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Sources of variability in zooplankton feeding experiments: The importance of accurate determination of algal growth rates*

ENRIC SAIZ

Institut de Ciències del Mar, CSIC, P. Nacional s/n, 08039 Barcelona, Spain

SUMMARY: The multiplicative models used to determine zooplankton feeding rates lead to increased uncertainty of the estimated grazing rate through the propagation of experimental error. The quantification of algal growth rates in control jars constitutes one of the main sources of error, which can account for most of the observed residual variability in zooplankton experimental data. This effect increases with increasing growth rate. Increasing the number of control jars and improving other methodological procedures should help to reduce this effect.

Key words: Methods, plankton, feeding

RESUMEN: FUENTES DE VARIABILIDAD EN EXPERIMENTOS DE ALIMENTACIÓN CON ZOOPLANCTON: IMPORTANCIA DE UNA CORRECTA ESTIMACIÓN DE LAS TASAS INTRÍNSECAS DE CRECIMIENTO DEL FITOPLANCTON. — Los modelos multiplicativos usados en la determinación de las tasas de alimentación del plancton, comportan una propagación de errores experimentales. En este artículo se demuestra que una de las principales fuentes de error radica en la determinación de las tasas de crecimiento intrínseco del alimento ofrecido mediante el uso de frascos control. Un análisis del tamaño muestra óptimo determina el número adecuado de replicados control a usar en función de la mencionada variabilidad.

Palabras clave: Métodos, plancton, alimentación.

INTRODUCTION

The important role of herbivorous zooplankton in the transfer of energy between producers and consumers in pelagic systems has lead to considerable effort, over the past three decades, to estimate the grazing rate of zooplankton, and especially of copepods. Most of these studies used the incubation method, which remains the most reliable of methods. This technique is based on the confinement of predators in a container and the measurement of the change in prey concentration after a certain period of incubation time. Control containers with prey alone provide a correction factor to account for intrinsic changes in the prey concentration. OMORI and IKEDA (1984), provide a description of the common methodological procedures.

Possible artifacts and sources of variability associated with the incubation method have been thoroughly studied, such as those related to the physiological status and previous life history of zooplankters (CHOW-FRASER, 1986, HASSETT and LANDRY, 1983) and to differences in the quality of the food offered (COWLES *et al.*, 1988, HOUDE and ROMAN, 1987, KIØRBOE, 1989). In addition, other sources of variability strictly associated with the methodology and experimental design can be considered: for example, the inaccurate quantification of the food offered in experiments (HARBINSON and MCALISTER, 1980, BAKKER *et al.*, 1985, BARETTA and MALSCHAERT,

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1985, KERSTING, 1985), the size of the containers used (OMORI and IKEDA, 1984, O'BRIEN, 1988), the prevention of settling of non-motile food using devices such as rotating wheels and plungers (OMORI and IKEDA, 1984, SAIZ *et al.*, 1992), and the statistical treatment of data (MARIN *et al.*, 1986).

The present paper examines another possible source of variability in feeding experiments, namely the multiplicative nature of the grazing equations, which yields a multiplicative propagation of errors through the different steps. It has not been contemplated how the different sources of error in conducting feeding experiments contribute, due to this multiplicative process, to the variability observed in the estimated feeding rates (TACK and VAN DE VRIE, 1985). Firstly, some of the sources of methodological error in feeding experiments and their respective magnitudes are addressed. It is shown that the error in estimating the algal growth coefficient, k, is considerable under the desirable experimental conditions. Finally, the contribution of this source of error to the variability of estimated grazing rates is studied.

