INSTITUT LOUIS MALARDE
Laboratory of Marine Biotoxins
BP30 Papeete Tahiti
98713 French Polynesia

STANDARD OPERATIONAL PROCEDURES (SOPS)

CIGUATERA ENVIRONMENTAL MONITORING

Field sampling and preparations of samples

Produced with support from the Food and Agriculture Organization of the United Nations Sub-regional Office for the Pacific (FAO SAP), 2022.

TABLE OF CONTENTS

INSTITUT LOUIS MALARDE
Laboratory of Marine Biotoxins
BP30 Papeete Tahiti
98713 French Polynesia

STANDARD OPERATING PROCEDURE (SOP) - 1

Assembly of a window screen device

Version : 2.0
Review date : 11.07.2022
Authors: Dr Mireille CHINAIN & Dr Clémence GATTI

Validation date : 22.07.2022

1/ List of equipments

- ❑ A lead weight of 0.5 kg, used as an anchor
- ❑ A monofilament fishing line or marine-grade rope of about 1m long, per set
- ❑ A plastic screw cap bottle of 250 mL used as a float
- ❑ A stainless steel swivel (size 2/0)
- ❑ A piece of fiberglass window screen of 15 x 10 cm (one per sample, not reusable)

2/ Window screen assembly:

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/

- ❑ Using the rope, connect the float, screen mesh and anchor as follows:
- ❑ Tie the rope to the weight
- ❑ Attach the float on the other end of the rope using a sliding knot
- ❑ Screw the cap on the plastic bottle.
- ❑ Fix the swivel on the rope, at about 35 cm from the weight and 20 cm from the float
- ❑ Clip the swivel onto one corner of the window screen mesh

3/ Hints

- ❑ The lead weight can be replaced by a bottle filled with sand, or a rock
- ❑ The 250 mL plastic bottle can be replaced by a polystyrene float or an empty bottle
- ❑ The float should be positioned subsurface
- ❑ Bright color paints can be used for an easier localization of the WSs in deployment sites
- ❑ Do not hang the swivel too close to the edge of the screen to avoid tearing of the WS mesh

INSTITUT LOUIS MALARDE
Laboratory of Marine Biotoxins
BP30 Papeete Tahiti
98713 French Polynesia

STANDARD OPERATING PROCEDURE (SOP) - 2

DEPLOYMENT AND COLLECTION OF WINDOW SCREEN DEVICES

Version : 2.0
Review date : 11.07.2022
Authors: Dr Mireille CHINAIN & Dr Clémence GATTI
Page(s) : 2
Validation date : 22.07.2022

A/ List of equipments

- ❑ A boat fully equipped with regulation safety equipment
- ❑ Snorkeling equipment and diving gear
- ❑ x5 fully-assembled window screen devices
- ❑ x5 plastic screw cap bottles (500 mL) used as containers for collected window screens
- ❑ A Global Positioning System (GPS) device for accurate localization of the sampling sites
- ❑ A waterproof thermometer
- ❑ A depth meter
- ❑ A diving slate and a pencil
- ❑ A permanent marker
- ❑ A cooler

B/ Deployment of WS devices

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/

- ❑ Label each window screen device with a permanent marker (note sample number and date of deployment)
- ❑ Record the GPS coordinates, seawater temperature and depth at the deployment site
- ❑ Collate all these information along with name of site, sample number, date and time of deployment on the diving slate
- ❑ Deploy the WS devices preferably at depths between 1.5 and 3 m
- ❑ For statistically robust information, at least 5 Window screen devices should be deployed per sampling site
- ❑ The deployment time should not exceed 24h, to avoid significant biofouling or grazing pressure

C/ Collection of Window screen devices following a 24h deployment period in the field

- ❑ Label each 500 mL plastic container with sample number, date and time of collection
- ❑ Unscrew the cap and fill the plastic bottle with seawater

- ❑ Gently unclip the screen from rope. Be careful not to shake or touch the screen to minimize the loss of material from the screen
- ❑ Gently fold and insert the screen in plastic bottle
- ❑ Cap the sampling bottle underwater and bring to surface
- ❑ Pick up the rest of the device

