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

Timo Tamminen & Jorma Kuparinen: On the measurement of heterotrophic activity in the aquatic environment using labelled substrates
Tiivistelmä: Vesistöjen bakteeriplanktonin aktiivisuuden mittauksesta radioaktiivisten merkkiaineiden avulla 4

Timo Tamminen: Linear transformations of the Michaelis-Menten kinetic equation in natural microbial communities research
Tiivistelmä: Michaelis-Menten -kinetiikan lineaarimuunnokset luonnon mikrobiyhteisöjen tutkimuksessa 11

Timo Tamminen, Seppo Kaitala & Ludmila V. Iliash: The heterotrophic glucose uptake potential of three marine dinoflagellates
Tiivistelmä: Kolmen merellisen dinoflagellaatin glukoosinottopotentiaali 21

Jorma Kuparinen, Kirsti Lahti, Tuija Talsi, Timo Tamminen & Anneli Virtanen: Determination of the Michaelis-Menten kinetic parameters with single concentration assays
Tiivistelmä: Bakteeriplanktonin glukoosinottoa kuvaavien kineettisten parametrien määritys yhden substraattipitoisuuden menetelmällä 26

Jorma Kuparinen, Kirsti Lahti, Ari Mäkelä, Seppo Rekolainen, Tuija Talsi, Timo Tamminen, Anneli Virtanen & Antti Uusi-Rauva: A practical approach to the measurement of microbial heterotrophic activity by the single concentration method
Tiivistelmä: Vesistöjen bakteeriplanktonin aktiivisuuden määritys yhden substraattipitoisuuden menetelmällä: käytännön suoritus 35

Tuija Talsi, Timo Tamminen & Jorma Kuparinen: Variability in planktonic heterotrophic activity and primary productivity assays in relation to sampling strategies
Tiivistelmä: Perustuotannon ja bakteeriplanktonin aktiivisuuden mittauksen tilastollinen luotettavuus näytteenoton suunnittelun perustana 42

VESIHALLITUS — NATIONAL BOARD OF WATERS, FINLAND
Helsinki 1984

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

Helsinki 1984. Valtion painatuskeskus

VARIABILITY IN PLANKTONIC HETEROTROPHIC ACTIVITY AND PRIMARY PRODUCTIVITY ASSAYS IN RELATION TO SAMPLING STRATEGIES

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TALSI, T., TAMMINEN, T. & KUPARINEN, J. 1984. Variability in heterotrophic activity and primary productivity assays in relation to sampling strategies. Publications of the Water Research Institute, National Board of Waters, Finland. No. 56.

The variation in planktonic heterotrophic activity and primary productivity assays was examined in a number of studies from Finnish lakes and coastal areas. Heterotrophic activity was measured as the turnover rate or maximal uptake velocity of labelled glucose. The coefficients of variation within water samples were of the same magnitude in turnover rate measurements (mean cv = 9.0 %, n = 635) as in primary productivity assays (mean cv = 6.8 %, n = 333). The coefficients of variation between replicate samples from a sampling site were in the order of 20 to 30 % with all parameters. To obtain ecologically representative values for these activity parameters, replication on the sampling level is important. The replicate samples can be pooled before conducting the assays, to maintain reasonable cost and effort in the determination of the parameters. A sampling strategy for heterotrophic activity and primary productivity assays in routine research is suggested.

Index words: Heterotrophic activity, primary productivity, plankton, variability, sampling strategies, glucose assimilation.

1. INTRODUCTION

Measurements of heterotrophic activity in the aquatic environment have become a widely applied tool in ecological and ecotoxicological studies since the 1960's (cf. Tamminen and Kuparinen 1984). However, quite little is known of the precision of the method, although information on the subject is essential for the evaluation of the results of assays. Another, related problem is the representativeness of a single assay for generalisations over water bodies, in other words, quantitative information on the distribution of active planktonic heterotrophic bacteria (patchiness).

