UNIVERSITAT DE BARCELONA
Facultat de Biologia
Departament d'Ecologia

Tesi doctoral

PROCESOS HETEROTRÒFICS MICROBIANS A L'EMBASSAMENT DE SAU

HETEROTROPHIC MICROBIAL PROCESSES IN THE SAU RESERVOIR

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"..no riñais a la niña por estar siempre absorta… de mayor será científica!"
Alfredo Comerma

A mis abuelos,
a mis padres,
a mis hermanos
y a Juan Carlos,

gracias por estar ahí.

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AGRAÏMENTS

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Talment com una cursa d'orientació… tot un repte! Una gran aventura que ja arribat a un final, que no la fi.

Un bon dia el Joan Armengol em va oferir un mapa per on fer una bonica i llarga cursa d'orientació, jo vaig acceptar i, de seguida, em van aconsellar la millor brúixola del mercat, en Karel Šimek. Un, quasi m'ha fet de pare, i, l'altre, m'ha donat molt més que una bona base científica, un punt de referència en tot moment i una molt bona amistat.

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En començar, davant del mapa tot et sembla igual. Però no, mirant i mirant, trobes aquells elements de referència que et fan situar i t'ajuden a prendre decisions. Aquí és on haig d'agrair a la gent del GREAC la seva bona acollida, el seu suport, la seva companyia. Al Sergi li vull agrair la mirada crítica al meu primer article, en vaig aprendre molt. Al Quico, les interessants converses per tornar cap a Mataró… La Isabel sempre m'ha animat molt. L'Helena, l'Anna Mª, l'Andrea, la Cesca, l'Eulàlia, el Joan, l'Enrique, la Núria, les Montses, Anna… buf! no sabeu tot el què he après amb vosaltres (a fer anar programes, a fer gràfics, a trobar les coses i treballar al laboratori, …), m'heu donat molta seguretat en el camí. Però em quedo amb lo més important, la vostra amistat. Als que heu vingut després, Esther, Susana, Rafa, Eli, David, Vicenç, Mari, Gora, Ainoha, … també us haig d'agrair molts bons moments. Sou un gran equip.

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INTRODUCCIÓ

LA XARXA TRÒFICA MICROBIANA

La visió clàssica del funcionament de la cadena tròfica planctònica es basa en la producció autòtrofa de matèria orgànica pel fitoplancton, que és consumida pel zooplancton, el qual pot ser consumit pels peixos. Mentre que les biomasses i les productivitats d'aquests organismes han estat sempre elements naturals als treballs d'ecologia aquàtica, els microorganismes no es van considerar fins molt més tard en l'anàlisi integral de l'ecosistema. Al 1974, POMEROY va ajudar a canviar aquest plantejament mitjançant la proposició d'un paradigma alternatiu per a la xarxa tròfica pelàgica marina. El nou plantejament argumentava que els microorganismes, la biomassa del quals és aproximadament igual a la del tradicional plàncton "de xarxa", podien mobilitzar més matèria i energia donat que les seves taxes metabòliques són mes elevades. Ben aviat va aparèixer el concepte de "microbial loop" (AZAM *et al.*, 1983). Al principi es va creure que el llaç microbià consistia en bacteris, flagel·lats fagotròfics i altres protozous com els ciliats, i que tenia la seva base als exudats de matèria orgànica del fitoplancton. En aquest llaç, el carboni del compartiment bacterià havia de fluir fins el zooplancton en successius nivells de predació: flagel·lats sobre bacteris, ciliats sobre flagel·lats, i zooplancton sobre flagel·lats i ciliats, incorporant així el carboni bacterià a la cadena tròfica clàssica. Aquests arguments foren igualment vàlids per les aigües dolces, les quals tenen fonamentalment la mateixa estructura planctònica. PORTER *et al.* (1979) i SHERR i SHERR (1988) van senyalar el paper dels protozous com enllaços dins de les xarxes tròfiques planctòniques a les aigües dolces, representant un important mecanisme pel qual el carboni orgànic dissolt (DOC), el carboni orgànic particulat (POC) i els bacteris passaven a formar part de la xarxa tròfica planctònica.

A la dècada dels anys 70, una metodologia específica desenvolupada per l'estudi dels microorganismes va canviar el concepte clàssic de la cadena tròfica, on els organismes més abundants, més

permanents, més diversificats i més àmpliament distribuïts havien estat ignorats (PEDRÓS-ALIÓ, C. i GUERRERO, R., 1994). La microscopia d'epifluorescència (PORTER and FEIG, 1980) i les tècniques amb isòtops radioactius (RIEMANN, 1984; KIRCHMANN *et al.*, 1985) van fer possible el descobriment de nous organismes més actius i abundants del que es pensava fins llavors. Des dels anys 80 fins a mitjans dels anys 90, un interès creixent es va abocar a la incidència, la taxonomia i la funcionalitat dels organismes microbians del plàncton. Malgrat que l'esforç es va fer a tota mena d'ambients aquàtics, els resultats més coherents van sorgir de l'estudi dels ecosistemes marins.

Les noves informacions sobre el paper que juguen els bacteris i el protozous planctònics revelen que les interaccions dels microorganismes són més complexes que el que es recollia en les descripcions inicials. Bona part de la producció primària és canalitzada a través de diversos compartiments del llaç microbià en forma de DOC i POC, abans de ser disponible per al zooplancton. En cossos d'aigua temperats i tropicals, els microorganismes poden arribar a consumir més de la meitat de l'energia fixada per la fotosíntesi (POMEROY i WIEBE, 1988). El DOC autòcton que és utilitzat pels bacteris no procedeix únicament dels fotosintats exudats per les algues (RIEMANN i S0NDERGARD, 1986), sinó que fan servir també DOC excretat pel zooplancton, derivat de l'activitat d'alimentació del zooplancton (LAMPERT, 1978), i el que prové de la lisi de bacteris i fitoplancton produïda per l'activitat dels virus (BRATBAK et al., 1992). Al llarg de la darrera dècada, les quantitats de virus reportats en diversos sistemes marins i d'aigua dolça han estat molt elevades (10^8 virus ml^{-1}; BERGH *et al*., 1989; HARA *et al*., 1991; MARANGER i BIRD, 1995), i sembla ser que constitueixen un component molt dinàmic de la comunitat microbiana. Mitjançant la infecció d'organismes cel·lulars i la subsegüent lisi, l'activitat dels virus alimenta la respiració i la producció heteròtrofa bacteriana. La predació dels protozous a les aigües dolces pot controlar les abundàncies bacterianes (BERNINGER *et al*., 1991). Els flagel·lats i els ciliats petits també poden regular la biomassa i l'espectre de mides del picofitoplancton (WEHR, 1991). Els ciliats més grans (>100 μm) s'alimenten en canvi de bacterioplancton i nanofitoplancton. Rotífers

(ARNDT, 1993) i copèpods (WICKMAN, 1995; DOBBERFUHL *et al.*, 1997) són consumidors eficients del fitoplancton i de tots el components del llaç microbià (bacteris, nanoflagel·lats heteròtrofs i ciliats). Algunes espècies de cladòcers (especialment *Daphnia*) s'alimenten de partícules de la mida de les bactèries i competeixen amb els protozous pel carboni i la resta de nutrients (SANDERS i PORTER, 1990; PACE et al., 1990; PACE i FUNKE, 1991). Resumint, els bacteris i els seus consumidors poden exercir efectes tant directes com indirectes a les relacions tròfiques, degut a que són a la vegada recursos alimentaris suplementaris, competidors, i depredadors, a tots els nivells de la xarxa tròfica clàssica. Per totes aquestes observacions, el concepte del llaç microbià va canviar des de la idea de "nivells ordenats de transferència de matèria i energia" fins a la idea de "una complexa xarxa tròfica". Així, es proposa que el llaç microbià és un component integral d'una xarxa tròfica més gran (SHERR i SHERR, 1988).

Tot i això, encara no està del tot clar quina rellevància tenen els microorganismes i les seves interaccions en els ecosistemes aquàtics eutròfics (WEISSE, 1991; WEISSE and STOCKNER, 1993; Del GIORGIO i GASOL, 1995). Encara que l'abundància dels microorganismes augmenta amb l'increment de les càrregues de nutrients i de la producció primària, es creu que la contribució relativa de la xarxa tròfica microbiana al flux del carboni decreix en augmentar l'eutròfia dels ecosistemes estudiats. Contràriament, RIEMANN i CHRISTOFFERSEN (1993) suggeriren un increment de la importància relativa del llaç microbià en comparació amb la cadena tròfica clàssica al llarg d'un gradient de productivitat creixent.

El nostre grup de recerca es va proposar avaluar la importància de la xarxa tròfica microbiana a l'embassament de Sau. Aquest ecosistema eutròfic es venia estudiant des dels anys 60, però no es coneixia la importància dels seus microrganismes. Aquesta tesi forma part d'un projecte més ampli que estudia els processos autòtrofs i la seva connexió amb les activitats heteròtrofes a l'epilimniom de l'embassament de Sau, posant un interès especial en els gradients longitudinals (des de l'entrada del riu Ter fins a la presa).

ZONACIÓ LONGITUDINAL ALS EMBASSAMENTS

Els embassaments acostumen a tenir una forma allargada i estreta, i així és el cas de Sau. L'entrada del tributari principal que aporta aigua i nutrients es troba allunyada del punt de sortida (generalment a la presa). Aquesta morfologia permet qualificar els embassaments com sistemes híbrids entre rius i llacs (MARGALEF, 1983), donat que mostren una progressiva transformació des de l'entrada del riu (amb característiques de riu) fins a la zona de la presa (amb característiques de llac). Els embassaments també ocupen una posició intermèdia entre els rius i els llacs naturals pel que fa a l'origen de la matèria orgànica que entra en aquests sistemes, així com per les seves característiques morfològiques i hidrològiques.

Normalment es distingeixen tres zones al llarg de l'eix longitudinal d'un embassament (KIMMEL *et al*., 1990):

1) La zona més propera a l'entrada del riu, **zona fluvial,** és un sistema lòtic. Es caracteritza per un moviment ràpid de l'aigua, temps de residència curts, nivells de nutrients i sòlids en suspensió elevats, i una penetració de la llum més baixa que a la resta de l'embassament.

2) La **zona** de **transició** es caracteritza per una elevada productivitat i biomassa del fitoplancton que es pot relacionar amb el descens de la velocitat de l'aigua (o increment del temps de residència, lligats a l'augment d'amplada i fondària), una important sedimentació de les argiles i llims des de les capes superficials, i un increment de la penetració de la llum. La zona de transició sovint es troba molt relacionada amb el punt d'enfonsament del riu sota la capa superficial d'aigua de l'embassament (veure Capítol 1).

3) La **zona lacustre** de l'embassament es l'àrea més propera a la presa, amb temps de residència de l'aigua elevats,

concentracions de nutrients dissolts i partícules inorgàniques en suspensió baixes, una major transparència de l'aigua i una capa fòtica més fonda que a la resta de l'embassament.

Les tres zones no són unitats discretes dins de l'embassament, altrament, són temporalment i espacialment dinàmiques. Les seves extensions varien en funció de l'escorrentia de la conca, les característiques dels aports (intensitat, densitat) i de la gestió que es faci de l'embassament (fondària i cabals de sortida). Qualsevol zona pot ser més o menys important en funció de les condicions hidrològiques. Com que no sempre és fàcil distingir aquestes zones, alguns autors (UHLMANN, 1991) prefereixen considerar l'embassament com un sistema homogeni i només tenir en compte les entrades i les sortides. Sota aquesta perspectiva, comparen el funcionament de l'embassament amb un quemostat.

