REEF RESTORATION & ADAPTATION PROGRAM

Optimizing large-scale systems for Symbiodiniaceae culture - Standard Operating Procedure

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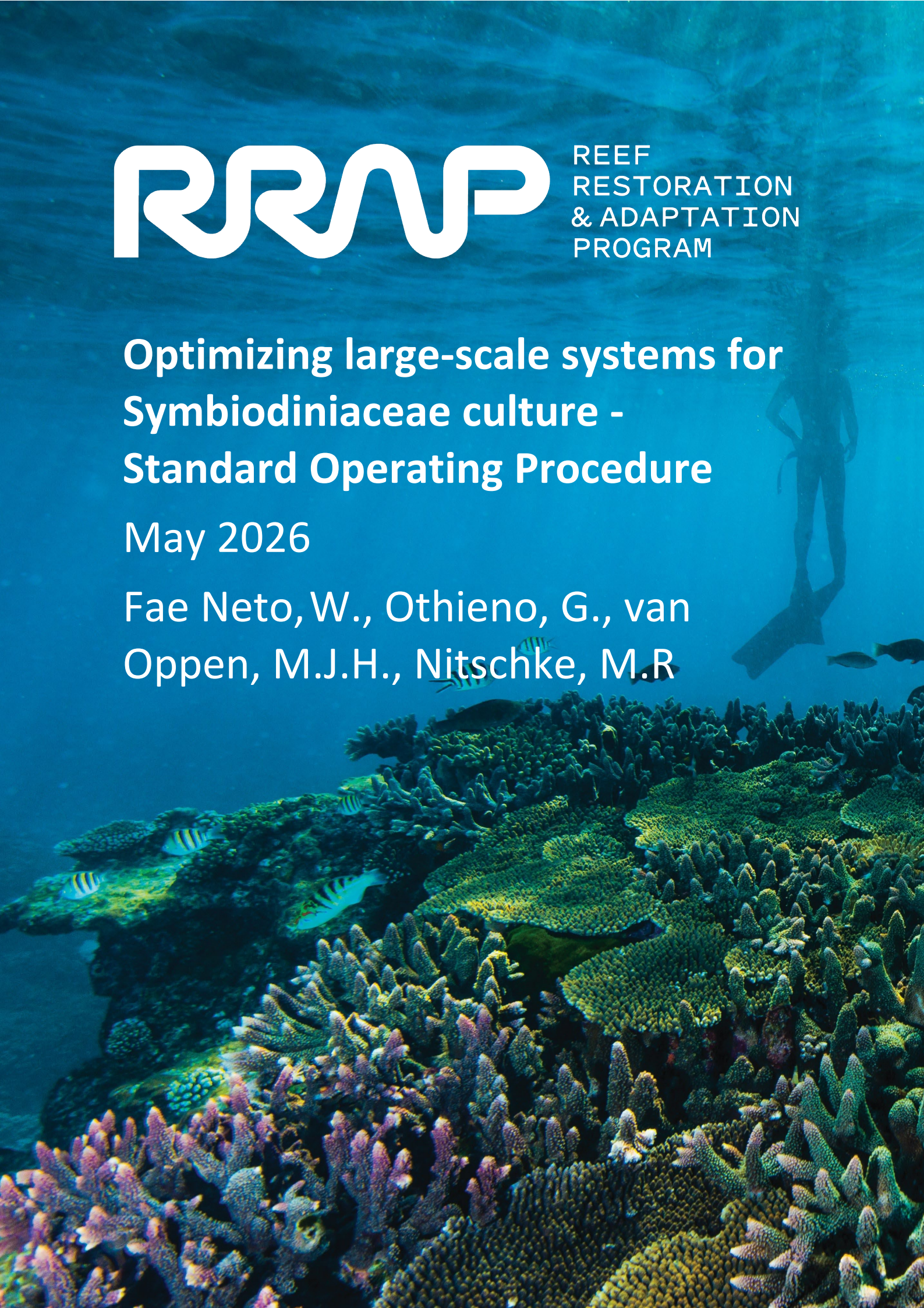

Optimizing large-scale systems for Symbiodiniaceae culture- Standard Operating Procedure

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Cover Images: Coral Reef, Credit: Gary Cranitch, Queensland Museum

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The RRAP partners acknowledge Aboriginal and Torres Strait Islander Peoples as the first marine scientists and carers of Country. We acknowledge the Traditional Owners of the places where RRAP works, both on land and in sea Country. We pay our respects to elders; past, present, and future; and their continuing culture, knowledge, beliefs, and spiritual connections to land and sea Country.

Where applicable: We specifically acknowledge and thank the following Traditional Owners of sea Country that this report relates to:

Location	**Traditional Owner Group**
The National Sea Simulator, Townsville	Bindal

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1 Executive Summary

This Standard Operating Procedure (SOP) outlines the process for culturing Symbiodiniaceae at volumes of 10 L to 400 L. These methods were optimised specifically for *Cladocopium proliferum* (SCF055) but are likely applicable to other species. Developed within the context of reef restoration efforts with reusable materials, this protocol provides a method for researchers and practitioners to cultivate microalgal cultures using bags and column systems commonly used for microalgae culturing with small modifications. These approaches are cost effective and require only limited dedicated infrastructure, making it easier to be used by programs from remote areas and/or developing countries, or in containerised systems. This method should be easily adaptable to use in existing infrastructure of aquarium or aquaculture industries. The SOP includes essential details on the adaptations made to commercial bags, aeration systems and general operational recommendations (e.g., inoculation volumes). It is intended for use by academic, government, and industry groups involved in coral aquaculture and algal biotechnology.

2 Background

This SOP builds upon recent experimental efforts aimed at increasing the culture volume of Symbiodiniaceae — photosynthetic microalgae critical to coral health and reef restoration efforts. In response to a rising demand for large-scale Symbiodiniaceae cultures, we focused on systems that have the potential for upscaling but also have low infrastructure demands, easing the path to adoption by partners. There is a general perception of shear-stress as a limiting factor in dinoflagellate culture, and early upscaling system ideas were designed around a perceived need to avoid strong aeration and prioritize culture vessel surface area for attached growth. However, such systems ultimately will prove challenging to maintenance, accurate sampling, and effective harvesting of cultures.

