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ABUNDÀNCIA I DINÀMICA DELS VIRUS EN ECOSISTEMES PLANCTÒNICS

Núria Guixa i Boixereu 1997

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Vist-i-plau del director de tesi

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A la meua família

"Cuando un hombre empieza a aprender, nunca sabe lo que va a encontrar. Su propósito es deficiente; su intención es vaga. Espera recompensas que nunca llegarán, pues no sabe nada de los trabajos que cuesta aprender"

Las enseñanzas de Don Juan, Carlos Castaneda

Índex

Agraïments

Ara que estic a la fi d'aguesta tesi, fai memòria i repasso amb una certa nostàlgia el passat des de l'estiu que vai estar a Galícia i vai assabentar-me que a Barcelona hi havia un Institut de Ciències del Mar. Gràcies al Miquel Vinyas, vai saber que el Carles Pedrós-Alió hi treballava, fent recerca en un camp gairebé desconegut per jo: ecologia microbiana. Quan vai venir a parlar amb ell, em va ensenyar el seu projecte dels virus i em va semblar molt "guai". Al Carles li agraïsso la llibertat que m'ha donat per portar a terme aguesta feina i així aprendre a espavilar-me entre els petits entrebancs que han anat sorgint, ell m'ha ensenyat a interpretar les dades amb claretat i m'ha ajudat a expressar en termes senzills les idees complicades. També li hai d'agrair que m'hagi donat l'oportunitat de participar en tantes campanyes, sobretot en les de l'Antàrtida. Després del Carles vai conéixer al Juani i vai intuir que aquí a l'Institut s'hi treballava molt bé, no em vai equivocar, el Juani ha estat el millor company de feina que podia tenir però sobretot un bon amic per compartir inquietuds, penes i alegries. Moltes de les dades de producció bacteriana utilitzades en aguesta tesi han estat obtingudes per ell.

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Al Cisó vai conéixer al Pep i al Ramon i vai pensar: que bons son aguestos!!!. El Pep m'ha fet sempre un espai, en la seua atapida agenda, per escoltar-me i solucionar-me tota mena de problemes, gràcies Pep per les teues crítiques i per fer-me pensar en les hipòtesi encara que fos un cop acabades les discussions. Amb el Ramon hem coincidit a l'inici i al final de la tesi, temps suficient per a rebre els seus consells en moments clau d'aguet aprenentatge.

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Moltes gràcies a tots vosaltres!!!

Resum

En el present treball hem estudiat l'abundància dels virus i el seu impacte sobre la comunitat bacteriana heterotròfica en diferents sistemes aquàtics. Donada l'especificitat característica de la predació vírica, la hipòtesi sobre la qual s'ha desenvolupat aquest treball ha estat la següent: L'abundància i l'impacte dels virus sobre les poblacions hospedadores hauria de disminuir a mesura que augmenta la diversitat biològica d'un sistema. El coneixement de la diversitat bacteriana en la natura es troba en fase de desenvolupament i en la majoria d'ambients aquàtics és encara una incògnita. Malgrat això, basant-nos en les condicions físico-químiques de diferents ambients i en els estudis realitzats en els mateixos sobre l'ecologia de la comunitat microbiana, vam poder escollir un seguit de sistemes sobre els quals constatar la nostra hipòtesi de partida. D'una banda hem estudiat sistemes amb un fort gradient físico-químic, capaç d'ocasionar grans canvis en la xarxa tròfica; ja sigui de manera temporal, com ha estat el cas de l'estudi d'una proliferació de fitoplàncton simulada al laboratori; o de manera permanent, com ha estat el cas de les salines costaneres, on la salinitat és el factor que inhibeix la presència de diferents organismes. D'altra banda, hem estudiat sistemes que malgrat presentar un cert gradient en alguna variable, aquest no és tan pronunciat com en els casos anteriors. Aquests ambients han estat la Mar Mediterrània, caracteritzada per la seva oligotròfia i l'Oceà Glacial Antàrtic, amb temperatures permanentment fredes.

El primer pas per a assolir el nostre objectiu va ser posar a punt la metodologia necessària per a quantificar el virioplàncton i per a estimar el seu impacte sobre les poblacions bacterianes. Per a la quantificació dels virus en ambients aquàtics s'han utilitzat dos mètodes de recompte diferents: microscòpia electrònica de transmissió (TEM) i microscòpia d'epifluorescència, prèvia tinció dels virus amb DAPI o amb YOPRO. Les estimes de l'abundància dels virus poden variar en un ordre de magnitud, depenent del mètode emprat. Per obtenir una calibració entre ells vam haver de realitzar diverses comparacions en diferents ambients. L'impacte dels virus sobre la comunitat bacteriana s'ha estimat utilitzant 2 mètodes: el recompte de cèl.lules visiblement infectades mitjançant microscòpia electrònica i la quantificació de les taxes de desaparició dels virus. Ambdós mètodes presenten un seguit d'inconvenients que depenen en part del sistema en el qual es realitza l'estudi. Així doncs, segons el sistema investigat hem utilitzat un mètode o l'altre. L'obtenció d'una calibració entre ells no ha estat possible.

L'impacte dels virus sobre les poblacions bacterianes des de l'inici fins a la fi d'una proliferació de fitoplàncton s'ha estudiat en un experiment realitzat al laboratori. Els principals objectius d'aquest treball van ser: quantificar la mortalitat de les poblacions bacte-

rianes causada pels virus i comparar-la amb l'ocasionada pels bacterívors en les diferents etapes de la proliferació. L'experiment es va realitzar afegint determinades quantitats de nutrients a l'aigua procedent de la costa de Masnou (Barcelona), filtrada per una xarxa de 150 μm. Durant 10 dies es va mesurar la concentració de clorofil.la i l'abundància d'organismes (flagel.lats, bacteris i virus). Al mateix temps, es va estimar la producció bacteriana heterotròfica, la taxa de bacterivoria i la lisi vírica sobre els bacteris, a l'inici, durant i un cop finalitzada la proliferació. Els resultats obtinguts indiquen que en el transcurs de la proliferació, mentre la bacterivoria anava disminuint, la lisi vírica augmentava. La màxima mortalitat ocasionada pels virus sobre els bacteris (60 % de la producció bacteriana) es va produir cap a la fi de l'experiment (9 dies). Mentre la bacterivoria presentava el màxim impacte sobre les poblacions bacterianes (80 % de la producció bacteriana heterotròfica) al cap de dos dies, quan els bacteris es trobaven en la seva màxima abundància. Aquests resultats semblen suggerir que ambdós factors exerceixen un tipus diferent de control sobre els bacteris. Els bacterívors exercirien un control sobre l'abundància bacteriana total, donat que la seva activitat es troba únicament regulada per l'abundància de la presa. Els virus actuarien per impedir que una especie esdevingués massa abundant, donat que la seva acció no tan sols depén del nombre de possibles hospedadors, sinò de la diversitat genètica dels mateixos.

L'estudi de l'impacte dels virus en un ambient natural amb un gradient físico-químic permanent s'ha investigat en les salines costaneres de La Trinitat (Delta de l'Ebre, Tarragona). Les Salines estan formades per basses amb diferents concentracions de sals, que plegades constitueixen un gradient que va des de 35 ‰ fins 300 ‰ de salinitat. Així, a mesura que la salinitat augmenta va disminuint el nombre d'organismes diferents que composen la xarxa tròfica. De manera que en les basses de salinitat més elevada (>250 ‰) tan sols hi trobem *archaea* i virus. En aquesta situació, l'objectiu del nostre treball va ser similar a l'anterior: conèixer l'abundància i impacte dels virus sobre la comunitat procariòtica al llarg del gradient de salinitat i comparar-lo amb l'impacte dels bacterívors. Per això es va quantificar l'abundància dels organismes (virus, procarionts i flagel.lats) i es va estimar la producció dels procarionts, la bacterivoria i el percentatge de cèl.lules infectades pels virus, en les diferents bases. Els resultats indiquen que en les basses on hi ha bacterívors, aquests són responsables d'un percentatge molt més elevat de la mortalitat procariòtica total que els virus. Alhora, el temps de duplicació de la comunitat procariòtica disminueix en absència de bacterívors (2.5-5 dies). Els virus, malgrat produir-se en un gran nombre, són responsables de menys del 10 % de la mortalitat procariòtica total, en les basses sense bacterívors. Aquest fet suggereix que els virus i procarionts han arribat a un equilibri en les basses d'elevada salinitat, i malgrat la baixa diversitat suposada de la comunitat procariòtica, la taxa de creixement dels hospedadors també determina el grau

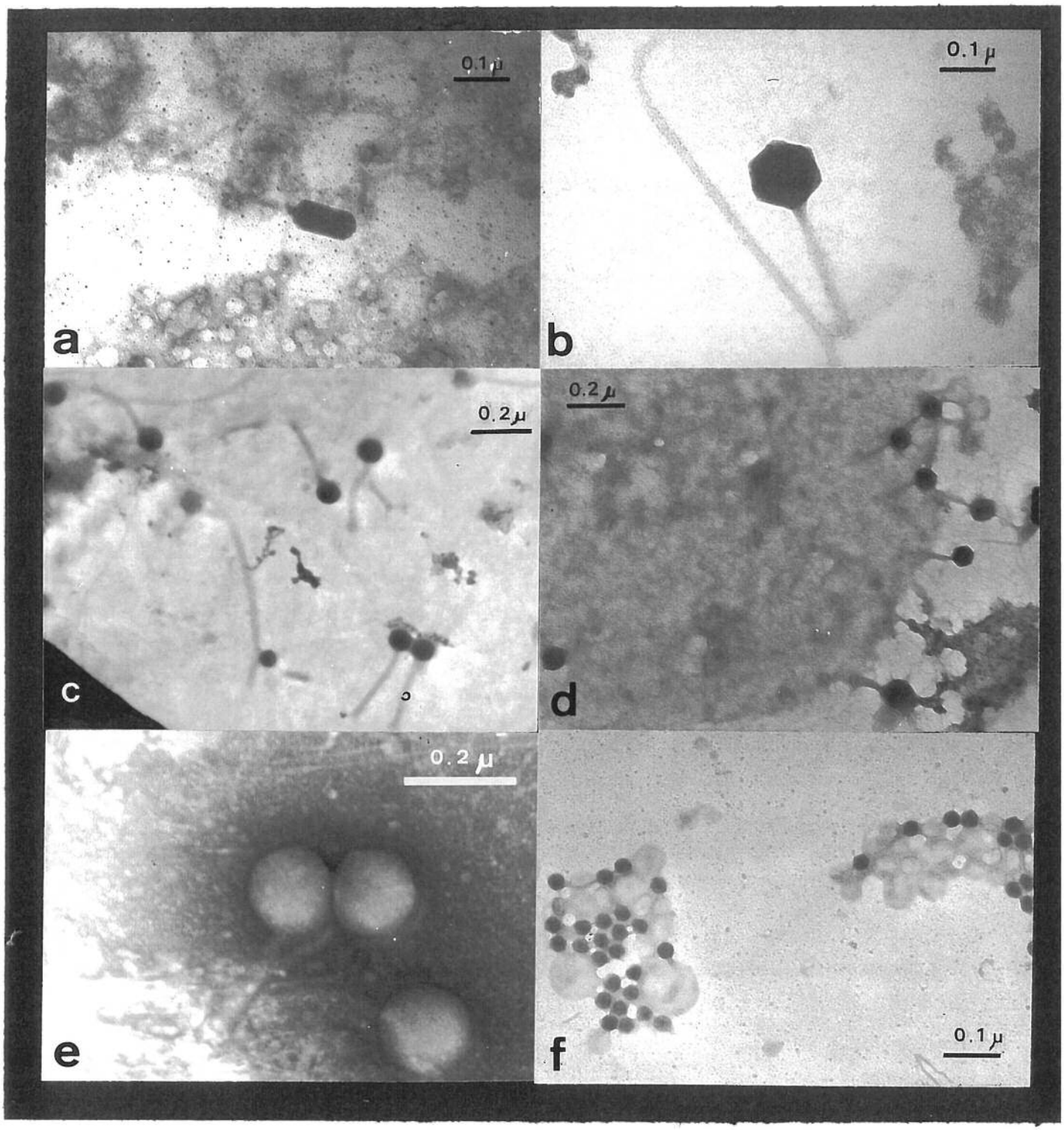
d'impacte dels virus sobre una comunitat. Això va complementar la nostra hipòtesi inicial.

El següent ambient estudiat ha estat la Mar Mediterrània en un transecte que va des de Barcelona a Mallorca durant la campanya VARIMED 95. Malgrat que la Mediterrània es considera un mar oligotròfic, en el transecte investigat destaquen tres zones (costanera, frontal i oceànica) on la producció es veu incrementada per diversos fenòmens físics. L'objectiu d'aquest estudi va ser investigar la distribució i l'impacte dels virus sobre la comunitat bacteriana en una situació d'oligotròfia, comparable a la que es dóna en la major part dels principals oceans del món. Es va realitzar la quantificació de l'abundància dels virus en perfils verticals de diferents estacions del transecte esmentat i es va investigar també la seva variabilitat al llarg del dia en les tres estacions considerades representatives. Al mateix temps es va intentar estimar l'impacte dels virus en aquestes estacions mitjançant el càlcul de les seves taxes de desaparició i el recompte de les cèl.lules infectades. La poca variabilitat observada en l'abundància dels virus, tant en el transecte des de la costa fins a mar obert com en els cicles dia-nit, és consistent amb la poca variabilitat que presenta l'abundància bacteriana en el mateix ambient. L'abundància dels virus en el transecte estudiat és una de les més baixes publicades per aigües marines. Això explica en part que els mètodes emprats per a quantificar l'impacte del virioplàncton es trobessin en el seu límit de detecció en aquest ambient. Aquests fets suggereixen, que malgrat l'existència d'un cert gradient tròfic, els virus en la Mediterrània són responsables d'un percentatge molt baix (<20 %) de la mortalitat bacteriana total.

L'últim ambient estudiat ha estat l'Oceà Glacial Antàrtic. Aquest estudi es va portar a terme durant les campanyes FRUELA 95 i 96, en el període corresponent a l'estiu austral. La zona investigada comprenia el sud del Passatge de Drake, l'oest de l'Estret de Bransfield i l'Estret de Gerlache. En l'ecosistema Antàrtic, la temperatura és el factor que condiciona amb major grau l'activitat dels organismes que hi habiten. A diferència dels ambients anteriors, la xarxa microbiana de l'Oceà Antàrtic no ha estat gairebé investigada, especialment en els processos que afecten a les perdues de la biomassa bacteriana heterotròfica. Els nostres objectius fonamentals van ser, d'una banda estudiar la variabilitat temporal i espaial de l'abundància dels virus i investigar el seu grau d'acoblament amb d'altres variables del sistema (clorofil.la i abundància de bacteris). I d'altra banda, quantificar l'impacte dels virus sobre la comunitat bacteriana a partir d'estimes de les seves taxes de desaparició. El percentage de mortalitat bacteriana deguda als virus es va comparar amb les pèrdues de bacterioplàncton ocasionades per la bacterivoria. La variabilitat temporal i espaial de l'abundància dels virus fou, en la totalitat de la zona estudiada, insignificant. Aquest fet coincideix amb el desacoblament observat entre l'abundància de virus i la concentració de clorofil.la i entre les abundàncies de virus

i bacteris. Respecte a l'impacte dels virus sobre la comunitat bacteriana, els resultats indiquen que són responsables d'un important percentatge de la mortalitat bacteriana total en la zona estudiada, els valors més elevats d'aquest percentage corresponen als llocs més eutròfics (>100 %) i els més baixos als llocs més oligotròfics (<50 %). Al comparar aquests valors amb les pèrdues bacterianes degudes als bacterívors, els virus representen una font de mortalitat bacteriana més elevada en les tres estacions on es van mesurar els dos processos. Aquest fet suggereix que la diferent resposta a la temperatura dels dos tipus d'activitat (bacterivoria i lisi vírica) podria ser decisiva a l'hora d'explicar les pèrdues de biomassa del bacterioplàncton en ambients freds.

Per últim s'han intentat trobar els patrons generals que expliquen la variabilitat de l'abundància dels virus en la natura. Per això vam recollir totes les dades publicades sobre distribucions de virus en ambients aquàtics i vam ajuntar-hi les nostres dades dels ambients esmentats anteriorment (més altres dades d'aigua dolça corresponents a llacs càrstics). Mitjançant regressions lineals s'ha relacionat l'abundància dels virus amb la temperatura, la concentració de clorofil.la, l'abundància dels bacteris i la producció i la taxa de creixement bacteriana, amb tot el conjunt de les dades recollides. El resultat de l'estudi posa de manifest que l'única variable que explica en un percentatge elevat (>50 %) la variabilitat en l'abundància dels virus, en un gran rang d'ecosistemes aquàtics, és l'abundància bacteriana. Això indirectament confirma que els bacteris són els hospedadors majoritaris dels virus en ambients aquàtics. El fet de disposar d'un seguit de dades d'ambients "peculiars" on la bacterivoria és gairebé inexistent (salines d'elevada salinitat i la part anaeròbica dels llacs càrstics), ens va permetre investigar si aquesta caracerística influia significativament sobre l'abundància dels virus i la seva relació amb els bacteris. Al comparar els dos ambients sense bacterívors s'ha vist que en cadascun d'ells l'abundància dels virus en relació amb l'abundància dels bacteris, segueix un patró diferent. Mentre que en les salines (>250 ‰) la relació virus/bacteri és similar a la dels ambients sense bacterívors (aproximadament 10 virus/bacteri), en la part anaeròbica dels llacs estratificats aquesta relació és significativament més baixa (aprox. 1-3 virus/bacteri). Això suggereix que altres factors (anaerobiosi, presència de sulfhídric) a més de l'absència de bacterívors, tenen un efecte decisiu sobre la producció dels virus en aquest sistema.



Fotografies obtingudes mitjançant microscòpia electrònica de transmissió (TEM) que mostren les morfologies dels virus observades en diferents ambients estudiats en aquest treball. Llacuna de'n Cisó (**a**), Mar Mediterrània (**b**), Llac del Tobar (**c**), Salines de Santa Pola (**d**), Llac de la Cruz (**e**), Mar de Weddell (**f**).

1. Introducció

La primera estima quantitativa de l'abundància dels virus en sistemes aquàtics va ser obtinguda per Torrella & Morita (1979), que van trobar una concentració de més de 10^4 virus mL^{-1} en aigües costaneres d'Oregon. No va ser, però, fins a finals dels anys 80, que diferents grups de recerca simultàniament, van posar de manifest que els virus eren els organismes més abundants dels ambients aquàtics (10^5-10^8 virus mL^{-1}, Berg *et al.* 1989, Proctor & Fuhrman 1990). Aquest fet va estimular l'interès per saber quins tipus d'organismes infecten, quin és el seu temps de renovació, els processos que els eliminen i la quantificació de la mortalitat sobre els seus hospedadors; amb l'objectiu de compendre millor l'ecologia i el flux de materials i energia a través de la xarxa tròfica tant marina com d'aigua dolça. La present tesi pretén donar resposta a algunes d'aquestes preguntes i està centrada en l'impacte dels virus sobre les poblacions bacteriana hetròtrofica.

L'estudi dels bacteris heteròtrofs en ambients aquàtics ha esdevingut fonamental en ecologia aquàtica des de que a final dels anys 70 es va posar de manifest el seu paper com a processadors d'una important part del carboni de l'oceà (Pomeroy 1974). Diferents factors determinen el creixement i l'abundància dels bacteris en ambients aquàtics. D'una banda influeix la disponibilitat de nutrients inorgànics (N & P) i la quantitat i qualitat del carboni orgànic disolt del medi. D'altra banda existeixen els factors responsables de les seves pèrdues. El més important d'aquests és la predació per bacterívors (Pace 1988, Sherr *et al.* 1989). Tanmateix, les pèrdues del bacterioplàncton no es poden explicar sempre per aquest procés (Sherr *et al.* 1989) i la lisi vírica ha resultat ser una causa important de mortalitat bacteriana en alguns dels ambients estudiats (Fuhrman & Noble 1995, Weinbauer & Peduzzi 1995a, Steward *et al.* 1996).

A continuació exposarem detalladament com s'inclouen els virus dins la xarxa tròfica planctònica, quins han estat els objectius plantejats en aquest treball i com els hem intentat resoldre.

Incorporació dels virus a la xarxa tròfica microbiana

Cicle de multiplicació

Per a comprendre el paper dels virus dins la xarxa tròfica planctònica ens hem de fixar en el seu cicle de multiplicació. Els virus són paràsits obligats i sumament específics sobre els seus hopedadors. Aquest fet determina que l'èxit del seu atac no sols depengui de la quantitat d'organismes presents en un ambient sino de la susceptibilitat d'aquests organismes a l'atac víric. Així doncs com més diversa és una comunitat d'organismes, la probabilitat de que es produeixi un atac víric serà més baixa. Un cop un virus ha aconseguit entrar dins una cèl.lula pot seguir un cicle lisogènic, lític o crònic (Fig.1.1).

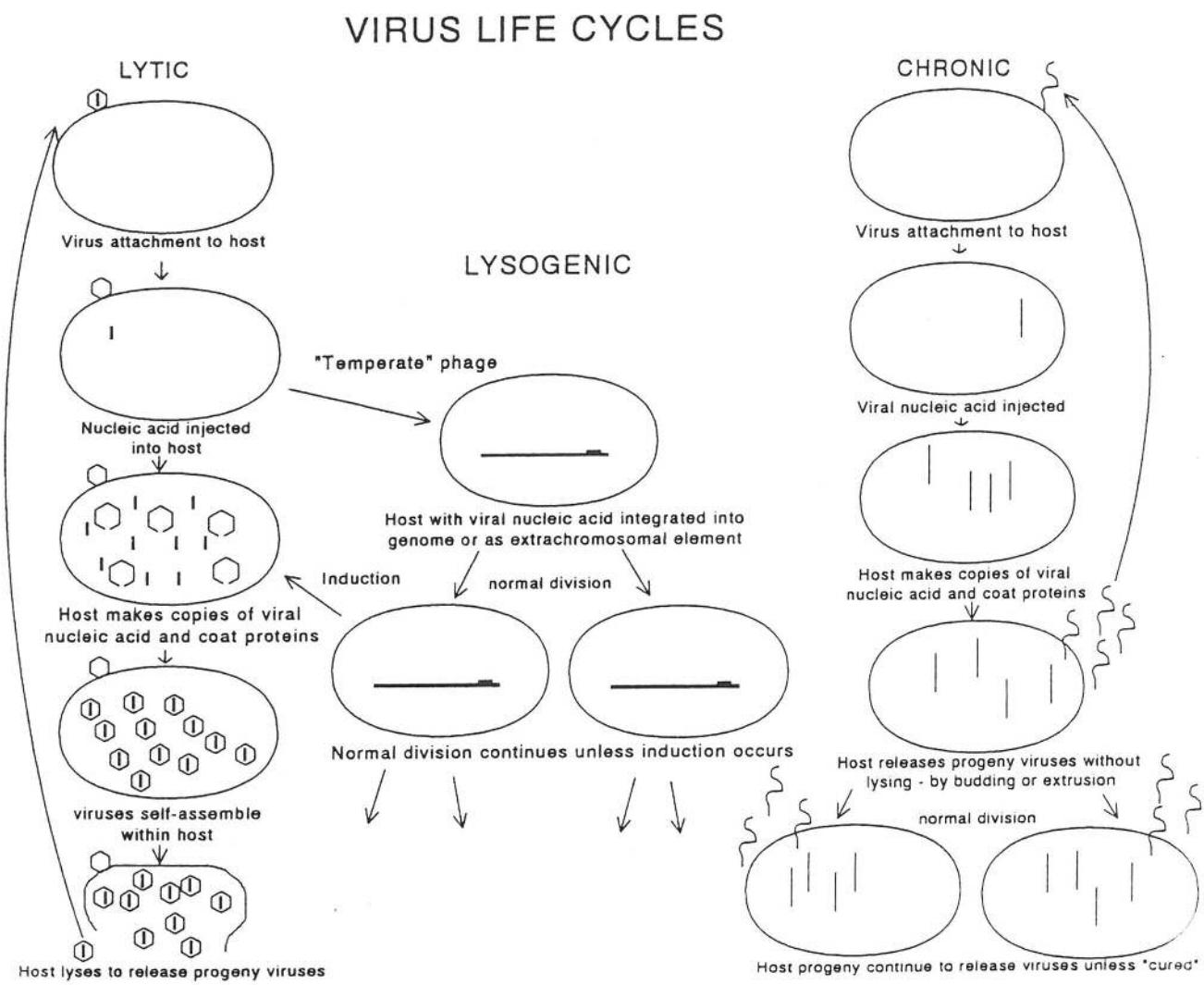


Fig.1.1. Cicles de multiplicació dels virus (Fuhrman & Suttle 1993). En el cicle lític, els virus utilitzen la maquinària enzimàtica de la cèl.lula hospedadora per a produir nous virions, que s'alliberen al medi ocasionant la lisi cel.lular. En el cicle lisogènic l'àcid nucleic del virus s'integra en el de la cèl.lula hospedadora, i es continua dividint sense ocasionar cap dany a la mateixa, a menys que es produeixi la inducció cap a un cicle lític. En la infecció crònica, es produeixen virions sense ocasionar la lisi cel.lular.

En el cicle lisogènic, l'àcid nucleic del virus s'integra en el de la cèl.lula hospedadora i es replica al mateix temps que aquesta. En aquestes condicions els virus no ocasionen cap mena de transtorn a la cèl.lula. D'un cicle lisogènic es pot induir un cicle lític per canvis en el medi (variacions en la concentració de nutrients) o per factors com la radiació ultraviolada o certs agens químics, com la mitomicina C. En el cicle lític, un cop els virus han entrat dins la cèl.lula hospedadora, aprofiten la maquinària enzimàtica d'aquesta per replicar el seu àcid nucleic i formar noves partícules víriques que acaben produint la lisi cel.lular. El temps transcorregut des de què un virus entra dins una cèl.lula fins que es produeix la seva lisi s'anomena període de latència. A l'inici del període de latència, mentre els virus repliquen el seu àcid nucleic, les partícules encara no són visibles dins la cèl.lula infectada, aquest temps s'anomena període d'eclipsi. El nombre de virions (partícules víriques) alliberats per una cèlula en cada cicle lític s'anomena tamany d'explosió. En alguns casos l'alliberació de partícules víriques es produeix sense lisar la cèl.lula hospedadora (infecció crònica). En els estudis realitzats fins al moment la lisogènia representa una via poc important per a la producció de virus a la natura (Wilcox & Fuhrman 1994, Weinbauer & Suttle 1996).

Abundància de virus en sistemes planctònics

La quantificació del nombre d'organismes en un ambient és el primer pas necessari per a poder abordar posteriors qüestions sobre la seva funció. Els recomptes de virus realitzats en ambients aquàtics ens permeten conèixer l'abundància de les partícules víriques o virions que es troben lliures en un sistema. Donat el grau d'incertesa en la identificació dels virions en sistemes naturals amb els diferents mètodes de recompte emprats, utilitzarem el terme de "partícules semblants a virions" (VLP, "virus like particles"), per a referir-nos a l'abundància dels virions i a la seva dinàmica, quan aquestes han estat mesurades amb les tècniques microscòpiques esmentades. L'abundància de les VLP en aigües oceàniques superficials oscil.la entre 10^5 i 10^7 VLP mL^{-1} (p. e. Cochlan *et al.* 1993), mentre que en aigües costaneres la seva abundància pot arribar fins 10^8 VLP mL^{-1} (p. e. Bratbak *et al.* 1990). Un dels principals inconvenients en la quantificació de l'abundància de les VLP amb les tècniques microscòpiques desenvolupades fins ara (microscòpia electrònica de transmissió, Heldal & Bratbak 1993 i microscòpia d'epifluorescència, Suttle 1993, Hennes & Suttle 1995) està en la incapacitat de poder diferenciar per criteris morfològics el tipus d'organisme al que parasiten. Malgrat això, els estudis realitzats per a conèixer l'abundància de les VLP tant en aigües dolces com marines ha servit per a poder establir unes pautes generals sobre les variables ecològiques amb qui es troben més estretament relacionades. Així doncs, en la majoria d'estudis, l'abundància de les VLP sembla estar correlacionada amb l'abundància dels bacteris (Boehme *et al.* 1993, Cochlan *et al.* 1993, Paul *et al.* 1993, Jiang & Paul 1994,

Weinbauer *et al.* 1995) i amb la concentració de clorofil.la a (Maranger & Bird 1995). Aquestes evidències juntament amb el fet de què els bacteris són els hospedadors potencials més abundants, indirectament suggereixen que la majoria de les VLP quantificades en ambients aquàtics infecten bacteris (Fuhrman & Suttle 1993) i que la seva abundància augmenta amb el grau d'eutrofia d'un sistema (Weinbauer & Peduzzi 1995a). Malgrat aquest fet, no es pot oblidar que s'han aïllat virus d'altres organismes marins, des de peixos fins a fitoplàncton (Fuhrman & Suttle 1993). En el cas dels cianòfags, els virus que infecten el gènere *Synechococcus* han estat ampliament estudiats (p. e. Suttle & Chan 1993), donada la importància del gènere com a productors primaris a l'oceà. Aquests estudis indiquen que les concentracions de virions que infecten una sola soca de *Synechococcus* van des de 1 fins a 10^5 per mL (Suttle 1992).

Donada la complexitat i diversitat del cicle de multiplicació dels virus, el coneixement de l'abundància de les VLP ens dona molt poca informació de la seva dinàmica i del seu impacte sobre la comunitat bacteriana i fitoplanctònica.

Dinàmica dels virus en l'ambient natural

L'estudi sobre l'activitat dels virus ha anat lligat a l'aparició de noves tècniques o a l'aplicació d'altres ja existents en altres camps de la virologia. En primer lloc resumirem les tècniques utilitzades per determinar la desaparició dels virus, i en segon lloc, aquelles utilitzades per estimar la seva producció.

La desaparició dels virus ha estat estudiada tant amb fags i els seus hospedadors aïllats de la comunitat en experiments de laboratori (Suttle & Chen 1992, Noble & Fuhrman 1997) com sobre la totalitat del virioplàncton (Heldal & Bratbak 1991, Mathias *et al.* 1995). En el cas dels virus s'ha de diferenciar entre la desaparició de les partícules víriques del medi i la pèrdua de la seva infectivitat, donat que ambdós processos poden no ocórrer simultàniament. La radiació ultraviolada i l'adsorció a partícules són els principals factors responsables de la pèrdua d'infectivitat dels virus (Fig. 1.2). Mentre que l'adsorció a partícules suposa al mateix temps la desaparició de les partícules víriques, la radiació ultraviolada pot ocasionar la pèrdua d'infectivitat (per danys en el material genètic) sense que els virions canviin el seu aspecte sota el microscopi. Altres processos com la ingestió per nanoflagelats han resultat ser quantitativament poc importants (Suttle & González 1993).

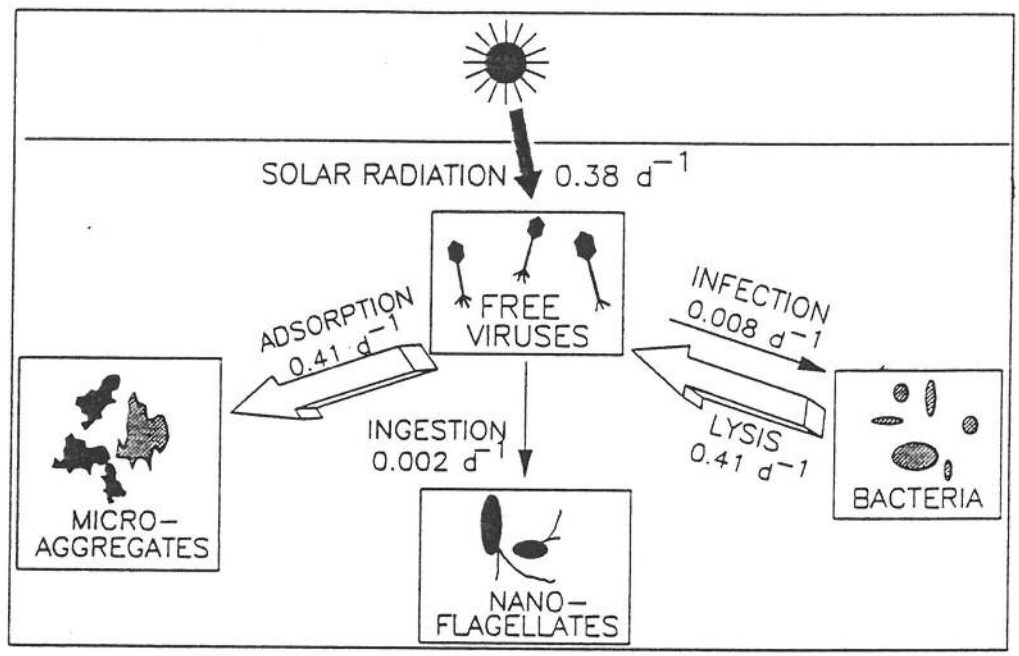


Fig. 1.2. Agents responsables de la desaparició dels virions en sistemes aquàtics i importància quantitativa de cadascun d'ells (Suttle & Chen 1992). La radiació ultraviolada és el factor més important a l'hora d'ocasionar la pèrdua d'infectivitat de les partícules víriques. Mentre que l'adsorció a partícules és el factor responsable de la major part de la desaparició dels virions.

Les taxes de desaparició de les VLP en comunitats naturals, han estat investigades amb incubacions de mostres d'aigua utilitzant cianur per inhibir l'activitat biològica (Heldal & Bratbak 1991). D'aquesta manera s'atura la producció de virions i es poden calcular les taxes de desaparició de les VLP, quantificant la disminució de la seva abundància al llarg del temps. Amb aquesta aproximació s'ha vist que les taxes de desaparició varien en funció de la mida de les VLP i del medi on es troben (Heldal & Bratbak 1991, Mathias *et al.* 1995). Les taxes de desaparició de les VLP estimades amb aquest mètode van de 0.4-1.1 h^{-1} (Heldal & Bratbak 1991) per a la fracció de virus <60 µm en aigües costaneres i de 0.06 a 0.08 h^{-1} en aigua dolça. Per a la fracció de virus de >60 µm aquestes taxes eren de 0.02-0.05 h^{-1}, en ambdós ambients (Mathias *et al.* 1995, Heldal & Bratbak 1991). El principal inconvenient d'aquest mètode és que tan sols mesura la desaparició de les VLP per causes físiques, donat que l'activitat biològica es troba inhibida. El càlcul de les taxes de desaparició ha servit també com a mesura de les taxes de producció de les VLP; donat que l'abundància de les VLP experimenta poques fluctuacions al llarg del temps en la major part dels ambients investigats, s'assumeix que les taxes de desaparició de les VLP són equival.lents a les seves taxes de producció.

La incorporació de molècules marcades radioactivament, com és el cas de la timidina o l'ortofosfat, s'ha utilitzat també per calcular la producció de virions en ambients aquàtics (Steward *et al.* 1992a, b). Amb aquest mètode s'han obtingut estimes de la producció de virus en un gradient des d'aigües costaneres fins a l'oceà, que van des de $<1x10^6$ fins $2.3x10^{11}$ virus L^{-1} h^{-1}. El principal inconvenient d'aquest mètode és la possibilitat d'infraestimar la producció de virus, donat que un cop incubada l'aigua amb la substància radioactiva s'ha de filtrar a través d'un filtre de 0.2 µm de tamany de porus per eliminar la fracció de radioactivitat incorporada en els bacteris i algues que no ha estat incorporada pels virus. Amb aquesta filtració es poden arribar a perdre el 50 % de les VLP que es troben en la mostra natural (Steward *et al.* 1992b).

No s'han realitzat estudis comparant ambdós mètodes (taxes de desaparició i incorporació de substracte radioactiu) en el mateix ambient, tanmateix, en el conjunt d'ambients on s'han utilitzat, les taxes de producció de partícules víriques obtingudes mesurant la incorporació de radioactivitat es troben en el rang més baix de les taxes obtingudes en els experiments de desaparició amb cianur.

Mortalitat causada pels virus sobre les poblacions bacterianes

L'evidència més clara de que els virus infecten una part del bacteriplànkton ha estat obtinguda mitjançant la visualització per microscòpia electrònica de bacteris infectats amb virus. Això s'ha posat de manifest amb observacions directes de les mostres naturals

(Weinbauer & Peduzzi 1994) o amb la realització de talls prims de les cèl.lules (Proctor & Fuhrman 1990). Aquestes observacions realitzades de forma quantitiativa han servit per estimar l'impacte dels virus sobre les poblacions bacterianes. L'inconvenient d'aquestes mesures és que els virus tan sols són visibles com a partícules dins l'hospedador durant un temps del període de latència (el període que no és el d'eclipsi) i part de la població bacteriana observada sense partícules víriques, pot estar infectada sense que aquestes siguin visibles. Proctor *et al.* (1993) van resoldre aquest problema estudiant detalladament alguns dels sistemes hospedador-virus presents en ambients marins. Van observar que gairebé tots els fags visibles intracel.lularment s'acumulaven durant l'últim 25 % del període de latència. D'aquesta manera van calcular uns factors de conversió (3.7- 7.15) per poder estimar el nombre total de bacteris infectats a partir del nombre de bacteris visiblement infectats (VIB "visibly infected bacteria"). Així, el percentatge de bacteris visiblement infectats observat en una determinada mostra, es multiplica per aquest rang de factors i obtenim el percentatge total de bacteris infectats. Per calcular el percentatge de la mortalitat bacteriana total causada pels virus a partir del percentage total de bacteris infectats es van utilitzar un seguit de consideracions:

1. El temps de duplicació dels bacteris hospedadors té una durada similar al període de latència dels virus que els infecten, això ha estat observat a partir de diferents estudis en determinats sistemes bacteri-bacteriòfag.

2. En una situació on els bacteris mantenen una abundància constant al llarg del temps, cada divisió cel.lular resulta en una cèl.lula que viu i en una cèl.lula que mort en el temps d'una generació (per mantenir l'abundància constant), aleshores qualsevol factor que produeixi la mort del 50 % de les cèl.lules d'una generació és responsable del 100 % de la mortalitat bacteriana. D'aquesta manera el percentatge total de cèl.lules infectades s'ha de multiplicar per 2 per obtenir el percentatge de la total mortalitat bacteriana ocasionada pels virus.

Malgrat que els esmentats factors han estat calculats a partir de la realització de talls prims, s'han aplicat també en els estudis on el recompte de les cèl.lules infectades s'ha realitzat amb l'observació directa de la mostra natural. Al comparar les dues aproximacions en un mateix ambient, el percentage de cèl.lules visiblement infectades amb l'observació directa de la mostra natural, sembla ser lleugerament inferior al mesurat a partir de la realització de talls cel.lulars prims (Fuhrman & Noble 1995). Això pot introduir un error en les estimes de mortalitat calculades a partir de la mostra natural sense realitzar talls, que en tot cas representarien una estima conservadora del percentage de la mortalitat bacteriana causada pels virus (Fuhrman & Suttle 1993). Amb aquests mètodes s'ha trobat que els virus poden ser responsables del 15 al 60 % de la mortalitat bacteriana total (Proctor &

Fuhrman 1990, Fuhrman & Noble 1995, Mathias *et al.* 1995, Weinbauer & Peduzzi 1995a, Steward *et al.* 1996). Els valors més baixos d'aquest rang correspondrien a llocs oligotròfics i els valors més elevats a llocs eutròfics (Weinbauer & Peduzzi 1995a).

El percentatge de mortalitat bacteriana deguda als virus, també s'ha pogut calcular a partir de les estimes de producció de virions i de les seves taxes de desaparició, descrites en paràgrafs anteriors. Per això es necessari conèixer el tamany d'explosió dels bacteris infectats. Aquest valor es difícil d'estimar en les poblacions bacterianes dels ambients naturals, donat que varia en funció del volum i de l'estat nutricional de la cèl.lula hospedadora (Weinbauer & Peduzzi 1994). En la majoria dels estudis realitzats s'assumeix un rang de tamanys d'explosió que va des de 50 a 100 virions, alliberats per cada bacteri infectat en cada cicle lític. Aquests valors s'han calculat induint la lisi dels bacteris amb estreptomicina (Heldal & Bratbak 1991). A partir de la mesura dels virions produits durant un període de temps (VLP $L^{-1}d^{-1}$) i dividint aquest valor pel tamany d'explosió, obtenim el nombre de bacteris lisats pels virus en el mateix temps (BN lisats L^{-1} d^{-1}). Es calcula aleshores, el percentatge de la producció heterotròfica bacteriana que es perd a causa de la lisi vírica:

(BN lisats $L^{-1}d^{-1}$x 100) / BN produits L^{-1} d^{-1}

En els sistemes on els bacteris mantenen una abundància constant al llarg del temps, el percentage obtingut correspodria al percentage de la mortalitat bacteriana total ocasionada pels virus.

Les estimes de mortalitat bacteriana calculades a partir de mesurar la producció dels virions amb la incorporació d'un substracte radioactiu, es troben dins el rang de les estimes calculades a partir del recompte de cèl.lules infectades (Steward *et al.* 1996). En un intent de comparar els dos mètodes en un mateix ambient s'ha vist que donen estimes de la mortalitat bacteriana similars (Fuhrman & Noble 1995).

Les estimes de mortalitat bacteriana calculades a partir de les taxes de desaparició de les VLP mesurades amb cianur, han estat comparades amb estimes de la mortalitat bacteriana calculades amb el recompte de cèl.lules infectades, en un mateix ambient (Mathias *et al.* 1995). Amb ambdós mètodes s'han obtingut resultats similars, encara que el percentatge de mortalitat causat pels virus sobre les poblacions bacterianes era lleugerament superior quan va estar calculat a partir de les taxes de desaparició (Mathias *et al.* 1995)

Les estimes de mortalitat bacteriana deguda als virus, malgrat anar envoltades d'un gran nombre d'assumpcions, confirmen que aquests, en certes ocasions, poden ser responsables d'una mortalitat bacteriana similar a la dels bacterívors. En els dos únics estudis on s'han comparat les pèrdues bacterianes degudes a virus i degudes a bacterívors, ambdós factors contribuien de manera similar a la mortalitat bacteriana (Fuhrman & Noble 1995, Steward *et al.* 1996).

Conseqüències de l'acció dels virus en la xarxa tròfica planctònica.

Malgrat que el nostre estudi està centrat en l'impacte dels virus sobre les poblacions bacterianes heterotròfiques, hem esmentat abans que els virus poden infectar a molts altres organismes de la xarxa tròfica i eliminar-los en un temps relativament curt. La possibilitat de que els virus fossin causants de l'acabament de proliferacions de fitoplàncton és una de les hipòtesis que ha despertat més interès. Així i tot existeixen poques dades que afavoreixin aquesta hipòtesi (Bratbak *et al.* 1990, Nagasaki *et al.* 1993, Brussard *et al.* 1996). La majoria de virus de fitoplàncton aïllats semblen influenciar més l'estructura genètica de la comunitat que la seva abundància (Fuhrman & Suttle 1993). L'especificitat característica de l'atac víric suggereix que els virus poden desenvolupar un important paper com a responsables del manteniment de la diversitat de les comunitats microbianes en sistemes planctònics (Thingstad *et al.* en prensa).

L'efecte dels virus en el flux de nutrients i carboni té especial interès en la qüestió de si els bacteris actuen com a "desaigüe o conexió" ("sink o link") en la xarxa tròfica. Quan els virus fossin responsables d'un percentage elevat de mortalitat bacteriana, el carboni orgànic disolt utilitzat pels bacteris retornaria novament al medi degut a la lisi d'aquests. En aquest cas, els sistemes bacteris-fags actuarien com a "desaigües", malgrat que els bacteris creixessin amb una elevada taxa (Fuhrman & Suttle 1993).

Recentment, Thingstad *et al.* (en prensa) han construit un model combinant l'efecte del substracte, els bacterívors i els virus sobre la comunitat bacteriana. Thingstad *et al.* (en prensa) suggereixen que l'acció específica dels virus actua com a control de l'abundància d'una espècie bacteriana que ha pogut creixer malgrat existir un substracte limitant. El control dels virus d'aquest "guanyador" permetria a les altres espècies bacterianes d'establir-se. Aquest tipus de control del bacterioplàcton rebria el nom de "side-in", en comparació amb els controls per substrat (bottom-up) i per predació (top-down, Fig. 1.3).

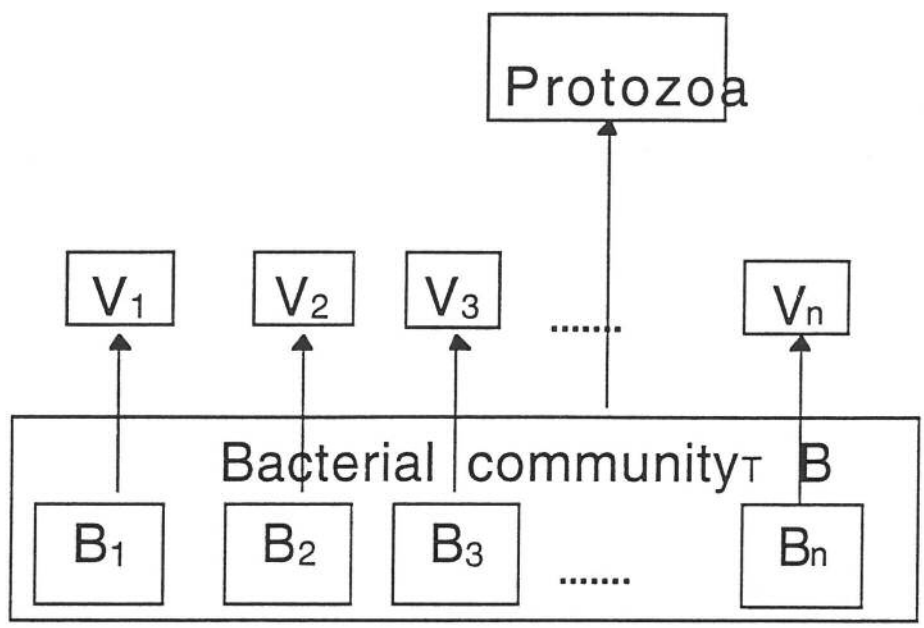


Fig. 1.3. Esquema que mostra la diferència de control que exercirien els protozous (top-down) i els virus (side-in) sobre les comunitats bacterianes segons Thingstad *et al.* (en prensa).

Objectius generals de la tesi

La intenció d'aquest treball ha estat estudiar l'abundància dels virus i el seu impacte sobre les poblacions bacterianes heterotròfiques en sistemes aquàtics. La hipòtesi sobre la qual s'ha desenvolupat l'estrategia de mostrejos de diferents ambients on portar a terme el nostre estudi ha estat la següent: Donada l'especificitat característica de la predació vírica, l'abundància i l'impacte dels virus haurien d'augmentar al disminuir la diversitat dels seus hospedadors. Malgrat que el coneixement de les "espècies" que composen les comunitats bacterianes en la natura actualment comença a estar a l'abast de les tècniques moleculars més recents, en la majoria d'ecosistemes de la Terra, és encara una incògnita. Així doncs hem aprofiat les característiques físico-químiques de determinats ambients, sobre els quals s'ha estudiat prèviament el funcionament de la xarxa tròfica microbiana, per aproximar-nos, en la mesura del possible, a la constatació de la hipòtesi de partida. Els objectius plantejats per a poder respondre a la mateixa han estat:

1. Estudiar l'impacte dels virus sobre les comunitats bacterianes heterotròfiques en ambients amb un fort gradient físico-químic, capaç de produir grans canvis en l'estructura de la xarxa tròfica. Això s'ha investigat en una situació temporal, com és el cas d'una proliferació de fitoplàncton, en un experiment realitzat al laboratori (Capítol 2). I en un gradient de salinitat, com és el cas de les salines de La Trinitat (Capítol 3).

2. Estudiar l'impacte dels virus sobre les comunitats bacterianes heterotròfiques en ambients fortament influenciats per una característica gairebé permanent. Aquest ha estat el cas de la Mar Mediterrània (Capítol 4), considerada com a oligotròfica, malgrat experimentar variacions espaials i temporals en la seva productivitat i l'Oceà Glaciar Antàrtic (Capítol 5), caracteritzat per les seves baixes temperatures al llarg de l'any.

3. Estudiar l'abundància i distribució de les VLP en sistemes aquàtics. Per això hem realitzat mostrejos en ambients de característiques molt diferents. Aprofitant diverses campanyes oceanogràfiques, hem obtingut estimes de l'abundància de les VLP a la Mar Mediterrània (FRONTS 1994 i VARIMED 1995) i a l'Antàrtida (ECOANTAR 1994, FRUELA 1995-96). S'han realitzat altres campanyes a llacs càrstics (Estanyol d'en Cisó, llacunes del Tobar i la Cruz) i a salines de la conca Mediterrània (salines de la Trinitat i salines de Santa Pola), on també hem quantificat l'abundància de les VLP. Amb les dades obtingudes i junt amb les d'altres estudis recollits en la literatura, hem investigat els factors ecològics que expliquen la variabilitat en l'abundància de les VLP en ecosistemes planctònics (Capítol 6).

Plantejament del treball i resposta als objectius

La tesi està dividida en capítols, coincidint amb els ambients estudiats, excepte l'últim, que correspon al recull de les dades presentades en els altres més les dades de l'abundància de les VLP extretes de la literatura. En cada capítol s'expliquen els corresponents mètodes utilitzats.

Capítol 2. Correspon a un experiment de laboratori realitzat amb aigua de la costa Mediterrània. En aquest treball hem pretés reproduir la situació d'una proliferació de fitoplàncton. En aquest experiment comparem les diferents tècniques de recompte per obtenir l'abundància de les VLP i quantifiquem l'acció dels virus i bacterívors sobre els bacteris en diferents etapes de la proliferació.

Capítol 3. Correspon a l'estudi de la distribució de les VLP i l'impacte del virioplàncton en unes salines costaneres (La Trinitat) amb un fort gradient de salinitat. D'aquesta manera hem pogut investigar com varia la lisi vírica dels bacteris al llarg del gradient. Al mateix temps hem comparat el percentatge de mortalitat bacteriana ocasionada pels virus i pels bacterívors en cada bassa.

Capítol 4. Correpon a la distribució de les VLP en un transecte de la Mar Mediterrània, desde la costa fins a mar obert, com a exemple d'ambient oligotròfic. En aquest treball s'ha estudiat la variabilitat de l'abundància de les VLP al llarg del transecte i en cicles dianit. Al mateix temps s'ha intentat quantificar l'impacte dels virus sobre les poblacions bacterianes en tres estacions del transecte.

Capítol 5. Correspon a la distribució i dinàmica de les VLP en un ecosistema fred com és el cas de l'Antàrtida. En aquest ambient hem estudiat la variabilititat temporal i espaial de l'abundància de les VLP en la zona compresa entre l'Estret de Brasfield, el Passatge de Drake i l'Estret de Gerlache. També hem investigat els factors implicats en la desaparició de les VLP i el percentatge de mortalitat que els virus ocasionen sobre la població bacteriana.

Capítol 6. En aquest capítol hem recollit totes les dades de l'abundància de les VLP obtingudes dels diferents mostrejos i hi hem afegit les procedents d'altres estudis. Mitjançant regressions, hem relacionat l'abundància de les VLP amb altres variables del sistema amb l'objectiu de conèixer els factors que millor expliquen la seva variabilitat.

2. Viral lysis and bacterivory during a phytoplankton bloom

Introduction

A few years ago bacterivory was considered to be the most important bacterial loss factor in aquatic environments (e. g. Pace 1988). More recently, however, viral infection has been found to account for a significant proportion of bacterial mortality in some aquatic environments (Fuhrman & Noble 1995, Weinbauer & Peduzzi 1995a, Steward *et al.* 1996).

The first attempt to incorporate viruses into the budget of microbial carbon transfer was done by Bratbak *et al.* (1992). In a mesocosm experiment, these authors measured viral lysis and bacterivory simultaneously. However, they could not balance bacterial losses with both factors. Viral lysis exceeded bacterial heterotrophic production (BHP) by a factor of 6 while bacterivory exceeded it by a factor of 2. Recently, two studies that have measured viral lysis and bacterivory simultaneously, have found that both factors accounted for the same proportion of bacterial mortality (Fuhrman & Noble 1995, Steward *et al.* 1996). However, another study has found a small contribution of viral lysis to bacterial mortality in comparison to bacterivory (Chapter 3). Thus, it is not clear in what situations viral lysis could prevail over bacterivory in controlling bacterial abundance. Weinbauer & Peduzzi (1995a), using a relationship between viral and bacterial abundance, concluded that viral infection could prevail over bacterivory at high bacterial abundance. Their results were in agreement with the uncoupling found between bacteria and heterotrophic nanoflagellates at high bacterial abundance (Gasol & Vaqué 1993).

All of these studies have been done in a steady-state situation where cells and viruses showed small temporal fluctuations. Large variations in the abundance of organisms in short periods of time have been reported during phytoplankton blooms. Bratbak *et al.* (1990) have studied the fluctuations in viral (VLP) abundance during a phytoplankton bloom. In their study, they found a fast increase in VLP abundance after the maximal bacterial abundance had been reached. These authors suggested that part of the bacterial population had been lysed by viruses. However, they did not quantify the proportion of bacterial mortality attributable to viral lysis during the bloom.

The objective of the present work was to investigate the different proportions of bacterial mortality attributable to viral lysis or bacterivory at different stages of a phytoplankton bloom. In order to avoid the difficulties associated with the drifting of water masses inherent to marine environments, we carried out a microcosm experiment. The appearance of a phytoplankton bloom was stimulated by adding nutrients to the natural water sample. Changes in chlorophyll a, flagellate, bacterial and VLP abundance were followed with time by different counting methods. Bacterial heterotrophic production,

bacterivory and viral lysis were also measured. The proportion of bacterial mortality due to viruses and to bacterivory was then estimated. In this work, we could asses whether one factor prevailed over the other, or whether both acted simultaneously at each stage of the bloom.

Materials and Methods

A microcosm experiment following the microbial populations during a phytoplankton bloom was performed in November 1995 with water from Masnou harbor, located in the Mediterranean coast (20 km north of Barcelona). The same input of inorganic nutrients was added to two polypropylene bottles with 20 L of the sampled water filtered throughout 150 μm pore size Nylon mesh. Both replicates of the enriched cultures were incubated during 12 days at a similar temperature as the original sample (16° C). Cultures were incubated with periods of 12 hours light and 12 hours dark. Light intensity was about 100-120 $\mu E\ m^{-2}\ s^{-2}$ during the light period. Samples for chlorophyll a concentration, bacterial, flagellate and VLP abundance were taken daily from both cultures. Bacterial heterotrophic production, bacterivory and percentage of visibly infected bacteria (VIB) were measured also at different times of the experiment (initial sample and after 2, 4, 7 and 9 days). At the same sampling days viral decay experiments were performed with water from the cultures.

Chlorophyll a was determined fluorometrically in 100 mL samples that were filtered through GF/F glass fiber filters and frozen. The filters were extracted overnight in 90 % acetone at 4 °C and fluorescence of the extract measured with a Turner Designs fluorometer (Yentsch & Menzel 1963).

Samples for bacterial, flagellate and VLP abundance were fixed with gluteraldehyde (2 % final concentration) in polypropylene bottles. Bacteria were stained with DAPI ($1\mu g\ mL^{-1}$ final concentration) and filtered onto black 0.2 μm pore size polycarbonate filters (Porter & Feig 1980), mounted on microscope slides and frozen. Bacterial abundance was determined with a Nikon epifluorescence microscope at a magnification of 1250x. About 200-300 bacteria were counted per sample. Flagellates were stained with DAPI ($1\mu g\ mL^{-1}$ final concentration) and filtered onto black 0.6 μm pore size polycarbonate filters mounted on microscope slides and frozen. Flagellate abundance was determined with a Nikon epifluorescence microscope at a magnification of 1250x. About 200-300 flagellates were counted per sample. Phototrophic nanoflagellates (PNFs) were distinguished from heterotrophic nanoflagellates (HNFs) based on the fluorescence of Chlorophyll a.

VLP abundance was determined by three different methods: YOPRO stain (Molecular Probes YO-PRO 1) and epifluorescence microscopy, DAPI stain and epifluorescence microscopy and by electron microscopy (TEM). Unfixed samples for VLP counting with YOPRO were immediately filtered (Hennes & Suttle 1995). 100 µL of sample were diluted with 700 µL of mili Q water filtered through a 0.02 µm pore size filter (Anodisc). Each diluted sample was gently filtered through a 0.02 µm pore size Anodisc 25 filter. The Anodisc filters with the filtered sample were laid on 80 µL of the staining solution (YO-PRO 1, 50 µM final concentration) in a Petri dish and incubated in the dark for two days at room temperature. The filters were then washed twice by filtering 800 µL of mili Q water through the membrane. Filters were transferred to glass slides, immediately covered with a drop of spectrophotometric-grade glycerol and a cover slip. Filters were stored at -20° C until counted.

VLP abundance was determined also using DAPI stain and counting the particles under the epifluorescence microscope (Suttle 1993). VLP were stained with DAPI (1 µg mL^{-1} final concentration) over night and filtered onto 0.02 µm pore size filters (Anodisc). Filters were mounted on microscope slides with non-fluorescent oil (R. P. Cargille Lab., Inc.) and frozen. Both YOPRO and DAPI stained samples were counted with a Nikon epifluorescence microscope at a magnification of 1250x. About 200-300 VLP were counted per sample.

In the samples for TEM, viruses were harvested onto the grids (400-mesh Ni electron microscope grids with carbon coated formvar film) using a Beckman SW41 swing-out rotor run at 40000 rpm for 30 minutes at 20° C (Bratbak & Heldal 1993, Suttle 1993). For each sample, duplicate grids were stained for 1 minute with uranyl acetate (2 % w/w). VLP were enumerated and sized in a Hitachi 600 transmission electron microscope (TEM) operated at 80 KV and at a magnification of 100000x. Fields were randomly selected and counted until the total counts exceeded 200 VLP. Because of the high acceleration voltage (80 KV) used in this study, we were able to identify cells containing mature phages on the same grids (Weinbauer *et al.* 1993). A cell was considered as infected when phage inside could be clearly recognized on the basis of shape and size (Bratbak *et al.* 1992, Weinbauer *et al.* 1993). The minimal number of phages found in an infected cell was 6. 500 cells at 20000x magnification were inspected for potential infection in each sample.

Bacterial heterotrophic production was measured by incorporation of ^{3}H-leucine into the cells (Kirchman 1993) with slight modifications. Two replicates and a formaldehyde (4 % final concentration) killed control were incubated with ^{3}H-leucine (40 nM final concentration) at the same temperature as the original cultures. Incubations

were terminated after 1:45 hours, by the addition of formaldehyde (4 % final concentration). The samples were then filtered through 0.22 μm cellulose acetate filters, rinsed twice in 5 % ice-cold TCA, and three times with 80 % ethanol. The filters were dissolved with 0.5 mL of ethyl acetate and 4.5 mL of Optiphase Hisafe II scillation cocktail was added before counting with a Beckman scillation counter. ^{3}H-leucine incorporated was converted to carbon produced using an empirical conversion factor estimated for coastal Mediterranean waters (Pedrós-Alió *et al.* submitted). Carbon produced was converted to cells produced dividing by the carbon content per cell. This was calculated with the equation reported by Norland (1993):

$$pgC\ cell^{-1} = 0.09 * (\mu m^3)^{0.9}$$

Cell volumes were determined with an image analysis system measuring at least 200 cells per sample. A Hammamatsu C2400-08 video camera was used to examine microscopic preparations. Objects occupying less than 7 pixels (equivalent to an sphere with a diameter less than 0.2 μm) were discarded. The remaining objects were measured and the volume calculated from area and perimeter measurements with the formula of Fry (1990). The system was calibrated with fluorescent latex beads and with natural bacterioplankton samples measured simultaneously by phase contrast microscopy and by epifluorescence (Massana *et al.* 1997).

Bacterivory by protists was measured with fluorescently labeled bacteria (FLB, Sherr *et al.* 1987), using the FLB disappearance method (Salat & Marrasé 1994). FLB were prepared from a heterotrophic bacterium isolated from the Mediterranean coast. 1 L samples were incubated at the same temperature as the original cultures in polycarbonate bottles in the dark. Incubations lasted 48 hours and were stopped by fixing subsamples with gluteraldehyde (final concentration 2 %). One experiment for each culture and a control killed with formaldehyde (final concentration 4 %) were done at the time indicated above.

All incubations for VLP decay experiments were carried out in 1.5 L polyethylene bottles. One experiment was carried out for each culture at the indicated time. Experiments were incubated at the same temperature as the original cultures during 48 hours. VLP decay was recorded after inhibiting production of new viruses by adding KCN to a final concentration of 2 nM (Heldal & Bratbak 1991). Samples for ^{3}H-leucine incorporation were taken at the beginning and at the end of each experiment in order to make sure that microbial activity was stopped by KCN. Viral decay rate (VDR) was calculated from the log-linear part of the decay curves using linear regression. Samples for counting VLP were taken at 1-2 hours intervals for the first 9-10 hours of the

experiment. After this time, samples were taken less frequently until the end of the experiments.

Results

VLP abundance determined with the three methods of counting is shown in Fig. 2.1. VLP abundance counted with YOPRO doubled from the beginning ($5x10^7$) to the end ($1x10^8$) of the experiment. Both replicate cultures showed similar numbers. The average coefficient of variation (CV) for YOPRO counts was 12 %. With the TEM method, VLP counts increased from $3x10^6$ at the beginning to $5x10^6$ at the end of the experiment. The average CV for TEM counts was 20 %. VLP abundance determined with DAPI gave the lowest counts. With this method, however, VLP increased one order of magnitude (from $1x10^5$ to $1x10^6$). The average CV for DAPI counts was 10 %. A linear function could be fitted to the log transformed VLP abundance with time, for each method. Analysis of covariance (ANCOVA) was used to test for significance of differences among the three methods of VLP counting. Methods gave significantly different results ($p < 0.001$, $n = 74$).

