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RESEARCH ARTICLE

Algird-Gordon Moigis

The clearance rate of microzooplankton as the key element for describing estimated non-linear dilution plots demonstrated by a model

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Abstract The objective of this study was to determine whether the clearance rate of grazers (as an individual response) was sensitive enough to describe non-linear plots estimated by dilution experiments for measuring the instant grazing rate of microzooplankton. The study was based on an initial analysis of a non-linear feeding pattern based on the food concentration dependence of clearance rate of microzooplankton. In contrast to the traditional assumption of a linear functional response, I assumed that the microzooplankton functional response was non-linear and that the dependence of the clearance rate can be sub-divided into four intervals of food concentration (Sections I–IV) as follows: in Section I clearance rate is zero; Section II is a transitional interval in which the clearance rate increases from zero to a maximum value; in Section III, the clearance rate is maximal and constant, and in Section IV, the clearance rate decreases from its maximum value due to saturated ingestion rate. A set of derived differential equations describes the phytoplankton growth rate in each section, leading to the possibility of comparing predicted non-linear dilution plots with observed non-linear dilution data, using only the specific solutions for Sections III and IV. One should evaluate the quality of fit provided by the non-linear and linear models, rather than uncritically accepting only the linear model for observed non-linear dilution data, using calculated expected non-linear and linear dilution plots as alternative hypotheses. It can be demonstrated that the non-linear model provided a better fit to estimated non-linear dilution data from the Red Sea, Rhode River Estuary (USA) and Kiel Fjord (Germany) than the standard linear model. Published dilution experiments which had a non-linear shape were also selected as illustrative examples to demonstrate the superior fit of the non-linear model.

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A.-G. Moigis
Leibniz-Instituts fuer Meereswissenschaften,
Duesternbrooker Weg 20, 24103 Kiel, Germany

A.-G. Moigis
Smithsonian Environmental Research Center,
P.O. Box 28, Edgewater, MD 21037, USA

Present address: A.-G. Moigis
Ulmenstr. 73, 24306 Ploen, Germany
E-mail: AMoigis@gmx.net

Introduction

Landry and Hassett (1982) introduced the dilution method for estimating the grazing rate of microzooplankton. As a method that is now used routinely in aquatic ecology, dilution based published studies have increased exponentially over the last decade with a "doubling time" of 2–3 years (see Fig. 2 of Dolan et al. 2000). The original method suggested a linear relationship between the apparent growth rate of phytoplankton (μ = true specific growth rate of phytoplankton minus instant grazing rate of microzooplankton) and dilution (D = fraction of unfiltered water), the slope of the linear regression analysis being the instant grazing rate of microzooplankton and the Y-axis intercepts the specific growth rate of phytoplankton. Hence, linear dilution plots (= LDP, Fig. 1) display a linear shape, as shown in several published dilution studies since then. As explicitly stated by Landry and Hassett (1982), this linearity is based on an assumed constant maximum clearance rate of grazers (c_{max}), indicating that the ingestion rate of grazers (I) has a linear dependence on food concentration (P). Consequently, in an LDP, I never has a maximum saturated value (I_{max}).

On the other hand, ever since Gallegos published his first dilution studies in 1989, several studies have been published since then showing "L-shaped" non-linear dilution plots. In such plots, data deviate from the above linear relationship, having a steep slope near the x-origin and no slope further from the origin (Fig. 1). According to Gallegos (1989), NLDP occur when microzooplankton feed at their maximum food-saturated ingestion

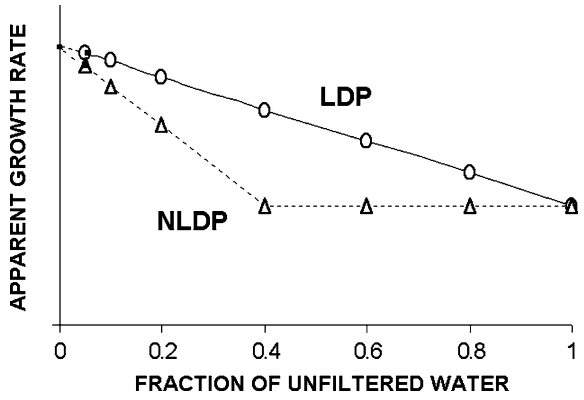

Fig. 1 Linear dilution plot (*LDP*) and non-linear (*NLDP*) dilution plot

rates (I_{max}). Based on hyperbolic Michaelis-Menten and hyperbolic tangent models to simulate saturated ingestion, Evans and Paranjape (1992) analyzed dilution data. In their analysis, the assumption of linearity of Landry and Hassett (1982) was relaxed so that the possibility of a curvature in the whole dilution range could be accepted. Evans and Paranjape (1992) demonstrated the possibility that non-linear models can fit better with experimental dilution data than the linear model. They noted specifically that non-linear models "will reveal some of the uncertainty in the rate estimates that the linear analysis conceals". Although Gallegos (1989) and Evans and Paranjape (1992) have demonstrated the difficulties associated with analyzing data stemming from NLDP, many authors continue to apply the linear regression analysis of the Landry and Hassett (1982) method, even when data are clearly non-linear. When a linear model may have been deemed statistically significant, many authors may fail to explicitly consider an alternative, perhaps superior, non-linear model. Accepting only the linear hypothesis automatically rejects the possibility that microzooplankton are feeding at their maximum ingestion rate, and there is a priori no reason to do this.

In the context of an analysis of dilution plots, I report the development of growth equations of phytoplankton as a model for describing NLDP according to plausible relationships between the clearance rate of microzooplankton and food concentration. As indicated above, the analysis of Evans and Paranjape (1992) was based on hyperbolic models which described the non-linear curvature in the whole dilution range. The dilemma is that one mathematical function cannot describe all of the relationships that exist between microzooplankton feeding and food concentration through the whole dilution range of NLDP. As demonstrated by Frost (1972, Fig. 2), a food concentration (P) may exist where the clearance rate is maximal (c_{max}) and the ingestion rate (I) a linear function of food, i.e., $I = c_{max}P$. However, at higher food, the ingestion rate may be maximal (I_{max}), defining an interval in which the clearance rate (c) declines monotonically with food as $c = I_{max}P^{-1}$ (Frost 1972, Fig. 2). Furthermore, at very low dilution, food clearance rate may be zero (Frost 1975; Rublee and Gallegos 1989), defining an interval in which there is no relationship between grazers' response and food concentration. To account for these differences, we can define four intervals of food concentration based on the unique parameters of maximum ingestion rate, maximum clearance rate and zero ingestion of microzooplankton. The objective of the analysis is to determine whether the clearance rate of microzooplankton, as a variable, is sensitive enough to describe NLDP. This exercise explores and demonstrates the potential of non-linear analysis of dilution data.

In this paper, I further report comparisons of predicted NLDP and LDP, the latter being an alternative hypothesis, with experimentally estimated non-linear dilution data. An expected NLDP was calculated by growth equations of phytoplankton developed in the above analysis, and an expected LDP was calculated by the standard linear regression procedure. Both predicted dilution plots were thereafter compared with estimated dilution data by calculating the squared correlation coefficients as quality of fit.

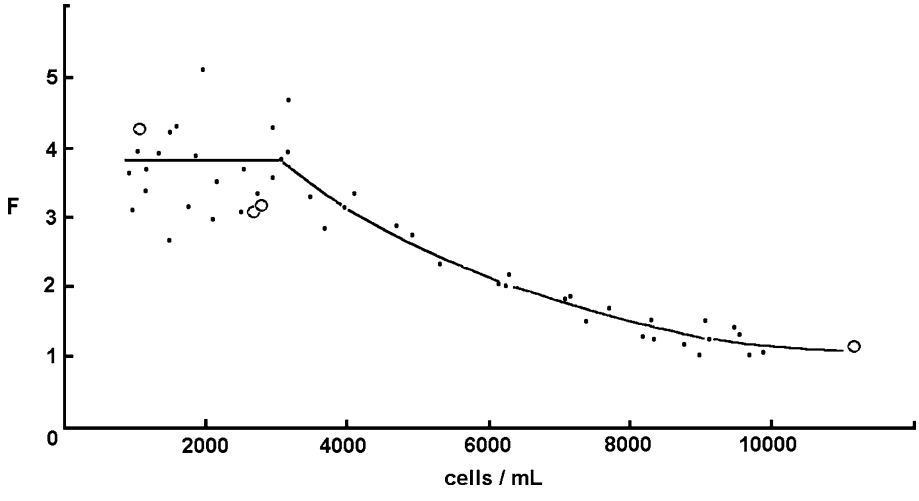

Fig. 2 Effect of cell concentration on volume swept clear (F in ml individual^{-1} h^{-1}), the latter terminology as used by Frost (1972), these results being from experiments made by him with batch culture (*dots*) and continuous culture (*circle*). This figure was redrawn from Frost (1972)

Last but not the least, I also report in this paper how the in situ value of g is calculated when a NLDP is estimated.

Theoretical analysis of dilution plots

The starting point for analyzing the effect of dilution (D) on the instant grazing rate (g) in a dilution plot is the basic Eq. 1:

$$g = cZ, \quad (1)$$

where c is the clearance rate of microzooplankton and Z the microzooplankton (or grazers) concentration. Table 1 shows the symbols used in the present analysis.

c has units of "volume of ambient water cleared of prey" individual^{-1} time^{-1}. c is equivalent to "volume swept clear" of Frost (1972) which is defined as the volume of ambient water from which food particles are completely removed by grazers to achieve a measured ingestion rate.

Z has units of individuals volume^{-1}. Z could alternatively be expressed in units of biomass volume^{-1}, and in such a case, c must consistently have units of "volume

Table 1 Notation of used symbols presented alphabetically. The first suffix within brackets refers to time (t) and the second suffix to dilution level (D)

c	Clearance rate of microzooplankton (volume individual^{-1} time^{-1} or volume (biomass of microzooplankton)$^{-1}$ time^{-1})
c_{avg}	Average clearance rate of microzooplankton
c_{max}	Maximum clearance rate of microzooplankton
$c_{(t,D)}$	Clearance rate of microzooplankton at time t and dilution D
$c_{(0,D)}$	Initial clearance rate at dilution D
Chl	Chlorophyll (μg l^{-1})
Chl_{cr}	Critical concentration of chlorophyll
$Chl_{(t,D)}$	Chlorophyll at time t and dilution D
$Chl_{(0,D)}$	Initial chlorophyll at dilution D
$Chl_{(t,1)}$	Final chlorophyll at undiluted level
$Chl_{(0,1)}$	Initial chlorophyll at undiluted level
D	Dilution level (fraction of unfiltered water)
D_{cr}	Critical dilution level
g	Grazing rate of microzooplankton (time^{-1})
g_{avg}	Average grazing rate of microzooplankton
$g_{in\ situ}$	In situ grazing rate
$g_{(t,D)}$	Instant grazing rate of microzooplankton at time t and dilution D
$g_{(0,D)}$	Initial instant grazing rate of microzooplankton at dilution D
$g_{(t,1)}$	Instant grazing rate of microzooplankton at time t and undiluted level
$g_{(0,1)}$	Initial instant grazing rate of microzooplankton at undiluted level
I	Ingestion rate of microzooplankton (biomass of food individual^{-1} time^{-1} or biomass of food (biomass of microzooplankton)$^{-1}$ time^{-1})
I_{max}	Maximum ingestion rate of microzooplankton
$I_{(t,D)}$	Ingestion rate of microzooplankton at time t and dilution D
$I_{(0,D)}$	Initial ingestion rate at dilution level D
k	Specific growth rate of phytoplankton (time^{-1})
$k_{in\ situ}$	In situ specific growth rate of phytoplankton
LDP	Linear dilution plot
NLDP	Non-linear dilution plot
P	Phytoplankton biomass (e.g. μg carbon l^{-1})
P_{avg}	Average phytoplankton biomass
P_{cr}	Critical concentration of algal food biomass
P_{th}	Threshold concentration of phytoplankton biomass
$P_{(t,D)}$	Phytoplankton biomass at time t and dilution D
$P_{(0,D)}$	Initial biomass of phytoplankton at dilution D
$P_{(t,1)}$	Phytoplankton biomass at time t at undiluted level
$P_{(0,1)}$	Initial biomass of phytoplankton at undiluted level
t	Time
μ	Apparent growth rate of phytoplankton (time^{-1})
μ_{avg}	Average apparent growth rate of phytoplankton
μ_D	Apparent growth rate of phytoplankton at dilution D calculated with the function $\ln(Chl_{(t,D)}/Chl_{(0,D)})/t$
μ_1	Apparent growth rate of phytoplankton at $D=1$ calculated with $\ln(Chl_{(t,D)}/Chl_{(0,D)})/t$
$\mu_{(t,D)}$	Apparent growth rate of phytoplankton at time t and diluted level
μ_{1-}	Apparent growth of phytoplankton without added nutrients at undiluted level
μ_{1+}	Apparent growth of phytoplankton with added nutrients at undiluted level
Z	Zooplankton concentration (or biomass) (individuals volume^{-3} or biomass volume^{-3})
Z_D	Zooplankton concentration or biomass at dilution D (constant value)
Z_1	Zooplankton concentration or biomass at undiluted level (constant value)

of ambient water cleared of prey" biomass^{-1} time^{-1} throughout the analysis.

g has units of time^{-1}. The expression "instant grazing rate" is equivalent to "instantaneous coefficient of grazing mortality" (g) of Landry and Hassett (1982), "grazing coefficient" of Gallegos (1989), "specific grazing rate" of Gallegos (1989) and Moigis and Gocke (2003), and refers to "instantaneous rate of phytoplankton mortality due to microzooplankton grazing".