D/ Hints

- ❑ Best deployment sites are those in low-energy environment (no current or wave action) nearby coral reefs colonized with macroalgae
- ❑ Screens should not rub against the lead weight
- ❑ Screens must remain suspended in the water column at all times
- ❑ Avoid touching or scratching the micro-algal cell mat attached on the screen
- ❑ Keep the samples near the ambient water temperature and avoid exposure to direct sunlight by placing the samples in a cooler until sample processing
- ❑ If you do not have a depth meter, you can make one using a rope attached to a lead weight and marked every 50 cm

E/ Personal Protection Equipment

- ❑ Wearing a pair of diving gloves during field deployment of WS devices is recommended

INSTITUT LOUIS MALARDE
Laboratory of Marine Biotoxins
BP30 Papeete Tahiti
98713 French Polynesia

STANDARD OPERATING PROCEDURE (SOP) - 3

MACROALGAL SAMPLING METHOD

Version : 2.0
Review date : 11.07.2022
Authors: Dr Mireille CHINAIN & Dr Clémence GATTI
Page(s) : 2
Validation date : 22.07.2022

A/ List of equipments

- ❑ A boat fully equipped with regulatory safety equipment
- ❑ Snorkeling equipment and diving gear
- ❑ A Global Positioning System (GPS) device for accurate localization of the sampling sites
- ❑ A waterproof thermometer
- ❑ A diving slate and a pencil
- ❑ Thick plastic bags (of 30 x 50 cm)
- ❑ Rubber bands
- ❑ A permanent marker
- ❑ An empty tank to collect seawater (or empty plastic bottles)
- ❑ A cooler

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. Label each plastic bag with a permanent marker (note the sample number and date of sampling)
2. Record the GPS coordinates of each sampling site, site name, sample number and date of sampling on the diving slate
3. Once in the water, fill the plastic bag with seawater
4. Collect about 100 g of macroalgae per sampling site at water depths between 1.5 and 3 m
5. Randomly pick small macroalgal tufts across the sampling site
6. Each plastic bag should contain only one species of macroalgae
7. Underwater, put the macroalgae within a plastic bag and seal it tightly with rubber bands
8. Before leaving the site, collect seawater in an empty tank (count approximately 1L per macroalgae sample)

9. Record the seawater temperature in the study site using a data logger or a waterproof field thermometer

C/ Hints

- ❑ Best sampling sites are those located in areas protected from current and wave actions
- ❑ Avoid collecting macrophytes in areas exposed to low tide
- ❑ Macroalgal sampling should be carried out underwater at all time
- ❑ Do not shake the macroalgae to avoid resuspension of microalgal cells in the water column
- ❑ When sampling, prefer calcareous or turf-like agglomerated filamentous macroalgae instead of laminar ones
- ❑ In the case of *Turbinaria* samples, preferably collect specimens colonized by micro-algal turf
- ❑ Select the same species across sampling locations to allow comparison between study sites
- ❑ If necessary, double the plastic bag to avoid leaking
- ❑ Keep the samples near the ambient water temperature and avoid exposure to direct sunlight by placing them in a cooler until sample processing

D/ Personal Protection Equipment

- ❑ Sampling boat should be equipped with all necessary safety equipment as per local regulations
- ❑ Wearing a pair of diving gloves is recommended during field sampling to avoid stings and/or bites by marine fauna

INSTITUT LOUIS MALARDE
Laboratory of Marine Biotoxins
BP30 Papeete Tahiti
98713 French Polynesia

STANDARD OPERATING PROCEDURE (SOP) - 4

PROCESSING OF WINDOW SCREEN SAMPLES

Version : 2.0
Review date : 11.07.2022
Authors: Dr Mireille CHINAIN & Dr Clémence GATTI
Page(s) : 3
Validation date : 22.07.2022

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

INSTITUT LOUIS MALARDE
Laboratory of Marine Biotoxins
BP30 Papeete Tahiti
98713 French Polynesia