These questions have been studied to some extent with state variables like plate counts (Niemelä 1972, Palmer et al. 1976, El-Shaarawi et al. 1981) and direct counts of bacteria (Jones and Simon 1980, Kirchman et al. 1982). Activity of bacteria is far more variable in time and space in the aquatic environment than these state variables. Variability of direct counts is astonishingly low, usually within one order of magnitude (10^6 cells ml^{-1}) in a diversity of aquatic environments (e.g. Jones and Simon 1980, Fuhrman et al. 1980, Larsson and Hagström 1982). With activity parameters, variations over three orders of magnitude are commonly encountered (e.g. Sepers 1977, Hoppe 1978). Therefore information on the distribution of bacteria, obtained with state variables is of limited value when considering the distribution (amount of patchiness) of bacterial activity in the aquatic environment. Information on this subject is neglible, as far as the authors

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know. Also, results concerning the precision of heterotrophic activity measurements with low molecular weight substrates are very scarce in the literature (Herbland and Pagès 1976).

This paper summarizes main results on the precision of the heterotrophic activity assays with labelled glucose, compiled from a number of studies from coastal and inland waters of Finland. These results are based on some 3000 activity measurements in 1978—1981. Some attention is also paid to the patchiness revealed by replicate sampling of heterotrophic activity in water bodies. The results are compared with parallel primary productivity assays.

2. MATERIAL AND METHODS

The study areas and sampling sites have been described in another paper of this issue (Kuparinen et al. 1984a).

Bacterial heterotrophic activity was measured as turnover rate (1/T) or maximal uptake velocity (V) of ^{14}C- or ^{3}H-labelled glucose with the single concentration technique (Kuparinen et al. 1984a, 1984b). Incubation volumes in turnover rate measurements were 50 or 100 ml and in maximal uptake velocity measurements, 10 ml. In addition to the heterotrophic activity measurements, parallel primary productivity assays with the standard ^{14}C technique were performed according to the Finnish standard SFS 3049, incubation volume being 100 ml. All radioactivity measurements were performed with a liquid scintillation counter (LKB-Wallac UltroBeta, 1215 RackBeta or Wallac Decem NTL 315) with sample channel ratio (^{3}H) or external standard (^{14}C, ^{3}H) channel ratio methods.

In most of the studies, heterotrophic activity was measured in replicate incubations (usually in triplicate) from a single water sample, to evaluate the within sample measurement variation. In some studies, several replicate samples were taken (successive casts) from a study site, and a single heterotrophic activity assay was performed from each sample. These results were used to calculate the between sample variation of the parameters. In one study, both replicate sampling and replicate incubations from each sample were performed (Bengtsår in 1979, 1980), and both heterotrophic activity and primary productivity were measured. Samples were taken with standard Ruttner, Sormunen or tube samplers, their volumes ranging from 1.5 to 7 l. The horisontal scale of replicate sampling was between 1 and 20 meters. Replicate samples were taken from surface waters (0 — 2 m), except for the studies from Bengtsår (in 1979) and Tvärminne (in 1980), where replicate samples originated from several depths from surface to bottom layers.

3. RESULTS

3.1 Variation within samples

The within sample variation in heterotrophic activity and primary productivity assays varied to some extent in different areas and research periods (Table 1). The coefficient of variation, describing the relative variation of the results ($CV\ \% = 100 \cdot SD \cdot \bar{x}^{-1}$), had the lowest mean value in primary productivity assays (6.8 %). With the glucose turnover rate it was slightly higher (9.0 %), and the maximal uptake velocity showed the highest mean value (12.3 %).

3.2 Variation between samples

The between sample variation was distinctly higher than the within sample variation, remaining over 20 % with all parameters (Table 2). In turnover rate measurements, the results from Bengtsår (in 1980) differ from other studies. It was observed in Kaskinen (in 1980) that the between sample variation was on several occasions higher in the oligotrophic sea zone than in the polluted coastal areas, with high bacterial activity prevailing. On the other hand, the results of each replicate sample are means of replicate incubations in the Bengtsår data. In other studies of Table 2, only a single assay was performed from each replicate sample, so that the total variation of these data consists of both within sample and between sample variation. The within sample variation has been, to some extent, diminished in the Bengtsår data, and the mean coefficient of variation describes thus mainly the real between sample variation (see Discussion).