With these problems identified, this protocol documents a scalable batch culture method capable of producing up to 400 L of Symbiodiniaceae in one single cultivation bag. The setup was adapted from a commercially available microalgae culturing system used to provide feed for the National Sea Simulator (SeaSim) and adapted to account for Symbiodiniaceae's slow growth rate, and nutritional needs.

Cultures were initially inoculated into 1 L flasks filled with culture medium and expanded through successive transfers into 10 L carboys, then into 50 L and 400 L bags. The culture in the 400 L bags was carried out in two stages, first at 200 L and later doubling in volume and complementation of nutrients. These systems were placed in a temperature-controlled room and illuminated with moderate to high light intensities to optimise growth. This SOP also includes steps to safely discard any leftover culture in the bag appropriately.

This culture system was designed to be a batch system, and it took between 4-6 months to achieve the final volume and desired cell density, starting from 1 L of culture. The system was tested for two strains of *Cladocopium proliferum* (SCF 055.01-10 and SCF164) and will probably require adjustments for other strains or species. SCF055.01-10 and SCF164 were previously identified as fast growing in systems up to 10 L (carboys), which made them good candidates for these trials.

3 Objectives and Scope

Objectives

The objective of this study was to provide standard procedures to scale up cultures of Symbiodiniaceae, specifically *Cladocopium proliferum*, from 10 L to 400 L, in systems like those used by the aquaculture industry. This SOP was also designed to test generally held assumptions around the shear-sensitive nature of dinoflagellates, which is a perceived barrier to application of in existing commercial systems.

Scope

Applicable to research laboratories, basic facilities in developing countries and industry partners cultivating Symbiodiniaceae for reef restoration and other research applications. This SOP is aimed at medium to large scales of culture, matching the need of the largest coral restoration activities to date (e.g., inoculating 10,000 – 100,000 coral recruits generated by sexual reproduction techniques at densities of 10,000 cells mL).

4 Pre-requisites

To effectively follow this SOP, it is important the user has some familiarity with working under sterile conditions and basic laboratory skills. Access to an autoclave and biosafety cabinet are important to avoid contamination (especially for the handling of small volume mother-cultures), but are not essential to the workflow. Appropriate Task Risk Assessments and Chemical Risk Assessments should be updated before carrying out any of the activities in this procedure and the local health and safety guidelines should be followed. For scaling up cultures a facility location with controlled temperature, lighting (150-200 µmol photons.m^{-2}.s^{-1}) and air lines, as well as a source of seawater with a cartridge filter of at least 0.5 µm is required. Here, cultivation was conducted at a temperature of 27°C ± 2.

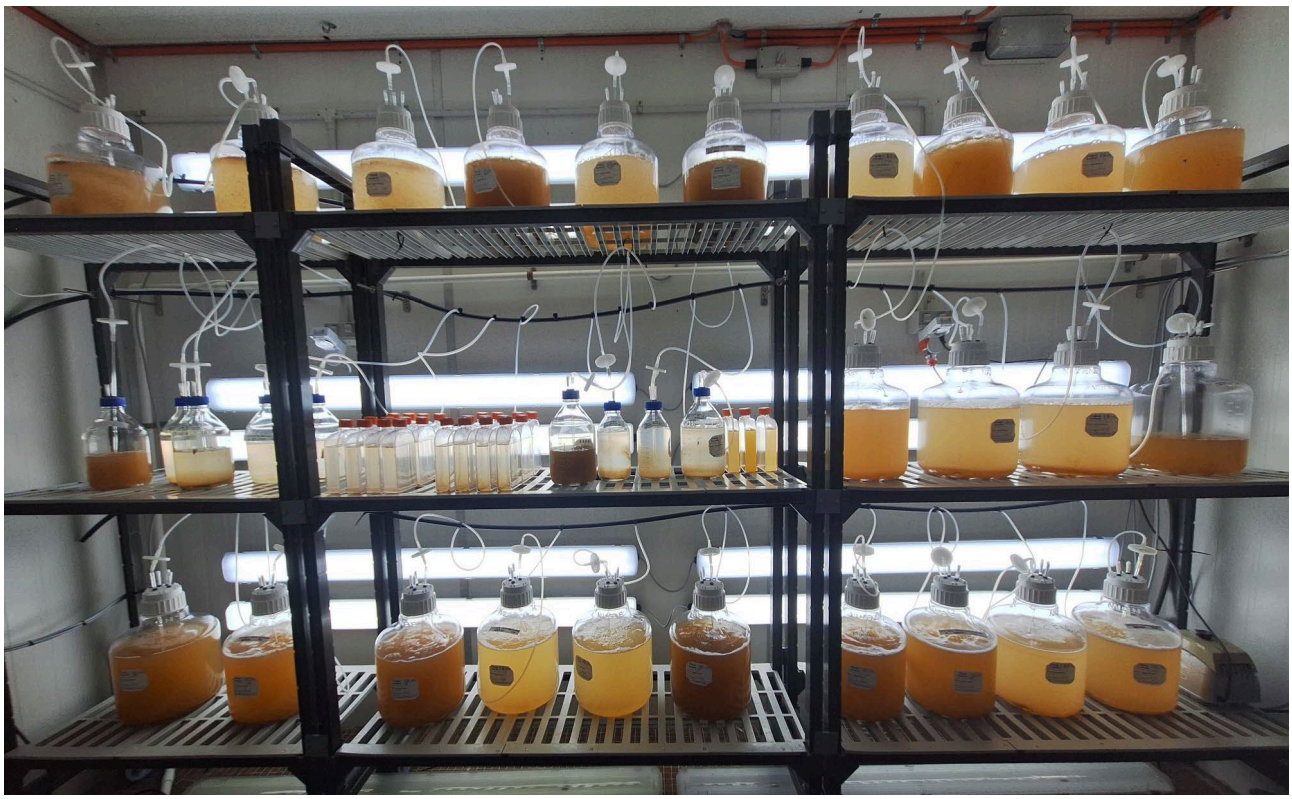

Figure 1- Cultures of various Symbiodiniaceae strains growing in carboys in a controlled temperature room with aeration and light system in place.

5 Identified Risks and Hazards

The following risks and hazards should be considered in large-scale cultivation of Symbiodiniaceae. Attention to sterile handling, safe chemical use, and physical ergonomics is essential for personnel safety.