Els embassaments poden desenvolupar gradients longitudinals molt acusats. Llavors, la condició tròfica de l'embassament canvia gradualment de l'eutròfica a l'oligotròfica, seguint el gradient de variacions físiques i químiques, i de processos biològics i fisiològics. Tal i com veurem al llarg dels capítols successius, aquest és el cas de l'embassament de Sau.

ÀREA D'ESTUDI I ANTECEDENTS

L'embassament de Sau es troba encaixat en una vall, cosa que li confereix una morfologia llarga (18.5 km de longitud) i estreta (fondària màxima de 84 m). Es localitza al tram mig del riu Ter, què neix als Pirineus, a 2500 m s. n. m., i flueix al llarg de 208 km fins el Mediterrani (SABATER *et al*., 1991).

Dintre de Catalunya, aquest és un embassament de capacitat intermèdia. Això, afegit a què es troba en una regió de clima mediterrani, fa que la hidrologia del sistema sigui molt variable entre anys. Dos dels anys del nostre període d'estudi (1999 i 2000) van ser clarament secs amb uns nivells de reserva d'aigua molt baixos.

Sau és la primera reserva d'aigua en una cascada de tres embassaments destinats a l'abastament d'aigua potable per a Barcelona i la seva àrea metropolitana. La seva importància socio-econòmica justifica que hagi estat estudiat des del primer emplenat al 1963. La sèrie de dades de què disposem (40 anys) és la més llarga d'aquest tipus a Espanya, recollint informació dels principals paràmetres químics i biològics (biomasses i diversitat del fitoplancton i zooplancton). Amb tot, el coneixement sobre l'ecologia d'aquest sistema aquàtic es fonamenta, principalment, en dades recollides mensualment, amb perfils verticals, dins l'àrea més propera a la presa.

Els estudis precedents descriuen Sau com un embassament monomíctic, amb un patró anual d'estratificació tèrmica a l'estiu (des d'abril fins a setembre) i de barreja a l'hivern (VIDAL, 1977). Alguns aspectes de l'estructura tèrmica i dels patrons de circulació interna han estat recentment estudiats (HAN *et al*., 2000), aplicant un model de simulació hidrodinàmica (1D, DYRESM). Les simulacions han demostrat la gran influència del riu Ter a l'estructura tèrmica a Sau. També s'ha demostrat que l'efecte del temps de residència sobre l'estructura tèrmica es manifesta en canvis a la fondària de la termoclina.

El procés d'eutrofització que ha patit l'embassament de Sau, des de 1963 fins 1990, també es troba ben documentat (VIDAL, 1977; VIDAL, 1993; ARMENGOL i VIDAL, 1988; ARMENGOL *et al*., 1994). A partir de 1991, es van construir i posar en funcionament diverses estacions depuradores d'aigües residuals a la part alta de la conca del Ter. Aquestes depuradores, en un principi, només disposaven de tractaments físico-químics. Aquest tipus de tractaments retiren el fòsfor dissolt de l'aigua força eficaçment, cosa que va provocar el descens de les càrregues d'aquest nutrient a l'embassament a partir del 1994. Fins a la implantació de tractaments biològics addicionals (1998) no es va apreciar una disminució de les càrregues de nitrogen. Tot i la reducció de l'entrada de nutrients, les dades recents mostren que les concentracions de clorofil·la *a* a l'embassament de Sau continuen sent pròpies de sistemes eutròfics (MASON, 1996). No es descarta una milloria en l'estrat tròfic de Sau en el futur. La recollida de dades endegada al 1963 continua efectuant-se mensualment.

En els estudis previs de l'ecologia del plancton a Sau, la comunitat microbiana no es va considerar. Aquests estudis es centraven en la taxonomia, i en les mesures de biomassa del fitoplancton i el zooplancton. La metodologia indicada per al fito- i zooplancton no era l'apropiada pels components microbians del plancton per diversos motius:

- es recollia les mostres amb xarxes que permeten el pas dels bacteris, els flagel·lats i la majoria de ciliats (53 μm∅);
- s'utilitzava fixadors extremadament agressius pels flagel·lats i ciliats, que resultaven en cèl·lules que no es podien reconèixer;
- les pressions aplicades a la filtració (>20mm Hg) de les mostres eren massa elevades pels protistes, provocant el trencament de les cel·lules;
- els augments de la microscopia òptica per enumerar fito- i zooplankton eren massa baixos per poder detectar els microorganismes.

Tan sols un projecte d'estudi dirigit a les poblacions microbianes es va dur a terme a l'agost de 1994. D'aquest treball es va publicar un article sobre la presència de virus de mida gran a l'embassament de Sau (SOMMARUGA *et al*., 1995). Després d'aquest treball es van obrir noves qüestions sobre l'ecologia de l'embassament, principalment pel que fa a la microbiologia i l'heterogeneïtat longitudinal (no tractada fins llavors en termes d'ecologia microbiana). Podem dir que va ser l'origen del present estudi. Així, vaig començar la tesi doctoral al gener de 1997, assumint l'estudi de l'ecologia microbiana a l'embassament de Sau. El Dr. Karel Šimek, que col·labora amb el nostre grup des de 1994, em va introduir en aquesta àrea i em va ensenyar la metodologia adequada.

OBJECTIUS DEL PRESENT ESTUDI

Fins l'any 1996, pocs estudis s'havien dut a terme a Sau sobre els micoorganismes planctònics i el seu paper en l'ecologia de l'embassament, tot i que molts altres aspectes d'aquest sistema es coneixien àmpliament. L'objectiu principal que ens vam marcar per la present tesi pretenia caracteritzar la xarxa tròfica microbiana d'aquest ecosistema eutròfic, amb elevades aportacions de carboni orgànic al·locton.

En primer lloc, volíem conèixer els rangs d'abundància i tenir una aproximació a la composició específica de bacteris i protistes (flagel·lats heteròtrofs i ciliats) a l'epilimnion de l'embassament. El nostre treball també es va centrar en l'estudi de les activitats microbianes: producció bacteriana i taxes de bacterivoria dels protozous.

Vam descobrir una marcada heterogeneïtat espacial en els paràmetres físics, químics i biològics des de el riu fins a la presa; pel que es va posar una atenció especial a aquests gradients. Volíem arribar a descriure les forces que controlaven la zonació longitudinal al llarg de l'embassament.

Després de 5 anys d'estudi (1996-2000), vam ser capaços d'avaluar la contribució de la xarxa microbiana pelàgica dins de l'embassament com a font de carboni per a nivells tròfics superiors.

Els resultats d'aquest treball es presenten en sis capítols. Cadascun ha estat escrit con un article independent, centrat en un tema particular amb la seva introducció i la seva discussió. Alguns d'ells estan publicats i altres estan en procés de publicació. He estat afortunada de que el nostre grup de treball hagi estat molt actiu durant el període 1996-2000, duent a terme moltes col·laboracions amb altres científics que han donat com a resultat una bona quantitat de publicacions a les que apareix-ho com coautora. En aquest treball, però, només es presenten els resultats que he processat personalment per tal d'assolir els objectius proposats. Les publicacions produïdes pel nostre grup han estat incloses com a

comentaris, amb les seves cites bibliogràfiques corresponents, per tal d'aclarir les qüestions relacionades amb la xarxa tròfica microbiana.

Els capítols han estat ordenats de manera lògica, començant per les principals forces en el control de la xarxa tròfica microbiana e introduint-hi qüestions més específiques relacionades amb les diferents particularitats en aquest embassament (els components més comuns i la seva activitat a l'epilimnion). Els objectiu específics en cada capítol es resumeixen a continuació:

- Capítol 1. Es fa una descripció de les principals forces físiques que actuen en l'eix longitudinal de l'embassament. L'objectiu va ser caracteritzar la variació estacional en el patró de circulació al llarg de l'embassament.
- Capítol 2. S'examinen els gradients químics longitudinals i la seva variació en funció de les condicions físiques. L'objectiu principal va ser aclarir la importància dels elevats aports al·lòctons de nutrients en la composició química de l'epilimnion, i com aquests nutrients són processats al llarg de l'embassament.
- Capítol 3. Es mostra un transecte longitudinal amb clars gradients en les activitats biològiques. Es descriu amb detall com els diferents grups d'organismes planctònics es desenvolupen seqüencialment a partir dels aports que fa el riu.
- Capítol 4. S'agrupen els diferents transectes per el seu estudi integrat. Es descriuen els patrons generals de distribució i les activitats microbianes en aquest embassament. També es detallen la diversitat i les activitats especifiques dels principals components del plancton microbià.
- Capítol 5. Es fa una recapitulació de tots els resultats en termes de biomassa i producció i es prova de donar una visió general dels fluxos de carboni en el context d'una successió longitudinal de la comunitat pelàgica. L'objectiu d'aquest estudi va ser avaluar la importància de la xarxa tròfica microbiana a Sau.
- Capítol 6. Es comparen les taxes específiques de creixentment dels nanoflagel·lats heteròtrofs i dels ciliats en un experiment amb

microcosmos. L'objectiu va ser conèixer aquestes taxes de creixement i comprovar si la xarxa tròfica microbiana es troba regulada per la depredació del zooplancton o per la disponibilitat de recursos.

INTRODUCTION

THE MICROBIAL FOOD WEB: COMPONENTS AND THEIR IMPORTANCE IN THE PELAGIC FOOD WEB

The classical view of the trophic functioning of the planktonic food chain is the autotrophic production of organic material by phytoplankton is consumed by zooplankton, which in turn is consumed by fish. While the size and the productivity of these large body organisms were natural elements in aquatic studies under this view, microorganisms were not integrated into the analysis of whole ecosystems. POMEROY (1974) challenged this point of view by proposing an alternative paradigm for the marine pelagic food web. He argued that microorganisms, whose biomass is approximately equal to that of the traditional "net" plankton, were potentially greater movers of energy and materials because of their higher metabolic rates. The "microbial loop" concept soon appeared (AZAM *et al.*, 1983). Initially the loop was believed to consist of bacteria, phagotrophic flagellates and other protozoans, such as ciliates, and based primarily on organic exudates of the phytoplankton. In this loop, bacterial carbon content would flow to zooplankton in successive predation steps of flagellates on bacteria, of ciliates on flagellates, and of zooplankton on flagellates and ciliates, thus reaching the classical food chain (Fig. I.1). These arguments were equally valid for freshwaters, which possess the same fundamental planktonic structure. In this way, PORTER *et al.* (1979) and SHERR and SHERR (1988) point out the role of protozoans as links in freshwater planktonic food chains, representing an important mechanism by which dissolved organic matter (DOC), particulated organic matter (POC) and bacteria enter planktonic food chains.

In studies from 1980 to 1995, increasing attention was paid to the occurrence, taxonomy and functional role of the microbial plankton

organisms, in all types of aquatic environments, although more comprehensive data from marine systems were collected.