I follow the standard terminology (introduced by Landry and Hassett 1982), and use the term dilution level D as a synonym for the phrase "fraction of unfiltered water".

Fundamentally, the analysis of the dilution method is based upon knowing the corresponding microzooplankton concentration (Z) at each dilution factor (D), and inferring a robust estimate of the clearance rate of microzooplankton (c). The central objective in this analysis is to determine whether a food concentration (P) dependence of c has a sufficient and dominant effect on g in a dilution experiment, in other words, if c is sensitive enough to describe a NLDP. The analysis is based upon an assumption that the relationship between c and food concentration can be subdivided into the following four intervals (Fig. 3).

Section I: c is zero. The highest value in this potential section is the threshold food concentration of microzooplankton (P_{th}). Zero ingestion was experimentally demonstrated by Frost (1975) and Rublee and Gallegos (1989). See also Steele (1974).

Section II: with increasing c (Frost 1980). The relationship between c and P can be approximated by the following linear equation (Eq. 2):

$$c = \underline{a}(P - P_{th}), \tag{2}$$

where$\underline{a}$ is the linear proportionality constant and P_{th} the threshold food concentration of microzooplankton.

Section III: with a constant maximum c ($=c_{max}$) according to Frost (1972, 1980, Fig. 2). The highest concentration of phytoplankton in Section III is defined according to Frost (1972) as critical food concentration (P_{cr}), this term being a synonym for "incipient limiting concentration" in other publications (e.g. Gallegos 1989; Elser and Frees 1995). Below P_{cr}, the ingestion rate of grazers (I) is not maximal, it decreases with decreasing P. At P_{cr} and above, I is maximal (I_{max}).

Section IV: with decreasing c according to Frost (1972, Fig. 2). In Section IV, I is maximal (I_{max}, Frost 1972; Gallegos 1989), hence the relationship between c and P is described by Eq. 3:

$$c = I_{max}P^{-1}. \tag{3}$$

In Section IV, a higher P is counterbalanced by a lower c. Because c depends on P, c can never be constant; it varies continuously with time because of the dependence of P on time. But even in Section IV,

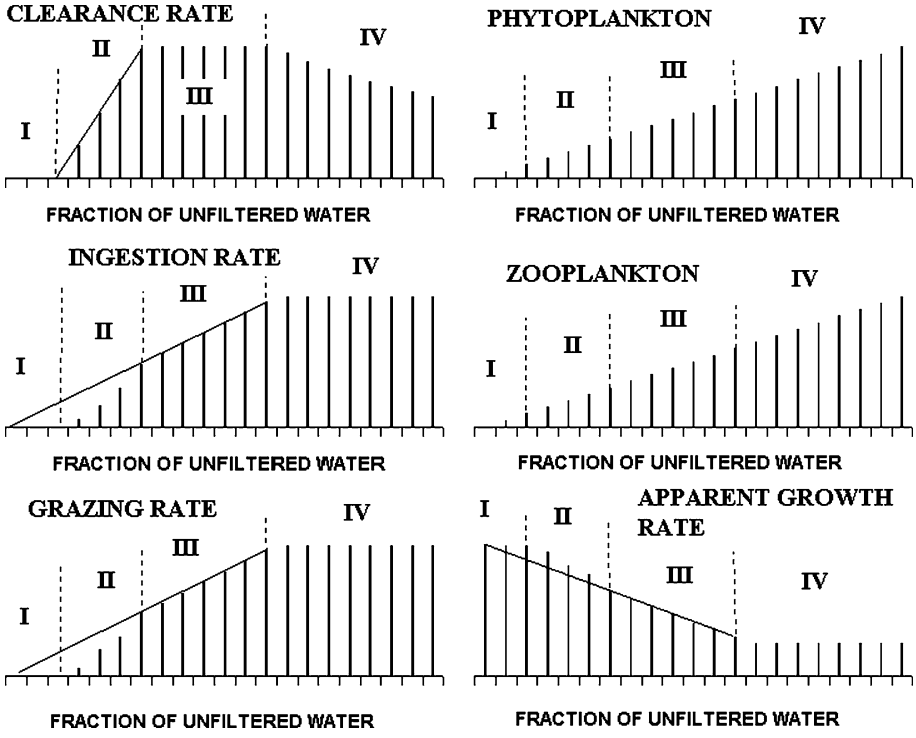

Fig. 3 Microzooplankton and phytoplankton concentrations (Z and P), clearance rate (c), instant grazing rate (g), apparent growth rate (μ) and ingestion rate of microzooplankton (I) as function of dilution level (D = fraction of unfiltered water). The plot of c, as starting point, is defined according to the assumptions made for Sections I, II, III and IV, and the plots of Z, P, g, μ and I (instantaneous values) are calculated ones. For the definitions of Sections I, II, III and IV and used equations see text

c would be constant in a precisely controlled chemostat system.

In this analysis, the specific growth rate of phytoplankton (k, in units of time^{-1}) is set equal at all dilution levels. It is assumed that added nutrients and comparable light regimes in all dilution treatments support an equal k in all the treatments during incubation. In this paper, the term specific growth rate of phytoplankton (k) is equivalent to "growth rate" of Landry and Hassett (1982).

Equations 1, 4 and 5 are used to analyze g, apparent growth rate (μ) and I for each section: g is defined by Eq. 1. μ is defined as:

$$\mu = k - g. \tag{4}$$

I is defined after Conover and Huntley (1980) as:

$$I = cP. \tag{5}$$

The central assumption of this analysis is that in a dilution experiment, P and Z are reduced at each dilution level D to $P_{(0,1)}D$ and Z_1D, respectively. The reduction of P is assumed to result in the grazers modifying their clearance rate. Figure 3 shows the assumed dependency of instantaneous values of c on D.

Development of growth equations

Equations describing the growth of the biomass of phytoplankton (P) are developed for each section as specific solutions of differential equations. The starting point of this analysis is the basic equation that describes the dynamic rate of change of P ($\mathrm{d}P/\mathrm{d}t$, Eq. 6), as introduced by Harvey (1937):

$$\frac{\mathrm{d}P}{\mathrm{d}t} = (k - g)P. \tag{6}$$

Specific differential equations describing the dynamic rate of change of P ($\mathrm{d}P/\mathrm{d}t$) are defined for each section (I–IV) by taking into account the above specific relationships between c and P, and assuming one population of Z:

Section I: c is zero, so we have

$$\frac{\mathrm{d}P}{\mathrm{d}t} = kP. \tag{7}$$

Section II: the relationship is $c = \underline{a}(P - P_{\mathrm{th}})$, so $g = \underline{a}(P - P_{\mathrm{th}})Z$, and we have

$$\frac{\mathrm{d}P}{\mathrm{d}t} = P[k - \underline{a}(P - P_{\mathrm{th}})Z]. \tag{8}$$

Section III: the relationship is $c = c_{\max}$, so $g = c_{\max} Z$, and we have

$$\frac{\mathrm{d}P}{\mathrm{d}t} = P(k - c_{\max}Z). \tag{9}$$

Section IV: the microzooplankton feed at $I_{\max}$, so $c = I_{\max}P^{-1}$, $g = I_{\max}P^{-1} Z$, and we have:

$$\frac{\mathrm{d}P}{\mathrm{d}t} = kP - I_{\max}Z. \tag{10}$$

For this analysis, I assume that Z does not vary with time ($\mathrm{d}Z/\mathrm{d}t = 0$, see comments below).

The above differential equations (Eqs. 7, 8, 9, 10) can be analytically solved to yield as specific solutions, for $t = 0$ $P_{(t,D)} = P_{(0,D)}$, the below equations which describe the growth of phytoplankton biomass:

Section I:

$$P_{(t,D)} = P_{(0,D)}\exp(k, t). \tag{11}$$

Section II:

$$P_{(t,D)} = \frac{P_{(0,D)}(k + \underline{a}P_{\mathrm{th}}Z_D)}{\underline{a}P_{(0,D)}Z_D + \left(k + \underline{a}P_{\mathrm{th}}Z_D - \underline{a}P_{(0,D)}Z_D\right)\exp(-kt)}. \tag{12}$$

Section III:

$$P_{(t,D)} = P_{(0,D)}\exp[(k - c_{\max}Z_D)t]. \tag{13}$$

Section IV:

$$P_{(t,D)} = P_{(0,D)}\exp(k\,t) + I_{\max}Z_D k^{-1}[1 - \exp(k\,t)]. \tag{14}$$

Conclusions of the analysis

There would be no other option than to subdivide the whole dilution interval into the above indicated four sections, should the dilution method be analyzed in the most appropriate way. Figure 3 shows calculated dependencies of c, Z, P, g, μ and I (initial instantaneous values at, let say, $t = 0$) on D. The instantaneous plot of c at $t = 0$, as a starting point, is according to the above relationships between c and P made for Sections I –IV, the plots of Z and P (the latter being initial instantaneous values at $t = 0$) are calculated with $Z_D = D\,Z_1$ and $P_D = D\,P_1$, respectively, g (instantaneous values at $t = 0$) is calculated with Eq. 1, μ (instantaneous values at $t = 0$) is calculated with Eq. 4), and I (instantaneous values at $t = 0$) is calculated with Eq. 5. In Fig. 3, k is assumed to be constant.

Conclusions that can be pointed out with regard to an instantaneous D dependency (at $t = 0$) are (Table 2; Figs. 3, 4): the initial ($t = 0$) undiluted instant grazing rate ($g_{(0,1)}$) in Section IV is $g_{(0,1)} = I_{\max}P_{(0,1)}^{-1}Z_1$ while the initial instant grazing rate at any other D within this section ($g_{(0,D)}$) is $g_{(0,D)} = I_{\max}(P_{(0,1)}D)^{-1}\,(Z_1D) = g_{(0,1)}$. This equality shows that in Section IV, $g_{(0,D)}$ does not

Table 2 Initial clearance rate ($c_{(0,D)}$), initial instant grazing rate ($g_{(0,D)}$) and initial ingestion rate ($I_{(0,D)}$) as functions of dilution D for Sections II, III and IV

	Section II	Section III	Section IV
Clearance ($c_{(0,D)}$)	$= \underline{a}(P_{(0,D)} - P_{\text{th}})$	$= c_{\max}$ = constant	$= I_{\max}\ P^{-1}_{(0,D)}$
Rate	$= \underline{a}(P_{(0,1)}D - P_{\text{th}})$		$= I_{\max}\ (P_{(0,1)}D)^{-1}$
Grazing ($g_{(0,D)}$)	$= c_{(0,D)}Z_D$	$= c_{\max}Z_D$	$= c_{(0,D)}Z_D$ = constant
Rate	$= \underline{a}(P_{(0,1)}Z_1D^2 - P_{\text{th}}Z_1D)$	$= c_{\max}Z_1D$	$= I_{\max}\ (Z_1D)\ (P_{(0,1)}D)^{-1}$
Ingestion ($I_{(0,D)}$)	$= c_{(0,D)}P_{(0,D)}$	$= c_{\max}P_{(0,D)}$	$= c_{(0,D)}P_{(0,D)} = c_{(0,D)}P_{(0,1)}D$
Rate	$= \underline{a}(P^2_{(0,1)}D^2 - P_{\text{th}}P_{(0,1)}D)$	$= c_{\max}P_{(0,1)}D$	$= I_{\max}$ = constant

$P_{(0,1)}$ is initial undiluted biomass and $P_{(0,D)}$ is initial diluted value. Z_1 is undiluted microzooplankton concentration, Z_{D} is the corresponding diluted value, and $c_{\max}$ is maximum clearance rate. The notation D denotes the corresponding dilution and the suffix 1 denotes the undiluted value. The symbol $\underline{a}$ denotes a linear proportionality constant (see text). For definition of Sections II, III and IV, see text

