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Nutrients and fish effects on plankton community in freshwater mesocosms

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Author
Daniele Ciuffa

Supervisor
Eleonora Ciccotti

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a Fabio

Key words

Trophic cascade, bottom-up, top-down, nutrients, phytoplankton, zooplankton, fish.

Summary

Eutrophication affects many lakes in the world. Two important mechanisms to solve this problem are nutrient and fish control.

The main aim of the present work was to study nutrient and fish effects on plankton community in freshwater shallow lakes. Two mesocosm experiments were performed, one in spring and the other in winter to test differences in response of planktonic organisms to nutrient and fish addition in different seasons. The results would be of extremely importance for the management of these ecosystems.

Mesocosms were polyethylene bags filled with lake water and placed into the lake. Three different nutrient additions and three different fish densities were considered. All the possible combinations between these two factors were tested. Each mesocosm had a replicate, for a total of eighteen mesocosms in both seasons. Lake water was also sampled to test any differences between mesocosms and the natural ecosystem. Both experiments lasted six weeks. In the first week mesocosms were filled with lake water and nutrient and fishes were added. Each week, after sampling nutrients (nitrate and orthophosphate) were added. Fish used was *Gambusia holbrooki* mainly because its largely recognized planktivory. After the last week, benthic macroinvertebrates were sampled and stomach contents of fishes were analyses.

On the basis of the many studies demonstrating the significance to take into account functional groups of zooplankton, instead of consider total zooplankton density, in the present work four groups (rotifers, cladocerans, copepods and nauplii) were considered with regards to the zooplankton community.

Nutrients addition was associated to increase in phytoplankton biomass both in spring and winter although this increase was higher during spring. Nutrients did not affect zooplankton density neither in spring nor in winter. High fish density was associated to increase in phytoplankton biomass in both seasons. On zooplankton community, fish had a composite effect. They did not increase total zooplankton

density neither in spring nor in winter. Fish changed zooplankton community. In spring, with increasing fish density rotifers increased while cladocerans and copepods decreased. In winter, zooplankton community was composed mainly by rotifers. Fish did not affect rotifers, but decreased cladocerans, copepods and nauplii. In spring, fishes could prey also on benthic macroinvertebrates. During winter, planktivory by fish was more intense due to the lack of benthic macroinvertebrates. Results show different response of plankton community between seasons. Results show the importance of considering as many variables as possible.

Riassunto

L'eutrofizzazione deteriora molti laghi nel mondo. Esistono, tuttavia, due importanti meccanismi per risolvere questo problema: il controllo della concentrazione dei nutrienti e il controllo della densità dei pesci nel lago.

Il principale obiettivo di questo lavoro è stato quello di studiare gli effetti dei nutrienti e dei pesci sulla comunità planctonica nei laghi. A questo scopo sono stati condotti due esperimenti in mesocosmi, in primavera e in inverno al fine di esaminare le differenze nella risposta degli organismi planctonici all'aggiunta di nutrienti e dei pesci in due stagioni diverse. I risultati ottenuti possono essere di estremo interesse per la gestione di questi ecosistemi.

I mesocosmi erano delle buste di plastica sorrette da una rete metallica e collocati all'interno del lago. Sono state considerate tre diverse concentrazioni di nutrienti e tre diverse densità di pesci. Tutte le possibili combinazioni di questi due fattori sono state analizzate (per un totale di nove combinazioni). Ogni mesocosmo aveva una propria replica per un totale di diciotto mesocosmi in ogni esperimento. Inoltre, parallelamente ai mesocosmi, è stata analizzata anche l'acqua di lago per controllare la naturalità di questi sistemi artificiali. Entrambi gli esperimenti sono durati due settimane. Nella prima settimana i mesocosmi sono stati riempiti con acqua di lago e successivamente sono stati aggiunti i pesci e i nutrienti. Questi ultimi sono stati aggiunti anche ogni settimana dopo i campionamenti. I nutrienti aggiunti sono stati azoto e fosforo, mentre la specie ittica utilizzata è stata *Gambusia holbrooki* che è ampiamente riconosciuta come una specie planctivora. Al termine di ciascun esperimento sono stati prelevati anche i macroinvertebrati bentonici e i pesci sono stati conservati per la successiva analisi dei contenuti stomacali.

Molti lavori hanno dimostrato l'importanza di prendere in considerazione i gruppi funzionali zooplanctonici piuttosto che considerare la densità dello zooplancton totale. In questo lavoro, infatti, sono stati considerati quattro gruppi funzionali dello zooplancton: rotiferi, cladoceri, copepodi e nauplii.

L'aumento dei nutrienti ha determinato un aumento della biomassa fitoplanctonica sia in primavera che in inverno. Questo aumento, tuttavia, è stato maggiore in primavera. I nutrienti non hanno influenzato la densità dello zooplancton né in primavera né in inverno.

La diversa densità dei pesci, invece, ha aumentato la biomassa fitoplanctonica nelle due stagioni. La densità ittica non ha influenzato la densità dello zooplancton totale né in inverno né in primavera. In primavera, tuttavia, all’aumentare della densità ittica, i rotiferi sono aumentati, mentre i cladoceri e i copepodi sono diminuiti. In inverno la comunità zooplanctonica era costituita principalmente da rotiferi. I pesci non hanno influito sui rotiferi, ma hanno diminuito la densità dei cladoceri, copepodi e nauplii.

In primavera i pesci hanno predato anche i macroinvertebrati bentonici, mentre in inverno probabilmente i pesci hanno predato più intensamente sullo zooplancton.

I risultati rivelano una diversa risposta della comunità planctonica nelle due stagioni. Inoltre questo lavoro dimostra l’importanza di esaminare quante più variabili possibili per avere una migliore conoscenza dei processi e delle interazioni fra gli organismi che avvengono all’interno di un lago.

Chapter 1. INTRODUCTION

1.1 Shallow lakes: generality

Shallow lakes constitute the bulk of the world's freshwater area (Wetzel, 1990); they have great importance in providing productive fisheries in Asia, Africa and South America (Dugan, 1994); they are of great conservation value, often for the migratory bird communities they support in the temperate world (Stephen *et al*., 2004a).

Many lakes in the world suffer from high loadings of nutrients of anthropogenic origin, mainly from sewage and agriculture (Moss, 2000). A conspicuous feature of eutrophicated lakes is high concentrations of algae, making the water green and turbid and sometimes toxic due to toxin-producing cyanobacteria (Lampert, 1981; Rohrlack, Henning & Kohl, 1999). Such turbid and toxic water makes the lake less valuable as a natural resource, for use as drinking water, and for recreation and fishing (Kitchell, 1992; Hansson & Bergman, 1998; Moss, 2000). Freshwater shallow lakes may be classified on the basis of their nutrient concentrations (**Table1.1**).

Trophic states	TN (mg/l)	TP (mg/l)	chl a (μg/l)
Oligotrophic	<0.35	<0.01	<3.5
Mesotrophic	0.35–0.65	0.01–0.03	3.5–9
Eutrophic	0.65–1.2	0.03–0.10	9–25
Hypertrophic	>1.2	>0.10	>25

Table1.1. Average characteristics of lakes (Nürnberg, 1996), of different trophic states.
The terms oligotrophic, mesotrophic, and eutrophic correspond to systems receiving low, intermediate, and high inputs of nutrients. Hypertrophic is the term used for systems receiving greatly excessive nutrient inputs. TN, total nitrogen; TP, total phosphorus; TIN, total inorganic nitrogen; chl a, chlorophyll a.

Shallow lakes are also very vulnerable to change. Many have lost the dominance of their submerged plants in clear water to a take-over by phytoplankton communities in turbid water with consequences for their biodiversity and fish communities (De Nie, 1987; Carvalho & Moss, 1995; Jeppesen *et al*., 1998, 1999, Stephen *et al*., 2004a).

Table1.2 below summarizes effects of eutrophication on lakes.

Increased biomass of freshwater phytoplankton and periphyton
Shifts in phytoplankton species composition to taxa that may be toxic or inedible (e.g. bloom-forming cyanobacteria)
Changes in vascular plant production, biomass, and species composition
Reduced water clarity
Decreases in the perceived aesthetic value of the water body
Taste, odor, and water supply filtration problems
Possible health risks in water supplies
Elevated pH and dissolved oxygen depletion in the water column
Increased fish production and harvest
Shifts in fish species composition towards less desirable species
Increased probability of fish kills

Table 1.2. Effects of eutrophication on lakes and reservoirs (modified from Smith, 1998)

Understanding how continental- or greater-scale variations in environmental factors and food-web structure influence these complex shallow lake systems is important because of the importance of these lakes to humanity as providers of diverse resources such as building materials, fisheries and water (Dugan, 1994). They are also important reservoirs of conservation and biodiversity value, not least for migratory and other water birds. Many shallow lakes have become severely damaged by eutrophication in combination with other forms of pollution, recreational impact, changing salinity and introduced species.

Both nutrient control and food web manipulation (biomanipulation) are used in combination in attempts to restore their biodiversity and other values (Moss, Madgwick & Phillips, 1996). The effectiveness of current restoration strategies could well be affected by climate as well as by local chemical conditions, but evidence from the Mediterranean is too little in comparison with that from the north-temperate region (Moss *et al.*, 2004). It is clear, however, that the differences in seasonality and in fish communities may have important consequences for warm lakes compared with cooler ones (Lazzaro *et al.*, 1992).

In this way the present work has critical significance as the experiments, in order to study the effects of nutrient addition and fish predation on plankton community, were performed in a Mediterranean region lake during two different seasons (spring and winter).

1.2 Hypothesis of alternative stable states

As nutrient loading increases submerged plants become vulnerable to the action of switch mechanisms (Stephen *et al*., 2004a).

One mechanism that helps stabilise plant communities is the harbouring of large numbers of daphnids and other cladocerans, that graze phytoplankton from adjacent waters, in and around the plant beds (Timms & Moss, 1984). Similar relationships exist among molluscs, periphyton and plants (Brönmark, 1985). These mechanisms are particularly influenced by fish predation, particularly on zooplankton, but also on periphyton grazers (Lauridsen *et al*., 1996).

Another mechanism for maintaining stability is restriction of the nitrogen availability in the water (Ozimek, *et al*., 1990), both through plant uptake and denitrification in the often-deoxygenated conditions within plant beds (Stephen *et al*., 2004a).

Much of the information on these processes has been pieced together into a general hypothesis of alternative stable states (Irvine *et al*., 1989; Scheffer *et al*., 1993; Jeppesen *et al*., 1998) from experiments and observations at many locations, although mainly in northern Europe (Stephen *et al*., 2004a).

In particular the alternative stable states model (Irvine, *et al*., 1989; Scheffer *et al*., 1993) has been proposed to explain the functioning of shallow lake ecosystems (Hietala *et al*., 2004).

The model suggests that at very high phosphorus concentrations a turbid, phytoplankton-dominated state may be the only possibility whereas at low phosphorus concentrations (<0.025 mg/l) a clear water state uniquely exists.

Between these extremes, both turbid, phytoplankton-dominated states and clear water, plant-dominated states are possible (Irvine *et al*., 1989; Scheffer *et al*., 1993). Both states are assumed to resist perturbations, such as changes in nutrient enrichment, because complex biological interactions buffer against community changes (Scheffer *et al*., 1993).

A switch from one state to the other may occur often following some catastrophic event. For instance, increase in the population of large *Daphnia* after a fish kill can cause a switch from turbid state to clear water state (Edmondson & Litt, 1982; Wright & Shapiro, 1984). Declines in macrophytes may have the opposite effect: a clear water lake turns into the turbid state following herbicide application (Van Donk *et al*., 1990). A switch may also occur as a result of severe nutrient enrichment (Schindler, 1977). When a threshold nutrient concentration is reached, macrophyte dominance may be replaced by phytoplankton dominance (Scheffer *et al*., 1993).

The basis of these assumptions derives from the trophic cascade hypothesis.

1.3 The trophic cascade

The trophic cascade hypothesis states that nutrient input sets the potential productivity of lakes and that deviations from the potential are due to food web effects (Carpenter *et al*., 1985). Nutrient and food web effects are complementary not contradictory, but they act at different time scales (Carpenter *et al*., 1985; Carpenter, 1988). Food web effects stem from variability in predator-prey interactions and their effects on community structure (Carpenter & Kitchell, 1987). Acting through selective predation, variability at the top of the food web cascades through zooplankton and phytoplankton to influence ecosystem processes (Carpenter *et al*., 1985).

The ideas about trophic cascade in lakes derive from two primary sources (Carpenter & Kitchell, 1993).

First is the extension of thermodynamic principles to ecology, which yields the expectation the organic production in lakes should be a function of nutrient status. Strong correlations exist between nutrient loading or nutrient concentration and primary production (Schindler, 1978). Flow of energy and matter upward through food chains is a central paradigm of the ecosystem approach (Lindeman, 1942; LeCren & Lowe-McConnell, 1981; Odum, 1969).

The second major element of the cascade idea derives from the evolutionary principles widely employed in contemporary population biology and community ecology. Consumers are typically selective in the types and sizes of resources they consume (Hall *et al*., 1976; Kerfoot & Sih, 1987). This reasonable extension of natural selection theory is embodied in aquatic ecology as the principles of size-selective predation (Hrbáček *et al*., 1961; Brooks & Dodson, 1965), the keystone predator concept (Paine, 1966) and theories of optimal foraging and habitat usage (Werner, 1986). In lake ecosystems, the result of selective predation plays a major role in community composition at each trophic level (de Bernardi, 1981). Piscivores determine the size and species composition of the planktivorous fish assemblage below them in a food web (Tonn & Magnuson, 1982). Selective planktivory by fishes and invertebrate predators profoundly influences the community of herbivorous zooplankton which, in turn, regulates the amount and species of phytoplankton that compete for nutrients (Brooks & Dodson, 1965; Sommer, 1989). Much of the available nutrient pool derives from recycling through excretion processes that are strongly size-dependent (Kitchell *et al*., 1979; Peters, 1983). Thus, the rates of primary production can be substantially influenced by a trophic cascade of size-selective predation processes that start at the top of the food web (Carpenter & Kitchell, 1993).

The components of the cascade argument have a crucial nexus at the zooplankton level (Carpenter & Kitchell, 1993) (**Figure 1.1**). Larger herbivores are consumed selectively by planktivorous fishes (Brooks & Dodson, 1965). Carnivorous zooplankton feed most heavily on smaller zooplankton (Hall *et al*., 1976). Abundant planktivorous fishes shift the zooplankton composition toward dominance by smaller individuals. When planktivorous fishes are absent, predation by planktivorous invertebrates and competition among herbivores shift the zooplankton toward larger individuals (Brooks & Dodson, 1965; Hall *et al*., 1976). Large herbivores such as *Daphnia* have a greater impact on phytoplankton because they consume a broad range of sizes and morphologies of algae (Burns, 1968; Gliwicz, 1980; Bergquist, Carpenter & Latino, 1985). Owing to their size, large zooplankton has lower mass-specific rates of nutrient excretion (Peters, 1983). In comparison with a small-bodied zooplankton assemblage of equal biomass, an assemblage dominated by large *Daphnia* should graze a broader spectrum of algae but recycle nutrients at lower rates. Thus, algal biomass and primary production should be less in *Daphnia*-dominated lakes than in lakes dominated by small zooplankton such as *Bosmina*, small calanoid copepods, or rotifers (Carpenter & Kitchell, 1993). Zooplankton biomass should be directly related to nutrient concentration but, for equivalent zooplankton biomass, algal biomass and production should be inversely related to mean zooplankton size (Carpenter & Kitchell, 1984).

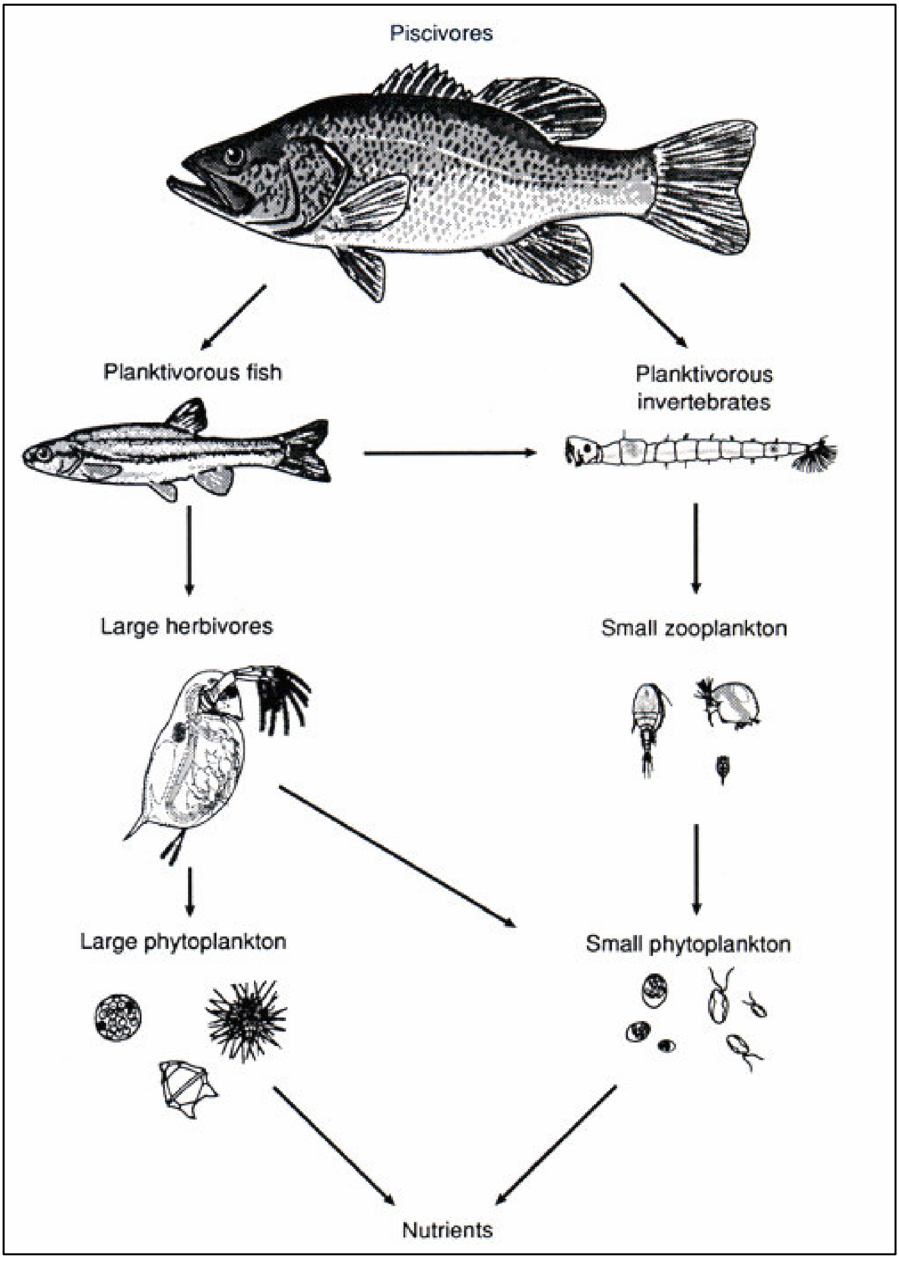


Figure 1.1. Major interactions of the trophic cascade illustrated with selected organisms. (From Carpenter & Kitchell, 1993).

Trophic cascade theory in aquatic systems suggest great importance of predation as a structuring force of food webs, stating that predators (for instance fish) can regulate lower trophic levels (Carpenter & Kitchell, 1993; Pace *et al*., 1999). Fish biology (*e.g.* abundance, age structure, prey selection, allometric relations) can play a key role in aquatic ecological processes (Vanni & Rutter, 1996). In aquatic environments, both direct effects of fish predation in reducing large zooplankton, as well as, indirect effects on primary producers by recycling or transport of nutrients have been described (*e.g.* Vanni, 1996; Hessen, 1997; Bertolo *et al*., 1999). Both direct and indirect effects can be more intense in eutrophic shallow lakes (Jeppesen *et al*., 1997). In shallow lakes, fish communities together with nutrient regime determine food web structure (Jeppesen *et al*., 1998; Moss, 1998; Scheffer, 1998). Zooplanktivorous fish usually exhibit selectivity in their diet dependent on physiology, predator-prey behaviour and environmental conditions. Consequently, zooplanktivorous fish reduce not only quantitatively zooplankton but change the demography, morphology and behaviour of the predated species (Gliwicz, 1990; Hansen & Jeppesen, 1992; Jacobsen *et al*., 1997; Moss, 1998). Remaining organisms may take advantage of the available resources, *e.g.* rotifers (Vanni, 1987a). Ontogenetic or facultative shifts in diet (*e.g.* to detritivory), may occur as responses to environmental variability, to exploit resources or avoid intraspecific competition (Lobón-Cerviá and Rincón, 1994; García-Berthou, 1999). Detritus (*e.g.* sediment material, plant debris, unidentifiable food particles) are often disregarded in dietary studies. Additionally, it is difficult to study fish predation owing to many uncontrolled factors (*e.g.*, environmental variability, prey availability) and experimental studies are required to understand the complexity of fish ecology and food webs.

For this reason in the present work, fish stomach contents were considered.

1.4 Functional groups approach to ecosystems analysis

Variation in the responses of aquatic plankton communities to nutrient enrichment has indicated the importance of considering not only the number of trophic levels but also the nature of the organisms within them (Leibold *et al*., 1997; Persson *et al*., 1996) and variation in the efficiency of consumers to exploit their prey (Power, 1992).

Hulot *et al*. (2000) argued that taking functional diversity within trophic levels into account is critical to understanding the response of lake ecosystems to environmental perturbations. They concluded that a functional group approach to ecosystems using careful analysis of major species and assumptions on their interactions may provide a better understanding of ecosystem functioning. This approach has important implications for ecosystem management.

Figure1.2 below represents morphologies of one cladoceran species (*Daphnia pulex*) and ten rotifers species.

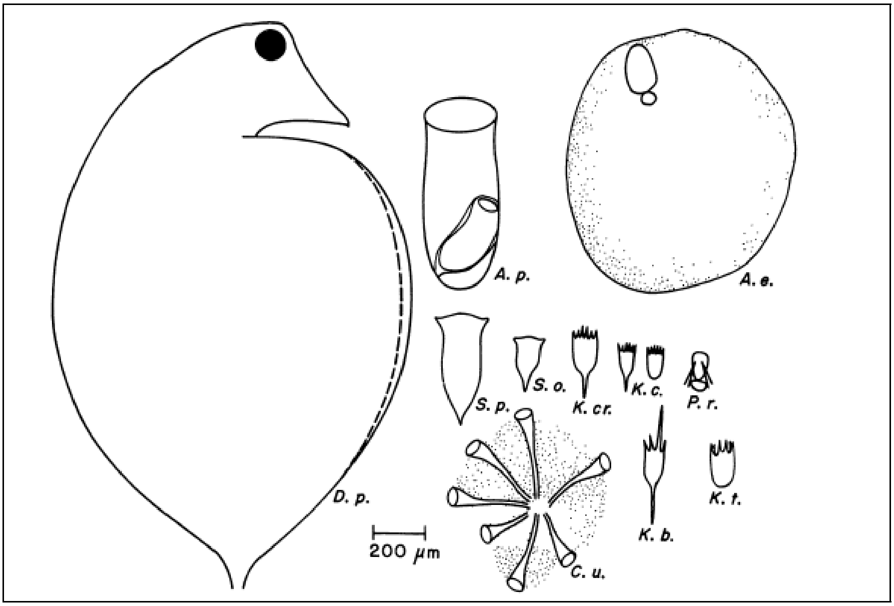


Figure 1.2. Morphologies of *Daphnia* and rotifers. *Daphnia pulex* (*D. p.*), *Ascomorpha ecaudis* (*A. e.*), *Asplanchna priodonta* (*A. p.*), *Conochilus unicornis* (*C. u.*), *Kellicottia bostoniensis* (*K. b.*), *Keratella cochlearis* (*K. c.*) (*typica* and *tecta* forms with and without a posterior spine), *K. crassa* (*K. cr.*), *K. testudo* (*K. t.*), *Polyarthra remata* (*P. r.*), *Synchaeta oblonga* (*S. o.*), *Synchaeta pectinata* (*S. p.*). (From Gilbert, 1988).

Lake eutrophication is characterized by increased algal abundance, decreased water transparency and a potential deficit of oxygen (Lacroix & Lescher-Moutoué, 1991). The classical prey-dependent linear food-chain model predicts that nutrient enrichment should increase algal biomass in lakes with an odd number of trophic levels. Therefore lake restoration usually seeks to eliminate trophic cascades either by direct suppression of zooplanktivorous fish or by addition of piscivorous fish. The varied success (DeMelo *et al.*, 1992) of these restorations is comprehensible in the context of this more complex model. Nutrient enrichment should not affect all algae in the same way. Successful ecosystem restoration needs a new theoretical approach that takes into account community structure and functional diversity (Hulot *et al.*, 2000).

Different phytoplankton species may have different demands of inorganic nutrients (Karjalainen *et al.*, 1996). These demands and the capability to take up nutrients differ according to abiotic conditions (Rhee & Gotham, 1981), the physiological status of phytoplankton (Lean & Pick, 1981) and the ecological strategy concerning the nutrition of the algae (autotrophymixotrophy) (Bird & Kalff, 1987; Nygaard & Tobiensen, 1993). Some experiments have shown that both herbivory and changes in the nutrient loading can change diversity of algae dramatically (Proulx *et al.*, 1996).

A well-defined theoretical basis exists with respect to food chains in terrestrial systems (Hairston, Smith & Slobodkin, 1960; Fretwell, 1977; Oksanen *et al.*, 1981). It has been extended to aquatic systems, linking nutrient loading and fish abundance to system variables, such as the total biomass of zooplankton or phytoplankton (Carpenter *et al.*, 1987; Persson *et al.*, 1988; Hairston & Hairston, 1993, 1997), and these theoretical predictions have repeatedly withstood empirical test (Carpenter *et al.*, 1987; Persson *et al.*, 1988; Hansson, 1992; Mazumder, 1994; Hansson *et al.*, 1998).

However, knowledge is limited regarding the validity of theoretical predictions when system variables are separated into finer taxonomic levels, such as genera or groups of genera. Some groups of organisms may constitute ‘keystone taxa’ with respect to their impact on food-web structure, whereas other groups may have a negligible impact (Hansson *et al.*, 2004).

Trophic interactions between primary producers and consumers are mediated through numerical predation but also through indirect pathways, such as nutrient regeneration (Elser & Urabe, 1999; Hillebrand & Kahlert, 2001; 2002). In interactions between primary producers and grazers, nutrient recycling plays an important role for primary production (Sterner, 1986). In more complex food webs with more than two trophic levels, indirect pathways via stoichiometric effects and nutrient recycling might be even more important. For example, it is well known, that predators recycle nutrients (Brabrand *et al.*, 1990, Attayde & Hansson, 1999; Vanni *et al.*, 2002) and affect primary producers, but it is not known how important food web complexity is in this context.

For lakes in temperate regions, a large body of evidence indicates that planktivory by fish is a major factor determining the biomass, structure and composition of both zooplankton and phytoplankton communities (Carpenter *et al.*, 1985; Vanni & Layne, 1997). Fish-induced increases of phytoplankton have most often been attributed to the trophic cascade hypothesis, which assumes strong top-down control between each pair of adjacent trophic levels (Carpenter & Kitchell, 1993; Brett & Goldman, 1997): fish affect the abundance and structure of zooplankton communities by selectively eliminating large bodied zooplankton prey (Gliwicz & Pijanowska, 1989), resulting in decreased and more selective grazing pressure on the phytoplankton (Carpenter & Kitchell, 1993; Declerck *et al*., 1997). However, several factors may weaken the link between trophic levels, and changes at higher trophic levels often do not fully cascade down the food web (McQueen *et al*., 1989; De Melo, France & McQueen, 1992). The link between trophic levels may, for instance, be weakened by nutrient deficiency in phytoplankton (Mackay & Elser, 1998), by shifts in the composition of the prey community towards less vulnerable taxa (Kerfoot & Sih, 1987; Gragnani *et al*., 1999), by adaptive anti-predator responses of prey species (Tollrian & Harvell, 1999), or by indirect effects of fish on phytoplankton via nutrient recycling (Attayde & Hansson, 2001).

In contrast to temperate lakes, few studies on food web dynamics of tropical lakes have been carried out. Most of these studies are descriptive and consider bottom-up control as the preponderant factor (Pinel-Alloul *et al*., 1998). It is unclear whether models developed for temperate systems, such as the trophic cascade hypothesis, are appropriate for the tropics (Lazzaro *et al*., 2003). From the information currently available, it seems that trophic cascade effects tend to be relatively weak in tropical lakes and several reviews have provided support for this idea (Lazzaro, 1997; Pinel-Alloul *et al*., 1998). Fish are probably important predators of zooplankton in neotropical lakes: zooplankton densities are in general very low, their communities are dominated by small species (Junk & Robertson, 1997), and the abundance of zooplanktivorous fish is rather high (Zaret, 1984; Rejas & Maldonado, 2000). However, simultaneously with predation-induced mortality, fish potentially can also positively affect zooplankton populations. Fish can have a strong impact on nutrient recycling (Schindler, 1992; Vanni & Layne, 1997; Attayde & Hansson, 1999) and improve the food availability for zooplankton by enhancing microbial and phytoplankton productivity (Attayde & Hansson, 2001).

Fish can also release the zooplankton community from predation by macroinvertebrate predators (Sorano, Carpenter & He, 1993). Furthermore, populations of small, inconspicuous zooplankton taxa may also benefit from size selective fish predation because they are released from competition with larger zooplankton (Vanni, 1986; Ślusarczyk, 1997). Therefore, high densities of zooplanktivorous fish will undoubtedly affect the general size structure and taxonomic composition of zooplankton communities, but once a community of small-bodied, predation-resistant zooplankton species is established, the direct negative effects of fish induced mortality on the dynamics of these populations may be rather limited (Vanni, 1987a; Declerck *et al.*, 2003), and may potentially be compensated by indirect positive effects (Attayde & Hansson, 2001).

Both top-down (predation) and bottom-up forces (resource availability) are important determinants of the distribution of biomass among trophic levels (Shurin *et al.*, 2002; Borer *et al.*, 2005). Less clear is how diversity within trophic levels influences the propagation of top-down and bottom-up effects (Fox, 2007). Several lines of evidence suggest that prey diversity may weaken top-down and bottom-up effects (King & Pimm, 1983; Leibold, 1989; Abrams, 1993; Leibold *et al.*, 1997; Chase *et al.*, 2000; Schmitz *et al.*, 2000). High prey species richness creates the potential for density compensation within the prey trophic level, since prey species often interact through exploitative and apparent competition (Leibold, 1996). Density compensation among prey can weaken the propagation of top-down and bottom-up effects by buffering total prey biomass (Leibold, 1989; 1996; Strong, 1992; Abrams, 1993). For this reason, results of predator removal and resource addition experiments often are better-predicted by models that explicitly incorporate details of food web structure than by models that assume homogeneous trophic levels (Briand & McCauley, 1978; Leibold, 1989; Wootton & Power 1993; Hulot *et al.*, 2000; Persson *et al.*, 2001; Jiang & Morin, 2005; Fox, 2007).

Hence in this work the zooplankton community was divided into groups of different size and ecology. The main groups considered were rotifers, cladocerans, copepods and nauplii.

Top predators can have substantial effects on species lower down in the food web (Power, 1990; Persson, 1999; Lövgren & Persson, 2002), either through numerical predation or through indirect pathways. Predators ingest prey and recycle nutrient, thereby increasing the nutrient availability of primary producers (Sterner & Elser, 2002). Furthermore, how different species are influenced by top predators depends also very much on the heterogeneity and shape of the food web (Menge, 1995). Some consumer species can be reduced through direct consumption by predators, thus resulting in positive effects on primary producer species, while other consumers increase in abundance through competitive release (Paine, 1966; 1969).

Grazing by zooplankton is central to determining algal biomass (Hietala *et al.*, 2004). Grazing efficiency is largely determined by zooplankton community structure; mean body size and taxonomic composition are the critical parameters (Cyr & Curtis, 1999). Heavy fish predation on zooplankton leads to low numbers of large-bodied zooplankton and low grazing pressure (Knoechel & Holtby, 1986). This favours the development of high algal biomass if nutrient concentrations are high (Carpenter *et al.*, 1987).

Many hypotheses have been advanced to explain the observed variability in the strength of trophic cascades. Principal among these is that top-down effects interact with bottom-up effects to produce results that are not predictable unless nutrient dynamics are also taken into account (Oksanen *et al.*, 1981; Polis, 1994). Several other factors, especially spatial heterogeneity (Scheffer, 1998), the prevalence of omnivory (Strong, 1992; Diehl, 1995), interference among predators (McCann *et al.*, 1998) and heterogeneity in prey edibility (Leibold, 1989; Bell, 2002), have been suggested to modify the outcome of food web manipulations under certain conditions (Polis *et al.*, 2000). However, it is not clear whether any of these factors is consistently important in determining the strength of trophic cascades.

Many ecologists attempted to improve the quality of freshwater shallow lakes damaged by eutrophication through manipulation of trophic cascade. In particular many works focused on nutrient control and fish predation in order to recover an eutrophicated lake.

The present work tried to understand the effects of nutrient control and fish predation on zooplankton community. Above all the experiments were performed in a Mediterranean lake where studies of this kind are very few.

Food chain theory predicts that with increasing nutrient levels, algal biomass does not necessarily increase as a greater part of the algal biomass production is removed by a growing herbivorous zooplankton community. However, if planktivorous fish are present, algal biomass will increase proportionately, as the planktivores will keep the herbivores at a low level (Carpenter *et al.*, 1987; Persson *et al.*, 1988; Hansson, 1992; Hairston & Hairston, 1993, 1997). This theory has predicted natural dynamics reasonably well with respect to broad system variables such as total abundance and biomass (Hairston

& Hairston, 1997; Hansson *et al.*, 1998). However, separation of system variables into finer taxonomic levels, such as to families or even genera, may be of extreme importance in developing water management plans, because some organism groups (e.g. *Daphnia*) are desired, whereas others (e.g. cyanobacteria) are generally not (Hansson *et al.*, 2004).

In order to improve the accuracy of management plans, and disentangle mechanisms behind nutrient and predation effects, the data from the mesocosm experiments were analysed both on a system level and on more detailed taxonomic and size-class levels.

The traditional equilibrium theory of food chain dynamics and its modifications (Hairston *et al.*, 1960; Fretwell, 1977; Oksanen *et al.*, 1981; Scheffer, 1991) emphasises the control of lower trophic levels by consumers, and predicts that effects of nutrient enrichment are transferred into biomass at the top trophic level and even-numbered levels below it. Accordingly, zooplankton biomass increases with enhanced primary productivity in two-level systems (phytoplankton and herbivorous zooplankton) but does not change in three-level systems (phytoplankton, herbivorous zooplankton and planktivorous fish).

1.5 Nutrient control and fish predation

Much effort has gone into development of techniques for restoration of plant-dominated clear water lakes through combinations of nutrient control and manipulation of fish communities (Shapiro *et al.*, 1975; Hosper, 1989; Moss, Madgwick & Phillips, 1996; Mehner *et al.*, 2002). It is not yet clear, however, how appropriate these might be outside the northern locations in which largely they have been developed (Stephen *et al.*, 2004a).

Understanding of the wider context is thus important, not least because in an analysis of the contribution of natural systems to the cash economy of the Earth, wetland systems, including shallow lakes, provided the greatest contribution to a very large sum (Costanza *et al.*, 1997).

Fish and nutrients are driving forces in controlling food webs in shallow lakes but the relative importance of one or the other factor is still a matter of discussion (McQueen *et al.*, 1986; Jeppesen *et al.*, 1997; 1998).

Most experimental studies have been carried out in temperate conditions and almost no data are available about top-down and bottom-up controls in warmer countries (Scheffer *et al*., 1993; Perrow *et al*., 1997; Drenner & Hambright, 1999; Mehner *et al*., 2002; Fernández-Aláez *et al*., 2004). In particular there has been little work on the potential roles of nutrients and fish in shallow lakes under Mediterranean climate (John, 1986; Galanti *et al*., 1990; Lazzaro, 1997; Talling & Lemoalle, 1998; Bachmann *et al*., 1999; Beklioglu *et al*., 2003).

Rapid shifts from clear to turbid phases have been observed when plant cover disappears from shallow lakes (Jeppesen *et al*., 1998; Fernández-Aláez *et al*., 2002), for example as a result of crayfish grazing (Rodríguez *et al*., 2003), but there has been little work on the potential roles of nutrients, fish and their combination in shallow lakes under Mediterranean climate.

Higher mean temperatures and productivity in the Mediterranean compared with temperate climate could affect fish or nutrient control of lake food webs in different ways (Fernández-Aláez *et al*., 1999; Jeppesen *et al*., 2003). For example, high annual temperatures influence fish growth and predation rates on zooplankton (Bachmann *et al*., 1996). In warmer lakes, zooplankton taxa, can be physiologically better adapted to higher temperatures (Atkinson, 1994; Moore *et al*., 1996). Therefore, in warmer lakes zooplankton may have weaker control of algal biomass (Fernando, 1994; Lazzaro, 1997; Benndorf *et al*., 2001). Furthermore, high zooplanktivore biomass and small-bodied zooplankters may enhance nutrient cycling and algal growth (Fernando, 1994; Lazzaro, 1997; Lazzaro *et al*., 2003). Greater amounts of solar energy hitting on lakes and higher temperature may further favour primary production (Camargo & Esteves, 1995; Talling & Lemoalle, 1998) in accordance with empirical evidence that cyanobacteria more often dominate phytoplankton communities at low latitudes (Komárek, 1985; Pollingher & Berman, 1991; Tallimg & Lemoalle, 1998).

Mediterranean shallow and clear lakes typically harbour higher plant densities (providing better refuge for zooplankton) and experience stronger water level fluctuations (resulting in higher light availability and stronger volume reductions) (Fernández-Aláez *et al*., 1999). Moreover, Mediterranean lakes are often smaller and more closed systems than northern lakes (Fernández-Aláez *et al*., 2004).

Nitrogen and phosphorus loading determine many features of lakes (Vollenweider, 1975), but evidence has emerged of a more complex situation, especially in shallow lakes, where the nature of the fish community can influence the zooplankton and thus indirectly the phytoplankton communities and even water chemistry (e.g. Irvine, Moss & Balls, 1989; Scheffer *et al*., 1993; Carpenter & Kitchell, 1993; Hansson *et al*., 1998). These effects may also have impacts on aquatic plants, which in turn may counterinfluence water chemistry and predator–prey relationships among fish and zooplankters (Jeppesen *et al*., 1998). A consensus has emerged that nutrients remain very important in shallow lakes, but that the extent to which their potential influence may be realised is very much a function of food-web structure and how it can be modified by nutrient loading (Moss *et al*., 2004).

Fish differences in dietary patterns observed among species inhabiting freshwaters are generally due to distinct feeding physiology, predator-prey behaviour and environmental constraints (e.g. O'Brian, 1987; Persson & Crowder, 1997). Fish do not only control quantitatively prey populations, but they can alter both diversity and demographic structure (and even morphology and behaviour) of prey (Vanni, 1986; Gliwicz, 1990; Hansen & Jeppesen, 1992; Moss, 1998; Jacobsen *et al*., 1997).Moreover, there is evidence that indirect effects can be determinant in trophic interactions, for instance fish can transport in the lakes nutrients vertically and horizontally, recycling nutrients though the food webs (Vanni, 1996; Hessen, 1997; Bertolo *et al*., 1999; Tátrai *et al*., 2003). Both direct and indirect effects influence trophic structure of lakes and can be specially intense in eutrophic shallow lakes (Persson *et al*., 1996; Jeppesen *et al*., 1997; Moss *et al*., 1996). The presence of omnivorous and detritivorous fishes in shallow lakes are commonly associated with changes related to eutrophication (e.g. Horppila & Kairesalo, 1990; Karjalainen *et al*., 1999), such as the growth of rotiferan communities or the development of algal populations, both planktonic and epiphytic, displacing macrophytes as primary producers (Jeppesen *et al*., 1997; Scheffer, 1998).

According to Lammens & Hoogenboezem (1991) fish diet in shallow lakes will relate to prey density, distribution and its availability.

Many works were carried out to understand the processes involved in the eutrophication of water. Several of these studies were performed in natural ecosystems. However many studies focused only on some variables (e.g. total zooplankton density, phytoplankton biomass) while holding other variables (e.g. nutrient loading, fish density) constant. These types of studies are only possible in mesocosm experiments. This is the reason why the present work considers experiments performed in mesocosms.

1.6 Why using mesocosms experiments

Mesocosms are laboratory or field enclosures used for experimental studies of selected ecosystem components or interactions. Because mesocosm experiments can be controlled and replicated, they are a powerful experimental tool for studying ecological mechanisms (Hairston, 1989; Mazumder, *et al*., 1990; Soto & Hurlbert, 1991). Because of their typically small size, mesocosms necessarily exclude some processes that may be important for predicting ecosystem dynamics (Frost *et al*., 1988; Carpenter & Kitchell, 1988). For example, inshore-offshore migration of fishes and hydrodynamic fluxes of nutrients cannot easily be included in enclosure experiments. Therefore, mesocosm results may not be directly transferable to the ecosystem scale and can, in fact, yield erroneous extrapolations (Frost *et al*., 1988).

Recognizing these limitation mesocosm experiments were used to study fish and nutrient effects on plankton community. System variables measured in the mesocosms were compared with those measured in the natural ecosystem.

Ecologists have a long tradition in manipulating simplified food webs to isolate general ecological mechanisms, many of which have been verified in the field (Carpenter *et al*., 1987, Brett & Goldberg, 1996; Shurin *et al*., 2002). However, species diversity and food web complexity are usually much higher in natural systems compared to laboratory studies and it has been shown that horizontal heterogeneity in food webs plays an important role for trophic interactions (Leibold, 1989; Polis & Strong, 1996).

Mesocosms are the only systems in which many of the ideas in ecology can be tested because of the difficulty and expense of manipulating particular variables while holding all others constant over large spatial and temporal scales. Rather than dismissing the results of such experiments as inadequate at the spatial scale of a whole lake or forest, it is necessary to identify the way in which trophic cascades are altered because of site alone. Such an approach has proved fruitful, for example in estimating the causes of differences in growth rates in larval anurans (Skelly & Kiesecker, 2001), and the effect of *Daphnia* on microzooplankton (Sarnelle, 1997).

One factor rarely considered is the impact of scale on the outcome of these experiments. For example, the consistent top-down effects that occur with the addition of a trophic level might eventually disappear if there is delayed compensation by species that are adapted to avoiding predators (Osenberg & Mittelbach, 1996). Furthermore, previous work has shown that the response ratios used in meta-analysis are often sensitive to experiment duration for a variety of ecological questions (Osenberg *et al*., 1997, 1999; Downing *et al*. 1999). They show that the conclusions drawn from short- and long-term experiments often differ considerably, and suggest weighting the log response ratio by the inverse of the experiment duration.

The effect of fish on zooplankton can be higher in mesocosms because macrophytes are most often absent in mesocosms, but abundant in small lakes and ponds (Bell *et al*., 2003). Macrophytes act as zooplankton refuges from fish predation (Jeppessen, 1998). Zooplanktivores might therefore be able to depress zooplankton to a greater extent in systems devoid of macrophytes, as is the case with many of the trophic cascade experiments in mesocosms.

On average, short-term experiments are adequate to predict the long-term pattern of trophic level biomass. For aquatic trophic cascades, short-term experiments appear to be of adequate duration to predict longer-term patterns (Bell *et al*., 2003).

However, the impact of predators on zooplankton communities cannot be evaluated clearly with field observations, because, in lakes, many environmental factors such as water temperature, oxygen concentration, and food abundance influence the prey–predator interactions. Mesocosm experiments are effective at evaluating the effect, since predator density can be controlled in a semi-natural environment.

Predation and resource abundance (and/or quality) can have important effects on the structure and dynamics of ecological communities (MacArthur, 1965; Paine, 1966; Harper, 1969; Connell, 1975; Lubchenco, 1978; Pianka, 1978; Pimm, 1982; Price *et al*., 1984). These factors also may interact. For example, predation can mediate the outcome of resource competition among prey species, as in rocky intertidal communities (Paine, 1984). Also, the level of food abundance and/ or quality for herbivores may determine the degree to which predators influence the dynamics and abundance of herbivores, as evidenced by terrestrial plant-insect interactions (Price *et al*., 1980) and phytoplankton- zooplankton interactions (Neill & Peacock, 1980; Neill, 1981). There have been few experimental studies in which predators and resources were manipulated simultaneously to assess the effects of these factors on community structure and dynamics (Price *et al*., 1980) because of the difficulties in conducting such experiments. Freshwater zooplankton communities are agreeable to such field experiments because populations large enough to be sampled repeatedly can be enclosed in the field (Lynch, 1979; DeMott & Kerfoot, 1982), predators and resources (e.g., food) can be manipulated, and the species in such communities have relatively short life-spans, allowing experiments to include several generations. Further, demographic and life history traits of zooplankton are easily obtained and allow one to interpret fluctuations in abundance in a mechanistic fashion.