MATERIAL AND METHODS

The data used in this paper are derived from experiments conducted to determine feeding rates for the calanoid copepod *Acartia clausi* fed the diatom *Thalasiossira weissflogii*. Part of these data have already been published for other purposes (SAIZ *et al.*, 1992). Briefly, the experiments consisted of the incubation in 920 ml jars of ca. 10 adult female copepods offered the above mentioned diatom. The algal suspension was prepared usually 1-2 hours prior to the experiment, and some nutrients (about 25 ml of f/10 medium per litre of suspension) were added to assure a supply of nutrients during the incubation. The suspensions were prepared in one or two 10-l containers, and after thorough mixing of the suspensions, the jars were filled by sequentially adding small amounts to each jar. The experiments were conducted at 20° C and 12h:12h dim light:dark, and the jars were kept on a 0.2 rpm rotating wheel (end over end) to prevent the algae from settling. The copepods were allowed to acclimate for a 24h period to the experimental food concentration and laboratory conditions prior to the test incubations. Four jars with copepods and algae (grazing) and 2 jars with only algae (control) were used for each experiment. Single or replicated initial and end samples were collected from each grazing and control jar, and particle concentration (as volume) was determined with a Coulter Multisizer particle counter in two to three 2-ml subsamples per replicate sample.

The equations employed to determine grazing rates were those provided by FROST (1972), the most commonly used in zooplankton ecology. These equations are based on the computation of the grazing coefficient g (time^{-1})

$$g = k - (ln\,[C_1 / C_0] / t_1 - t_0), \qquad (1)$$

where C_0 and C_1 are the food concentrations in the grazing jars respectively at the initial time t_0 and at the final time t_1, and k (time^{-1}) is the estimated algal growth rate computed from the control jars. The clearance rate F (volume swept clear individual^{-1} time^{-1}) is computed

$$F = V \cdot g / n \qquad (2)$$

where V is the volume of the containers used in the incubation, g is the grazing coefficient, and n the number of copepods employed. Finally, the ingestion rate I (biomass ingested individual^{-1} time^{-1}) is

$$I = F \cdot C \qquad (3)$$

where C is the average food concentration in grazing jars during the experiment, calculated as follows

$$C = (C_0[\exp\,\{(k\text{-}g)(t_1 - t_0)\} - 1])/\{(k\text{-}g)(t_1 - t_0)\} \qquad (4)$$

The functional response of observed ingestion rates to food concentration was described by means of Ivlev's equation without critical concentration ($I = I_{max} \cdot [1 - e^{\{b \cdot C\}}]$). The equation was fitted using log-transformed ingestion rates to reduce variance at high food concentrations (Fig.1). Although other equations (i.e. with critical concentration, Michaelis-Menten) explained similar amounts of variance, their estimates of the fit of the parameters were not as precise. For this analysis the data used corresponded to a set of 13 experiments performed with *Acartia clausi* over a range of food concentrations from ca. 0.2 to 2.2 mm^3 l^{-1} of *Thalassiosira weissflogii*. The equation parameters (for the log-transformed data) obtained were $I_{max} = 1.43 \pm 0.040$ SE and $b = -3.31 \pm 0.299$ SE. The non-linear model explained only 66% of the variance in feeding rates in the experiments.

The hypothesis that residual variability (i.e. not explained by the functional model) derives from error in estimating algal growth rate (k), was examined by evaluating the sensitivity of feeding rate estimates to small changes in k. Twenty four-hour experiments conducted in 1-l jars with 10 adult females of *Acartia clausi* were assumed. Random normally distributed values of the algal growth rate were generated assuming estimate errors corresponding to coeffi-

