VESIENTUTKIMUSLAITOKSEN JULKAISUJA
PUBLICATIONS OF THE WATER RESEARCH INSTITUTE **56**

VESIHALLITUS — NATIONAL BOARD OF WATERS, FINLAND
Helsinki 1984

ISBN 951-46-8080-4
ISSN 0355-0982

Helsinki 1984. Valtion painatuskeskus

FOREWORD

This series of methodological articles on the measurement of aquatic heterotrophic activity originates from several independent studies of Finnish lakes and coastal areas, carried out during 1978—1982. Most of these studies were financed by the National Board of Waters with water protection funds, and they were carried out in Water Districts of Vaasa, Tampere, Helsinki, Kokkola and Kainuu. The authors wish to thank the Water Research Institute and all the Water District organisations for support of these studies. Especially we thank the head of the Research Division of the Vaasa Water District, Mr. Pertti Sevola, whose continuing encouragement has been essential from the very beginning.

The major part of the radioactivity measurements were performed in the Isotope Laboratory of the Faculty of Agriculture and Forestry, University of Helsinki. The authors wish to address their sincere gratitude to the director of the Laboratory, Mr. Antti Uusi-Rauva, without whom these studies would not have been possible.

ON THE MEASUREMENT OF HETEROTROPHIC ACTIVITY IN THE AQUATIC ENVIRONMENT USING LABELLED SUBSTRATES

Timo Tamminen[1] & Jorma Kuparinen[1]

TAMMINEN, T. & KUPARINEN, J. 1984. On the measurement of heterotrophic activity in the aquatic environment using labelled substrates. Publications of the Water Research Institute, National Board of Waters, Finland, No. 56.

The development of heterotrophic activity assay methodology in hydrobiological research is briefly reviewed. Different methodological approaches to the utilization of radioactive substrates in heterotrophic activity measurements are described. Emphasis is placed on the application of these methods in the study of the structure and function of the aquatic ecosystem as well as on applications in ecotoxicological research. A summary of related Finnish research is also presented.

Index words: Heterotrophic activity, Bacterioplankton, labelled substrates, monitoring, ecotoxicology, pelagic ecosystem.

1. INTRODUCTION

The re-evaluation of the trophic role of microbial heterotrophic organisms in the food web of the aquatic ecosystem has been a major development in hydroecological research during the past decade. The traditional view underestimated or totally ignored the role of microheterotrophic organisms in the marine pelagic ecosystem (Steele 1974), although evidence from limnological studies has emphasized their significance in the cycles of matter in the aquatic environment since the early works of e.g. Kuznetsov (1959), Kuznetsov et al. (1966), Romanenko (1966), Sorokin (1965) and Sorokin and Paveljeva (1972). Sorokin (1971a, 1971b) has also presented similar results from the marine environment.

Perhaps the best review of the state of the art has been presented by Williams (1981a). Methods that are capable of adequately measuring the dynamics of microheterotrophic processes are urgently needed and are presently under rapid development. Heterotrophic bacteria represent only one component of the "microbial complex" (Williams 1981a), along with colourless nanoflagellates etc., but their metabolism is undoubtly of great importance for the fluxes of matter in the pelagic ecosystem as a whole.

Traditional microbiological methods — state variable measurements such as plate counts, MPN etc. — are inadequate to provide the required information on heterotrophic processes. Bacterial numbers and biomass can be determined by epifluorescence microscopy directly from water

1) Tvärminne Zoological Station, University of Helsinki, SF-10850 Tvärminne, Finland.

samples (Hobbie et al. 1977). This state variable gives valuable information, but it gains full significance only in combination with relevant activity parameters.

One approach to the study of the role of microheterotrophs in the aquatic carbon cycle is to measure community metabolism by oxygen analysis (Bryan et al. 1976). Respiration can be divided into different size fractions in order to estimate the contribution of micro-organisms to the total respiration (Williams 1981b) and to relate respiration to microbial state parameters such as direct counts.

Another approach is to measure the activity of microheterotrophs as the assimilation of radioactively labelled substrates. Since Parsons and Strickland (1962) launched the utilization of radioactive organic substrates in hydrobiology, a vast amount of data has accumulated concerning diverse heterotrophic processes. This article attempts briefly to summarize the main trends in this field of research and to discuss the value of this approach to ecological and ecotoxicological studies of the aquatic environment.