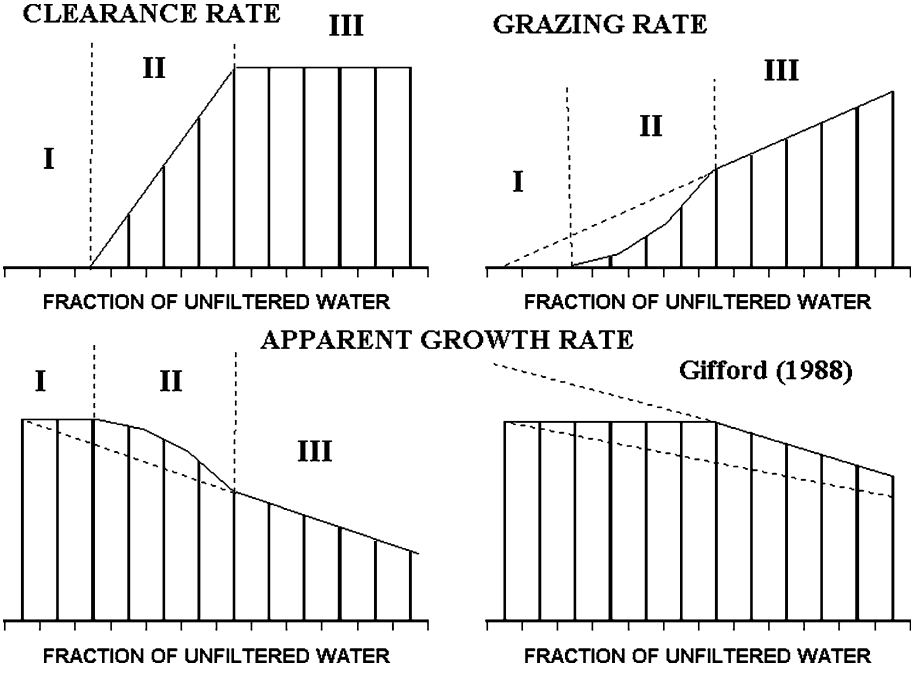

Fig. 4 As Fig. 3, but shown at the lower interval of D for clearance rate, instant grazing rate and apparent growth rate. Also is shown a redrawn figure from Gifford (1988)

depend on D. In Section III, $g_{(0,D)}$ depends on D according to $g_{(0,D)} = c_{\max}Z_1D$, in Section II, $g_{(0,D)}$ depends on D according to $g_{(0,D)} = \underline{a}[P_{(0,1)}Z_1D^2 - P_{\text{th}}Z_1D]$, and in Section I, $g_{(0,D)}$ is zero because c is zero. In Section IV, the initial apparent growth rate ($\mu_{(0,D)}$) does not depend on D because $g_{(0,D)}$ does not depend on D. In Section III, there is a linearity between $\mu_{(0,D)}$ and D according to $\mu_{(0,D)} = k - c_{\max}Z_1D$ (Figs. 3, 4). In Section II, there is a non-linear decrease of $g_{(0,D)}$ with decreasing D caused by a simultaneous decrease of Z and c, hence $\mu_{(0,D)}$ always exceeds the extrapolated linearity of Section III into Section II (Figs. 3, 4). In Section II, $\mu_{(0,D)}$ approaches asymptotically the specific growth rate of phytoplankton (k) with decreasing D. In Section I, $\mu_{(0,D)}$ is equal to k. In Section IV, the initial ingestion rate ($I_{(0,D)}$) is independent of D because of being maximal, in Section III, $I_{(0,D)}$ is linearly proportional to D, in Section II, $I_{(0,D)}$ depends on D according to $I_{(0,D)} = \underline{a}(P^2_{(0.1)}D^2 - P_{\text{th}}P_{(0.1)}\text{D})$ and in Section I, $I_{(0,D)}$ is zero. Figures 3 and 4 shows the dependencies of c, μ, g and I on D, and Table 2 provide the corresponding equations which describe these dependencies.

Conclusions that can be pointed out with regard to a time dependency are (Table 3), as long as P remains in the corresponding section over the course of time: in Section IV, $g_{(t,D)}$ varies with time because c depends on P which, in turn, varies with time (see more comments in Discussion). It can be demonstrated with the developed equation for Section IV that $P_{(t,D)} = P_{(t,1)}D$, and from this, it can be deduced that within Section IV, $g_{(t,D)}$ does not depend on D over the course of time because of the identity $g_{(t,D)} = I_{\max}P^{-1}_{(t,D)}Z_D = I_{\max}(P_{(t,1)}D)^{-1}\ (Z_1D) = I_{\max}P^{-1}_{(t,1)}Z_1 = g_{(t,1)}$. Consequently, all instantaneous values of $g_{(t,D)}$ within Section IV are always the same at every moment of the course of time. The same conclusion is also valid for $\mu_{(t,D)}$, all the instantaneous values of $\mu_{(t,D)}$ within Section IV are always the same at every moment of the course of time. In Section III, $g_{(t,D)}$ does not vary with time ($g_{(0,D)} = g_{(t,D)}$) because of the constant value of $c_{\max}$ in the relationship

Table 3 Clearance rate ($c_{(t,D)}$), instant grazing rate ($g_{(t,D)}$) and ingestion rate ($I_{(t,D)}$) as function of $c_{(t,D)}$, showing these relationships their dependencies on time (t) for Sections I, II, III and IV. The corresponding dilution is given by the notation D. The symbol $\underline{a}$ denotes a linear proportionality constant (see text)

	Section I	Section II	Section III	Section IV
Clearance rate ($c_{(t,D)}$)	0	$c_{(t,D)} = \underline{a}(P_{(t,D)} - P_{\text{th}})$	$c_{(t,D)} = c_{\max}$	$c_{(t,D)} = I_{\max}P^{-1}_{(t,D)}$
Grazing rate ($g_{(t,D)}$)	0	$g_{(t,D)} = \underline{a}(P_{(t,D)} - P_{\text{th}})Z_D$	$g_{(t,D)} = c_{\max}Z_D$	$g_{(t,D)} = I_{\max}Z_D P^{-1}_{(t,D)}$
Ingestion rate ($I_{(t,D)}$)	0	$I_{(t,D)} = \underline{a}(P^2_{(t,D)} - P_{\text{th}}P_{(t,D)})$	$I_{(t,D)} = c_{\max}P_{(t,D)}$	$I_{(t,D)} = I_{\max}$

$g_{(t,D)} = g_{(0,D)} = c_{\max}Z_1D$. Hence, $\mu_{(t,D)} = k - g_{(t,D)} = k - g_{(0,D)} = \mu_{(0,D)}$ which, in turn, means that $\mu_{(t,D)}$ only depends on D according to the relationship $\mu_{(t,D)} = k - c_{\max}Z_1D$. So, $\mu_{(t,D)}$ does not vary with time. According to the latter relationship, the extrapolated linearity from Section III into the Y_0 intercept corresponds to k. In Section II, $g_{(t,D)}$ depends on time because of the dependency of c on P (Table 3). Consequently, in Section II, $\mu_{(t,D)}$ varies with time. In Section I, $g_{(t,D)}$ is always zero because c is zero, so $\mu_{(t,D)}$ is always equal k. $I_{(t,D)}$ is constant only in Section IV because of being maximal, while in Section II and III, $I_{(t,D)}$ varies with time. In Section I, $I_{(t,D)}$ is zero. Table 3 provides the corresponding equations describing these dependencies.

As indicated above, in Sections II and IV, c varies with time, so μ varies with time which means that the instantaneous dilution plot (μ vs.D) also varies with time. Figure 5 shows a (μ vs. D) dilution plot calculated with Eqs. 11, 12, 13 and 14, and thereafter with the logarithm function $\ln(P_{(t,D)}/P_{(0,D)})/t$ which would correspond to a dilution plot estimated by a dilution experiment that has initial and final values of P. Although this calculated dilution plot (Fig. 5) is not identical with an instantaneous dilution plot, as shown in Fig. 3, the shapes of both the dilution plots are similar, both being L-shaped. A conclusion that can be pointed out here is that a L-shaped NLDP can basically be described with only the growth equations developed for Sections III ($I < I_{\max}$) and IV ($I = I_{\max}$) (Fig. 5). Sections I and II should solely be seen as theoretical ones

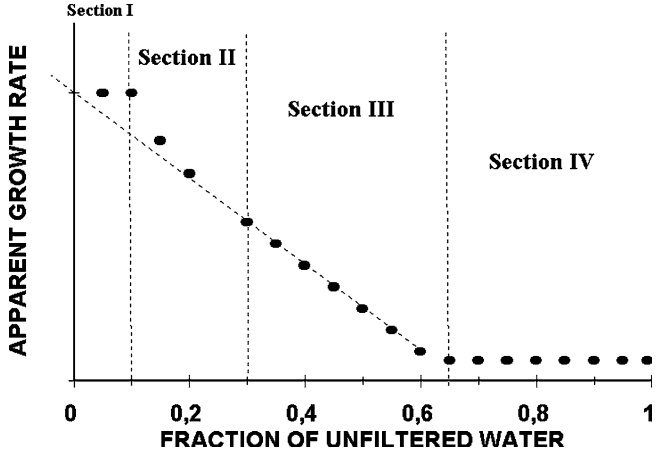

Fig. 5 Dilution plot calculated by means of Eq. 11 (Section I), Eq. 12 (Section II), Eq. 13 (Section III) and Eq. 14 (Section IV)

in dilution experiments because the chance to have a potential zero ingestion rate of grazers would presumably only occur at unusually extreme dilutions of ambient phytoplankton (see more comments in Discussion).

According to the above observations, μ and D are linearly related only in Section III. Thus, a correct value of k will only be calculated if a regression is restricted to the data stemming from this part of the curve. This remains true even when there is a threshold food concentration below which feeding ceases. The latter comment is in contradiction to the statement given by Gifford (1988), but it should be remarked that in Gifford's figure (Gifford 1988, redrawn in Fig. 4), there was an omission of the transitional interval between zero ingestion and linear plot. Consequently, in her figure, k was placed too low.

Based on the latter observations, it can be further concluded that having estimated a NLDP, the undiluted g can only be calculated as the difference of k estimated by extrapolation with μ values stemming from the linear interval of the dilution plot and undiluted μ_1. Since g in Section IV varies with time, g calculated as difference in a dilution experiment denotes an average value over the course of an incubation (see more comments in Discussion).

Comparison of predicted NLDP with estimated data

Dilution plots showing a non-linear shape, that is, with no slope further from x-origin (= NLDP) were selected as illustrative examples for the purpose of determining whether these could be better described by the non-linear model than by the linear model.

Calculation of expected dilution plots

For each selected dilution experiment, expected NLDP and LDP were calculated. Depending on where the initial biomass was ($P_{(0,D)} < P_{\text{cr}}$ or $P_{(0,D)} > P_{\text{cr}}$), the expected non-linear final biomass ($P_{(t,D)}$) was calculated for each D by using the corresponding equations for Sections III and IV [Eqs. 13, 14, respectively, or alternative equations (see below), = non-linear model]. As indicated above, a L-shaped NLDP can basically be described with these specific equations developed for Sections III and IV. Sections I and II were not considered because of the above given comments. Having

calculated for all D, the expected non-linear values of final biomass ($P_{(t,D)}$), the corresponding expected non-linear values of μ were thereafter calculated by means of the relationship ln $(P_{(t,D)}/P_{(0,D)})/t$. The expected linear values of μ (= alternative linear model) were calculated by means of the linear regression procedure between estimated μ and D. These calculated non-linear and linear μ data were thereafter compared with the estimated data of μ by the dilution experiment. A calculated squared correlation coefficient was used as the criterion to decide which model conformed better to the measured data (see below).

The expected non-linear values of $P_{(t,D)}$ [i.e. $\mathrm{Chl}_{(t,D)}$] are calculated as follows:

With microzooplankton data (Z)

$I_{\max}$, $c_{\max}$ and P_{cr} are required for the calculation of final $P_{(t,D)}$ (or $\mathrm{Chl}_{(t,D)}$) in each D with either Eq. 13 ($P_{(0,D)} < P_{\mathrm{cr}}$) or Eq. 14 ($P_{(0,D)} > P_{\mathrm{cr}}$), as indicated above. The corresponding initial value of diluted $P_{(0,D)}$ is calculated as $P_{(0,1)}$ *D. $I_{\max}$, $c_{\max}$ and P_{cr} are calculated as follows:

Maximum ingestion rate ($I_{\max}$)

Rearranging Eq. 14 to $D=1$, we get Equation to calculate $I_{\max}$ (Eq. 15):

$$I_{\max} = \left[P_{(t,1)} - P_{(0,1)}\exp(k\,t)\right]k\{Z_1[1-\exp(k\,t)]\}^{-1}. \quad (15)$$

$P_{(t,1)}$ and $P_{(0,1)}$ are the initial and final values of undiluted P estimated by the dilution experiment. k is estimated as indicated below, and Z_1 is the estimated undiluted concentration of grazers.