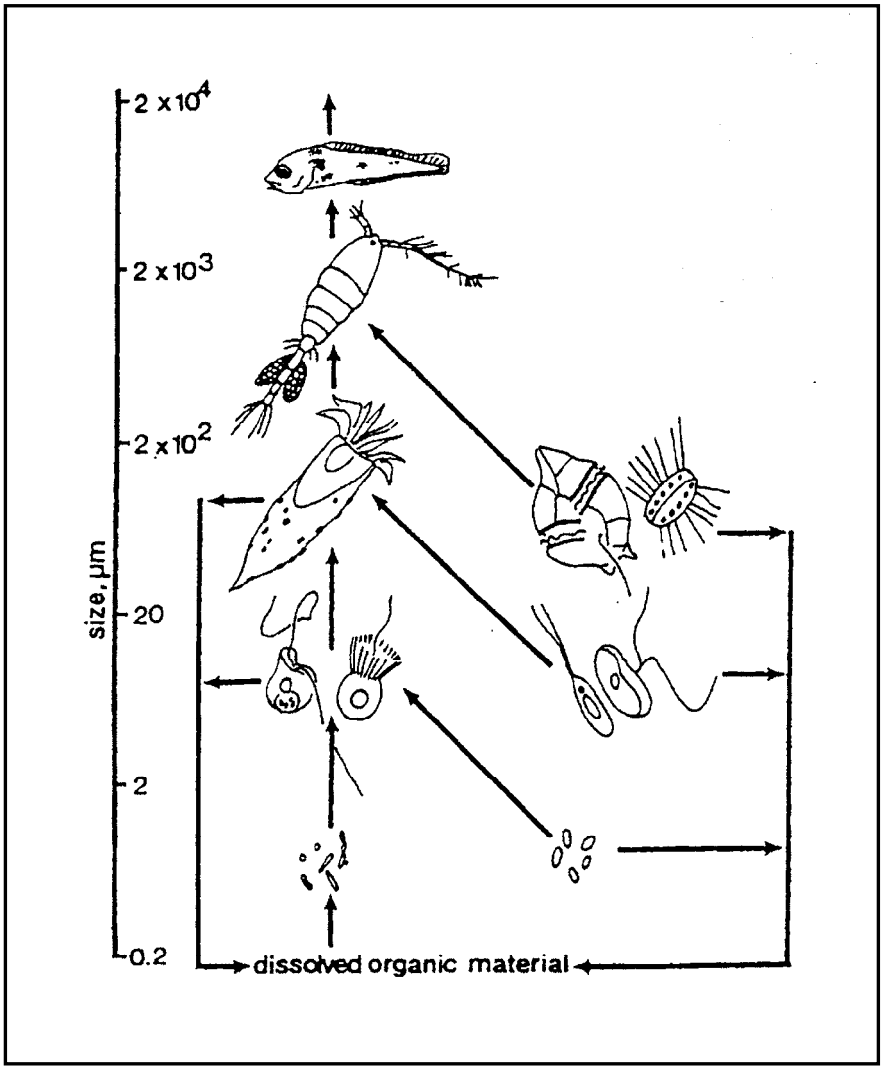

Figure I.1
Schematic representation of the classical food chain and microbial loop (FENCHEL, 1988). Autotrophic organisms are on the right, and heterotrophic organisms on the left. Organisms are vertically ordered by their ranges of size.

Specific methodology developed for the study of microbes in the nineteen seventies changed the classical limnological concept of pelagic trophic chains, in which the most diverse, permanent, and widespread organisms were being ignored (PEDRÓS-ALIÓ, C. and GUERRERO, R., 1994). Epifluorescence microscopy (PORTER and FEIG, 1980) and radioactive isotope (RIEMANN, 1984; KIRCHMANN *et al.*, 1985)

techniques allowed to discover new organisms more abundant and active than previously thought. Nowadays, the suite of techniques available to aquatic microbial ecologists is very large (see *Handbook of methods in Aquatic Microbial Ecology* edited by KEMP, P. *et al.* in 1993), reflecting the increasing relevance of microbial studies in Limnology.

Increasing information about the trophic role of planktonic bacteria and protists is revealing that microbial interactions are more complex than initial descriptions. Most primary production is chanelled through various compartments of the microbial loop as DOC and POC, before becoming available for zooplankton. Microorganisms in temperate and tropical waters often use half or more of the energy fixed by photosynthesis (POMEROY and WIEBE, 1988). The pool of autochthonous DOC utilized by bacteria, however, derives not only from extracellular release of photosynthates by algae (RIEMANN and S0NDERGARD, 1986), but also from DOC excreted by zooplankton, spill from zooplankton feeding activity (LAMPERT, 1978), and lysis of bacteria and phytoplankton due to viral activity (BRATBAK et al., 1992). Over a decade ago, viruses were reported in high number (10^8 virus ml^{-1}; BERGH *et al*., 1989; HARA *et al*., 1991; MARANGER and BIRD, 1995) in several marine and freshwater systems, where they apparently represent a dynamic component of microbial communities. By infecting cellular organisms and the subsequent lysis, viral activity fuels heterotrophic bacterial production and respiration and may reduce transfer of organic matter to the upper trophic levels, which is mediated by the microbial loop. Protozoan grazing in freshwaters can control bacterial abundances (BERNINGER *et al*., 1991). Flagellates and small ciliates (prostomatids) can also regulate the biomass and size structure of picoalgae (WEHR, 1991). Larger ciliates (>100 μm) feed in turn on bacterioplankton and nanophytoplankton. Rotifers (ARNDT, 1993) and copepods (WICKMAN, 1995; DOBBERFUHL *et al.*, 1997) are efficient consumers of phytoplankton and of all components of the microbial loop (i. e. bacteria, heterotrophic nanoflagellates and ciliates). Some cladoceran species (especially *Daphnia*) feed on bacteria-sized particles and interfere with protozoans for carbon and supply of nutrients (SANDERS and PORTER, 1990; PACE et al., 1990; PACE and FUNKE, 1991). Summarizing,

bacteria and their consumers can exert both direct and indirect effects on trophic relations, as they are supplemental food resources, competitors, and predators, at all levels of the classic food chain. Therefore, consumers of bacterial production can exert top-down and lateral, as well as bottom-up control (PORTER, 1996). The microbial loop concept has changed from “orderly level transfers of nutrients and energy from bacteria to metazoans” to a “complex trophic web”. The microbial loop is a component and integral part of a larger microbial food web (SHERR and SHERR, 1988) which includes all pro- and eucaryotic unicellular organisms, both autotrophic and heterotrophic, and all are integrated in a general planktonic food web (PORTER, 1996). This web concept is represented in Figure I.2.

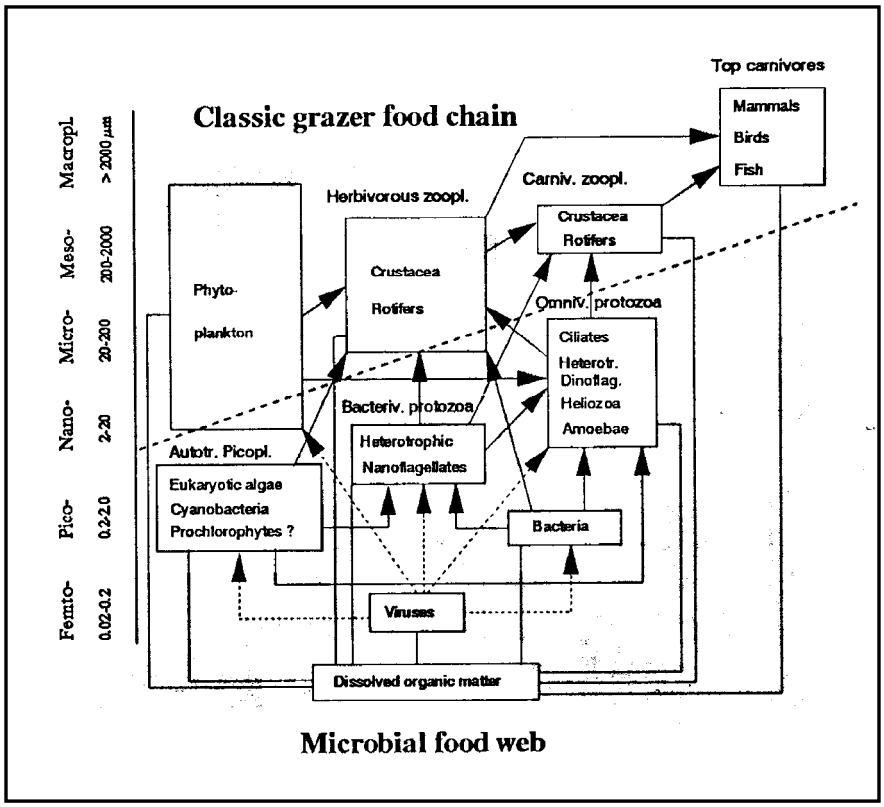

Figure I.2
The contemporary view of microbial food web structure (below the dotted line) in relation to the classic food chain (above the dotted line) in the pelagic zone of lakes (from WEISSE and STOCKNER, 1992). Boxes are vertically ordered by ranges of size of the organisms. Full lines and arrows indicate feeding interactions, broken lines and arrows, viral infection. The pool of dissolved organic matter is replenished by various release processes (excretion, exudation, cell lysis, sloppy feeding) from each compartment and used as substrate by bacteria.

It has been established that microbes play a key role in nutrient cycling and energy flows in aquatic ecosystems. Typical abundances of

main groups (i. e. picoalgae, viruses, bacteria, HNF and ciliates) in oligotrophic and eutrophic lakes are summarized in Table I.1. The ecological constraints on the functioning of microbial communities include a variety of interactions that differ in intensity over time and space, see RIEMANN and CHRISTOFFERSEN (1993) and references listed therein.

Table I.1
Abundances of main groups of microbial plankton in lakes depending on degree of trophy. From RIEMANN and CHRISTOFFERSEN (1993) and references listed therein, excepting viruses (from HARA *et al.*, 1991 and SOMMARUGA *et al.*, 1995).

Abundances of main groups of microbial plankton

	oligotrophic lakes	eutrophic lakes
Picoalgae	10^4 ml^{-1} (8% of the total chlorophyll)	$>10^6$ ml^{-1} (1% of the total chlorophyll)
Viruses	10^6 ml^{-1}	$> 10^8$ ml^{-1}
Bacteria	10^5 ml^{-1}	10^7 ml^{-1}
HNF	10^2 ml^{-1}	10^5 ml^{-1}
Ciliates	10 ml^{-1}	10^3 ml^{-1}

WEISSE *et al.* (1990) described in mesotrophic Lake Constance the importance of microbial loop during early phytoplankton blooms. During clear water phase in the mesotrophic Římov Reservoir (ŠIMEK and STRAŠKRABOVÁ) protozoans decreased significantly, and thus their role in bacterivory was negligible. NIXDORF and ARNDT (1993) described a shift within the metabolic interactions of the microbial food web from winter/spring to summer, indicating a high significance of the protozooplankton as a regulator on bacteria during the colder season, whereas from early summer the influence of metazooplankton dominated by cladocerans was evident in the eutrophic Lake Müggelsee. During massive bloom of cyanobacteria in eutrophic shallow lake, CHRISTOFFERSEN *et al.* (1990) found that microzooplankton and metazooplankton were more important than HNF in controlling the bacterial production. There are, however, other examples where the role of ciliates in the carbon budgets is minor (PACE *et al.*, 1990). The trophic state of the system is high important to explain these differences between systems. As an example, ŠIMEK *et al*. (1999) described that ciliates

become as bacterivores as HNF with increasing trophy of three reservoirs studied.

It is still under discussion, however, what relevance do microorganisms and their interactions have along the gradient of lakes of different trophy (WEISSE, 1991; WEISSE and STOCKNER, 1993; Del GIORGIO and GASOL, 1995). Although the abundance of microorganisms increases with higher nutrient load and primary production (Table I.1), it is believed that the relative contribution of the microbial food web to the carbon flux decreases along eutrophication gradients. But RIEMANN and CHRISTOFFERSEN (1993) in their analysis of the microbial loop along an increasing productivity gradient suggested increasing importance compared to the classical grazer food chain.

