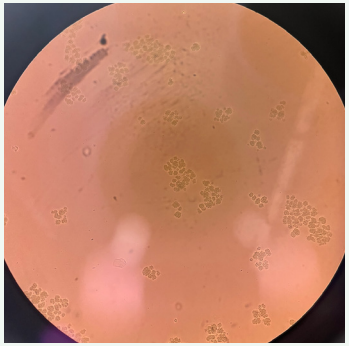
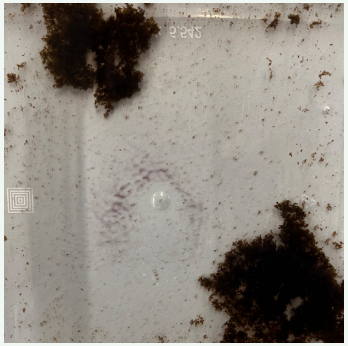
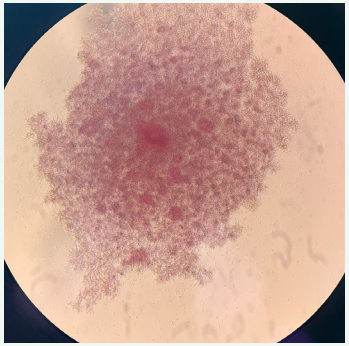
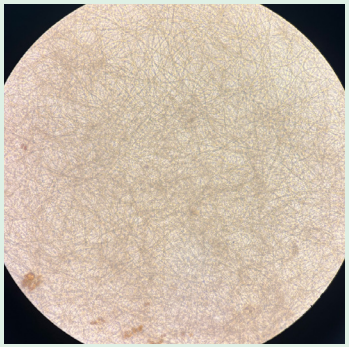
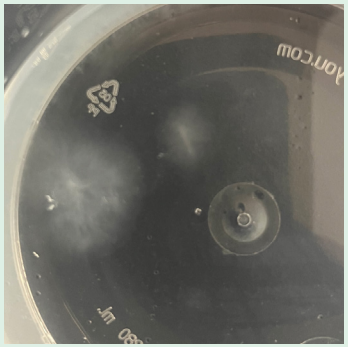
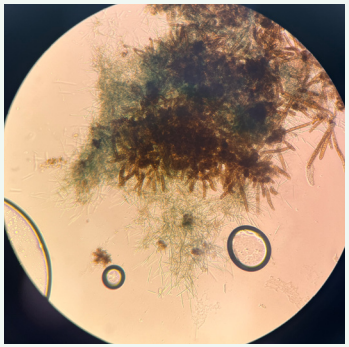
Contamination Identification for Gametophyte Cultures

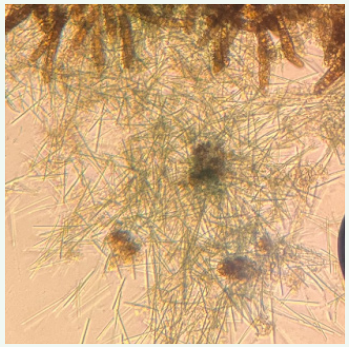
Bacteria	 	Grey/purple free-floating bacteria
	  	Pink attached bacteria
Fungi	 	

HORTIMARE GREENWAVE

Cyanobacteria

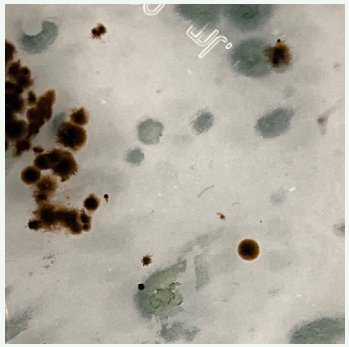
Cyanobacteria are a subset of gram-negative bacteria that use photosynthesis. While they are technically bacteria, they present unique risks to the health of cultures so we group them separately from other bacteria.