5.1 Biological Hazards

Risk	Description	Mitigation
Contamination	Introduction of bacteria, fungi, or competing microalgae is especially detrimental in slow-growing cultures	- Use sterile techniques throughout - Filter-sterilize air and media - Use closed, aseptic transfer systems or spaces such as biosafety cabinets
Aerosol exposure	Aerosols can be generated during bag inflation, media handling, or sampling	- Perform small transfers inside biosafety cabinet - Wear eye protection during all liquid handling
Potential algal bioactives	Some dinoflagellates produce bioactive compounds (though *Cladocopium* is generally considered non-toxic)	- Avoid direct skin or eye contact - Use PPE (gloves, lab coat, goggles)

5.2 Chemical Hazards

Substance	Hazard	Mitigation
Sodium hypochlorite (bleach)	Corrosive; causes skin and eye irritation	- Use gloves and eye protection - Work in well-ventilated areas
Ascorbic acid	May causes skin, eye, and respiratory tract irritation.	-Wear appropriate PPE
Sodium thiosulfate	May causes skin, eye, and respiratory tract irritation.	- Wear appropriate PPE - Use masks or respirators if dealing with high dust concentrations
Ethanol (80%)	Flammable: vapor inhalation can irritate respiratory tract	- Keep away from flames and heat sources - Use in well-ventilated areas - Store in flammables cabinet
Media components (trace metals, vitamins)	May irritate skin or mucosa in concentrated form	- Handle with gloves - Avoid inhalation or ingestion
pH adjusters (NaOH, HCl)	Corrosive; can cause chemical burns	- Add slowly under stirring - Use appropriate PPE - Store properly and label clearly

5.3 Physical Hazards

Risk	Description	Mitigation
Bag rupture or overpressure	Excessive air pressure can rupture culture bags	- Use regulated air/gas flow (typically <0.2 vvm) - Ensure ports and connections are secure - Do not overinflate - Make sure air outlet is not blocked
Spills of large volumes	Spills can create slip hazards, contamination risk	- Place culture bags on spill trays/Nally bins or near drains - Have spill kits on hand - Disinfect spills with bleach immediately
Manual lifting of heavy culture bags	Full 50 L bags may weigh >50 kg; improper handling can cause back or joint injuries	- Do not attempt to lift heavy bags manually - Use carts, trolleys, or lift-assist equipment - Always work with a second person for large volumes
Electric shock or short-circuiting	Risk of electric shock or equipment damage when peristaltic pumps, air systems, or lights are used near wet surfaces or spilled media	- Keep all electrical equipment elevated and away from wet areas - Never handle plugs or cables with wet hands - Regularly inspect cords and power strips for damage
UV sterilization systems (if used)	Can cause eye and skin damage	- Avoid direct exposure - Use shielding and operate only with protective covers in place

5.4 Environmental Hazards

Risk	Description	Mitigation
Release of non-native or genetically distinct algae	Could disrupt local marine ecosystems if unintentionally released	- Sterilize all culture waste before disposal (e.g., 10% bleach for ≥30 minutes) - Follow institutional and environmental waste protocols - Use secondary containment for bags near drains

Release of bleach and other chemicals	Could disrupt local marine ecosystems if unintentionally released	- Make sure to neutralize any used chemicals

5.5 Personnel Safety Guidelines

- Always Wear PPE: lab coat, gloves, eye protection, mask as needed.
- Avoid working alone when handling large volumes.
- Ensure all personnel are trained in spill response and lifting ergonomics.
- Maintain a clean, organized workspace to prevent accidents.

6 Equipment and Materials

6.1 Procedure equipment

- Carboy (10 L) with aeration ports
- Measuring cylinder (1 L and 2 L)
- Nalgene rapid-flow filter (0.22 µm)
- Pump
- Falcon tube (50 mL)
- Air filter 0.22 µm
- Cartridge filter 0.5 µm
- Erlenmeyer (2L)
- Schott bottle (2 L) – 3
- Nally bin
- 50 L Liquid-handling plastic bag (Sartorius)(Annexure)
- Flow meter
- Pippett and tips (1000 µL, 200 µL, 20 µL)
- Hemocytometer
- Microscope (20-40x magnification)
- Clear algae bag (double sealed 200x100 cm)
- Cage (150 cm high and 60cm diameter with a curved in base).
- 0.01 µm mini filters
- 50 mL syringe barrel with narrow end cut off
- Chucks cloth
- Duct tape
- Needle
- Airlines
- Scissors
- Silicone tube (0.5 cm diameter)
- 0.2 cm diameter John guest tube
- DPD chlorine testing tablets
- Glass vial
- Funnel
- Water drum with tap
- Sodium thiosulfate
- IMK Daigo's media
- Bleach (10-15% active chlorine)
- Ascorbic acid

6.2 Personal protective equipment (PPE) and other safety equipment

It is important to wear closed shoes, protective goggles, lab coat, and nitrile gloves. The use of a biosafety cabinet is essential if you intend to retain the sterility of the mother culture and the culturing vessels during the early stages of the upscaling (up to 10 L) to keep the culture healthy and prevent contamination, and the use of a fume hood is advised when working with certain chemicals (refer to CRAs and SDS).

7 Steps for Implementation

7.1 Carboy culture (10 L)

7.1.1 Autoclave carboy

In a vertical autoclave, sterilize the 10 L carboy, with the carboy lid slightly open, at 121°C for 15 min. Follow the safety procedures in place for the use of autoclave, with proper training and PPE.

7.1.2 Filter water

Fill a clean water drum (30 L) with sea water filtered through a 0.5 µm cartridge filter and adjust the salinity using reverse osmosis/distilled water, for *Cladocopium proliferum* it is recommended between 33-35 ppm. (This can be used to fill multiple carboys or prepare smaller volumes for other culturing/uses)

In a biosafety cabinet, following working under sterile conditions practices, place the Nalgene Rapid-Flow filter (0.22 µm) apparatus, the Millipore pump, a sterile Schott bottle of 2 L, a 1 L graduated measuring cylinder and a sterile 10 L carboy. Screw the filtering apparatus into the Schott bottle and connect the hose from the Millipore pump to the filtering apparatus.