Maximum clearance rate ($c_{\max}$)

Instant grazing rate (g_{III}) in a dilution treatment within Section III (D_{III}) is $c_{\max}$ multiplied by the corresponding concentration of grazers Z_{III} in D_{III}($=Z_1D_{\mathrm{III}}$). Likewise, g_{III} is the difference of k and the corresponding μ_{III} in D_{III}. Combining both, we have the relationship (Eq. 16):

$$g_{\mathrm{III}} = k - \mu_{\mathrm{III}} = c_{\max}Z_1D_{\mathrm{III}}. \quad (16)$$

Rearranging Eq. 16, we get Eq. 17 to calculate $c_{\max}$:

$$c_{\max} = g_{\mathrm{III}}[Z_1D_{\mathrm{III}}]^{-1} \text{ or}_{\max} = [k-\mu_{\mathrm{III}}][Z_1D_{\mathrm{III}}]^{-1}. \quad (17)$$

Critical food concentration (P_{cr})

P_{cr} that limits Sections III and IV is the food concentration at which I becomes its maximum value ($I_{\max}$), and c begins to decrease from its maximum value ($c_{\max}$). This relationship is described by Eq. 18:

$$I_{\max} = P_{\mathrm{cr}}c_{\max}. \quad (18)$$

Rearranging Eq. 18, we get Eq. 19 to calculate P_{cr}:

$$P_{\mathrm{cr}} = I_{\max}c_{\max}^{-1}. \quad (19)$$

Without grazers' data

In those dilution experiments without indicated grazers' data, P_{cr} and $P_{(t,D)}$ can alternatively be calculated by the following equations: for the calculation of P_{cr}, we have the alternative Eq. 20:

$$P_{\mathrm{cr}} = \left[P_{(t,1)} - P_{(0,1)}\exp(k,t)\right]k\,D_{\mathrm{III}}\{g_{\mathrm{III}}[1-\exp(k\,t)]\}^{-1}. \quad (20)$$

The corresponding value of g_{III} in D_{III} is calculated from the relationship $k - \mu_{\mathrm{III}}$. Therefore, the corresponding values of μ_{III} and D_{III} can be selected from any one of the dilution levels within Section III (= linear interval of the dilution plot). $P_{(0,1)}$ and $P_{(t,1)}$ are the initial and final values of undiluted P estimated by the dilution experiment.

For the calculation of $P_{(t,D)}$ in Section III ($P_{(0,D)} < P_{\mathrm{cr}}$), we have the alternative Eq. 21:

$$P_{(t,D)} = P_{(0,D)}\exp\left[\left(k - g_{\mathrm{III}}DD_{\mathrm{III}}^{-1}\right)t\right]. \quad (21)$$

$P_{(0,D)}$ is the initial value of diluted P calculated as $P_{(0,1)}$*D, $P_{(0,1)}$ being the initial undiluted value of P estimated by the dilution experiment. For the calculation of each $P_{(t,D)}$ in Section III, g_{III} ($=k-\mu_{\mathrm{III}}$) and the corresponding D_{III} are constant values which are selected from only one dilution level in Section III.

For the calculation of $P_{(t,D)}$ in Section IV ($P_{(0,D)} > P_{\mathrm{cr}}$), we have alternative Eq. 22:

$$P_{(t,D)} = P_{(0,D)}\exp(k\,t) + \left[P_{(t,1)} - P_{(0,1)}\exp(k\,t)\right]D. \quad (22)$$

As indicated above, $P_{(0,1)}$ and $P_{(t,1)}$ are the initial and final values of undiluted P estimated by the dilution experiment, these being constant values for the calculation of each $P_{(t,D)}$ in Section IV. The initial value of diluted $P_{(0,D)}$ is calculated as $P_{(0,1)}$*D.

Specific growth rate of phytoplankton: (k)

k is in any case needed for the calculation of $P_{(t,D)}$ in either Section III or in Section IV (with or without microzooplankton data). In the below indicated Red Sea dilution experiments, k was estimated by the 3-point extrapolation procedure to the Y-axis of Gallegos (1989), using, therefore, the averages of the values of μ_D estimated in $D=0.05$ and $D=0.10$ (Fig. 6, left). In the below selected published dilution experiments, k was alternatively estimated by a similar extrapolation procedure, but achieved with all the μ values which showed to be within the linear interval of the dilution plot (Fig. 6, right).

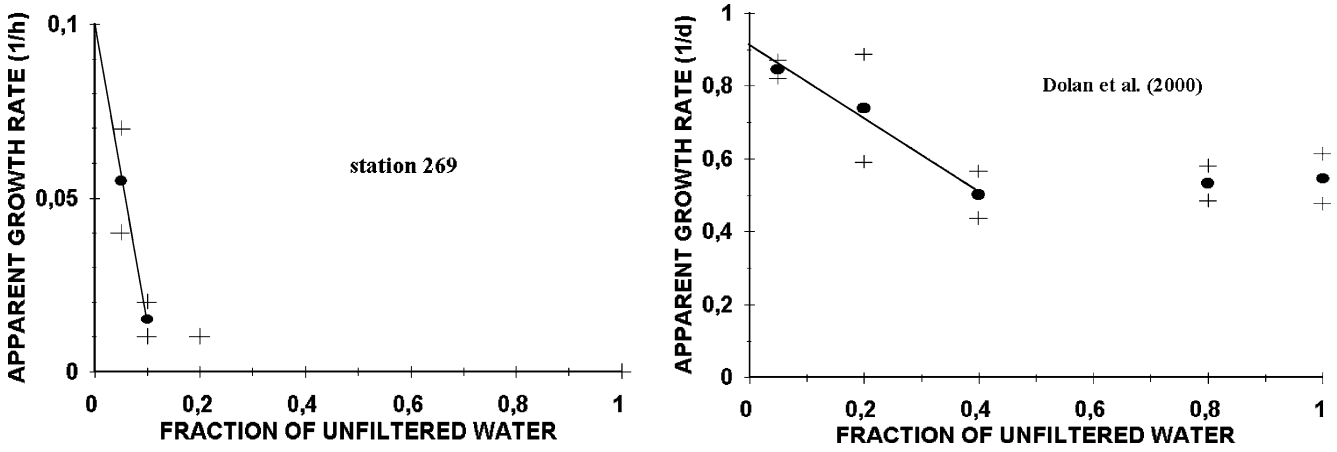

Fig. 6 *Left* 3-Point extrapolation procedure of Gallegos (1989) for estimating the specific growth rate of phytoplankton k (station 269). *Right* Extrapolation procedure for estimating k, using, therefore, all the μ data stemming from the linear part of the dilution plot (Dolan et al. 2000)

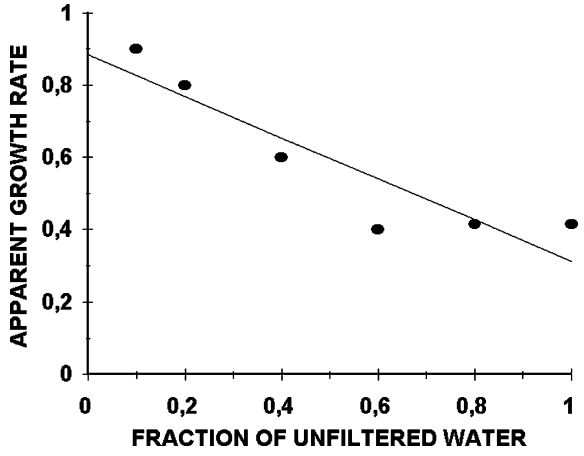

Fig. 7 A NLDP calculated with the growth equations developed for the Sections III and IV, showing a calculated significant linear correlation coefficient ($R = 0.907$), demonstrating this an error type II of accepting a false linear hypothesis

Statistics

Squared correlation coefficients (r_{xy}^2)

Some published dilution plots which show a non-linear shape (e.g., see references below) were evaluated by only considering the linear model. The calculated statistical linear tests always showed to be significant. However, L-shaped NLDP can also have a linear significance (Fig. 7). Table 4 shows, as illustrative examples, calculated linear correlation coefficients of a set of NLDP that were calculated by means of the above developed equations. These dilution plots (not all shown in Fig. 7) were calculated in such a way that they had a decreasing D_{cr}, so that the degree of the non-linear L-shape could be increased. In almost all of the cases, the linear correlation coefficients are significantly different from zero, even when $D_{cr} = 0.40$. Further calculations also show that the chance to obtain a significance increases with increasing sample size (N) (Table 4), which implies that a linear significance is not so forbidding to be calculated. N has only to be increased. This is a fundamental problem with the traditional significance test, and is one of the reasons why some statisticians advocate the use of interval test. Hence, assuming that the non-linear hypothesis were true, a linear test would show a high error type II, that is, the probability to accept a false hypothesis, which in this case would be the linear model. This demonstrates that a statistical linear test would be worthless if an alternative non-linear hypothesis were not also tested.

Until date no test exists to evaluate a NLDP. Thus, dilution data are tested by first calculating the "square of the standard error of estimate" (= index of error) according to Welkowitz et al. (1988). Therefore, the

Table 4 Calculated linear correlation coefficients (R) of hypothetical NLDP (Fig. 7)

N	D_{cr}	R	s (%)	$R_{5\%}$	$R_{1\%}$
10	0.80	0.958	1	0.632	0.765
10	0.70	0.907	1	0.632	0.765
10	0.50	0.753	5	0.632	0.765
10	0.40	0.659	5	0.632	0.765
6	0.80	0.965	1	0.811	0.917
6	0.70	0.912	5	0.811	0.917
6	0.50	0.782	NS	0.811	0.917
6	0.40	0.783	NS	0.811	0.917

D_{cr} is the critical dilution level, s is the criterion of significance of R, N is the sample size, and $R_{5\%}$ and $R_{1\%}$ are the respective 5 a-nd 1% table values of R, *NS* not significant

linear and non-linear models are stated as alternative hypothesis, and LDP and NLDP are calculated as outlined above. Having calculated the expected linear and non-linear data, the corresponding values of index of error are first calculated by means of Eq. 23 (Welkowitz et al. 1988):

$$\text{Index of error} = \sigma^2_{y\prime} = \sum \frac{(Y - Y')^2}{N}, \tag{23}$$

where Y is estimated μ, Y' expected μ calculated by a model and N sample size. All the individual estimated μ in each D should be used in this calculation if the topic variance for each D is to be taken into account.

The lowest value of $\sigma^2{}_{y\prime}$, could already be used as the criterion for accepting a model as the most appropriate. Nonetheless, having a calculated value of $\sigma^2{}_{y\prime}$, the squared correlation coefficients (r^2_{xy}, Eq. 24) can be calculated as the definitive test value for both models according to Welkowitz et al. (1988):

$$r^2_{xy} = \frac{\left(\sigma^2_y - \sigma^2_{y\prime}\right)}{\sigma^2_y}, \tag{24}$$

where σ^2_y is the variance of all estimated Y values (square of the standard deviation of estimated Y). The model with the highest r^2_{xy} is used as the criterion for accepting it as the most appropriate. It is worth pointing out that r^2_{xy} calculated for the linear model is identical to the usual square of the linear correlation coefficient.

Case studies of assessment of dilution plots

Case studies: Red Sea and Gulf of Aden

Dilution experiments were made in open waters of the Red Sea and Gulf of Aden. The areas of study are located within the confined waters of Sudan in the Red Sea and within the territorial waters of (formerly South) Yemen in the Gulf of Aden (Table 5). The experiments took place during the MINDIK cruise in February/March 1987 (Lenz et al. 1988; Nellen et al. 1996).

Surface water used for the dilution experiments was taken alongside the research vessel with a bucket. The following dilution levels were selected: 1.00 (one bottle), 0.20 (one bottle), 0.10 (two bottles) and 0.05 (two bottles). These values were chosen according to previous experiments made in the MINDIK cruise. The incubations were carried out in 2.2 dm^3 glass bottles. Filtered sea water was prepared with Whatman GF/F filters. Filtrate which was filtered again with GF/F filters showed no fluorescence. No nutrients were added. Chlorophyll a (Chl) was selected as a biomass indicator of phytoplankton. The bottles were placed in a water bath located on deck, and were cooled with running surface water. After the incubation, a volume of 1 dm^3 taken twice from each of the 1.00 and 0.20 incubation bottles was filtered and the average was calculated. A higher volume of 2 dm^3 taken from each of the 0.10 and 0.05 bottles was filtered so that the chlorophyll measurement made with a Turner 112 fluorometer could reach its detection (using the 30× window opening). The fluorometer was calibrated with a chlorophyll a standard (Sigma) according to the method of Parsons et al. (1985). Chlorophyll was calculated according to Parsons et al. (1985). GF/F filters were used. The data of μ were reported by Lenz et al. (1988). $Chl_{(0,1)}$ was determined, from case to case, two to five times and an average value was calculated. μ_D was calculated with Eq. 25:

$$\mu_D = \frac{\ln\left(Chl_{(t,D)}/Chl_{(0,D)}\right)}{t}, \tag{25}$$

where $Chl_{(0,D)}$ and $Chl_{(t,D)}$ are initial and final chlorophylls at dilution level D, respectively, and t the incubation time. The incubation times were between 2.8 and 4.1 h (Table 6). Equations 20, 21 and 22 were used to calculate the expected NLDP.