Several studies have examined the role of fish predation on freshwater zooplankton community structure (Hrbatek, 1962; Brooks & Dodson, 1965; Lynch 1979, Hurlbert & Mulla, 1981). Almost all previous experimental studies have involved the introduction of planktivorous fish to previously fishless lakes or enclosures within such lakes (Hrbatek, 1962; Lynch, 1979). When fish are stocked at high density, the large bodied zooplankton species in these lakes, which have life histories adapted to living in the absence of these exotic predators (Lynch, 1980), are eradicated by size selective predation and replaced by a completely different set of smaller species capable of coexisting with planktivorous fish. Because of this response of large species zooplankton communities to fish, fish predation is viewed as a dominant factor structuring zooplankton communities (Zaret, 1980). However, little is known about the effect of fish predation on small zooplankton species that are able to coexist with fishes that prey upon them (Lynch *et al*., 1981; Bartell, 1982; Wright & O'Brien, 1984). Population densities of small species may be controlled by fish predation even though the small species are not driven to extinction by fish. DeMott and Kerfoot (1982) found that densities of small species coexisting with rainbow trout were not limited by ambient levels of fish predation; few trout were present and they fed mainly on littoral and benthic prey. Many lakes contain high densities of obligate planktivores, or fish that prefer to feed on planktonic organisms (Werner *et al.* 1983). The role of fish predation in determining the dynamics of small zooplankton species under these circumstances is unknown. The role of resource limitation in determining zooplankton community structure is also unclear. Recent experimental work has shown that food abundance and/or quality can greatly influence zooplankton community structure and population dynamics (Neill & Peacock, 1980; DeMott & Kerfoot, 1982; DeMott, 1983; Neill, 1984). In all of these cases fish were either absent or relied heavily upon non-planktonic prey. Thus the relative importance of food limitation and fish predation to zooplankton has not been ascertained in cases in which fish predation is likely to be intense.

1.7 Plankton seasonal dynamics

Zooplankton community structure and biomass are determined by food availability, temperature, predation and interactions between different zooplankton organisms, including inter-specific and interference competition (Gliwicz, 1985; Sterner, 1989; Gliwicz, 2003).

Accordingly, zooplankton community structure varies seasonally. The plankton ecology group (PEG) model (Sommer *et al.*, 1986) describes a major increase in cladoceran abundance in late spring, which in some lakes often leads to a high grazing pressure and a clear water phase. During summer, the grazing pressure may decline as a result of predation or propagation of inedible algae and high abundance of cyanobacteria. In autumn, the grazing pressure on phytoplankton may rise again, but without reaching the level of the peak observed in late spring (Sommer *et al.*, 1986). This pattern does not, however, apply to all lake types. Jeppesen *et al.* (1997) have shown that concurrently with increasing nutrient levels, or increased predation pressure from fish on zooplankton, the spring and autumn peaks decline and ultimately disappear at the highest nutrient and fish densities. Other investigations (Deneke & Nixdorf, 1999) and minimal models (Scheffer *et al.*, 1997) also lend support to the hypothesis that decreasing zooplankton grazing occurs at high fish densities. Conversely, fish kills or low fish recruitment may lead to high grazing pressure on phytoplankton and clear water conditions throughout the summer (Kubecka & Duncan, 1994; Gliwicz, 2003).

Little is known of the relative importance of resource and predator control of zooplankton during winter. Usually resource control is considered to be the most important factor (Jeppesen *et al.*, 2004). The PEG model states that (i) 'herbivore biomass decreases as a result of reduced fecundity because of lower food concentration as well as decreasing temperature', and (ii) 'towards the end of winter, nutrient availability and stronger light permit unlimited growth of phytoplankton'. The suggested lack of grazer control in spring is often attributed to a resource and temperature mediated delay in zooplankton growth (Sommer *et al.*, 1986), but it could also be a result of high predation on zooplankton by fish. In fact, some experimental studies (Tempte *et al.*, 1988; Vanni *et al.*, 1990; Rudstam, Lathrop & Carpenter, 1993) and minimal model exercises (Scheffer *et al.*, 1997) suggest that the spring clear water phase might occur earlier in the season if planktivorous fish density is low. Similarly, Jeppesen *et al.* (1997) showed that potential zooplankton grazing pressure on phytoplankton in eutrophic lakes with high densities of planktivorous fish was low, not only during summer but throughout the year. This also indicates fish control of zooplankton during winter.

Whether the regulating influence of fish on trophic structure is more important in summer than winter is unknown. Attention may be drawn both to factors reducing and increasing the predation on zooplankton during winter (Jeppesen *et al.*, 2004). Among the factors reducing the predation risk is aggregation of planktivorous fish, such as bream (*Abramis brama* L.), near the bottom, in the littoral zone or in adjacent streams (Jepsen & Berg, 2002). Secondly, their activity on a diel basis may be reduced (Jacobsen *et al.*, 2002). Thirdly, a gradual decline in abundance of underyearling fish usually occurs during autumn because of fish predation and, accordingly, the predation pressure on zooplankton most likely decreases. Finally, the food intake by fish declines during winter (Kitchell *et al.*, 1977), although the growth of prey zooplankton is also reduced (Bottrell *et al.*, 1976).

By contrast, several factors may enhance the predation pressure on zooplankton during winter (Jeppesen *et al.*, 2004). First, transparency is higher during winter when phytoplankton biomass is low, and this may increase the predation risk from visually hunting fish. Secondly, as a result of an autumn overturn in summer-stratified lakes, zooplankton cannot use the hypolimnion as a refuge against potential predators. Thirdly, a major loss of refuges for zooplankton may also occur in lakes with high summer coverage of submerged macrophytes (clear shallow lakes and some small-sized deep lakes) when plants senesce in autumn. Unfortunately, most studies of fish-zooplankton interactions and the cascading effects on phytoplankton are restricted to the summer season. Outdoor field experiments with zooplanktivorous fish *Menidia beryllina* (Cope) conducted in Texas, U.S.A., showed depression of large-bodied zooplankton during all seasons, including winter when the temperature occasionally dropped to 3–4 °C (Drenner *et al*., 1986). These results indicate that fish potentially may depress zooplankton grazing during winter. Several field studies have revealed low zooplankton grazing in winter. Haney (1973) recorded grazing rates of zooplankton by fish in Heart Lake, U.S.A., of <10% per day in winter and early spring, while the rates were comparatively high in autumn (20–50% day^{-}1). Gulati (1978) found grazing rates of 4.5% day^{-}1 in autumn and winter and 22% in early spring (March to April). Yet, it is noteworthy that zooplankton grazing, expressed as the percentage of phytoplankton biomass, was reduced from summer to winter. This does not, however, necessarily imply lower grazer control of phytoplankton, as the growth rate of phytoplankton also decreases in winter. Thus, Garnier & Mourelatos (1991) showed that zooplankton consumption only declined from 85 to 43% of the potential phytoplankton production, suggesting a high grazing pressure also during winter, although the filtering rate of zooplankton underwent a fivefold reduction from summer to winter.

To further elucidate the role of nutrient and fish in determining zooplankton community structure, mesocosm experiments were repeated in winter.

Since the present work focus mainly on the effects of nutrient addition and fish density on zooplankton community, next paragraph presents the characteristics of some zooplankton groups.

1.8 Zooplankton: generality

The animals found in fresh waters are extremely diverse and are represented by nearly all phyla. Evaluation of their functional roles within aquatic systems requires a balanced understanding between the general modes and timing of growth and reproduction in relation to the availability and utilization of food. The population dynamics and certain important adaptive behavioural characteristics that influence these dynamics regulate the productivity of individual species populations and entire communities. Underlying all evaluations of productivity of the animals are their food of trophic relations with plants and other animals and competitive and predatory interactions that lead to a greater success of one species over another (Wetzel, 1983).

Truly planktonic animals are dominated by three major groups: Rotifera and two subclasses of the Crustacea, the Cladocera and Copepoda. Under most circumstances, rotifers and especially the limnetic crustaceans are overwhelmingly the dominants of zooplanktonic productivity (Wetzel, 1983).

1.8.1 Rotifers

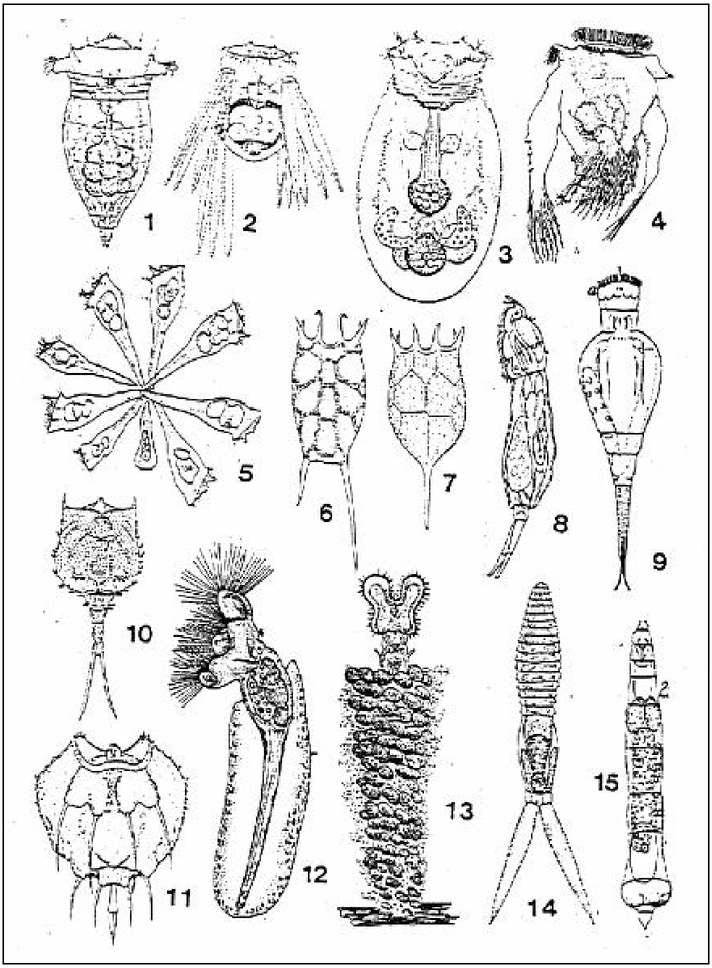


Figure 1.3. Diversity of morphological adaptation in rotifers to different environments. Planktonic: 1 - *Synchaeta*, 2 - *Polyarthra* (fam. Synchaetidae); 3 - *Asplanchna* (fam. Asplanchnidae); 4 - *Hexarthra* (fam. Hexarthridae); 5 - *Conochilus* (fam. Conochilidae); 6, 7 - *Keratella* (fam. Brachionidae). Psammonic: 8 - *Dicranophorus*, 9 - *Wierzejskiella* (fam. Dicranophoridae). Phytophylic: 10 - *Trichotria*, 11 - *Macrochaetus* (fam. Trichotriidae). Periphytonic: 12 - *Collotheca* (fam. Collothecidae); 13 - *Floscularia* (fam.Flosculariidae). Parasitic: 14 - *Claria* (fam. Clariaidae); 15 - *Balatro* (fam. Dicranophoridae). (2000, Annual Reports of the Zoological Institute RAS, from different authors).

Rotifera are typically of freshwater lake; only two significant genera and a few species are marine. About three-quarters of the rotifers are sessile and associated with littoral substrates. Approximately 100 species are completely planktonic, and these rotifers form a significant component of the zooplankton (Wetzel, 1983).

Rotifers are regarded as opportunistic organisms, mainly because of their high tolerance to changes in environmental conditions and their high reproductive rates (Allan, 1976). Their feeding strategy consists of filtering of water and capturing the prey in the crown of cilia. The prey selection being dependent on particle size. This type of feeding may be classified as generalists or specialists (Gilbert & Bogdan, 1984) depending on extent of selection of food particles. The selection of particles is related, among other factors, to the great morphological diversification of the buccal apparatus. Arndt (1993) considered that bacteria, heterotrophic flagellates and small ciliates constitute a large part of the food resources that may be used by rotifers. Branco & Senna (1996) and Caleffi (1998) have shown the importance of bacteria and phytoplankton as a food resource for rotifers in reservoirs. Also Ooms-Wilms (1997) emphasized the importance of bacteria as a food resource for rotifers, and highlighted the relationship between the size of the bacteria and the rotifer species that consume them. Thus, rotifers play an important role in the transfer of energy in the trophic webs and the nutrient recycling. Spatial and seasonal variations in food supply can affect rotifer distribution. Sanders *et al*. (1989) who investigated rotifer seasonality in a temperate lake discussed the relationship between the abundance of some rotifer species that fed mainly on bacteria, and the density of bacterioplankton over a period of 8 months, which included both stratification and de-stratification periods. Mazumder *et al*. (1992), in a 2-year study of a lake, found that rotifers consumed mainly phytoplankton. These findings do not necessarily apply to lakes in different geographical regions, especially to tropical lakes, which are characterized by high temperatures and near-lack of seasonal variations, except for two extreme seasonal periods (dry and rainy seasons).

Stemberger & Gilbert (1987) reviewed the works on rotifer defences against predators, and Havel (1987) discussed morphological, chemical and behavioural defences induced by predators. Recently, also Walz (1993, 1995) reviewed strategies of rotifer life history and Snell (1998) summarized chemical ecology of rotifers, including chemical signals in feeding and induction of defences against predators.

1.8.2 Cladocerans

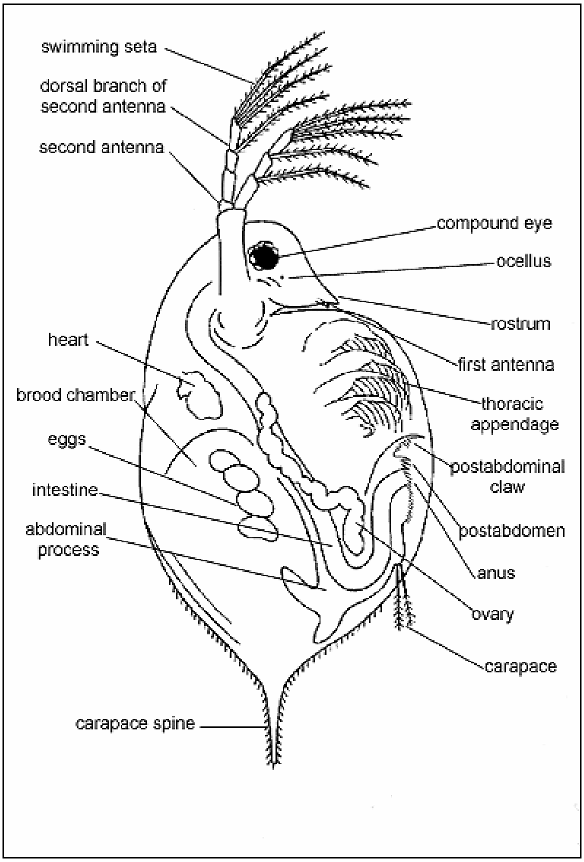


Figure 1.4. Cladoceran morphology (1997, Province of British Columbia).

Cladocerans are mainly microzooplankrton. With exception of two species, nearly all cladocerans range in size from 0.2 to 3.0 mm. All have a distinct head and the body is covered by a bivalve cuticular carapace. Light-sensitive organs usually consist of a large, compound eye and a smaller ocellus. The second antennae are large swimming appendages and constitute the primary organs of locomotion. The mouthparts consist of: (1) large, chitinized mandibles that grind food particles, (2) a pair of small maixillules, used to push food between the mandibles, and (3) a median labrum that covers the other mouthparts (Wetzel, 1983).

Cladocerans usually have five pairs of legs attached to the ventral part of the thorax. The legs are flattened and bear numerous hairs and long setae. Complex movements of these setose legs create a current of water through the valves. This current oxygenates the body surface and forces a stream of food particles anteriorly. The food particles, filtered by the setae, collect in a ventral food groove between the bases of the legs and are impelled forward toward the mouth to be mixed there with oral secretions (Wetzel, 1983).

Reproduction in the cladocerans is parthenogenetic during a greater part of the year. Until interrupted by sexual reproduction, females produce eggs that develop into more parthenogenetic females. The number of eggs produced per clutch varies from two or more in the Chydoridae to as many as 40 in the larger Daphnidae. The eggs are deposited into a brood chamber, or pouch, a cavity dorsal to the body bounded by the valves of the carapace. The eggs develop in the brood pouch and hatch as a small form of the parent. As a result there are no free-living larval forms among the cladocerans. One clutch of eggs is normally released into the brood pounch during each adult instar (Wetzel, 1983).

The seasonal succession of the Cladocera is quite variable, both among species and within a species living in different lake conditions. Some species are perennial, and overwinter in low population densities as adults (parthenogenetic females) rather than as resting eggs. These species may exhibit one, two, or more irregular maxima. Some perennial species exhibit maxima in surface layers only during colder periods in the spring, and in the cooler hypolimnetic and metalimnetic strata during summer stratification. The aestival species that have a distinct diapause in a resting egg stage commonly develop population maxima in the spring and summer when the water is relatively warm. Although one population maximum is general, a second population peak often occurs in the autumn (Wetzel, 1983).

Seasonal polymorphism occurs in the Cladocera perhaps more conspicuously than in any other group. This phenomenon has been assumed to be of major adaptive significance to these organisms. Early investigators assumed that cyclomorphic forms would have greater resistance to sinking because the viscosity of the water decreases at higher epilimnetic temperatures. Cyclomorphosis is confined almost completely to species that inhabit the epilimnion, or at least migrate to it daily. Epilimnetic turbulence, however, decreases the small advantage gained from form resistance (Jacobs, 1967). Furthermore, such resistance would be somewhat disadvantageous to cladocerans swimming during vertical diurnal migration.

1.8.3 Copepods

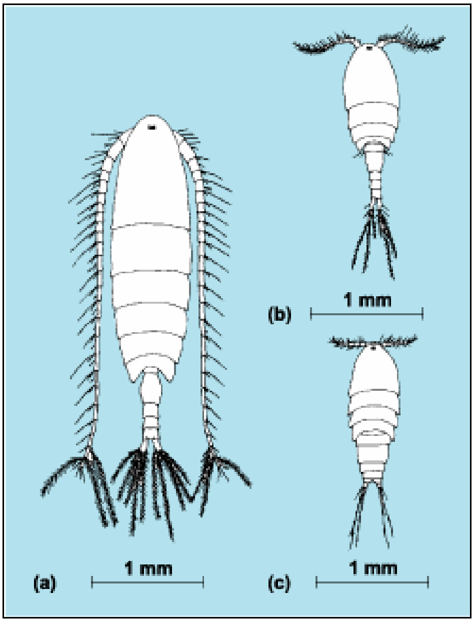


Figure 1.5. Free-living copepods. (a) *Calanus finmarchicus* (calanoid) (b) *Cyclops* sp. (cyclopoid) (c) *Harpacticus* (harpacticoid).

Copepoda are separable into three distinct groups: Calanoida, Cyclopoida and Harpacticoida. Although accurate identification is based largely on morphological details of appendages, several general characteristics delineate the major groups. The body consists of the anterior metasome (cephalothorax), which is divided into the head region, bearing five pairs of appendages representing antennae and mouthparts, and the thorax, with six pairs of mainly swimming legs. The posterior urosome consists of abdominal segments, the first of which is modified in females as the genital, and terminal caudal rami bearing setae (Wetzel, 1983).

The three groups of free-living copepods can be distinguished by the general structure of the first antennae, urosome, and fifth leg. The harpacticoid copepods are almost exclusively littoral, habituating macrovegetation, mosses in particular, and the littoral sediments. Although the cyclopoid copepods are primarily littoral benthic species, those few members that are predominantly form major components of the copepod zooplankton, especially in small, shallow lakes. The calanoid copepods are almost exclusively planktonic (Wetzel, 1983).

Copepods are extensively represented in the seas where most of about 10,000 known species and a similar number of so far undescribed species (Hairston & Bohonak, 1998) are widely distributed. Huys & Boxshall (1991), evaluating their abundance, diversity and size, call them ‚the insects of the seas’’. However, some groups of copepods successfully colonize inland waters and together with Cladocera and Rotifera are quite abundant in freshwater planktonic communities. Although herbivorous at least during some life stages, copepods also represent one of the main groups of invertebrate predators in both limnetic and littoral regions of inland waters. Rotifers, due to their usually much smaller size and restricted mobility, become often preferred food items in the diet of freshwater copepods. Williamson (1983) reviewed and summarized information on invertebrate predation on planktonic rotifers. Greene (1983, 1985) also published a detailed study on selective predation and on prey selection in zooplankton.

1.9 Main aims

The present work attempts to evaluate the relative roles of fish predation and food availability on phytoplankton biomass and zooplankton community, using controlled, replicated in situ manipulation experiments.

The null hypothesis was that predation and food availability would not influence phytoplankton biomass and zooplankton abundance and dynamics. The alternative hypothesis was that phytoplankton biomass and zooplankton abundance and dynamics are controlled by fish predation and food availability. The null hypothesis was considered rejected if population densities and/or demographic traits were significantly affected by manipulations of food and/or predator levels.

Although the results of such an experiment depend on the specific levels of predation and food availability employed, by using conditions within the range of those found in natural lake systems, some useful inferences on the effects of predation and food availability on phytoplankton biomass and zooplankton abundance and dynamics can be made.

Eighteen mesocosm experiments with different nutrients loadings and fish densities were performed in a shallow lake situated in the Experimental Ecology Laboratory and Aquaculture of the University of Rome “Tor Vergata”. The experiment was repeated in two different seasons: spring and winter to test different effects of nutrient and fish on plankton community. Experiments were thus executed in a Mediterranean region lake where information on these arguments is lacking.

The founding supposition was that there would be shifts in the relative importance of top-down and bottom-up effects between seasons. This hypothesis was based on the assumption that algal growth would benefit from extended growing seasons during spring.

Nutrient shortage would be more likely to set in when temperature is higher and the growing season longer (Talling & Lemoalle, 1998), leading to greater importance of nutrient control in spring. Moreover algal growth, being inherently faster than that of zooplankton (Fogg & Thake, 1987), would outstrip development of herbivorous zooplankton at higher temperatures. In turn, the influence of planktivorous fish on zooplankton populations would become less relevant for control of phytoplankton at higher temperatures. The hypothesis was of a greater importance of nutrients in warmer season and a greater importance of fish effects under cooler conditions.

The most important variables such as: temperature, pH, dissolved oxygen, oxide-reduction potential, specific conductivity, turbidity, nitrite, nitrate, ammonium, orthophosphate, chlorophyll a, zooplankton composition and zooplankton density were measured.

Data were appropriately transformed (log, square root or arcsine transformations, depending on the variable) to meet the statistical requirements. Data were analysed separately for the two experiments with fish and nutrients as treatment variables.

Similar studies were carried out in a European mesocosm experiment in 1998 and 1999 in six different localities throughout Europe (Fernández-Aláez *et al*, 2004; Hietala *et al*., 2004; Hansson *et al*., 2004; Moss *et al*., 2004; Romo *et al*., 2004; Stephen *et al*., 2004a;

Stephen *et al.*, 2004b; Vakkilainen *et al.*, 2004; Van De Bund *et al.*, 2004; Van De Bund & Van Donk, 2004).

The results from these experiments were that the phytoplankton community is mainly affected by nutrients addition and the fish additions influenced the larger zooplankton organisms.

A main problem resulted from these experiments is the difference between the two years of study. Such difference arises from the different starting conditions of the shallow lakes mainly due to the presence of macrophytes.

In the present study there are at least two important differences respect of those experiments. First of all the bottom of each mesocosm is not sealed into the sediment and so there is not the problem of the regeneration of nutrient from the sediment. The second difference is the absence of submerged macrophytes, which can assimilate nutrients and provide refuge for zooplankton against fish predation, inside each mesocosm.

In this way the assumption was that only nutrients and fish would affect the planktonic community and not other variables.

Before performing the experiments some hypothesis were postulated:

1. Top-down and bottom-up controls can be different between seasons.

2. Nutrient addition can increase phytoplankton biomass and thus zooplankton density and structure.

3. Fish addition can decrease zooplankton density and increase phytoplankton biomass.

Chapter 2. MATERIAL AND METHODS

2.1 Study site

Experiments were carried out in a shallow lake located in the wetland of the Experimental Ecology Laboratory and Aquaculture, Biology Department, University of Rome "Tor Vergata" which has an experimental aquaculture system.

The system (**Figure2.1**) is made up of three shallow freshwater lakes interconnected of 200, 900 and 800 m^2 respectively that supply eight tanks for fish intensive rearing. The outlet of these tanks flows again into the lakes. The experiments were carried out in third lake. During the experiment no fish rearing occurred.

The shores of the lake (**Figure 2.2**) are surrounded by a belt of *Phragmites australis*, *Typha latifolia*, *Salix alba* and *Populus nigra*.

Fish community is composed by several species such as *Cyprinus carpio*, *Carassius carassius*, *Gambusia holbrooki*, *Psudorasbora parva* and *Micropterus salmoides*.

The lake was studied from July 2002 to June 2003 and results are reported in **tables 2.1-5**.

Lake water was well oxygenated and pH was slightly basic demonstrating a high photosynthesis activity. Concentration of soluble reactive phosphorus was high. Phytoplankton community was mainly composed by bacillariophyta and chlorophyta species. Zooplankton community was mainly predominated by rotifer species.

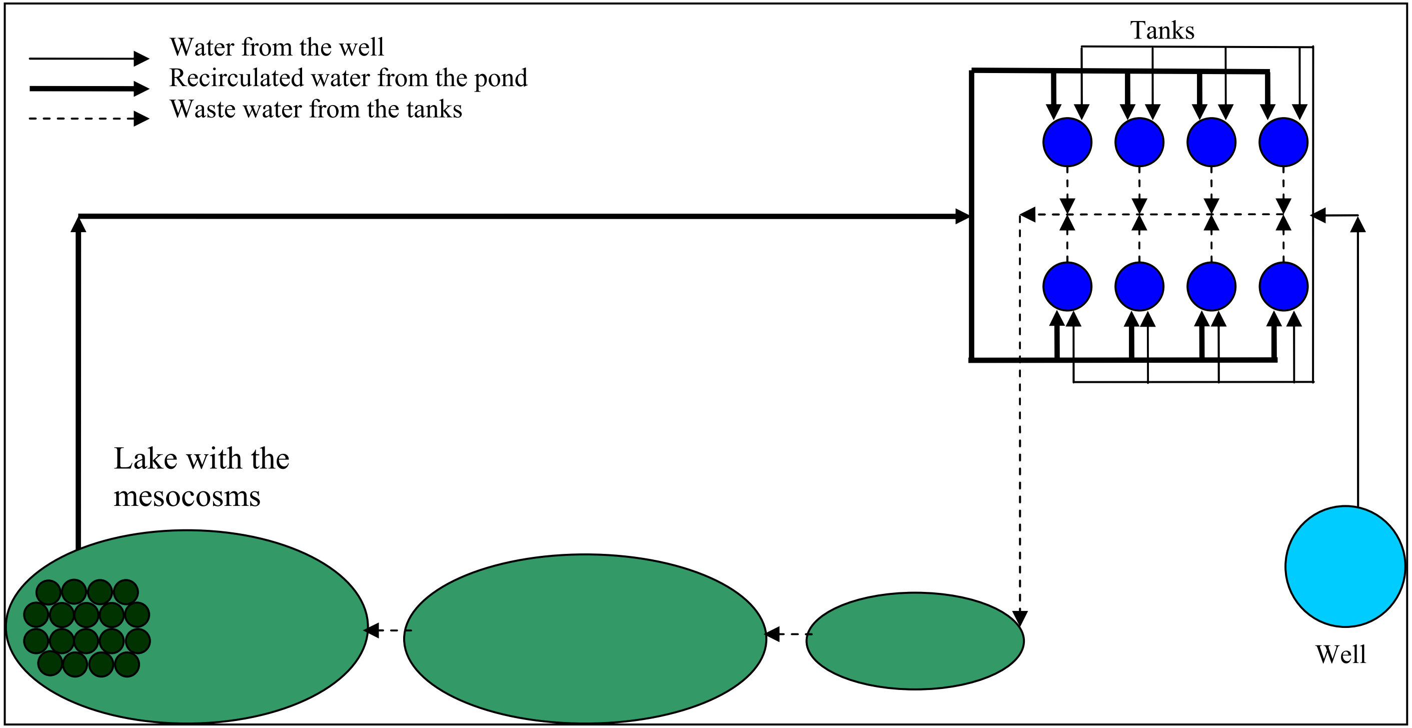


Figure 2.1. Experimental aquaculture system of the Experimental Ecology Laboratory and Aquaculture, Biology Department, University of Rome "Tor Vergata" where the mesocosm experiments took place.

Temperature (°C)	18±1
Dissolved oxygen (mg/l)	11.1±0.6
Dissolved oxygen (%)	118±6
pH	8.42±0.07
N-NO2 (mg/l)	0.010±0.002
N-NO3 (mg/l)	1.7±0.2
N-NH4 (mg/l)	0.14±0.02
PO4 (mg/l)	0.16±0.02

Table 2.1. Water chemistry of the lake measured from July 2002 to June 2003

Phylum	Species
Alveolata	*Gymnodinium* sp.
Bacillariophyta	*Achnanthes laceolata*
	Nitzschia sp.
	Gomphonema sp.
	Navicula sp.
	Amphora ovalis
	Chaetoceros sp.
	Fragilaria sp.
	Synedra sp.
Chlorophyta	*Chlorococcum* sp.
	Pediastrum sp.
	Coelastrum microporum
	Crucigenia sp.
	Scenedesmus quadricauda
	Scenedesmus sp.
	Golenkiniopsis longispina
	Kirchneriella sp.
	Monoraphidium sp.
	Micractinium sp.
Streptophyta	*Cosmarium* sp.
	Staurastrum sp.

Table 2.2. Phytoplankton community composition of the lake observed from July 2002 to June 2003.

TOTAL (org/ml)	10171±2126
Bacillariophyta (org/ml)	7825±2005
Chlorophyta (org/ml)	1625±201
Streptophyta (org/ml)	171±81
Alveolata (org/ml)	550±144

Table 2.3. Phytoplankton phyla densities of the lake measured from July 2002 to June 2003.

Phylum	Species
Rotifera	*Brachionus angularis*
	Brachionus calyciflorus
	Brachionus quadridentatus
	Brachionus urceolaris
	Brachionus sp.
	Keratella sp.
	Keratella cochlearis
	Trichotria sp.
	Synchaeta sp.
	Polyarthra sp.
	Lecane sp.
	Trichocerca sp.
	Cephalodella sp.
	Filinia sp.
	Rotaria sp.
	Asplanchna sp.
	Hexarthra mira
Cladocera	*Bosmina longirostris*
	Daphnia sp.
Copepoda	Nauplii
	Cyclops sp.

Table 2.4. Zooplankton community composition of the lake observed from July 2002 to June 2003.

TOTAL (org/l)	68±15
Rotifera(org/l)	67±15
Cladocera (org/l)	0.8±0.3
Copepoda (org/l)	0.019±0.009
Nauplii (org/l)	0.19±0.04

Table 2.5. Zooplankton groups densities of the lake measured from July 2002 to June 2003.

Figure 2.2. The lake used as natural ecosystem.

2.2 Experimental design

Spring experiment was carried out between May 19 and July 23 in 2005, while winter experiment was carried out between February 21 and March 28 in 2006.

Sets of 18 mesocosms were placed in each experiment in area of the lake around 1 m deep (**Figure 2.3**).

Each mesocosm consisted of flexible polyethylene bags (diameter 0.48 m, surface 0.181 m^2, depth 1.1 m; volume 197 l) filled with lake water (**Figure 2.4**). The mesocosms, which were closed at the bottom and open at the top, were surrounded by a protecting cage (**figure 2.5**). Mesocosms were filled with lake water through a pump (**Figure 2.6**). To prevent biases in zooplankton densities because of migration behaviour in the lake, water was shaken before fill the mesocosms.

Figure 2.3. Disposition of the mesocosms in the lake.

Figure 2.4. One mesocosm.

Figure 2.5. Protecting cages of the polyethylene bags

Bags were collocated inside the lake to prevent alteration of temperature of the water during the experiment and to replicate natural conditions.

Fish were captured in the lake shortly before introduction to the mesocosms. The mesocosms were checked at least two times during the day. Dead or unhealthy looking fish were immediately replaced to prevent nutrient enrichment by fish decomposition, however this event never occurred.

There was no a notable fall in water levels owing to evaporation neither a rise owing to rain during the experiments.

Figure 2.6. Filling of the mesocosms with lake water through a pump.

Experiments involved addition of nutrient and fishes in nine combinations with twofold replication to a total of eighteen mesocosms in each experiment.

Three fish densities (0, 4 and 20 g fresh mass/m^2) and three nutrient loadings (weekly additions of N-P in mg/l: 0-0, 0.3-0.03 and 3-0.3) were applied.

Table 2.6 reports values of nutrient additions and fish densities used in the mesocosms. The corresponding values were calculated considering volume and surface of the mesocosms 197 l and 0.181 m^2 respectively.

Nutrient additions			
Label	*Description*	*Addition*	*Corresponding to*
N0	No nutrient addition	Nitrogen 0 mg/l	0 g
		Phosphorus 0 mg/l	0 g
N1	Low nutrient addition	Nitrogen 0.3 mg/l	0.0591 g
		Phosphorus 0.03 mg/l	0.00591 g
N2	High nutrient addition	Nitrogen 3 mg/l	0.591 g
		Phosphorus 0.3 mg/l	0.0591 g
Fish densities			
Label	*Description*	*Density*	*Corresponding to*
F0	No fish	0 g/m^2	0 g (0 fishes)
F1	Low fish density	4 g/m^2	0.717 g (4 fishes)
F2	High fish density	20 g/m^2	3.583 g (20 fishes)

Table 2.6. Values of nutrient additions and fish densities used in the mesocosm experiments.

Figure 2.7 below represents disposition of the mesocosms inside the lake with the nine different combinations of nutrient and fish treatments reported.

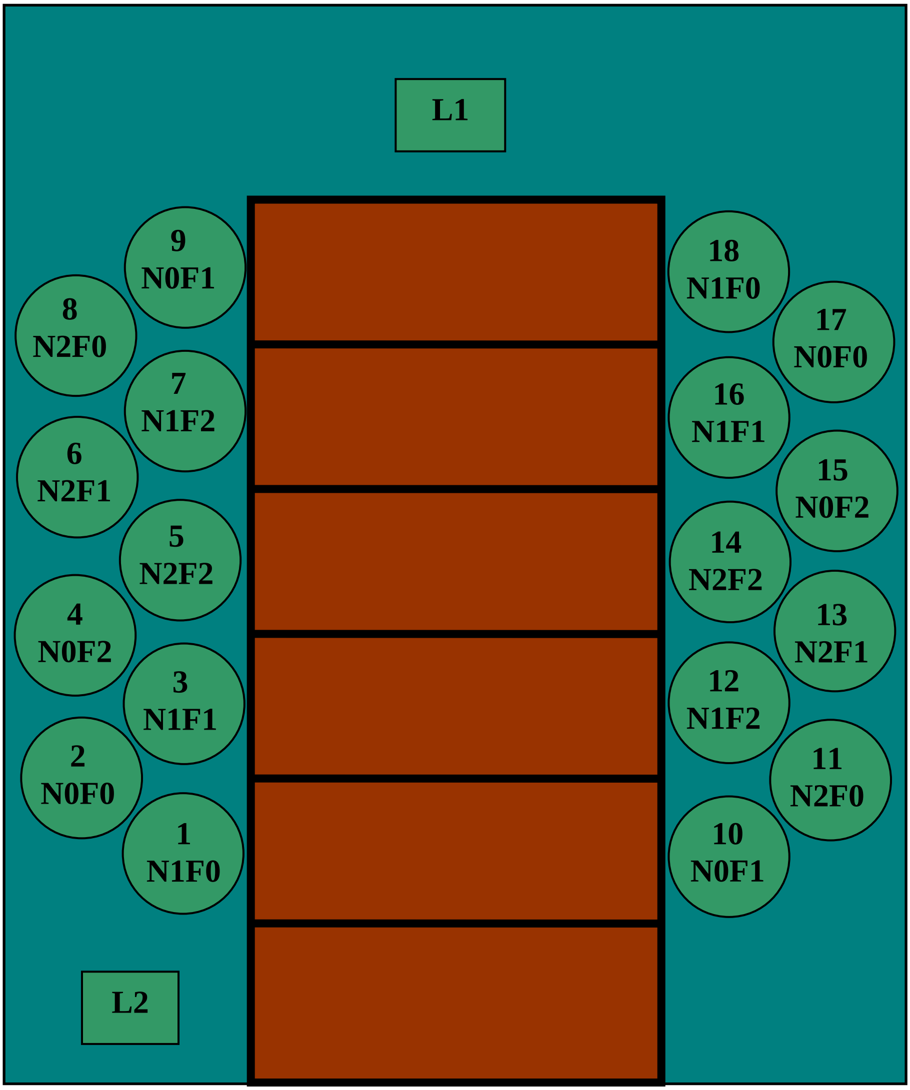


Figure 2.7. Disposition of the mesocosms in the lake with the different combination of nutrient and fish treatment reported.

Fish average weights slightly differed between the two seasons (**Table 2.7**). Although in winter experiment fish average weight was slightly lower (0.17±0.01 g) than in spring experiment (0.18±0.02 g) the same number of fishes were used. In this way both experiments had the same number of zooplanktivorous fishes preyed over planktonic organisms.

	Spring Experiment	*Winter Experiment*
Average weight (g)	0.18±0.02	0.17±0.01
Average total length (cm)	2.85±0.04	2.61±0.04

Table 2.7. Average weight and average total length of fishes used in mesocosm experiments (*n* = 144 in both experiments).

Unfortunately it is not known fish abundance in the lake, thus it is not known precisely how the fish densities used in the mesocosms compared with those of lake. Anyway densities used in the mesocosms (4 and 20 g/m^2) are well recognized to reproduce fish densities in natural ecosystems.

Post & McQueen (1987) and Perrow *et al*. (1999) stated that a fish biomass as low as 2–5 g fresh mass m^{-2}, or 0.2 individual m^{-2}, might be sufficient to remove *Daphnia* populations.

Nutrients were added as mixtures of sodium nitrate and potassium dihydrogen phosphate and they were added each week after sampling.

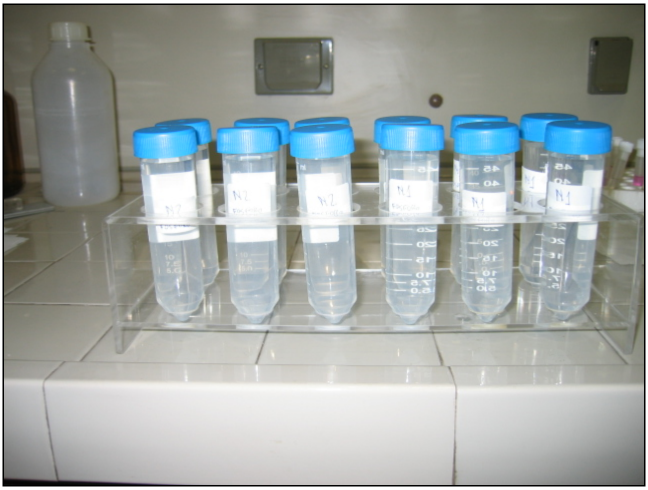


Figure 2.8. Test-tubes containing the mixtures of sodium nitrate and potassium dihydrogen phosphate.

Table 2.8 below summarizes activities executed in each experiment. Dates of sampling days for both experiments are also reported.

Number of week	**Date**		**Activity**
	Spring Experiment	*Winter Experiment*	
Week 0	May 19 2005	February 21 2006	Filling mesocosms, fish addition, sampling and nutrients addition
Week 1	May 26 2005	February 28 2006	Sampling and nutrients addition
Week 2	June 2 2005	March 7 2006	Sampling and nutrients addition
Week 3	June 9 2005	March 14 2006	Sampling and nutrients addition
Week 4	16 June 2005	March 21 2006	Sampling and nutrients addition
Week 5	23 June 2005	March 28 2006	Sampling, emptying mesocosms, sampling of sediment and fishes

Table 2.8. Sampling dates and activities.

The fish species introduced in the mesocosms was *Gambusia holbrooki*. Four individuals were used for the low density (F1) and twenty individuals for the high density (F2). To avoid reproduction during experiment only male individuals of *Gambusia holbrooki* were used.

Since results of both experiments depend on *Gambusia holbrooki* biology, next paragraph describes the fish species.

2.3 *Gambusia holbrooki* Girard, 1859

Gambusia holbrooki Girard, 1859 (Family *Poeciliidae*) is a small fish native to coastal drainages of the south eastern United States (**Figure 2.9**). It was introduced in Europe from the ’20 as a biological agent for the aquatic life stages of mosquitoes (Dawes, 1991). Two species of the genus *Gambusia* exist, the western mosquitofish, *Gambusia affinis*, and the eastern mosquitofish, *Gambusia holbrooki*.

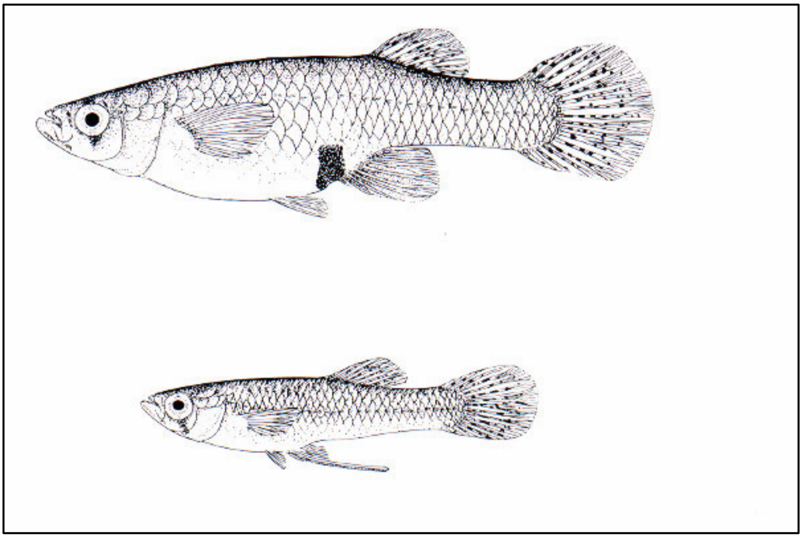


Figure 2.9. *Gambusia holbrooki* Girard, 1859. Female (high) and male (down).

The earliest recorded introductions were of *G. affinis* from Texas to Hawaii and subsequently to the Philippines in 1905 (Seale, 1917) shortly after the discovery that mosquitoes transmit malaria and yellow fever, and the distribution for either species now includes 74 countries throughout the tropical and temperate world (Ling, 2004) (**Figure 2.10**).

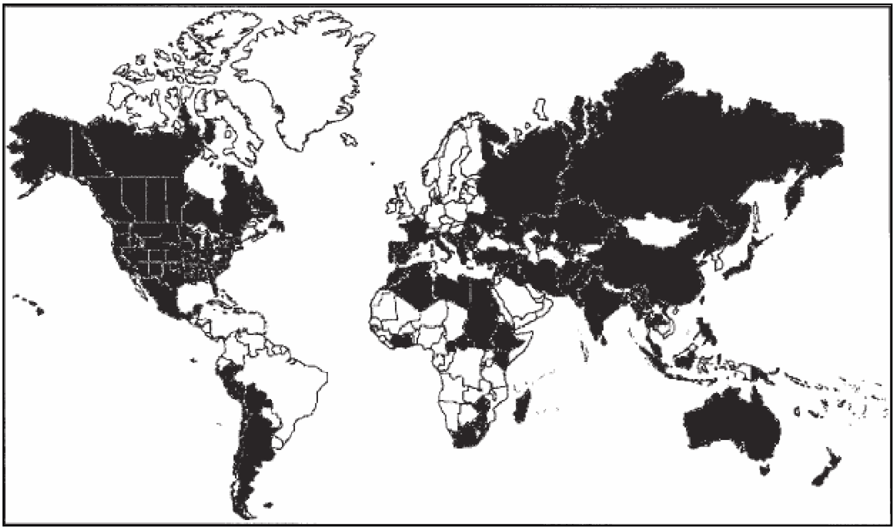


Figure 2.10. Countries of the world where *Gambusia* has been introduced (from Ling, 2004).

From 1920 it was introduced in Spain. Grassi introduced it in some lakes in Latium, Italy (Ronchetti, 1968). In 1924 it was introduced in some place of ex URSS, Corsica, southern France; in Algeria in 1926, in Morocco in 1928, in Libya in 1931. Following it was introduced in other countries in the world such as Germany, Dalmatian, Albany, Greece, Cyprus, Syria, Egypt, Tunisia, Sudan, eastern Africa, Madagascar, Iran, India, Indochina, China, Japan, Australia, New Zealand, Canada, Mexico and Argentina. For its food plasticity *Gambusia* survived in many countries of temperate, subtropical and tropical areas (Ronchetti, 1968).

Gambusia has been implicated in the displacement, decline, or elimination of numerous native fish and amphibian species in many countries where they have been introduced. Those species most affected are small, surface-feeding, littoral-zone fishes and the egg and larval stages of amphibians (Ling, 2004).

Gambusia holbrooki has ecological advantages related to fast growth, early maturity, viviparity (Vargas & Sostoa, 1996) and food consumption rates, that can reach 83% of fish weight per day (Wurtsbaugh & Cech, 1983).

They have therefore gained a reputation as a significant aquatic pest, earning them titles such as “fish destroyer” (Myers, 1965), “damnbusia” (McCullough, 1998), and “plague minnow” (Pyke &

White, 2000). However, some of the evidence for direct impacts of *Gambusia* on native species is patchy and circumstantial (Lloyd *et al*. 1986) and often associated with other factors causing decline in indigenous species such as habitat disturbance (Jansen & Healey, 2003). Disturbed or polluted habitats tend to favour many invasive exotic species because of their broad environmental tolerances (Arthington, 1991). *Gambusia* is tolerant of extremely variable environmental conditions making them ideal colonist species (Lloyd *et al*., 1986).

Gambusia has an evident sexual dimorphism. Female can reach a length of 5-6 cm, male 2.5-3 cm. Colour is grey for both sex. Female has a dark blotch next the anal fin especially during pregnancy. Male has the anal fin modified in gonopodium used during coupling.