Fill a clean 1 L Schott bottle with water from the drum, take it inside the biosafety cabinet, measure one litre in a measuring cylinder, from the cylinder pour in small aliquots into the filter. After filling 2 L into the Schott bottle, empty it in the 10 L carboy, repeat until having 9 L of filtered sea water in the carboy.

7.1.3 Prepare IMK stock solution

Place a 2 L Erlenmeyer on a magnetic stirrer plate with a stirring rod inside. Pour 500 mL of RO water into a 1 L measuring cylinder, add 2 packages of IMK Daigo's media for 100 L each, and top up to 1 L with RO water. Cover the mouth of the measuring cylinder with a piece of parafilm and invert it a couple of times, transfer the solution to the 2 L Erlenmeyer. Add another litre of RO water to the cylinder and mix it well to remove any leftover IMK powder, transfer to the 2 L Erlenmeyer. Let the solution mixing for about 30 min.

In the biosafety cabinet, using a new Nalgene rapid-flow filter (0.22 µm) and a 2 L Schott bottle, filter the IMK solution from the 2 L Erlenmeyer into the 2 L Schott bottle.

This procedure will need to be repeated, larger volumes of IMK Daigo's stock solution can be prepared at once. This stock solution needs to be used at 10 $mL.L^{-1}$ of water.

7.1.4 Add IMK to Carboy

Following working with sterility techniques in a biosafety cabinet, use a sterile measuring cylinder or sterile serological pipette to add 100 mL of the IMK stock solution into the Carboy. Close the Carboy and mix it well.

7.1.5 Inoculate Carboy

Take a sample of the 1 L microalgae culture and check under the microscope for contamination. Counting in a Neubauer chamber can also be carried out at this point in case the inoculation requires a specific starting cell density.

Under sterile conditions, transfer the desired volume of culture to the 10 L Carboy; it is common practice when scaling up cultures to use the inoculum volume as 10% of the final volume when the culture is approaching the end of the exponential growth phase. Take a sample from the now inoculated carboy for cell counting under the microscope using a hemocytometer.

7.1.6 Growing conditions within the carboy

Place the carboy in a room with controlled temperature of 27±2 °C, irradiance of 150-200 µmol photons$\cdot m^{-2} \cdot s^{-1}$ and provide constant aeration to the culture, with a medium amount of air bubbles promoting a constant uplift in the centre of the carboy. It is recommend to manually pick up and rotate the

carboys in a circular motion by hand a few days a week also assist the culture into suspension. This seems to improve growth rates, especially when cell densities are higher. During the growing phase in the carboys, it is important to check weekly for contaminants and keep track of the cell density by doing cell counts. Samples should be taken in the most sterile conditions possible, preferably inside a biosafety cabinet or over a flame (if safe to do so). The cultures should be kept growing for about a month, or when the cell density reaches more than 1 x 10^6 cells.mL^{-1}; the time to reach this density depends on the initial cell density, with the ideal starting density around 2 x 10^5 cells.mL^{-1} (i.e, a fivefold increase in density over 30 days)

7.2 Culture in commercially available bags with small modifications (50 L)

7.2.1 Modifying the bag

Some adaptations were made to Sartorius 50 L bags (Flexsafe® 2D Bag - MPC - Tri-Clamp - 50 L, FLS130164) to allow aeration. The bags were reused from previous trials (see below for cleaning/sterilizing protocol), with no operational issues. The hose with the MPC port was cut about 3 cm from the bag and an aeration rod was inserted through. An extra piece of silicone tubing can be used to help fit the aeration rod, and an air filter should be attached to the tip of the aeration rod using some silicone tubing (Fig. 2). The superfluous sampling port can be cut above the pinch clamp allowing enough space to insert an airline filter (0.22 µm) that will function as air outlet. The third hose, with the septic valve (Tri-clamp), can be shortened to facilitate sampling (with a 5 mL serological pipette), while it can also be used to fill the bag, add nutrients and any other chemicals necessary.

Hazard: Using a glass aeration tube can be hazardous, with risk of breaking and perforating the bag. Take extra care.

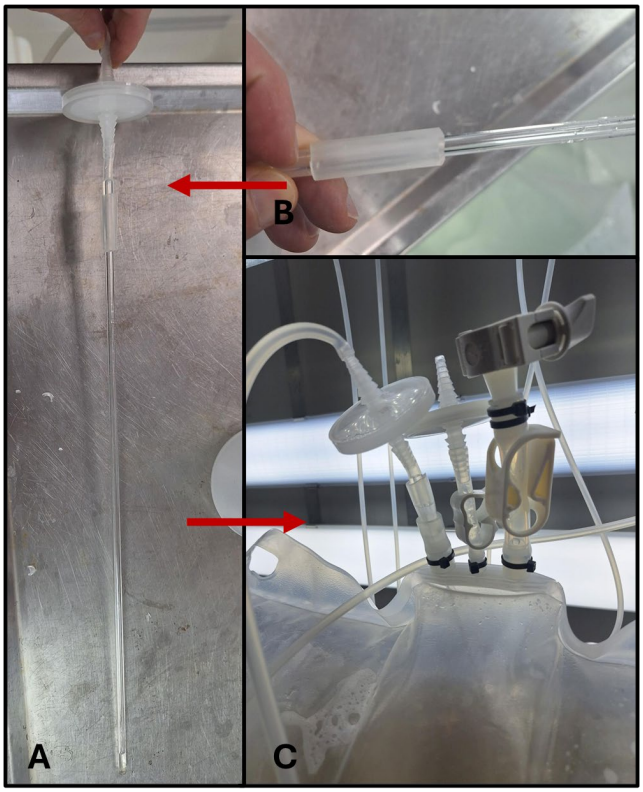

Figure 2 - Modifications made to a 50L sartorius bag. A) Aeration rod with air filter (0.22 µm) in place, B) piece of silicone hose to better fit aeration rod in the bag and C) bag valves after modifications, from left to right, air inlet with aeration rod inserted in, air outlet with pinch clamp, and septic valve shortened with pinch clamp.

7.2.2 Preparing the bag and a large volume of sterile seawater

Place the empty 50 L culture bag inside a Nally bin. Using the available connection on the bag, connect to a sea water line, with water passing through a 0.5 µm (or smaller mesh size) cartridge filter and completely fill the bag with water.

Note: If possible, fill bag in the desired place for culture to avoid moving/lifting the heavy bag. If necessary to move, make sure to have help and use trolleys to move it. This is a 2-3 person task.

