Journal of Experimental Marine Biology and Ecology 403 (2011) 65–74

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Experimental simulation of trophic interactions among omnivorous copepods, heterotrophic dinoflagellates and diatoms

Mianrun Chen, Hongbin Liu *

Division of Life Science, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong

ARTICLE INFO

Article history:
Received 28 July 2010
Received in revised form 11 April 2011
Accepted 12 April 2011
Available online 8 May 2011

Keywords:
Diatoms
Grazing
Heterotrophic dinoflagellates
Omnivorous copepods
Trophic cascade
Trophic interactions

ABSTRACT

Trophic cascades in the marine pelagic food web, especially in the microbial food web in which copepods are the top controllers, are difficult to quantify and are generally overlooked. In this study, we simulated a simple pelagic food web in laboratory feeding experiments to demonstrate the trophic interactions among copepods, heterotrophic dinoflagellates and chain-forming diatoms and to quantify the trophic cascade effect and the true feeding rate of copepods. Results from the study showed that heterotrophic dinoflagellates play a central role in the lower trophic level of the marine food web by consuming diatoms and by serving as a quality food source for copepods, especially during the period of diatom blooms. Copepod omnivory was mediated by the concentration of diatoms, with the highest ingestion of diatoms occurring at intermediate diatom abundances and the selectivity toward dinoflagellates increasing as the diatom concentration increased. The trophic cascade was surprisingly low and even negative in treatments with low diatom concentrations, suggesting a competition effect when there was not enough diatom food. In this situation, the omnivorous copepods together with heterotrophic dinoflagellates exhibited strong herbivory and were able to control diatoms. In contrast, a high trophic cascade effect occurred at high diatom concentrations in which copepods had a high ingestion of heterotrophic dinoflagellates. We conclude that the role of copepods in the food web structure is mediated by the concentrations of diatoms, but one of the most important biological factors that determine the strength of trophic cascades is the ingestion rates of both copepods and heterotrophic dinoflagellates.

1. Introduction

The diets of pelagic copepods are characteristically broad (Kleppel, 1993) and strict herbivory of copepods rarely exists in nature. Most copepods prefer feeding on microzooplankton due to their large size, easy perception, as well as the relatively high food quality (Batten et al., 2001; Gifford et al., 2007; Campbell et al., 2009). Moreover, copepods are capable of switching their feeding behavior depending on the prey composition, and particularly on the relative abundance of phytoplankton and microzooplankton (Landry, 1981). Thus, copepods in marine ecosystems link the grazing food chain and the microbial loop and transfer materials and energy from producers to higher trophic levels (Stibor et al., 2004). Recently, the feeding impact of omnivorous copepods on different prey components is of increasing interest as it is a key to understand the dynamics of marine planktonic food web.

One of the oldest methods that is still widely used to measure the feeding rate of copepods is the bottle incubation method by adding a number of copepods into the treatments and determining the prey concentrations at the beginning and the end of the experiments. The feeding rate of herbivores is then estimated by directly comparing the phytoplankton growth rates in treatment bottles with those in control bottles according to the equations of Frost (1972). However, this estimation would definitely underestimate the feeding rates of omnivores on phytoplankton and on smaller particles (nano- and picoplankton) without considering the different grazing rates of microzooplankton between the treatments and controls because both the concentration and grazing activity of microzooplankton likely change in the presence of omnivores. In recent years, many studies have revealed that microzooplankton are the most important consumers of phytoplankton and other microbes within the microbial food web due to their comparatively high abundance and higher grazing rates than copepods (Calbet and Landry, 2004; Liu et al., 2005a). When microzooplankton are consumed by copepods, grazing by microzooplankton on phytoplankton and other smaller food particles is reduced.