Surface temperature and geographical position of each station (Table 5) were taken from the standard station protocol of the German research vessel Meteor.

Case studies: Rhode River Estuary, Chesapeake Bay (USA)

Two dilution experiments which were made within the compound of the Smithsonian Environmental Research Center (= SERC experiments) located at the Rhode River Estuary, a subestuary of the Chesapeake Bay in Maryland (USA), are presented as case studies. The corresponding protocols of these experiments are in Dolan et al. (2000) and Moigis and Gocke (2003). The first SERC dilution experiment has been previously published as the second referred dilution experiment of

Table 5 Location and date of the stations in the Red Sea and Gulf of Aden, and temperature as °C

Station	Date (1987)	Latitude N	Longitude E	Temperature
Sudan				
130	16 Feb	21°19.9′	37°50.8′	25.0
133	17 Feb	19°37.4′	37°15.3′	26.9
137	18 Feb	19°59.2′	38°27.6′	26.1
143	19 Feb	19°58.0′	38°07.6′	26.1
165	22 Feb	19°03.2′	39°07.4′	26.4
Gulf of Aden				
269	13 Mar	13°09.8′	47°05.4′	26.4

Table 6 Specific growth rate of phytoplankton (k), initial undiluted chlorophyll ($Chl_{(0,1)}$), critical chlorophyll concentration (Chl_{cr}) calculated with Eq. 22, critical dilution level (D_{cr}) and incubation time at the indicated stations in the Red Sea and Gulf of Aden

Station	Specific growth rate	$Chl_{(0,1)}$	Chl_{cr}	D_{cr}	Incubation time	% <2 μm
130	0.32	0.106	0.016	0.150	2.8	–
133	0.26	0.213	0.022	0.103	3.0	–
137	0.38	0.086	0.009	0.105	3.7	85
143	0.17	0.123	0.042	0.341	3.7	–
165	0.16	0.089	0.008	0.090	3.3	84
269	0.10	0.109	0.013	0.119	4.1	73
Mean	0.23	0.121	0.018	0.151	3.4	80
Standard Deviation	0.10	0.047	0.013	0.095	0.5	7

k is given in unit of h^{-1}, the incubation time is given in unit of h, $Chl_{(0,1)}$ and Chl_{cr} are given in units of μg l^{-1}, and D_{cr} is without units given as fraction of unfiltered water. The last column gives the values of the contribution of the picoplankton size fraction (<2 μm) to total chlorophyll (from Lenz et al. 1988)

Dolan et al. (2000), and it will be referred to as the Dolan et al. (2000) experiment. The concentration of ciliates (in units of individuals per liter) reported in Table 2 of Dolan et al. (2000, see comments below) was selected as microzooplankton data for the evaluation of this dilution plot. The expected NLDP was calculated by using Eqs. 13 and 14. The second SERC dilution has been previously published as the R dilution experiment

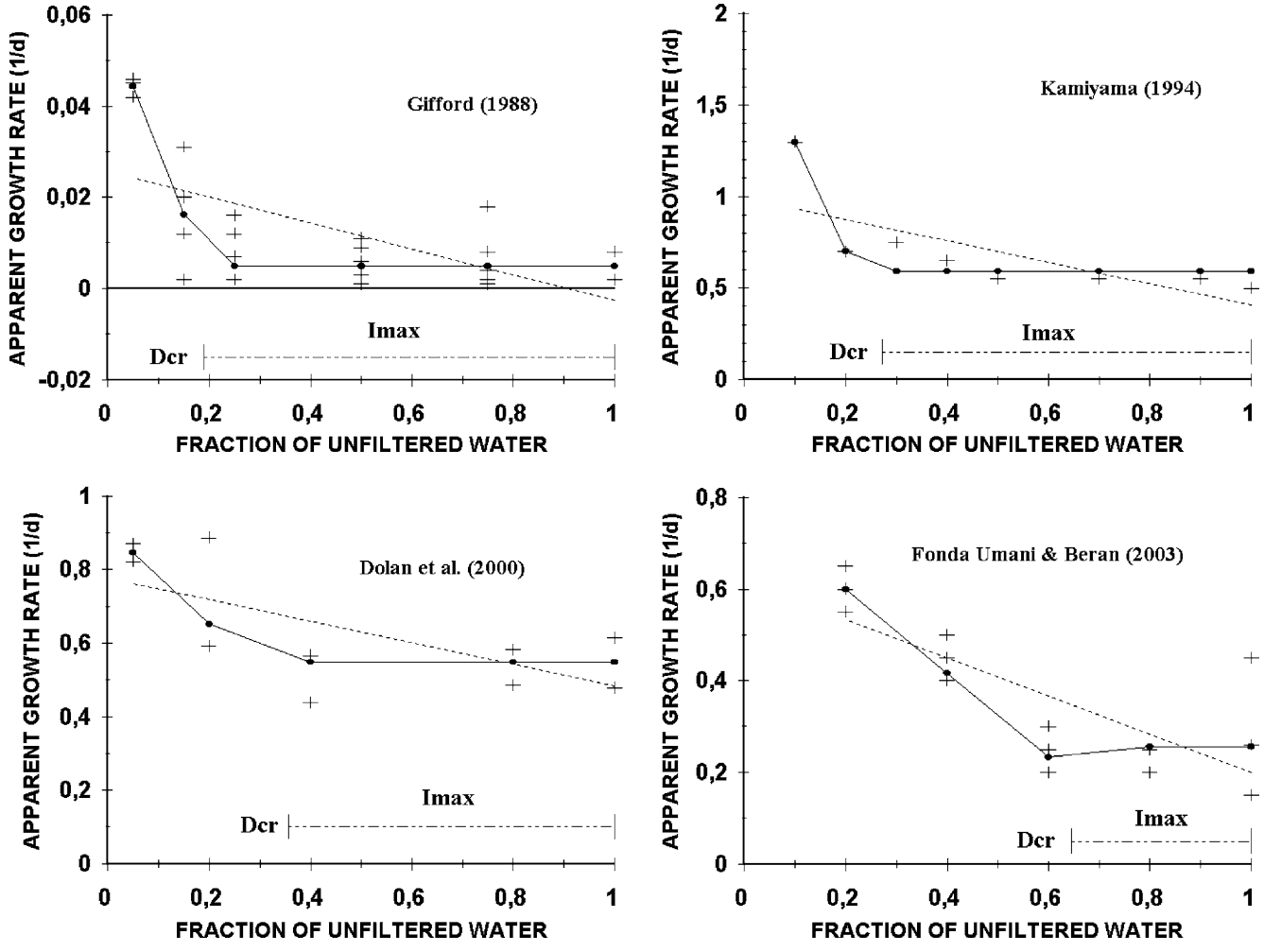

Fig. 8 Dilution plots of published experiments. With the exception of the experiment of Dolan et al. (2000), these plots were redrawn from the indicated sources. The individual values of estimated apparent growth rate (μ_D) are shown by the symbol +. The *dotted line* is the calculated linear model, *full circles* are calculated values of μ according to the non-linear model, the *continuous line* merely connects these calculated μ. D_{cr} is the calculated critical dilution level. The dilution interval with the maximum ingestion rate of microzooplankton (I_{max}) is shown as *horizontal line*

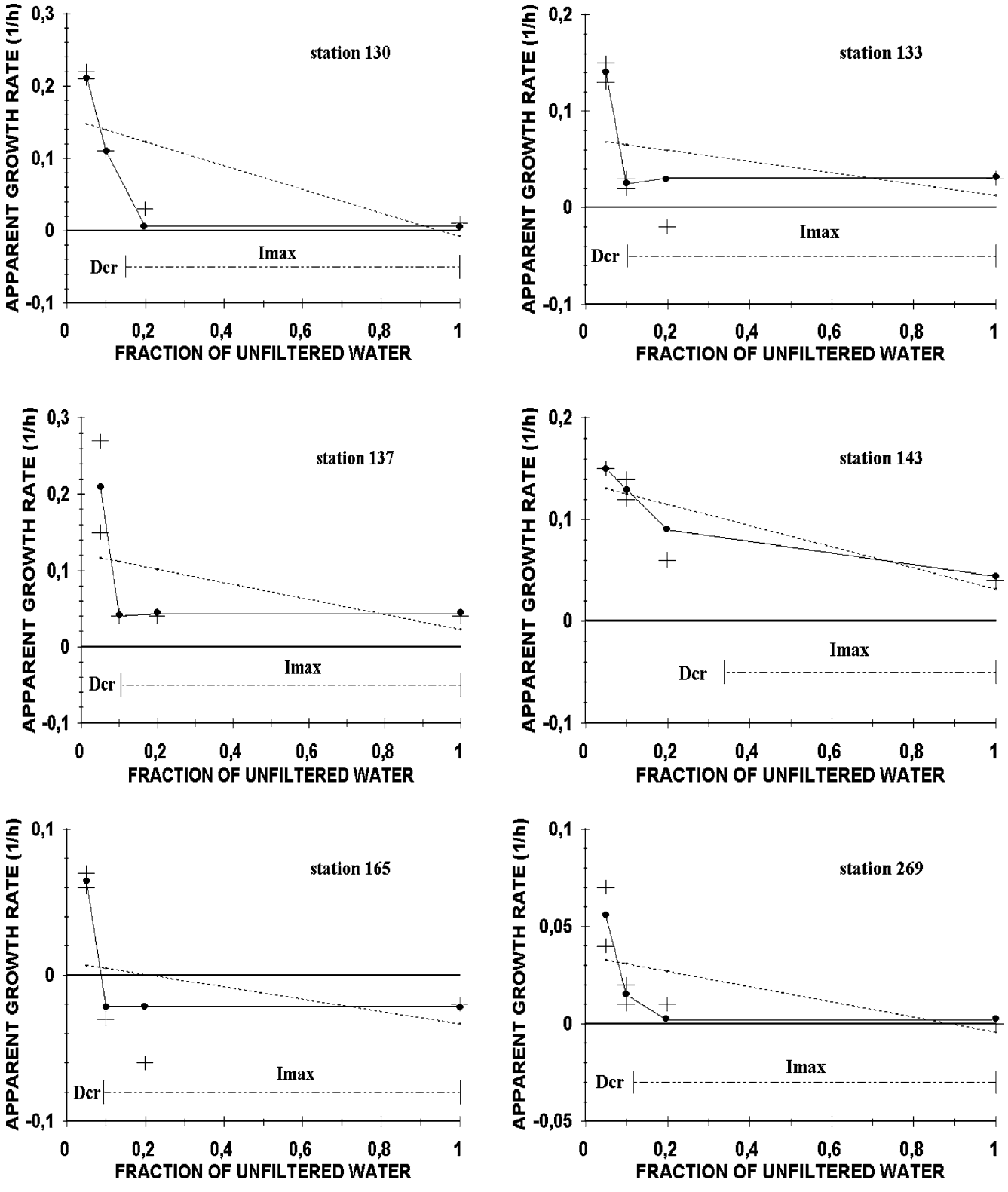

Fig. 9 Dilution plots of experiments made in the Red Sea and Gulf of Aden. The individual values of estimated apparent growth rate (μ_D) are shown by the symbol +. The *dotted line* is the calculated linear model, *full circles* are values of μ calculated according to the non-linear model, the *continuous line* merely connects these calculated μ. D_{cr} is the calculated critical dilution level. The dilution interval with the maximum ingestion rate of microzooplankton (I_{max}) is shown as a *horizontal line*. The dilution interval with the maximum ingestion rate of microzooplankton (I_{max}) is shown as a *horizontal line*

