forestry, fisheries
& the environment
Department:
Forestry, Fisheries and the Environment
REPUBLIC OF SOUTH AFRICA

Standard Operating Procedure: Phytoplankton Sampling, Identification and Enumeration using Inverted Microscopy

Branch: Fisheries Management
Chief Directorate: Aquaculture Development and Freshwater Fisheries
Directorate: Sustainable Aquaculture Management

Issue 6: July 2024

TITLE

Standard Operating Procedure: Phytoplankton sampling, identification and enumeration using inverted microscopy

COMMENCEMENT

This Standard Operating Procedure comes into force on 1 July 2024.

REVOCATION

This programme issue revokes and replaces Standard Operating Procedure: Phytoplankton Sampling and Enumeration for Phytoplankton Laboratories (Issue 5) as well as any previous issues of the document.

STANDARD OPERATING PROCEDURES ISSUED

Issue	Date of issue
1	06 March 2014
2	1 January 2019
3	1 March 2021
4	1 May 2023
5	1 April 2024

ISSUING AUTHORITY

This Standard Operating Procedure is issued by the Environmental Officer Specialised Production of the Directorate Sustainable Aquaculture Management of the Department of Forestry, Fisheries and the Environment in terms of the Aquacultured Marine Fish Food Safety Programme (AMFFSP).

Environmental Officer Specialised Production

DATE: 01/07/2024

Contents

1. DOCUMENT CONTROL

The Standard Operating Procedure (SOP): Standard Operating Procedure: Phytoplankton sampling, identification and enumeration using inverted microscopy was compiled by Department of Forestry, Fisheries and the Environment: Food Safety Office (FSO) of the Directorate Sustainable Aquaculture Management. The SOP is administered by the FSO and will be reviewed and updated as relevant new information becomes available.

A detailed record of all amendments shall be maintained, and the latest version will be made available at the FSO and will be loaded onto the DFFE website. Suggestions for alterations that would significantly improve the document are welcomed. These should be forwarded to the coordinator, Mr John Foord and enquiries can be directed to Mr Mayizole Majangaza (Appendix 1).

2. SCOPE

This document covers the procedures for the phytoplankton sampling and enumeration as required in terms of the Aquacultured Marine Fish Food Safety Programme (AMFFSP). The procedures include phytoplankton sampling, phytoplankton identification & enumeration and the reporting of the analysis results to the Department of Forestry, Fisheries and the Environment (DFFE). Sampling of the phytoplankton takes place at various production areas located between Port Nolloth in the Northern Cape, and Haga Haga in the Eastern Cape, South Africa.

3. BACKGROUND

The Department of Forestry, Fisheries and the Environment (DFFE) is the managing and regulatory authority for the undertaking of aquaculture activities that include farming, harvesting and transporting of fish for wholesale trading stipulated in the permit conditions issued in terms of the Marine Living Resources Act, 1998 (Act No. 18 of 1998) and associated regulations. The Directorate: Sustainable Aquaculture Management (D: SAM) of the Fisheries Branch of DFFE is responsible for the development, management and regulation of a sustainable aquaculture industry that contributes towards job creation, food security, rural development and economic growth. D: SAM aims to achieve the above-mentioned strategic objectives through the development and implementation of relevant enabling legislation, policies and programmes as well as be responsive and compliant to international obligations and agreed standards. The Food Safety Office (FSO) within D: SAM is responsible for the development and management of food safety programmes stipulated in the permit conditions issued in terms of the Marine Living Resources Act, 1998 (Act No. 18 of 1998) including the AMFFSP and the associated National Residue Control Programme (NRCP). The objectives of the food safety programmes include providing guarantees to domestic and international markets and consumers that South African cultured fish products are safe for human consumption.

The risks to food safety of cultured fish include environmental residues (heavy metals, pesticides, polychlorinated biphenyl, dioxins, perfluoroalkyl substances, polycyclic aromatic hydrocarbons and radionuclides) and veterinary medicine residues (hormones, antibiotics and anthelmintics), the accumulation of biotoxins (Paralytic Shellfish Toxins (PST), Lipophilic Shellfish Toxins (LST) and Amnesic Shellfish Toxins (AST)) and microbiological contamination in shellfish indicated by the presence of *E. coli*.

