Handbook of Algal Pests

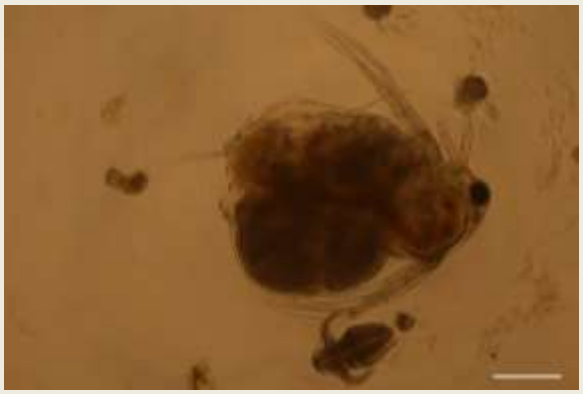
v.2 (for *Nannochloropsis* cultures)

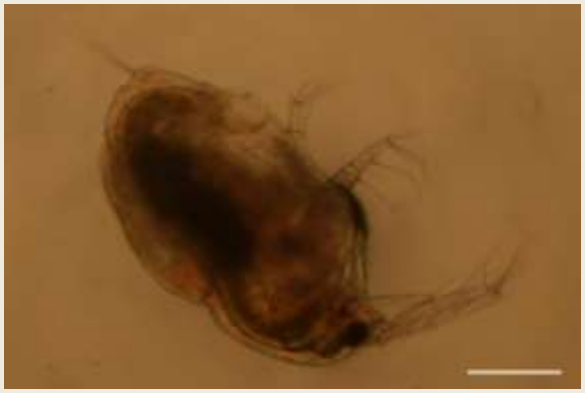
Arthropods

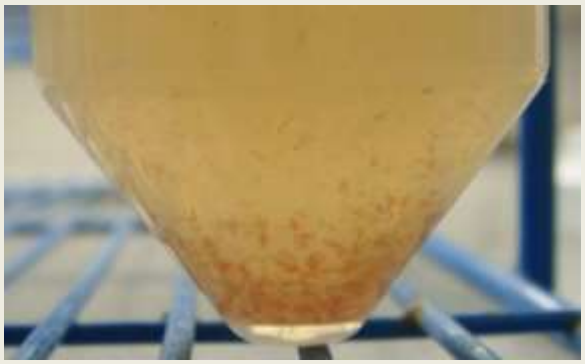
Arthropods are invertebrate animals with exoskeletons, segmented bodies, and jointed appendages. This group includes insects, spiders, and crustaceans. Although many arthropod pests can consume algae, some such as shoreflies and mosquitoes do not consume algae but are nuisances in and around ponds. Control measures may be necessary for both types of pests. In this section, details are provided for water fleas and midge flies, which have been identified as problem pests at Sapphire.

Water Fleas

Water fleas (order Cladocera, aka cladocerans) are group of small crustaceans with >600 species. They are common in inland freshwater habitats and can be found in other aquatic environments.







Immature Water Fleas
Size: 0.1-0.5 mm
Body: Ovoid, motile

Adult Water Fleas
Size: 0.5-1.5mm
Body: Shape varies. Water fleas are semi-transparent, but may take on varying shades of brown or red depending upon environmental conditions. Adult water fleas move quickly and appear to jump erratically -- leading to their common name.
Water fleas can be seen with the naked eye. Under a microscope, eggs or parts of fleas are best seen at 400X magnification.

Reproduction
Water fleas can reproduce asexually and sexually. During most of their active period, which occurs in the ice-free season in northern temperate climates, they reproduce asexually through parthenogenesis. Sexual reproduction may begin towards the end of the active period and leads to hatching of males and gamogenetic females, which results in fertilized resting eggs.

Symptoms of Infection
Water fleas are considered a pest if they reduce the productivity of an algal culture. Water fleas utilize algae and detritus as their main food source. Currently, the only sign of infection is visually observing pond samples for the physical presence of water fleas.

Identification and Treatment
ITS 1 & 2 sequencing exists for a single native water flea. The data closely link it to the genus *Chydorus*, likely a previously unidentified species. No qPCR primers are currently available for water fleas at Sapphire. Bravo appears to be most effective at managing populations of water fleas in ponds, although repeated dosing is needed to maintain recurring infections.

Midge Flies

Midge flies are a group of insects from several families of Diptera. Many are prey for insectivores and others are detritivores.

Scale bar = 1000 µm

Larvae
Size: 0.25-5 mm
Body: Larvae are elongate and cylindrical, with distinct segmentation and a hard sclerotized head. They have no true legs, but do have a pair of unjointed "prolegs" on the first segment of the thorax. The presence of this pair of prolegs, the absence of true legs, and the structure of the head are key traits for identifying larvae. Color varies widely among larvae, most are tan or brown.

Adult Fly
Size: 1-10mm
Body: Adults are small, slim, long-legged flies. They resemble and are often confused with mosquitoes, but they do not bite and have no scales on their wings. Many species rest on their hind two pairs of legs and hold their forelegs out in front of them. Generally, this pest is dark colored, usually brown or black.