2. METHODOLOGICAL APPROACHES TO THE UTILIZATION OF RADIOACTIVE SUBSTRATES IN THE STUDY OF HETEROTROPHY

The method of Parsons and Strickland (1962) was based on the measurement of heterotrophic assimilation of radioactively labelled organic substrate (^{14}C-glucose). The introduction of radioactive substrates was an essential improvement in the precision of measurement of heterotrophic processes. This offers the possibility to study heterotrophic processes *in situ* or in relatively undisturbed laboratory conditions. Wright and Hobbie (1965, 1966) were the first to analyze kinetically substrate uptake results, and this analysis resulted in wide applications of the methodology in hydrobiological research.

The original method of Parsons and Strickland (1962) and Wright and Hobbie (1965) measured only net assimilation of the substrate (incorporation into microbial cells), and therefore represented an underestimate of the total assimilation. Hobbie and Crawford (1969) improved the method by taking into account the respired fraction of assimilated substrate and therefore measuring total heterotrophic uptake of the substrate.

The kinetic analysis of uptake measurements provides three parameters describing the efficiency of heterotrophic utilization of the substrate: maximal uptake velocity (V); concentration sum (K + S) of the half-saturation constant (K) and ambient substrate concentration (S); and turnover time (T). The application of the kinetic method is laborious, since it requires replicate incubations of each sample at several substrate addition levels. Therefore, the so-called single concentration method of measuring either the turnover time (or turnover rate, 1/T) (Williams and Askew 1968, Azam and Holm-Hansen 1973) or maximal uptake velocity (Wright 1978) has been developed to evaluate heterotrophic activity with a more reasonable amount of effort (Kuparinen et al. 1984a). Azam and Holm-Hansen (1973) introduced the use of high specific activity ^{3}H-labelled substrates. These enable tracer level additions of the substrate and the natural turnover rate (1/T) of the substrate can thus be measured even in oligotrophic conditions.

The assimilation measurement of a single organic compound offers only one indicator of the total heterotrophic activity. By definition, total heterotrophy cannot be measured as assimilation of any single substrate. Some attempts have been made to overcome this restriction. Sorokin (1955) and Romanenko (1964) proposed that dark CO_2 assimilation would represent about 6 % of the total carbon assimilation of aerobic heterotrophic bacteria. Therefore, this constant has been used to calculate approximate total heterotrophy from dark $^{14}CO_2$ assimilation measurements (see Romanenko 1979). Another alternative has been proposed by Monheimer (1974). This method is based on the measurement of $^{35}SO_4$ assimilation and the assumption of a constant S/C ratio in heterotrophic assimilation. Both approaches have met with serious criticism (Overbeck 1979, Monheimer 1978), but they are still actively studied and no final agreement concerning their limits of applicability has been reached.

Perhaps the most actively developing field of research at the moment is the combination of state variable and activity measurements. These approaches can be divided into three categories. An early strategy was the application of the autoradiographical technique, which produces the number of cells actively metabolizing the assayed substrate (Brock 1967, Brock and Brock 1968, Hoppe 1976). This approach has also been combined with the determination of total bacterial counts to yield the fraction of active cells in the community (Meyer-Reil 1978, Tabor and Neihof 1982).

Secondly, some heterotrophic activity measurement (e.g. turnover rate of glucose) can be combined with total counts to obtain an activity index of the bacterioplankton community (activity per cell; Wright 1978).

Thirdly, perhaps the most promising alternative is to measure the incorporation of a radioactively labelled RNA or DNA precursor (thymidine, adenine, uridine) and combine this with experimentally determined ratios of RNA or DNA to cell carbon (Brock 1967, Tobin and Anthony 1978, Karl 1979, Fuhrman and Azam 1980, 1982). This approach yields bacterial production, or in connection with bacterial counts, generation times of bacteria. Although the approach is widely debated at the moment (e.g. Karl 1982), it offers a promising possibility for the quantification of microheterotrophic activity in the aquatic environment.

It is rather obvious that quite different aspects of the metabolic activity of aquatic microheterotrophic organisms are described by each of the approaches referred to above. The term "heterotrophic activity" or "microheterotrophic activity" (when confusion with higher trophic levels is possible) should be used in a broad sense, referring to each parameter describing relevant aspects of the total metabolism of aquatic microbial heterotrophs. The method must be chosen in each case according to the problem under study. Both indicator level parameters as well as parameters attempting to estimate the total quantity of microheterotrophy — production of microheterotrophs — certainly have wide potential applications in hydrobiological research.

