Recovering growth and grazing rates from nonlinear dilution experiments

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Abstract

Biological rate measurements provide critical information for understanding key processes and modeling future states of marine ecosystems. Experimentally derived rates can be challenging to interpret when methodological assumptions are untested or potentially violated under variable natural conditions, such as the assumed linear grazing response of the dilution technique for estimating rates of phytoplankton growth and microzooplankton grazing impact. Here, we show that grazing nonlinearity can be related to the ratio of initial phytoplankton biomass to the half-saturation parameter in the *Holling II* model, while not being affected by varying grazer biomass during dilution experiments. From this, we present theory to recover growth and grazing rates from multi-treatment dilution experiments with nonlinear grazing results. We test our analyses with data collected during the California Current Ecosystem-Long-Term Ecological Research (CCE-LTER) program. We show that using a linear regression in 2-treatment dilution experiments may lead to underestimates of microzooplankton grazing rates, particularly in high-phytoplankton-biomass coastal regions where grazing can be saturated. Using the *Holling II* grazing model and a correction factor, growth and grazing rates from 2-treatment experiments can also be estimated, as illustrated by application to Lagrangian water-tracking studies of growth and grazing dynamics in the CCE.

Since its introduction more than three decades ago, the dilution technique (Landry and Hassett 1982) has been a widely used experimental approach for estimating the rates of phytoplankton growth and microzooplankton grazing on phytoplankton in the oceans (Calbet and Landry 2004). Although the technique has been shown experimentally to work well under certain field conditions (e.g., Landry and Calbet 2005), its critical assumptions are often untested in the various systems in which it is applied. Inaccuracies may thus arise from nonlinear grazing responses associated with saturated grazing (Gallegos 1989; Evans and Paranjape 1992; Redden et al. 2002; Sanderson et al. 2012; Chen et al. 2014), treatment effects on grazer abundance, activity or community structure (Landry et al. 1995; Gallegos et al. 1996; Dolan et al. 2000; Moigis 2006; First et al. 2009), and trophic cascades (Calbet and Saiz 2013).

To build the connection between experimentally measured grazing rates and actual rates in the ocean, interpretation of the data from dilution experiments would benefit

from a theoretical framework that accounts for the nonlinear grazing kinetics of microzooplankton. In previous studies addressing this (e.g., Evans and Paranjape 1992), data and model comparisons have invoked nonlinear feeding models to represent the functional responses of microzooplankton feeding with respect to phytoplankton concentration (e.g., Holling 1959). However, the resulting models generally do not have simple mathematical interpretations or are analytically intractable (Redden et al. 2002). In addition, there are few experimental datasets to parameterize and verify these grazing formulas properly (Franks 2009). Application of such formulations to dilution experiments requires adequate measurements of not only net growth rates, but also ancillary measurements of plankton community biomass and structure, which are often ignored or difficult to make in dynamic ocean ecosystems subject to strong physical forcing and biological interactions (Li et al. 2012).

Here, we investigate the solution to a first-order differential equation coupling phytoplankton and zooplankton dynamics to develop theory for recovering growth and grazing rates from dilution experiments that show nonlinear grazing response curves. We explore this theory using dilution-experiment data collected during process cruises of

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the California Current Ecosystem-Long-Term Ecological Research (CCE-LTER) program. Our goal is to use these data to investigate the efficacy of the dilution technique for understanding phytoplankton grazing dynamics and to examine the utility of experimental dilution data for parameterizing microzooplankton grazing models. To recover the underlying growth and grazing rates from nonlinear multi-treatment dilution experiments, we incorporate the dilution-experiment data into a theoretical model and determine the rates and model parameters using nonlinear data fitting. Based on these analyses, we develop a method for correcting 2-treatment dilution-experiment data assuming an underlying *Holling II* grazing function. Finally, we apply these methods to data from CCE-LTER Lagrangian studies to quantify phytoplankton growth and grazing dynamics in southern California coastal and oceanic waters.

Theoretical development

Dilution experiments: the linear case

To calculate phytoplankton growth rate μ and microzooplankton grazing rate m from a multi-treatment dilution experiment, we begin with Landry and Hassett's (1982) equation for the rate of change of chlorophyll a (Chl) concentration in an incubation vessel:

$$\frac{d\text{Chl}}{dt} = \text{Chl}(\mu - Dm). \tag{1}$$

Here, D is the fraction of undiluted seawater in the incubation vessel, μ is the phytoplankton intrinsic growth rate, and m is the phytoplankton mortality rate due to grazing. In the undiluted seawater treatment, assuming constant μ and m during experiments, we can calculate the net chlorophyll growth rate ε from the initial (Chl$_0$) and final (Chl$_t$) chlorophyll concentrations and the incubation time Δt:

$$\varepsilon = \frac{1}{\Delta t} \ln \left(\frac{\text{Chl}_t}{\text{Chl}_0} \right) = \mu - m. \tag{2}$$

Similarly, assuming that grazing rate m decreases linearly in proportion to the dilution factor D, the net chlorophyll growth rate ε_d in diluted treatments can be calculated from the initial diluted chlorophyll concentration Chl$_0^d$ and the final diluted concentration Chl$_t^d$:

$$\varepsilon_d = \frac{1}{\Delta t} \ln \left(\frac{\text{Chl}_t^d}{\text{Chl}_0^d} \right) = \mu - mD. \tag{3}$$

It should be noted that μ and m in Eqs. 2, 3 are the actual (potentially dilution-dependent) rates. The Landry and Hassett (1982) technique then estimates the phytoplankton growth rate μ_L and microzooplankton grazing rate m_L using linear regression of net growth rates ε and ε_d vs. D, where μ_L is the intercept and m_L the magnitude of the slope of the regression. Hereafter, we will use μ_L and m_L to denote the

rates derived from the experimental manipulations in the linear case, in order to make them different from μ and m in nonlinear dilution models.

