ELSEVIER

Journal of Experimental Marine Biology and Ecology 341 (2007) 176–183

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

A cautionary note: Examples of possible microbial community dynamics in dilution grazing experiments

Martin Agis, Angelica Granda [1], John R. Dolan *

Université Pierre et Marie Curie-Paris6, Laboratoire d'océanographie de Villefranche, 06230, Villefranche-sur-Mer, France
CNRS, Marine Microbial Ecology Group, Laboratoire d'océanographie de Villefranche, Station Zoologique, B.P. 28, 06230 Villefranche-Sur-Mer, France

Received 19 May 2006; received in revised form 21 August 2006; accepted 12 September 2006

Abstract

Dilution experiments are used commonly to provide estimates of grazing pressure exerted on phytoplankton and bacterioplankton as well as estimate their growth rates. However, very little attention has been given to the dynamics of grazers, especially heterotrophic nanoflagellates (HNF), in such experiments. We found temporal changes in concentrations of ciliates and HNF in a dilution experiment using water from the oligotrophic N.W. Mediterranean Sea. Ciliates decreased markedly over 24 h when held in seawater diluted with particle-free water (60% and 20% final conc whole seawater) while HNF increased in concentration in the same treatments. Using a time-course approach in a second experiment, we monitored changes in HNF and bacterioplankton concentrations in 20% whole seawater (80% particle-free seawater). Both HNF and heterotrophic bacteria displayed stable concentrations for the first 12 h and then grew rapidly, especially HNF, from 12 to 24 h. Examination of bacterial community composition using denaturing gel gradient electrophoresis (DGGE) showed a change in community composition over the 24 h incubation period. Dilution can have differential effects on the distinct components of the marine microbial food web.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Bacterioplankton; Grazing; Microzooplankton; Nanozooplankton

1. Introduction

Dilution is a time-honored method in plankton ecology. Kirchman et al. (1982) proposed diluting natural planktonic communities with consumer or predator-free water to estimate growth in bacterioplankton. Landry and Hassett (1982) introduced the use of a dilution series to measure phytoplankton growth in situ and grazing losses. It is the most widely employed method to estimate grazing of microzooplankton and usually chlorophyll concentrations are employed as a metric of phytoplankton biomass (e.g., Bamstedt et al., 2000). In recent years, it has become very common to employ the dilution method of Landry and Hassett (1982) to estimate growth and grazing losses of picoplankton, especially heterotrophic bacteria but also that of phototrophic prokaryotes *Synechococcus* or *Prochlorococcus*. In 2005 reports alone, dilution grazing experiments were used to estimate grazing losses and growth rates in a large variety of marine and estuarine systems ranging from estuaries and coastal areas to open ocean waters (e.g., Bec et al., 2005; Berninger and Wickham, 2005; Collos et al., 2005; Fileman and Leakey, 2005; Garces et al., 2005; Jochem

* Corresponding author.
E-mail address: dolan@obs-vlfr.fr (J.R. Dolan).
[1] Present address: Department of Ecology, University of Oviedo.

0022-0981/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2006.09.002

et al., 2005; Leising et al., 2005; Strzepek et al., 2005; Troussellier et al., 2005; Umani et al., 2005; Yokokawa and Nagata, 2005).

The dilution method, like any other method, can yield uninterpretable results. For dilution grazing experiments, this can take the form of insignificant correlation between prey growth rate estimates and dilution factor; variable proportions of uninterpretable results have been reported (Dolan et al., 2000; Worden and Binder, 2003). Attempts have been made to refine the dilution grazing method by selecting certain data, (either 3 or 2 dilution treatments) when the dilution series gives a non-linear result (e.g., Gallegos, 1989; Evans and Paranjape, 1992; Worden and Binder, 2003).