STANDARD OPERATING PROCEDURE (SOP) - 5

PROCESSING OF MACROALGAL SAMPLES

Version : 2.0
Review date : 11.07.2022
Authors: Dr Mireille CHINAIN & Dr Clémence GATTI
Page(s) : 3
Validation date : 22.07.2022

A/ List of equipments

- ❑ 125 µm, 45 µm and 20 µm mesh size sieves
- ❑ 50 mL screw cap tubes and rack
- ❑ A thin permanent marker
- ❑ A reservoir filled with seawater collected *in situ* from sampling sites
- ❑ A squeeze bottle with filtered seawater
- ❑ A small funnel
- ❑ 2 x 100 mL graduated cylinders
- ❑ Paraffin tape
- ❑ 5 mL plastic pipettes and pump
- ❑ A notebook and a pen
- ❑ Formalin solution (37 % weight in water)
- ❑ A micropipet (P1000) with suitable disposable tips
- ❑ Plastic Pasteur pipettes
- ❑ A portable scale
- ❑ A brush (3 cm wide)

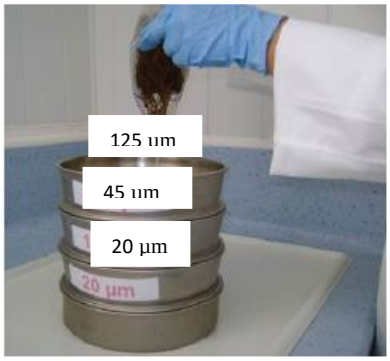
B/ Procedure

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. Stack the 3 sieves in decreasing order of mesh size (from 125 µm to 20 µm)
ii. Label the 50 mL screw cap tubes with site name, sample number, date of sampling, and fraction size, i.e. “F.45 µm” or “F.20 µm” using a permanent marker, and place them on the tube rack

iii. After creating some air and space on top of the plastic bag, vigorously shake then gently knead the macroalgal sample for 30s to dislodge microalgal cells from the macroalgal substrate
iv. Progressively pour the seawater out of the plastic bag before filtration through the nested sieves
v. To resolve clogging, hold the sieves and tap them gently until complete filtration of seawater.
vi. Fill the bag with additional seawater until the macroalgae are completely covered, then shake again and process sample as described above.
vii. Remove the 125 µm sieve
viii. Retrieve the detrital residue on the 45 µm sieve by tilting the sieve, and bring all the residual material on one side of the sieve using the squeeze bottle filled with filtered seawater
ix. Transfer the entire 45 µm fraction in the graduated cylinder using a small funnel, and bring the sample to a total volume of 40 mL



x. Cover the top of the graduated cylinder with paraffin and invert it 2-3 times to resuspend the cells, then immediately transfer the sample into the 50mL screw cap tube labelled "F.40 µm"
xi. Proceed identically with the 20 µm sieve and fraction residue using another graduated cylinder, and transfer the 20 µm fraction into the50mL screw cap tube labelled "F.20 µm"
xii. Blot the macroalgae sample on paper towel and weigh them using a portable scale
xiii. Record the species and weight of each macroalgal sample in the notebook (this value will be needed to determine the number of micro-algae cells / g of macroalgae dry weight)
xiv. Before processing the next sample, remove debris from the 125 µm mesh sieve using a small brush, and wash the sieves, funnel and graduate cylinders under tap water, then rinse with deionized water and allow to dry

2. SAMPLE PRESERVATION

i. *For routine screening/examination of samples*, preserve samples in Lugol's neutral iodine solution by adding several 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 1.6 mL of the formalin solution in each tube (or 38 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")
ii. Seal the tubes tightly with paraffin tape
iii. Store samples at room temperature until cell enumeration under the microscope

C/ Hints

- ❑ Do not knead the macroalgal sample too vigorously to avoid lysis of micro-algal cells
- ❑ During filtration, proceed gradually to avoid spillage of the 45 µm and 20 µm fractions from sieves

- ❑ If cell isolation and culturing experiments are planned, it is imperative to remove a small aliquot (approximately 5 mL) from the 45 µm fraction sample using a plastic pipette and pump before sample preservation. In this case, carefully measure and record the final residual volume indicated on the graduated cylinder
- ❑ 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