

RESEARCH ARTICLE

# Disentangling niche competition from grazing mortality in phytoplankton dilution experiments

Stephen J. Beckett<sup>1\*</sup>, Joshua S. Weitz<sup>1,2\*</sup>

**1** School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia, United States of America, **2** School of Physics, Georgia Institute of Technology, Atlanta, Georgia, United States of America

\* [stephen.beckett@biology.gatech.edu](mailto:stephen.beckett@biology.gatech.edu) (SJB); [jsweitz@gatech.edu](mailto:jsweitz@gatech.edu) (JSW)



**OPEN ACCESS**

**Citation:** Beckett SJ, Weitz JS (2017) Disentangling niche competition from grazing mortality in phytoplankton dilution experiments. PLoS ONE 12(5): e0177517. <https://doi.org/10.1371/journal.pone.0177517>

**Editor:** Hans G. Dam, University of Connecticut, UNITED STATES

**Received:** February 10, 2017

**Accepted:** April 29, 2017

**Published:** May 15, 2017

**Copyright:** © 2017 Beckett, Weitz. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** Code associated with the manuscript is available at <https://github.com/sjbeckett/DilutionMethod-NicheCompetition> and is archived at <http://doi.org/10.5281/zenodo.264468>. doi: [10.5281/zenodo.264468](https://doi.org/10.5281/zenodo.264468).

**Funding:** JSW was awarded SCOPE Award ID 329108 from the Simons Foundation (<https://www.simonsfoundation.org/>). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** The authors have declared that no competing interests exist.

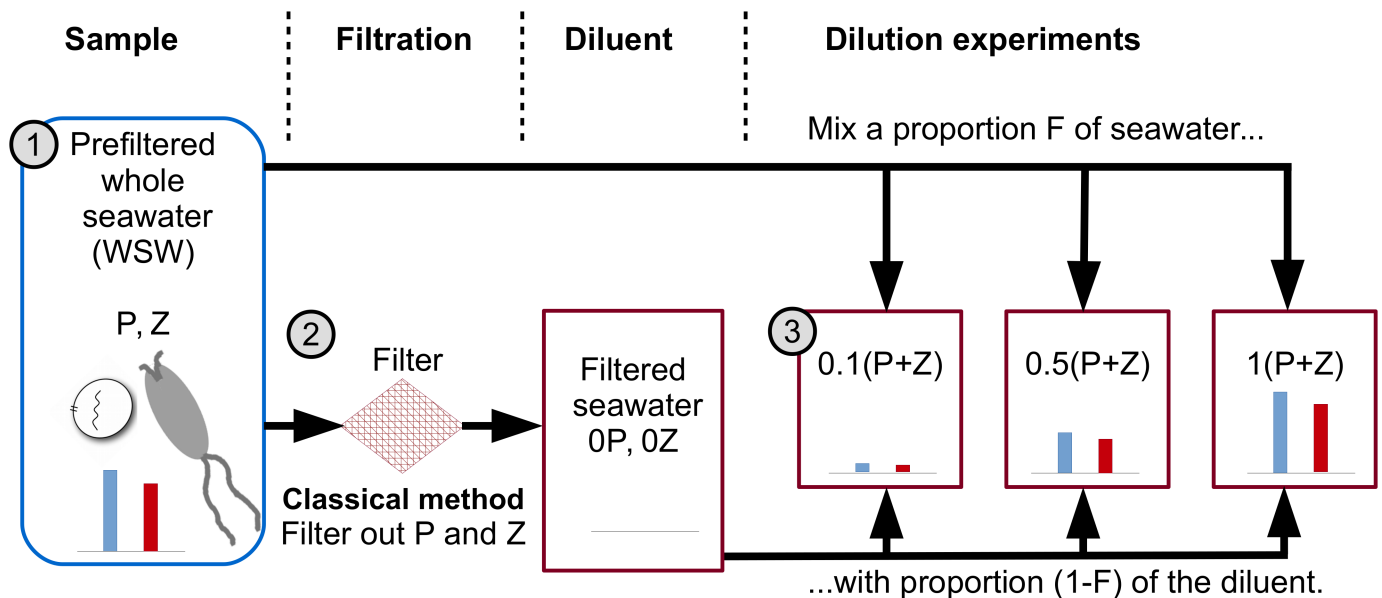
## Abstract

The dilution method is the principal tool used to infer *in situ* microzooplankton grazing rates. However, grazing is the only mortality process considered in the theoretical model underlying the interpretation of dilution method experiments. Here we evaluate the robustness of mortality estimates inferred from dilution experiments when there is concurrent niche competition amongst phytoplankton. Using a combination of mathematical analysis and numerical simulations, we find that grazing rates may be overestimated—the degree of overestimation is related to the importance of niche competition relative to microzooplankton grazing. In response, we propose a conceptual method to disentangle the effects of niche competition and grazing by diluting out microzooplankton, but not phytoplankton. Our theoretical results suggest this revised “Z-dilution” method can robustly infer grazing mortality, regardless of the dominant phytoplankton mortality driver in our system. Further, we show it is possible to independently estimate both grazing mortality and niche competition if the classical and Z-dilution methods can be used in tandem. We discuss the significance of these results for quantifying phytoplankton mortality rates; and the feasibility of implementing the Z-dilution method in practice, whether in model systems or in complex communities with overlap in the size distributions of phytoplankton and microzooplankton.

## Introduction

Phytoplankton form the base of the ocean food web and are drivers of ocean biogeochemical cycles. Microzooplankton grazing is thought to be one of the dominant drivers of phytoplankton mortality [1, 2]; and is a core process within the marine microbial loop [3, 4]. However, other processes such as nutrient limitation, sinking and viral lysis compete and interact with grazers as sources of phytoplankton mortality [5–8]. Understanding the dynamics of microbial food webs is therefore key to understanding their role in oceanic biogeochemical fluxes [9–11].