Non-linear relations between prey growth rates and dilution factor are typically ascribed to changes in the feeding behavior of grazers when prey availability is altered (e.g., Moigis, 2006). However, dilution may be associated with more than changes in feeding behaviour of grazers. For herbivorous ciliates, changes in the concentrations and composition of ciliate microzooplankton in typical dilution experiments have been documented in estuarine (Dolan et al., 2000) and marine communities (Dolan and McKeon, 2005). Dilution is known to affect heterotrophic bacteria. Changes occur in the community composition of bacterioplankton in seawater diluted with particle-free water over periods ranging from 24 to 48 h (e.g., Franklin et al., 2001; Beardsley et al., 2003; Fuchs et al., 2000). Surprisingly, there is to our knowledge no data on the responses of heterotrophic nanoflagellates to dilution. In fact, to our knowledge, the dynamics of all the three 'microbial loop' populations, ciliates, heterotrophic nanoflagellates and bacteria, have not been examined together in these experiments.

Here we present results from an examination of the dynamics of the three major components of the microbial loop: ciliates, heterotrophic nanoflagellates (HNF), generally assumed to be the major grazers of picoplankton, and heterotrophic bacteria (HB) in 2 dilution experiments. Our goal was examine the dynamics of microbial populations when diluted with particle-free seawater. In our experiments we found that nanoflagellate concentrations may, over a 24 h incubation time, be unrelated to dilution factor (but perhaps related to ciliate growth or mortality) and the community composition of the bacterioplankton can shift during a dilution experiment.

2. Materials and methods

The study was carried out in September–October 2004. Water for experiments was collected at 10 m depth from 'Point B', a standard oceanographic station at the mouth of the Bay of Villefranche (43°41′10′′N, 7°19′0′′E) using Niskin bottles. Filtered sea water was prepared using GF/F and 0.2 μm filters and a peristaltic pump and then mixed with appropriate volumes of whole seawater. The seawater used was not filtered to remove metazoan zooplankton for 2 reasons. Firstly, screening can damage ciliate microzooplankton and secondly, copepod abundances were very low, 0.2 individuals l^{-1}, based on plankton tows taken nearly simultaneously (Gasparini and Antajan, in press). For each 'dilution level' 10 l was prepared in a single carboy; the water was gently mixed and a set of 3 polycarbonate incubation bottles filled with 2.4 l of the solution.

2.1. Experiment 1

The first experiment, performed the 20–21st of September, consisted of 3 dilution levels: 20%, 60% and 100% whole seawater. From each dilution level carboy, a single sample was taken for the determination of initial chlorophyll concentration. Individual bottles were sampled immediately prior to (t_0) and at the end (t_{24}) of the incubation period, to determinate the abundance of ciliate microzooplankton, heterotrophic nanoflagellate (HNF) and heterotrophic bacteria (HB).

Incubations took place on the dock of the Station Zoologique. Bottles were placed in a flow-through seawater bath, covered with a neutral density filter yielding 50% incident illumination (measured inside filled incubation bottles using a LICOR instrument) similar to 10 m depth light conditions at the Pt B sample point (Dolan, unpublished observations). After 24 h, samples were taken from each bottle to determine t_{24} chlorophyll *a* concentration and abundance of micro-organisms.

Samples for fluorometric determination of chlorophyll *a* were filtered through GF/F filters, frozen and extracted in 10 ml acetone 90% (Lorenzen, 1967). Chlorophyll *a* concentration was determined by fluorimetry on a Turner Designs fluorometer. The apparent growth rate of phytoplankton (k) and grazing rate of microzooplankton (g) were calculated following Landry and Hassett (1982).

To determine concentrations of ciliate microzooplankton, 200 ml samples were taken from each dilution bottle, at t_0 and t_{24}, fixed in Lugol's solution (2% final conc) and refrigerated. Either aliquots (100 ml) or the entire sample was settled and material examined using inverted microscopy. To determine concentrations of HNF and heterotrophic bacteria, 200 ml samples were taken, fixed with EM-grade gluteraldehyde (1% final conc), and stored refrigerated. Aliquots (20–100 ml) of these samples were stained with diamidino-2-phenylindole (DAPI), filtered onto 0.2 μm black polycarbonate

Download English Version:

https://daneshyari.com/en/article/4397760

Download Persian Version:

https://daneshyari.com/article/4397760

Daneshyari.com