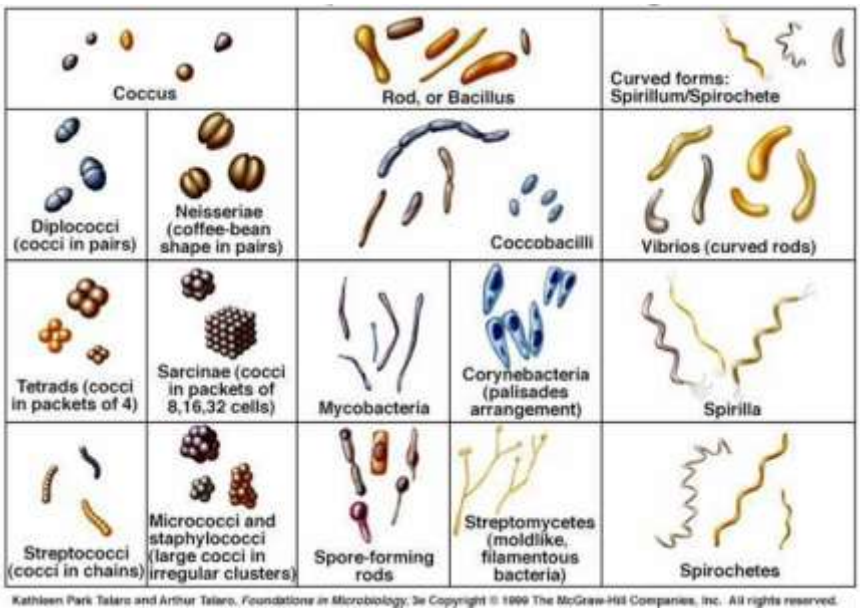
Reproduction
This pest reproduces sexually and mates in flight.
After mating and while in flight, the female releases her eggs into to water, where they sink to the bottom.

Symptoms of Infection
Currently, infections are detected by assessing the abundance of adults or larvae in ponds or pond samples.
Common signs of infection include:

- Presence or development of worms in pond or pond samples, most often at the bottom
- Presence of hatching adults on the pond surface

Identification and Treatment
Currently no ITS 1 & 2 sequencing or qPCR primers are available for this pest. AquaBac (BT.israeliensis) is used to treat cultures with related dipteran pests with varying rates of success. The Columbus site had some success treating unrelated pests with Bravo, but has had no experience with AquaBac.

Bacteria

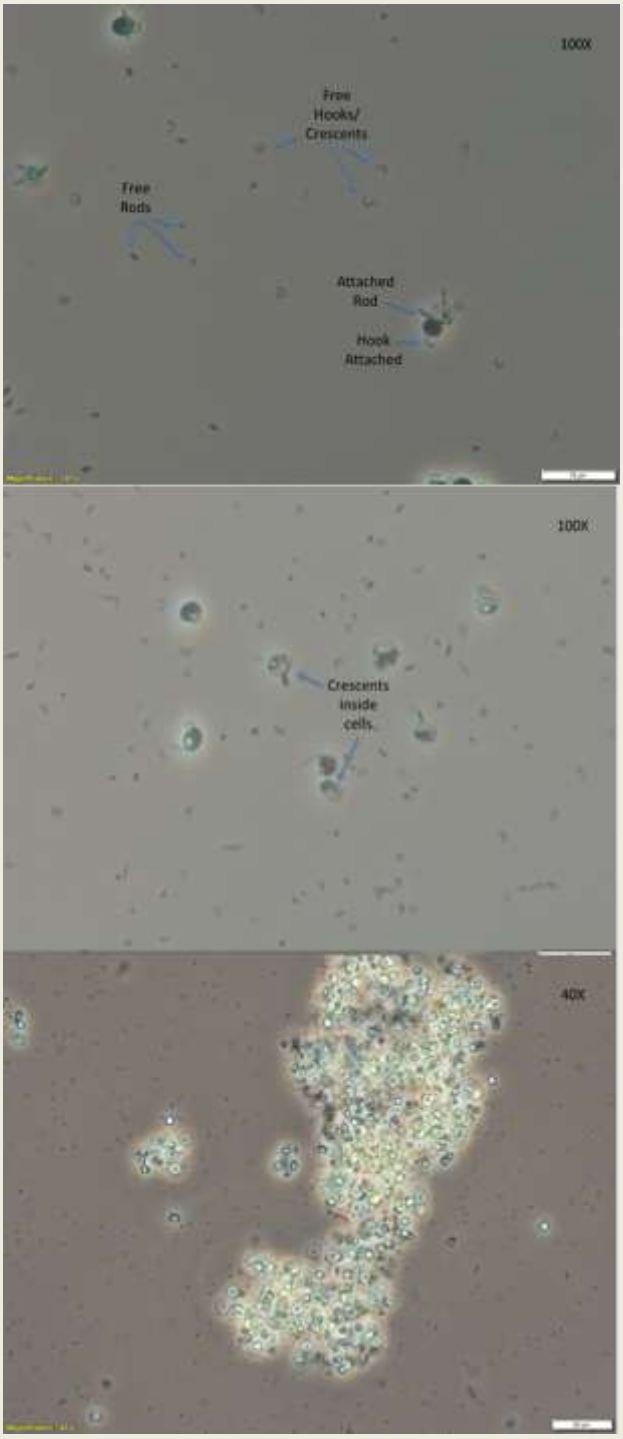


A wide variety of bacteria live in open ponds. Bacteria are prokaryotic microorganisms a few micrometers in length with varying shapes (e.g., spherical, rod, spiral). They can exist as individual cells or groups (clusters) of cells. They can only be seen under high magnification. Unlike cells of animals and other eukaryotes, bacteria do not contain a nucleus and rarely have membrane-bound organelles. They are also often unpigmented (black when seen through a microscope). Bacteria do include the phylum Cyanobacteria, a group of bacteria that are pigmented and photosynthesize. Cyanobacteria are often (incorrectly) called blue-green algae, as the term “algae” refers to a group of eukaryotes. In this guide, we separate our discussion of cyanobacteria from other bacteria.

Bacteria-algal interactions can be positive, negative, or neutral. Research at LCTS has focused primarily on predatory bacteria due to the negative effects that these can have on culture health and biomass; however, the large number of benign or potentially beneficial bacteria, along with presently indistinguishable phenotypes of parasitic bacteria present in ponds, complicates pest capture and identification. Below we describe the bacterium designated ‘FD111’ which has been a consistent *Nannochloropsis* pest that has been managed with crop protection actions in outdoor raceway ponds.