Note: In case it is desired to adjust the salinity, before filling the bag measure the salinity of the sea water line and adjust the volume of seawater and RO water accordingly.

Note: Extra care must be taken to avoid the contact of the air valves with water.

While the seawater has been cartridge filtered, it is not guaranteed to be sterile. With the bag filled, remove as much air as possible and add 0.2 mL of bleach/ L of water. The bag should be left in the dark and without aeration for 24 hr. After 24 hr, prepare a solution of ascorbic acid in RO water by mixing 1.5 g into 50 mL of RO water and then inject through the needleless sampling port (final concentration should be 30 $mg.L^{-1}$ of water). Turn light and gentle aeration on and leave it for 24 hr to neutralize the bleach and remove any free chlorine. After 24 hr, take a sample of the water to test for free chlorine with DPD tablets (place the tablet in 10 mL of the sample in a glass vial and ensure its all clear without any pink or purple coloration.

Hazard: PPE must be worn when handling bleach.

7.2.3 Inoculation

With the help of a graduated bucket/container and a clean (UV sterilised or autoclaved) tube, remove about 15 L of the bag water. A pipette controller and serological pipette can be used to help create flow for siphoning water out of the bag. A peristaltic pump can also be used.

Transfer 500 mL of the IMK stock solution into the bag, trying to keep it as sterile as possible. Use 80% ethanol on surfaces and hands. After IMK has been transferred to the bag, keeping the transfer as sterile as possible, syphon culture from a 10 L carboy to the bag (Fig.3).
Hazard: When using electrical equipment near water sources make sure to follow health and safety guidelines.

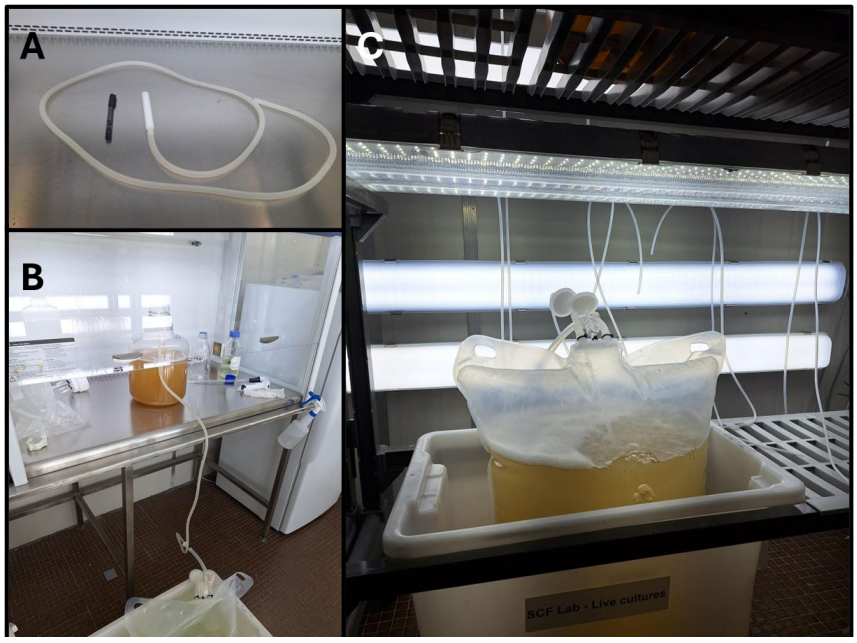

Figure 3 - Inoculation process for the 50 L bag, A) detail on silicone hose with a 3D printed piece that helps keeping the hose on the bottom of the carboy while syphoning, B) transfer from the carboy into the 50 L bag, C) culture bag inside a Nally bin, lights behind and above the bag allows for irradiance of 200 µmol photon.m^{-2}.s^{-1}.

7.2.4 Growth and maintenance

Cultivation should be performed in a controlled temperature room with temperature of 27 ± 1-2 °C, irradiance of 150-200 µmol photons·m^{-2}·s^{-1} (14L:10D) and constant aeration of the culture. Manually rocking the bags every couple of days can be helpful to keep the cells in suspension, taking care to not obstruct the air valves. During the growing stage it is important to check weekly for contaminants and keep track of the cell density by doing haemocytometer cell counts. Samples should be taken in the most sterile conditions possible, making sure to always clean surfaces and hands with 80% ethanol. The use of a 5 mL serological pipette through the septic port should be used to sample the culture (Fig. 4). Before opening the port, it is important to close aeration and wait for the pressure inside the bag to go down, this avoids aerosols to escape when opening the septic port. Before closing the septic port, close the stopper, spray the port and the cap with ethanol before putting it on, and fixing it in place. As for the carboys, the cultures should be kept growing for about a month, or when the cell density reaches more than 1 x 10^6 cells.mL^{-1}; the time to reach this density depends on the initial cell density, with the ideal starting density around 2 x 10^5 cells.mL^{-1} (i.e, a fivefold increase in density over 30 days)[1].

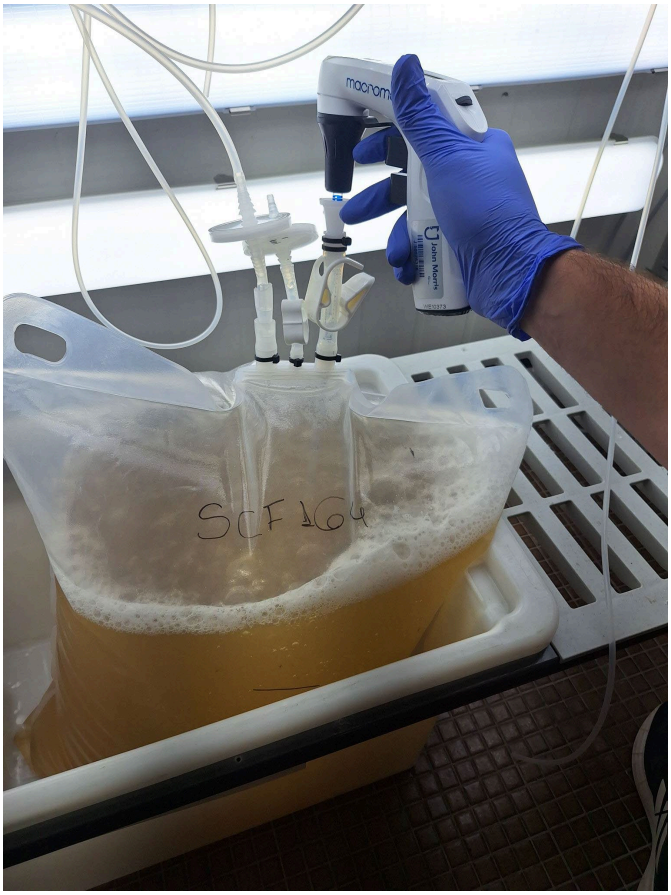

Figure 4 - Sampling a 50 L culture bag with a pipette controller and a 5 mL serological pipette.