In the special case of a 2-treatment dilution experiment (Landry et al. 2008), the growth and grazing rates can be estimated as

$$m_L = \frac{\varepsilon_d - \varepsilon}{1 - D} \quad \text{and} \quad \mu_L = \frac{\varepsilon_d - D\varepsilon}{1 - D}. \tag{4}$$

Including the effects of nonlinear grazing in dilution experiments

The Landry and Hassett (1982) formulation to recover phytoplankton growth rate μ_L and microzooplankton grazing rate m_L is a simplified form of a more general equation for phytoplankton grazing mortality that involves a grazing functional response, $f(P)$, of the zooplankton Z to changes in the phytoplankton P concentration:

$$\frac{dP}{dt} = \mu P - g_{\max} f(P) Z, \tag{5}$$

where $g_{\max}$ is the maximum grazing rate of microzooplankton and P and Z are expressed as nitrogen biomass.

Equating the right-hand sides of Eqs. 1, 5, we obtain (for raw water, $D = 1$, replacing symbol Chl in Eq. 1 by P for "phytoplankton")

$$mP = g_{\max} f(P) Z, \tag{6}$$

Here, μ and m in Eqs. 5, 6 are the "instantaneous," potentially time-dependent, growth and grazing mortality rates of phytoplankton community in the model, which may differ from the measured rates of μ_L and m_L.

We choose a varying zooplankton Z during the experiment rather than the constant Z assumed in the original theory (e.g., Landry and Hassett 1982; Gallegos 1989). For simplification, we allow zooplankton to change as $Z = Z_0 e^{vt}$, with Z_0 the initial zooplankton biomass and v the net zooplankton growth rate which we assume to be constant during the experiments. We also assume a *Holling II* functional form $f(P) = P/(K + P)$ for zooplankton grazing on phytoplankton, with K the half-saturation constant, being mathematically equivalent to the *Michaelis-Menten* function and often an accurate description of the dependence of the grazer ingestion rate of prey at different densities in laboratory experiments (Fenchel 1988).

From Eq. 6 with $f(P)$ replaced by the *Holling II* formula, the instantaneous grazing rate, m thus can be written as

$$m = g_{\max} \frac{Z}{K + P}. \tag{7}$$

Now we have a first-order differential equation from Eq. 5:

$$\frac{dP}{dt} = \mu P - g_{\max} \frac{P}{K + P} Z_0 e^{vt}. \tag{8}$$

Dividing each side of Eq. 8 by P and integrating from 0 to Δt, we have

$$\frac{1}{\Delta t}\ln\left(\frac{P_t}{P_0}\right)=\mu-\frac{1}{\Delta t}\int_0^{\Delta t} g_{\max}\frac{Z_0 e^{vt}}{K+P}dt, \tag{9}$$

where P_0 is the initial phytoplankton biomass and is related to Chl_0 with the chlorophyll-to-nitrogen ratio. Equation 9 can be written as

$$\varepsilon=\mu-\bar{m}, \tag{10}$$

where $\varepsilon=\frac{1}{\Delta t}\ln\left(\frac{P_t}{P_0}\right)$ and $\bar{m}=\frac{1}{\Delta t}\int_0^{\Delta t} g_{\max}\frac{Z_0 e^{vt}}{K+P}dt$. Incubation duration Δt is a constant, usually a day. The mean grazing mortality rate over the experiment ($\bar{m}$) is potentially different from the instantaneous grazing mortality rate (m) that can vary with t due to changes in P and Z during experiments. Similarly, we have $\varepsilon_d=\mu-\bar{m}_d$ for diluted treatments with $\bar{m}_d$ the mean grazing rate during each diluted incubation.

Impact of dilutions on grazing mortality rates

In the next few paragraphs, focusing on Eq. 8, we will first investigate dilution effects under two special cases of saturated grazing (Case 1) and unsaturated grazing (Case 2) and then address the general situation when zooplankton grazing varies during the experimental incubations. In contrast with most previous studies (e.g., Gallegos 1989), we allow the clearance rates and microzooplankton biomass to vary during all dilutions, assuming that the microzooplankton community alters its clearance rate in response to the initial dilution (DP_0 and DZ_0 after dilution).

Case 1: When zooplankton grazing is saturated for all diluted and undiluted treatments, we have $K \ll P$. This gives $P/(P+K) \cong 1$ and Eq. 8 can be simplified to

$$\frac{dP}{dt}=\mu P-g_{\max}Z_0 e^{vt}. \tag{11}$$

The solution to this equation (when $v\neq\mu$) is

$$P=\left(P_0+\frac{g_{\max}Z_0}{v-\mu}\right)e^{\mu\Delta t}-\frac{g_{\max}Z_0}{v-\mu}e^{v\Delta t}, \tag{12}$$

and the formula for mean grazing rate ($\bar{m}$) is

$$\bar{m}=\frac{1}{\Delta t}\left\{\ln\left[\frac{P_0}{g_{\max}Z_0}(v-\mu)\right]-\ln\left[\frac{P_0}{g_{\max}Z_0}(v-\mu)+1-e^{(v-\mu)\Delta t}\right]\right\}. \tag{13}$$