In order to evaluate the importance of microbial food web in the Sau Reservoir our group of research purposed to measure abundances and activities of microbes as well as phytoplankton and zooplankton. This thesis forms part of a general project, considering autotrophic processes in the epilimnion of the Sau Reservoir, and their connection with the heterotrophic activities, summarised in this study. Consequently, the study focuses on the epilimnetic community. More attention has been paid to horizontal gradients in the reservoir rather than vertical heterogeneity. In addition, the complex methodology for sample processing required by summer hypolimnetic samples of anaerobic conditions, is another reason.

SPATIAL HETEROGENEITY IN RESERVOIRS, LONGITUDINAL ZONATION

Reservoirs, such as the Sau Reservoir, are often large and narrow, receiving water and nutrient inputs from a single large tributary, which is distant from the point of discharge. These characteristics allows to define

reservoirs as hybrid systems between rivers and lakes (MARGALEF, 1983), because they exhibit a progressive transformation from lotic systems (river inflow) to lake systems (nearer the dam). Reservoirs also occupy an intermediate position between rivers and natural lakes in regard to their organic matter sources, as well as their morphologic and hydrodynamic characteristics.

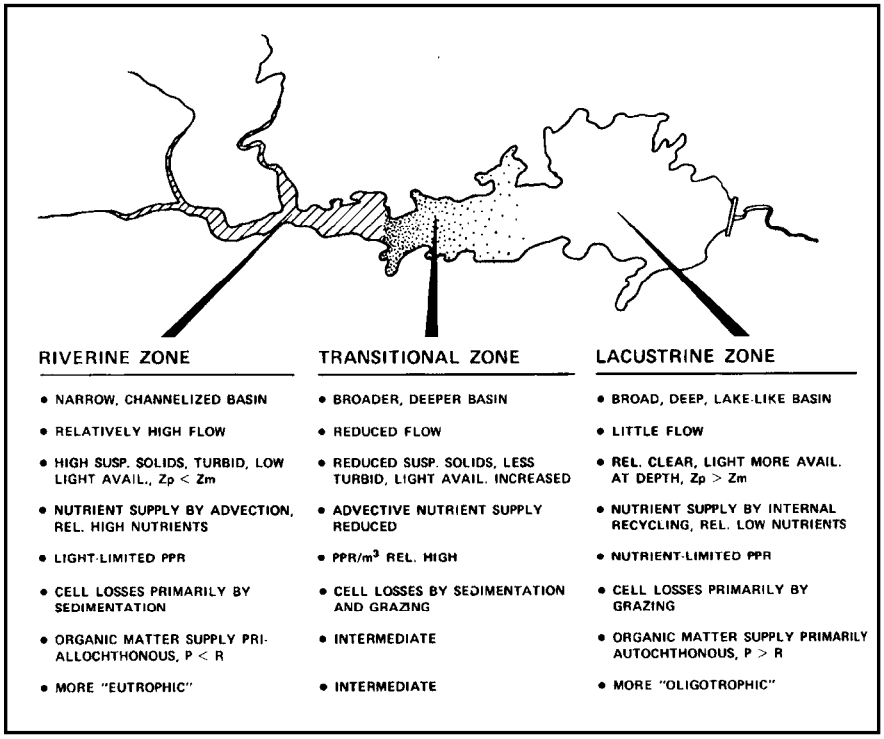

Figure I.3
Longitudinal zonation in environmental factors controlling light and nutrient availability for phytoplankton production, organic matter supply, and trophic status in an idealized reservoir (from KIMMEL *et al*., 1990).

Typically, 3 zones can be distinguished along the longitudinal axis of a reservoir (KIMMEL *et al*., 1990; see Fig. I.3), i. e.

1) The uplake **riverine zone** is a lotic environment. It is characterized by higher flow, shorter water residence time, and higher levels of available nutrients, suspended solids, and light extinction relative to the downstream portions of the reservoir.

2) The **transition zone** is characterized by high phytoplankton productivity and biomass in conjunction with increasing basin

breadth, decreasing flow velocity, increased water residence time, large sedimentation of silt and clay particles from near-surface waters, and increased light penetration. The transition zone is often associated with the plunge point (see Chapter 1), where the river inflow plunge beneath the water surface.

3) The **lacustrine zone** of the reservoir is an area near the dam, with usually longer water residence time, lower concentration of dissolved nutrients and suspended inorganic particles, higher water transparency, and a deeper photic layer than other areas of the reservoir.

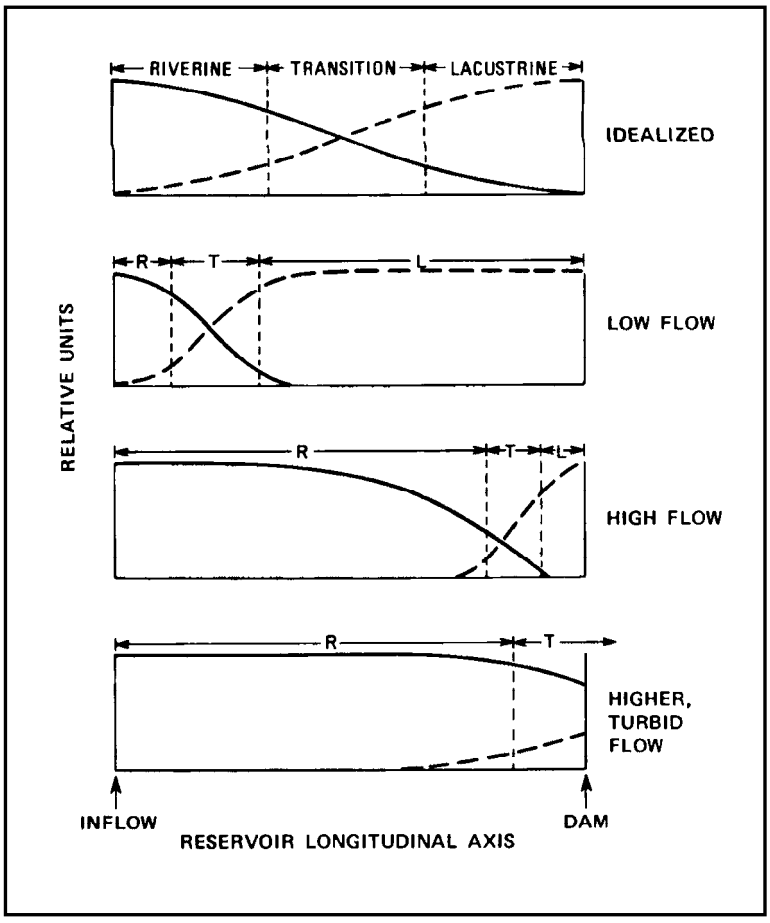

Figure I.4
Variation in the longitudinal zonation of environmental conditions within reservoir basins. Solid and dashed lines represent the prevalence of riverine and lacustrine conditions, respectively (from KIMMEL *et al*., 1990).

The three zones are not discrete units in the reservoir, but spatially and temporally dynamic. Their area fluctuates in response to watershed runoff, inflow characteristics, density flow behaviour, and reservoir operations (Fig. I.4). Any zone takes more or less importance depending on hydrological conditions. Because are not always easily distinguishable, some authors (e. g. UHLMANN, 1991) prefer to consider reservoirs as a homogeneous system, taking in consideration only inflows and outflows. This school of thought compares reservoir functioning to a chemostat.

Marked longitudinal gradients may develop in reservoirs. Then, trophic state gradually changes from eutrophic to oligotrophic conditions down the gradient owing to physical, chemical and biological-physiological processes. It is the case of the Sau Reservoir, due to its special morphology, long and narrow, as we will see in the following section.

STUDY SITE AND STATE OF THE ART

Sau Reservoir is canyon-shaped, 18.5 km long and deep (z_{max}=84m). It is located in the middle stretch of the River Ter (Catalonia, NE Spain). The River Ter has its source in the Pyrenees at 2500m a. s. l. and flows 208 km to the Mediterranean Sea (SABATER *et al*., 1991; Fig. I.5).

The reservoir is the first of a cascade of three reservoirs supplying drinking water to the region of Barcelona. Because of its socio-economic importance, it has been monitored for the past 40 years, since it was first filled in 1963. The data series is the longest of its kind in Spain and it contains chemical and biological (phytoplankton and zooplankton abundance, biomass and diversity) information. The body of knowledge upon which our understanding from this reservoir is based has been acquired primarily from studies on monthly vertical gradients at the area close to the dam (station 1, see Fig. I. 5).

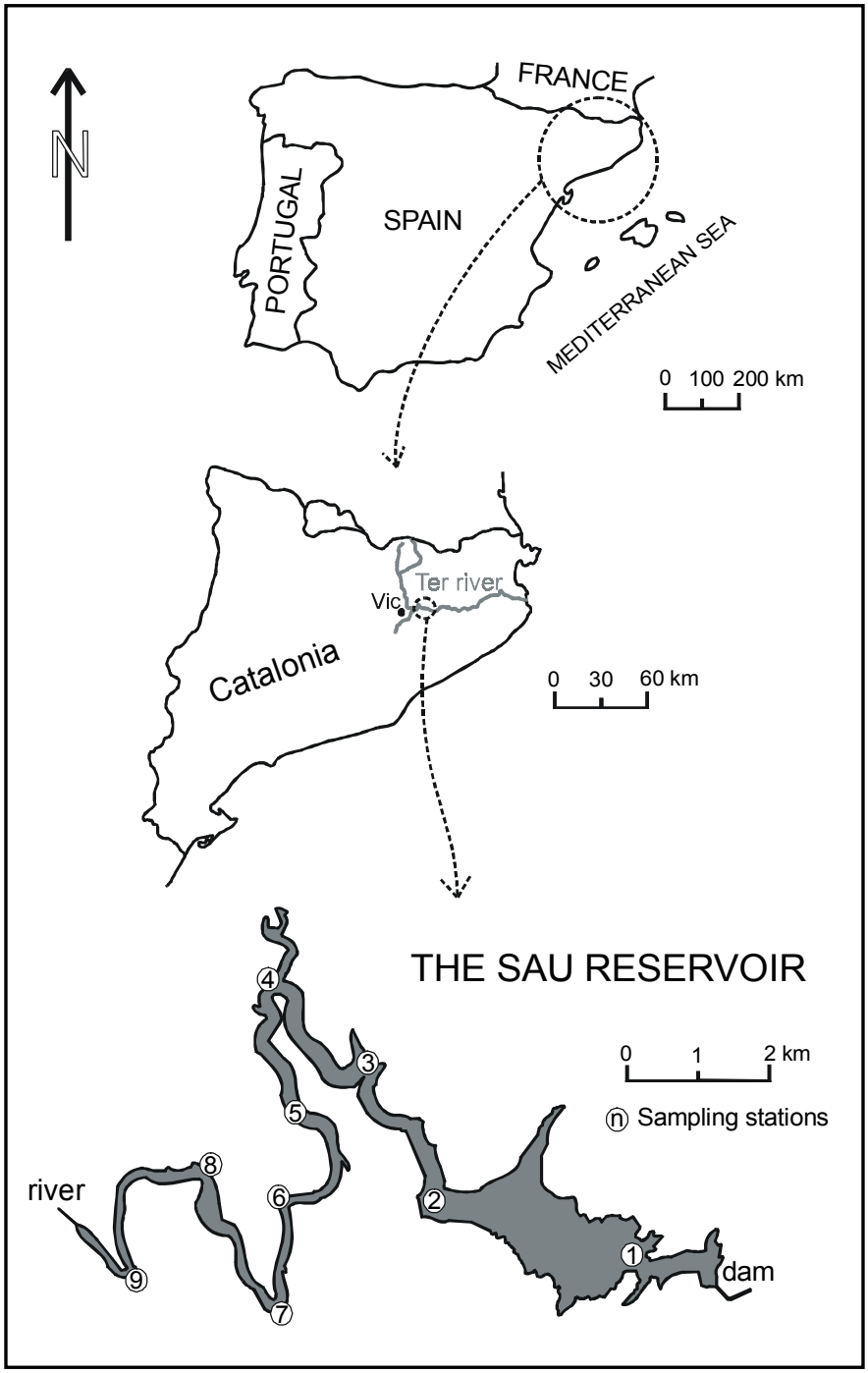

Figure I.5
Study location in Spain (upper) map. Location of the River Ter and the Sau Reservoir in Catalonia (middle map). Location of the nine sampling stations along the longitudinal axis of the reservoir (bottom map).

