Summary of sample collections in the Kurshio region in 2020 Cruise

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According to the plan of Japan cruies, 4 to 6 transect lines will be sampled in July’s cruise. We plan to obtain **nanoflagellate and bacteria community** at each station and carry out **incubation experiments** at 3 stations in each transect line (priority: inshore station, offshore station and middle station between inshore and offshore stations).

1. The experiments of Nanoflagellate and bacteria community (for each station)
2. Experiment of species diversity of nanoflagellate and bacteria community (DNA)
3. Experiment of nanoflagellate abundance (Gluta)
4. Experiment of bacteria abundance (Para)
5. The incubation experiments (for 3 stations in each transect lines, totally 18 stations)
6. Secondary production and growth rate
7. Copepod mass-specific growth rate and biomass (Exp. 1a)
8. Heterotrophic nanoplankton growth rate, biomass and mortality (Exp. 1b)
9. Microbial trophic interactions
10. Heterotrophic nanoplankton ingestion on bacteria (Exp. 2)

**Sampling water volume at each station**

|  |  |  |
| --- | --- | --- |
| Sampling depth | Needed seawater (L) | Experiment |
| Surface | 25 | I. |
| The depth of Chla max | 25 | I. |
| 5 meter-depth | 160 | II-1a |
| Surface | 40 | II-1b+II-2 |
|  |  |  |

The spent time of trawl net

|  |  |  |  |
| --- | --- | --- | --- |
| Sampling depth | Mesh-size | Spend-time (minutes) | Experiment |
| 5-m (vertical tow) | 50 μm Norpac | 25  (The nets were set to 5 m depth and allowed to drift with the ship for 20 minutes) | II-1a |
| 5-m (vertical tow) | 100 μm Norpac | 25  (The nets were set to 5 m depth and allowed to drift with the ship for 20 minutes) | II-1a |
| From 200-m to surface (oblique tow) | 50 μm Norpac with Flowmeter | 40-50 | II-1a |

Tools

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Experiment (Overall)** | **Item** | **Type** | **amount** | **Prepare** |
|  | Sharpie marker | Black/Blue | 10 |  |
| (40 stations) | Electrical Tapes | 19 mm(width)x10 m | 8 |  |
|  | 20 μm nylon net | Big/Small | 5~8 |  |
|  | Container box | DNA/flow | 5 |  |
|  | Silicone tube 1 for Glo-Flo or Niskin petcock assy | 30 meters long (need to know the diameter of petcock assy) | 1 |  |
|  | cable tie/zip tie | 3.6 mm(width) x 150 mm | 30 |  |
|  | cable tie/zip tie | big | 30 |  |
|  | Aluminum foil | 75-150 square foot roll | 2~3 packs |  |
|  | Curved extra fine tip tweezer |  | 6 |  |
|  | Scissors |  | 3 |  |
|  |  |  |  |  |

1. The equipment of nanoflagellate and bacteria community (If we sample at 40 stations)

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| --- | --- | --- | --- | --- |
| **Experiment (DNA)** | **Item** | **Type** | **amount** | **Prepare** |
|  | Carboys | 10-20L | 6 |  |
| (40 stations, 2 depths, no replicates) | 1.2 μm Polycarbonate filter # | 1.2 μm (D = 142 mm) | 3 boxes (300) |  |
| # needs to match | 0.2 μm Polycarbonate filter # | 0.2 μm (D = 142 mm) | 3 boxes (300) |  |
| ∆ needs to match | 5 mL cryogenic tube | 5mL tube | 200 |  |
|  | 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 |  |
|  | 50 mL 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%)** | 15mL x 3 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)

1. The incubation experiments (for 3 stations in each transect line, totally 18 stations in 6 transect lines)

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| --- | --- | --- | --- | --- |
| **1a Copepod GR** | **Item** | **Type** | **amount** | **Prepare** |
|  | 20 L cubitainers | 20 L | 24 (6 per set) |  |
| (18 stations) | 100 L dark incubation tanks | 100L | 8 (6 for GR and 2 for dilution) |  |
|  | 50 μm Norpac net | with Flowmeter | 15 or more |  |
|  | 100 μm Norpac net |  | 5 or more |  |
|  | Flowmeter for Norpac |  | 2 |  |
|  | Cod End for Norpac |  | 5 |  |
|  | 80 μm sieves for reverse filtration | diameter 50 cm | 2 |  |
|  | 150 μm sieves for reverse filtration | diameter 50 cm | 2 |  |
|  | 50 μm nylon filter | 2m x 2m | 2 |  |
|  | 50 um hand-made sieve |  | 2 |  |
|  | 20 L plastic buckets | 20 L | 2 |  |
|  | 100 mL plastic bottles | 100 mL | 160 (8 per set) |  |
|  | 40% formalin or  10% formalin |  | 3 L (140 mL per set)  12 L (560 mL per set) |  |
|  | Glutaraldehyde |  | 160 mL (8 mL per set) |  |
|  | Kimwipes Kimtech | 11 x 21cm  (280 papers/box) | 1 box |  |
|  | 75% Ethanol |  | 0.5 L |  |
|  | Alcohol thermometer |  | 2 |  |
|  | Cleaning Sponge Scouring Pad |  | 2 |  |
|  | Pressure sprayer  (<https://tg.pe/rJu>) |  | 2 |  |
|  | Wash bottle |  | 3 |  |
|  |  |  |  |  |

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| **1b nanoplankton GR** | **Item** | **Type** | **amount** | **Prepare** |
|  | 2L carboys | 2L | 6 |  |
| (18 stations) | 20 μm filters | 2m x 2m | 2 |  |
|  | 50 mL centrifuge tube | 50 mL | 160 |  |
|  | Glutaradehyde | 8mL each set | 200 mL |  |
|  |  |  |  |  |
|  |  |  |  |  |
| **2 Dilution** | **Item** | **Type** | **amount** | **Prepare** |
|  | Peristaltic pump | with LS25 tube X 4  (2 for backup) | 2 sets with 4 tubes∆ |  |
| (18 stations) | Filter holders # |  | 2 |  |
|  | 20L carboys |  | 4 |  |
| # needs to match | 2L carboys |  | 26 |  |
| ∆ needs to match | 1.2 μm filter # | 1.2 μm polycarbonate filter (D = 142 mm) | 1 boxes |  |
|  | 0.2 μm filter # | 0.2 μm polycarbonate filter (D = 142 mm) | 1 boxes |  |
|  | Silicone tubes 2 | For pump rotate | 4 tubes∆ |  |
|  | Silicone tubes 3 | For pump to filter holder | 4 tubes∆ |  |
|  | Liquid nitrogen |  |  |  |
|  | 2 mL cryogenic tubes | 2 mL | 960 |  |
|  | Paraformaldehyde | 960 μL per set | 20 mL |  |
|  |  |  |  |  |