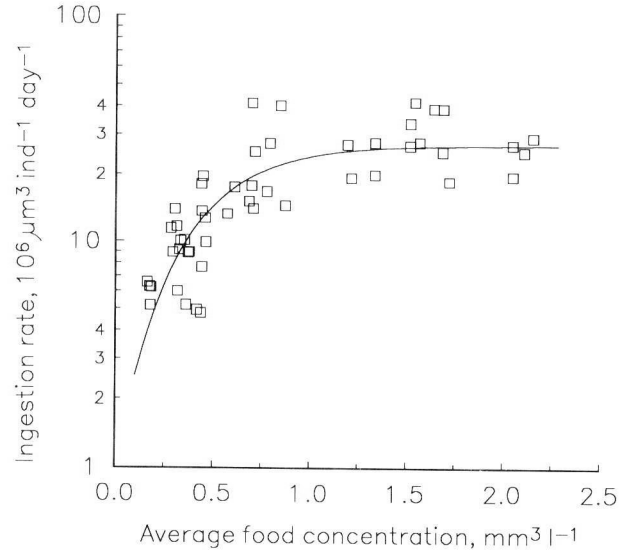

FIG. 1. — Relationship between food concentration and ingestion rates of *Acartia clausi*. Average food concentration (C) is expressed as $mm^3\ l^{-1}$; ingestion rate (I) is expressed as $10^6\ \mu m^3\ ind^{-1}\ day^{-1}$. The continuous line represents the Ivlev's model fitted to the data.

cients of variation (CV) ranging from 10 to 40% (this includes the average value of 19% observed in the experiments). The corresponding ingestion estimates were computed using the model parameters obtained for the experimental data. Simulations were computed for values of k ranging from almost no growth (k=0.001 h^{-1}) to a rate of 0.030 h^{-1} (slightly higher than a duplication per day), which includes the average value of 0.02 h^{-1} observed in the experiments.

To estimate the relative contribution of this source of variability in a real process (i.e. the experimental data presented), simulations were made for each of the 13 experiments. Two values of k (the two control jars) were sampled randomly from a normal distribution of k values with mean equal to that empirically obtained in each experiment, and a standard deviation computed from the average coefficient of variation for the whole set of experiments (corrected for bias, CV = 21.36). For the set of 13 experiments this process was simulated 100 times, and for each simulation the respective Ivlev's fit was calculated. To compare the error observed in the original data with the contribution due to the error in k-estimates, the quotient "(MS_{resid} observed)/(average MS_{resid} in simulations)" was computed.

RESULTS AND DISCUSSION

Analysis of the sources of error in the experiments

Here I report the magnitude and contribution to variance of some major procedures or steps when conducting feeding experiments. The experiments studied were not designed specifically to test hypotheses on sources of error. This fact made it impossible to use the same set of experiments for all the analyses. Consequently, this part will be descriptive, conducting the different analyses on those experiments performed under the same conditions.

1. Filling procedure. Variability in the initial food concentration.

Data comes from 16 experiments where sets of 6 jars were filled from a single batch suspension of algae. The suspension was well mixed and the water added in small amounts sequentially to the jars in order to distribute the water evenly. The coefficients of variation in the initial food concentration for each set of jars were computed. The average coefficient was 5.9 % (range: 3.2-13.6). A nested ANOVA test showed that the differences between jars within experiments were significant ($P<0.01$). Sixty nine per cent of this variability was the consequence of real differences between jars, while the remainder of the variation was due to subsampling and counting error. The high variability in the initial food concentration due to differences between replicated jars was unexpected due to the previous thorough mixing of the suspensions. This variability in the filling procedure might preclude the use of the equations proposed by MARIN *et al.* (1986) to estimate grazing rates, which require the same initial food concentration in all the jars. This fact also clearly indicates that the common procedure of not taking initial samples from each experimental jar can significantly increase the error in estimates.

2. Error in estimates due to subsampling and counting.

Data corresponded to 60 samples from different experiments which were subsampled in duplicate, and each subsample counted twice. A nested ANOVA test showed that the variance component added by subsampling was null, all the variability within samples being due to counting error. The counting error (standard error/mean × 100; based on the 2 duplicated counts of each subsample) averaged 4 % for the 60 pairs of subsamples.

3. Differences in the algal growth rate between experiments.

TABLE 1. — Quotient g/k as a function of the algal growth coefficient k, the duration of the experiment (incubation time) and the maximum reduction in food concentration that we want to accept (10 %, 25 % and 50 %).

