## **Johor Straits Sampling Checklist**

| Date: |  |
|-------|--|
|       |  |

| Preparations in the Lab — qty is per trip  10x sterivex (4 per site, +2 extra)  4x 10ml syringes  RNA Later  Blue tack  Toilet roll   5x 15ml tubes, acid washed (1x acid, 3x RO water), dry (2 per site, +1 extra)  10 cryovials (4 per site, +2 extra)  Pre-label with sample ID, location, date, sample type  Pre-aliquot 20ul of 25% glutaraldehyde into each vial  Store in 4C fridge until the morning of sampling  Bring for sampling inside cooler + ice pack  4x GF/F (2 per site)  8x 50ml syringe (4 per site)  filter holder   | <ul> <li>What to do in the Field</li> <li>Run autosampler for 10 minutes with local water</li> <li>Collect each sterivex sample for 15 minutes, then remove water by pushing air using syringe</li> <li>Fill each sterivex sample with 2ml RNA Later using pipette</li> <li>Put sterivex samples in Dryshipper.</li> <li>Use mid-stream sterivex filtrate.</li> <li>Rinse each tube 3 times with sample before collection</li> <li>Place in Dryshipper</li> <li>Add 980ul of water sample inside each vial, accordingly to each sample type</li> <li>Fix samples in the cooler for 15-30 minutes</li> <li>Flash freeze in Dryshipper</li> <li>For each sample, do 2 for 150um pre-filtrate &amp; 2 for 0.22um filtrate (total 4 per site).</li> <li>Place GF/F in filter holder using forceps</li> <li>Gently, filter 50ml of sample</li> </ul> | Back in the Lab  Store sterivex in -80C  Rinse Autosampler with tap water for 10 minutes  [Once a week, rinse the autosampler with 5L 10% bleach, then 10 mins with tap water.]  Store tubes in -20C.  Store cryovials in -80C.   |
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| <ul> <li>10x sterivex         (4 per site, +2 extra)</li> <li>4x 10ml syringes</li> <li>RNA Later</li> <li>Blue tack</li> <li>Toilet roll</li> <li>5x 15ml tubes, acid washed         (1x acid, 3x RO water), dry         (2 per site, +1 extra)</li> <li>10 cryovials         (4 per site, +2 extra)</li> <li>Pre-label with sample ID,         location, date, sample type</li> <li>Pre-aliquot 20ul of 25%         glutaraldehyde into each vial</li> <li>Store in 4C fridge until the         morning of sampling</li> <li>Bring for sampling inside         cooler + ice pack</li> <li>4x GF/F (2 per site)</li> <li>8x 50ml syringe (4 per site)</li> <li>filter holder</li> </ul> | with local water  Collect each sterivex sample for 15 minutes, then remove water by pushing air using syringe  Fill each sterivex sample with 2ml RNA Later using pipette  Put sterivex samples in Dryshipper.  Use mid-stream sterivex filtrate.  Rinse each tube 3 times with sample before collection  Place in Dryshipper  Add 980ul of water sample inside each vial, accordingly to each sample type  Fix samples in the cooler for 15-30 minutes  Flash freeze in Dryshipper  For each sample, do 2 for 150um pre-filtrate & 2 for 0.22um filtrate (total 4 per site).   | Rinse Autosampler with tap water for 10 minutes  [Once a week, rinse the autosampler with 5L 10% bleach, then 10 mins with tap water.]  Store tubes in -20C.  |