7.3 Culturing up to 400 L

The set up of the 400 L bag must be done in the location where culturing will be carried out as the system cannot be moved. Light irradiance should be around 200 µmol photon m^2 s^{-1} and temperature of 27 ± 2°C.

7.3.1 Prepare culture bag

Check the bag for any lose/weak ends/seams (Figure 5, Left) that could potentially cause leaks. Insert the bag into the cage and the ensure the bottom corners are well positioned. Cut a 'Chucks' cloth in half, fold one half into three equal parts. Roll the folded Chucks and tape one end. Spray the untaped end with ethanol and insert into a modified 50 mL syringe barrel where the pipetting end has been removed (Figure 5, Centre). Cut

the bag at the top corner, attach the modified 50 mm syringe barrel with chucks cloth and tape the cut end of bag on to the syringe barrel (Figure 6, Left).

Figure 5 – Left – visually inspect the bag seams for any deformities or weaknesses, Centre – a modified syringe with sterilisable cloth cap, Right – the syringe/cloth cap taped into the corner of a bag.

Adjust the bag to fit perfectly into the cage with the bottom corners poking out of the lowest points (Figure 6, Left). Insert a 10 cm airline onto a silicon tube, connect this tube to filtered air source at one end and insert the other end through the syringe barrel and chucks cloth to inflate the bag (Figure 6, Centre). After the bag has inflated, attach tape on the top end of the bag and pierce a small hole through the centre of the taped part. Attach a 1000 µL pipette tip to a silicone tube (0.5 cm diameter) and connect to the outlet of a 0.01 µm mini filter (Figure 6, Right). Secure the connection between the silicon tube and the filter with a zip tie.

Figure 6 – Left – 400 L bag with the bottom corners protruding from the cage, Centre – filtered air being delivered to inflate the bag, Right – mini filter set-up through which filtered seawater will be delivered to the bag.

7.3.2 Filling the bag, aeration set up and sterilization

Connect a 5 cm long silicon tube on one end of the mini filter to a 0.2 cm diameter John guest tube (Figure 7, Left). Secure connections with a zip tie and insert the John guest fitting into the seawater supply. Open the valve and start filling the bag with seawater (Image H). When the bag fills to 200 L (approximately half the cage height), cut 5 cm of duct tape and place at the bottom corner of the bag where it was previously pulled through and protruding from the cage. Cut off 1 cm of the pointy end of the 1000 µL pipette tip and attach to a new filtered airline (see airline attachment to bag bottom in Figure 7, Right). Make a small hole at each bottom corner of the bag with sterile needle and attach the airline with the pipette tip through the hole. Ensure there is no leakage. To create a sampling point, cut a 5 cm length of duct tape and attach to the bag about 30 cm below the water level in the bag (Figure 7, Right). Pierce through the attached duct tape and attach a 1000 µL pipette tip connected to a 5 cm diameter silicon tube with a flow stopper. Finally, to sterilize the seawater, add bleach (0.2 mL L^{-1} of water) through the Chucks syringe barrel and leave for 24 hrs,

preferably in the dark. After 24 hrs, add ascorbic acid at a concentration of 30 mg L^{-1} of water and leave for 24 r with gentle aeration and lights on, to neutralize the bleach and remove any excessive chlorine. After the ascorbic acid has neutralized the chlorine, spray the sample collection area with 80% ethanol, unclip the stopper and let it flash for a few seconds. Take a sample of the water and clip the stopper. Test for free chlorine with DPD tablets (place the tablet in 10 mL of the sample in a glass vial and ensure its all clear without any pink or purple coloration).

Figure 7 – Left – example of the mini-filter and John Guest seawater tap feeding filtered sweater into a bag, Right – aeration (bottom right) and sampling ports created with pipette tips and duct tape.

7.3.3 Inoculation and scaling up

Rinse a funnel with hot water and spray with ethanol, remove the Chucks cloth from the syringe barrel and place on a paper towel, then insert the funnel into the syringe barrel placed on top of the bag. Pour the Daigo's IMK stock solution (10 $mL.L^{-1}$) through the funnel. The culture inoculum can be transferred from the 50 L bag to the 400 L bag using a pump or syphoning smaller volumes to a clean container (e.g., autoclaved carboy) and pouring into the funnel (Figure 8). Remove the funnel, spray the Chucks cloth with ethanol and insert back into the syringe barrel. Take samples routinely at least twice a week to monitor health and perform a haemocytometer count.

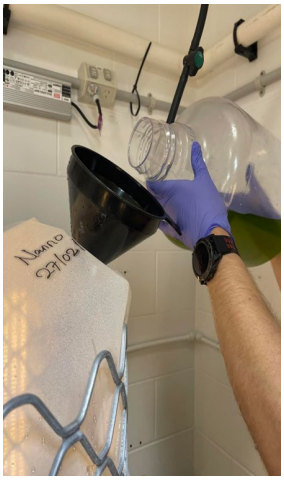

Figure 8 – Example pouring of a culture in a carboy into a funnel which is positioned in the syringe barrel.

After 32 days, the cell counts were observed to reach 8 x 10^5 cells mL^{-1} at which point the bag was filled to its maximum capacity of 400 L (Figure 9), and an appropriate amount of IMK stock solution added for this 200 L of extra seawater. At this scale of 400 L, the culture was only observed for another 12 days, at which

point the experiment was completed and the culture was discarded. Rather then this two-step process of 200 L and then upscaling to 400 L, it is likely that going straight from 2 x 10 L carboys or a 50 L bag straight into 400 L will be more desirable due to reduced amount of handling required during upscaling.