FD111

FD111 is a bacterium that has not yet been isolated or identified to the genus level. This bacterium causes rapid health decline and crash of *Nannochloropsis* cultures.



Size

~2 µM long by 0.3 µM wide

Morphology

Two bacterial phenotypes, 'rods' (straight, columnar bacteria) and 'hooks' (crescent shaped bacteria), are associated with cells experiencing pigment recession and death during FD111 crashes in *Nannochloropsis* cultures. The rods move erratically, the hooks appear to spin in place. It is presently unknown whether the different forms are lifecycle stages of the same pest or multiple species.

Infected cells undergo rapid pigment loss after bacteria attachment. Flocculation increases in most cases.

Symptoms of Infection

Common signs of infection include the following:

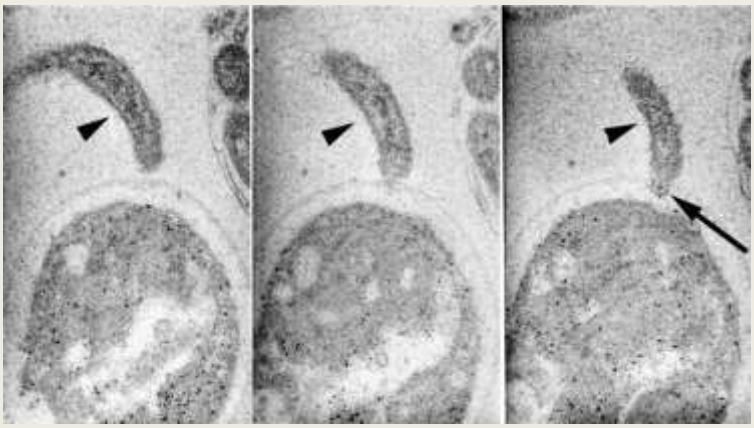
- Rod and crescent shaped bacteria become prevalent, attached to cells and free swimming
- 'Hollowed out' cells with single bright blue/green structure appear
- Analysis of qPCR data show a decline in the FD111 Ct value (decline in values for multiple days resulting in a <34 Ct at normal operating densities)
- Photosynthetic yield (via PAM fluorometry) decreases rapidly
- Flocculation occurs

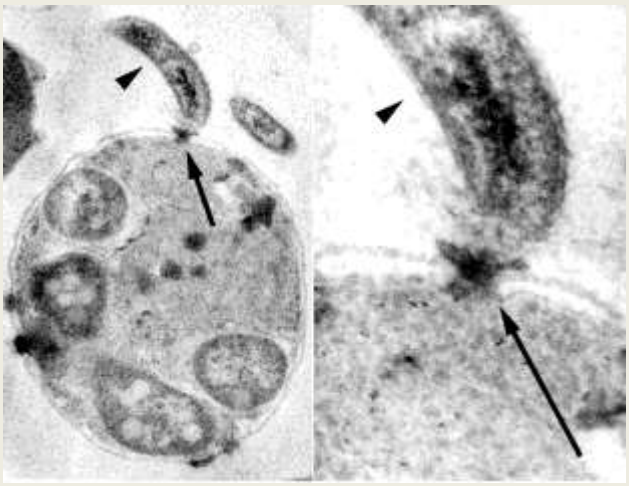
Identification and Treatment

Microscopic observations and qPCR can be used to confirm an FD111 infection. Bleach is the primary treatment option for FD111. Because FD111 can cause a rapid pond crash, a bleach treatment schedule is ideal to prevent FD111 crashes. In some situations, additional/higher bleach doses might be required.

Life Cycle

Experiments at LCTS and with outside collaborators have contributed to our knowledge of the FD111 life cycle and mode of infection. Electron microscopy has shown attachment of bacteria to *Nannchloropsis* cells and development of progeny inside the infected cell. Control efforts utilizing bleach are often scheduled as it is hypothesized that therapeutic levels of bleach dosing are ineffective in controlling stages of the pest protected inside of infected cells. The images to the right show stages of infection as described below.





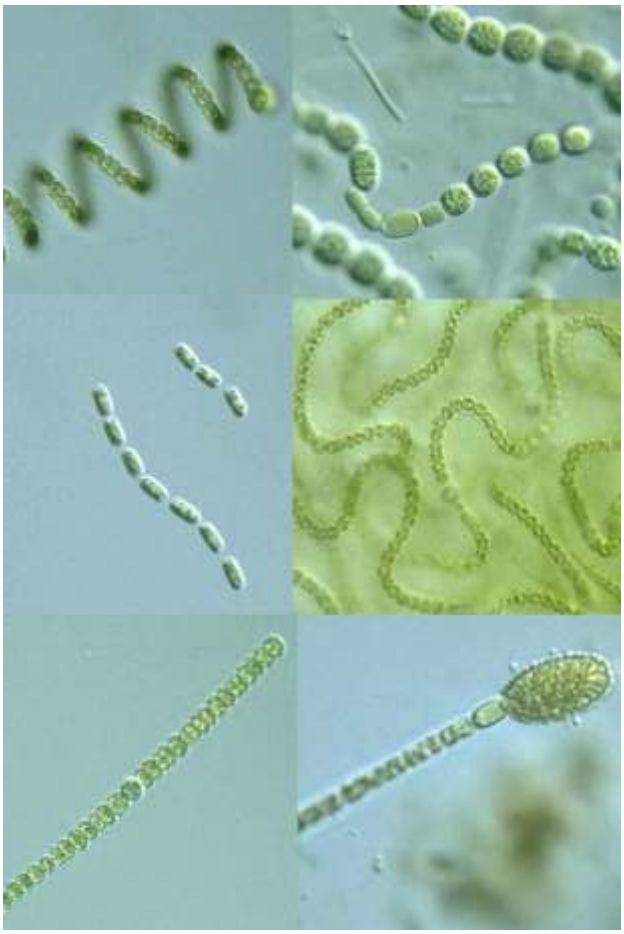
Top: Tunneling electron micrographs of FD111 infected cells show bacteria attaching to cell surface.

