**Biodiversity and abundance of bacteria and nanoflagellates in the Kuroshio Region**

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In marine food web, bacteria and nanoflagellates are the linkage between microbial loop and grazing food chain, playing an important role for energy transfer and biochemical cycling. However, our understanding on community structure of marine microbial food web and its control factors were largely confined within trophic level. Relative few studies have considered the joint effects of bacteria and nanoflagellates diversity and abundance across trophic levels. Here, we examined the biodiversity relationship and its consequences on trophic interaction between nanoflagellates and bacteria in the Kuroshio region. We obtained the nanoflagellates and bacterial biodiversity data through the sequences of 18S and 16S rDNA respectively with illumina Miseq, and abundance data by using epifluorescence microscopic and flow cytometry counting, respectively.

**Sample collections**

To obtain nanoflagellates and bacteria community, seawater samples were collected from 5-m depth and subsurface chlorophyll maximum (SCM) layers using a CTD-General Oceanic Rosette assembly with X-Niskin bottles at every station.

Samples for examining genetic and species diversity of nanoflagellates and bacteira, 9 L of seawater were filtered sequentially through a 20 μm mesh, then a 1.2 μm, and finally a 0.2 μm pore size polycarbonate filter using a peristaltic pump. The 0.2 μm and 1.2 μm pore size polycarbonate filters were frozen in liquid nitrogen on-board. Samples for estimating nanoflagellates and bacteria abundance, 2 ml and 50ml of seawater was filtered through a 20 μm mesh and fixed with paraformaldehyde solution to final concentration 0.2% and glutaraldehyde to final concentration 1%, respectively. All samples were preserved at -20 °C.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Experiment (Overall)** | **Item** | **Type** | **amount** | **Prepare** |
|  | Sharpie marker | Black/Blue | 5~5 |  |
| (40 stations) | Electrical Tapes | Gluta Sample | 8 |  |
|  | 20um nylon net | Big/Small | 5~8 |  |
|  | Container box | DNA/flow | 5 |  |
|  | Scissors |  | 1~2 |  |
|  | cable tie/zip tie | 3.6 mm(width) x 150 mm | 20 |  |
|  | cable tie/zip tie | big | 20 |  |
|  | Aluminum foil | 75-150 square foot roll | 1~2 pack |  |
|  |  |  |  |  |
| **Experiment (DNA)** | **Item** | **Type** | **amount** | **Prepare** |
|  | Carboys | 10-20L | 6 |  |
| (40 stations, 2 depths, no replicates) | Polycarbonate filter # | 1.2μm (D = 142 mm) | 3 boxes (300) |  |
| # needs to match | Polycarbonate filter # | 0.2μm (D = 142 mm) | 3 boxes (300) |  |
| ∆ needs to match | 5mL cryogenic tube | 5mL | 200 |  |
|  | Tweezer | For arranging filter | 4 |  |
|  | Silicone tubes 1 (mesh) | Long (CTD- 20L Bottle) | 6 |  |
|  | peristaltic pump | with LS25 tube X 4  (2 for backup) | 2 set with 4 tubes∆ |  |
|  | Filter holder # | hold the 142 mm filter | 3 sets |  |
|  | Silicone tubes 2 | For pump rotate | 4 tubes∆ |  |
|  | Silicone tubes 3 | For pump to filter holder | 4 tubes∆ |  |
|  | Silicone gel | For pump-tube | 1 |  |
|  |  |  |  |  |
| **Experiment (Gluta)** | **Item** | **Type** | **amount** | **Prepare** |
|  | Silicone tubes 1 (mesh) | Short (CTD-50mL) | 2 |  |
| (40 stations, 2 depths, replicates A/B) | Plastic dropper | 3mL | 5~10 |  |
|  | 50mL Centrifuge Tubes | 50mL | 200 |  |
|  | Labels | Print Out | 200 |  |
|  | Basket | Market type | 1 |  |
|  | Metal rank | For 50mL x 9 tubes | 1 |  |
|  | Latex gloves | M | 2 boxes |  |
|  | Neoprene rubber Glove | M (for preventing gluta) | 1 pair |  |
|  | **Glutaraldehyde (50%)** | **1mL each sample (final 1%)** | **>200 mL** |  |
|  |  |  |  |  |
| **Experiment (Flow)** | **Item** | **Type** | **amount** | **Prepare** |
|  | Soft Tubes with mesh | Short (CTD-50mL) | 2 |  |
| (40 stations, 2 depths, replicates A/B) | 2 mL Cryogenic tubes | 2mL | 200 |  |
|  | Spray bottle (for Ethanol) | 200mL-400mL | 1 |  |
|  | Ethanol | 75% for sterilizing | 1L-2L |  |
|  | Pipette | 1000uL | 3 |  |
|  | Pipette | 20uL | 3 |  |
|  | Tip | 1000uL | 6 boxes |  |
|  | Tip | 20uL | 6 boxes |  |
|  | Liquid Nitrogen?? | ??? | for a month? Dip & freeze? |  |
|  | **Paraformaldehyde (10%)** | **2uL each sample (final 0.2%)** | 15mLx3 tubes |  |

＊How to preserve -20 samples on board? (storage size for NF samples)

＊How to deliver -20 samples from cruise to NTU laboratory? (shipping method and fee for all samples)