Title:

Chain response of microbial loop to the decay of a diatom bloom in the East China Sea

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Abstract

Algal bloom has been regarded as one of the key causes for the summer hypoxia phenomena in the bottom water adjacent to the Yangtze River estuary in the East China Sea. Although a series of biological processes within microbial loop are involved in the development of oxygen depletion during the bloom decay, little has been known about the dynamics of microorganisms in response to the decaying process of the bloom through trophic interaction context. Here, we report some preliminary results of our observations about the response of microbial loop to the bloom decay, based on the onboard incubation experiments for 10 days during a diatom bloom near the Yangtze River estuary in August, 2011. Light and dark incubations were conducted to simulate the bloom decay inside and below the euphotic layer, respectively. In the first stage of bloom decay (Day 0 to Day4), rapid response was found in heterotrophic bacteria (HB) and ciliate growth, which was in accordance with the decrease of total Chl *a*, indicating a “bottom-up” control at the early stage of bloom decay. However, the increase of heterotrophic nanoflagellates (HNF) abundance was rather inconspicuous, suggesting predation pressure on HNF from ciliate or other predator at this stage. In the second stage (Day 4 to Day8), HB and ciliate decreased rapidly with the increase of HNF, revealing the release of HNF from ciliate predation, which suggested a “top-down” control. In the last stage of our experiment (Day 8 to 10), the trophic interactions were more complex, but it also implied a “top-down” control within the microbial loop. Meanwhile, virus had been monitored in the whole process of our incubations. It was found that virus lysed microalgae at the first stage, and lysed HB at the second stage. In addition, the

bacterial mortality was principally caused by HNF grazing in the light-sufficient incubations and by viral lysis in the light-insufficient incubations. Our results suggest tight trophic interactions within the microbial loop in the decaying process of the algal bloom, which may assist our understanding of the role of microbial loop in hypoxia formation in coastal waters.

Keywords: chain response, microbial loop, bloom decay, trophic interaction

1. Introduction

The Yangtze River estuary is a seasonally hypoxic zone in the East China Sea (ECS) (Chen et al., 2007; Wei et al., 2007), where massive phytoplankton blooms have been observed almost every summer (Chen et al., 2003; Gao and Song, 2005). A vast amount of the primary production and nutrients fixed by phytoplankton were directed to the microbial loop (Kamiyama et al., 2000; Weisse et al., 1990), which is a key role in controlling carbon cycling and nutrient flow in the marine ecosystems (Azam, 1998; Gilbert et al., 1998; Jumars et al., 1989). Dissolved organic matter (DOM) released from phytoplankton through lysis or grazing is the major energy sources for heterotrophic bacteria (HB) in oceans and lakes, which may be responsible for microbial degradation processes (Arrigo, 2004; Azam, 1983; Brussaard et al., 1995). A large amount of studies about the changes of bacterial communities in phytoplankton blooms have been reported in most of aquatic ecosystems (Fandino et al., 2001; Larsen et al., 2004; Rink et al., 2007; Yager et al.,

2001) and it has been confirmed that HB is a significant biotic factor in the process of biogeochemical cycling in aquatic ecosystems (Azam, 1983; Cole et al., 1988). As a major predator of HB, heterotrophic nanoflagellate (HNF) is an important link between HB and other higher trophic levels (such as ciliate) in microbial loop (Taylor, 1982). Through grazing on bacteria, HNF has a great influence on remineralization and **plays** a crucial role in carbon and nutrient recycling in pelagic ecosystems (Azam, 1983; Burns and Gilbert, 2006; Jürgens et al., 1999; Lim et al., 2005).