It is an ovovivipary species and can give birth up to 100 fry each delivery, concluding 4-5 deliveries each season. Sexual ratio is 1:1 (Bisazza *et al.*, 1990). There is a correlation between weight, length, female native place and number of fry (Naama & Al-Hassan, 1989). Optimal temperature for fry growth in case of high availability of food is between 25 and 35°C. In case of limited food condition temperature can decrease to 25°C. (Wurtsbaugh & Cech Jr., 1983).

Gambusia can tolerate water temperature higher than 35 °C and lower than 6-7°C (Castleberry & Cech, 1987). It can survive up to dissolved oxygen tension lower than 7 mmHg (Castleberry & Cech, 1987). It can resist to ammonium and nitrate concentrations toxic for other fish species (Beesley, 1986).

Its diet is made up of several crustacean and insect species. (Bence & Murdoch, 1986; Linden & Cech Jr., 1990). Among crustacean it feeds on cladocerans (Blaustein & Karban, 1990), ostracods and copepod (Kramer *et al*., 1987). Among insects it feeds on culicids and chironomids larvae, Notonectidae, corixidae, omoctera, odonota, coleopteran and ephemeroptera (Farley & Younce, 1977; Kramer *et al.*, 1987; Walters & Legner, 1980).

Some workers have used this species to test pelagic trophic interactions (*e.g.* Hurlbert *et al*., 1972; Lancaster and Drenner, 1990), the optimal foraging theory (Bence & Murdoch, 1986) and toxicant bioaccumulation in wetlands (Rawlik, 2001). Some dietary studies have focused on its role in control of insects (Cech & Moyle, 1983; Walton & Mulla, 1991; Linden & Cech, 1990). However, few studies (Crivelli & Boy, 1987) have analysed the dietary patterns of mosquitofish in shallow Mediterranean lakes, where the introduction of this species maybe rapidly adapt and affect the food web. Additionally, most experiments on food web ecology and fish only study the dynamics of prey populations without analysis of fish stomach content to determine direct top-down effects (Blanco *et al.*, 2004).

The present work considers mosquitofish predation on plankton community in spring and winter and investigates differences between the two seasons.

2.4 Sampling methods

All mesocosms were sampled prior to introducing fish and adding nutrients. Subsequent samples were taken at weekly intervals, immediately before the next nutrient addition, starting 1 week after the beginning of the experiment.

Samples were taken in the 18 mesocosms and at two points of the natural ecosystem (L1 and L2).

The physico-chemical variables measured were temperature, pH, dissolved oxygen, oxide-reduction potential, specific conductivity, turbidity, nitrogen nitrite, nitrogen nitrate, nitrogen ammonia and orthophosphate.

Temperature, pH, dissolved oxygen, oxide-reduction potential, specific conductivity, turbidity, and chlorophyll a were measured using Datasonde Hydrolab 4a (**Figure 2.11**).

Figure 2.11. Hydrolab Data sonde 4a used for sampling water chemistry and chlorophyll a in the mesocosms and the natural system.

Nitrogen nitrite, nitrogen nitrate, nitrogen ammonia and orthophosphate were measured using Standard Methods (A.P.H.A.).

Twenty litres of water were passed trough a 100 µm mesh to determine abundance a diversity of zooplankton organisms.

At the end of the experiments zoobenthos samples were taken in each mesocosm.

Sampling was executed every week at the same hour (9 a. m.).

Data were reported in figures and tables. Only for statistical computation data were appropriately transformed (log, square root or arcsine transformations, depending on the variable) to meet the statistical requirements. Data were analysed separately for the two experiments with fish and nutrients as treatment variables.

Chapter 3. RESULTS

3.1 Water chemistry

No differences in the values of the measured variables were found among mesocosms before adding fish and nutrients in either experiment.

Figures below represents variables values for each mesocosm replicate.

3.1.1 Spring experiment

Temperature in the natural ecosystem ranged from 21.30 to 26 °C (**Figure 3.1**). In the mesocosms it ranged from 20.50 to 25.93 °C (**Figure 3.2**). No differences in the values of temperature were found among replicates during the experiment. The highest temperature was registered on the forth week with a mean temperature of 25.82 °C in the mesocosms and 21.35 °C in the natural ecosystem.

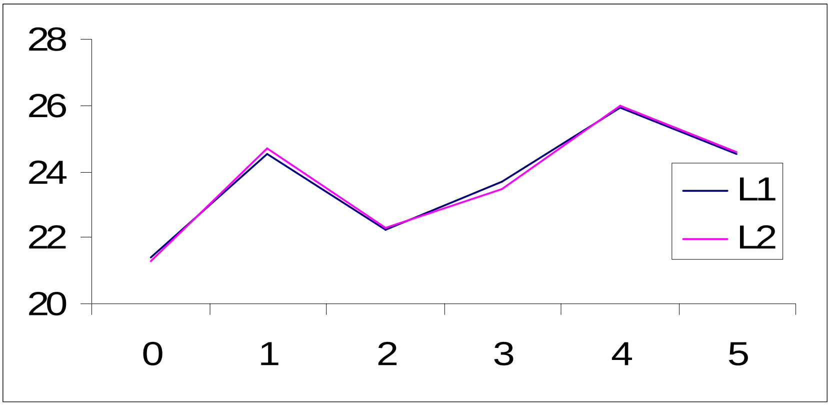


Figure 3.1. Temperature values (°C) in the natural ecosystem during the spring experiment.

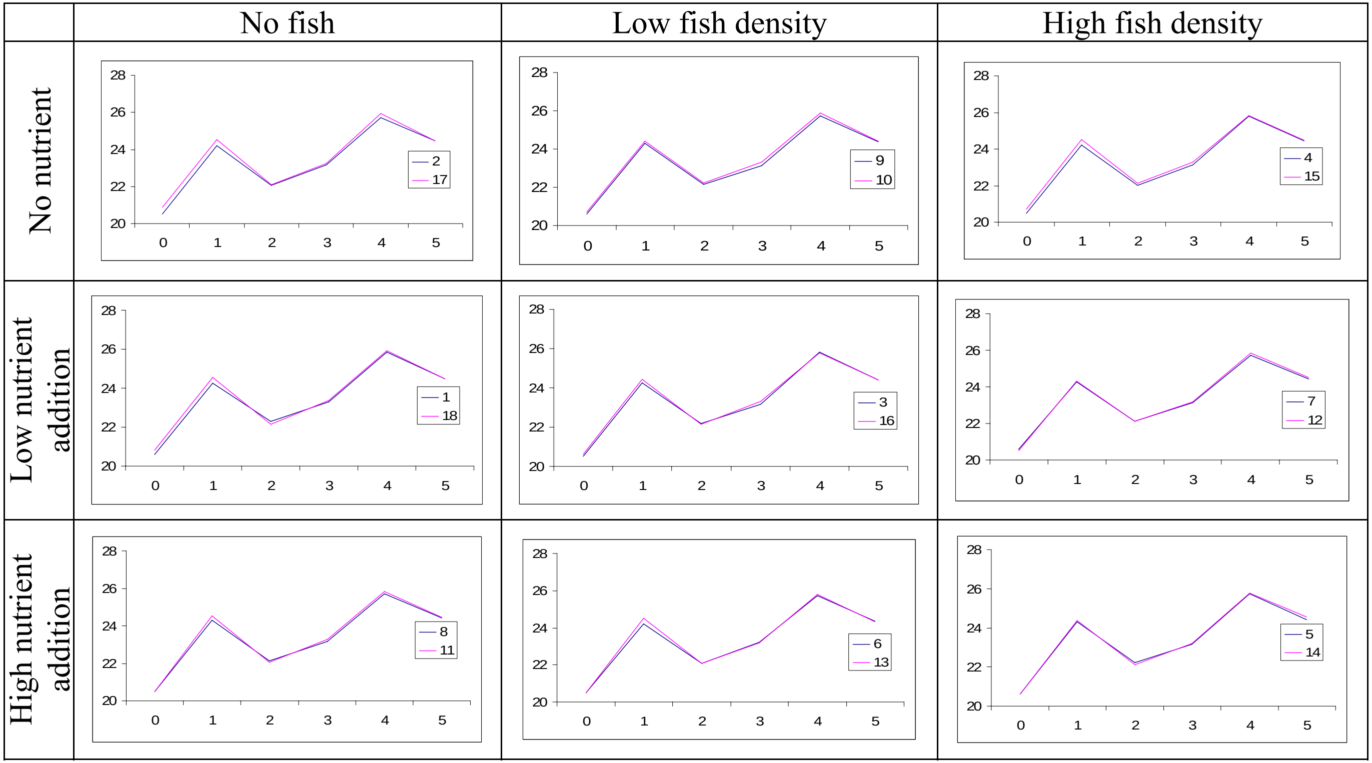


Figure 3.2. Temperature values (°C) at different nutrient additions and fish densities in the 18 mesocosms during the spring experiment.

The pH values in the natural ecosystem ranged from 8.05 to 9.46 (**Figure 3.3**). In the mesocosms pH values ranged from 8.43 to 10.34 (**Figure 3.4**). Higher pH was found in the mesocosms with the highest nutrient addition. pH values gradually increased during the study in each mesocosm.

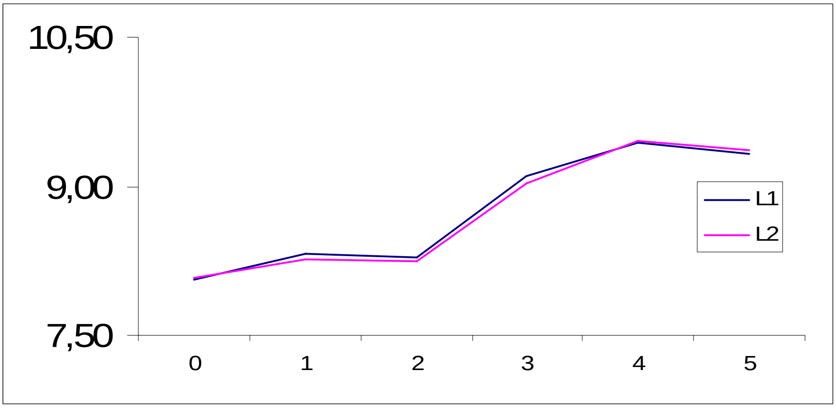


Figure 3.3. pH values in the natural ecosystem during the spring experiment.

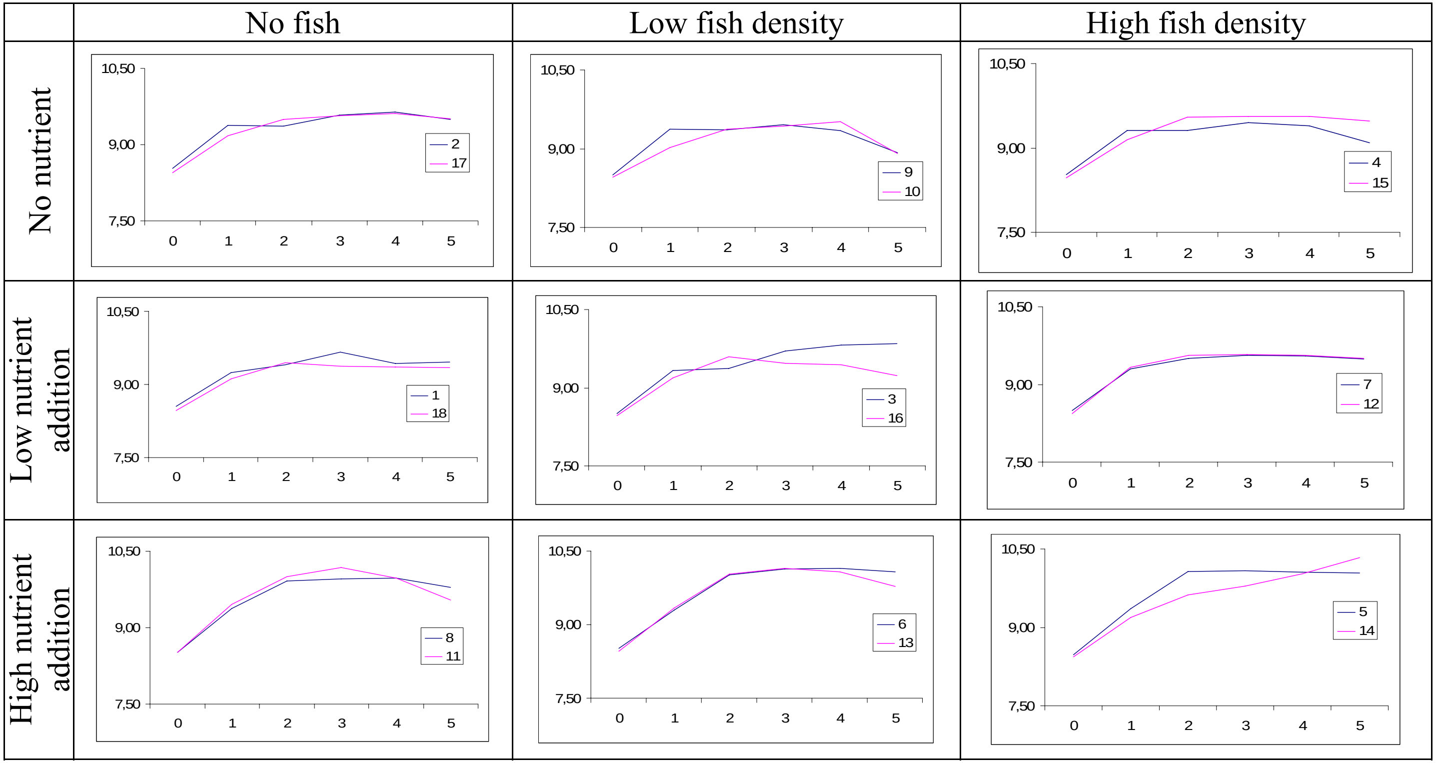


Figure 3.4. pH values at different nutrient additions and fish densities in the 18 mesocosms during the spring experiment.

In the natural system dissolved oxygen ranged from 66.1 to 179.0 mg/l. higher values were observed in the third and fourth week, while in the fifth week dissolved oxygen percentage decreased (**Figure 3.5**). In the mesocosms dissolved oxygen ranged from 76.5 to 150 mg/l. Especially dissolved oxygen percentage was high in mesocosms with high nutrient addition (**Figure 3.6**). In the last week the percentage of dissolved oxygen decreased in all the mesocosms.

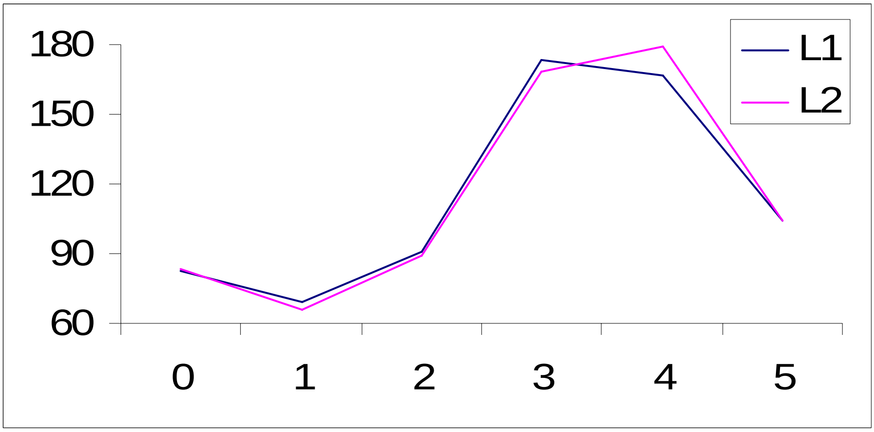


Figure 3.5. Dissolved oxygen percentage (%) in the natural ecosystem during the spring experiment.

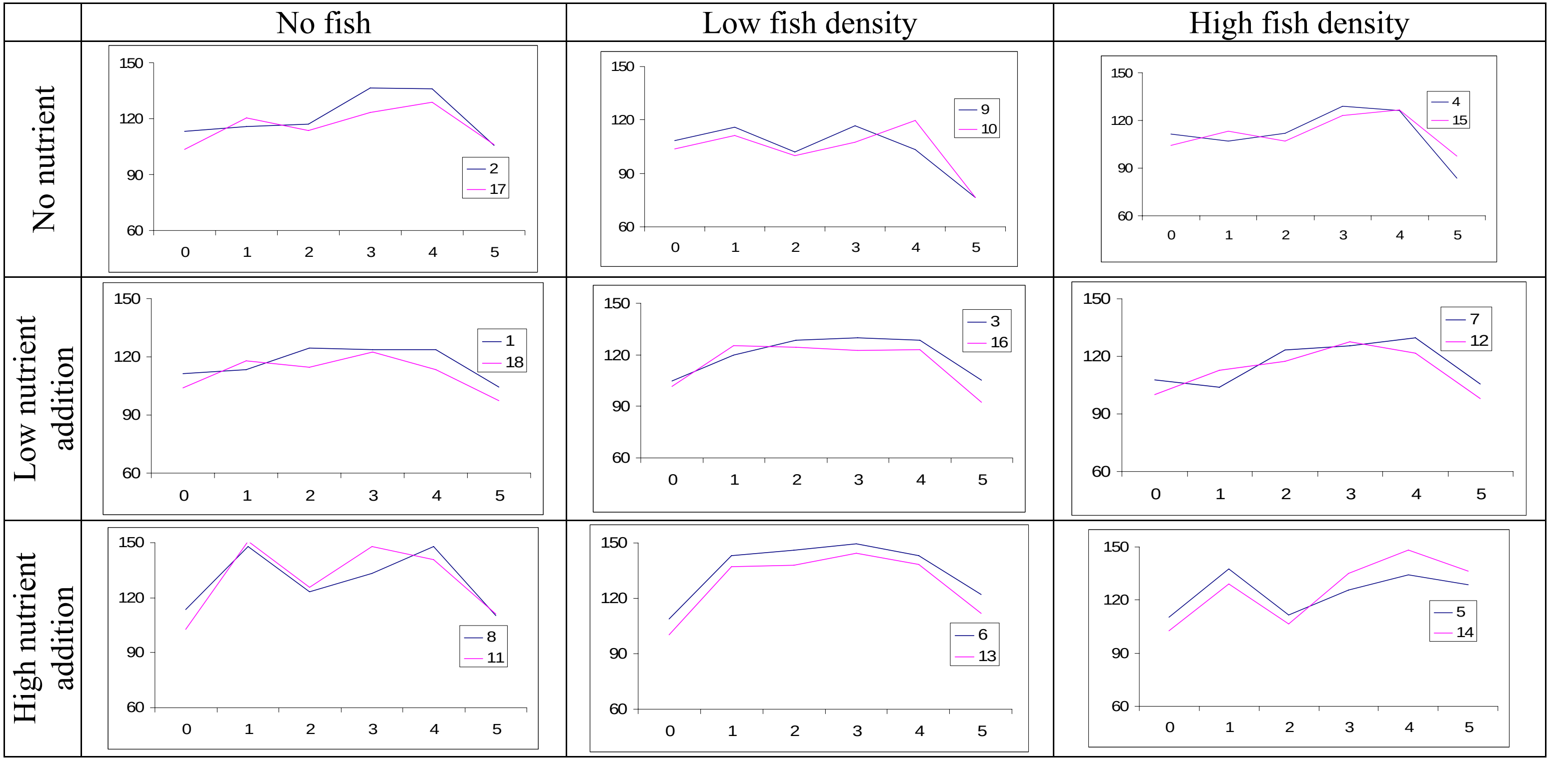


Figure 3.6. Dissolved oxygen percentage (%) at different nutrient additions and fish densities in the 18 mesocosms during the spring experiment.

In the natural ecosystem values of oxide-reduction potential were high and gradually decreased during the experiment. They ranged from 116 to 171 mV (**Figure 3.7**). In the mesocosms they ranged from 85 to 153 mV (**Figure 3.8**). Maximum values of oxide-reduction potential were reached in the first week in all the mesocosms.

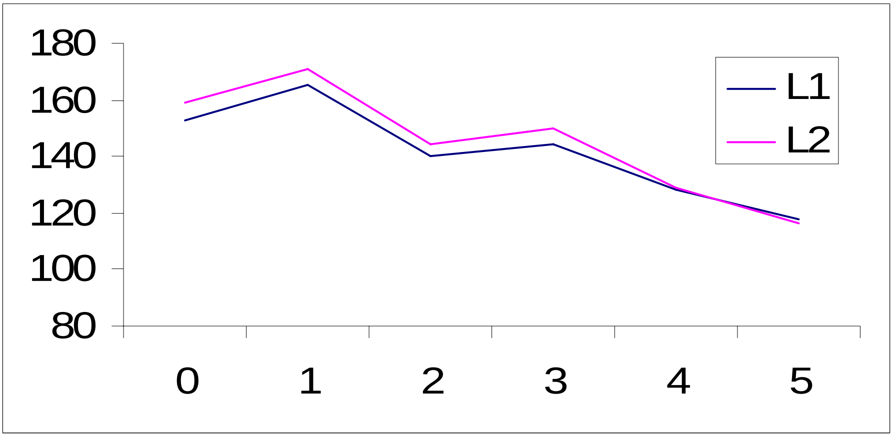


Figure 3.7. Oxide-reduction potential values (mV) in the natural ecosystem during the spring experiment.

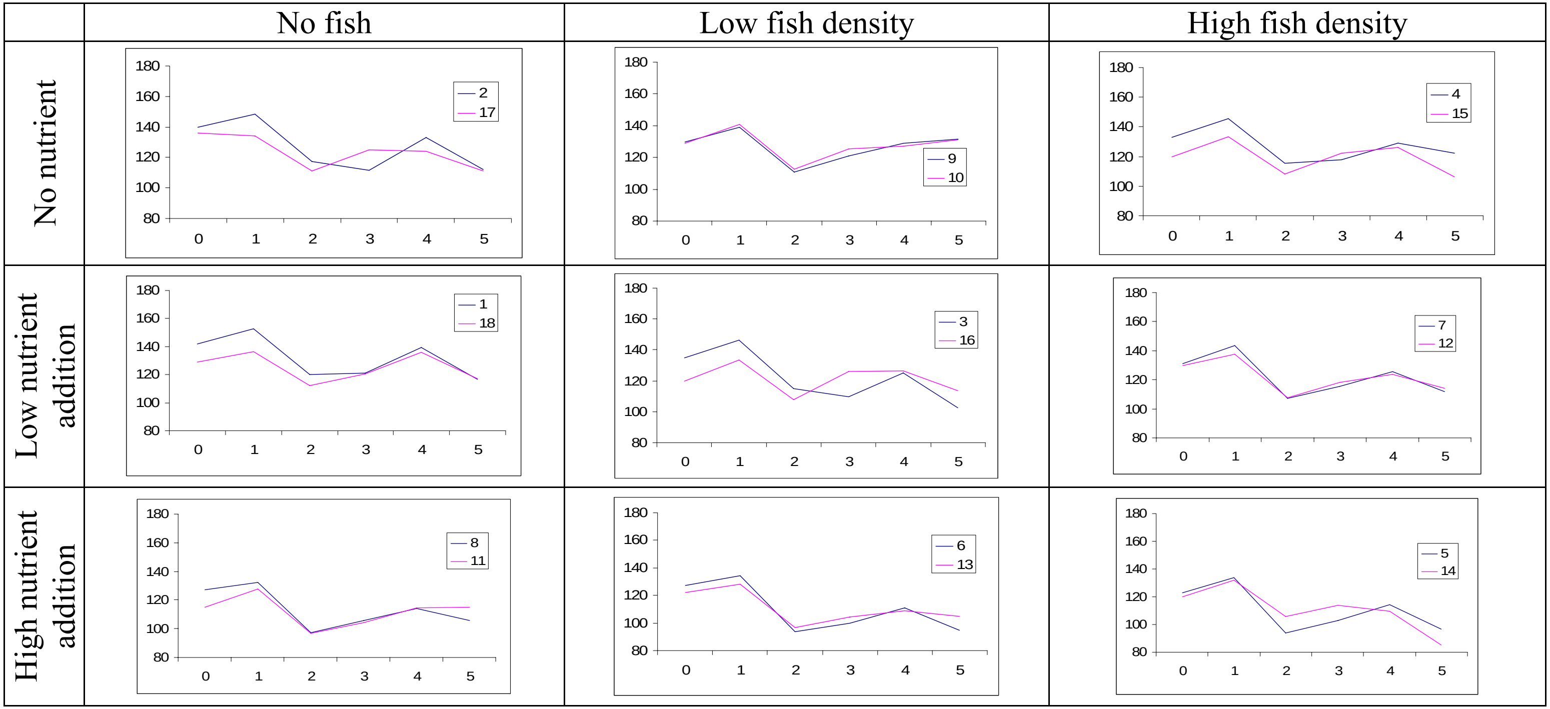


Figure 3.8. Oxide-reduction potential values (mV) at different nutrient additions and fish densities in the 18 mesocosms during the spring experiment.

In the natural ecosystem specific conductivity values gradually decreased during the experiment from 680 to 559 µS/cm (**Figure 3.9**). The two replicates (L1 and L2) were similar except last week when they differed of 33 µS/cm. Specific conductivity values in the mesocosms ranged from 607 to 672 µS/cm (**Figure 3.10**).

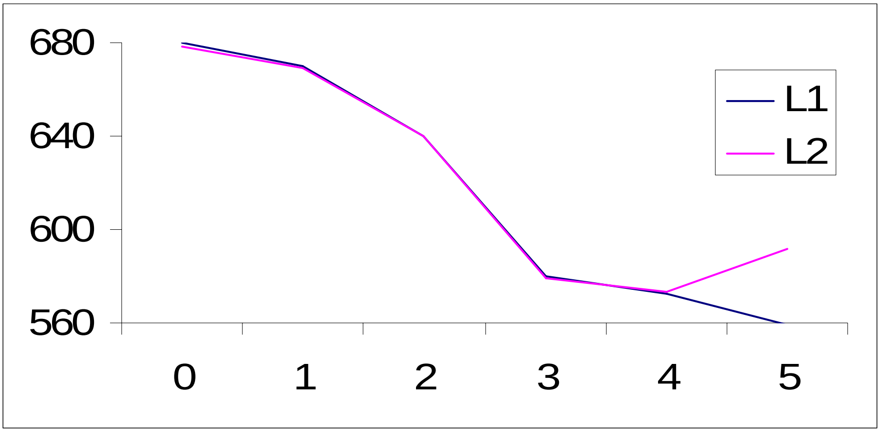


Figure 3.9. Specific conductivity values (µS/cm) in the natural ecosystem during the spring experiment.

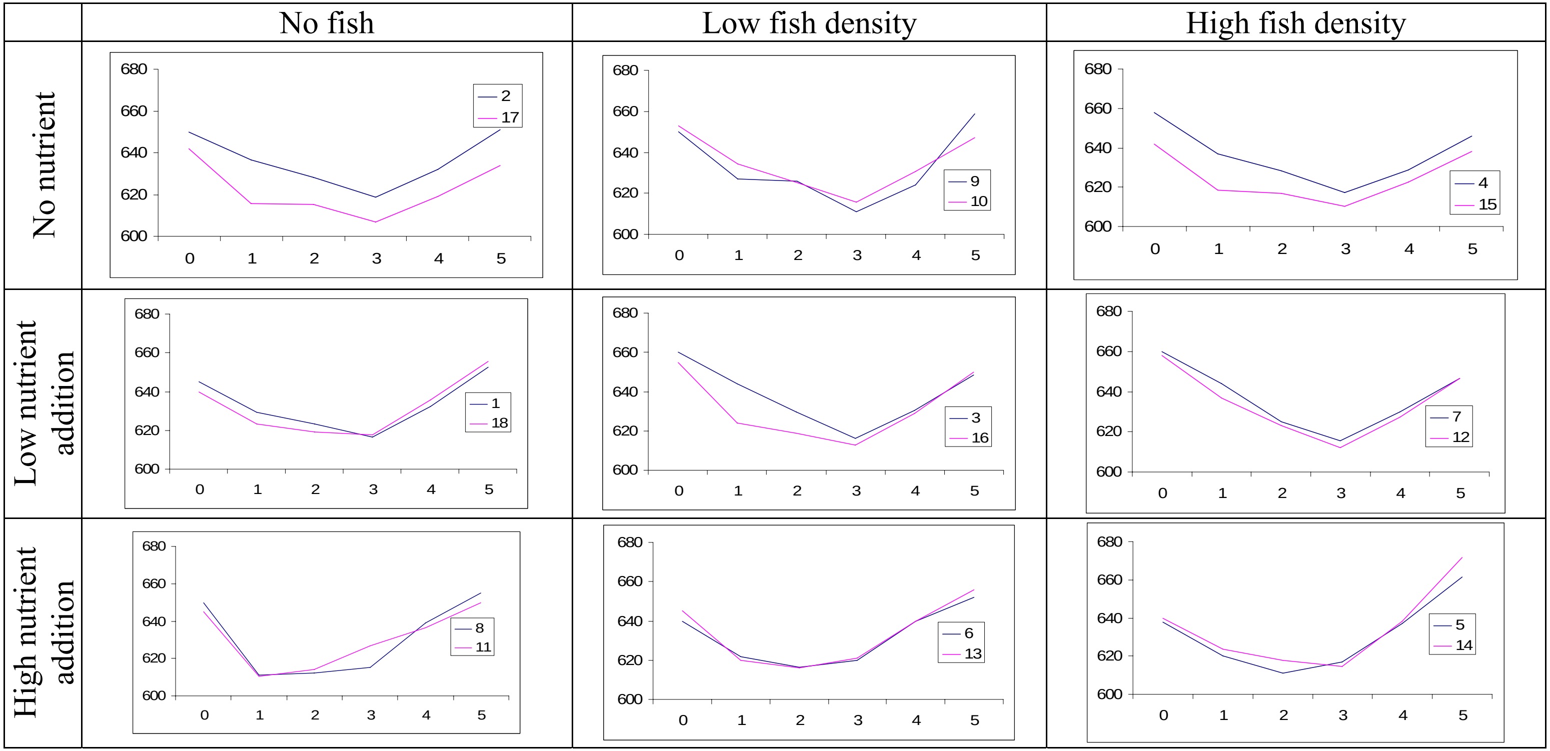


Figure 3.10. Specific conductivity values (μS/cm) at different nutrient additions and fish densities in the 18 mesocosms during the spring experiment.

In the natural ecosystem turbidity values were high and they ranged from 11 to 80 NTU (**Figure 3.11**). Turbidity values in the mesocosms were lower and they ranged from 3 to 26 NTU (**Figure 3.12**).

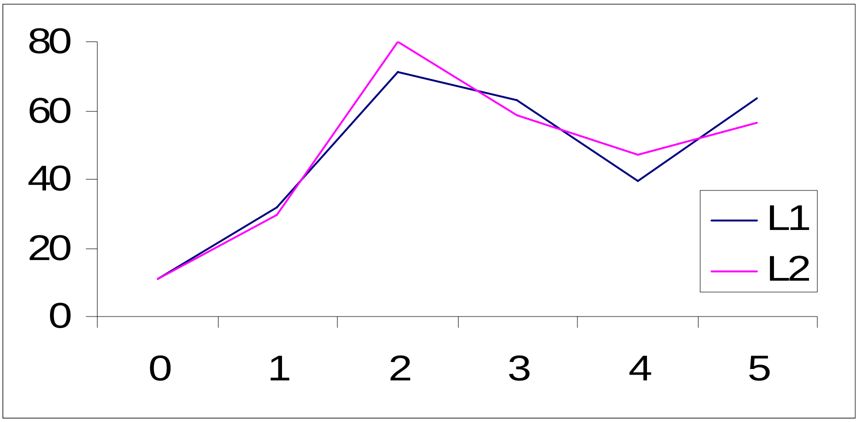


Figure 3.11. Turbidity values (NTU) in the natural ecosystem during the spring experiment.

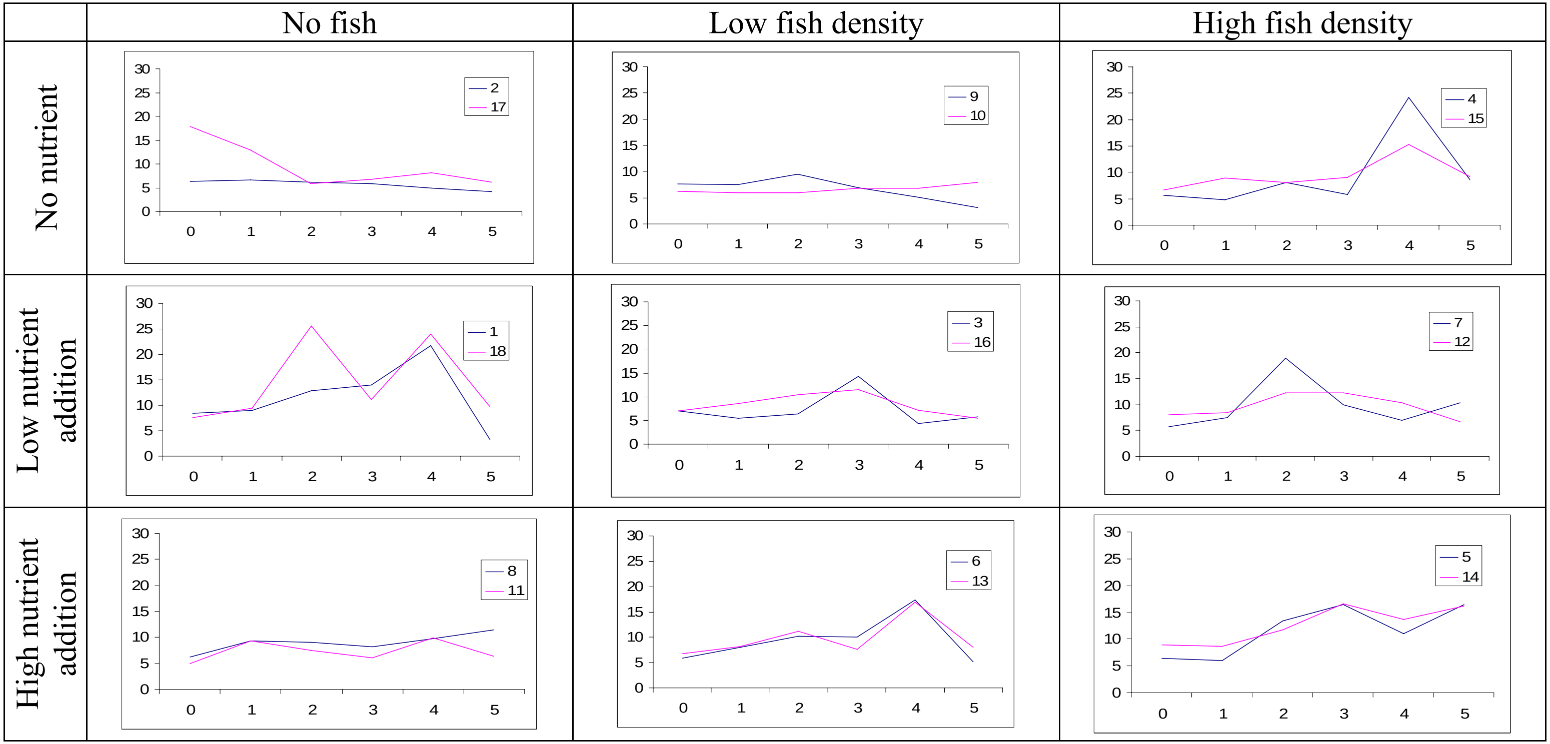


Figure 3.12. Turbidity values (NTU) at different nutrient additions and fish densities in the 18 mesocosms during the spring experiment.

In the natural ecosystem nitrogen nitrite concentration values were high only in the first week and they ranged from 0.001 to 0.023 mg/l (**Figure 3.13**).

Nitrogen nitrite concentrations ranged from 0.003 to 0.036 mg/l in the mesocosms (**Figure 3.14**). These values were high only in the first week, and then they were always below 0.001 mg/l in all the mesocosms.

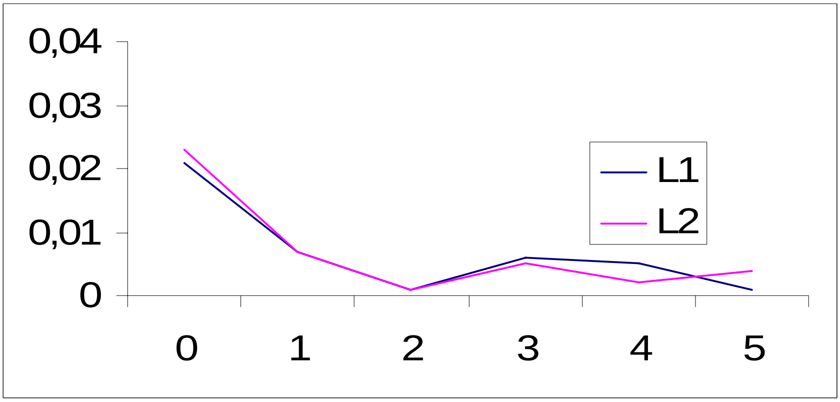


Figure 3.13. N-NO2 concentrations (mg/l) in the natural ecosystem during the spring experiment.

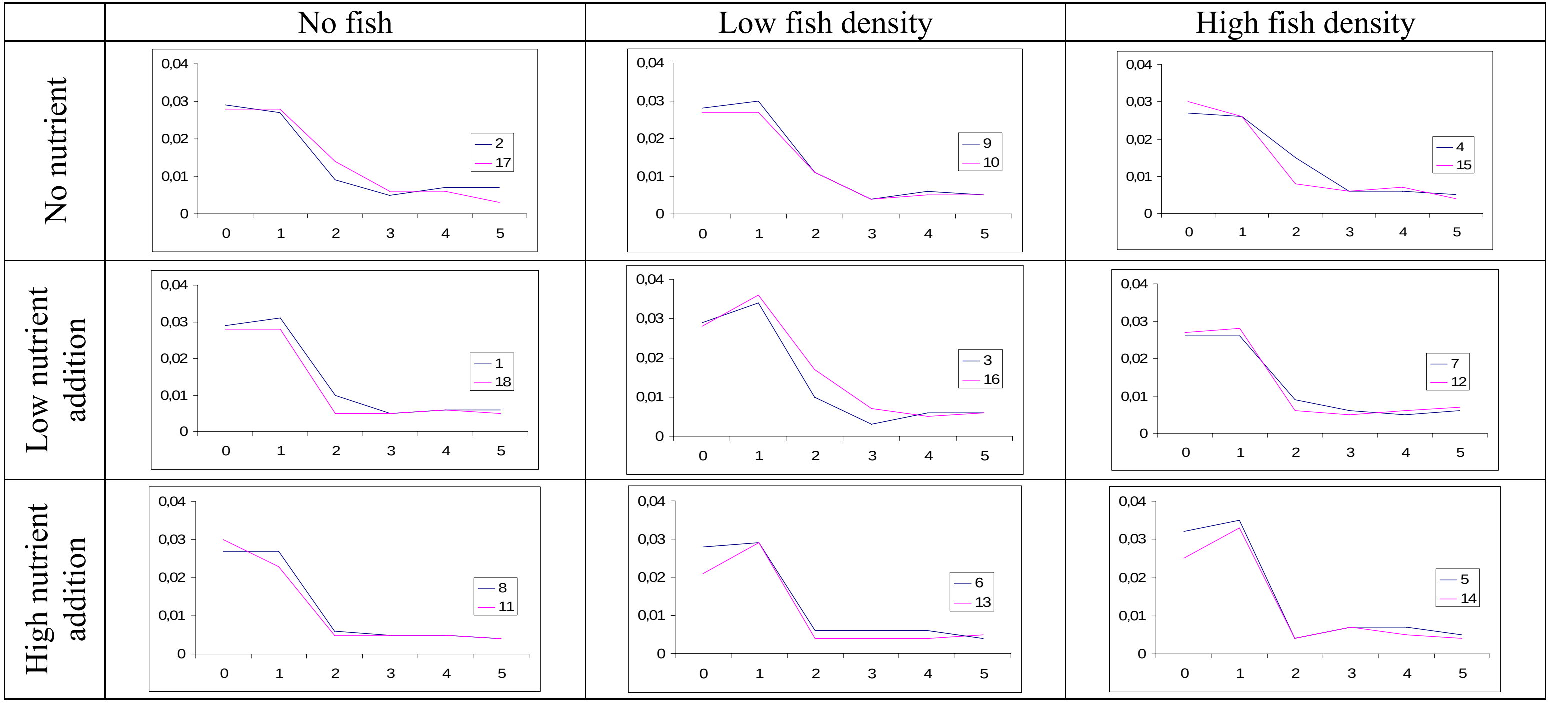


Figure 3.14. N-NO2 concentrations (mg/l) at different nutrient additions and fish densities in the 18 mesocosms during the spring experiment.

In the natural ecosystem nitrogen nitrate concentrations ranged from 0.5 to 3.7 mg/l (**Figure 3.15**). The minimum concentration was observed in the second week and then it increased during the study period.

Nitrogen nitrate concentrations ranged from 1.2 to 3.9 mg/l in the mesocosms (**Figure 3.16**). Nutrient addition slightly influenced the nitrate concentrations that increased only in the mesocosms with the high nutrient addition.

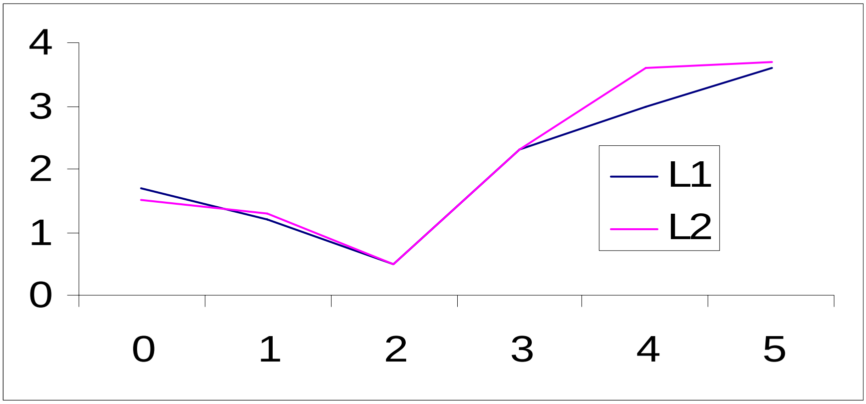


Figure 3.15. N-NO3 concentrations (mg/l) in the natural ecosystem during the spring experiment.

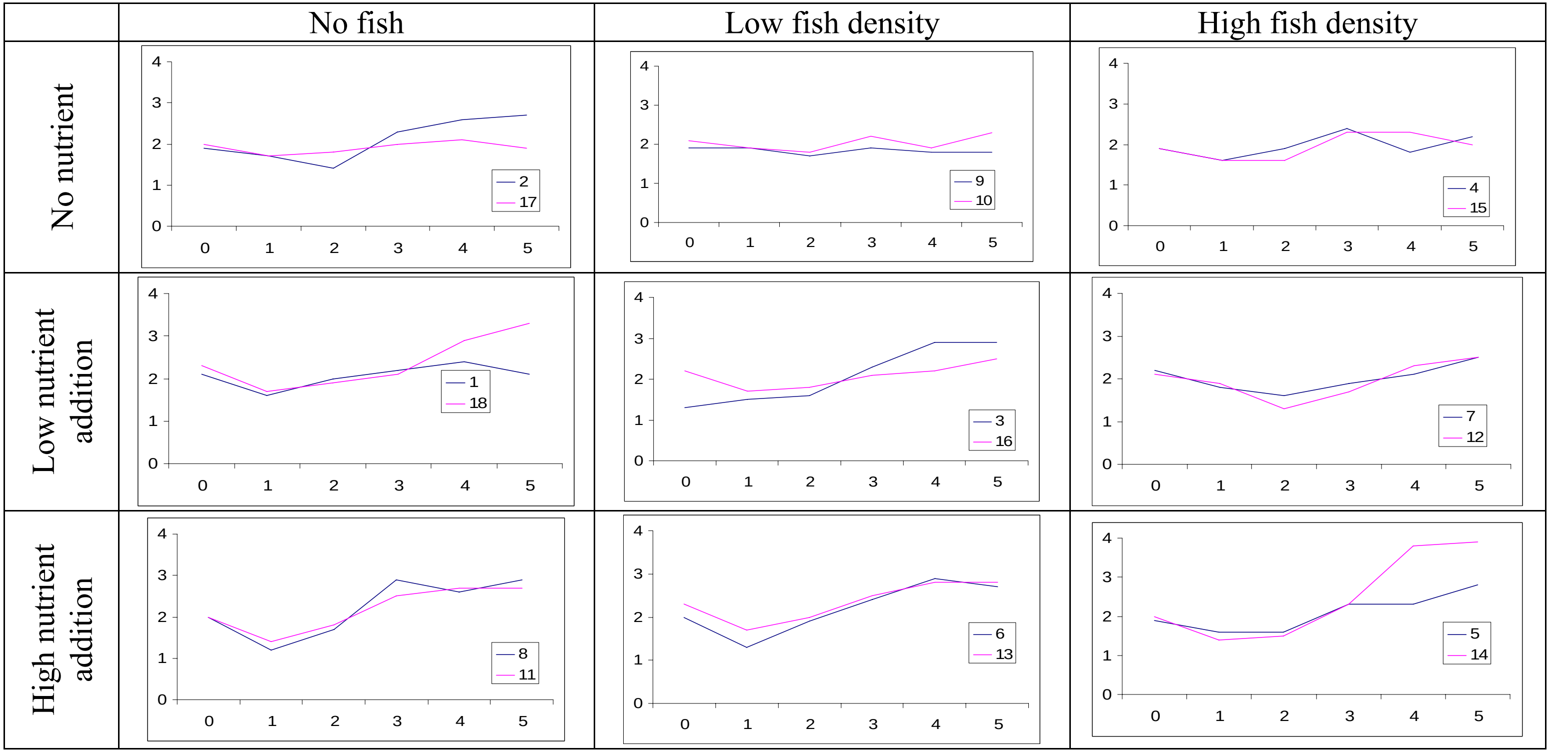


Figure 3.16. N-NO3 concentrations (mg/l) at different nutrient additions and fish densities in the 18 mesocosms during the spring experiment.