3.3 Estimation of the required level of replication

If the mean coefficient of variation of a method is known, an estimation of the amount of replication

required to achieve a certain level of precision (reliability of the mean) can be performed. This can be done according to the following equation (cf. Eberhardt 1978):

$$n = \frac{t^2 SD^2}{p^2 \bar{x}^2} = \frac{t^2 CV^2}{p^2}$$

where

n = the number of replicates
CV = mean coefficient of variation, % (known from previous studies)
p = the required precision, as the relation of confidence limits to the mean
t = t-value on the chosen risk level

Table 1. Variation within samples for heterotrophic activity ($1/T = \%h^{-1}$ and $V = \mu g$ gluc. l^{-1} h^{-1}) and primary productivity (PP = mg C m^{-3} d^{-1}) assays in different study sites. n1 = number of subsamples, n2 = number of samples, CV % = mean coefficient of variation, $\bar{x}$ = mean value for the period.

Study	Period	Sample volume (l)	1/T				V				PP			
			$\bar{x}$	n1	CV %	n2	$\bar{x}$	n1	CV %	n2	$\bar{x}$	n1	CV %	n2
Kaskinen 1978	May — Oct.	1.0	3.19	3	6.1	34	0.22	3	7.0	24				
Kaskinen 1979	May — Sept.	1.0	4.33	3	8.6	42								
Bengtsår 1979	June — Oct.	1.0	7.53	3	6.1	80					170	3	5.0	79
Bengtsår 1980	April — June	2.0	7.10[1]	3	5.9	141					83[1]	3	8.5	71
Mänttä 1979	June — Oct.	2.0	2.02	3	18.5	24								
Lake Tuusulanjärvi 1979—80	June — June	1.0	7.54	3	7.3	46					505	3	4.4	40
Tvärminne, (Långskär) 1979—80	June — June	1.0	2.86	3	7.5	92					88	3	9.1	87
Tvärminne, (Storgadden) 1980	May — Nov.	5.0[2]	1.01	4	14.2	18	0.04	4	8.9	12				
1981	Feb. — Dec.	5.0	1.22	4	17.6	74					85[1]	4	5.3	56
Raahe 1981	June — July	2.0	6.62	3	7.6	66								
River Kajaanijoki 1981	Aug. — Sept.	2.0	3.68	3	16.8	18	2.58	3	21.0	18				
Mean					9.0				12.3				6.8	
Total						635				54				333

[1] Incubation *in situ*, others *in vitro*
[2] Sample volume in the field; divided into 1 l portions in the laboratory.

Table 2. Variation between samples for heterotrophic activity ($1/T = \%h^{-1}$ and $V = \mu g$ gluc. $l^{-1}h^{-1}$) and primary productivity (PP = mg C $m^{-3}d^{-1}$) assays in different study sites. n1 = number of subsamples, n2 = number of samples, CV % = mean coefficient of variation, $\bar{x}$ = mean value for the period.

Study	Period	Sample volume (l)	1/T				V				PP			
			$\bar{x}$	n1	CV %	n2	$\bar{x}$	n1	CV %	n2	$\bar{x}$	n1	CV %	n2
Kaskinen 1980	May — Sept.	2.0	3.48	3	28.3	108	0.26	3	25.5	96				
Bengtsår 1980	April — June	2.0	8.87[1]	3	7.8	14					130	3	19.8	7
Tvärminne (Storgadden) 1980	May — Nov.	0.5	3.59	5	28.2	30	0.08	5	16.2	30				
Mean					26.4				23.3				19.8	
Total						152				126				7

[1] Incubation *in situ*, others *in vitro*

The degrees of freedom of the t-value in equation 1 are chosen according to the number of replicates in the determination of the mean CV %, and the number of replicates (n) is achieved through iteration. In this study, a 5 % risk level was chosen.

Within sample variation. It was assumed that the mean CV % values of the methods (within sample variation, Table 1) are sufficiently reliable for the procedure. With the turnover rate and primary productivity assays that is obviously the case, as the results are based on large data sets. The iteration procedure was performed also for maximal uptake velocity data, although the mean CV % value is not satisfactorily determined. The results of the iteration on different precision levels (p) are presented in Table 3 for the within and between sample variation.

With each parameter, a triplicate measurement was sufficient to maintain the precision below 20 % of the mean. If a 40—50 % precision is considered sufficient, only a single measurement is needed.