3. APPLICATIONS TO ECOLOGICAL AND ECOTOXICOLOGICAL STUDIES

Monitoring. Labelled compounds of small molecular weight have been in extensive use in monitoring and describing variations of heterotrophic activity with respect to time and space. Variations over three orders of magnitude with respect to geographic location, trophic status of the water body, temperature and depth of the study site have been reported (Sepers 1977, Hoppe 1978). As a monitoring parameter, heterotrophic activity measurements have revealed trends in the heterotrophy of a water body and changes in heterotrophy-autotrophy relations (Kuparinen et al. 1981). Measurements of turnover rate with a single substrate (e.g. glucose) offer a simple method valid for monitoring purposes.

Ecosystem research. Knowledge of microbial heterotrophic activity in the aquatic environment is of limited interest *per se.* Heterotrophic activity measurements are needed particularly in order to understand the role of microheterotrophs in the structure and function of the aquatic ecosystem. This aim can be reached in two principal ways: through the quantitative energy flow approach, or through multivariate analysis of indicator level parameters. Both approaches can ultimately lead to modelling of the ecosystem, usually with energy flow models (based on differential equations) or with structural equation models (based on covariance between parameters).

In statistical multivariate analyses of ecological data, heterotrophic activity parameters have been used to describe the functional role of microheterotrophy in the ecosystem, especially the relationships of heterotrophy to autotrophy, nutrient cycling or water quality parameters (Bölter 1982, Tamminen 1982c).

When quantifying the flows of energy and carbon through the pelagic food web, the flow from autotrophs to the dissolved organic carbon (DOC) pool (exudation) has received increasing attention (Williams 1981a, Wolter 1982, Larsson and Hagström 1982, Riemann et al. 1982). This pathway leads back to higher trophic levels via bacterial secondary production and has gone unnoticed in earlier studies of the aquatic food web (e.g. Steele 1974). Although the total DOC pool remains rather constant in aquatic systems, a small fraction of it is subject to rapid cycling (e.g. Derenbach and Williams 1974, Smith et al. 1977, Wiebe and Smith 1977, Larsson and Hagström 1979). Turnover rate measurements of compounds of this fraction, coupled with quantitative analyses of their pool sizes, have emphasized the significance of these compounds as a carbon source for bacterial production (Bölter 1981). Also, these results have lent support to the use of a single model substrate (e.g. glucose) as an indicator of this rapidly cycling fraction of DOC (Bölter 1981).

Ecotoxicology. In ecotoxicological studies, heterotrophic activity measurements can be applied to screening tests. These tests allow a rapid and precise measurement of the effect of a pollutant on natural bacterioplankton communities, and the single concentration method is especially appropriate (Azam and Holm-Hansen 1973). Tests have been applied to screening the effects of a variety of single pollutants or combined effluents (Albright et al. 1972, Kuparinen 1980, Kuparinen 1984b,

Kaitala et al. 1983, Tamminen 1983a). The drawback of using microheterotrophs in toxicological tests is their ability to adapt rather quickly to the test situation through physiological or genetic adaptation if the exposure time exceeds several generation times of the organisms (Menzel 1977). Therefore, ecologically relevant results can be obtained only by testing acute reactions, preferably coupled with field data on the pollutant and activity parameters.

4. HETEROTROPHIC ACTIVITY MEASUREMENTS IN FINLAND

Heterotrophic activity measurements were adopted in fresh water studies in Finland in the late nineteen-sixties. In the pioneering works of Leppänen (1970), Seppänen and Ojanen (1973), Lehmusluoto and Leppänen (1974) and Leppänen (1982), a kinetic approach was used to monitor glucose uptake in different water bodies. Leppänen (1982) also discussed glucose uptake kinetics in the context of dark CO_2 assimilation measurements. Salonen (1981) has estimated bacterioplankton production in the oligotrophic lake Pääjärvi with the dark CO_2 assimilation measurement.

Glucose assimilation has also been used as an indicator of heterotrophic activity in all later investigations, except for the study of Mäkelä (1981), in which pyruvate and an amino acid mixture were used to monitor heterotrophic activity in a pelagic brackish water ecosystem.