Besides prokaryotic and eukaryotic microorganisms, virus is considered to be a new component of the microbial loop (Bergh et al., 1989; Bratbak et al., 1990; Proctor and Fuhrman, 1990; Suttle et al., 1990). Over the years, numerous reviews have pointed out that virus acts as an important biological control on the mortality of phytoplankton (particularly during blooms) and bacteria (Brussaard et al., 1996; Gobler et al., 1997; Reisser, 1993; Short, 2012; Suttle, 1994). Although grazing by HNF seems responsible for the mortality of bacteria, viral lysis is also considered as a potential factor for bacterial loss (Proctor and Fuhrman, 1990; Suttle et al., 1990). Moreover, recent studies have also implicated that virus has an effect on the termination or control of various algal blooms (Brussaard et al., 1995; Castberg et al., 2001; Gastrich et al., 2004; Tomaru et al., 2007; Van Boekel et al., 1992).

In addition, light is one of the most important factors in determining the microbial communities in the aquatic ecosystems (Jiao et al., 2002). During bloom decay, when nutrients are depleted, phytoplankton cells will form aggregates, which will eventually sink out of the euphotic layer to the deep water. However, due to the wave

94 action, a large portion of the aggregates may suspend and remain in the euphotic layer
95 for quite a period of time. Till now, little is known about the difference in the
96 responses of microbial loop to bloom decay inside and below the euphotic layer of the
97 water column.

98 It can be expected that most of the organic carbon accumulated in algal blooms
99 will be combusted within microbial loop, accompanying with a significant reduction
100 of oxygen concentration in the water column, which in turn may affect the
101 composition of microbial loop (Dagg et al., 2007; 2008). Thus, algal bloom has been
102 suggested as one of the key causes for the summer hypoxia phenomena in the bottom
103 water adjacent to the Yangtze River estuary in the ECS (Li et al., 2002; Rabouille et
104 al., 2008; Zhu et al., 2011), where microbial organisms (including prokaryote,
105 eukaryote and virus) are active and have a fluctuation in their abundance during the
106 oxygen depletion process (Baird et al., 2004; Brk-Laitinen et al., 2012; Park and
107 Cho, 2002). During the last decades, the microbial loop have been widely
108 investigated in fresh water and marine ecosystems (Chen et al., 2008; Chen et al.,
109 2009; Chen et al., 2010; Lefranc et al., 2005; Moon-van Der Staay et al., 2001).
110 However, little attention has been paid on the trophic interactions within microbial
111 loop during the bloom decay, especially the “top-down” control, and thus the role of
112 microbial loop (from HB to ciliate and virus as well) in the fate of algal bloom is still
113 poorly understood.

114 In the current study, our aims were to investigate the response of microbial loop
115 to the decay of algal bloom (as well as the process of oxygen depletion), and figure

out the different role of virus in different stage of the bloom decay. Meanwhile, we also try to identify the difference of the above response between inside and below the euphotic layer. For this purpose, the incubation experiments accompanied with multiple measures were adopted allowing for a detailed description of changes in the microorganisms (*i.e.* phytoplankton, heterotrophic bacteria, nanoflagellate, ciliate and virus) and the associate parameters (*i.e.* nutrients, dissolved organic matter and dissolved oxygen).

2. Materials and Methods

A multidisciplinary investigation, aboard *R.V. Beidou*, on the bottom water oxygen depletion phenomenon around the ESC coastal waters, was carried out from August 10 to 31, 2011. The scope of survey area (27.3–33.4°N, 121.9–126.1°E) is shown in Fig. 1. High concentration ($\sim 8 \text{ mg/m}^3$) of Chl *a* in the surface water was detected at Stn.F4 (29.0°N, 123.2°E) on August 19. The microscopic examination revealed that it was a diatom bloom at the beginning of declining phase. In order to track the succession of microbial loop along with the process of bloom decay in the water, the incubation experiments were conducted from August 19 to 29. The surface seawater at Stn.F4 was collected by 10 L Niskin bottles on a “Sea Bird” CTD, then transferred into 14 polycarbonate carboys (20L) and incubated in a tank on the forecastle of the vessel. Six of the incubation carboys were wrapped in thick black plastic bags to simulate light-insufficient condition in the water below the euphotic layer (D-group). The others were directly exposed to sun light to simulate

light-sufficient condition in the euphotic layer (L-group). Water bath was achieved by pumping seawater continuously from the sea surface to the incubation tank. Sampling was conducted at 10.00~11.00 a.m. on Days 0, 4, 8 and 10 from the start of incubation (Day 0). Samples were duplicated, and each carboy served as a sample.