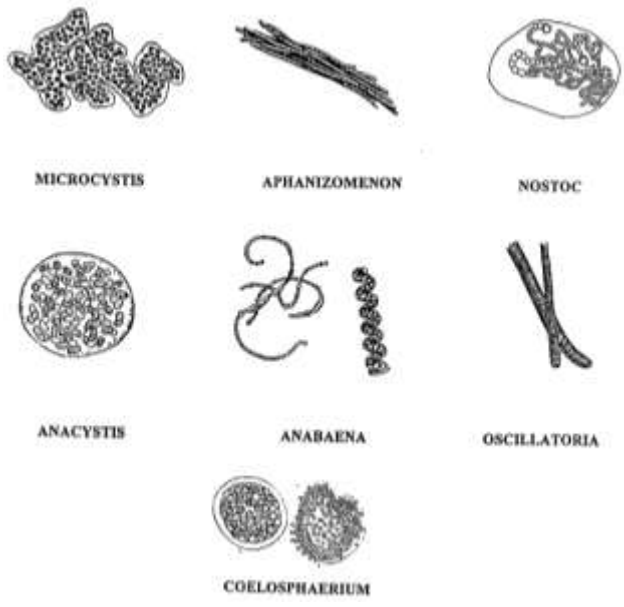
Middle: Pest penetrates cells wall and reproduction continues inside of the *Nannochloropsis* cell.

Bottom: Cross sections of infected cells show length-wise and cross-cut bacteria inside of an infected *Nannochloropsis* cell.

Cyanobacteria

Cyanobacteria are single celled or colonial photosynthetic bacteria found commonly in freshwater and brackish habitats – lakes, rivers, and estuaries. In natural environments, cyanobacteria blooms can reduce light and oxygen availability to other aquatic organisms. Notably, many cyanobacteria produce toxins that – at bloom concentrations – can be harmful to humans, affecting the liver (hepatotoxins), the nervous system (neurotoxins) and skin (dermatotoxins). In algal ponds, cyanobacteria can compete with production strains for resources or can have other effects (e.g., flocculation). Some low level of filamentous cyanobacteria is normal in production ponds, but large 'mats' could indicate treatment is needed. Due to their high growth rates and wide environmental tolerances, cyanobacteria are often the first photosynthetic organism to predominate after a culture has crashed or abiotic conditions (e.g., high temperature or alkalinity) begin to get out of normal ranges.





Specific control for cyanobacteria in actively growing culture has not been carried out to date in outdoor cultures at Sapphire. As previously mentioned, high levels of cyanobacteria have been an indicator of some other abiotic or biotic problem which has caused slowing or cessation of growth of the cultivation strain.

SE60993

SE60993 is a strain of the genus Leptolyngbya. The genus Leptolyngbya is the largest and most clearly polyphyletic (distantly related organisms with similar phenotypes placed into common taxa before the use of genomics) cyanobacterium genus.

Size
10-300 µm

Morphology
This strain is non-motile and blue-green in color. It is filamentous with long, solitary filaments or filaments coiled into clusters and fine mats. Cells divide by crosswise binary fission. It is best seen under 400X magnification.

Prevalence
SE60993 and morphologically similar cyanobacteria are common invaders of multiple algal cultures. It is a native pest and is ubiquitous in the local environment and visible throughout the year. SE60993 tends to proliferate when there is a lack of natural predation (e.g., from amoeba).

Identification and Treatment
16S sequencing is available for this pest. Omega 500F or Defiant are effective deterrents. SE60993, like other flocculating strains, is susceptible to DAF harvest and may be selectively removed with low polymer dose harvesting.

Scale bar = 10 µM

Synechocystis and –like organisms

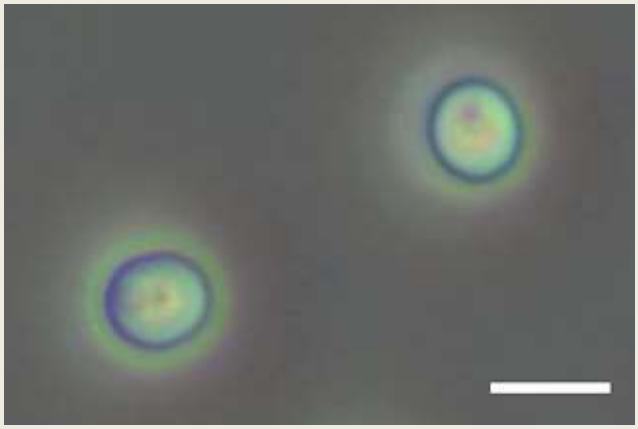
Synechocystis is a genus of unicellular cyanobacteria that is able to grow both phototropically during the day and heterotrophically at night. This organism can effectively anticipate transitions between the light and dark phases, allowing for ecological success. It is a ubiquitous taxon in both freshwater and marine environments.