The second period of heterotrophic activity measurements started in late nineteen-seventies with the adoption of the single concentration method in the study of heterotrophic activity near the sediment surface (Kuparinen 1978). During this period, the main interest was in the study of single concentration methodology (Kuparinen and Uusi-Rauva 1980, Tamminen 1980, Kuparinen and Tamminen 1982, Kuparinen et al. 1984b, Talsi et al. 1984) and its application to pollution monitoring, ecotoxicological and ecosystem studies. The more laborious kinetic approach has been used mainly to determine which substrate concentrations should be used in the single concentration assays (Kuparinen et al. 1984b).

Monitoring. Because of the neglible amount of data on heterotrophic processes in Finnish lakes and coastal brackish waters, monitoring studies have been continuously emphasized. Particular attention has been paid to monitoring the effects of pulp and paper mill effluents on different water bodies (Kuparinen 1980, 1981, 1984b,, Kuparinen et al. 1981, Talsi 1981, 1982a, 1982b, Lahti 1982, Talsi and Rekolainen 1982, Rekolainen and Talsi 1982, Tamminen 1983b). Monitoring of heterotrophic activity in the Gulf of Finland (Virtanen 1981, Tamminen 1983c) and in eutrophic lake (Tamminen 1982a) has also been conducted.

Ecotoxicology. The ulitization of the single concentration assay has enabled screening-type toxicity testing. Within a few recent years, a number of studies have been carried out on the effects of waste waters from pulp and paper mills (Talsi 1981, 1982a, 1982c, Lahti 1980, Kuparinen 1981, 1982, 1983b, Kuparinen and Niemi 1981) and other industrial effluents (Tamminen 1982b, 1983a). Some studies concerning the toxicity of single pollutants have also been performed (Kuparinen 1981, Kaitala et al. 1983, Talsi 1983).

Ecosystem research. Some attention has been paid to the study of the role of heterotrophic micro-organisms in the structure and function of the ecosystem. Tamminen (1983d) discussed the connection between phyto- and bacterioplankton through the exudate pathway. Talsi and Rekolainen (1982) and Tamminen (1982c, 1983a, 1983c) have utilized statistical multivariate techniques to study the connections between heterotrophic and autotrophic processes and the relations of these processes with standard water quality parameters. Kuparinen et al. (1984c) and Kuparinen (1983a) attempted to quantify the role of bacterial production and glucose mineralization in relation to the total carbon mineralization (measured by oxygen consumption) in a pelagic brackish water ecosystem.

LOPPUTIIVISTELMÄ

Tässä työssä käsitellään radioaktiivisten merkkiaineiden käyttöön perustuvien vesistön heterotrofiamittausten kehitystä viime vuosikymmeninä. Hiilidioksidin pimeäsitoutumisen sekä sulfaatin, yksinkertaisten orgaanisten yhdisteiden ja RNA- tai DNA-prekursorien oton mittausten tuottamaa tietoa esitellään lyhyesti. Lisäksi käsitellään radioaktiivisten merkkiaineiden avulla tehtyjen aktiivisuusmittausten yhdistelemistä perinteiseen bakteerimikroskopointiin tai muihin tilaparametreihin, joko autoradiografian avulla tai erilaisten bakteeriyhteisöä kuvaavien indeksien, yhteisön generaatioaikojen tai tuotannon määrittämiseksi.

Heterotrofisen aktiivisuuden mittausten soveltamista seurantatutkimuksiin, ekologisiin tai ekotoksikologisiin tutkimuksiin käsitellään, ja erityisesti korostetaan heterotrofisen aktiivisuuden mittausten tarpeellisuutta vesiekosysteemin rakenteen ja toiminnan selvittelyssä. Lopuksi esitellään menetelmien sovellutuksia suomalaisessa tutkimuksessa.

REFERENCES

Albright, L.J., Wentworth, J.W. & Wilson, E.M. 1972. Technique for measuring metallic salt effects upon the indigenous heterotrophic microflora of a natural water. Water Res. 6: 1581—1596.

Azam, F. & Holm-Hansen, O. 1973. Use of tritiated substrates in the study of heterotrophy in seawater. Mar. Biol. 23: 191—196.

Brock, T.D. 1967. Bacterial growth rate in the sea: direct analysis by thymidine autoradiography. Science 155: 81—83.