Replacing P_0 and Z_0 with DP_0 and DZ_0, we get the mean grazing mortality rate of the individual dilution treatments ($\bar{m}_d$)

$$\bar{m}_d=\frac{1}{\Delta t}\left\{\ln\left[\frac{DP_0}{g_{\max}DZ_0}(v-\mu)\right]-\ln\left[\frac{DP_0}{g_{\max}DZ_0}(v-\mu)+1-e^{(v-\mu)\Delta t}\right]\right\}. \tag{14}$$

We thus have $\bar{m}_d=\bar{m}$, which says that the mortality rate is not affected by dilution when grazing is saturated (we will get the same conclusion when $v=\mu$). Indeed, the dilution results of Redden et al. (2002) indicated that the apparent growth rate remained constant for $D>0.5$ in the Dee Why Lagoon due to saturated microzooplankton grazing when phytoplankton concentration exceeded 3.83 μg Chl L^{-1}.

Case 2: If zooplankton grazing is never saturated, then $K \gg P$. This gives $P/(P+K) \cong P/K$, and the Eq. 8 can be simplified to

$$\frac{dP}{dt}=\mu P-\frac{g_{\max}Z_0}{K}Pe^{vt}. \tag{15}$$

The solution to Eq. 15, when $v\neq0$, is

$$P=P_0 e^{\left[\mu\Delta t-\frac{g_{\max}Z_0}{K}\left(\frac{e^{v\Delta t}-1}{v}\right)\right]}, \tag{16}$$

with the mean grazing rate

$$\bar{m}=\frac{g_{\max}Z_0}{K}\left(\frac{e^{v\Delta t}-1}{v\Delta t}\right). \tag{17}$$

Replacing Z_0 with DZ_0, we will obtain $\bar{m}_d$ as

$$\bar{m}_d=\frac{g_{\max}DZ_0}{K}\left(\frac{e^{v\Delta t}-1}{v\Delta t}\right). \tag{18}$$

We now have $\bar{m}_d=D\bar{m}$, indicating that the grazing rate varies linearly with dilution D when grazing is never saturated (we will have the same conclusion for $v=0$). This is the case for the classic linear dilution theory (Landry and Hassett 1982).

In reality, a rapid increase of phytoplankton biomass could lead to saturated grazing, even when zooplankton grazing is unsaturated at the beginning of the experimental incubations. In addition, zooplankton grazing can become unsaturated by dilution even when it was originally saturated in the raw seawater. Now, we consider zooplankton grazing varying between unsaturation and saturation during the dilution experiment. Since the Eq. 8 cannot be solved analytically, we approach its solution as an approximation using the Picard iteration method (*see* the Supporting Information for details). The final approximate solution is written as

$$P=P_0 e^{\left[\mu\Delta t-\frac{g_{\max}Z_0}{(K+P_0)}\left(\frac{e^{v\Delta t}-1}{v}\right)\right]} \tag{19}$$

and

$$\bar{m}=\frac{g_{\max}Z_0}{(K+P_0)}\left(\frac{e^{v\Delta t}-1}{v\Delta t}\right). \tag{20}$$

For the dilution treatments, we get

$$\bar{m}_d=\frac{g_{\max}DZ_0}{(K+DP_0)}\left(\frac{e^{v\Delta t}-1}{v\Delta t}\right). \tag{21}$$

Therefore, we have

$$\bar{m}_d = D \frac{K + P_0}{K + DP_0} \bar{m}. \qquad (22)$$

This equation basically says that the mortality rate varies nonlinearly with the dilution factor D (we will get the same conclusion for $v = 0$). It is interesting to note that the nonlinearity is related to the half-saturation constant K and the initial phytoplankton biomass P_0, but it is not influenced by changing zooplankton biomass Z during the experiments. This arises from our assumption that the zooplankton growth rate v does not depend on the dilution D.

Estimating nonlinear grazing from multi-treatment dilution experiments

The dilution equation ($\varepsilon_d = \mu - \bar{m}_d$) can now be rewritten as

$$\varepsilon_d = \mu - D \left(\frac{1 + \frac{P_0}{K}}{1 + D\frac{P_0}{K}} \right) \bar{m}, \qquad (23)$$

where μ, $\bar{m}$, and P_0/K are all assumed constant during each dilution series. By applying Eq. 23 to dilution series of ε_d and D with the best fit chosen to minimize the sum of squared residuals of the modeled and observed apparent net growth rates, we can estimate μ and $\bar{m}$, as well as P_0/K for any multi-treatment dilution result. Equation 23 can be rewritten as $\varepsilon_d = \mu - [f(DP_0)/f(P_0)]\bar{m}$, according to the *Holling II* formulation $f(P) = P/(P + K)$. For other grazing function forms, such as the *Ivlev* function, the final nonlinear dilution equation will be quite similar except for $f(P)$ being replaced by the *Ivlev* formulation, which is addressed in detail in the Supporting Information.

A modeled dilution experiment with $\mu = 0.5$ d^{-1} and $\bar{m} = 0.4$ d^{-1} shows that the nonlinearity of dilution curves depends mainly on the ratio P_0/K in the model (Fig. 1). Strongly nonlinear relationships are observed when P_0/K is high. These are conditions under which we expect grazing to be saturated, at least for less-edible components of the phytoplankton community, such as might occur in coastal regions or during dense blooms. For low P_0/K conditions typical of offshore pelagic regions, the relationship between net growth rate and dilution factor will be approximately linear. Nonlinear dilution curves occasionally found in the oligotrophic offshore waters can be a result of nutrient addition, which affects functional responses of grazers by altering their prey quality and quantity (Worden and Binder 2003).