Between sample variation. Our information on the between sample variation (Table 2) is limited when compared to the within sample variation data (Table 1). However, also an estimation of the required number of replicate samples was performed on the basis of the mean CV % values from Table 2. These results can be considered only as indicatory, especially in the case of primary productivity, but the general result is rather obvious (Table 3). To achieve a certain confidence level, far more replicate samples must be assayed than with replicate subsamples (Table 3). For example, to achieve a 20 % precision, altogether seven replicate samples from a sampling site must be taken. With three or four replicates, a precision level of 40 to 50 % would be achieved. This conclusion applies to all the three parameters, which behaved rather similarly both on within sample and between sample levels. It is therefore concluded that the within sample variation (specific for each method) is clearly lower with all parameters than the actual variation observed in the nature (between sample variation).

4. DISCUSSION

The average within sample variation for glucose turnover rate (9.0 %) and maximal uptake velocity (12.3 %) are in agreement with the 6.2 % found by Herbland and Pagès (1976) for heterotrophic activity. Our within sample variation for primary productivity (6.8 %) is clearly below the mean value (17.9 %) of natural phytoplankton studies summarized in Herbland and Pagès (1976). They considered it impossible to achieve CV % values below 10 %, partly on the basis on the early results of Cassie (1962). In that study, primary productivity was measured with a Geiger-Müller counter, which obviously was a major source for the relatively high within sample variation observed. With the application of liquid scintillation counting, our CV % values for primary productivity were consistently below 10 % (Table 1).

The coefficients of variation (within sample) in heterotrophic activity and primary productivity measurements were below 10 % in most studies (Table 1). The within sample variation is specific

Table 3. The number of replicates required to achieve a certain confidence level (the measured mean is ± % of "actual") in heterotrophic activity (1/T, V) and primary productivity (PP) assays at 5 % risk level.
a) = number of replicates for within sample precision
b) = number of replicates for between sample precision.

	Confidence level						
Parameter	10 %	20 %	30 %	40 %	50 %	90 %	120 %
a)							
1/T	5—7	3—4	2	1	1		
V	9	3—7*	2—4[1)]	1—2	1—2		
PP	3—10*	3	1	1	1		
b)							
1/T	20	7	3—10[1)]	2—6	3—4		1
V	19—20	7	3—9[1)]	3	3—4	1	
PP	18	6—7	3—9[1)]	2—5	3	1	

1) = a "dead end" in iteration

for each method. In these methods, it consists of several sources in the course of the assay. Volume errors in the subsample division and pipetting of the radioactivity, registration errors of the incubation time, errors in filtration and radioactivity measurements all add to the overall variability within a single sample.

We have observed with standard radioactive solutions that the pipetting and radioactivity measurement errors are consistently below 1 %, when micropipettes (e.g. Finnpipette) and liquid scintillation counting are applied and a sufficient number of counts (> 10 000) are measured. The errors in incubation time registration are minutes at most, so that in incubations running a couple of hours the error is seldom over 1 %. If standard laboratory dispensers are used to subsample division, the volume errors are clearly below 1 %. It is difficult to evaluate the filtration error precisely, because several factors are involved (filtration pressure, leakage of the equipment, insufficient washing of the filter, quality of filters). The filtration errors are hardly very significant, if gentle pressure (less than 100 mmHg) is maintained and good quality equipment is used, as when applying the same routine, different CV % values have been obtained for different water bodies. This suggests that the main source of the observed over 10 % within sample variations would be the inhomogenous distribution of organisms in the subsamples from a sample container.

It is a generally accepted view in the literature that a major part (80—95 %) of planktonic bacteria are free-living cells (e.g. Williams 1970, Allen 1971, Berman 1975, Gocke 1975, Azam and Hodson 1977). Therefore, it can be assumed that in subsampling a thoroughly shaken water sample, the bacteria are distributed evenly in the subsamples. Our data shows that in sample bottle volumes of 1 l, the homogenization of the sample has succeeded (CV below 10 %) in all study areas (Table 1). With 2 or 5 sample volumes, the CV % has been clearly over 10 % in several studies, with the exception of Bengtsår (in 1980) and Raahe (in 1981). In the case of Tvärminne (in 1980), the 5 l original sample was subsampled (1 l) before the final subsample division, so that the low CV % value of V should be compared with the 1 l sample results. The patchiness revealed by higher CV % values in some 2 to 5 l volumes is obviously connected with the quality of the water sample, mainly with levels of particulate organic matter (increasing bacterial aggregation on particles). With primary productivity assays, no patchiness was observed in subsample assays (CV steadily below 10 %), so that the homogenisation of algae in the sample water before subsample division succeeded better than with bacteria.