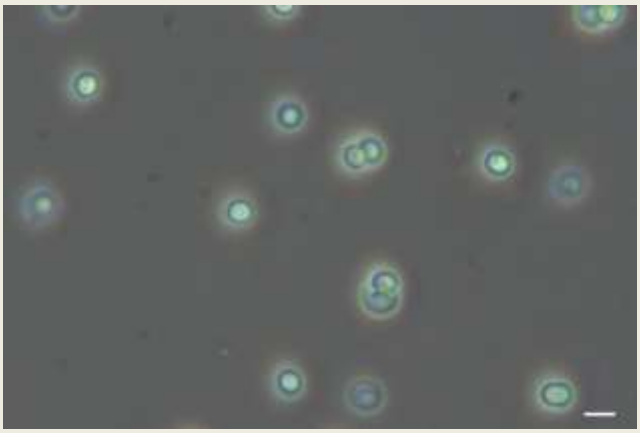
Size
3-5 µm

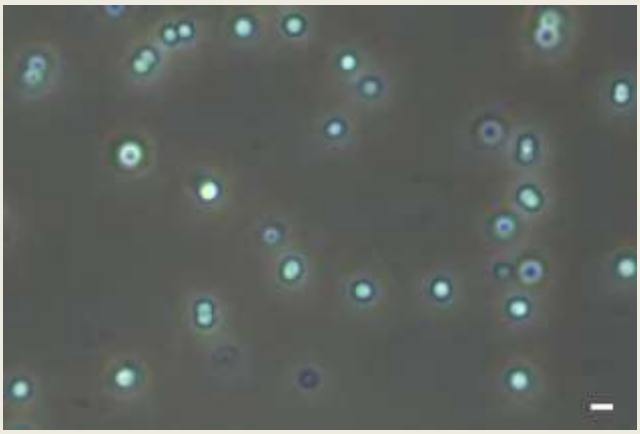
Morphology
Typically spherical/round. Ovoid when dividing, non-motile. Blue-green pigmentation. Cells normally seen individually or in pairs when dividing. Best seen under 400X magnification.

Prevalence
Synechocystis is a common invader of multiple algal cultures and has been consistently documented in cultures growing at the LCTS. It tends to proliferate after crashes of the target cultures.

Identification and Treatment
16S sequencing and qPCR primers are available for this pest. Defiant can control proliferation of *Synechocystis* species.







Scale bar = 4 µM

Chlorophytes

Chlorophytes form a division of green algae containing both unicellular and multicellular species. Most species live in aquatic (particularly freshwater) habitats, but some can be terrestrial. Due to their high growth rates, these algae can compete with desired strains for resources including space, nutrients, and sunlight. Green algae invaders, often termed "weeds", are common in open algae ponds. Many weed-algae have been captured from ponds in Las Cruces and Columbus and subsequently characterized. Some invading algae have proven to be useful production strains for different end products; however, most organisms require crop protection efforts because the invading species lack the desired phenotypes of the production strains (e.g., high EPA levels). Mechanical, chemical, cultural, and biological control methods have been validated for weed-strains and must often be used in conjunction to achieve effective control.

All open algae ponds are prone to (either gradual or rapid) contamination by chlorophyte species. Previously observed infestations have occurred as cultivation methods have favored the growth of the weed. For example, a harvest method effective for a cultivated strain will necessarily give an advantage to an invader resistant to that harvest method. Similarly, nutrients used more efficiently by an invader than by a cultivated strain will cause a gradual increase in relative concentration of weeds.

Control methods for these ubiquitous pests must take action thresholds into account and consider the integration of chemical, mechanical, and cultural control as well as the impact on the control of other non-algal pests. Previously successful weed control efforts at various scales have included:

- Addition of cultivated rotifers capable of eating the weed but not the production strain
- Temporary changes in harvest technique
- Settling of weeds by slower motivation of ponds
- Herbicides
- Changes in salinity/alkalinity

SE60992

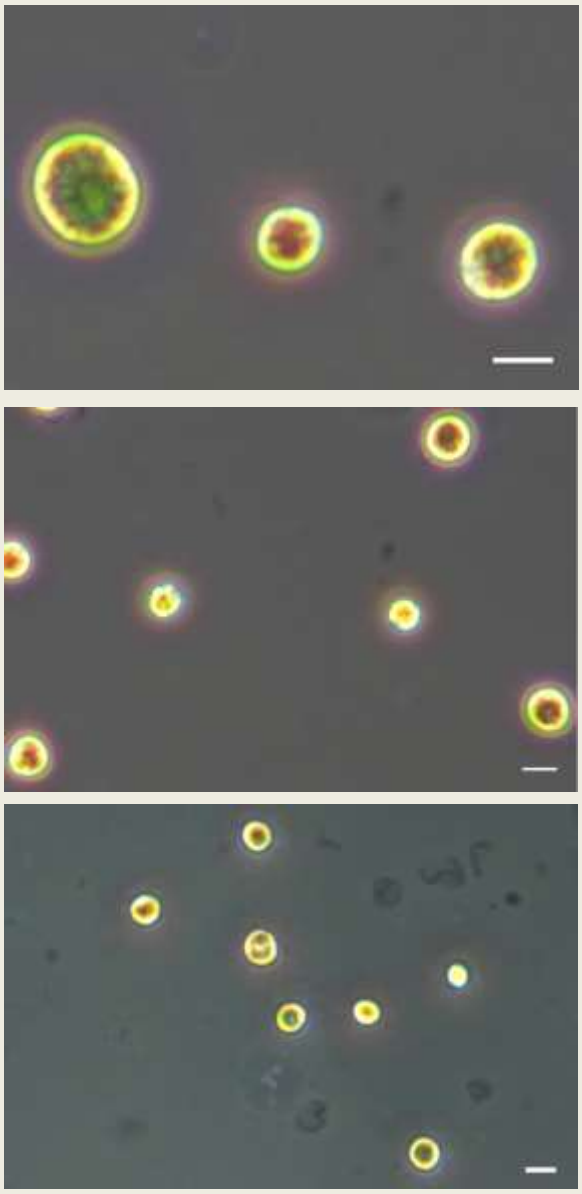
SE60992 strain was identified as Chlamydomonas mexicana via ITS1/2 sequencing. Chlamydomonas is a group of green algae consisting of unicellular flagellates, found in a variety of aquatic environments. In the field, this taxon has not presented with flagella. Chlamydomonas is a polyphyletic group within Volvocales, and many "Chlamydomonas" lineages are to be reclassificated.

Chlamydomonas previously captured at LCTS and Columbus are related to soil-dwelling algae common in local agroecosystems.

