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

In this cruise, we plan to measure (1) the secondary production, and (2) the ingestion rates of heterotrophic plankton. For secondary production of <200 μm plankton, we will measure the biomass and growth rates of nano- and microzooplankton by incubation of predator-removed seawater. For secondary production of mesozooplankton (mainly copepods), we will measure the biomass and growth rates by artificial cohort incubations. To fully disentangle the trophic interactions in the plankton food web in the Kuroshio, we will size-fractionated the plankton community and conducted dilution experiments on nanozooplankton, microzooplankton, and mesozooplankton grazers.

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, (b) 20-200 μm (ciliates and large flagellates) microzooplankton, and (b) 2-20 μm (flagellates) heterotrophic nanoplankton. GR and B of the three zooplankton group will be measured.

1. Copepod mass-specific growth rate

20 L cubitainers will be filled with 18 L incubation water with plankton < 50 μm. Nauplii and copepodites 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 and bring back to Taiwan for analysis.

[Equipment]

* 20L cubitainers, 6 per set of experiment
* 100L dark incubation tanks or 100L portable coolers with lid, 2 per set of experiment
* 80 and 150 μm sieves for reverse filtration
* 100mL plastic bottles, 8 per set of experiment
* formalin

1. Microzooplankton growth rate

Prepare 10 L 200 μm filtered seawater. Filled 2L carboys with 200 μm filtered seawater and incubate for 24 hrs. The body mass distribution of fresh microzooplankton before and after dilution incubation will be measured recorded by FlowCAM (ideally).

[Equipment]

* Portable FlowCAM with fluorescent trigger
* Portable pump
* 10L carboys, 1 per set of experiment
* 2L carboys, 3 per set of experiment
* 200 μm filters

1. Heterotrophic nanoplankton growth rate

Prepare 10 L 20 μm filtered seawater. Filled 2L carboys with 20 μm filtered seawater and incubate for 24 hrs. The body mass distribution of fresh microzooplankton before and after dilution incubation will be measured recorded by FlowCAM (ideally).

[Equipment]

* Portable FlowCAM with fluorescent trigger
* Portable pump
* 10L carboys, 1 per set of experiment
* 2L carboys, 3 per set of experiment
* 20 μm filters

Biomass of copepods will be estimated by zooplankton community collected by 50 μm plankton net. Biomass of microzooplankton and nanoplankton will be numeralized by glutaraldehyde- and paraformaldehyde-fixed water samples.

[Equipment]

* 50 μm plankton net
* 20 μm sieve
* paraformaldehyde
* glutaraldehyde

1. Ingestion rates

Basically, we will use size-fractionated dilution experiments to estimate the ingestion rates of meso-, micro- and nanozooplankton. We will need at least 2 dark incubation tanks for all the carboys used for the whole set of dilution experiments.

1. Mesozooplankton ingestion

Prepare 10 L 20 μm filtered seawater, which contains protist prey. Seawater with mesozooplankton grazer is prepared by 20-min vertical tow(s) of 50 μm plankton net; the net-concentrated grazers will be suspended in 20 L 50 μm-filtered seawater. Record the water volume flowing through the net by flowmeter to back-calculate the *in situ* density of grazers.

Fill 2L carboys with 100/75/50/25% seawater with mesozooplankton grazers and fill the rest volume with20 μm filtered seawater. Enrich each carboy with nutrients and incubate for 24 hrs. Prepare an additional incubation of 100% seawater with grazers without nutrient enrichment. The body mass and abundance of fresh microplankton (<200 μm) before and after dilution incubation will be measured recorded by FlowCAM.

Net-concentrating index: *I*net = *F* /*V*cod (*F* is the water volume (liter) recorded by flowmeter; *V*cod is the volume of codend).

Suspending dilution index: *I*sus = *I*net/21 (21 L is the end-volume of seawater with mesozooplankton grazers).

Real grazer density: *N*meso = *N*0, meso/*I*sus. (*N*0,meso is the concentration of mesozooplankton in the seawater with grazer at *t*0)

[Equipment]

* Portable FlowCAM with fluorescent trigger
* Portable pump
* 10L carboy, 1 per set of experiment
* 2L carboys, 3 replicates for each dilution treatment, 15 per set of experiment
* Formalin (mesozooplankton preservation)

1. Microzooplankton ingestion

Prepare 10 L 200 μm filtered and 0.2 μm filtered, particle-free seawater. Fill 2L carboys with 100/75/50/25% 200 μm filtered seawater and fill the rest volume with 0.2 μm filtered water. Enrich each carboy with nutrients and incubate for 24 hrs. Prepare an additional incubation of 100% 200μm filtered seawater without nutrient enrichment. The body mass and abundance of fresh nanoplankton (<20 μm) before and after dilution incubation will be measured recorded by FlowCAM.

[Equipment]

* Portable FlowCAM with fluorescent trigger
* Portable pump
* 10L carboys, 2 per set of experiment
* 2L carboys, 3 replicates for each dilution treatment, 15 per set of experiment
* 0.2 μm filters

1. Heterotrophic nanoplankton ingestion

Prepare 10 L 20 μm filtered and 0.2 μm filtered, particle-free seawater. Filled 2L carboys with 100/75/50/25% 20 μm filtered seawater and fill the rest volume with 0.2 μm filtered water. Enrich each carboy with nutrients and incubate for 24 hrs. The body mass and abundance of fresh nanoplankton (<20 μm) before and after dilution incubation will be measured recorded by FlowCAM (ideally). Bacteria will be filtered on 0.2 μm cellulous filter and immediately frozen for quantitative sequencing.

[Equipment]

* Portable FlowCAM with fluorescent trigger
* Portable pump
* 10L carboys, 2 per set of experiment
* 2L carboys, 12 per set of experiment
* 0.2 μm filters