The between sample variation ($\bar{x}$ of CV over 20 %) in bacterial activity measurements exceeded considerably the 4,6 % variation in bacterial counts found by Jones and Simon (1980). This supports the view that activity parameters vary in time and space far more than the state parameters such as direct counts (Jones and Simon 1980) or plate counts (Palmer et al. 1976). The results on the between sample variation show clearly that patchiness of both bacterial activity and primary productivity was encountered in the water bodies in magnitudes well exceeding the within sample variation. The patchiness of planktonic algae has been widely studied (e.g. Platt et al. 1970, Harris 1980, Therriault and Platt 1981), and it has been shown that patchiness occurs in scales ranging from meters to kilometers (macropatches). Our results indicate, because of the sampling techniques, patchiness in the scale of 1 to 20 meters. Hydrological phenomena undoubtly dominate the formation, scales and duration of the patches in the aquatic environment (Therriault and Platt 1981), so that results on patchiness must be discussed in relation to each study area and period of time.

Although our results on the phytoplankton patchiness are scarce, an interesting feature could be pointed out (Table 2). In the Bengtsår data (year 1980), the phytoplankton patchiness was significantly larger (CV = 19.8 %) than that of bacterial activity (CV = 7.8 %) measured from exactly the same incubations (simultaneous addition of ^{3}H and ^{14}C labelled substrates). As already discussed, the bacterial CV % value was exceptionally low in this study, but it could be concluded that the existence of bacterial and phytoplankton patches can be relatively independent at a certain moment of time. This is naturally no contradictory evidence to the very likely causal connection between these patches, with a certain time lag involved.

The within- and between sample variation comparison brings us to the main topic of this article, that is, the suitable sampling strategy to obtain results of these activity parameters that are valid for generalisations over the water body or the study site. Our results clearly show that the main source of variation is the between sample variation. On a general level of experimental design, Sokal and Rohlf (1969) have stated that replication on the highest hierarchical level is essential to improve the reliability of the results. This phenomenon was observed also with our results. Replication should be concentrated on the sampling level.

However, a cost-benefit analysis should be

performed in each study before rushing to the field. The cheapest — in terms of both cost and effort — and most reliable information on a study site can obviously be obtained by replicate sampling over a relatively large area at a single site (in a 10 m scale) and by combining these samples in a larger container, which is then homogenized by thorough shaking before conducting the assays. By increasing the number of original samples from one to seven, the precision of the assays increased from about 100 % to 20 % (Table 3) with all the parameters. Replicate incubations improve the precision far less, but triplicate incubations nevertheless increase the precision on the measurement level from 40 to 20 % (Table 3). It is therefore suggested that a suitable sampling strategy for routine measurements of both heterotrophic activity and primary productivity (Fig. 1) should include the combination of seven original samples from a study site into a pooled sample. This should then be assayed in duplicate or triplicate to further increase the precision, but also, and perhaps even more importantly, to ensure a measured value for the parameter in case of some disturbance in the sample processing during the incubation and filtration procedure. If the cost of the analysis is critical and limits the number of samples to be handled, the separate incubations can then be pooled either during or after the filtration, because the radioactivity measurement, which is by far the most expensive part of these assays, adds only a neglible amount of variation to the final result.

LOPPUTIIVISTELMÄ

Tässä työssä tarkasteltiin vesistöjen heterotrofisen aktiivisuuden ja perustuotannon mittausten rinnakkaisvariaatiota sekä sisävesi- että rannikkoalueilla. Variaatiota mitattiin sekä rinnakkaisnäytteiden (-nostojen) että laboratoriossa jaettujen alanäytteiden tasolla. Heterotrofista aktiivisuutta mitattiin glukoosin kiertonopeutena (1/T) tai maksimaalisena ottonopeutena (V).