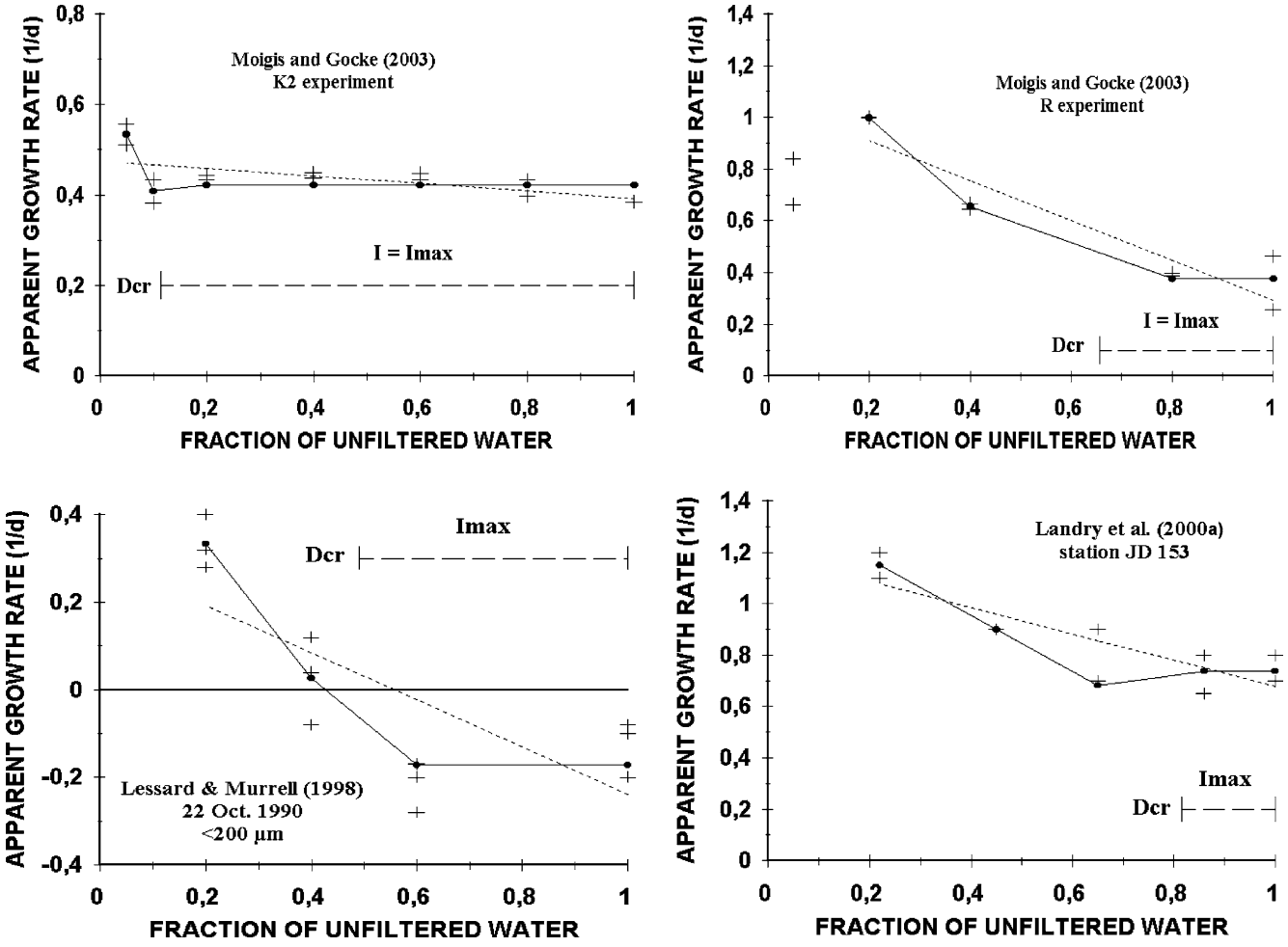

Fig. 10 Dilution plots without grazers' data. The upper plots were redrawn from the indicated sources by using original experimental data, the lower plots were redrawn from the indicated publications. The individual values of estimated apparent growth rate (μ_D) are shown by the symbol +. The *dotted line* is the calculated linear model, *full circles* are calculated values of μ according to the non-linear model, the *continuous line* merely connects these calculated μ. D_{cr} is the calculated critical dilution level. The dilution interval with the maximum ingestion rate of microzooplankton (I_{max}) is shown as *horizontal line*

of Moigis and Gocke (2003), and this plot will be referred to as the Moigis and Gocke R-experiment. In this experiment, no microzooplankton data were estimated. Accordingly, the expected NLDP was calculated by using Eqs. 20, 21 and 22. In both dilution experiments, an additional filtered water sample was incubated as well. To this filtered water sample, the same amounts of nutrients were added as to the water samples of the dilution set. The chlorophyll concentration of the filtered water sample estimated at the end of the incubation was used to correct the measured final chlorophyll values of the dilution set. This correction was made according to Moigis and Gocke (2003). Hence, the procedure of evaluation of the first SERC dilution plot

Table 7 Source of selected dilution experiments and reported microzooplankton data. The indicated figures and tables are the corresponding ones in the indicated sources. Corresponding units of reported microzooplankton data are given in Table 10

Source	Reported dilution experiment	Source of reported dilution experiment	Source of reported zooplankton data
Gifford (1988)	March 11, 1985	Fig. 2	Table 2
Kamiyama (1994)	April 17, 1991 (station 5, unfractioned seawater)	Fig. 9	Fig. 6
Lessard and Murrell (1998)	October 22, 1990	Fig. 2	–
Landry et al. (2000a)	Station JD 153	Fig. 2	–
Fonda-Umani and Beran (2003)	May 1999 (site C1, total phytoplankton)	Fig. 2	Tables 3 or 4

was different from the one indicated by Dolan et al. (2000). These dilution plots were drawn based on our own experimental data (Figs. 8, 10).

Case study: Kiel Fjord, Baltic Sea (Germany)

The K2 dilution experiment of Moigis and Gocke (2003) made in the Kiel Fjord in Germany was selected as an additional case in order to demonstrate the evaluation without having any microzooplankton data, and it will be referred to as the Moigis and Gocke K2-experiment (K for Kiel). The protocol of this dilution experiment is described in detail in Moigis and Gocke (2003). Accordingly, the evaluation of this dilution plot was made by using Eqs. 20, 21 and 22. A filtered water sample with the same added amount of nutrients as given to the dilution set was also incubated, and the final estimated chlorophyll value was used to correct the estimated final chlorophyll values of the dilution set. This correction was made according to Moigis and Gocke (2003). This dilution plot was drawn based on our own experimental data (Fig. 10).

Case studies: published dilution plots selected from the literature

It can be observed in the literature that some published dilution plots which showed a typical non-linear shape (L-shaped) were solely evaluated by the traditional linear regression procedure. Some of these dilution plots were selected at random as additional case studies to demonstrate how these can alternatively be described better by the non-linear model. Dilution plots were taken from Gifford (1988), Kamiyama (1994), Lessard and Murrell (1998), Landry et al. (2000a) and Fonda-Umani and Beran (2003). The protocols of these dilution experiments are in the corresponding publication therein. These published dilution plots were redrawn as best as possible in the Figs. 8 and 10. Expected NLDP and LDP were calculated and compared with the estimated data. With exception of Lessard and Murrell (1998) and Landry et al. (2000a), data of microzooplankton reported in these publications allowed the calculation of I_{max}, c_{max} and P_{cr}. Having these parameters, the expected NLDP could thereafter be calculated with Eqs. 13 and 14. Even when some of the selected data of microzooplankton were seen as non-representative grazers, the use of these data should only demonstrate, as illustrative examples, the possibility to calculate additional feeding parameters of microzooplankton. The use of I_{max} and c_{max} does not have any influence on the calculation of the expected NLDP made with Eqs. 13 and 14 because the same expected NLDP would likewise be calculated without any microzooplankton data by using the alternative Eqs. 20, 21 and 22. These latter equations were used for the evaluation of the dilution plots of Lessard and Murrell (1998) and Landry et al. (2000a).

Table 7 shows the selected dilution experiments with its corresponding sources as well as the sources of selected microzooplankton data. With the exception of Fonda-Umani and Beran (2003), microzooplankton data were always reported in units of individuals per liter. Fonda-Umani and Beran (2003) reported their microzooplankton data in units of µg C-biomass per liter. Their reported phytoplankton biomass in units of µg C l^{-1} was used for the calculation of I_{max} and c_{max}. In almost of the selected published dilution experiments, initial values of undiluted chlorophyll ($Chl_{(0,1)}$) or biomass of phytoplankton ($P_{(0,1)}$) were taken from the respective table or figure. For the dilution experiment JD153 of Landry et al. (2000a) which was made during IronExII, the specific chlorophyll data was taken from the Fig. 4 of Landry et al. (2000b), $Chl_{(0,1)}$ being ~ 0.5 µg l^{-1}.

Corresponding undiluted final values of chlorophyll ($Chl_{(t,1)}$) or biomass ($P_{(0,1)}$) needed for the calculations were calculated afterwards with the relationship $Chl_{(t,1)} = Chl_{(0,1)}\exp(\mu t)$ or $P_{(t,1)} = P_{(0,1)}\exp(\mu t)$, where μ is the average of those μ values which were likewise to be in the constant interval adjacent to the undiluted step of the dilution plot indicated in the reference.

Results

Case studies: Red Sea and Gulf of Aden

Figure 9 shows the dilution plots of the experiments made in the Red Sea and Gulf of Aden. All these dilution plots had a non-linear shape, values of μ adjacent to undiluted μ_1 corresponded to μ_1. Only increased values of μ could be detected in $D = 0.10$ and $D = 0.05$. Values of μ in $D = 0.2$ showed to be similar to μ_1. The calculated one-tailed t test showed that the means of undiluted μ and μ in $D = 0.2$ were not significantly different ($t = -0.33$, t table value 5% = 2.228, $df = 10$).

k ranged from 0.10 to 0.38 h^{-1}, with an average of 0.23 ± 0.10 h^{-1} (Tables 6, 8). g calculated as the difference of k and μ_1 ranged between 0.10 and 0.34 h^{-1}, with an average of 0.21 ± 0.10 h^{-1} (Table 8). Chl ranged from 0.089 to 0.213 µg l^{-1}, the average value being 0.121 ± 0.047 µg l^{-1} (Table 6). The last column of Table 6 gives the contribution of the picoplankton size fraction (< 2 μm) to total chlorophyll (from Lenz et al. 1988) which ranged from 73 to 85%, the average value being 80 ± 7%. Water temperature ranged from 25.0 to 26.4°C, the mean value being 26.2 ± 0.7°C (Table 5).

The expected NLDP are shown in Fig. 9 with the corresponding calculated LDP. Table 9 shows the values of $\sigma^2_{y'}$ and r^2_{xy} calculated for the expected NLDP and LDP. The non-linear values of r^2_{xy} are always higher than the linear ones. All the non-linear values of r^2_{xy} were > 0.8.

Table 8 Specific grazing rate ($g_{(D=1)}$) calculated as the difference in specific growth rate of phytoplankton (k) and undiluted apparent growth rate (μ_1) estimated by the dilution experiment for the indicated stations in the Red Sea and indicated references

Station or reference	k	μ_1	$g_{(D=1)}$	μ_1^-	$g_{\text{in situ}}$	$k_{\text{in situ}}$
130	0.32	0.01	0.31			
133	0.26	0.04	0.22			
137	0.38	0.04	0.34			
143	0.17	0.04	0.13			
165	0.16	−0.02	0.18			
269	0.10	0.01	0.09			
Gifford (1988)	0.058	0.005	0.053	–	–	–
Kamiyama (1994)	1.90	0.50	1.40	–	–	–
Lessard and Murrell (1998)	0.64	−0.13	0.77	–	–	–
Landry et al. (2000a)	1.39	0.75	0.64	0.75	0.64	1.39
Dolan et. al. (2000)	0.91	0.55	0.36	−0.12	0.44	0.32
Fonda-Umani and Beran (2003)	0.78	0.29	0.49	–	–	–
Moigis and Gocke (2003) (R experiment)	1.34	0.37	0.97	−0.09	1.23	1.14
Moigis and Gocke (2003) (K2 experiment)	0.66	0.39	0.27	0.41	0.27	0.68

μ_1^- is undiluted apparent growth rate without added nutrients, $g_{\text{in situ}}$ is in situ grazing rate (without added nutrients) calculated with Eq. 30, $k_{\text{in situ}}$ is in situ specific growth rate of phytoplankton. Specific growth rate of phytoplankton and instant grazing rate are given in unit of h^{-1} for the Red Sea and Gifford (1988) experiments, and in unit of day^{-1} for the rest

Case studies: selected dilution plots (SERC-Maryland, IfM-Kiel and published ones)

Figure 8 shows the NLDP redrawn from the selected published dilution plots with reported microzooplankton data (Gifford 1988; Kamiyama 1994; Dolan et al. 2000; Fonda-Umani and Beran 2003). The calculated expected NLDP shown in Fig. 8 fitted well to the estimated data. The corresponding calculated values of k, D_{cr}, Chl_{cr}, $I_{\max}$ and $c_{\max}$ are shown in Table 10. Figure 8 shows that the calculated values of D_{cr} fitted well to the dilution intervals where the estimated μ began to increase. Even when all the calculated linearities in Fig. 8 were significant, the calculated values of non-linear r^2_{xy} were always higher than the linear ones (Table 9).