The accumulation of biotoxins is caused by toxic phytoplankton blooms that are present in the vicinity of aquaculture farms. Phytoplankton sampling needs to be undertaken frequently and in close proximity to aquaculture farms in order to address the inherent temporal and spatial variability in phytoplankton communities along the South African coast. The phytoplankton monitoring programme requires that phytoplankton samples are taken in close proximity to the relevant farms and that the potentially toxic species are identified, and the concentrations calculated to determine the potential risk of the bloom on the farm in terms of food safety. If harmful species are found, contingency measures must be implemented to prevent the harvesting and marketing of toxic shellfish to consumers. Additional samples may be taken from distant areas where blooms are known to develop before being advected to within close proximity to farms. This would serve as an early warning system.

4. SAMPLING REQUIREMENTS

The following sampling requirements shall be adhered to:

- Samples are to be taken by a government official or an independent service provider approved by the DFFE.
- Samples should be taken daily between 10:00 to 15:00 when phytoplankton are more likely to migrate to near the surface.
- Sampling shall be done at fixed sampling stations accessible during most weather conditions.
- The length of the Lund-tube used for the sampling of phytoplankton in open water systems should be determined by the depth of the water column at the sampling station, the depth to which the shellfish is suspended and the limnology within the production area. The Lund-tube should not reach the seabed to avoid contamination of the water sample by benthic material.
- The sampling point should be as close to the intake as possible in land-based systems or where the water is discharged from the intake pipe for the first time.
- There should be no intermediate filter finer than 200 µm that could filter out phytoplankton in land-based systems.
- Remote sensing should be used to determine if there are blooms in the vicinity of production areas to increase sampling frequency at the sampling stations when required, particularly in those regions where samples are not taken on a daily basis. Satellite imagery can be obtained from:
  - Tumblr: https://csiroceancolour.tumblr.com/
  - OCIMS: https://www.ocims.gov.za/
  - HAB DeST: https://www.ocims.gov.za/hab/

DFFE have determined official sampling stations from which the phytoplankton samples shall be taken (Table 1). The sampling stations for the land-based farms are in close proximity to the water intake pipe or where the water is first discharged onto the farm if the intake site is inaccessible. The sampling stations for the sea-based farms are in close proximity to the production areas on the side that would typically be first exposed to an approaching bloom.

Table 1: Official phytoplankton sampling stations

Station	Represented Farms	Coordinates
PSSA1	Diamond Coast Abalone	29°40'10.66"S, 17° 2'40.63"E
PSSB5	Doring Bay Abalone	31°48'57.64"S, 18°13'58.51"E
PSSB1	Blue Ocean Mussels, Saldanha Bay	33° 2'14.66"S, 17°58'33.41"E
PSSB2	Outer Bay North, Saldanha Bay	33° 2'25.59"S, 17°56'36.25"E
PSSB3	Big Bay, Saldanha Bay	33° 2'16.78"S, 18° 0'20.72"E
PSSB4	Small Bay, Saldanha Bay	33° 0'52.75"S, 17°57'39.10"E
PSSB6	West Coast Abalone	32°43'2.16"S. 17°55'28.03"E
PSSB7	Jacobsbaai Sea Products	32°57'47.77"S, 17°53'10.14"E
PSSC1	Abagold, Aqunion Whalerock, HIK & Tuna Marine	34°43'56.21"S, 19°22'12.06"E
PSSC2	Premier Fishing	34°35'22.51"S; 19°20'17.56"E
PSSC3	Aqunion Romansbaai	34°36'10.43"S; 19°20'14.18"E
PSSC4	Irvin & Johnson	34°37'37.13"S; 19°17'48.29"E
PSSC5	Buffelsjags Abalone, South Cape Abalone	34°44'39.11"S; 19°36'6.46"E

Station	Represented Farms	Coordinates
PSSD1	Zwembesi Farms	33°56'40.85"S, 25°37'45.78"E
PSSD2	Zwembesi Farms	33°57'20.02"S, 25°37'48.81"E
PSSD3	Ulwandle Fishing	34° 1'58.39"S, 25°41'59.32"E
PSSD4	Wild Coast Abalone	32˚45'06"S, 28˚16'32"E

5. SAMPLE SUBMISSION

Water samples shall be submitted to SeeWise, Amanzi Biosecurity or Nelson Mandela University laboratories for toxic phytoplankton identification and enumeration. The laboratory addresses are included below:

SeaWise laboratory
19 Main Road
Saldanha
7395

Amanzi Biosecurity laboratory
45 Jan van Riebeek Crescent
Sandbaai
Hermanus
7200

Nelson Mandela University laboratory:
Botany Department, Building 12, Ground Floor, Room 002
University Way, Summerstrand
Gqeberha
6031

Samples are required to be submitted to the laboratory once a week, except for high-risk areas where samples are required to be submitted at least three times a week for analysis. The DFFE shall determine which the high-risk areas are based on a risk assessment.

