Disentangling the interactions between microbial loop and grazing food web at the southern ECS

In the marine food web, bacteria and bacterivorous nanoflagellates (NF) are the two critical trophic levels of the microbial loop. Within the microbial loop, the energy and nutrients are transferred from bacteria to nanoflagellate and cycle back to bacteria. When phytoplankton production is low in oligotrophic environment, nanoflagellates are alternative prey to small size copepods (e.g. nauplii and copepodites) and the energy and nutrients can be transferred from microbial to the classic food web. Consequently, the microbial loop plays an important role for biochemical cycling and energy transfer in the marine food web. In the marine food web, bacteria and bacterivorous nanoflagellates (NF) are the two critical trophic levels of the microbial loop. Within the microbial loop, the energy and nutrients are transferred from bacteria to nanoflagellate and cycle back to bacteria. When phytoplankton production is low in oligotrophic environment, nanoflagellates are alternative prey to small size copepods (e.g. nauplii and copepodites) and the energy and nutrients can be transferred from microbial to the classic food chain. Consequently, the microbial loop plays an important role for biochemical cycling and energy transfer in the marine food web. However, most of the studies only focus on the individual controlling factors of microbial loop and grazing food web activity. Few studies have investigated the trophic interactions among bacteria, heterotrophic nanoflagellates (HNF), phytoplankton and microzooplankton. Here, we design two modified dilution experiment to estimate the trophic interactions between HNF and bacteria (Exp I) and between HNF/phytoplankton and microzooplankton (nauplii and copepodites) (Exp II). To further estimate the trophic transfer efficiency between phytoplankton and microzooplankton, we design artificial cohort incubation for microzooplankton growth rate and sample the microzooplankton community biomass to calculate the secondary production of microzooplankton (Exp III).

**(Exp I) HNF ingestion, growth and mortality**

1. HNF ingestion and growth: Prepare 12 L 20 μm-filtered and 12 L-0.2 μm filtered, particle-free seawater (10 m depth). Fill 2L carboys with 2, 1.6, 1.2, 0.8, 0.4, 0 L 20 μm-filtered seawater and 0, 0.4, 0.8, 1.2, 1.6, 2 L 0.2 μm-filtered water, and incubate in dark 100 L tank for 12 hrs. There are two replicates for each dilution treatment. Bacteria community before and after incubation will be preserved in 0.2 % paraformaldehyde and frozen in liquid nitrogen immediately on-board. The ingestion of HNF on bacteria is calculated by the regression between the decrease of bacteria biomass and dilution ratio.

The incubation with 2L 20 μm-filtered (predator-free) seawater will be used for calculating the growth rate of HNF. The body mass and abundance of HNF before and after dilution incubation will be preserved in 1% glutaraldehyde and frozen in -20 °C on-board.

1. HNF mortality: Fill two replicates of 2L carboys with non-filtered seawater (with predator) and incubate for 12 hrs. The body mass and abundance of HNF before and after dilution incubation will be preserved in 1% glutaraldehyde and frozen in -20 °C on-board.

[Equipment]

* 20L carboys, 3 per set of experiment
* 2L carboys, 14 per set of experiment
* 2 Silicone tubes covered with 20 μm mesh
* Peristaltic pump
* 1.2 and 0.2 μm filters, 2 and 1 pieces per set of experiment.
* 100L dark incubation tanks, 0.5 per set of experiment

[Chemicals]

* 10% paraformaldehyde, 1.12 mL per set of experiment.
* 50% glutaraldehyde, 8 mL per set of experiment

[Consumables]

* 2 mL cryogenic tubes, 56 tubes per set of experiment
* 50 mL centrifuge tubes, 16 tubes per set of experiment

[Seawater volume]

4L whole water + 12L 20μm-filtered water + 12L 0.2μm-filtered water = 28 L (at least).