The increase in phytoplankton and other small planktonic organisms due to the grazing of copepods on microzooplankton is defined as a trophic cascade (Pace et al., 1999) and it may result in an underestimation of copepod feeding rate. The term trophic cascade was first introduced by Paine (1980) for the interactions among food webs of benthic ecosystems. Carpenter et al. (1985) applied the term of cascading trophic interactions in studies of limnetic plankton and showed that biomasses of adjacent trophic levels are negatively correlated in the

* Corresponding author. Tel.: +852 23587341; fax: +852 23581559.
E-mail address: liuhb@ust.hk (H. Liu).

0022-0981/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.jembe.2011.04.009

scenario of top-down control. Efforts to estimate the effect of trophic cascades in the marine pelagic food web are still quite limited (Sommer, 2008); studies on the microbial food web in which copepods are the top controllers are especially rare (Liu et al., 2005a; Sommer and Sommer, 2006; Zöllner et al., 2009). In the previous research on marine food webs, the trophic cascade was generally overlooked unless a "negative" feeding rate of copepods was observed (Leising et al., 2005b). In fact, in the bottle incubation method, the observed feeding rates determined by directly comparing the prey concentration between the control and treatments are the net result of two opposite factors, the direct consumption of copepods (negative) and the trophic cascade (positive) induced by copepod ingestion of intermediate protist grazers. Thus, the negative feeding rates of copepods on phytoplankton and on small prey particles would be expected if the trophic cascade effects outweigh the direct ingestion of copepods. In order to accurately estimate mesozooplankton herbivory and the roles of copepods and microzooplankton as consumers in controlling phytoplankton blooms, there is a need for accurate quantification of both the trophic cascade and the direct feeding of copepods on phytoplankton.

Nejstgaard et al. (2001) attempted to correct the underestimation of copepod herbivory on natural phytoplankton caused by trophic cascades by introducing a correction term for the loss of microzooplankton grazing (k_p) into the equation of Frost (1972). The microzooplankton grazing rate in their approach was achieved by a separate dilution experiment (Landry and Hassett, 1982). The authors assumed that microzooplankton grazing was only dependant on the mean concentration of microzooplankton during the incubation period and the decrease of microzooplankton grazing in copepod treatment bottles was proportional to the decrease of microzooplankton abundance in the treatment bottles as compared to that in the control bottles. This assumption is achieved if the grazing activities of microzooplankton in treatments and controls are the same. However, the grazing activities of microzooplankton are seldom the same because of possible changes in the functional response of microzooplankton grazing in response to the changes in phytoplankton concentrations during the incubation which can be significantly different between treatments and controls. To reduce the laborious work associated with dilution experiments, Klaas et al. (2008) attempted to determine the correction based on a simple ecosystem model without a separate dilution experiment. However, the amount or proportion of phytoplankton that is increased by copepod suppression of microzooplankton grazing is still not clearly stated by the model.

Therefore, in order to quantify the effect of a trophic cascade in incubation experiments, a simple but reliable way to estimate microzooplankton grazing that combines the functions of both mean concentrations of phytoplankton and microzooplankton must be established. In this study, we applied a simple pelagic food web in laboratory feeding experiments to demonstrate the trophic interactions among copepods, microzooplankton and phytoplankton and to quantify the trophic cascade effect and the real feeding rate of copepods. The food web included a copepod (omnivorous mesozooplankton predators), a heterotrophic dinoflagellate (intermediate microzooplankton grazers) and a diatom (phytoplankton primary producer). The trophic cascade for chain-forming diatoms is rarely reported, although the potential important roles of heterotrophic dinoflagellates in grazing chain-forming diatoms and in providing good nutritional quality food for copepod reproduction have been recognized (Veloza et al., 2006; Sherr and Sherr, 2007). Jeong et al. (2004) reported that a heterotrophic dinoflagellate *Protoperidinium bipes* isolated from temperate coastal waters off Korea had a considerable grazing impact on the chain-forming diatom *Skeletonema costatum*.