In the natural ecosystem nitrogen ammonia concentrations ranged from 0.2 to 0.6 mg/l and they remained almost constant during the study except the forth week (**Figure 3.17**). Nitrogen ammonia concentrations ranged from 0.02 to 0.56 mg/l in the mesocosms (**Figure 3.18**). Almost in all mesocosms these values increased during the study.

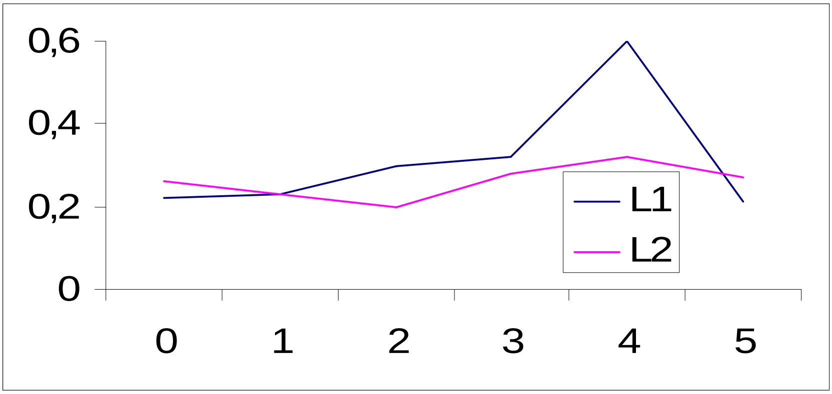


Figure 3.17. N-NH3 concentrations (mg/l) in the natural ecosystem during the spring experiment.

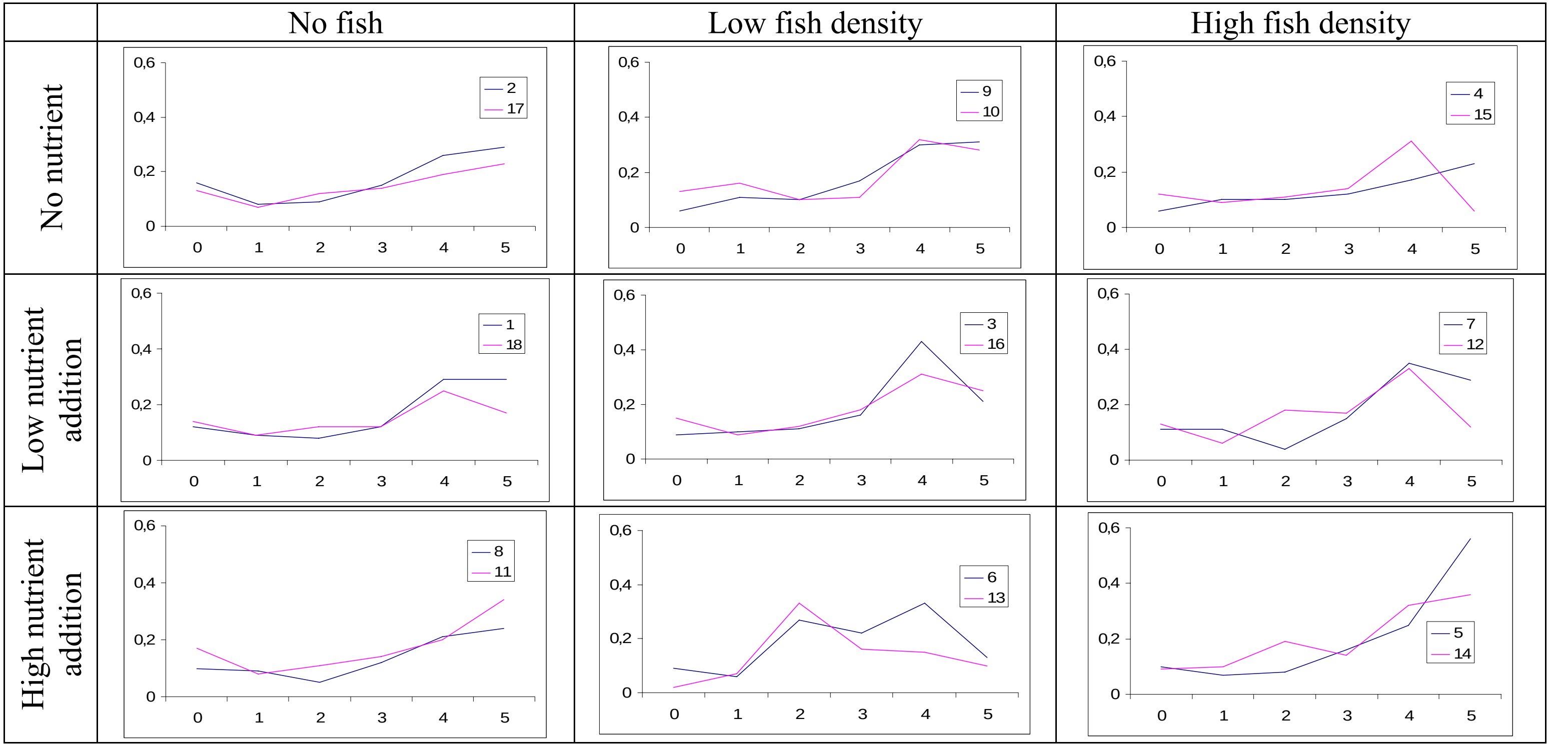


Figure 3.18. N-NH3 concentrations (mg/l) at different nutrient additions and fish densities in the 18 mesocosms during the spring experiment.

In the natural ecosystem orthophosphate concentrations gradually decreased from 0.2 to 0.01 mg/l (**Figure 3.19**).

Orthophosphate concentrations ranged from 0.01 to 0.19 mg/l in the mesocosms (**Figure 3.20**). The mesocosms with high nutrient addition had higher orthophosphate concentrations.

Table 3.1 summarizes all physical and chemical variables considered at nutrient and fish different treatment. Values are reported as mean ± SE.

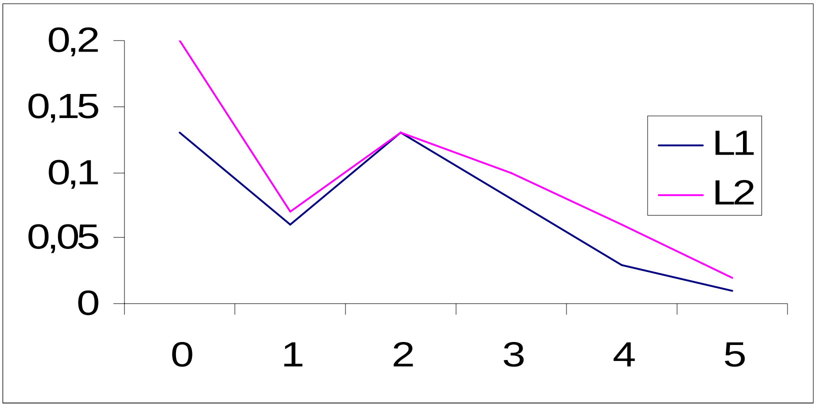


Figure 3.19. PO4 concentrations (mg/l) in the natural ecosystem during the spring experiment.

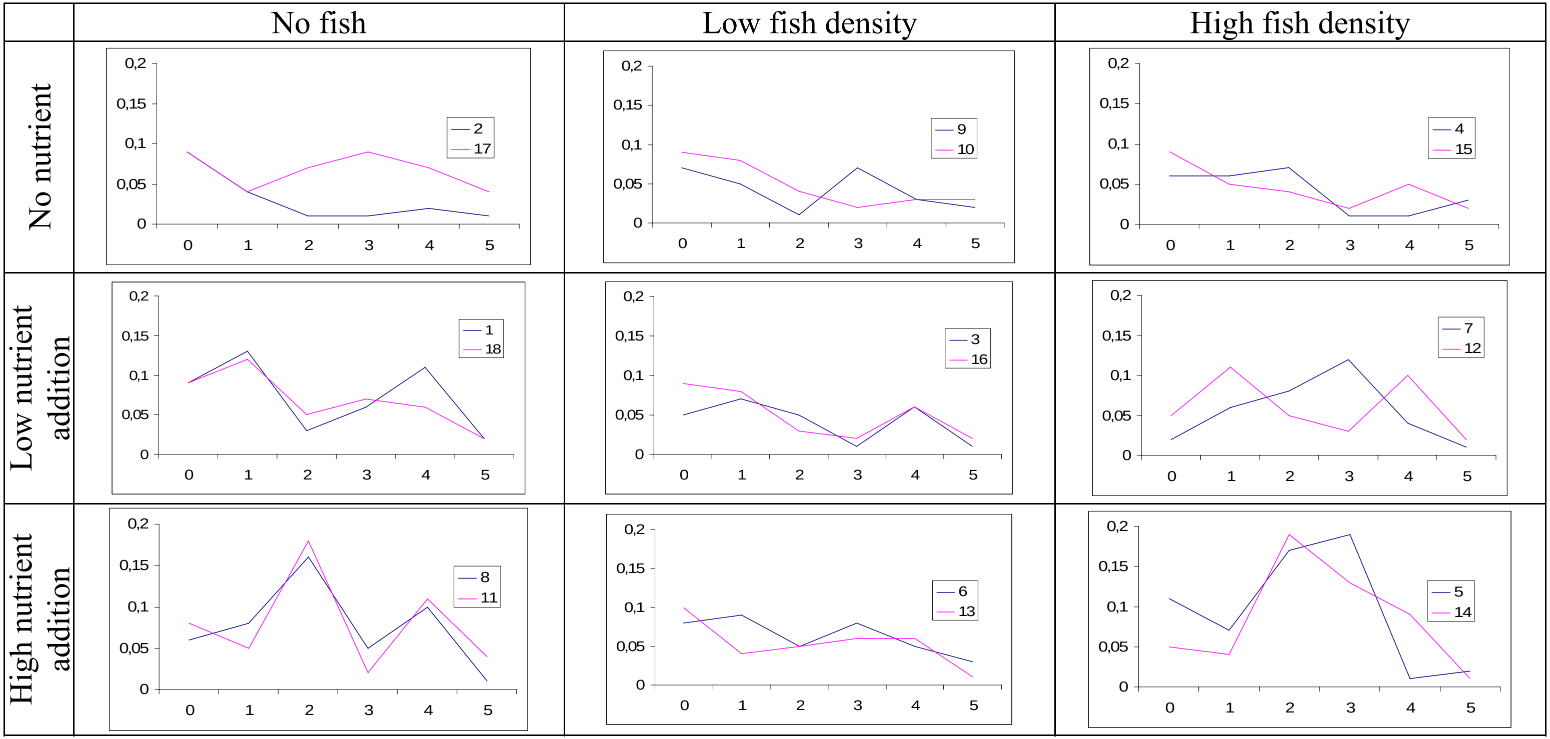


Figure 3.20. PO4 concentrations (mg/l) at different nutrient additions and fish densities in the 18 mesocosms during the spring experiment.

	N0	N1	N2	F0	F1	F2	L
Temperature [°C]	24.0±0.2	24.0±0.2	24.0±0.2	24.0±0.2	24.0±0.2	24.0±0.2	24.2±0.4
pH [Units]	9.38±0.04	9.46±0.03	9.86±0.06	9.56±0.05	9.56±0.07	9.58±0.06	8.9±0.2
DO% [Sat]	112±3	117±2	134±2	123±3	120±3	119±3	121±14
DO [mg/l]	9.4±0.2	9.9±0.2	11.2±0.2	10.4±0.2	10.1±0.3	10.1±0.2	10±1
ORP [mV]	124±2	123±2	109±2	120±3	118±3	117±3	140±6
SpCond [µS/cm]	627±2	630±2	630±3	628±3	630±3	629±3	607±13
Turbidity [NTU]	8±1	12±1	11±1	10±1	8±1	12±1	54±5
N-NO2 (mg/l)	0.011±0.002	0.011±0.002	0.010±0.002	0.010±0.002	0.011±0.002	0.011±0.002	0.004±0.001
N-NO3 (mg/l)	2.0±0.1	2.1±0.1	2.3±0.1	2.2±0.1	2.1±0.1	2.1±0.1	2.2±0.4
N-NH3 (mg/l)	0.17±0.02	0.18±0.02	0.19±0.02	0.16±0.01	0.19±0.02	0.18±0.02	0.32±0.05
PO4 (mg/l)	0.04±0.00	0.06±0.01	0.07±0.01	0.06±0.01	0.04±0.00	0.06±0.01	0.07±0.01

Table 3.1. Mean values ± SE for variables measured under different nutrient additions and fish densities in the 18 mesocosms during the spring experiment (*n* = 30 for mesocosms and *n* = 10 for lake, time 0 values were not considered; N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

3.1.2 Winter experiment

In the natural ecosystem temperature ranged from 7.61 to 13.95 °C (**Figure 3.21**). Temperature ranged from 7.65 to 14.01 °C in the mesocosms (**Figure 3.22**). Both in the mesocosms and natural ecosystem temperature reached its minimum value in the third week and the maximum value in the last week.

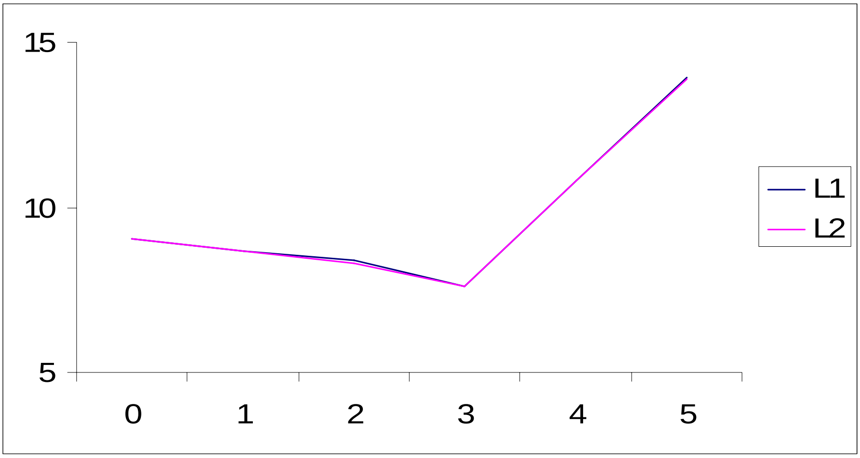


Figure 3.21. Temperature values (°C) in the natural ecosystem during the winter experiment.

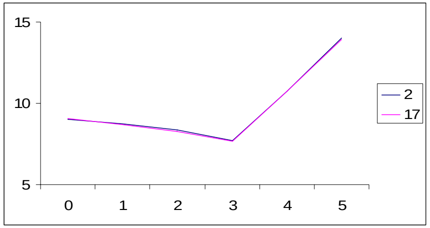
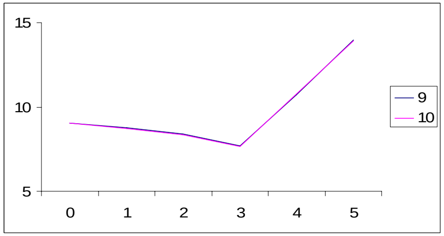
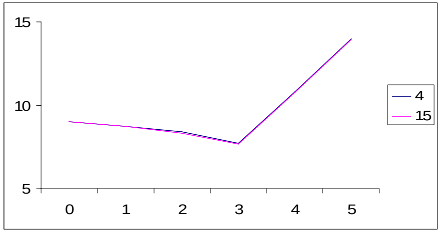
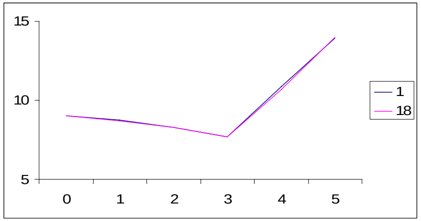
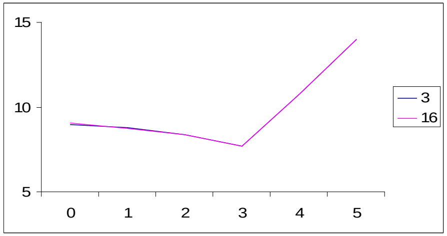
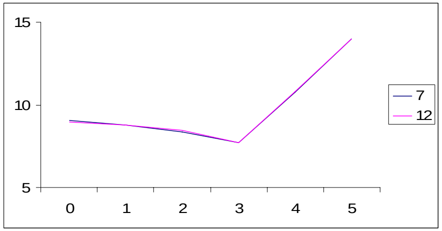
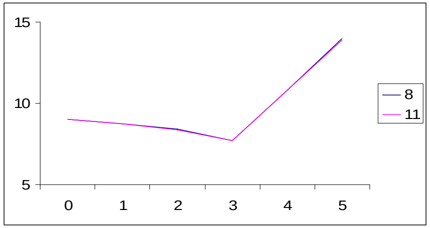
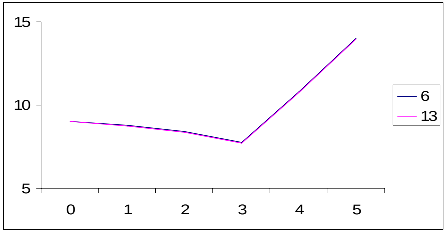
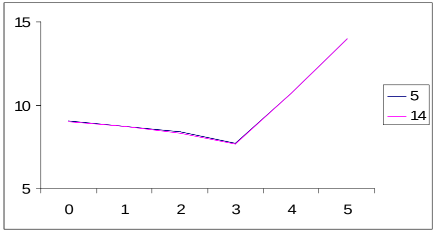
	No fish	Low fish density	High fish density
No nutrient			
Low nutrient addition			
High nutrient addition			

Figure 3.22. Temperature values (°C) at different nutrient additions and fish densities in the 18 mesocosms during the winter experiment.

In the natural ecosystem pH values ranged between 7.87 and 8.95. Maximum value was reached in the first sampling date and the minimum value was reached in the first week (**Figure 3.23**).

The pH values in the mesocosms with no nutrient addition ranged from 8.82 to 9.30 (**Figure 3.24**). In the mesocosms with high nutrient addition pH reached higher values and ranged from 8.89 to 10.21.

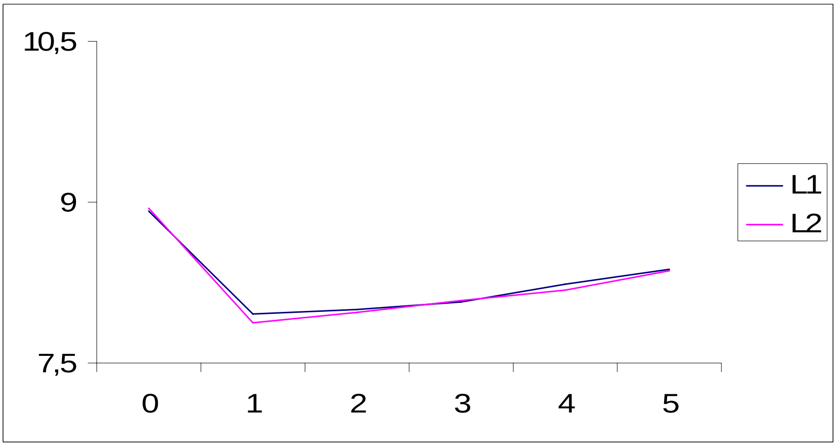


Figure 3.23. pH values in the natural ecosystem during the winter experiment.

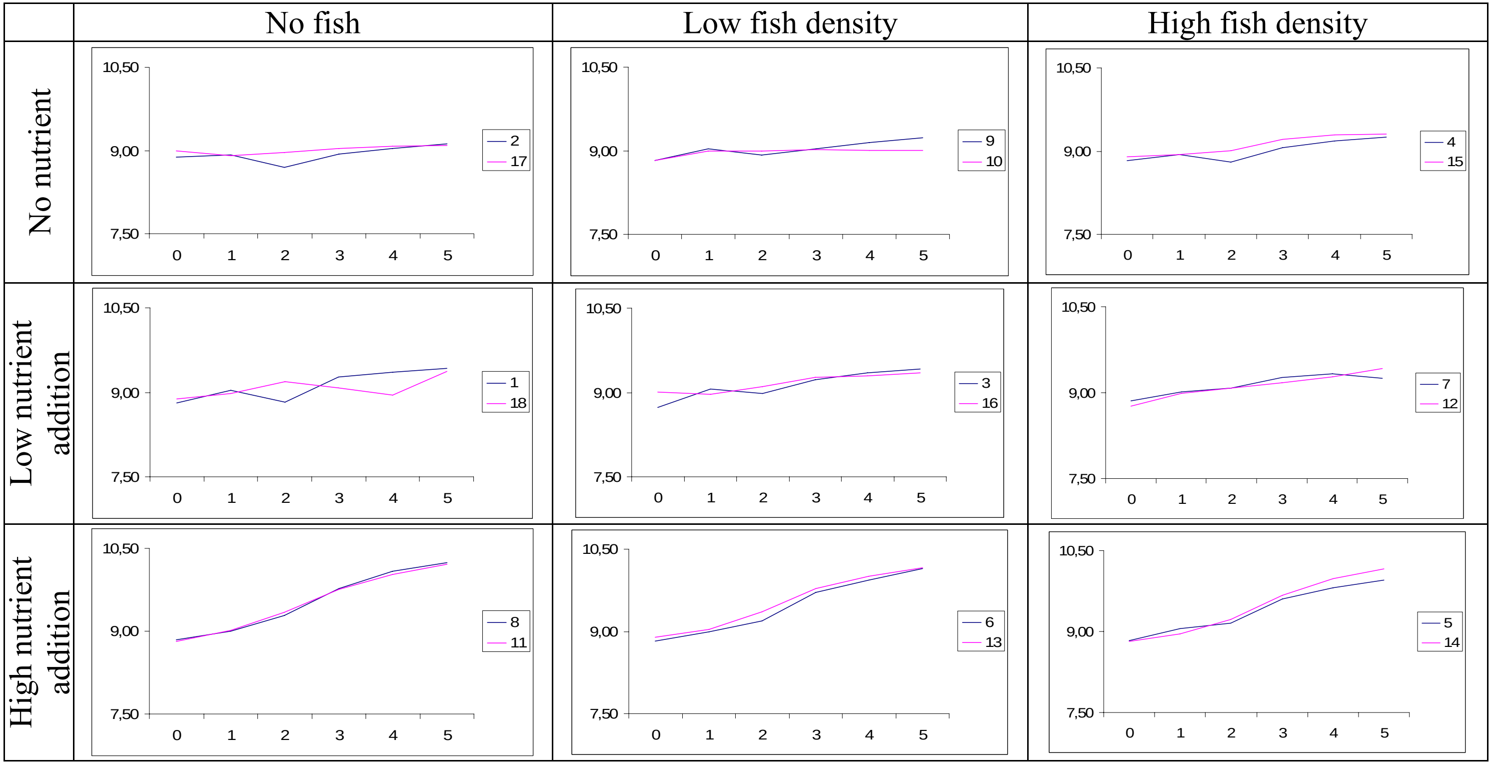


Figure 3.24. pH values at different nutrient additions and fish densities in the 18 mesocosms during the winter experiment.

In natural ecosystem dissolved oxygen percentage ranged from 73 to 104 (**Figure 3.25**).

Dissolved oxygen percentage in the mesocosms was lower than the spring experiment and ranged from 77 to 113 (**Figure 3.26**). There are no differences between mesocosms.

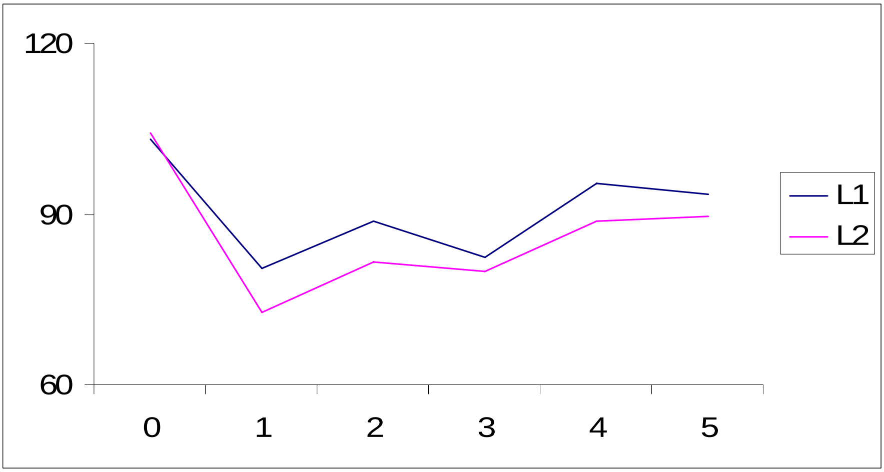


Figure 3.25. Dissolved oxygen percentage (%) in the natural ecosystem during the winter experiment.

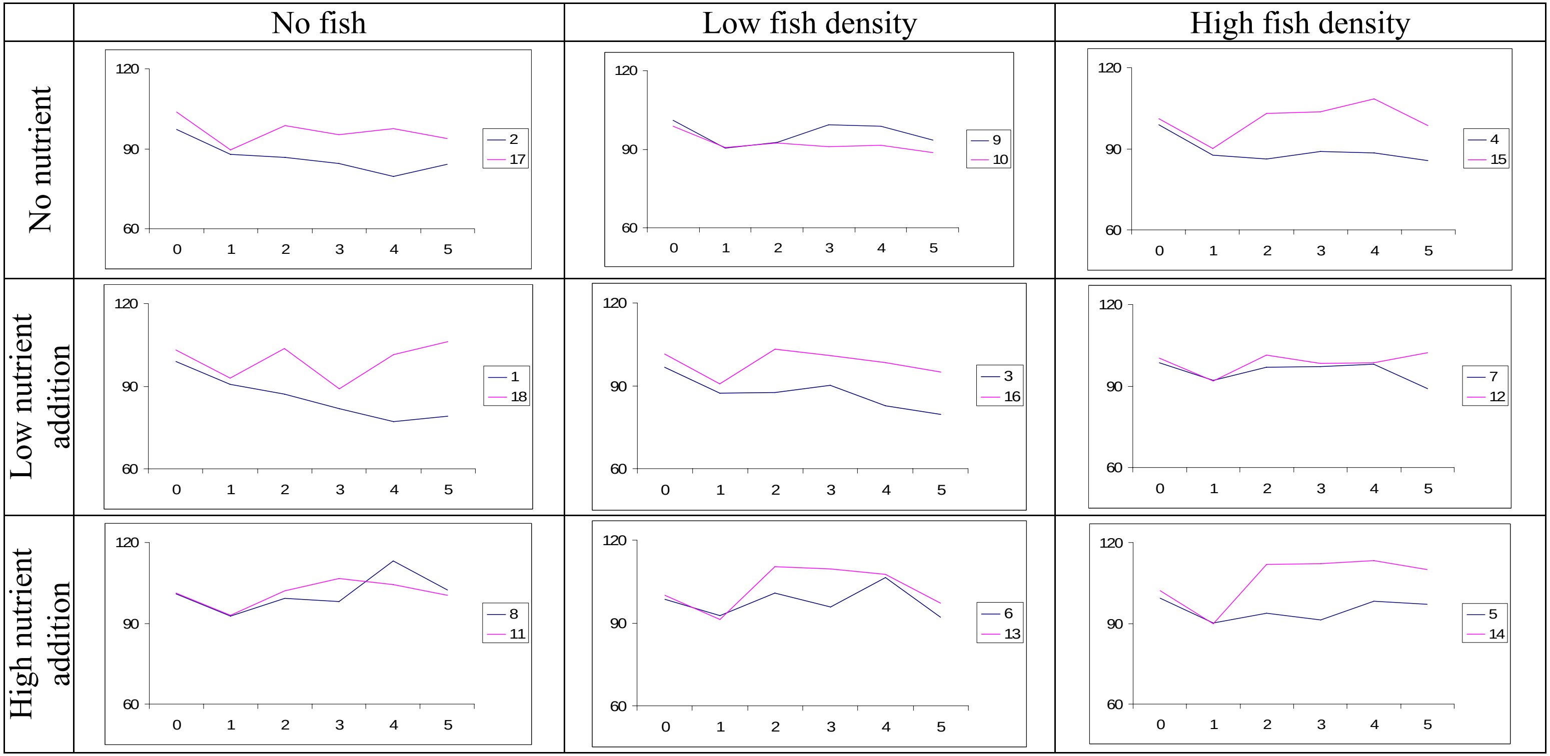


Figure 3.26. Dissolved oxygen percentage (%) at different nutrient additions and fish densities in the 18 mesocosms during the winter experiment.

In natural ecosystem, oxide-reduction potential values ranged from 182 to 257 mV (**Figure 3.27**). They reached their maximum values in the third week.

In the mesocosms oxide-reduction potential values were much higher than spring experiment and ranged from 161 mV to 252 mV (**Figure 3.28**).

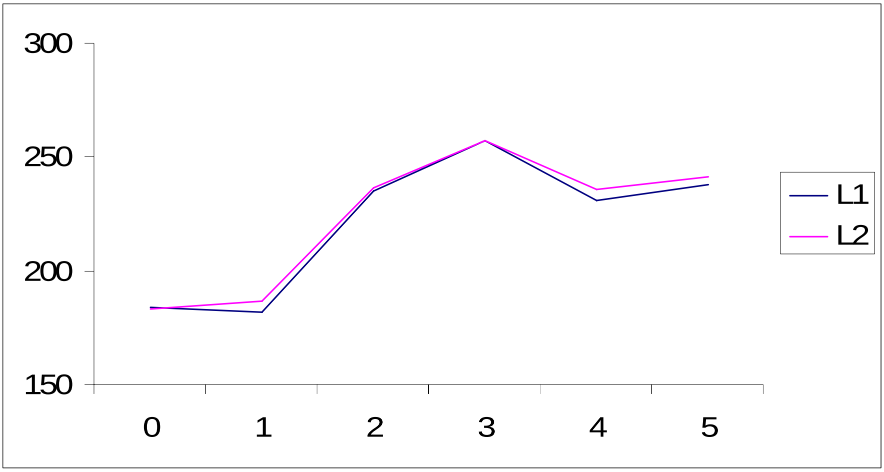


Figure 3.27. Oxide-reduction potential values (mV) in the 18 mesocosms during the winter experiment.

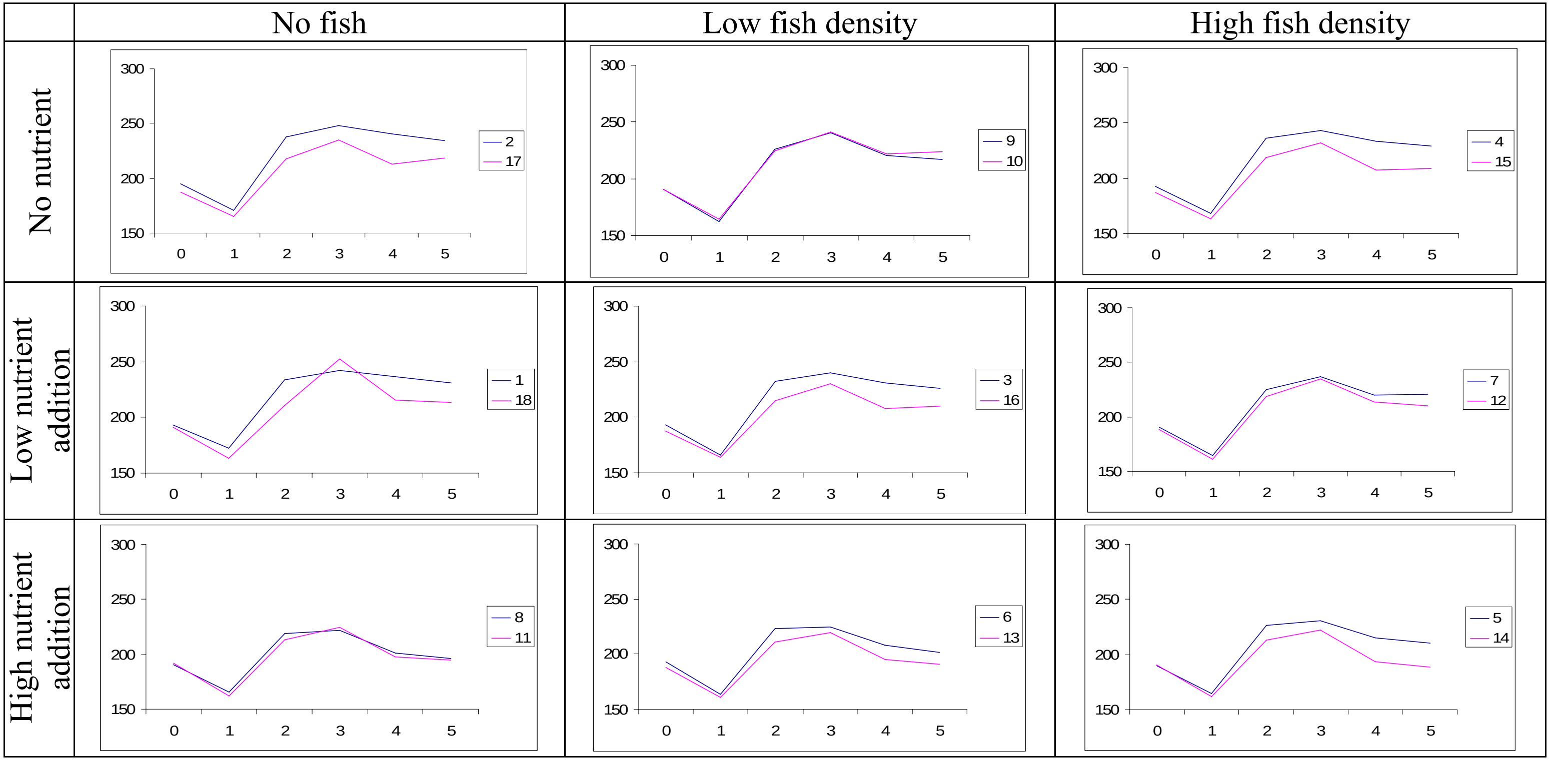


Figure 3.28. Oxide-reduction potential values (mV) at different nutrient additions and fish densities in the 18 mesocosms during the winter experiment.

In natural ecosystem specific conductivity values ranged from 631 to 684 µS/cm, and they gradually decreased during the experiment (**Figure 3.29**).

Specific conductivity values decreased in all the mesocosms from 687 to 527 µS/cm (**Figure 3.30**). In the spring experiment specific conductivity values decreased in the first week and then they increased until the last week.

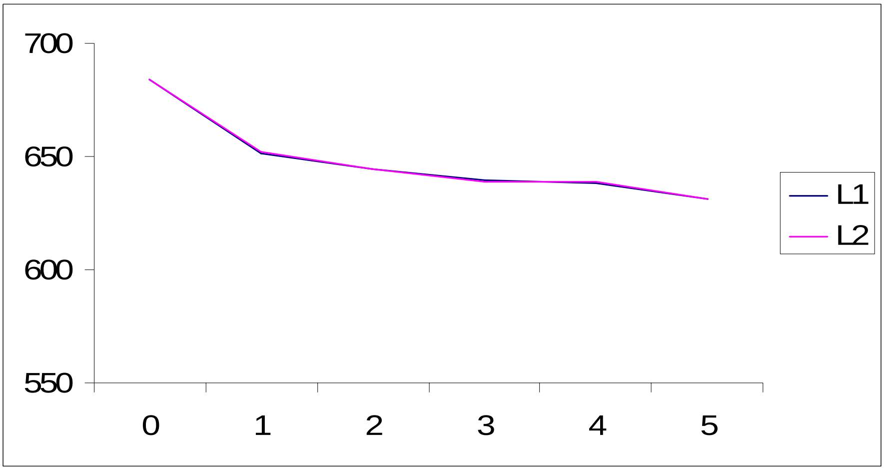


Figure 3.29. Specific conductivity values (µS/cm) in the natural ecosystem during the winter experiment.

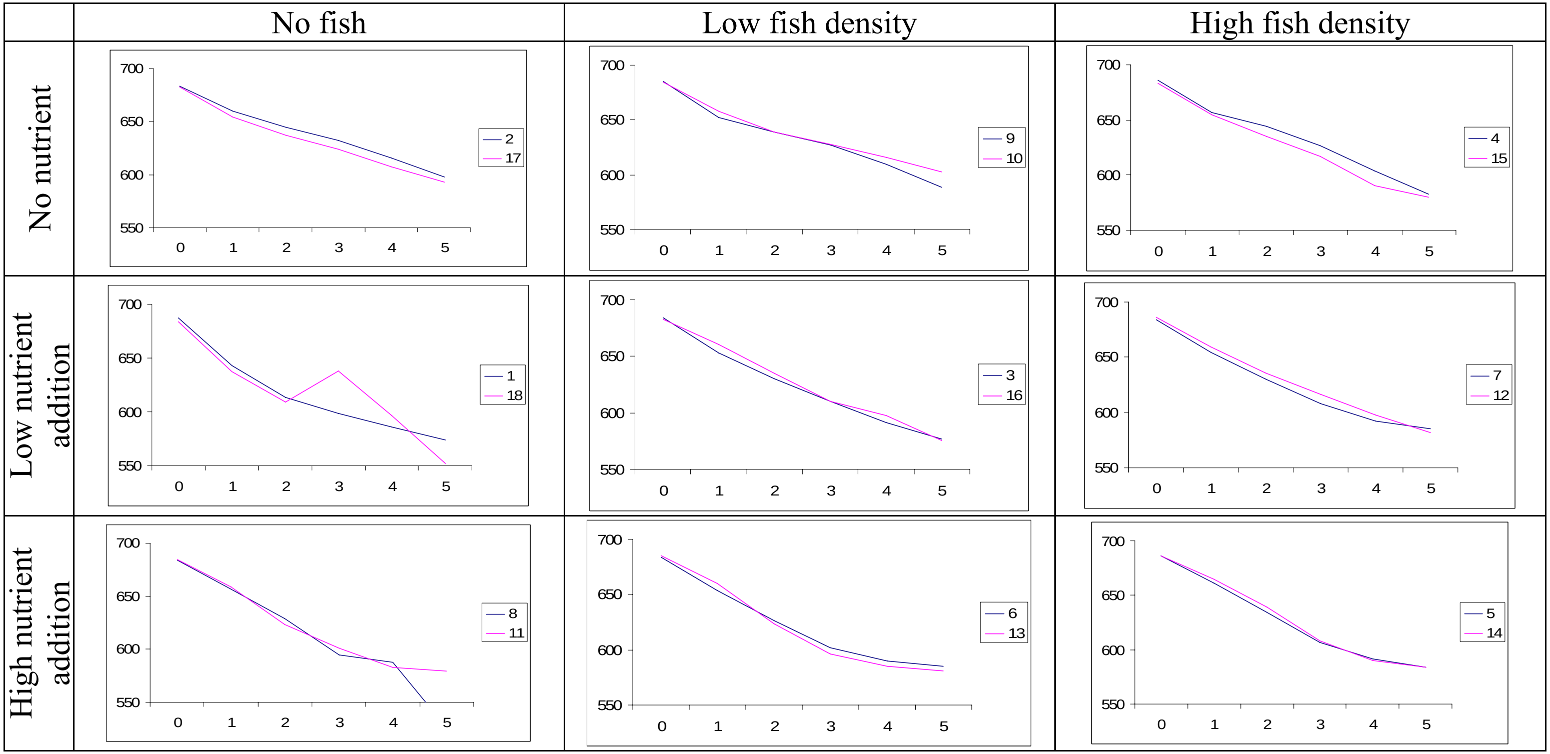


Figure 3.30. Specific conductivity values (µS/cm) at different nutrient additions and fish densities in the 18 mesocosms during the winter experiment.

In natural ecosystem turbidity values ranged from 11 to 25 NTU (**Figure 3.31**).

Turbidity values in all the mesocosms ranged from 3 to 47 NTU (**Figure 3.32**).

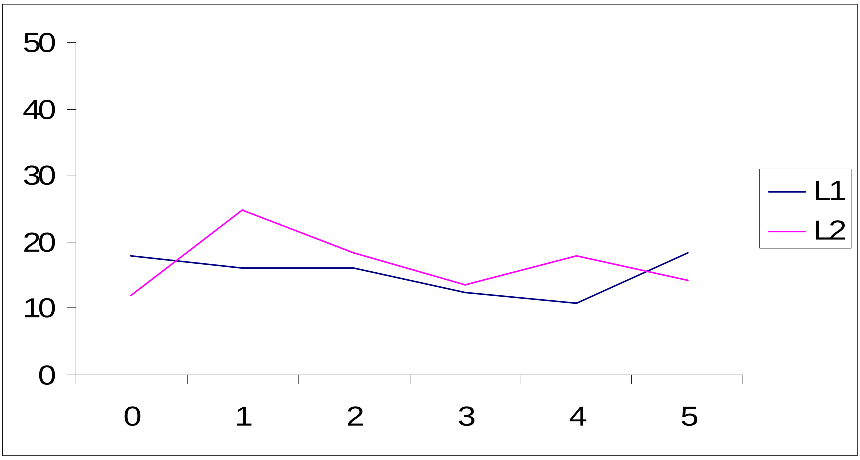


Figure 3.31. Turbidity values (NTU) in the natural ecosystem during the winter experiment.

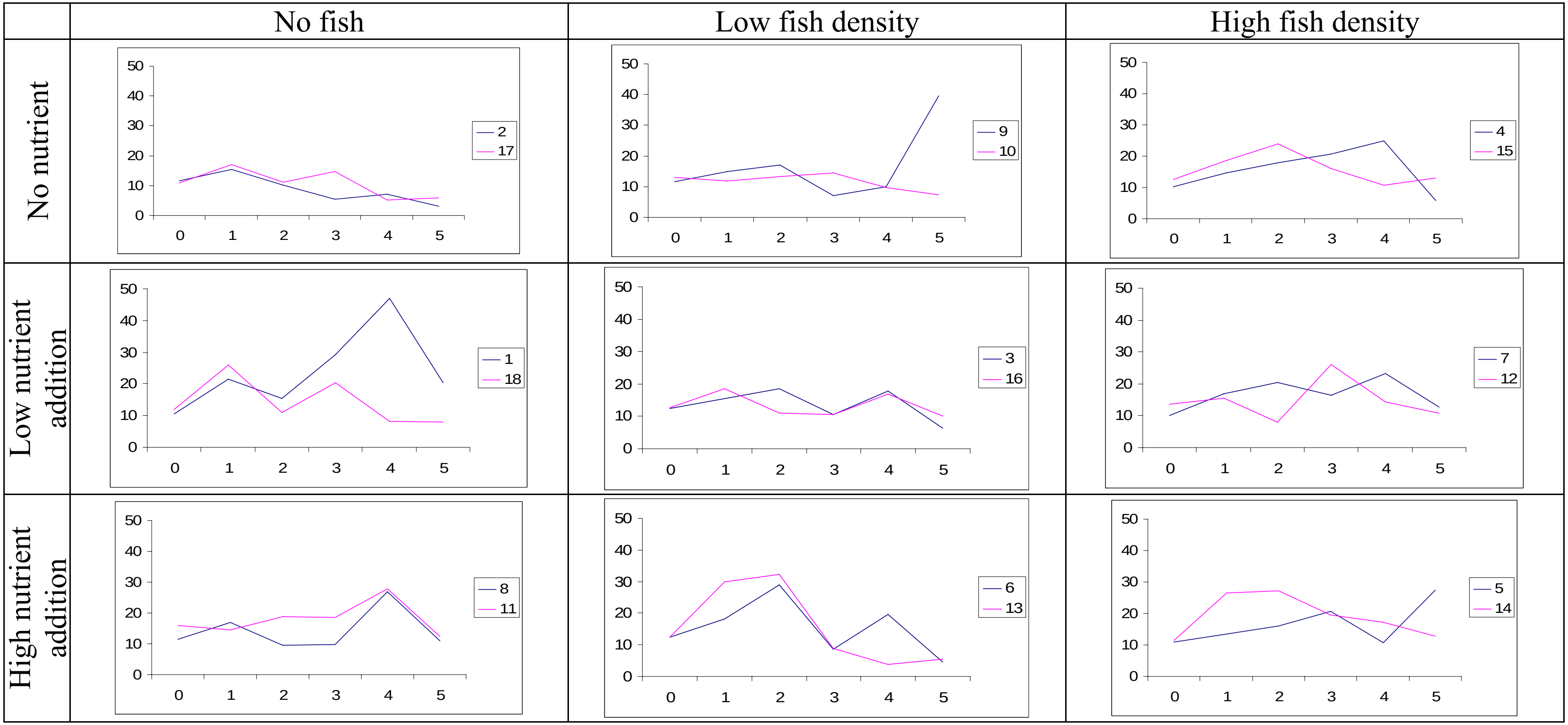


Figure 3.32. Turbidity values (NTU) at different nutrient additions and fish densities in the 18 mesocosms during the winter experiment.

In the natural ecosystem nitrogen nitrite concentrations ranged from 0.005 to 0.013 mg/l (**Figure 3.33**). Minimum value was reached in the second week.

Nitrogen nitrite concentrations in all the mesocosms ranged from 0.006 to 0.021 mg/l (**Figure 3.34**).

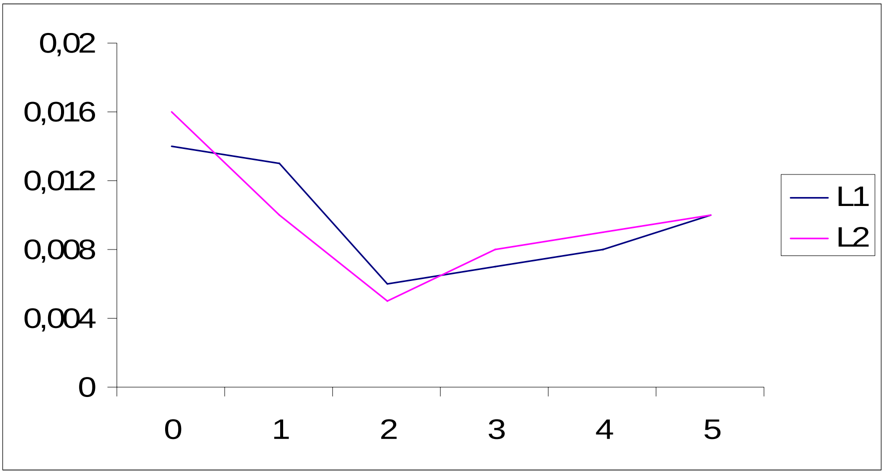


Figure 3.33. N-NO2 concentrations (mg/l) in the natural ecosystem during the winter experiment.