Estimates of the relative importance of grazing versus other mortality drivers depends on the quality and robustness of experimental techniques. The dilution method [12] is a



**Fig 1. Schematic of the classical dilution method of Landry and Hassett.** **1. Sample** Environmental samples are prefiltered to focus on microbial communities—this is termed whole seawater (WSW). Dilution method theory assumes WSW contains microzooplankton and the phytoplankton they graze upon. **2. Filtration** The classic dilution method filters some WSW to create a diluent containing no phytoplankton or microzooplankton. **3. Dilution series** A series of bottles are filled with a proportion  $F$  of WSW and mixed with a proportion  $(1 - F)$  of the diluent creating a dilution series. The blue and red bars represent the relative abundance of phytoplankton and microzooplankton. Apparent growth rates are calculated by measuring the differences in phytoplankton population sizes in each bottle across the dilution series at two time points (the beginning and end of an incubation period). The microzooplankton grazing rate is estimated by finding the gradient of a linear regression model between the dilution level  $F$  and the apparent growth rate.

<https://doi.org/10.1371/journal.pone.0177517.g001>

long-established and popular technique used to measure the impact of micro- and nano-zoo-plankton on phytoplankton communities. The method is outlined in Fig 1. A sample of seawater is taken and prefiltered so only the microbial fraction remains. Some of this whole seawater (WSW) sample is further filtered, to remove phytoplankton and microzooplankton, creating a diluent. The dilution method procedure creates a series of bottles each containing a different proportion of WSW mixed with diluent to create a dilution series.

By measuring the differences in phytoplankton population sizes between two time points within each bottle, the corresponding approximate per capita growth rates can be calculated. The dilution curve represents a plot of the approximate growth rates within each bottle against the dilution level (the proportion of WSW within a bottle). The phytoplankton growth rate and the grazing rate are reported as the intercept and slope, respectively, of the dilution curve. This is due to the underlying theoretical model of phytoplankton dynamics presumed to operate in the bottle. This model, introduced by Landry and Hassett [12], is:

$$\frac{dP}{dt} = \overbrace{rP}^{\text{growth}} - \overbrace{aPZ}^{\text{grazing}} \quad (1)$$

in which  $P$  is the density of phytoplankton, which grow at rate  $r$ ; and are grazed upon by microzooplankton  $Z$  at a rate of  $a$ . Consider that the whole seawater sample initially contains a phytoplankton density of  $P_0$  and a microzooplankton density of  $Z_0$ . A particular bottle within the dilution series containing a proportion  $F$  of whole seawater will initially contain phytoplankton and microzooplankton densities of  $FP_0$  and  $FZ_0$  respectively. Evaluating the apparent growth rate, calculated as the rate of population change per capita, under these conditions

shows that:

$$\frac{1}{P} \frac{dP}{dt} \Big|_{FP_0, FZ_0} = r - (aZ_0)F. \tag{2}$$

Eq (2) implies that the relationship between dilution level and apparent growth rate will be linear with an intercept equal to the intrinsic per capita growth rate and a gradient equal to the initial per capita grazing mortality rate. However, the model given by Eq (1) may be an insufficient description of bottle phytoplankton dynamics for several reasons.

Microzooplankton grazing may respond non-linearly with respect to phytoplankton densities in the bottle for example due to feeding saturation. This could significantly alter the shape of the dilution curve and how grazing mortality estimates can be made [13–15]. Additional sources of phytoplankton mortality such as losses due to sinking or viral lysis (e.g. [16, 17]) are not accounted for in this model, yet incorporating these processes may be important for interpreting empirical data from dilution experiments. We also note that the model intends to describe bulk community dynamics. Diversity is not considered as phytoplankton and microzooplankton communities are each treated as a single population, neglecting potential important functional differences between species. Calbet et al. [18] consider trophic chains; but overall limited attention has been given to diversity.

Another critique of the method is that it does not take into account the resource requirements for phytoplankton growth. In the absence of microzooplankton ( $Z_0 = 0$ ) this model predicts the phytoplankton population will grow without bounds to infinity as niche competition between phytoplankton is not considered. Subsequent modifications of the dilution method included a nutrient enrichment step so that phytoplankton could grow near their idealized maximum rates [14]. Even if nutrients are added to bottles to attempt to keep phytoplankton growing in the exponential growth phase, this does not eliminate the potential of competition occurring between phytoplankton. While many potential limitations exist (some of which are included in the discussion), in this paper we choose to focus on how the inclusion of niche competition may alter the interpretation of dilution experiment measurements.

A common way to represent niche competition between phytoplankton is by using a logistic growth model [19]. Logistic growth is a phenomenological model used to implicitly represent competition for resources, whilst not tracking those resources explicitly. It is a common tool in mathematical ecology and has been used to describe phytoplankton growth dynamics (e.g. [20]). Using a logistic growth function has the effect of bounding phytoplankton populations to a carrying capacity  $K$ . We note that the inclusion of logistic growth could be interpreted to mean that phytoplankton mortality increases as  $P$  approaches  $K$  or that phytoplankton growth decreases as  $P$  approaches  $K$ , or a combination thereof. A candidate model considering the effect of niche competition is:

$$\frac{dP}{dt} = \overbrace{rP \left( 1 - \frac{P}{K} \right)}^{\text{logistic growth}} - \overbrace{aPZ}^{\text{grazing}}. \tag{3}$$

Using Eq (3) as the description for phytoplankton dynamics we find the apparent growth rate within a bottle in the dilution series is predicted as:

$$\frac{1}{P} \frac{dP}{dt} \Big|_{FP_0, FZ_0} = r - \left( \frac{rP_0}{K} + aZ_0 \right) F. \tag{4}$$

The model of Eq (3), including niche competition, also predicts a linear relationship between dilution level and apparent niche growth rates, as shown in Eq (4). Here, the intercept of

the dilution curve represents the maximum growth rate, just as in the classical model. However, the slope is now interpreted as the *combined* effect of niche competition and grazing, both of which necessarily have positive signs. Therefore, dilution experiments may overestimate grazing rates should niche competition be important. We hypothesize that niche competition will be important when the functional form of phytoplankton growth is better approximated by a saturating growth function than by an unbounded growth function. In this paper we explore this hypothesis and ways to improve dilution experiments when both grazing and niche competition operate concurrently.