We can also estimate other parameters such as g_{max} and K. Reorganizing Eq. 20, we find

$$g_{max} = \bar{m} \cdot \left(\frac{K + P_0}{Z_0} \right) \cdot \left(\frac{v\Delta t}{e^{v\Delta t} - 1} \right). \qquad (24)$$

We calculate the half-saturation parameter K from P_0/K. The zooplankton growth rate v can be determined by the change
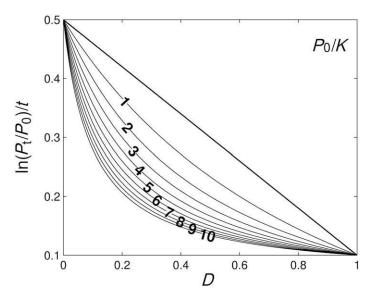

Fig. 1. Relationship of apparent net growth rate (*dlnPdt*) and dilution factor D in an ideal dilution experiment (with $\mu = 0.5$ d^{-1} and $m = 0.4$ d^{-1}) for different P_0/K values (labeled on contour lines). P and K are in units of μM nitrogen.

of zooplankton over the duration of incubation as $v = \frac{1}{\Delta t} \ln\left(\frac{Z_t}{Z_0}\right)$. For the case of $v = 0$, we simply have $g_{max} = \bar{m}(K + P_0)/Z_0$. Therefore, the maximal grazing rate g_{max} can be estimated from $\bar{m}$, K, v, P_0, and Z_0.

It is important to bear in mind that the rate $\bar{m}$ derived from dilution experiments is only a mean grazing rate during the incubation, while the instantaneous grazing rate m potentially varies throughout the incubation, depending on the biomasses of P and Z as given by Eq. 7. From the parameter v, we can further estimate the instantaneous grazing rate m_0 at the beginning of the experiment ($t = 0$, with P_0 and Z_0) from Eq. 7:

$$m_0 = \frac{g_{max} Z_0}{K + P_0} = \bar{m} \cdot \left(\frac{v\Delta t}{e^{v\Delta t} - 1} \right). \qquad (25)$$

This m_0 is actually the rate of microzooplankton grazing that most biological oceanographers want to know, since it represents the instantaneous grazing rate in the seawater sample when it was initially sampled. When $v = 0$, we have $m_0 = \bar{m}$, indicating that the mean grazing rate is equal to the initial grazing rate when microzooplankton biomass remains constant during the experiments.

To estimate the nonlinear growth and grazing rates μ and $\bar{m}$, and grazing half-saturation constant K, we fit the model (Eq. 23) to the data. We accomplished this in Matlab using *fminsearch*, which employs multidimensional unconstrained nonlinear optimization. The Matlab function computes the minimum of a given function using the Nelder-Mead simplex method. The nonlinear relationship of dilution factor D and net growth rate ε is modeled as $\varepsilon = h(D, A)$, where h is

Table 1. Summary of four multi-treatment dilution experiments for nonlinear fitting exercises. Units of P_0, Z_0, and K are in μM nitrogen; R^2 and p value of the nonlinear fitting for each experiment are also shown; S_K is the sensitivity of apparent growth rate to K with the unit of d^{-1} μM^{-1}, *see* text for detail.

Cycle	Chl$_0$ (μg L^{-1})	P_0 (μM)	Z_0 (μM)	μ (d^{-1})	$\bar{m}$ (d^{-1})	P_0/K	K (μM)	R^2	p	S_K
0601	6.03 ± 0.45	2.68 ± 0.21	0.75 ± 0.16	0.90 ± 0.17	0.64 ± 0.16	5.05 ± 2.02	$0.53 \pm 0.38^*$	0.61	<0.01	0–0.25
0603	5.48 ± 0.59	3.29 ± 0.22	1.20 ± 0.89	0.64 ± 0.03	0.51 ± 0.02	3.94 ± 0.72	$0.84 \pm 0.37^*$	0.66	<0.01	0–0.12
0604	1.05 ± 0.16	0.69 ± 0.08	0.22 ± 0.02	0.72 ± 0.01	0.57 ± 0.01	0.56 ± 0.03	$1.24 \pm 0.12^*$	0.92	<0.01	0–0.04
0704	1.27 ± 0.12	0.43 ± 0.04	0.13 ± 0.05	0.92 ± 0.02	0.67 ± 0.02	2.15 ± 0.29	$0.20 \pm 0.08^*$	0.91	<0.01	0–0.57

* The progressive error contributed from both P_0 and P_0/K.

an unknown function, and A is a set of problem-dependent parameters, which are to be determined. For this model, we have a measured data set of $[D_1, \varepsilon_1]$, $[D_2, \varepsilon_2]$,..., $[D_n, \varepsilon_n]$. Operationally, we first define a misfit or cost function $\frac{1}{n-1} \sum_{i=1}^{n} (h(D_i,A)-\varepsilon_i)^2$ to be minimized. Second, we assign an initial set of the parameters A from which to begin the search. The routine *fminsearch* finds the minimum of the cost function, starting at the initial estimate, and iterates the computations to return parameter values that minimize the cost function. To test this data-fitting technique, we randomly assigned different initial guess values for *fminsearch* and found that the final optimal parameters were unchanged. R^2 and p-value of the nonlinear fitting can also be computed as well as the uncertainties of the optimized parameters (Table 1).