Nutrient concentrations showed a depletion of NO_3 and SiO_3 after day 2. PO_4 decreased slowly along the experiment (data not shown). Chlorophyll a concentration increased more than 10 times, after nutrient addition up to the second day of the experiment. After this day chlorophyll a decreased until the end of the experiment (Fig. 2.2A). Bacterial abundance reached the maximal concentration at day 5. Bacteria started to increase after the peak of chlorophyll a had been reached. Bacteria increased their initial abundance by a factor of three. After day 5, bacteria decreased to levels similar to the initial value (Fig. 2.2A).

Phototrophic nanoflagellates (PNFs) followed the same pattern than chlorophyll a. Their concentration from the beginning of the experiment to the second day increased 10 times. After this day their abundance started to decrease until day 5 and remained constant afterwards (Fig. 2.2B). Heterotrophic nanoflagellates (HNFs) showed oscillations with three peaks of abundance (days: 0, 2 and 8) through the experiment (Fig. 2.2B). One of these peaks (8 days) occurred after the peak of maximal bacterial abundance.

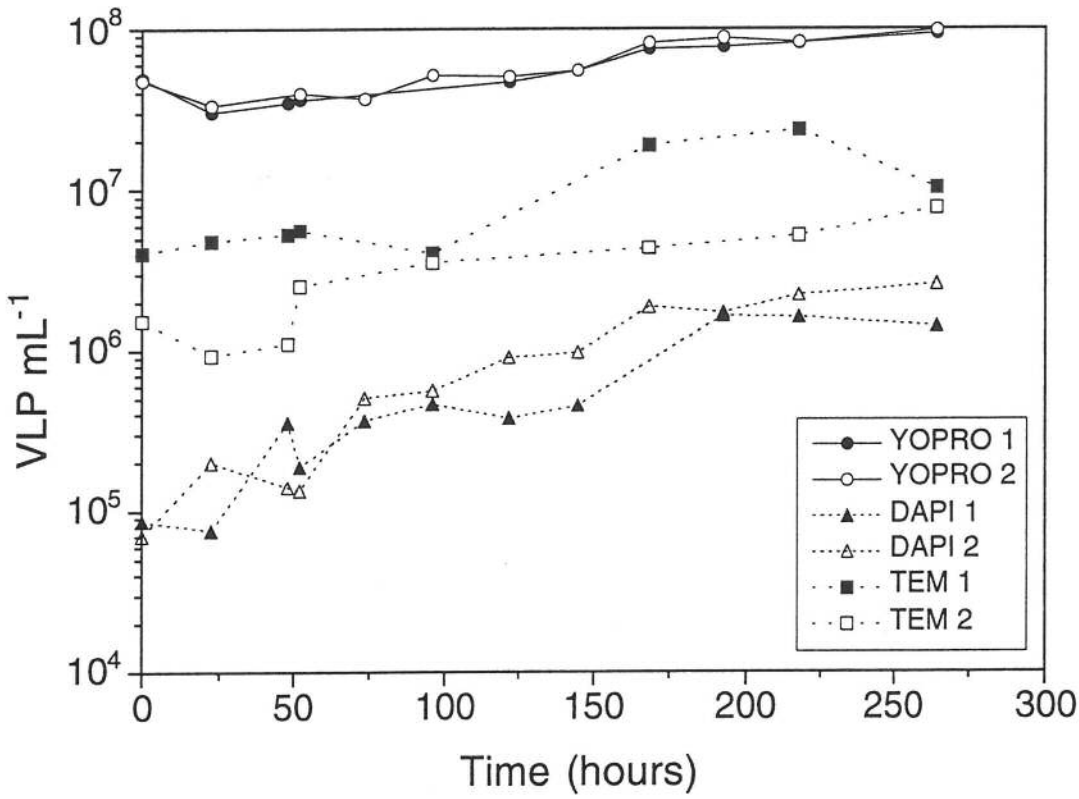


Fig. 2.1. VLP abundance along the experiment according to different counting methods.

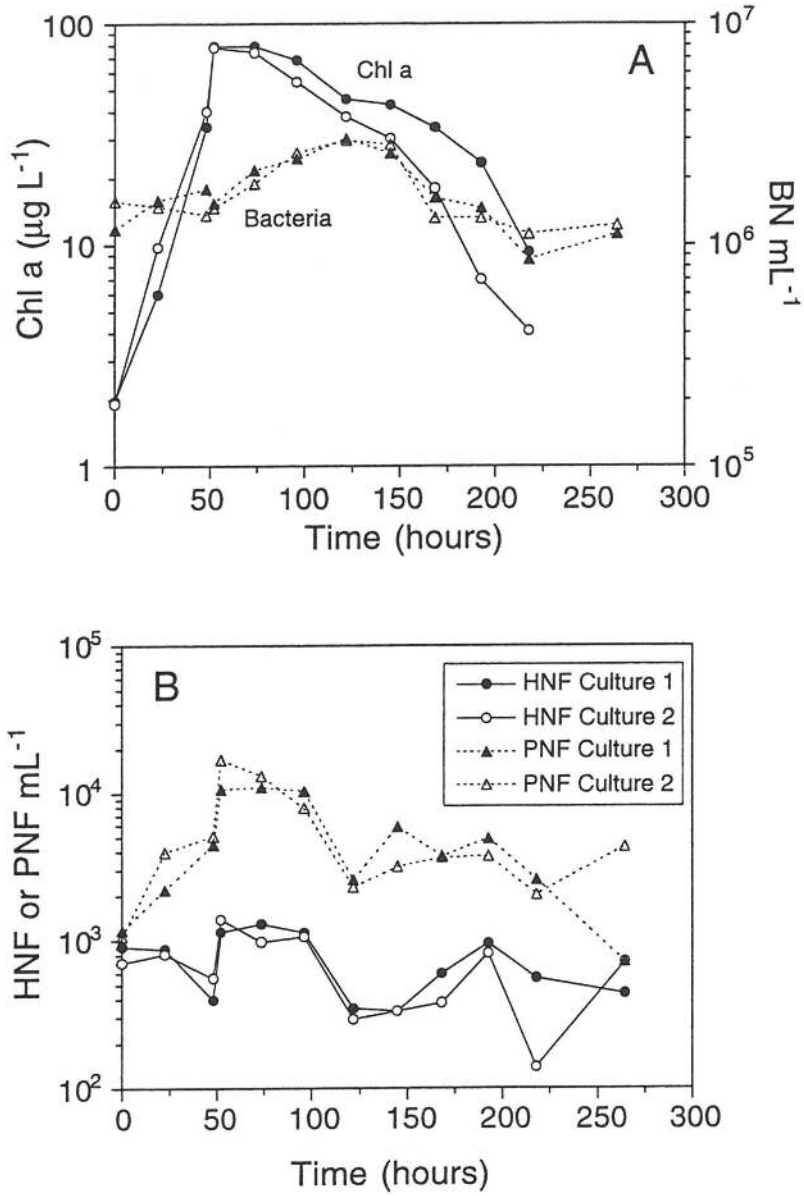


Fig. 2.2. **A-** Chlorophyll a (Chla) and bacterial abundance (BN) throughout the experiment. **B-** Heterotrophic (HNF) and phototrophic (PNF) nanoflagellate abundance throughout the experiment.

Table 2.1. Bacterial volumes (average ± SE) at different times of the experiment for both cultures.

Time (days)	Culture	Volume (μm^3)
0	1	0.030 ± 0.010
	2	0.039 ± 0.016
1	1	0.036 ± 0.016
	2	0.042 ± 0.019
2	1	0.037 ± 0.021
	2	0.038 ± 0.018
4	1	0.033 ± 0.012
	2	0.036 ± 0.014
7	2	0.047 ± 0.023
9	1	0.064 ± 0.034
	2	0.050 ± 0.027

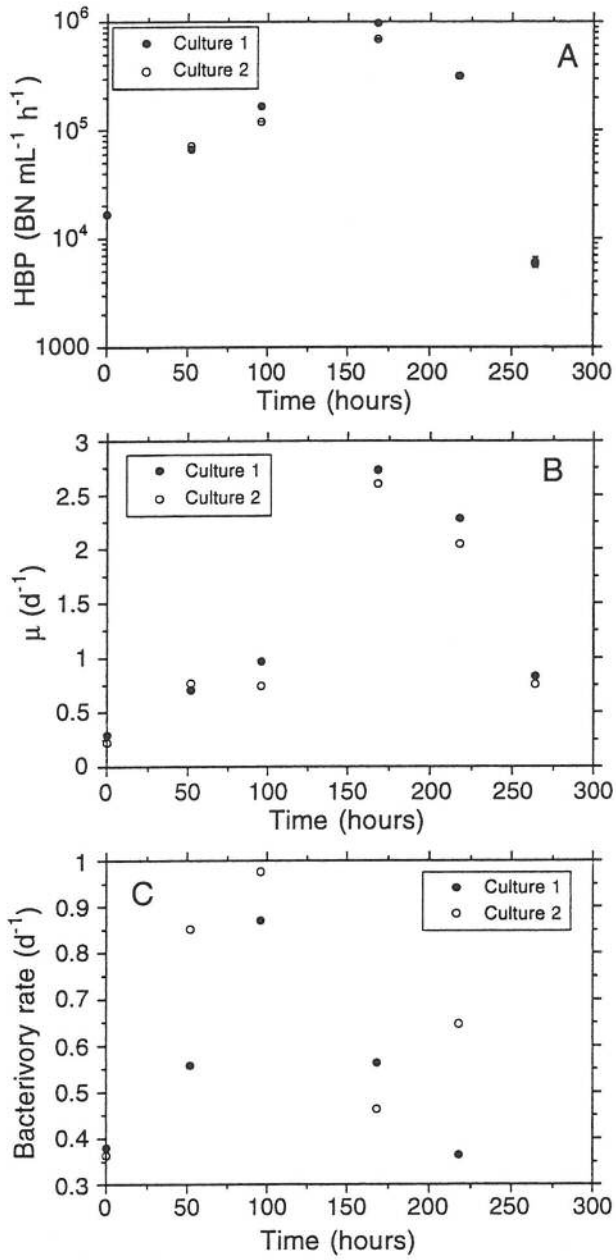


Fig. 2.3. **A-** Bacterial heterotrophic production (BHP) along the experiment. Bars indicate standard errors based on two replicates for each culture. When no bars are visible, errors are smaller than marker points. **B-** Specific growth rate (μ) of the bacteria along the experiment. **C-** Bacterivory rate along the experiment.

Bacterial heterotrophic production (BHP) increased from the beginning of the experiment until day 7. During this period, bacterial production increased 100 fold. After 9 days, BHP showed a fast decrease until the end of the experiment (Fig. 2.3A). Bacterial cell volumes along the experiment are shown in Table 1. Volumes measured were used to convert BHP from carbon to cells (see methods).

Specific bacterial growth rate (μ) showed a similar pattern to that of BHP. Values of μ at day 7 were 5 times higher than those at the beginning of the experiment (Fig. 2.3B). The bacterivory rate increased about 3 times from the beginning to day 4. The maximal bacterivory rate appeared three days before the maximal specific bacterial growth rate. Between days 4 and 7 bacterivory showed a fast decrease (Fig. 2.3C).

VLP decay experiments showed a similar pattern of VLP abundance through time (Fig. 2.4). VLP showed a fast decrease, initially (<10 hours) and afterwards either decreased more slowly (experiment carried out at day 2, Fig. 2.4B) or remained constant (remaining experiments). In the original sample VLP abundance showed a fast decrease from the beginning to the first thirty minutes and a slower decrease from this point to 10 hours (Fig. 2.4A). Viral decay rates (VDR) are shown in Table 2.2 and Fig. 2.5A. VDR found for the original water showed the lowest value. VDR at 9 days showed the maximal values. VDR for both replicate cultures were similar (Fig. 2.5A).

The percentage of visibly infected bacteria (% VIB, Fig. 2.6), was always lower than 1 % (Table 3). We converted visibly infected bacteria to total infected bacteria with the factors reported by Proctor *et al* . (1993). The resulting percentage was still lower than 5 %. At the original sample and at day 2, no infected cells could be detected. The maximal value of VIB was found at day 7.

Viral production rates calculated from VDR or from % VIB are shown in Fig. 2.5B. To convert % VIB to viral production we assumed that viral latent period had a similar length to host generation time (Proctor *et al.* 1993, Chapter 3) and we used a range of burst sizes between 100 and 300 viruses released per lysed cell. Viral production calculated using VDR increased exponentially from the beginning to day 9 (Fig. 2.5B). Viral production at this day was about one order of magnitude higher than that at the beginning of the experiment. Viral production calculated from %VIB showed a different pattern, with the maximal production at day 7 (Fig. 2.5B). Using this approach viral production measured was at least ten times lower than when measured using VDR.

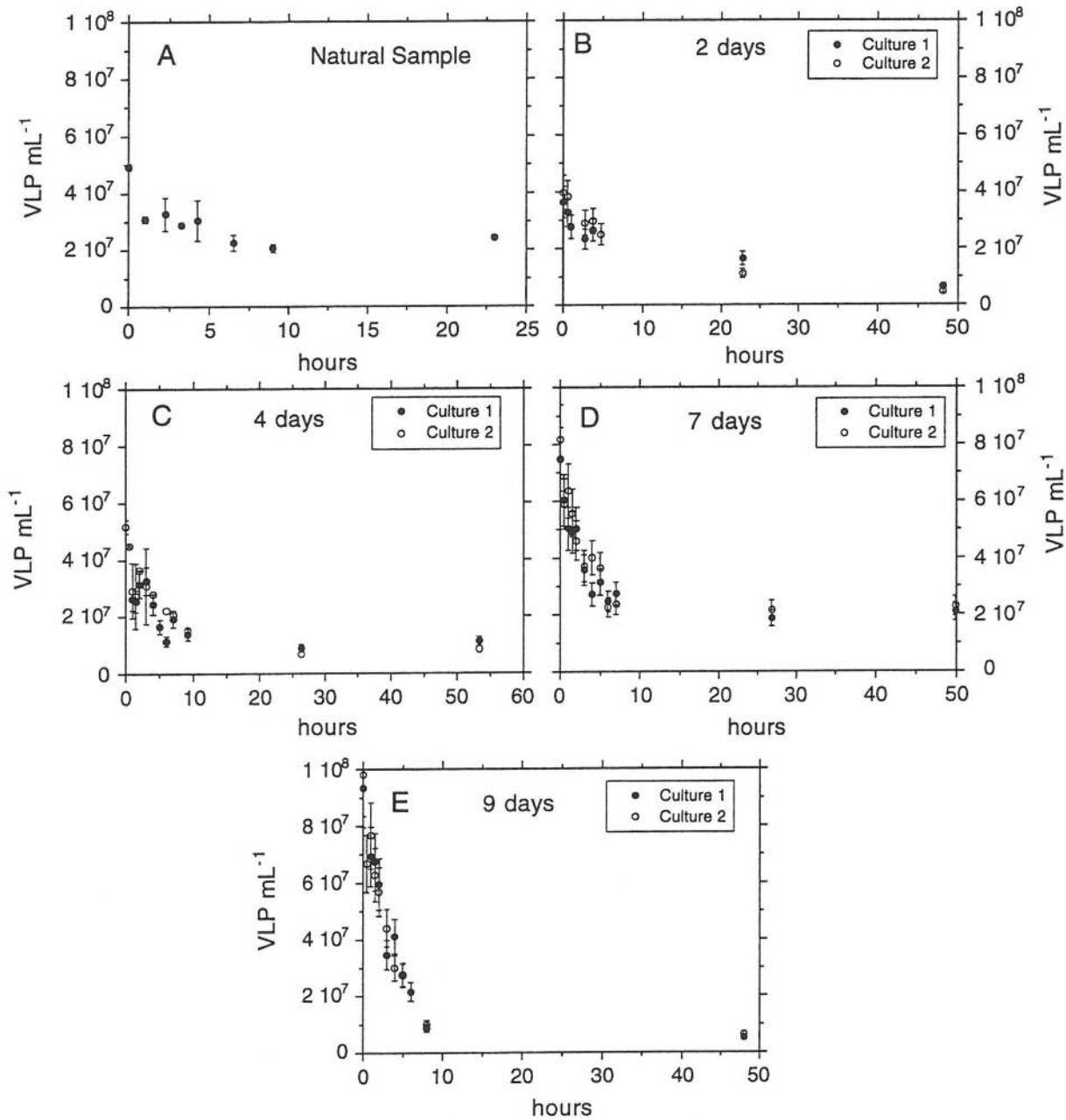


Fig. 2.4. Viral decay experiments performed after 0 (**A**) , 2 (**B**), 4 (**C**), 7 (**D**) and 9 (**E**) days. At day 0, viral decay experiment was performed with the natural sample. Bars indicate standard errors based on two replicates for each experiment. When no bars are visible, errors are smaller than marker points.

Table 2.2. Statistical parameters of the slopes (VDR) obtained from the decay experiments performed on different days and for both cultures (1 and 2).

Time (days)	Culture	VDR (h^{-1})	n	p	r^2	hours
0	1&2	0.049	6	0.002	0.975	0-9
2	1	0.091	5	0.045	0.675	0-5
	2	0.096	5	0.004	0.955	0-5
4	1	0.104	9	0.012	0.611	0-10
	2	0.108	10	<0.001	0.830	0-10
7	1	0.104	9	0.003	0.722	0-9
	2	0.166	10	<0.001	0.917	0-9
9	1	0.277	9	<0.001	0.955	0-9
	2	0.263	10	<0.001	0.980	0-9

Table 2.3. Percentage of visibly infected bacteria (% VIB) in the different sampling days and for both cultures. Infected cells were not detected (ND) during the first days of the experiment

Time (days)	Culture	% VIB
0	1 & 2	ND
1	1 & 2	ND
2	1 & 2	ND
4	1	0.2
	2	0.2
7	1	0.5
	2	0.6
9	1	0.4
	2	0.4

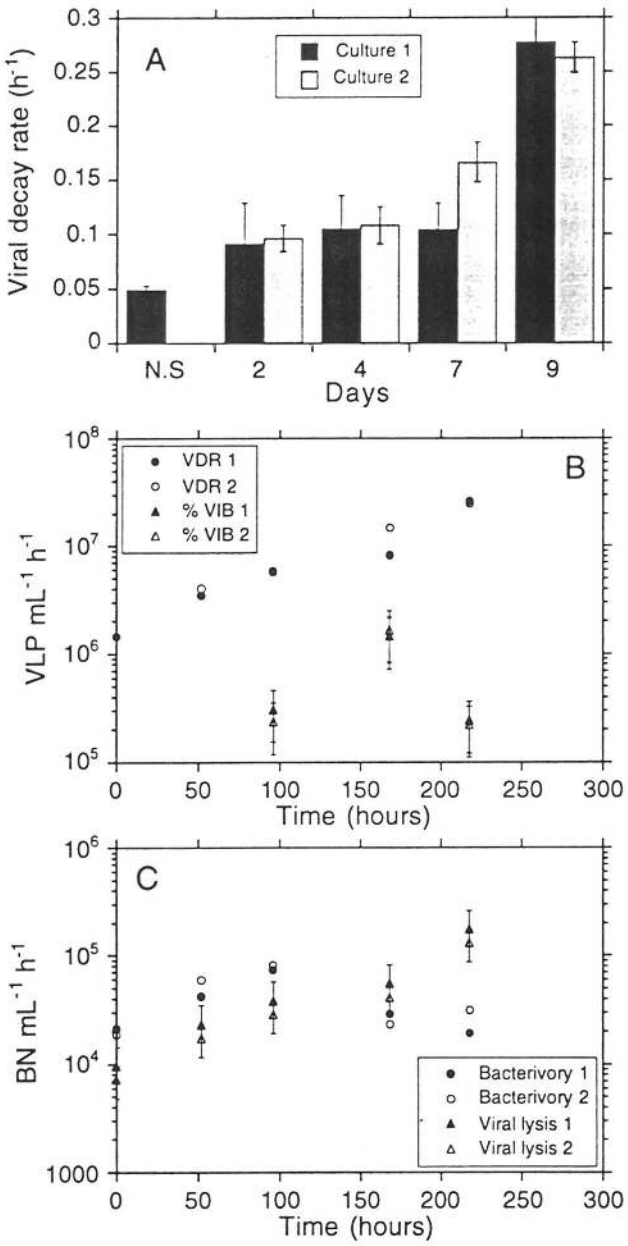


Fig. 2.5. **A**- Viral decay rate (VDR) calculated as the slope of the log-linear part of each decay experiment. Bars indicate standard error for each slope. Significance and r^2 of this slopes and interval of hours used to calculate them are shown in Table 2.2. **B**- Viruses produced per mL and per hour (VLP mL^{-1} h^{-1}), calculated according to VDR and according to the percentage of infected cells (% VIB). In the last case we have assumed a burst size between 100-300 VLP per cell. Error bars correspond to the standard error of the estimated values using this range of burst sizes. **C**- Bacterial cells lost per hour and per mL due to viral lysis and due to bacterivory. Bacterial mortality due to viral lysis corresponds to the values calculated from VDR. Error bars correspond to the standard error of the estimated values using a range of burst size between 100-300 VLP per cell.

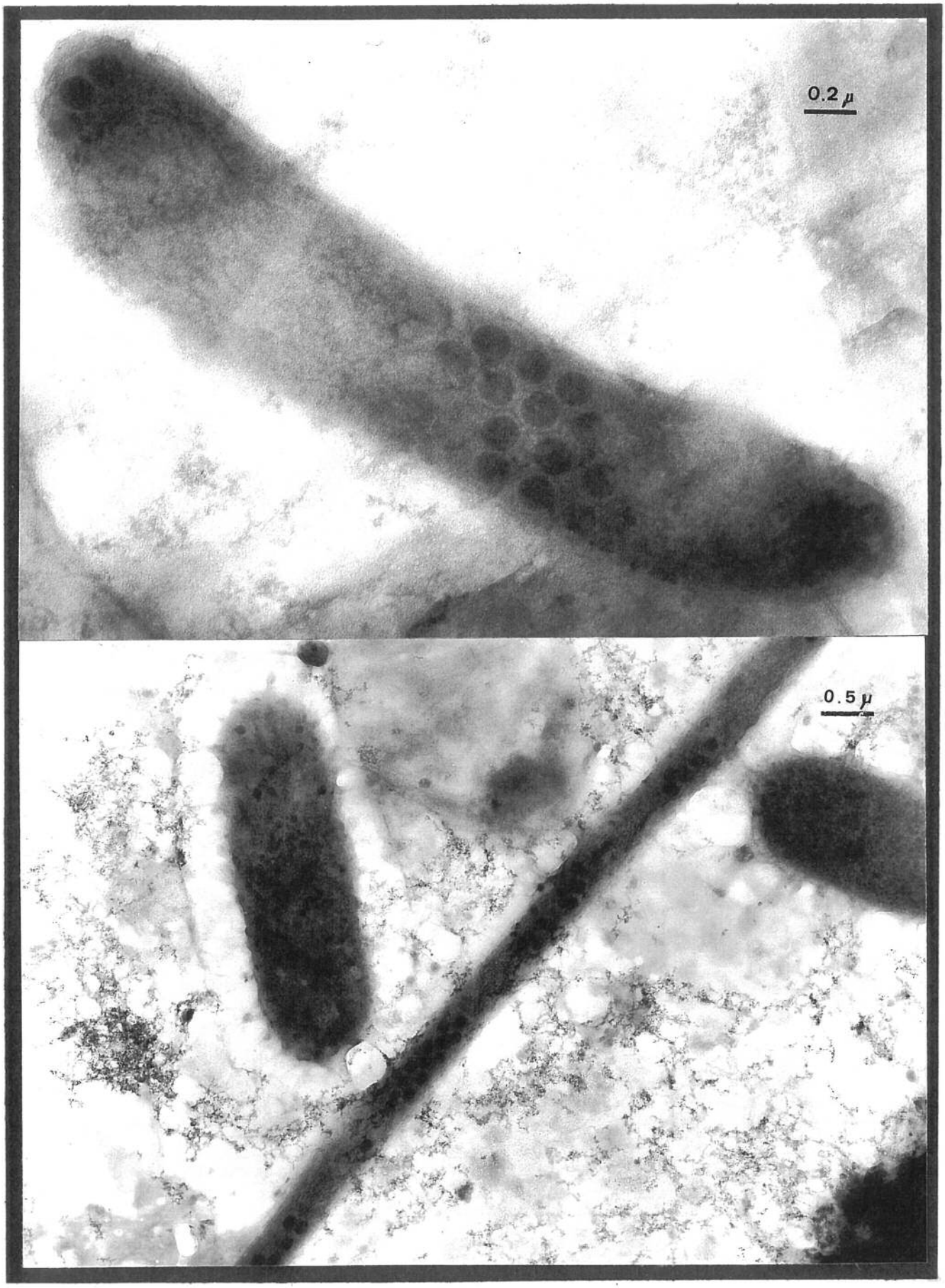


Fig. 2.6. Visibly infected bacteria at day 7 of the experiment.

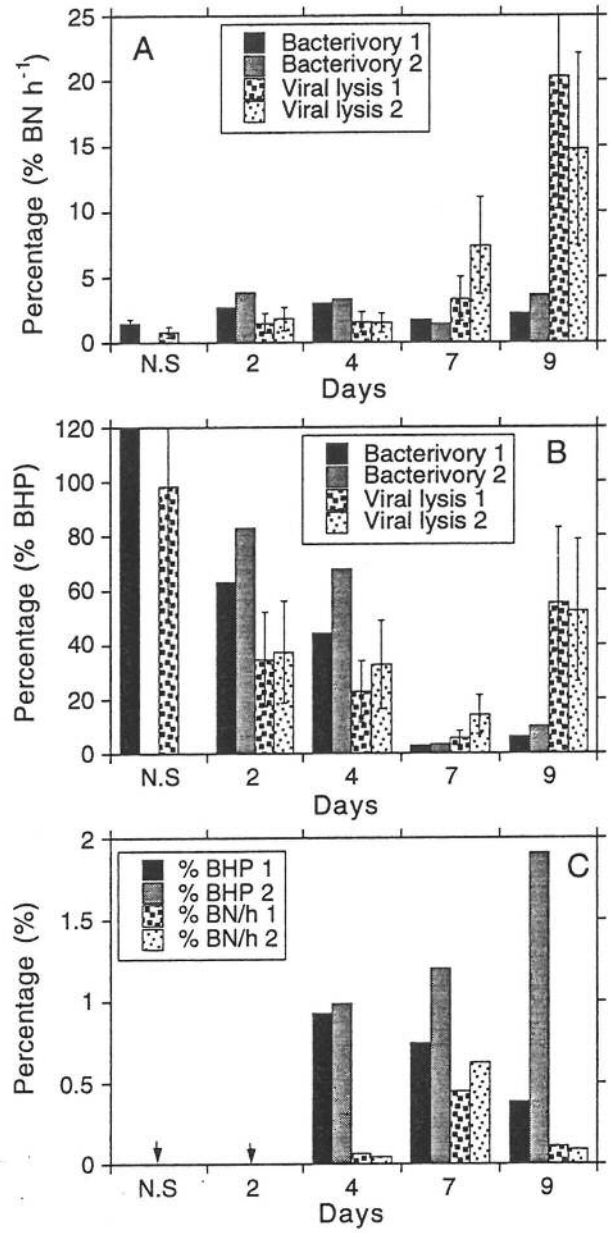


Fig. 2.7. **A-** Percentage of bacterial abundance (% BN) ingested by bacterivores or lysed by viruses per hour at different days of the experiment. The values of viral lysis were calculated from VDR. N. S., Natural Sample. Error bars indicate the lowest and the highest values of bacteria lysed by viruses calculated assuming a range of burst sizes between 100-300 VLP released per cell. **B-** Percentage of bacterial heterotrophic production (% BHP) ingested by bacterivores or lysed by viruses at different days of the experiment for both cultures. The values of viral lysis were calculated from VDR. N. S., Natural Sample. Error bars indicate the lowest and the highest values of bacteria lysed by viruses calculated assuming a range of burst sizes between 100-300 VLP released per cell. **C-** Percentage of bacterial heterotrophic production (BHP) and abundance (BN) ingested by bacterivores or lysed by viruses per hour at different days of the experiment. Bacteria lysed by viruses have been calculated from the percentage of infected cells. The two arrows indicate samples where infected cells could not be detected.

Bacterial losses per mL and per hour due to bacterivory or to viral lysis (using VDR), are shown in Fig. 2.5C. In order to calculate the bacteria lysed per mL and per hour from VDR we used the same range of burst sizes as above. Bacteria ingested by bacterivores showed a maximum peak at day 4, while bacteria lysed by viruses increased exponentially from the beginning the end of the experiment.

The percentages of bacterial abundance and production lost due to viral lysis or to bacterivory are shown in Fig. 2.7. The percentage of bacteria ingested by bacterivores per hour was always lower than 5 % (Fig. 2.7A). Maximal values corresponded to days 2 and 4. The percentage of bacteria lysed by viruses per hour (calculated from VDR) was lower than that ingested by bacterivory during the first sampling days (Fig. 2.7A). At days 7 and 9 however, the percentage of bacterial mortality due to viral infection was higher than that due to bacterivory.

The percentage of BHP ingested by bacterivores was higher than 100 % at the original sampling (Fig. 2.7B). At days 2 and 4 it was about 40-80 %. And at days 7 and 9 it was lower than 10 %. The percentage of BHP lysed by viruses (calculated using VDR) was also higher at the original sample (100 %). At days 2 and 4 this percentage was slightly lower than the percentage of BHP ingested by bacterivores (about half). At day 7, viral lysis and bacterivory accounted for a similar percentage of BHP, although viral lysis accounted for a slightly higher percentage than bacterivores. At day 9 viral lysis accounted for a much larger fraction of BHP than bacterivory (Fig. 2.7B).

The percentage of bacterial losses due to viral infection using the approach of % VIB is shown in Fig. 2.7C. This percentage was always lower than 5 % of BHP. Bacterial abundance lost per hour was always lower than 1 %. At day 9 differences between both replicate cultures were about 4 times.

Discussion

Methods of viral counting

VLP abundance found changed by an order of magnitude or more depending of the method used. The slopes of the increase in abundance with time found with each method were significantly different. However, using the data presented here plus other data from different environments, we have found a significant linear relationship between DAPI & TEM counts and between YOPRO & TEM counts (Chapters 4 and 6). Thus, for a broad range of environments it seems safe to assume that VLP abundance shows a proportional difference among methods used (Hennes & Suttle 1995, Weinbauer & Suttle 1997).

However, the differences found in the present study suggest that in highly productive environments the methods of counting VLP could be influenced to a different extent by the particulate material in the sample, since the slopes are not equal.

DAPI counts seem to underestimate the number of VLP (Suttle 1993), mostly because the small DAPI stained VLP are not visible under the epifluoresence microscope with this dye. It has been also reported that TEM counts underestimate VLP abundance, to a degree depending of the amount of organic matter present in the sample (Hennes & Suttle 1995). Moreover, this method presents the highest CV (20 % in this study) compared to DAPI counts (CV: 10 %) and YOPRO counts (CV: 12 %). The YOPRO counting method has some advantages over TEM (Hennes & Suttle 1995). Unfortunately, there is no test that can absolutely eliminate the possibility that viral-size particles other than viruses are stained by YOPRO. Hennes & Suttle (1995) investigated this possibility and concluded that the discrepancy between TEM and YOPRO estimates of viral abundance stems from the TEM protocol underestimating VLP concentration. Thus, for the rest of the discussion we will refer to the VLP abundance obtained with the YOPRO method alone.

Limitations of the measurement of bacterial mortality due to viral lysis

We used two approaches to estimate bacterial mortality due to viral infection: counting of the percentage of visibly infected bacteria (% VIB) and the measurement of the viral decay rate (VDR) in cyanide amended cultures. The two methods showed different results.

The direct count of infected cells presents several problems (Proctor *et al.* 1993, Weinbauer & Peduzzi 1994, Steward *et al.* 1996). First, viruses are only visible in infected bacteria during a part of their latent period. To be able to convert the visibly infected cells to total infected cells, Proctor *et al* . (1993) calculated a conversion factor derived for some specific host-virus systems. The factor, however, was calculated for conversion of %VIB in thin sections and not in whole cells as we determined % VIB. Second, to convert total infected cells to bacterial mortality per time, the length of the viral latent period must be known. At the moment there is not way to measure this period in natural samples. However, for some host-virus systems investigated in cultures it has been reported that the latent period is similar to the host generation time (Proctor *et al.* 1993).

Finally, the most important problem that we found in the present study to quantify % VIB was the fact that a high percentage of bacteria appeared opaque under TEM in spite of using a high acceleration voltage (80 KV, Weinbauer *et al.* 1993). Thus, our estimates of bacterial mortality from % VIB are clearly underestimates. Therefore, we will not refer to them in the rest of the discussion.

Calculations of VDR inhibiting viral production with KCN (Heldal & Bratbak 1991) also presents problems (Heldal & Bratbak 1991, Suttle & Fuhrman 1992, Chapter 5). VDR values obtained with this method, represent a minimal estimation of viral decay, because only abiotic loss factors are considered. However, VDR found in some studies with this method exceeded BHP several fold and thus, the bacterial assemblage would have to disappear in a short period of time (Bratbak *et al.* 1992, Chapter 5). This is unrealistic and, therefore, it seems that results from this method have to be examined with caution. In the present study viral production calculated with this method showed results consistent with the parallel estimations of bacterial production and bacterivory. Likewise, in an other study (Mathias *et al.* 1995), a good agreement was found between this method and %VIB. Thus, even accepting that this method presents some uncertainties, we consider the results obtained here as reasonable.

Another difficulty of converting VDR to bacterial mortality is that the burst size must be know. The method of counting the VLP inside the VIB has been used in some studies to estimate the burst size in natural environments (Weinbauer & Peduzzi 1994, Fuhrman & Noble 1995, Chapter 3). However, this method could underestimate the burst size because the stage of the latent period to which the VLP observed in the cells correspond, is unknown. Also phages lying on top of each other may be counted as one phage (Weinbauer & Peduzzi 1994). Different studies have used a range of burst sizes between 10 and 300 phages released per lysed bacteria (Heldal & Bratbak 1991) to calculate viral impact on the bacterial assemblage from viral production rates (Steward *et al.* 1992b). Other authors have used an average burst size of 50 to calculate viral production from VIB (Steward *et al.* 1996). Burst size has been shown to be dependent on bacterial growth rate (Probst-Ricciuti 1972) and bacteria cell volume (Weinbauer & Peduzzi 1994, Chapter 3). This implies that nutrient supply may indirectly determine the total number of phage set free per bacterium (Weinbauer & Peduzzi 1994). Børsheim *et al.* (1993) calculated an average burst size of 185 phages in cultured marine bacteria. Given the conditions of our experiment (high nutrient supply in enclosure cultures) we used the upper range of the burst sizes reported in the literature (100-300) to convert our estimated VDR to bacterial mortality.

Viral lysis and bacterivory as factors of bacterial mortality during a phytoplankton bloom

Bacterial growth in this experiment was expected not to be substrate limited because of the high amount of organic carbon from the phytoplankton bloom. Thus, we expected bacterivores and/or viruses to control bacterial abundance and growth. Bacterivory rate was maximal before the peaks of bacterial heterotrophic production and bacterial specific growth rate. By this time the phytoplankton bloom was declining (day 7). Immediately after the maximal peak of bacterivory, heterotrophic nanoflagellates decreased and increased only slightly after three days. Phototrophic nanoflagellates followed the pattern of chlorophyll a up to day 4, and increased slightly after the peak of bacterivory. This could indicate that part of the phototrophic nanoflagellate assemblage was mixotrophic. After a diatom bloom, when mineral nutrients are depleted the diatoms will sink out, mainly as cysts. In these periods mixotrophy has been shown to be a successful strategy for the small algae to retain a C:N:P ratio close to the Redfield ratio (Nygaard & Tobiesen 1993)

The fact that heterotrophic nanoflagellates increased only slightly after the peak of bacterivory could suggest that not all the bacterivory was due to the heterotrophic nanoflagellate assemblage. Mixotrophy is one possibility but ciliates could also be responsible for a portion of bacterivory during the experiment. Ciliates would pass throught the 150 µm filters used to set up the cultures. In spite of their expected low initial concentration (Vaqué *et al.* 1997), they might have grown during the experiment and become important bacterivores in an advanced stage of the bloom. At the same time, ciliates that ingest flagellates could have been also responsible for the slight fluctuations in flagellate abundance along the experiment.

VLP abundance increased from the first day up to the end of the experiment. This was a consequence of an increase in viral production. Maximal viral production appeared 2 days after the maximal heterotrophic production. This could indicate that a part of the actively growing bacterial assemblage was susceptible to viral attack.

Part of the viral assemblage could be infecting phytoplankton. However, our results showed a clear decrease in chlorophyll a when the nutrient concentration was depleted, while VLP increased until the end of the experiment. This suggests that the VLP were not produced by phytoplankton.

Bacterivory and viral lysis did not present their maximal values at the same stage of the bloom. While bacterivory was maximal immediately after the bloom when bacterial abundance was maximal, viral production showed maximal values when the

phytoplankton bloom had declined. This could be a consequence of the different strategies of both groups of organisms: The host-selective predators (viruses) determine the abundance of bacteria in each host-virus system, while the non selective predators (HNF) determine the size of the total bacterial assemblage (Thingstad *et al.* in press)

Balance between bacterial losses and bacterial production

Bacterivory plus viral lysis accounted for all the BHP before, during and immediately after the bloom period. From the beginning to day 4, bacterivory accounted for a higher percentage of the bacterial production and abundance per hour than viral lysis. From day 7 onwards bacterial heterotrophic production could not be balanced by viral lysis plus bacterivory. In the post bloom period, bacterivory accounted for a lower percentage of BHP and bacterial abundance per hour than viral lysis.

In this experiment bacterial cells did not show a constant abundance through time. Thus, for each period of time, net changes in bacterial abundance should be balanced by heterotrophic bacterial production minus bacterial losses (if these were the only factors responsible of bacterial mortality). Therefore, bacterial abundance (BN) plus bacterial heterotrophic production (BHP) measured for a fixed day (d_i) minus the losses due to bacterivory (BTV) and minus the losses due to viral lysis (VL) measured during the same day, would be equivalent to the bacterial abundance observed in the next sampling time (d_f):

$$BN_{df} = BN_{di} + (BHP - BTV - VL)_{di} \qquad \text{where } d_f = d_{i+2} \text{ (days)}$$

Because the interval of sampling activities (BHP, BTV and VL) was 2 days (except from day 4 to 7) we calculated the balance for this time intervals. In doing this, however, we are probably introducing an error, because BHP and viral lysis were measured over a period of a few hours, while bacterivory was measured over a 2 day period. This error could be specially important in a system such as this, which is changing continuously. Thus, in order to be able to compare these processes we used the averaged BHP and the averaged viral lysis measured at the beginning and at the end of this 2 day period. The results presented in Fig. 8, therefore, correspond to the following equation:

$$BN_{(di+2)} = BN_{(di)} + \text{Average } BHP_{(di,\, di+2)} - \text{Average } VL_{(di,\, di+2)} - BV_{(di)}$$

Where $di = 0, 2, 4$ and 7 (days)

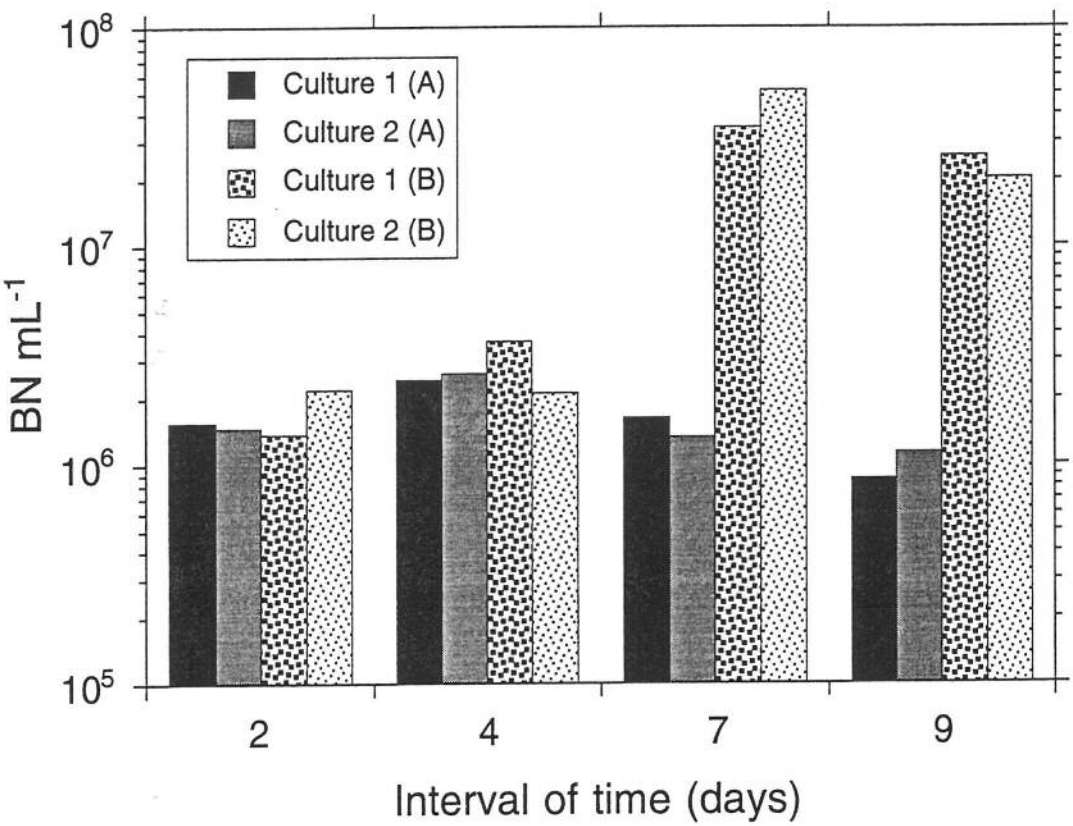


Fig. 2.8. Bacterial abundance (BN mL^{-1}) determined by epifluorescence microscopy at the end of each time interval (BN $_{(di+2)}$ = A) and bacterial abundance calculated according to: BN $_{(di)}$ + Average HBP $_{(di, di+2)}$ - Average VL $_{(di, di+2)}$ - BV $_{(di)}$= B. Where i = 0, 2, 4 and 7 days. Values have been calculated for each culture separately.

In the intervals of the first 4 days of the experiment (0-2, 2-4), viral lysis and bacterivory balanced bacterial losses with the net changes observed in bacterial abundance. During this period of time, although bacterivory and viral production increased, BHP increased faster. From days 4 to 7, bacterivory and viral lysis did not balance the zero increase in bacterial abundance. Subtracting losses due to bacterivory and viral lysis from the BHP, bacterial abundance should have been about 30 times higher than the observed abundance. Between days 7 and 9, we did not find a balance either: bacterial abundance was about 20 times higher than the expected value if viral lysis and bacterivory were the only factors causing bacterial mortality.

A factor of 20-30 is difficult to reconcile unless at this time other factors were responsible for bacterial mortality. Attachment to algae could be an important factor of bacterial losses during this period. It has been reported that bacterial cells attach mainly to moribund algae (Azam *et al.* 1993). In this period (day 7-8) mineral nutrients were depleted and diatoms could sink out, mainly as cysts (Macquoid & Hobson 1996). If bacteria attached to them, they would be removed from the water column and they would not be counted. At the same time, it has been reported that attached bacteria show higher values of production than free living bacteria (Pedrós-Alió & Brock 1983). In the method to measure BHP we would not distinguish between production corresponding to attached or free living bacteria. Thus, in this case BHP could be overestimated with respect to bacterial abundance and bacterivory as measured by microscopy. The percentage of VIB has also been reported to be higher in attached bacteria than in free-living bacteria (Proctor & Fuhrman 1991). With the viral decay method to measure viral production we could only detect decay of free VLP. Thus viral lysis may be also underestimated in relation to the BHP.

The experiment cannot be extrapolated to nature, because the food web was simplified by eliminating organisms higher than 150 µm. However, it gives an indication of the kind of control that viruses could exert over the bacterial assemblage in a non steady-state situation such as a phytoplankton bloom. As has been pointed out before (Bratbak *et al.* 1990) viruses show intense activity in environments that have received a load of nutrients. In such systems, bacteria will be able to grow at a fast rate because of the increased amount of carbon provided by the phytoplankton. Bacterivores will respond to the increase in bacterial biomass. They will exert an unespecific control on bacterial abundance and, at the same time, they will stimulate growth of part of the bacterial assemblage able to take the organic carbon released by their activity. At this time viruses will have their maximal impact, preventing any particular species from becoming too dominant (Thingstad *et al.* in press).

3. Viral lysis and bacterivory as prokaryotic loss factors along a salinity gradient

Introduction

Understanding of the processes controlling prokaryotic assemblages in aquatic systems and measurement of the carbon fluxes through them has been hampered by methodological difficulties. Thus, the appearance of a reliable technique to measure bacterivory (Sherr *et al.* 1987) resulted in several studies indicating that predation of bacterioplankton by protozoans was the principal fate of bacterial production in different environments (Pace 1988, Sherr *et al.* 1989, Vaqué *et al.* 1993). However, once the consistent methodology to measure both parameters simultaneously was established, protozoan bacterivory did not always account for 100 % of bacterial production (Sherr *et al.* 1989). In order to explain the constant cell concentration of bacterioplankton commonly found in natural waters over long periods of time, alternative loss factors of bacterioplankton had to be considered (Vaqué *et al.* 1993). Recently, viral lysis has been recognized as an important cause of bacterial mortality in both marine (Proctor & Fuhrman 1990) and fresh waters (Hennes & Simon 1994).

High percentages of bacterial mortality have been attributed to viral lysis in some environments (Heldal & Bratbak 1991, Proctor & Fuhrman 1990, 1992, Steward *et al.* 1992b, Weinbauer & Peduzzi, 1994, 1995a). However very few studies have attempted to balance bacterial production on the one hand, and mortality including both protist predation and virus infection, on the other (Bratbak *et al.* 1992, Fuhrman & Noble 1995).

The objective of this work was to determine the influence of viruses on prokaryotic abundance and growth rate relative to that of bacterivores. Two approaches are possible to compare both sources of mortality simultaneously. In one approach, a natural community can be enclosed in a mesocosm, where all factors can be measured simultaneously under controlled conditions. Fuhrman & Noble (1995) have used this approach and have found that bacterivory and viral lysis accounted for about 50 % each of bacterial mortality in water sampled in coastal California. In another approach, measurements can be done in a natural environment. This has the advantage of avoiding possible changes in the microbial community due to enclosure, the so called bottle effects, which can be occasionally very important for viruses (Bratbak *et al.* 1992). The problem, on the other hand, is that the system may not be in steady state, and that advection or other uncontrolled factors may complicate interpretation of results. We decided to look for a natural system which resembled a mesocosm as much as possible. Further, we looked for a series of systems where we could expect the relative importance of bacterivory and viral lysis to change with respect to each other, so that the effects of each factor could be analyzed separately.

The systems chosen for our purpose were two different solar salterns. These consist of a series of shallow ponds connected in a sequence of increasingly saline brines, used for the commercial production of salt from sea water. During evaporation of sea water, sequential precipitation of calcium carbonate, calcium sulfate and finally sodium chloride occurs. As the salinity increases, the multiple connections in the microbial loop decrease. The increase in salinity results in a gradual disappearance of large forms of life and marine prokaryotes at salinities lower than 100 ‰. From this salinity up to 200 ‰ prokaryotic assemblages are dominated by moderately halophilic organisms. From 200 ‰ salinity onwards changes affecting the microbial populations can be followed by the changes in the aspect of the water: the green-brown color of the water changes to red color in the crystallizer ponds. Such ponds become almost monospecific cultures of halophilic archaea (Rodríguez-Valera 1988). The different ponds in the salterns are maintained under constant conditions over long periods of time, enabling the microbial populations to reach an equilibrium at each pond (Rodríguez-Valera 1988, Pedrós-Alió *et al.*, in preparation). This common feature among salterns allowed us to assume that the microbial populations living in them were in a steady-state.

We quantified the distribution of virus like particles (VLP), the frequency of infected cells and the burst size throughout the salinity gradient. Using conversion factors from the literature (Proctor *et al.* 1993), we estimated prokaryotic mortality due to viral lysis and the production rate of viruses. Prokaryotic heterotrophic activity and bacterivory were measured to compare the two sources of mortality (due to virus and to bacterivores).

The finding of high abundance of "square archaea" and the fact that they were the only visibly infected cells in the saltiest ponds allowed a study of them separately from the rest of the prokaryotic community.

We demonstrate that viruses have a small effect in controlling the whole prokaryotic abundance under steady-state and that their influence on prokaryotic growth rates is minimal in these systems.

Materials and methods

Terminology

In order to be consistent with the currently accepted terminology for the prokaryotes we have used the term "prokaryote" when referring to all the community of prokaryotes and the term "archaea" when referring to this particular domain (Woese *et al.* 1990). The problem arises when referring to "grazing" or "bacterivory" since neither one is appropriate to refer to predation on prokaryotes including a mixture of bacteria and archaea. In order to avoid cumbersome terms, however, we have retained the words bacterivore and bacterivory when referring to predation on prokaryotes. For the "square cells" we have used the term "square archaea", because it is accepted that they belong in the archaeal domain (Stoeckenius 1981).

Solar salterns

Samples were collected on 28-29 July 1994 from the multi-pond solar saltern "La Trinitat" located in Delta de l'Ebre (Tarragona, Spain, 40° 35' N, 0° 41' E). The water salinity of the nine ponds sampled represented the increasing gradient from sea water salinity (37 ‰) to the crystallizers (370 ‰). The ponds are shallow, about 0.5 m deep, and their area varies from 200 Ha for the less saline to just 3 Ha in the crystallizers. A very persistent breeze keeps the water well mixed. Samples were taken with a bucket at the end of a pole, collecting mostly subsurface water. The corners of the square ponds were avoided, since the wind accumulates organic matter scums and debris in those areas. Some data were also collected in "Braç del Port" salterns (Santa Pola, Alacant, Spain, 38° 12' N, 0° 36' W) on 29-31 July 1993. The range of salinities in the latter was from 38 ‰ to 382 ‰.

Abundance of prokaryotic cells and viruses

In each pond, 3 samples of 25 mL were collected in polypropylene bottles and fixed immediately with formaldehyde (4 % final concentration). Viral and prokaryotic abundance were determined using ultracentrifugation and transmission electron microscopy (TEM, Børsheim *et al.* 1990). To avoid interference of the high prokaryotic numbers in viral counts, we diluted the samples four times at salinities lower than 250 ‰ and 10 times at higher salinities. The dilution was done with distilled water filtered through an Anopore filter (pore size 0.02 μm). Previously, we had checked that both cells and virus-like particles (VLP) remained intact after dilution.

Cells and viruses were harvested onto the grids (400-mesh Ni electron microscope grids with carbon coated formvar film) using a Beckman SW41 swing-out rotor run at 40000 rpm for 30 minutes at 20° C (Bratbak & Heldal 1993, Suttle 1993). For each sample, duplicate grids were stained for 1 minute with uranyl acetate (2 % w/w). VLP were enumerated and sized in a Jeol 100CX transmission electron microscope (TEM) operated at 80 KV and at a magnification of 100000x. Fields were randomly selected and counted until the total counts exceeded 200 VLP. Prokaryotic cells were enumerated in the same preparations at 20000x magnification. Viral abundance was also determined using DAPI stain and counting the particles under the epifluorescence microscope (Suttle 1993). We used epifluorescence in order to compare viral counts between two different salterns, since this technique is much faster for routine use.

Frequency of cells containing mature phage

Because of the high acceleration voltage (80 KV) used in this study, we were able to identify cells containing mature phages (Heldal & Bratbak 1991). A cell was considered as infected when phage inside could be clearly recognized on the basis of shape and size (Bratbak et al 1992, Weinbauer *et al.* 1993). The minimal number of phages found in an infected cell was 6. At least 200 fields at 20000x magnification were inspected for potential infection in each sample. The number of mature phages inside cells gave an estimation of the burst size (Weinbauer *et al.* 1993).

Because of the low abundance of infected cells in all the samples we did not calculate the frequency of cells with mature phages separately for each morphotype, except in the case of the infected square archaea at salinities higher than 180 ‰, where this morphotype became more abundant.

Prokaryotic heterotrophic activity

Prokaryotic heterotrophic activity was measured through the incorporation of [methyl-^{3}H] thymidine (TdR) (Bell 1993). Samples of 10 mL were incubated in the dark at *in situ* temperature for 45 min with 20 nM TdR. Two replicates plus a formaldehyde killed control were incubated for each pond. To end the incubation, formaldehyde was added and the vials kept in a cooler with ice until return to the laboratory. The samples were filtered and counted in a liquid scintillation counter (Pedrós-Alió *et al.*, in preparation).

Bacterivory

Bacterivory by protists was measured with fluorescently labeled bacteria (FLB, Sherr *et al.* 1987), using the FLB disappearance method (Pace *et al.* 1990, Salat & Marrasé 1994). FLB were prepared from a heterotrophic bacterium isolated from the Mediterranean coast (1 x 0.8 μm, biovolume: 0.42 μm^2). This cell size is a compromise between the average cell volume at the lowest salinity ponds (0.084 μm^3) and at the highest salinity where bacterivory was measured (0.916 μm^3). Bacterivory experiments were carried out in all ponds except for the two crystallizers (with salinities >300 ‰). At these high salinities no predators were present, since neither flagellates nor ciliates can live in the crystallizers. 1 L samples were incubated at *in situ* temperature in polycarbonate bottles in the dark. Two replicates and a control killed with formaldehyde (final concentration 1 %) were done at each pond. Incubations lasted 48 hours and were stopped by fixing subsamples with gluteraldehyde (final concentration 2 %).

Results

Abundance of prokaryotic cells and VLP

Total counts of prokaryotes increased with salinity by one order of magnitude from the less saline pond (37.5 ‰) up to the most saline (370 ‰, Fig. 3.1A). Abundance of VLP increased by almost two orders of magnitude in the same range of salinities (Fig. 3.1A). Prokaryotic and VLP abundance were significantly correlated ($p < 0.001$, $r = 0.97$, $n = 9$, Fig. 3.1B).

Because of their particular shape, size and abundance at salinities higher than 250 ‰, it was possible to quantify square archaea separately (Table 3.1). In order to avoid possible confusion with odd-shaped prokaryotes that could occasionally appear square in the harvested samples, we used the criterion of Stoeckenius (1981) to classify them: Square archaea were identified as flat and rectangular cells with perfectly straight edges, measuring a few micrometers on the side (Fig. 3.2A). The smallest cells were square, measuring 2 by 2 μm, whereas the largest cells were often rectangular with mean side lengths of 1.87 ± 0.56 and 2.83 ± 0.53 μm. In the saltiest ponds the square archaea constituted around 25 % of the total number of prokaryotes (Table 3.1).

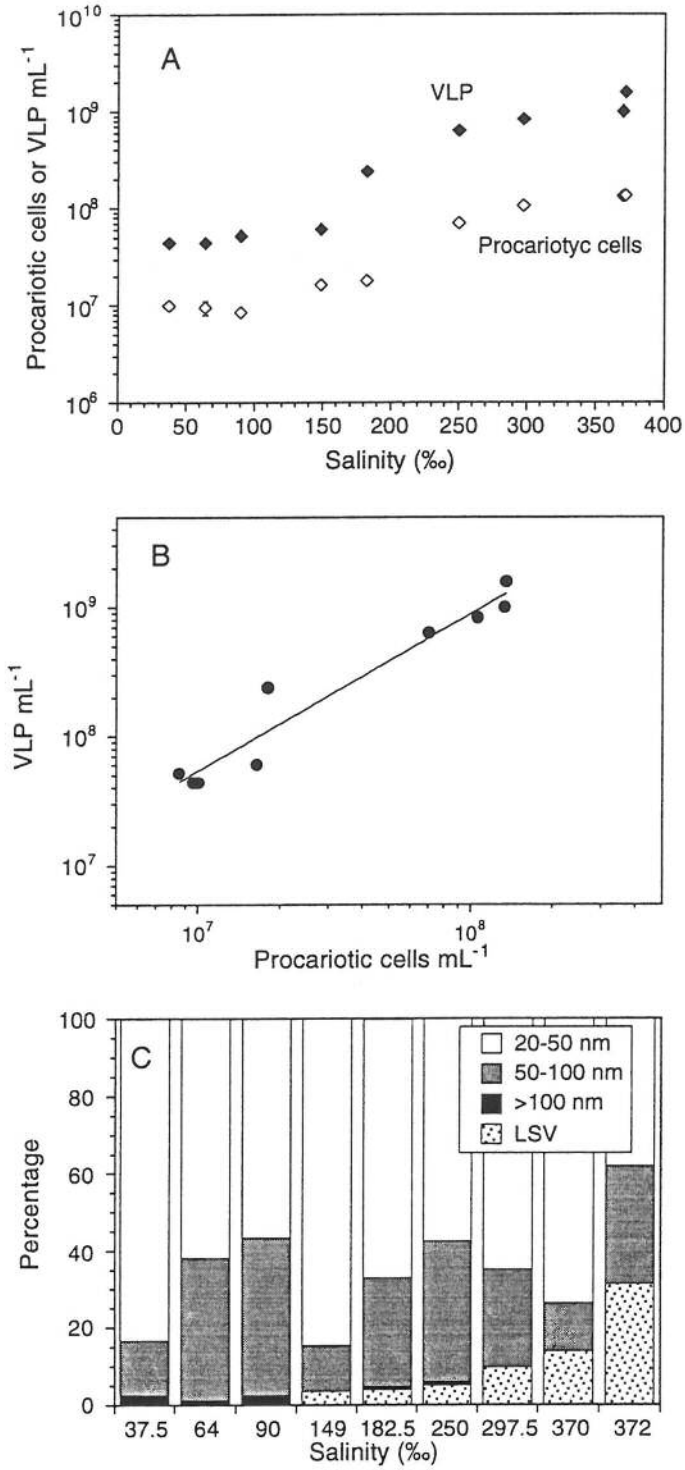


Fig. 3.1. **A-** Prokaryotic and viral abundance along the salinity gradient. Bars indicate standard errors based on two replicates. When no bars are visible, errors are smaller than marker points. **B-** Relationship between prokaryotic and viral abundance. **C-** Viral size class distribution represented as percentage of the total in each pond. LSV: Lemon-shaped viruses.

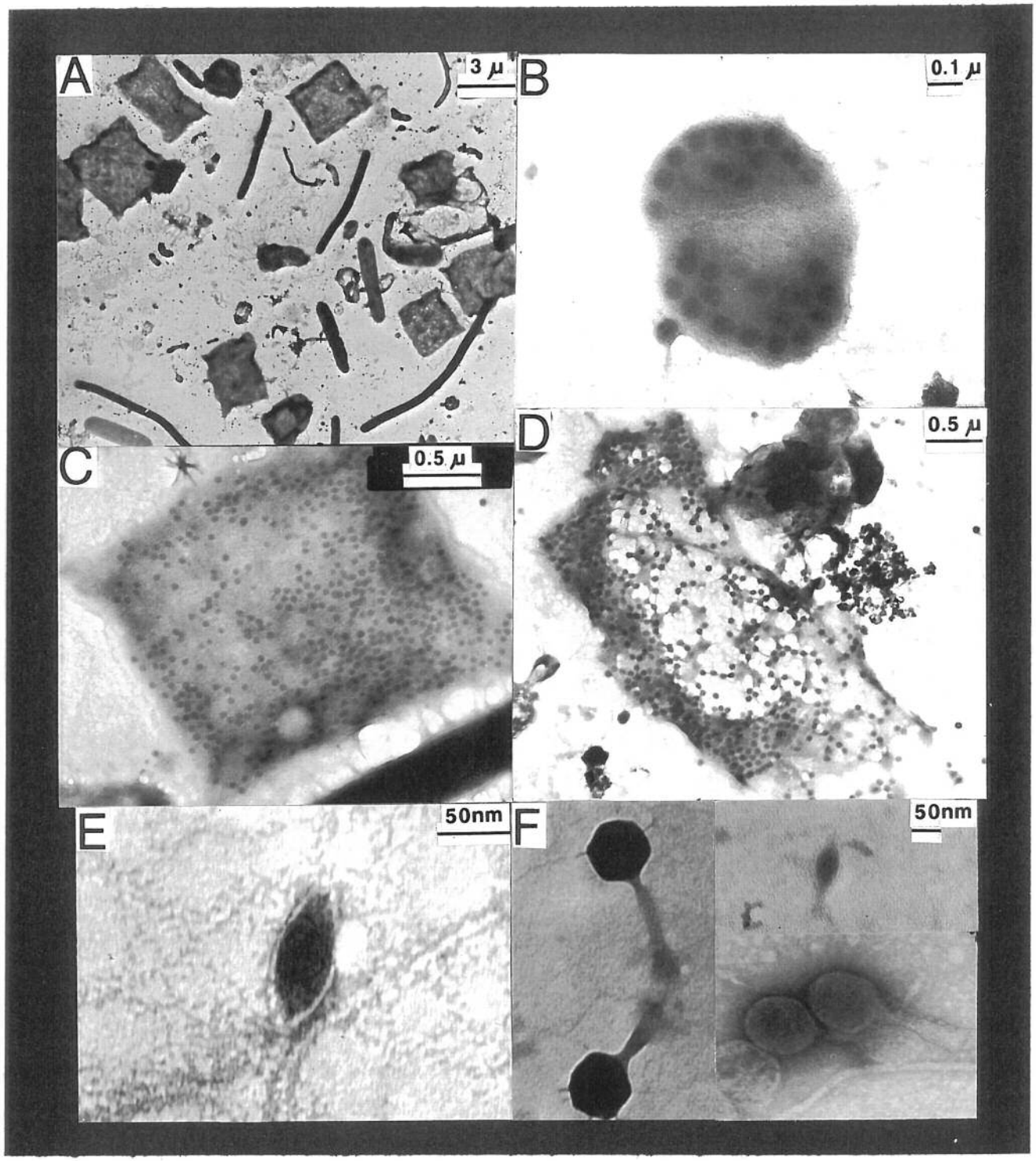


Fig. 3.2. **A-** Prokaryotic cells in the crystallizer ponds (370 ‰ salinity), the sample was diluted 10 times (see materials and methods). **B-** Prokaryotic cell with phage particles, head diameter ca. 60 nm. **C-** Square archaea with phage particles, head diameter ca. 40 nm. **D-** Square archaea lysed by phages, head diameter ca. 50 nm. **E-** Lemon-shaped virus. **F-** Different types of phages, including two icosahedral, tailed types and a lemon-shaped virus.