6. SEA BASED SAMPLING

The sampling procedure outlined below applies to farms that are sea based such as the bivalve farms.

6.1. Sampling Equipment

The sampling equipment required to take a representative phytoplankton sample from a water column for a sea-based farm consists of:

a) In Saldanha Bay a 6 m flexible tube (Lund tube) is used for Small Bay and a 10 m tube for Big Bay and Outer Bay North. In Algoa Bay a 5 m tube is used for Zwembesi Farms. The flexible tube should have an internal diameter of approximately 15 mm and a weight at the bottom end.
b) Clean bucket
c) Phytoplankton sample bottles

6.2. Sampling Procedure

The samples for sea-based farms shall be taken according to the procedure outlined below:

1. Lower the sampling tube into the water until the top of the tube is just above the water surface.
2. Close off the top end of the tube with thumb or stopper.
3. Pull the sampling tube out until the bottom end is just below the surface and lift bottom end out of water with free hand, tilting it upwards to prevent water from running out.
4. Drain the water into a clean bucket.
5. Gently mix the sample in the bucket well and sub-sample with sample bottle containing formalin/ lugols fixative, being careful not to pour out the fixative. Leave a header space for mixing and ensure that the cap is tightly screwed on afterwards.
6. Use clean, watertight bottles supplied by the relevant phytoplankton laboratory.
7. Should there be an obvious bloom, live samples should also be taken.
8. For live samples, use same procedure as described above for collecting the sample and sub-sample with a sampling bottle that does not contain fixative. Keep the live sample separate and cool at approximately 5˚C.
9. Keep all samples out of direct sunlight.

7. LAND BASED FARM AND RANCHING SAMPLING

Samples for land-based farms and ranching areas should ideally be taken out at sea as described above as wave action and pumps may damage phytoplankton cells. Due to the turbulent nature of the close inshore environment these samples also tend to include substantial amounts of organic debris and suspended solids. However, where this is not practical, the procedure outlined below should be used.

7.1. Sampling Equipment

The sampling equipment required to take a representative phytoplankton sample from a water column for a sea-based farm consists of:

a) Clean bucket
b) Rope if required to reach water at the sampling point
c) Phytoplankton sample bottles

7.2. Sampling Procedure

The samples for land-based farms shall be taken according to the procedure outlined below:

1. A clean bucket (with a rope attached if necessary) is used to scoop the water from near the intake; from the holding tank; the dam where the water is first discharged or at the specified site for ranching areas.
2. Sub-sample with the sample bottle, being careful not to pour out formalin/ lugols fixative and leave a header space for mixing. Ensure that the cap is tightly screwed on afterwards.
3. Use clean, watertight sample bottles supplied by the phytoplankton laboratory.
4. Should there be an obvious bloom, live samples should also be taken and submitted within 24 hours.
5. For live samples, use same procedure as described above for collecting the sample and sub-sample with a sampling bottle that does not contain fixative. Keep the live sample separate and cool at approximately 5˚C.
6. Keep all samples out of direct sunlight.

8. PHYTOPLANKTON IDENTIFICATION AND ENUMERATION

8.1. Background

The Utermöhl method (Hasle, 1978) is utilized to identify and enumerate phytoplankton samples collected in accordance with AMFFSP. A list of all organisms indicated in the programme shall be identified and

their respective concentrations are to be reported to the Food Safety Office of the Directorate Sustainable Aquaculture Management. The laboratory is required to be SANAS accredited based on ISO 17025 (General requirements for the competence of testing and calibration laboratories) and ISO 15204 (Water quality. Guidance standard on the enumeration of phytoplankton using inverted microscopy - Utermöhl technique) or working towards accreditation.

8.2. Laboratory equipment

The laboratory equipment required for the settling of the phytoplankton samples and the identification of the toxic phytoplankton species include:

- Inverted microscope.
- Settling units:
  - Polycarbonate sedimentation table
  - Counting chambers which consist of a sedimentation cylinder (Volume ranging from 5 to 50 ml) and bottom plate of coverslip thickness.
  - Rectangular glass cover plates for covering the sample once the settling chamber is removed.

8.3. Sample settling procedure

The settling of the phytoplankton samples for analysis includes the following steps:

1. The daily phytoplankton sample is mixed by gentle, repeated inversions of the fixed sample bottle for 60 seconds.
2. If there is a high sediment load the sample can first be filtered through a 200 µm mesh to remove the sediment.
3. Sub-sample by pouring the required volume directly into the selected settling chamber. Leave to settle for 24 hours.
4. After settling, carefully slide the sedimentation cylinder off the bottom plate and safely dump overlying sample water into a glass beaker. Simultaneously slide the rectangular cover slip over the sample well.
5. Dry the area around the cover slip with paper towel.