Recovering rates from 2-treatment dilution experiments

The nonlinear fitting approach is not directly applicable to 2-treatment dilution experiments because we cannot assess nonlinearity from two data points. If we define r as a nonlinear dilution factor and make

$$r = D\frac{K+P_0}{K+DP_0} \tag{26}$$

we will have from Eq. 28

$$\varepsilon_d = \mu - \bar{m}r. \tag{27}$$

Combining with the raw water case ($\varepsilon = \mu - \bar{m}$), we obtain the true growth and grazing mortality rates as

$$\mu = \frac{\varepsilon_d - r\varepsilon}{1-r} \quad \text{and} \quad \bar{m} = \frac{\varepsilon_d - \varepsilon}{1-r} \tag{28}$$

Because r approaches D as P_0/K decreases (Fig. 2), the original linear assumption in dilution experiments (Landry and Hassett 1982) is clearly only appropriate in regions of low P_0/K. In coastal regions, where the ratio P_0/K is expected to be high, the value of r may be substantially larger than D.

The relationship between the experimental parameters (μ_L, m_L, D) and the underlying nonlinear parameters (μ, $\bar{m}$, r) for a 2-treatment dilution experiment is demonstrated in Fig. 3. The nonlinear correction is a translation of the

dilution point (D, ε_d) to the right, to the point (r, ε_d); the new intercept and slope then give the nonlinear growth and grazing mortality rates. We should be able to recover the nonlinear mortality rates ($\bar{m}$, Eq. 28) from the measured mortality rates (m_L, Eq. 4) by

$$\bar{m} = \phi \cdot m_L, \tag{29}$$

where the conversion factor $\phi = (1-D)/(1-r) = 1 + D(P_0/K)$. Similarly, we can recover the nonlinear growth rate (μ) from the measured (linear) rates (μ_L and m_L) by

$$\mu = \mu_L + m_L \cdot (\phi - 1). \tag{30}$$

Thus, the underlying nonlinear growth and grazing mortality rates can be recovered from the measured rates in a 2-treatment dilution experiment if we know the value of K, which is assumed a parameter of the local microzooplankton community and can be estimated from a multi-treatment experiment conducted in the same water. The approach will be particularly useful in Lagrangian studies of growth and grazing dynamics while following a water parcel (in this case, a large number of rate estimates of the same water are needed both vertically and temporally), as saturated grazing may develop or disappear during the evolution of the tracked plankton ecosystem.

Materials and methods

Semi-Lagrangian process experiments of 3–5 d duration were conducted on CCE-LTER cruises in May 2006 (Cycles 0601, 0603, 0604) and April 2007 (0704) in the waters along the CalCOFI (California Cooperative Oceanic Fisheries Investigations) line 80 off of Point Conception, California. Cycles 0601, 0603, and 0704 were of the richer upwelling-influenced waters that lie shoreward of the main axis of the southward flowing California Current, and Cycle 0604 was located offshore at the transition zone between the coastal upwelling regions and the oligotrophic open ocean. For each experimental cycle, water-column sampling and in situ incubations were conducted while following a water parcel marked by a satellite-tracked surface drifter drogued at 15 m (Landry et al. 2009; Li et al. 2011). The fieldwork included
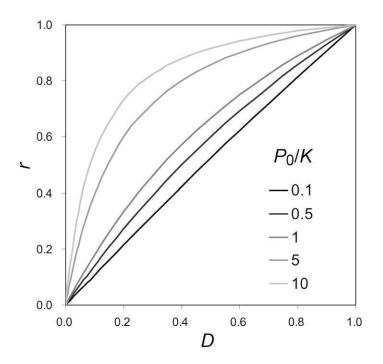
Fig. 2. Relationship of D, P_0/K, and r in a *Holling II* functional form. Labeled lines represent the values of nonlinear dilution factor r.

Fig. 3. Recovering growth and grazing rates from a 2-treatment dilution experiment with knowledge of the nonlinear grazing response. The thick black line shows the linear dilution with the intercept μ_L and the slope m_L; the red curve is the nonlinear dilution with the intercept μ and the mean slope $\overline{m}$; the nonlinear correction is a translation of the dilution point (D, ε_d) to the ideal point (r, ε_d) with D and r the linear and nonlinear dilution factors.

both hydrographic and biomass measurements (phytoplankton and microzooplankton carbon biomass) as well as experiments quantifying biological rates (phytoplankton growth, zooplankton grazing, and particle export). In situ incubations were performed by hanging bottles on a line below the drifting buoy.

Phytoplankton growth and grazing mortality rates

Rate estimates of phytoplankton community growth and grazing mortality were assessed by 2-treatment dilution experiments conducted daily following the drifter path, using water collected from two depths (5 m and 12 m) in the mixed layer. Standard multi-treatment dilution experiments were also performed using mixed layer seawater samples (5–12 m). These experiments were set up similarly to the 2-treatment, but contained nutrient addition treatments and starting with seawater collected in the early evening in the vicinity of the experimental drifter. Experimental protocols of two-point dilution experiments and multi-treatment dilution experiments are presented in the Supporting Information.