| <ul> <li>Blue tack</li> <li>Toilet roll</li> <li>5x 15ml tubes, acid washed (1x acid, 3x RO water), dry (2 per site, +1 extra)</li> <li>10 cryovials (4 per site, +2 extra)</li> <li>Pre-label with sample ID, location, date, sample type</li> <li>Pre-aliquot 20ul of 25% glutaraldehyde into each vial</li> <li>Store in 4C fridge until the morning of sampling</li> <li>Bring for sampling inside cooler + ice pack</li> <li>4x GF/F (2 per site)</li> <li>8x 50ml syringe (4 per site)</li> <li>filter holder</li> </ul>   | <ul> <li>pushing air using syringe</li> <li>Fill each sterivex sample with 2ml RNA Later using pipette</li> <li>Put sterivex samples in Dryshipper.</li> <li>Use mid-stream sterivex filtrate.</li> <li>Rinse each tube 3 times with sample before collection</li> <li>Place in Dryshipper</li> <li>Add 980ul of water sample inside each vial, accordingly to each sample type</li> <li>Fix samples in the cooler for 15-30 minutes</li> <li>Flash freeze in Dryshipper</li> <li>For each sample, do 2 for 150um pre-filtrate &amp; 2 for 0.22um filtrate (total 4 per site).</li> <li>Place GF/F in filter holder using forceps</li> </ul>  | tap water for 10 minutes  [Once a week, rinse the autosampler with 5L 10% bleach, then 10 mins with tap water.]  Store tubes in -20C.  Store cryovials in -80C.   |
| <ul> <li>5x 15ml tubes, acid washed (1x acid, 3x RO water), dry (2 per site, +1 extra)</li> <li>10 cryovials (4 per site, +2 extra)</li> <li>Pre-label with sample ID, location, date, sample type</li> <li>Pre-aliquot 20ul of 25% glutaraldehyde into each vial</li> <li>Store in 4C fridge until the morning of sampling</li> <li>Bring for sampling inside cooler + ice pack</li> <li>4x GF/F (2 per site)</li> <li>8x 50ml syringe (4 per site)</li> <li>filter holder</li> </ul>   | RNA Later using pipette - Put sterivex samples in Dryshipper.  - Use mid-stream sterivex filtrate Rinse each tube 3 times with sample before collection - Place in Dryshipper - Add 980ul of water sample inside each vial, accordingly to each sample type - Fix samples in the cooler for 15-30 minutes - Flash freeze in Dryshipper - For each sample, do 2 for 150um pre-filtrate & 2 for 0.22um filtrate (total 4 per site).  - Place GF/F in filter holder using forceps  | autosampler with 5L 10% bleach, then 10 mins with tap water.] Store tubes in -20C.  Store cryovials in -80C.  |
| <ul> <li>(1x acid, 3x RO water), dry (2 per site, +1 extra)</li> <li>10 cryovials (4 per site, +2 extra)</li> <li>Pre-label with sample ID, location, date, sample type</li> <li>Pre-aliquot 20ul of 25% glutaraldehyde into each vial</li> <li>Store in 4C fridge until the morning of sampling</li> <li>Bring for sampling inside cooler + ice pack</li> <li>4x GF/F (2 per site)</li> <li>8x 50ml syringe (4 per site)</li> <li>filter holder</li> </ul>  | <ul> <li>Rinse each tube 3 times with sample before collection</li> <li>Place in Dryshipper</li> <li>Add 980ul of water sample inside each vial, accordingly to each sample type</li> <li>Fix samples in the cooler for 15-30 minutes</li> <li>Flash freeze in Dryshipper</li> <li>For each sample, do 2 for 150um pre-filtrate &amp; 2 for 0.22um filtrate (total 4 per site).</li> <li>Place GF/F in filter holder using forceps</li> </ul>   | Store cryovials in -80C.  |
| <ul> <li>(4 per site, +2 extra)</li> <li>Pre-label with sample ID, location, date, sample type</li> <li>Pre-aliquot 20ul of 25% glutaraldehyde into each vial</li> <li>Store in 4C fridge until the morning of sampling</li> <li>Bring for sampling inside cooler + ice pack</li> <li>4x GF/F (2 per site)</li> <li>8x 50ml syringe (4 per site)</li> <li>filter holder</li> </ul>   | each vial, accordingly to each sample type  - Fix samples in the cooler for 15-30 minutes  - Flash freeze in Dryshipper  - For each sample, do 2 for 150um pre-filtrate & 2 for 0.22um filtrate (total 4 per site).  - Place GF/F in filter holder using forceps  |   |