Brock, M.L. & Brock, T.D. 1968. The application of microautoradiographic techniques to ecological studies. Mitt. int. Ver. Limnol. 15: 1—29.

Bryan, J.R., Riley, J.P. & Williams, P.J. leB. 1976. A Winkler procedure for making precise measurements of oxygen concentration for productivity and related studies. J. Exp. Mar. Biol. Ecol. 21: 191—197.

Bölter, M. 1981. DOC-turnover and microbial biomass production. Kieler Meeresforsch. Sonderh. 5: 304—310.

Bölter, M. 1982. Seasonal variation in microbial biomass production and related carbon flux in Kiel Fjord. ICES C.M. 1982/No. 25 (manuscript).

Derenbach, J.B. & Williams, P.J. leB. 1974. Autotrophic and bacterial production: fractionation of plankton populations by differential filtration of samples from the English Channel. Mar. Biol. 25: 263—269.

Fuhrman, J.A. & Azam, F. 1980. Bacterioplankton secondary production estimates of coastal waters of British Columbia, Antarctica and California. Appl. Environ. Microbiol. 39: 1085—1095.

Fuhrman, J.A. & Azam, F. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol. 66: 109—120.

Hobbie, J.E. & Crawford, C.C. 1969. Respiration corrections for bacterial uptake of dissolved organic compounds in natural waters. Limnol. Oceanogr. 14: 528—532.

Hobbie, J.E., Daley, R.J. & Jasper, S. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33: 1225—1228.

Hoppe, H. 1976. Determination and properties of actively metabolizing heteotrophic bacteria in the sea, investigated by means of microautoradiography. Mar. Biol. 36: 291—302.

Hoppe, H. 1978. Relations between active bacteria and heterotrophic potential in the sea. Neth. J. Sea Res. 12: 78—98.

Kaitala, S., Kuparinen, J. & Maximov, V.N. 1984. The investigation of heavy metal and pentachlorophenolate stress on heterotrophic activity and primary productivity by means of factorial experiment. In: Liu, D. & Dutka, B. (eds.) Toxicity screeming procedures using bacterial systems. Marcel Dekker, inc. New York.

Karl, D.K. 1979. Measurement of microbial activity and growth in the ocean by rates of stable ribonucleic acid synthesis. Appl. Environ. Microbiol. 38: 850—860.

Karl, D.M. 1982. Selected nucleic acid precursors in studies of aquatic microbial ecology. Appl. Environ. Microbiol. 44: 891—902.

Kuparinen, J. 1978. Aerobisten heterotrofisten mikrobien aktiivisuuden mittauksesta. 77 p. Helsinki. Graduate thesis. University of Helsinki, Department of Limnology.

Kuparinen, J. 1980. Heterotrofisten bakteerien aineenvaihdunta-aktiivisuuden mittauksen hyväksikäyttö puunjalostusteollisuuden jätevesien tarkkailussa. National Board of Waters, Finland. Report 189: 1—62.

Kuparinen, J. 1981. Sulfiittiselluloosatehtaan jätevesien ja eräiden raskasmetalli- ja orgaanisten yhdisteiden vaikutus vesistön bakteeritoimintaan. National Board of Waters, Finland. Report 204: 1—55.

Kuparinen, J. 1982. The influence of sulphite mill effluents on heterotrophic activity as measured by glucose assimilation. Hydrobiologia 86: 115—119.

Kuparinen, J. 1984a. Glucose assimilation: its contribution to carbon flux in the pelagial and use in monitoring heterotrophic activity. Arch. Hydrobiol. Beih. Ergebn. Limnol. 19: 15—22.

Kuparinen, J. 1984b. The effects of effluents from sulphite cellulose factory on microbial heterotrophic activity in the fresh water. Oikos (in print).

Kuparinen, J. & Uusi-Rauva, A. 1980. A simplified method to measure respiration rates of aerobic heterotrophic populations. Hydrobiologia 75: 113—115.

Kuparinen, J., Rekolainen, S., Talsi, T. & Tamminen, T. 1981. The effect of kraft pulp mill effluents on heterotrophic activity and primary productivity. P. 138—142. Postersession abstracts, vol. 1. Bottenviksseminariet, Luleå, Sweden.

Kuparinen, J. & Niemi, M. 1981. Heterotrophic activity as an indicator of toxicity in aquatic environments. Report 31. NORDFORSK. Helsinki. Ecotoxikologiska metoder för akvatisk miljö.