### Materials and methods

In this paper we utilize ecological models of phytoplankton dynamics analyzed *in silico*. For the *in silico* dilution experiments, whole seawater is mixed with the diluent at 10 dilution levels (with proportions  $F = 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9$  and 1 of WSW) to create a dilution series with 10 bottles. The apparent growth rate,  $\tilde{r}$ , within each bottle is calculated from recordings of the simulated population dynamics as:

$$\tilde{r} = \frac{1}{T} \log \left( \frac{P_T}{P_0} \right) \tag{5}$$

where  $P_0$  is the phytoplankton density at the beginning of the simulation and  $P_T$  is the phytoplankton density after time  $T$  [12]. Here the apparent growth rate is calculated using an incubation time of 24h in length. Dilution method estimates of grazing mortality are then calculated by finding the slope of the linear regression fit of apparent growth rates within each simulated bottle in the dilution series (response) against the proportion,  $F$ , of WSW it contains (predictor). The intercept of this regression is understood as representing phytoplankton growth rate, whilst the slope is interpreted as the grazing mortality rate. Model parameters for *in silico* dilution experiments are shown in Table 1. Code for the following analyses is available [21].

### Assessment

#### Top-down pressure as indicator of control of phytoplankton by grazers

In the absence of microzooplankton the steady state solution for phytoplankton population density within the bottle is  $P_A^* = K$ . Similarly, the steady state in the presence of microzooplankton is  $P_Z^* = K \left( 1 - \frac{aZ_0}{r} \right)$ . Neither of these densities are necessarily reached during the dilution experiment. Nonetheless, these densities provide a means to quantify the relative importance of grazing. To see why, note that increasing microzooplankton pressure will lead to a reduction in  $P_Z^*$ , away from  $P_A^*$ . Microzooplankton pressure is maximised when the per

Table 1. Ecological parameters used in this study.

Symbol	Description	Value	Units
$r$	Intrinsic per capita growth rate of phytoplankton	1	day <sup>-1</sup>
$K$	phytoplankton carrying capacity	$2.2 \times 10^7$	phytoplankton ml <sup>-1</sup>
$a$	microzooplankton filtering rate	$4.8 \times 10^{-5}$	ml/(microzooplankter·day)
$Z_{0L}$	Initial microzooplankton density at low pressure	1000	microzooplankton ml <sup>-1</sup>
$Z_{0I}$	Initial microzooplankton density at intermediate pressure	10000	microzooplankton ml <sup>-1</sup>
$Z_{0H}$	Initial microzooplankton density at high pressure	20000	microzooplankton ml <sup>-1</sup>
$P_0$	Initial phytoplankton density	$K \left( 1 - \frac{aZ_0}{r} \right)$	phytoplankton ml <sup>-1</sup>

<https://doi.org/10.1371/journal.pone.0177517.t001>

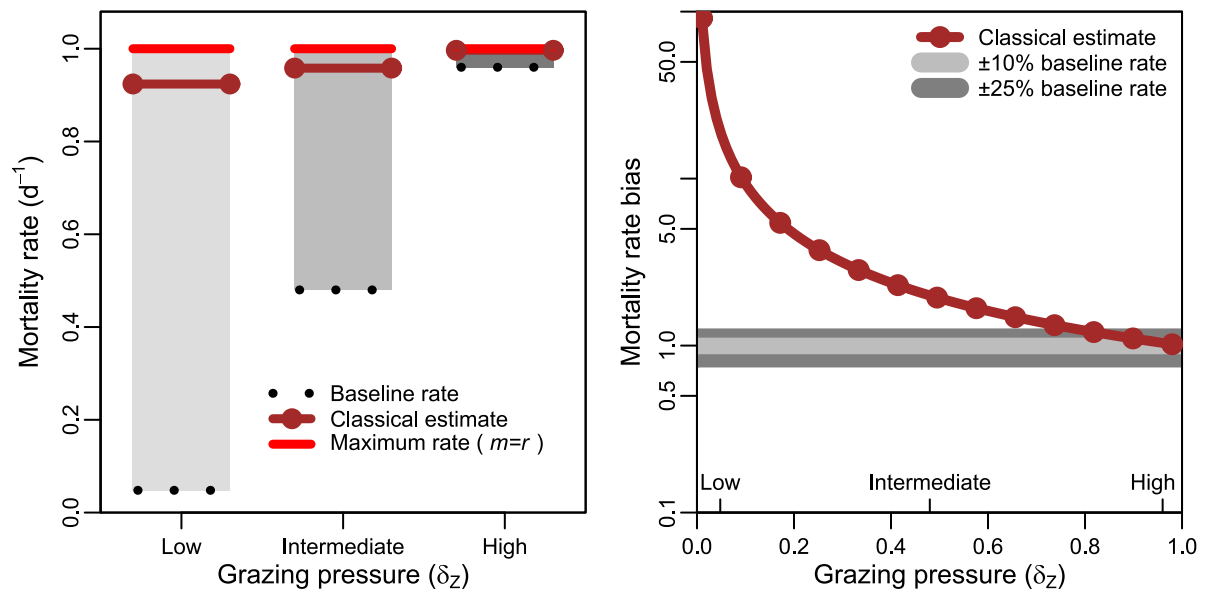
capita mortality rate due to grazing,  $aZ_0$ , is equal to the per capita intrinsic phytoplankton growth rate,  $r$ . We can quantify top-down control of phytoplankton by the microzooplankton by using  $\delta_Z$  as a measure of **grazing pressure**:

$$\delta_Z = 1 - \frac{P_Z^*}{P_A^*} = \frac{aZ_0}{r}. \tag{6}$$

In the absence of microzooplankton, no grazing occurs and  $\delta_Z = 0$ . If top-down microzooplankton grazing were to drive steady state phytoplankton density to half of the resource limited density then  $\delta_Z = 0.5$ . When microzooplankton grazing results in the phytoplankton population being drawn to extinction ( $P_Z^* = 0$ ), then top-down control by microzooplankton is maximised and  $\delta_Z = 1$ . We use three specific levels of grazing pressure (**low**:  $\delta_Z = 0.048$ ,  $aZ_0 = 0.002 \text{ day}^{-1}$ , **intermediate**:  $\delta_Z = 0.48$ ,  $aZ_0 = 0.02 \text{ day}^{-1}$ , **high**:  $\delta_Z = 0.96$ ,  $aZ_0 = 0.04 \text{ day}^{-1}$ ) to highlight the performance and sensitivity of the dilution method to the dominance of microzooplankton grazing as a driver of phytoplankton mortality.