Table 3.1. Frequency of cells containing mature phages and relative abundance of square archaea. Values are means calculated from the two grids observed from each pond. Range of values shown in parentheses.

Salt Pond	Salinity (‰)	Cells with mature phage (% of total)	Square archaea (% total cells)	Square archaea with mature phages (% of Square archaea)
TR1	37.5	<0.04	0	NP[a]
TR2	64	<0.05	<3	NP
TR3	90	<0.06	<3	NP
TR7	149	1.28 (0.35-2.21)	<5	NP
TR5	182.5	1.30 (0.26-2.33)	<5	NP
TR4	250	1.88 (0.00-3.76)	8.53 (8-8.9)	1.57 (1.19-1.95)
TR6	297.5	0.85 (0.51-1.20)	31.51 (28-34)	2.81 (1.67-3.94)
TR8	370	1.30 (0.86-2.71)	28.09 (15-40)	6.73 (3.26-10.2)
TR9	372	0.62 (0.50-0.74)	21.78 (17-30)	2.57 (2.08-3.07)

[a]NP: Not Present.

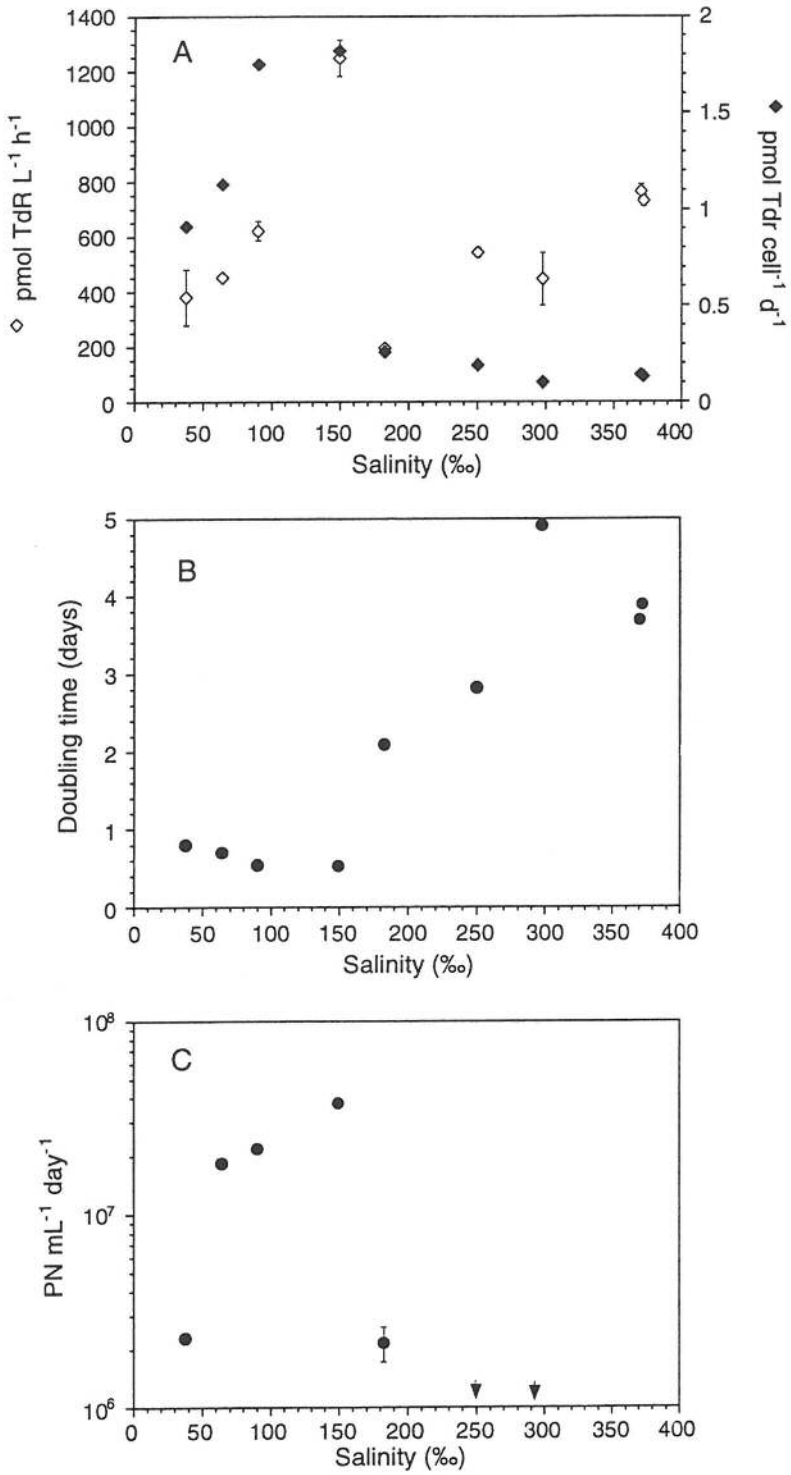


Fig 3.3. **A-** Incorporation of [methyl-^{3}H]thymidine (TdR) along the salinity gradient (empty symbols) and TdR incorporation per cell (filled symbols). Bars indicate standard errors based on two replicates. When no bars are visible, errors are smaller than marker points. **B-** Doubling times of the prokaryotic assemblage in each pond. **C-** Prokaryotes ingested by bacterivores (PN mL^{-1} day^{-1}) along the salinity gradient. Bars represent standard errors of two replicates. The two arrows indicate ponds where bacterivory was found to be zero.

Size distribution of viruses

VLP were classified into four classes based on shape and the diameter of their heads (Fig. 3.1C, Fig. 3.2E, F). Most VLP in all ponds had icosahedral heads of 20-50 nm. The percentage of VLP in each class did not follow any pattern with increasing salinity, except for the lemon- shaped viruses (LSV, Fig. 3.2E, F). These LSV showed a progressive increase in abundance with increasing salinity, reaching 31.4 % of the VLP in the most salt concentrated ponds. The size of these viruses, apparently untailed, was 40 x 80 nm. The abundance of LSV was significantly correlated with that of square archaea ($p < 0.03$, $r = 0.7$, $n = 9$). These viruses were seen infecting square archaea, but the number of infected cells was too low for statistically significant counts. Thus, we could not provide infection percentages for LSV-square archaea separately. The square archaea, however, were often infected by viruses with other morphologies (Fig. 3.2C).

Prokaryotic heterotrophic activity and bacterivory

Thymidine incorporation (Fig. 3.3A) did not show a clear pattern with salinity increase or with VLP numbers. The activity per cell showed the highest values at salinities lower than 170 ‰ (Fig. 3.3A). Data on thymidine incorporation rates, combined with the abundance of prokaryotes, enabled calculations of prokaryotic doubling times, using a theoretical conversion factor of $1.5x10^{18}$ cell mol^{-1} TdR (Pedrós-Alió *et al.* in preparation). Doubling times became higher than 2 days at salinities higher than 170 ‰ (Fig. 3.3B).

Bacterivory (ingested prokaryotes mL^{-1} d^{-1}) showed the highest values in ponds with salinities between 78 ‰ and 150 ‰ (Fig. 3.3C). In the two more concentrated ponds where bacterivory was measured we did not find any disappearance of FLBs. Prokaryotic heterotrophic activity (pmol TdR L^{-1} h^{-1}) was significantly correlated with bacterivory ($p < 0.001$, $r = 0.866$, $n = 10$). And conversely, doubling times were longer when bacterivory was lowest.

Frequency of infected prokaryotes and burst size

In the ponds with values of total salinity lower than 150 ‰ we did not find any infected cell in 200 fields screened for each grid, with an average of 10 cells per field. In the ponds with higher salinities the overall frequency of prokaryotes visibly infected with phages ranged between 0-3.76 % (Table 3.1). No significant differences were found among the different ponds (ANOVA, $p > 0.05$, $n = 12$). The frequency of infected cells for the square archaea, ranged between 1.19-10.2 %, showing the highest values in pond

TR8 (Table 3.1). Again, among the ponds where these archaea were present, differences were not significant (ANOVA, $p > 0.05$, $n = 8$). In the ponds with salinities higher than 350 ‰ (TR8 and TR9) all the infected prokaryotes that we could see belonged to this morphotype. The LSV were seen infecting only square archaea.

The number of viruses inside the infected prokaryotes (square archaea excluded, Fig. 2B) ranged between 6 and 35 phages per host cell (mean, 22 ±7). The number of phages found in the infected square archaea (Fig. 3.2C, D) was significantly different (Student's t test, $p < 0.001$) from that in other infected prokaryotes. It ranged between 100 and 380 (mean 203 ± 77). We did not find differences of the burst size for the same morphotype among the ponds.

Losses of prokaryotic abundance and production

In order to estimate the impact of viral attack on the prokaryotic assemblages we had to use a series of assumptions. In the first place, we calculated the total number of infected prokaryotes (TIP) from the number of visibly infected prokaryotes (VIP) and the range of conversion factors (f:3.7-7.14) provided by Proctor *et al.* (1993): TIP = VIP x f. We then reasoned that this number of infected cells is the number of cells that would die within a time equivalent to the latent period (L) of the phage infection. Thus, the prokaryotes lysed per mL per day because of viral infection (v) is: v = TIP / L. The latent period L, however, is unknown for a natural assemblage which may combine several phage-prokaryote systems. An approach used by Proctor *et al.* (1993) was based on the similitude between latent periods (L) and doubling times (Dt) of the host-prokaryotic systems. Under this assumption L = Dt and thus:

$$v = TIP / Dt = (VIP \times f) / Dt$$

The results of these calculations are shown in Fig. 3.4A. It is apparent that despite very different doubling times (Fig. 3.3B) in the ponds where viral infection was measurable, the number of prokaryotes lysed per mL and per day was fairly similar throughout the salinity gradient.

From the number of prokaryotes lysed per day and the burst size of the infected prokaryotes we could calculate the number of viruses produced per day. The number of viruses produced markedly increased with salinity (Fig. 3.4A), due to the larger burst size of the prokaryotes in the most saline ponds.

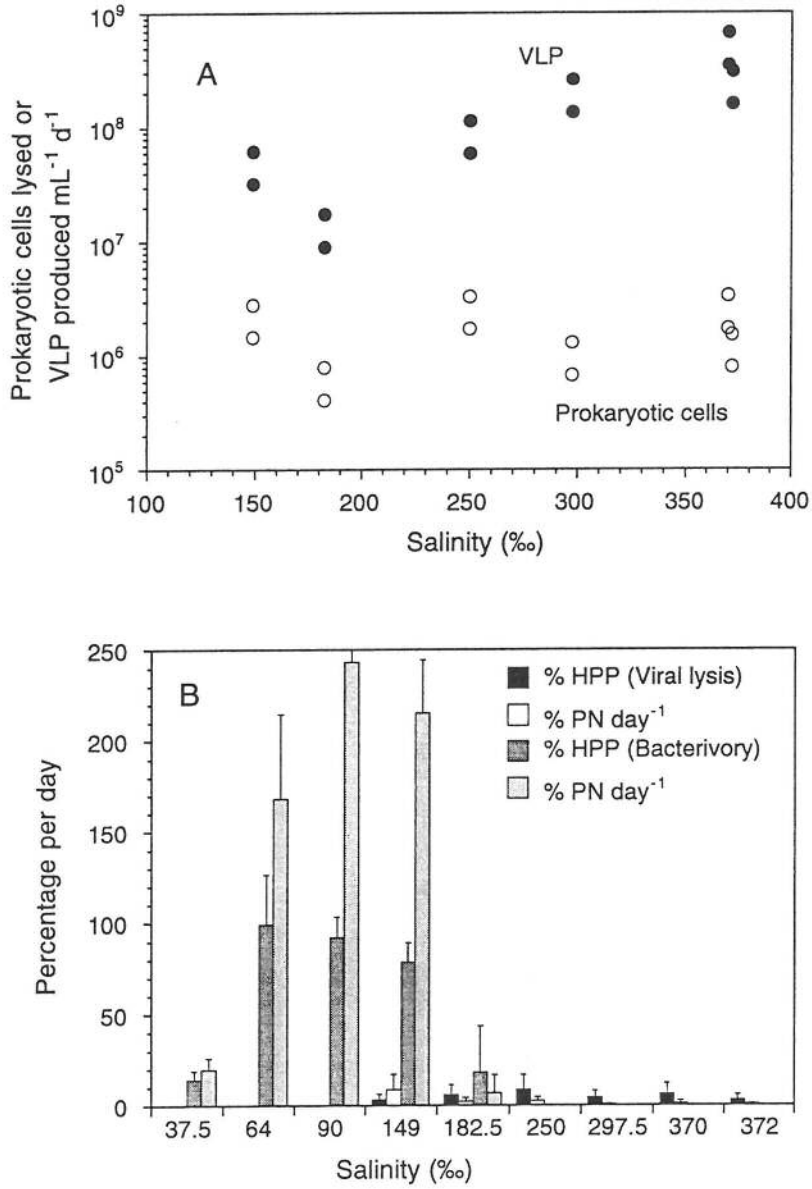


Fig. 3.4. **A-** Prokaryotic lysis rates (Bacteria mL^{-1} day^{-1}) and viral production rates (VLP mL^{-1} day^{-1}) along the salinity gradient. The two points for each salinity represent the values found after applying the range of conversion factors (Proctor *et al.* 1993) to the average percentage of visibly infected cells (Table 3.1). **B-** Percentage of heterotrophic prokaryotic production (HPP) and abundance (PN) ingested by bacterivores and lysed by viruses per day in the different ponds. Error bars (only the upper one shown) indicate the lowest and the highest calculated values.

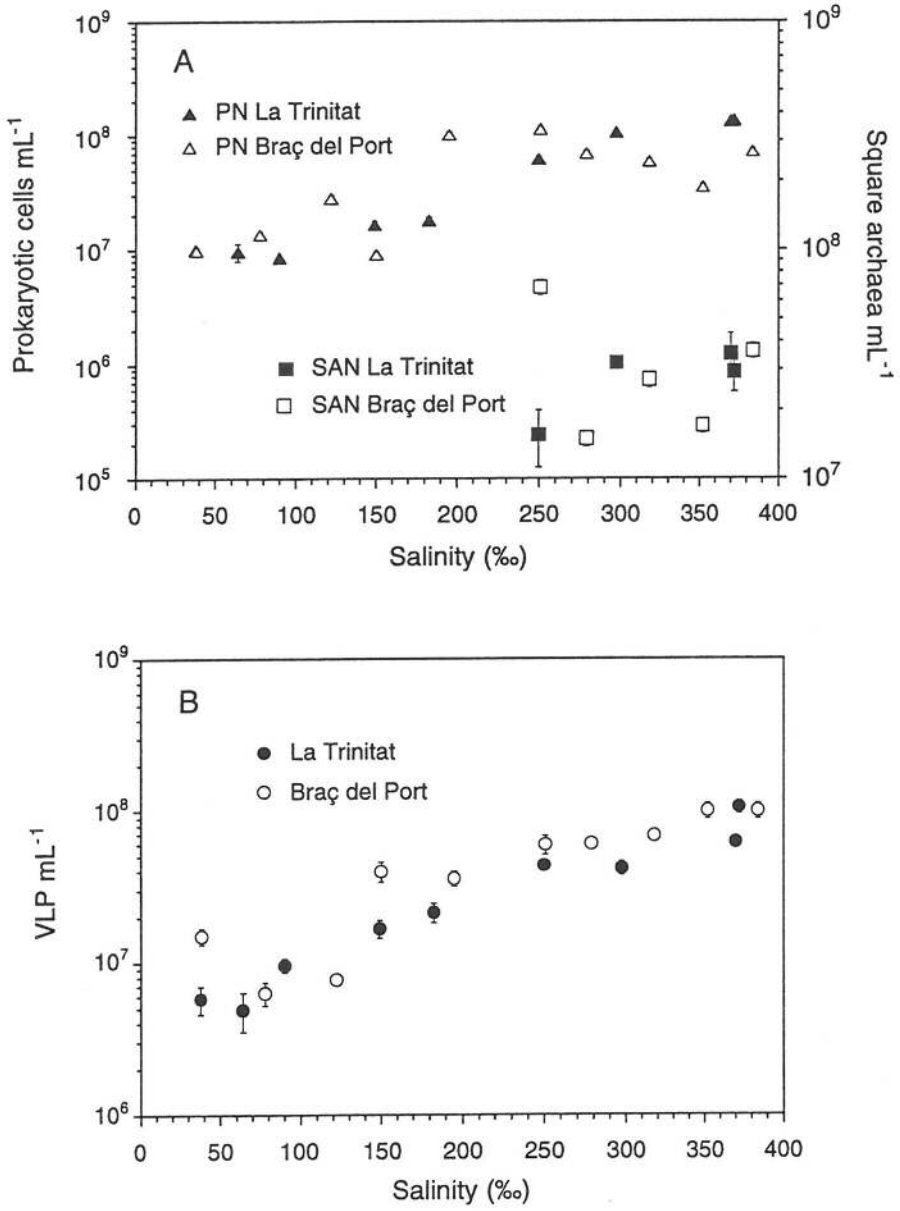


Fig 3.5. A- Abundance of prokaryotes (PN) and square archaea (SAN) along the salinity gradient from two salterns ("Braç del Port" and "La Trinitat"). Bars represent standard errors of two replicates. **B-** Viral abundance from the two salterns, determined with DAPI stain and epifluorescence microscopy. Bars represent standard errors of six replicates in "Braç del Port" and of two replicates in "La Trinitat". When no bars are visible errors are smaller than marker points.

The importance of viruses and bacterivores in controlling prokaryotic abundance and production is shown in Fig. 3.4B. The percentage of prokaryotic losses attributed to viral lysis was always lower than 20 % of both biomass and production. Bacterivory, on the other hand, accounted for a much higher fraction of prokaryotic abundance and production losses at salinities lower than 180 ‰. In the first pond, with a salinity similar to that of sea water, both the percentages of prokaryotic abundance and production ingested by bacterivores were lower than 30 %. In the ponds with salinities ranging from 64 ‰ to 150 ‰, bacterivory accounted for about 100 % of the prokaryotic production. This represented more than 100 % of the prokaryotic abundance per day, due to the fact that prokaryotes in these ponds doubled approximately twice a day. Viral infection could be first detected at 150 ‰ salinity. At this salinity, both the percentage of bacterial abundance and production losses attributed to viral lysis were almost thirty times lower than the percentage attributed to bacterivory. At 182 ‰ salinity, viral lysis and bacterivory reached similar low percentages, although bacterivory was still three times higher than viral lysis. At this salinity, prokaryotic doubling times (Fig. 3.3B) increased about two-fold with respect to doubling times at lower salinities.

From 250 ‰ salinity onwards, bacterivory disappeared and viral infection acted alone. The percentage of prokaryotic abundance losses per day attributed to viral lysis, was lower than 5 %. At the saltiest ponds (from 250 ‰ to 372 ‰ of salinity), prokaryotic doubling times reached values ranging between 2.5 and 5 days (Fig. 3.3B).

Comparison between salterns

Salterns derived from sea water have the same types of salts in solution all over the world. Due to this characteristic, organisms and ecological relationships among different salterns are similar (Rodríguez-Valera 1988, Oren 1994). In order to confirm this similarity for the viruses we compared prokaryotic and viral abundance between "La Trinitat" and "Braç del Port" salterns, located in different places along the Mediterranean coast (Fig. 3.5). In "Braç del Port" bacterial and viral abundance were determined using DAPI stain and epifluorescence microscopy. In order be able to compare both salterns, we also determined viral abundance in "La Trinitat" with epifluorescence microscopy. Although in "La Trinitat" salterns viral abundance determined with epifluorescence microscopy (Fig. 3.5B) was 2-20 times lower than that found with TEM (Fig. 3.1A), viral counts done with DAPI were highly reproducible. From 6 replicate filters for each pond from "Braç del Port", the percentage of variability around the mean was always lower than 10 %. Thus, despite the lower sensitivity of DAPI-epifluorescence microscopy with respect to TEM to estimate viral abundance, the method should be useful to compare different samples with similar physico-chemical characteristics.

Prokaryotic abundance in both salterns showed a similar pattern (Fig. 3.5A). The abundance was lower than $4x10^7$ prokaryotic cells mL^{-1} at salinities lower than 200 ‰ and increased until $1x10^8$ prokaryotic cells mL^{-1} from this salinity on. Square archaea started to be considerably abundant at 250 ‰ salinity in both salterns (Fig. 3.5A). Viruses followed also a similar pattern in both salterns and they reached the maximal abundance at salinities higher than 250 ‰ (Fig. 3.5B).

Discussion

Advantages of solar salterns for the study of virus-prokaryotic interactions

The solar salterns provide a range of environments specially suited to test hypotheses in microbial ecology. Limited species diversity, high cell abundance and short food chains (Rodríguez-Valera 1988, Oren 1994) make the system progressively simple and easy to analyze. The gradient of salinity, from sea water to saturated sodium chloride brines, allows examination of microbial processes as more and more components disappear as their upper tolerance limit to salt is reached. One last advantage of this system is that each pond can be considered to be at a steady-state. Cell abundance does not change on different years (Pedrós-Alió *et al.*, in preparation) and except for the rare heavy rainfalls, there are essentially no alterations of the system. This allowed simultaneous calculation of the total mortality due to bacterivory and viruses.

We also knew that bacterivory would decrease and finally disappear as salinity increased (Pedrós-Alió *et al.*, in preparation), while viral lysis could potentially be present throughout the gradient. Therefore, we could check whether viral mortality and bacterivory co-varied, varied in opposite directions or did not show any relationship to each other.Finally, these ecosystems are markedly similar all over the world, as far as they are derived from the evaporation of sea water, and the conclusions drawn from the study of specific salterns should have a more general validity (Oren 1994).

Abundance of VLP and percentage of infected cells

The numbers of VLP found in the salt ponds (10^7-10^9 mL^{-1}) are some of the highest reported in planktonic systems (Fuhrman & Suttle 1992, Maranger & Bird 1995). The variability in viral abundance among the ponds was largely explained by the abundance of prokaryotic cells (PN). Several studies have reported that the strength of the VLP/PN relationship found, indirectly suggested that the majority of viruses in these environments were of prokaryotes (Proctor & Fuhrman 1990, Cochlan *et al.* 1993,

Maranger & Bird 1995). Our data support this idea, since VLP numbers were significantly correlated with prokaryotic abundance but no correlation was found between VLP and chlorophyll a concentrations (data not shown).

In one study it has been also suggested that there is a correlation between cell abundance and frequency of cells containing mature phages (Weinbauer *et al.* 1993), but in an other study this correlation was not found (Steward *et al.* 1996). From our data, the percentage of infected cells was not different among the ponds. However, the production of viruses increased with prokaryotic cell abundance due to the different burst sizes of the prokaryotes in the different ponds. A great variability in the burst size of whole marine bacterial assemblages (10-300), has been reported in other studies (Heldal & Bratbak 1991, Steward *et al.* 1996). Considerable variability in the burst size of different bacterial morphotypes has been found (Weinbauer & Peduzzi 1994). Other factors such as growth rate or cell volume could also influence the burst size of the prokaryotes.

Phage production rates may be used to calculate phage-induced bacterial mortality when the burst size is known. Some studies have used the minimum (10), the maximum (300) and the average (50) burst sizes reported for marine environments (Steward *et al.* 1992b). The range of prokaryotic mortality rates reported in this way change by an order of magnitude, depending on the assumed burst sizes. These studies have advised caution about the uncertainty of their mortality estimates (Steward *et al.* 1992b). Our data are a clear example of the importance of determining the burst size of the prokaryotes in each environment in order to get accurate estimates of bacterial mortality due to viral lysis, when the latter are estimated from phage production rates. Despite some uncertainties in the determination of burst sizes with current methods (Weinbauer & Peduzzi 1994), this is the most direct way available to estimate viral mortality.

We have calculated viral production from direct measurement of burst sizes and number of infected cells. The viral production in the different ponds was within the range of values reported for other environments (Steward *et al.* 1992b). Assuming that the ponds are at steady-state, the VLP number should not change with time. Thus, our estimated virus production rates should be equal to decay rates. These rates varied between 0.04-0.7 h^{-1}, which also fall within the range of published values (Heldal & Bratbak 1991, Bratbak *et al.* 1992, Mathias *et al.* 1995).

We conclude that it is possible to support a high phage production rate with low percentages of prokaryotic mortality in Nature. The low percentage of prokaryotic mortality due to viral infection and the high bacterial biomass suggest that viruses do not control total prokaryotic abundance.

Prokaryotic mortality: virus infection versus bacterivory

The factors that control growth rate and mortality of prokaryotes in aquatic environments have received considerable attention. However, very few studies have included direct measurements of viral lysis. In one study losses due to bacterivory and viral lysis were determined simultaneously in a mesocosm (Bratbak *et al.* 1992). These authors measured primary production, bacterial production, viral decay rates and bacterivory. In their results, predation exceeded bacterial production estimates by a factor of 2. Viral lysis exceeded bacterial production by a factor of 6. Therefore, a balance could not be found. Fuhrman & Noble (1995) found a balanced budget between bacterial losses and production including bacterivory and viral infection. They used independent lines of evidence to conclude that viruses and protists caused comparable rates of bacterial mortality in two mesocosm experiments. Recently, Steward *et al.* (1996) reported comparable rates of bacterial mortality due to viral lysis and flagellate bacterivory in Arctic waters. They did not find a balance between bacterial production and these two sources of bacterial mortality, suggesting that other sources of bacterial mortality were present.

We have quantified the two factors that have been reported to account for most of prokaryotic mortality in Nature. Taking advantage of the steady-state dynamics present in the salterns, we could assume a balance between prokaryotic heterotrophic production and losses due to bacterivory and viral lysis. In our study, in the ponds where bacterivory was present, prokaryotic production balanced prokaryotic losses in three of the ponds (from 64 ‰ to 149 ‰ of salinity). In the other two ponds (37.5 ‰ and 182.5 ‰ of salinity) prokaryotic production was 2-4 times higher than prokaryotic losses. In the higher salinity ponds, bacterial production exceeded losses by viral infection by a factor >10. Since production was higher than the measured losses in the higher salinity ponds, there must exist other sources of mortality, perhaps flocculation and sedimentation. Some characteristics of the halophilic archaea such as the production of salt-requiring bacteriocins that inhibit the growth of other halophilic archaea, have been reported as a source of mortality in laboratory cultures (Oren 1994).

Moreover, special characteristics of several halophage-halophilic archaea systems, such as carrier-states, could also contribute to explain this uncoupling. A carrier-state has been defined as a situation in which the host persistently carrying and continually producing viruses, survives and multiplies (Pauling 1982, Zillig *et al.* 1988). In this carrier-state, infected bacteria continue to multiply at the same rate as uninfected bacteria (Torsvik & Dundas 1980). Thus, infected cells, could produce phages without increasing mortality.

We do not know the relative importance of these different processes (flocculation, sedimentation, presence of bacteriocins and establishment of carrier-states). Studies in the laboratory suggest that they could be potentially important in the salterns. The fact that prokaryotic heterotrophic production exceeded losses by viral infection in the saltiest ponds supports this hypothesis. Another possible explanation could be an underestimation of viral lysis. This is discussed in a later section.

Prokaryotic growth rate and viral lysis

There is evidence that the supply of organic matter may control bacterial growth in many aquatic environments (Billen *et al.* 1990). It is also known that bacterivores are important contributors to the DOM pools in the oceans (Taylor *et al.* 1985, Nagata & Kirchman 1992). This recycling of nutrients increases prokaryotic growth rates in the presence of bacterivores. A similar effect has been suggested for viral lysis of bacteria (Fuhrman & Suttle 1993, Weinbauer & Peduzzi 1995b). From our results, the doubling times of prokaryotes were longer in the ponds where bacterivory was absent and viral lysis acted alone, and shorter in ponds where both bacterivory and viral lysis were present. Thus, we can conclude that viruses had an effect smaller than bacterivores in increasing prokaryotic growth rates. However viral lysis may play a significant role in maintaining moderate prokaryotic growth rates in the saltiest ponds.

Hypersaline environments are often rich in nutrients, and in the salterns the temperature is close to the optimal for growth of halophilic prokaryotes (Oren 1994). Other requirements, such as ionic composition of the ponds could determine the growth rate of the different prokaryotic assemblages. Therefore, the growth stimulation due to bacterivores and viruses may be partially masked by these other factors.

Critical assumptions in the calculation of bacterial mortality

We estimated bacterial mortality caused by two different factors: bacterivory and viral lysis. Both estimates are subject to errors. If the errors were very high, some of the conclusions would be invalid. The FLB technique to determine bacterivory has been used many times in different environments and is usually taken to give reasonable estimates (Sherr *et al.* 1989). In our case the use of a single bacterial culture to prepare FLB which were then used in all ponds may have introduced some error. The FLB were larger than average prokaryotic volume in the lower salinity ponds and smaller than the average in the higher salinity ponds (see Methods). Bacterivores are known to have preferential feeding on larger bacteria, as long as they fall within a certain size range. Thus, our bacterivory estimates could be overestimates at lower salinities and underestimates at higher salinities.

We think, however, that this was not a major problem. According to this line of reasoning the 37 ‰ salinity pond should have shown the highest overestimation. Yet, bacterivory in this pond was relatively low. In fact, it showed the same value as in the 180 ‰ salinity pond, where average bacterial volume was similar to that of the FLB. Thus, other factors different from size were having a much stronger impact on predation estimates. Further, grazing estimates were very reasonable in all cases. They showed a covariation with bacterial specific thymidine incorporation rates and decreased down to zero as salinity increased in parallel to the abundance of ciliates and flagellates. Thus, we conclude that the large differences in grazing between ponds are real, even if the absolute value is subject to some error.

The critical assumptions used to calculate viral mortality from visibly infected cells by TEM are: the ultracentrifugation step, the conversion factors to convert visibly infected cells to total number of infected cells, and the assumption that the latent periods are similar in length to the host doubling times.

During the ultracentrifugation process there is the possibility that we lost phage-infected prokaryotes by disruption of cells (Weinbauer & Peduzzi 1994). Thus, the frequency of infected cells reported would tend to be conservative.

The conversion factor used to get the total number of infected cells was derived from thin sections (Proctor *et al.* 1993), and we applied it to samples observed directly by negative staining. The total number of cells with mature phages may be higher than the visibly infected cells but perhaps not in the same proportion as in thin sections (Fuhrman & Suttle 1993). Fuhrman & Noble (1995) used both methods to estimate the percentage of infected cells. Although they only had two measurements to compare both methods, the number of visibly infected cells found with thin sections was higher than that counted with direct observations of infected bacteria. Thus, our estimates of infection for the whole prokaryotic community could be considered conservative (but not those for the square archaea). The special morphology of this group (flat and square), made it possible to avoid some of the inconveniences of the direct observation of infected cells (variation in staining and cell thickness, Fuhrman & Noble 1995). Moreover, the conversion factor derived using the whole cell method in natural communities (M. Weinbauer, cited in Steward *et al.* 1996) was within the range calculated by Proctor *et al.* (1993).

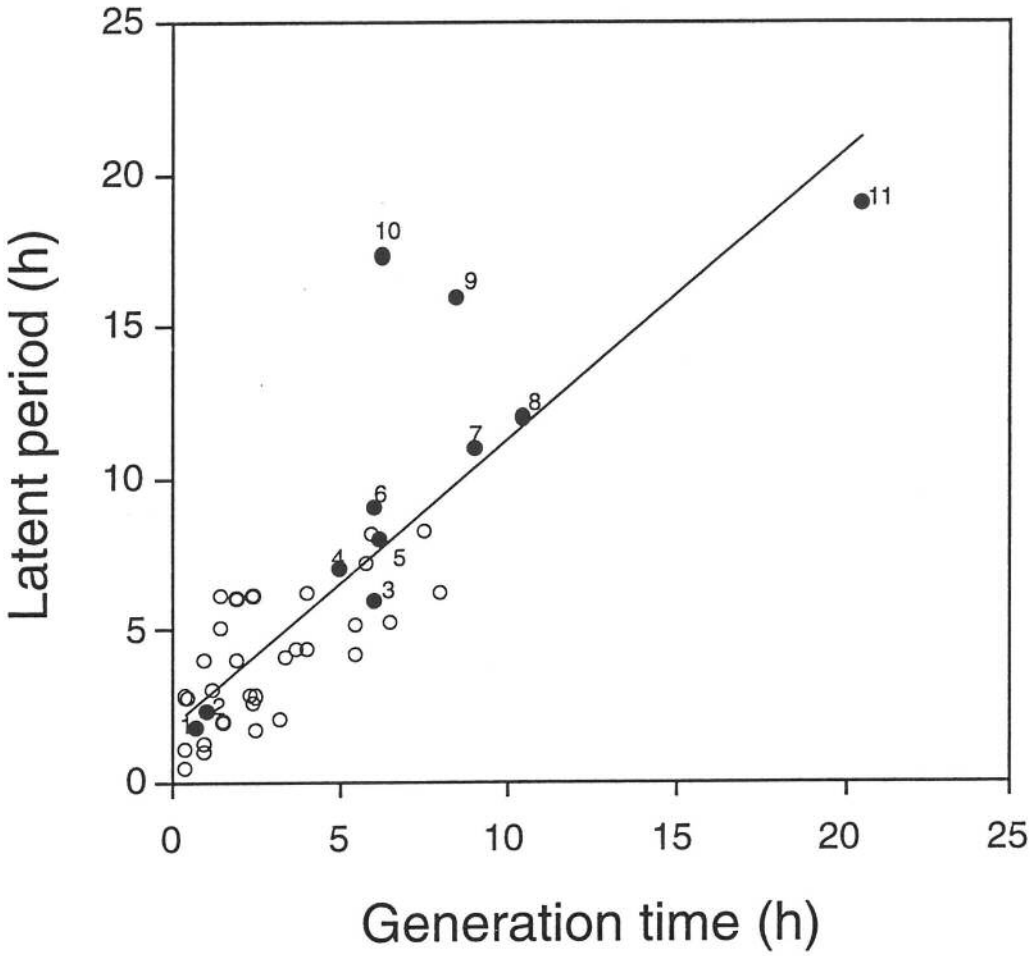


Fig 3.6. Generation time of uninfected cells vs. latent period of infected hosts. Open circles present data from Proctor *et al.* (1993). Filled circles present data from the halotolerant /halophilic prokaryote-phage systems reported in the literature: [1&2] *Deleya halophila* /F19 from Calvo *et al.* (1988), [3] *Halobacterium halobium* /Ja1 from Wais *et al.* (1975), [4] *Halobacterium halobium* str.R1/ϕH from Schnabel *et al.* (1982), [5&8] *Halobacterium halobium*/Hh3, Hh1 from Pauling (1982), [6&7] *Halobacterium curtirubrum* NRC 34001/S5100 from Daniels and Wais (1990), [9,10&11] *Halobacterium salinarium* /HS1 from Torsvik & Dundas (1980).

The most critical step in our calculations is the assumed equivalence between the generation times of the prokaryotes and the latent periods of the viruses. To test this assumption, Proctor *et al.* (1993) compared latent periods and generation times of marine isolates cultured with a range of nutrients. We have added data from the few studies done with halotolerant and halophilic prokaryotes and their phages in a range of salinities, to their data set (Fig.3. 6). The regression with the added data was significant ($p < 0.001$, $r^2 = 0.68$, $n = 42$), and the slope of the regression was not significantly different from 1 (Student t test, $p > 0.05$). Therefore, it seems safe to assume similar lengths for the latent periods and the generation times.

Another possible error in our calculations is the assumption that all the infected prokaryotes will lyse during the time of the latent period. It has been suggested that it is possible that bacterivores ingest infected bacteria, even at a higher rate than non-infected bacteria (Weinbauer & Peduzzi 1995a). This could be the reason why we did not find any visibly infected cell in the lower salinity ponds. If this were the case, our data of mortality due to viral lysis would be still lower in the ponds where the bacterivory was present. This would not matter, however, in the ponds with higher salinities, where bacterivory was zero.

We can not give the quantitative importance of these sources of error. Except for the first one and partially for the second, however, all the other would cause our estimates to be overestimates, and therefore the conclusion of very low impact of viral lysis on prokaryotes in salterns would not be affected.

Square archaea and lemon-shaped viruses

The square archaea were first observed by Walsby (1980) in the salt-saturated brines of a sabkha (salt plain) in the Southern Sinai Peninsula. Since then these cells have been found in other hypersaline habitats in Israel, Spain, California and Senegal. These prokaryotes have not been grown in pure cultures (Oren 1994), but there is no doubt that these organisms are halophilic archaea (Stoeckenius 1981). In the absence of any convincing laboratory cultures, it is not possible to tell whether more than one type exists (Grant & Larsen 1988).

In samples from salt-saturated brines, it is common to find a series of odd morphologies, including cup-shaped, square, rectangular and triangular cells. Although several box-shaped archaea have been isolated in pure cultures from similar environments (Javor *et al.* 1982), they were more variable in appearance and smaller than Walsby's square archaea and they lacked gas vacuoles (Parkes & Walsby 1981). No information is available about the phages of any of these halophilic archaea.

We found a clear pattern of increasing square archaeal abundance with increasing salinity. In the saltiest ponds this morphotype formed a large percentage of the cell number (25 %). Thus, they could be expected to play a significant role in the ecosystem.

We did not find any significant difference among the percentages of visibly infected square archaea with increases in their abundance. This indicates a constant proportion between uninfected cells and infected cells We could not estimate *in situ* growth rates specifically for this group, thus we could not calculate rates of mortality for them as we did before with the whole prokaryotic community. However, applying Proctor *et al.*'s conversion factors to the percentage of square archaea visibly infected we could estimate the total percentage of infection for the square bacteria. Thus, from 5 % to 48 % of the square archaea could be infected by viruses at any moment. Of course, the conversion factors of Proctor *et al.* (1993) were calculated for a marine *Vibrio*.. The real factor for the square archaea could be completely different. In any case, the number of square archaea are rather constant in salterns: we have found the same abundance in different years and in different salterns (Fig.3.6). The persistence of these numbers with relatively high infection suggests that the viruses do not have a strong impact on the abundance of square archaea.

Different mechanisms may work in the maintenance of this equilibrium. It has been already mentioned that carrier-states can be very important in the salterns. Since the square archaea were the only infected morphotype in the most salty ponds, this may indicate that they were the most metabolically active component of the prokaryotic assemblage. Moreover, they are halophilic archaea and in the saltiest ponds they find the best conditions for growth (Oren 1994). From the halophage-halophilic archaea pairs studied in cultures, it is known that when the halophilic archaea reach their optimal growth conditions, a carrier-state is often established. This situation seems to protect halophilic archaea from extensive phage-induced lysis, and provides for the perpetuation of the phage (Torsvik & Dundas 1980). Thus, a high percentage of infected square archaea would not necessarily cause high mortality in their population.

We have also found a particular morphotype of virus infecting the square archaea, the "lemon-shaped viruses". Similarly shaped viruses have been isolated from other archaea: *Sulfolobus B12* and *Methanococcus voltae* (Zillig *et al.* 1988), but had not been isolated or described in halophilic archaea. It is interesting that it has been suggested that this type of VLP could have arisen before the separation of the major branches of the archaeal domain (Zillig *et al.* 1988).

Throughout the salinity gradient we have been able to follow the viral infection from a situation where bacterivores and viruses interacted with prokaryotes up to a high salinity environment where only prokaryotes and viruses were present. This study of viruses in salterns supports a general conclusion about their role in steady-state situations: the viruses are not important in determining overall bacterial abundance. However, more work is necessary in order to know how the populations can remain in such high abundance. Studies related to the diversity and activity of the different bacterial assemblages are necessary. The salterns seem to be an appropriate environment to test some of these hypotheses.

4. Low viral impact on bacteria in an oligotrophic marine system: the northwestern Mediterranean Sea

Introduction

Open oligotrophic waters constitute the largest portion of the world ocean. Virus-like particles (VLP), were found to be surprisingly abundant in such waters only eight years ago, when isolated samples from different environments were examined for viral concentration (Berg *et al.* 1989, Proctor & Fuhrman 1990, Hara *et al.* 1991). Some additional open waters samples have been examined for VLP abundance in the context of development of methods (Steward *et al.* 1992b, Hara *et al.* 1996). However, only two studies (Cochlan *et al.* 1993, Boehme *et al.* 1993) have determined virus-like particles (VLP) distribution in open ocean waters.

Viruses have been shown to account for a high percentage of bacterial mortality in some marine environments (Proctor & Fuhrman 1990, Weinbauer & Peduzzi 1995a), similar to that due to heterotrophic nanoflagellates when both sources of bacterial losses have been measured simultaneously (Fuhrman & Noble 1995, Steward *et al.* 1996). Viral lysis of bacterioplankton causes not only removal of bacterial cells. It has been reported that mortality of microbes due to viral infection may have consequences for nutrient and energy cycling (Middelboe *et al.* 1996), for the control of species diversity (Thingstad *et al.* in press) and for the exchange of genetic materials among bacteria in the marine environments (Chiura 1997).

Although VLP abundance itself gives little information about viral impact on the host populations, concentration of viruses in seawater is an essential information for the ecological study of an aquatic environments. Thus, VLP abundance has been shown to be closely coupled to the trophic characteristics of the system (Maranger & Bird 1995, Chapter 6). VLP abundance seems to decrease with increasing distance from shore as has been reported in Florida coastal waters (Boehme *et al.* 1993) and in the California coast (Cochlan *et al.* 1993). However, an opposite trend was found in the estuarine Gulf of Bothnia (Cochlan *et al.* 1993). At the same time, mortality of bacteria due to viral infection has been shown to be higher in eutrophic than in oligotrophic environments (Weinbauer & Peduzzi 1995a). Therefore, it seems that VLP abundance indirectly reflects viral impact on the host populations. Yet, almost none of this information has been gathered from open ocean environment. Thus, almost nothing is known about the abundance and impact of viruses in one of the most extensive ecosystems on Earth.

Common characteristics found in the major oceanic gyres of the Atlantic and Pacific oceans are the presence of a deep chlorophyll a maximum (DCM) and permanent oligotrophic conditions (Cullen 1991). The Mediterranean Sea offers the same characteristics of oligotrophy and presence of a DCM within a short distance from shore (Estrada *et al.* 1993). Thus, the Mediterranean constitutes a model to study viral distribution and impact in oligotrophic open sea waters.

The objectives of this work were a) to determine VLP distribution in a transect from the coast to offshore in the western Mediterranean, b) to study relationships between VLP abundance and other components of the food webs, c) to investigate possible changes in VLP abundance along diel cycles in representative stations of this transect and d) to estimate the impact of viruses on the bacterial assemblage.

Materials and Methods

Samples were collected along a transect in the northwestern Mediterranean Sea between June 6 and June 24 (1995) on board BIO Hespérides (Fig. 4.1). Three stations were selected along the transect to study diel cycles. These stations represented the three main zones: the shelf coastal waters (station C), the shelf-break frontal zone (station S) and the offshore "deep" zone (Station D). The characteristics of the microbial populations at these three stations have been described in Pedrós-Alió *et al.* (submitted). Two diel cycles were followed at each station. Vertical profiles of temperature, salinity and fluorescence were taken with a MarkII CTD. Water samples were taken with 12 L Niskin bottles in a rosette.

Chlorophyll a was determined fluorometrically in 100 mL samples that were filtered through GF/F glass fiber filters and frozen. The filters were extracted overnight in 90 % acetone at 4° C and fluorescence of the extract measured with a Turner Designs fluorometer (Yentsch & Menzel 1963).

Samples for bacterial abundance were fixed with gluteraldehyde (2 % final concentration) in polypropylene bottles. Bacteria were stained with DAPI (1 $\mu g\ mL^{-1}$ final concentration) and filtered onto black 0.2 μm pore size polycarbonate filters (Porter & Feig 1980), mounted on microscope slides and frozen. Bacterial abundance was determined with a Nikon epifluorescence microscope at a magnification of 1250x. About 200-300 bacteria were counted per sample.

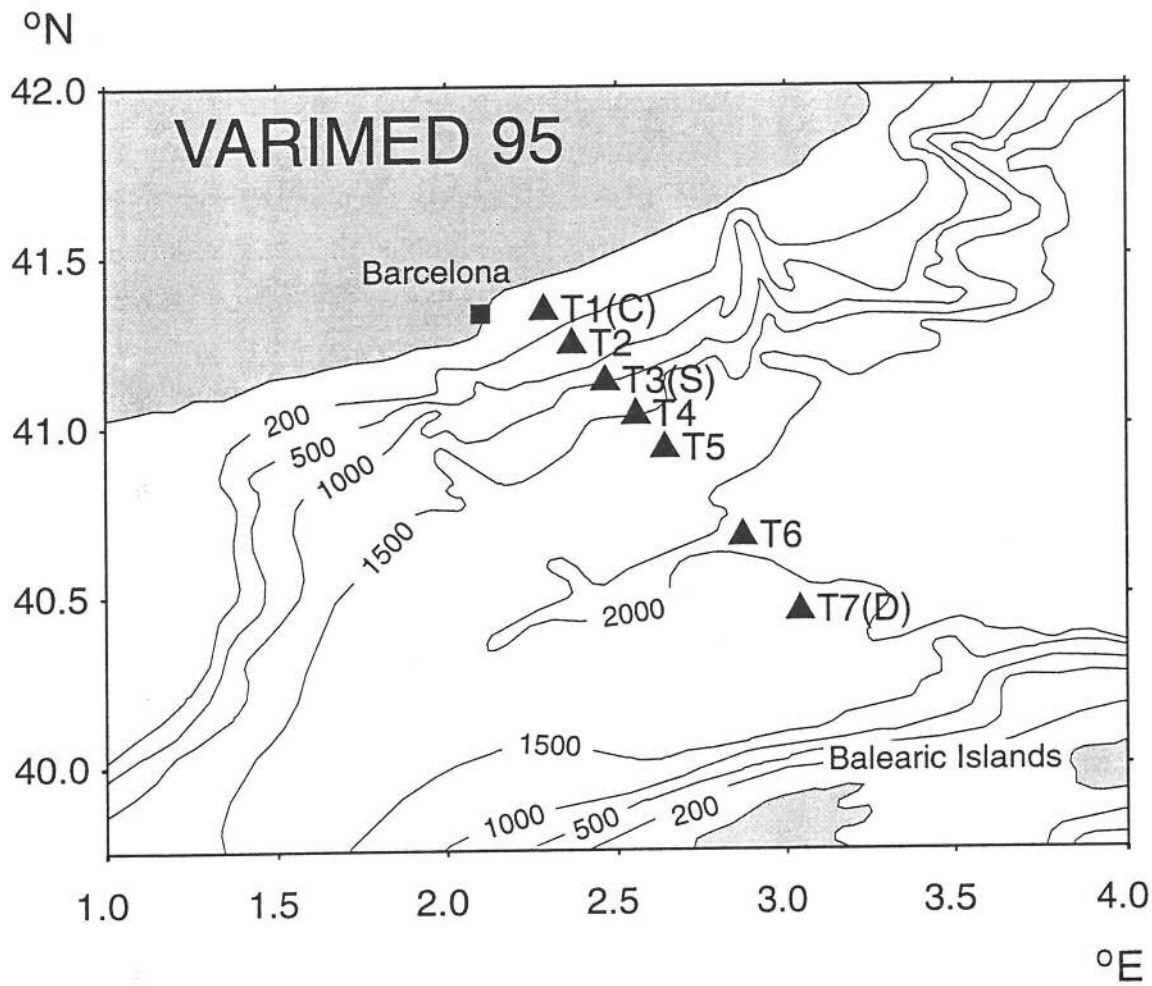


Fig. 4.1. Map of the area studied during the cruise VARIMED 95. The transect follows a line between the city of Barcelona and the channel between the islands of Mallorca and Menorca. The stations where vertical profiles were studied are marked as T1 to T7. In T1 (C: coastal), T3 (S: slope) and T7 (D: deep) diel cycles were investigated.

Abundance of VLP was determined by YOPRO (YO-PRO 1, Molecular Probes) stain and epifluorescence microscopy (Hennes & Suttle 1995). Unfixed 100 µL samples were immediately diluted with 700 µL of mili Q water filtered through a 0.02 µm pore size Anodisc filters. Each diluted sample was gently filtered through a 0.02 µm pore size Anodisc 25 filter. The Anodisc filters with the filtered sample were laid on 80 µl of the staining solution (YO-PRO 1, 50 µM final concentration) in a Petri dish and incubated in the dark for two days at room temperature. The filters were then washed twice by filtering 800 µL of mili Q water through the membrane. Filters were transferred to glass slides, immediately covered with a drop of spectrophotometric-grade glycerol and a cover slip. Filters were stored at -20° C until counted. VLP abundance was determined with an epifluorescence microscope at a magnification of 1250x with blue light excitation and a barrier filter.

Most of the studies in the literature have used transmission electron microscopy (TEM) to estimate VLP abundance in nature. In order to be able to compare our data counted by epifluorescence microscopy with those reported in other works, we converted our YOPRO counts to TEM counts using a regression line (Log VLP-YOPRO = -0.32 + 1.1 Log VLP-TEM) obtained from the comparison between both methods in different environments. The data used to build this regression line corresponded to a microcosm experiment (Chapter 2) plus data from the only two studies that compared both methods (Hennes & Suttle 1995, Weinbauer & Suttle 1997).

Results

Depth profiles of chlorophyll a, bacterial and VLP abundance are shown in Fig. 4.2. The coastal station (C) showed a uniform abundance in VLP and bacteria throughout the water column. The other stations followed a different pattern, with peaks of chlorophyll a, bacterial and VLP abundance at different depths. The peak of chlorophyll a (DCM) was usually sharp and could be found between 40 and 70 m depending on the station. The peak of bacteria was less sharp and it was usually found slightly above the DCM. The distribution of viruses was more irregular from one station to another. In some cases (T4, T6, T7) a peak of VLP was coincident with the bacterial peak. At other stations (T3, T5), VLP concentration decreased smoothly with depth from the surface. Finally, no clear pattern was detected, in some profiles (T1, T2). Peaks of bacterial abundance and chlorophyll a concentration were coincident in four stations (T2, T5, T6 and T7). The peak of VLP abundance was just below or above the peak of bacterial abundance and chlorophyll a maximum.

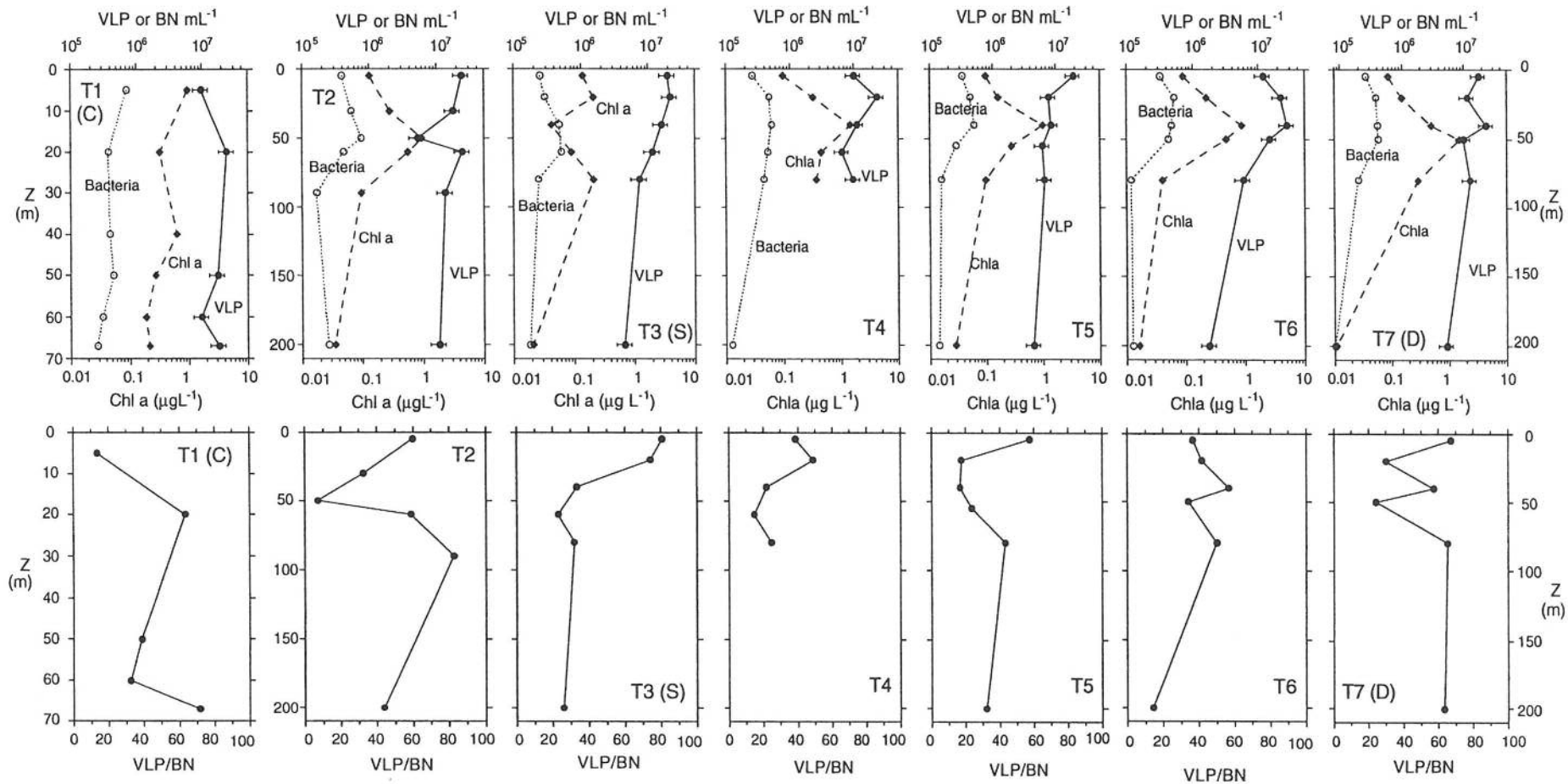


Fig. 4.2. The upper panels show the vertical profiles of VLP abundance (VLP mL-1), bacterial abundance (BN mL-1) and chlorophyll a concentration (µg Chla L-1) at the stations sampled in the transect (T1 to T7). The lower panels show the vertical profiles of the VLP/BN ratio at the same stations. Error bars indicate the standard error, which was calculated as percentage of the mean in selected triplicate samples.

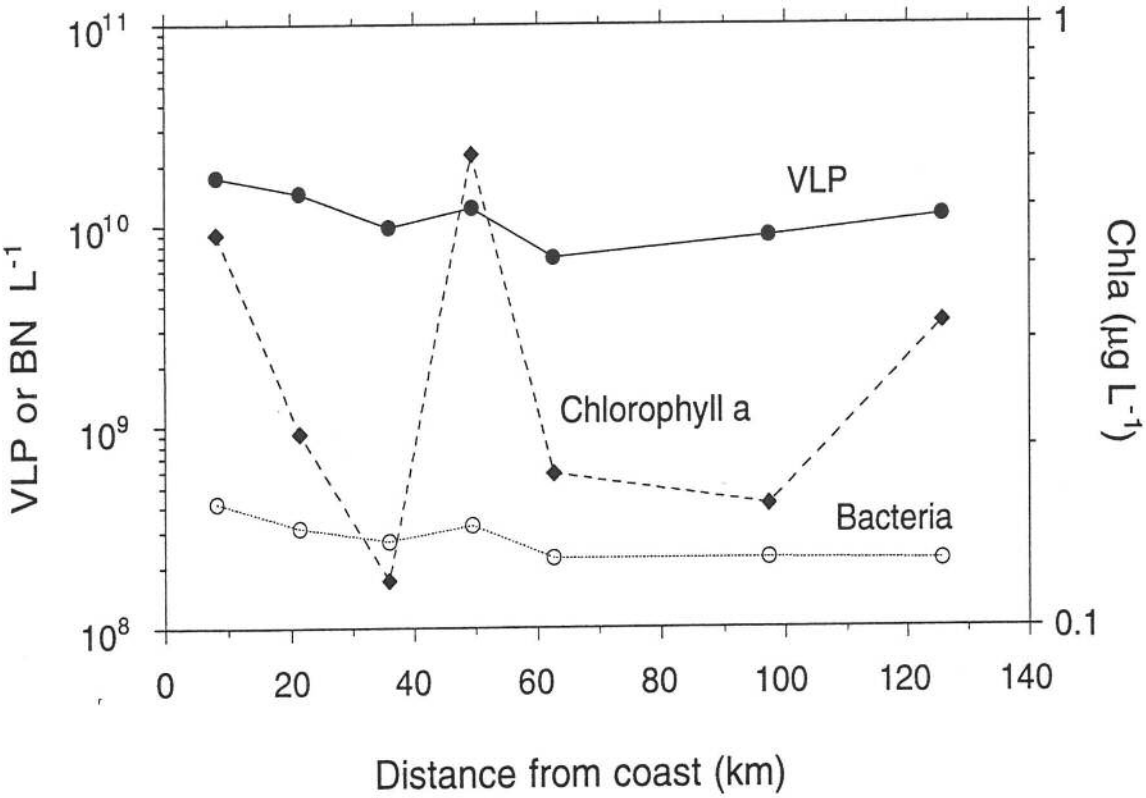


Fig. 4.3. Integrated values of the vertical profiles of VLP (VLP L^{-1}) and bacterial abundance (BN L^{-1}) and chlorophyll a (Chla µg L^{-1}) along the transect (km from the coast). Values for the whole water column were integrated and the result divided by the integration depth, thus obtaining weighted averages.

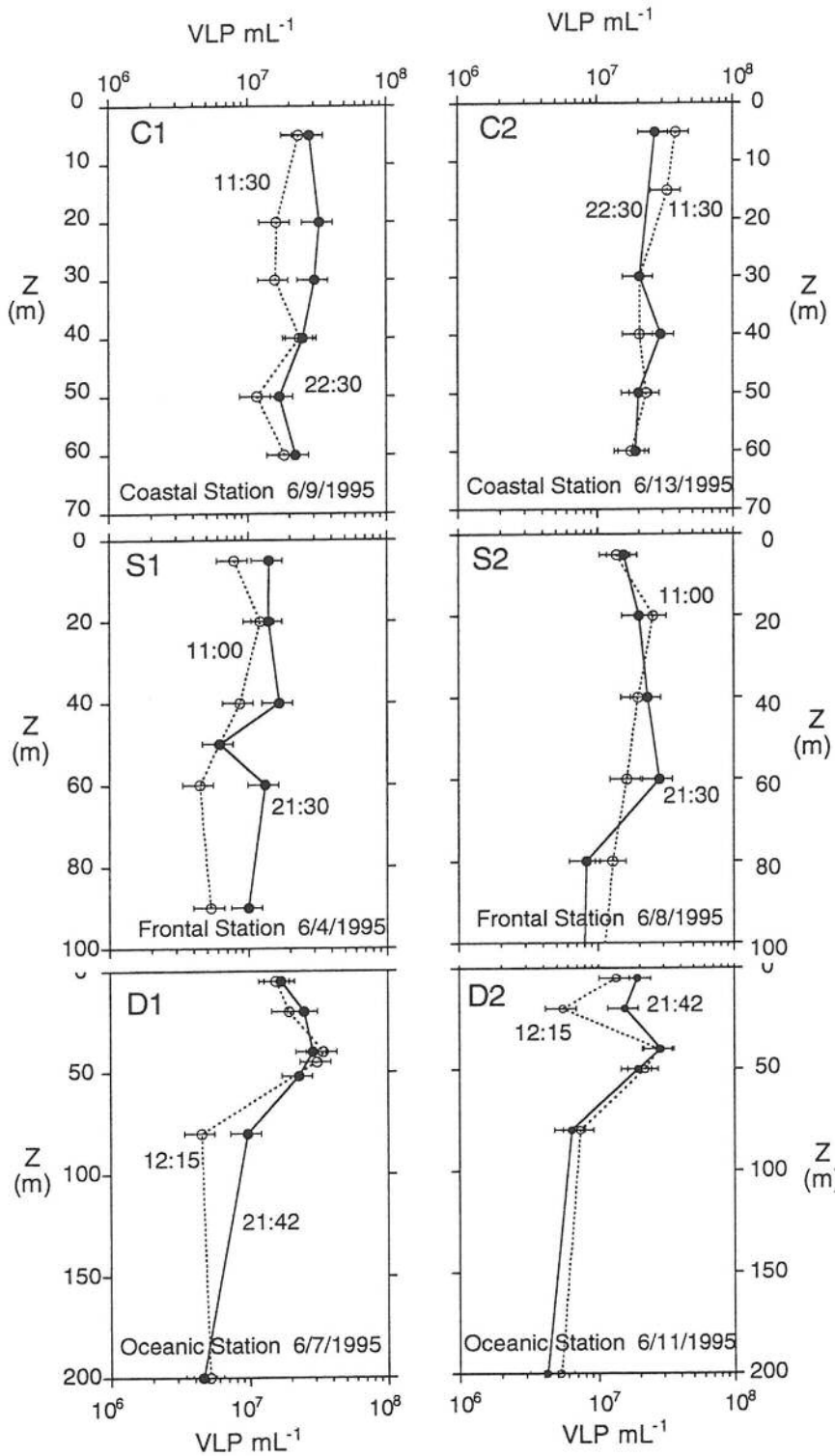


Fig. 4.4. Variability in VLP abundance during the diel cycles (1 and 2) investigated at the three stations: C, S and D. Error bars indicate the standard error, which was calculated as the percentage of the mean in selected triplicate samples and later extrapolated to all samples.

Values of VLP abundance ranged between $5x10^6$ and $3x10^7$ along the transect. Changes in VLP abundance were minimal at depths higher than 60 m in all the stations. The VLP/BN ratio ranged from 10 to 80 (Fig. 4.2). The depth distribution of VLP/BN was parallel to that of VLP abundance.