Kuparinen, J. & Tamminen, T. 1982. Respiration of tritiated substrates in heterotrophic activity assays. Appl. Environ. Microbiol. 43: 806—809.

Kuparinen, J., Lahti, K., Mäkelä, A., Rekolainen, S., Talsi, T., Tamminen, T., Virtanen, A. & Uusi-Rauva, A. 1984a. A practical approach to the measurement of microbial heterotrophic activity by the single concentration method. Helsinki. Publ. Wat.Res. Inst., National Board of Waters, Finland. 56: xx-xx. (This issue).

Kuparinen, J., Lahti, K., Talsi, T., Tamminen, T. & Virtanen, A. 1984b. Determination of the Michaelis-Menten kinetic parameters with single concentration assays. Helsinki. Publ. Wat. Res. Inst., National Board of Waters, Finland. 56: xx—xx. (This issue).

Kuparinen, J., Leppänen, J.-M., Sarvala, J., Sundberg, A. & Virtanen, A. 1984c. Production and utilization of organic matter in a Baltic ecosystem off Tvärminne, SW coast of Finland. Rapp. P.-v. Réun. Cons.int. Explor. Mer. 183:180—192.

Kuznetsov, S.I. 1959. Die Rolle der Mikro-organismen im Stoffkreislauf der Seen. 301 p. Berlin.

Kuznetsov, S.I., Romanenko, V.I. & Karpova, N.S. 1966. Cislennoçt bakterij i produkcija organiceskogo veščestvo v vodnoi masse Rybinskogo vodohranilišča v 1963 i 1964 gg. Producirovanie i krugavorot organiceskogo veščestvo vo vnutrennih vodoemah. Moscow—Leningrad. P. 123—132.

Lahti, K. 1980. Eräiden bakteeri- ja levämyrkyllisyystestien vertailu puunjalsotusteollisuuden jätevedellä. 103 p. Helsinki. Graduate thesis, University of Helsinki. Department of Limnology.

Lahti, K. 1982. Rautaruukki Oy:n Raahen tehtaiden jätevesien myrkyllisyyden ja vaikutusalueen kartoitus bakteerien ja levien avulla. Helsinki. Vesihallituksen monistesarja 116: 5—27. (mimeograph, in Finnish, National Board of Waters).

Larsson, U. & Hagström, Å. 1979. Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. Mar. Biol. 52: 199—206.

Larsson, U. & Hagström, Å. 1982. Fractionated phytoplankton primary production, exudate release and bacterial production in a Baltic eutrophication gradient. Mar. Biol. 67: 57—70.

Lehmusluoto, P.O. & Leppänen, T. 1974. Humusveden bakteeritoiminnan aktiivisuudesta. (English summary: On bacterial activity in a humic lake). Limnologisymposio 1971. Suomen limnologinen yhdistys. P. 22—34.

Leppänen, T. 1970. Tutkimuksia bakteerien glukoogin käytöstä Hakojärvessä ja siihen sijoitetuissa koealtaissa. 103 p. Helsinki. Graduate thesis, University of Helsinki. Department of Limnology.

Leppänen, T. 1982. Bakteeriplanktonin biomassa, tuotanto ja heterotrofinen aktiivisuus Hakojärvessä ja kahdella Saimaan alueella. 183 p. Helsinki. Licenciate thesis, University of Helsinki, Department of Limnology.

Menzel, D.W. 1977. Summary of experimental results: Controlled ecosystem pollution experiment. Bull. Mar. Sci. 27: 142—145.

Meyer-Reil, L-A. 1978. Autoradiography and epifluorescence microscopy combined for the determination of number and spectrum of actively metabolizing bacteria in natural waters. Appl. Environ. Microbiol. 36: 506—512.

Monheimer, R.H. 1974. Sulfate uptake as a measure of planktonic microbial production in freshwater ecosystems. Can. J. Microbiol. 20: 825—831.

Monheimer, R.H. 1978. Difficulties in interpretation of microbial heterotrophy from sulfate uptake data: laboratory studies. Limnol. Oceanogr. 23: 150—154.

Mäkelä, A. 1981. Murtoveden heterotrofisten bakteerien aktiivisuus aminohapposeoksella ja palorypälehapolla mitattuna. 62 p. Helsinki. Graduate thesis, University of Helsinki, Department of Limnology.