### Sensitivity of mortality rate estimates to niche competition

The ability of the dilution method to estimate microzooplankton associated mortality rates is potentially affected by the relative level of bottom-up (i.e., niche competition) and top-down (i.e., microzooplankton) pressures. This is highlighted in Fig 2A using simulations with the three chosen levels of grazing pressure. Each level of grazing pressure corresponds to a steady state microzooplankton density (see Table 1) from which a steady state phytoplankton density,  $P^*$ , is calculated while keeping the growth rate,  $r$ , fixed at 1 per day. The grazing mortality rate was estimated using the dilution experiment, in order to compare to the baseline mortality rate  $aZ_0$ . Estimates made using the classical dilution method were closest to the baseline rates



**Fig 2. The classical dilution method may overestimate rates of mortality via grazing.** (A) Expected baseline microzooplankton associated mortality rates and rates estimated using the classical dilution method for three levels of grazing pressure; low grazing pressure (1000 microzooplankton ml<sup>-1</sup>), intermediate grazing pressure (10000 microzooplankton ml<sup>-1</sup>) and high grazing pressure (20000 microzooplankton ml<sup>-1</sup>). The maximum mortality rate is calculated for the condition when total mortality,  $m$ , is equal to the phytoplankton growth rate  $r$ . (B) Mortality rate bias across the full gradient of grazing pressure. The grazing pressure associated with each of the examples given in (A) are shown on the x-axis.

<https://doi.org/10.1371/journal.pone.0177517.g002>

when grazing pressure was high, but substantially higher than the expected baseline rates when grazing pressure was low. Mortality rate bias is calculated as the estimated grazing mortality rate (by the dilution method) divided by the baseline grazing mortality rate. Examining the resulting bias in mortality rates (Fig 2B) we find that baseline mortality is overestimated by a factor of  $\approx 20$  at the highlighted low grazing pressure, a factor of  $\approx 2$  at the highlighted intermediate grazing pressure and by less than 5% at the highlighted high grazing pressure. Thus mortality rate bias is lowest when grazing pressure is highest, corresponding to situations where phytoplankton densities approach 0. This suggests that the classical dilution method provides more accurate estimates of microzooplankton associated mortality rates when grazing pressure is high i.e., *when grazing is significantly more important than niche competition*.

### Conflating grazing with population growth inhibition

Grazing mortality rates are overestimated by the classical dilution method when microzooplankton grazing pressure is low. Indeed, the estimated grazing mortality rates appear closer to the maximum phytoplankton growth rate (see Fig 2A). This feature of the classical dilution method arises due to the inability of the method to disentangle grazing and niche competition as shown in Eq (4). As a simple modification, we assume that the WSW sample is dynamically at steady state;  $P^*$  and  $Z^*$  denoting the steady state densities in the environment. Therefore, the initial conditions for our *in silico* experiments for a bottle with dilution factor  $F$  are  $P = FP^*$  and  $Z = FZ^*$ , where  $Z^* = Z_0$  and  $P^* = K(1 - \frac{aZ_0}{r})$ . Substituting these quantities into Eq (4) we find:

$$\left. \frac{1}{P} \frac{dP}{dt} \right|_{FP^*, FZ^*} = r(1 - F). \tag{7}$$

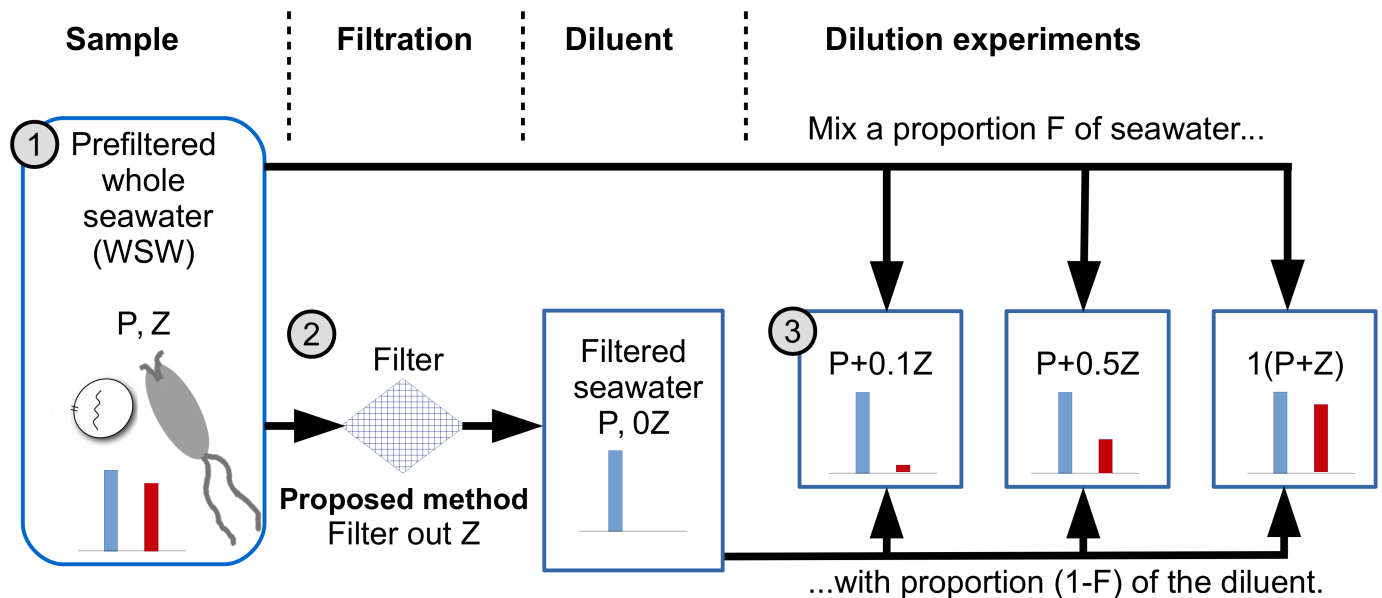
In the limit when  $F = 0$ , we find that the per capita rate of change is  $r$ ; whilst at  $F = 1$ , the per capita rate of change is 0. At steady state phytoplankton growth is balanced by phytoplankton mortality. When microzooplankton grazing pressure is high, mortality due to grazing is much greater than mortality due to niche competition and so  $r \approx aZ_0$ . However, as grazing pressure decreases the relative importance of mortality due to competition between phytoplankton increases and  $r$  is no longer a good estimate of grazing mortality. Thus, an alternative interpretation for the slope calculated by the classical dilution method is that it represents **the combined rate of phytoplankton mortality by both microzooplankton grazing and by niche competition**.