A) Incubation time 6 hours

	Reduction in food concentration		
k (h^{-1})	10 %	25 %	50 %
0.0001	176.6	480.5	1156.2
0.001	18.6	48.9	116.5
0.005	4.5	10.6	24.1
0.01	2.8	5.8	12.6
0.015	2.2	4.2	8.7
0.02	1.9	3.4	6.8
0.025	1.7	2.9	5.6
0.03	1.6	2.6	4.9

B) Incubation time 24 hours

	Reduction in food concentration		
k (h^{-1})	10 %	25 %	50 %
0.0001	44.9	120.9	289.8
0.001	5.4	13.0	29.9
0.005	1.9	3.4	6.8
0.01	1.4	2.2	3.9
0.015	1.3	1.8	2.9
0.02	1.2	1.6	2.4
0.025	1.2	1.5	2.2
0.03	1.1	1.4	2.0

The mean algal growth rate for the 13 experiments averaged 0.020 h^{-1} (range 0.013 to 0.031 h^{-1}; CV = 26 %). A nested ANOVA test showed that the differences between experiments were significant ($P < 0.05$). Of this variability, 48 % was due to real differences between experiments (for instance, that the state of the batch culture was not the same) and the remaining 52 % was due to differences within experiments (between the two replicate control jars). These differences between replicates included counting error (quite small, as stated above) and other uncontrollable factors that constitute the experimental error (small differences in illumination and temperature, real variability, etc).

4. Error in the estimates of the algal growth rate.

The variability in the estimation of k from two replicated jars was studied for 13 experiments. The average coefficient of variation of these 2-jar based k-estimates was 19 % (range: 0.2 % to 46 %, $n = 13$), which resulted in a 13 % error in the estimates of the average.

From these considerations, it is evident that the estimation of the algal growth rates during grazing incubations can hold a considerable amount of variability and, therefore, it might constitute a major source of error in the experiments studied.

Furthermore, the importance of this source of error is more remarkable because of the fact that the algal growth coefficient is an essential step in computing grazing rates. Although mathematically the importance of the variance in k can be negligible for values of $k << g$, this situation is not usually advisable. The grazing coefficient reflects the decrease in food concentration in the grazing bottles. One way to keep $k << g$ is to increase the density of animals, but the effects of crowding on grazing rates probaby are very important, although they have not been well quantified (OMORI and IKEDA 1984). There are also practical reasons; researchers do not want the food concentration to change much during experiments (rule of thumb 25-30 %) in order to make the average food concentrations reliable. This is essential in studies on the functional response of the individual. Table 1 shows the quotient g/k as a function of algal growth rate, duration of the incubation and maximum acceptable reduction in food concentration. For growth rates of algae ranging between 0.01 and 0.03 h^{-1} (generation times from 3 to 1 days respectively), the quotient g/k oscillates between 1 and 6 for reductions in food concentration up to 25 %. Consequently, for the most recommended situations, k is clearly of the same magnitude as g.

Contribution of k to the variance in feeding rates

The sensitivity of feeding rates to the error in k is dependent on the absolute value of k and the food concentration. In Fig. 2 the coefficient of variation (based on log-transformed ingestion rates) of feeding estimates as a function of food concentration, the average value of k and the error in k-estimates is shown. In 176 of the 14080 simulations performed a negative ingestion was obtained. These data were discarded to allow for log-transformation (see below). The consequences of variation in k were noticeable at both low and high food concentrations. The contribution was negligible for low variance in k-estimates, but for coefficients of variation of 20 % (similar to the average observed in the experiments studied) or above, the effect was considerable even for the log-transformed data. The importance of this effect was dependent on the magnitude of the algal growth rates, increasing substantially as the growth rate increases.