To ensure that the same species that was isolated from subtropical coastal waters of Hong Kong is also one of the potential grazers of chain-forming diatoms and to establish the relationship between ingestion and prey concentration, we first conducted a feeding experiment to measure the growth and ingestion rate of *P. bipes*, as a function of prey concentrations. Copepod feeding selectivity and trophic cascades were then assessed in a feeding experiment using *Acartia erythraea* as the grazer in the presence of *P. bipes* and a range of different concentrations of *S. costatum*. The degree of omnivory for *Acartia* varies with environmental conditions and in particular its feeding mode shifts with the proportion of microzooplankton and phytoplankton in the food (Gifford and Dagg, 1988). Hence, the effect of a trophic cascade would change as the ratio of heterotrophic dinoflagellates and diatoms changes. Our goal was to elucidate the general pattern of interactions between predator and prey with the appearance of an intermediate grazer and to quantify the scale of trophic cascades under different food concentrations through a laboratory simulation experiment.

2. Materials and methods

2.1. Preparation of experimental organisms

The diatom *Skeletonema costatum* was maintained in f/2 medium under a 14:10 light:dark cycle of approximately 100 μmol photons $m^{-2}s^{-1}$ at 24 °C. Cultures were kept in exponential growth phase and used for maintaining heterotrophic dinoflagellates and in feeding experiments. The median cell volume of *S. costatum* (67 μm^3) was determined by measuring the width and length of 200 cells under microscope and converted to carbon content (8.7 pg C $cell^{-1}$) according to Menden-Deuer and Lessard (2000).

The heterotrophic dinoflagellate *Protoperidinium bipes* was isolated from Port Shelter on the east coast of Hong Kong in April 2008. The isolates were placed in 1.2 L polycarbonate (PC) bottles and *S. costatum* was added as food to a final concentration of 5000 cells ml^{-1} and 100 ml f/2 medium was added to support the growth of *S. costatum*. The bottles were placed on a Grant OLS200 orbital/linear shaking bath at 20 °C and a shaking speed of 80–90 rotations min^{-1}. Three days later, aliquots of the enriched cultures were transferred to several Petri dishes and *P. bipes* was isolated and placed with diatom food (5000 cells ml^{-1}). A culture of *P. bipes* was then established by two serial single cell isolations. The median cell volume of *P. bipes* (1123 and 1211 μm^3 with and without copepod predation, respectively) was calculated according to Jeong et al. (2004) by measuring cell length and maximum width of 200 cells under a inverted microscope, and the cell volume was converted to carbon content (192–205 pg C $cell^{-1}$) according to Menden-Deuer and Lessard (2000).

The calanoid copepod *Acartia erythraea* was collected with a net tow from eastern Hong Kong waters (Port Shelter) in July 2008. Contents of the cod-end were transferred to a 10 L plastic container enriched with both *P. bipes* and *S. costatum* and continuously aerated. Healthy, active adult females of *A. erythraea* were sorted next day from the mixture for feeding experiments.

2.2. Feeding experiment of P. bipes on S. costatum

Usually 2 days before the feeding experiments were conducted we stopped adding *S. costatum* to the stock cultures of *P. bipes* and transferred the upper 80% volume of the culture to a 10 L PC carboy to obtain a large volume of *P. bipes* stock with high density, but containing a low concentration of the target prey *S. costatum*. This process helped to minimize the effect of residual growth during the batch culture (Jeong et al., 2004). To determine the abundance of *P. bipes* and its prey in the stocks of *P. bipes* and *S. costatum*, triplicate 10 ml and 1 ml aliquots were taken from the carboy and flask, fixed with 5% Lugol's acid solution. Triplicate 1 ml aliquots were also taken from the carboy containing *P. bipes* and fixed to determine the residual amount of *S. costatum* that was carried over in the feeding experiments. *P. bipes* and *S. costatum* were counted under an inverted microscope in a 24-well and a 96-well plate, respectively.

At the beginning of the experiments, 6 treatments (each with triplicates) containing mixtures of a known concentration of grazers