Figure 10 shows the NLDP redrawn from Lessard and Murrell (1998), Landry et al. (2000a) and Moigis and Gocke (2003, K2 and R experiments). Because no specific data of grazers were reported for these dilution experiments in their respective papers (or elsewhere), $c_{\max}$ and $I_{\max}$ could not be calculated. Figure 10 shows the calculated expected LDP and NLDP. The latter figure also shows that the calculated values of D_{cr} fitted well to the dilution intervals where the estimated μ began to increase. The corresponding calculated values of k, D_{cr} and Chl_{cr} are shown in Table 10. In all these cases, the calculated values of non-linear r^2_{xy} were higher than the linear ones (Table 9).

For all NLDP, g calculated as the difference of k and μ_1 are shown in Table 8.

Discussion

Linear dilution plots and NLDP have to be seen as the two extreme cases of possible dilution plots which can be estimated by dilution experiments. LDP will only be estimated when one or more populations of grazers with non-saturated feeding have a dominant role in the community grazing impact. The main conclusion that

Table 9 Square of index of error ($\sigma^2_{y'}\times1{,}000$) and square of correlation coefficient (r^2_{xy}) calculated for the linear and non-linear models

Station number (Red Sea) or reference	$\sigma^2_{y'}$		r^2_{xy}	
	Linear	Non-linear	Linear	Non-linear
130	3.31	0.10	0.48	0.98
133	3.40	0.48	0.10	0.87
137	6.50	1.20	0.15	0.84
143	0.68	0.18	0.65	0.90
165	2.17	0.28	0.09	0.88
269	0.38	0.10	0.32	0.83
Gifford (1988)	0.12	0.04	0.37	0.79
Kamiyama (1994)	133.60	4.68	0.53	0.91
Lessard and Murrell (1998)	21.84	4.55	0.54	0.91
Dolan et. al. (2000)	13.92	8.71	0.44	0.65
Landry et al. (2000)	7.70	6.94	0.72	0.75
Fonda-Umani and Beran (2003)	10.42	5.09	0.58	0.79
Moigis and Gocke (2003) (R experiment)	9.03	2.87	0.87	0.96
Moigis and Gocke (2003) (K2 experiment)	1.39	0.59	0.35	0.72

All the linear correlation coefficients calculated for the Red Sea dilution experiments are non significant, while all the corresponding ones calculated for the dilution experiments reported in the below references therein are significant

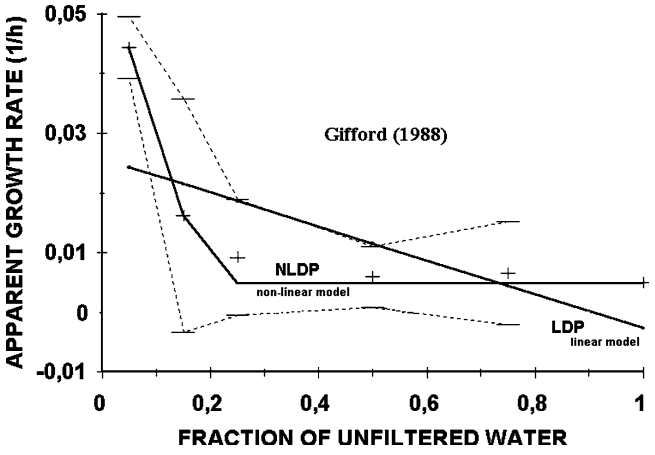

Fig. 11 Confidence interval (5%) for the population mean of estimated apparent growth rate (μ) (*horizontal lines*) calculated in each $D<1$ of the experiment of Gifford (1988). The upper and lower confident limits (*horizontal lines*) are simply connected by *dotted lines*. The cross denotes the average value of μ in each D. NLDP and LDP are the calculated non-linear and linear dilution plots, respectively

can be made for estimated LDP is that the whole grazers' community has non-saturated feeding. In the other extreme case, NLDP will only be estimated when one or more populations of grazers with saturated feeding have a dominant role in the community grazing impact. A NLDP is based solely on saturated feeding of the whole grazers' community. In any case, LDP and NLDP should be contemplated as two possible alternative dilution data which have a chance to be estimated by a dilution experiment.

The present comparisons between predicted and estimated data show that a model which is primarily based on a food depending clearance rate of grazers that, in turn, reflects saturated and non-saturated feeding, fits better to the above specific estimated NLDP than the traditional linear model. The criteria for the statement of better fit is the higher calculated r^2_{xy} of the non-linear model than the corresponding linear one. Furthermore, Fig. 11 shows the 5% confidence intervals for the population mean of estimated μ ($x \pm t_{(N\text{-}1),\alpha/2}s/N^{1/2}$, where x is sample mean, $t_{(N\text{-}1),\alpha/2}$ the corresponding t value, s sample standard deviation and N sample size, respectively) which could be calculated for each D (with exception of $D=1$, where $N=2$) of the dilution experiment of Gifford (1988). All the non-linear μ values calculated by the non-linear model are within the corresponding confidence intervals of the estimated data, whereas two calculated linear μ values are outside of the corresponding confidence intervals. This is an additional argument for accepting the non-linear model as the more adequate one for this specific dilution plot. Statistically stating, in two cases of D ($D=0.05$ and 0.50), the upper or lower limits of the 95% confidence interval for the population mean of estimated μ could not reach these calculated linear μ. Thus, it was not likely to estimate these specific calculated linear μ values. Their probabilities to be estimated were lower than 5%.

The comparison between predicted and estimated data was especially made with selected dilution plots which showed a non-linear shape (L-shaped), that is, in particular, having no slope further from the x-origin, in addition to the steep slope near the x-origin. The above analysis shows that a NLDP does not have a slope further from the x-origin because of a constant μ in Section IV. In Section IV, μ is constant because g is constant, this constancy being a reflection of I_{max} in the identity $g_{(t,D)} = I_{max}\ (P_1D)^{-1}\ (Z_1D) = I_{max}\ P_1^{-1}\ Z_1 = g_{(t,1)}$. Thus, the non-linear model reflects a functional response curve Type I within the predator-prey dynamics (Holling 1959) which, in turn, means that there is no handling time in the food consumption

Table 10 Specific growth rate of phytoplankton (k), maximum ingestion rate (I_{max}), maximum clearance rate (c_{max}), critical chlorophyll concentration (Chl_{cr}) and critical dilution level (D_{cr}) calculated for the indicated published experiments (1–8)

Reference	K	$Chl_{(0,1)}$ or $P_{(0,1)}$*	t	I_{max}	Z	g_{III}	D_{III}	c_{max}	Chl_{cr}or P_{cr}*	D_{cr}
1	0.06	0.300	12 h	1.45	11,800	0.014	0.05	25.3	0.057	0.191
2	1.90	2	1 day	2.9	1,100	0.600	0.10	5.4	0.54	0.271
3	0.91	12.7	1 day	193	30,400	0.065	0.05	42.7	4.52	0.356
4	0.78	147*	1 day	10.3	8.46	0.183	0.20	0.108	94.8*	0.645
5	0.64	0.071	1 day	–	–	0.310	0.20	–	0.035	0.491
6	1.39	0.500	1 day	–	–	0.239	0.22	–	0.407	0.815
7	1.34	14.8	1 day	–	–	0.342	0.05	–	9.7	0.657
8	0.66	10.2	1 day	–	–	0.125	0.05	–	1.33	0.130

Z is the microzooplankton concentration, $Chl_{(0,1)}$ or $P_{(0,1)}$* is the initial undiluted chlorophyll or biomass of phytoplankton, g_{III} is the selected instant grazing rate in D_{III}. Chl (initial and critical) is given in units of $\mu g\ l^{-1}$, P*(initial and critical values which are denoted with an *, only in reference 4) is given in units of $\mu g\ C\ l^{-1}$, Z is given in the references 1, 2 and 3 in units of individuals l^{-1} and in reference 4 in units of $\mu g\ C\ l^{-1}$. I_{max} is given in reference 1 in units of pg chl individual^{-1} h^{-1}, in reference 2 in units of ng chl individual^{-1} day^{-1}, in reference 3 in units of pg chl individual^{-1} day^{-1} and in reference 4 in units of $\mu g\ C\ \mu g\ C^{-1}\ day^{-1}$. c_{max} is given in reference 1 in units of μl individual^{-1} h^{-1}, in reference 2 in units of ml individual^{-1} day^{-1}, in reference 3 in units of μl individual^{-1} day^{-1} and in reference 4 in units of $\mu l\ pg\ C^{-1}\ day^{-1}$. Reference 1 is Gifford (1988), reference 2 is Kamiyama (1994), reference 3 is Dolan et al. (2000), reference 4 is Fonda-Umani and Beran (2003), reference 5 is Lessard and Murrell (1998), reference 6 is Landry et al. (2000a), reference 7 is the R-experiment of Moigis and Gocke (2003) and reference 8 is the K2-experiment of Moigis and Gocke (2003)

(Begon et al. 1996; Cotgreave and Forseth 2002), the latter being plausible for planktonic grazers. Rigler (1961) had experimentally demonstrated that the feeding of a planktonic species like Daphnia sp. can have a functional response Type I. However, it should be pointed that there is a departure of the linearity between I and P, as prey concentration, at the lower dilution interval (Fig. 3).

Predicted data was only calculated by means of the growth equations developed for Sections III and IV since the corresponding relationships between c and P were beyond any doubt demonstrated by Frost (1972, Fig. 2). By means of these equations, it could be demonstrated that the better fit of the non-linear model to estimated dilution data reflected more a maximum ingestion rate (I_{max}) of grazers in $D = 0$.

One of the outcomes of the above analysis is that an extrapolation procedure for estimating k would only give a correct value if all the μ values belonged alone to Section III where g is only D dependent. Hence, there would be a potential systematic error by showing an overestimated k value if one μ value belonged to Section I or II. Figures 3, 4 and 5 show that values of μ in Section I and II are above those linear ones extrapolated from Section III where this linearity is restricted.

A zero clearance rate (Section I) is still controversial within the scientific community. The review of Strom et al. (2000) reported several studies which supports the existence of a threshold food concentration only for copepods (see the referred studies of Parsons et al. 1967, 1969; Frost et al. 1983; Price and Paffenhofer 1986; Paffenhofer 1988; Wlodarczyk et al. 1992 in Strom et al. 2000). However, Strom et al. (2000) also referred that there is no evidence for such a threshold concentration for protists. On the other hand, Dolan et al. (2000) and Dolan and McKeon (2004) demonstrated in an assessment of the dilution method a reduction of ciliates concentration at the highest dilution level, this reduction being indicative for zero ingestion caused by starvation. Ciliates might merely have expired at these low food concentrations, and according to the above comments, including a corresponding μ in an extrapolation procedure would overestimate k. Hence, g would be overestimated, a conclusion that would comply with the comments of Dolan and McKeon (2004). The existence of Section I could easily be demonstrated with dilution experiments made in the laboratory by having only one grazer species, and by determining whether in the lowest dilution interval the dilution plot becomes parallel to the X-axis (Figs. 3, 4, 5).

Section II, consequential of Section I and thus also controversial, could be caused by a weakness of grazers raised by very extreme low ingestion rates (Fig. 3). Perhaps, the swimming velocity of grazers which are still alive is intensely reduced by this hunger, hence, the encounter rate between grazers and phytoplankton is additionally reduced in this section.

The continuous increase of the apparent growth rate with decreasing fraction of unfiltered water which can be observed in the whole dilution range of the dilution plots of Landry and Hassett (1982) illustrates an undiluted phytoplankton concentration that was below a critical food concentration. This, in turn, indicates that the ingestion rate of microzooplankton was not maximal. It can be concluded from their LDP that the ingestion of microzooplankton was limited by the food concentration during the experiments. It should be pointed out, however, that this conclusion is not representative for coastal waters with relative high Chl values. Since Gallegos (1989), NLDP have also been estimated in similar coastal waters (e.g. McManus and Ederington-Cantrell 1992; Moigis and Gocke 2003).

On the other hand, from the evaluated experiments of Gifford (1988, made in coastal waters, $Chl_{cr} = 0.057$ µg l^{-1}, $D_{cr} = 0.191$), Lessard and Murrell (1998, made in open waters, $Chl_{cr} = 0.035$ µg l^{-1}, $D_{cr} = 0.491$), and from the Red Sea experiments made in open waters (average $Chl_{cr} = 0.018$ µg l^{-1}, average $D_{cr} = 0.151$, Table 6), the conclusion can be drawn that even though Chl has low values in either coastal or open waters, the feeding of grazers in these waters can in specific cases likewise be saturated. Saturated ingestion depends, among other things, on the nature of grazers, the size being a likely characteristic (nanozooplankton in the Red Sea and microzooplankton in coastal waters). This conclusion, however, should not be contemplated as being representative for any waters which have low Chl.