Fig. 3 shows the simulation of the experiments performed on *Acartia clausi*. For each experiment, two control jars were simulated and the feeding rates for the respective grazing jars computed. A few of the simulated k values resulted in negative or extremely

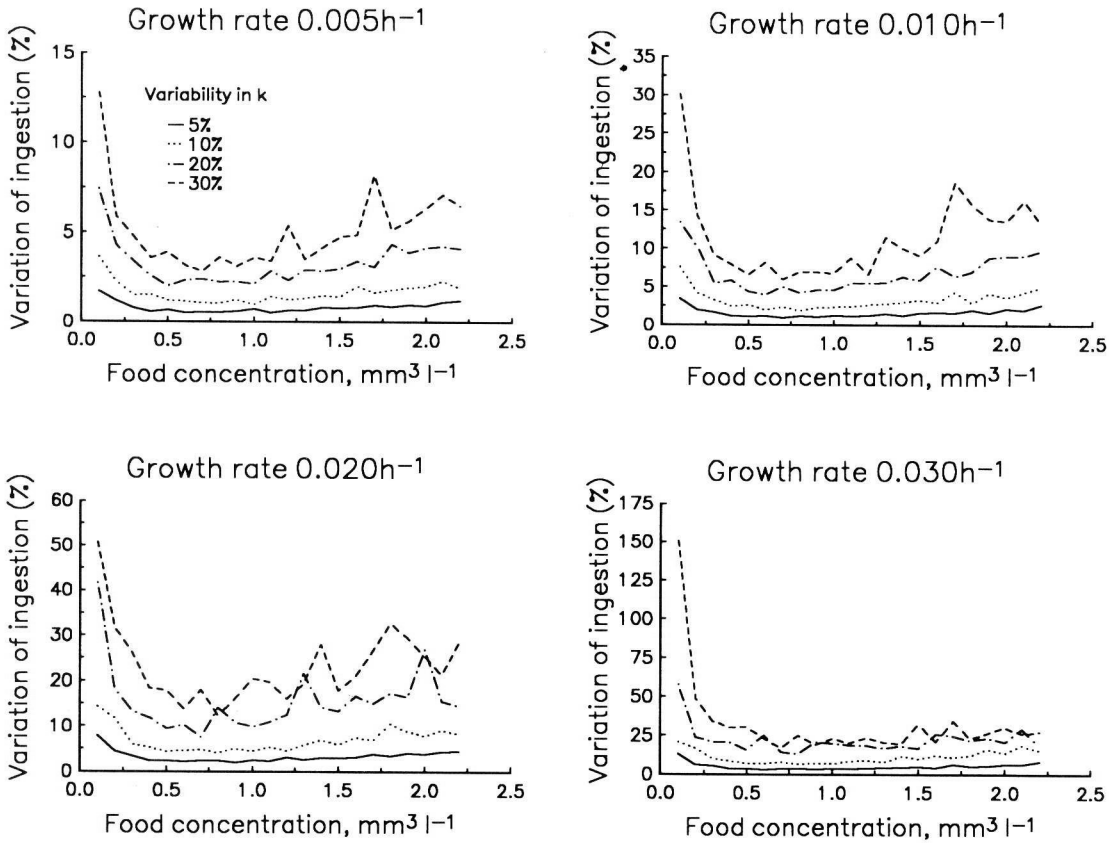

FIG. 2. — Sensitivity of feeding rate estimates to error in estimating algal growth rates (k) as function of food concentration (C), average value of k and its variability (coefficient of variation, CV). The coefficient of variation of the predicted feeding rates (log-transformed) is shown in ordinates. Food concentration in abscissae. The lines correspond to different degrees of variability in k (coefficients of variation from 5 to 30 %). Forty simulations were performed for each food concentration (at 0.1 intervals).