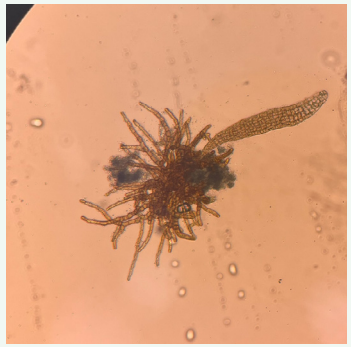


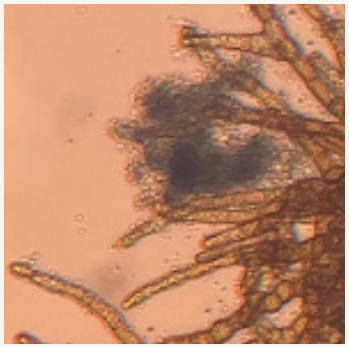


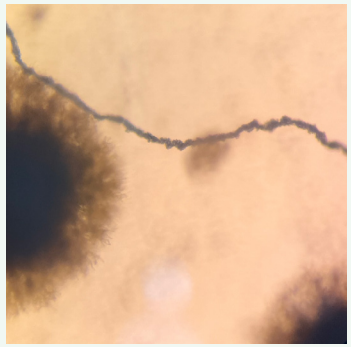
Green cyanobacteria

- Long cells with no pattern
- Green color
- Often found attached to gametophytes
- Macroscopically, looks like small green clusters







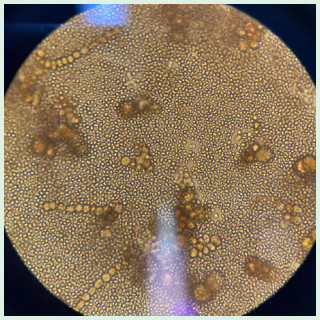


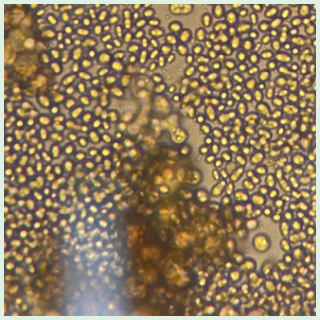
Blue-green cyanobacteria

- Small, round cells that are appear in clumps or chains
- Macroscopically, looks like small spots of blue-green cells or sometimes as light streaks on the sides of containers (images 1 & 2)
- Low abundance is just a few spots on container sides
- High abundance is when gametophytes are entangled with contamination clumps (images 3 & 4)

Microalgae

Microalgae are single-celled organisms, although they often exist in chains or groups. Single cells are not big enough to be visible by eye, but groups of them are. Most microalgae we find in cultures are blue-green.



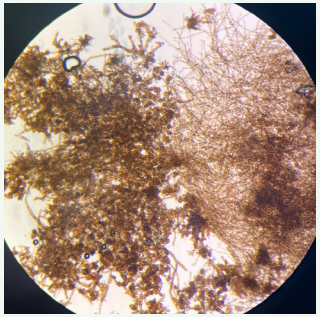


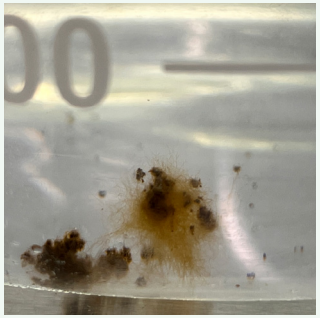
Other protists - green algae

- Visible microscopically, or sometimes create a “shiny” look in a culture
- Move around freely and quickly in the culture
- Smaller than gametophyte cells
- Images show very high abundance

Ectocarpus

Ectocarpoid-like are filamentous brown algae are a complex of different species that look similar to *Ectocarpus*. They look similar to kelp gametophytes when growing in a culture (image 1). When the ectocarpoid-like tufts exponentially grow they are visible macroscopically. You will need to look at a culture under the microscope to determine if there are ectocarpoid-like algae entwined with the kelp.

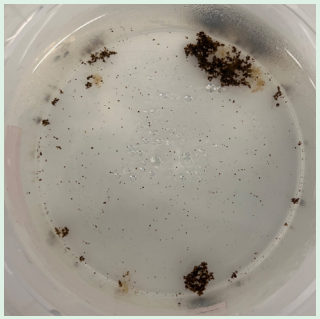




You can distinguish between kelp and ectocarpoid-like algae in three main ways:

1. Cell fill - ectocarpoid-like cells look “emptier” than kelp
2. Color - ectocarpoid-like is a lighter color than healthy kelp gametophytes
3. Morphology - both kelp gametophytes and ectocarpoid-like algae branch in similar ways, but ectocarpoid-like algae haves more long single filamentous (uniseriate) that branch out from the main cluster of filaments