Generally, the samples should be settled in a 50 ml settling chamber to improve the concentration determination resolution. Samples with high densities of phytoplankton may be settled in 25 ml chambers if it is impractical to enumerate when settled in a 50 ml settling chamber.

8.4. Identification and enumeration

The identification of samples should be undertaken by trained personnel. Basic training is provided by DFFE and more advanced training is provided by the Intergovernmental Oceanographic Commission (IOC) of United Nations Educational, Scientific and Cultural Organization (UNESCO). Resources indicated below should also be considered, when required, to identify species. When necessary, photos of species that are difficult to identify can be sent to phytoplankton specialists at the Sea Point Research Aquarium in Cape Town for assistance.

Phytoplankton species identification resources:

1. IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (http://www.marinespecies.org/hab/index.php)
2. Phytoplankton Identification: a look at the tiny drifters along the California coast (http://oceandatacenter.ucsc.edu/home/outreach/PhytoID_fullset.pdf)
3. Hansen G. et al. 2001. Potentially harmful microalgae of the Western Indian Ocean: a guide based on a preliminary survey. IOC Manuals and Guides No.41. IOC of UNESCO (http://archive.iwlearn.net/bclme.org/factfig/HAB%20workshop/Books/Hansenetal2001.pdf)

4. Marine Phytoplankton Atlas of Kuwait's Waters. Published in Kuwait. 2009. Kuwait Institute for Scientific Research (https://www.researchgate.net/publication/246990259_Oceanographic_Atlas_of_Kuwait's_Waters).
5. Tomas C. R. 1997. Identifying Marine Phytoplankton. Academic Press
6. Hallegraeff G.M. et al. 2003. Manual on Harmful Marine Microalgae
7. Lassus P. et al. 2016. Toxic and Harmful Microalgae of the World Ocean. Denmark. ISSHA
8. OCHABs-IOC Science and Communication Centre for HABS phytoplankton proficiency test (Henrik Enevoldsen Email: hab.ioc@unesco.org)

Phytoplankton are identified to species level or at least to genus level if the species cannot be accurately identified. All harmful algal species listed in the AMFFSP shall be recorded and enumerated.

The enumeration for each relevant species shall be undertaken as follows:

1. Samples are examined with an inverted microscope using an objective magnification of at least X20 for routine scanning and at least X40 for confirming species identification when required.
2. The entire slide is assessed from one side to the other, starting at the top of the slide and moving down to the bottom in a snake-like pattern to not neglect any part of the slide.
3. The toxin producing species listed in the AMFFSP are counted and the concentration determined using the following formula:

   cell count / volume of chamber (ml) X 1 000 ml= cells/L

   Example: 200 cells / 50 X 1000 = 4 000 cells/L

The data record shall be completed with the following information:

- Sample reference number
- Phytoplankton sampling station number
- Date and time of collection
- Temperature
- Name of laboratory technician analysing the sample
- Volume settled
- Concentration of each of the toxic species present in the sample

Additional useful information would include:

- Temperature, Salinity and Turbidity recorded.

8.5. REPORTING RESULTS

The results from the phytoplankton laboratories shall be emailed to the Food Safety Office (Email: SAMSanitation@dffe.gov.za) within 24 hours of completing the analysis. Should there be a significant increase of more than 10% in the concentration of a potentially toxin-producing species the Food Safety Office and the relevant farms shall be notified on the same day.

9. STAFF HEALTH AND SAFETY AND WASTE DISPOSAL

Laboratory managers are required to ensure that appropriate Health and Safety procedures are implemented, and that staff are adequately trained in terms of the procedure requirements. Personal protective equipment (PPE) should be worn in the laboratory while handling and preparing samples for analysis including a laboratory coat, closed shoes and when required specialized mask for formalin fumes and safety glasses. If there is a fume hood in the laboratory, then a specialized mask does not have to be used while working with formalin in the fume hood.

Formalin is a hazardous substance as it is carcinogenic. Staff are required to be familiar with the Material Safety Datasheet requirements for formalin when handling and disposing of waste formalin and materials

contaminated with formalin. All hazardous waste should be placed in a designated and labeled hazardous waste containers and disposed of as hazardous waste.

10. REFERENCES

Department of Forestry, Fisheries and the Environment. 2024. Aquacultured Marine Fish Food Safety Programme (AMFFSP). Cape Town. Issue 8, 1-67.

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Appendix 1: Contact Information

Food Safety Office
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Sea Point Research Facility
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