Figure 1

Dissolved oxygen (DO) was measured with Winkler method as described in Zhu et al., (2011). Water samples (500 ml) for chromophoric dissolved organic matter (CDOM) detection were filtered with Whatman GF/F filters and the absorption spectra measurement was performed following the protocol of Guo et al. (2011). The absorption coefficient at 280 nm (a_{280}) was chosen as an index of CDOM concentration. Samples (300 ml) for Chl *a* analyses were filtered on to 0.2 µm Millipore polycarbonate membranes filters and extracted with 90% acetone and determined fluorometrically (Parsons et al., 1977). Samples (100 ml) for nutrients determination were filtered through Whatman GF/F filters and analyzed using an Auto-Analyzer Skalar SAN^{plus} (Liu et al., 2010).

Samples (250ml) for phytoplankton identification and cell counting were fixed with formaldehyde (1%, final conc.) and analyzed in the laboratory with inverted microscope as described by Utermöhl (1958). Samples (1L) for ciliate identification and enumeration were fixed with Lugol's solution (1%, final conc.) and counted using an inverted microscope (Olympus IX51) at 100× or 200× magnification as described in Yu et al. (2013). The sampling and enumeration of nanoflagellate principally followed the protocol of Lin et al. (2013). The fluorescently labeled bacteria (FLB)

was used to quantify bacteria mortality caused by HNF grazing (Sherr and Sherr, 1987). One milliliter of FLB suspension (1×10^7 FLB/ml) was added to 100 ml seawater, which was pre-filtered through a nylon mesh of 20- μm pore size by gravity. After FLB addition, samples were incubated in the dark at the same temperature as the original incubation. Subsamples (20ml) were fixed with glutaraldehyde (1%, final conc.) at selected time intervals (0 and 15 min of incubation, respectively) and filtered onto 0.2- μm pore size Millipore black polycarbonate membrane filter at low vacuum pressure (<100 mm Hg), then stained with DAPI (10 $\mu\text{g}/\text{ml}$, final conc.) for 10 min and examined in the laboratory via epifluorescence microscopy (Leica DM4500B) at 1000 $\times$ magnification. The enumeration of HB was run with a FACSVantage SE flow cytometer (Becton Dickinson) equipped with a water-cooled Argon laser (488 nm, 1 W, Coherent) and the counting of virus stained with SYBR Green I (Molecular Probes) was using epifluorescence microscopy (Olympus BX51); more detail is described in Zhao et al. (2013).

The two-tail t-test was used to compare changes in the biotic and non-biotic parameters during the experiments, and one-way ANOVA was used to test for the differences in the above parameters between the treatments.

3. Results

3.1 Process of the blooms decay

Throughout the incubation, phytoplankton was dominated by diatoms, which approximately contributed to 98% of algal cell density, while dinoflagellates and

chrysophytes were present at a far lower level. The maximum density of phytoplankton was found at the start of the incubation (1.2×10^3 cell/ml), then it continuously decreased, and more rapidly in D-group than in L-group (Fig. 2A). *Skeletonema* sp. dominated the phytoplankton community in the beginning. In L-group, *Pseudo-nitzschia pungens* became the predominant phytoplankton species from Day 4 to 8, followed by *Thalassiosira* sp., then *Cylindrotheca closterium* at the end of the incubation (Day 10). In D-group, the phytoplankton community was dominated by *Pseudo-nitzschia pungens* and *Skeletonema* sp. throughout the incubation (Table 1).