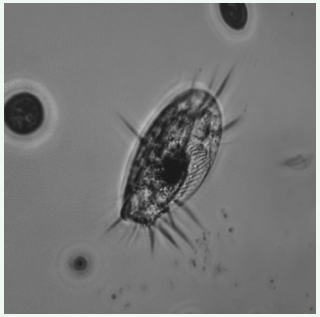
Ectocarpus continued

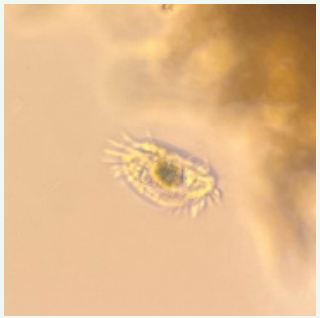


Since kelp and ectocarpoid-like are both brown alga, it is not possible to kill the ectocarpoid-like algae without damaging the kelp. Instead, you can treat low level ectocarpoid-like algae infections with manual measures. For cultures that look like image 4, you can pipette out any clumps of ectocarpoid-like thalli and move the remaining kelp gametophytes into a new container. Continue to monitor the culture until the ectocarpoid-like thalli are no longer present. Cultures that look like image 5 are not treatable because there are so many new tufts of ectocarpoid-like algae forming, and the kelp gametophytes are all entwined with them.

Protozoa

Protozoa/protists/Chytrids are single-celled organisms that can be free-living or parasitic. They feed on organic matter in cultures and are motile. Most single-celled contaminants that are moving in a culture can be considered protozoans. Euplotes and ciliates are common. We do not have any non-manual treatments to deal with protists so we resort to filtering them out since they are typically smaller than kelp gametophyte cells





Euplotes

- Visible microscopically
- Move around freely in the culture using legs
- Much larger than gametophyte cells
- Type of ciliate
- Images show low abundance

Dinoflagellate

Dinoflagellates are protists; we list them in a separate category due to their unique shape. They are similar in width to male gametophytes. Like other protists, they move using flagella and graze on organic matter. Dinoflagellates do not present a major threat to cultures, but can cause issues if left unchecked.

Photo from https://www.biologycorner.com/resources/dino.jpg

Photo from https://www.reef2reef.com/threads/dinoflagellates-%E2%80%93-are-you-tired-of-battling-altogether.293318/page-90

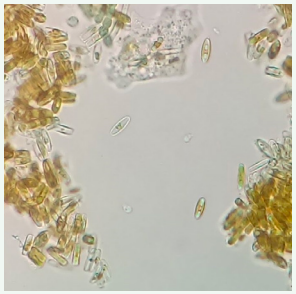
Dinoflagellates have many shapes and sizes. These are the two most common shapes we see. This first shape is very distinguishable as a dinoflagellate, but the second shape is less so because of its similarity to kelp cells.

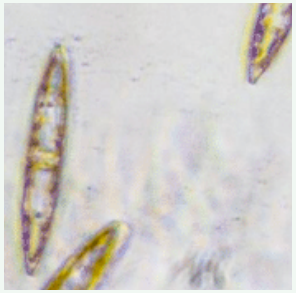
Dinoflagellates can be identified by:

1. Their cell walls are made of separate “plates” so you can often see gaps between them
2. They are not usually perfectly round

Diatoms

Diatoms are a type of protist; we list them separately because they are treated in a different way than other protists. Diatoms appear in cultures in a few different morphologies. Diatoms are a threat to cultures because they grow much faster than the gametophytes and can smother them.





We typically see two main shapes of diatoms in gametophyte cultures, which are shown here, but there are many more possible shapes.

There are a few main ways to identify diatoms:

1. Diatoms are based on their shape: either being pennate (like a shoe-box) or centric (like a Petri-dish).
2. Diatoms can be filamentous and form long chains.

Because of the unique cell wall structure of diatoms, there is a chemical way to treat them. Dosing a culture with germanium dioxide causes the diatoms to die by preventing them from creating new cell walls. It is important to treat diatoms early because the GeO2 treatment does not kill already existing diatoms, but instead prevents new growth.