**(Exp II) Microzooplankton (mainly nauplii and copepodites) ingestion**

Prepare 20 L 20 μm-filtered seawater (10 m depth), which contains HNF prey. Seawater with mesozooplankton grazer is prepared by 10-min vertical tow(s) of 50 μm Norpac net with a flowmeter at 10 m depth. The water volume flowing through the net by flowmeter is recorded to back-calculate the *in situ* density of grazers. The net-concentrated grazers will be resuspended in 20 L 50 μm-filtered seawater. Fill 2L carboys with 2, 1.6, 1.2, 0.8, 0.4, 0 L of seawater with mesozooplankton grazers and 0, 0.4, 0.8, 1.2, 1.6, 2 L of 20 μm filtered seawater. and incubate in dark 100 L tank for 24 hrs. The body mass and abundance of fresh HNF and phytoplankton before and after dilution incubation will be measured recorded by FlowCAM. The community of microzooplankton (mainly nauplii and copepodites) in the beginning of incubation will be preserved in 5% formalin for estimating grazer composition and density.

 Ratio of Norpac net-concentration: *I*net = *F* /*V*cod (*F* is the water volume (liter) recorded by flowmeter; *V*cod is the volume of codend).

 Ratio of concentration after resuspension: *I*res = *I*net/(20+ *V*cod) (20 L is the volume of 50 μm-filtered seawater for resuspension).

 Real microzooplankton density: *N*meso = *N*0, meso/*I*res. (*N*0,meso is the density of microzooplankton in the beginning of the incubation).

We will calculate the real microzooplankton in the natural seawater by:

[Equipment]

* 20L carboys, 1 per set of experiment
* 2L carboys, 12 per set of experiment
* 2 Silicone tubes covered with 20 μm mesh
* 1 Silicone tube covered with 50 μm mesh
* Portable FlowCAM with fluorescent trigger
* 2L carboys, 12 per set of experiment
* 20L bucket, 1 for the cruise
* 50μm Norpac net
* flowmeter
* 100L dark incubation tanks, 0.5 per set of experiment

[Chemicals]

* 40 % formalin, 5mL or 10% formalin , 20mL per set of experiment

[Consumables]  
- 100 mL plastic bottle, 1 per set of experiment.

[Seawater volume]

12L 20μm-filtered water + 20L 50μm-filtered water = 32 L (at least)

**(Exp III) Microzooplankton (mainly nauplii and copepodites) growth and production**

Secondary production of microplankton group *i* (*SPi*) is defined as

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where *GRi* is the mass-specific growth rate and *Bi* is the biomass of group *i*. We will incubate two microzooplankton size classes: 50-80 μm (nauplii) and 100-150 μm (copepodites).

20 L cubitainers will be filled with 18 L 50 μm-filtered incubation water (at 10m depth) with plankton < 50 μm. Nauplii and copepodites will be collected by 10-min vertical tow of 50 and100 μm plankton nets at 10 m depth and gently resuspended in a bucket of 20 L 50 μm-filtered incubation water. Fill the remaining space of cubitainers with nauplii/copepodites grazer by reverse filtering the seawater with nauplii/copepodites through 80/150 μm sieves. Nauplii and copepodites will be incubated for 24 and 48 hrs, respectively. Microzooplankton community before and after incubation will be preserved in 5% formalin for analysis.

[Equipment]

* 20L cubitainers, 6 per set of experiment
* 100L dark incubation tanks, 2 per set of experiment
* 80 and 150 μm sieves for reverse filtration

[Chemicals]

* 40 % formalin, 40mL or 10% formalin , 160mL per set of experiment

[Consumables]

* 100mL plastic bottles, 8 per set of experiment

[Seawater volume]

120L 50μm-filtered water (cubitainers) + 40L 50μm-filtered water (buckets )= 160 L (at least)

**\* Time for net tows per station**

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| Sampling depth | Mesh-size | Spend-time (minutes) | Experiment |
| 10-m vertical tow | 50 μm Norpac with a flowmeter | 15 (The net will be set to 10 m depth and allowed to drift with the ship for 10 minutes) | II |
| 10-m vertical tow | 50 μm Norpac\*2 and 100 μm Norpac attached on ring | 15 (The net will be set to 10 m depth and allowed to drift with the ship for 10 minutes) | III |
| From bottom to surface oblique tow | 50 μm Norpac with Flowmeter | 15-50 min, depending in the bottom depth | II |

**\* Go-Flo bottle water collection per station**

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| Sampling depth | # of Go-Flo (20L) | Experiment |
| 10-m | 2 | I |
| 10-m | 2 | II |
| 10-m | 8 | III |