Phytoplankton and microzooplankton biomasses

Community abundances and biomasses of phytoplankton and microzooplankton were determined by a combination of flow cytometry and epifluorescence microscopy (Taylor et al. 2011). Detailed methodologies for determining phytoplankton and microzooplankton biomasses are presented in the Supporting Information.
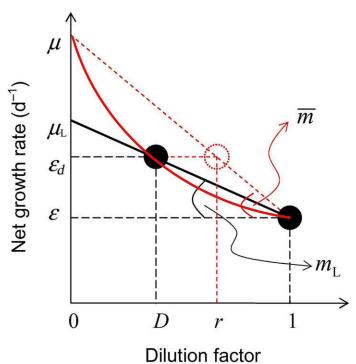
Results and discussion

Assessing nonlinearity of dilution experiments in the California coastal and pelagic ecosystems

Multi-treatment dilution experiments were conducted during four field cycle studies (0601, 0603, and 0604 during May 2006 and 0704 during April 2007) from CCE-LTER using samples acquired within the mixed layer (Landry et al. 2009) providing opportunities for quantifying the nonlinearity of microzooplankton grazing in these waters. Cycles 0601, 0603, and 0704 were located near Point Conception off the Southern California coast with large differences in initial surface Chl a concentrations: higher in Cycles 0601 ($\sim$ 6.03 μg L^{-1}) and 0603 ($\sim$ 5.48 μg L^{-1}) but lower in Cycle 0704 ($\sim$ 1.27 μg L^{-1}). Cycle 0604 was located offshore with typically lower Chl a concentration ($\sim$ 1.05 μg L^{-1}). Nonlinear fitting results of the multi-treatment dilution experiments are shown in Fig. 4 and summarized in Table 1. The initial biomasses of P_0 and Z_0 of each experiment were estimated from Chl concentration based on the ratio of chlorophyll to phytoplankton carbon and the carbon ratio of microzooplankton to phytoplankton in the raw seawater where the incubation samples were taken. Nonlinear fitting of Cycle 0603 (Fig. 4c) was performed by excluding the two negative
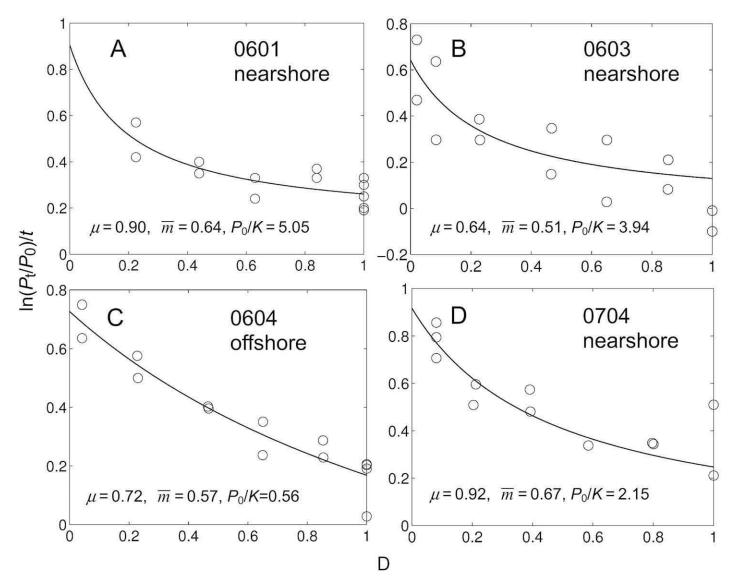
Fig. 4. Fitting the nonlinear model Eq. 23 to the 1-d multi-treatment dilution experiments in the California Current Ecosystem for Cycle 0601 (**A**), Cycle 0603 (**B**), and Cycle 0604 of the 2006 cruise (**C**) and Cycle 0704 of the 2007 cruise (**D**). *P* and *K* are in units of μM nitrogen.

net growth rates in undiluted treatments ($D = 1$). If these points are included, P_0/K will reduce from 3.94 to 0.92 leading to a higher K of 3.57 μM.

To compare the difference between coastal and offshore ecosystems, we focus in particular on two cycles: 0704 (nearshore) and 0604 (offshore). Although the initial chlorophyll concentration for the experiments was slightly higher in 0704 than in 0604, the phytoplankton biomass P_0 was lower in 0704 than 0604 due to elevated chlorophyll to carbon ratios of the surface phytoplankton community nearshore (Li et al. 2010). Our results suggest that both the nonlinear growth and grazing mortality rates were much higher in Cycle 0704 than in Cycle 0604. The rates of Cycle 0604 estimated by nonlinear regression ($\mu = 0.72$ d^{-1}, $\bar{m} = 0.57$ d^{-1}) are not much different from those estimated by linear

regression ($\mu_L = 0.68$ d^{-1}, $m_L = 0.53$ d^{-1}), consistent with the low P_0/K there. However, estimates of growth and grazing mortality rates for Cycle 0704 from the nonlinear model ($\mu = 0.92$ d^{-1}, $\bar{m} = 0.67$ d^{-1}) are substantially higher than those estimated using the linear model ($\mu_L = 0.71$ d^{-1}, $m_L = 0.39$ d^{-1}). Stronger nonlinearity in the multi-treatment dilution experiment in Cycle 0704 ($P_0/K = 2.15$), compared to Cycle 0604 ($P_0/K = 0.56$), gives a lower estimated K of 0.20 μM for Cycle 0704 but 1.23 μM for Cycle 0604. These results indicate that different plankton communities in nearshore and offshore ecosystems could be associated with substantially different K, though their initial chlorophyll concentrations were similar. These values (Table 1) are within the ranges of K estimated from laboratory feeding experiments (Hansen et al. 1997) for ciliates (0.06–2.46 μM) and

heterotrophic dinoflagellates (0.2–11 μM), the two major microzooplankton groups in the California Current Ecosystem (Landry et al. 2009). The larger K for offshore experiments underscores the important role of microzooplankton grazing in structuring the South California Current pelagic ecosystem. In contrast, microzooplankton grazing in the coastal marine ecosystem is more easily saturated by phytoplankton prey, which may allow larger grazers, such as mesozooplankton, to become competitors with microzooplankton for phytoplankton prey.

