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 (prior: 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 nanflagellate 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 (Exp. 1a)
8. Heterotrophic nanoplankton growth rate and mortality (Exp. 1b)
9. Microbial trophic interactions
10. Heterotrophic nanoplankton ingestion on bacteria (Exp. 2)

**Sampling water volume in each station**

Sampling water at each station

|  |  |  |
| --- | --- | --- |
| Sampling depth | Needed seawater (L) | Experiment |
| Surface | 25 | I. |
| The depth of Chla max | 25 | I. |
| 10 meter-depth | 160 | II-1a |
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The spent time of trawl net

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| --- | --- | --- | --- |
| Sampling depth | Mesh-size | Spend-time (minutes) | Experiment |
| 5-m (vertical tow) | 50 μm Norpac | 25 | II-1a |
| 5-m (vertical tow) | 100 μm Norpac | 25 | II-1a |
|  |  |  |  |

Tools

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| **Experiment (Overall)** | **Item** | **Type** | **amount** | **Prepare** |
|  | Sharpie marker | Black/Blue | 10 |  |
| (40 stations) | Tape | Tube/Sample | 8 |  |
|  | 20 μm mesh | Big/Small | 5~8 |  |
|  | Container box | DNA/flow | 5 |  |
|  | Silicone tubes | 30 metres | 2 |  |
|  | Electrical Tapes | 19 mm(width)x10 m | 5 |  |
|  | cable tie/zip tie | 3.6 mm(width) x 150 mm | 20 |  |
|  | Aluminum foil | 75-150 square foot roll | 1 pack |  |
|  | Curved extra fine tip tweezer |  | 5 |  |
|  | 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** |
|  | Water bottle | 10-20L | 6 |  |
| (40 stations, 2 depths, no replicates) | 1.2 μm filter | 1.2 μm filter (D = 142mm) | 3 boxes (300) |  |
|  | 0.2 μm filter | 0.2 μm filter (D = 142mm) | 3 boxes (300) |  |
|  | 5mL tube | 5mL tube | 200 |  |
|  | Soft Tubes with 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 set |  |
|  | 3 or 5 c.c. plastic dropper |  | 5 |  |
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| **Experiment (Gluta)** | **Item** | **Type** | **amount** | **Prepare** |
|  | Soft Tubes with mesh | Short (CTD-50mL) | 2 |  |
| (40 stations, 2 depths, replicates A/B) | Dropper | 3mL | 5~10 |  |
|  | Tubes | 50mL | 200 |  |
|  | Labels | Print Out | 200 |  |
|  | **Glutaraldehyde (50%)** | **1mL each sample (final 1%)** | **>200 mL** |  |
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|  |  |  |  |  |
| **Experiment (Flow)** | **Item** | **Type** | **amount** | **Prepare** |
|  | Soft Tubes with mesh | Short (CTD-50mL) | 2 |  |
| (40 stations, 2 depths, replicates A/B) | Tubes | 2mL | 200 |  |
|  | Pipette | 1000mL | 3 |  |
|  | Pipette | 20mL | 3 |  |
|  | Tip | 1000mL | 6 boxes |  |
|  | Tip | 20mL | 6 boxes |  |
|  | Liquid Nitrogen?? | ??? | for a month? Dip & freeze? |  |
|  | **Paraformaldehyde (10%)** | **2uL each sample (final 0.2%)** | 15mL x 3 tubes |  |

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 | 6 |  |
|  | 50 μm Norpac net |  | 10 or more |  |
|  | 100 μm Norpac net |  | 3 or more |  |
|  | 80 μm sieves | diameter 50 cm | 2 |  |
|  | 150 μm sieves | diameter 50 cm | 2 |  |
|  | 50 μm nylon filter | 2m x 2m | 2 |  |
|  | 20 L plastic buckets | 20 L | 2 |  |
|  | 100 mL plastic bottles | 100 mL | 160 (8 per set) |  |
|  | 40% formalin or  10% formalin |  | 1 L (40 mL per set)  4 L (160 mL per set) |  |
|  | Glutaraldehyde |  |  |  |
|  | Kimwipes Kimtech | 11 x 21cm  (280 papers/box) | 1 box |  |
|  | 75% Ethanol |  | 200 mL |  |
|  | Alcohol thermometer |  | 2 |  |
|  | Cleaning Sponge Scouring Pad |  | 2 |  |
|  |  |  |  |  |
|  |  |  |  |  |
| **1b nanoplankton GR** | **Item** | **Type** | **amount** | **Prepare** |
|  | 2L carboys | 2L | 12 |  |
| (18 stations) | 20 μm filters | 2m x 2m | 2 |  |
|  | 50 mL centrifuge tube | 50 mL | 160 |  |
|  | Glutaradehyde | 8mL each sample | 200 mL |  |
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| **2a ingestion** | **Item** | **Type** | **amount** | **Prepare** |
|  | Portable pump | Short (CTD-50mL) | 2 |  |
| (18 stations) | Filter holders |  | 2 |  |
|  | 20L carboys |  | 4 |  |
|  | 2L carboys |  | 36 |  |
|  | 1.2 μm filter | 1.2 μm filter (D = 142 mm) | 1 boxes |  |
|  | 0.2 μm filter | 0.2 μm filter (D = 142 mm) | 1 boxes |  |
|  | Liquid nitrogen |  |  |  |
|  | 2 mL cryogenic tubes | 2 mL | 960 |  |
|  | Paraformaldehyde | 960 μL per set | 20 mL |  |
|  |  |  |  |  |