Figure 2

Table 1

As with the drop of phytoplankton cell density, Chl *a* decreased from 7.14 mg/L to 0.59 mg/L in both groups, but faster in D-group than in L-group (Fig. 2B).

The variation of CDOM showed a similar pattern in both groups. The decrease of a_{280} from the beginning of the incubation to Day 4 indicated a reduction of CDOM concentration, after that the concentration of CDOM increased. In general, the CDOM concentration was a little lower in D-group than in L-group (Fig. 2C).

The concentration of dissolved oxygen (DO) drastically declined during the bloom decay in both groups, from 9.22 mg/L to 7.31 mg/L and 6.53 mg/L in L-group and D-group, respectively (Fig. 2D). Although the DO concentration did not decrease to the hypoxic level (the threshold value is commonly defined as < 3 mg/L) at the end of the incubation, the t-test result showed a significant difference between L-group

and D-group ($t = 3.57$, $P < 0.05$).

The concentration of nitrate decreased from $4.27 \mu\text{M}$ to $< 1 \mu\text{M}$ in L-group but quite stable in D-group throughout the incubation (Fig. 2E). On the other hand, the concentration of phosphorous was low ($< 0.1 \mu\text{M}$) in L-group and a faint increase was observed at the end of the incubation, while a strong increase was detected in D-group on Day 4 (Fig. 2F).

3.2 Dynamics within the microbial loop

In both incubation groups, the ciliate assemblage was characterized by nano-sized aloricate ciliates (Fig. 3) and the nanoflagellate community was dominated by HNF (Fig. 4). HNF in the size class of $2\text{-}5 \mu\text{m}$ was the most abundant HNF throughout the incubation (Fig. 5).

The abundance of ciliate dramatically fluctuated during the incubation, which increased significantly in the early stage (Day 0 to 4) of the bloom decay in L-group from $3.5 \times 10^3 \text{ ind./L}$ to a maximum of $7.2 \times 10^3 \text{ ind./L}$, and then continuously decreased to the end of the incubation (Fig. 6A). In D-group, however, the abundance of ciliates showed a weak fluctuation around $3.3 \times 10^3 \text{ ind./L}$ (Fig. 6A).

The abundance of HNF in L-group was low from Day 0 to Day 4, about $1.7 \times 10^3 \text{ cells/ml}$, and rose drastically to a level of $10.3 \times 10^3 \text{ cells/ml}$ from Day 4 to Day 8, then decreased rapidly at the end of the incubation (Fig. 6B). In D-group, HNF was rather constant at a lower abundance of about $1.8 \times 10^3 \text{ cell/ml}$ during the incubation (Fig. 6B).

The bacterial abundance ranged from $6.6 \times 10^5 \text{ cells/ml}$ to $20.4 \times 10^5 \text{ cells/ml}$ and

226 4.4×10^5 cells/ml to 14.8×10^5 cells/ml in L-group and D-group, respectively (Fig. 6C).

227 In L-group, the abundance of bacteria increased to a peak value at Day 4 and then
228 decreased dramatically but rose again towards the end of the incubation. In D-group,
229 bacterial abundance showed a roughly similar pattern with L-group before Day 8, but
230 the increase was not observed in the last 2 days of the incubation.

231 The viral abundance ranged from 8.7×10^6 cells/ml to 25.9×10^6 cells/ml and
232 18.7×10^6 cells/ml in L-group and D-group, respectively (Fig. 6D). In L-group, the
233 abundance of virus increased to a peak at Day 4, then slightly decreased, and finally
234 slightly rose again at the end of the incubation. In D-group, however, the viral
235 abundance increased to a peak at Day 8 and then decreased.

Figure 7

237 The grazing rate of HNF on bacteria was $11.2 \text{ cells h}^{-1} \text{ ind}^{-1}$ on the onset of the
238 experiment, then rose to $137.6 \text{ cells h}^{-1} \text{ ind}^{-1}$ and $41.9 \text{ cells h}^{-1} \text{ ind}^{-1}$ at Day 4 in the
239 L-group and D-group, respectively. After that, the grazing rate dropped to a low level
240 in both groups, then rose again at the end of the incubation (Fig. 7A).