Relationships between bacterial and VLP abundance and between chlorophyll a and VLP abundance were not significant for coastal stations (T1, T2, $p = 0.4$, $n = 11$) and intermediate stations (T3, T4, $p = 0.2$, $n = 11$). For the offshore stations (T5, T6, T7), VLP and bacterial abundance showed a significant relationship ($p < 0.0009$, $r^2 = 0.506$, $n= 18$). The relationship between chlorophyll a and VLP abundance was also significant in the later stations ($p < 0.0061$, $r^2 = 0.38$, $n = 18$).

Integrated values of chlorophyll a concentration, bacterial and VLP abundance from surface to 70 m (coastal station) or 200 m (frontal and open sea stations) showed a similar pattern (Fig. 4.3). Peaks with maximal concentration of these parameters were coincident in T1, T4 and T7.

No clear pattern in diel variability of VLP abundance was found at any of the stations (Fig. 4.4). At the coastal and frontal stations VLP abundance showed small changes with depth in both diel cycles. At the coastal station, VLP was slightly higher at night than during the day in the first diel cycle (Fig. 4.4 C1, C2). At the frontal station each diel cycle presented a different pattern (Fig. 4.4 S1, S2). During the day the peak of maximal VLP abundance at 20 m appeared both times. At night, however, the first diel cycle showed a minimum at 50 m and the second a maximum at 60 m. At the deep station (Fig. 4.4 D1, D2), no diel cycles were found. However, VLP abundance showed slightly higher values at night in the upper mixed layer. In this station, patterns of VLP abundance with depth were similar in both cycles.

Discussion

The values of VLP abundance reported in the present work are difficult to compare directly with similar studies because of the different methodology used to count VLP (Table 4.1). Thus, we have converted our YOPRO counts to TEM counts using the relationship found between both methods (see materials and methods). After conversion of the VLP abundance found with YOPRO to TEM, values of VLP abundance were 2-5 times lower than those found with YOPRO. This was in agreement with Suttle & Hennes (1995), who compared viral abundance from different environments with both methods. These converted values of VLP abundance in the Mediterranean were in the lower range of the reported numbers for open sea environments.

Table 4.1. Values of VLP abundance, counting method used in each study, and VLP/BN from different marine environments. Data between parenthesis correspond to the transformed VLP abundance from YOPRO to TEM according to the regression line: Log VLP-YOPRO = -0.32 + 1.1 Log VLP-TEM.

	VLP mL^{-1}				
Location	**Filter[2]+Spin[3] + TEM**	**Spin[3] + TEM**	**YOPRO + Epifluorescence**	**VLP/BN**	**Reference**
Gulf of Mexico[1]					Boheme et al. 1993
coast	2.60×10^6			3.1	
off shore	3.45×10^5			2.1	
Southern California Bight[1]					Cochlan et al.1993
coast		7.20×10^6		6.8	
off shore		1.11×10^6		2.4	
Gulf of Bothnia					Cochlan et al. 1993
coast		3.20×10^7		14.7	
off shore		3.15×10^7			
Western Mediterranean[1]					This study
coast		(3.53×10^6)	1.60×10^7	49.9 (10.1)	
off shore		(2.87×10^6)	1.24×10^7	39.7 (8.3)	
North Atlantic		1.50×10^7		49.7	Berg et al. 1989
Barents sea		$<6.00 \times 10^4$		3.0	Berg et al. 1989
Sargasso sea		3.00×10^3			Proctor & Fuhrman 1990
Kuroshiro Bay		8.60×10^6		22.6	Hara et al. 1991

[1] Values corresponded to the mean from different vertical profiles
[2] Ultrafiltration
[3] Ultracentrifugation

Values from some very oligotrophic seas, however, such as the Sargasso Sea and the Barents Sea, showed the lowest VLP abundance values reported, two to three orders of magnitude lower than values for the Mediterranean. Since such extremelly low values have been found only twice, however their general significance cannot be evaluated.

Boehme *et al.* (1993) found also a lower VLP abundance value in an offshore than in a inshore station in the Gulf of Mexico (Table 4.1). However, they concentrated the viruses from the natural sample by vortex flow filtration (30-100 kDa pore size) and viral losses have been reported during this process (Paul *et al.* 1993, Suttle & Chan 1994). Obviously, the method used to count viruses must be taken into account in order to compare results from different studies. It has been suggested that TEM counts could underestimate VLP abundance in natural environments (Hennes & Suttle 1995, Weinbauer & Suttle 1997). However, at the time being, there are very few studies that have used YOPRO to estimate viral abundance in aquatic environments. All together, VLP abundance in the western Mediterranean was one of the lowest reported in marine environments.

VLP abundance did not show a marked decrease from coast to open sea. However we found some differences between coast and open sea in the vertical distribution of VLP. The coastal station (C) was different from the rest, because it did not show variability in VLP abundance through the water column. The slight variability in VLP abundance with depth in coastal samples was close to the average coefficient of variation (CV) of viral counting by YOPRO in this environment (average CV = 25 %). Thus, we were not able to detect any statistically significant relationship between VLP and other components of the food web in coastal samples. This is reasonable because bacteria and chlorophyll a also showed very small changes with depth. This could be the reason why the correlation between VLP abundance and chlorophyll a and between VLP and bacterial abundance were only significant in the offshore samples. The fact that both relationships explained a low percentage of VLP variability in open sea could indicate that part of the viral assemblage would be bacteriophages and the rest phytoplankton viruses.

Although bacteria and VLP were only correlated in the offshore samples, the integrated values of both parameters showed the same pattern along the transect. This indicates that VLP abundance is coupled to the other components of the microbial food web.

No clear diel pattern was found in any of the stations. The slight differences found in VLP concentration between night and day at the coastal station are difficult to interpret because they only occurred once. In station S both diel cycles followed different patterns.

However both cycles presented a minimum in VLP abundance close to the surface during the day, perhaps reflecting decay due to sunlight irradiation. In station D no diel cycles were found. Gasol *et al.* (in press) in their study of diel variability on bacterial activity during the same cruise, concluded that diel cycles in bacterial heterotrophic production were present in the offshore station while no diel pattern was found at the coastal station. Despite changes in activity, bacterial abundance did not present a clear pattern (Gasol *et al.* in press). We only had two profiles, twelve hours apart, per diel cycle. More profiles could have potentially shown a more significant pattern of variation. However, we think it unlikely, since bacterial abundance was measured six times during the same cycles and no significant differences could be found (Gasol *et al.* in press). Likewise, studies in other coastal areas (Jiang & Paul 1994, Weinbauer *et al.* 1995) have not shown diel variations in VLP. These corresponded to eutrophic environments where concentration of bacteria and VLP were about an order of magnitude higher than those found in the Mediterranean.

In order to measure the impact of viruses on the bacterial assemblage in the Mediterranean, we tried two different methods: the counting of visibly infected bacteria (VIB, Weinbauer *et al.* 1993) and the measurement of viral decay rates in KCN amended cultures (Heldal & Bratbak 1991). We tried to count infected bacteria by observing the whole cells by TEM at 80 KV acceleration, according to Weinbauer *et al.* (1993). We found no infected cells after counting 300 cells per sample in more than 10 samples. This means that the percentage of visibly infected bacteria was lower than 0.3 %. The whole cell method (Weinbauer *et al.* 1993) to detect infected cells has the advantage that the whole cell is examined, while in thin sections (Proctor *et al.* 1993), infected cells may appear as noninfected just because the particular section examined happened not to cut through any viral particle. On the other hand, the whole cells sometimes appear opaque to the electrons and this may cause some infected cells to be scored as noninfected, just because the viral particles are masqued by the dark cell. The only direct comparison between the two methods is that carried out by Fuhrman & Noble (1995), who compared the two options in one of their two mesocosms. They found 3.3-4.6 % visibly infected cells in thin sections and 0.7-1.5 % in whole bacteria. Thus, the thin section method gave percentages which were between 3 and 5 times larger in these two cases. Therefore, as a maximum, we would have 0.3 % times 5 = 1.5 % infected cells. Proctor *et al.* (1993) provided a range of factors (7.4 to 14.3) to convert percent of infected cells to bacterial mortality. Using these factors with our infection values, viruses would be responsible for 11.1-21.5 % of the whole bacterial mortality.

We also tried to measure VLP decay rates in bottle incubations using cyanide as inhibitor of biological activity, according to Heldal & Bratbak (1991). This method was

used by Heldal & Bratbak (1991) in Raunefjorden and in Lake Kalandsvannet and by Mathias *et al.* (1995) in a backwater system of the Danube river. Heldal & Bratbak (1991) could not estimate the percentage of the total bacterial mortality caused by viral infection with the cyanide method because they did not measure bacterial heterotrophic production. The lowest decay rate measured by Mathias et al (1995) was 0.06 h^{-1}, implying that viruses could account for 15 % of total bacterial mortality. In our experiments no significant decreases could be detected in a total of 6 viral decay experiments performed in stations C, S and D (two in each one, Fig. 4.1), using the cyanide method. If the maximal possible impact of viruses estimated from the VIB method were true (21.5 %), viral decay rates would be, in all the samples, always lower than 0.02 h^{-1}. The fact that our estimated viral decay rates were not significantly different from 0 ($p > 0.05$), means that viral decay rates were still lower than these values. Thus, the impact of viruses should be certainly lower than 21.5 % of the total bacterial mortality in the Mediterranean Sea.

There is another way to estimate viral impact on the bacterial assemblage (Steward *et al.* 1992a, b). These authors measured viral production by incorporation of tritiated thymidine into viral DNA. With this method a viral production lower than 10^9 VLP L^{-1} d^{-1} would not be detected. In the offshore stations where they applied this method, only 2 out 8 samples showed detectable values of viral production. With the maximal impact of viral mortality over the bacterial population possible in the Mediterranean (21.5 %), viral production would be 8×10^7 VLP L^{-1} d^{-1} as a maximum. This value is not detectable by the method of Steward *et al.* (1992b).

In summary, bacterial mortality due to viral infection was found to be below the detection limits of current methods in the northwestern Mediterranean. Thus, viral impact in the Mediterranean has to be necessarily small, certainly much smaller than 20 % of the total bacterial mortality. This is probably the case in most open sea waters, although development of finer techniques is needed before this point can be definitively proven.

5. Viral distribution and activity in Antarctic waters

Introduction

Viruses have been shown to be present at high abundance, and to account for a significant percentage of the bacterial mortality, in different temperate marine habitats (e. g. Proctor & Fuhrman 1990, Steward *et al.* 1992b, Weinbauer & Peduzzi 1995a). However, little is know about viral distribution and activity in cold marine environments. Virus-like particles (VLP) have been counted in surface waters in Drake Passage (Smith *et al.* 1992) and in Arctic sea ice (Maranger *et al.* 1994). And more recently, Steward *et al.* (1996) have measured viral production in pelagic environments from the Arctic (Bering and Chukchi Seas). In this study, viruses were active members of the food web and accounted for an important percentage of bacterial mortality in the most productive waters of the investigated area (Steward *et al.* 1996).

The study of the Southern Ocean has a special interest because a markedly seasonal phytoplankton bloom sustains the entire Antarctic food chain. This is particularly true for coastal ecosystems, where a large variation of rates of primary production exists along the year (Karl 1993). The coastal shelf region comprised between the Bransfield Strait and the Bellinghausen Sea is though to be specially important because it supports an extensive spring bloom of phytoplankton and is a nursery area for Antarctic krill (Karl 1993).

The only interdisciplinary study that has investigated microbiological processes in this area was conducted by the RACER program from December 1986 to March 1987 and later in October and November 1989 (Karl 1993). During the first stage of the phytoplankton bloom, bacterial activity did not respond to the sudden increase in primary production (December). By January, the spring phytoplankton bloom had finished and bacteria showed the highest values of activity in the northern Gerlache Strait and the lowest values in Drake Passage. By February, only a station in Gerlache Strait maintained the higher levels of microheterotrophic activity. In March, microheterotrophic activity was low throughout the studied area. Although bacterivory and viral activity were not measured, an uncoupling between autotrophic and heterotrophic processes was observed (Bird & Karl 1991). This uncoupling seems to be a general characteristic of the high latitude oceans (Pomeroy & Deibel 1986, Karl 1993)

The mechanisms responsible for the apparent suppression of the microbial activity in the first stages of the phytoplankton bloom are not still well understood. It has been suggested that low temperature could have differential effects on the different components of the microbial plankton (Pomeroy & Deibel 1986). At the same time, other components

of the microbial loop that have been shown to control bacterial activity and abundance in more temperate latitudes are not well documented in Antarctic waters. Few data are available about protozoan grazing (Vaqué *et al.* in preparation) and, as pointed before, the role of viruses is practically unknown.

The goal of our work was to determine the variability in VLP abundance and dynamics in several representative habitats of this coastal region close to the Antarctic Peninsula (southern Drake Passage, western Bransfield Strait and Gerlache Strait) during the austral spring and summer. First, we described the spatial and temporal distribution of VLP abundance in the region. Second, we measured viral decay in some representative stations and used this parameter as an indicator of viral activity. We also investigated the influence of organic matter and temperature on viral decay rates and the abiotic factors responsible for viral decay. Finally, we calculated bacterial mortality due to viral infection and compared its importance to protozoan bacterivory.

Materials and Methods

Study area and sampling

Samples were collected during two cruises of the BIO Hespérides from December 1995 to January 1996 (FRUELA 95) and from January to February 1996 (FRUELA 96). This period of time corresponded to late spring and summer. The area studied and the sampling sites are presented in Fig. 5.1 and Table 5.1. This area includes several representative Antarctic waters: 1) two different deep-water zones (the western basin of the Bransfield Strait and the southern Drake Passage); 2) a frontal zone between the Bellinghausen Sea and the Bransfield Strait water masses and 3) the eutrophic and mesotrophic coastal region of the Gerlache Strait. Seawater samples for depth profiles and diel cycles were collected with 10 L Niskin bottles mounted on a rosette with a mark II CTD.

Abundance of microorganisms

Chlorophyll a was measured by fluorometry of acetone extracts (Yentsch & Menzel 1963). Between 25 and 200 cm^3 of seawater were filtered through GF/F filters. The filters were subsequently stored for 24 hours in 90% acetone. The fluorescence of the chlorophyll extracts was determined by means of a Turner Designs fluorometer.

Samples for bacterial counting were preserved in 2 % gluteraldehyde and stored in polypropylene bottles until they could be processed. Bacteria were stained with DAPI (1μg mL^{-1} final concentration) and filtered onto black 0.2 μm pore size polycarbonate filters (Porter & Feig 1980) on board, mounted on microscopy slides and frozen. Bacterial abundance was determined with an epifluorescence microscopy at a magnification of 1250x.

Samples for VLP counting were immediately filtered and stained with YO-PRO 1 (Hennes & Suttle 1995). 100 μL of unfixed sample were diluted with 700 μl of mili Q water filtered through a 0.02 μm pore size filter (Anodisc). Each diluted sample was gently filtered through a 0.02 μm pore size Anodisc 25 filter. The Anodisc filter with the filtered sample was laid on 80 μL of the staining solution in a Petri dish and incubated in the dark for two days at room temperature. The filters were then washed twice by filtering 800 μL of mili Q water through the membrane. Filters were transferred to glass slides, immediately covered with a drop of spectrophotometric-grade glycerol and a cover slip. Filters were stored at -20° C until counted. VLP abundance was determined with an epifluorescence microscopy at a magnification of 1250x.

Bacterial heterotrophic production

Bacterial heterotrophic production was determined by ^{3}H-leucine incorporation (Kirchman *et al.* 1985) as modified for micro-centrifugation by Smith & Azam (1992). Four replicates and two killed controls with trichloroacetic acid (TCA, 5 % final concentration) for each sample were incubated in the dark at *in situ* temperature with 20 nM Leu in 2 mL capacity microcentrifuge tubes. Incubations were terminated by addition of TCA (5 % final concentration) after four hours. The tubes were centrifuged for ten minutes at 16000 x g and the supernatant aspirated. The samples were washed by the addition of 1.5 mL of 5 % TCA and vortex mixing. Samples were centrifuged again (10 min, 16000 x g) and aspirated. ^{3}H-leucine incorporated was converted to carbon produced using an empirically determined conversion factor estimated for each different area (Pedrós-Alió *et al.* in preparation). In order to calculate the viral impact on the bacterial heterotrophic production, carbon produced was converted to cells produced. For this purpose carbon content per cell was estimated from the average bacterial volume of 0.07 μm^3 was used (Calderón-Paz 1997), in the equation: pg C = 0.09 * $(\mu m^3)^{0.9}$ (Norland *et al.* 1993).

Table 5.1. A- Date and location of the stations sampled in FRUELA 95 with integrated values (from surface to 80-100 m) of bacterial (BN) and VLP abundance, VLP/BN ratio and chlorophyll a.

Station	Date	Lat	Long	BN/L	VLP/L	VLP/BN	Chla (µg/L)
5	12/4/95	63° 51' 06''	60° 10' 37''	3.44×10^{11}	1.14×10^{13}	33.21	0.74
8	12/4/95	63° 02' 32''	61° 30' 55''	4.85×10^{11}	9.17×10^{12}	18.88	0.73
12	12/5/95	61° 57' 51''	63° 08' 29''	2.33×10^{11}	1.05×10^{13}	45.23	2.09
15	12/6/95	62° 49' 30''	63° 32' 13''	2.87×10^{11}	2.33×10^{13}	81.42	3.57
17	12/6/95	63° 19' 29''	62° 41' 00''	3.94×10^{11}	1.83×10^{13}	46.46	1.08
29	12/8/95	63° 33' 03''	65° 47' 26''	2.32×10^{11}	8.20×10^{12}	35.32	2.83
34	12/9/95	64° 54' 06''	64° 29' 20''	1.82×10^{11}	1.23×10^{13}	67.61	1.60
39	12/10/95	64° 52' 01''	64° 29' 20''	2.55×10^{11}	1.41×10^{13}	55.29	1.94
40	12/10/95	64° 37' 50''	62° 52' 54''	3.06×10^{11}	1.75×10^{13}	57.23	3.43
47	12/11/95	64° 03' 09''	61° 46' 03''	4.64×10^{11}	1.41×10^{13}	30.40	2.28
72	12/13/95	63° 58' 31''	61° 01' 39''	4.58×10^{11}	1.10×10^{13}	24.12	1.12
79	12/14/95	63° 19' 54''	61° 40' 23''	3.40×10^{11}	1.29×10^{13}	38.00	0.63
81	12/14/95	63° 07' 17''	61° 52' 59''	6.03×10^{11}	2.01×10^{13}	33.35	0.85
94	12/15/95	63° 52' 34''	60° 16' 33''	3.97×10^{11}	1.47×10^{13}	36.93	
97	12/15/95	63° 31' 21''	60° 30' 00''	3.67×10^{11}	1.00×10^{13}	27.29	0.89
123	12/17/95	62° 50' 22''	60° 26' 57''	4.90×10^{11}	9.92×10^{12}	20.24	0.53
140	12/18/95	63° 07' 49''	59° 25' 31''	3.90×10^{11}	1.42×10^{13}	36.42	1.78
142	12/18/95	62° 55' 03''	59° 37' 14''	4.95×10^{11}	1.30×10^{13}	26.15	0.91
37	12/20/95	64° 51' 27''	63° 54' 59''	3.86×10^{11}	1.21×10^{13}	31.35	1.66
36	12/20/95	64° 54' 20''	64° 15' 56''	3.10×10^{11}	1.22×10^{13}	39.29	
156	12/21/95	64° 57' 28''	63° 31' 42''	1.76×10^{11}	1.02×10^{13}	58.08	1.93
168	12/26/95	63° 24' 28''	60° 37' 16''	3.36×10^{11}	1.56×10^{13}	30.55	1.63
169	12/28/95	64° 48' 54''	63° 13' 50''	5.18×10^{11}	2.20×10^{13}	42.63	2.82

Table 5.1. B- Date and location of the stations sampled in FRUELA 96 with the integrated values (from surface to 80-100 m) of bacterial (BN) and VLP abundance, VLP/BN ratio and chlorophyll a .

Station (µg/L)	Date	Lat	Long	BN/L	VLP/L	VLP/BN	Chla
186	1/19/96	64° 03' 11''	61° 45' 46''	5.65×10^{11}	1.01×10^{13}	17.88	
187	1/19/96	64° 53' 47''	64° 30' 11''	5.88×10^{11}	1.04×10^{12}	17.69	1.49
189	1/20/96	64° 51' 20''	63° 54' 42''	7.76×10^{11}	1.08×10^{13}	13.99	2.28
191	1/20/96	64° 52' 08''	63° 14' 39''	5.90×10^{11}	1.09×10^{13}	18.56	4.22
193	1/20/96	64° 34' 01''	62° 35' 27''	5.72×10^{11}	1.12×10^{13}	19.64	2.24
195	1/21/96	64° 22' 46''	61° 54' 05''	4.65×10^{11}	1.08×10^{12}	23.27	1.07
197	1/21/96	62° 43' 12''	65° 27' 13''	4.25×10^{11}	1.01×10^{13}	23.78	0.07
198	1/22/96	62° 57' 48''	65° 02' 41''	3.22×10^{11}	1.39×10^{13}	26.58	0.23
200	1/24/96	63° 41' 13''	63° 51' 02''	2.78×10^{11}	7.64×10^{12}	27.53	0.38
203	1/23/96	64° 08' 47''	63° 05' 33''	3.22×10^{11}	1.39×10^{13}	43.03	0.39
204	1/23/96	63° 48' 28''	61° 53' 09''	3.60×10^{11}	1.40×10^{13}	38.33	0.46
206	1/23/96	63° 19' 43''	62° 40' 57''	2.69×10^{11}	1.33×10^{13}	49.57	0.32
208	1/24/96	62° 49' 42''	63° 32' 04''	2.23×10^{11}	7.12×10^{12}	31.96	0.11
210	1/24/96	32° 21' 39''	32° 23' 07''	3.19×10^{11}	1.03×10^{12}	32.26	
213	1/25/96	62° 30' 20''	62° 23' 07''	2.82×10^{11}	7.44×10^{13}	26.36	0.34
215	1/26/96	63° 02' 43''	61° 29' 36''	2.98×10^{11}	1.02×10^{12}	34.13	0.37
217	1/26/96	63° 34' 37''	60° 39' 25''	3.12×10^{11}	1.01×10^{13}	32.35	0.65
219	1/26/96	63° 39' 26''	59° 32' 50''	3.37×10^{11}	1.44×10^{13}	42.70	0.72
220	1/27/96	63° 20' 12''	59° 56' 22''	3.44×10^{11}	1.02×10^{13}	29.60	0.45
221	1/27/96	63° 01' 01''	60° 16' 40''	2.77×10^{11}	1.28×10^{13}	46.16	0.88
222	1/27/96	62° 41' 49''	60° 36' 27''	4.02×10^{11}	1.22×10^{13}	30.26	0.29
225	2/1/96	64° 03' 39''	61° 17' 01''	6.69×10^{11}	1.55×10^{13}	23.15	3.49
226	2/3/96	64° 51' 30''	63° 59' 17''	5.91×10^{11}	1.06×10^{13}	17.90	5.29

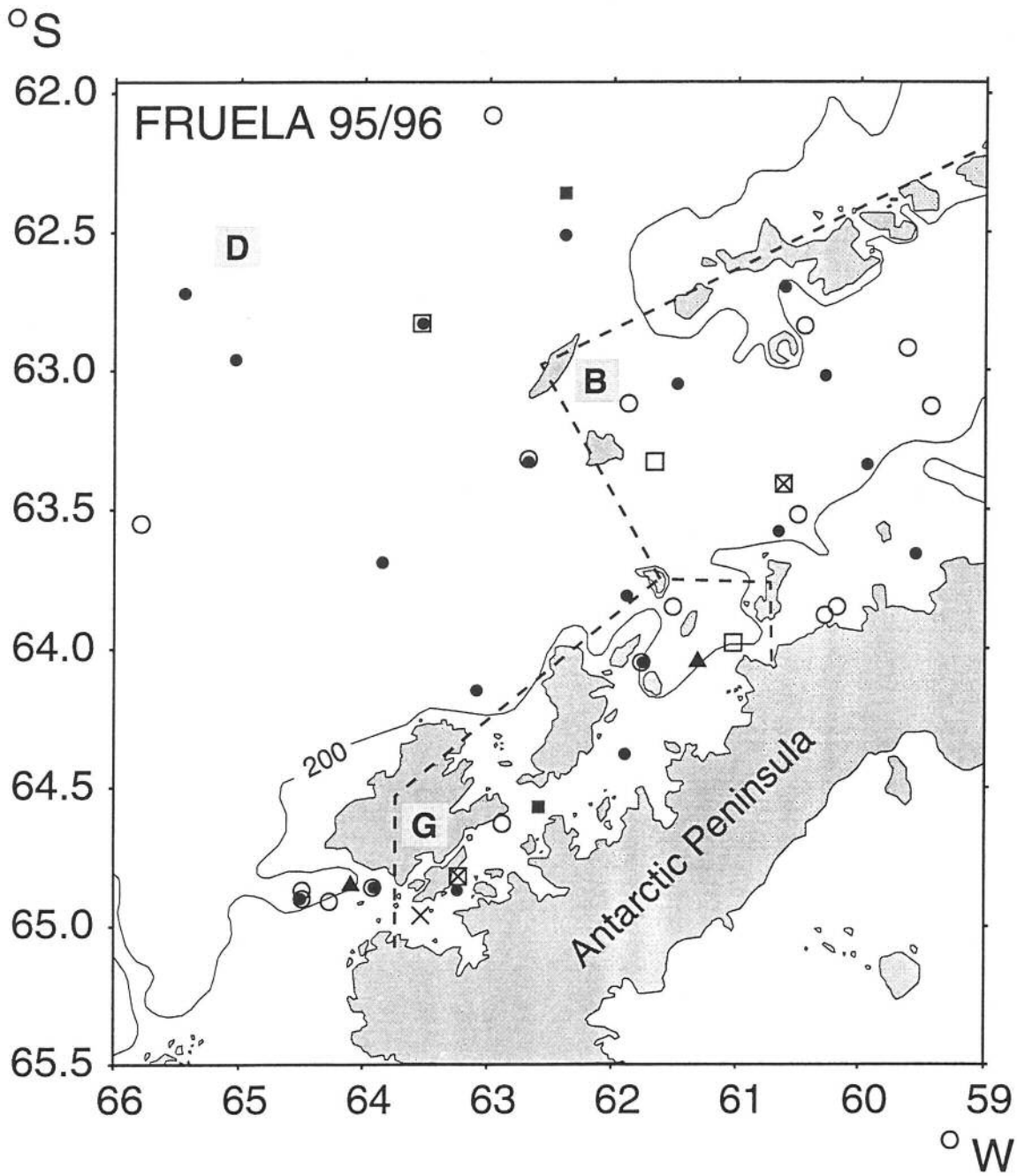


Fig. 5.1. Study area in the southern Drake Passage (D), Bransfield (B) and Gerlache (G) Straits with location of the stations used for VLP counts: shallow vertical profiles (circles), deep vertical profiles (triangles), diel cycles (crosses) and decay rate experiments (squares). Empty symbols indicate stations sampled during FRUELA 95 and full symbols during FRUELA 96.

Viral decay rates

Water samples for these determinations were collected from representative stations in the three areas sampled: Gerlache and Bransfield Straits and Drake Passage (Fig. 5.1 and Table 5.1). All incubations were made in polyethilene bottles in the dark. Different volumes (from 100 mL to 1 L) were used in different experiments. Previously, we had checked that the different volumes used did not influence the rate of viral decay obtained. Viral decay rate was recorded after inhibiting production of new viruses by adding KCN to a final concentration of 2 nM (Heldal & Bratbak 1991). Samples for ^{3}H-leucine incorporation were taken at the beginning and at the end of each experiment in order to make sure that the bacterial activity had been inhibited by the KCN. The viral decay rate (VDR) was calculated from the log-linear part of the decay curves using linear regression. Two types of experiments were carried out:

Type I experiments were designed to investigate viral decay attributed to adsorption to particles. These experiments were done in three stations from Gerlache Strait (72, 169 and 193), two stations from Bransfield Strait (168 and 79) and two stations from Drake Passage (15 and 210, Fig. 5.1). Stations 15, 72, 79, 168 and 169 were occupied during FRUELA 95 and stations 193 and 210 during FRUELA 96 (Fig. 5.1, Table 5.1). In each experiment, a 2 L sample was taken from 5 m deep water (20 m in station 15) and divided into two parts: 1 L was filtered through 0.8 μm polycarbonate filters and the other liter through 50 μm net filter. Decay rate experiments were carried out with the filtered samples as described before. The filtered water was incubated at *in situ* temperature after addition of cyanide (inactive cultures). Samples for VLP counts were retrieved frequently (2-3 hours) during the first 12 hours and occasionally afterwards up to 48 hours. Cyanide was added again after 24 hours to insure an inhibiting concentration of KCN during FRUELA 95, but not in the two FRUELA 96 experiments. In some of these experiments (stations 72, 168 and 15) we had parallel incubations of water without cyanide (active cultures).

Type II experiments were carried out to examine the influence of temperature and organic matter concentration on viral decay. 8 L of 5 m deep water from a station in Gerlache Strait (72, Fig. 5.1, Table 5.1A) and a station in Bransfield Strait (168, Fig. 5.1, Table 5.1A) were filtered through 0.8 μm polycarbonate filters to remove bacterivores. Filtered water was dispensed into 1 L, acid washed, autoclaved, Pyrex bottles. The resulting eight batch cultures were divided into two groups: one group received a glucose addition (10 μM final concentration) and the other was used as control. Each pair of batch cultures was incubated at different temperatures: -1°, 0°, 4°, 5° C (station 72) and

-1°, 0°, 4°, 8° C (station 168). Thus, for each temperature, we had two cultures: control and glucose addition. Samples for bacterial and VLP abundance and ^{3}H-leucine incorporation were taken every day during 5-6 days. On the third sampling day, viral decay rate experiments were performed with 100 mL aliquots from each batch culture as described before. The incubations for determining viral decay rates were done at the same temperature as the original batch cultures. In station 168 viral decay rate determinations were carried out in the batch cultures incubated at -1° and 8° C.

Results

Viral abundance and distribution

Vertical distribution of VLP and bacterial abundance were investigated from the surface to the bottom in stations 225 and 226 (Table 5.1B, Figs. 5.1 and 5.2). The maximal abundance of VLP was found at the surface in both stations and was coincident with the maxima of bacterial abundance and chlorophyll a. Minimal abundances of both parameters were found below 300 m. Both VLP and bacterial abundance decreased with depth. The correlation between VLP and depth (z) with the data from both stations was:

$$\ln VLP = 26.23 - 1.39 \ln z \quad r^2 = 0.84 \quad p < 0.0001$$

And the correlation between bacterial abundance (BN) and depth (z) was:

$$\ln BN = 14.43 - 0.41 \ln z \quad r^2 = 0.92 \quad p < 0.0001$$

The VLP/BN ratio was minimal (10/1) at the deepest points in both stations and maximal at the surface in station 225 (50/1) and at 200 m in station 226 (32.5/1).

Correlations between VLP and bacterial abundance and between VLP abundance and chlorophyll a were investigated with the data from all the stations and depths shown in Fig. 5.1 and in Table 5.1. VLP abundance was significantly correlated with both parameters (Table 5.2). These correlations were also calculated for each cruise separately (Table 5.2). In FRUELA 95, VLP and bacterial abundance were not significantly correlated. VLP abundance, however, was correlated with chlorophyll a. In FRUELA 96 both parameters were significantly correlated with VLP abundance. We also calculated correlations for each area (Drake, Bransfield and Gerlache) separately (Table 5.2). VLP were significantly correlated with bacterial abundance and chlorophyll a in Drake Passage. In Bransfield Strait no significant correlations were found for these parameters.

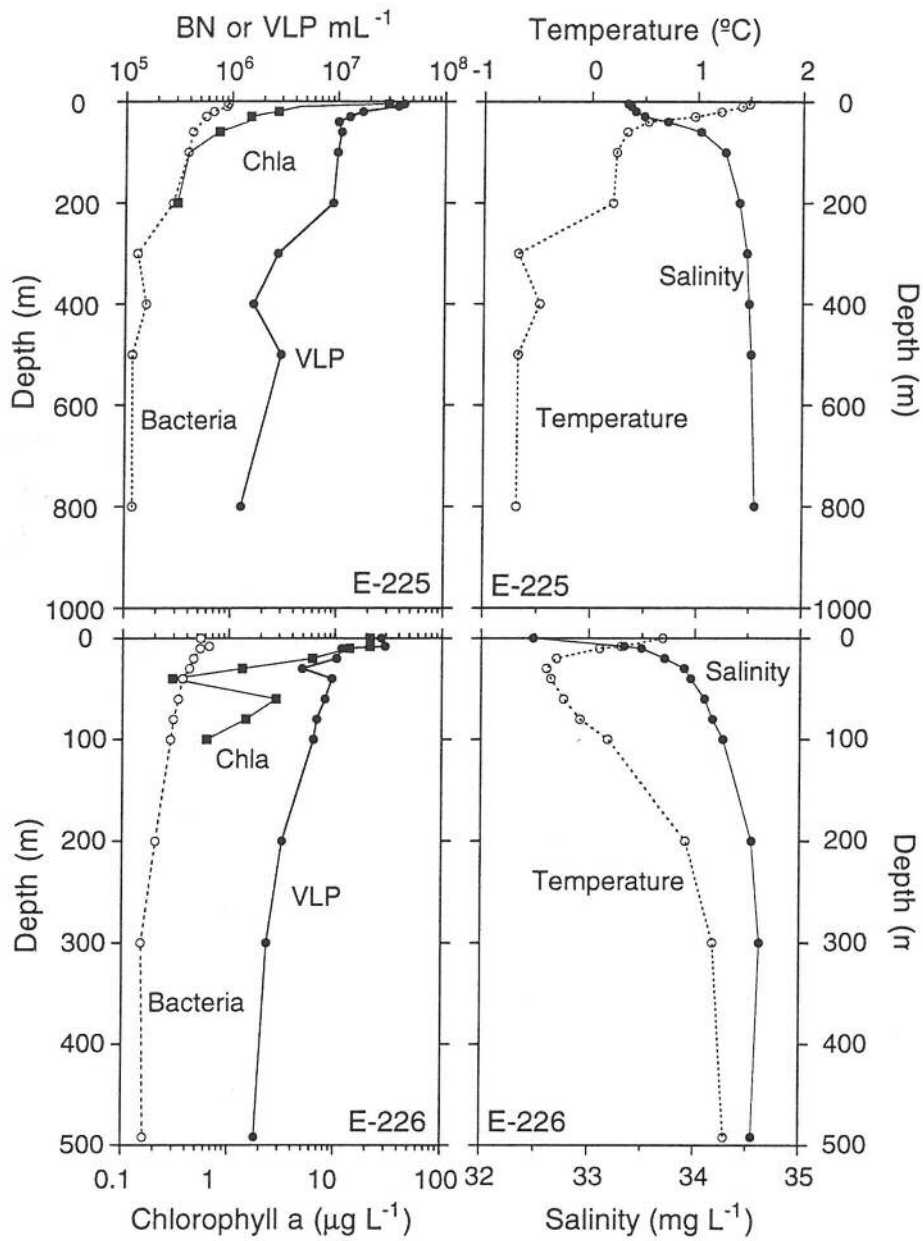


Fig. 5.2. Deep vertical profiles from stations 225 (upper panels) and 226 (lower panels) of temperature, salinity, chlorophyll a concentration, bacterial and VLP abundance.

Table 5.2. Correlations between VLP abundance and bacterial abundance (BN) or chlorophyll a concentration (Chla).

	BN		Chla	
	r^2 (n)	p	r^2 (n)	p
Total	0.17 (254)	<0.001	0.21 (208)	<0.001
FRUELA 95	- (115)	0.06	0.13 (86)	0.001
FRUELA 96	0.31 (139)	<0.001	0.22 (122)	<0.001
Drake	0.17 (74)	<0.001	0.16 (61)	<0.001
Bransfield	- (109)	0.057	- (82)	0.124
Gerlache				
Total	0.30 (155)	<0.001	0.18 (113)	<0.001
FRUELA 95	- (69)	0.09	0.22 (38)	<0.001
FRUELA 96	0.44 (84)	<0.001	0.23 (53)	<0.001

In Gerlache Strait bacterial abundance and chlorophyll a were significantly correlated with VLP abundance. When these correlations were calculated for each area in each cruise separately, in Bransfield Strait and in Drake Passage correlations were not different from those presented with the data of both cruises together. In FRUELA 96, VLP abundance in Gerlache Strait was significantly correlated with bacterial abundance and with chlorophyll a. In FRUELA 95, VLP abundance in Gerlache Stratit was not significantly correlated with bacterial abundance but it was significantly correlated with chlorophyll a (Table 5.2).

Distribution of VLP abundance was compared among the three areas using the integrated values from surface to 80 or 100 m (Table 5.1). VLP abundance was not significantly different among the three areas studied for any of the cruises (ANOVA, FRUELA 95, $p = 0.817$, $n = 20$, FRUELA 96, $p = 0.317$, $n = 23$, Fig. 5.3). For the VLP/BN ratio however, ANOVA revealed significant differences between the three areas investigated in FRUELA 96 ($p < 0.001$, $n = 22$). A post hoc Tukey test indicated that the significant differences were those between Gerlache Strait and the other areas ($p < 0.001$, Fig. 5.3). For the cruise FRUELA 95 we also found significant differences in the VLP/BN ratio ($p = 0.037$, $n = 20$). A post hoc Tukey test revealed that the significant differences were those between Drake Passage and Bransfield Strait ($p = 0.037$, Fig. 5.3).

Seasonal distributions of VLP abundance and the ratio VLP/BN for each area were also checked. Significant differences in the integrated VLP abundance were found only in Gerlache Strait when comparing both cruises ($p < 0.001$). Differences in the VLP/BN ratio were found between FRUELA 95 and 96 in Gerlache Strait ($p < 0.001$, $n = 15$) and in Drake Passage ($p = 0.04$, $n = 12$). Values were higher in FRUELA 95 than in FRUELA 96 (Fig. 5.3).

Diel cycles of VLP abundance were followed in two stations from Gerlache Strait (Fig. 5.1, 156 and 169) and one station from Bransfield Strait (168). In all the stations, very slight variations in VLP abundance were found along the day (Fig. 5.4). In stations 168 and 169 there was a similar pattern, with a maximum of VLP abundance at the beginning and at the end of the cycle, at 10 m depth in station 168 and at 20 m in station 169 and a minimum at 2 hours at the same depths (Fig. 5.4B, C). The maximal changes in VLP abundance took place in the photic layer. In station 156, the depth distribution of VLP abundance was similar but the maximum occurred at 6:00 hours and the minimum at 18:00 (Fig. 5.4A). In the rest of the depths VLP abundance changed very little during the day.

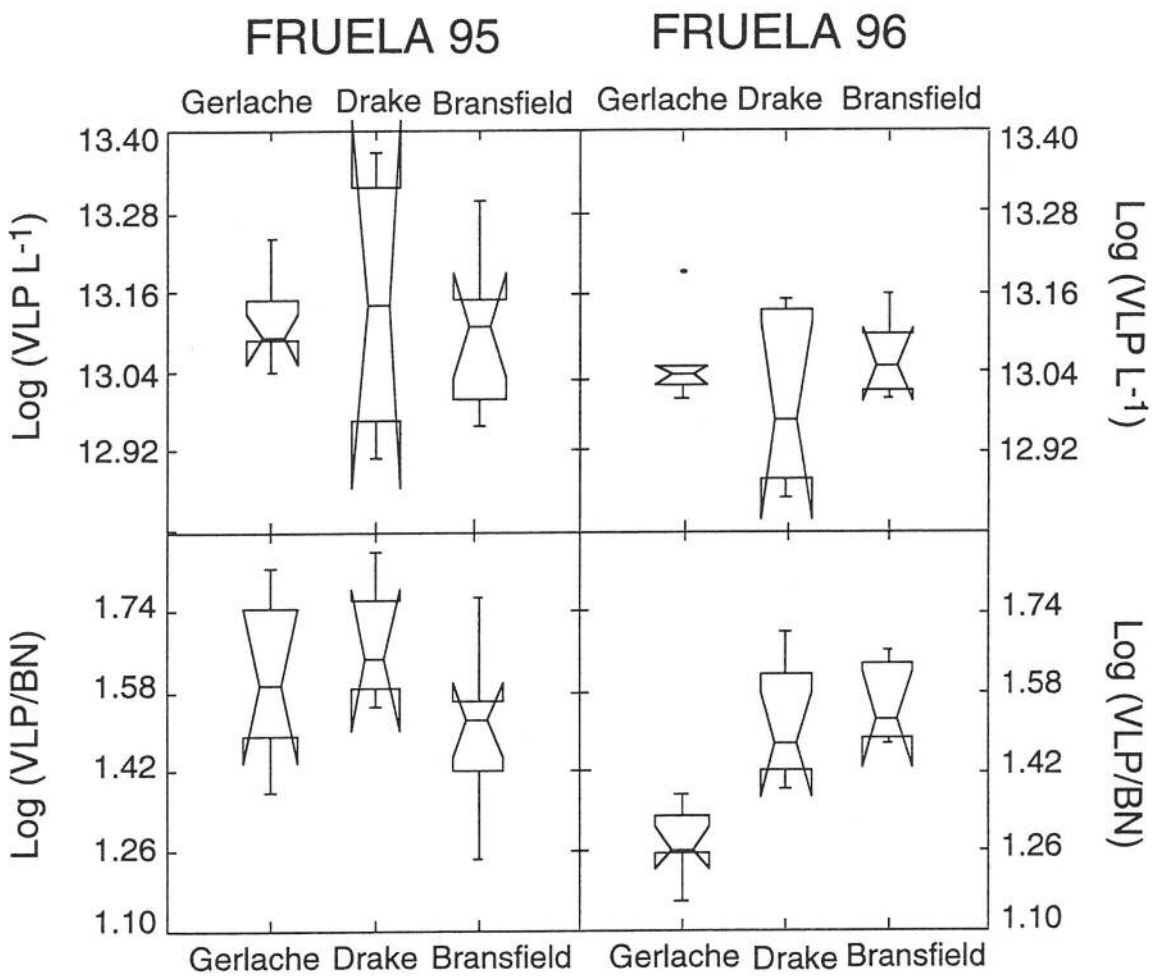


Fig. 5.3. Box-and-whiskers plots of integrated values (from the surface to 80-100 m) of log transformed VLP abundance (Log VLP L^{-1}) and log transformed VLP/BN ratio (Log VLP/BN) for the three areas studied in each cruise (FRUELA 95 and FRUELA 96).

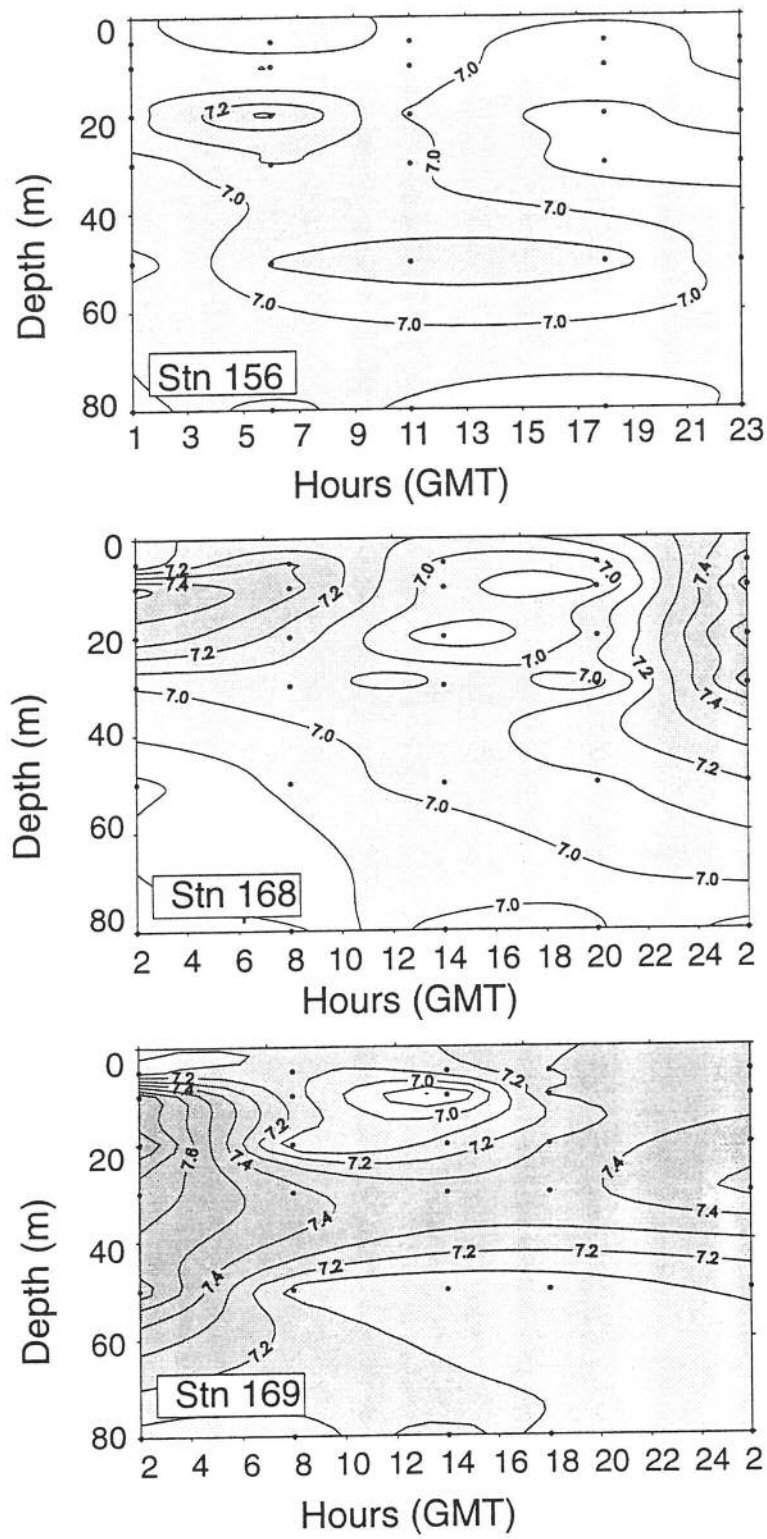


Fig. 5.4. Diel changes in VLP abundance (Log transformed data) at stations: 156 (upper panel), 168 (middle panel), and 169 (lower panel). Filled circles indicate the sampling depths.

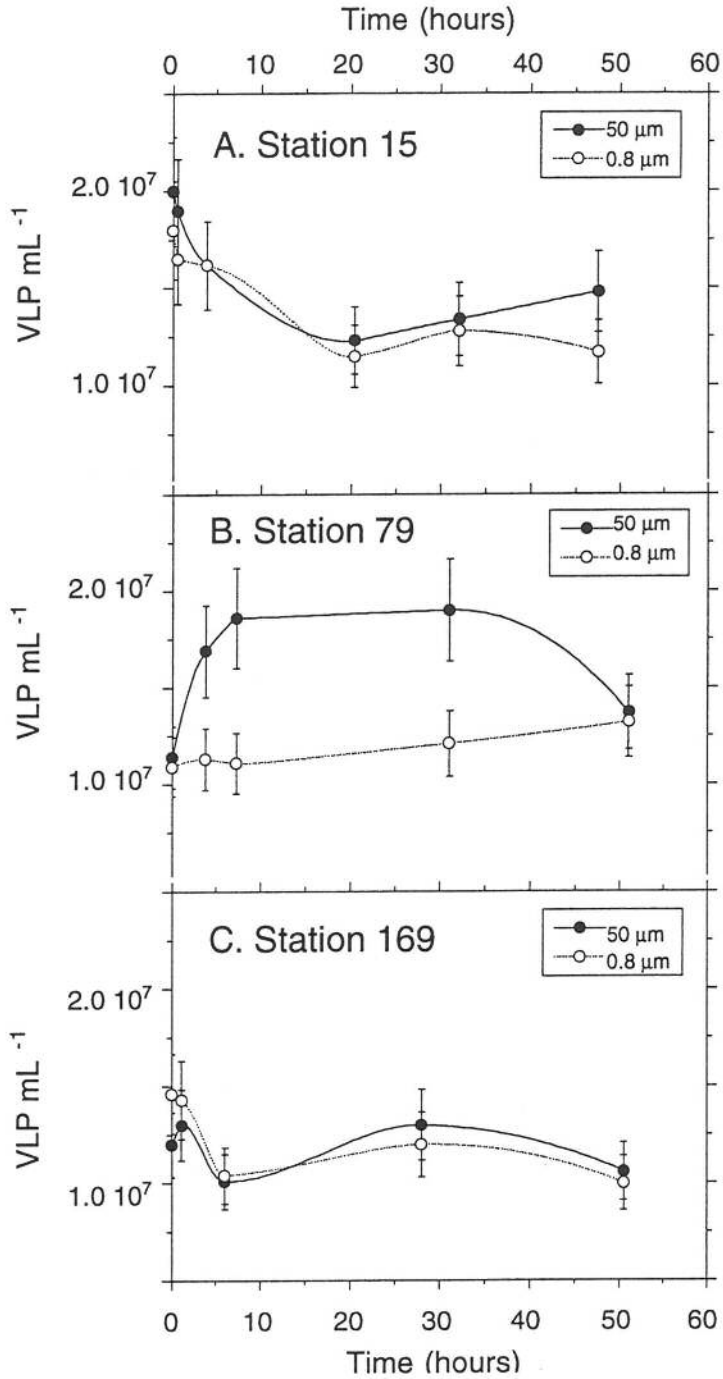


Fig. 5.5. Changes in VLP abundance (VLP mL^{-1}) in the active cultures from type I decay rate experiments performed at stations 15 (**A**), 79 (**B**) and 169 (**C**). Error bars indicate the standard error which was calculated as the percentage of the mean in triplicate samples at time = 0, for each experiment, and later extrapolated to all the samples.

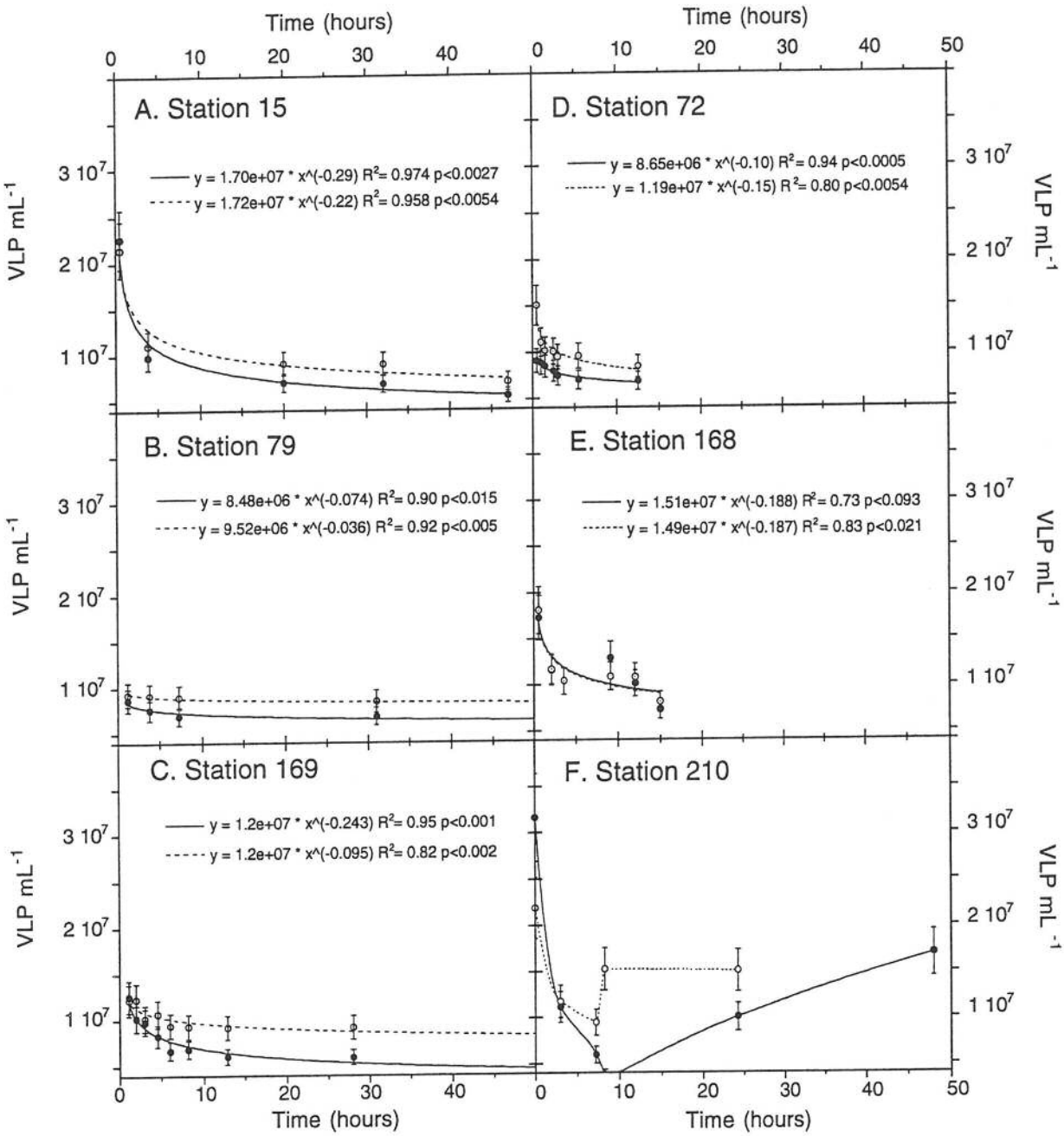


Fig. 5.6. Changes in VLP abundance in the inactive cultures from type I experiments fitted by a power function (except for station 210) for both size fractions: <50 μm (filled symbol) and <0.8 μm (empty symbols) for stations 15 (**A**), 79 (**B**), 169 (**C**), 72 (**D**), 168 (**E**) and 210 (**F**). Error bars indicate the standard error which was calculated as the percentage of the mean in triplicate samples at time = 0, for each experiment, and later extrapolated to all the samples.

Table 5.3. Viral decay rates (VDR) calculated for the first and second parts of the decay curves, shown in Fig. 5.6. VDR that are not significantly different from zero are shown in italics.

	Station	Fraction[a]	VDR(h^{-1})	StErr[b]	p	r^2	n[c]	hours[d]
A- VDR 1								
	15	50	*0.208*	0.054	0.162	0.94	3	<4
	15	0.8	*0.152*	0.062	0.247	0.86	3	<4
	210	50	0.235	0.032	0.017	0.96	4	<8
	210	0.8	*0.111*	0.043	0.230	0.87	3	<8
	72	50	0.074	0.007	0.002	0.97	5	<3
	72	0.8	0.114	0.026	0.021	0.87	6	<3
	169	50	0.122	0.048	0.001	0.95	6	<6
	169	0.8	0.066	0.014	0.001	0.85	6	<6
	193	50	0.260				2	<4
	193	0.8	0.207				2	<4
	79	50	0.036	0.001	0.022	0.96	3	<8
	79	0.8	*0.006*	0.001	0.055	0.99	3	<8
	168	50	*0.308*	0.034	0.071	0.99	3	<4
	168	0.8	0.173	0.035	0.039	0.92	4	<4
B- VDR 2								
	15	50	0.0126	0.0022	0.029	0.94	4	4-48
	15	0.8	0.0099	0.0018	0.030	0.94	4	4-48
	72	50	*0.0080*	0.0052	0.367	0.70	3	3-15
	72	0.8	*0.0139*	0.0047	0.207	0.89	3	3-15
	169	50	0.0070	0.0018	0.031	0.83	5	6-48
	169	0.8	0.0021	0.0004	0.027	0.94	4	6-48
	79	50	*0.0020*	0.0010	0.300	0.80	3	8-48
	79	0.8	0.0023	0.0001	0.042	0.99	3	8-48
	168	50	0.0950	0.0087	0.056	0.99	3	8-15
	168	0.8	*0.0500*	0.0270	0.315	0.75	3	8-15

[a] Cultures filtered through 50 μm mesh or 0.8 μm polycarbonate filters
[b] Standard error of the slope of the regression
[c] Number of points used in the regression
[d] Periods used in the regression

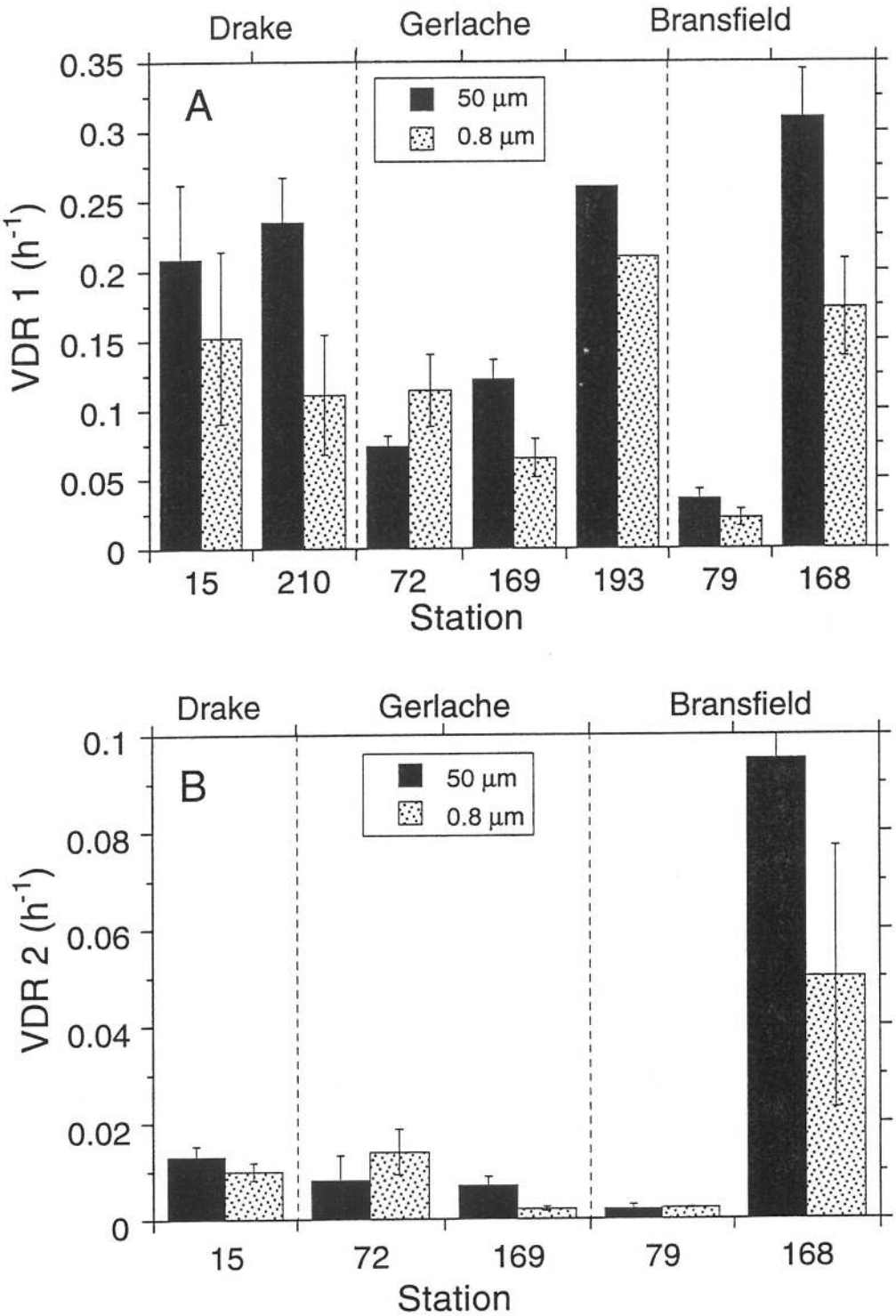


Fig. 5.7. **A**- VDR1 (Viral decay rate from the first hours of type I experiments for the different stations and for both size fractions (<50 µm and <0.8 µm). Error bars indicate the standard error of the slope (VDR) of the linear regression. When no bars are present, VDR has been estimated from two points of the regression line only. **B**- VDR 2 (Viral decay rate from the last time points of type I experiments) for the different stations and both size fractions (<50 µm and <0.8 µm). Error bars indicate the standard error of the slope (VDR) of the linear regression.

Type I viral decay rate experiments

Changes in VLP abundance with time for the active cultures are shown in Fig. 5.5. In the experiments carried out at stations 15 and 169, VLP abundance in the active cultures showed slight changes over the 48 hours the experiments lasted (Fig. 5.5A, C). In the experiment at station 79, viral abundance at the <50 μm size fraction, increased slightly (less than twice) during the first 10 hours of the experiment.

The VLP changes with time for the inactive cultures appear in Fig. 5.6. In general, the same decay pattern was observed in all the experiments, with a fast decrease in VLP numbers during the first 3-8 hours (Fig. 5.6A-F). After 3-8 hours this decrease slowed down, leading to a second rate of decay (Fig. 5.6A-E). Thus, two different viral decay rates (VDR 1 and VDR 2) were calculated for each experimental bottle (Table 5.3). VDR 1 corresponded to the first 3-8 hours of the experiment. VDR 2 was calculated from 3-8 hours to the end of the experiment. In some cases, rates were not significantly different from 0 ($p > 0.05$, Table 5.3). In the experiments performed in FRUELA 96 (stations 193 and 210), only VDR 1 could be calculated from the first 3-6 hours of the experiment (e. g. Fig. 5.6F, Table 5.3). The VLP followed an erratic course afterwards apparently caused by the loss of effectiveness of KCN after 24 hours. An ANCOVA was used to compare VDR (1 and 2) for both size fractions in each experiment. Although the rates were slightly higher in the <50 μm size fraction in all the stations except for station 72 (Fig. 5.7A, B), these differences were significant only for station 169 ($p = 0.021$, $n = 12$ for VDR 1 and $p = 0.062$, $n = 9$, for VDR 2).