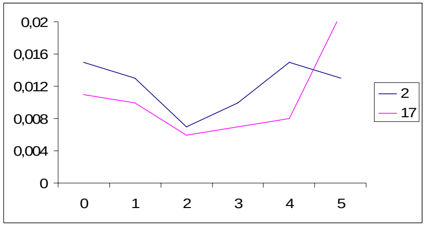
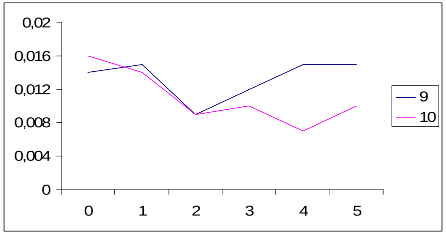
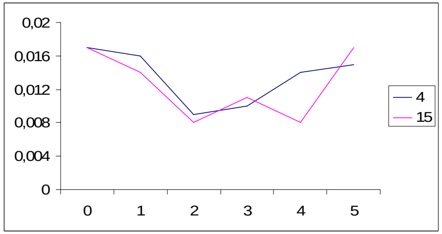
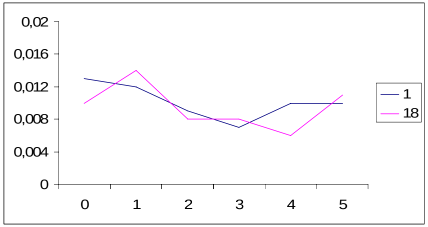
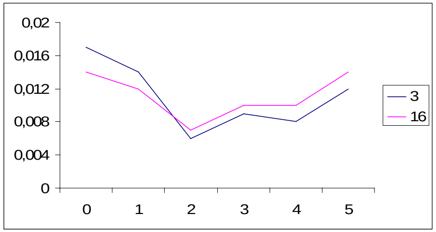
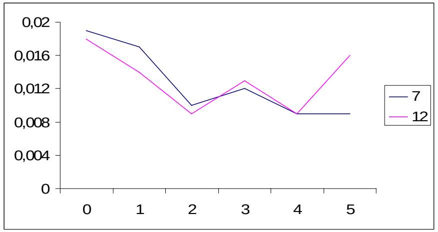
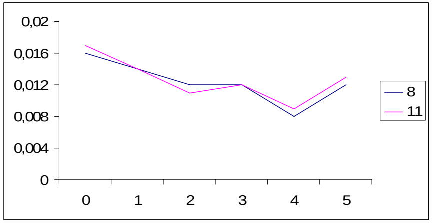
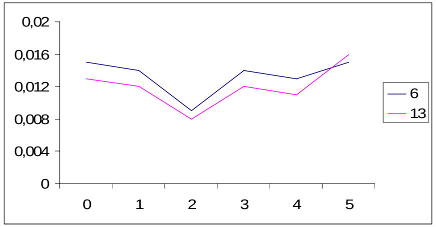
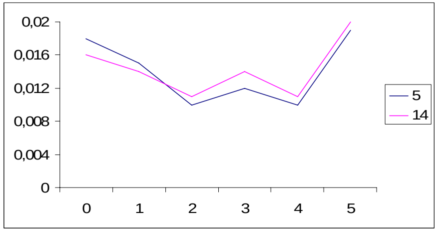
	No fish	Low fish density	High fish density
No nutrient			
Low nutrient addition			
High nutrient addition			

Figure 3.34. N-NO2 concentrations (mg/l) at different nutrient additions and fish densities in the 18 mesocosms during the winter experiment.

In natural ecosystem, nitrogen nitrate concentrations ranged from 1.20 to 2.80 mg/l (**Figure 3.35**). Minimum value was reached in the second week.

Nitrogen nitrate concentrations in all the mesocosms ranged from 1.90 to 3.60 mg/l (**Figure 3.36**) and they were higher than concentrations in spring experiment.

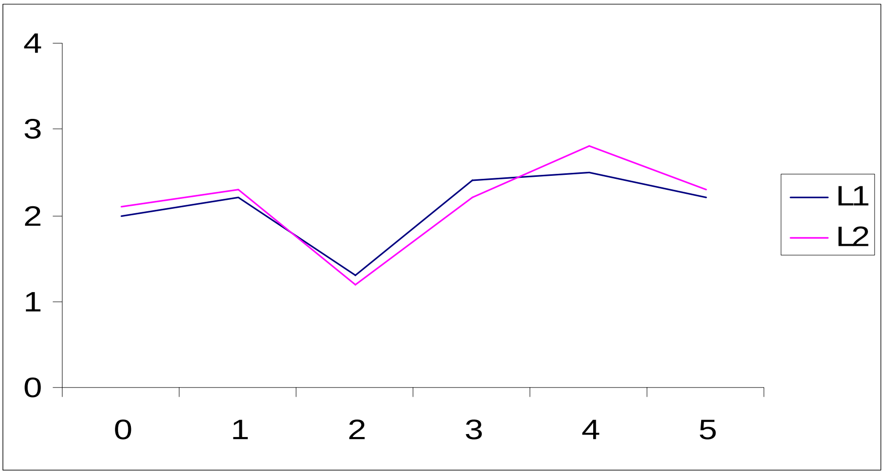


Figure 3.35. N-NO3 concentrations (mg/l) in the natural ecosystem during the winter experiment.

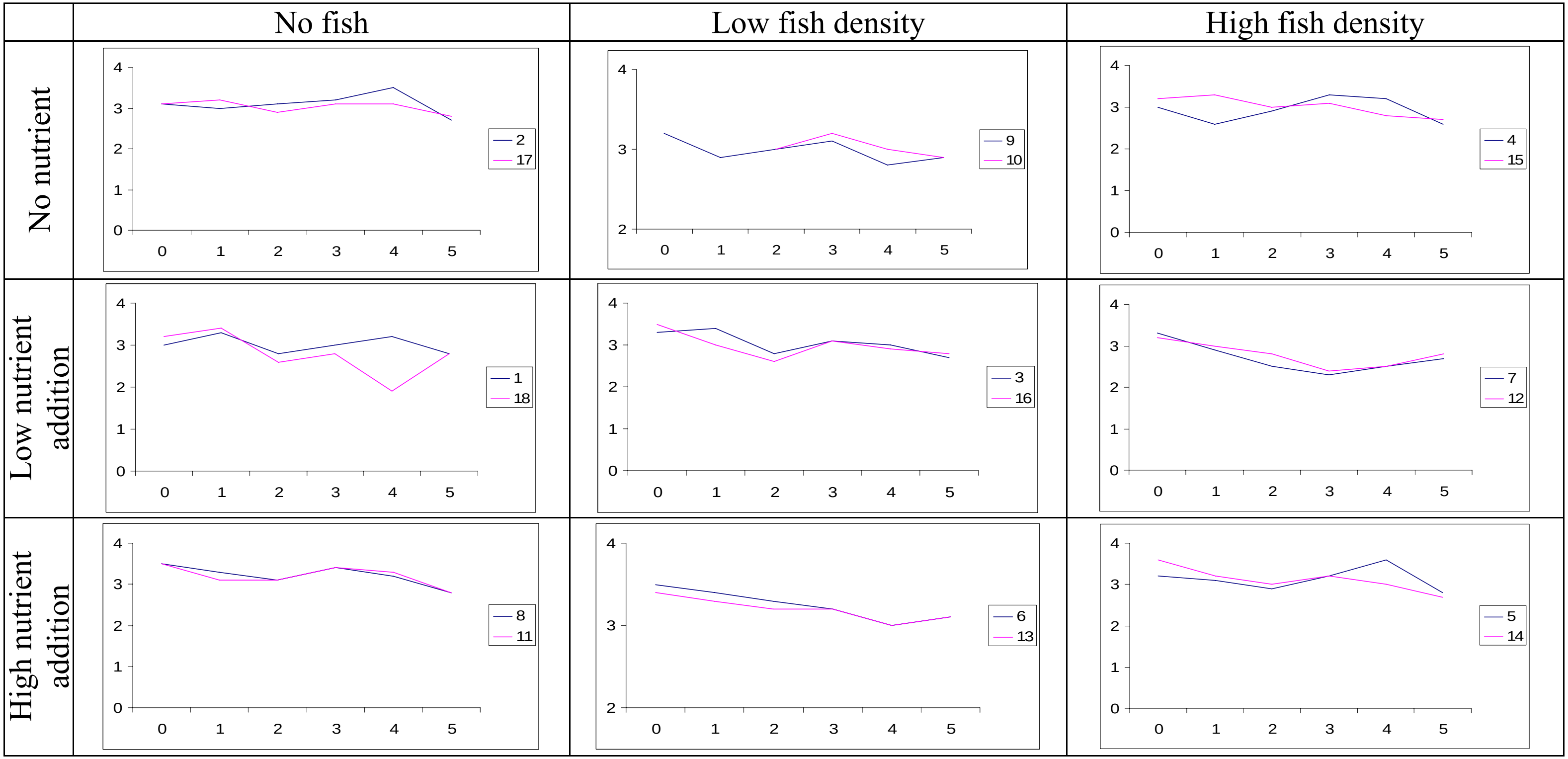


Figure 3.36. N-NO3 concentrations (mg/l) at different nutrient additions and fish densities in the 18 mesocosms during the winter experiment.

In natural ecosystem nitrogen ammonia concentrations ranged from 0.13 to 0.45 mg/l (**Figure 3.37**).

Nitrogen ammonia concentration in the mesocosms ranged from 0.09 to 0.61 mg/l (**Figure 3.38**).

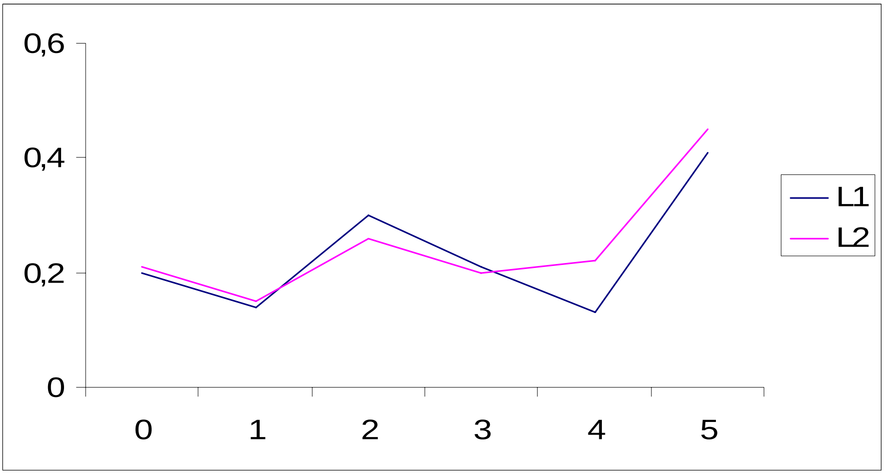


Figure 3.37. N-NH3 concentrations (mg/l) in the natural ecosystem during the winter experiment.

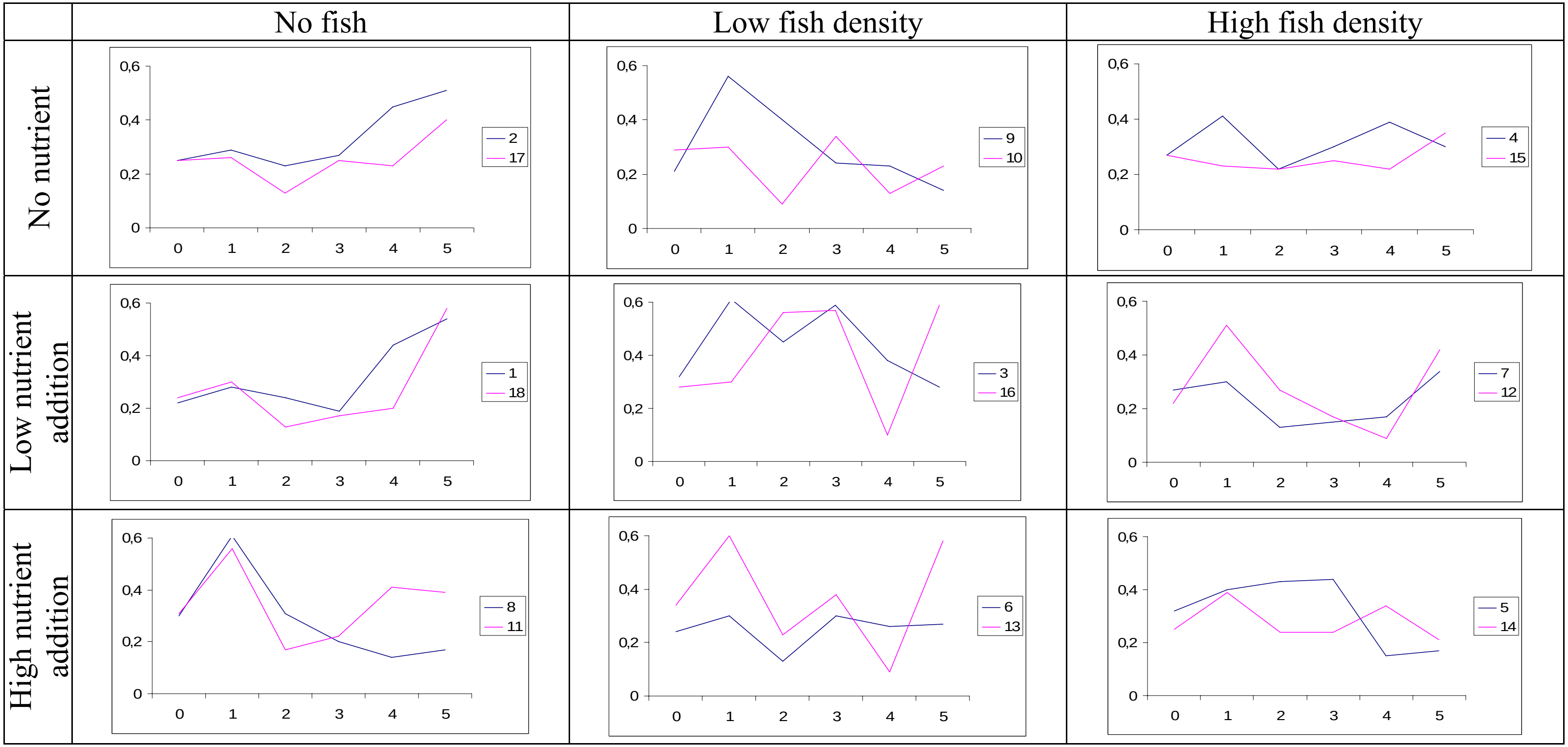


Figure 3.38. N-NH3 concentrations (mg/l) at different nutrient additions and fish densities in the 18 mesocosms during the winter experiment.

In natural ecosystem orthophosphate concentrations ranged from 0.06 to 0.22 mg/l (**Figure 3.39**).

Orthophosphate concentrations in all the mesocosms ranged from 0.02 to 0.38 mg/l (**Figure 3.40**). Both in natural ecosystem and mesocosms orthophosphate concentrations were higher than concentrations in spring experiment.

Table 3.2 summarizes all physical and chemical variables considered at nutrient and fish different treatment. Values are reported as mean ± SE.

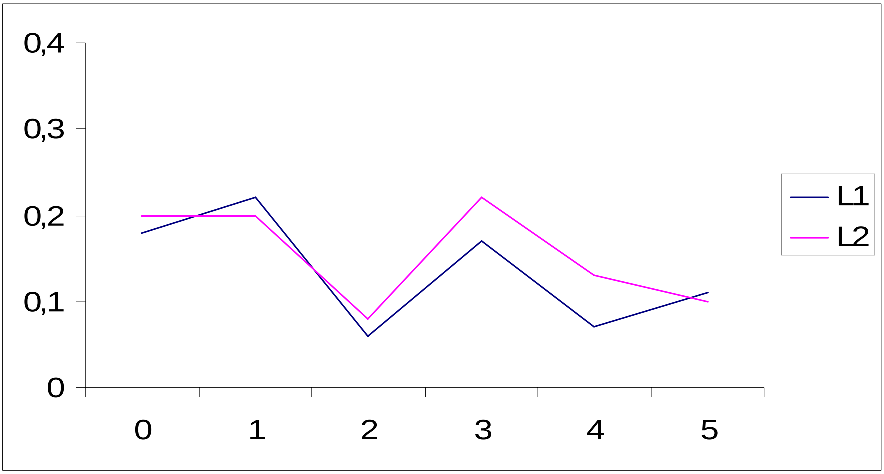


Figure 3.39. PO4 concentrations (mg/l) in the natural ecosystem during the winter experiment.

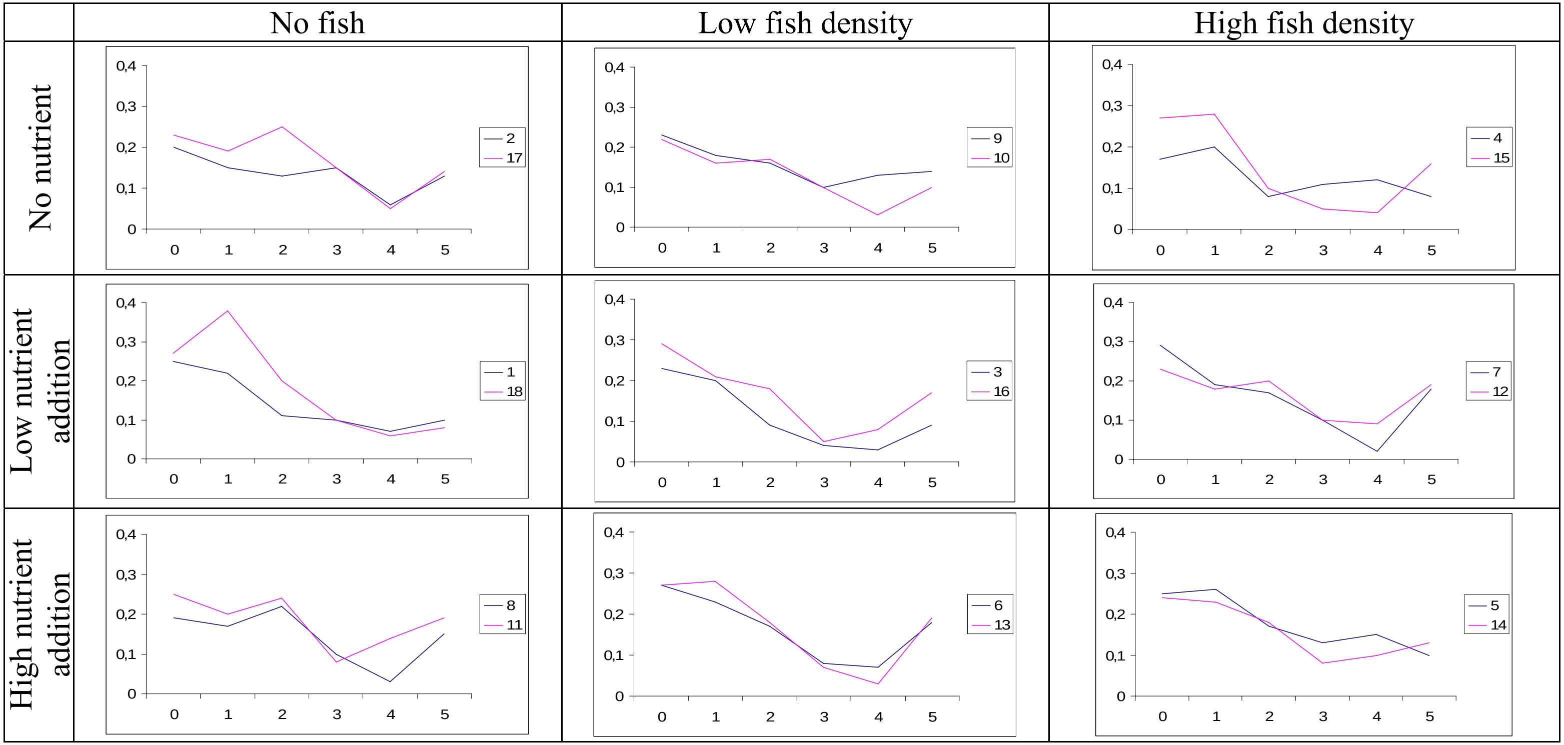


Figure 3.40. PO4 concentrations (mg/l) at different nutrient additions and fish densities in the 18 mesocosms during the winter experiment.

	N0	N1	N2	F0	F1	F2	L
Temperature [°C]	9.9±0.4	9.9±0.4	9.9±0.4	9.9±0.4	9.9±0.4	9.9±0.4	9.9±0.8
pH [Units]	9.04±0.03	9.18±0.03	9.62±0.08	9.27±0.08	9.29±0.07	9.28±0.06	8.11±0.05
DO% [Sat]	92±1	93±1	101±1	94±2	95±1	97±1	85±2
DO [mg/l]	10.4±0.2	10.5±0.2	11.4±0.2	10.7±0.2	10.7±0.2	11.0±0.2	9.6±0.2
ORP [mV]	215±5	213±5	201±4	212±5	209±5	209±5	230±8
SpCond [µS/cm]	624±4	612±5	610±6	612±6	617±5	617±5	641±2
Turbidity [NTU]	14±1	17±1	17±1	16±2	15±2	17±1	17±1
N-NO2 (mg/l)	0.012±0.001	0.011±0.001	0.013±0.001	0.011±0.001	0.011±0.001	0.013±0.001	0.009±0.001
N-NO3 (mg/l)	3.00±0.04	2.81±0.06	3.13±0.04	3.02±0.06	3.04±0.04	2.89±0.06	2.1±0.2
N-NH3 (mg/l)	0.29±0.02	0.34±0.03	0.31±0.03	0.31±0.03	0.34±0.03	0.28±0.02	0.27±0.04
PO4 (mg/l)	0.13±0.01	0.13±0.01	0.15±0.01	0.14±0.01	0.13±0.01	0.14±0.01	0.14±0.02

Table 3.2. Mean values ± SE for variables measured under different nutrient additions and fish densities in the 18 mesocosms during the winter experiment (*n* = 30 for mesocosms and *n* = 10 for lake, time 0 values were not considered; N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

3.2 Phytoplankton

3.2.1 Spring experiment

Chlorophyll a concentrations in all the mesocosms ranged from 8.41 to 270.91 μg/l, while in the natural ecosystem it ranged from 20.49 to 113.95 μg/l (**Figure 3.41**).

Mean values of chlorophyll a were high in the mesocosms with the highest fish density (F2) during the experiment (**Figure 3.42**).

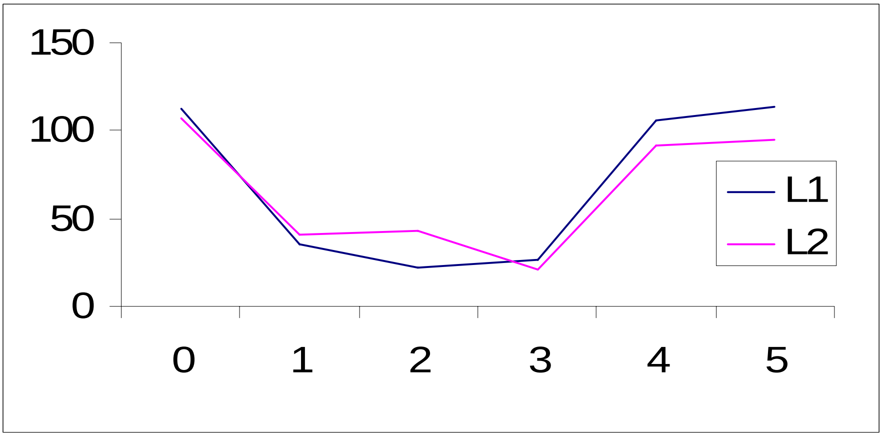


Figure 3.41. Chlorophyll a concentrations (μg/l) in the natural ecosystem during the spring experiment.

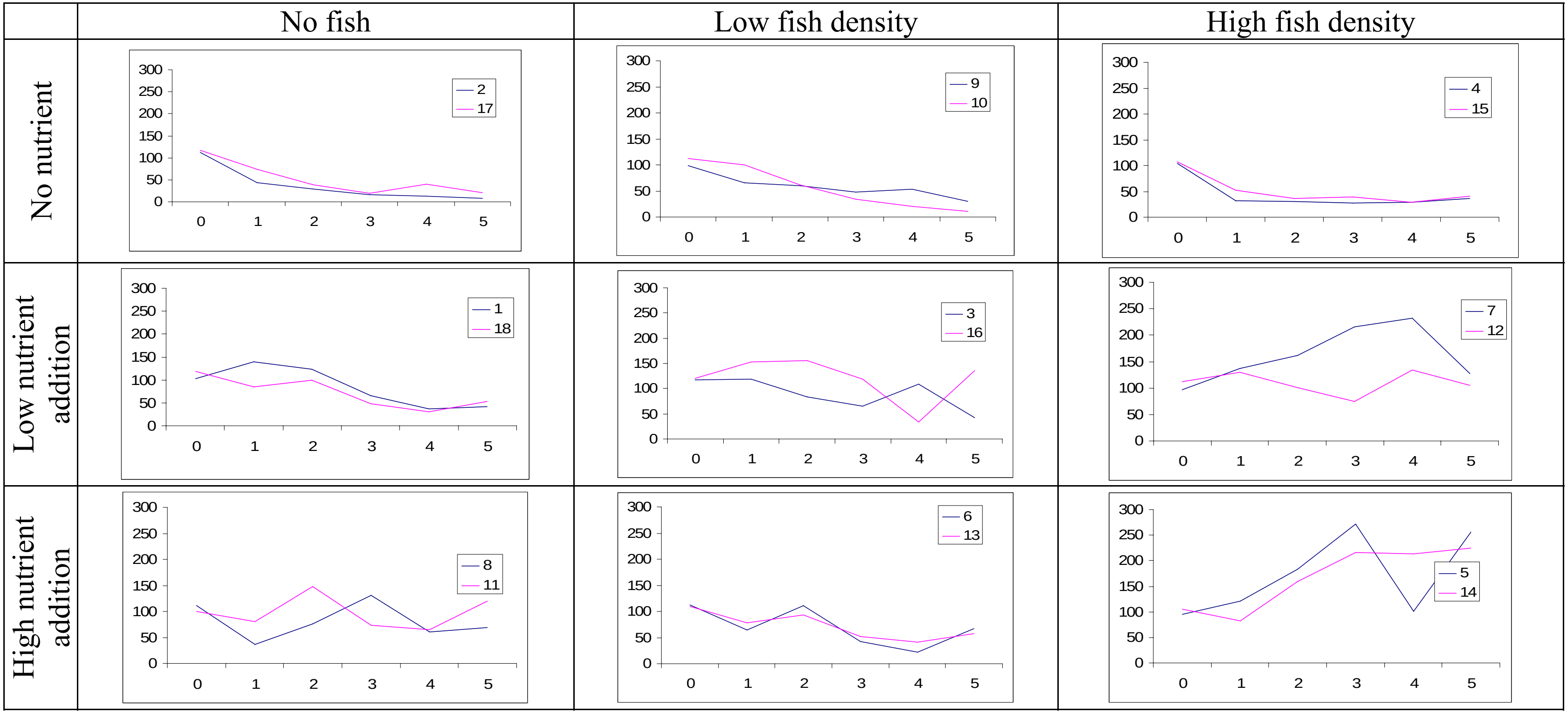


Figure 3.42. Chlorophyll a concentrations (μg/l) at different nutrient additions and fish densities in the 18 mesocosms during the spring experiment.

Chlorophyll a concentration was high in mesocosms with high fish density (F2) throughout the experiment (**Figure 3.43**).

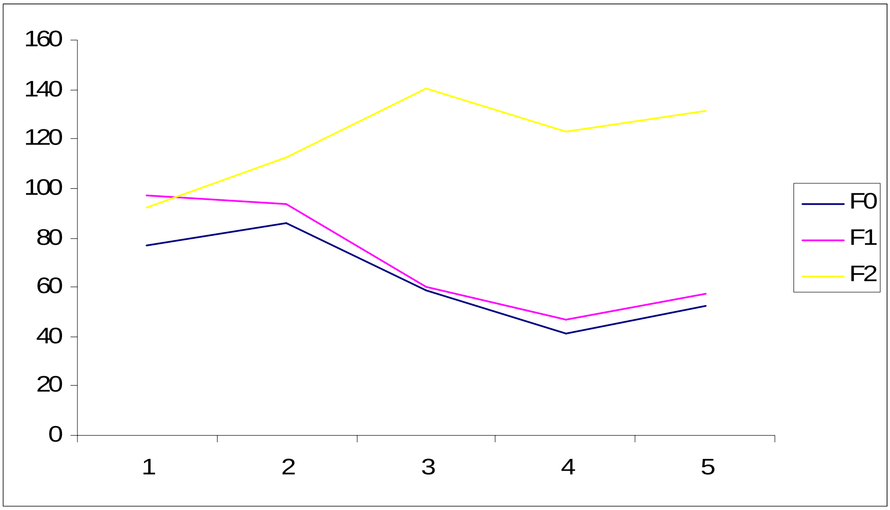


Figure 3.43. Mean values of chlorophyll a concentrations (μg/l) in the 18 mesocosms in different fish densities (F0, F1 and F2) during the spring experiment (F0 = no fish addition, F1 = low fish density, F2 = high fish density).

Chlorophyll a concentrations in the mesocosms increased with increasing nutrient addition (*n*=30, F=1.234, p=0.575, t=-6.756, p<0.001, data log transformed) and increasing fish density (*n*=30, F=1.169, p=0.676, t=-3.111, p<0.01, data log transformed) (**Figure 3.44**).

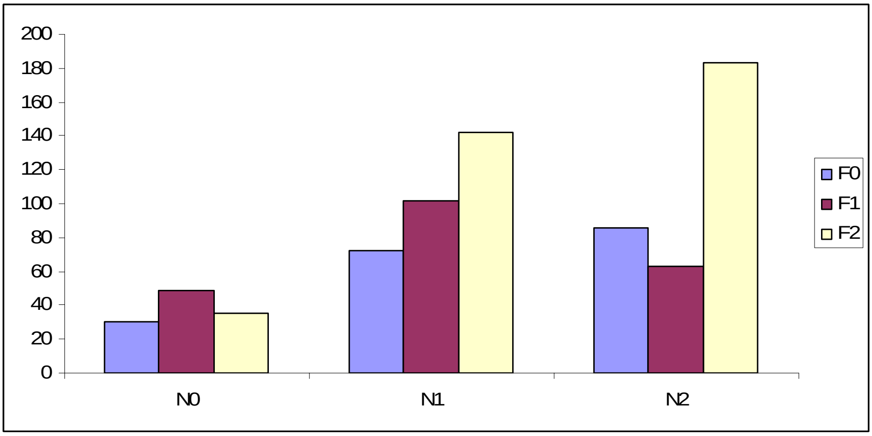


Figure 3.44. Mean values of chlorophyll a concentrations (µg/l) in the 18 mesocosms at different nutrient additions (N0, N1 and N2) and fish densities (F0, F1 and F2) during the spring experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

Chlorophyll a concentrations increased of 66% from no nutrient addition (N0) to high nutrient addition (N2) and it increased of 48% from no fish (F0) to high fish density (F2) (**Table 3.3**).

	N0	N1	N2	F0	F1	F2	L
Chlorophyll a [µg/l]	38±4	105±9	111±13	63±7	71±7	120±14	60±12

Table 3.3. Mean values ± SE of chlorophyll a concentrations under different nutrient additions and fish densities in the 18 mesocosms during the spring experiment (*n* = 30 for mesocosms and *n* = 10 for lake, time 0 values were not considered; N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

3.2.2 *Winter experiment*

Chlorophyll a concentrations in all the mesocosms ranged from 3.95 to 42.40, while in the natural ecosystem they ranged from 36.29 to 89.87 μg/l (**Figure 3.45**).

Mean values of chlorophyll a were high in the mesocosms with the highest fish density (F2) during the experiment (**Figure 3.46**).

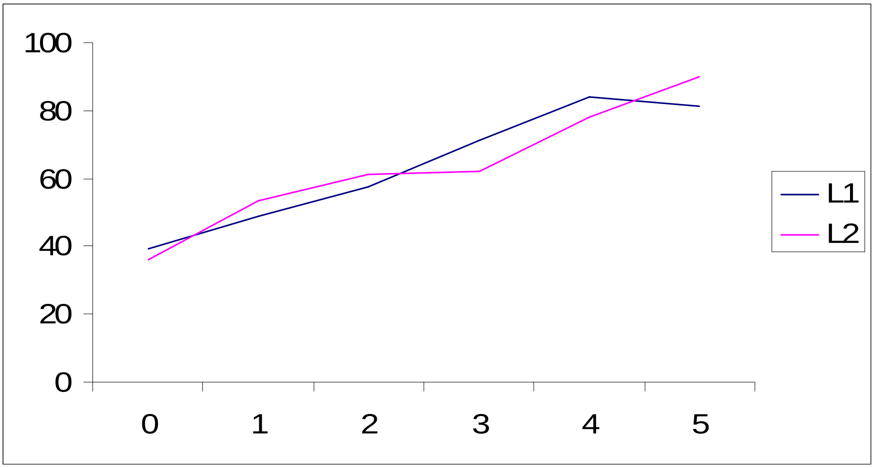


Figure 3.45. Chlorophyll a concentrations (μg/l) in the natural ecosystem during the winter experiment.

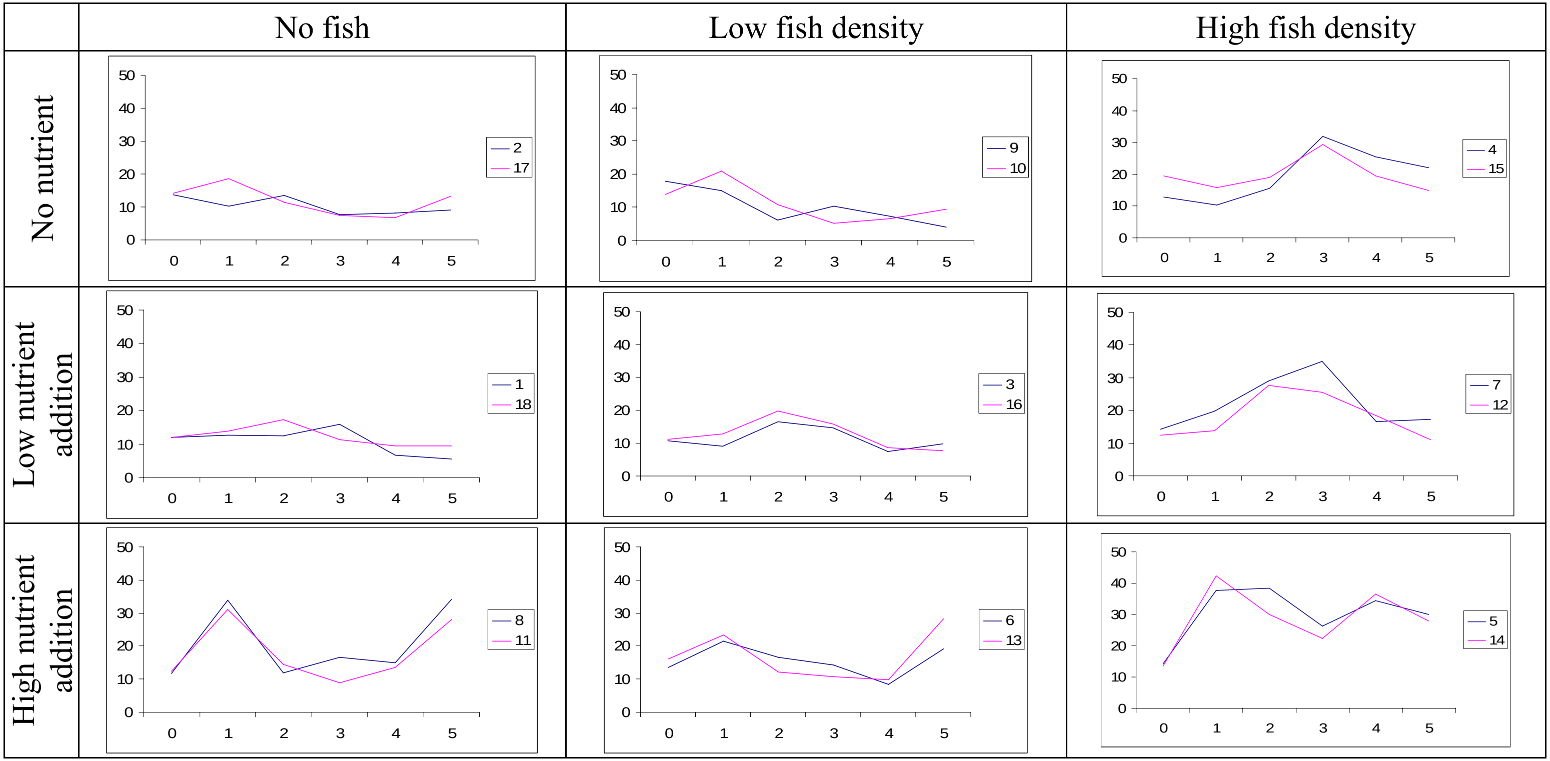


Figure 3.46. Chlorophyll a concentrations (µg/l) at different nutrient additions and fish densities in the 18 mesocosms during the winter experiment.

Chlorophyll a concentration was high in mesocosms with high fish density (F2) throughout the experiment (**Figure 3.47**).

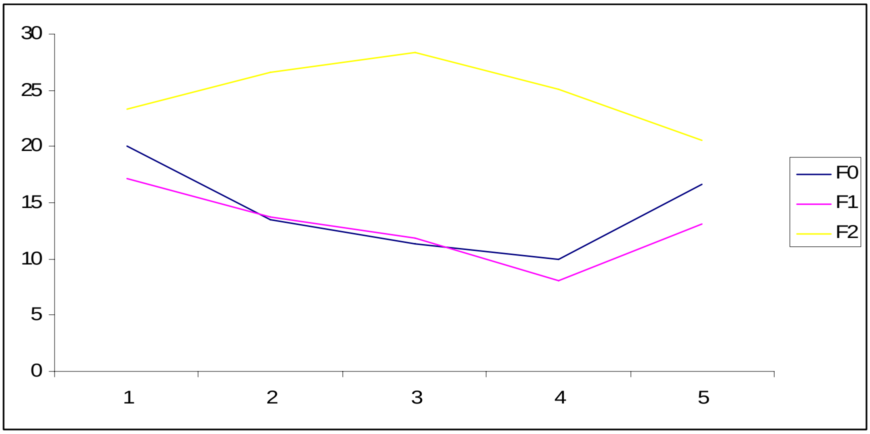


Figure 3.47. Mean values of chlorophyll a concentrations (μg/l) in the 18 mesocosms in different fish densities (F0, F1 and F2) during the winter experiment (F0 = no fish, F1 = low fish density, F2 = high fish density).

Chlorophyll a concentrations in the mesocosms increased with increasing nutrient addition (n=30, F=1.163, p=0.687, t=-4.354, p<0.001, data log transformed) and increasing fish density (n=30, F=1.581, p=0.223, t=-5.461, p<0.001, data log transformed) (**Figure 3.48**).

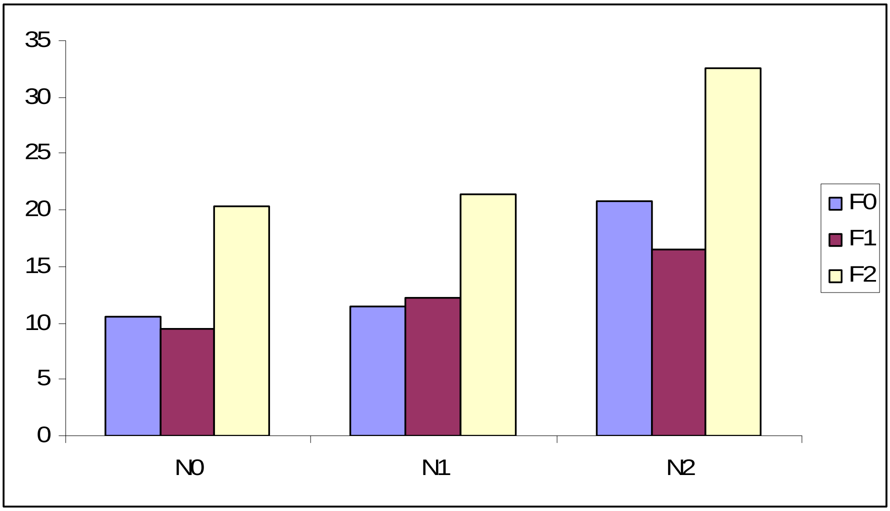


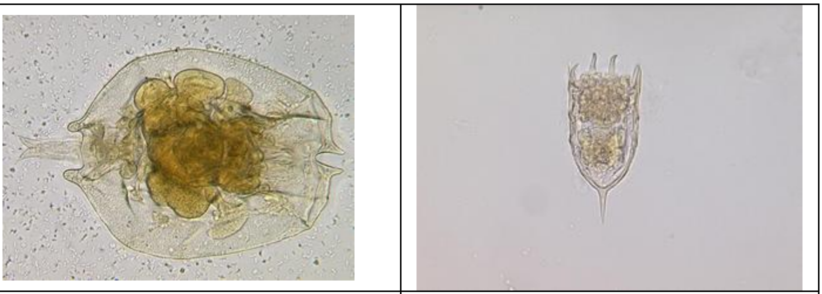
Figure 3.48. Mean values of chlorophyll a concentrations (µg/l) in the 18 mesocosms in different nutrient concentrations (N0, N1 and N2) and fish density (F0, F1 and F2) during the winter experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

Chlorophyll a concentrations increased of 39% from no nutrient addition (N0) to high nutrient addition (N2) and it increased of 44% from no fish (F0) to high fish density (F2) (**Table 3.4**).

	N0	N1	N2	F0	F1	F2	L
Chlorophyll a [µg/l]	14±1	15±1	23±2	14±1	13±1	25±2	69±4

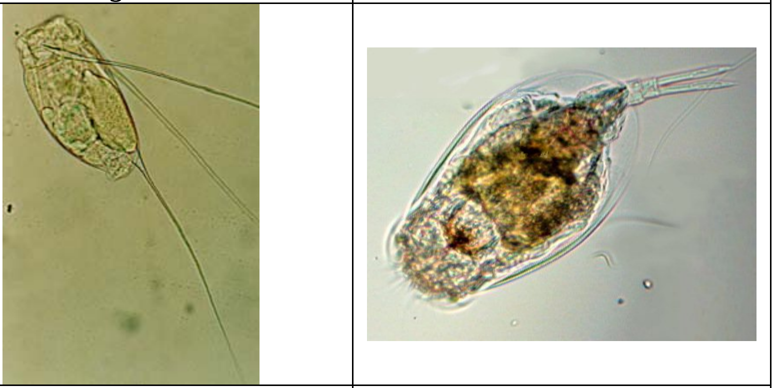
Table 3.4. Mean values ± SE of chlorophyll a concentrations under different nutrient additions and fish densities in the 18 mesocosms during the winter experiment (*n* = 30 for mesocosms and *n* = 10 for lake, time 0 values were not considered; N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

3.3 Zooplankton



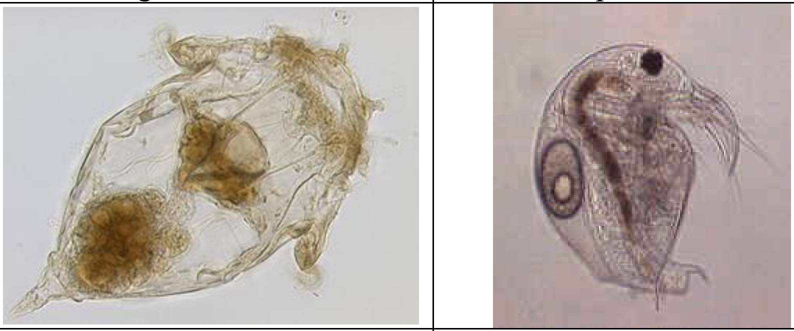
Brachionus angularis

Keratella cochlearis



Filinia longiseta

Euchlanis sp.



Synchaeta sp.

Bosmina longirostris

Figure 3.49. Some specimens of zooplankton species observed in the mesocosms during the two experiments.

3.3.1 Spring experiment

The mean zooplankton density in all the mesocosms in the pretreatment week was 1066 ± 400 org/l. Rotifers (84.6%) was the dominant group in terms of density. Nauplii were 9%, Copepods 6.1% and cladocerans 0.3% (**Figure 3.50**).

Among rotifers *Brachionus angularis* was the main species, with a mean density of 831 org/l. *Keratella cochlearis* reached 59.4 org/l. *Polyarthra remata* reached 7.4 org/l. *Trichocerca* sp. and *Synchaeta* sp. reached 3.3 and 0.28 org/l respectively.

In the natural ecosystem mean zooplankton density was 386 ± 34 org/l. Rotifers were the dominant group with a percentage of 87.4%. Nauplii were 6.5%, copepods 5.5% and cladocerans 0.6% (**Figure 3.51**).