To address the dependence of the nonlinear estimate on the variation of K, a one-at-a-time sensitivity analysis (Hamby 1994) was performed by calculating the sensitivity (S_K) as the partial derivative of the apparent growth rate (Eq. 23) with respect to K:

$$S_K = \frac{\partial \varepsilon_d}{\partial K} = \bar{m} P_0 \cdot \frac{D(1-D)}{(K+DP_0)^2} \qquad (31)$$

The maximal sensitivity is reached when $dS_K/dD = 0$. Therefore, we get the maximal S_K of $\bar{m}P_0/[4K(K+P_0)]$ for D equal to $K/(P_0 + 2K)$. Equation 31 also demonstrates that the apparent growth rate will not be sensitive to K ($S_K = 0$) when D is approaching 0 or 1. The final results of the sensitivity analyses for the multi-treatment dilution experiments during the field studies are shown in Table 1. There are various ranges of S_K for the four different cycle studies, with the smallest in Cycle 0604 (0–0.04 μM^{-1} d^{-1}) and the largest in Cycle 0704 (0–0.57 μM^{-1} d^{-1}). The maximal sensitivity of 0.57 μM^{-1} d^{-1} in Cycle 0704 means that, for a 0.1 μM increase in K, the apparent growth rate increases by 0.06 d^{-1}.

Because the nonlinear model has more parameters than the linear model, it can give a closer fit to nonlinear data. To test whether the additional parameters significantly improved the model-data fit, we used the Akaike information criterion (AIC) with correction for small sample sizes to compare the nonlinear and linear dilution models. For Cycle 0704 in the coastal region, the AIC of the nonlinear fit was -58.8, which is lower than the AIC of -52.6 from the linear regression, suggesting that even with the additional parameters, the nonlinear model better described the data. However, for Cycle 0404, the AIC of the nonlinear model (-72.5) was not much different from the linear model (-73.6), suggesting that the linear regression was adequate for estimating phytoplankton growth and grazing rates in offshore regions.

Because we lack measurements of microzooplankton biomass at the end of the dilution experiments to determine the net growth rates of the grazers, we are not able to recover the true maximal grazing rates g_{max} (Eq. 24) and the instantaneous mortality rate m_0 (Eq. 25). Net growth rates of microzooplankton (ν) could vary substantially from less than -1 d^{-1} to more than 1 d^{-1} during dilution experiments (First et al. 2009; Modigh and Franze 2009). However, if we assume $\nu = 0$ (no change of microzooplankton during experiments), we would roughly estimate g_{max} of 3.16 d^{-1} for Cycle 0704 and 4.99 d^{-1} for Cycle 0604, which are within the ranges of $g_{max} = 2.4$–11.5 d^{-1} for ciliates and $g_{max} = 0.26$–4.08 d^{-1} for heterotrophic dinoflagellates (Hansen et al. 1997). Future studies should quantify microzooplankton biomasses both before and after dilution experiments to more fully understand phytoplankton growth and grazing dynamics.

While saturated feeding is one of the most plausible explanations for nonlinear dilution curves, we cannot exclude other mechanisms, such as the shading effect in turbid waters, contamination, and varying microzooplankton grazing activity and community structure during experiments (Landry et al. 1995; Gallegos et al. 1996; Dolan et al. 2000; Moigis 2006; First et al. 2009). Complex interactions, such as mixtrophy, omnivory, and intraguild predation may not be adequately described by a single prey-predator model. Trophic cascades may explain some of the nonlinear dilution results (Calbet and Saiz 2013), though the nongrowth assumption in their model would always lead to negative net growth rates. As dilution experiments deal with community-level processes, responses to dilution may differ substantially among individual phytoplankton groups. Strong nonlinear responses for *Prochlorococcus*, but linear responses for *Synechococcus* were reported in nutrient-amended dilution experiments conducted in the oligotrophic Sargasso Sea (Worden and Binder 2003). Nonlinearity of dilution curves was also observed in a mesocosm experiment with oligotrophic waters in the Mediterranean Sea, where the system showed large increases in autotrophic dinoflagellates and heterotrophic ciliates over 6 d of incubations (Calbet et al. 2012).

Application of 2-treatment correction method during Lagrangian studies

We now apply our nonlinear correction to the data of 2-treatment dilution experiments conducted during Lagrangian studies of Cycles 0704 and 0604. Water parcels were carefully tracked for several days using subsurface floats, with 2-treatment dilution experiments performed each day, with multi-treatment dilution experiments (described above) only performed on the first day. As pointed out above, we apply the K estimated from multi-treatment dilution experiments to recover the underlying nonlinear growth and grazing rates of the same water parcel from the time series of 2-treatment dilution experiments (Table 2), assuming that K remains constant over the 4–5 days' evolution of the tracked water parcel.

In the coastal waters of Cycle 0704, phytoplankton biomass decreased with sampling time, consistent with the initial patch originating from the waters upwelled some time before. The nonlinear dilution factor r of Cycle 0704 (0.46–0.60) was higher than the linear dilution factor of $D = 0.33$ used in our 2-treatment dilution experiments (Table 2).