### Diluting microzooplankton alone enhances grazing rate estimates

In the classical dilution method the incubated samples contain diluted levels of both phytoplankton and microzooplankton [12]. Rather than enriching the medium (see [14]) we propose a different approach: altering the filter to exclude microzooplankton but not phytoplankton cells. Thus, altering the proportion of WSW used within each treatment will only change the initial microzooplankton density—representing a linear gradient between no microzooplankton and ambient levels of microzooplankton; whilst maintaining ambient levels of phytoplankton. We first explore this approach conceptually and revisit the practical constraints of implementation in the Discussion. This “Z-dilution” method, is depicted in Fig 3.

In this case, we expect the per capita bottle population dynamics for a dilution level  $F$  to be:

$$\left. \frac{1}{P} \frac{dP}{dt} \right|_{P_0, FZ_0} = r \left( 1 - \frac{P_0}{K} \right) - (aZ_0)F \tag{8}$$



**Fig 3. Proposed revision to the classical dilution method.** Whilst the classical dilution method (see Fig 1) uses a filter excluding phytoplankton and microzooplankton, the proposed method instead uses an alternative filter, able to exclude microzooplankton, but through which phytoplankton can pass. Thus constituent levels of microzooplankton and phytoplankton within each bottle, shown by red and blue bars respectively, differ to those in the classical dilution experiment.

<https://doi.org/10.1371/journal.pone.0177517.g003>

where the slope of the dilution curve is here found as the grazing rate. This slope is precisely the baseline grazing mortality rate we hope to estimate using the dilution method. This analytical result suggests this revised method should accurately estimate grazing mortality rates, regardless of the level of grazing pressure.

As before, we can investigate the expected per capita growth rate *in silico* using the Z-dilution method under steady state dynamics. By substituting the initial conditions for this revised dilution method,  $P = P^*$  and  $Z = FZ^*$ , into Eq (4) we obtain the per capita phytoplankton rate of change as:

$$\left. \frac{1}{P} \frac{dP}{dt} \right|_{P^*, FZ^*} = aZ_0(1 - F). \tag{9}$$

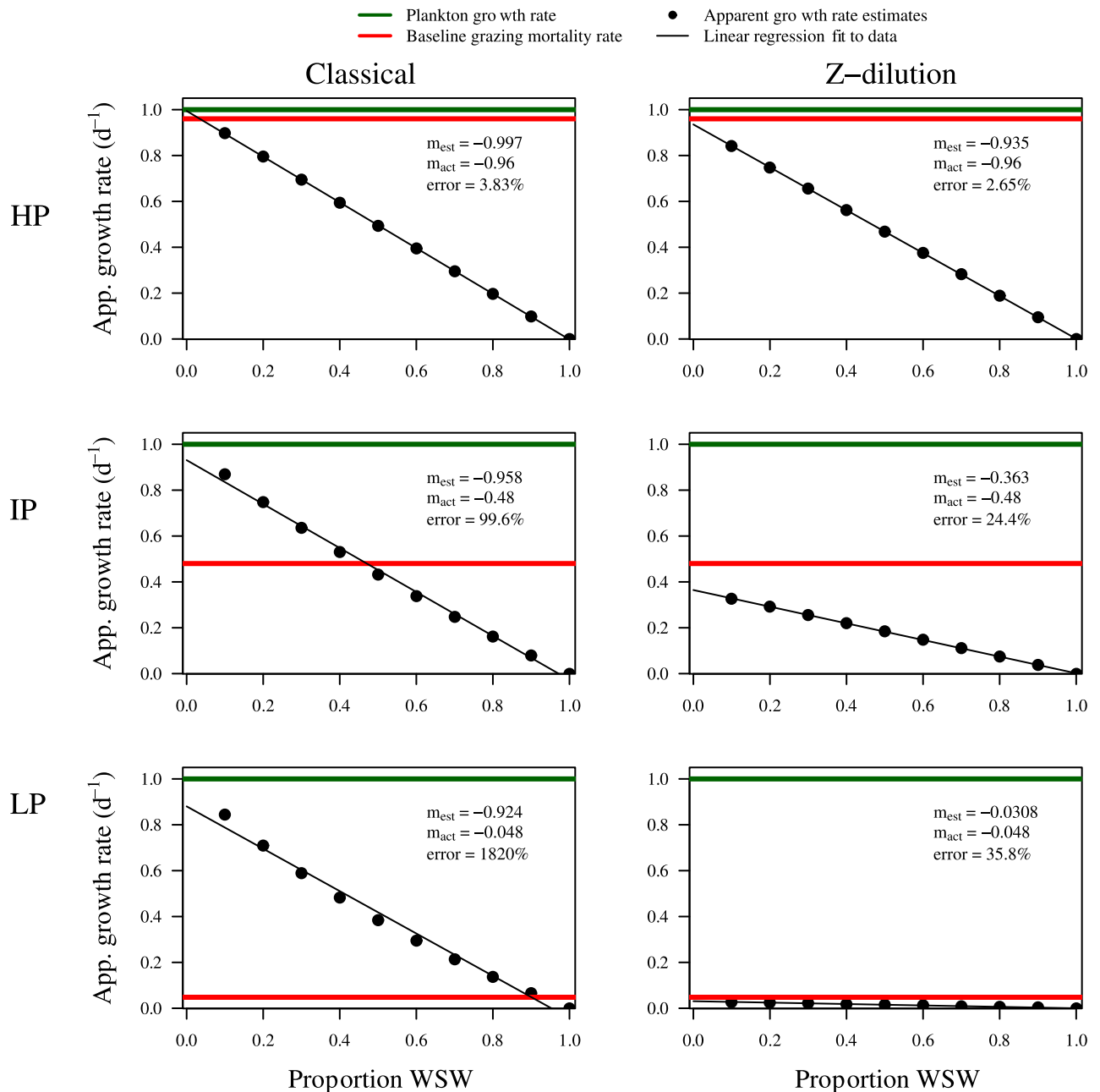
Under steady state conditions we expect the dilution curve to have a slope of  $aZ_0$  and an intercept of  $aZ_0$ .