In Catalonia, this is a reservoir of intermediate capacity and highly variable in its hydrology between years, due to the Mediterranean climate

(see Table I.2). Last two years of this study, 1999 and 2000, have been very dry in comparison with historical data.

Table I.2
Main morphometric and hydrologic descriptors of the Sau Reservoir from 1995 to 2000, and averages from historical data (1965-90).

	1965-1990	1996	1997	1998	1999	2000
Height above sea level (m)	416	416	417	409	398	401
Surface (ha)	466	482	489	410	303	323
Volume (hm^3)	117	123	126	95	54	63
Mean depth (m)	25	26	26	23	18	19
Inflow (hm^3)	541	1009	558	204	310	259
Outflow (hm^3)	540	1007	536	269	308	223
Residence time (days)	79	45	84	170	63	89

Annual pattern of thermal stratification in summer (from April to September) and vertical mixing in winter have been observed in Sau since 1963, describing it as a monomictic aquatic system (VIDAL, 1977). Some aspects in thermal structure and water circulation were recently studied (HAN *et al.*, 2000), applying 1D model of reservoir hydrodynamics DYRESM. Simulations demonstrate that the inflow with high temperature is the main factor controlling the thermal structure in the Sau reservoir and demostrate the effect of residence time on thermal stratification is manifested mainly by the changes in the depth of thermocline.

Its also well documented how the Sau Reservoir became progressively eutrophied from 1963 to 1990 (VIDAL, 1977; VIDAL, 1993; ARMENGOL and VIDAL, 1988; ARMENGOL *et al.*, 1994), cf. Fig. I.6. Several waste treatment plants were installed upstream to the reservoir in 1991-92 (Fig. I.6a), which initially made only physic-chemical treatment of water. This treatment is high efficient removing dissolved phosphorous by flocculation as we observed in decreasing SRP loads from 1994. Until the installation of additional biological treatments (1998), no efficient elimination of dissolved nitrogen was observed (Fig. I.6b). In spite of this reduction in nutrient loads, recent data show the reservoir to have

chlorophyll *a* concentrations (Fig. I.6c) typical of highly eutrophic systems (according with MASON, 1996).

Only little aspects about phosphorous content in the sediment of the Sau Reservoir were studied several years ago (ARMENGOL *et al.*, 1986; ARMENGOL and VIDAL, 1988), and this matter could also explain this no reduction in chlorophyll *a* concentrations although external loads have been reduced. Probably, we must to follow reservoir dynamics, next years, in order to see an improvement in its trophic status. Historical monthly sampling in station 1 is still made in this reservoir.

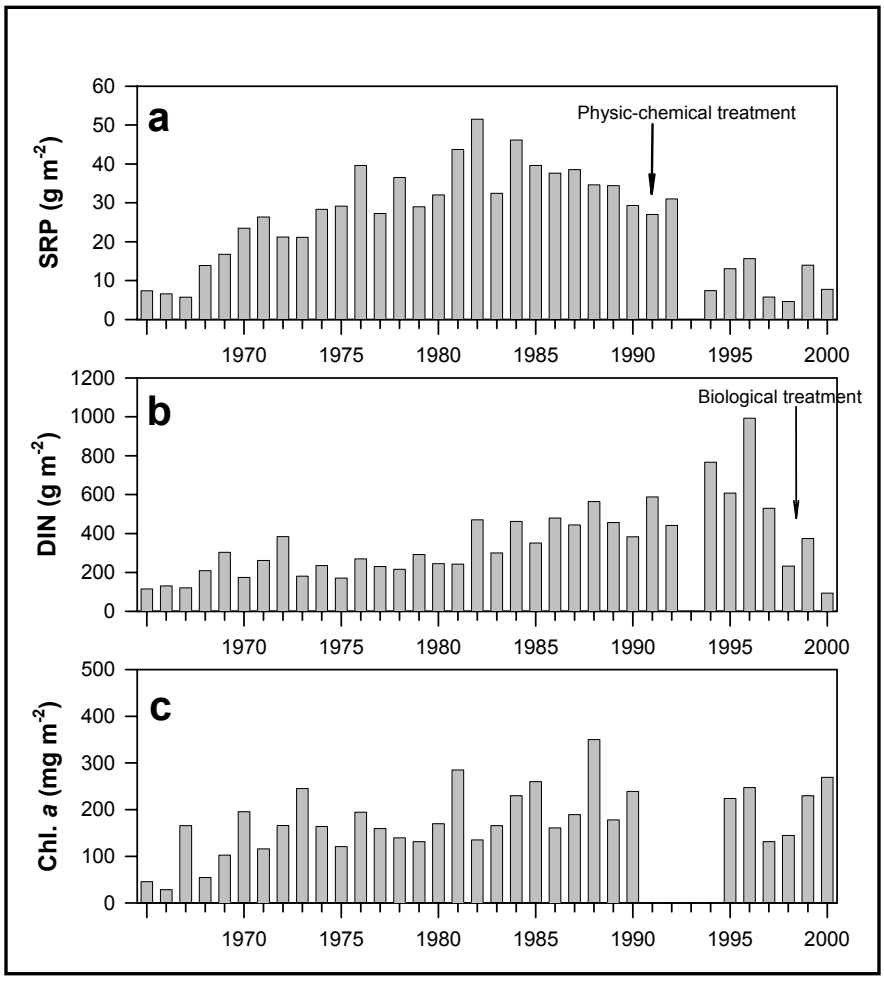

Figure I.6
Annual nutrient per area loads and phytoplankton biomass in the Sau Reservoir, from 1965 to 2000.

a) Soluble reactive phosphorous loads (SRP).

b) Dissolved inorganic nitrogen (DIN).

c) Chlorophyll a concentration (Chl. *a*).

Historically, in studies of plankton ecology from the Sau Reservoir, microbial communities have been ignored. Most studies on the plankton community have focused on the taxonomy and biomass determinations of phyto- and zooplankton. Microbial plankton groups have been overlooked in studies from Sau due to the methodology used for both phytoplankton and zooplankton samples:

- to sample (p.e. nets through which bacteria, flagellates and most ciliates pass, 53μm∅),
- to preserve (extremely disruptive to flagellates and ciliates, and render their cells unrecognisable),
- to filtrate (microbial groups are very sensitive to pressures >20mm Hg)
- and to enumerate (low optical magnifications).

Only one microbiological project was conducted in August 1994, when several scientists meet in this reservoir. They published a paper about the presence of large virus-like particles in the Sau (SOMMARUGA *et al*., 1995). From that sampling new questions about ecology of Sau where constructed, mainly on microbiological point of view and following longitudinal heterogeneity (not described until then in terms of microbial ecology). We could say that it was the origin of the present study.

I started my PhD in January 1997, assuming a new area of study and methodology in the Sau Reservoir, the microbial ecology. I was helped by Dr. Karel Šimek, who has collaborated with our group of research since 1994.

To study longitudinal changes in chemistry and biology along the main axis of the reservoir, nine sampling stations were established for the present study at ca. 1.8 km intervals (see Fig. I. 5).

OBJECTIVES OF THIS STUDY

Although many aspects about ecosystem have been extensively studied until 1996 in this warm, eutrophic and monomictic reservoir, as yet relatively little studies have been conducted about protozoan components of the plankton and their role in this system. The main objective was to characterize microbial food web in this eutrophic ecosystem, with high organic allocthonous inputs.

First we would know ranged abundances and specific composition of bacteria and protozoa (hetrotrophic nanoflagellates and ciliates) in the epilimnion of the Sau Reservoir. We also focused our study in microbial activities: bacterial production and grazing rates on bacteria by protozoa.

We discovered a marked spatial heterogeneity in chemical, physical and biotic parameters from the river inflow to the dam; and we paid more attention to these gradients. Then we wanted to describe main forces controlling longitudinal zonation through the reservoir.

From five years of study (1996-2000), we were able to evaluate the contribution of pelagic microbial food web in the reservoir, as a carbon flow and a source to higher trophic levels (zooplankton, fishes).

Results from this study are presented in six chapters. Each chapter has been written as an independent article focused on a particular topic with its own introduction and discussion. Some of them are published in one or several papers and other are submitted or in press. I have been lucky that our group of research has been very active in the 1996-2000 period, making several collaborations with other scientists resulting in high amount of published papers in which I am co-author. Nevertheless, I have summarized in this thesis only the data directly processed by me following my own objectives. The papers published by our group have been introduced as comments (with its quotations) to understand all questions related to microbial food web.

The chapters have been arranged following a logical order, starting in major forces controlling microbial food web and introducing more specific questions related to its particularity in this reservoir (common components and their activity in the epilimnion). The main objectives of each chapter are summarized as follows:

- Chapter 1 is a description of the main physical forces acting in the longitudinal axis of the reservoir. The objective of this section was to characterize seasonal variation in circulation patterns through the reservoir.

- Chapter 2 examines chemical longitudinal gradients and its variation depending on physical conditions. The main objective was to elucidate the relevance of high allochthonous nutrient inputs on the epilimnion chemical water composition of the reservoir and how they are processed from the river to the dam.

- Chapter 3 shows a clear longitudinal transect in the epilimnion where high gradients in biological activity were found. This study describes in detail how planktonic groups develop from the source of river inputs.

- Chapter 4 gathers up all longitudinal transects under a general point of view, describing general patterns and specific microbial activities in this reservoir. Diversity and species-specific activities of the main components in microbial plankton are also detailed.

- Chapter 5 is a summary of all data in terms of biomass and production, and attempts to give an overall view on the carbon fluxes within the context of longitudinal pelagic community succession. The objective of this study was to evaluate the importance of microbial food web in the Sau Reservoir.

- Chapter 6 compares heterotrophic nanoflagellates and specific ciliate growth rates calculated in a microcosm experiment. Our

goal was to know these growth rates and to test top-down control versus bottom-up control on microbial food web.

MATERIAL AND METHODS

SAMPLINGS

In order to study physical, chemical and biological longitudinal gradients in the Sau Reservoir, nine sampling points were stablished from the river inflow to the dam. At every point station (see Figure I.1) we made a profile from the surface to the bottom with a multiparametric probe and collected integrated water samples from the epilimnion for chemical and biological analysis. Usually we spent two or three days sampling, but ever we took water samples more quickly than water moved through the reservoir.

Photo M.1
We collected water samples using two types of sampler:
a) Plankton tube sampler.
a) Limnological water sampler (UWITEC).