Overbeck, J. 1979. Dark CO_2 uptake-biochemical background and its relevance to *in situ* bacterial production. Arch. Hydrobiol., Beih. 12: 38—47.

Parsons, T., & Strickland, J.D.H. 1962. On the production of particulate organic carbon by heterotrophic processes in sea water. Deep-Sea Res. 8: 211—222.

Rekolainen, S. & Talsi, T. 1982. Kaskisten merialueella ja Oy Metsä-Bothnia Ab:n jätevesien vaikutusalueella tehtyjen vesistötutkimusten vertailu. 27 p. Vaasa. Vesihallituksen monistesarja 103 (mimeograph, in Finnish).

Riemann, B., Søndergaard, M., Schierup, H-H., Bosselmann, S., Christensen, G., Hansen, J. & Nielsen, B. 1982. Carbon metabolism during a spring diatom bloom in the eutrophic lake Mossø. Int.Revue ges. Hydrobiol. 67: 145—185.

Romanenko, V.I. 1964. Geterotrofnaja assimilacija CO_2 bakterialnoj flory vody. Mikrobiologija 33: 679—683.

Romanenko, V.I. 1966. The comparative characteristics of the microbiological processes in water reservoirs of different types. Trans. Inst. Biol. Fresh. Waters Acad. Sci. USSR, No 9 (12a;) 293.

Romanenko, V.I. 1979. Microbiology of the Volga. Mordukhai-Boltovskoi (ed.). The river Volga and its life. Monographiae Biologicae 33. Junk Publishers, The Hague. P. 106—136.

Salonen, K. 1981. The ecosystem of the oligotrophic Lake Pääjärvi 2. Bacterioplankton Verh. Internat. Verein. Limnol. 21:448—453.

Sepers. A.B.J. 1977. The utilization of dissolved organic compounds in aquatic environments. Hydrobiologia 52: 39—54.

Seppänen, H. & Ojanen, T. 1973. Glucose-14 uptake in lake Hiidenvesi. Aqua Fennica 1973: 95—102.

Smith, W.O.Jr., Barber, R.T. & Huntsman, S.A. 1977. Primary production off the coast of northwest Africa: excretion of dissolved organic matter and its heterotrophic uptake. Deep-Sea Res. 24: 35—47.

Sorokin, Yu. I. 1955. Opredelenic velicin hemosinteza v vode Rybinskogo vodohranilisca s primeneniem ^{14}C. Doklady AN SSSR 105: 1343—1345.

Sorokin, Yu. I. 1965. On the trophic role of chemosynthesis and bakterial biosynthesis in water bodies. Mem. Ist. Ital. Idrobiol. 18 (suppl.): 187—205.

Sorokin, Yu. I. 1971a. Bacterial populations as components of oceanic ecosystems. Mar. Biol. 11: 101—105.

Sorokin, Yu. I. 1971b. On the role of bakteria in the productivity of tropical oceanic waters. Int. Revue ges. Hydrobiol. 56: 1—48.

Sorokin, Yu. I. and Paveljeva, E.B. 1972. On the quantitative characteristics of the pelagic ecosystem of Dalnee lake (Kamchatka). Hydrobiologia 40: 519—552.

Steele, J.H. 1974. The structure of marine ecosystems. 128 p. Harvard University Press, Cambridge, Mass.

Tabor, P.S. & Neihof, R.A. 1982. Improved microautoradiographic method to determine individual

micro-organisms active in substrate uptake in natural waters. Appl. Environ. Microbiol. 44: 945—953.

Talsi, T. 1981. Bakteerien glukoosinotto selluteollisuuden jätevesien vaikutusten arviointimenetelmänä. 98 p. Vaasa. Vesihallituksen monistesarja 83. (mimeograph, in Finnish, Vaasa Water District Office).

Talsi, T. 1982a. Rautaruukki Oy:n Raahen tehtaiden jätevesien vaikutuksesta merialueen bakteeritoimintaan. Oulu. Vesihallituksen monistesarja 116: 28—60. (mimeograph, in Finnish, Oulu Water District Office).

Talsi, T. 1982b. Kajaani Oy:n sulfiittisellu- ja paperitehtaan jätevesien vaikutukset vastaanottavan vesistön bakteeritoimintaan. 35 p. Kajaani. Vesihallituksen monistesarja 129. (mimeograph, in Finnish, Kainuu Water Oistrict Office).