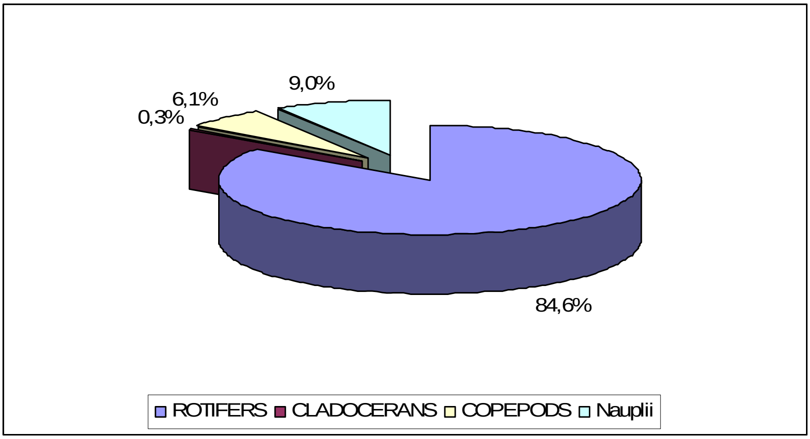


Figure 3.50. Zooplankton composition in the pretreatment week in all the mesocosms during the spring experiment.

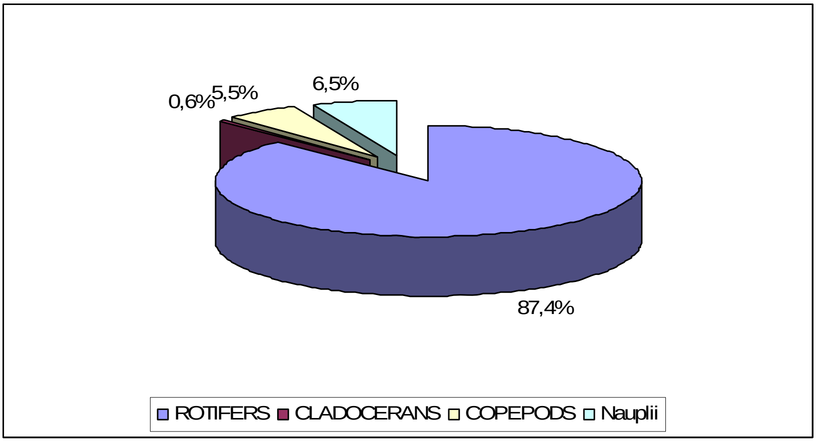
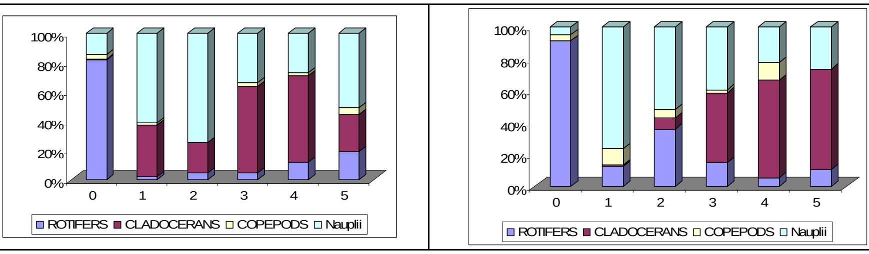


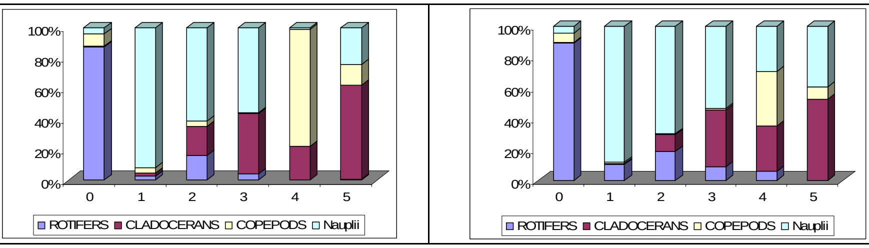
Figure 3. 51. Zooplankton composition in the pretreatment week in the natural ecosystem during the spring experiment.

Zooplankton community structure changed during the experiment. Rotifers decreased and the other groups increased their percentage. In particular nauplii increased their density from the first week. In mesocosms with high fish density rotifers in the last week became the principal group (**Figure 3.52**).

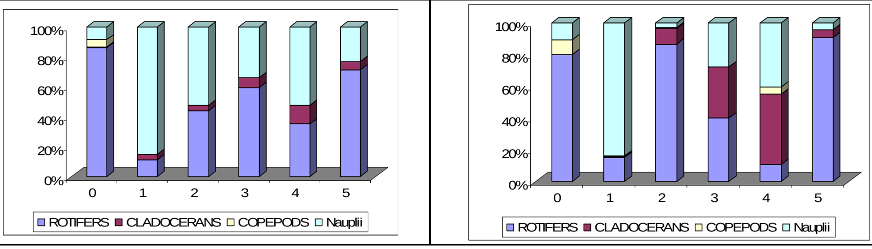
In the natural ecosystem cladocerans became the principal group (**Figure 3.53**).



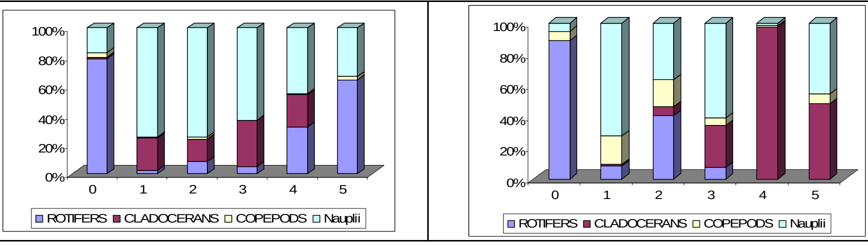
No nutrient and no fish



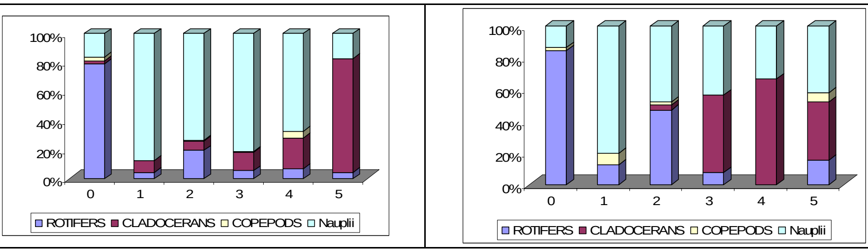
No nutrient and low fish density



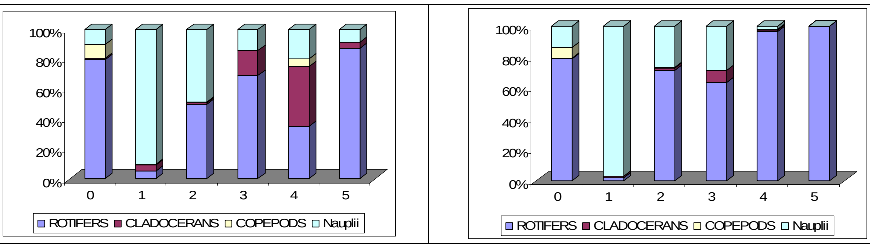
No nutrient and high fish density



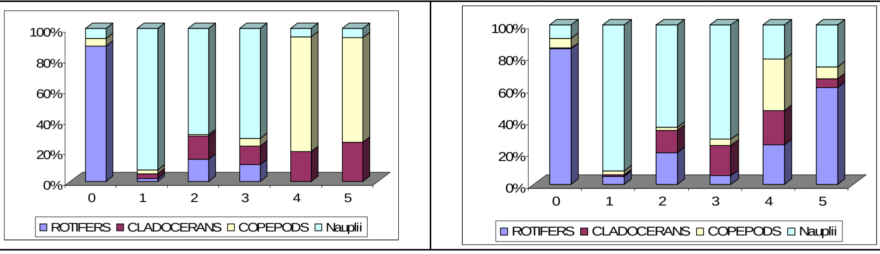
Low nutrient addition and no fish



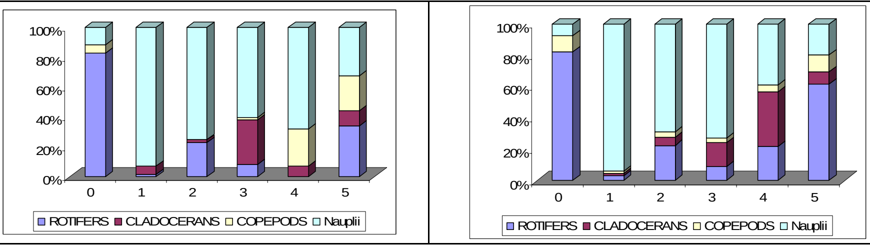
Low nutrient addition and low fish density



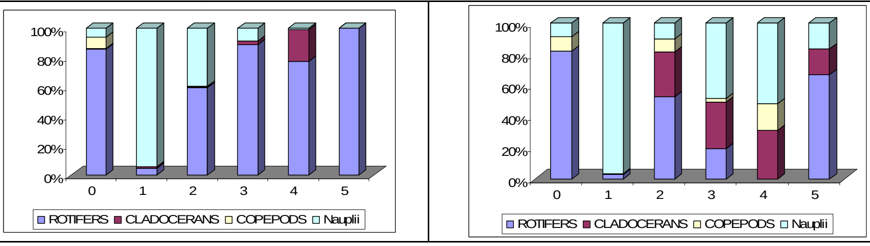
Low nutrient addition and high fish density



High nutrient addition and no fish

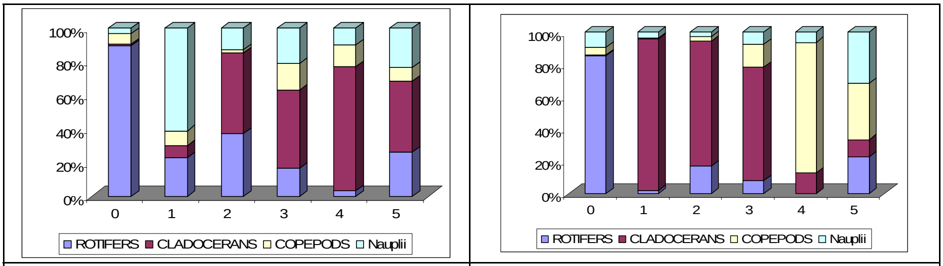


High nutrient addition and low fish density



High nutrient addition and high fish density

Figure 3.52. Percentage density of the main zooplankton groups at different nutrient additions and fish densities in the 18 mesocosms during the spring experiment.



L1 L2

Figure 3.53. Percentage density of the main zooplankton groups in the natural ecosystem during the spring experiment.

Zooplankton community was composed by different species (**Table 3.5**). Some species were observed almost in all the mesocosms such as *Brachionus angularis*, *Euchlanis* sp., *Keratella cochlearis*, *Polyarthra remata*, *Trichocerca* sp., *Bosmina longirostris*, *Cyclops* sp. Some other species were never observed in the mesocosms during the spring experiment such as *Brachionus calyciflorus*, *Filinia longiseta*, *Daphnia magna*.

A similar zooplankton composition was observed in the natural ecosystem (**Table 3.6**). *Brachionus angularis*, *Brachionus urceolaris*, *Keratella cochlearis*, *Polyarthra remata*, *Synchaeta* sp., *Trichocerca* sp., *Bosmina longirostris* were the species always observed. *Brachionus calyciflorus*, *Filinia longiseta* and *Daphnia magna* were never observed.

	N_F_	N0F0		N0F1		N0F2		N1F0		N1F1		N1F2		N2F0		N2F1		N2F2	
	Mesocosm	2	17	9	10	4	15	1	18	3	16	7	12	8	11	6	13	5	14
ROTIFERS	*Brachionus angularis*	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Brachionus calyciflorus																		
	Brachionus urceolaris		■	■		■	■		■		■	■	■	■	■	■		■	■
	Euchlanis sp.	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■
	Filinia longiseta																		
	Keratella cochlearis	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Lecane sp.	■					■	■			■	■	■						
	Polyarthra remata		■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■
	Synchaeta sp.					■										■		■	
	Trichocerca sp.	■	■	■	■	■	■	■		■	■	■	■	■	■		■	■	■
CLADOCERANS	*Bosmina longirostris*	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Daphnia magna																		
	Cladoceran	■	■	■	■	■		■		■	■	■		■	■	■	■	■	■
COPEPODS	Calanoid	■	■	■		■	■		■			■				■	■	■	■
	Cyclops sp.	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

Table 3.5. Zooplankton community species in the 18 mesocosms during the spring experiment (N0 = no nutrient, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish, F1 = low fish density, F2 = high fish density).

	Station	L1	L2
ROTIFERS	*Brachionus angularis*	■	■
	Brachionus calyciflorus		
	Brachionus urceolaris	■	■
	Euchlanis sp.	■	
	Filinia longiseta		
	Keratella cochlearis	■	■
	Lecane sp.		■
	Polyarthra remata	■	■
	Synchaeta sp.	■	■
	Trichocerca sp.	■	■
CLADOCERANS	*Bosmina longirostris*	■	■
	Daphnia magna		
	Cladoceran	■	■
COPEPODS	Calanoid	■	
	Cyclops sp.	■	■

Table 3.6. Zooplankton community species in the natural ecosystem during the spring experiment.

Figure 3.54 represents total zooplankton density during the experiment as the average value for the three different fish density in each week. Total zooplankton density decreased during the experiment. Maximum zooplankton density was reached during the first week and it was 2562 org/l in the mesocosms with low fish density (F1). Minimum value was 135 org/l in the mesocosms with low fish density (F1).

Rotifers (**Figure 3.55**) decreased their density in the first week, but in the mesocosms with high fish density in the second week rotifers density increased.

Cladocerans (**Figure 3.56**) increased their density for the first three weeks and then they decreased.

Copepods (**Figure 3.57**) density was between 0 and 98 org/l during the experiment.

Nauplii (**Figure 3.58**) reached their zooplankton maximum density in the first week and they were responsible of the total zooplankton maximum density.

Shannon-Weaver index (**Figure 3.59**) increased during the experiment.

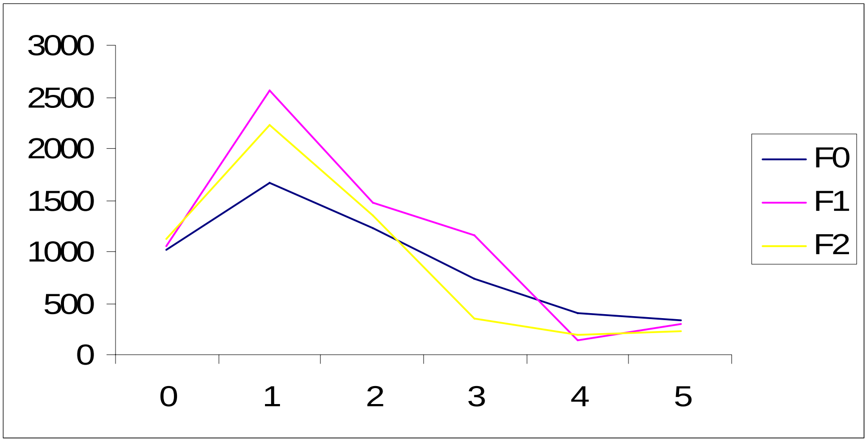


Figure 3.54. Total zooplankton density (org/l) in the 18 mesocosms during the spring experiment in different fish densities (F0 = no fish addition, F1 = low fish density, F2 = high fish density).

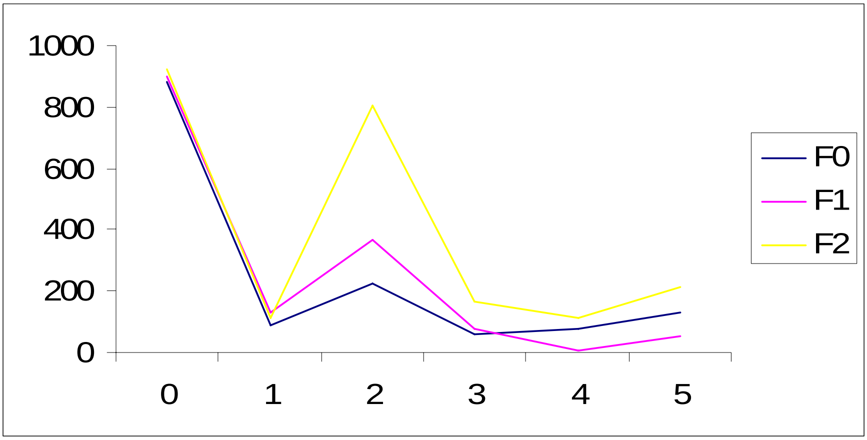


Figure 3.55. Rotifers density (org/l) in the 18 mesocosms during the spring experiment in different fish densities (F0 = no fish addition, F1 = low fish density, F2 = high fish density).

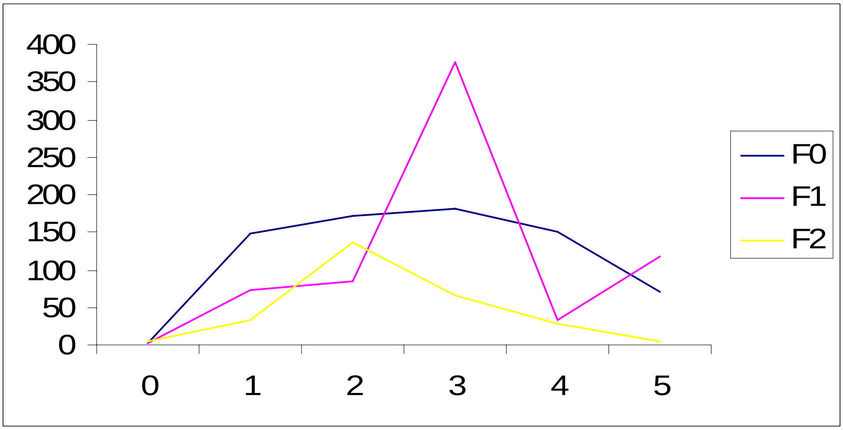


Figure 3.56. Cladocerans density (org/l) in the 18 mesocosms during the spring experiment in different fish densities (F0 = no fish addition, F1 = low fish density, F2 = high fish density).

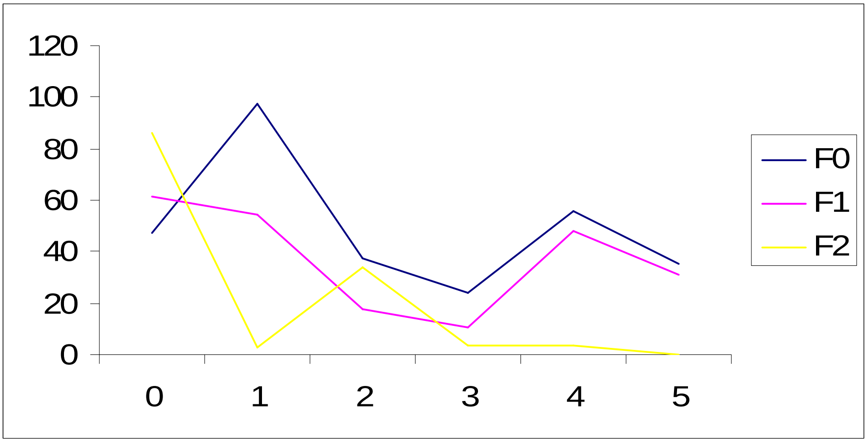


Figure 3.57. Copepods density (org/l) in the 18 mesocosms during the spring experiment in different fish densities (F0 = no fish addition, F1 = low fish density, F2 = high fish density).

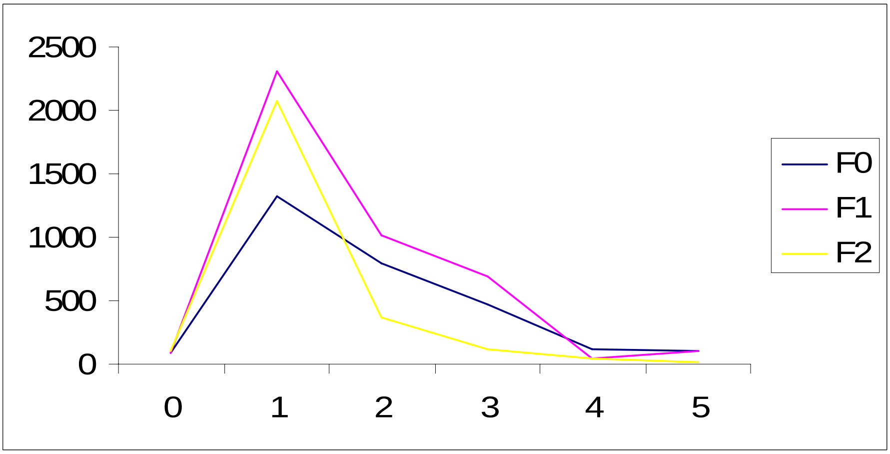


Figure 3.58. Nauplii density (org/l) in the 18 mesocosms during the spring experiment in different fish densities (F0 = no fish addition, F1 = low fish density, F2 = high fish density).

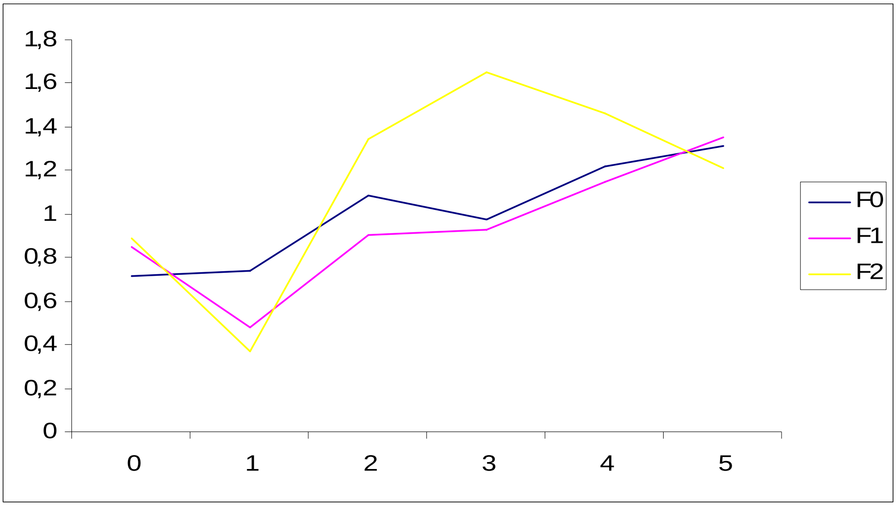


Figure 3.59. Shannon-Weaver index in the 18 mesocosms during the spring experiment in different fish densities (F0 = no fish addition, F1 = low fish density, F2 = high fish density).

In the natural ecosystem total zooplankton density was high for the first two weeks and then decreased (**Figure 3.60**). Rotifers density was high only in the second week when it reached 601 org/l (**Figure 3.61**). Cladocerans density was high for the first two weeks and determined total zooplankton density (**Figure 3.62**). Copepods density was between 21 and 61 org/l during the experiment (**Figure 3.63**). Nauplii density was high only in the first week when it reached 331 org/l (**Figure 3.64**).

Shannon-Weaver index increased from 0.81 to 1.63 (**Figure 3.65**).

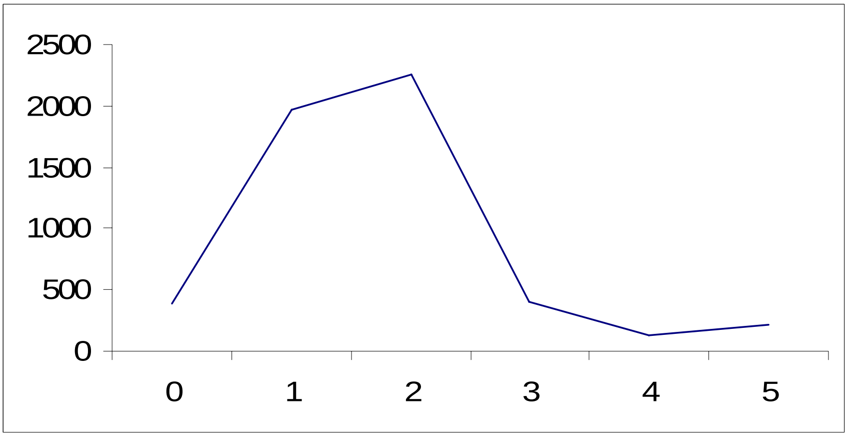


Figure 3.60. Total zooplankton density (org/l) in the natural ecosystem during the spring experiment.

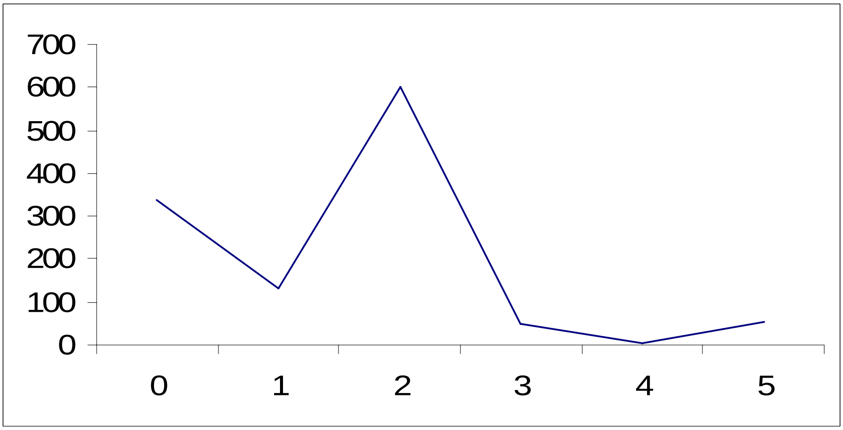


Figure 3.61. Rotifers density (org/l) in the natural ecosystem during the spring experiment.

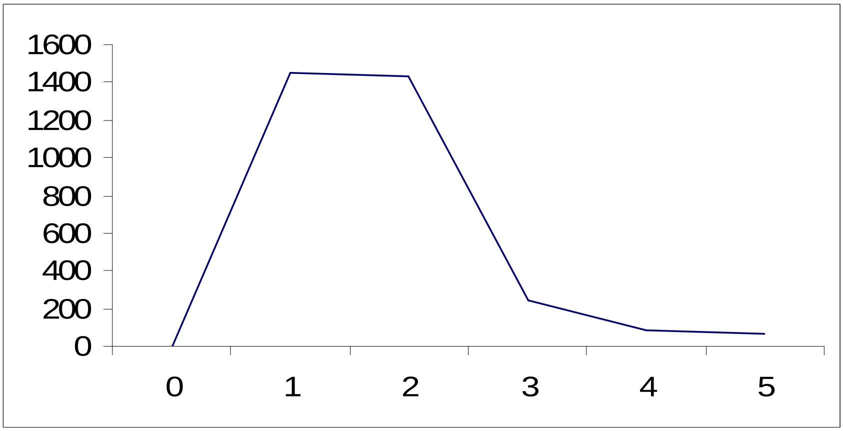


Figure 3.62. Cladocerans density (org/l) in the natural ecosystem during the spring experiment.

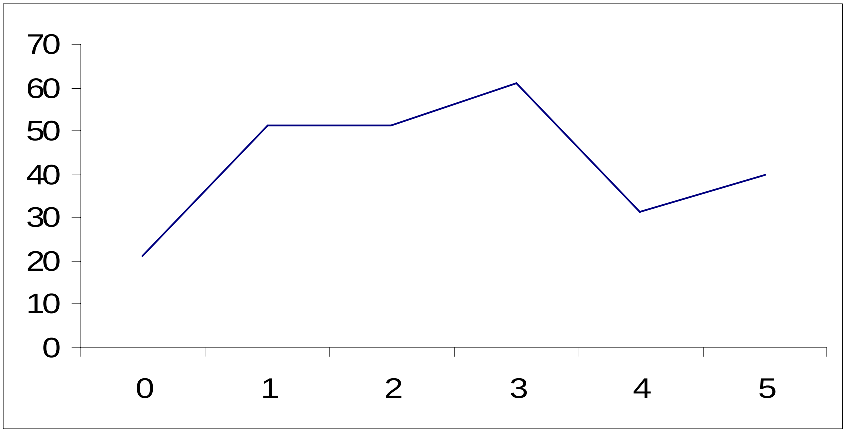


Figure 3.63. Copepods density (org/l) in the natural ecosystem during the spring experiment.

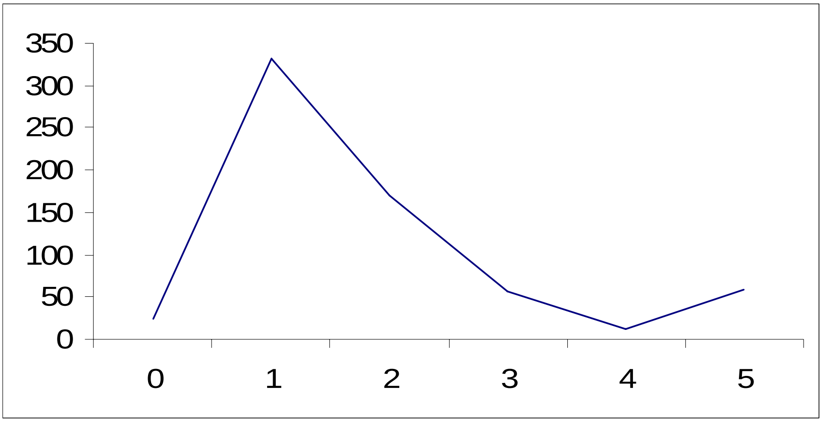


Figure 3.64. Nauplii density (org/l) in the natural ecosystem during the spring experiment.

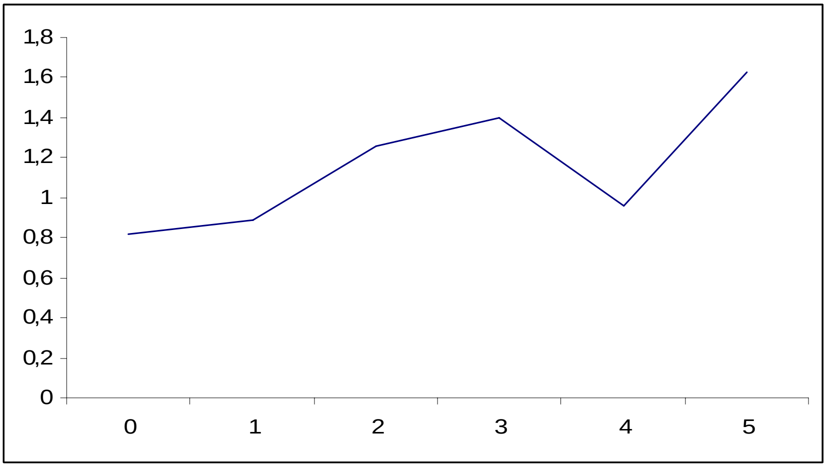


Figure 3.65. Shannon-Weaver index in the natural ecosystem during the spring experiment.

Figures below represent the influence of the three fish density on total zooplankton density for the three nutrient additions.

Difference in mean total zooplankton density between no nutrient addition (N0) and high nutrient addition (N2) was not statistically significative (*n*=30, F=1.347, p=0.428, t=-1.549, p=0.127, data log transformed). Total zooplankton density not even changed with increasing fish density from no fish (F0) to high fish density (F2) (*n*=30, F=1.586, p=0.220, t=1.001, p=0.321, data log transformed) (**Figure 3.66**).

Nutrient addition did not affect rotifers density (*n*=30, F=1.449, p=0.323, t=-1.046, p=0.300, data log transformed), but it was affected by fish density (*n*=30, F=5.013, p<0.001, t=-2.389, p<0.05, data log transformed) (**Figure 3.67**).

Cladocerans density was not influenced by nutrient addition (*n*=30, F=1.243, p=0.562, t=0.739, p=0.463 data log transformed) but it decreased with increasing fish density (*n*=30, F=1.189, p=0.644, t=4.067, p<0.001data log transformed) (**Figure 3.68**).

Copepods density was not influenced by nutrient addition (*n*=30, F=1.060, p=0.877, t=-1.854, p=0.069, data log transformed), but was highly influenced by fish density (*n*=30, F=1.469, p=0.306, t=5.630, p<0.001, data log transformed) (**Figure 3.69**).

Nauplii density was not influenced by nutrient addition (*n*=30, F=1.656, p=0.180, t=-0.670, p=0.506, data log transformed) and only slightly influenced by fish density (n=30, F=1.858, p=0.101, t=1.943, p=0.057, permutation test p=0.044, data log transformed) (**Figure 3.70**).

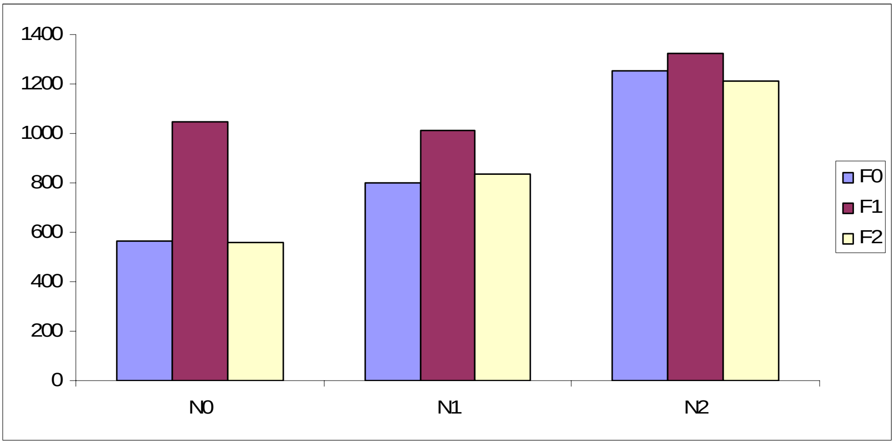


Figure 3.66. Total zooplankton density (org/l) in the 18 mesocosms during the spring experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

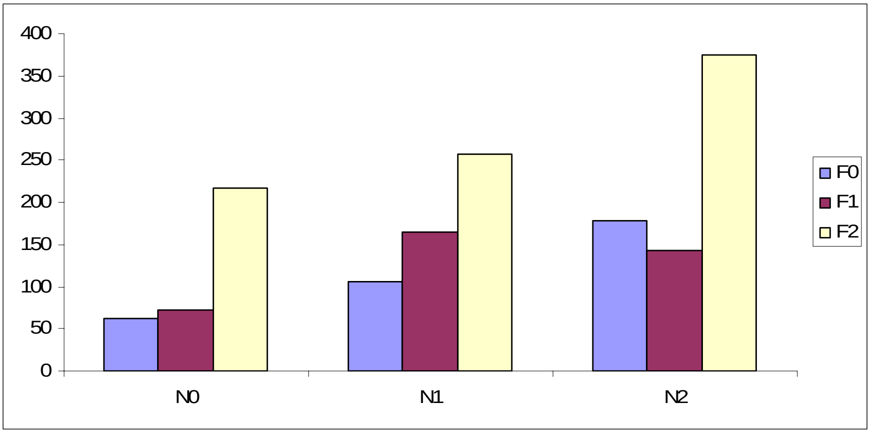


Figure 3.67. Rotifers density (org/l) in the 18 mesocosms during the spring experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

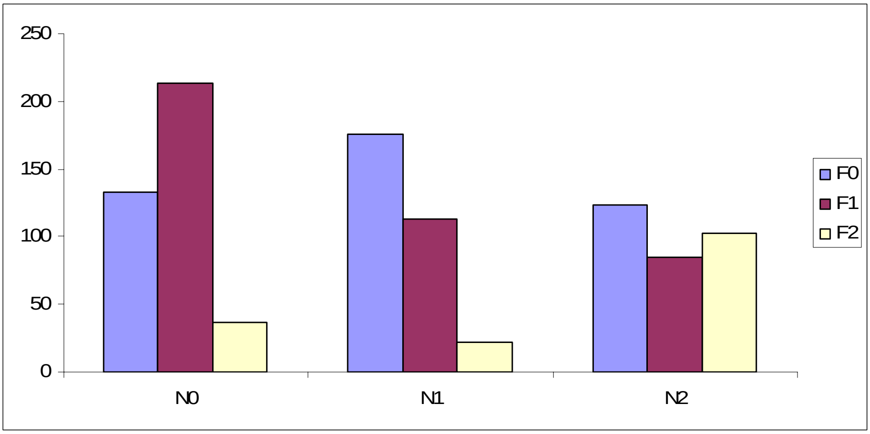


Figure 3.68. Cladocerans density (org/l) in the 18 mesocosms during the spring experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

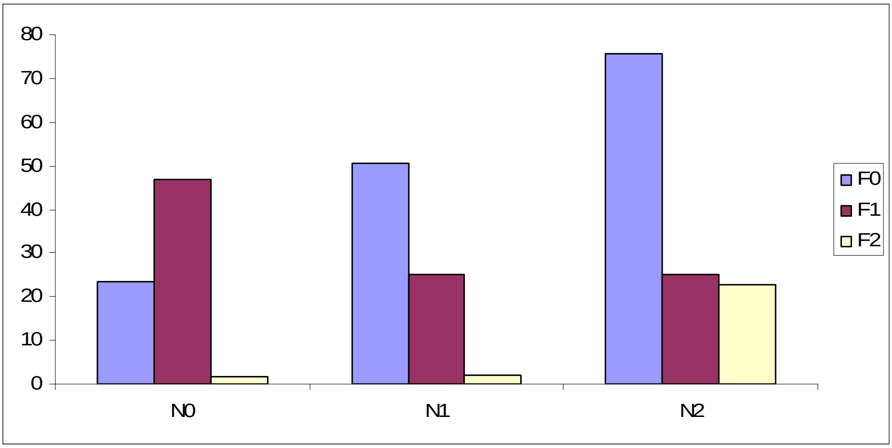


Figure 3.69. Copepods density (org/l) in the 18 mesocosms during the spring experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

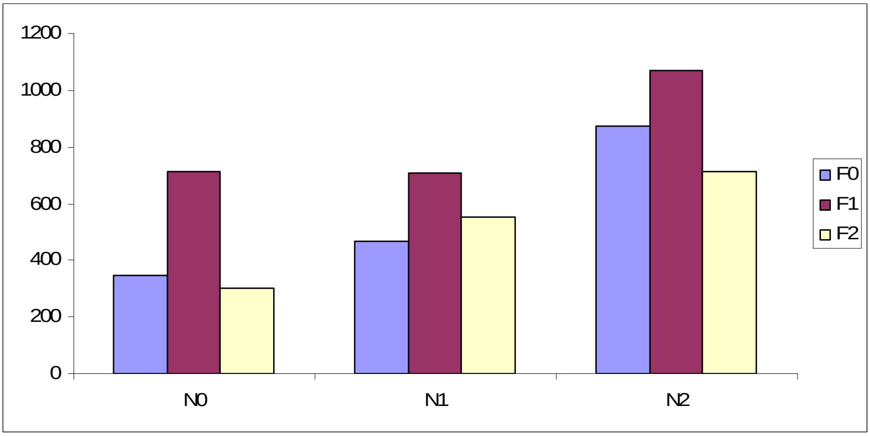


Figure 3.70. Nauplii density (org/l) in the 18 mesocosms during the spring experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

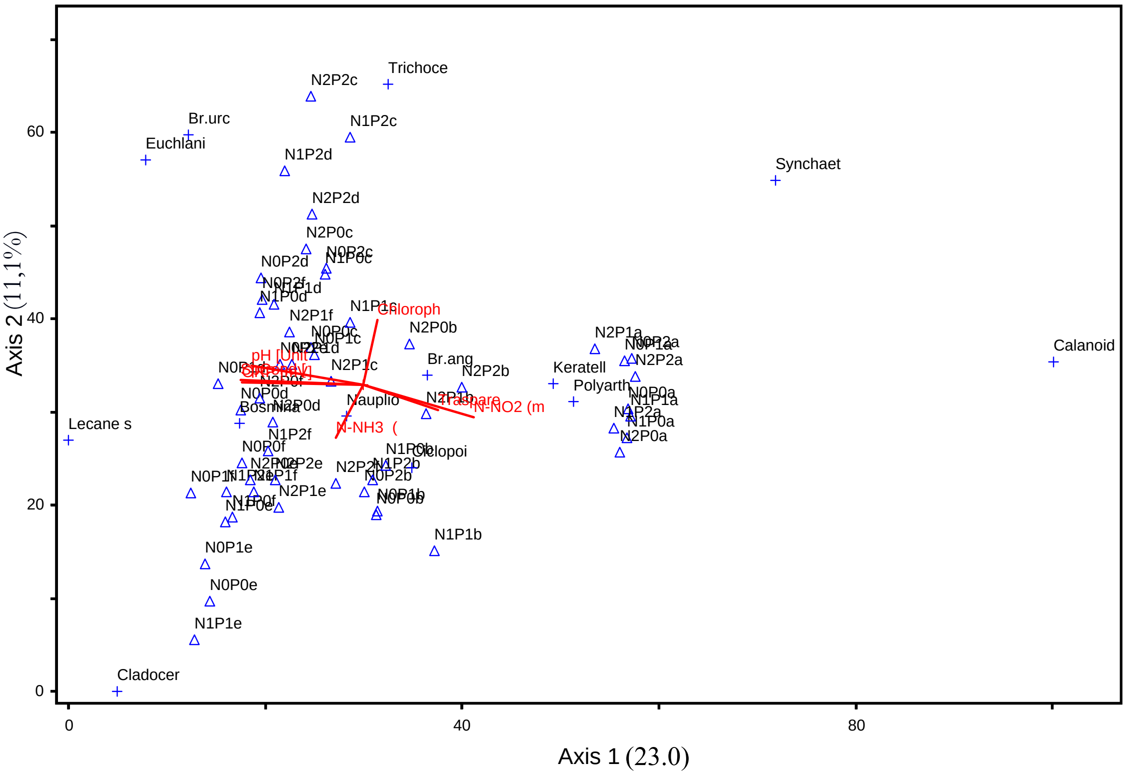
Rotifers density increased of 59% from no fish (F0) to high fish density (F2). Cladocerans and copepods density decreased of 63% and from 82% respectively from no fish (F0) to high fish density (F2). Nauplii density was high at low fish density.

(org/l)	**N0**	**N1**	**N2**
TOTAL	723±129	881±146	1262±208
Rotifers	117±31	176±46	232±56
Cladocerans	128±37	104±22	104±26
Copepods	24±8	51±12	76±10
Nauplii	454±107	576±130	885±202
(org/l)	**F0**	**F1**	**F2**
TOTAL	871±124	1127±193	868±179
Rotifers	115±29	127±30	283±64
Cladocerans	144±24	137±36	54±22
Copepods	50±14	32±8	9±6
Nauplii	562±106	830±175	523±170
(org/l)	**L**		
TOTAL	996±344		
Rotifers	168±80		
Cladocerans	655±305		
Copepods	47±7		
Nauplii	126±53		

Table 3.7. Mean values ± SE for zooplankton groups and total zooplankton density (org/l) measured under different nutrient additions and fish densities in the 18 mesocosms during the spring experiment experiment (*n* = 30 for mesocosms and *n* = 10 for lake, time 0 values were not considered; N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

CCA (ter Braak, 1986, 1994) was performed to investigate correlation between environmental variables and zooplankton species. Data were log transformed. All environmental variables were used except temperature (**Figure 3.71**). In the first week all mesocosm had a similar zooplankton community. Around the third and fourth week mesocosms with high fish density (F2) had a zooplankton community rich in rotifers (*Trichocerca* sp., *Brachionus urceolaris*, *Euchlanis* sp.).

Table 3.8 shows a significative correlation between environmental variables and zooplankton species.


Trichoce
N2P2c
Br.urc
N1P2c
Euchlani
N1P2d
Synchaet
N2P2d
N2P0c
N0P2c
N1P0c
N0P2d
N0P2f
N1P1d
N1P0d
N1P1c
Chloroph
N2P1f
N2P0b
N0P0c
N0P1c
N2P1a
N0P2a
N0P1a
N2P2a
Calanoid
N2P1d
pH [Unit
Br.ang
N0P1d
N2P1c
N2P2b
Keratell
N2P0f
Polyarth
N0P0a
N1P1a
N0P0d
N2P1b
Trasparе
N-NO2 (m
Nauplio
Bosmina
N2P0d
N1P2a
N1P0a
Lecane s
N-NH3 (
N2P0a
N1P2f
N0P0f
N1P0b
Ciclopoi
N2P0e
N0P2e
N2P2f
N1P2b
N0P1f
N1P2e
N2P1f
N0P2b
N2P1e
N1P0f
N0P1b
N0P0b
N1P0e
N1P1b
N0P1e
N0P0e
N1P1e
Cladocer
Axis 2 (11,1%)
60
40
20
0
0
40
80
Axis 1 (23.0)

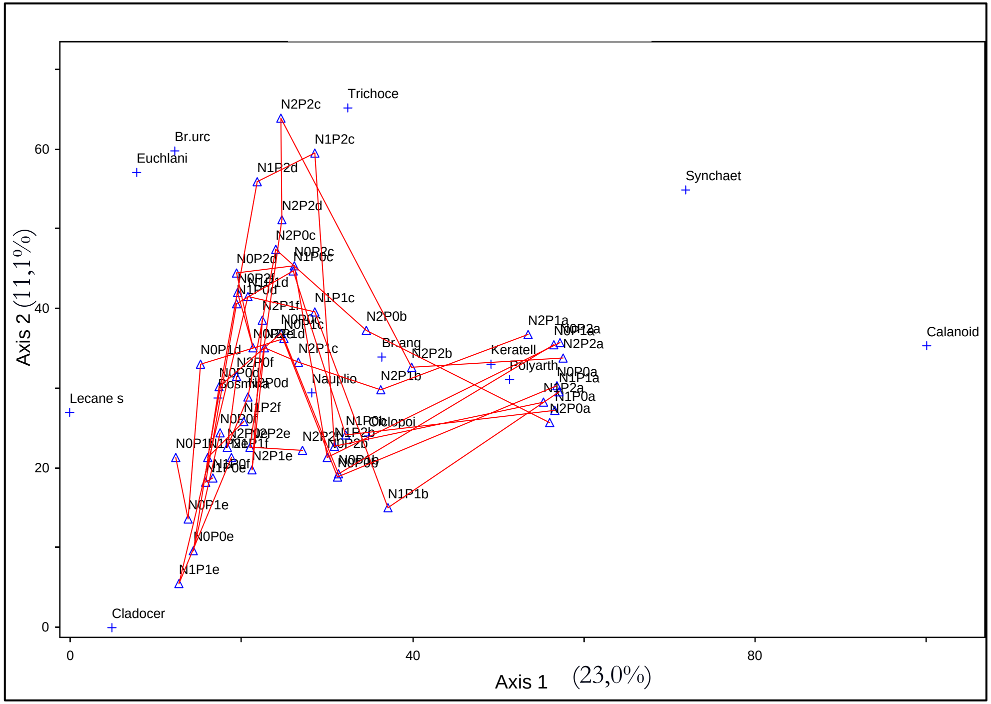


Figure 3.71. CCA results from correlation between environmental variables and zooplankton species during spring experiment

Real data		Randomized data Monte Carlo test, 99 runs			
Axis	Spp-Envt Corr	Mean	Minimum	Maximum	p
1	0.872	0.445	0.346	0.567	0.100
2	0.641	0.383	0.295	0.479	0.100
3	0.584	0.357	0.265	0.501	0.100

Table 3.8. Results from Canonical Correspondence Analysis (CCA).