242 4. Discussions

243 4.1 Response of microbial loop to the bloom decay

244 Heterotrophic bacteria (HB) constitutes an important part of the pelagic food web
245 by utilizing DOM and converting DOM into particulate biomass available to higher
246 trophic levels through the microbial loop (Azam, 1983). Hence, the dynamics of
247 bacterial community is not merely controlled by resources (bottom-up control), such

as the quality of DOM (Kirchman et al., 2000), but also by predation (top-down control), such as the grazing by HNF. HNF is thought to be a major trophic link in the microbial loop as dominant consumers of prokaryotes in marine waters (Strom, 2000). Their abundance may be regulated by either the availability of food resources (bacteria and picoeukaryotes) or the predation by ciliates (Weisse and Scheffell-Möser, 1991) and even copepods (Stoecker and Capuzzo, 1990). Thus, ciliates can be considered as a trophic link between nanoplankton and mesozooplankton (Calbet and Saiz, 2005).

In our incubation experiments, the relatively high abundance of each component (i.e. HB, HNF and ciliates) within the microbial loop in both incubation groups (i.e., light sufficient L-group and light insufficient D-group) suggests that the DOM produced by (or released from) phytoplankton enhanced the flow of energy from HB through HNF to ciliates.

Table 2

In the first stage of bloom decay (Day 0 to Day 4), the increases in bacterial abundance was observed in both groups (Fig. 6C), which coincided with a decrease in CDOM concentration (Fig. 2C). However, the abundance of HNF were relatively constant in both groups (Fig. 6B), despite the increasing grazing activity of HNF upon HB in this stage (Fig. 7A). This may be due to strong predation pressure on HNF from ciliates, which showed an rapid increase in abundance from Day 0 to Day 4 (Fig. 6A). All of these results suggest a bottom-up control within the microbial loop during the first stage of bloom decay (Table 2).

In the second stage of bloom decay (Day 5 to Day 8), the abundance of ciliate decreased in both groups (Fig. 6A), suggesting a predation control on ciliates by mesozooplankton (Calbet and Saiz, 2005). Along with the decrease in ciliate abundance, HNF grew to the peak in L-group, while merely a slight increase in D-group (Fig. 6B). In spite of this, the decrease of bacterial abundance was detected in both groups (Fig. 6C) and related to an increase in CDOM concentration (Fig. 2C), indicating a strong depression control from HNF grazing or viral lysis. These results indicate a prevailing top-down control in microbial loop during the second stage of bloom decay (Table 2).

In the last stage of the incubation (Day 9 to Day 10), the situation was much more complicated. A simultaneous decrease in the abundance of ciliate and HNF was observed in L-group, implying that HNF and ciliate may both have been depressed by the mesozooplankton predation. Nonetheless, the decrease of HNF abundance in L-group clearly corresponded with an increase of bacterial abundance, revealing a top-down control, though less obviously, within the microbial loop.

4.2 Effects of viral lysis in bloom decay

Viral lysis is proved or regarded as an additional factor accounting for the bacterial mortality (Fuhrman and Suttle, 1993; Fuhrman and Noble, 1995; Steward et al., 1996; Suttle, 1994; Weinbauer et al., 1995; Weinbauer and Höfle, 1998) and a potential reason for phytoplankton bloom decline (Gobler et al., 2007; Tomaru et al., 2007) in aquatic systems. Some researchers have also discussed the host specificity of viral infection in plankton communities (Mann, 2006; Nagasaki, 2008). Virus to

bacteria ratio (VBR) is an important parameter indicating virus-host interactions (Wommack and Colwell, 2000). Low VBR values could suggest a limited virus effect on bacteria, while high VBR values suggest that bacterial abundance is controlled by viruses through infection (Barros et al., 2010; Bettarel et al., 2006).