We collected integrated epilimnion water samples using plankton tube sampler three or five meters long in some cases (Photo M.1). When epilimnion was deeper than five meters, we used a 5-liter, dark, limnological water sampler UWITEC (Photo M.1) taking five samples in

several depths and mixing them in a 25-liter, dark, polyethylene bottle. The water was stored in 5-liter or 25-liter, dark, polyethylene bottles awaiting analyses.

PHYSICAL AND CHEMICAL PARAMETERS

Temperature, conductivity, pH, redox, oxygen and turbidity

We measured temperature (ºC), conductivity ($\mu S\ cm^{-2}$), pH, redox (mV), oxygen saturation percentage, oxygen concentration ($mg\ l^{-1}$) and nephelometric turbidity (ntu) using two kinds of multiprobe provided both with a cable 100 meters long.

The first one was a YSI-Grant Water Quality Logging System (model 3600). Since December 1997 we used a Turo Water Quality Analyser (model T-611, see Photo M.2). Both multiparametric probe sensors are detailed in Table M.1.

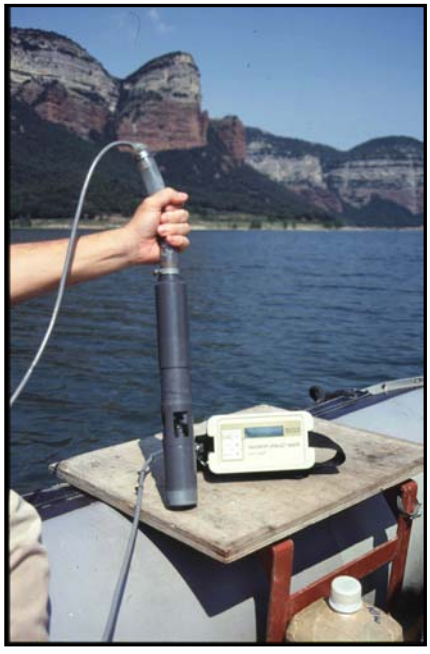

Photo M.2
Multiparametric probe Turo.

We measure profiles meter by meter from the surface to the bottom at each sampling station. The data from profiles showed us at what levels there were strong changes in some parameter. On the boat and with the result profiles we could evaluate where the stratification or mixing layers (epilimnion, metalimnion and hipolimnion) were located in order to choice the depths where we took the samples.

Table M.1
Main characteristics of sensors from both multiprobes used.

YSI-Grant sensors	
Sensor	**Type**
Temperature	Thermistor of sintered metallic oxide, which changes in resistance as T[a] changes.
Conductivity	Pure nickel electrodes.
pH	Silver/silver chloride wire electrode in special solution and covered by a glass membrane.
Redox	Reference electrode, part of the pH probe.
Oxygen	Clark-type polarographic electrode.
Turbidity	Nephelometric measurement from a 180 sensor with pulsed infra-red light source.
Turo sensors	
Sensor	**Type**
Temperature	PN junction in stainless steel sleeve.
Conductivity	Four electrode cell.
pH	Combination silver/silver chloride type with sintered Teflon junction.
Redox (ORP)	Combination bare metal electrode common reference junction with pH electrode.
Oxygen	Polargraphic sensor with Teflon membrane and built-in stirrer.
Turbidity	Nephelometric measurement from a 90 sensor with pulsed infra-red light source.

Secchi depth

We used 30cm diameter, and black-white dish to measure the Secchi depth (cm).

Meteorological data

Since May 1997 we have a meteorological station Campbell Scientific installed on the building of the Sau yacht club (Photo M.3).

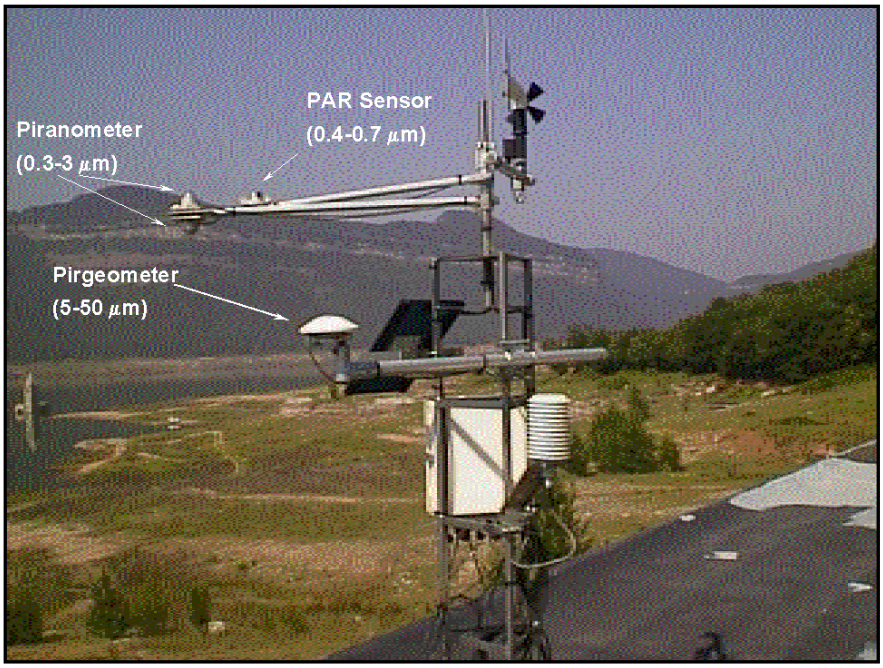

Photo M.3
Meteorological station in the Sau Reservoir.

This station is provided by the following sensors:

- Temperature and relative humidity Vaisala (HMP35AC).
- Atmospheric pressure Vaisala (PTB101B).
- Large infrared radiation: pirgeometer Kipp&Zonen CG1 (5000-50000nm range).

- Sun radiation: piranometer Kipp&Zonen CM3 (300-3000nm range).
- Photosynthetic active radiation (PAR): Skye SKP215 (400-700nm range).
- Anemometer –weather vane (RM Young 05103).
- Rain gauge (Munro R102).

Chemical analyses

Total particulate nitrogen and **carbon** were measured via elemental analysis in a 1500 Carlo Erba analyser. Vanadium pentaoxid is used as catalyst. Specific volume of sample is filtered through precombusted Whatman GF/F glass microfibre filter (24mm ∅) which is analysed.

Particulate material (PM), or suspended solids, in the water were measured by the difference in weight of a Whatman GF/F glass microfibre filter (47mm ∅) before and after filtration of a specific water volume. Filters were dried in a furnace at 60ºC during 24h.

We measured directly from non-filtered water **total phosphorous** (Total P) and **total nitrogen** (Total N). We followed the protocol described by GRASSHOFF (1983): samples were digested with an oxidizing reagent inside Teflon tubes (110ºC, $1^1/_2$h.) in order to transform Total P and Total N to soluble forms. After oxidation we measured these nutrients by colorimetry as soluble reactive P and nitrate explained bellow.

Water filtered through Whatman GF/F glass microfibre filters (47mm ∅), pre-combusted at 450ºC, was used to measure solutes: soluble reactive phosphorous (SRP), total dissolved phosphorous (TDP), nitrate, nitrite, ammonium, total dissolved nitrogen (TDN), silicate, chloride, sulphate, dissolved organic carbon and alkalinity.

Soluble reactive phosphorous (SRP) was measured according to the method described by MURPHY and RILEY (1962) using a Shimadzu (UV-1201) spectrophotometer at 890nm. **Total dissolved phosphorous**

(TDP) was oxided like Total P and both two were measured by the same way than SRP.

Nitrate, **chloride** and **sulphate** were analized in a Konik (model KNK 500-A) liquid crhomatograph supplied with two sensors: a WESCAN conductivity sensor and Kontron (model 332) UV/V sensor. Column used is Waters IC-Pack Anion.

Nitrite was measured by colorimetry in a Shimadzu (UV-1201) spectrophotometer at 540nm (GRASSHOFF, 1983).

Ammonium was measured following the method described by Solorzano (1969) using a Technicon AutoAnalyzer at 630nm.

Total dissolved nitrogen (TDN) was oxidated like Total N and both two were measured by the method described by GRASSHOFF (1983) using a Shimadzu (UV-1201) spectrophotometer. We measured the samples absorvance at 220nm (NO^-_3) and we deduct from them the absorvance at 275nm (dissolved organic material).

Silicate was measured by colorimetry in a Shimadzu (UV-1201) spectrophotometer at 810nm following the method described by Koroleff (GRASSHOFF, 1983).

Dissolved organic carbon (DOC) was determined via combustion in a Shimatzu (model TOC-5000) total carbon analyzer provided with an infrared sensor.

Alkalinity was measured following Gran method. We used an automatic Metrohm titrationer (model Titrino SM 702) provided with a pH electrode and HCl (0.01N) as titulator. We followed MACKERETH *et al*. method (1978) to obtain dissolved inorganic carbon (DIC), bicarbonates (HCO_3^-), and carbonates ($CO_3^=$) concentrations, and CO_2 partial pressure.

BIOLOGICAL PARAMETERS

Bacterial abundance, cell volume and production

Subsamples, from an integrated epilimnion water sample, were fixed with formaldehyde (2% final concentration), stained with the fluorochrome DAPI (4', 6-Diamidino-2-phenylindole) at 0.2 % wt/vol (PORTER and FEIG, 1980), filtered on 0.2 μm black nucleopore filters and enumerated by epifluorescence microscopy (Reichert-Jung POLYVAR).

Subamples (50 ml) were fixed *in situ* and stored at 4ºC until their filtration in the laboratory. We filtered 1-5 ml subsample on 0.2 μm nucleopore policarbonate filters precolorized with Irgalan Black (for details see HOBBIE *et al*., 1977). Some millilitres of sample were filtered and 60μl of DAPI (from a solution of 0.1 mg ml^{-1}) were added on the rest sample volume (cf. Figure M.2). After 4 minutes from DAPI addition all water was filtered. Filtration was made bellow 20 mmHg pressure. Filter was placed on a slide, which we had moistened with our breath. We put on the filter a drop of non-fluorescence immersion oil (as assembly medium), the cover and other drop of immersion oil.

Filters were immediately checked under epifluorescence using UV light filter. We checked every filter under 500 magnification to confirm a homogeneous distribution of bacteria (see Photo M.4). Under 1250 magnification several fields were at random counted per filter for a minimum 400 bacteria. **Bacterial abundance** (BA; 10^6 bacteria ml^{-1}) was calculated as follows:

$$BA = \frac{N_b / f}{V} \cdot F \qquad F = \frac{A_1}{A_2} \cdot d \qquad \text{Equation M.1}$$

where N_b is the number of bacteria counted, f is the number of fields checked, V is the volume of sample filtered, and F is the correction factor.

A_1 is the area of filtration on the filter, A_2 is the area of one field checked in microscope and d is the dilution of the sample by fixatives.

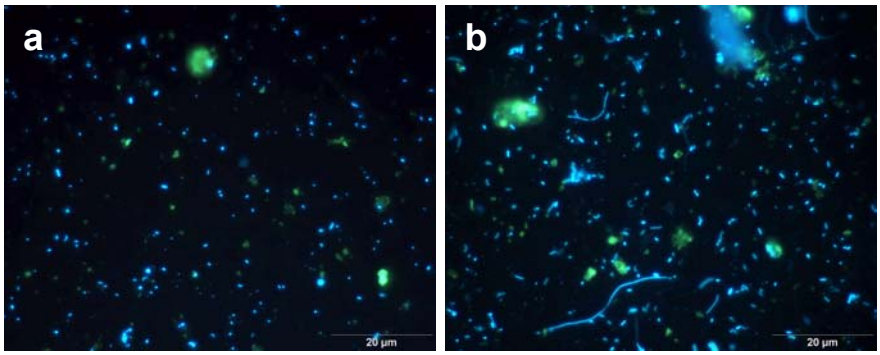

Photo M.4
Photographs under fluorescent microscope of DAPI stained bacterial preparations from the Sau Reservoir.
a) Sample collected at station C1 in February 1998, near to the dam.
b) Sample collected at station C5 in March 2000, near to the river inflow, because reservoir water level was low.