Size
4-8µm

Morphology
Typically, spherical/round. Ovoid when dividing, usually non-motile. Bright green pigmentation. Divide rapidly and will cluster into small aggregates. Best visualized under 400X magnification in bright field or phase II contrast.

Prevalence
Chlamydomonas species are common invaders of multiple algal cultures at the LCTS. They may be visible at very low levels throughout the year and tend to proliferate when suboptimal conditions exist for the target strain and alkalinity is low. Infestation has not been noted at high salinities.



Scale bar = 4 µm

Identification and Treatment
ITS sequencing and qPCR primers are currently not available for this pest. Increasing alkalinity levels in increments of 2,500 ppm may control proliferation of SE60992. 0.5 ppm Omega 500F is also an effective deterrent.

SE60994

This strain is a chlorophyte morphologically similar to Scenedesmus and Desmodesmus. It has not been genetically identified.

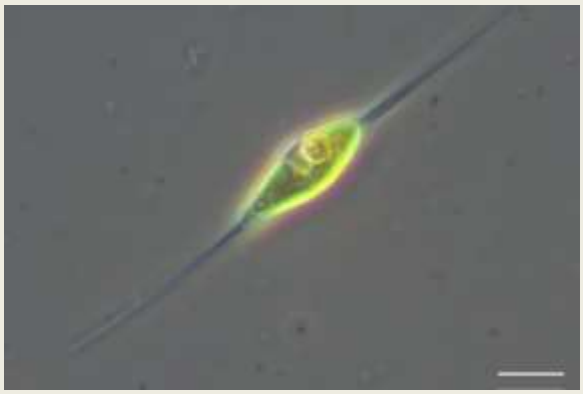
Size
10-30 µm

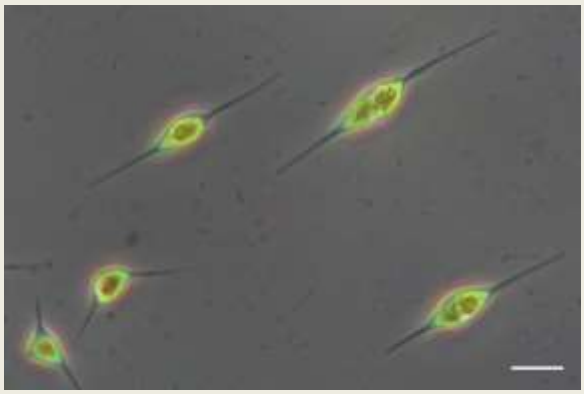
Morphology
Long, ovoid cells that taper at ends into elongated spikes; non-motile. Bright green pigmentation. Cells normally seen individually. Best visualized with 100X to 400X magnification.

Prevalence
SE60994 was discovered in media designed for *Spirulina* cultivation placed at Columbus in the summer of 2011. Based on experiments at the LCTS, SE60994 tends to proliferate under warmer temperatures and when:

- there is a lack of natural predation from chytrids and amoeba
- alkalinity concentrations increase above 10,000 ppm

Identification and Treatment
ITS sequencing is available for this pest. qPCR primers, however, are not currently available. 0.5 ppm Omega 500F is an effective deterrent of SE60994. Settling through lower motivation may also contribute to control.





Scale bar = 10 µm

SE60995 “Halfmoon”

This strain is a chlorophyte belongs to the Selenastraceae flamily. It was identified via ITS1/2 sequencing.

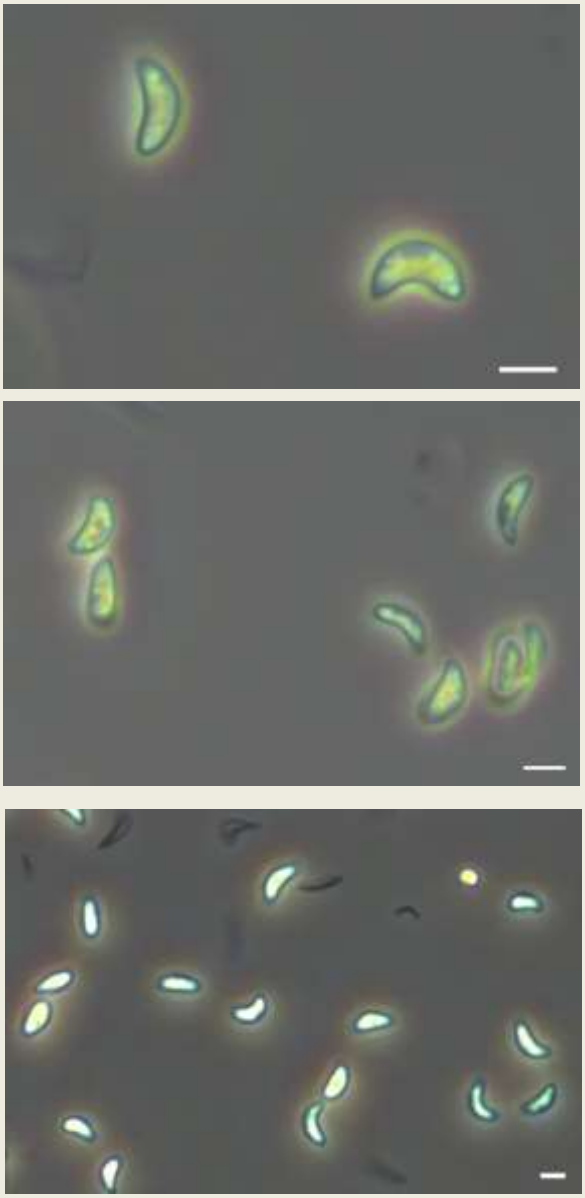
Size
4-8 µm

Morphology
Small, slender, crescent shaped cells; non-motile Bright green pigmentation. Cells normally seen individually. Best seen with 400X magnification.