3.3.2 Winter experiment

The mean zooplankton density in all the mesocosms in the pretreatment week was 1737 ± 439 org/l. Rotifers (99.27%) was the dominant group in terms of density. Nauplii were 0.58%, Copepods 0,12% and cladocerans 0.03% (**Figure 3.72**).

Among rotifers *Polyarthara remata* and *Keratella cochlearis* were the main species, with a mean density of 956 and 766 org/l respectively. *Filinia longiseta* reached 0.97 org/l while *Brachionus angularis* 2.01 org/l.

In the natural ecosystem mean zooplankton density was 1024 ± 688 org/l. Rotifers were the dominant group with a percentage of 98.29%. Nauplii were 1.22%, copepods 0.37% and cladocerans 0.12% (**Figure 3.73**).

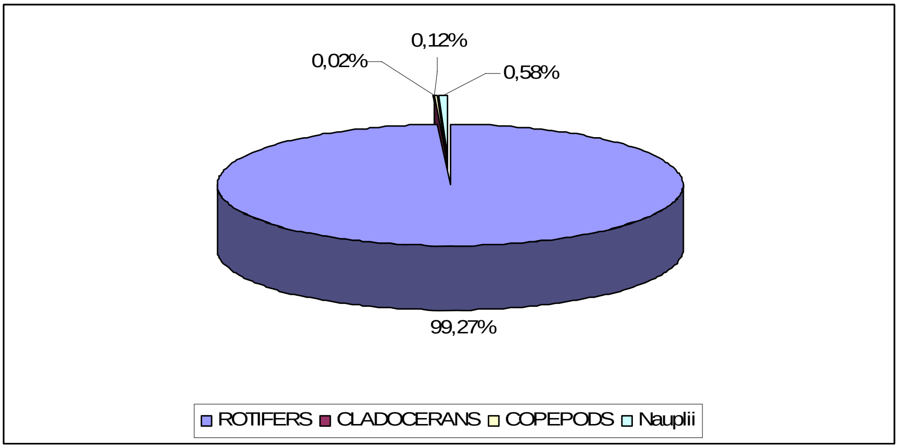


Figure 3.72. Zooplankton composition in the pretreatment week in all the mesocosms during the winter experiment.

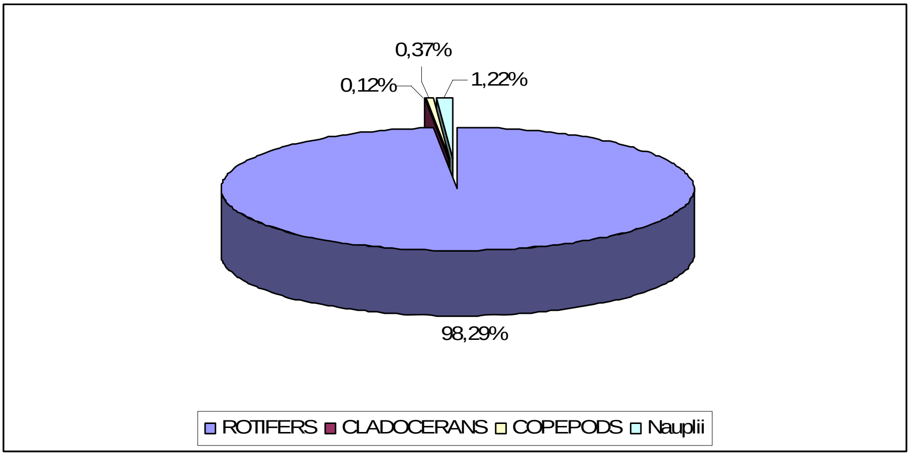
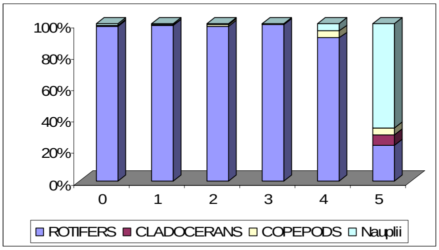
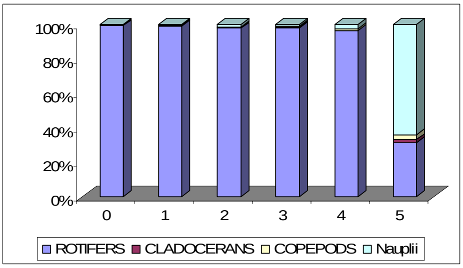


Figure 3.73. Zooplankton composition in the pretreatment week in the natural ecosystem during the winter experiment.

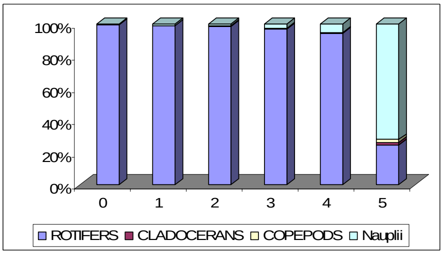
Zooplankton community structure was composed mainly by rotifers (**Figure 3.74**).

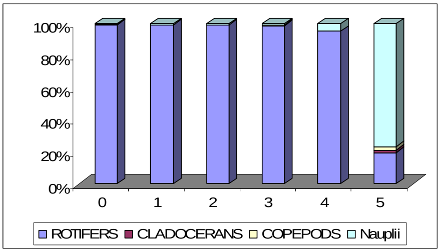
Also in the natural ecosystem zooplankton community structure was composed mostly by rotifers (**Figure 3.75**).



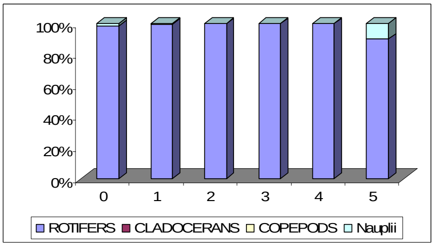


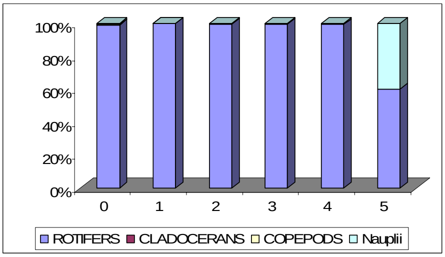
No nutrient and no fish



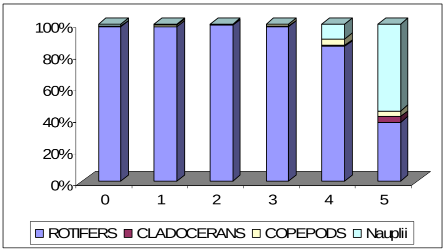


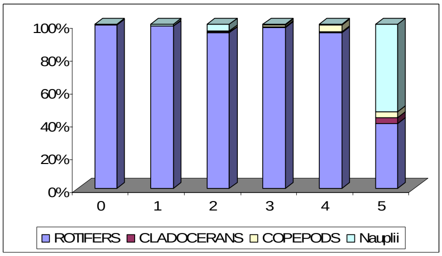
No nutrient and low fish density



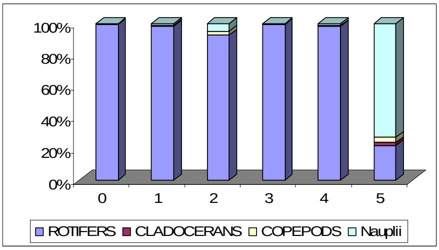


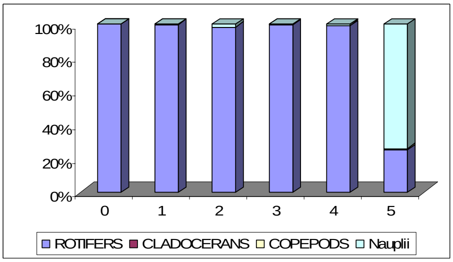
No nutrient and high fish density



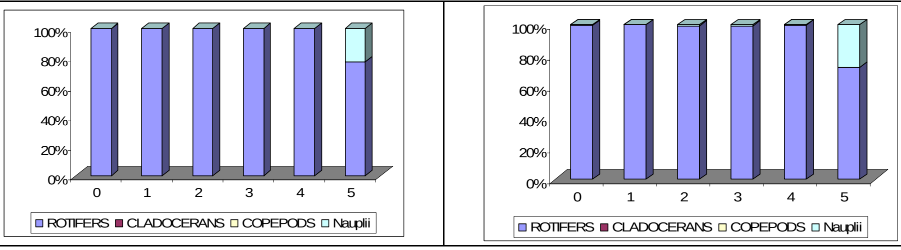


Low nutrient addition and no fish

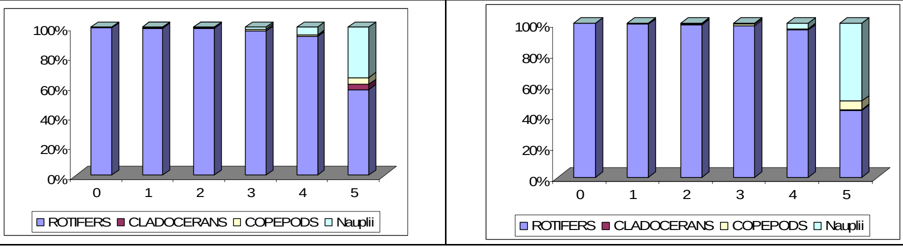




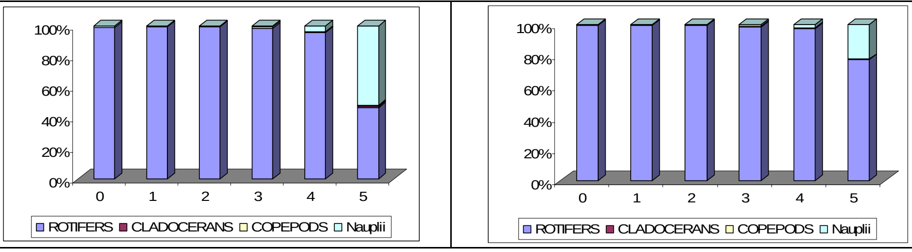
Low nutrient addition and low fish density



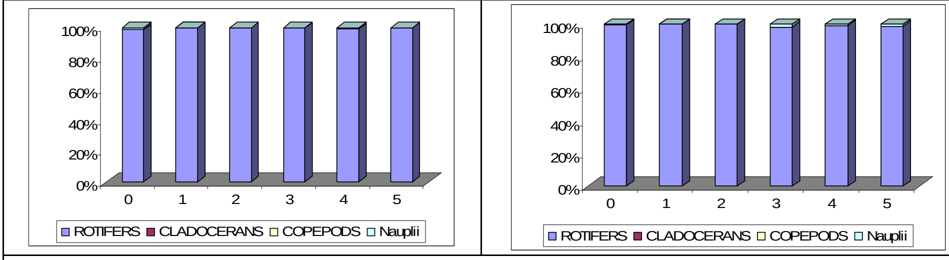
Low nutrient addition and high fish density



High nutrient addition and no fish

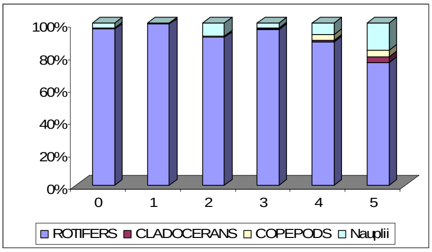


High nutrient addition and low fish density

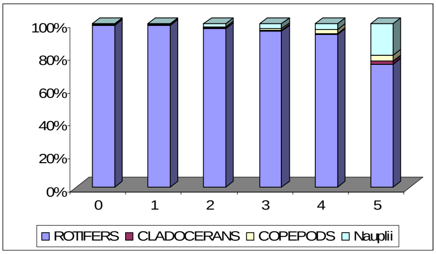


High nutrient addition and high fish density

Figure 3.74. Percentage density of the main zooplankton groups at different nutrient additions and fish densities in the 18 mesocosms during the winter experiment.



L1



L2

Figure 3.75. Percentage density of the main zooplankton groups in the natural ecosystem during the winter experiment.

Zooplankton community (**Table 3.9**) was characterized by some species. *Brachionus calyciflorus*, *Filinia longiseta*, *Keratella cochlearis*, *Polyarthra remata* and *Cyclops* sp. were observed in all the mesocosms. *Brachionus angularis*, *Brachionus urceolaris*, *Euchlanis* sp., *Lecane* sp., *Trichocerca* sp. and cladoceran were never observed.

In the natural ecosystem (**Table 3.10**) some species were always observed such as *Brachionus calyciflorus*, *Filinia longiseta*, *Keratella cochlearis*, *Polyarthra remata*, *Synchaeta* sp., *Daphnia magna* and *Cyclops* sp. Other species were never observed such as *Brachionus angularis*, *Brachionus urceolaris*, *Euchlanis* sp., *Lecane* sp., *Trichocerca* sp. Cladoceran and Calanoid.

	N_F_	N0F0		N0F1		N0F2		N1F0		N1F1		N1F2		N2F0		N2F1		N2F2	
	Mesocosm	2	17	9	10	4	15	1	18	3	16	7	12	8	11	6	13	5	14
ROTIFERS	*Brachionus angularis*																		
	Brachionus calyciflorus	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Brachionus urceolaris																		
	Euchlanis sp.																		
	Filinia longiseta	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Keratella cochlearis	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Lecane sp.																		
	Polyarthra remata	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Synchaeta sp.	■		■		■	■		■	■	■	■	■	■	■	■	■	■	■
	Trichocerca sp.																		
CLADOCERANS	*Bosmina longirostris*								■					■	■	■			
	Daphnia magna	■	■	■	■			■	■	■				■	■	■	■	■	
	Cladoceran																		
COPEPODS	Calanoid	■	■																
	Cyclops sp.	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

Table 3.9. Zooplankton community species in the 18 mesocosms during the winter experiment (N0 = no nutrient, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish, F1 = low fish density, F2 = high fish density).

	Station	L1	L2
ROTIFERS	*Brachionus angularis*		
	Brachionus calyciflorus	■	■
	Brachionus urceolaris		
	Euchlanis sp.		
	Filinia longiseta	■	■
	Keratella cochlearis	■	■
	Lecane sp.		
	Polyarthra remata	■	■
	Synchaeta sp.	■	■
	Trichocerca sp.		
CLADOCERANS	*Bosmina longirostris*	■	
	Daphnia magna	■	■
	Cladoceran		
COPEPODS	Calanoid		
	Cyclops sp.	■	■

Table 3.10. Zooplankton community species in the natural ecosystem during the winter experiment.

Figures below represent density of total zooplankton and density of each group during the experiment as the average value for the three different fish density in each week. Total zooplankton density decreased during the experiment (**Figure 3.76**). It reached its maximum value in the first week with a density of 4837 org/l in the mesocosms with no fish addition. Minimum value was reached in the last week with a density of 309 org/l. Total zooplankton density was due by rotifers that had the same trend (**Figure 3.77**). Cladocerans density was very low during the experiment (**Figure 3.78**). Copepods density was very high in mesocosms with no fish addition respect of the other two fish densities (**Figure 3.79**). Nauplii density was low during the experiment and only in the last week in mesocosms with no fish addition and low fish density it increased (**Figure 3.80**).

Shannon-Weaver index (**Figure 3.81**) was under 1 during all the experiment. It decreased until the forth week.

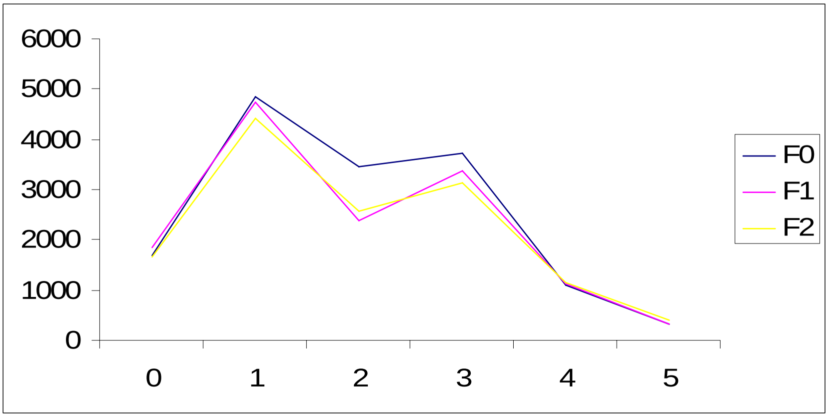


Figure 3.76. Total zooplankton density (org/l) in the 18 mesocosms during the winter experiment in different fish densities (F0 = no fish addition, F1 = low fish density, F2 = high fish density).

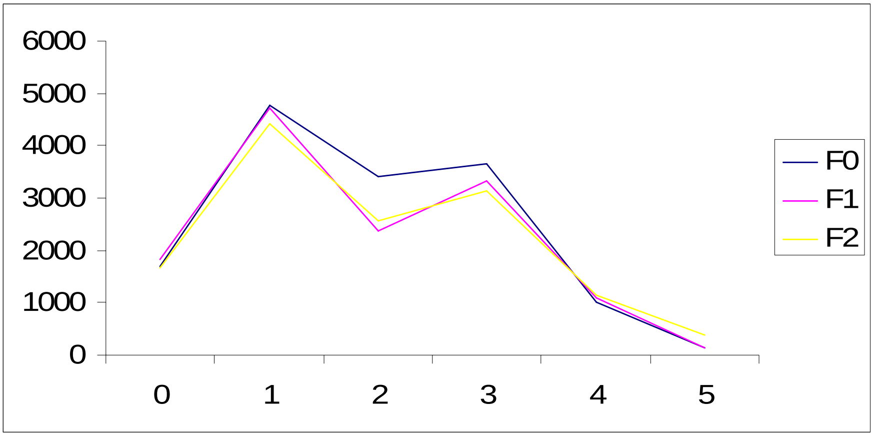


Figure 3.77. Rotifers density (org/l) in the 18 mesocosms during the winter experiment in different fish densities (F0 = no fish addition, F1 = low fish density, F2 = high fish density).

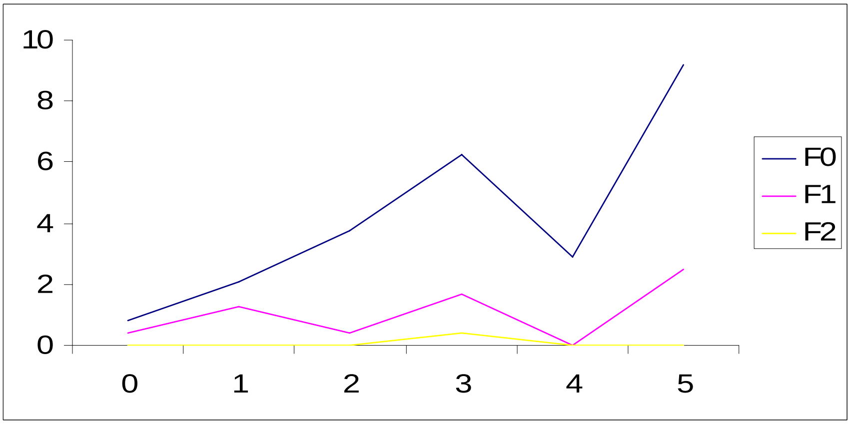


Figure 3.78. Cladocerans density (org/l) in the 18 mesocosms during the winter experiment in different fish densities (F0 = no fish addition, F1 = low fish density, F2 = high fish density).

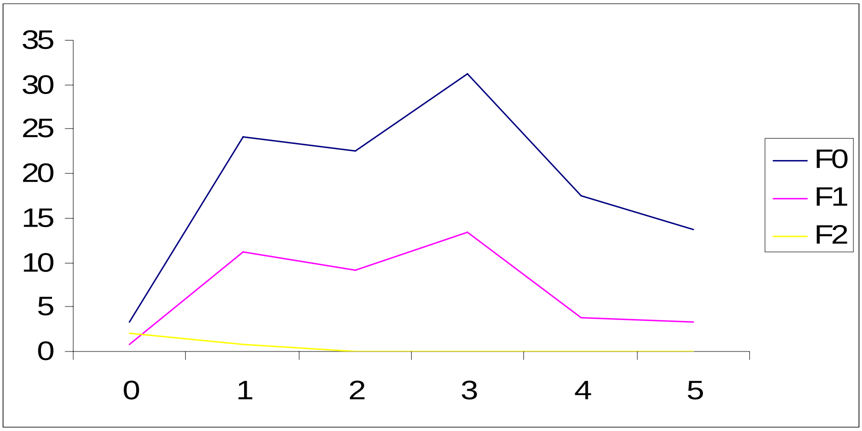


Figure 3.79. Copepods density (org/l) in the 18 mesocosms during the winter experiment in different fish densities (F0 = no fish addition, F1 = low fish density, F2 = high fish density).

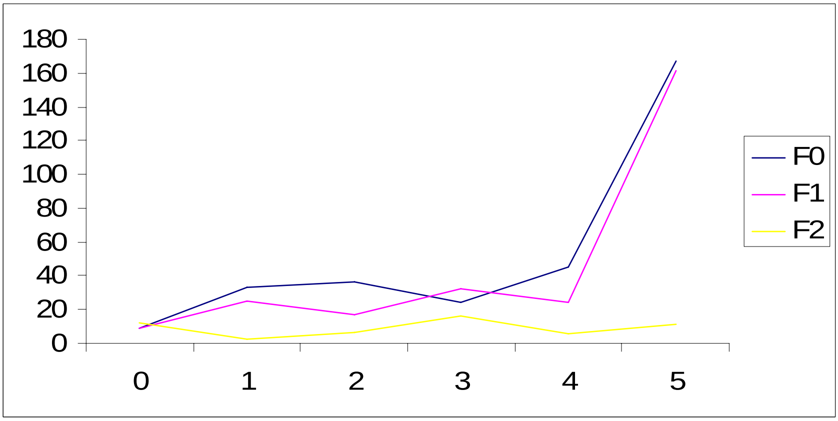


Figure 3.80. Nauplii density (org/l) in the 18 mesocosms during the winter experiment in different fish densities (F0 = no fish addition, F1 = low fish density, F2 = high fish density).

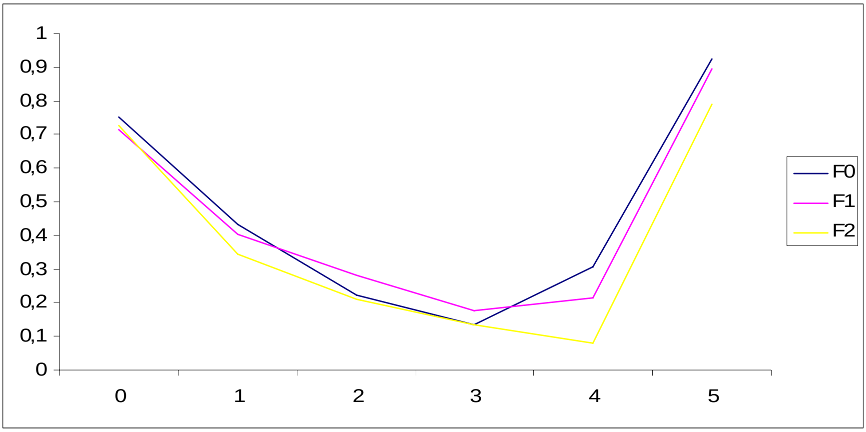


Figure 3.81. Shannon-Weaver index in the 18 mesocosms during the winter experiment in different fish densities (F0 = no fish addition, F1 = low fish density, F2 = high fish density).

Total zooplankton density reached its maximum value in the first week with a density of 3126 org/l (**Figure 3.82**). Rotifers density had the same trend of the total zooplankton density (**Figure 3.83**). Cladocerans density was low during the experiment and only in the last week was high (**Figure 3.84**). Copepods density slightly increased during the experiment (**Figure 3.85**). Also nauplii density increased during the experiment (**Figure 3.86**).

Shannon-Weaver index (**Figure 3.87**) was between 0.31 and 1.03.

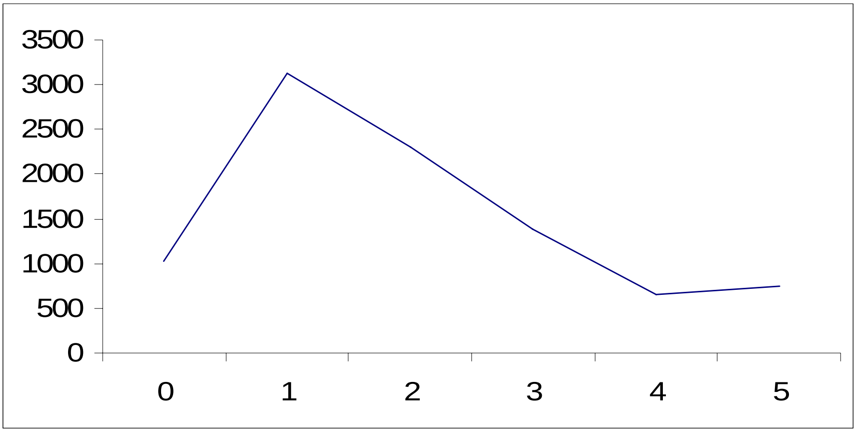


Figure 3.82. Total zooplankton density (org/l) in the natural ecosystem during the winter experiment.

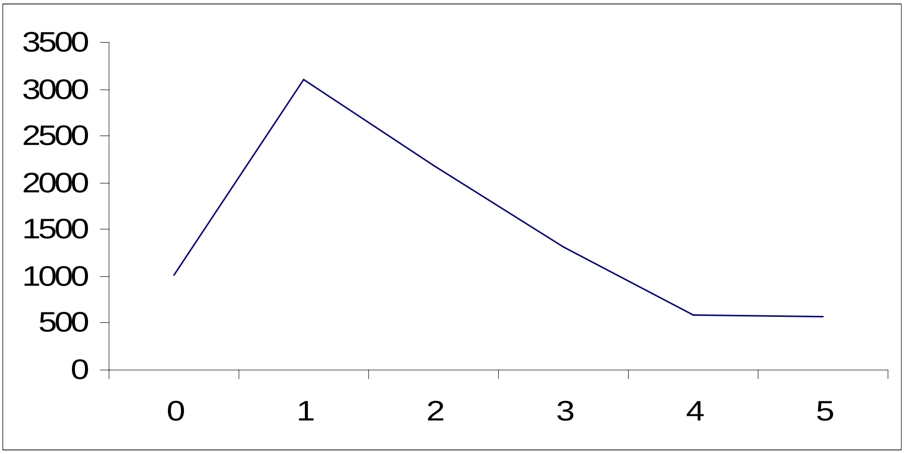


Figure 3.83. Rotifers density (org/l) in the natural ecosystem during the winter experiment.

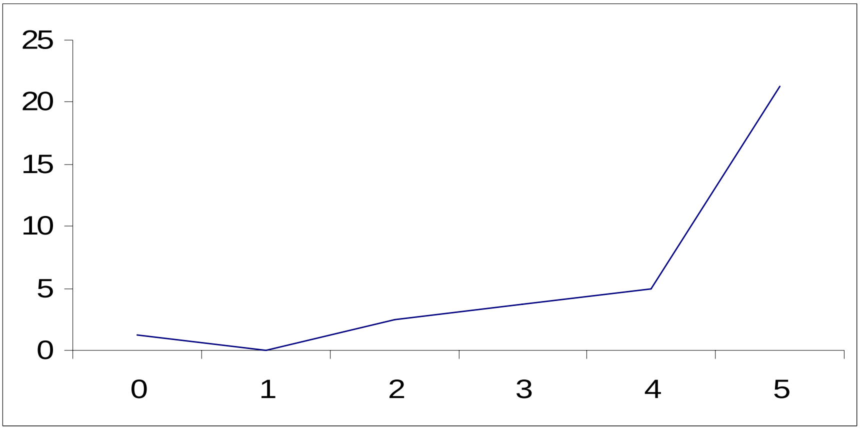


Figure 3.84. Cladocerans density (org/l) in the natural ecosystem during the winter experiment.

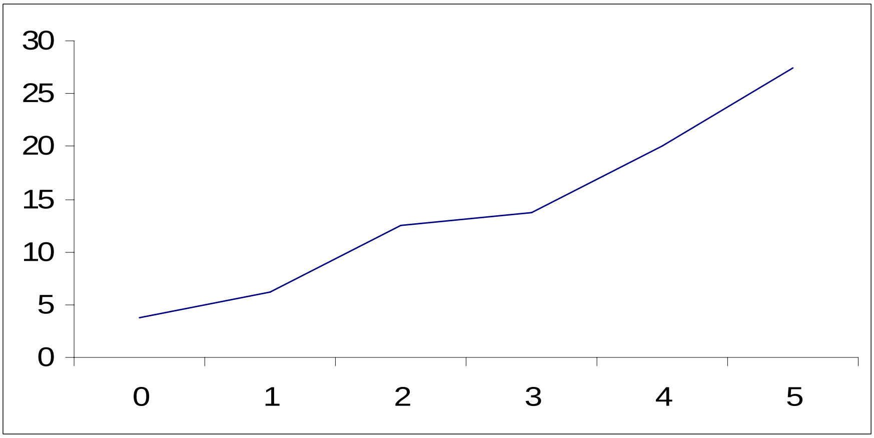


Figure 3.85. Copepods density (org/l) in the natural ecosystem during the winter experiment.

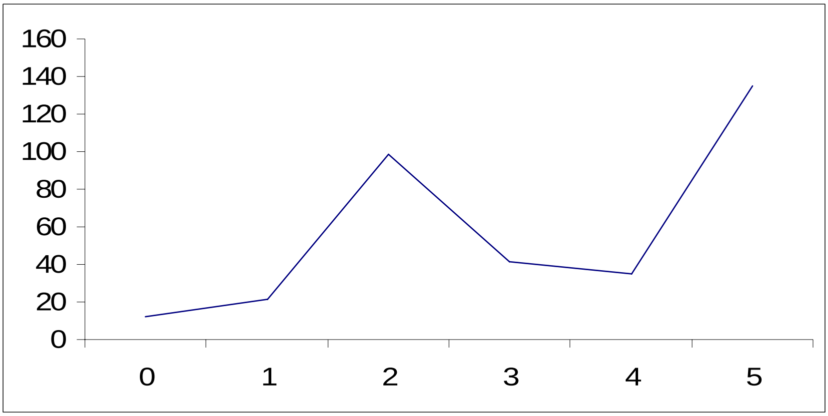


Figure 3.86. Nauplii density (org/l) in the natural ecosystem during the winter experiment.

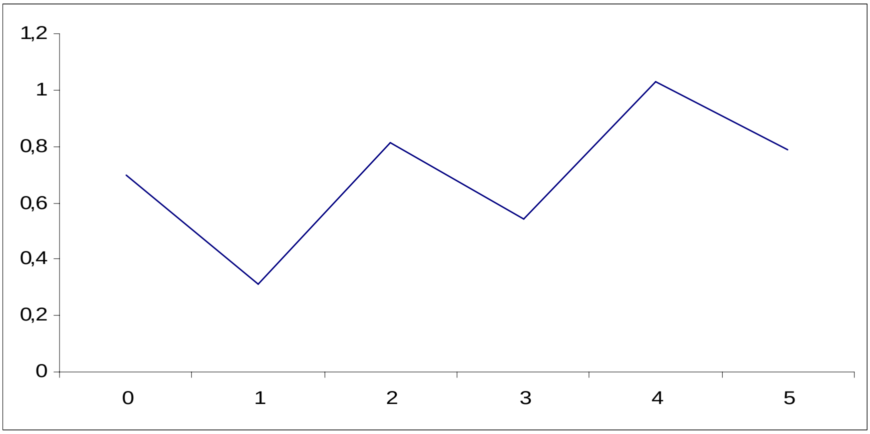


Figure 3.87. Shannon-Weaver index in the natural ecosystem during the winter experiment.

Figures below represent the influence of the three fish densities on total zooplankton density for the three nutrient additions.

Total zooplankton density did not show differences between no nutrient (N0) and high nutrient addition (N2) (*n*=30, F=2.053, p=0.057, t=-1.200, p=0.235, data log transformed) and between no fish (F0) and high fish density (F2) (*n*=30, F=1.067, p=0.207, t=0.716, p=0.477, data log transformed) (**Figure 3.88**).

Also rotifers density did not show differences in nutrient addition (*n*=30, F=2.564, p=0.014, t=-1.328, p=0.190, data log transformed) and fish density (*n*=30, F=1.050, p=0.897, t=0.140, p=0.889, data log transformed) (**Figure 3.89**).

Cladocerans density did not change in nutrient addition (*n*=30, F=1.044, p=0.909, t=-0.575, p=0.567, data log transformed) but showed difference in fish density (*n*=30, F=14.77, p<0.001, t=8.506, p<0.001, data log transformed) (**Figure 3.90**).

Difference in copepods density between no nutrient and high nutrient addition was not statistically significative (*n*=30, F=1.097, p=0.806, t=-0.573, p=0.569, data log transformed) but showed difference in fish density (*n*=30, F=2.971, p=0.005, t=24.97, p<0.001, data log transformed) (**Figure 3.91**).

Nauplii density was not different between no nutrient (N0) and high nutrient addition (N2) (*n*=30, F=1.416, p=0.354, t=0.759, p=0.451, data log transformed) but showed difference in fish density (*n*=30, F=1.022, p=0.955, t=6.975, p<0.001, data log transformed) (**Figure 3.92**).

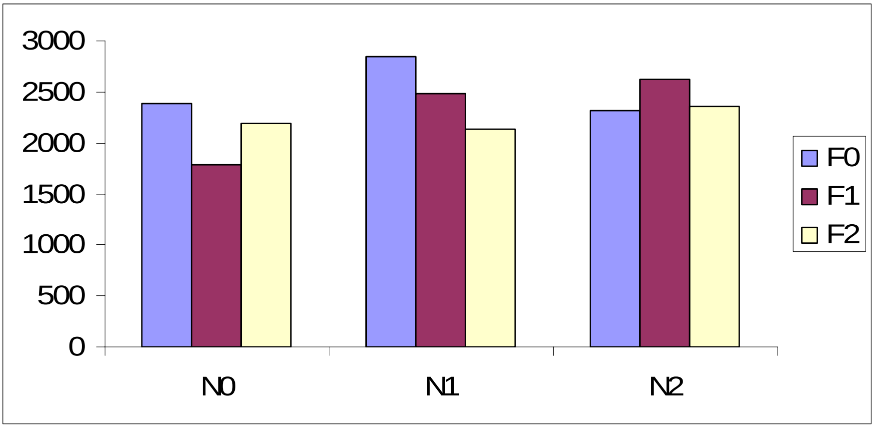


Figure 3.88. Total zooplankton density (org/l) in the 18 mesocosms at different nutrient additions and fish densities during the winter experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

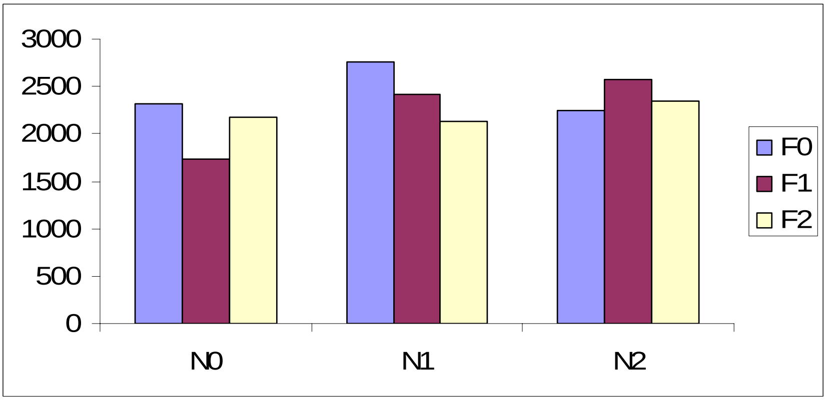


Figure 3.89. Rotifers density (org/l) in the 18 mesocosms at different nutrient additions and fish densities during the winter experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

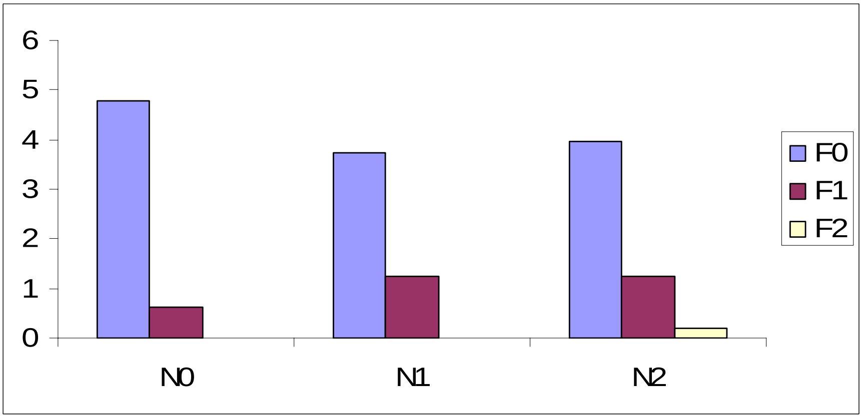


Figure 3.90. Cladocerans density (org/l) in the 18 mesocosms at different nutrient additions and fish densities during the winter experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

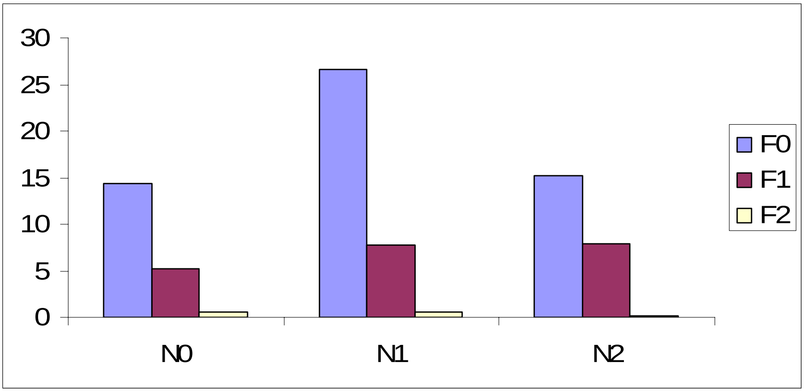


Figure 3.91. Copepods density (org/l) in the 18 mesocosms at different nutrient additions and fish densities during the winter experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

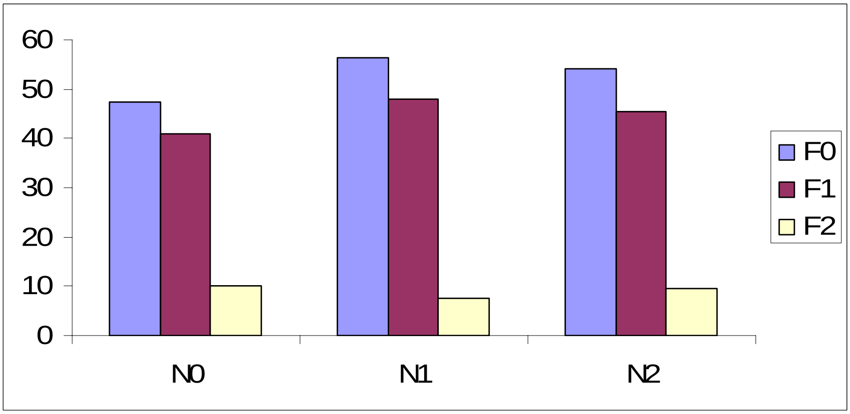


Figure 3.92. Nauplii density (org/l) in the 18 mesocosms at different nutrient additions and fish densities during the winter experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

Cladocerans, copepods and nauplii density decreased of 98%, 99% and 87% respectively from no fish (F0) to high fish density (F2).

(org/l)	**N0**	**N1**	**N2**
TOTAL	2188±284	2630±383	2590±325
Rotifers	2142±287	2571±385	2537±331
Cladocerans	2±1	2±1	2±1
Copepods	8±2	14±3	9±2
Nauplii	37±8	43±10	41±13
(org/l)	**F0**	**F1**	**F2**
TOTAL	2683±359	2386±343	2339±297
Rotifers	2595±364	2325±349	2330±297
Cladocerans	5±1	1.2±0.4	0.1±0.1
Copepods	22±3	8±1	0.2±0.1
Nauplii	61±11	52±12	8±2
(org/l)	**L**		
TOTAL	1642±344		
Rotifers	1553±353		
Cladocerans	7±3		
Copepods	16±3		
Nauplii	66±15		

Table 3.11. Mean values ± SE for zooplankton groups and total zooplankton density (org/l) measured under different nutrient additions and fish densities in the 18 mesocosms during the winter experiment (*n* = 30 for mesocosms and *n* = 10 for lake, time 0 values were not considered; N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

CCA (ter Braak, 1986, 1994) was not performed for winter experiment as zooplankton community was composed mainly by rotifers throughout the experiment.

3.4 Benthic macroinvertebrates

Some specimens of benthic macroinvertebrates observed in the spring and winter experiments are shown in **Table 3.12**.

	
Orthetrum sp.	*Micronecta* sp.
	
Trichoptera specimen	Gastropod specimen

Table 3.12. Some specimens of benthic macroinvertebrates observed in the mesocosms during the two experiments.

3.4.1 Spring experiment

During the spring experiment different benthic macroinvertebrates were found (**Table 3.13**). Chironomid larvae were found in all the mesocosms. Other benthic macroinvertebrates were found sporadically in the mesocosms. Also some gastropods were found.

No differences among mesocosms due to different nutrient addition or fish density were significative.

	Chironomid larvae	*Orthetrum*	Trichoptera	Ostracods	*Micronecta*	Other insects	Gastropods
N1F0	■	■	■	■		■	
N0F0	■	■	■	■	■	■	
N1F1	■						
N0F2	■						
N2F2	■			■			
N2F1	■						
N1F2	■	■					
N2F0	■	■	■	■			■
N0F1	■	■					

Table 3.13. Benthic macroinvertebrates found in the 18 mesocosms during the spring experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

3.4.2 Winter experiment

During the winter experiment only chironomid larvae were found in the mesocosms (**Table 3.14**). Other insects were found accidentally only in one mesocosm.

	Chironomid larvae	*Orthetrum*	Trichoptera	Ostracods	*Micronecta*	Other insects	Gastropods
N1F0	■						
N0F0	■						
N1F1	■					■	
N0F2	■						
N2F2	■						
N2F1	■						
N1F2	■						
N2F0	■						
N0F1	■						

Table 3.14. Benthic macroinvertebrates found in the 18 mesocosms during the winter experiment (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

3.5 Stomach contents of fishes

3.5.1 Spring experiment

Diet of fishes in the mesocosms consisted mainly of chironomid larvae, copepods and cladocerans (**Figure 3.93**). No differences were found between the mesocosms.

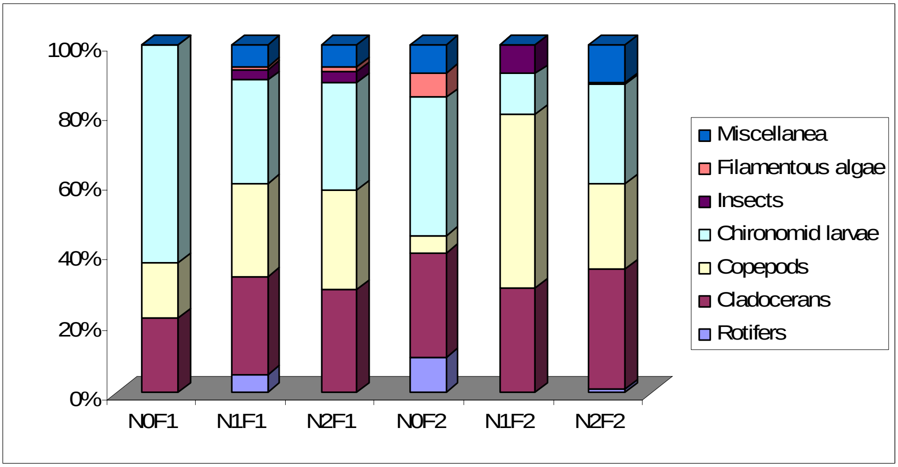


Figure 3.93. Stomach contents of fishes in the 18 mesocosms during the spring experiment calculated as the percentage volume of each food item per the degree of fullness of the stomach (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

3.5.2 Winter experiment

During the winter experiment the diet of fishes was mainly composed by copepods (**Figure 3.94**). No differences were found between the mesocosms.