When the power functions for both size fractions were compared, the ANCOVA revealed significant differences for the experiments from station 169 ($p < 0.001$, $n = 17$) and marginally significant differences for station 79 ($p = 0.057$, $n = 10$). These power functions will be used in the last section. The overall viral decay rate for each experiment could be fitted by a power function (Fig. 5.6A-E).

Influence of temperature and glucose addition on viral activity. Type II experiments.

Decay patterns for all the experiments incubated at different temperatures were similar (two examples are shown in Fig. 5.8A, B). VLP abundance decreased at a constant rate from the beginning to 10-25 hours, depending of the experiment. Thus, in these experiments only one VDR was calculated, using linear regression. The resulting VDR are shown in Fig. 5.8C, grouped by incubation, temperature and station.

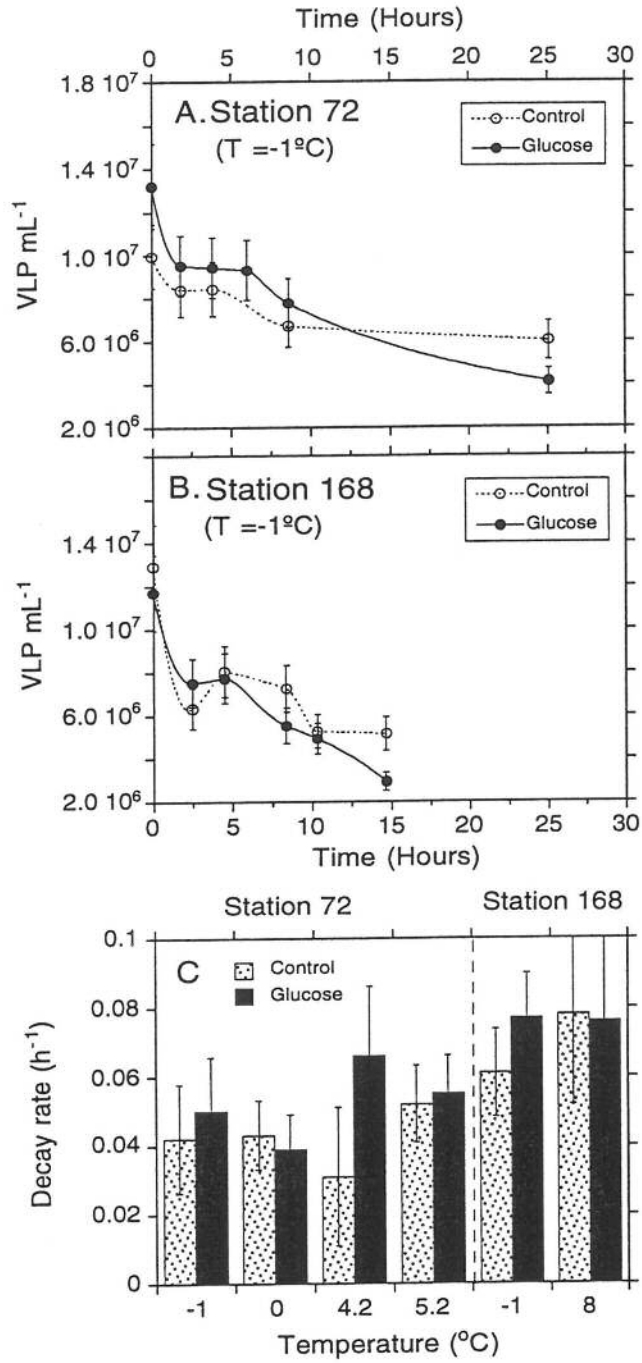


Fig. 5.8. **A**- Decrease in VLP abundance with time in the type II decay experiment performed at station 72 at -1° C for both treatments (glucose addition and control). Error bars indicate the standard error which was calculated as the percentage of the mean in triplicate samples at time = 0 and later extrapolated to all samples. **B**- Decrease in VLP abundance with time in the type II experiment performed at station 168 at -1° C for both treatments (glucose addition and control). Error bars indicate the standard error which was calculated as the percentage of the mean in triplicate samples at time = 0 and later extrapolated to all samples. **C**- VDR estimated in type II experiments performed in stations 72 and 168 at different temperatures and for both treatments (glucose addition and control). Error bars indicate the standard error of the slope (VDR) of the linear regression.

Table 5.4. Effects of temperature and glucose addition on viral decay rates. Results of the ANCOVA.

	Station	Treatment	ANCOVA	
			n	p
A- Factor: Temperature				
	72	Control	20	0.462
	72	Glucose	23	0.078
	168	Control	12	0.983
	168	Glucose	11	0.780
B- Factor: Glucose adittion				
	72	-1	10	0.989
	72	0	10	0.680
	72	4.2	11	0.068
	72	5.2	9	0.780
	168	-1	11	0.326
	168	8	11	0.635

In most cases no significant effects of either temperature or glucose addition could be detected (Table 5.4). The only two exceptions where the effect of temperature on the glucose amended cultures at station 72 and the effect of glucose amendment on the cultures incubated at 4.2° C also in station 72, where the significance was marginal.

Bacterial mortality due to viral infection

In the stations where we performed viral decay experiments with the natural sample filtered through 50 μm, we could calculate the impact of viral infection on bacterial abundance and production. We assumed that the rate of viral decay measured was equivalent to the rate of viral production (Heldal & Bratbak 1991). To convert viral production to percentage of bacterial mortality due to viral infection we assumed a range of burst sizes between 50 and 100 (Bratbak *et al.* 1990). We calculated the percentage of bacterial abundance and production that could be removed by viral lysis in three different ways (Fig. 5.9).

1. Using VDR 1 (Table 5.3A) we found that bacterial mortality due to viral infection exceeded 100 % of the bacterial production in all stations (Fig. 5.9 A). The percentage of bacterial abundance lysed per hour with this rate of viral production ranged between 1.5 % in station 79 to 37 % in station 168 (Fig.5.9B).

2. Using VDR 2 (Table 5.3B), viral lysis accounted for more than 100 % of bacterial heterotrophic production only in station 168. In the remaining stations this percentage ranged between 3-50 % (Fig. 5.9C). The rate of bacteria lysed per hour was lower than 0.9 %, except for station 168 where it was about 15% (Fig. 5.9D).

3. A third approach was used to calculate viral impact on the bacterial assemblage (except at stations 193 and 210), assuming again that the pattern of viral decay would be similar to the pattern of viral production. We calculated the total number of VLP after 25 hours using the power function found for each experiment (considering the exponent of the independent variable as positive) and we subtracted from this the number of VLP that were present at the first hour of the experiment, calculated with the same function (Fig. 5.9E, F). The resultant VLP concentration would be the number of VLP produced during a period of 24 hours. According to this approach, viral infection would account for less than 100 % of the bacterial production in three of the stations (72, 79 and 168) and for more than 100 % in the other two. Around 50-60 % of the bacterial abundance would be lysed by viruses daily at stations 72, 168 and 169. At station 79 this percentage was lower than 10 % and in station 15 it was close to 100 %.

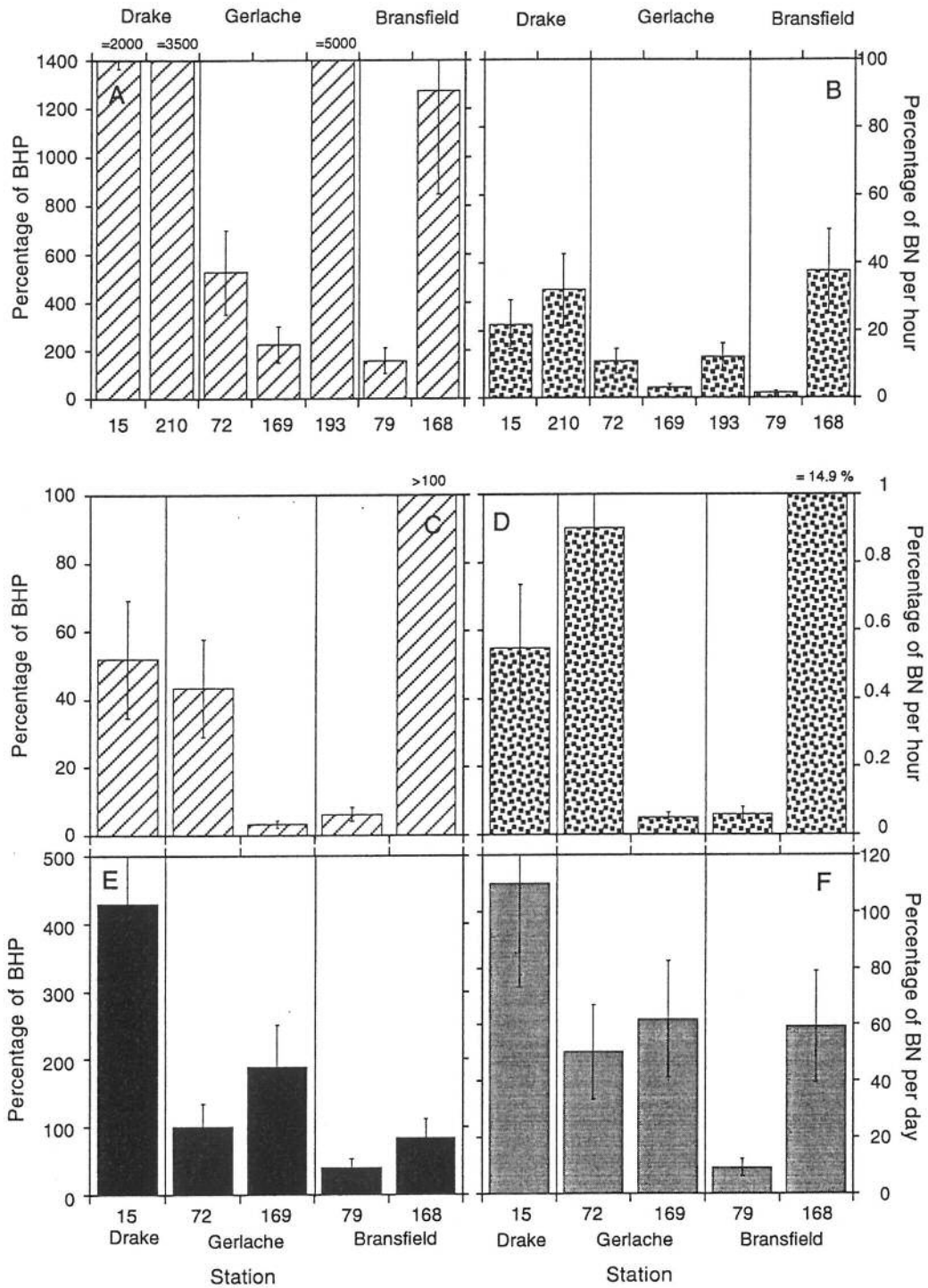


Fig. 5.9. Percentage of bacterial heterotrophic production (BHP) and abundance (BN) lysed by viruses per hour (or day) in different stations according to VDR 1 (**A-B**), to VDR 2 (**C-D**) and to the power function (**E-F**). Error bars indicate the range of values calculated using a range of burst sizes (50-100).

Discussion

Temporal and spatial distribution of viral abundance

Individual values of VLP abundance, ranged between $1x10^6$ and $8x10^7$ VLP mL $^{-1}$. The maximal value was found at the surface in station 169 in Gerlache Strait and the minimal values in the deep layers of the vertical profiles. However ninety percent of the viral counts were between $7x10^6$-$2x10^7$. It is difficult to compare these values with those from other studies because in most of them VLP abundance was measured with transmission electron microscopy (TEM). It has been shown that counting YOPRO stained viruses with fluorescence microscopy gives higher estimates of VLP abundance than those obtained with the TEM (Hennes & Suttle 1995, Weinbauer & Suttle 1997). In order to be able to compare our data with those from other studies, we transformed our VLP-YOPRO counts to VLP-TEM counts, using a relationship (Log VLP-YOPRO = -0.32 + 1.1Log VLP-TEM) found between both methods (Chapter 4). The transformed values ranged between $5.5x10^5$ and $3x10^7$ VLP mL $^{-1}$. This range is much wider than that observed in a previous study of Drake Passage (Smith *et al.* 1992) and is similar to the range of VLP abundance found by Steward *et al.* (1996) in the Arctic. However, as we pointed out before, a large percentage of samples showed a range of values smaller than an order of magnitude.

A general trend for lower VLP abundances in FRUELA 96 than in FRUELA 95 was apparent in the three regions. Due to the variability within each region, however, only the values for Gerlache Strait were significantly different. There were no significant differences in VLP among the three zones in either cruise. Taking all data together, variability in VLP abundance was relatively low. No significant relationships were found neither with bacterial abundance nor with chlorophyll a. Only when the Gerlache Strait data were considered alone was a significant relationship found between VLP and bacterial abundance. The latter variable explained about 50 % of the variability of the former.

Small scale variability was investigated in vertical profiles and diel cycles. Both stations where we analysed deep vertical profiles presented similar patterns of VLP and bacterial abundance, with the highest concentrations of both at the surface. In both stations, VLP abundance showed a faster decrease with depth than bacterial abundance for the first 30 m. From surface to this depth VLP abundance followed the decrease pattern of chlorophyll a. The VLP/BN ratio also decreased with depth. This suggests that part of the viruses at the surface were phytoplankton viruses. The vertical profile reported by Hara *et al.* (1996) in a station of the subartic, also showed the maximal viral

concentrations in the photic zone. However the VLP/BN ratio fluctuated throughout the water column. The one order of magnitude decrease in VLP together with the decrease in the VLP/BN ratio with depth, would suggest a lower activity of viruses in the deep layers. This is consistent with the lower bacterial activity at lower depths.

Diel cycles of VLP abundance showed, in general, very slight variability. However, in stations 168 and 169 the largest changes occurred at the surface (0-20m) and the maximal VLP abundance was found at night (2:00), coincident with the minimal solar radiation. This could be due to inhibition of viral activity by sunlight (Suttle & Chen 1992, Noble & Fuhrman 1997). In a very dynamic system such as the surface waters of the Gerlache Strait, this could be reflected in a decrease of VLP abundance. In station 156, the pattern was different, and the minimal VLP abundance was reached 5-6 hours after the maximal solar radiation.

Factors responsible of viral decay

Viral decay has been investigated in some marine environments with the whole viral assemblage (Heldal & Bratbak 1991, Mathias *et al.*. 1995) and with some specific phage-host systems (Suttle & Chen 1992, Noble & Fuhrman 1997). The difference between viral decay of infectiviy and viral decay of particles should be beared in mind. It has been reported that sunlight is one of the most important factors responsible for loss of viral infectivity (Suttle & Chen 1992, Noble & Fuhrman 1997). In the dark, adsorption to heat labile particles has been reported to be the factor responsible for both processes: decay of particles and infectivity (Suttle & Chen 1992, Noble & Fuhrman 1997). Other factors such as grazing by nanoflagellates (González & Suttle 1993) and enzymatic activity have been reported to be quantitatively less important.

The fate of viruses when bacterial respiration is inhibited by cyanide is unknown (Heldal & Bratbak 1991). However, losses of viral particles can be only due to abiotic factors. These include adsorption to particles and release of nucleic acids from the viral particle (Heldal & Bratbak 1991). Thus, it seems the factor responsible for viral decay in our experiments was adsorption to particles. This process is dependent of the particle concentration and could be irreversible or reversible (Murray & Jackson 1992). From the pattern found in all our viral decay experiments, it seems viral losses arrived at a plateau after 8-15 hours of the experiment. The remaining viral concentration in all of them was similar ($5\text{-}7 \times 10^6$ VLP mL^{-1}). Thus, it is possible that during the fast decay along the first hours of these experiments, viruses irreversibly bound to particles (VDR 1). Then, either binding became reversible when viral concentration reached a threshold or all the

available binding sites were occupied, and viral decay slowed down (VDR 2). A balance between adsorption and desorption could be established at this point.

The origin of the particles responsible of viral adsorption is unknown. Suttle & Chen (1992) found that the element responsible for viral decay of infectivity in the absence of sunlight was heat labile and could be only partially attributable to bacteria or protists. Viruses attached to cells would not be counted by the YOPRO method, because of the small size of the viruses in comparison to cells. However, viruses attached to organic matter aggregates would still be counted because these particles shown orange fluorescence with YOPRO, while nucleic acids shown green-yellow fluorescence (Hennes & Suttle 1995). Therefore, we suggest that viral adsorption to cells was the factor responsible for viral decay observed in the experiments with cyanide.

In six out of seven experiments, VDR were higher in the <50 μm size fraction than in the <0.8 μm size fraction. These differences, however, were only significant for VDR 1 in station 169. Thus, it seems that particles larger than 0.8 μm were involved in viral decay in station 169. When this station was sampled there was a high phytoplankton abundance mostly composed by *Phaeocystis*. (J. Rodríquez, J.M. Blanco and F. Jiménez, personal comunication). Studies have described large numbers of viral particles attached to slime associated with a collapsing diatom bloom (Bratbak *et al.* 1990). We suggest that viral adsorption to *Phaeocystis* cells in station 169 could be responsible for the higher VDR found in the <50 μm size fraction than in the <0.8μm.

In station 168 patterns of viral decay for <0.8 μm and <50 μm size fractions were similar. In this station the most abundant component of phytoplankton biomass was *Cryptomonas* (J. Rodríquez, J.M. Blanco and F. Jiménez personal comunication). These algae are about 10 μm long and would not pass through 0.8 μm pore size filters. However this alga breaks easily and we have observed pieces of this alga broken in the <0.8 μm size-fraction at high concentrations. Thus, viruses could attach to cells and cell pigments with the same efficiency, resulting in similar decay rates in both size fractions.

In the other experiments (at station 15, 72 and 79) we did not find any significant difference between rates in both size fractions. However, a slightly higher decay of VLP can be observed on the <50 μm size fraction. The average coefficient of variation (CV) of VLP counts in these experiments was about 14 %. The power functions fitted for <0.8 and <50 μm size fractions in each experiments overlapped with this percentage of error. Thus, the method was not sensitive enough to detect slight differences between both size fractions.

Influence of temperature and organic matter on viral activity

No significant differences among the same treatments (control or glucose addition) incubated at different temperatures were found. Significant differences between both treatments for each temperature were neither found in any of the stations. The fact that significant difference were not found could be due to the method used to measure viral activity. As discussed above, the cyanide method would only measure viral decay due to adsorption to particles, most likely to cells. Thus, we could detect differences in viral activity if only bacteria or other particles were found in different amounts in the batch cultures (filtered by 0.8 μm pore size) from which the viral decay rate experiments were carried out. In the batch cultures, ^{3}H-leucine incorporation started to increase after the second sampling day in the glucose addition treatment incubated at the highest temperatures (>4° C) and later in the other treatments. Bacterial abundance, however, did not increase after the third sampling day (Pedrós-Alió *et al.* in preparation). Thus, we suggest that when decay experiments were performed, changes in bacterial abundance were not enough to be reflected in significantly higher viral adsorption. Consequently, the cyanide method, might have not been sensitive enough to detect differences in viral activity due to either temperature or organic matter addition.

Bacterial mortality due to viral lysis estimated from decay experiments. Limitations of the methodology

Viral decay experiments using KCN as inhibitor of biological activity have been used previously to measure viral production rates (Heldal & Bratbak 1991, Bratbak *et al.* 1992, Mathias *et al.* 1995). In this approach it is assumed that the VLP abundance in the system is maintained constant through time. In order to test this assumption, we also incubated active cultures where VLP abundance showed slight fluctuations through time. Thus, it seems that the viruses lost by decay were actually replaced by viruses released from lysed bacterial cells.

The first drawback of the method is that it could underestimate viral decay rates because biotic processes are not considered. Thus, viral production estimated with cyanide would represent, *a priori*, a conservative approach to the truly viral production in aquatic environments.

Another disadvantage of the method is that to transform viral production rate to bacterial mortality, an estimate of burst size is need. A wide range of values of burst size from bacteria in natural environments has been found (Weinbauer & Peduzzi 1994). A

lower burst size would mean an overestimation of viral mortality. Steward *et al.* (1996) found that bacteria from the Arctic contained phages in a range between 6 and 270. However, these authors used an average burst size of 50 to convert the estimates of viral production to bacterial mortality. We assumed a range of burst sizes between 50 and 100 as representative of marine bacteria (Bratbak *et al.* 1990).

All the calculations to convert viral decay rates to bacterial mortality, have been done assuming that all VLP were bacteriophages. As pointed out before, an important part of the VLP could be phytoplankton viruses and our bacterial mortality values would then be overestimates.

The main problem of the cyanide method is the difficult interpretation of viral decay experiments. Curves obtained from these experiments showed a fast rate at the first hours and a slow rate afterwards (24-48 hours). This pattern was also found by Heldal & Bratbak (1991) and Mathias *et al.* (1995). In this situation there are three options: 1, to consider the first rate as valid and the second as an artifact of the incubations. 2, to consider the first as an artifact of enclosing samples in bottles and the second rate as the valid one. And 3, to fit a power function to the whole data set and to use this to calculate losses in a given period of time.

1. This option is the one taken by Heldal & Bratbak (1991) and Mathias *et al.* (1995). In this case, the assumption is that as the experiment proceeds, all the possible sites for phage adsorption became full and therefore the asymptotic part of the curve is not valid for nature. The viral decay rates found during the first hours of the experiments (VDR 1) in Antarctic waters ranged between 0.006-0.3 h^{-1}. These values showed a higher range of variability than those found by Mathias *et al.* (1995, 0.06-0.1 h^{-1}) in samples from the Danube River. The upper values of our VDR 1 are in the range of rates estimated by Heldal & Bratbak (1991) in a Norwegian fjord.

Bacterial mortality necessary to maintain the viral production estimated with VDR 1 was always higher than 100% of the bacterial production. The lowest impact of viruses on the bacterial assemblage derived from VDR 1 was found at stations 79 and 169 (1-3% of BN per hour). Even assuming that viral lysis were the only factor responsible for bacterial mortality in these stations, bacteria would have to grow at a rate twice of that found to maintain a constant abundance. This result is difficult to accept because although values of bacterial heterotrophic production could be underestimated (Calderón-Paz 1997), other causes of bacterial mortality are certainly present (Vaqué *et al.*, in preparation). At the other stations the imbalance is even worse. Thus, the percentage of bacterial

mortality due to viral infection obtained with the VDR 1 seems totally unrealistic. This is in agreement with the results found by Bratbak *et al.* (1991) in a diel mesocosm experiment, where viral lysis exceeded bacterial production by a factor of 6. We think that cyanide addition may affect the natural community not just by stopping its activity. Processes favoring formation of aggregates could be involved that would artificially increase the rate of viral decay. In some environments this may not be the case. Thus, Mathias *et al.* (1995) used two approaches (percentage of infected bacteria and viral decay after cyanide addition) to measure bacterial mortality due to viral lysis. The values obtained with both methods were overlapping, giving estimates of bacterial mortality due to viral infection between 10.8% to 43.2% of the whole bacterial mortality (considering balanced bacterial growth).

2. In the second approach the assumption is that the initial fast decay is due to the bottle enclosure and only after a few hours is the natural decay rate restored. An alternative interpretation is that two "kinds" of viruses with different kinetics of disappearance are present. In fact Mathias *et al.* (1995) obtained some evidence of this. They observed that smaller viruses (<60 nm) showed a faster decay (VDR 1) while larger viruses decreased slowly (VDR 2). Thus, the VDR 2 seems to correspond to a part of the initial viral assemblage. Using VDR 2 to calculate bacterial mortality due to viral infection, results are more realistic. However, it is not possible to use this rate to measure the whole viral impact on the natural bacterial assemblage because the smaller viral particles with a faster decay would be ignored (Mathias *et al.* 1995).

3. The last approach is more pragmatic. If we assume there are two or more different virus population with different decay rates, an exponential decrease can not be expected. The use of the power function found for each experiment to calculate bacterial mortality is statistically the most satisfying approach. However, this has two problems. First, long incubations are needed and cyanide seems to decrease its effect after 24 hours (see for example Fig. 5.6F). This could be the reason why in stations 193 and 210, where we did not add cyanide again after 24 hours, we did not obtain a clear pattern in the decay experiments. And second, with this approach an exponential viral decay rate can not be calculated. Still, the total number of viruses produced per day can be calculated as has been explained in the results. Production and decay take place continuously in nature. To manipulate the natural community blocking one of these processes for a long period of time may give results that are far away from what is really occurring in nature. Control incubations of active cultures, however, seemed to indicate that net changes of viruses were zero throughout the 48 hours that the experiments lasted. Thus, the

approach of using the data from the whole decay experiment to estimate bacterial mortality seems the most appropriate.

Lets, then, consider the results of bacterial mortality due to viral infection derived from the power function. Viral impact on the natural bacterial assemblage would account for a significant percentage of production. In station 79, viral lysis (40 % of BHP) and bacterivory (3 % of BHP) together accounted for less than 50 % of the bacterial production. In station 72 and 168, viral impact accounted for 100% of the bacterial production. In these stations viruses would be responsible for all the bacterial losses possible with the experimentally determined specific growth rate (0.41 d^{-1} and 0.53 d^{-1} respectively). In station 15 and station 169 bacteria should grow at a rate faster than the measured one (twice in station 169 and three times in station 15) to be able to maintain a constant abundance.

In Arctic waters viral infection has been found to account for 10-23 % of the bacterial heterotrophic production in average (Steward *et al.* 1997). The highest values reported in this study (36 %) are equivalent to the lowest values found in the present work. The viral impact found by Steward *et al.* (1997) was estimated by counting visibly infected bacteria (VIB) in whole cell preparation (Weinbauer & Peduzzi 1994). It has been reported that this method could underestimate bacterial mortality due to viral infection because of the possibility of disruption of cells during ultracentrifugation (Weinbauer & Peduzzi 1994, Steward *et al.* 1996) and because some cells that could be infected appear opaque under TEM (Chapters 2 and 4). Moreover, to convert VIB to bacterial mortality it is necessary to use a range of conversion factors that have been calculated experimentally for two marine phage-bacteria host systems by thin section (Proctor *et al.* 1993). Thus, there are some uncertainties in the method of counting VIB that seem to cause underestimations of viral impact on the bacterial assemblage (Steward *et al.* 1996). As has been pointed out above, the cyanide method also has disadvantages and in some cases it seems to give estimations of viral impact difficult to reconcile in nature (stations 15 and 169 in this study, Bratbak & Heldal 1991, Bratbak *et al.*. 1992). Thus, more work is needed to improve both methods of estimating bacterial mortality due to viruses. For the cyanide method it would be necessary to know the mechanisms involved in the disappearance of viruses observed.

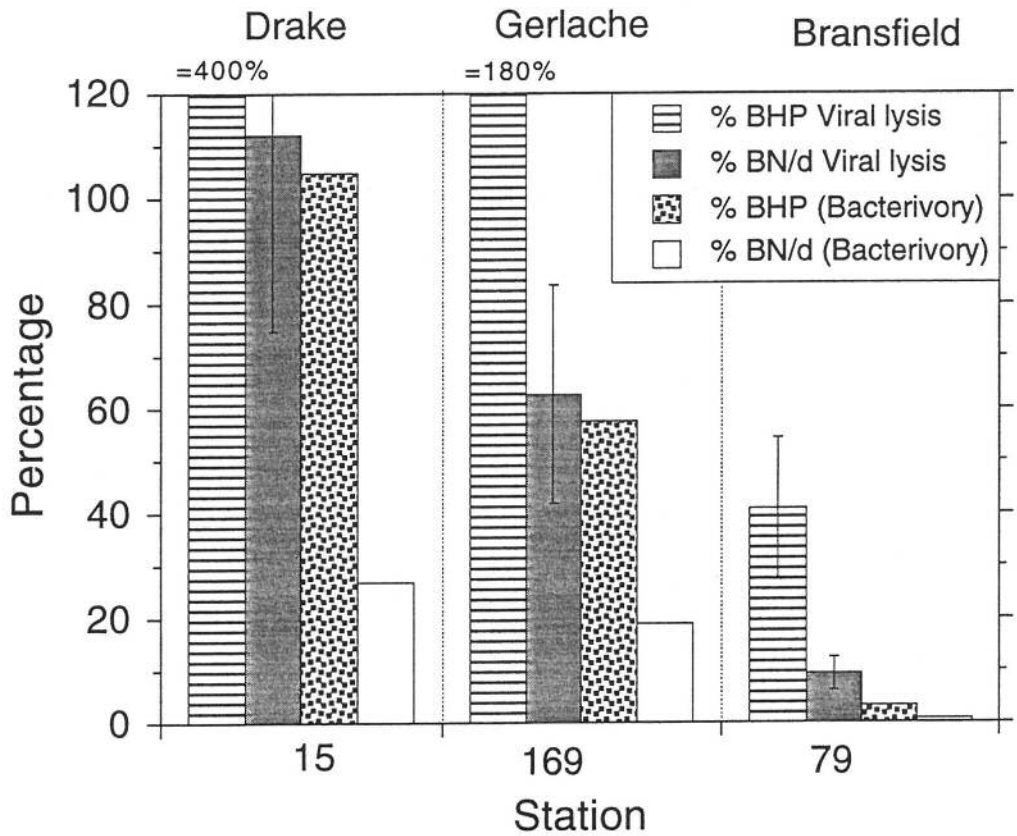


Fig. 5.10. Percentage of bacterial heterotrophic production (BHP) and abundance (BN) ingested by bacterivores and lysed by viruses per day in different stations. Error bars indicate the range of values calculated using a range of burst sizes (50-100).

Despite the uncertainties in our estimations of bacterial mortality due to viruses some tentative conclusion appears reasonable. Viral lysis presented the highest impact in the most eutrophic station (15) and the lowest impact in the most oligotrophic station (79). This is in agreement with other studies that found that viral mortality of bacteria was higher in more eutrophic waters (Weinbauer & Peduzzi 1995, Steward *et al.* 1996).

When comparing viral lysis and bacterivory at the stations where both processes were measured (Fig. 5.10), viruses always accounted for a percentage of the bacterial production and abundance larger than bacterivores. This result is different from that presented by Steward *et al.* (1996) in the Arctic. These authors found that viral lysis and bacterivory accounted for a similar percentage of bacterial mortality (25 % each). A differential response of microbial processes to temperature has been suggested to occur in cold waters (e. g. Pomeroy & Deibel 1986). This feature has been studied for bacterioplankton and phytoplankton. If this characteristic was general, uncoupling could occur between any two trophic links in the microbial food webs. Recently, Vaqué *et al.* (in preparation) have found extremely low values of bacterivory in Antarctic waters. Bacterivory increased only after bacterial abundance and activity were stimulated artificially (Calderón-Paz *et al.* in preparation). Thus, bacterivory and bacterial activity seem to be uncoupled in cold environments. We could expect viruses to be coupled to bacterial activity because they need the cell enzymes to multiply. Thus, in cold environments, viruses could cause a higher impact on bacterial mortality than bacterivores, at temperatures where those showed a lower activity than bacteria. Clearly, more work is necessary to test this hypothesis.

6. Relationships between viral abundance and environmental factors across diverse aquatic environments

Introduction

The abundance of virus-like particles (VLP) has been determined in several aquatic environments (Table 6.1). Total counts of VLP, however, are the result of the balance between viral production and viral decay and, thus, do not reveal their role in the environment. One way to study such role is to measure the impact of viruses on their host populations directly. This has been attempted by measuring viral decay rates (Bratbak & Heldal 1991), viral production rates (Steward *et al.* 1992a, b) or percentage of infected cells (Proctor *et al.* 1993, Weinbauer & Peduzzi 1994). Alternatively, the role of viruses can be approached indirectly. Data can be gathered from a wide range of ecosystems and the quantitative relationships between viral abundance and those ecological parameters that, *a priori*, should influence their dynamics can be determined empirically (Maranger & Bird 1995).

The relationship between VLP and bacterial abundance has been determined for a few specific environments (Wommack *et al.* 1992, Boehme *et al.* 1993, Cochlan *et al.* 1993, Paul *et al.* 1993, Jiang & Paul 1994, Weinbauer & Peduzzi 1995a). Other factors such as temperature and salinity have been analyzed only twice (Paul *et al.* 1993, Jiang & Paul 1994). The general empirical approach favored here has been used only once (Maranger & Bird 1995). This study however was limited by the small data base available at the time, by the narrow range of aquatic systems analyzed, and by the limited number of factors considered: bacterial abundance and chlorophyll a.

The main objective of the present work was to find the factors that control and covary with viral abundance in nature. For this purpose, we sampled a diverse set of environments including both marine and fresh water ecosystems. Among the first we included two solar salterns from the Spanish coast, and transects in the Mediterranean and the Weddell Seas. The fresh water systems sampled included several karstic lakes with anoxic hypolimnia. In these environments we measured salinity, temperature, oxygen, VLP concentration, bacterial abundance and cell volume, chlorophyll a, bacterial heterotrophic production and growth rate, and bacterivory. We also collected VLP abundance data in natural environments reported in the literature (Table 6.1) to extend the study of Maranger & Bird (1995). We then used this data base to determine the empirical relationships between VLP abundance and all the other variables.

We were particularly interested in including data from anaerobic and hypersaline environments. In these extreme environments, the bacterial assemblage is composed of large cells, with a low impact of bacterivory, and a low growth rate. These systems provide natural experiments in which a given environmental factor, for example bacterivory,

can be isolated from the others. Comparison of data from these systems with more "normal" systems allows to test specific hypothesis about the nature of the relationships between viral abundance and other environmental variables.

Our second objective was to identify the factors that determine the impact of viral infection on natural bacterial assemblages. Some studies have suggested a direct relationship between the percentage of visibly infected bacteria (% VIB) with total bacterial abundance (in the Adriatic Sea, Weinbauer *et al.* 1993) or with bacterial production (in the Bering and Chukchi Seas, Steward *et al.* 1996). We investigated the relationships between VIB and bacterial abundance, heterotrophic production and specific growth rate using the whole data base (Table 6.1).

Materials and methods

Data collection

We collected data from studies of VLP abundance, distribution and dynamics in natural environments, published between 1989 to 1996 (Table 6.1). We excluded data from mesocosm experiments in order to avoid changes in the microbial community due to confinement. To study the variables that could influence VLP abundance in nature we followed the following strategy. First, we looked for a general relationship using the data as presented in the original reports (Table 6.1). These included: depth profiles, oceanic transects, diel and seasonal cycles, integrated samples and isolated depths, depending on the study. Despite the risk of some degree of autocorrelation among the data, we used the individual data points without integrating or averaging, since the relevant time and space scales of variability for VLP abundance in each study were unknown. Second, marine and fresh water environments were studied separately, using the same data set as for the general relationship. Third, we examined the relationships in each type of marine system separately: salt ponds, pelagic areas and ice, because of the very different physico-chemical characteristics of the ecosystems investigated. Last, we analyzed the relationships in each type of fresh water system separately. Only a few fresh water environments had enough data to carry out such analysis: temperate Lake Constance (Hennes & Simon 1995), Canadian lakes (Maranger & Bird 1995), and the Danube River backwaters (Mathias *et al.* 1995). The data from these environments belonged either to isolated depths within the euphotic layer (Mathias *et al.* 1995) or were taken using an integrated column water sampler (Hennes & Simon 1995, Maranger & Bird 1995) and the karstic lakes studied here.

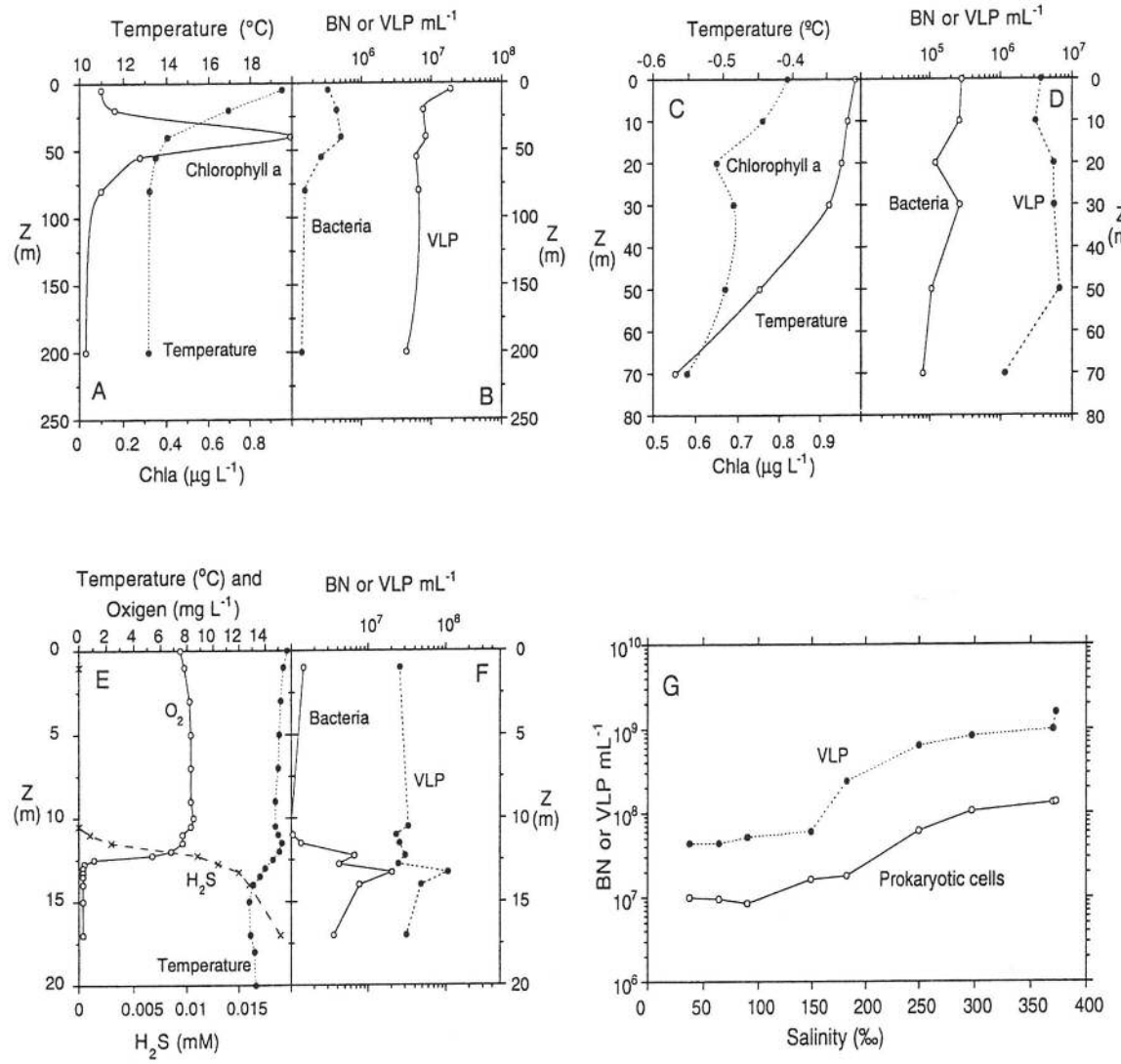


Fig. 6.1. A, B- Depth profiles of temperature (° C), chlorophyll a (Chla µg L^{-1}), bacterial (BN mL^{-1}) and VLP abundance (VLP mL^{-1}) from a oceanic station sampled during the cruise VARIMED 95 in the Mediterranean Sea. **C, D**- Depth profiles of temperature (° C), chlorophyll a (Chla µg L^{-1}), bacterial (BN mL^{-1}) and VLP abundance (VLP mL^{-1}) from a station sampled during the cruise ECOANTAR 94 in the Weddell Sea. **E, F**- Depth profiles of temperature, oxygen concentration, chlorophyll a (Chla µg L^{-1}), bacterial (BN mL^{-1}) and VLP abundance (VLP mL^{-1}) from Lake Tobar sampled during a period of stratification. **G**- VLP and bacterial abundance along a salinity gradient from La Trinitat Salterns.

Table 6.1. Set of data used in the present study.

Reference	Site	Envir.	Mean VLP^t	n	method	BN	Chla	HNF	Sal	T	BHP	μ	Vol	BV	VIB
This study	Weddell Sea	Ocean	7.65×10^6	54	1	+	+	+	+	+	+	+	+	+	-
	Mediterranean Sea	Ocean	2.90×10^6	52	3	+	+	+	+	+	+	+	+	+	-
		Coast	2.50×10^6	16	3	+	+	+	+	+	+	+	+	+	-
		Salt Ponds	3.70×10^8	19	1&2	+	+	+	+	+	+	+	+	+	-
	Lake Cisó	Karstic lake	1.27×10^8	32	1&2	+	+	+	+	+	+	+	+	-	-
	Lake Tobar	Karstic lake	3.87×10^8	9	1	+	+	+	+	+	+	+	+	-	-
	Lake La Cruz	Karstic lake	1.59×10^8	8	1	+	+	+	+	+	+	+	+	-	-
Berg *et al.* 1989	Lake Plusee	Fresh water	1.01×10^7	1	1	+	-	-	-	-	-	-	-	-	-
	Chesapeake Bay	Coast	6.10×10^6	1	1	+	-	-	-	-	-	-	-	-	-
	Korsfjorden	Coast	9.90×10^6	1	1	+	-	-	-	-	-	-	-	-	-
	Raunefjorden	Coast	4.80x106	1	1	+	-	-	-	-	-	-	-	-	-
	North Atlantic	Ocean	1.50×10^7	1	1	+	-	-	-	-	-	-	-	-	-
	Barents Sea	Ocean	6.00×10^4	1	1	+	-	-	-	-	-	-	-	-	-
Bratbak *et al.* 1990	Raunefjorden	Coast	3.70×10^6	11	1	+	-	+	-	-	-	-	+	-	-
Heldal & Bratbak 1991	Raunefjorden	Coast	4.60×10^7	3	1	+	-	-	-	+	-	-	-	-	-
	Bergen	Harbour	1.95×10^7	2	1	+	-	-	-	+	+	+	-	-	-
	Lake Kalandsvannet	Fresh water	1.25×10^8	3	1	+	-	-	-	+	-	-	-	-	-
Hara *et al.* 1991	Osaka Bay	Coast	1.83×10^8	1	2	+	-	-	-	-	-	-	-	-	-
	Otsuchi Bay	Coast	1.43×10^7	5	2	+	-	-	-	-	-	-	-	-	-
	Sagami Bay	Coast	4.04×10^7	6	2	+	-	-	-	-	-	-	-	-	-
	Kuroshiro	Ocean	8.70×10^6	3	2	+	-	-	-	-	-	-	-	-	-
Nygaard *et al.* 1991	Oslofjord	Coast	5.90×10^7	8	1	+	+	+	+	+	+	+	+	+	-
Steward *et al.* 1992	California coast	Coast	1.59×10^8	5	1	+	+	-	-	-	+	+	-	-	-
	Pacific Ocean	Ocean	9.75×10^6	8	1	+	+	-	-	-	+	+	-	-	-
Wommack *et al.* 1992	Chesapeake Bay	Coast	2.53×10^7	55	1	+	-	-	-	-	-	-	-	-	-

Table 6.1. (Continuation)

Reference	Site	Envir.	Mean VLP[t]	n	method	BN	Chla	HNF	Sal	T	BHP	μ	Vol	BV	VIB
Cochlan *et al.* 1993	California coast	Coast	7.20×10^6	12	1	+	+	-	-	-	-	-	-	-	-
	Pacific Ocean	Ocean	1.11×10^6	9	1	+	+	-	-	-	-	-	-	-	-
	Gulf of Bothnia	Coast	3.20×10^7	4	1	+	+	-	-	-	-	-	-	-	-
Paul *et al.* 1993	Key Largo	Coast		8	4	+	-	-	+	-	-	-	-	-	-
Boheme *et al.* 1993	Gulf of Mexico	Coast		12	4	+	+	-	-	-	-	-	-	-	-
		Ocean		16	4	+	+	-	+	+	-	-	-	-	-
Weinbauer *et al.* 1993	Northern Adriatic Sea	Coast	1.97×10^7	35	1	+	-	-	-	-	-	-	-	-	-
Jiang&Paul 1994	Tampa Bay	Coast		27	4	+	+	-	+	+	-	-	-	-	-
Maranger *et al.* 1994	South of Resolute	Ice	4.50×10^7	12	1	+	+	-	-	-	+	+	-	-	-
		Coast	5.99×10^6	3	1	+	+	-	-	-	+	+	-	-	-
Hennes&Simon 1995	Lake Constance	Fresh water	1.84×10^7	19	1	+	+	-	-	-	-	+[a]	-	-	+
Mathias et al. 1995	Danube River	Fresh water	2.50×10^7	23	1	+	+	-	-	+	+[b]	+[b]	-	-	+[a]
Hennes&Suttle 1995	Gulf of Mexico	Coast	3.48×10^7	14	1&3	+	+	-	-	+	-	-	-	-	-
Weinbauer et al. 1995	Northern Adriatic sea	Coast	1.32×10^7	71	1	+	+	+	-	-	-	-	-	-	-
Maranger&Bird 1995	Canadian Lakes	Fresh water	9.10×10^7	18	1	+	+	-	-	-	+	+	+	-	-
Steward *et al.* 1996	Artic	Ocean	2.09×10^6	11	1	+	+	+	-	-	+	+	-	-	+
		Coast	4.60×10^6	11	1	+	+	+	-	-	+	+	-	-	+
Maranger *et al.* 1996	Lake Gilbert	Sediment	3.22×10^9	10	1	+	+	-	-	-	-	-	-	-	-
		Fresh water	4.01×10^7	13	1	+	+	-	-	+	-	-	-	-	-
Bratbak *et al.* (unpub.)	Lake Saelenvatnet	Anaerobic lake	2.00×10^8	23	1	+	-	+	+	+	+	-	+	-	-

[t] Average calculated after transforming the data using the regressions shown in Fig. 6.1.

[a] Data are monthly averages.

[b] Data extrapolated from a relation with temperature.

Description of the environments sampled

The northwestern Mediterranean is an oligotrophic sea characterized by the presence of a deep chlorophyll a maximum during a large part of the year (Estrada *et al.* 1993). The data used in our work corresponded to a transect between Barcelona and Mallorca sampled during June 1995 (cruise VARIMED 95, Chapter 4). Seawater samples for depth profiles were collected with 10 l Niskin bottles mounted on a CTD rosette. An example of a vertical profile is shown in Fig. 6.1A, B.

Samples from the Weddell Sea corresponded to a transect occupied during January 1994 (cruise ECOANTAR 94). The transect, between the ice-edge and the Weddell-Scotia confluence, presented a spatial segregation of activity and biomass of the different components of the microbial food web. Across the transect, we found a spatial succession from the ice-edge to the open ocean in this order: Initiation of the phytoplankton bloom followed by a maximum of macrozooplankton grazing, post-bloom dominated by secondary production of bacteria and protozoans, finally minimal activity of the microbial loop (Calderón-Paz *et al.* in preparation). Temperature across the transect fluctuated between -1.7° C and 2.4° C. Seawater samples were collected with 10 L Niskin bottles mounted on a CTD rosette. An example of a vertical profile is shown in Fig. 6.1C, D.

Karstic lakes are characterized by a peculiar morphometry and high mineralisation of the bottom waters that favor the stratification of the water layers during a part of the year (Miracle *et al.* 1992). The data presented here correspond to the sampling of three lakes located in two karstic areas in Spain: the Cuenca mountains (Lake Tobar and Lake La Cruz) and the Banyoles area (Lake Cisó). They were sampled during summer 1994 in a period of stratification with a sharp gradient of oxygen and sulfide. The epilimnia become aerobic during summer stratification (April to September) and opposite gradients of oxygen and sulfide appear in the metalimnia. During this period the lakes present three different communities: aerobic in the epilimnion, microaerophilic in the upper metalimnion and anaerobic in the lower metalimnion and the hypolimnion. Water samples were taken with a cone-shaped weight connected to a peristaltic pump (Miracle *et al.* 1992). An example of a vertical profile is shown in Fig. 6.1E, F.

Solar salterns consist of a series of shallow ponds connected in a sequence of increasingly saline brines. Samples were collected in July 1994 from "La Trinitat" (Delta de l'Ebre, Spain) and from "Braç del Port" salterns (Santa Pola, Spain) in July 1993. The different ponds in the salterns are maintained under constant conditions over long periods of time, enabling the microbial populations to reach an equilibrium at each pond

(Rodríguez-Valera 1988, Chapter 3). Samples were taken with a bucket at the end of a pole, collecting mostly subsurface water. An example of VLP and bacterial distribution with salinity is shown in Fig. 6.1G, H.

VLP abundance

The most frequently used method to quantify VLP abundance in aquatic systems is to pellet the viruses directly onto formvar coated grids and to count them with a transmission electron microscopy (TEM) after staining with uranyl acetate (method 1, Table 6.1, Børsheim *et al.* 1990, Bratbak & Heldal 1993, Suttle 1993). Some studies have used epifluorescence microscopy. Initially viruses were stained with DAPI (method 2, Table 6.1, Suttle 1993) and more recently with YOPRO (method 3, Hennes & Suttle 1995). A new stain has recently been used to count viruses by epifluorescent microscopy (SYBR green, J.A. Fuhrman personal communication), however no data had been published when our study was carried out. A third approach consists of concentrating viruses by ultrafiltration before enumeration by electron microscopy (method 4, Table 6.1, Paul *et al.* 1991).

It is well know that these methods give different absolute numbers when simultaneously used in any given system (Suttle & Chan 1994, Hennes & Suttle 1995, Weinbauer & Suttle 1997). This is due to the different fraction of the viral particles detected by each method. Thus, in principle, only numbers obtained with the same method should be used to derive general relationships. Since the data base (Table 6.1) is very limited, however, we tried to convert as many data sets as possible to numbers obtained with a standard method.

To compare methods 1 and 2 we used our own data corresponding to two solar salterns, Lake Cisó and a microcosm experiment (Fig. 6.2A). Hara *et al.* (1991) also compared VLP counts by DAPI and by TEM, but we did not include their data in our comparison because TEM methodology used in the latter study was different from method 1. In our comparison DAPI counts were always lower than the TEM counts. However, we found a significant relationship between these two methods ($p < 0.0001$, $r^2 = 0.796$, $n = 34$, Fig. 6.2A).

To compare methods 1 and 3 we used the data of Hennes & Suttle (1995) and we added our own data corresponding to a microcosm experiment (Fig. 6.2B). The YOPRO counts were always higher than the TEM counts, and the relationship found was also significant ($p < 0.0001$, $r^2 = 0.698$, $n = 20$, Fig. 6.2B).

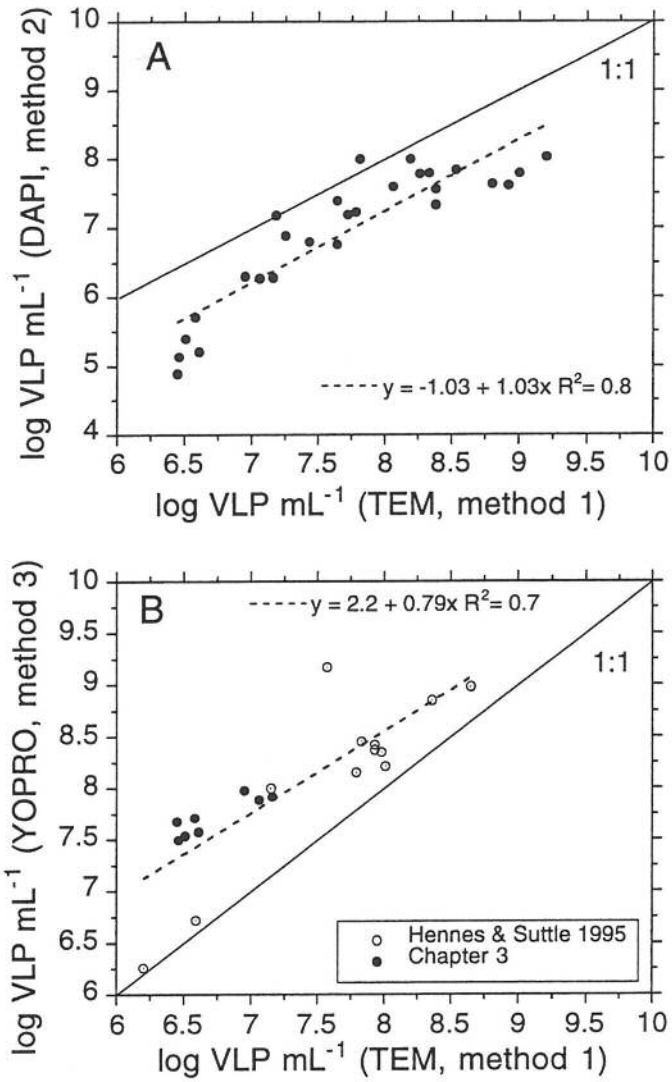


Fig. 6.2. **A**- Relationship between viral particles (log VLP mL^{-1}) counted by electron microscopy (TEM, method 1) and that counted by epifluorescence microscopy previously stained with DAPI (method 2). Solid line: relationship of 1:1. **B**- Relationship between viral particles (log VLP mL^{-1}) counted by electron microscopy (TEM, method 1) and that counted by epifluorescence microscopy previously stained with YOPRO (method 3). Solid line: relationship of 1:1.

Note: Recently, Weinbauer & Suttle (1997) have compared epifluorescence (DAPI and YOPRO) and electron microscopy in natural marine waters. However we did not include their data in our comparisons because they were published after the present analysis had been completed.

We used the relationships shown in Fig. 6.2 to transform the data of VLP abundance obtained with YOPRO and DAPI to numbers comparable to those obtained with method 1.

Several studies have reported that recovery of viruses using method 4 is not 100 % efficient (Paul *et al.* 1993, Suttle & Chan 1994), but the percentage of losses is not constant, thus we did not use the data found with method 4.

Other variables

Samples for bacterial abundance were fixed with gluteraldehyde (2 % final concentration) or formaldehyde (4 % final concentration). Bacteria were stained with DAPI (1µg mL^{-1} final concentration) and filtered onto black 0.2 µm pore size polycarbonate filters (Porter & Feig 1980). Bacterial abundance was determined with an epifluorescence microscopy at a magnification of 1250x.

Heterotrophic bacterial production was determined by tritiated thymidine (^{3}H-Tdr) or tritiated leucine (^{3}H-Leu) incorporation (Calderón-Paz 1997) depending on the system. In the solar salterns, karstic lakes and Mediterranean Sea both methods were used to measure bacterial production. In the remaining systems bacterial production was measured by ^{3}H-Leu incorporation alone. Because thymidine incorporation gives a reasonable estimation of cell production and most of the reports in the data base used this method, we used our thymidine data whenever possible. In the remaining systems, leucine incorporation data were converted to cells produced L^{-1} d^{-1}. The ^{3}H-Leu incorporated was converted to carbon (Kirchman 1993) and the empirical relationship found by Simon &Azam (1989), recalculated by Norland (1993), was used to convert carbon to cells. This was possible because the bacterial cell volume was measured simultaneously (Calderón-Paz 1997, J.M. Gasol personal communication).

Chlorophyll a was measured by fluorometry in acetone extracts in all the marine samples (Estrada *et al.* 1993). Seawater volumes from 25 to 200 cm^3 were filtered through GF/F filters. The filters were subsequently homogenized in 90 % acetone, and the suspension was cleared by ultracentrifugation. The fluorescence of the extracts was determined with a Turner Designs fluorometer. In the fresh water samples chlorophyll a was measured spectrophotometrically. Known volumes from each depth were filtered on small pore size glass fiber filters. Pigments were extracted by placing the filters in 5 mL of 90% acetone supersaturated with $MgCO_3$. Absorption spectra were determined in a spectrophotometer between 350 and 850 nm (Miracle *et al.* 1992).

Bacterivory by protists was measured with fluorescently labeled bacteria in the solar salterns (Chapter 3) or minicells (Mediterranean and Weddell Seas), using the disappearance of marked cells method (Pace *et al.* 1990, Salat & Marrasé 1994). Samples were incubated at *in situ* temperature in polycarbonate bottles in the dark. Two replicates and a control killed with formaldehyde (final concentration 4 %) were carried out for each experiment. Incubations lasted 48 hours and were stopped by fixing subsamples with gluteraldehyde (final concentration 2 %).

Statistical analyses

Data were analyzed by least square regressions, analysis of variance (ANOVA) and analysis of covariance (ANCOVA). Data were transformed to fulfill the normality assumptions of least squares regression analysis. All the variables, except percentage of infected cells and temperature, required log transformation.

Results

Relationship between viral abundance and different variables

Physico-chemical variables. Physico-chemical variables studied included temperature, oxygen concentration and salinity. Temperature could affect viral abundance indirectly, through its influence on the biological activity of the host cells. In fact, a significant relationship between temperature and viral abundance has been found in Florida coastal waters (Jiang & Paul 1994) and the northern Adriatic Sea (Weinbauer *et al.* 1995). We also found a significant relationship between VLP abundance and temperature ($p < 0.001$, $r^2 = 0.120$, $n = 296$). Oxygen concentration and salinity did not fulfill the normality assumptions (Fig. 6.3A, B) necessary for least square regression analysis, even after several transformations of the data (data not shown). Therefore, we could not estimate a general relationship. In some of the investigated environments, however, salinity (in the solar salterns) or oxygen concentration (in the anaerobic hypolimnia of karstic lakes) presented extreme values. In these cases we could analyze the impact of these factors on viral abundance qualitatively (see section on bacterivory below).

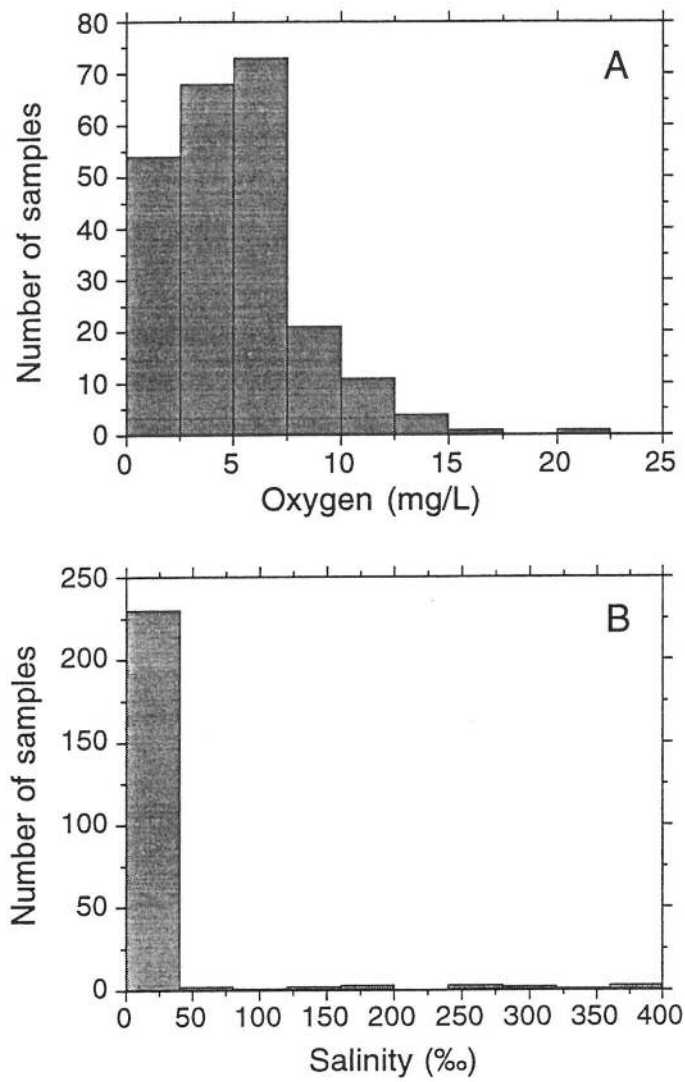


Fig. 6.3. **A-** Distribution of oxygen concentration in the whole data base.
B- Distribution of salinity data in the whole data base.

Bacterial abundance. A strong relationship was found between viral and bacterial abundance (Table 6.2, Fig. 6.4A). The slope of this regression was slightly, although significantly, smaller than 1 ($t_{0.05} = 1.969$, $p < 0.001$). An analysis of covariance (ANCOVA) did not find a significant difference between the slopes of the viral-bacterial relationships in freshwater and in marine systems ($p = 0.676$, $r^2 = 0.662$, $n = 80$) but a significant difference was found in the intercept ($p < 0.003$, $r^2 = 0.660$, $n = 580$). The intercept for fresh water environments, however, was only 1.2 times that for marine environments. According to the general relationship, bacterial abundance explained 66 % of the variability in VLP abundance.

Bacterial abundance was a moderately good predictor of viral abundance in all marine environments (Table 6.2, Fig. 6.4B). For fresh water studies the relation was not always significant (Table 6.2, Fig. 6.4C). An analysis of covariance revealed a non significant difference between the slopes for the different marine environments ($r^2 = 0.555$, $n = 431$, $p = 0.706$), but the intercept was significantly different between ocean and ice ($p < 0.014$). There was a significant difference among the slopes of fresh water environments ($p < 0.029$, $r^2 = 0.556$, $n = 68$). A Tukey post hoc test revealed that the significant differences were those between Lake Constance and the Canadian lakes ($p < 0.008$).

Bacterial biovolume. Bacterial cell volume (μm^3 $cell^{-1}$) was measured in addition to bacterial abundance in environments such as the solar salterns and karstic lakes. We investigated the relationship between VLP abundance and bacterial biovolume (μm^3 mL^{-1}) in these environments to check whether the general relationship with bacterial abundance could be improved by converting cell number to biovolume. In the case of solar salterns, biovolume was a better predictor of VLP abundance than bacterial abundance ($p < 0.001$, $r^2 = 0.822$, $n = 12$). However, in karstic lakes the regression did not improve by using biovolume instead of bacterial abundance ($p < 0.001$, $r^2 = 0.228$, $n = 24$). Since the data base for cell volume is much smaller than that for cell numbers we did not pursue this point.