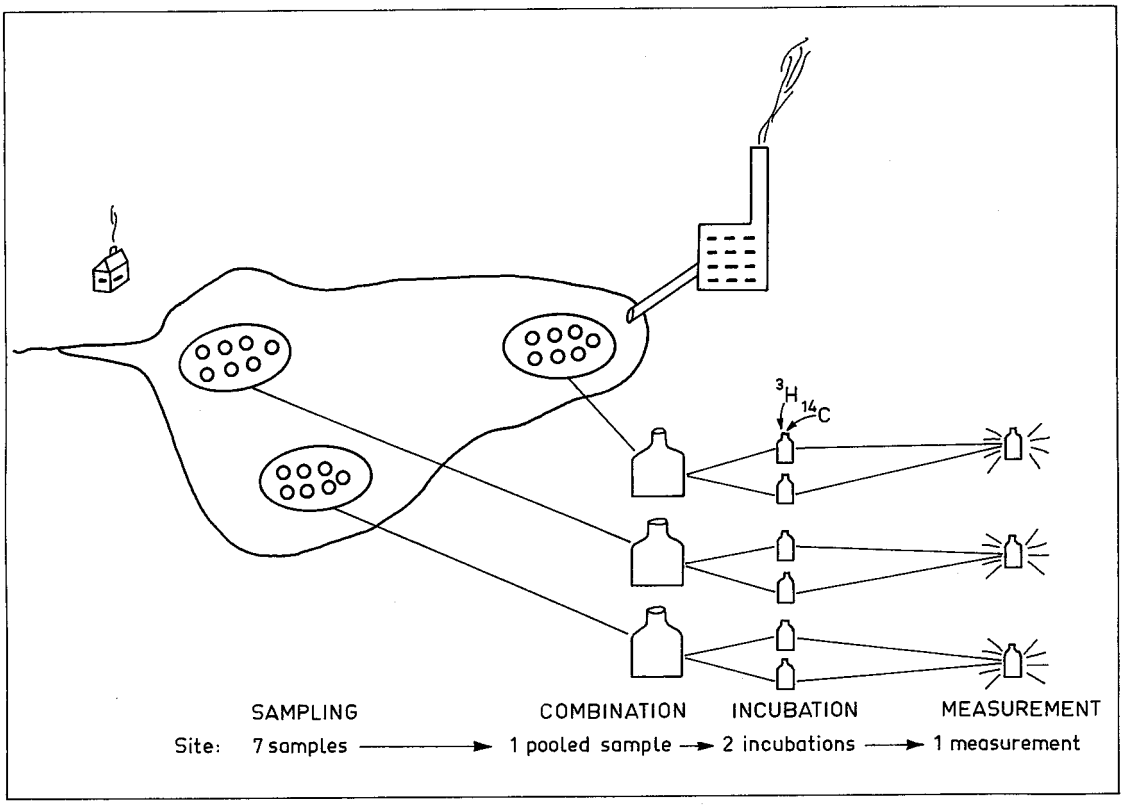

Fig. 1. Sampling strategy for routine measurements of heterotrophic activity and primary productivity.

Glukoosin kiertoajan keskimääräinen variaatiokerroin (CV = $s/\bar{x} \cdot 100$ %) oli samaa tasoa (9.0 %, n = 635) kuin perustuotantokykymittauksissa (6.8 %, n = 333) laboratoriossa jaettujen alanäytteiden välillä. Rinnakkaisnäytteiden (-nostojen) väliset variaatiokertoimet olivat luokkaa 20—30 % kaikilla mitatuilla muuttujilla. Rinnakkaisnäytteiden (-nostojen) käyttö lisää siis huomattavasti tuloksen edustavuutta sekä heterotrofisen aktiivisuuden että perustuotannon mittauksissa. Erilliset nostot voidaan yhdistää kokoomanäytteeksi ennen muuttujien mittausta, jolloin työmäärä ja kustannukset pysyvät kohtuullisina. Käytännön työskentelyyn ehdotetaan seitsemän rinnakkaisnoston yhdistämistä kokoomanäytteeksi, jonka jälkeen heterotrofinen aktiivisuus ja/tai perustuotanto voidaan mitata kahtena rinnakkaismittauksena.

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