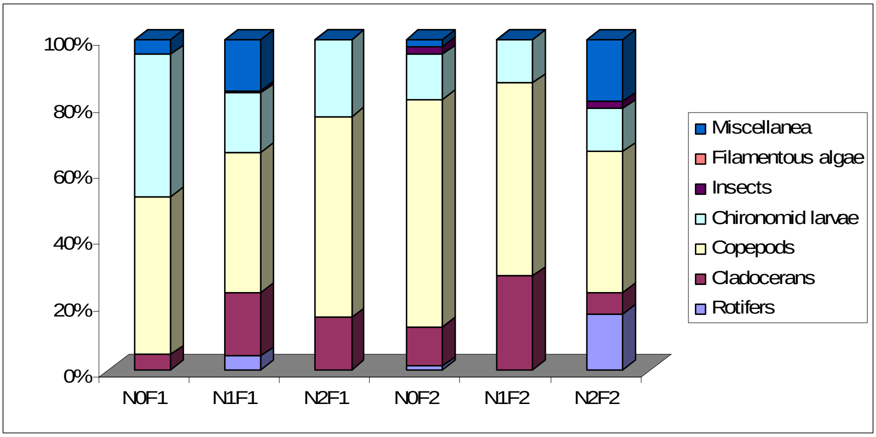


Figure 3.94. Stomach contents of fishes in the 18 mesocosms during the winter experiment calculated as the percentage volume of each food item per the degree of fullness of the stomach (N0 = no nutrient addition, N1 = low nutrient addition, N2 = high nutrient addition, F0 = no fish addition, F1 = low fish density, F2 = high fish density).

Chapter 4. DISCUSSION

The results obtained in the two mesocosm experiments can lead to several considerations.

Starting conditions were equal in all the mesocosms for all the variables considered.

Mesocosms control with no nutrient and no fish addition were similar to the natural ecosystem for several variables. Also zooplankton species found in mesocosms were the same observed in the natural system.

Mesocosm replicate did not differ strongly each other and so it was possible to consider average values between every couple of mesocosms.

Water chemistry was influenced by nutrient addition suggesting a rapid uptake from algae. Total phytoplankton biomass was more influenced by nutrients than by the presence of planktivorous fish. On the contrary zooplankton density was controlled much more by top-down control. Differences in the response of zooplankton community emerged between the two seasons. In particular during winter fish predation was higher due to the absence of benthic macroinvertebrates.

Following, results from the analyses of water chemistry, phytoplankton biomass and zooplankton community are discussed and compared to results from other works.

Figure 3.95 below represents summary of the results of both experiments. Dotted lines represent no-significative effects, while thin lines represent significative effects. Red lines represents spring experiment, while blue lines represent winter experiment. Increasing effects are represented by a plus (+) while decreasing effects are represented by a minus (–).

Total zooplankton is distinguished in rotifers, cladocerans, copepods and nauplii since these are the functional groups considered in the present work.

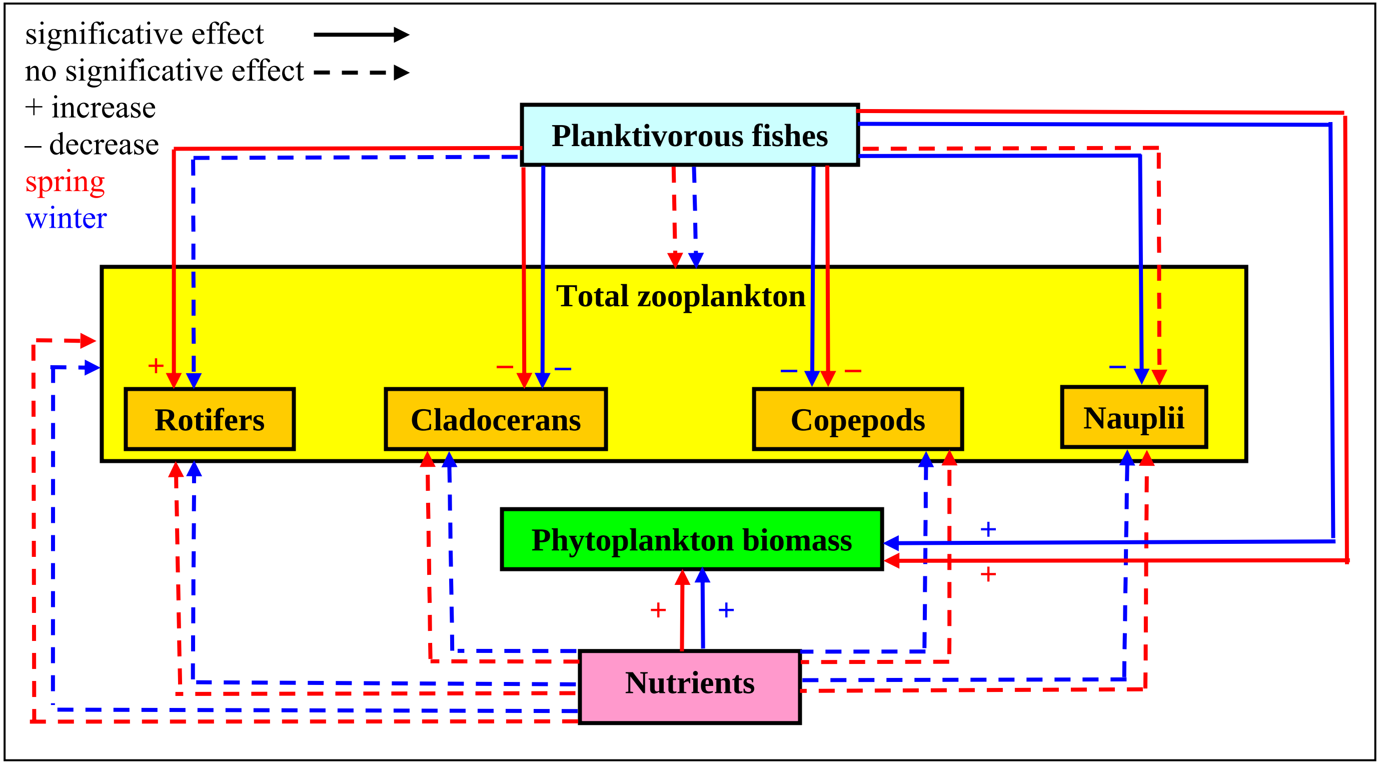


Figure 3.95. Summary of responses of zooplankton community and phytoplankton biomass to nutrient enrichment and fish predation in 18 mesocosm experiments during spring and winter.

4.1 Water chemistry

Temperature is one of the most important physical variables. Water temperature increase determines biochemical reactions enhance. Biochemical reactions reach their maximum speed between 33 and 35 °C. Speed reactions increase determines algal blooms and higher oxygen consumption. In spring experiment maximum temperature was 26°C suggesting a high biochemical reactions speed.

In natural ecosystems pH value is affected by dissolved CO_2 concentrations which can originate from atmosphere, calcium carbonate dissolution and mainly from aquatic organisms respiration. Decreasing CO_2 concentrations due to photosynthesis determines high pH values. No-lethal pH range for aquatic organisms is between 5 and 9 (Floccia *et al.*, 1985), but several polluting compound may be dangerous at higher or lower values. For example high pH values increase toxic NH_3 concentrations compared to NH^+_4 (Alabaster *et al.*, 1972). pH values both in spring and winter were higher in mesocosms than in the natural ecosystems even in no nutrient and no fish addition mesocosms.

Dissolved oxygen concentrations depends on atmospheric pressure and temperature. Dissolved oxygen percentage below 60% may injure aquatic organisms, while values over 100% suggest high photosynthetic activity. In the mesocosms dissolved oxygen percentage was higher than in the natural ecosystem both in spring and winter.

Redox potential is higher than 200 mV in high dissolved oxygen concentrations, while in presence of sulfurs it is below –200 mV. Redox potential values were higher in winter both in mesocosms and lake probably due to the lack of sulphur producing bacteria.

Specific conductivity indicates capability of water to conduct electric current. Its value depends on dissolved ions concentrations. It reveals trophic status of water since is trend is influenced by biological activity. Specific conductivity values above 1500 – 1800 µS/cm may damage aquatic organisms. In mesocosm experiments these values were always low and did not exceed 700 µS/cm neither in spring nor in winter.

Turbidity is due to the presence of thin suspending particles. It may depend on natural process or discharges from human activity In mesocosms and natural ecosystems turbidity values were very variables during the experiments.

Nitrogen nitrite is the more unstable inorganic nitrogen form since it is intermediate between ammonium and nitrogen nitrate. Nitrites are toxicant compounds especially for fishes due to their oxidant activity. Maximum value is 0.1 mg/l. Nitrogen nitrite concentrations were below this value both in mesocosms and natural ecosystem.

Nitrogen nitrate is the more oxidised inorganic nitrogen form and the more easily used for uptake by algae. In spring experiment nitrogen nitrate concentrations were always lower than in winter suggesting a higher uptake by algae during spring.

Nitrogen ammonia depends on biological degradation of nitrogen compounds. Both in spring and winter nitrogen ammonia concentrations were very variables during experiments.

Orthophosphate is the phosphorus form directly assumed by algae and it is the most important limiting factor in lakes. In spring experiment orthophosphate concentrations were lower than in winter suggesting a higher uptake by algae during spring.

Nutrient addition did not increase nutrient concentrations in no one of the two experiments. The same results were also found in Van de Bund & Van Donk (2004) who studied effects of fish and nutrient additions in a charophyte-dominated lake. Nutrient concentrations in the enclosures did not exceed levels of the weekly additions, indicating that there was no net accumulation of nutrients in the water, not even at the highest level of addition.

Moss *et al.* (2004) summarized the results on continental-scale patterns of nutrient and fish effects on shallow lakes in a pan-European mesocosm experiment. The chemical variables they considered were: total phosphorus (TP; µg/l), soluble reactive phosphorus (SRP; µg/l), nitrate-N (mg/l), ammonium-N (µg/l), pH, alkalinity (meq/l), total suspended solids (TSS; mg/l), the latter two in 1998 only. In 1998 and 1999 fish significantly influenced none of the chemical variables. In 1998 nutrient loading influenced five of the seven chemical variables, always by increasing values, while in 1999 nutrient additions positively influenced all the chemical variables. Nutrient additions thus had a much greater effect on water chemistry, whilst fish addition had no effect on nutrients.

In spring experiment temperature was at least ten degrees higher than in winter experiment. This difference was probably the main cause of the observed results in the two experiments.

In winter experiment orthophosphate and nitrate concentrations were always higher than those in spring experiment. This is probably due to the lack of algae uptake.

4.2 Phytoplankton

In the mesocosms phytoplankton biomass was influenced by nutrient addition and fish predation. This effect was more evident when both treatments were present.

In the present work during spring only in mesocosms with no nutrient addition chlorophyll a remained below 100 µg/l. In mesocosms with high nutrient addition and high fish density chlorophyll a could reach 300 µg/l.

Other authors. (Karjalainen *et al.*, 1998; Scheffer & Jeppesen, 1998; Spencer & Ellis, 1998) found that nutrient additions stimulate phytoplankton growth and that this effect would be enhanced by the presence of fish (Scheffer, 1991).

In winter experiment chlorophyll a concentrations remained low. Only in mesocosms with high fish density chlorophyll a reached 40 µg/l.

Hietala *et al.* (2004) conducted field mesocosms experiment in 1998 and 1999 to study community responses to nutrient enrichment at different fish densities. Although nutrient enrichment resulted in similar ranges of total phosphorus concentrations in both years, the response of phytoplankton to nutrient enrichment clearly differed between the two study periods in mesocosm experiments. In 1998, a clear water state with visible lake bottom occurred only in no nutrient enrichment/fish-free and no nutrient enrichment/low-fish mesocosms with phosphorus concentrations <0.06 mg/l. Chlorophyll a concentration increased linearly with phosphorus concentration up to a maximum of approximately 200 µg/l.

In 1999, on the contrary, chlorophyll a concentrations remained below 100 µg/l, and a clear water state prevailed over the whole nutrient gradient.

Stephen *et al.* (2004a) summarized the results of eleven parallel mesocosms carried out in six lakes across Europe. Phytoplankton biomass was influenced much more by nutrients than fish, with increases being found with increasing nutrient loading in all locations, although fish shifted the community composition towards smaller algae, especially chlorophytes and cyanobacteria, presumably by removing large grazers that would otherwise have fed on these small species. The contribution of cyanobacteria also increased with increasing temperature.

Van de Bund & Van Donk (2004) investigated the effects of fish and nutrient addition on food web stability in a charophyte dominated lake in the Netherlands. In 1998 top-down control of phytoplankton biomass appeared to play an important role, especially in the low-nutrient treatments. This was possible because zooplankton was dominated by large cladocerans from the start, with *Diaphanosoma brachyurum* as the most abundant species. Addition of fish greatly reduced the biomass of these large and effective grazers, resulting in an increase in phytoplankton biomass. In 1999 zooplankton was dominated by small taxa, with *Ceriodaphnia pulchella* and *Chydorus sphaericus* as the dominant species. Grazing on algae by large zooplankton therefore appears to have played a minor role in the 1999 experiment from the start. Even in the absence of fish the large zooplankters never reached high densities during the experiment. At the start of the experiment, densities were very low, and the 6-week experiment was probably too short for the large zooplankton to build up high densities. This is consistent with the fact that addition of rudd (*Scaridinius erythrophtalmus*) had no significant effects in 1999, whereas effects on zooplankton were mostly highly significant in 1998.

Mesocosms experiments on nutrient and fish effects on shallow lake food webs in a Mediterranean climate were carried out by Romo *et al*. (2004). They found that nutrients controlled biomass of phytoplankton and periphyton, while zooplankton, regulated by planktivorous fish, influenced the relative percentages of the dominant phytoplankton species.

Fernández-Aláez *et al*. (2004) in a two year experimental study on nutrient and predator influences on food web constituents in a shallow lake of north west Spain found different outcomes each year depending on the initial conditions, especially of macrophyte biomass. In 1998 estimated initial ‘per cent water volume inhabited’ (PVI) by submerged macrophytes was about 35%. Phytoplankton biomass estimated as chlorophyll a was strongly controlled by fish, whereas effects of nutrient enrichment were not significant. In 1999 estimated PVI was 80%, no fish effect was observed on phytoplankton biomass, but nutrients had significant effects.

In the present work to avoid this kind of trouble the bottom of each mesocosm was not open to the sediments so macrophytes were not present.

Moss *et al.* (2004) summarized the results on continental-scale patterns of nutrient and fish effects on shallow lakes in a pan-European mesocosm experiment. Fish increased chlorophyll a concentration and biovolume of Cryptophyceae in 1998 but not in 1999, when instead fish increased dinoflagellate biomass. Nutrient loading influenced five of the primary producer variables in 1998. It increased chlorophyll a concentration, total phytoplankton biomass, and biomasses of Chlorophyceae and Cryptophyceae, and decreased the biomass of small algae (GALD <50 μm).

In 1999 nutrient additions increased chlorophyll a concentration, total phytoplankton biomass, the proportion of small algae and periphytic chlorophyll a, but decreased the diversity (Shannon–Wiener) index of phytoplankton. Thus in 1998 nutrients significantly influenced five of 13 primary producer variables. Nutrient loading increased phytoplankton growth, often selectively increasing the proportions of green algae and cryptomonads, and decreasing the proportions of small algae and phytoplankton diversity.

Results obtained in the present work suggest the importance of nutrients and fish to manipulate phytoplankton community both in spring and winter in a Mediterranean region lake.

4.3 Zooplankton

The results of the two mesocosm experiments emphasise the importance of consumer control over resource control in determining zooplankton biomass in shallow lake systems. Thus, these outcomes are in agreement with the general view that large herbivores respond strongly to predator manipulations in aquatic food webs (Brett & Goldman, 1997; Mehner *et al.*, 2002; Shurin *et al.*, 2002). Rotifers increased with fish treatments. Large herbivores such as copepods and cladocerans decreased their density with increasing fish addition. Small zooplankton species apparently benefited from the mostly negative responses of their larger competitors and raptorial predators to the presence of fish. Thus, within zooplankton communities different functional groups responded inconsistently to the treatments. Such compensation suggests a ratio-dependent functional response (Arditi & Ginzburg, 1989; Leibold *et al*., 1997).

Zooplankton community was not influenced by nutrient addition. Neither total zooplankton density nor any of the zooplankton groups increased or decreased. Only fish had an important effect on zooplankton community. In particular total zooplankton density did not change with increased fish density, but community structure changed. Increased fish density increased rotifers in spring, but decreased cladocerans and copepods in both seasons. Nauplii decreased only during winter with increasing fish density.

Also Stephen *et al.* (2004a) in eleven parallel mesocosm experiments on nutrient and fish effects on shallow lakes found that fish had their greatest effect on zooplankton communities (increasing the biomasses of smaller cladocerans and rotifers and decreasing that of large cladocerans).

Other ecologists found a relation between nutrient and zooplankton biomass. For example in Hansson *et al.* (2004) total zooplankton biomasses showed only a tendency towards a positive response to increased nutrient levels in the presence of fish. Nutrient additions in the absence of fish affected the total biomass of zooplankton significantly. Cyclopoid copepods showed the highest biomass at intermediate nutrient concentrations in the treatments with no fish and low fish abundances. At high fish abundances, however, cyclopoid copepods showed an exponentially increasing trend with nutrient addition. Rotifers and copepod nauplii generally showed significantly higher biomass in the presence, than in the absence, of fish. Rotifers especially had very low biovolumes in the absence of fish, irrespective of nutrient treatment. However, at the highest fish density, rotifers declined after a maximum biovolume at intermediate nutrient levels.

In spring experiment copepods disappeared almost completely in the presence of high fish density, whereas rotifers generally were most abundant. Rotifers showed a positive response to fish which may be because of a reduction in predation from copepods as illustrated by Gilbert (1988) and Jack & Gilbert (1994). In any case, it is clear that not all zooplankton groups respond similarly to the presence of fish. For the largest (copepods), and smallest (rotifers) zooplankters there was even a gradual, although opposite, response to fish biomass.

These data suggest that the general predictions of strong negative effects of planktivorous fish on herbivorous zooplankton may not be valid for all taxonomic groups and size classes of zooplankton.

In winter experiment rotifers density was almost equal in the three fish treatment and there was not a positive response to fish. Copepods density was higher in the fish less mesocosms than in the mesocosms with high fish density. Also cladocerans had a negative response to fish density that was not clear in the spring experiment.

Fish did not decrease rotifers density probably because in winter rotifers were very abundant (up to 5000 org/l) and reached their maximum density even in the presence of large herbivores.

Simple models have indicated that nutrient enrichment produces an increase in zooplankton density when fish are absent, while not necessarily affecting phytoplankton density (Scheffer, 1991). This idea is well supported by empirical evidence (Shapiro, 1995; Spencer & Ellis, 1998; Stephen *et al*., 1998). It is also in accordance with results from the experimental study on nutrient and predator influences on food web constituents in a shallow lake of North West Spain of Fernández-Aláez *et al*. (2004). In their fishless enclosures nutrient addition produced a significant increase in zooplankton biomass, indicating that grazing was responsible for maintaining relatively low phytoplankton biomass at all nutrient levels, although cyclopoid copepods rather then the more effectively grazing large cladocerans were most affected by nutrient enrichment. Spencer & Ellis (1998) concluded that with nutrient enrichment, the phytoplankton community has the potential to increase to high levels but that this potential may not be realised if zooplankton densities are high.

In the mesocosms of the present work nutrient enrichment did not increase neither total zooplankton density nor any of the zooplankton groups. Probably nutrient addition affects only the biomass of the zooplankton community and not zooplankton density.

In their mesocosm experiments Romo *et al*. (2004) found that planktivorous fish markedly influence zooplankton community structure in shallow Mediterranean lakes, removing larger zooplankters and increasing the abundance of smaller species, particularly rotifers. This affected algal community composition more than algal biomass. Removal of cladocerans by mosquito-fish (*Gambusia holbrooki*) was complete even at the lower fish density, whereas cyclopoid copepods were less affected by fish, especially at intermediate nutrient levels. Escape movements of cladocerans are less effective than those of copepods (Drenner & MacComas, 1980; Gliwicz, 2002). Similar results have been reported in enclosure experiments involving other fish species (Bertolo *et al*., 2000b).

Schriver *et al*. (1995) observed in a mesocosm experiment that at increasing fish densities, zooplankton dominance shifted from large-sized cladocerans to cyclopoids.

Vakkilainen *et al*. (2004) summarized the result of the response of zooplankton to nutrient enrichment and fish predation studied in 1998 and 1999 by carrying out eleven parallel mesocosm experiments in six lakes across Europe. Nutrient enrichment had a marginally significant multivariate effect on zooplankton only in 1998, when it increased the biomass of small crustacean grazers and, in all sites except Valencia, decreased that of raptorials. The responses of large crustacean grazers to nutrient enrichment were mostly negligible in fish-free treatments, and negative in fish treatments. Thus, neither the main effect of nutrients nor the joint effect of nutrients and fish were significant on large grazers. Fish had a highly significant multivariate effect on zooplankton in both years. In general, the biomass of rotifers increased, and those of all other functional groups decreased with increasing fish density. In the absence of fish, crustacean grazers and raptorials responded positively to nutrient enrichment, rotifers mostly negatively.

In contrast to the traditional equilibrium theory of food chain dynamics and its modifications (Hairston *et al*., 1960; Fretwell, 1977; Oksanen *et al*., 1981; Scheffer, 1991), the higher zooplankton biomass observed by Vakkilainen *et al*. (2004) in fish treatments compared with fish-free treatments, especially in 1998, shows that total zooplankton biomass may increase with nutrients independently of the number of trophic links in the food chain. Several field and laboratory studies of aquatic food chains have shown similar increases with nutrient enrichment in both two- and three-level systems (Mazumder, 1994; Leibold *et al*., 1997; Persson *et al*., 2001). In Vakkilainen *et al*. (2004), available food resources appeared to determine total zooplankton biomass, whereas fish regulated relative abundances of different functional groups. This emphasises the importance of compositional changes within trophic levels and reveals the limitation of simple food-chain and ratio-dependent models to capture such changes, as pointed out by Leibold *et al*. (1997) and Hulot *et al*. (2000). In addition, the result that algal biomass was positively related with TP concentration even in the presence of efficient grazers has often been observed in previous studies (Hansson, 1992; Mazumder, 1994; Brett & Goldman, 1997; Leibold *et al.*, 1997; Shuter & Ing, 1997; Persson *et al.*, 2001). It reflects the positive response of adjacent trophic levels and, therefore, is not consistent with traditional food-chain theory.

In the present work increase in the biomass of rotifers was generally associated with an increase in chlorophyll a, indicating the low ability of small zooplankton to control total phytoplankton biomass. This is also observed in Vakkilainen *et al.* (2004).

Rotifers are highly specialised suspension-feeders and are not able to control total algal biomass, at least in eutrophic systems, because they feed mainly on nanoplankton such as bacteria, flagellates, ciliates, and small algae (Pourriot, 1977; Gilbert & Bogdan, 1984). In addition, rotifers probably recycle nutrients more effectively than larger crustaceans (Vanni, 2002) and therefore further enhance the availability of nutrients to algae.

The proliferation of rotifers observed in the present experiments and in those of Vakkilainen *et al.* (2004) was probably related to the lower susceptibility to fish predation by rotifers. Additionally, their shorter generation times compared with larger crustaceans allowed the smaller zooplankton to respond quickly to nutrient enrichment.

Thus, results of the present work as well as those of other works show that high fish biomass (20 g dry mass/m^2) leads to generally low grazer biomass and consistently to high algal biomass with increasing nutrient availability. However, when fish biomass is low (4 g dry mass/m^2) grazer biomass can be high and control algal biomass in shallow-lake systems. Fish had a greater role than nutrients in regulating zooplankton density and especially relative abundances of zooplankton functional groups. Cladocerans and copepods were of crucial importance in controlling algal biomass even under high nutrient addition.

In the mesocosms nutrients did not affect zooplankton density in no one of the three fish densities. A reason may be that many algae in the mesocosms are not edible for many zooplankton species.

Moss *et al.* (2004) summarized the results on continental-scale patterns of nutrient and fish effects on shallow lakes in a pan-European mesocosm experiment. There were no interactions of fish and nutrients in either year so the picture that emerges is of a strong effect of nutrients on which are superimposed lesser but still substantial effects of fish. Fish influenced fewer variables than nutrients. Fish also significantly increased rotifer biomass, but reduced biomass of large and small Cladocera and raptorial zooplankters in 1998. In 1999 they decreased the total zooplankton biomass, but increased the proportion of open-water filterers, increased the rotifer biomass and decreased that of large Cladocera. In 1998 and 1999 fish significantly influenced four of the nine zooplankton variables. The influence of fish was thus apparently greater on the upper levels of the food web than on the lower. Nutrients significantly increased total zooplankton biomass and that of open water filterers in 1998, although the increases were small. Nutrients increased total zooplankton biomass, both cyclopoid and calanoid copepod biomasses and in consequence decreased the biomass of open-water filterers in 1999 but increased that of raptorial species. They had no significant effect on other zooplankton variables. Thus in 1998 and 1999 nutrients significantly influenced two and five of nine zooplankton variables respectively. Chi-squared tests showed that the ratios of number of chemical: primary producer: zooplankton variables significantly influenced by either fish or nutrients did not differ between years for either fish effects (χ^2 for fish effects = 1, d.f. = 2, $P > 0.5$) or nutrient effects (χ^2 for nutrient effects = 2.6, d.f. = 2, $P > 0.2$) or all effects (fish and nutrient) taken together ($\chi^2 = 3.6$, d.f. = 5, $P > 0.5$). In percentage terms, the effects of nutrients on chemical, primary producer and zooplankton variables were, on average, 83, 39 and 39. Through food availability, it sometimes increased zooplankton biomass but had lesser influence on community composition. Fish tended to remove the Cladocera, particularly the larger ones (Gliwicz, 2004) and increase the rotifer biomass and sometimes increase the chlorophyll a concentration.

Environmental variables (e.g. temperature) were probably more important than nutrient and fish additions in structuring zooplankton community. During winter zooplankton community is dominated by rotifers in any nutrient and fish treatments.

Jeppesen *et al.* (2004) analysed data from 34 lakes studied for 8–9 years divided into three seasons: summer, autumn/spring and winter, and four lake classes: all lakes, shallow lakes without submerged plants, shallow lakes with submerged plants and deep lakes. They found that fish predation pressure during autumn/spring and winter is as high as during summer, and maybe even higher during winter in macrophyte-rich lakes.

This founding is contrast from what observed in the present work. Spring zooplankton community was completely different from winter one. Probably the lack of benthic macroinvertebrates during winter enhanced fish predation over zooplankton organisms.

Liess *et al.* (2006) manipulated the complexity of trophic interactions by using simple laboratory food webs and complex field food webs in enclosures in Lake Erken. In the complex food web fish probably increased the amount of dissolved nutrients, thus enhancing phytoplankton growth. Fish decreased zooplankton abundance, thus reducing nutrient recycling by zooplankton and nutrient availability of periphyton. Lövgren and Persson (2002) found that fish affected zooplankton nutrient recycling. In Liess *et al.* (2006) however, fish led only to an insignificant decrease in zooplankton biomass. Several studies have found that fish exert a top- down effect on periphyton and phytoplankton through predation on herbivores, and facilitate periphyton and phytoplankton growth through nutrient recycling (McCollum *et al.* 1998; Romare *et al.* 1999; Andre *et al.* 2003). Fish increased dissolved nutrient concentrations and phytoplankton biovolume in pelagic habitats (Attayde & Hansson, 2001). However, this has grater importance in nutrient limiting conditions. In eutrophic lakes fish predation on zooplankton may affect the structure and dynamics of plankton more than fish excretion (Attayde & Hansson, 2001).

This finding may explain the reason why nutrient addition in the mesocosms did not affect zooplancton density. Mesocosms were eutrophic ecosystem, thus a further increase in nutrient addition did not affect phytoplankton growth.

Rejas *et al.* (2005) carried out two enclosure experiments in Laguna Bufeos, a neotropical lake located in the floodplain of River Ichilo (Bolivia). They found that the presence of fish in the enclosures had a strong effect on the community composition of crustacean zooplankton and on the population dynamics of individual species. With the exception of cyclopoid copepods, all crustacean zooplankton species reached higher densities in the enclosures with fish. This indicates that the positive influence of indirect effects of fish were more important than the direct negative effect of mortality because of predation.

In the present work fish predation only increased rotifers density. Crustacean organisms increase observed by Rejas *et al.*

(2005) was probably due to a higher fish predation on benthic macroinvertabrates.

Vanni (1987b) investigated the effects of fish predation and food availability on population densities and demography of zooplankton in Dynamite Lake, Illinois, USA, a lake with a high density of size-selective planktivorous fish and low food levels. Overall, population densities of zooplankton were affected much more by manipulations of food availability than by manipulations of fish predation. The cladocerans *Bosmina longirostris*, *Ceriodaphnia lacustris*, and *Diaphanosoma birgei* were greatly increased in density by elevated phytoplankton levels, in the presence and absence of fish. Demographic analysis revealed that increased densities in response to elevated food levels resulted from both an increase in birth rates (*Bosmina*, *Diaphanosoma*) and a decrease in mortality rates (*Ceriodaphnia*, *Diaphanosoma*). The rotifers *Lecane* and *Monostyla* also increased dramatically in response to elevated phytoplankton densities. Copepods were less responsive to manipulations of food levels, but several taxa exhibited increases in density in response to increased phytoplankton abundance. Few species were reduced in density by fish predation. *Ceriodaphnia* density was reduced by fish more than any other species in the entire community, and the density of even this species was much more affected by food availability.

Ślusarczyk (1997) in seven enclosure experiments analyzed the short-term effects of exposing *Bosmina longirostris* to high levels of fish predation. The density of *Bosmina* did not change significantly in the experimental enclosures, suggesting no direct fish impact on *Bosmina* demography.

However, detailed studies revealed strong fish effects on some demographic and life history parameters of *Bosmina*. While the mean size of individual *Bosmina* decreased, reproduction enhanced in the enclosures with fishes.

The structuring role and predation impact of cyclopoid copepod *Cyclops vicinus* on the rotifer community was studied using *in situ* enclosure experiments in Římov Reservoir by Devetter & Seda (2006). The most preferred, *Synchaeta*, was replaced by *Polyarthra* after *Synchaeta* became extinct and these species were replaced by *Keratella* when both soft-bodied forms were not available in sufficient quantities. Loricated species (*K. cochlearis* and *K. longispina*) were consumed at a slower rate.

Arvola & Salonen (2001) studied the impact of *Daphnia longispina* on the plankton food web in a polyhumic lake where this species comprised almost all zooplankton biomass. They found that *Daphnia* fed heavily of *Keratella cochlearis*. The high density of *Keratella cochlearis* in the mesocosms both in spring and winter may be due to the very low density of *Daphnia*.

Matveev *et al*. (2000) analyzed the relative roles of direct fish effects vs. zooplankton grazing in mesocosm experiments by adding to natural communities large grazing zooplankton (*Daphnia carinata*) and small planktivorous fish (mosquitofish or juveniles of Australian golden perch).

They found that the addition of mosquitofish to communities containing *Daphnia* further reduced the abundance of some small zooplankton (*Moina*, *Keratella*), but increased the numbers of *Daphnia* and adult *Boeckella*. In spite of the likely increase in grazing due to *Daphnia*, the abundance of total phytoplankton and dominant alga *Volvox* did not decline in the presence of mosquitofish but was maintained at a significantly higher level than in control. These results suggest that mosquitofish can promote the growth of phytoplankton in a direct way, probably by recycling nutrients, and even in the presence of large grazers.

These findings are in contrast from what observed in the mesocosms and from many other works.

Predation impact differs depending on the prey species and their highly selective predation has a strong influence on diversity of prey communities (Nagata & Hanazato, 2006).

The present study has demonstrated that the cyclopoid copepods have significant impacts on species composition and diversity of zooplankton community especially in spring. It may be suggested therefore that, in the fish-abundant lakes, the dominance of the small-sized species in the zooplankton communities is induced by fish, and that species composition and diversity of the communities are often controlled by the cyclopoid copepods. These results are also confirmed by Nagata & Hanazato (2006) who used mesocosms to analyze predation impacts and prey community structures by two cyclopoid copepod species coexisting with small-sized herbivorous zooplankton species in a fish-abundant lake.

During the experiments were found different zooplankton species both in mesocosms and lake. Some of these species were indifferent to seasons (e.g. *Keratella cochlearis* and *Polyarthra* *remata*), other were found only in spring (e.g. *Brachionus angularis*) and other in winter (e.g. *Brachionus calyciflorus*, *Filinia longiseta* and *Synchaeta* sp.). The different behaviour may be due to food reason or to susceptibility to predators. Predation impact differs depending on the prey species and their highly selective predation has a strong influence on diversity of prey communities (Nagata & Hanazato, 2006).

K. cochlearis is found year-round, with an optimum growth rate at 15 °C (Walz, 1983). It consumes mainly living algae smaller than 15 μm, especially small green algae (*Stichococcus*), chrysomonads and cryptomonads (Stemberger, 1981), but also detritus (Pourriot, 1977). Bacteria are reported to be a significant food source for *Keratella* spp. and *K. longispina* (Bogdan *et al*., 1980; Ooms-Wilms, 1991; Arndt, 1993; Walz, 1995). *K. cochlearis* tolerates low oxygen concentrations and can reach high densities under these conditions (Elliot, 1977; Ruttner-Kolisko, 1977; Mikschi, 1989).

In contrast, *Polyarthr*a spp., although were found both in spring and winter are particulate feeders, generally feeding on flagellates larger than 15 μm (Bogdan & Gilbert, 1987).

Both *K. cochlearis* and *Polyarthra* spp. consume high-quality food, while the former is a generalist feeder and the latter more specialized.

Heterotrophic nanoflagelates are not the only food source for any planctonic rotifer, but may be an important part of the diet of *Synchaeta* and *Polyarthra* and also *Keratella* (Buikema *et al*., 1978; Stemberger, 1981; Bogdan & Gilbert, 1982; Arndt, 1993; Jurgens *et al*., 1996).

The diets of *F. longiseta* seem to consist of low-quality food.

The rotifers may also differ in their vulnerability to predation pressure. Both the presence of a lorica and the small size of the loricate species (*K. cochlearis*) offer protection against predation, whereas both *Polyarthra* and *F. longiseta*, with their soft cuticle and larger size, are less protected (Stemberger & Gilbert, 1987).

K. cochlearis has a rigid lorica and can produce a posterior spine in the presence of predatory copepods and *Asplanchna* (Stemberger & Gilbert, 1984). Both *Polyarthra* and *F. longiseta* often avoid predators with a rapid movement of their paddle-like or spine-like appendages (Stemberger & Gilbert, 1987). It is expect low vulnerability to predation pressure for *K. cochlearis* and high vulnerability for the soft-bodied rotifers. Predation on *K. cochlearis* is expected to be lower than on bigger aloricate rotifers, such as *F. longiseta* and *Polyarthra*. If both prey types are present, the aloricate, soft-bodied species should be preferred by cyclopoid copepods (Stemberger, 1985).

These results show the importance to consider not only total zooplankton density but also densities of the different zooplankton groups in Mediterranean region lake especially during spring.

4.4 Stomach contents and benthic macroinvertebrates

Eastern mosquitofish *Gambusia holbrooki* is one of the most widely distributed visually-feeding fishes. Mosquitofish was first described as a surface zooplanktivore (Crivelli & Boy, 1987; Hubbs, 1991) and introduced as a species for controlling insects, but it seems to fail in this task and prefers plankton (Blanco *et al*., 2004).

Blanco *et al*. (2004) studied the diet of the eastern mosquitofish *Gambusia holbrooki* with *in situ* experimental mesocosms located in a shallow lake under different nutrient concentrations (phosphorus and nitrogen) and fish population densities. Their experimental results agree with some field data concerning preference of mosquitofish on microinvertebrates (copepods, cladoceran, ostracods and rotifers) (Hurlbert *et al*., 1972; Soto & Hurlbert, 1991; Hansson & Carpenter, 1993; Cabral *et al*., 1998; García-Berthou, 1999), with scarce predation on insect larvae (Rodríguez, 1989).

Also Ciuffa (2003) and Ciuffa *et al*., 2006 studied mosquito fish diet in a semi-natural ecosystem. They found that mosquitofish could prey over microzooplankton and macroinvertebrates as well.

This species was also reported to feed on benthic animals (Cech *et al*., 1992), but Blanco *et al*. (2004) experimental research and some other field data (Cabral *et al*., 1998; Stober *et al*., 1998) confirm that benthic animals (*e.g.* chironomids) are a small fraction of mosquitofish diet. In their study, Blanco *et al*. (2004) found that nutrient loading or fish density scarcely affected predation on macroinvertebrates, although nutrients affected macroinvertebrate populations (Miracle *et al*., 2006). Mosquitofish fed mainly on rotifers, copepods and ostracods. Cladocerans, even though scarce in the plankton, were positively selected especially by females. Removal of large efficient filter feeders favoured significantly rotifer dominance in the plankton and by top-down cascade that of small cyanophytes (Romo *et al*., 2004).

In mesocosms of the present work nutrient had no influence on fish predation. Nutrients addition did not increase zooplankton density.

In Blanco *et al.* (2004) nutrient concentrations had a low influence on mosquitofish predation. Fertilization led to an increment of prey density (macro- and microinvertebrates) in their experiments and enhanced phosphorus levels in fish mesocosms, especially in 1998, when turbid states predominated (Romo *et al*., 2004). Then it is likely that fish enhanced prey number by means of indirect trophic effects (*e.g.* phosphorus recycling). Data show that mosquitofish had mainly an omnivorous diet under a wide range of nutrient gradient (0–1 mg/l P and 0–10 mg/l N), with an important consumption of algae during the study.

Only in the Florida Everglades was recorded a similar dietary pattern with algae constituting more than half of the gut content biomass (Stober *et al*., 1998; Rawlik, 2001). Main algal groups ingested were similar to those found in the phyto- and epiphyton, suggesting a non-selective predation but a “passive feeding” as has been noticed in other planktivorous species (O’Brien, 1987; Sanderson *et al*., 1998). Detrital matter was also an abundant food component. Mosquitofish is known to feed on plant matter and detritus (Stober *et al*., 1998; García-Berthou, 1999). In other fish species, detritivory may mean absence or inaccessibility of more profitable prey, and often involves lower feeding efficiency and enhancement of indirect effects (Lobón-Cerviá & Rincón, 1994), which can contribute to maintain an algal turbid state in shallow lakes.

Blanco *et al*. (2004) found that in 1998, mosquitofish shifted from detritivory to zooplanktivory with higher fish stocks, suggesting that there was no food limitation despite dense populations. This is also consistent with the observed absence of an effect of fish number on microinvertebrate electivity. The hypothesis that higher fish stock should increase predation on prey is confirmed but, according to the optimal foraging theory (see, *e.g.*, Bence & Murdoch, 1986), they should select larger prey, which did not occur. Even more mosquitofish size was unrelated to prey body-size. This species behaviour was opportunistic taking the most abundant and visible prey; in both years, preferred ostracods followed by cyclopoids and cladoceran. Motion of cladoceran has disadvantages to escape fish predation compared with that of copepods (Drenner & McComas, 1980). Higher fish stock did no significantly affect macroinvertebrate consumption on macroinvertebrates.

Margaritora *et al.* (2001) studied predatory impacts of the mosquitofish on zooplankton in a pond at Tenuta di Castelporziano over two periods (1985-1987; 1995-1998). They found that, in most habitats, *Gambusia* feeds selectively on conspicuous zooplankton, selecting large and actively moving individuals (Zaret, 1980) and consequently influences the structure and dynamics of aquatic communities (Margaritora, 1990). In 1985-87, no zooplankters longer than 1 mm were found in the pond studied. The dominance of small zooplankters, especially rotifers and larval copepods, is probably related to the presence of *Gambusia* and its selective predation of the large-bodied species. In fact, although mosquitofish are generalist feeders, they demonstrate some prey size selectivity (Miura *et al.*, 1979; Farley 1980; Garcia-Berthou, 1999). In most habitats, mosquitofish diet consists of zooplankton, namely ostracods and copepods (Soto & Hurlbert, 1991), with a preference for cladocerans in many biotopes, such as seminatural ponds (Miura *et al.*, 1979), rice fields (Blaustein & Karban, 1990), and drainage channels (Crivelli & Boy, 1987). In 1995-98, because of the elimination of fish predation, there was a marked increase in large-bodied species, e.g. Daphnidae and adult calanoids, and a corresponding decrease in the population density of the smaller organisms. The larger species, more efficient in collecting small organic particles and capable of collecting larger particles as well, succeeded in competitively excluding the smaller ones. The elimination of fish predation thus shows that *Gambusia* can influence the zooplankton community both directly, by killing the larger individuals and indirectly, by altering competitive forces within the herbivorous zooplankters.

Hurlbert & Mulla (1981) investigated the effects of mosquitofish (*Gambusia affinis*) predation in 12 experimental ponds in southern California over a period of 10 months. *Gambusia* essentially eliminated *Daphnia pulex* and *Ceriodaphnia* sp. populations, reduced *Diaptomus pallidus* and *Keratella quadrata* populations, had little impact on *Cyclops vernalis*, and caused large increases in *K. cochlearis*, *Polyarthra* sp., *Synchaeta* sp., and *Trichocerca* spp. populations and in total phytoplankton. *Gambusia* caused a decrease in the PIE (probability of interspecific encounter) of the planktonic crustaceans and an increase in the PIE of the planktonic rotifers. Hemiptera, such as neustonic *Microvelia* sp. and nektonic

Buenoa sp. and *Notonecta* sp., and *Hyla regilla* tadpoles were absent from fish ponds but sometimes abundant in control ponds. *Gambusia* caused higher pH and oxygen levels, presumably via its effect on the phytoplankton. The impact of *Gambusia* on the pond ecosystems was less in winter, when fish numbers and feeding rates were low, than in summer.

Similar results were found in the present work were Gambusia had no effects on zooplankton community during winter

Cech *et al.* (1992) in their laboratory experiments demonstrated that mosquitofish preyed all invertebrate species preferring *Culex* and *Daphnia* instead of Corixidae and amphipidos.

Diet of *Gambusia affinis* in natural systems in southern France was considered by Crivelli & Boy (1987). In no-reproductive period (October-May) copepods and cladocerans (*Chyrodidae* and *Daphnidae*) were the preferred preys, while during reproductive period insects represented the 80% of the diet of mosquitofish.

In an experiment performed by Hurlbert *et al.* (1972) in plastic tanks, *Gambusia* reduced densities of *Daphnia pulex*, Culicidae and Chironomidae until their complete disappearance allowing rotifers to increase. Afterwards the lacking of preferred preys *Gambusia* preyed on rotifers determining a rapid collapse. Subsequently algal bloom determined turbid water.

In small ponds (36 m^2) *Gambusia* reduced densities of Dytiscidae, Chaoboridae, Notonectidae and Odonata (Walton & Mulla, 1991). In experimental mesocosms *Gambusia* determined blooms of diatoms, filamentous cyanobacteria and green algae, increase of rotifers and decrease of copepods, cladocerans and chironomidae (Lancaster & Drenner, 1990).

In rice-field *Gambusia* reduced Odonata, Corixidae, Notonectidae and Dytiscidae, copepods, cladocerans and ostracods (Kramer *et al.*, 1988). Same results were obtained in a fitodepuration lagoon for wastewater treatment (Casteberry & Cech, 1990).

Miura *et al.* (1984) studied impacts of *Gambusia affinis* on rice-field. Addition of 0.022 g/m^2 of fish did not affect densities of copepods, ostracods, Corixidae and Odonata, but reduced densities of cladocerans, Ephemeroptera, Notonectidae and Chironomidae.

Blaustein (1992) in rice-field with an addition of 2318 *Gambusia* individuals/ha observed significative reduction of Notonectidae, while Ephemeroptera, Coleoptera, copepods, cladocerans and ostracods did not reduce their density.

The view that warmer lakes are predominantly controlled by bottom-up mechanisms (Lazzaro, 1997), was in part supported by results of Romo *et al*. (2004), since with similar functional fish species, clear water phases were maintained in those lakes with submerged plants and low nutrient levels. Overall, more comparative studies from tropical, subtropical and warmer areas, such as the Mediterranean are needed.

In conclusion, experiments showed a greater impact of mosquitofish on macroinvertebrates than on zooplankton in spring. On the contrary mosquitofish had a greater impact on zooplankton in winter.

Removal of large filter feeding animals such as cladocerans and copepods favoured dominance of rotifers. In general, nutrient concentrations had a low influence on mosquitofish predation.

Chapter 5. CONCLUSIONS

Results obtained from these mesocosms experiments may lead to several considerations. First of all nutrients and fish had different effects on plankton community. Moreover these effects were different between seasons.

Phytoplankton biomass estimated as chlorophyll a increased both in increasing nutrient loading and fish density, both in spring and winter. The increase was more evident in spring. In particular chlorophyll a increased in high nutrient addition and high fish density.

Nutrients did not increase neither total zooplankton density nor density of any zooplankton groups. This was true in spring and in winter.

Fish did not increase total zooplankton density neither in spring nor in winter. Otherwise zooplankton community structure changed. In spring, with increasing fish density rotifers increased, while cladocerans and copepods decreased. In winter with increasing fish density rotifers did not change their density, but cladocerans, copepods and nauplii decreased. Probably planktivory is enhanced in winter because benthic macroinvertebrates are scarce and fishes prey mainly on planktonic organisms.

Some significant considerations emerge from these results.

Nutrients had no effect on zooplankton community contradicting many other studies carried out in northern countries.

Fishes increased phytoplankton biomass both decreasing herbivores and enhancing nutrient regeneration.

Fish predation did not affect total zooplankton density, but had diverse effect on zooplanktonic groups.

In addition presence of benthic macroinvertebrates was very important to the outcome of the experiments.

These results enhance the importance to study nutrient and fish additions in freshwater lakes to improve knowledge on these ecosystems.

In conclusion nutrients and fish control can have different effects on plankton community. These considerations should be taken into account for the the restoration of eutrophicated lakes.

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