Chlorophyll a concentration. The regression between VLP abundance and chlorophyll a was significant, although a low percentage of the variability was explained (Fig. 6.5A, Table 6.3). An ANCOVA revealed significant differences between the slopes found for marine and freshwater environments ($p < 0.001$, $r^2 = 0.442$, $n = 296$, Table 6.2, Fig. 6.5A). In fact, the regression for fresh water systems was very poor, explaining only 8 % of the variance. Only in the ice and in the Canadian lakes was chlorophyll a a good predictor of VLP abundance (Table 6. 3, Fig. 6.5B).

Table 6.2. Relationship between VLP and bacterial abundance in different environments (log VLP = (a ± SE) + (b ± SE) log BN).

Data set	a Intercept	b Slope	r^2	n	p
Total Data	1.525 ± 0.168	0.918 ± 0.027	0.657	580	<0.001
Marine	1.813 ± 0.225	0.866 ± 0.038	0.546	431	<0.001
Salt Ponds	1.162 ± 1.249	0.948 ± 0.166	0.659	19	<0.001
Ocean	1.836 ± 0.301	0.860 ± 0.052	0.409	389	<0.001
Ice	1.423 ± 0.572	0.992 ± 0.097	0.905	10	<0.001
FW	2.210 ± 0.427	0.828 ± 0.064	0.536	149	<0.001
Karstic Lakes[a]	3.580 ± 1.316	0.649 ± 0.99	0.638	8	<0.017
Lake Constance	-1.119 ± 0.215	1.325 ± 0.348	0.461	19	<0.001
Canadian lakes	5.683 ± 0.971	0.331 ± 0.144	0.035	18	0.035
Danube River				23	0.087

Table 6.3. Relationship between VLP abundance and chlorophyll a in different environments (log VLP = (a ± SE) + (b ± SE) log Chla).

Data set	a Intercept	b Slope	r^2	n	p
Total data	7.018 ± 0.035	0.698 ± 0.052	0.385	296	<0.001
Marine	7.008 ± 0.043	0.766 ± 0.070	0.334	239	<0.001
Salt Ponds				19	0.209
Ocean	6.828 ± 0.040	0.551 ± 0.065	0.258	211	<0.001
Ice	7.539 ± 0.073	1.023 ± 0.188	0.808	9	<0.001
FW	7.450±0.103	0.240 ± 0.107	0.083	57	0.029
Karstic Lakes[a]					0.079
Lake Constance				19	0.976
Canadian lakes	7.482 ± 0.090	0.506 ± 0.099	0.621	18	<0.001

[a] Integrated data from the epilimnion

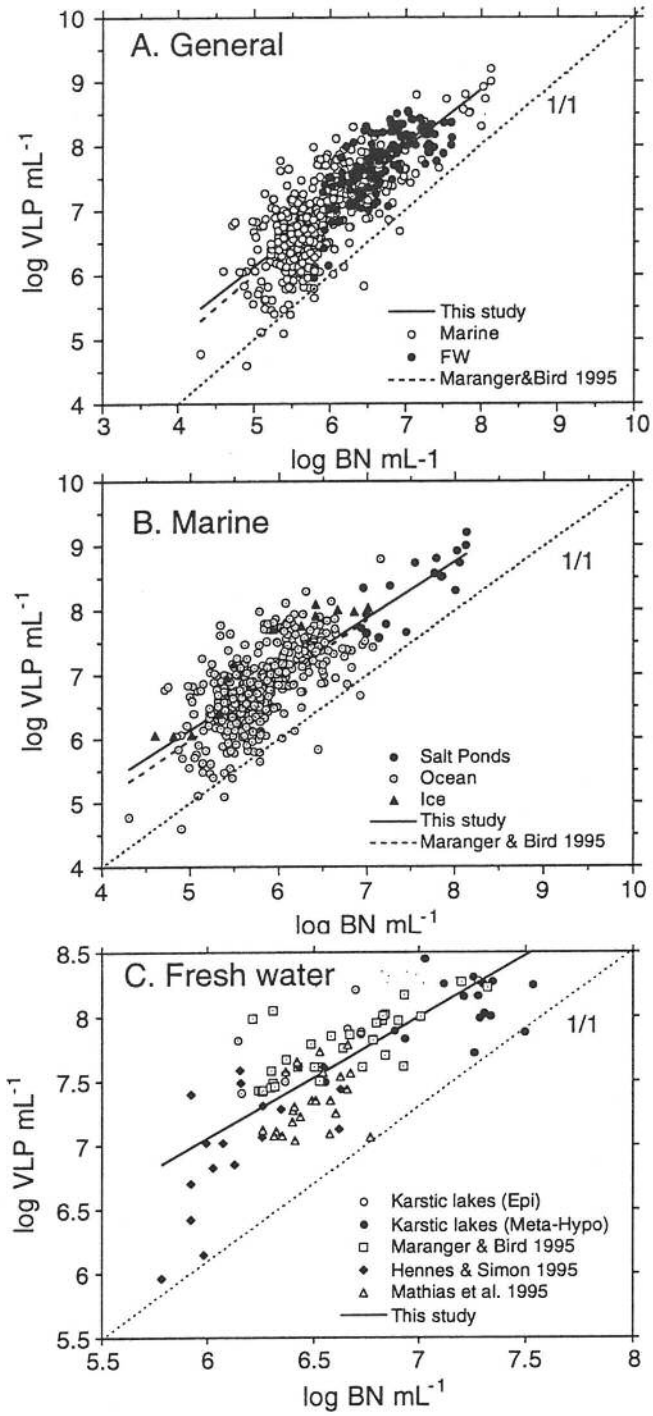


Fig. 6.4. **A**- Relationship between VLP (log VLP mL^{-1}) and bacterial abundance (log BN mL^{-1}) with all the data collected from fresh water (FW) and marine environments (fitted solid line). The relationship found by Maranger & Bird (1995) is also shown. The dotted line represents a hypothetical 1:1 relationship. **B-** Relationship between VLP and bacterial abundance in marine environments (solid line). The relationship found by Maranger & Bird (1995) is also shown. The dotted line represents a hypothetical 1:1 relationship. **C-** Relationship between VLP and bacterial abundance in fresh water environments (solid line). The dotted line represents a hypothetical 1:1 relationship. Maranger & Bird 1995 did not find a significant relationship between both parameters. Data from karstic lakes have been separated into integrated values from the epilimnion (Epi), metalimnion plus hypolimnion (Meta-Hypo).

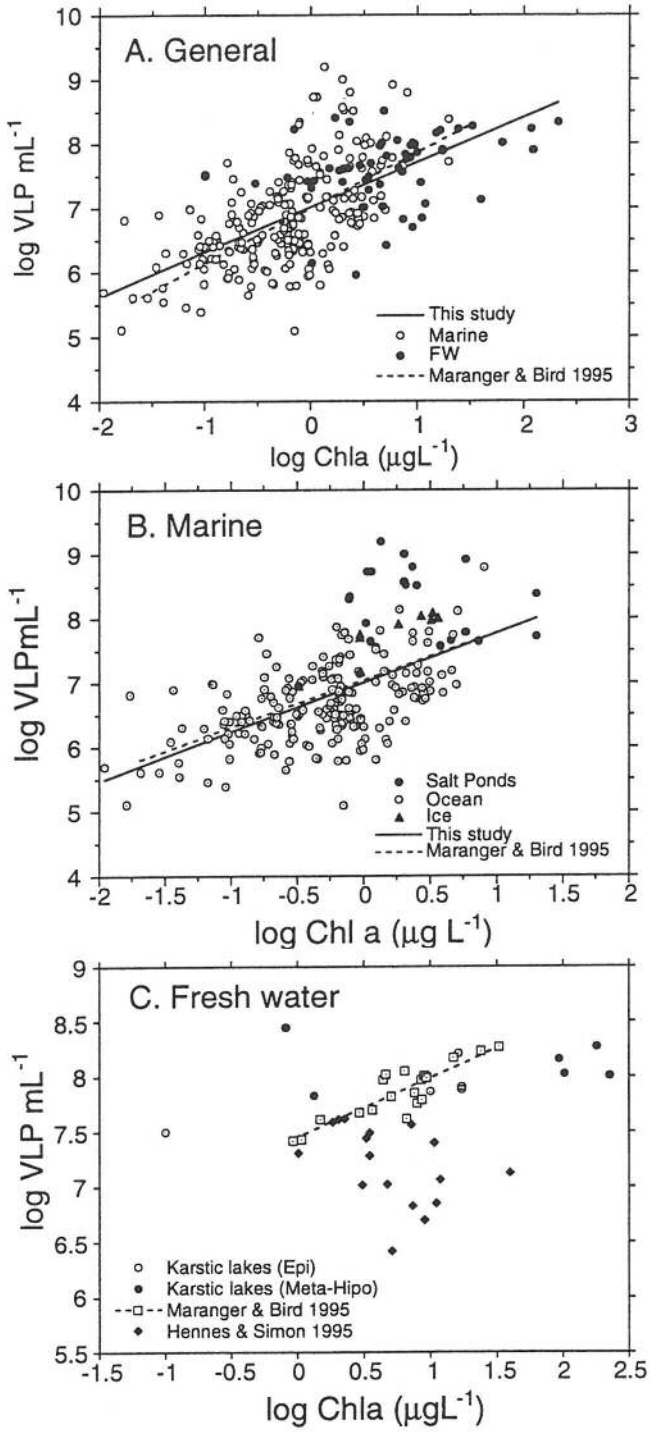


Fig. 6.5. **A**- Relationship between VLP abundance (log VLP mL^{-1}) and chlorophyll a (log Chla µg L^{-1}) from the whole data set (solid line). The relationship found by Maranger & Bird (1995) is also shown. **B**- Relationship between VLP abundance and chlorophyll a in marine environments (solid line). The relationship found by Maranger & Bird (1995) is also shown. **C**- Relationship between VLP abundance and chlorophyll a in fresh water environments (solid line). The relationship found by Maranger & Bird (1995) is also shown. Data from karstic lakes have been separated into integrated values from the epilimnion (Epi), metalimnion plus hypolimnion (Meta-Hypo).

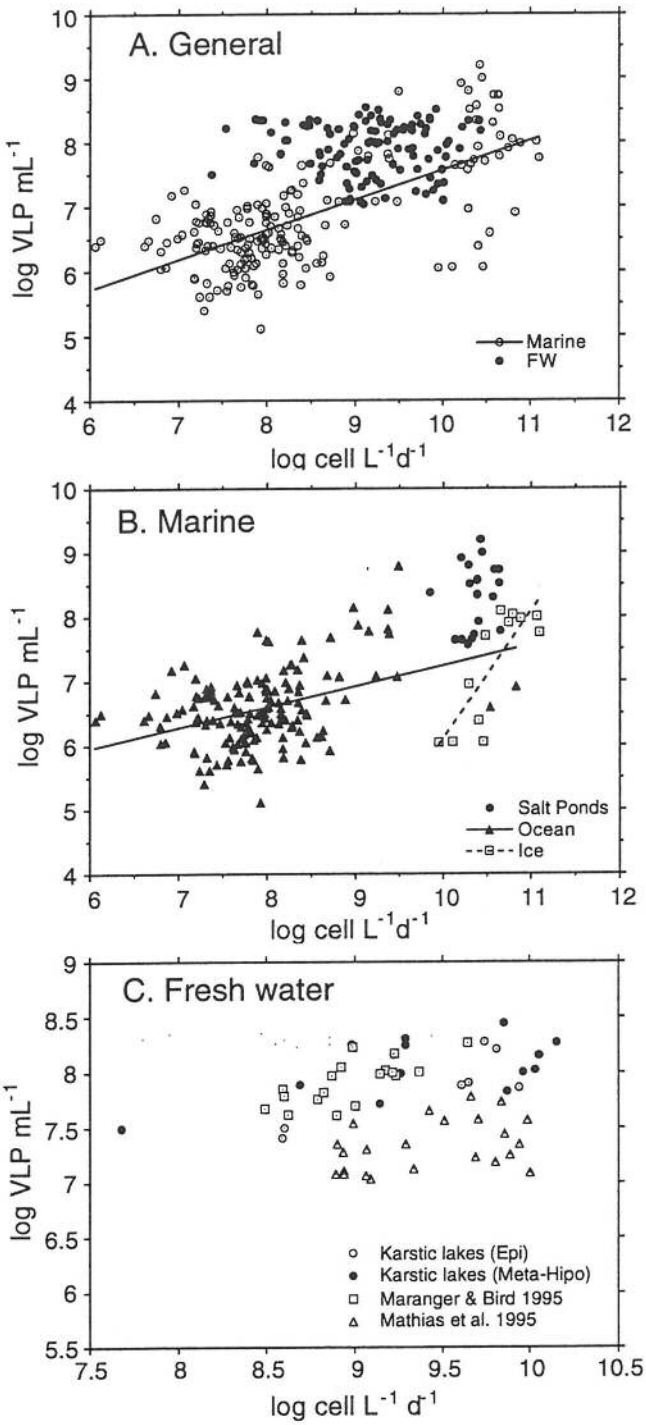


Fig. 6.6. A- Relationship between VLP abundance (log VLP mL^{-1}) and heterotrophic bacterial production (log cell L^{-1} d^{-1}) in marine environments (solid line). Fresh water environments did not show a significant relationship between both variables. **B-** Relationship between VLP abundance and heterotrophic bacterial production in the different marine environments studied. The dotted line represents the relationship found in the ice. The solid line represents the relationship found in oceanic environments. Salt Ponds did not show a significant relationship between both variables. **C-** Data from karstic lakes have been separated into integrated values from the epilimnion (Epi), metalimnion plus hypolimnion (Meta-Hypo).

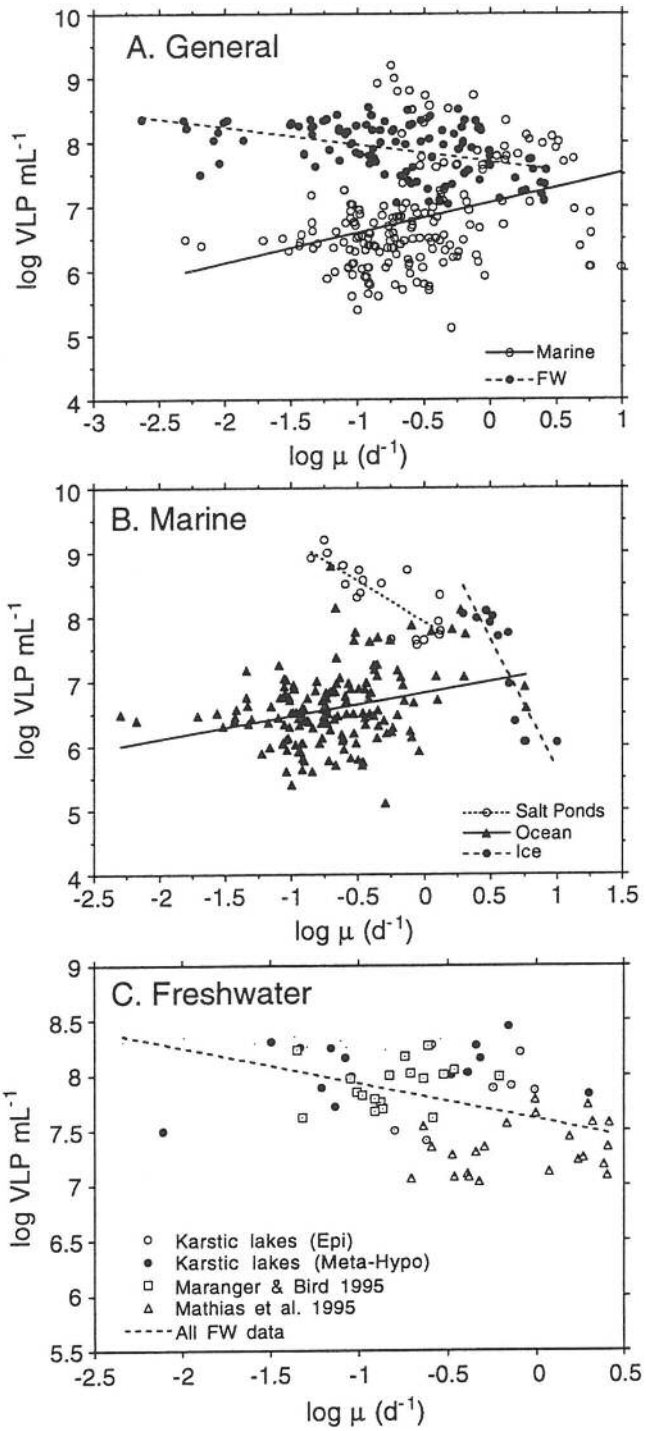


Fig. 6.7. **A**- Relationship between VLP abundance and bacterial specific growth rate (log μ) in marine (solid line) and in fresh water environments (dotted line). **B**- Relationship between VLP abundance and bacterial specific growth rate in the different marine environments investigated. The big dotted line represents the relationship found in oceanic environments. The solid line represents the relationship found in Salt Ponds. The small dotted line represents the relationship found in the ice. **C**- Relationship between VLP abundance and specific bacterial growth rate in fresh water environments. Data from karstic lakes have been separated into integrated values from the epilimnion (Epi), metalimnion plus hypolimnion (Meta-Hypo).

Table 6.4. Relationship between VLP abundance and heterotrophic bacterial production (cells L^{-1} d^{-1}) in different environments (log VLP = (a ± SE) + (b ± SE) log HBP)

Data set	a Intercept	b Slope	r^2	n	p
Total Data	2.747 ± 0.316	0.515 ± 0.036	0.413	289	<0.001
Marine	2.966 ± 0.322	0.460 ± 0.043	0.444	183	<0.001
Salt Ponds				19	0.444
Ocean	4.037 ± 0.487	0.321 ± 0.061	0.154	152	<0.001
Ice	-13.644 ± 4.719	1.975 ± 0.446	0.662	12	<0.001
FW				106	0.574
Karstic Lakes[a]	3.320 ± 1.251	0.482 ± 0.133	0.726	7	0.015
Canadian lakes	3.649 ± 1.021	0.475 ± 0.114	0.035	18	0.035
Danube River	5.296 ± 1.021	0.216 ± 0.113	0.148	23	0.070

Table 6.5. Relationship between VLP abundance and growth rate (μ, d^{-1}) in different environments (log VLP = (a ± SE) + (b ± SE) log μ).

Data set	a Intercept	b Slope	r^2	n	p
Total Data				289	0.798
Marine	7.066 ± 0.081	0.468 ± 0.103	0.103	183	<0.001
Salt Ponds	7.907 ± 0.107	-1.279 ± 0.241	0.624	19	<0.001
Ocean	6.820 ± 0.082	0.352 ± 0.098	0.080	152	<0.001
Ice	9.641 ± 0.452	-4.006 ± 0.752	0.753	12	<0.001
FW	7.701 ± 0.053	-0.266 ± 0.054	0.189	106	<0.001
Karstic Lakes[a]				7	0.233
Canadian lakes				18	0.387
Danube River				23	0.196

[a] Integrated data from the epilimnion

Bacterial heterotrophic production. Bacterial heterotrophic production was not as good a predictor of VLP abundance as bacterial abundance (Table 6.4, Fig. 6.6A). Among the marine environments, only in the ice did bacterial production seem to be well correlated with VLP abundance. Although in the ocean the regression was statistically significant, the r square was very low (Table 6.4, Fig. 6.6B). In fresh water the general relationship was not significant except for karstic lakes (Table 6.4, Fig. 6.6C).

Bacterial specific growth rate. VLP abundance was not significantly related with Bacterial specific growth rate in the global data set (Fig. 6.7A, Table 6.5). In the environments, where this relationship was significant, it explained a low percentage of VLP variability (Fig. 6.7), except for the ice and the solar salterns (Table 6.5, Fig. 6.7B). In spite of the low percentage of variability explained by specific growth rate (Table 6.5, Fig. 6.7A), the regressions for marine and fresh water environments were significant and the slopes were negative in the case of freshwater systems (Table 6.5, Fig. 6.7A). This suggests that less VLP were present when bacteria were more active.

Bacterivory. Bacterivory, expressed as ingested bacteria mL^{-1} d^{-1}, was a significant predictor of viral abundance ($p < 0.004$, $n = 40$), but only explained 21 % of the variability in VLP abundance. However, for each particular environment where bacterivory was measured (Table 6.1), none of the regressions with VLP abundance was significant ($p = 0.144$). When we used grazing rate (d^{-1}) as independent variable, the regression was not significant either ($p = 0.08$, $n = 40$).

As mentioned before, in some of the sampled environments the presence or absence of bacterivory was determined by extreme physico-chemical characteristics of the medium. Salinity was determinant in the solar salterns (Chapter 3) and oxygen and sulfide concentrations in some anaerobic lakes (karstic lakes and Lake Saelenvatnet). Thus, in these environments we could treat bacterivory as a categorical variable with three levels: 1, when bacterivory was detectable or at least presence of bacterivores was reported; in this level we included the epilimnia of stratified lakes and the ponds with low salinity (<150 ‰). 2, when bacterivory was very low, in this level we included the metalimnia of the stratified lakes and ponds with intermediate values of salinity (150 ‰-250 ‰). 3, when bacterivory was not detectable, in this level we included the saltiest ponds (>250 ‰) and the hypolimnia of the stratified lakes. An analysis of variance (ANOVA) for each environment separately, revealed that VLP abundance was not significantly different for the three levels of bacterivory in stratified lakes with anaerobic hypolimnia ($p = 0.809$, $n = 72$, Fig. 6.8A), but it was significantly different in the solar salterns ($p < 0.029$, $n = 19$, Fig. 6.8B).

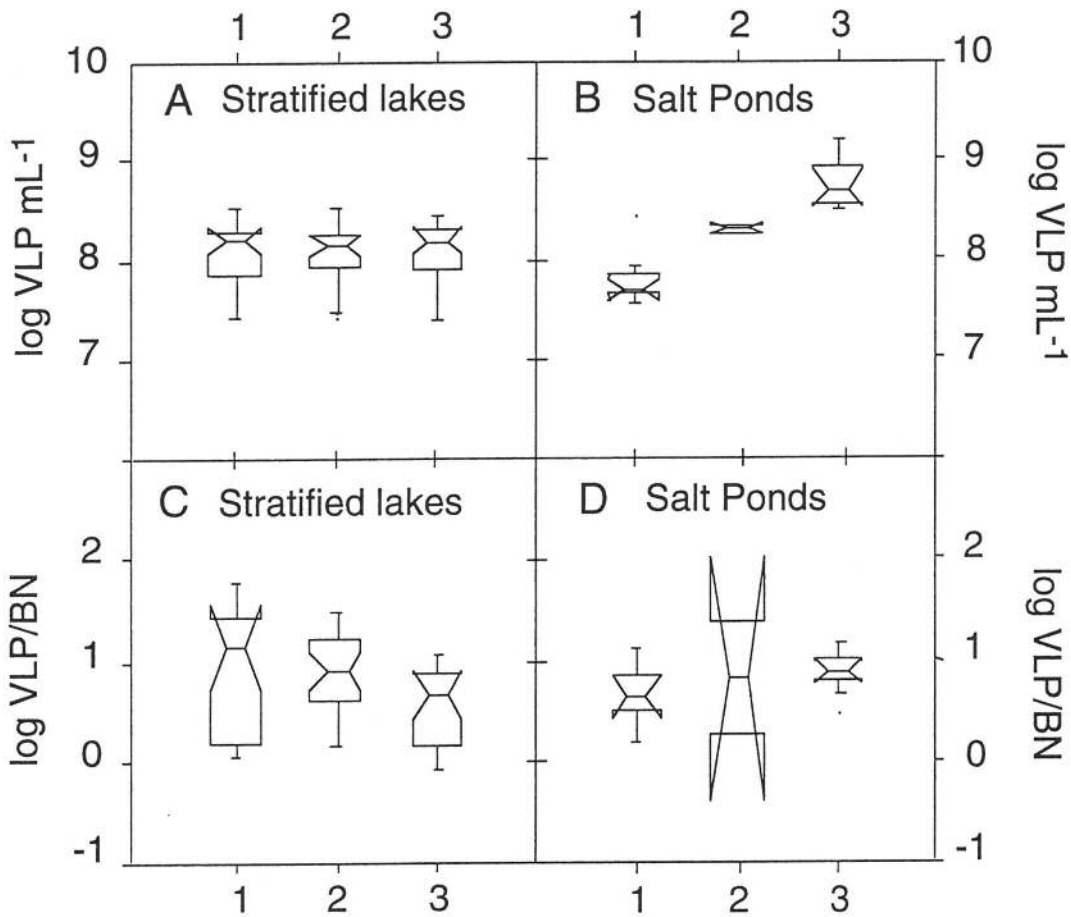


Fig. 6.8. Box-and-whiskers plots of VLP abundance (log VLP mL^{-1}, **A**, **B**) and VLP/BN ratio (log VLP/BN, **C**, **D**) in stratified anaerobic lakes and salt ponds. For each environment data were grouped according to different bacterivory pressure (See text): 1- Presence of bacterivores, 2- Low impact of bacterivores, 3- Bacterivory undetectable.

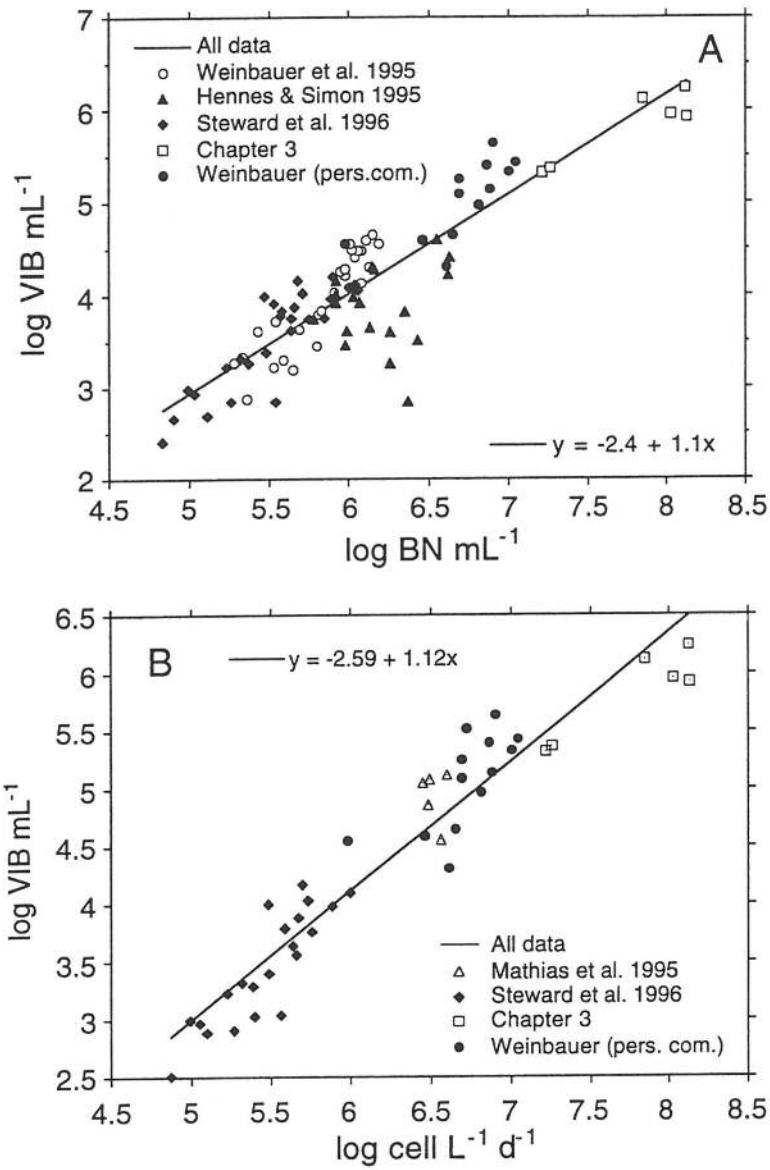


Fig. 6.9. **A**- Relationship between bacterial abundance (log BN mL^{-1}) and visibly infected bacteria (log VIB mL^{-1}). Solid line represents the relationship found with the whole data set. **B**- Relationship between bacterial heterotrophic production (log cell L^{-1} d^{-1}) and visibly infected bacteria (log VIB mL^{-1}). Solid line represents the relationship found with the whole data set.

A Tukey post hoc test indicated that the significant differences occurred between levels 1 and 3 ($p < 0.027$) in this environment.

In the stratified lakes we observed that the VLP/BN ratio was lower where bacterivory was undetectable than in places with measurable bacterivory. An ANOVA revealed that VLP/BN was significantly different among the three levels of bacterivory ($p < 0.001$, Fig. 6.8C, $n = 72$) in stratified lakes. A Tukey post hot test revealed than the significant differences were between levels 1 and 3 ($p < 0.006$) and between levels 2 and 3 ($p < 0.021$). Levels 1 and 2 were not significantly different ($p = 0.726$). In the solar salterns, the VLP/BN ratio was not significantly different for the three levels ($p = 0.052$, $n = 19$, Fig. 6.8D).

Relationships between visibly infected bacteria (VIB) and different variables.

Bacterial abundance was significantly correlated with the total number of VIB ($p < 0.001$, $r^2 = 0.779$, $n = 93$, Fig. 6.9A), but not with the percentage of VIB ($p = 0.500$, $n = 93$). The percentage of VIB did not seem to increase linearly with bacterial numbers, except for the samples from the Adriatic Sea.

The total number of VIB was significantly correlated with heterotrophic bacterial production ($p < 0.001$, $r^2 = 0.775$, $n = 31$, Fig. 6.9B). The relationship between heterotrophic bacterial production and percentage of VIB was not statistically significant ($p = 0.072$, $n = 37$). Bacterial specific growth rate was neither significantly correlated with the total number of infected cells ($p = 0.873$, $n = 31$) nor with the percentage of VIB ($p < 0.1121$, $n = 31$).

Discussion

General Relationships

Bacterial abundance was the variable that explained VLP abundance better when considering the whole data set (66 % of the variability). A significant relationship between viral and bacterial abundance has been found in several studies from particular environments (Boehme *et al.* 1993, Cochlan *et al.* 1993, Paul *et al.* 1993, Jiang & Paul 1994, Weinbauer & Peduzzi 1995), suggesting indirectly that bacteria are the most abundant potential hosts for the viruses. The fact that the strength of this relationship is maintained over the wide range of ecosystems included in the present study seems to confirm this suggestion.

The percentage of variance explained was lower than that found by Maranger & Bird (1995). The slopes found in both studies were also significantly different ($t_{0.05}$, 1.960, $p < 0.001$). In fact, Maranger & Bird (1995) found than chlorophyll a was a better predictor of viral abundance than bacterial abundance. In our study, viral abundance also showed a significant regression with chlorophyll a, but the regression coefficient was very low ($r^2 = 0.385$) indicating that algal viruses were probably of small importance in the data base. The two studies should be compared with caution because of the new data, especially from fresh water environments, reported since 1995. The data used by Maranger & Bird (1995) were either from the photic zone or from the upper 30 m, where algal and cyanobacterial viruses could be more abundant than in deeper layers. This could be the reason why in their study VLP abundance was highly correlated with chlorophyll .

In our study, viral abundance also showed significant regressions with heterotrophic bacterial production. Again, this regression explained less than 45 % of the variability in VLP abundance. Using chlorophyll a in multiple regressions did not increase significantly the percentage of variability explained just with bacterial abundance. Inclusion of temperature and bacterial abundance, or heterotrophic bacterial production and bacterial abundance, as independent variables did increase the percentage of variability explained slightly ($p < 0.001$, $r^2 = 0.740$, $n = 296$, for bacterial abundance and temperature, $p < 0.001$, $r^2 = 0.748$, $n = 289$, for bacterial abundance and heterotrophic bacterial production).

Uncoupling between bacterial and viral abundance

The slope of the regression between viral and bacterial abundance was significantly lower than 1. This indicates that viral abundance did not increase at the same rate as bacterial abundance. As a consequence, the VLP/BN ratio tended to decrease when bacterial abundance increased. Thus, for a bacterial abundance of 10^5 bacteria per mL, the VLP/BN ratio would be 10.8, while for a bacterial abundance of 10^7 bacteria per mL, this ratio would be 7.4. Before discussing the possible factors responsible for this uncoupling we should consider two points: First, although the slope of this relationship was significantly different from 1, this difference was small and perhaps when more data become available, the slope would change. Second, part of the VLP assemblage could be phytoplankton viruses. At the moment, however, there is no way of knowing what proportion of the VLP abundance corresponds to phages or to phytoplankton viruses. Thus, we will assume that most of the VLP abundance corresponded to bacteriophages. Leaving these caveats aside, several reasons could explain the uncoupling in the empirical relationship between VLP and bacterial abundance found in the present study.

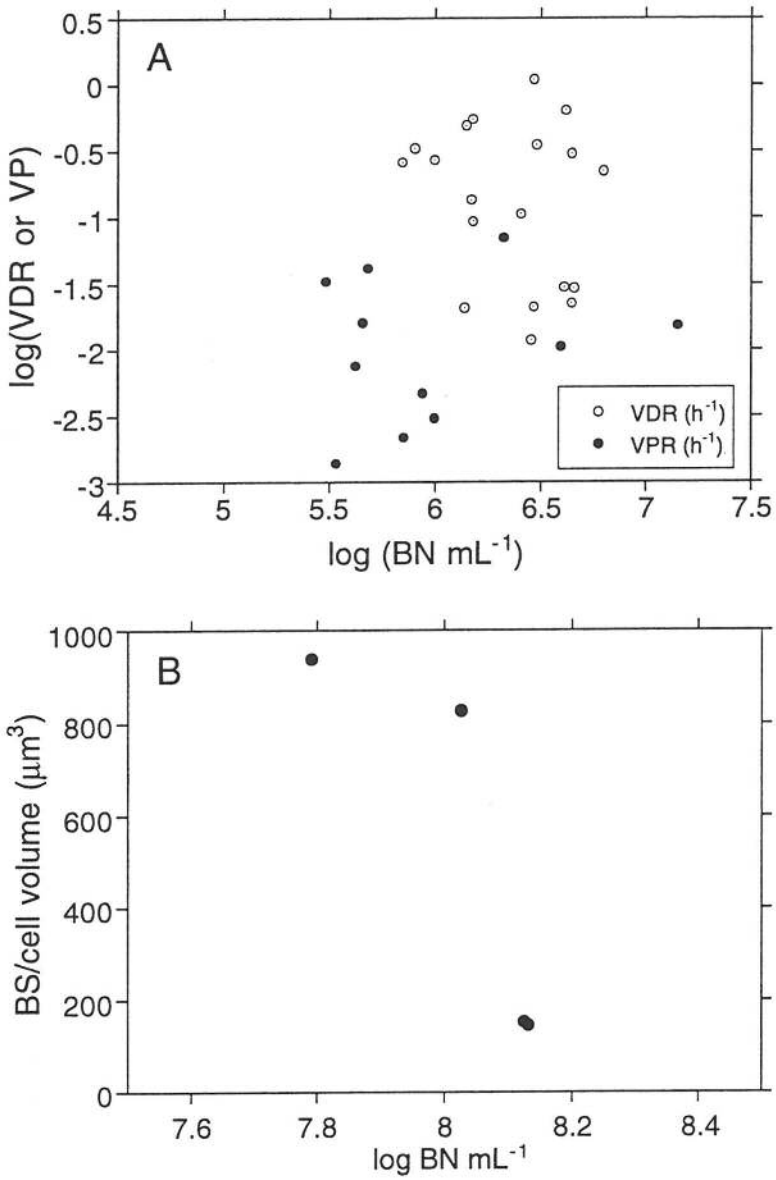


Fig. 6.10. **A**- Relationship between viral decay rate (VDR) or viral production rate (VPR) with bacterial abundance. **B**- Relationship beween efficience of viral infection: Burst size per unit of volume (BS/V) in the samples from the salterns.

1. VLP abundance in an environment is the result of the balance between VLP produced and VLP lost. In a steady-state situation production and losses are in equilibrium, thus the rate of either process over the abundance would give the VLP turnover in an environment. The uncoupling found between VLP and bacterial abundance could be explained if VLP turnover increased with increasing bacterial abundance. In this case, the VLP/BN ratio would tend to be lower at high bacterial abundance because VLP would remain relatively less time as free particles than at low bacterial abundance. Several of the data collected in the present study correspond to steady-state situations. Therefore, we checked, if either viral production or decay rates increased with increasing bacterial abundance (Fig. 6.10A). No significant relationship between production or decay rates and bacterial abundance was found ($p > 0.05$, Fig. 6.10A). Thus, a difference in turnover rates with bacterial abundance must be rejected as the cause of the observed slope.

2. In our data base, the number of infected cells increased proportionally to the total number of cells (the slope of the relationship was 1). If VLP/BN ratio tends to be lower when increasing bacterial abundance, the efficiency of the infection (VLP released per cell volume) should be higher at lower bacterial abundance. If this was true, the average burst size divided by the average cellular volume (BS/V) in an environment would tend to be lower with increasing bacterial abundance. Unfortunately, the only relevant data to explore this relationship between BS/V with bacterial abundance are those from the salterns (Fig. 6.10B). The data are consistent with a decreased efficiency at higher cell abundance. However, a bigger data base would be needed to confirm this hypothesis.

Marine and fresh water environments

In both marine and fresh water environments bacterial abundance was the factor that best explained variation in VLP abundance. Although the intercepts of the marine and freshwater relationships were slightly different, it seems that VLP abundance follows the same pattern with respect to bacteria in both types of environments. Maranger & Bird (1995) found significant differences in both the slopes and the intercepts between marine and fresh water environments. However, in their study, fresh water data corresponded only to some Canadian lakes. Thus, their conclusion of significant differences between marine and freshwater environments is probably due to the extremely limited freshwater data base available at the time, which showed the peculiarities of a given type of lake.

The remaining variables showed different relationships to VLP abundance in both type of systems. However, most of these relationships had very low regression coefficients. That is, the relevance of the independent variables to explain changes in VLP abundance was so little that it would be unwarranted to pay much attention to such

differences. Therefore, we conclude that VLP abundance did not follow different patterns in marine and fresh water environments.

The relationships between bacterial and VLP abundance were very similar in all the marine environments (ocean, salt ponds and ice). The remaining variables, however, showed completely different relationships in the different types of marine systems. The regression coefficients were always high in the ice samples and very low in the oceanic samples. In the solar salterns only the regression coefficient for growth rate was high. The large number of oceanic studies with respect to other marine environments gave to this group of data a strong impact on the general regressions. Thus, the general relationships were heavily weighted towards oceanic environments. Since these environments are the most extensive in nature, however this is not necessarily bad.

In an attempt to improve the relationships found for each particular environment, we included the variables in multiple regressions. Only in oceanic samples did the relationship improve using either chlorophyll a and bacterial abundance ($p < 0.001$, $r^2 = 0.607$, $n = 211$), or heterotrophic bacterial production and bacterial abundance ($p < 0.001$, $r^2 = 0.645$, $n = 152$) as independent variables.

Bacterial abundance explained 50 % of the variability of VLP abundance in fresh water environments. This relationship, however was very variable among the different types of freshwater systems and not always significant. Since the data sets for each type of freshwater habitat were limited (around 20 points at most) the generally positive relationship between bacteriophage and bacteria could be hidden by time lags between bacterial and viral blooms in particular habitats. Thus, within a specific data set the relationship could be negative or non significant in the short term, as Maranger & Bird (1995) found for their summer samples of Canadian lakes. The other variables investigated explained a very small percentage of VLP abundance or were not significant.

In spite of having less studies for fresh water than for marine systems, we think the results found here are representative because of the wide range of variability in the degree of trophy, latitude and sampling season of the fresh water bodies included. This heterogeneity among fresh water systems could be the reason why the percentage of variability of VLP abundance explained with all the variables analysed was always lower than in marine systems. Another reason could be the fact that about 10 % of the data corresponded to anaerobic layers of lakes (karstic lakes, Lake Saelenvatnet) and the relationship between VLP and bacteria could be different in these habitats. This feature could bias the relationship for fresh water systems. When we calculated the relationships between VLP abundance and the other variables in fresh waters without the data

corresponding to anaerobic layers, however, we did not find any increase in the variability of VLP abundance explained. The slopes and the intercepts found with the reduced data set were not different from those presented in the results (data not shown). Thus, data from anaerobic environments did not seem to influence the general relationships.

Anaerobic water bodies and solar salterns

One of the objectives of our work was to study environments with low impact of bacterivory in order to see whether the relationships between viruses and hosts followed a similar pattern as in systems with higher bacterivory rates. High salinity ponds (>250 ‰) the anaerobic layers of stratified lakes presented some similar characteristics: high bacterial abundance ($1x10^7$-$1.35x10^8$ cells mL^{-1}), low specific bacterial growth rate (0.002-0.07 d^{-1}), large bacterial cell volume (0.10-0.96 μm^3), high VLP abundance ($1x10^8$-$1.58x10^9$ VLP mL^{-1}) and insignificant bacterivory pressure (Massana & Pedrós-Alió 1994, Chapter 3). However, there were differences in both the total VLP abundance and in the VLP/BN ratio. In solar salterns VLP abundance increased with salinity, and there were no differences in the VLP/BN ratio along the salinity gradient (Fig. 6.1G). In anaerobic lakes VLP abundance was not different among the three layers (epilimnion, metalimnion and hypolimnion) during the stratification period, but the VLP/BN ratio was lower in the hypolimnia (Fig. 6.1F). Thus, the nature of the relationship between bacteria and viruses must be different in the two types of environments despite the similarities in microbial processes pointed out above. As mentioned before VLP abundance is the result of the balance between viral production and decay. At the same time, viral production is dependent on the % VIB, the latent period and the burst size. Thus, some or all of these variables could influence the differences found between the saltiest ponds and the anaerobic lakes in the VLP/BN ratio.

In the salterns the % VIB was very low (< 5 %), viral latent period was supposed to be long because of the long doubling times of the host populations (Proctor *et al.* 1993, Chapter 3). However, the burst size was very high (above 300 viruses released per bacteria). Burst size has been reported to be influenced by the volume of the hosts (Weinbauer & Peduzzi 1994). In agreement with this, the relationship between biovolume and VLP abundance in salt ponds improved the relationship found between bacterial and VLP abundance. Thus, the VLP/BN ratio found in the salterns could reflect the high burst sizes despite the low viral impact on the total bacterial mortality. In anaerobic lakes we would also expect long latent periods because of the long generation times of the cells.

However, we could not count infected bacteria with the method of Weinbauer & Peduzzi (1994), because bacteria appeared opaque under TEM. As a consequence we could not determine the burst size. However, the inclusion of biovolume did not improve the relationship found between VLP and bacterial abundance. Thus, the low VLP/BN found in the anaerobic layers could be the consequence of a small burst size. We suggest that anaerobiosis reduces burst sizes despite the high volume of the bacterial hosts. Anaerobiosis and temperature have been shown to influence the replication of at least two bacteriophages in laboratory experiments (Zachary 1977). In the latter study, lower temperatures resulted in longer latent periods and reduced burst sizes. Replication under anaerobic conditions resulted in longer latent periods. However, the burst sizes could be reduced or increased, depending on the phage. Temperature is highly variable among the anaerobic lakes used in this study, with values from 3º C to 23º C. An ANOVA of the variability of VLP/BN observed in anaerobic lakes with both temperature and oxygen as factors, increased to 50 % the percentage of explanation from that when only oxygen was used as a factor. Our results are in agreement with Zachary's experiment, but data about the percentage of infection and burst sizes of viral hosts in these lakes are needed to substantiate a conclusion about viral production under anaerobiosis.

In summary, bacterial abundance is the variable that better explains the variability on VLP abundance across a huge range of aquatic environments with a similar relationship in most of them. 50 % of the variability in VLP numbers is explained by bacterial numbers. No differences were found between marine and freshwater environments for the slope of this relationship. Bacterial and VLP abundance showed a slight uncoupling that could be due to a low efficiency (burst size per unit of volume) of the viral infection at high bacterial abundance. In particular environments such as the anaerobic layers of stratified lakes, burst size could be influenced by the physico-chemical characteristics of the medium. Thus, burst size appear as a key variable in the dynamics of viral infection in nature. Its measurement in as wide a range of habitats as possible seems a promising source of information.

Conclusions

Les conclusions d'aquest estudi sobre l'abundància dels virus i el seu impacte en les comunitats bacterianes dels sistemes planctònics, són les següents:

1. En situacions on la comunitat bacteriana presenta taxes de creixement elevades com en el cas de proliferacions de fitoplàncton, l'atac víric és responsable d'un percentatge de la mortalitat bacteriana total similar a l'ocasionat per la bacterivoria. Les màximes taxes d'activitat dels virus i dels bacterívors no coincideixen en el temps, les primeres es donen un cop la proliferació ha finalitzat i les segones en el moment en que aquesta es troba en el seu màxim.

2. En ambients caracteritzats per un fort gradient físico-químic, l'abundància de les VLP experimenta grans variacions al llarg del mateix. En situacions on les comunitats bacterianes presenten taxes de creixement baixes malgrat acumular una gran biomassa, com el cas de les salines costaneres, els virus són responsables d'un percentage baix de la mortalitat bacteriana total respecte als bacterívors. Els virus, malgrat trobar-se en gran abundància, tenen un efecte insignificant sobre la mortalitat bacteriana total. La predicció inicial de què l'impacte dels virus augmentaria en disminuir la diversitat dels hospedadors no es compleix en aquest sistema, degut a l'efecte de les baixes taxes de creixement.

3. L'impacte dels virus sobre la comunitat bacteriana heterotròfica en el cas d'un sistema oligotròfic com la Mar Mediterrània, és troba en els límits de detecció de la metodologia actual. Això suggereix que els virus exerceixen un baix control sobre la biomassa bacteriana en aquests ambients. Aquesta conclusió es pot extendre possiblement a la major part dels oceans oligotròfics de zones temperades.

4. En el cas de l'ecosistema Antàrtic, l'activitat dels virus sembla estar relacionada amb la quantitat de material particulat del medi, essent aquest més elevat en els llocs més eutròfics del mateix. L'atac dels virus sobre la comunitat bacteriana, en aquest cas, sembla ser més important que la bacterivoria, que podria estar fortament inhibida per les baixes temperatures. Malgrat això, la quantificació del seu impacte necessita ser contrastada amb algun altre mètode per poder donar una conclusió més rotunda.

5. La quantificació de l'abundància de les VLP ens dóna poca informació del seu efecte sobre la comunitat hospedadora. Malgrat això, mitjançant les relacions amb altres variables del sistema, l'abundància de bacteris sembla ser la variable que explica amb un major percentatge la variabilitat de l'abundància de les VLP. El grau d'acoblament entre les abundàncies d'ambós grups d'organismes sembla estar condicionat pel tamany d'explosió.

6. El tamany d'explosió ha estat identificat com una de les variables més importants en la dinàmica dels virus en medis naturals. La seva determinació en diferents hàbitats és una línia de recerca fonamental pel futur.

7. La hipòtesi de partida sobre la que es va planificar aquest treball no ha pogut ser totalment contrastada. Malgrat això les conclusions anteriors indiquen que l'impacte dels virus sobre les comunitats bacterianes heterotròfiques en ambients aquàtics va més encaminada al manteniment de la diversitat de les mateixes que al control de la seva abundància total. Al mateix temps, l'acció dels virus en la natura sembla dependre no tan sols de la quantitat i diversitat d'hospedadors sinò també de la taxa de creixement dels mateixos. Els virus seran temporalment responsables d'un percentatge elevat de la mortalitat bacteriana, en els casos on es donen les tres condicions favorables per a la seva multiplicació (elevat nombre d'hospedadors, poques espècies diferents i taxes de creixement elevades).

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Anex

Base de dades utilitzades en el capítol 6

Estudi	Data	LLOC	Z (m)	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
Aquesta tesi	ago-94	La Trinitat			mg/L	mg/L	μg/L				μgC/L/d	cell/L/d			cell/L/d
Salines		TR1		32	37.50	21.2	1.12	1.00E+07	4.40E+07	1.30E+04	355.37	1.37E+10	0.862	0.804	1.15E+06
		TR2		32.6	64.00	15.17	7.30	9.60E+06	4.40E+07		931.32	1.63E+10	0.991	0.699	9.20E+06
		TR3		34	90.00	9.71	19.84	8.52E+06	5.20E+07	8.16E+04	1065.08	2.24E+10	1.288	0.538	1.10E+07
		TR7		34	149.00	12.54	5.82	1.64E+07	6.07E+07	1.07E+05	1121.69	4.49E+10	1.318	0.884	1.89E+07
		TR5		31	182.50	10.53	19.83	1.80E+07	2.40E+08		687.08	7.06E+09	0.331	2.094	1.09E+06
		TR4		36.7	250.00	4.86	2.32	6.17E+07	6.35E+08		669.50	1.95E+10	0.246	1.964	
		TR6		33	297.50	5.06	5.82	1.06E+08	8.35E+08		776.11	1.61E+10	0.141	3.349	
		TR8		37	370.00	5.26	2.00	1.33E+08	1.00E+09		912.70	2.75E+10	0.188	3.687	
		TR9		37.2	372.50	5.67	1.34	1.35E+08	1.58E+09		690.64	2.63E+10	0.178	3.894	
	ago-94	Sta Pola													
		SP1		35.5	384.00	0.89	2.05	7.17E+07	1.00E+08		125.01	4.37E+10	0.48	1.46	
		SP2		37.0	352.00	1.12	1.14	3.50E+07	1.00E+08		109.54	3.83E+10	0.74	0.94	
		SP3		29.0	168.00	4.09	0.78	9.05E+06	4.00E+07		69.31	2.42E+10	1.30	0.53	
		SP4		32.0	279.00	2.10	2.51	6.90E+07	6.10E+07		57.84	2.02E+10	0.26	2.70	
		SP5		31.5	122.00	3.94	4.56	2.81E+07	7.78E+06		60.24	2.10E+10	0.56	1.24	9.93E+06
		SP6		30.5	38.00	11.02	1.04	9.83E+06	1.50E+07		72.18	2.52E+10	1.27	0.55	1.12E+07
		SP7		36.0	318.00	1.15	2.00	5.86E+07	6.90E+07		69.38	2.42E+10	0.35	2.00	
		SP8		40.5	251.30	0.03	1.06	1.13E+08	6.00E+07		123.04	4.30E+10	0.32	2.15	
		SP9		32.0	195.00	0.90	0.78	1.01E+08	3.60E+07		106.21	3.71E+10	0.31	2.22	9.41E+06
		SP10		30.5	78.00	6.70	3.76	1.36E+07	6.32E+06		54.64	1.91E+10	0.88	0.79	9.65E+06
ECOANTAR 94	ene-94	Mar de Weddell													
Transecte		0W7	0.0	-1.410	33.51	3.35	3.02	2.19E+05	4.17E+07	2.26E+02	1.34	1.04E+08	0.389	0.878	8.46E+04
		10W7	10.0	-1.370	33.69	3.08	3.02	1.77E+05	1.49E+07	5.50E+01	1.98	1.55E+08	0.628	0.595	
		20W7	20.0	-1.549	34.04	2.84	2.33	2.21E+05	5.91E+07	6.88E+01	1.01	7.85E+07	0.304	1.083	
		40W7	40.0	-1.722	34.33	2.55	0.67	2.51E+05	5.18E+06	5.70E+01	0.33	2.56E+07	0.097	3.028	
		50W7	50.0	-1.725	34.39	2.48	0.55	1.38E+05	1.82E+07	5.90E+01	0.15	1.16E+07	0.080	3.609	
		60W7	60.0	-1.731	34.40	2.41	0.35	1.68E+05	1.11E+07	4.31E+01	0.20	1.56E+07	0.089	3.287	4.50E+04
		0W119	0.0	-1.269	33.56	3.25	2.33	2.84E+05	4.40E+07	1.33E+03	1.26	9.80E+07	0.297	1.106	
		10W119	10.0	-1.267	33.56	3.24	2.40	2.36E+05	8.75E+06	8.57E+02	3.00	2.34E+08	0.688	0.553	
		20W119	20.0	-1.271	33.56	3.21	2.47	4.83E+05	5.59E+06	2.11E+02	1.26	9.86E+07	0.186	1.668	
		30W119	30.0	-1.416	34.01	2.89	2.74	2.33E+05	7.66E+06	3.84E+02	1.28	1.00E+08	0.358	0.943	
		50W119	50.0	-1.468	34.10	2.83	1.56	1.88E+05	2.50E+06	8.64E+02	0.32	2.47E+07	0.123	2.419	
		80W119	70.0	-1.537	34.26	2.62	0.71	1.65E+05	2.50E+06	6.77E+02	0.27	2.12E+07	0.121	2.469	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
		0W8	0.0				2.06	1.15E+06	2.50E+06	1.97E+03	2.08	1.62E+08	0.132	2.274	8.95E+04
		10W8	10.0	-0.349	34.00	3.12	1.99	7.61E+05		1.72E+03	5.11	3.98E+08	0.421	0.823	
		20W8	20.0	-0.348	34.00	3.19	1.91	8.26E+05		9.36E+02	2.80	2.18E+08	0.234	1.356	
		40W8	40.0	-0.345	34.03	3.13	1.69	7.55E+05	3.77E+06	1.06E+03	1.84	1.43E+08	0.174	1.769	2.29E+05
		50W8	50.0	-0.397	34.08	2.98	2.14	6.83E+05		1.95E+03	3.72	2.90E+08	0.354	0.951	
		70W8	70.0	-1.413	34.27	2.60	0.23	6.83E+05			0.87	6.76E+07	0.094	3.108	
		0W9	0.0	-0.371	34.44	2.55	0.32	2.33E+05	3.00E+06	1.50E+03	0.06	4.57E+06	0.019	14.338	
		10W9	10.0	-0.370	34.44	2.51	0.23	2.07E+05	4.16E+06	7.49E+02	0.19	1.49E+07	0.070	4.144	
		21W9	20.0	-0.377	34.44	2.60	0.29	1.41E+05	7.33E+06	1.55E+03	0.30	2.33E+07	0.153	1.987	
		40W9	40.0	-0.381	34.44	2.51	0.27	2.52E+05	8.42E+06	6.01E+02	0.30	2.34E+07	0.089	3.287	
		50W9	50.0	-0.400	34.45	2.52	0.26	1.88E+05	7.72E+06		0.27	2.07E+07	0.104	2.824	
		70W9	70.0	-0.403	34.45	2.51	0.28	2.00E+05	5.86E+06		0.26	2.01E+07	0.096	3.060	
		0W10	0.0	-0.258	34.41	2.75	0.82	2.83E+05	3.70E+06	3.60E+03	0.14	1.10E+07	0.038	7.409	1.72E+05
		10W10	10.0	-0.269	34.41	2.71	0.76	2.64E+05	3.12E+06	1.43E+03	0.02	1.33E+06	0.005	137.903	
		20W10	20.0	-0.278	34.41	2.75	0.65	1.21E+05	5.58E+06	2.15E+03	0.22	1.71E+07	0.132	2.269	
		30W10	30.0	-0.297	34.41	2.70	0.69	2.67E+05	5.63E+06	2.15E+03	0.29	2.28E+07	0.082	3.543	1.98E+05
		50W10	50.0	-0.398	34.42	2.63	0.67	1.07E+05	6.68E+06	2.43E+03	0.28	2.17E+07	0.185	1.677	
		70W10	70.0	-0.519	34.43	2.59	0.58	7.97E+04	1.15E+06	2.01E+03	0.09	7.17E+06	0.086	3.384	
		0W11	0.0	0.647	34.13	2.71	0.91	1.78E+05	1.50E+07	3.92E+03	0.11	8.27E+06	0.045	6.246	0.00E+00
		20W11	20.0	0.646	34.13	2.72	0.95	1.95E+05	2.82E+06	3.46E+03	0.09	7.40E+06	0.037	7.570	
		30W11	30.0	0.612	34.14	2.67	1.00	2.03E+05	2.04E+06	7.26E+02	0.08	6.25E+06	0.030	9.261	
		40W11	40.0	0.591	34.16	2.67	0.87	1.67E+05	2.13E+06	9.33E+02	0.26	2.04E+07	0.115	2.582	0.00E+00
		50W11	50.0	0.537	34.20	2.59	0.78	3.49E+05	3.28E+06	3.23E+03	0.12	9.66E+06	0.027	10.259	
		70W11	70.0	0.520	34.23	2.60	0.80	3.13E+05	2.66E+06	1.58E+03	0.21	1.61E+07	0.050	5.682	
		0W12	0.0	0.888	34.19	2.80	0.78	4.55E+05	3.27E+06	4.76E+03	0.61	4.75E+07	0.099	2.959	
		10W12	10.0	0.890	34.20	2.78	1.02	3.82E+05	1.34E+06	2.14E+03	0.49	3.78E+07	0.094	3.105	
		20W12	20.0	0.830	34.24	2.69	0.65	4.47E+05	7.00E+06	4.55E+03	1.26	9.79E+07	0.198	1.575	
		30W12	30.0	0.635	34.26	2.59	0.69	5.04E+05	2.18E+06	4.75E+03	0.60	4.66E+07	0.088	3.301	
		50W12	50.0	0.409	34.30	2.53	0.59	4.65E+05	2.94E+06	4.96E+03	1.15	8.96E+07	0.176	1.748	
		81W12	70.0	0.394	34.32	2.12	0.21	1.74E+05	2.50E+06	7.70E+02	0.01	1.16E+06	0.007	104.608	
		0W13	0.0	2.429	33.85	2.81	0.23	6.21E+05	2.26E+06	4.57E+03	0.38	3.00E+07	0.047	6.028	1.51E+05
		20W13	20.0	2.375	33.85	2.80	0.17	5.96E+05	9.77E+05	3.31E+03	0.54	4.19E+07	0.068	4.238	
		40W13	40.0	2.251	33.85	2.76	0.26	6.16E+05	4.41E+05	5.07E+03	1.02	7.92E+07	0.121	2.464	
		60W13	60.0	0.459	33.96	2.92	0.95	4.47E+05	8.80E+05	1.08E+04	0.55	4.32E+07	0.092	3.173	
		70W13	70.0	0.075	33.97	2.92	1.24	5.26E+05	6.39E+05	7.63E+03	0.87	6.77E+07	0.121	2.462	2.09E+05
		80W13	80.0	-0.284	33.95	2.90	1.30	3.96E+05	1.95E+06	5.82E+02	0.80	6.25E+07	0.147	2.065	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
		0W1	0.0	-0.486	33.97	3.14	0.67	4.22E+05	8.88E+06	7.52E+02	0.59	4.60E+07	0.103	2.852	
		10W1	10.0	-0.490	33.97	3.13	0.58	4.66E+05	7.85E+06	6.70E+02	2.27	1.77E+08	0.322	1.030	
		30W1	30.0	-0.655	34.17	3.06	0.82	5.11E+05	7.00E+06	1.20E+03	1.56	1.22E+08	0.214	1.470	
		50W1	50.0	-1.197	34.34	2.67	1.62	2.48E+05	9.40E+06	6.96E+02	0.78	6.04E+07	0.218	1.445	
		80W1	80.0	-1.654	34.37	2.54	0.68	2.30E+05	2.35E+06	2.65E+02	0.20	1.59E+07	0.067	4.311	
		100W1	100.0	-1.691	34.40	2.46	0.61	2.04E+05	2.80E+06	1.72E+02	0.22	1.69E+07	0.079	3.653	
		Mediterrani													
FRONTS 94	jun-94	Sof 2	5.0	23.3	38.06		0.07	2.12E+05			2.48	1.68E+08	0.560	0.674	
	Mar obert	Sof 2	25.0	20.43	38.05		0.04	2.83E+05	7.85E+06		1.42	9.67E+07	0.281	1.209	
		Sof 2	50.0	15.95	38.16		0.21	5.60E+05	5.03E+06		1.62	1.10E+08	0.171	1.884	
		Sof 2	70.0	13.43	38.13		0.53	3.36E+05	2.80E+06		2.24	1.52E+08	0.357	0.983	
		Sof 2	90.0	13.17	38.26		0.17	1.31E+05	5.84E+06		0.93	6.29E+07	0.374	0.945	
		Sof 2	200.0	13.25	38.50			5.17E+04	5.96E+06		0.24	1.59E+07	0.256	1.308	
		Sof 4	5.0	25.1	38.14		0.07	3.46E+05	9.70E+06		2.39	1.62E+08	0.367	0.960	
		Sof 4	20.0	18.54	38.19		0.07	2.69E+05	9.50E+06		1.30	8.80E+07	0.270	1.252	
		Sof 4	40.0	14.68	38.16		0.41	3.52E+05	1.14E+07		1.43	9.72E+07	0.232	1.427	
		Sof 4	55.0	13.47	38.29		0.34	3.34E+05	1.02E+07		0.88	6.00E+07	0.157	2.032	
		Sof 4	75.0	13.16	38.35		0.09	9.53E+04	6.79E+06		0.72	4.89E+07	0.396	0.899	
		Sof 4	200.0	13.35	38.52		0.02	5.62E+04	6.60E+06		0.08	5.52E+06	0.089	3.453	
VARIMED 95	jun-95	T1	5.0	18.77	37.93	6.357	0.97	7.23E+05	1.00E+07	1.69E+03	4.06	2.76E+08	0.309	1.113	3.29E+05
Transecte	costa	T1	20.0	15.5	37.94	6.595	0.33	3.83E+05	2.44E+07		0.64	4.37E+07	0.103	3.019	
		T1	40.0	14.23	37.97	6.479	0.64	4.04E+05			1.99	1.35E+08	0.275	1.232	
		T1	50.0	13.54	37.99	6.23	0.28	4.54E+05	1.76E+07	1.08E+03	1.30	8.82E+07	0.169	1.901	5.82E+05
		T1	60.0	13.43	38.01	5.98	0.19	3.08E+05	1.00E+07		0.85	5.77E+07	0.164	1.958	
		T1	67.0	13.41	38.01	5.773	0.22	2.56E+05	1.84E+07		0.58	3.95E+07	0.136	2.312	
		T2	5.0	19.25	37.83	5.715	0.13	3.93E+05	2.35E+07		2.09	1.42E+08	0.294	1.160	
		T2	30.0	15.02	37.88	6.564	0.28	5.42E+05	1.76E+07		1.66	1.13E+08	0.180	1.794	
		T2	50.0	14.14	37.94	6.47	0.95	7.59E+05	2.00E+07		1.41	9.58E+07	0.113	2.756	
		T2	60.0	13.68	37.98	6.276	0.57	4.14E+05	2.44E+07		0.74	5.04E+07	0.109	2.850	
		T2	90.0	13.13	38.10	5.922	0.10	1.64E+05	1.35E+07		0.88	5.98E+07	0.297	1.149	
		T2	200.0	13.03	38.24	5.684	0.03	2.46E+05	1.08E+07		1.05	7.12E+07	0.242	1.375	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	Front	T3	5.0	18.87	38.00	5.696	0.14	2.45E+05	1.98E+07		0.89	6.07E+07	0.211	1.556	
		T3	20.0	16.58	37.96	6.208	0.21	2.91E+05	2.16E+07		1.14	7.74E+07	0.225	1.471	
		T3	40.0	14.73	38.08	6.662	0.04	4.78E+05	1.59E+07		0.75	5.11E+07	0.097	3.196	
		T3	60.0	13.82	38.10	6.46	0.09	5.09E+05	1.17E+07		2.21	1.50E+08	0.247	1.353	
		T3	80.0	13.26	38.15	5.829	0.21	2.35E+05	7.49E+06		0.22	1.52E+07	0.059	5.072	
		T3	200.0	13.11	38.39	5.286	0.02	1.72E+05	4.46E+06		0.25	1.72E+07	0.091	3.381	
		T4	5.0	19.38	38.12	5.568	0.10	2.58E+05	9.98E+06		0.51	3.44E+07	0.119	2.623	
		T4	20.0	16.22	37.92	6.302	0.33	4.74E+05	2.33E+07		0.94	6.38E+07	0.120	2.605	
		T4	40.0	14.66	38.12	6.691	1.47	5.13E+05	1.12E+07		0.76	5.15E+07	0.091	3.383	
		T4	60.0	13.52	38.25	6.308	0.45	4.49E+05	6.56E+06		2.25	1.53E+08	0.279	1.214	
		T4	80.0	13.24	38.31	5.844	0.37	3.91E+05	9.70E+06		0.69	4.71E+07	0.108	2.873	
		T4	200.0	13.19	38.48	4.939		1.22E+05			0.63	4.25E+07	0.285	1.191	
	Mar obert	T5	5.0	19.52	37.97	5.436	0.10	3.28E+05	1.90E+07		3.28	2.22E+08	0.495	0.746	
		T5	20.0	16.94	38.13	6.067	0.16	4.37E+05	7.70E+06		0.85	5.77E+07	0.118	2.653	
		T5	40.0	14.05	38.07	6.497	0.99	5.06E+05	8.42E+06		2.22	1.51E+08	0.248	1.345	
		T5	55.0	13.5	38.19	5.821	0.28	2.62E+05	6.16E+06		0.54	3.69E+07	0.125	2.506	
		T5	80.0	13.22	38.33	5.572	0.10	1.53E+05	6.63E+06		0.31	2.11E+07	0.123	2.554	
		T5	200.0	13.17	38.48	4.914	0.03	1.38E+05	4.45E+06		0.33	2.25E+07	0.144	2.202	
		T7	5.0	19.45	37.83	5.477	0.09	3.21E+05	1.18E+07		6.16	4.18E+08	0.804	0.505	
		T7	20.0	17.35	37.90	6.098	0.22	5.22E+05	2.18E+07		4.17	2.83E+08	0.415	0.865	
		T7	40.0	14.5	38.16	6.84	0.88	4.84E+05	2.75E+07		1.53	1.04E+08	0.186	1.746	
		T7	50.0	13.63	38.29	6.899	0.48	4.29E+05	1.47E+07		1.45	9.86E+07	0.197	1.654	
		T7	80.0	13.04	38.30	5.501	0.04	1.18E+05	5.90E+06		0.75	5.12E+07	0.344	1.013	
		T7	200.0	13.15	38.45	4.798	0.02	1.23E+05	1.77E+06		1.26	8.53E+07	0.505	0.734	
		T9	5.0	19.77	37.51	5.309	0.09	2.63E+05	1.77E+07		0.85	5.75E+07	0.188	1.721	
		T9	20.0	17.42	37.95	5.874	0.15	3.83E+05	1.15E+07		1.12	7.57E+07	0.172	1.872	
		T9	40.0	14.75	37.99	6.315	0.49	4.07E+05	2.34E+07		1.32	8.96E+07	0.190	1.713	
		T9	50.0	14.12	38.09	6.217	1.51	4.12E+05	1.00E+07		0.83	5.64E+07	0.122	2.567	
		T9	80.0	13.1	38.17	5.566	0.29	1.96E+05	1.28E+07		0.83	5.62E+07	0.241	1.383	
		T9	200.0	13.11	38.38	5.003	0.01	8.25E+04	5.25E+06		0.52	3.56E+07	0.343	1.017	
C 24 hores	dia	E2 C1.3	5.0	18.72	37.77	5.818	0.17	3.32E+05	7.88E+06	6.01E+02	7.60	5.15E+08	0.905	0.460	3.14E+05
(Mediterrani)	nit	E2 C1.3	50.0	13.78	38.04	6.05	0.64	3.15E+05	6.15E+06	1.61E+02	1.04	7.05E+07	0.192	1.690	7.99E+04
	dia	E2 C1.6	5.0	16.78	37.94	5.916	0.29	3.43E+05	1.41E+07	7.89E+02	6.34	4.30E+08	0.783	0.515	2.33E+04
	nit	E2 C1.6	50.0	14.12	38.06	6.31	0.74	6.12E+05	6.20E+06	4.28E+02	3.55	2.41E+08	0.317	1.089	1.03E+05