Talsi, T. 1983. Sköldvikin alueen teollisuuslaitosten jätevesien myrkkyvaikutukset. 53 p. Helsinki. Vesihallituksen monistesarja 161. (mimeograph, in Finnish, Helsinki Water District Office).

Talsi, T. & Rekolainen, S. 1982. Bakteerien glukooginotto selluteollisuuden jätevesien vaikutusten ja leviämisen seurannassa. 51 p. Helsinki. National Board of Waters, Finland. Report 218: 1—51. (in Finnish).

Talsi, T., Tamminen, T. & Kuparinen, J. 1984. Variability in planktonic heterotrophic activity and primary productivity assays in relation to sampling strategies. Helsinki. Publ. Wat. Res. Inst., National Board of Waters, Finland. 56: xx-xx. (This issue).

Tamminen, T. 1980. Radioaktiivisten merkkiaineiden käyttömahdollisuudet vesistöjen hajotustoiminnan mittauksessa. 79 p. Helsinki. Vesihallituksen monistesarja 42. (mimeograph in Finnish, Helsinki Water District Office).

Tamminen, T. 1982a. Winter microbial activity in Lake Tuusulanjärvi. Hydrobiologia 86: 109—113.

Tamminen, T. 1982b. Effects of ammonium effluents on planktonic primary production and decomposition in a coastal brackish water environment. I. Nutrient balance of the water body and effluent tests. Neth. J. Sea Res. 16: 455—464.

Tamminen, T. 1982c. Effects of ammonium effluents on planktonic primary production and decomposition in a coastal brackish water environment. II. Interrelations between biotic and abiotic components of the planktonic ecosystem. Neth. J. Sea Res. 15: 349—361.

Tamminen, T. 1983a. Ammonium effluent tests with natural communities of phytoplankton and bacterioplankton and with test algae. Oikos (manuscript).

Tamminen, T. 1983b. Sulfaattisellutehtaan jätevesien vaikutus murtoveden planktiseen hajotustoimintaan. 46 p. Helsinki. National Board of Waters, Report 238. (in Finnish).

Tamminen, T. 1983c. Vertical distribution of planktonic primary productivity, heterotrophic activity and ATP during an annual cycle by the SW coast of Finland. (manuscript).

Tamminen, T. 1983d. Relations between planktonic heterotrophy and photoautotrophy in simultaneous light and dark incubations during a spring bloom. Bactériologie Marine, Editions du CNRS, Paris 1984: 125—131.

Tamminen, T. 1983e. Ammoniumtyppikuormituksen vaikutus murtoveden planktiseen perustuotantoon ja hajotustoimintaan. 94 p. Helsinki. National Board of Waters, Report 230. (in Finnish).

Tobin, R.S. & Anthony, D.H.J. 1978. Tritiated thymidine incorporation as a measure of microbial activity in lake sediments. Limnol. Oceanogr. 23: 161—165.

Wiebe, W.J. & Smith, D.F. 1977. Direct measurement of dissolved organic carbon release by phytoplankton and incorporation by microheterotrophs. Mar. Biol. 42: 213—223.

Williams, P.J.leB. 1981b. Microbial contribution to overall marine plankton metabolism: direct measurements of respiration. Oceanologica Acta 4: 359—364.

Williams, P.J. leB. 1981a. Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food web. Kieler Meeresforsch., Sonderh. 5: 1—28.

Williams, P.J. leB. & Askew, C. 1968. A method of measuring the mineralization by micro-organisms of organic compounds in sea-water. Deep-Sea Res. 15: 365—375.

Virtanen, A. 1981. Studies on the decomposition of organic matter in the Gulf of Finland. Meri 9: 71—106.

Wolter, K. 1982. Bacterial incorporation of organic substances released by natural phytoplankton populations. Mar. Ecol. Prog. Ser. 7: 287—295.

Wright, R.T. 1978. Measurement and significance of specific activity in the heterotrophic bacteria of natural waters. Appl. Environ. Microbiol. 36: 297—305.

Wright, R.T. & Hobbie, J.E. 1965. The uptake of organic solutes in lake water. Limnol. Oceanogr. 10: 22—28.

Wright, R.T. & Hobbie, J.E. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology 47: 447—464.