Saturated ingestion not only depends on food concentration, but also on food size (Frost 1972). The experiments of Frost (1972, see Fig. 6 therein) made with only one species of grazers showed that the larger the cell size of phytoplankton (= food) was, the lower was the critical food concentration. Consequently, the chance to estimate a NLDP increases with a bigger cell size of food. This might explain why the JD153 plot of Landry et al. (2000a) showed to be a NLDP ($D_{cr} = 0.82$). JD153 was made after the second enrichment of iron during IronEx II. During the phase of additions in the patch, there was a dramatic increase of diatoms and dinoflagellates and a modest increase of larger prymnesiophytes (Landry et al. 2000b), indicating this an increase of food size for grazers. During this phase there was also observed a dramatic increase of chlorophyll concentration, from about 0.1 to 1.7 µg l^{-1}, and phytoplankton biomass concentration, from about 25 to 120 µg carbon l^{-1} (Landry et al. 2000b). Hence, both observations (bigger food size and higher food concentration) support the estimation of NLDP which, in that case, showed to be specific for the dilution experiments made in the patch until JD153. The previous experiment JD151 also has a calculated D_{cr} value of 0.77, and the corresponding calculated non-linear r^2_{xy} is higher than the linear one (calculations not shown here). Dilution experiments made in control waters which had a lower Chl showed LDP (Landry et al. 2000a). Nevertheless, LDP which were also estimated in the patch after JD153 during the peak of the bloom (from JD 155 to JD 163) might have been caused by a development to a signifi-

cant assemblage of >40 μm protistan grazers, as ciliates and dinoflagellates (Landry et al. 2000b), indicating this a varying size composition of microzooplankton.

The standard dilution procedure of Landry and Hassett (1982) is done with a dilution interval from undiluted level to $D = 0.20$. For instance, the dilution experiments made in the Red Sea by Reckermann and Veldhuis (1997) had such a dilution interval from undiluted level to $D = 0.25$, but the present dilution experiments made by me in the Red Sea had a lower average D_{cr} of 0.151 ± 0.095. The conclusion that can be drawn from this is that when a dilution experiment is made with standard dilution levels, there is always a risk of failing to hit the dilution interval where μ begins to increase. This omission might explain why some published dilution plots reported zero or low grazing (e.g. Kamiyama 1994; Lessard and Murrell 1998; Murrell and Hollibaugh 1998; Fonda-Umani and Beran 2003).

A further conclusion of the above analysis of the dilution method is that D_{cr} could be calculated as a test value for assessing estimated LDP. Empirical calculations showed that calculated values of D_{cr} with Eq. 20 of LDP calculated with Eq. 13 would always be >1, and this invalidates the assumption made for the non-linear model which was that the undiluted level is in Section IV. A calculated $D_{cr} > 1$ would, indeed, be a contradiction. Hence, a criteria whether to accept the linear model directly could be a calculated $D_{cr} > 1$.

I have followed the common practice and assumed Z to be constant during the course of a dilution experiment. As indicated above, the clearance rate was sensitive enough to control the dilution experiments so that the Z constancy will be an adequate assumption, but Dolan et al. (2000) discuss alternatives.

Estimation of in situ grazing rate when a NLDP is measured

The above analysis showed that when a LDP is estimated, a value of g estimated by linear regression is identical with the in situ value of g ($g_{\text{in situ}} = g$ at undiluted level without added nutrients). In such a case, the undiluted level is in Section III, so c is constant (c_{max}) and g does not depend on P. However, how can $g_{\text{in situ}}$ be estimated when a NLDP is estimated, in other words, when the whole grazers community is having a saturated feeding at the undiluted level?

As previously demonstrated, in Section IV, the clearance rate ($c_{(t,D)}$) either decreases with increasing $P_{(t,D)}$ or increases with decreasing $P_{(t,D)}$, consequently the instant grazing rate ($g_{(t,D)}$) also varies over the course of time, $g_{(t,D)}$ being in this way time dependent (Fig. 12, left). Since in Section IV, $g_{(t,D)}$ varies over the course of time, the instant value of $\mu_{(t,D)}$ also varies over the course of time according to the relationship $\mu_{(t,D)} = k - g_{(t,D)}$. The above analysis also demonstrated that in Section IV, $g_{(t,D)}$ is constant with regard to D, thus $\mu_{(t,D)}$ is constant with regard to D (Fig. 12, right).

By means of empirical calculations, it can be demonstrated that a value of apparent growth rate which is calculated with the standard logarithm function $\mu_D = \ln(\text{Chl}_{(t)}/\text{Chl}_{(0)})/t$ (the subscript D refers to a value calculated with the logarithm function), corresponds to a calculated average value of μ_t which varies over the course of time (μ_{avg}), as $\mu_D = \mu_{avg}$ = average of μ_t. The same empirical calculations further show that a value of μ_{avg} corresponds to the difference of a constant k and a calculated average value of g_t which varies over the course of time (g_{avg}) according to the relationship $\mu_{avg} = k - g_{avg}$. Hence, a value of μ_D that is calculated with the above logarithm function corresponds to μ_{avg} according to the relationship $\mu_D = \mu_{avg} = k - g_{avg}$, μ_{avg} being an average of μ_t, and g_{avg} an average of g_t.

As commented above, in Section IV, $g_{(t,D)}$ is constant with regard to D, so the average value of $g_{(t,D)}$ ($= g_{avg}$) is constant with regard to D. Hence, μ_{avg} ($= k - g_{avg}$) in Section IV is constant with regard to D (Fig. 12, right continuous line), μ_{avg} being equal to any μ_D in this section. Consequently, having estimated a NLDP, the difference of a constant k and the undiluted $\mu_{(D=1)}$ which is calculated with the logarithm function $\mu_{(D=1)} = \ln(\text{Chl}_{(t,1)}/\text{Chl}_{(0,1)})/t$ ($= \mu_{avg}$) is equal to g_{avg}, according to the relationship $g_{avg} = k - \mu_{(D=1)} = k - \mu_{avg}$. As above, the value of g_{avg} calculated at $D = 1$ is constant with regard to D in Section IV.

In a dilution experiment, nutrients are added to the water samples of the dilution set with the only purpose to maintain the maximum specific growth rate of phytoplankton so that a constant value of k can be obtained. Thus, a value of grazing rate which is estimated in a NLDP as the difference of k and μ_1 must not correspond to the one in the same undiluted water sample without added nutrients. Because of the added nutrients, the value of k in the water sample with added nutrients can be higher than the corresponding value of k in the same water sample without added nutrients. If this were the case, then the apparent growth rate ($\mu = k - g$) in the water sample with added nutrients would also be higher. Hence, in such a case, P would grow faster in the water sample with added nutrients than in the water sample without added nutrients. As indicated above, c and g in Section IV depend on P, hence c and g in the water sample with added nutrients would be lower than the corresponding ones in the water sample without added nutrients. Consequently, having estimated a NLDP (with added nutrients), g_{avg} calculated as the difference of k and $\mu_{(D=1)}$ would thus underestimate the in situ value of $g_{\text{in situ}}$ ($D = 1$, without added nutrients). g_{avg} would not correspond to $g_{\text{in situ}}$.

The only common feature of both undiluted samples (with and without added nutrients) is I_{max} because of both undiluted levels being in Section IV. In an undiluted water sample in Section IV, the average grazing rate over the course of time (g_{avg}) is:

$$g_{avg} = c_{avg} Z_1, \tag{26}$$

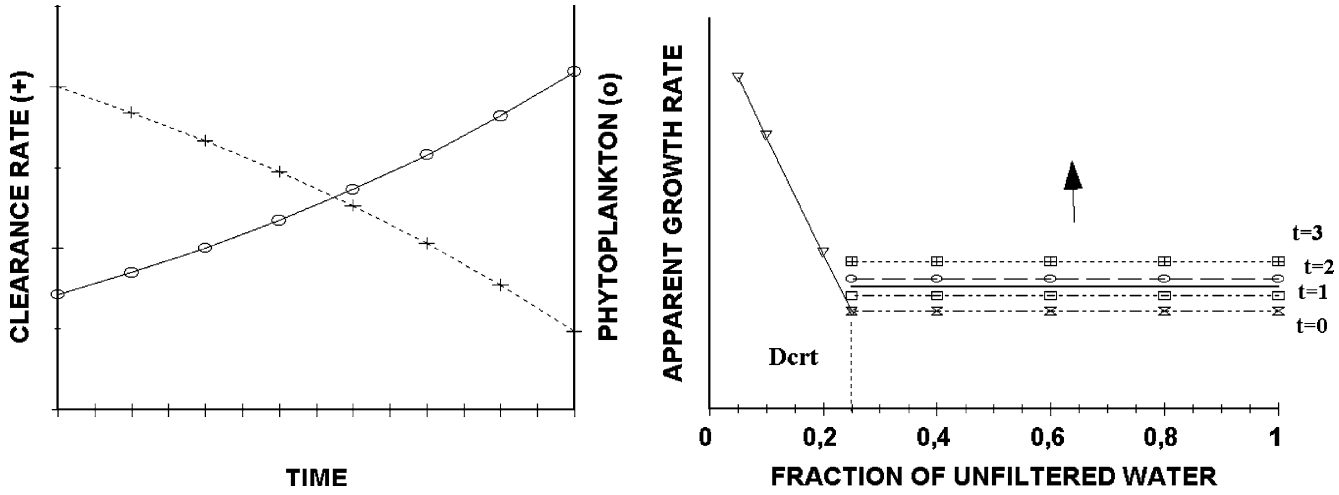

Fig. 12 *Left* Time sequence of clearance rate of microzooplankton (c, +) and phytoplankton biomass as food for microzooplankton (P, o) in Section IV. When P increases, c decreases, and so the instant grazing rate (g). *Right* Time sequence of a NLDP over the course of an incubation. In Section IV, the apparent growth rate (μ) increases with time, while in Section III, μ does not change. The *continuous line* denotes the average μ from $t=0$ to $t=3$, as it would be estimated by a dilution experiment. Both figures should clarify that an estimated value of g in Section IV corresponds to an average value of varying g that is caused by varying c which, in turn, is caused by varying P

where c_{avg} is average clearance rate over the incubation time in an undiluted water sample, and Z_1 undiluted concentration of microzooplankton. c_{avg} can be approximated by:

$$c_{\text{avg}} = I_{\max} P_{\text{avg}}^{-1}, \tag{27}$$

where P_{avg} is average concentration of phytoplankton over the course of the incubation time in an undiluted water sample. P_{avg} can be approximated by Eq. 28:

$$P_{\text{avg}} = (\mu_1 t)^{-1} P_{(0,1)} [\exp(\mu_1 t) - 1], \tag{28}$$

where μ_1 is estimated apparent growth rate at undiluted level. Combining Eqs. 26, 27 and 28 we have g_{avg} for Section IV (Eq. 29):

$$g_{\text{avg}} = I_{\max} Z_1 \mu_1 t \left\{ P_{(0,1)} [\exp(\mu_1 t) - 1] \right\}^{-1}. \tag{29}$$

Equation (29) is applicable to undiluted water samples with ($g_{\text{avg}+}$) and without added nutrients ($g_{\text{avg}-}$). From Eq. 29 we have the ratio of average grazing rate without added nutrients ($g_{\text{avg}-} = g_{\text{in situ}}$) to average grazing rate with added nutrients ($g_{\text{avg}+}$), hence the equation to calculate $g_{\text{in situ}}$ is:

$$g_{\text{in situ}} = g_{\text{avg}-} = g_{\text{avg}+} \frac{\mu_{1-} [\exp(\mu_{1+} t) - 1]}{\mu_{1+} [\exp(\mu_{1-} t) - 1]}, \tag{30}$$

where μ_{1-} and μ_{1+} are the respective estimated undiluted apparent growth rates without and with added nutrients. This development demonstrates that in a NLDP the undiluted in situ value of g without added nutrients ($g_{\text{avg}-} = g_{\text{in situ}}$) can only be calculated with Eq. 30. This equation has been previously introduced by Moigis and Gocke (2003).

Table 8 shows the values of $g_{\text{in situ}}$ of the R-experiment of Dolan et al. (2000), the R and K2 experiments of Moigis and Gocke (2003) and the experiment of Landry et al. (2000a) calculated with Eq. 30.

To properly estimate the in situ grazing rate ($g_{\text{in situ}}$), an additional undiluted water sample without added nutrients must be included within the protocol of dilution experiments. With exception of the dilution experiments of Dolan et al. (2000), Landry et al. (2000a) and Moigis and Gocke (2003) all the above published experiments were achieved without an additional or reported incubation of a water sample without added nutrients. Under such circumstances, it is impossible to retrospectively derive an estimate of $g_{\text{in situ}}$, using Eq. 30.

In situ values of k ($k_{\text{in situ}}$) can additionally be calculated with Eq. 31:

$$k_{\text{in situ}} = \mu_{1-} + g_{\text{in situ}}. \tag{31}$$

Table 8 shows values of $k_{\text{in situ}}$ estimated by the dilution experiments of Dolan et al. (2000), Landry et al. (2000a) and Moigis and Gocke (2003).

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