### Comparison of classical and revised dilution methods

We now compare the performance and robustness of the classical and Z-dilution methods. We do so by varying the strength of grazing pressure. Fig 4 shows the dilution curves measured for both methods after 24h incubation for the three highlighted conditions of grazing pressure. The linear regression fits for the classical dilution method have a slope (and intercept) close to the intrinsic growth rate under all three conditions. In contrast, the slope (and intercept) found by the Z-dilution method is closer to the identified baseline rates of microzooplankton associated mortality rate. This provides numerical evidence confirming the analytical results found in Eqs (7) and (9).

As a consequence, the classical and Z-dilution methods give substantially different estimates of grazing mortality rates (Fig 5). When microzooplankton grazing pressure is high



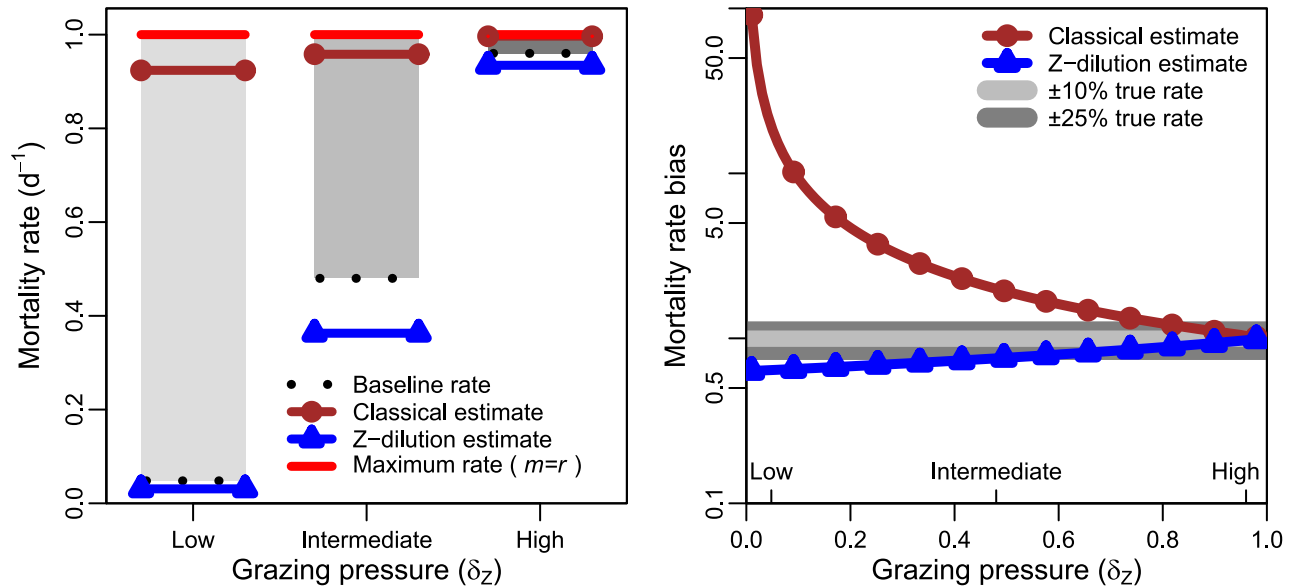


**Fig 4. Dilution curves show the classical dilution method is insensitive to niche competition.** Apparent growth rates are plotted against the proportion of whole seawater for each bottle in the *in silico* dilution series after a 24h incubation period when using the classical dilution method and the Z-dilution method respectively. Three cases, each with different microzooplankton grazing pressure conditions (LP: Low pressure, IP: Intermediate pressure and HP: high pressure, as defined in Fig 2) are shown. The estimated mortality rate ( $m_{est}$ ) found as the linear regression slope, the baseline mortality rate ( $m_{act}$ ) and the percentage error in estimation are shown for each subplot (all rounded to 3 s.f.).

<https://doi.org/10.1371/journal.pone.0177517.g004>

estimates made by both methods are within 10% of the true mortality rate after a 24h incubation period. However, the ability to estimate mortality rates declines with reduced grazing pressure. The classical dilution method overestimates microzooplankton associated mortality. The revised method, in which only microzooplankton are subject to dilution, tends to underestimate the true rate. The degree to which the revised method underestimates the expected





**Fig 5. A comparison of classical and Z-dilution method estimates.** (A) Mortality rates and their estimates at three levels of grazing pressure after 24h incubation period. The maximum mortality rate is calculated for the condition when the mortality,  $m$ , is equal to the phytoplankton growth rate  $r$ . Baseline mortality rates are shown for each condition. (B) Mortality rate bias is plotted against the level of grazing pressure ( $\delta_z$ )—the three conditions shown in (A) are marked on the x-axis. Bands indicating  $\pm 10\%$  and  $\pm 25\%$  differences from the true mortality rate in the sample are shown.

<https://doi.org/10.1371/journal.pone.0177517.g005>

rates is substantially less than that by which the classical dilution method overestimates the expected rates. This is particularly evident when grazing pressure is low.