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	dia	E3 C1.3	5.0	19.33	37.86	5.389	0.06	2.30E+05	1.56E+07	3.00E+00	2.78	1.89E+08	0.576	0.660	3.29E+05
	nit	E3 C1.3	45.0	13.62	38.28	6.662	0.58	1.00E+05	3.10E+07		1.99	1.35E+08	0.825	0.494	5.82E+05
	dia	E3 C1.6	5.0	19.55	38.00	5.327	0.17	2.93E+05	1.70E+07	8.39E+02	2.00	1.36E+08	0.364	0.966	9.59E+04
	nit	E3 C1.6	52.0	13.86	38.29	6.755	0.66	3.00E+05	2.29E+07	7.12E+02	1.40	9.48E+07	0.262	1.284	4.14E+04
	dia	E2 C2.2	5.0	19.68	38.08	5.394	0.12	2.90E+05	1.37E+07		3.42	2.32E+08	0.564	0.671	
	nit	E2 C2.2	60.0	14.81	38.10	6.125	0.84	3.19E+05	1.63E+07		2.85	1.93E+08	0.454	0.802	
	dia	E1 C1.4	5.0	19.5	37.91	5.765	0.31	5.51E+05	2.34E+07	5.32E+02	4.07	2.76E+08	0.388	0.914	7.28E+04
	nit	E1 C1.4	50.0	14.04	37.96	6.384	0.64	6.27E+05	1.17E+07	2.10E+03	5.40	3.66E+08	0.441	0.822	1.27E+05
	dia	E3 C2.2	5.0	19.49	38.04	5.887	0.13	9.24E+04	1.34E+07	8.82E+02	0.98	6.67E+07	0.521	0.715	6.69E+04
	nit	E3 C2.2	40.0	14.21	38.22	7.16	0.52	3.15E+05	2.75E+07	8.64E+02	0.63	4.30E+07	0.122	2.575	1.28E+05
	dia	E1 C2.3	5.0	17.83	37.70	6.061	0.35	7.00E+05	2.66E+07	1.04E+03	1.45	9.83E+07	0.125	2.510	1.29E+05
	nit	E1 C2.3	40.0	14.82	37.93	6.355	0.58	5.61E+05	2.92E+07	5.90E+02	0.41	2.81E+07	0.046	6.449	
		Ciso													
perfil vertical	jul-94	C 1.00	1.0	22.25		6.76		1.90E+07	1.90E+08		90.22	5.52E+09	0.258	1.313	
		C 2.25	2.3			1.69		2.40E+07	1.52E+08		36.74	1.49E+09	0.073	4.440	
		C 2.50	2.5	17		1.85		4.14E+07	1.35E+08		66.80	2.71E+09	0.081	3.958	
		C 2.75	2.8			0.7		4.00E+07	2.30E+08		47.01	1.91E+09	0.059	5.435	
		C 5.00	5.0	10.5		0		1.80E+07	2.03E+08		57.46	1.96E+09	0.032	27.589	
C. 24h (Cisó)	ago-94	CA1 24h	0.5	23.1		14.85	10.00	5.26E+06	7.30E+07		121.23	8.72E+09	0.977	0.426	
Hores	14:00	CB1 24h	2.5	20.4		8.41	210.00	2.21E+07	2.14E+08		474.97	2.57E+10	0.772	0.535	
	14:00	CC1 24h	2.6			6.08	0.00	2.23E+07	2.17E+08		326.74	1.95E+10	0.629	0.622	
	14:00	CD1 24h	3.0	18.5		2.57	0.00	2.21E+07	1.54E+08		81.31	3.52E+09	0.148	2.368	
	14:00	CE1 24h	3.5	15.5		1.81	0.00	1.93E+07	9.80E+07		40.29	1.84E+09	0.091	3.689	
	18:00	CA3 24h	0.5	23.7		13.16	16.25	4.96E+06	1.62E+08		85.09	6.42E+09	0.804	0.496	
	18:00	CB3 24H	2.5	21.9		5.84	122.60	1.96E+07	7.86E+07		287.36	1.56E+10	0.584	0.672	
	18:00	CC3 24H	2.7			2.87	0.00	2.10E+07	9.08E+07		193.52	1.09E+10	0.438	0.837	
	18:00	CD3 24H	3.0	18.5		1.92	0.00	2.08E+07	1.56E+08		66.45	2.99E+09	0.130	2.679	
	18:00	CE3 24H	3.5	16		0.69	0.00	1.90E+07	1.46E+08		36.62	1.67E+09	0.084	3.969	
	2:00	CA5 24h	0.5	23		11.49	17.18	5.29E+06	7.65E+07		66.54	4.07E+09	0.571	0.670	
	2:00	CB5 24H	2.5			3.86	62.69	1.37E+07	1.02E+08		215.12	1.16E+10	0.615	0.644	
	2:00	CC5 24H	2.6			1.81	0.00	1.79E+07	1.52E+08		394.86	2.68E+10	0.840	0.493	
	2:00	CD5 24H	3.0	18.6		1.26	0.00	1.85E+07	1.88E+08		53.06	2.30E+09	0.117	2.948	
	2:00	CE5 24H	3.5	16		1.26	0.00	2.00E+07	1.79E+08		25.05	9.55E+08	0.047	7.395	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	6:00	CA7 24h	0.5	24		12.63	17.18	4.54E+06	8.03E+07		66.48	4.51E+09	0.719	0.541	
	6:00	CB7 24H	2.5	22.1		4.08	118.42	2.00E+07	1.71E+08		307.78	1.67E+10	0.606	0.652	
	6:00	CC7 24H	2.6				0.00	2.56E+07	5.86E+07		186.97	1.08E+10	0.362	0.985	
	6:00	CD7 24H	3.0	19		1.25	0.00	2.09E+07	7.85E+07		67.55	3.09E+09	0.131	2.652	
	6:00	CE7 24H	3.5	12		0.90	0.00	1.82E+07	5.25E+07		30.48	1.37E+09	0.074	4.517	
perfil (Ciso)	jun-94	CR 1	1.0	19.25		5.6		1.39E+06	6.60E+07						
		CR 2.4	2.4			3.5		3.62E+06	8.70E+07						
		CR 2.5	2.5	12		3.1		3.50E+06	1.05E+08						
		CR 2.6	2.6			2.3		9.27E+06	1.04E+08						
		CR 2.7	2.7			3.2		2.36E+07	9.50E+07						
		CR 2.8	2.8			2.4		4.00E+07	1.03E+08						
		CR 4	4.0	10.25		1.5		3.14E+07	7.60E+07						
		Llacuna del Tobar													
	sep-94	TB A	1.0	15.4		7.8	0.00	1.44E+06	2.57E+07		5.19	3.92E+08	0.241	1.336	
		TB B	10.5	14.8		8.3	0.10	9.88E+05	3.30E+07		12.91	1.02E+09	0.635	0.604	
		TB C	11.0	15		7.7	4.40	1.05E+06	2.31E+07		151.57	1.03E+10	2.379	0.216	
		TB D	11.5	15.3		7.7	2.00	1.34E+06	2.55E+07		28.56	1.73E+09	0.828	0.498	
		TB E	12.3			5.4	0.60	6.65E+06	3.01E+07		27.23	1.55E+09	0.210	1.605	
		TB F	12.8			0.4	0.30	4.19E+06	2.45E+07		25.60	1.35E+09	0.278	1.272	
		TB G	13.3			0.3	0.00	2.04E+07	1.07E+08		3.56	1.68E+08	0.008	38.801	
		TB H	14.0	13.1		0.3	0.00	7.74E+06	4.80E+07		1.63	7.12E+07	0.009	36.244	
		TB I	17.0	12.9		0.3	0.00	3.58E+06	3.14E+07		0.45	2.36E+07	0.006	107.428	
	sep-94	**Llacuna de La Cruz**													
		CRUZ A	1.0	17.7		9.6	0.10	2.32E+06	3.16E+07		5.94	4.03E+08	0.160	1.971	
		CRUZ B	10.3				0.70	2.82E+06	1.70E+08		52.16	3.59E+09	0.821	0.491	
		CRUZ C	11.5	10		0.7	1.70	1.23E+07	2.55E+08		84.50	5.11E+09	0.347	1.019	
		CRUZ D	11.8				4.80	1.09E+07	3.26E+08		129.69	8.34E+09	0.568	0.667	
		CRUZ E	12.0	9.3		0.5	2.30	8.03E+06	2.24E+08		121.90	7.11E+09	0.634	0.620	
		CRUZ F	12.3				0.00	8.66E+06	1.35E+08		120.19	6.14E+09	0.528	0.741	
		CRUZ G	13.0	7.3		0.4	0.00	7.87E+06	5.21E+07		20.18	1.08E+09	0.129	2.553	
		CRUZ H	15.0	6.2		0.4	0.00	7.65E+06	7.75E+07		9.42	4.91E+08	0.062	5.142	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
		Llac Saelenvannet (Noruega)													
perfil vertical	oct-94	B 0	0.0	10.5	1.00	12		3.05E+06	1.81E+08			3.00E+08	0.098	7.047	
		B 1	1.0	10	1.80	12		3.44E+06	1.59E+08			6.40E+08	0.186	3.731	
		B 1.3	1.3					8.19E+06	2.10E+08			1.30E+09	0.159	4.370	
		B 1.6	1.6	12	13.00	9		7.60E+06	3.16E+08			1.80E+09	0.237	2.927	
		B 2	2.0	14	14.00	2.5		1.10E+07	1.33E+08			5.10E+08	0.046	15.066	
		B 3	3.0	17	15.00	1		1.24E+07	1.45E+08			1.10E+08	0.009	78.136	
		B 4	4.0	17	15.50	0		8.70E+06	2.22E+08			9.00E+07	0.010	67.003	
perfil vertical		B 0	0.0	4	1.00	8		3.50E+06	6.58E+07			1.40E+08	0.040	17.329	
		B 1	1.0	8	3.50	9		1.08E+07	3.50E+08			1.30E+09	0.120	5.758	
		B 1.25	1.3	10.5	12.50	12		1.02E+07	1.50E+08			4.40E+09	0.431	1.607	
		B 1.5	1.5	12.5	15.00	2		8.38E+06	2.20E+08			2.50E+09	0.298	2.323	
		B 1.75	1.8	14	16.00	0		1.18E+07	2.60E+08			1.50E+09	0.127	5.453	
		B 2	2.0	15	14.50	0		1.56E+07	2.70E+08			1.10E+09	0.071	9.830	
		B 3	3.0	15	15.50	0		1.64E+07	2.05E+08			1.60E+08	0.010	71.048	
		B 4	4.0	15	15.50	0		1.16E+07	1.09E+08			1.60E+08	0.014	50.254	
perfil vertical	Març-95	B 0	0.0	3	10.00	11.3		6.62E+06	2.25E+08			2.98E+08	0.044	15.718	
		B 1	1.0	3	10.00	11.3		6.38E+06	2.17E+08			3.69E+08	0.056	12.325	
		B 1.5	1.5	3	10.00	9.5		6.32E+06	1.68E+08			4.80E+08	0.073	9.462	
		B 2	2.0	3.5	10.00	8.8		7.62E+06	1.79E+08			2.81E+08	0.036	19.137	
		B 2.5	2.5	5	19.00	5		8.00E+06	1.86E+08			2.54E+08	0.031	22.200	
		B 3	3.0	6.5	17.00	0		3.20E+07	2.30E+08			7.39E+07	0.002	300.428	
		B 3.5	3.5	9	19.00	0		1.69E+07	2.21E+08			8.18E+07	0.005	143.467	
		B 5	5.0	10	20.50	0		6.69E+06	1.68E+08			3.41E+07	0.005	136.226	
Berg. et al. 89	may-88	LlacPlußsse	0.2					6.20E+06	2.54E+08						
	may-88	Cheasapeake bay	1.0					3.20E+06	1.01E+07						
	oct-88	Korsfjorden	1.0					1.10E+06	6.10E+06						
	ago-89	Raunefjorden	1.0					2.00E+05	9.90E+06						
	feb-89	Raunefjorden	1.0					5.00E+05	1.00E+04						
	mar-89	Raunefjorden	1.0					5.00E+05	4.80E+06						
	may-88	North Atlantic	10.0					3.00E+05	1.49E+07						
	ene-89	Barents sea	30.0					2.00E+04	6.00E+04						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
Bratbak et al 90	mar-89	Raunefjorden	1.5					1.25E+05	8.02E+05	5.78E+02					
Bloom	mar-89	(Norway)	1.5					1.40E+05	4.22E+05	1.05E+03					
C. estacional	mar-89		1.5					3.44E+05	5.58E+06	8.05E+02					
	mar-89		1.5					2.09E+05	4.86E+06	1.19E+03					
	mar-89		1.5					4.35E+05	7.29E+06	1.85E+03					
	mar-89		1.5					4.33E+05	1.56E+06	7.99E+02					
	mar-89		1.5					2.43E+05	9.98E+06	5.59E+02					
	mar-89		1.5					2.42E+05	6.39E+06	2.42E+03					
	mar-89		1.5					6.11E+05	1.25E+06	2.67E+03					
	abr-89		1.5					6.15E+05	1.87E+06	3.34E+03					
	abr-89		1.5					2.88E+05	6.51E+05	2.88E+03					
Heldal&Bratbak91	jun-90	Dept MB	S	12				4.40E+06	4.40E+07			2.45E+08	0.55	1.26	
	jun-90	Bergen Harbour	1.5	13				2.90E+06	5.90E+07						
	jun-90	Raunefjoreden	S	14				1.40E+06	3.50E+07						
	jun-90	Raunefjoreden	5.0	14				1.50E+06	2.80E+07						
	jun-90	Raunefjoreden	25.0	8				7.00E+05	1.10E+07						
	ago-90	Lake Kalandsvan	0.5	17	1.00			3.00E+06	2.02E+08						
	ago-90	Lake Kalandsvan	1.0	16	1.00			4.10E+06	1.54E+08						
	ago-90	Lake Kalandsvan	25.0	5	1.00			8.00E+05	1.90E+07						
Hara et al.91	sep-87	Osaka Bay	0.0					2.00E+06	3.50E+07						
	jun-90	Otsuchi Bay1	0.0					3.40E+05	2.60E+06						
		Otsuchi Bay2	0.0					1.90E+05	1.70E+06						
		Otsuchi Bay2	15.0					2.70E+05	4.10E+06						
		Otsuchi Bay2	60.0					3.10E+05	2.20E+06						
		Otsuchi Bay3	0.0					1.90E+05	1.70E+06						
	ago-90	Sagami bayA	0.0					2.90E+06	1.40E+07						
		Sagami bayC	0.0					2.50E+06	1.00E+07						
		Sagami bayC	10.0					3.10E+06	7.70E+06						
		Sagami bayC	50.0					2.40E+06	5.60E+06						
		Sagami bayC	100.0					1.30E+06	3.70E+06						
		Sagami bayC	200.0					9.30E+05	2.70E+06						
	abr-88	Kuroshiro1	10.0					3.70E+05	2.00E+06						
		Kuroshiro2	10.0					4.10E+05	1.20E+06						
		Kuroshiro3	10.0					3.90E+05	1.20E+06						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
Nygaard et al 91		Oslofjorden													
		Perfil	0.0	16.31	22.22	7.4	4.71	1.15E+06	5.48E+07	3.70E+03		2.42E+09	2.03	0.49	8.91E+04
			2.0	16.1	22.31	7.4	5.10	1.20E+06	1.29E+08	5.76E+03		2.34E+09	1.88	0.53	1.35E+05
			4.0	15.84	22.47	7.7	1.33	1.40E+06	6.44E+07	4.58E+03		2.34E+09	1.61	0.62	9.60E+04
			6.0	16.01	22.65	7	0.72	1.23E+06	6.01E+07	4.63E+03		1.41E+09	1.12	0.90	1.56E+05
			8.0	16.18	23.20	6.8	0.63	1.32E+06	7.42E+07	3.31E+03		1.07E+09	0.80	1.26	5.80E+04
			12.0	14.54	26.14	3.4	0.77	1.15E+06	4.81E+07	2.54E+03		5.18E+08	0.45	2.24	6.25E+04
			16.0	10.46	29.29	2.8	0.66	1.17E+06	2.35E+07	4.80E+03		2.57E+08	0.22	4.58	1.13E+05
			30.0	7.72	31.25	1.9	0.22	4.40E+05	1.78E+07	1.15E+03		1.84E+08	0.41	2.41	8.77E+04
Steward et al 92b	nov-91	Peñasquitos	0.0				8.00	1.41E+07	6.20E+08			3.10E+09	0.20	3.49	
cosat(California)	nov-91	Mission Bay	0.0				1.85	3.90E+06	1.37E+08			9.40E+08	0.22	3.21	
	nov-91	Scripps Pier	0.0				0.59	6.90E+05	1.17E+07			6.50E+08	0.66	1.04	
mig(California)	sep-91	301	10.0				0.93	2.10E+06	1.37E+07						
	dic-91	2	2.0				0.64	4.80E+05	1.17E+07			3.00E+09	1.98	0.35	
Oceà(California)	dic-91	5	2.0				0.42	3.40E+05	1.83E+07			1.90E+08	0.44	1.56	
	dic-91	8	2.0				0.49	4.20E+05	1.53E+07			2.20E+08	0.42	1.65	
	sep-91	305	5.0				0.18	8.70E+05	1.24E+07			4.80E+08	0.44	1.58	
	sep-91	305	10.0				0.28	7.10E+05	1.20E+07			1.70E+09	1.22	0.57	
	sep-91	305	30*					8.10E+05	1.07E+07			7.80E+07	0.09	7.54	
	sep-91	305	50*					4.50E+05	5.70E+06			2.10E+07	0.05	15.20	
	sep-91	305	700.0				0.00	7.00E+04	1.10E+06			6.30E+06	0.09	8.04	
	sep-91	305	900.0				0.00	1.10E+05	2.50E+06			4.10E+06	0.04	18.94	
Cochlan et al. 93	sep-90	Costa California	0.0				1.43	2.23E+06	2.83E+07						
Perfils			10.0				2.58	2.31E+06	1.88E+07						
			20.0				1.97	1.49E+06	7.18E+06						
			30.0				0.18	5.19E+05	7.99E+06						
			40.0				0.12	3.64E+05	3.13E+06						
			50.0				0.13	4.01E+05	3.72E+06						
		Mig	0.0				0.69	1.88E+06	5.52E+06						
			10.0				0.39	1.09E+06	1.03E+06						
			20.0				0.78	8.57E+05	3.16E+06						
			30.0				0.28	6.21E+05	1.16E+06						
			40.0				0.09	6.24E+05	3.86E+06						
			50.0				0.09	3.73E+05	2.58E+06						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
		Oceà	0.0				0.30	1.52E+06	1.33E+06						
			10.0				0.17	6.88E+05	2.13E+06						
			20.0				0.12	5.04E+05	2.48E+06						
			30.0				0.47	4.67E+05	2.02E+06						
			40.0				0.09	2.98E+05	2.46E+05						
			50.0				0.04	2.84E+05	3.50E+05						
			100.0					2.70E+05	8.67E+05						
			200.0					2.44E+05	2.95E+05						
			400.0					1.39E+05	3.01E+05						
Cochlan et al. 93	ago-91	Gulf of Bothnia	0.0				1.24	2.24E+06	3.31E+07						
(continuació)		Costa	4.0				1.23	2.50E+06	3.60E+07						
			8.0				1.23	1.97E+06	3.30E+07						
			20.0				0.66	2.05E+06	2.58E+07						
Wommack et al. 92		Chesapeake bay													
Costa	sep-90	908	Super.					5.87E+06	2.30E+07						
			Fons					4.21E+06	2.12E+07						
		858	S					6.67E+06	2.40E+07						
			F					3.47E+06	1.62E+07						
		845	S					5.93E+06	7.21E+06						
			F					3.12E+06	1.15E+07						
		818	S					8.39E+06	4.78E+06						
			F					1.79E+06	5.44E+06						
		804	S					6.72E+06	1.29E+07						
			F					2.21E+06	1.10E+07						
	abr-91	908	S					2.21E+06	2.28E+07						
			F					3.62E+06	2.39E+07						
		858	S					2.77E+06	2.84E+07						
			F					3.43E+06	4.43E+07						
		845	S					3.28E+06	7.51E+06						
			F					2.99E+06	3.81E+07						
		818	S					2.84E+06	1.48E+07						
			F					2.14E+06	2.24E+07						
		804	S					2.47E+06	2.07E+07						
			F					2.47E+06	3.28E+07						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	ago-91	908	S					2.00E+06	3.54E+07						
			F					2.58E+06	2.82E+07						
		858	S					3.01E+06	3.43E+07						
			F					3.01E+06	2.36E+07						
		845	S					2.35E+06	2.78E+07						
			F					2.06E+06	1.54E+07						
		818	S					2.12E+06	4.14E+07						
			F					1.61E+06	1.80E+07						
		804	S					4.37E+06	5.17E+07						
			F					1.93E+06	1.70E+07						
		756	S					2.25E+06	4.91E+07						
			F					1.85E+06	1.85E+07						
Wommacket al. 92	jun-91	908	S					8.83E+06	2.92E+07						
			F					5.82E+06	2.47E+07						
		858	S					1.17E+07	2.69E+07						
			F					4.14E+06							
		845	S					9.49E+06	3.36E+07						
			F					3.30E+06	3.17E+07						
		818	S					5.50E+06	1.92E+07						
			F					4.43E+06	8.86E+06						
		804	S					2.42E+06	1.68E+07						
			F					2.02E+06	2.73E+07						
		756	S					2.09E+06	2.05E+07						
			F					9.52E+05	3.28E+07						
	oct-91	908	S					2.36E+06	2.15E+07						
			F					2.87E+06	2.55E+07						
		858	S					2.06E+06	3.01E+07						
			F					2.50E+06	2.28E+07						
		845	S					2.31E+06	2.88E+07						
			F					1.95E+06	3.53E+07						
		818	S					2.34E+06	2.69E+07						
			F					1.03E+06	5.23E+07						
		804	S					7.34E+05	3.29E+07						
			F					8.05E+05	1.61E+07						
		756	S					5.82E+05	2.23E+07						
			F					7.25E+05	5.02E+07						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
Paul et al. 93		Key Largo (Florida)													
		Blakwater sound		16.99	26.39		0.45	9.35E+05	1.20E+07						
		Tarpon sound		17.86	28.96		0.25	1.71E+06	8.37E+06						
		1		14.53	33.99		0.72	1.12E+06	4.97E+06						
		2		14.15	34.00		2.82	6.72E+05	3.18E+06						
		3		13.65	35.33		0.38	8.46E+05	3.19E+06						
		Moskito Bank		13.21	35.67		0.34	9.58E+05	1.78E+06						
		French Reef		19.57	34.20		0.48	3.52E+05	1.60E+06						
		6		19.73	34.09		0.46	6.13E+05	1.48E+06						
Boehme et al. 93	jun-92	Golf de Mèxic													
Transecte		OffS 9	3.0				0.03	1.58E+05	5.06E+05						
oceà		OffS 9	113.8				0.08	8.81E+04	5.35E+05						
		OffS 8	3.0				0.08	3.00E+05	5.05E+05						
		OffS 8	82.1				0.20	1.85E+05	4.60E+05						
		OffS 8	2457.0				0.08	5.52E+03	3.63E+04						
Boehme et al. 93		OffS 10	3.0	28.49	36.10	6.05	0.08	2.57E+05	6.09E+05						
		OffS 10	21.5	26.52	36.34	6.45	0.08	2.65E+05	5.12E+05						
		OffS 10	47.0	21.79	36.20	7	0.23	2.47E+05	8.47E+05						
		OffS 10	64.0	21	36.20	6.6	0.23	1.70E+05	5.03E+05						
		OffS 10	97.0	19.09	36.40	4.55	0.05	5.73E+04	3.72E+05						
		OffS 10	442.9					4.15E+04	5.47E+04						
		OffS 10	935.7					1.53E+04	6.83E+04						
		OffS 10	2436.0					3.04E+03	1.01E+04						
mig		NS 7	3.0				0.00	1.71E+05	4.86E+05						
		NS 7	314.3				0.00	2.90E+04	2.48E+04						
		NS 7	1407.0				0.10	3.31E+04	1.25E+04						
		NS 6	3.0				0.00	1.42E+05	4.84E+05						
		NS 6	98.3				0.08	6.47E+04	7.52E+04						
		NS 6	196.1				0.11	1.19E+05	3.99E+04						
		NS 5	3.0				0.00	1.42E+05	7.44E+05						
		NS 5	98.4				0.05	5.05E+05	7.54E+05						
costa		C 4	3.0				0.11	2.02E+05	3.22E+05						
		C 4	56.0				0.38	3.68E+05	6.28E+05						
		C 3	3.0				0.12	4.07E+05	4.65E+05						
		C 3	29.5				0.53	9.04E+05	1.73E+06						
		C 2	3.0				1.70	1.54E+06	3.67E+06						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
		C 2					1.75	3.48E+06	6.16E+06						
		C 1	3.0				8.28	5.32E+06	1.61E+07						
C. estacional	may-91	SJ107	0.5					3.72E+05	1.32E+07						
		SJ105	0.5					4.23E+05	5.64E+06						
		SJ103	0.5					4.93E+05	3.27E+06						
		SJ101	0.5					9.11E+05	7.37E+07						
		SJ108	0.5					1.05E+06	5.50E+07						
	jul-91	SJ107	0.5					7.56E+05	1.24E+07						
		SJ105	0.5					1.11E+06	2.09E+07						
		SJ103	0.5					6.07E+05	2.91E+07						
		SJ101	0.5					6.34E+05	1.01E+07						
		SJ108	0.5					1.21E+06	3.68E+07						
	ago-91	SJ107	0.5					3.95E+05	4.72E+06						
		SJ105	0.5					4.28E+05	5.67E+06						
		SJ103	0.5					5.34E+05	1.27E+07						
		SJ101	0.5					1.25E+06	4.26E+07						
		SJ108	0.5					1.89E+06	8.68E+07						
	may-92	SJ107	0.5					2.22E+05	1.45E+06						
		SJ105	0.5					2.45E+05	5.54E+06						
		SJ103	0.5					2.08E+05	3.75E+06						
		SJ101	0.5					2.90E+05	5.29E+06						
		SJ108	0.5					4.15E+05	2.94E+07						
Boehme et al. 93	jun-92	SJ107	0.5					8.10E+05	1.34E+07						
C. estacional		SJ105	0.5					6.65E+05	1.05E+07						
(continuacio)		SJ103	0.5					6.40E+05	6.71E+06						
		SJ101	0.5					1.07E+06	1.44E+07						
		SJ108	0.5					9.74E+05	3.15E+07						
	ago-92	SJ107	0.5					4.38E+05	1.07E+07						
		SJ105	0.5					3.34E+05	5.73E+06						
		SJ103	0.5					6.82E+05	9.37E+06						
		SJ101	0.5					3.56E+05	5.03E+06						
		SJ108	0.5					1.92E+06	2.33E+07						
	nov-92	SJ107	0.5					1.21E+06	2.85E+07						
		SJ105	0.5					1.55E+06	4.20E+07						
		SJ103	0.5					1.85E+06	1.32E+07						
		SJ101	0.5					9.62E+05	3.17E+06						
		SJ108	0.5					1.15E+06	1.59E+07						

Jiang & Paul 94		Tampa Bay (Superficie)														
C. Estacional	dic-90	Tampa Bay		23.96	29.27		1.62	3.66E+06	5.81E+06							
	dic-90	Tampa Bay	S	17.74	30.61		2.16	3.17E+06	5.20E+06							
	ene-91	Tampa Bay	S	22.20	30.06		4.32	3.36E+06	3.63E+06							
	ene-91	Tampa Bay	S	20.24	29.15		3.68	2.56E+06	6.05E+06							
	feb-91	Tampa Bay	S	19.27	30.12		3.19	1.83E+06	6.53E+06							
	feb-91	Tampa Bay	S	19.15	30.43		4.27	2.19E+06	2.66E+06							
	mar-91	Tampa Bay	S	19.15	30.43		3.78	1.87E+06	4.96E+06							
	mar-91	Tampa Bay	S	19.09	32.07		3.28	2.43E+06	2.42E+06							
	abr-91	Tampa Bay	S	25.18	29.70		1.56	2.24E+06	3.87E+06							
	abr-91	Tampa Bay	S	24.57	30.79		3.38	1.80E+06	5.08E+06							
	may-91	Tampa Bay	S	26.16	30.18		1.95	3.94E+06	5.44E+06							
	may-91	Tampa Bay	S	28.17	31.28		3.72	3.14E+06	9.44E+06							
	jun-91	Tampa Bay	S	29.33	29.82		4.26	4.92E+06	2.26E+07							
	jun-91	Tampa Bay	S	28.23	29.15		3.42	4.11E+06	9.44E+06							
	jul-91	Tampa Bay	S	30.18	29.76		5.38	4.18E+06	1.67E+07							
	jul-91	Tampa Bay	S	29.27	26.22		11.53	5.60E+06	1.82E+07							
	ago-91	Tampa Bay	S	29.70	26.34		7.49	5.60E+06	1.36E+07							
	ago-91	Tampa Bay	S	30.55	26.28		10.20	5.11E+06	1.10E+07							
	sep-91	Tampa Bay	S	28.78	24.88		8.77	4.35E+06	3.01E+07							
	sep-91	Tampa Bay	S	31.04	21.22		12.90	5.47E+06	2.90E+07							
	sep-91	Tampa Bay	S	26.52	24.21		12.16	3.10E+06	2.64E+07							
	oct-91	Tampa Bay	S	27.13	26.22		7.44	3.01E+06	2.19E+07							
	oct-91	Tampa Bay	S	25.24	23.17		6.45	3.57E+06	3.25E+07							
	nov-91	Tampa Bay	S	24.21	26.16		3.69	2.56E+06	8.35E+06							
	nov-91	Tampa Bay	S	21.22	27.20		3.05	2.54E+06	7.26E+06							
	dic-91	Tampa Bay	S	21.16	29.15		2.36	3.10E+06	3.51E+06							
	dic-91	Tampa Bay	S	19.33	29.70		2.51	2.22E+06	4.96E+06							

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
Hennes&Simon95	abr-92	Llac Constance	0-8m				1.03	9.58E+05	1.40E+06						
C. estacional	abr-92	Constance	0-8m				2.66	6.08E+05	9.11E+05						
	abr-92	Constance	0-8m				5.12	8.32E+05	2.64E+06						
	abr-92	Constance	0-8m				9.01	8.35E+05	5.01E+06						
	abr-92	Constance	0-8m				39.57	4.16E+06	1.34E+07						
	may-92	Constance	0-8m				10.67	8.37E+05	2.50E+07						
	may-92	Constance	0-8m				7.39	1.06E+06	6.71E+06						
	may-92	Constance	0-8m				11.89	1.80E+06	1.16E+07						
	may-92	Constance	0-8m				3.28	4.23E+06	2.76E+07						
	jun-92	Constance	0-8m				2.26	2.67E+06	4.17E+07						
	jun-92	Constance	0-8m				1.85	1.42E+06	3.87E+07						
	jun-92	Constance	0-8m				1.02	1.81E+06	2.05E+07						
	jun-92	Constance	0-8m				3.49	2.22E+06	1.93E+07						
	jun-92	Constance	0-8m				2.05	3.53E+06	4.09E+07						
	jul-92	Constance	0-8m				3.48	1.43E+06	3.09E+07						
	jul-92	Constance	0-8m				3.07	1.18E+06	1.04E+07						
	jul-92	Constance	0-8m				4.72	9.86E+05	1.05E+07						
	jul-92	Constance	0-8m				7.18	2.33E+06	3.67E+07						
	jul-92	Constance	0-8m				11.09	1.34E+06	7.08E+06						
Mathias et al.95	ene-92	Danube river	0.4	0.73				2.24E+06	1.19E+07			7.76E+08	0.34	2.01	
C. Estacional	mar-92	Danube river	0.4	0.94				3.09E+06	2.25E+07			7.93E+08	0.26	2.71	
	abr-92	Danube river	0.4	5.21				2.57E+06	1.09E+07			1.23E+09	0.47	1.46	
	nov-92	Danube river	0.4	2.95				4.21E+06	3.46E+07			9.76E+08	0.23	3.00	
	dic-92	Danube river	0.4	1.67				2.53E+06	1.89E+07			8.55E+08	0.34	2.06	
	ene-93	Danube river	0.4	1.83				2.10E+06	1.29E+07			8.70E+08	0.41	1.69	
	mar-93	Danube river	0.4	4.59				5.87E+06	1.16E+07			1.16E+09	0.20	3.53	
	nov-93	Danube river	0.4	4.69				2.55E+06	2.01E+07			1.17E+09	0.45	1.53	
	dic-93	Danube river	0.4	1.76				2.05E+06	1.19E+07			8.63E+08	0.42	1.66	
	abr-93	Danube river	0.4	9.7				3.79E+06	2.24E+07			1.96E+09	0.51	1.36	
	abr-92	Danube river	0.4	10.74				1.81E+06	1.33E+07			2.18E+09	1.18	0.59	
	oct-93	Danube river	0.4	12.61				2.63E+06	4.50E+07			2.64E+09	0.98	0.70	
	oct-92	Danube river	0.4	14.6				4.70E+06	3.67E+07			3.25E+09	0.68	1.02	
	may-93	Danube river	0.4	17.98				4.58E+06	6.07E+07			4.60E+09	0.98	0.70	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	sep-92	Danube river	0.4	18.56				2.72E+06	1.69E+07			4.88E+09	1.73	0.40	
	sep-93	Danube river	0.4	18.93				2.32E+06	3.78E+07			5.07E+09	2.09	0.33	
	may-92	Danube river	0.4	21.15				2.50E+06	1.53E+07			6.38E+09	2.42	0.29	
	jun-93	Danube river	0.4	21.89				3.37E+06	5.42E+07			6.89E+09	1.96	0.35	
	jul-93	Danube river	0.4	22.31				4.53E+06	2.75E+07			7.19E+09	1.54	0.45	
	ago-92	Danube river	0.4	22.99				4.02E+06	1.79E+07			7.71E+09	1.85	0.38	
	jun-92	Danube river	0.4	24.19				3.23E+06	2.24E+07			8.73E+09	2.56	0.27	
	jul-92	Danube river	0.4	25.55				3.77E+06	1.23E+07			1.00E+10	2.53	0.27	
	ago-93	Danube river	0.4	25.15				3.50E+06	3.71E+07			9.64E+09	2.61	0.27	
Hennes & Suttle 95		Golf de Mèxic													
		GM1.592	Superfic.		28.85		0.19	1.27E+06	1.33E+08						
		GM13.21	S		28.81		0.50	5.24E+06	1.22E+08						
		GM24.9	S		27.49		0.44	2.79E+06	6.72E+06						
		GM33.75	S		29.15		0.79	2.80E+06	1.29E+08						
		GM44.03	S		27.84		0.65	1.70E+06	1.04E+08						
		GM53.77	S		27.63		0.62	2.63E+06	1.11E+08						
		GM62.99	S		28.15		0.50	3.17E+06	7.72E+07						
		GM73.89	S		29.57		0.50	1.42E+06	6.85E+07						
		GM83.6	S		35.44		0.11	1.80E+06	2.26E+07						
		GM93.36	S		33.32		0.65	2.98E+06	4.54E+07						
Weinbauer et al.95	jun-91	Adriàric	0.5					9.15E+05	7.51E+06						
C. Estacional		Adriàric	2.9					8.30E+05	5.75E+06						
		Adriàric	6.0					1.11E+06	1.02E+07						
		Adriàric	9.0					5.53E+05	5.31E+06						
	jul-91	Adriàric	0.5					1.22E+06	9.29E+06						
		Adriàric	4.0					1.31E+06	1.47E+07						
		Adriàric	8.1					1.65E+06	2.50E+07						
		Adriàric	12.0					9.04E+05	9.19E+06						
	ago-91	Adriàric	0.5					1.12E+06	1.91E+07						
		Adriàric	4.0					1.01E+06	9.33E+06						
		Adriàric	8.0					1.94E+06	3.78E+07						
		Adriàric	12.2					1.21E+06	1.11E+07						
	may-92	Adriàric	0.5					4.05E+05	6.25E+06						
		Adriàric	2.9					4.06E+05	7.59E+06						
		Adriàric	6.0					4.31E+05	7.59E+06						
		Adriàric	9.0					4.00E+05	6.25E+06						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	nov-92	Adriàric	0.6					1.23E+06	6.51E+07						
		Adriàric	4.9					7.93E+05	6.27E+07						
		Adriàric	10.0					8.66E+05	6.52E+07						
		Adriàric	15.1					6.86E+05	6.24E+07						
	feb-92	Adriàric	0.5					3.34E+05	1.80E+06						
		Adriàric	5.0					3.12E+05	2.24E+06						
		Adriàric	9.9					3.01E+05	2.24E+06						
		Adriàric	15.0					2.67E+05	1.35E+06						
	jun-91	Arusinia	0.0					4.85E+05	7.80E+06	1.51E+03					
	ago-91	Arusinia	0.0				0.32	7.84E+05	9.18E+06	4.12E+03					
	oct-91	Arusinia	0.0				2.08	1.08E+06	1.41E+07	6.99E+03					
	dic-91	Arusinia	0.0				0.69	1.53E+06	5.47E+07	4.21E+03					
	feb-92	Arusinia	0.0				0.07	1.70E+05	2.88E+05	1.75E+03					
	abr-92	Arusinia	0.0				0.70	2.45E+05	1.26E+05	2.09E+03					
	jun-92	Arusinia	0.0				0.20	6.59E+05	6.14E+06	1.15E+03					
	may-92	Rovinj	0.0				2.51	6.60E+05	1.41E+07	1.00E+03					
	jun-92	Rovinj	0.0				0.46	3.22E+05	3.58E+06	6.89E+03					
	ago-92	Rovinj	0.0				0.16	6.77E+05	5.13E+07	6.86E+03					
	nov-92	Rovinj	0.0				3.07	7.45E+05	6.23E+07	4.20E+03					
	feb-92	Rovinj	0.0				0.07	1.42E+05	1.37E+06	1.96E+03					
C.diari (Hora)	12.00	Adriàtic	20.0				0.62	5.53E+05	2.21E+06	1.36E+03					
jun-92	12.00	Adriàtic	20.0				0.63	6.19E+05	1.89E+06						
	16.00	Adriàtic	20.0				0.67	8.91E+05	3.01E+06	1.89E+03					
	18.00	Adriàtic	20.0				0.78	7.20E+05	2.89E+06						
	20.00	Adriàtic	20.0				0.61	3.24E+05	5.01E+06	2.52E+03					
	22.00	Adriàtic	20.0				0.61	3.33E+05	4.51E+06						
	24.00	Adriàtic	20.0				0.60	5.24E+05	3.90E+06	3.55E+03					
	2.00	Adriàtic	20.0				0.78	6.21E+05	4.00E+06						
	4.00	Adriàtic	20.0				0.71	3.89E+05	3.09E+06	3.18E+03					
	6.00	Adriàtic	20.0				0.60	5.22E+05	3.90E+06						
	8.00	Adriàtic	20.0				0.62	6.06E+05	3.21E+06	1.74E+03					
	10.00	Adriàtic	20.0				0.56	6.15E+05	4.50E+06						
	12.00	Adriàtic	20.0				0.56	5.30E+05	4.89E+06	1.74E+03					
	12	Adriàtic	5.0				5.00	1.90E+06	9.29E+06	6.18E+03					
	14	Adriàtic	5.0				4.57	1.12E+06	1.53E+07						
	16	Adriàtic	5.0				3.88	9.01E+05	1.32E+07	3.60E+03					
	18	Adriàtic	5.0				1.64	7.40E+05	1.30E+07						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	20	Adriàtic	5.0				3.14	8.13E+05	1.03E+07	3.18E+03					
	22	Adriàtic	5.0				2.79	8.26E+05	5.21E+06						
	24	Adriàtic	5.0				2.85	1.26E+06	6.93E+06	2.96E+03					
	2	Adriàtic	5.0				2.29	1.10E+06	8.25E+06						
	4	Adriàtic	5.0				3.14	7.94E+05	7.40E+06	1.29E+03					
	6	Adriàtic	5.0				2.83	1.11E+06	6.93E+06						
	8	Adriàtic	5.0				1.73	1.01E+06	8.52E+06	1.48E+03					
	10	Adriàtic	5.0				2.91	6.20E+05	5.61E+06						
	12	Adriàtic	5.0				2.75	7.48E+05	8.33E+06	4.11E+03					
	14	Adriàtic	5.0				0.78	5.97E+05	7.21E+06						
	16	Adriàtic	5.0				2.39	8.01E+05	5.87E+06	2.77E+03					
	18	Adriàtic	5.0				1.84	1.30E+06	6.71E+06						
	20	Adriàtic	5.0				3.95	1.47E+06	7.03E+06	3.20E+03					
	22	Adriàtic	5.0				1.44	9.76E+05	1.50E+07						
	24	Adriàtic	5.0				0.49	9.07E+05	1.41E+07	3.06E+03					
	2	Adriàtic	5.0				1.41	1.10E+06	1.52E+07						
	4	Adriàtic	5.0				1.24	9.55E+05	1.02E+07	4.09E+03					
	6	Adriàtic	5.0				0.57	9.95E+05	1.13E+07	2.39E+03					
Steward et al 96	Aug-Sep 92	Chukchi Sea	0.0	6.12	32.06			3.84E+05	4.46E+06	1.32E+03					
Artic		Est 1	5.0	6	32.04			5.38E+05	3.37E+06	9.56E+02					
		Est 1	10.0	5.28	32.04			3.03E+05	5.21E+06	4.37E+02	15.09	7.55E+08	1.25	0.55	
		Est 1	15.0	4.25	32.24			4.69E+05	4.94E+06	4.83E+02					
		Est 1	20.0	4.13	32.24			4.97E+05	4.42E+06	1.34E+03					
		Est 1	25.0	4.05	32.31			4.54E+05	5.13E+06	3.01E+03	9.2	4.60E+08	0.70	0.99	
		Est. 17	0.0	4.33	28.66			2.08E+05	3.27E+06	1.38E+02	1.71	8.55E+07	0.34	2.01	
		Est. 17	17.3	2.83	29.32			1.67E+05	3.04E+06	5.04E+01	2.9	1.45E+08	0.63	1.11	
		Est. 17	36.9	-1.23	32.32			5.70E+05	5.09E+06	4.03E+02	3.39	1.70E+08	0.26	2.66	
		Est. 17	57.0	-1.1	32.44			7.64E+05	4.69E+06	2.19E+02	4.45	2.23E+08	0.26	2.71	
		Est. 17	97.2	-1.18	32.49			9.92E+05	6.97E+06	3.87E+02	4.49	2.25E+08	0.20	3.40	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
		Est 32	4.9	2.27	28.22			3.07E+05	4.38E+06	1.39E+01					
		Est 32	28.0	-1.04	30.34			4.32E+05	3.58E+06	9.35E+01	4.86	2.43E+08	0.45	1.55	
		Est 32	53.9	-1.44	32.24			2.43E+05	3.77E+06	9.03E+01					
		Est 32	100.2	-1.52	32.71			3.64E+05	5.80E+06	8.34E+01	0.72	3.60E+07	0.09	7.35	
		Est 32	131.6	-0.99	33.50			2.49E+05	2.79E+06	9.72E+00					
		Est 32	158.8	-0.68	34.41			1.85E+05	2.50E+05	7.55E+01	0.39	1.95E+07	0.10	6.92	
		Est 32	193.4	-0.44	34.60			7.42E+04	6.85E+05	1.12E+00					
		Est 32	228.1	-0.13	34.66			1.25E+05	8.17E+05	1.01E+01	0.3	1.50E+07	0.11	6.12	
		Est 32	263.3	0.12	34.78			8.04E+04	3.98E+04	5.76E+00					
		Est 32	344.2	0.42	34.86			9.82E+04	3.57E+05	8.26E+00					
		Est 32	403.4	0.48	34.88			1.13E+05	5.13E+05	8.86E-02	0.55	2.75E+07	0.22	3.18	
Maranger&Bird 95	Estiu93	Llacs de Canada													
		Croche	Euphotic				7.64	3.81E+06	7.03E+07		8.5920	3.91E+08	0.10	7.09	
		Cromwell	Euphotic				14.94	8.47E+06	1.48E+08		36.9600	1.68E+09	0.18	3.82	
		Triton	Euphotic				4.41	6.68E+06	9.43E+07		37.7760	1.72E+09	0.23	3.02	
		Echo	Euphotic				24.13	2.11E+07	1.69E+08		21.3360	9.72E+08	0.05	15.39	
		PinRouge	Euphotic				8.57	7.93E+06	9.35E+07		16.2720	7.41E+08	0.09	7.76	
		Connelly	Euphotic				7.97	4.35E+06	5.76E+07		13.5120	6.16E+08	0.13	5.24	
		Orignal	Euphotic				4.60	6.90E+06	1.04E+08		32.5440	1.48E+09	0.19	3.56	
		Dufresne	Euphotic				8.61	3.06E+06	6.15E+07		8.7600	3.99E+08	0.12	5.65	
		Manitou	Euphotic				6.43	2.03E+06	1.13E+08		18.2880	8.33E+08	0.34	2.02	
		Gilbert	Euphotic				3.67	6.88E+06	4.98E+07		22.1280	1.01E+09	0.14	5.07	
		Orford	Euphotic				1.50	2.66E+06	4.10E+07		17.3280	7.89E+08	0.26	2.67	
		Waterloo	Euphotic				32.80	1.59E+07	1.85E+08		96.7200	4.41E+09	0.24	2.83	
		Massawippi	Euphotic				6.60	8.43E+06	4.20E+07		9.1680	4.18E+08	0.05	14.33	
		Magog	Euphotic				8.97	1.02E+07	9.92E+07		36.0720	1.64E+09	0.15	4.64	
		Bowker	Euphotic				2.92	2.35E+06	4.68E+07		6.7440	3.07E+08	0.12	5.64	
		Bromont	Euphotic				9.05	6.70E+06	1.02E+08		51.2160	2.33E+09	0.30	2.32	
		QuinnBay	Euphotic				5.09	6.02E+06	6.54E+07		14.5920	6.65E+08	0.10	6.62	
		Quenouilles	Euphotic				9.38	1.63E+06	9.68E+07		30.6000	1.39E+09	0.62	1.12	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
Maranger et al. 94	abr-92	Artic (Gel)	4 cm				0.95	3.15E+05	1.47E+07						
C estacional	abr-92		4 cm				0.94	8.67E+05	5.05E+07			3.05E+10	3.59	0.19	
	abr-92		4 cm				0.94	1.79E+06	5.67E+07			1.25E+11	4.26	0.16	
	may-92		4 cm				1.82	2.60E+06	8.05E+07			5.55E+10	3.11	0.22	
	may-92		4 cm				3.30	2.58E+06	1.23E+08			4.50E+10	2.91	0.24	
	may-92		4 cm				3.63	4.64E+06	1.03E+08			1.16E+11	3.26	0.21	
	may-92		4 cm				2.69	1.03E+07	1.09E+08			6.21E+10	1.95	0.36	
	may-92		4 cm				3.21	7.12E+06	9.63E+07			7.65E+10	2.46	0.28	
Perfil	may-92		1.5-4 cn					6.52E+04	1.13E+06			8.88E+09	9.95	0.07	
	may-92		0.5-1.5 cn					3.98E+04	1.15E+06			1.28E+10	5.78	0.12	
	may-92		0-0.5 cn					1.04E+05	1.14E+06			2.86E+10	5.62	0.12	
	may-92		interfase					2.10E+05	2.47E+06			2.56E+10	4.81	0.14	
	may-92	aigua	4m					2.26E+05	8.13E+06			6.71E+10	5.70	0.12	
	may-92		8m					1.06E+05	3.85E+06			3.40E+10	5.78	0.12	
Maranger et al. 96	ago-92	Llac Gilbert	2.0			9.23		7.10E+08	2.90E+09						
		Sediment (1 cm)	4.0			9.26		5.66E+08	1.80E+10						
			6.0			12.47		1.05E+09	2.20E+09						
			7.0			4.93		1.07E+09	1.99E+09						
			8.0			0.46		9.43E+08	1.78E+09						
			9.0			0		1.78E+09	1.39E+09						
			10.0			0.06		1.67E+09	1.68E+09						
			11.0			0		9.73E+08	9.88E+08						
			12.0			0		9.02E+08	6.07E+08						
			13.0			0		1.17E+09	6.68E+08						
		perfil aigua	0.0	19		9.2	1.08	1.74E+06	2.69E+07						
			1.0	18.99		9.18		2.02E+06	3.11E+07						
			2.0	19.02		9.16	0.93	1.80E+06	2.65E+07						
			3.0	19		9.2		1.83E+06	2.71E+07						
			4.0	18.87		9.18		1.98E+06	2.79E+07						
			5.0	15.94		9.27		2.07E+06	2.87E+07						
			6.0	12.45		10.94		2.00E+06	3.82E+07						
			7.0	8.96		12.37		3.38E+06	3.18E+07						
			8.0	7.32		4.91		3.36E+06	4.06E+07						
			9.0	5.9		0.46		3.18E+06	4.06E+07						
			10.0	5.26		0		5.36E+06	4.07E+07						
			11.0	5.08		0		4.67E+06	7.19E+07						
			12.0	5.11		0		6.26E+06	8.99E+07						