| <ul><li>8x 50ml syringe (4 per site)</li><li>filter holder</li></ul>   | forceps   | Store GFF at -20C.  |
| <ul> <li>Forceps</li> <li>Aluminium foil</li> <li>For each site, do 1 sample for completely unfiltered water, 1 for 150um pre-filtrate</li> </ul>  | through the GFF, then rinse filter with 50ml of DI water  - Push air out of syringe to dry filter  - Fold in 2 (sample side in), place in aluminium foil  - Put in Dryshipper   |   |
| <ul> <li>Aquaflash + glass cuvette</li> <li>Kimwipes</li> <li>6x 50ml tubes (3 per site, 1 each for completely unfiltered, 150um prefiltered, 0.22um filtered)</li> </ul>  | <ul> <li>Aliquot 2ml of sample into glass cuvette</li> <li>Measure &amp; record.</li> <li>Do for completely unfiltered, 150um pre-filtered &amp; 0.22um filtered sample</li> </ul>  |   |
| - Bring Secchi disk  | <ul> <li>Lower disk until it just disappears.</li> <li>Record depth.</li> <li>Pull disk until it just appears.</li> <li>Record depth.</li> <li>Repeat 5 times</li> </ul>  | - Average the 10<br>numbers, that is the<br>Secchi Depth  |
| - Put ice packs in -80   | - Record date, sampling location,   | - Thaw/re-freeze ice  |
| -  |   | packs - Clean cooler  |
| <ul> <li>Prepare sampling materials</li> <li>At least 1l of DI Water</li> <li>1000ul pipette &amp; tips</li> <li>Gloves, 70% EtOH, tissue</li> <li>Salinity &amp; temperature probe</li> </ul>   | - Record measurements: - Fluorescence & yield - Secchi depth - Salinity & Temperature - Number of samples of each kind taken  | <ul> <li>Clean cooler</li> <li>Remove all samples to proper storage places</li> <li>Dispose remaining DI Water</li> <li>Fully charge the Dryshipper when not</li> </ul>   |
|  | completely unfiltered water, 1 for 150um pre-filtrate  - Aquaflash + glass cuvette - Kimwipes - 6x 50ml tubes (3 per site, 1 each for completely unfiltered, 150um pre-filtered, 0.22um filtered)  - Bring Secchi disk  - Put ice packs in -80 - Prepare cooler - Prepare Dryshipper - Prepare sampling materials - At least 1l of DI Water - 1000ul pipette & tips - Gloves, 70% EtOH, tissue  | completely unfiltered water, 1 for 150um pre-filtrate  - Aquaflash + glass cuvette - Kimwipes - 6x 50ml tubes (3 per site, 1 each for completely unfiltered, 150um pre-filtered, 0.22um filtered, 0.22um filtered, 0.22um filtered sample  - Bring Secchi disk - Bring Secchi disk - Put ice packs in -80 - Prepare Cooler - Prepare Dryshipper - Prepare sampling materials - At least 1l of Dl Water - 1000ul pipette & tips - Gloves, 70% EtOH, tissue - Salinity & temperature probe - Aliquot 2ml of sample into glass cuvette - Aliquot 2ml of sample into glass cuvette - Measure & record Measure & record Do for completely unfiltered, 150um pre-filtered & 0.22um filtered sample - Lower disk until it just disappears. Record depth Pull disk until it just appears. Record date, sampling location, sampling time, weather, sampling personnel - Record measurements: - Fluorescence & yield - Salinity & Temperature - Number of samples of each |

Completely unfiltered = from bypass filter, turn red knob

<sup>150</sup>um pre-filtered = from the outhose, the one that spews water very far