## Discussion

Estimated microzooplankton grazing rates are central to efforts to understand the relative importance of top-down vs. bottom-up forces in the global oceans. We find that the performance of mortality rate estimates is dependent on the filtering apparatus used by the dilution method and to the relative amount of top-down control. In this paper we reviewed the robustness of the classical dilution method and a proposed alternative, the “Z-dilution” method. Classical dilution theory works well when niche competition is low relative to microzooplankton grazing. But, in circumstances when niche competition is an important control of phytoplankton populations, we predict that the classical method will lead to an overestimation of grazing mortality. Instead, we find that diluting microzooplankton, but not phytoplankton, represents a conceptually feasible approach to isolate the effects of grazing from niche competition. In doing so, the method goes beyond efforts to estimate grazing exclusively—whether in the classical dilution method or in extensions that include enrichment [12, 14].

We found that the slope of the dilution curve in the classical dilution method is expected to be equivalent to the combined effect of niche competition and microzooplankton grazing (Eq (4)). Our work suggests that if the actual population dynamics within each dilution bottle includes both grazing mortality and niche competition, then finding the differences between the slopes (or equivalently, using this model, the intercepts) of the classical and the Z-dilution methods would provide a way to quantify the effects of niche competition. We note that an alternative and complementary approach for assessing the relative impact of niche competition could be found by measuring the differences between the slopes of the classical [12] and

nutrient-enriched [14] dilution experiments. If niche competition is unimportant, then one should expect to recover the same dilution curve for the classical, the nutrient-enriched, and the Z-dilution methods. It is important to highlight the fact that in the Z-dilution method we assume the use of a filter that can exclude microzooplankton, but allow all phytoplankton to pass through. In practice, it will be important to evaluate the extent to which size- or feature-specific filtering can be achieved when they share similar and overlapping size-spectra. The method can be applied to well-characterized artificial or lab ecosystems with non-overlapping size distributions. Doing so may challenge the interpretations made by the classical dilution method. However, even if full separation of grazers and phytoplankton is not possible *in situ*, this does not necessarily preclude the Z-dilution from being useful. If limited size-spectra overlap exists the Z-dilution method may still provide estimates close to that it would have attained if full phytoplankton-grazer separation was possible. Developing novel filtering methods and evaluating the robustness of the Z-dilution method to partial overlap represent future challenges.

This is not the first time that the use of the dilution method has been critically assessed (e.g. [13–15, 18, 22]) or suggested to overestimate the impact of grazing. Indeed, a recent meta-analysis found that many studies erroneously applied tests that assessed the significance of mortality rates based on whether they were greater than or less than zero, rather than strictly greater than zero [23, 24]. Other studies have also suggested that grazing rates may be overestimated due to changes within the grazer communities [25]. In addition to statistical and measurement uncertainty it is important to address model uncertainty. As populations are only measured twice during dilution experiments, pre- and post-incubation, it is unknown to what extent the assumption of exponential growth given by classical dilution theory is held. Assuming grazer communities are constant on the timescale of dilution experiments we found that microzooplankton associated mortality rates, found via the classic dilution method, may be overestimated in environments when microzooplankton grazing pressure is low. However, we suggest that previous rate estimates made by the classical dilution method could potentially be used as an upper bound for the true grazing mortality rate. Similarly, estimates made via nutrient-enhanced approaches could be compared to those here as a means to gauge the *relative* importance of grazing with respect to niche competition. One may expect nutrient-limited oligotrophic regions to experience strong niche competition, but whether the relative importance of niche competition with respect to grazing in these environments is also strong is an open question. It might be, counter to expectation, that the relative strength of niche competition may be low with respect to grazing even in oligotrophic conditions. As we do not know the degree of grazing pressure *a priori* when performing dilution experiments, this will be important to assess—and the Z-dilution method provides a conceptual approach to do so.

The model presented in our manuscript is purposefully simple in order to convey our key message: classical grazing rate estimates may be conflated with niche competition. The ability to which it is possible to critically measure ecological properties using dilution experiments hinges on the appropriate formulation of the underlying ecological model used to interpret the data. Future theory work should focus on more realistic model descriptions of phytoplankton interactions and dynamics. Investigating community diversity e.g. differences in prey selectivity, allelopathic competition, virus host range and other important ecological traits, could be important for accurately interpreting the bulk community rates obtained by performing dilution experiments [26]. It may also be important to further develop resource explicit models to account for nutrient limited phytoplankton growth and the dynamics of resources during dilution incubations [27, 28]. Another important focus could be the relevance of mixotrophic lifestyles [29, 30] to dilution rate measurements. We encourage those who use the dilution method to consider how the Z-dilution method can improve quantitative understanding of

the importance of niche competition. Additionally, we encourage researchers to consider the response of other components of phytoplankton mortality with respect to dilution. The present analysis further supports the need to combine theory and experiments together to improve understanding of ecosystem functioning of marine microbes.

## Acknowledgments

This work was supported by a grant from the Simons Foundation (SCOPE Award ID 329108, J.S.W.). We thank David Caron, Debbie Lindell, Mick Follows and anonymous reviewers for feedback that has improved this manuscript.