One limitation of this technique was described by SOMMARUGA *et al.* (1995), inspecting samples collected in August 1994 from the Sau Reservoir: separation between small coccoid bacteria and large virus-like particles (LVLP) is not possible under epifluorescence microscope. This fact may produce an overestimation on bacterial abundance when LVLP are very abundant. But SOMMARUGA *et al*. (1995) determined a very low frequency of LVLP, around 7%, and our results show mean bacterial sizes higher than LVLP sizes. So we concluded no relevant overestimation of bacterial abundance in our samples.

More than 400 bacteria were sized by semiautomatic image analysis (Lucia, Laboratory Imaging, Prague, for more details see ŠIMEK *et al*. 1995). Bacterial biomass was calculated by applying the allometric relationship between cell volume and carbon content according to NORLAND (1993), see Table 5.1 (Chapter 5).

Bacterial production was measured at *in situ* temperature following the method described by BELL (1993), using the protocol of thymidine incorporation into total macromolecules (Cold TCA Insoluble Fraction). There is a scheme of this protocol in Figure M.1. From an integrated epilimnion water sample, we incubated four 5-ml subsamples for 30 min at *in situ* temperature with 20 nM of [*methyl*-^{3}H]thymidine and then we fixed them with formaldehyde (2% final concentration). Two blanks prefixed with 2% formaldehyde were processed in parallel.

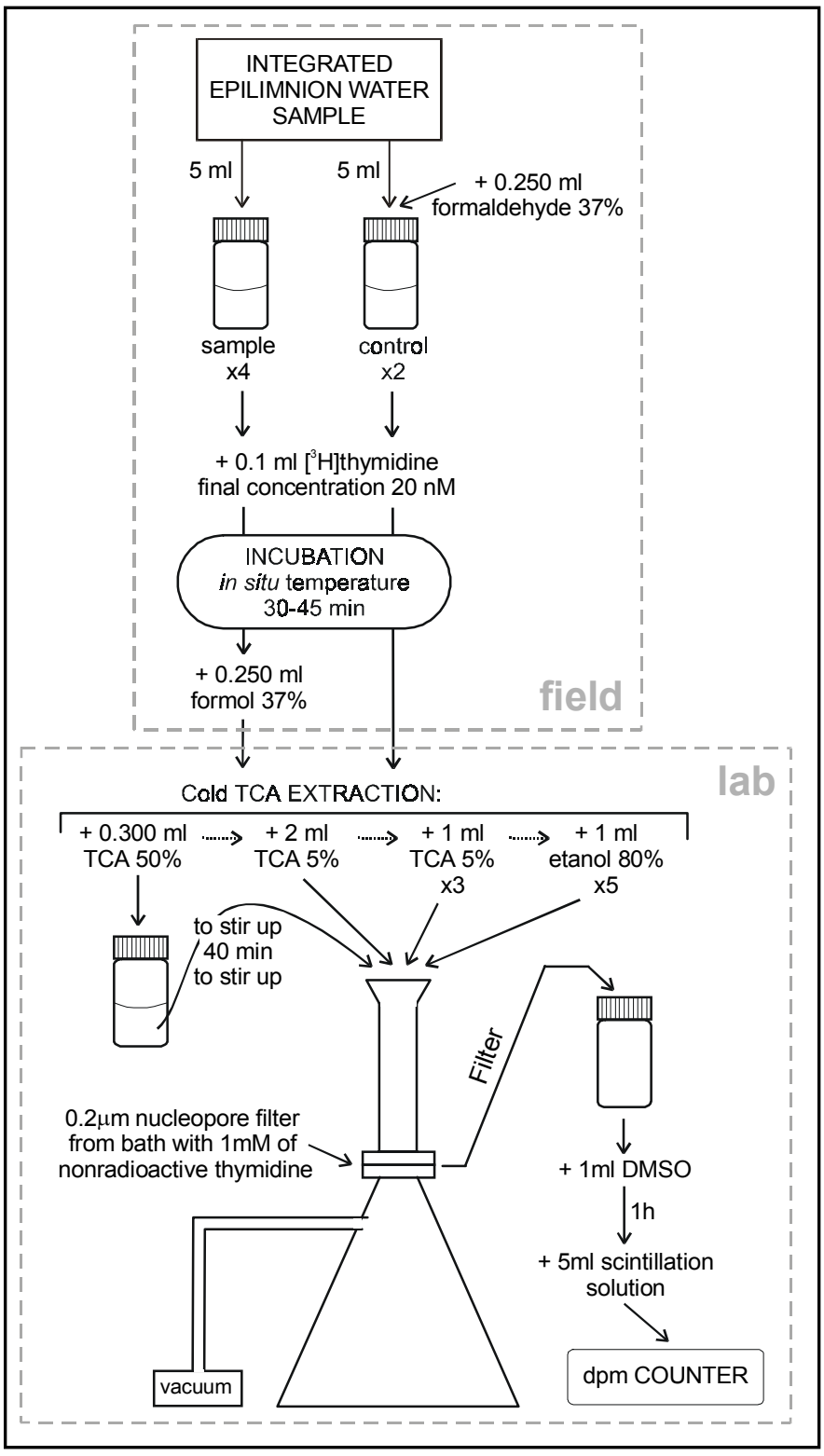

Figure M.1
Schematic protocol of bacterial production via icorporation of tritiated thymidine, described by BELL (1993). Samples were incubated in the field, and processed in the laboratory by Cold TCA Insoluble Fraction treatment.

In the laboratory, we followed protocol of cold TCA (trichloroacetic acid) extraction. Prior filtration filters were placed in a solution of nonradioactive thymidine (≈1 mM) in a Petri dish. 300 μl of cold TCA were added to subsamples and blanks, and after shake them well, they were kept on ice for 40 min. The manifold was mounted with filters and ice-cold funnels. Samples were placed into the corresponding funnels and filtered at a pressure <100 mmHg. Vials were rinsed once with 2 ml ice-cold 5 % TCA, and 3 times with 1ml portions of ice-cold 5 % TCA, adding each rinse to the funnels. Funnels and filters were rinsed 5 times with 1 ml portions of ice-cold 80 % ethanol to remove thymidine taken into the cells but not incorporated into DNA. When filters were dry the filtration was stopped. Filters were placed in plastic scintillation vials, adding 1 ml DMSO (Dimetil Sulfoxid) to dissolve the filters shaking well. After 1 hour, 5 ml of scintillation solution was added. Radioactivity was measured in a Packard tri-carb 1500 liquid scintillation analyser.

Bacterial production (BP; moles thymidine l^{-1} h^{-1}) was calculated as follows:

$$BP = \left(\frac{\left[dpm_{sample} - dpm_{blank} \right] \cdot (4.5 \cdot 10^{-13})}{SA \cdot t \cdot v} \right) \cdot (10^{-3}) \qquad \text{Equation M.2}$$

where $4.5 \cdot 10^{-13}$ is the number of curies per dpm; SA is the specific activity of the [^{3}H]thymidine solution in curies per mmol; t is the incubation time in h; v is the filtered volume in l; and 10^{-3} is mmol per mole.

The conversion of moles incorporated to cells produced per unit time requires a conversion factor (TCF = **thymidine conversion factor**). The most widely used TCF is $2 \cdot 10^{18}$ cells mol^{-1} (BELL, 1993). We used it in our first sampling (Chapter 3). We decided to measure empirically the thymidine conversion factor in future samplings through the Sau Reservoir (almost at three points: riverine, transitional and lacustrine zones). Grazer-free mixed bacterial population from the reservoir was obtained by filtration through 1 μm nucleopore filter, and incubated (1 l) at *in situ* temperature. Subsamples were taken at 0, 6, 12, 18 and 24 h, fixed with formaldehyde and counted via epifluorescence microscopy. At 6 and 18 h of incubation we also took samples to measure bacterial

production via thymidine incorporation. The rate of bacterial growth (cells l^{-1} h^{-1}) from linear part of curve was divided by thymidine incorporation (moles thymidine l^{-1} h^{-1}) in the same period of time.

Protistan abundance, cell volume and grazing

To estimate bacterivory or grazing by heterotrophic nanoflagellates (HNF) and ciliates we used fluorescently labelled bacterioplankton (**FLB**)[1], prepared according to the protocol of SHERR and SHERR (1993) modified by ŠIMEK *et al*. (1995). This protocol is summarized in Figure M.2. We determined HNF and ciliate uptake rates on bacterioplankton in short-time FLB direct uptake experiments where tracer amounts of FLB accounted for 5-20% of natural bacterial abundances.

[1] FLB were supplied by Dr. Šimek from Hydrobiological Institute CAS (Czech Republic). Natural bacterioplankton collected from the Římov Reservoir (Czech Republic) was used to prepare FLB, which were stained with DTAF (5-[(4,6-dichlorotriazin-2-yl) amino] fluorescein). For details see ŠIMEK and STRAŠKRABOVÁ (1992).

We took a 300 ml subsample from the integrated epilimnetic water sample and incubated it in Pyrex bottles with FLB. Samples were incubated at *in situ* temperature inside a portable thermobox, and 50-ml subsamples were taken at times 0, 10, 20 and 30 min. after tracer addition. In summer, when water temperature and microbial activities are higher, we took subsamples more often (0, 3, 6 and 10 min). The subsamples were preserved with alkaline Lugol's solution (0.5%), followed by formaldehyde (2%), and decolorized with a few drops of sodium thiosulphate (3%) (SHERR and SHERR, 1993). Subsamples fixed at time zero were counted for the initial abundances of bacterial, FLB, HNF, and of ciliates. We recorded at each time the number of FLB per protozoan cell ingested (HNF and ciliates).

Abundances of HNF (10^3 cells ml^{-1}) and ciliates (cells ml^{-1}) and uptake rates on bacterioplankton were determined in 10-30 ml subsamples stained with DAPI (60 μl from a solution of 0.1 mg ml^{-1}), filtered through 1 and 2 μm black nucleopore filters, respectively, and inspected via epifluorescence microscopy (Reichert-Jung, Polyvar). HNF and ciliates were counted under 1250 and 500 magnification, respectively, using an UV light filter. Several fields were counted per filter for a minimum 100 HNF and 50 ciliates. The protocol followed and the

calculation of protistan abundances were the same than the explained above for bacterial abundance (cf. Equation M.1). See also scheme in Figure M.2 and specifications in Table M.2.