Estudi	Data	LLOC	Z (m)	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
Aquesta tesi	ago-94	La Trinitat			mg/L	mg/L	μg/L				μgC/L/d	cell/L/d			cell/L/d
Salines		TR1		32	37.50	21.2	1.12	1.00E+07	4.40E+07	1.30E+04	355.37	1.37E+10	0.862	0.804	1.15E+06
		TR2		32.6	64.00	15.17	7.30	9.60E+06	4.40E+07		931.32	1.63E+10	0.991	0.699	9.20E+06
		TR3		34	90.00	9.71	19.84	8.52E+06	5.20E+07	8.16E+04	1065.08	2.24E+10	1.288	0.538	1.10E+07
		TR7		34	149.00	12.54	5.82	1.64E+07	6.07E+07	1.07E+05	1121.69	4.49E+10	1.318	0.884	1.89E+07
		TR5		31	182.50	10.53	19.83	1.80E+07	2.40E+08		687.08	7.06E+09	0.331	2.094	1.09E+06
		TR4		36.7	250.00	4.86	2.32	6.17E+07	6.35E+08		669.50	1.95E+10	0.246	1.964	
		TR6		33	297.50	5.06	5.82	1.06E+08	8.35E+08		776.11	1.61E+10	0.141	3.349	
		TR8		37	370.00	5.26	2.00	1.33E+08	1.00E+09		912.70	2.75E+10	0.188	3.687	
		TR9		37.2	372.50	5.67	1.34	1.35E+08	1.58E+09		690.64	2.63E+10	0.178	3.894	
	ago-94	Sta Pola													
		SP1		35.5	384.00	0.89	2.05	7.17E+07	1.00E+08		125.01	4.37E+10	0.48	1.46	
		SP2		37.0	352.00	1.12	1.14	3.50E+07	1.00E+08		109.54	3.83E+10	0.74	0.94	
		SP3		29.0	168.00	4.09	0.78	9.05E+06	4.00E+07		69.31	2.42E+10	1.30	0.53	
		SP4		32.0	279.00	2.10	2.51	6.90E+07	6.10E+07		57.84	2.02E+10	0.26	2.70	
		SP5		31.5	122.00	3.94	4.56	2.81E+07	7.78E+06		60.24	2.10E+10	0.56	1.24	9.93E+06
		SP6		30.5	38.00	11.02	1.04	9.83E+06	1.50E+07		72.18	2.52E+10	1.27	0.55	1.12E+07
		SP7		36.0	318.00	1.15	2.00	5.86E+07	6.90E+07		69.38	2.42E+10	0.35	2.00	
		SP8		40.5	251.30	0.03	1.06	1.13E+08	6.00E+07		123.04	4.30E+10	0.32	2.15	
		SP9		32.0	195.00	0.90	0.78	1.01E+08	3.60E+07		106.21	3.71E+10	0.31	2.22	9.41E+06
		SP10		30.5	78.00	6.70	3.76	1.36E+07	6.32E+06		54.64	1.91E+10	0.88	0.79	9.65E+06
ECOANTAR 94	ene-94	Mar de Weddell													
Transecte		0W7	0.0	-1.410	33.51	3.35	3.02	2.19E+05	4.17E+07	2.26E+02	1.34	1.04E+08	0.389	0.878	8.46E+04
		10W7	10.0	-1.370	33.69	3.08	3.02	1.77E+05	1.49E+07	5.50E+01	1.98	1.55E+08	0.628	0.595	
		20W7	20.0	-1.549	34.04	2.84	2.33	2.21E+05	5.91E+07	6.88E+01	1.01	7.85E+07	0.304	1.083	
		40W7	40.0	-1.722	34.33	2.55	0.67	2.51E+05	5.18E+06	5.70E+01	0.33	2.56E+07	0.097	3.028	
		50W7	50.0	-1.725	34.39	2.48	0.55	1.38E+05	1.82E+07	5.90E+01	0.15	1.16E+07	0.080	3.609	
		60W7	60.0	-1.731	34.40	2.41	0.35	1.68E+05	1.11E+07	4.31E+01	0.20	1.56E+07	0.089	3.287	4.50E+04
		0W119	0.0	-1.269	33.56	3.25	2.33	2.84E+05	4.40E+07	1.33E+03	1.26	9.80E+07	0.297	1.106	
		10W119	10.0	-1.267	33.56	3.24	2.40	2.36E+05	8.75E+06	8.57E+02	3.00	2.34E+08	0.688	0.553	
		20W119	20.0	-1.271	33.56	3.21	2.47	4.83E+05	5.59E+06	2.11E+02	1.26	9.86E+07	0.186	1.668	
		30W119	30.0	-1.416	34.01	2.89	2.74	2.33E+05	7.66E+06	3.84E+02	1.28	1.00E+08	0.358	0.943	
		50W119	50.0	-1.468	34.10	2.83	1.56	1.88E+05	2.50E+06	8.64E+02	0.32	2.47E+07	0.123	2.419	
		80W119	70.0	-1.537	34.26	2.62	0.71	1.65E+05	2.50E+06	6.77E+02	0.27	2.12E+07	0.121	2.469	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
		0W8	0.0				2.06	1.15E+06	2.50E+06	1.97E+03	2.08	1.62E+08	0.132	2.274	8.95E+04
		10W8	10.0	-0.349	34.00	3.12	1.99	7.61E+05		1.72E+03	5.11	3.98E+08	0.421	0.823	
		20W8	20.0	-0.348	34.00	3.19	1.91	8.26E+05		9.36E+02	2.80	2.18E+08	0.234	1.356	
		40W8	40.0	-0.345	34.03	3.13	1.69	7.55E+05	3.77E+06	1.06E+03	1.84	1.43E+08	0.174	1.769	2.29E+05
		50W8	50.0	-0.397	34.08	2.98	2.14	6.83E+05		1.95E+03	3.72	2.90E+08	0.354	0.951	
		70W8	70.0	-1.413	34.27	2.60	0.23	6.83E+05			0.87	6.76E+07	0.094	3.108	
		0W9	0.0	-0.371	34.44	2.55	0.32	2.33E+05	3.00E+06	1.50E+03	0.06	4.57E+06	0.019	14.338	
		10W9	10.0	-0.370	34.44	2.51	0.23	2.07E+05	4.16E+06	7.49E+02	0.19	1.49E+07	0.070	4.144	
		21W9	20.0	-0.377	34.44	2.60	0.29	1.41E+05	7.33E+06	1.55E+03	0.30	2.33E+07	0.153	1.987	
		40W9	40.0	-0.381	34.44	2.51	0.27	2.52E+05	8.42E+06	6.01E+02	0.30	2.34E+07	0.089	3.287	
		50W9	50.0	-0.400	34.45	2.52	0.26	1.88E+05	7.72E+06		0.27	2.07E+07	0.104	2.824	
		70W9	70.0	-0.403	34.45	2.51	0.28	2.00E+05	5.86E+06		0.26	2.01E+07	0.096	3.060	
		0W10	0.0	-0.258	34.41	2.75	0.82	2.83E+05	3.70E+06	3.60E+03	0.14	1.10E+07	0.038	7.409	1.72E+05
		10W10	10.0	-0.269	34.41	2.71	0.76	2.64E+05	3.12E+06	1.43E+03	0.02	1.33E+06	0.005	137.903	
		20W10	20.0	-0.278	34.41	2.75	0.65	1.21E+05	5.58E+06	2.15E+03	0.22	1.71E+07	0.132	2.269	
		30W10	30.0	-0.297	34.41	2.70	0.69	2.67E+05	5.63E+06	2.15E+03	0.29	2.28E+07	0.082	3.543	1.98E+05
		50W10	50.0	-0.398	34.42	2.63	0.67	1.07E+05	6.68E+06	2.43E+03	0.28	2.17E+07	0.185	1.677	
		70W10	70.0	-0.519	34.43	2.59	0.58	7.97E+04	1.15E+06	2.01E+03	0.09	7.17E+06	0.086	3.384	
		0W11	0.0	0.647	34.13	2.71	0.91	1.78E+05	1.50E+07	3.92E+03	0.11	8.27E+06	0.045	6.246	0.00E+00
		20W11	20.0	0.646	34.13	2.72	0.95	1.95E+05	2.82E+06	3.46E+03	0.09	7.40E+06	0.037	7.570	
		30W11	30.0	0.612	34.14	2.67	1.00	2.03E+05	2.04E+06	7.26E+02	0.08	6.25E+06	0.030	9.261	
		40W11	40.0	0.591	34.16	2.67	0.87	1.67E+05	2.13E+06	9.33E+02	0.26	2.04E+07	0.115	2.582	0.00E+00
		50W11	50.0	0.537	34.20	2.59	0.78	3.49E+05	3.28E+06	3.23E+03	0.12	9.66E+06	0.027	10.259	
		70W11	70.0	0.520	34.23	2.60	0.80	3.13E+05	2.66E+06	1.58E+03	0.21	1.61E+07	0.050	5.682	
		0W12	0.0	0.888	34.19	2.80	0.78	4.55E+05	3.27E+06	4.76E+03	0.61	4.75E+07	0.099	2.959	
		10W12	10.0	0.890	34.20	2.78	1.02	3.82E+05	1.34E+06	2.14E+03	0.49	3.78E+07	0.094	3.105	
		20W12	20.0	0.830	34.24	2.69	0.65	4.47E+05	7.00E+06	4.55E+03	1.26	9.79E+07	0.198	1.575	
		30W12	30.0	0.635	34.26	2.59	0.69	5.04E+05	2.18E+06	4.75E+03	0.60	4.66E+07	0.088	3.301	
		50W12	50.0	0.409	34.30	2.53	0.59	4.65E+05	2.94E+06	4.96E+03	1.15	8.96E+07	0.176	1.748	
		81W12	70.0	0.394	34.32	2.12	0.21	1.74E+05	2.50E+06	7.70E+02	0.01	1.16E+06	0.007	104.608	
		0W13	0.0	2.429	33.85	2.81	0.23	6.21E+05	2.26E+06	4.57E+03	0.38	3.00E+07	0.047	6.028	1.51E+05
		20W13	20.0	2.375	33.85	2.80	0.17	5.96E+05	9.77E+05	3.31E+03	0.54	4.19E+07	0.068	4.238	
		40W13	40.0	2.251	33.85	2.76	0.26	6.16E+05	4.41E+05	5.07E+03	1.02	7.92E+07	0.121	2.464	
		60W13	60.0	0.459	33.96	2.92	0.95	4.47E+05	8.80E+05	1.08E+04	0.55	4.32E+07	0.092	3.173	
		70W13	70.0	0.075	33.97	2.92	1.24	5.26E+05	6.39E+05	7.63E+03	0.87	6.77E+07	0.121	2.462	2.09E+05
		80W13	80.0	-0.284	33.95	2.90	1.30	3.96E+05	1.95E+06	5.82E+02	0.80	6.25E+07	0.147	2.065	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
		0W1	0.0	-0.486	33.97	3.14	0.67	4.22E+05	8.88E+06	7.52E+02	0.59	4.60E+07	0.103	2.852	
		10W1	10.0	-0.490	33.97	3.13	0.58	4.66E+05	7.85E+06	6.70E+02	2.27	1.77E+08	0.322	1.030	
		30W1	30.0	-0.655	34.17	3.06	0.82	5.11E+05	7.00E+06	1.20E+03	1.56	1.22E+08	0.214	1.470	
		50W1	50.0	-1.197	34.34	2.67	1.62	2.48E+05	9.40E+06	6.96E+02	0.78	6.04E+07	0.218	1.445	
		80W1	80.0	-1.654	34.37	2.54	0.68	2.30E+05	2.35E+06	2.65E+02	0.20	1.59E+07	0.067	4.311	
		100W1	100.0	-1.691	34.40	2.46	0.61	2.04E+05	2.80E+06	1.72E+02	0.22	1.69E+07	0.079	3.653	
		Mediterrani													
FRONTS 94	jun-94	Sof 2	5.0	23.3	38.06		0.07	2.12E+05			2.48	1.68E+08	0.560	0.674	
	Mar obert	Sof 2	25.0	20.43	38.05		0.04	2.83E+05	7.85E+06		1.42	9.67E+07	0.281	1.209	
		Sof 2	50.0	15.95	38.16		0.21	5.60E+05	5.03E+06		1.62	1.10E+08	0.171	1.884	
		Sof 2	70.0	13.43	38.13		0.53	3.36E+05	2.80E+06		2.24	1.52E+08	0.357	0.983	
		Sof 2	90.0	13.17	38.26		0.17	1.31E+05	5.84E+06		0.93	6.29E+07	0.374	0.945	
		Sof 2	200.0	13.25	38.50			5.17E+04	5.96E+06		0.24	1.59E+07	0.256	1.308	
		Sof 4	5.0	25.1	38.14		0.07	3.46E+05	9.70E+06		2.39	1.62E+08	0.367	0.960	
		Sof 4	20.0	18.54	38.19		0.07	2.69E+05	9.50E+06		1.30	8.80E+07	0.270	1.252	
		Sof 4	40.0	14.68	38.16		0.41	3.52E+05	1.14E+07		1.43	9.72E+07	0.232	1.427	
		Sof 4	55.0	13.47	38.29		0.34	3.34E+05	1.02E+07		0.88	6.00E+07	0.157	2.032	
		Sof 4	75.0	13.16	38.35		0.09	9.53E+04	6.79E+06		0.72	4.89E+07	0.396	0.899	
		Sof 4	200.0	13.35	38.52		0.02	5.62E+04	6.60E+06		0.08	5.52E+06	0.089	3.453	
VARIMED 95	jun-95	T1	5.0	18.77	37.93	6.357	0.97	7.23E+05	1.00E+07	1.69E+03	4.06	2.76E+08	0.309	1.113	3.29E+05
Transecte	costa	T1	20.0	15.5	37.94	6.595	0.33	3.83E+05	2.44E+07		0.64	4.37E+07	0.103	3.019	
		T1	40.0	14.23	37.97	6.479	0.64	4.04E+05			1.99	1.35E+08	0.275	1.232	
		T1	50.0	13.54	37.99	6.23	0.28	4.54E+05	1.76E+07	1.08E+03	1.30	8.82E+07	0.169	1.901	5.82E+05
		T1	60.0	13.43	38.01	5.98	0.19	3.08E+05	1.00E+07		0.85	5.77E+07	0.164	1.958	
		T1	67.0	13.41	38.01	5.773	0.22	2.56E+05	1.84E+07		0.58	3.95E+07	0.136	2.312	
		T2	5.0	19.25	37.83	5.715	0.13	3.93E+05	2.35E+07		2.09	1.42E+08	0.294	1.160	
		T2	30.0	15.02	37.88	6.564	0.28	5.42E+05	1.76E+07		1.66	1.13E+08	0.180	1.794	
		T2	50.0	14.14	37.94	6.47	0.95	7.59E+05	2.00E+07		1.41	9.58E+07	0.113	2.756	
		T2	60.0	13.68	37.98	6.276	0.57	4.14E+05	2.44E+07		0.74	5.04E+07	0.109	2.850	
		T2	90.0	13.13	38.10	5.922	0.10	1.64E+05	1.35E+07		0.88	5.98E+07	0.297	1.149	
		T2	200.0	13.03	38.24	5.684	0.03	2.46E+05	1.08E+07		1.05	7.12E+07	0.242	1.375	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	Front	T3	5.0	18.87	38.00	5.696	0.14	2.45E+05	1.98E+07		0.89	6.07E+07	0.211	1.556	
		T3	20.0	16.58	37.96	6.208	0.21	2.91E+05	2.16E+07		1.14	7.74E+07	0.225	1.471	
		T3	40.0	14.73	38.08	6.662	0.04	4.78E+05	1.59E+07		0.75	5.11E+07	0.097	3.196	
		T3	60.0	13.82	38.10	6.46	0.09	5.09E+05	1.17E+07		2.21	1.50E+08	0.247	1.353	
		T3	80.0	13.26	38.15	5.829	0.21	2.35E+05	7.49E+06		0.22	1.52E+07	0.059	5.072	
		T3	200.0	13.11	38.39	5.286	0.02	1.72E+05	4.46E+06		0.25	1.72E+07	0.091	3.381	
		T4	5.0	19.38	38.12	5.568	0.10	2.58E+05	9.98E+06		0.51	3.44E+07	0.119	2.623	
		T4	20.0	16.22	37.92	6.302	0.33	4.74E+05	2.33E+07		0.94	6.38E+07	0.120	2.605	
		T4	40.0	14.66	38.12	6.691	1.47	5.13E+05	1.12E+07		0.76	5.15E+07	0.091	3.383	
		T4	60.0	13.52	38.25	6.308	0.45	4.49E+05	6.56E+06		2.25	1.53E+08	0.279	1.214	
		T4	80.0	13.24	38.31	5.844	0.37	3.91E+05	9.70E+06		0.69	4.71E+07	0.108	2.873	
		T4	200.0	13.19	38.48	4.939		1.22E+05			0.63	4.25E+07	0.285	1.191	
	Mar obert	T5	5.0	19.52	37.97	5.436	0.10	3.28E+05	1.90E+07		3.28	2.22E+08	0.495	0.746	
		T5	20.0	16.94	38.13	6.067	0.16	4.37E+05	7.70E+06		0.85	5.77E+07	0.118	2.653	
		T5	40.0	14.05	38.07	6.497	0.99	5.06E+05	8.42E+06		2.22	1.51E+08	0.248	1.345	
		T5	55.0	13.5	38.19	5.821	0.28	2.62E+05	6.16E+06		0.54	3.69E+07	0.125	2.506	
		T5	80.0	13.22	38.33	5.572	0.10	1.53E+05	6.63E+06		0.31	2.11E+07	0.123	2.554	
		T5	200.0	13.17	38.48	4.914	0.03	1.38E+05	4.45E+06		0.33	2.25E+07	0.144	2.202	
		T7	5.0	19.45	37.83	5.477	0.09	3.21E+05	1.18E+07		6.16	4.18E+08	0.804	0.505	
		T7	20.0	17.35	37.90	6.098	0.22	5.22E+05	2.18E+07		4.17	2.83E+08	0.415	0.865	
		T7	40.0	14.5	38.16	6.84	0.88	4.84E+05	2.75E+07		1.53	1.04E+08	0.186	1.746	
		T7	50.0	13.63	38.29	6.899	0.48	4.29E+05	1.47E+07		1.45	9.86E+07	0.197	1.654	
		T7	80.0	13.04	38.30	5.501	0.04	1.18E+05	5.90E+06		0.75	5.12E+07	0.344	1.013	
		T7	200.0	13.15	38.45	4.798	0.02	1.23E+05	1.77E+06		1.26	8.53E+07	0.505	0.734	
		T9	5.0	19.77	37.51	5.309	0.09	2.63E+05	1.77E+07		0.85	5.75E+07	0.188	1.721	
		T9	20.0	17.42	37.95	5.874	0.15	3.83E+05	1.15E+07		1.12	7.57E+07	0.172	1.872	
		T9	40.0	14.75	37.99	6.315	0.49	4.07E+05	2.34E+07		1.32	8.96E+07	0.190	1.713	
		T9	50.0	14.12	38.09	6.217	1.51	4.12E+05	1.00E+07		0.83	5.64E+07	0.122	2.567	
		T9	80.0	13.1	38.17	5.566	0.29	1.96E+05	1.28E+07		0.83	5.62E+07	0.241	1.383	
		T9	200.0	13.11	38.38	5.003	0.01	8.25E+04	5.25E+06		0.52	3.56E+07	0.343	1.017	
C 24 hores	dia	E2 C1.3	5.0	18.72	37.77	5.818	0.17	3.32E+05	7.88E+06	6.01E+02	7.60	5.15E+08	0.905	0.460	3.14E+05
(Mediterrani)	nit	E2 C1.3	50.0	13.78	38.04	6.05	0.64	3.15E+05	6.15E+06	1.61E+02	1.04	7.05E+07	0.192	1.690	7.99E+04
	dia	E2 C1.6	5.0	16.78	37.94	5.916	0.29	3.43E+05	1.41E+07	7.89E+02	6.34	4.30E+08	0.783	0.515	2.33E+04
	nit	E2 C1.6	50.0	14.12	38.06	6.31	0.74	6.12E+05	6.20E+06	4.28E+02	3.55	2.41E+08	0.317	1.089	1.03E+05

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	dia	E3 C1.3	5.0	19.33	37.86	5.389	0.06	2.30E+05	1.56E+07	3.00E+00	2.78	1.89E+08	0.576	0.660	3.29E+05
	nit	E3 C1.3	45.0	13.62	38.28	6.662	0.58	1.00E+05	3.10E+07		1.99	1.35E+08	0.825	0.494	5.82E+05
	dia	E3 C1.6	5.0	19.55	38.00	5.327	0.17	2.93E+05	1.70E+07	8.39E+02	2.00	1.36E+08	0.364	0.966	9.59E+04
	nit	E3 C1.6	52.0	13.86	38.29	6.755	0.66	3.00E+05	2.29E+07	7.12E+02	1.40	9.48E+07	0.262	1.284	4.14E+04
	dia	E2 C2.2	5.0	19.68	38.08	5.394	0.12	2.90E+05	1.37E+07		3.42	2.32E+08	0.564	0.671	
	nit	E2 C2.2	60.0	14.81	38.10	6.125	0.84	3.19E+05	1.63E+07		2.85	1.93E+08	0.454	0.802	
	dia	E1 C1.4	5.0	19.5	37.91	5.765	0.31	5.51E+05	2.34E+07	5.32E+02	4.07	2.76E+08	0.388	0.914	7.28E+04
	nit	E1 C1.4	50.0	14.04	37.96	6.384	0.64	6.27E+05	1.17E+07	2.10E+03	5.40	3.66E+08	0.441	0.822	1.27E+05
	dia	E3 C2.2	5.0	19.49	38.04	5.887	0.13	9.24E+04	1.34E+07	8.82E+02	0.98	6.67E+07	0.521	0.715	6.69E+04
	nit	E3 C2.2	40.0	14.21	38.22	7.16	0.52	3.15E+05	2.75E+07	8.64E+02	0.63	4.30E+07	0.122	2.575	1.28E+05
	dia	E1 C2.3	5.0	17.83	37.70	6.061	0.35	7.00E+05	2.66E+07	1.04E+03	1.45	9.83E+07	0.125	2.510	1.29E+05
	nit	E1 C2.3	40.0	14.82	37.93	6.355	0.58	5.61E+05	2.92E+07	5.90E+02	0.41	2.81E+07	0.046	6.449	
		Ciso													
perfil vertical	jul-94	C 1.00	1.0	22.25		6.76		1.90E+07	1.90E+08		90.22	5.52E+09	0.258	1.313	
		C 2.25	2.3			1.69		2.40E+07	1.52E+08		36.74	1.49E+09	0.073	4.440	
		C 2.50	2.5	17		1.85		4.14E+07	1.35E+08		66.80	2.71E+09	0.081	3.958	
		C 2.75	2.8			0.7		4.00E+07	2.30E+08		47.01	1.91E+09	0.059	5.435	
		C 5.00	5.0	10.5		0		1.80E+07	2.03E+08		57.46	1.96E+09	0.032	27.589	
C. 24h (Cisó)	ago-94	CA1 24h	0.5	23.1		14.85	10.00	5.26E+06	7.30E+07		121.23	8.72E+09	0.977	0.426	
Hores	14:00	CB1 24h	2.5	20.4		8.41	210.00	2.21E+07	2.14E+08		474.97	2.57E+10	0.772	0.535	
	14:00	CC1 24h	2.6			6.08	0.00	2.23E+07	2.17E+08		326.74	1.95E+10	0.629	0.622	
	14:00	CD1 24h	3.0	18.5		2.57	0.00	2.21E+07	1.54E+08		81.31	3.52E+09	0.148	2.368	
	14:00	CE1 24h	3.5	15.5		1.81	0.00	1.93E+07	9.80E+07		40.29	1.84E+09	0.091	3.689	
	18:00	CA3 24h	0.5	23.7		13.16	16.25	4.96E+06	1.62E+08		85.09	6.42E+09	0.804	0.496	
	18:00	CB3 24H	2.5	21.9		5.84	122.60	1.96E+07	7.86E+07		287.36	1.56E+10	0.584	0.672	
	18:00	CC3 24H	2.7			2.87	0.00	2.10E+07	9.08E+07		193.52	1.09E+10	0.438	0.837	
	18:00	CD3 24H	3.0	18.5		1.92	0.00	2.08E+07	1.56E+08		66.45	2.99E+09	0.130	2.679	
	18:00	CE3 24H	3.5	16		0.69	0.00	1.90E+07	1.46E+08		36.62	1.67E+09	0.084	3.969	
	2:00	CA5 24h	0.5	23		11.49	17.18	5.29E+06	7.65E+07		66.54	4.07E+09	0.571	0.670	
	2:00	CB5 24H	2.5			3.86	62.69	1.37E+07	1.02E+08		215.12	1.16E+10	0.615	0.644	
	2:00	CC5 24H	2.6			1.81	0.00	1.79E+07	1.52E+08		394.86	2.68E+10	0.840	0.493	
	2:00	CD5 24H	3.0	18.6		1.26	0.00	1.85E+07	1.88E+08		53.06	2.30E+09	0.117	2.948	
	2:00	CE5 24H	3.5	16		1.26	0.00	2.00E+07	1.79E+08		25.05	9.55E+08	0.047	7.395	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	6:00	CA7 24h	0.5	24		12.63	17.18	4.54E+06	8.03E+07		66.48	4.51E+09	0.719	0.541	
	6:00	CB7 24H	2.5	22.1		4.08	118.42	2.00E+07	1.71E+08		307.78	1.67E+10	0.606	0.652	
	6:00	CC7 24H	2.6				0.00	2.56E+07	5.86E+07		186.97	1.08E+10	0.362	0.985	
	6:00	CD7 24H	3.0	19		1.25	0.00	2.09E+07	7.85E+07		67.55	3.09E+09	0.131	2.652	
	6:00	CE7 24H	3.5	12		0.90	0.00	1.82E+07	5.25E+07		30.48	1.37E+09	0.074	4.517	
perfil (Ciso)	jun-94	CR 1	1.0	19.25		5.6		1.39E+06	6.60E+07						
		CR 2.4	2.4			3.5		3.62E+06	8.70E+07						
		CR 2.5	2.5	12		3.1		3.50E+06	1.05E+08						
		CR 2.6	2.6			2.3		9.27E+06	1.04E+08						
		CR 2.7	2.7			3.2		2.36E+07	9.50E+07						
		CR 2.8	2.8			2.4		4.00E+07	1.03E+08						
		CR 4	4.0	10.25		1.5		3.14E+07	7.60E+07						
		Llacuna del Tobar													
	sep-94	TB A	1.0	15.4		7.8	0.00	1.44E+06	2.57E+07		5.19	3.92E+08	0.241	1.336	
		TB B	10.5	14.8		8.3	0.10	9.88E+05	3.30E+07		12.91	1.02E+09	0.635	0.604	
		TB C	11.0	15		7.7	4.40	1.05E+06	2.31E+07		151.57	1.03E+10	2.379	0.216	
		TB D	11.5	15.3		7.7	2.00	1.34E+06	2.55E+07		28.56	1.73E+09	0.828	0.498	
		TB E	12.3			5.4	0.60	6.65E+06	3.01E+07		27.23	1.55E+09	0.210	1.605	
		TB F	12.8			0.4	0.30	4.19E+06	2.45E+07		25.60	1.35E+09	0.278	1.272	
		TB G	13.3			0.3	0.00	2.04E+07	1.07E+08		3.56	1.68E+08	0.008	38.801	
		TB H	14.0	13.1		0.3	0.00	7.74E+06	4.80E+07		1.63	7.12E+07	0.009	36.244	
		TB I	17.0	12.9		0.3	0.00	3.58E+06	3.14E+07		0.45	2.36E+07	0.006	107.428	
	sep-94	**Llacuna de La Cruz**													
		CRUZ A	1.0	17.7		9.6	0.10	2.32E+06	3.16E+07		5.94	4.03E+08	0.160	1.971	
		CRUZ B	10.3				0.70	2.82E+06	1.70E+08		52.16	3.59E+09	0.821	0.491	
		CRUZ C	11.5	10		0.7	1.70	1.23E+07	2.55E+08		84.50	5.11E+09	0.347	1.019	
		CRUZ D	11.8				4.80	1.09E+07	3.26E+08		129.69	8.34E+09	0.568	0.667	
		CRUZ E	12.0	9.3		0.5	2.30	8.03E+06	2.24E+08		121.90	7.11E+09	0.634	0.620	
		CRUZ F	12.3				0.00	8.66E+06	1.35E+08		120.19	6.14E+09	0.528	0.741	
		CRUZ G	13.0	7.3		0.4	0.00	7.87E+06	5.21E+07		20.18	1.08E+09	0.129	2.553	
		CRUZ H	15.0	6.2		0.4	0.00	7.65E+06	7.75E+07		9.42	4.91E+08	0.062	5.142	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
		Llac Saelenvannet (Noruega)													
perfil vertical	oct-94	B 0	0.0	10.5	1.00	12		3.05E+06	1.81E+08			3.00E+08	0.098	7.047	
		B 1	1.0	10	1.80	12		3.44E+06	1.59E+08			6.40E+08	0.186	3.731	
		B 1.3	1.3					8.19E+06	2.10E+08			1.30E+09	0.159	4.370	
		B 1.6	1.6	12	13.00	9		7.60E+06	3.16E+08			1.80E+09	0.237	2.927	
		B 2	2.0	14	14.00	2.5		1.10E+07	1.33E+08			5.10E+08	0.046	15.066	
		B 3	3.0	17	15.00	1		1.24E+07	1.45E+08			1.10E+08	0.009	78.136	
		B 4	4.0	17	15.50	0		8.70E+06	2.22E+08			9.00E+07	0.010	67.003	
perfil vertical		B 0	0.0	4	1.00	8		3.50E+06	6.58E+07			1.40E+08	0.040	17.329	
		B 1	1.0	8	3.50	9		1.08E+07	3.50E+08			1.30E+09	0.120	5.758	
		B 1.25	1.3	10.5	12.50	12		1.02E+07	1.50E+08			4.40E+09	0.431	1.607	
		B 1.5	1.5	12.5	15.00	2		8.38E+06	2.20E+08			2.50E+09	0.298	2.323	
		B 1.75	1.8	14	16.00	0		1.18E+07	2.60E+08			1.50E+09	0.127	5.453	
		B 2	2.0	15	14.50	0		1.56E+07	2.70E+08			1.10E+09	0.071	9.830	
		B 3	3.0	15	15.50	0		1.64E+07	2.05E+08			1.60E+08	0.010	71.048	
		B 4	4.0	15	15.50	0		1.16E+07	1.09E+08			1.60E+08	0.014	50.254	
perfil vertical	Març-95	B 0	0.0	3	10.00	11.3		6.62E+06	2.25E+08			2.98E+08	0.044	15.718	
		B 1	1.0	3	10.00	11.3		6.38E+06	2.17E+08			3.69E+08	0.056	12.325	
		B 1.5	1.5	3	10.00	9.5		6.32E+06	1.68E+08			4.80E+08	0.073	9.462	
		B 2	2.0	3.5	10.00	8.8		7.62E+06	1.79E+08			2.81E+08	0.036	19.137	
		B 2.5	2.5	5	19.00	5		8.00E+06	1.86E+08			2.54E+08	0.031	22.200	
		B 3	3.0	6.5	17.00	0		3.20E+07	2.30E+08			7.39E+07	0.002	300.428	
		B 3.5	3.5	9	19.00	0		1.69E+07	2.21E+08			8.18E+07	0.005	143.467	
		B 5	5.0	10	20.50	0		6.69E+06	1.68E+08			3.41E+07	0.005	136.226	
Berg. et al. 89	may-88	LlacPlußsse	0.2					6.20E+06	2.54E+08						
	may-88	Cheasapeake bay	1.0					3.20E+06	1.01E+07						
	oct-88	Korsfjorden	1.0					1.10E+06	6.10E+06						
	ago-89	Raunefjorden	1.0					2.00E+05	9.90E+06						
	feb-89	Raunefjorden	1.0					5.00E+05	1.00E+04						
	mar-89	Raunefjorden	1.0					5.00E+05	4.80E+06						
	may-88	North Atlantic	10.0					3.00E+05	1.49E+07						
	ene-89	Barents sea	30.0					2.00E+04	6.00E+04						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
Bratbak et al 90	mar-89	Raunefjorden	1.5					1.25E+05	8.02E+05	5.78E+02					
Bloom	mar-89	(Norway)	1.5					1.40E+05	4.22E+05	1.05E+03					
C. estacional	mar-89		1.5					3.44E+05	5.58E+06	8.05E+02					
	mar-89		1.5					2.09E+05	4.86E+06	1.19E+03					
	mar-89		1.5					4.35E+05	7.29E+06	1.85E+03					
	mar-89		1.5					4.33E+05	1.56E+06	7.99E+02					
	mar-89		1.5					2.43E+05	9.98E+06	5.59E+02					
	mar-89		1.5					2.42E+05	6.39E+06	2.42E+03					
	mar-89		1.5					6.11E+05	1.25E+06	2.67E+03					
	abr-89		1.5					6.15E+05	1.87E+06	3.34E+03					
	abr-89		1.5					2.88E+05	6.51E+05	2.88E+03					
Heldal&Bratbak91	jun-90	Dept MB	S	12				4.40E+06	4.40E+07			2.45E+08	0.55	1.26	
	jun-90	Bergen Harbour	1.5	13				2.90E+06	5.90E+07						
	jun-90	Raunefjoreden	S	14				1.40E+06	3.50E+07						
	jun-90	Raunefjoreden	5.0	14				1.50E+06	2.80E+07						
	jun-90	Raunefjoreden	25.0	8				7.00E+05	1.10E+07						
	ago-90	Lake Kalandsvan	0.5	17	1.00			3.00E+06	2.02E+08						
	ago-90	Lake Kalandsvan	1.0	16	1.00			4.10E+06	1.54E+08						
	ago-90	Lake Kalandsvan	25.0	5	1.00			8.00E+05	1.90E+07						
Hara et al.91	sep-87	Osaka Bay	0.0					2.00E+06	3.50E+07						
	jun-90	Otsuchi Bay1	0.0					3.40E+05	2.60E+06						
		Otsuchi Bay2	0.0					1.90E+05	1.70E+06						
		Otsuchi Bay2	15.0					2.70E+05	4.10E+06						
		Otsuchi Bay2	60.0					3.10E+05	2.20E+06						
		Otsuchi Bay3	0.0					1.90E+05	1.70E+06						
	ago-90	Sagami bayA	0.0					2.90E+06	1.40E+07						
		Sagami bayC	0.0					2.50E+06	1.00E+07						
		Sagami bayC	10.0					3.10E+06	7.70E+06						
		Sagami bayC	50.0					2.40E+06	5.60E+06						
		Sagami bayC	100.0					1.30E+06	3.70E+06						
		Sagami bayC	200.0					9.30E+05	2.70E+06						
	abr-88	Kuroshiro1	10.0					3.70E+05	2.00E+06						
		Kuroshiro2	10.0					4.10E+05	1.20E+06						
		Kuroshiro3	10.0					3.90E+05	1.20E+06						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
Nygaard et al 91		Oslofjorden													
		Perfil	0.0	16.31	22.22	7.4	4.71	1.15E+06	5.48E+07	3.70E+03		2.42E+09	2.03	0.49	8.91E+04
			2.0	16.1	22.31	7.4	5.10	1.20E+06	1.29E+08	5.76E+03		2.34E+09	1.88	0.53	1.35E+05
			4.0	15.84	22.47	7.7	1.33	1.40E+06	6.44E+07	4.58E+03		2.34E+09	1.61	0.62	9.60E+04
			6.0	16.01	22.65	7	0.72	1.23E+06	6.01E+07	4.63E+03		1.41E+09	1.12	0.90	1.56E+05
			8.0	16.18	23.20	6.8	0.63	1.32E+06	7.42E+07	3.31E+03		1.07E+09	0.80	1.26	5.80E+04
			12.0	14.54	26.14	3.4	0.77	1.15E+06	4.81E+07	2.54E+03		5.18E+08	0.45	2.24	6.25E+04
			16.0	10.46	29.29	2.8	0.66	1.17E+06	2.35E+07	4.80E+03		2.57E+08	0.22	4.58	1.13E+05
			30.0	7.72	31.25	1.9	0.22	4.40E+05	1.78E+07	1.15E+03		1.84E+08	0.41	2.41	8.77E+04
Steward et al 92b	nov-91	Peñasquitos	0.0				8.00	1.41E+07	6.20E+08			3.10E+09	0.20	3.49	
cosat(California)	nov-91	Mission Bay	0.0				1.85	3.90E+06	1.37E+08			9.40E+08	0.22	3.21	
	nov-91	Scripps Pier	0.0				0.59	6.90E+05	1.17E+07			6.50E+08	0.66	1.04	
mig(California)	sep-91	301	10.0				0.93	2.10E+06	1.37E+07						
	dic-91	2	2.0				0.64	4.80E+05	1.17E+07			3.00E+09	1.98	0.35	
Oceà(California)	dic-91	5	2.0				0.42	3.40E+05	1.83E+07			1.90E+08	0.44	1.56	
	dic-91	8	2.0				0.49	4.20E+05	1.53E+07			2.20E+08	0.42	1.65	
	sep-91	305	5.0				0.18	8.70E+05	1.24E+07			4.80E+08	0.44	1.58	
	sep-91	305	10.0				0.28	7.10E+05	1.20E+07			1.70E+09	1.22	0.57	
	sep-91	305	30*					8.10E+05	1.07E+07			7.80E+07	0.09	7.54	
	sep-91	305	50*					4.50E+05	5.70E+06			2.10E+07	0.05	15.20	
	sep-91	305	700.0				0.00	7.00E+04	1.10E+06			6.30E+06	0.09	8.04	
	sep-91	305	900.0				0.00	1.10E+05	2.50E+06			4.10E+06	0.04	18.94	
Cochlan et al. 93	sep-90	Costa California	0.0				1.43	2.23E+06	2.83E+07						
Perfils			10.0				2.58	2.31E+06	1.88E+07						
			20.0				1.97	1.49E+06	7.18E+06						
			30.0				0.18	5.19E+05	7.99E+06						
			40.0				0.12	3.64E+05	3.13E+06						
			50.0				0.13	4.01E+05	3.72E+06						
		Mig	0.0				0.69	1.88E+06	5.52E+06						
			10.0				0.39	1.09E+06	1.03E+06						
			20.0				0.78	8.57E+05	3.16E+06						
			30.0				0.28	6.21E+05	1.16E+06						
			40.0				0.09	6.24E+05	3.86E+06						
			50.0				0.09	3.73E+05	2.58E+06						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
		Oceà	0.0				0.30	1.52E+06	1.33E+06						
			10.0				0.17	6.88E+05	2.13E+06						
			20.0				0.12	5.04E+05	2.48E+06						
			30.0				0.47	4.67E+05	2.02E+06						
			40.0				0.09	2.98E+05	2.46E+05						
			50.0				0.04	2.84E+05	3.50E+05						
			100.0					2.70E+05	8.67E+05						
			200.0					2.44E+05	2.95E+05						
			400.0					1.39E+05	3.01E+05						
Cochlan et al. 93	ago-91	Gulf of Bothnia	0.0				1.24	2.24E+06	3.31E+07						
(continuació)		Costa	4.0				1.23	2.50E+06	3.60E+07						
			8.0				1.23	1.97E+06	3.30E+07						
			20.0				0.66	2.05E+06	2.58E+07						
Wommack et al. 92		Chesapeake bay													
Costa	sep-90	908	Super.					5.87E+06	2.30E+07						
			Fons					4.21E+06	2.12E+07						
		858	S					6.67E+06	2.40E+07						
			F					3.47E+06	1.62E+07						
		845	S					5.93E+06	7.21E+06						
			F					3.12E+06	1.15E+07						
		818	S					8.39E+06	4.78E+06						
			F					1.79E+06	5.44E+06						
		804	S					6.72E+06	1.29E+07						
			F					2.21E+06	1.10E+07						
	abr-91	908	S					2.21E+06	2.28E+07						
			F					3.62E+06	2.39E+07						
		858	S					2.77E+06	2.84E+07						
			F					3.43E+06	4.43E+07						
		845	S					3.28E+06	7.51E+06						
			F					2.99E+06	3.81E+07						
		818	S					2.84E+06	1.48E+07						
			F					2.14E+06	2.24E+07						
		804	S					2.47E+06	2.07E+07						
			F					2.47E+06	3.28E+07						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	ago-91	908	S					2.00E+06	3.54E+07						
			F					2.58E+06	2.82E+07						
		858	S					3.01E+06	3.43E+07						
			F					3.01E+06	2.36E+07						
		845	S					2.35E+06	2.78E+07						
			F					2.06E+06	1.54E+07						
		818	S					2.12E+06	4.14E+07						
			F					1.61E+06	1.80E+07						
		804	S					4.37E+06	5.17E+07						
			F					1.93E+06	1.70E+07						
		756	S					2.25E+06	4.91E+07						
			F					1.85E+06	1.85E+07						
Wommacket al. 92	jun-91	908	S					8.83E+06	2.92E+07						
			F					5.82E+06	2.47E+07						
		858	S					1.17E+07	2.69E+07						
			F					4.14E+06							
		845	S					9.49E+06	3.36E+07						
			F					3.30E+06	3.17E+07						
		818	S					5.50E+06	1.92E+07						
			F					4.43E+06	8.86E+06						
		804	S					2.42E+06	1.68E+07						
			F					2.02E+06	2.73E+07						
		756	S					2.09E+06	2.05E+07						
			F					9.52E+05	3.28E+07						
	oct-91	908	S					2.36E+06	2.15E+07						
			F					2.87E+06	2.55E+07						
		858	S					2.06E+06	3.01E+07						
			F					2.50E+06	2.28E+07						
		845	S					2.31E+06	2.88E+07						
			F					1.95E+06	3.53E+07						
		818	S					2.34E+06	2.69E+07						
			F					1.03E+06	5.23E+07						
		804	S					7.34E+05	3.29E+07						
			F					8.05E+05	1.61E+07						
		756	S					5.82E+05	2.23E+07						
			F					7.25E+05	5.02E+07						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
Paul et al. 93		Key Largo (Florida)													
		Blakwater sound		16.99	26.39		0.45	9.35E+05	1.20E+07						
		Tarpon sound		17.86	28.96		0.25	1.71E+06	8.37E+06						
		1		14.53	33.99		0.72	1.12E+06	4.97E+06						
		2		14.15	34.00		2.82	6.72E+05	3.18E+06						
		3		13.65	35.33		0.38	8.46E+05	3.19E+06						
		Moskito Bank		13.21	35.67		0.34	9.58E+05	1.78E+06						
		French Reef		19.57	34.20		0.48	3.52E+05	1.60E+06						
		6		19.73	34.09		0.46	6.13E+05	1.48E+06						
Boehme et al. 93	jun-92	Golf de Mèxic													
Transecte		OffS 9	3.0				0.03	1.58E+05	5.06E+05						
oceà		OffS 9	113.8				0.08	8.81E+04	5.35E+05						
		OffS 8	3.0				0.08	3.00E+05	5.05E+05						
		OffS 8	82.1				0.20	1.85E+05	4.60E+05						
		OffS 8	2457.0				0.08	5.52E+03	3.63E+04						
Boehme et al. 93		OffS 10	3.0	28.49	36.10	6.05	0.08	2.57E+05	6.09E+05						
		OffS 10	21.5	26.52	36.34	6.45	0.08	2.65E+05	5.12E+05						
		OffS 10	47.0	21.79	36.20	7	0.23	2.47E+05	8.47E+05						
		OffS 10	64.0	21	36.20	6.6	0.23	1.70E+05	5.03E+05						
		OffS 10	97.0	19.09	36.40	4.55	0.05	5.73E+04	3.72E+05						
		OffS 10	442.9					4.15E+04	5.47E+04						
		OffS 10	935.7					1.53E+04	6.83E+04						
		OffS 10	2436.0					3.04E+03	1.01E+04						
mig		NS 7	3.0				0.00	1.71E+05	4.86E+05						
		NS 7	314.3				0.00	2.90E+04	2.48E+04						
		NS 7	1407.0				0.10	3.31E+04	1.25E+04						
		NS 6	3.0				0.00	1.42E+05	4.84E+05						
		NS 6	98.3				0.08	6.47E+04	7.52E+04						
		NS 6	196.1				0.11	1.19E+05	3.99E+04						
		NS 5	3.0				0.00	1.42E+05	7.44E+05						
		NS 5	98.4				0.05	5.05E+05	7.54E+05						
costa		C 4	3.0				0.11	2.02E+05	3.22E+05						
		C 4	56.0				0.38	3.68E+05	6.28E+05						
		C 3	3.0				0.12	4.07E+05	4.65E+05						
		C 3	29.5				0.53	9.04E+05	1.73E+06						
		C 2	3.0				1.70	1.54E+06	3.67E+06						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
		C 2					1.75	3.48E+06	6.16E+06						
		C 1	3.0				8.28	5.32E+06	1.61E+07						
C. estacional	may-91	SJ107	0.5					3.72E+05	1.32E+07						
		SJ105	0.5					4.23E+05	5.64E+06						
		SJ103	0.5					4.93E+05	3.27E+06						
		SJ101	0.5					9.11E+05	7.37E+07						
		SJ108	0.5					1.05E+06	5.50E+07						
	jul-91	SJ107	0.5					7.56E+05	1.24E+07						
		SJ105	0.5					1.11E+06	2.09E+07						
		SJ103	0.5					6.07E+05	2.91E+07						
		SJ101	0.5					6.34E+05	1.01E+07						
		SJ108	0.5					1.21E+06	3.68E+07						
	ago-91	SJ107	0.5					3.95E+05	4.72E+06						
		SJ105	0.5					4.28E+05	5.67E+06						
		SJ103	0.5					5.34E+05	1.27E+07						
		SJ101	0.5					1.25E+06	4.26E+07						
		SJ108	0.5					1.89E+06	8.68E+07						
	may-92	SJ107	0.5					2.22E+05	1.45E+06						
		SJ105	0.5					2.45E+05	5.54E+06						
		SJ103	0.5					2.08E+05	3.75E+06						
		SJ101	0.5					2.90E+05	5.29E+06						
		SJ108	0.5					4.15E+05	2.94E+07						
Boehme et al. 93	jun-92	SJ107	0.5					8.10E+05	1.34E+07						
C. estacional		SJ105	0.5					6.65E+05	1.05E+07						
(continuacio)		SJ103	0.5					6.40E+05	6.71E+06						
		SJ101	0.5					1.07E+06	1.44E+07						
		SJ108	0.5					9.74E+05	3.15E+07						
	ago-92	SJ107	0.5					4.38E+05	1.07E+07						
		SJ105	0.5					3.34E+05	5.73E+06						
		SJ103	0.5					6.82E+05	9.37E+06						
		SJ101	0.5					3.56E+05	5.03E+06						
		SJ108	0.5					1.92E+06	2.33E+07						
	nov-92	SJ107	0.5					1.21E+06	2.85E+07						
		SJ105	0.5					1.55E+06	4.20E+07						
		SJ103	0.5					1.85E+06	1.32E+07						
		SJ101	0.5					9.62E+05	3.17E+06						
		SJ108	0.5					1.15E+06	1.59E+07						

Jiang & Paul 94		Tampa Bay (Superficie)														
C. Estacional	dic-90	Tampa Bay		23.96	29.27		1.62	3.66E+06	5.81E+06							
	dic-90	Tampa Bay	S	17.74	30.61		2.16	3.17E+06	5.20E+06							
	ene-91	Tampa Bay	S	22.20	30.06		4.32	3.36E+06	3.63E+06							
	ene-91	Tampa Bay	S	20.24	29.15		3.68	2.56E+06	6.05E+06							
	feb-91	Tampa Bay	S	19.27	30.12		3.19	1.83E+06	6.53E+06							
	feb-91	Tampa Bay	S	19.15	30.43		4.27	2.19E+06	2.66E+06							
	mar-91	Tampa Bay	S	19.15	30.43		3.78	1.87E+06	4.96E+06							
	mar-91	Tampa Bay	S	19.09	32.07		3.28	2.43E+06	2.42E+06							
	abr-91	Tampa Bay	S	25.18	29.70		1.56	2.24E+06	3.87E+06							
	abr-91	Tampa Bay	S	24.57	30.79		3.38	1.80E+06	5.08E+06							
	may-91	Tampa Bay	S	26.16	30.18		1.95	3.94E+06	5.44E+06							
	may-91	Tampa Bay	S	28.17	31.28		3.72	3.14E+06	9.44E+06							
	jun-91	Tampa Bay	S	29.33	29.82		4.26	4.92E+06	2.26E+07							
	jun-91	Tampa Bay	S	28.23	29.15		3.42	4.11E+06	9.44E+06							
	jul-91	Tampa Bay	S	30.18	29.76		5.38	4.18E+06	1.67E+07							
	jul-91	Tampa Bay	S	29.27	26.22		11.53	5.60E+06	1.82E+07							
	ago-91	Tampa Bay	S	29.70	26.34		7.49	5.60E+06	1.36E+07							
	ago-91	Tampa Bay	S	30.55	26.28		10.20	5.11E+06	1.10E+07							
	sep-91	Tampa Bay	S	28.78	24.88		8.77	4.35E+06	3.01E+07							
	sep-91	Tampa Bay	S	31.04	21.22		12.90	5.47E+06	2.90E+07							
	sep-91	Tampa Bay	S	26.52	24.21		12.16	3.10E+06	2.64E+07							
	oct-91	Tampa Bay	S	27.13	26.22		7.44	3.01E+06	2.19E+07							
	oct-91	Tampa Bay	S	25.24	23.17		6.45	3.57E+06	3.25E+07							
	nov-91	Tampa Bay	S	24.21	26.16		3.69	2.56E+06	8.35E+06							
	nov-91	Tampa Bay	S	21.22	27.20		3.05	2.54E+06	7.26E+06							
	dic-91	Tampa Bay	S	21.16	29.15		2.36	3.10E+06	3.51E+06							
	dic-91	Tampa Bay	S	19.33	29.70		2.51	2.22E+06	4.96E+06							

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
Hennes&Simon95	abr-92	Llac Constance	0-8m				1.03	9.58E+05	1.40E+06						
C. estacional	abr-92	Constance	0-8m				2.66	6.08E+05	9.11E+05						
	abr-92	Constance	0-8m				5.12	8.32E+05	2.64E+06						
	abr-92	Constance	0-8m				9.01	8.35E+05	5.01E+06						
	abr-92	Constance	0-8m				39.57	4.16E+06	1.34E+07						
	may-92	Constance	0-8m				10.67	8.37E+05	2.50E+07						
	may-92	Constance	0-8m				7.39	1.06E+06	6.71E+06						
	may-92	Constance	0-8m				11.89	1.80E+06	1.16E+07						
	may-92	Constance	0-8m				3.28	4.23E+06	2.76E+07						
	jun-92	Constance	0-8m				2.26	2.67E+06	4.17E+07						
	jun-92	Constance	0-8m				1.85	1.42E+06	3.87E+07						
	jun-92	Constance	0-8m				1.02	1.81E+06	2.05E+07						
	jun-92	Constance	0-8m				3.49	2.22E+06	1.93E+07						
	jun-92	Constance	0-8m				2.05	3.53E+06	4.09E+07						
	jul-92	Constance	0-8m				3.48	1.43E+06	3.09E+07						
	jul-92	Constance	0-8m				3.07	1.18E+06	1.04E+07						
	jul-92	Constance	0-8m				4.72	9.86E+05	1.05E+07						
	jul-92	Constance	0-8m				7.18	2.33E+06	3.67E+07						
	jul-92	Constance	0-8m				11.09	1.34E+06	7.08E+06						
Mathias et al.95	ene-92	Danube river	0.4	0.73				2.24E+06	1.19E+07			7.76E+08	0.34	2.01	
C. Estacional	mar-92	Danube river	0.4	0.94				3.09E+06	2.25E+07			7.93E+08	0.26	2.71	
	abr-92	Danube river	0.4	5.21				2.57E+06	1.09E+07			1.23E+09	0.47	1.46	
	nov-92	Danube river	0.4	2.95				4.21E+06	3.46E+07			9.76E+08	0.23	3.00	
	dic-92	Danube river	0.4	1.67				2.53E+06	1.89E+07			8.55E+08	0.34	2.06	
	ene-93	Danube river	0.4	1.83				2.10E+06	1.29E+07			8.70E+08	0.41	1.69	
	mar-93	Danube river	0.4	4.59				5.87E+06	1.16E+07			1.16E+09	0.20	3.53	
	nov-93	Danube river	0.4	4.69				2.55E+06	2.01E+07			1.17E+09	0.45	1.53	
	dic-93	Danube river	0.4	1.76				2.05E+06	1.19E+07			8.63E+08	0.42	1.66	
	abr-93	Danube river	0.4	9.7				3.79E+06	2.24E+07			1.96E+09	0.51	1.36	
	abr-92	Danube river	0.4	10.74				1.81E+06	1.33E+07			2.18E+09	1.18	0.59	
	oct-93	Danube river	0.4	12.61				2.63E+06	4.50E+07			2.64E+09	0.98	0.70	
	oct-92	Danube river	0.4	14.6				4.70E+06	3.67E+07			3.25E+09	0.68	1.02	
	may-93	Danube river	0.4	17.98				4.58E+06	6.07E+07			4.60E+09	0.98	0.70	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	sep-92	Danube river	0.4	18.56				2.72E+06	1.69E+07			4.88E+09	1.73	0.40	
	sep-93	Danube river	0.4	18.93				2.32E+06	3.78E+07			5.07E+09	2.09	0.33	
	may-92	Danube river	0.4	21.15				2.50E+06	1.53E+07			6.38E+09	2.42	0.29	
	jun-93	Danube river	0.4	21.89				3.37E+06	5.42E+07			6.89E+09	1.96	0.35	
	jul-93	Danube river	0.4	22.31				4.53E+06	2.75E+07			7.19E+09	1.54	0.45	
	ago-92	Danube river	0.4	22.99				4.02E+06	1.79E+07			7.71E+09	1.85	0.38	
	jun-92	Danube river	0.4	24.19				3.23E+06	2.24E+07			8.73E+09	2.56	0.27	
	jul-92	Danube river	0.4	25.55				3.77E+06	1.23E+07			1.00E+10	2.53	0.27	
	ago-93	Danube river	0.4	25.15				3.50E+06	3.71E+07			9.64E+09	2.61	0.27	
Hennes & Suttle 95		Golf de Mèxic													
		GM1.592	Superfic.		28.85		0.19	1.27E+06	1.33E+08						
		GM13.21	S		28.81		0.50	5.24E+06	1.22E+08						
		GM24.9	S		27.49		0.44	2.79E+06	6.72E+06						
		GM33.75	S		29.15		0.79	2.80E+06	1.29E+08						
		GM44.03	S		27.84		0.65	1.70E+06	1.04E+08						
		GM53.77	S		27.63		0.62	2.63E+06	1.11E+08						
		GM62.99	S		28.15		0.50	3.17E+06	7.72E+07						
		GM73.89	S		29.57		0.50	1.42E+06	6.85E+07						
		GM83.6	S		35.44		0.11	1.80E+06	2.26E+07						
		GM93.36	S		33.32		0.65	2.98E+06	4.54E+07						
Weinbauer et al.95	jun-91	Adriàric	0.5					9.15E+05	7.51E+06						
C. Estacional		Adriàric	2.9					8.30E+05	5.75E+06						
		Adriàric	6.0					1.11E+06	1.02E+07						
		Adriàric	9.0					5.53E+05	5.31E+06						
	jul-91	Adriàric	0.5					1.22E+06	9.29E+06						
		Adriàric	4.0					1.31E+06	1.47E+07						
		Adriàric	8.1					1.65E+06	2.50E+07						
		Adriàric	12.0					9.04E+05	9.19E+06						
	ago-91	Adriàric	0.5					1.12E+06	1.91E+07						
		Adriàric	4.0					1.01E+06	9.33E+06						
		Adriàric	8.0					1.94E+06	3.78E+07						
		Adriàric	12.2					1.21E+06	1.11E+07						
	may-92	Adriàric	0.5					4.05E+05	6.25E+06						
		Adriàric	2.9					4.06E+05	7.59E+06						
		Adriàric	6.0					4.31E+05	7.59E+06						
		Adriàric	9.0					4.00E+05	6.25E+06						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	nov-92	Adriàric	0.6					1.23E+06	6.51E+07						
		Adriàric	4.9					7.93E+05	6.27E+07						
		Adriàric	10.0					8.66E+05	6.52E+07						
		Adriàric	15.1					6.86E+05	6.24E+07						
	feb-92	Adriàric	0.5					3.34E+05	1.80E+06						
		Adriàric	5.0					3.12E+05	2.24E+06						
		Adriàric	9.9					3.01E+05	2.24E+06						
		Adriàric	15.0					2.67E+05	1.35E+06						
	jun-91	Arusinia	0.0					4.85E+05	7.80E+06	1.51E+03					
	ago-91	Arusinia	0.0				0.32	7.84E+05	9.18E+06	4.12E+03					
	oct-91	Arusinia	0.0				2.08	1.08E+06	1.41E+07	6.99E+03					
	dic-91	Arusinia	0.0				0.69	1.53E+06	5.47E+07	4.21E+03					
	feb-92	Arusinia	0.0				0.07	1.70E+05	2.88E+05	1.75E+03					
	abr-92	Arusinia	0.0				0.70	2.45E+05	1.26E+05	2.09E+03					
	jun-92	Arusinia	0.0				0.20	6.59E+05	6.14E+06	1.15E+03					
	may-92	Rovinj	0.0				2.51	6.60E+05	1.41E+07	1.00E+03					
	jun-92	Rovinj	0.0				0.46	3.22E+05	3.58E+06	6.89E+03					
	ago-92	Rovinj	0.0				0.16	6.77E+05	5.13E+07	6.86E+03					
	nov-92	Rovinj	0.0				3.07	7.45E+05	6.23E+07	4.20E+03					
	feb-92	Rovinj	0.0				0.07	1.42E+05	1.37E+06	1.96E+03					
C.diari (Hora)	12.00	Adriàtic	20.0				0.62	5.53E+05	2.21E+06	1.36E+03					
jun-92	12.00	Adriàtic	20.0				0.63	6.19E+05	1.89E+06						
	16.00	Adriàtic	20.0				0.67	8.91E+05	3.01E+06	1.89E+03					
	18.00	Adriàtic	20.0				0.78	7.20E+05	2.89E+06						
	20.00	Adriàtic	20.0				0.61	3.24E+05	5.01E+06	2.52E+03					
	22.00	Adriàtic	20.0				0.61	3.33E+05	4.51E+06						
	24.00	Adriàtic	20.0				0.60	5.24E+05	3.90E+06	3.55E+03					
	2.00	Adriàtic	20.0				0.78	6.21E+05	4.00E+06						
	4.00	Adriàtic	20.0				0.71	3.89E+05	3.09E+06	3.18E+03					
	6.00	Adriàtic	20.0				0.60	5.22E+05	3.90E+06						
	8.00	Adriàtic	20.0				0.62	6.06E+05	3.21E+06	1.74E+03					
	10.00	Adriàtic	20.0				0.56	6.15E+05	4.50E+06						
	12.00	Adriàtic	20.0				0.56	5.30E+05	4.89E+06	1.74E+03					
	12	Adriàtic	5.0				5.00	1.90E+06	9.29E+06	6.18E+03					
	14	Adriàtic	5.0				4.57	1.12E+06	1.53E+07						
	16	Adriàtic	5.0				3.88	9.01E+05	1.32E+07	3.60E+03					
	18	Adriàtic	5.0				1.64	7.40E+05	1.30E+07						

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
	20	Adriàtic	5.0				3.14	8.13E+05	1.03E+07	3.18E+03					
	22	Adriàtic	5.0				2.79	8.26E+05	5.21E+06						
	24	Adriàtic	5.0				2.85	1.26E+06	6.93E+06	2.96E+03					
	2	Adriàtic	5.0				2.29	1.10E+06	8.25E+06						
	4	Adriàtic	5.0				3.14	7.94E+05	7.40E+06	1.29E+03					
	6	Adriàtic	5.0				2.83	1.11E+06	6.93E+06						
	8	Adriàtic	5.0				1.73	1.01E+06	8.52E+06	1.48E+03					
	10	Adriàtic	5.0				2.91	6.20E+05	5.61E+06						
	12	Adriàtic	5.0				2.75	7.48E+05	8.33E+06	4.11E+03					
	14	Adriàtic	5.0				0.78	5.97E+05	7.21E+06						
	16	Adriàtic	5.0				2.39	8.01E+05	5.87E+06	2.77E+03					
	18	Adriàtic	5.0				1.84	1.30E+06	6.71E+06						
	20	Adriàtic	5.0				3.95	1.47E+06	7.03E+06	3.20E+03					
	22	Adriàtic	5.0				1.44	9.76E+05	1.50E+07						
	24	Adriàtic	5.0				0.49	9.07E+05	1.41E+07	3.06E+03					
	2	Adriàtic	5.0				1.41	1.10E+06	1.52E+07						
	4	Adriàtic	5.0				1.24	9.55E+05	1.02E+07	4.09E+03					
	6	Adriàtic	5.0				0.57	9.95E+05	1.13E+07	2.39E+03					
Steward et al 96	Aug-Sep 92	Chukchi Sea	0.0	6.12	32.06			3.84E+05	4.46E+06	1.32E+03					
Artic		Est 1	5.0	6	32.04			5.38E+05	3.37E+06	9.56E+02					
		Est 1	10.0	5.28	32.04			3.03E+05	5.21E+06	4.37E+02	15.09	7.55E+08	1.25	0.55	
		Est 1	15.0	4.25	32.24			4.69E+05	4.94E+06	4.83E+02					
		Est 1	20.0	4.13	32.24			4.97E+05	4.42E+06	1.34E+03					
		Est 1	25.0	4.05	32.31			4.54E+05	5.13E+06	3.01E+03	9.2	4.60E+08	0.70	0.99	
		Est. 17	0.0	4.33	28.66			2.08E+05	3.27E+06	1.38E+02	1.71	8.55E+07	0.34	2.01	
		Est. 17	17.3	2.83	29.32			1.67E+05	3.04E+06	5.04E+01	2.9	1.45E+08	0.63	1.11	
		Est. 17	36.9	-1.23	32.32			5.70E+05	5.09E+06	4.03E+02	3.39	1.70E+08	0.26	2.66	
		Est. 17	57.0	-1.1	32.44			7.64E+05	4.69E+06	2.19E+02	4.45	2.23E+08	0.26	2.71	
		Est. 17	97.2	-1.18	32.49			9.92E+05	6.97E+06	3.87E+02	4.49	2.25E+08	0.20	3.40	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
		Est 32	4.9	2.27	28.22			3.07E+05	4.38E+06	1.39E+01					
		Est 32	28.0	-1.04	30.34			4.32E+05	3.58E+06	9.35E+01	4.86	2.43E+08	0.45	1.55	
		Est 32	53.9	-1.44	32.24			2.43E+05	3.77E+06	9.03E+01					
		Est 32	100.2	-1.52	32.71			3.64E+05	5.80E+06	8.34E+01	0.72	3.60E+07	0.09	7.35	
		Est 32	131.6	-0.99	33.50			2.49E+05	2.79E+06	9.72E+00					
		Est 32	158.8	-0.68	34.41			1.85E+05	2.50E+05	7.55E+01	0.39	1.95E+07	0.10	6.92	
		Est 32	193.4	-0.44	34.60			7.42E+04	6.85E+05	1.12E+00					
		Est 32	228.1	-0.13	34.66			1.25E+05	8.17E+05	1.01E+01	0.3	1.50E+07	0.11	6.12	
		Est 32	263.3	0.12	34.78			8.04E+04	3.98E+04	5.76E+00					
		Est 32	344.2	0.42	34.86			9.82E+04	3.57E+05	8.26E+00					
		Est 32	403.4	0.48	34.88			1.13E+05	5.13E+05	8.86E-02	0.55	2.75E+07	0.22	3.18	
Maranger&Bird 95	Estiu93	Llacs de Canada													
		Croche	Euphotic				7.64	3.81E+06	7.03E+07		8.5920	3.91E+08	0.10	7.09	
		Cromwell	Euphotic				14.94	8.47E+06	1.48E+08		36.9600	1.68E+09	0.18	3.82	
		Triton	Euphotic				4.41	6.68E+06	9.43E+07		37.7760	1.72E+09	0.23	3.02	
		Echo	Euphotic				24.13	2.11E+07	1.69E+08		21.3360	9.72E+08	0.05	15.39	
		PinRouge	Euphotic				8.57	7.93E+06	9.35E+07		16.2720	7.41E+08	0.09	7.76	
		Connelly	Euphotic				7.97	4.35E+06	5.76E+07		13.5120	6.16E+08	0.13	5.24	
		Orignal	Euphotic				4.60	6.90E+06	1.04E+08		32.5440	1.48E+09	0.19	3.56	
		Dufresne	Euphotic				8.61	3.06E+06	6.15E+07		8.7600	3.99E+08	0.12	5.65	
		Manitou	Euphotic				6.43	2.03E+06	1.13E+08		18.2880	8.33E+08	0.34	2.02	
		Gilbert	Euphotic				3.67	6.88E+06	4.98E+07		22.1280	1.01E+09	0.14	5.07	
		Orford	Euphotic				1.50	2.66E+06	4.10E+07		17.3280	7.89E+08	0.26	2.67	
		Waterloo	Euphotic				32.80	1.59E+07	1.85E+08		96.7200	4.41E+09	0.24	2.83	
		Massawippi	Euphotic				6.60	8.43E+06	4.20E+07		9.1680	4.18E+08	0.05	14.33	
		Magog	Euphotic				8.97	1.02E+07	9.92E+07		36.0720	1.64E+09	0.15	4.64	
		Bowker	Euphotic				2.92	2.35E+06	4.68E+07		6.7440	3.07E+08	0.12	5.64	
		Bromont	Euphotic				9.05	6.70E+06	1.02E+08		51.2160	2.33E+09	0.30	2.32	
		QuinnBay	Euphotic				5.09	6.02E+06	6.54E+07		14.5920	6.65E+08	0.10	6.62	
		Quenouilles	Euphotic				9.38	1.63E+06	9.68E+07		30.6000	1.39E+09	0.62	1.12	

Estudi	Data	LLOC	Z	°C	Sal	Oxigen	Chla	BN/ml	VLP/ml	HNF/ml	HBPa	HBPb	μ (d-1)	DT(d)	Bacteriv.
Maranger et al. 94	abr-92	Artic (Gel)	4 cm				0.95	3.15E+05	1.47E+07						
C estacional	abr-92		4 cm				0.94	8.67E+05	5.05E+07			3.05E+10	3.59	0.19	
	abr-92		4 cm				0.94	1.79E+06	5.67E+07			1.25E+11	4.26	0.16	
	may-92		4 cm				1.82	2.60E+06	8.05E+07			5.55E+10	3.11	0.22	
	may-92		4 cm				3.30	2.58E+06	1.23E+08			4.50E+10	2.91	0.24	
	may-92		4 cm				3.63	4.64E+06	1.03E+08			1.16E+11	3.26	0.21	
	may-92		4 cm				2.69	1.03E+07	1.09E+08			6.21E+10	1.95	0.36	
	may-92		4 cm				3.21	7.12E+06	9.63E+07			7.65E+10	2.46	0.28	
Perfil	may-92		1.5-4 cn					6.52E+04	1.13E+06			8.88E+09	9.95	0.07	
	may-92		0.5-1.5 cn					3.98E+04	1.15E+06			1.28E+10	5.78	0.12	
	may-92		0-0.5 cn					1.04E+05	1.14E+06			2.86E+10	5.62	0.12	
	may-92		interfase					2.10E+05	2.47E+06			2.56E+10	4.81	0.14	
	may-92	aigua	4m					2.26E+05	8.13E+06			6.71E+10	5.70	0.12	
	may-92		8m					1.06E+05	3.85E+06			3.40E+10	5.78	0.12	
Maranger et al. 96	ago-92	Llac Gilbert	2.0			9.23		7.10E+08	2.90E+09						
		Sediment (1 cm)	4.0			9.26		5.66E+08	1.80E+10						
			6.0			12.47		1.05E+09	2.20E+09						
			7.0			4.93		1.07E+09	1.99E+09						
			8.0			0.46		9.43E+08	1.78E+09						
			9.0			0		1.78E+09	1.39E+09						
			10.0			0.06		1.67E+09	1.68E+09						
			11.0			0		9.73E+08	9.88E+08						
			12.0			0		9.02E+08	6.07E+08						
			13.0			0		1.17E+09	6.68E+08						
		perfil aigua	0.0	19		9.2	1.08	1.74E+06	2.69E+07						
			1.0	18.99		9.18		2.02E+06	3.11E+07						
			2.0	19.02		9.16	0.93	1.80E+06	2.65E+07						
			3.0	19		9.2		1.83E+06	2.71E+07						
			4.0	18.87		9.18		1.98E+06	2.79E+07						
			5.0	15.94		9.27		2.07E+06	2.87E+07						
			6.0	12.45		10.94		2.00E+06	3.82E+07						
			7.0	8.96		12.37		3.38E+06	3.18E+07						
			8.0	7.32		4.91		3.36E+06	4.06E+07						
			9.0	5.9		0.46		3.18E+06	4.06E+07						
			10.0	5.26		0		5.36E+06	4.07E+07						
			11.0	5.08		0		4.67E+06	7.19E+07						
			12.0	5.11		0		6.26E+06	8.99E+07						