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STANDARD OPERATING PROCEDURE (SOP) - 4

PROCESSING OF WINDOW SCREEN SAMPLES

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A/ List of equipment

- ❑ A 1L graduated cylinder
- ❑ A 200-300 µm pore size mesh sieve
- ❑ A funnel large enough to fit the 200-300 µm sieve
- ❑ Some 20 µm nylon mesh pieces cut into 4 cm squares
- ❑ A filtration unit composed of a 200 mL or 500 mL filter funnel, a 25 mm diameter filter base, a stopper, and a 500 mL or 1 L side-arm flask
- ❑ A hand vacuum pump
- ❑ 2 pairs of flat forceps
- ❑ A squeeze bottle with filtered seawater
- ❑ 15mL conical screw cap tubes and rack
- ❑ Formalin solution (37 % weight in water)
- ❑ Pure ethanol
- ❑ 10 mL plastic pipettes in individual packaging
- ❑ A micropipet (P1000) with suitable disposable tips
- ❑ Plastic Pasteur pipettes
- ❑ A thin permanent marker
- ❑ A notebook and a pen
- ❑ Paraffin tape

B/ Procedure

The detailed procedure can be viewed on the video tutorial available online on the CIGUAWATCH platform : https://ciguawatch.ilm.pf/ciguatera-environmental-monitoring-methodology-2/

1. SAMPLE FILTRATION

i. Place the funnel and the 200-300 µm sieve on top of the graduated cylinder
ii. Pour a small portion of the sample from the 500 mL plastic bottle through the sieve to create head space

iii. Invert the plastic bottle several times and shake vigorously for 5 to 10 seconds to detach cells from window-screens and disperse cell clumps, then remove the window-screen from the container and discard it
iv. Pour the entire sample volume through the 200-300 µm sieve to remove large sediments and detritus
v. Rinse the bottle using a squeeze bottle filled with filtered seawater and filter the rinsing seawater
vi. Record the "Total Sample Volume" on the graduated cylinder in a notebook.
vii. Assemble the filtration unit by placing a piece of 20 µm nylon mesh on top of the filter base, held in place by a 250 mL – 500 mL filter funnel
viii. Connect the hand vacuum pump to the side-arm flask
ix. Place a piece of paraffin on the top of the graduated cylinder and invert 2-3 times to resuspend the cells, then immediately filter the whole sample, proceeding in small volumes. Apply low pressure from time to time using the hand vacuum pump to speed up filtration
x. If due to the progressive clogging of the 20 µm nylon mesh only a subsample is filtered, record the "Remaining Volume" on the graduated cylinder
xi. Record the "Volume filtered" which corresponds to (Total Sample Volume - Remaining Volume) in the notebook
xii. To complete the (sub)sample filtration, rinse the filter funnel using a squeeze bottle filled with filtered seawater to collect the residual cellular material onto the 20 µm nylon mesh, then let the rinsing seawater to gravity filter

2. SAMPLE PRESERVATION

i. Fill the conical tubes with 15 mL of filtered seawater
ii. Disconnect the funnel from its base and gently take the edge of the 20 µm nylon mesh using flat forceps, then fold the mesh so that the cells are on the inside
iii. Immediately transfer the 20 µm nylon mesh to 15mL screw cap tubes filled with filtered sea water.
iv. Cap the sample then tap the tube downward against table top so that the 20 µm mesh is fully immersed in seawater
v. Shake the tube vigorously to dislodge cells from the 20 µm nylon mesh
vi. *For routine screening/examination of samples*, preserve samples in Lugol's neutral iodine solution by adding 1-2 drops of Lugol's solution in the 15 ml tube until a light tea-color is obtained. Cap and shake to mix sample. Store the sample in the dark until microscope observation for cell enumeration. Note that Lugol's will fade with time, so it is necessary to periodically add a small amount of Lugol's solution (if the color fades away, the sample is no longer preserved !)
For long-term storage of samples, formaldehyde should be preferred as a fixative: add 600 µl of the formalin solution in each tube (or 15 drops using a plastic Pasteur pipette). Due to the high toxicity and carcinogenicity of formaldehyde, precautions should be taken while handling this fixative (see section "D/ Personal Protection Considerations")
vii. Seal the tube tightly with paraffin tape

viii. Label the tube with date, site name, sample number, sample volume and volume filtered using a thin permanent marker. Make sure this information is also recorded in the notebook
ix. Store samples at room temperature until cell enumeration under the microscope
x. Before processing the next window-screen sample, remove debris from the 200-300 µm sieve, then wash the sieve, funnel, graduated cylinder and the entire filtration unit under tap water, followed by rinse with deionized water. Blot on a clean paper towel

C/ Hints

- ❑ If samples are going to be used for molecular analysis (quantitative PCR), preserve samples in pure ethanol instead of formalin by filling the conical screw cap tubes directly with 15 mL of pure ethanol (instead of filtered seawater) before transferring the 20 µm nylon mesh
- ❑ If samples are going to be used for molecular analysis, <u>all</u> the material (sieves, funnel, lab ware, forceps, etc.) should be soaked in 10% bleach for 10mn to eliminate DNA carryover, then rinsed in deionized water and allowed to air dry
- ❑ Microscope(s) should be kept in a cool, air-conditioned room to ensure longer lifespan of this(ese) costly equipment(s)

D/ Personal Protection Considerations

- ❑ Use a pair of safety glasses at all time
- ❑ Sample preservation: if formaldehyde is used as a fixative, work **under a laboratory fume hood or in a ventilated area**
- ❑ Sample preservation: wear gloves when handling formaldehyde and tube samples