Figure 9 – SCF055 culture in the 400 L format.

7.4 Harvesting biomass

7.4.1 Carboys

For harvesting the biomass from carboys, it is recommended to use a biosafety cabinet, especially if the biomass is not used all at once (e.g. multiple days of inoculation). Use gloves and make sure to clean all surfaces and gloves with 80% ethanol. Remove the carboy lid and place it on a clean surface, avoid touching the inside of the cap or the aeration rod. Using a sterile cell scraper, resuspend the cells growing attached to the bottom and the walls of the carboy, careful not to rub your hand inside the carboy. Tilt the carboy gently to be able to look at the bottom of the carboy checking for any large clumps or patches of cells. Give the carboy a swirl and, taking care not to touch the inner surface of the carboy with your hand, use a pipette controller to help resuspend cells and remove other larger patches from the bottom by carefully pipetting up and down. You can then use the pipette to harvest small volumes, or you can pour from the carboy into a separate graduated clean container (volumetric cylinders, beakers or jugs/jars) for larger volumes.

7.4.2 50 L Bags

To harvest biomass from the 50 L bags, first close all the pinch clamps, give the bag a good swirl/shake so that most of the biomass is resuspended, carefully look at the bottom of the bag for patches of attached growth and tap/slap those spots to dislodge the patches of Symbiodiniaceae. Close the aeration and wait for the pressure inside the bag to reduce. Open the tri-clamp valve, place the septic valve stopper onto a clean surface, open the pinch clamp and harvest the culture by carefully pouring culture into a smaller clean container. Remember to use ethanol to disinfect your gloves and any other tool used in the process, as well as surfaces. Another option is, using clean tweezers, remove the filter from a 5 mL serological pipette and attach a tube/hose on top. Insert it through the septic valve and syphon, using the pipette controller and another serological pipette to create flow, the culture into a smaller container.

7.4.3 400 L Bags

To harvest the biomass from the 400 L bags use the sample port installed around the middle section of the bag to harvest the biomass into smaller vessels. It is important to use ethanol (80%) to clean the port, hands and surfaces. Another option is to use the insert created for aeration in one of the bottom corners or carefully create another port on the bottom of the bag following as it is described in the set-up section.

7.5 Discarding culture excess (or contaminated cultures)

In the case where the culture needs to be destroyed prior to discarding, it is recommended to bleach it to avoid any environmental contamination. The suggested protocol is as follows:

- Add 250 mL of bleach for every 50 L liter of culture (approx. 0.5% v/v final concentration).
- Leave it for 24 hr and check if the biomass is pale (Fig. 6)
- Prepare a sodium thiosulfate solution by dissolving 75 g of sodium thiosulfate in 1 L of warm RO water (it is easier to dissolve the thiosulfate in warm water).
- Neutralize the bleach with 300 mL of sodium thiosulfate solution for every 250 mL of bleach used, this does not take long (15-30 min).
- Take 10 mL of the water in a glass vial and test with a DPD tablet, if there are no signs of chlorine, the water can be discarded safely through the drain.

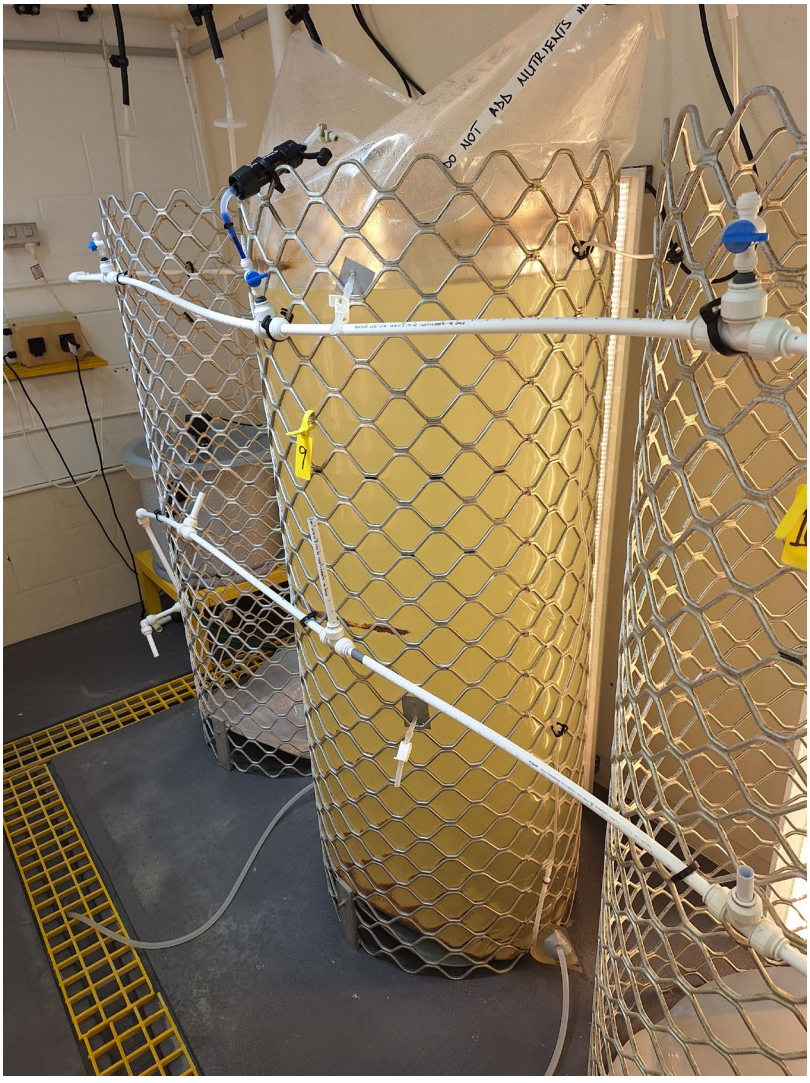

Figure 10 – Example of a culture that has been subjected to bleach for 24 hr.

8 Monitoring Cultures for Contamination

During standard hemocytometer counting of cultures to monitor for changes in cell density, care should be taken to observe any unusual cell types that could indicate culture contamination. The majority of Symbiodiniaceae cells (Figure 11, Left) should be golden brown, round, and approximately 10 µm in diameter. This is the general morphology of all Symbiodiniaceae cultures, with small variations in diameter from species to species (+- 2 µm). Other cell morphologies are occasionally visible (Figure 7, Right).