In this study, the change of virus abundance between L-group and D-group was quite different or even inversed after four days of incubation (Fig. 6D). Despite of the different change of virus abundance, the VBR exhibited the similar tendency in both groups (Fig. 7B). In the first stage (Day 0 to 4), the VBR values in both group were relatively low (6.7 to 12.7 and 7.7 in L-group and D-group, respectively), indicating a low level of viral-mediated bacterial mortality. However, in this stage, the abundance of virus in the water significantly increased (especially in L-group), accompanied by the decrease of phytoplankton abundance (Fig. 2A). Thus it was most likely that the increased virus abundance came from the lysis of phytoplankton infected by virus. In the second stage (Day 5 to Day 8), VBR rose dramatically to the peak in both groups (31.8 and 45.7 in L-group and D-group, respectively), which coincided with a decrease in bacterial abundance, suggesting that some portion of the increased bacterial mortality might have been caused by viral infection (Similar explanation may be found in Wilcox and Fuhrman, 1994).

In addition, HNF grazing and viral lysis may have played a different role in different incubation groups. Grazing experiments indicated that bacterial mortality in light-sufficient incubations (L-group) was mainly caused by HNF grazing. According to the high grazing rate of HNF on bacteria in L-group (Fig. 7A), the decrease in

bacteria abundance (Fig. 6C) is thus more easily explained as a result of predation by HNF. This inference is coincided with a significant negative correlation between bacteria and HNF abundance in L-group ($r = -0.82$, $P < 0.05$). In D-group, however, we found a strong negative correlation between the abundance of bacterial and virus ($r = -0.81$, $P < 0.01$), which suggests a strong control of viral lysis on the bacterial population in light-insufficient incubations (D-group).

4.3 Effects of light on the response of microbial loop during the bloom decay

Light is considered as an important driver of energy scaling from low trophic levels up to higher trophic levels. Although the illuminance was not measured in our incubation experiments, we still can find some clues based on our data.

In light-sufficient L-group, HB was provided with more available substrate from the photosynthesis of phytoplankton, which may be easily inferred from the depletion of nutrients (Figs. 2E & F) and the slower decline of DO (Fig. 2D) in L-group during the incubation. A significant correlation relationship between HB and HNF abundance was observed ($r = -0.82$, $P < 0.05$), which implying that HNF rapidly responded to the variation of HB under the condition of enough light. In contrast, the photosynthetic production **was** reduced due to light limitation in light-insufficient D-group, which resulted in lower secondary production of HB. The abundances of HNF and ciliate in D-group showed slight fluctuations throughout the incubation. It could be explained by lower growth rates of HNF and ciliate or higher grazing pressure from microzooplankton in the light limiting environment, which leading to the inconspicuous response of microbial loop.

It also should be noted that the structure of ciliate and nanoflagellate community changed differently in the L-group and D-group. For ciliate, the tintinnids accounted for a small proportion at the beginning of the incubation, and then the proportion of tintinnids increased during the incubation in both groups. However, significant difference was found in the proportion of tintinnids between L-group (about 37.5%) and D-group (about 17.5%) ($t=2.98$, $P<0.05$) at the end of our experiment (Fig. 3). For nanoflagellate, the abundance ratio of HNF to PNF was about 1:1 at the beginning of the incubation, while the ratio was about 3:2 in the L-group and 4:1 in the D-group during the incubation (Fig. 4). All of this implied that the light condition could affect the community structure of microzooplankton, which further have an influence on the response of microbial loop.