Table 2. Evolution of phytoplankton growth and grazing in the surface mixed layer during Lagrangian studies in Cycle 0604 and Cycle 0704 of the southern California Current Ecosystem. 2-treatement dilution experiments are corrected based on Eqs. 29, 30 with K values from Table 1; Error bounds are standard deviations.

Cycle	Date	P_0 (μM)	μ_L (d^{-1})	m_L (d^{-1})	r	ϕ	μ (d^{-1})	$\bar{m}$ (d^{-1})
0604	26 May 2006	0.574 ± 0.015	0.536 ± 0.131	0.145 ± 0.055	0.393 ± 0.002	1.104 ± 0.003	0.543 ± 0.124	0.157 ± 0.061
	27 May 2006	0.768 ± 0.022	0.413 ± 0.041	0.215 ± 0.046	0.411 ± 0.002	1.138 ± 0.005	0.435 ± 0.044	0.241 ± 0.050
	28 May 2006	0.773 ± 0.020	0.602 ± 0.057	0.436 ± 0.019	0.412 ± 0.002	1.140 ± 0.004	0.655 ± 0.059	0.488 ± 0.032
	30 May 2006	0.486 ± 0.006	0.675 ± 0.104	0.314 ± 0.053	0.383 ± 0.001	1.085 ± 0.002	0.691 ± 0.106	0.336 ± 0.056
0704	16 Apr 2007	0.396 ± 0.012	0.602 ± 0.098	0.209 ± 0.071	0.596 ± 0.005	1.660 ± 0.020	0.703 ± 0.149	0.339 ± 0.113
	17 Apr 2007	0.382 ± 0.032	0.638 ± 0.001	0.345 ± 0.127	0.591 ± 0.013	1.637 ± 0.053	0.849 ± 0.097	0.561 ± 0.223
	18 Apr 2007	0.337 ± 0.043	0.582 ± 0.228	0.281 ± 0.271	0.580 ± 0.011	1.596 ± 0.041	0.741 ± 0.372	0.444 ± 0.434
	19 Apr 2007	0.168 ± 0.033	0.500 ± 0.068	0.310 ± 0.115	0.465 ± 0.004	1.253 ± 0.009	0.570 ± 0.058	0.382 ± 0.144

Large values of ϕ from 1.25 to 1.66, increasing in proportion to phytoplankton biomass, suggest that the usual linear regression of the 2-treatment dilution data might significantly underestimate phytoplankton growth and grazing rates in these waters. The recovered growth and grazing rates decreased with time as the patch moved, and grazing mortality by microzooplankton constituted a large percentage of the loss of phytoplankton (revealed by the relatively high but constant ratios of $\bar{m}/\mu$). In contrast, in the offshore waters of Cycle 0604, we found the values of $r \sim 0.4$ (note: $D = 0.33$) and $\phi \sim 1.12$, suggesting that the linear regression gave accurate estimates of growth and grazing rates from 2-treatment dilution experiments in this region. The phytoplankton patch followed during Cycle 0604 showed increases and then decreases in both biomass and grazing mortality rate, though the phytoplankton community grew faster as the patch evolved. The decrease of phytoplankton biomass on 30 May 2006 may indicate an increase of mesozooplankton grazing pressure, since microzooplankton grazing was low at that time.

One particularly notable feature of the growth and grazing estimates (Table 2) is that the rate ratios ($\bar{m}/\mu$) for the recovered nonlinear rate data substantially exceed those of the original (linear) estimates. For 0604, the difference in the mean values (0.519 vs. 0.495, respectively) is relatively small, but still represents an increase of 5%. The larger mean difference for 0704 (0.603 vs. 0.497, respectively) corresponds to an increase in the rate ratio of 21%. The m/μ ratio reflects the proportion of phytoplankton productivity consumed by microzooplankton, for which the global database average computed from uncorrected dilution data is 67% (Calbet and Landry 2004). Based on the present analysis, nonlinearities in dilution experiments suggest that this global estimate of microzooplankton grazing impact on phytoplankton is likely conservative to the extent that nonlinearities have not been incorporated in the dilution database.

In summary, we developed theory to extract and parameterize nonlinear grazing functional responses from estimates of phytoplankton apparent growth rates obtained from multi-treatment dilution experiments. Using numerical data-fitting techniques, our approach allows us to recover underlying growth and grazing mortality rates from nonlinear data of multi-treatment dilution experiments. By assuming a functional form for grazing and microzooplankton growth, we can also recover the parameters of the nonlinear grazing function. In addition, using parameters estimated from multi-treatment dilution experiments, we can assess nonlinearities in 2-treatment experiments conducted in the same waters. Our results suggest that the 2-treatment dilution technique gives accurate estimates of the grazing mortality rates on phytoplankton in oligotrophic offshore oceanic waters, but may underestimate grazing mortality rates in more eutrophic coastal areas where phytoplankton are more abundant and grazing more likely to be saturated. Application of our nonlinear correction method to 2-treatment dilution experiments conducted during two Lagrangian process studies by the CCE-LTER program reveals the complex interactions between phytoplankton growth and grazing dynamics in the southern California Current Ecosystem. Such results also suggest that previous estimates of the proportion of phytoplankton primary productivity consumed by microzooplankton, especially in richer coastal environments, are conservative.

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Conflict of Interest

None declared.