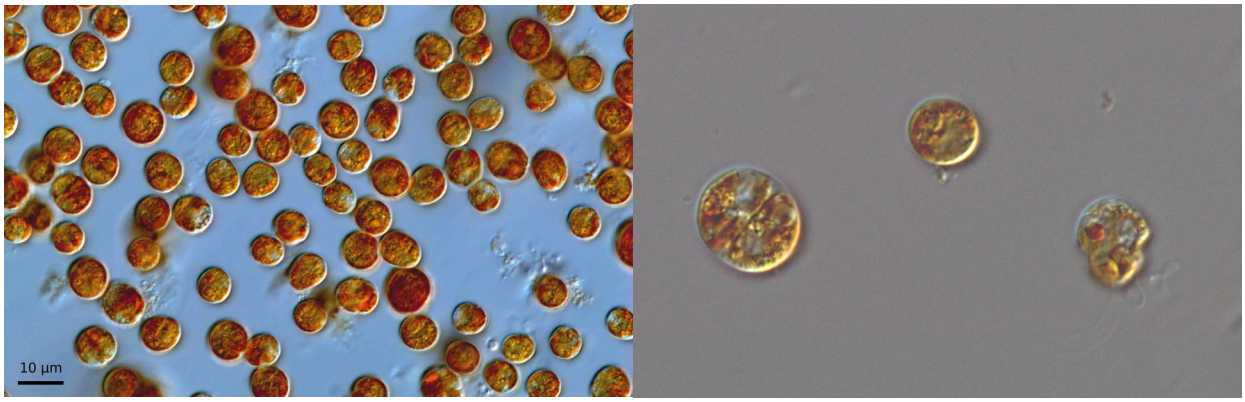

Figure 11. Representative images of SCF055. Left: this image is taken under 63x magnification on an oil-immersion objective and shows mostly round cell types. Right: other cell morphologies are occasionally visible including round, dividing cells with a central cleavage furrow (left cell), and swimming mastigote cells with asymmetrical shape (apical top slightly larger than antapical bottom) and flagella (right cell).

However, there are other micro-algae that have this same golden-brown colour. Diatoms, especially pennate diatoms, can grow very rapidly (much faster than Symbiodiniaceae) and have very similar pigmentation and can therefore contaminate cultures and go undetected until significant proliferation has occured. Contaminations with such diatoms (Figure 12) must be confirmed using microscopy. In the case of contamination with diatoms, germanium dioxide may be used at a concentration of 11 $mg.L^{-1}$ to reduce the contamination, however, elimination can be challenging.

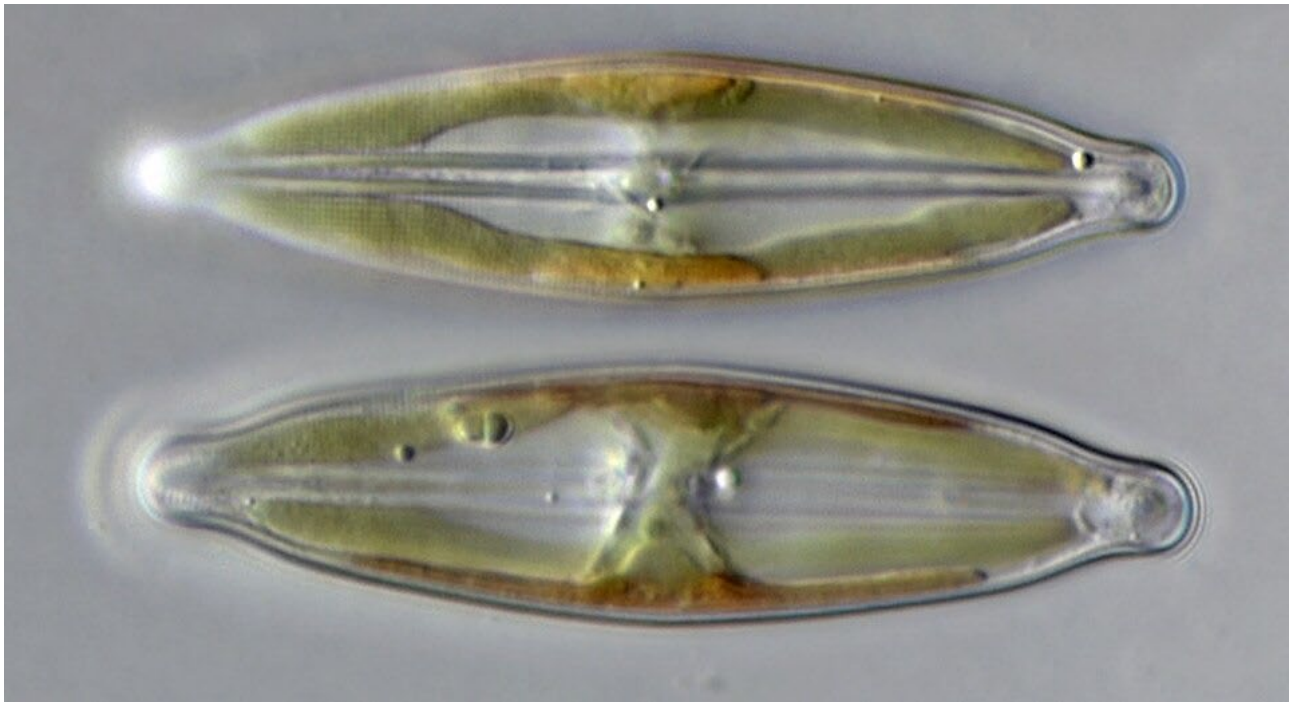

Figure 12: Likely contaminants of cultures include pennate diatoms, which have the same general golden-brown coloration as Symbiodiniaceae and can only be identified with microscopy. Photo by Djpmapfer, used under the

conditions of Creative Commons Attribution-Share Alike 4.0 International. Accessed from WikiCommons (File:Naviculoid diatom.jpg - Wikimedia Commons).

9 Acronyms

PPE – Personal Protective Equipment
SOP – Standard Operating Procedure
RO – Reverse Osmosis
CRA – Chemical Risk Assessment
TRA – Task Risk Assessment
SDS – Safety Data Sheet

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