Prevalence
SE60095 are common invaders of multiple algal cultures. They are believed to have first appeared in cultures of *Spirulina*. They may be visible at low levels throughout the year and tend to proliferate when:

- there is lack of natural predation from grazing protozoa (e.g. ciliates and amoeba) and rotifers
- alkalinity concentrations decrease below 10,000ppm

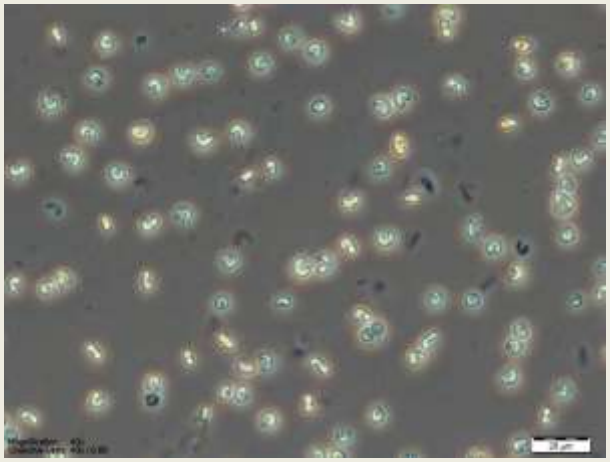
Identification and Treatment
ITS sequencing and qPCR primers are available for this pest. Pyraclostrobin (Headline) is also an effective deterrent.



Scale bar = 4 µm

SE61268 “Chubby Half Moon"

SE61268 is a chlorophyte in the family Selenastraceae. It was identified via ITS1/2 sequencing.



Scale bar = 20 µm

Size

~5 µm across, composed of an ~2 µm cylinder folded in a ‘C’ shape

Morphology

SE61268 presents as a blue green “half-moon” or crescent shape which may vary in the distance between its apical points. SE61268 can be differentiated by other similar forms of this taxon by its thickness of the cell giving its common name “chubby half-moons”.

Prevalence

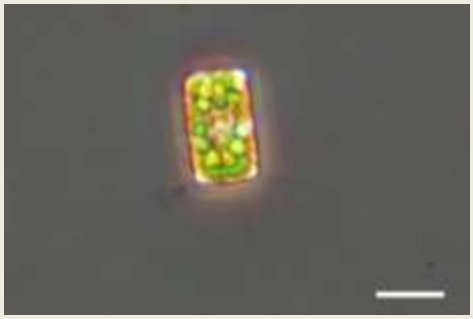
In 2013, SE61268 appeared concurrently at the LCTS and Colubmus ponds. It is a common invader of multiple algal cultures. It has been consistently seen in cultures growing at the LCTS and Columbus sites. It is visible throughout the year and tends to proliferate when:

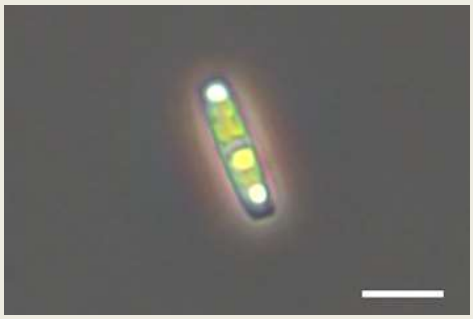
- ideal conditions are not available for the predominant strain (i.e. infection specific to the cultivation strain)
- there is a lack of natural predation/parasites potentially due to chemical control of similar pest taxa infecting the cultivated strain.
- the harvest strategy favors removal of the cultivated strain, but not invaders (this weed is not easily removed during polymer based harvest processes, resulting in rapid accumulation of the weed in cultures).

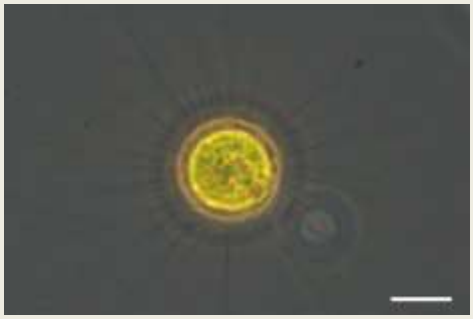
Identification and Treatment

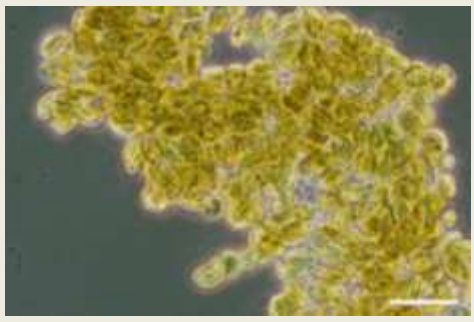
ITS 1 & 2 sequencing exists for a single native genotype. There are several phenotypes of this “weed” and not all may belong to SE61268. Chemical suppression of this weed can be achieved using Bravo or Omega, but the best results are seen by using a combination of 2ppm Bravo + 1ppm Omega.

Diatoms









Scale bar = 10µM

Diatoms, a major group of algae in the class Bacillariophyceae, are unicellular with forms adapted to be planktonic (suspended in the water column) or benthic (attached to a substrate). Diatoms have a characteristic siliceous frustule that can range in form, symmetry, and thickness depending on morphological as well as environmental characteristics. Diatoms can form colonies of various shapes (e.g., ribbons, fans, zigzags, stars) with connecting siliceous structures, chitin, or mucus. Diatoms are non-motile, but have flagellated sexual life stages. They occur as either pennate or centric forms. In both forms, the cell consists of two halves (smaller hypotheca, larger epitheca) separated by a girdle band.

