Secondary production and microbial trophic interactions along Kuroshio from subtropical to temperate area

In this cruise, we plan to measure (1) secondary production of copepods and heterotrophic nanoplankton, (2) microbial food web activity, and (3) energy transfer efficiency of heterotrophic nanoplankton,. To estimate the secondary production of mesozooplankton (mainly copepods), we will measure the biomass and growth rates by artificial cohort incubations (Exp. 1a). To estimate the secondary production of <20 μm heterogrophic nanoplankton (HNF), we plan to estimate the biomass of HNF from the sea water and measure the growth rate via predator removal experiments (Exp. 1b). From the predator removal experiments, we will also be able to estimate the predation mortality from predators that are > 20 μm in ESD. To estimate the microbial trophic interactions between <20 μm HNF and bacteria in the Kuroshio, we will conduct dilution experiments with HNF as the grazers (Exp. 2). From Exp. 1a and Exp. 2, we can estimate the energy transfer efficiency of heterotrophic nanoplankton.

1. Secondary production and growth rate

Secondary production of plankton 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 assign three main zooplankton groups by size (a) 100-150 μm (copepodites) and 50-80 μm (nauplii) copepods, and (b) 2-20 μm (flagellates) heterotrophic nanoplankton.

1. Copepod mass-specific growth rate (Exp. 1a)

Three replicates of 20 L cubitainers will be filled with 18 L incubation water from 5 m depth with plankton < 50 μm for each copepod size class (50-80 and 100-150 μm). Nauplii and copepodites in the surface water at 5 m depth will be collected by 20-min vertical tow of 50/100 μm plankton nets and gently suspended in 20 L incubation sea water. Fill the remaining space of cubitainers with nauplii/copepodites grazer by reverse filtering the sea water with copepods through 80/150 μm sieves. Nauplii and copepodites will be incubated for 24 and 48 hrs, respectively. Copepods before and after incubation will be preserved in 5% formalin and bring back to Taiwan for analysis.

Biomass of copepods will be numeralized by formalin- fixed 50 μm net samples collected by oblique tow (200 m to Surface with Flowmeter, 40-50 min).

[Equipment]

* 20L cubitainers, 6 per set of experiment (3+1 sets for the cruise)
* 100L dark incubation tanks or 100L portable coolers with lid, 2 per set of experiment (3 sets for the cruise)
* 50 μm and 100 μm Norpac net (10/3 for the cruise)
* codends (5 for the cruise)
* Flowmeter
* 80 and 150 μm sieves (diameter 50 cm) for reverse filtration
* Silicone tubes covered by 50 μm nylon filter
* Long (~1.5 m) silicone tube
* 20 L plastic buckets, 2 for the cruise
* 50 μm nylon hand-made sieves for collecting incubated copepods (20cm height cylinder sieve; 2 for the cruise)
* Pressure sprayer
* Wash bottle
* 100mL plastic bottles, 8 per set of experiment
* 1000L plastic bottles, 1 per set of experiment.

[Chemicals]

* ~140mL 40% formalin or ~560mL 10% formalin per set of experiment

[Seawater volume]

20L\*6 for cubitainers + 20L\*2 for buckets =160 L

1. Heterotrophic nanoplankton growth rate and mortality (Exp. 1b)

* Growth rate: Filled two 2L carboys with 20 μm filtered surface seawater (2-5m) and incubate for 12 hrs. This equals to 100% (not diluted) incubation in the dilution experiment. >20 μm flagellates will be collected for this experiment.
* Mortality: Filled two 2L carboys with non-filtered whole surface seawater and incubate for 12 hrs.

The nanozooplankton community before and after incubation will be preserved by 1% glutaldehyde and frozen before analysis.

Biomass of nanoplankton will be numeralized by glutaraldehyde- fixed water samples collected in the beginning of incubation.

[Equipment]

* 2L carboys, 4 per set of experiment (3 sets for the cruise)

Note: 2 carboys for nanoplankton growth are included in the dilution experiment. In the end we only need 2 carboys here.

* Silicone tubes covered by 20 μm filters
* 50 mL centrifuge tube, 8 per set of experiment

[Chemicals]

* Glutaraldehyde 8mL per set of experiment

[Seawater volume]

2L\*2 = 4 L for mortality incubation (unfiltered surface seawater, need to collect first).

1. Microbial trophic interactions
2. Heterotrophic nanoplankton ingestion on bacteria (Exp. 2)

Prepare 20 L 20 μm-filtered and 20 L 0.2 μm-filtered, particle-free surface seawater. Filled two replicates of 2L carboys with 100/80/60/40/20/0% 20 μm-filtered seawater and fill the volume left with 0.2 μm-filtered water. Incubate the seawater for 12 hrs.

Bacteria community before and after incubation will be preserved in 0.2 % paraformaldehyde and instantly frozen by liquid nitrogen.

[Equipment]

* 100L dark incubation tanks or 100L portable coolers with lid, 1 per set of experiment (2 for the cruise)
* Peristaltic pump
* Filter holders for 1.2 and 0.2 μm filter
* 20L carboys, 2 per set of experiment
* 2L carboys, 12 per set of experiment (3 sets for the cruise)
* (1-2 containers)
* 1.2 and 0.2 μm filters (3+3 per set of experiment)
* 2 mL cryogenic tubes, 48 per set of experiment

[Chemicals]

* Paraformaldehyde 960 μL per set of experiment

[Seawater volume]

2L\*3\*2 (20 μm-filtered surface seawater) + 2L\*3\*2 (0.2 μm-filtered surface seawater) = 24 L

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Equi/Che | 2L Carboy | 20 L Carboy | Silicone tube with 20 μm filter | 1.2/0.2 μm cellulous filter | 50 mL centrifuge tube | 2 mL cryogenic tube | Glutaraldehyde  (mL) | Paraformaldehyde  (μL) |
| Nanoflagellate production | -- (dilution) | --  (dilution) | --  (dilution) | --  (dilution) | 4 | 0 | 4 | 0 |
| Nanoplankton mortality | 2+1§ | 0 | 0 | 0 | 4 | 0 | 4 | 0 |
| Nanoplankton dilution | 12+1 | 2 | 2 | 3/3 | 0 | 48 (2 replicates) | 0 | 960 |

**Equipment and chemicals for seawater incubation (nanoflagellate incubation and dilution) per station**

Exp

§ For nanoflagellate mortality incubation, fill the two 2L carboys with unfiltered whole water before preparing 20 μm-filtered water for production and dilution experiments.

[Seawater volume]

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

Need 1-2 dark incubation tank for all these carboys.

**The total seawater volume for Exp 1 and Exp 2 is 160L + 28L = 188 L surface seawater (2-5m, following the sampling of bacteria community).**