## Author Contributions

**Conceptualization:** SJB JSW.

**Data curation:** SJB.

**Formal analysis:** SJB JSW.

**Funding acquisition:** JSW.

**Investigation:** SJB.

**Methodology:** SJB JSW.

**Project administration:** SJB JSW.

**Software:** SJB.

**Writing – original draft:** SJB JSW.

**Writing – review & editing:** SJB JSW.

## References

1. Sherr EB, Sherr BF. Significance of predation by protists in aquatic microbial food webs. *Antonie van Leeuwenhoek*. 2002; 81(1-4):293–308. <https://doi.org/10.1023/A:1020591307260> PMID: 12448728
2. Calbet A, Landry MR. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnology and Oceanography*. 2004; 49(1):51–57. <https://doi.org/10.4319/lo.2004.49.1.0051>
3. Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F. The ecological role of water-column microbes in the sea. *Marine Ecological Progress Series*. 1983; 10:257–263. <https://doi.org/10.3354/meps010257>
4. Turner JT. Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. *Progress in Oceanography*. 2015; 130:205–248. <https://doi.org/10.1016/j.pocean.2014.08.005>
5. Barbeau K, Moffett JW, Caron DA, Croot PL, Erdner DL. Role of protozoan grazing in relieving iron limitation of phytoplankton. *Nature*. 1996; 380:61–64. <https://doi.org/10.1038/380061a0>
6. Kirchman DL. *Microbial ecology of the oceans*. vol. 36. John Wiley & Sons; 2010.
7. Weitz JS, Wilhelm SW. Ocean viruses and their effects on microbial communities and biogeochemical cycles. *F1000 Biol Rep*. 2012; 4:17. <https://doi.org/10.3410/B4-17> PMID: 22991582
8. Brum JR, Morris JJ, Décima M, Stukel MR. Mortality in the oceans: causes and consequences. *Association for the Sciences of Limnology and Oceanography*. 2014; p. 16–48.
9. Falkowski PG, Barber RT, Smetacek V. Biogeochemical controls and feedbacks on ocean primary production. *Science*. 1998; 281(5374):200–206. <https://doi.org/10.1126/science.281.5374.200> PMID: 9660741
10. Follows MJ, Dutkiewicz S, Grant S, Chisholm SW. Emergent biogeography of microbial communities in a model ocean. *Science*. 2007; 315(5820):1843–1846. <https://doi.org/10.1126/science.1138544> PMID: 17395828

11. Lima-Mendez G, Faust K, Henry N, Decelle J, Colin S, Carcillo F, et al. Determinants of community structure in the global plankton interactome. *Science*. 2015; 348(6237):1262073. <https://doi.org/10.1126/science.1262073> PMID: 25999517
12. Landry MR, Hassett RP. Estimating the grazing impact of marine micro-zooplankton. *Marine Biology*. 1982; 67(3):283–288. <https://doi.org/10.1007/BF00397668>
13. Evans GT, Paranjape MA. Precision of estimates of phytoplankton growth and microzooplankton grazing when the functional response of grazers may be nonlinear. *Marine Ecology Progress Series*. 1992; 80(2):285–290. <https://doi.org/10.3354/meps080285>
14. Landry MR, Kirshtein J, Constantinou J. A refined dilution technique for measuring the community grazing impact of microzooplankton, with experimental tests in the central equatorial Pacific. *Marine Ecology Progress Series*. 1995; 120(1):53–63. <https://doi.org/10.3354/meps120053>
15. Chen B, Laws EA, Liu H, Huang B. Estimating microzooplankton grazing half-saturation constants from dilution experiments with nonlinear feeding kinetics. *Limnology and Oceanography*. 2014; 59(3): 639–644. <https://doi.org/10.4319/lo.2014.59.3.0639>
16. Evans C, Archer SD, Jacquet S, Wilson WH. Direct estimates of the contribution of viral lysis and microzooplankton grazing to the decline of a *Micromonas spp.* population. *Aquatic Microbial Ecology*. 2003; 30(3):207–219. <https://doi.org/10.3354/ame030207>
17. Kimmance SA, Brussaard CPD. Estimation of viral-induced phytoplankton mortality using the modified dilution method. *Limnology and Oceanography: Methods*. 2010; 7:65–73.
18. Calbet A, Saiz E. Effects of trophic cascades in dilution grazing experiments: from artificial saturated feeding responses to positive slopes. *Journal of Plankton Research*. 2013; 35(6):1183–1191. <https://doi.org/10.1093/plankt/ftb067>
19. Verhulst PF. Recherches mathématiques sur la loi d'accroissement de la population. *Nouveaux Mémoires de l'Académie Royale des Sciences et Belles-Lettres de Bruxelles*. 1845; 18:14–54.
20. Lewis ND, Breckels MN, Archer SD, Morozov A, Pitchford JW, Steinke M, et al. Grazing-induced production of DMS can stabilize food-web dynamics and promote the formation of phytoplankton blooms in a multitrophic plankton model. *Biogeochemistry*. 2012; 110(1-3):303–313. <https://doi.org/10.1007/s10533-011-9649-0>
21. Beckett SJ, Weitz JS. Code for: Disentangling the Relative Strength of Niche Competition from Grazing-Induced Phytoplankton Mortality. Zenodo. 2017;.
22. Agis M, Granda A, Dolan JR. A cautionary note: examples of possible microbial community dynamics in dilution grazing experiments. *Journal of Experimental Marine Biology and Ecology*. 2007; 341(2): 176–183. <https://doi.org/10.1016/j.jembe.2006.09.002>
23. Latasa M. Comment: A potential bias in the databases of phytoplankton growth and microzooplankton grazing rates because of the improper formulation of the null hypothesis in dilution experiments. *Limnology and Oceanography*. 2014; 59(3):1092–1094. <https://doi.org/10.4319/lo.2014.59.3.1092>
24. Landry MR. On database biases and hypothesis testing with dilution experiments: Response to comment by Latasa. *Limnology and Oceanography*. 2014; 59(3). <https://doi.org/10.4319/lo.2014.59.3.1095>
25. Dolan J, McKeon K. The reliability of grazing rate estimates from dilution experiments: Have we overestimated rates of organic carbon consumption by microzooplankton? *Ocean Science*. 2005; 1:1–7. <https://doi.org/10.5194/os-1-1-2005>
26. Strom SL. Microbial ecology of ocean biogeochemistry: a community perspective. *Science*. 2008; 320(5879):1043–1045. <https://doi.org/10.1126/science.1153527> PMID: 18497289
27. Andersen T, Schartau AKL, Paasche E. Quantifying external and internal nitrogen and phosphorous pools, as well as nitrogen and phosphorous supplied through remineralization, in coastal marine plankton by means of a dilution technique. *Marine Ecology Progress Series*. 1991; 69:67–80. <https://doi.org/10.3354/meps069067>
28. Mills MM, Ridame C, Davey M, La Roche J, Geider RJ. Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature*. 2004; 429:292–294. <https://doi.org/10.1038/nature02550> PMID: 15152251
29. Ward BA, Follows MJ. Marine mixotrophy increases trophic transfer efficiency, mean organism size, and vertical carbon flux. *Proceedings of the National Academy of Sciences*. 2016; 113(11):2958–2963. <https://doi.org/10.1073/pnas.1517118113>
30. Caron DA. Mixotrophy stirs up our understanding of marine food webs. *Proceedings of the National Academy of Sciences*. 2016; 113(11):2806–2808. <https://doi.org/10.1073/pnas.1600718113>