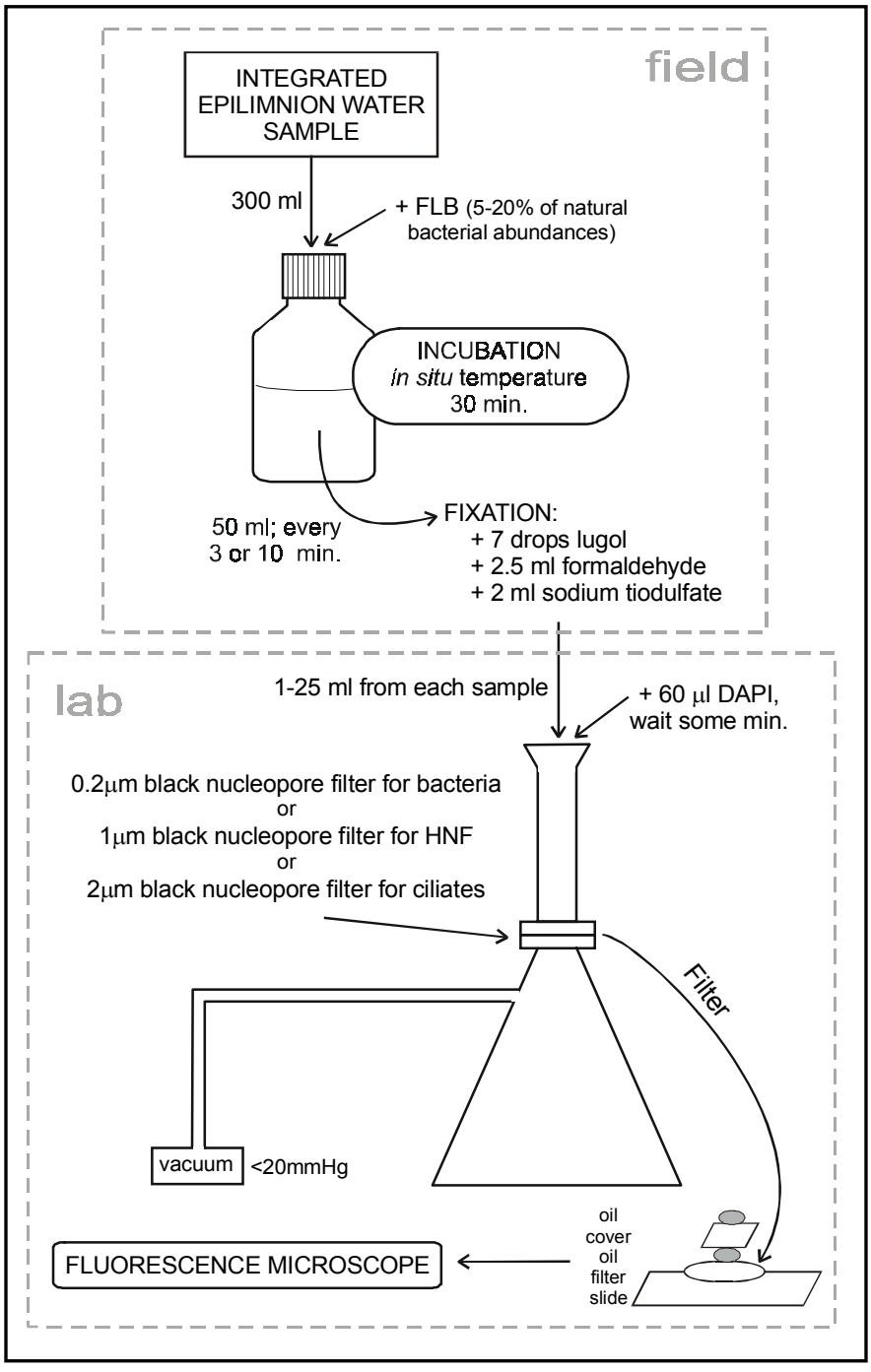

Figure M. 2
Schematic protocol for protozoan grazing incubations in the field. Samples were stained with DAPI in the laboratory and counted by epifluorescence microscope.

Filtration pressure below 80 mm Hg (in our case a pressure of 20 mm Hg was used) has been reported to avoid cell breakage (GASOL and MORAN, 1999; and references therein).

Table M.2
Amount of sample (ml) and staining time with DAPI for the several inspections of these samples via epifluorescence microscope.

TO INSPECT:	SAMPLE FILTERED (ml)	TIME WITH DAPI (min.)
Bacterial abundance	1-5	4
HNF abundance	10-20	2-4
Ciliates abundance	10-30	1-4
FLB inside HNF	10-20	0.75
FLB inside ciliates	10-30	0.75

To estimate ciliate and HNF **mean cell volumes**, we measured length and width with an ocular micrometer, of at least 20 individuals of each well-mixed subsample (preserved samples inspected for tracer uptake). The volumes were calculated by approximation to prolate spheroids:

$$V = \frac{\pi}{6} \cdot l \cdot w^2 \qquad \text{Equation M.3}$$

where l is cell length and w width. Their corresponding biomasses were calculated by applying the conversion factors (volume to carbon content) for HNF and ciliates, detailed in Table 5.1 (Chapter 5).

The ciliate community structure was analysed on DAPI-stained samples by epifluorescence microscopy. However, the latter approach was also completed by live sample observation and, in selected cases, protargol staining (SKIBBE, 1994) was also applied as in MACEK *et al.* (1996). We based our identifications on the keys published in FOISSNER and BERGER (1996) and detailed references listed therein. Live samples were recorded by a video system coupled with microscope, in order to facilitate ciliate identifications.

Individual grazing rates (IGR; bacteria ingested protist^{-1} h-^{1}) were estimated searching protists on filters under UV light filter (cf. Photo M.5),

and then changing to blue light filter to count how many FLB had each ciliate inside. The **total protistan grazing rate** (TGR; 10^6 bacteria ingested h^{-1} ml^{-1}) was obtained by multiplying the average uptake rates of HNF and ciliates by their total *in situ* abundances. **Mean clearance rates** on bacteria (nl^{-1} $protist^{-1}$ h^{-1}) were also calculated for the main groups of ciliates by dividing the individual grazing rates by the *in situ* bacterial abundances. Volume-specific clearance rates on bacteria resulted from mean clearance rates divided by mean cell volumes. Results can be seen in Table 4.2 (Chapter 4).

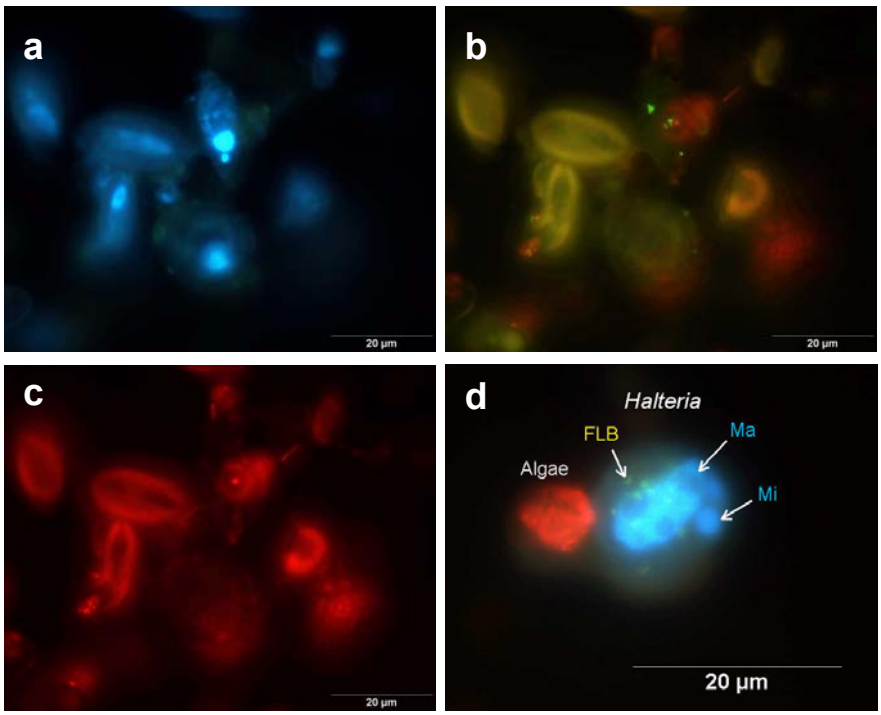

Photo M.5
Images from epifluorescence microscope. From a to c: the same field under three different light filters. **a)** UV light filter. There is a *Cyclidium* sp. with macro- and micronucleus stained by DAPI (in blue). Algal DNA is also stained but less bright. **b)** Blue light filter. The FLB (bright yellow) ingested by this ciliate. **c)** Specific light filter for Chlorophyll *a* allowed us to differentiate autotrophic organisms from the heterotrophic ones. In the image, lateral plastids of several *Cryptomonas* sp. can be seen. **d)** *Halteria* sp. in a integrated image from the same field at the three kind of light filters. Ma: macronucleus. Mi: micronucleus.

The method requires microscope inspection of numerous subsamples, therefore, a considerable amount of time and labor is required. On average, we consumed 1 and 2 hours inspecting one subsample of HNF and ciliates, respectively. The advantage was that most components of protozooplankton were directly identified.

Chlorophyll *a*

The chlorophyll *a* content in phytoplankton collected on Whatman GF/F glass microfibre filters was analysed by using the trichromatic method of JEFFREY and HUMPHREY (1975).

Zooplankton abundance

Integrated zooplankton samples were collected using a 53 μm mesh Apstein net (Photo M.6) in 20 m vertical hauls at each sampling station. When depth was <20 m, we collected the zooplankton hauling from bottom to surface. Taking depth integrated zooplankton samples mitigates the effect of the vertical migration of zooplankton and heterogeneous distribution in the water column on density estimates. Samples were preserved in 4% formaldehyde (final concentration).

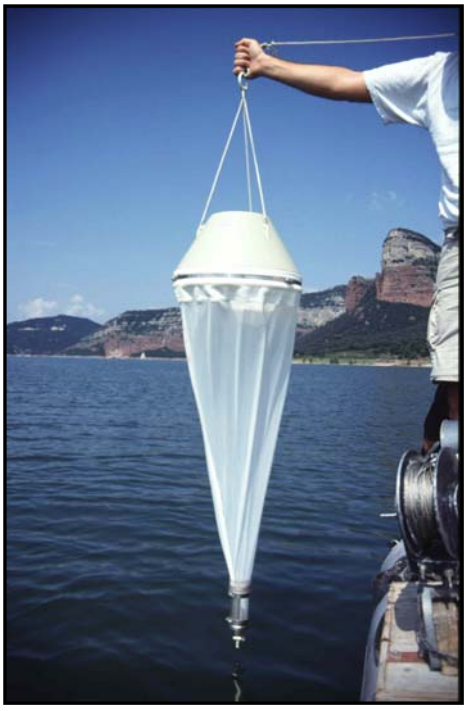

Photo M.6
The 53 μm mesh Apstein net used to collect zooplankton in the Sau Reservoir.

We also obtained a live replicate sample for direct observation in order to identify rotifer species. The fixed samples were immediately sieved in the laboratory. Six mesh sizes (53, 100, 150, 250, 500 and 710 μm) were used to define the size distribution of the sample. Each subsample was sedimented in a sedimentation chamber and quantified in an inverted microscope (OLYMPUS T041) by counting at least 60 individuals from the main species (McCAULEY, 1984).

The zooplankton biomass in the epilimnion was calculated as the proportional biomass of the total in the haul present in the epilimnion. Dry weights (DW) were estimated by geometric approximations in RUTTNER-KOLISKO (1977) for rotifers, considering an approximate density of ρ = 1 g cm^{-3} and that DW was about 10 % of fresh weight (LATJA and SALONEN, 1978) except for *Asplachna* (4 %; DUMONT *et al*., 1975). Mean weights for crustacean species were estimated from regression equations in BOTTRELL *et al*. (1976) and McCAULEY (1984). The biomass of eggs was not considered.