5. Conclusions

In this study, tight trophic interactions were observed within microbial loop during the decay of a diatom bloom. Our results indicate a prevailingly bottom up control in the early stage of the bloom decay, while top down control in the later stages. It was also found that virus tend to infect and lyse microalgae at the first stage of the bloom decay, and infects and lyses HB at the second stage. During bloom decay, different light conditions (i.e., light-sufficient or light-insufficient) may lead to significant difference in oxygen consumption of the water and even has a great influence on the components of microbial loop (i.e., HB, HNF and ciliate). In addition, the bacterial mortality was principally caused by HNF grazing in light-sufficient

incubations and by viral lysis in light-insufficient incubations. This study provides an insight into the trophic interactions within the microbial loop in response to the decay of a diatom bloom in the ESC, which may assist our understanding of the role of microbial loop in hypoxia formation in coastal waters. For comprehensive understanding of such roles, further studies should focus on the changes in metabolic physiology, biochemistry and phylogenetic composition of microbial loop in the process of bloom decay.

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579

580 **Table legends**

581

582 Table 1

583 The dominant phytoplankton species and their relative abundance (RA) during the
584 incubation

585

586 Table 2

587 The variation of the main components of microbial loop and CDOM in different
588 stages of the incubation. The arrows represent increase (up arrows), decrease (down
589 arrows) and steady (horizontal arrows) in abundance or concentration.

590

Figure legends

Figure 1

The investigation area and the location of Stn.F4 in the ECS. Stn.F4 was about 150 km to the south of Hangzhou bay and at the edge of a bottom water hypoxia area in the East China Sea which reported by Chen et al.(2007) and Wei et al.(2007). The water depth of the station was 72.4m.

Figure 2

Variations of the abundance of phytoplankton (A) and the concentrations of Chl *a* (B), CDOM (C), DO (D), nitrate (E) and phosphate (F) during the incubation.

Figure 3

Relative abundance of tintinnid and aloricate ciliate in L-group and D-group during the incubation.

Figure 4

Relative abundance of HNF and PNF in L-group and D-group during the incubation.

Figure 5

Relative abundance of three size categories of HNF in L-group and D-group during the incubation.

Figure 6

Variations of the abundance of ciliate (A), HNF (B), HB (C) and virus (D) during the incubation.

Figure 7

Grazing rate of HNF on bacteria (A) and VBR values (B) during the incubation.

Table 1

The dominant phytoplankton species and their relative abundance (RA) during the incubation

Day	L-Group		D-Group	
	Species	RA	Species	RA
0	<i>Skeletonema</i> sp.	70.4%	<i>Skeletonema</i> sp.	70.4%
	<i>Pseudo-nitzschia pungens</i>	10.8%	<i>Pseudo-nitzschia pungens</i>	10.8%
4	<i>Pseudo-nitzschia pungens</i>	38.6%	<i>Pseudo-nitzschia pungens</i>	67.6%
	<i>Thalassiosira condensata</i>	30.8%	<i>Thalassiosira condensata</i>	9.8%
8	<i>Pseudo-nitzschia pungens</i>	35.6%	<i>Pseudo-nitzschia pungens</i>	56.7%
	<i>Cylindrotheca closterium</i>	20.8%	<i>Skeletonema</i> sp.	18.5%
	<i>Leptocylindrus danicus</i>	11.2%		
10	<i>Cylindrotheca closterium</i>	66.7%	<i>Pseudo-nitzschia pungens</i>	52.5%
	<i>Pseudo-nitzschia pungens</i>	20.6%	<i>Skeletonema</i> sp.	21.8%

625

626 Table 2

627 The variation of the main components of microbial loop and CDOM in different
628 stages of the incubation. The arrows represent increase (up arrows), decrease (down
629 arrows) and steady (horizontal arrows) in abundance or concentration.

630

Components	Day 0 to 4		Day 5 to 8		Day 9 to 10	
	L	D	L	D	L	D
Ciliate	↗	↗	↘	↘	↘	↗
HNF	→	→	↗	→	↘	↘
HB	↗	↗	↘	↘	↗	→
CDOM	↘	↘	↗	↗	↗	↗

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634 Figure 1

Figure 2

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639 Figure 3

Figure 4

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Figure 6

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649 Figure 7