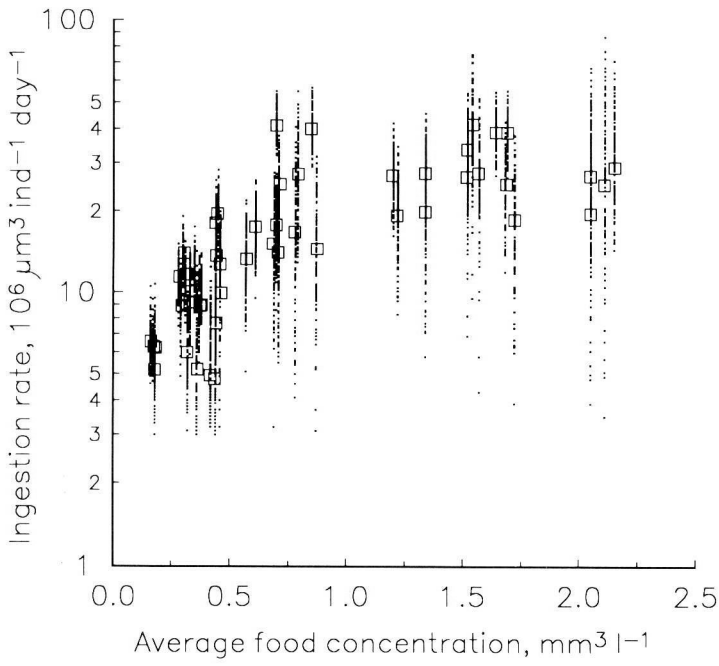

FIG. 3. — Scatterplot of the 100 simulations performed for the set of 13 experiments considered. Units as in Fig. 1. The simulations were performed generating random normally-distributed algal growth coefficients for each control jar. The hollow squares represent the empirical data.

TABLE 2. — Some representative papers dealing with the feeding of zooplankton. The number of grazing and control bottles used are presented. 'Not clear' or 'not stated' means that the number of controls is not specified for all the experiments or that is not mentioned, respectively.

Control Jars	Grazing Jars	Comment	Reference
2	2	—	RICHMAN and ROGERS, *Limnol. Oceanogr.* 14: 701-709, 1969
1	1	—	ALLAN *et al.*, *Mar. Biol.* 43: 317-331, 1977.
—	—	not stated	PAFFENHÖFER and KNOWLES, *Mar. Biol.* 48: 143-152, 1978.
up to 4	—	not clear	DONAGHAY and SMALL, *Mar. Biol.* 52: 129-136, 1979.
—	—	not stated	ROMAN and RUBLEE, *Limnol. Oceanogr.* 25: 982-990, 1980.
1	1	—	HUNTLEY, *Limnol. Oceanogr.* 26: 831-842, 1981.
2 to 3	6 to 8	—	KIØRBOE *et al.*, *Ophelia* 21: 181-194, 1982.
1	2	—	GLASSER, *J. Plankton Res.* 6: 553-569, 1984.
2	3	—	MILLER and LANDRY, *Mar. Biol.* 78: 265-270, 1984.
2	2 to 3	—	MARIN *et al.*, *Mar. Biol.* 93: 49-58, 1986.
—	—	not stated	AYUKAI, *Mar. Biol.* 94: 579-587, 1987.
up to 4	—	not clear	IVES, *J. Exp. Mar. Biol. Ecol.* 112: 131-145, 1987.
2	6	—	MOBLEY, *J. Exp. Mar. Biol. Ecol.* 114: 199-216, 1987.
2	3	—	BERGREEN *et al.*, *Mar. Biol.* 99: 341-352, 1988.
—	—	not stated	PAFFENHÖFER and STEARNS, *Mar. Ecol. Prog. Ser.* 42: 33-38, 1988.
3	4 to 6	—	KIØRBOE, *Mar. Ecol. Prog. Ser.* 55: 229-234, 1989.
1	3	—	TURNER and TESTER, *J. Exp. Mar. Biol. Ecol.* 126: 21-43, 1989.
3	4	—	DURBIN *et al.*, *Mar. Ecol. Prog. Ser.* 68: 23-45, 1990.
2	3	—	STØTTRUP and JENSEN, *J. Exp. Mar. Biol. Ecol.* 141: 87-105, 1990.
1	2 to 3	—	TURNER and GRANELI, *J. Exp. Mar. Biol. Ecol.* 157: 19-31, 1992.

low ingestion rates. This resulted in extremely low fits for Ivlev's equations for that set of experiments. Consequently, a minimum ingestion threshold of $3 \cdot 10^6$ μm^3 ind^{-1} day^{-1} was established to keep the simulation valid. Only 102 of the 1300 simulations of experiments had to be repeated. Although this procedure reduced the variability, it was necessary to compute the logarithmic fit of the data and to obtain reasonable values. This fact also suggested that some of the abnormal or negative ingestion rates that sometimes are obtained in the laboratory (and attributed to "bad shape" of the food offered and/or of the zooplankters) might be induced by high variability in the estimates of algal growth rates.

