbeiter am Institut für Limnologie an der Universität von San Simon, Cochabamba, Bolivien
09 / 1996 - 10 / 1997	Wissenschaftlicher Mitarbeiter am Botanischen und Ökologischen Forschungszentrum an der Universität von San Simon, Cochabamba, Bolivien

Sprachen	Deutsch, Spanisch, Englisch und Französisch
EDV Kenntnisse	Microsoft Word, Excel und PowerPoint SAS, Statistika, SPSS, PC-Ord und Systat Deltagraph, Adobe Photoshop und Illustrator