CONCLUSIONS

The average residual mean squares of the simulations was 0.040 (range: 0.017-0.069). The quotient of the residual mean squares between the simulated data and the real data is smaller than 1 (observed 0.026 vs. simulated 0.040). This fact means that the variability in our k-estimates was large enough to explain most of the residual variability observed in the experimental data, as the initial hypothesis stated.

The data related to control jars is rarely reported in the zooplankton feeding literature. Growth rates in experiments are seldom reported, and even the number of controls is very often omitted. In Table 2 a list of some representative papers in zooplankton feeding ecology and the number of control jars used are reported. In general two control jars per experiment are used. The appropriate number of controls for an experiment depends on the experimental procedures (i.e. filling procedure, counting accuracy) and the algal growth rate. For instance, an exponentially-growing batch culture of *Thalassiossira weisflogii* has a growth rate k (biomass, per hour) of about 0.015-0.027. In this range, the contribution of the variability in k-estimates can be significant. Some authors report negligible growth of algae in their experiments (that is, $k = 0$, for instance MARIN *et al.* 1986); then this source of error is avoided, but the physiological state of the algae might not be optimum for the copepods. Taxonomic and size dependence of growth rates should also be taken into account.

A compromise exists between acceptable error and the number of jars one can manage in an experiment. Fig. 4 shows the minimum sample size required to obtain estimates of k with different precision (capacity to discern a significant difference in the value of k; significance level α=0.05 and power 1-ß=0.90). This value depends on the variability in k. For a coefficient of variation of 20 % in k, the minimum sample size required to obtain a precision of 20 % and 30 % in the computed estimate of k is 11 and 5 replicate control jars respectively. If it were possible to reduce the coefficient of variation to 15 % (for instance, by improving the counting procedure), a reasonably appropriate number of control jars to manage in the experiments with a significant reduction in scattering is approximately 4 jars.

It has been shown that the initial hypothesis is

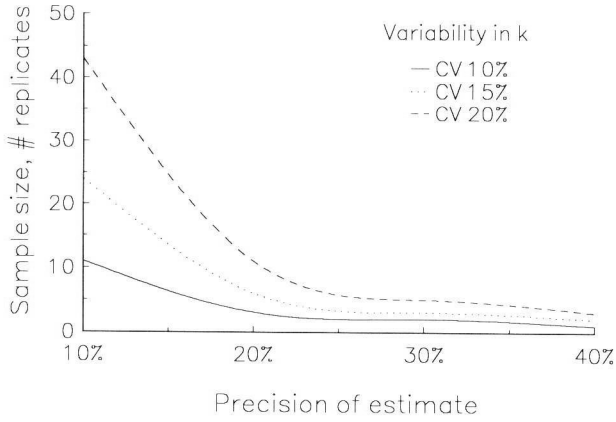

FIG. 4. — Sample size (number of replicates; in ordinates) required to detect a significant percentage of difference in the value of the algal growth rate *k* (precision of estimate, as percentage; in abscissae). The different lines correspond to coefficients of variation of 10, 15 and 20 % in the algal growth rate (k).

well supported, i.e. much of the residual variability observed in experimental data can be a consequence of the inaccurate determination of algal growth rates in experiments. This source of error has not been considered before and appears to be very important, especially when the experiments are performed with algae growing in the exponential phase. Increasing the number of control jars can significantly improve the accuracy of experiments. When designing feeding experiments, preliminary studies must be performed to determine the number of grazing jars required to obtain the appropriate significance level (and power) in the statistical test. It is proposed that this kind of analysis should also optimize the number of control jars required. In the future, more attention should be paid to the growth of algae in zooplankton feeding experiments which, coupled with better-designed experiments, will help to obtain better estimates of zooplankton feeding rates.

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