Diatoms are mainly photosynthetic, but some are heterotrophic. Benthic diatoms are ubiquitous in outdoor algae cultures, as there is unoccupied substrate (i.e., walls of the ponds, paddlewheels) for them to occupy, reducing competition with the planktonic production strains. While diatoms compete with production strains for resources other than space (e.g., light, nutrients), they typically do not dominate cultures. They can create floating 'mats'.

Size & Morphology

2-200 µm. Various shapes and sizes. Pigmentation ranging from yellow to green to brown. Cells can occur individually or in aggregated mats (see general description above). Best seen with 100X to 400X magnification.

Prevalence and Symptoms of Infection

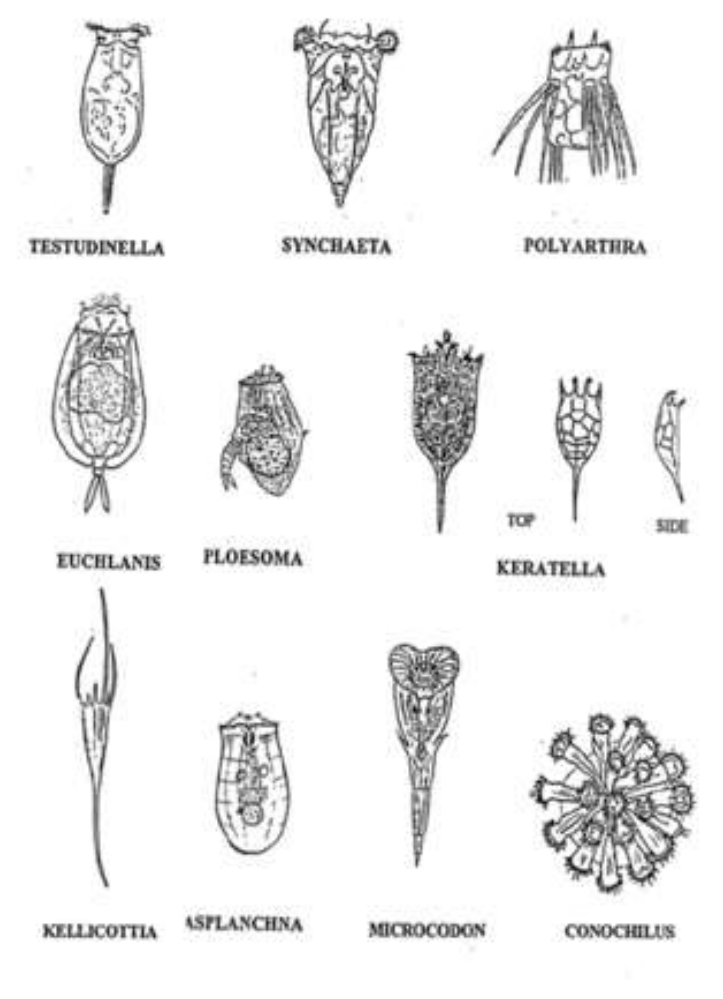
Diatoms are common invaders of multiple algal cultures and are commonly found as benthic mats on pond substrate (e.g., paddlewheels, walls). They have been consistently observed in cultures grown at the LCTS. Certain genera begin to appear in late fall and will remain until late spring, whereas others are predominantly present in summer months. They tend to dominate when ideal conditions aren't available for the predominant strain (e.g. temperatures cooling for green algae cultures).

Identification and Treatment

To date, no diatoms have been isolated for ITS sequencing or qPCR primer design. Diatoms can be controlled, to some degree, with bleach and hydrogen peroxide.

Rotifers

Rotifers are small grazing animals with bilateral symmetry and wheel-like mouths that sweep in microbes. Rotifers can be seen without a microscope but are very small. Rotifers can consume large amounts of algae and adjust their lifecycle speed and strategy based on food availability. The genus Brachionus has mostly been found in ponds. Once a population is established, rotifers can be difficult to manage because of their high production rate and the formation of cysts. Cysts are rotifer eggs that contain a very hard shell and can remain dormant for extended periods before hatching. They can desiccate and are wind-transported.



Cysts

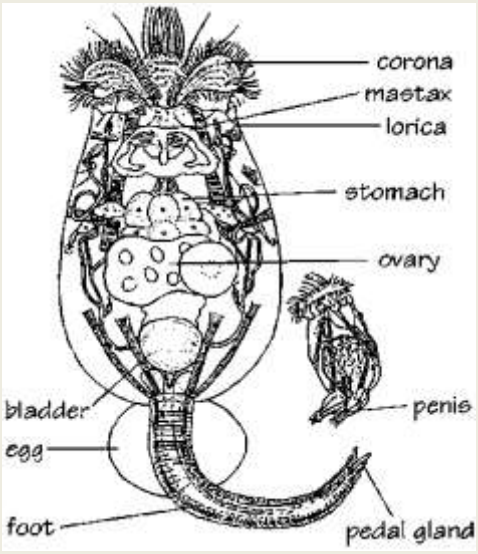
Size: 50-200 µm

Morphology: Ovoid, non-motile. Cysts are dormant rotifer eggs. Most have a brown hue. They are best seen with 100 X to 200X magnification .

Adults

Size: 40-500 µm

Morphology: Shape varies. Most rotifers are semi-transparent. Green algae will be noticeable in the stomach if they are feeding. Can be found swimming or anchored by their foot. Best seen with 4-10X objectives.



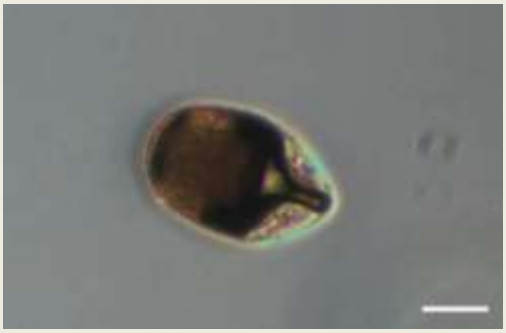
Symptoms of Infection

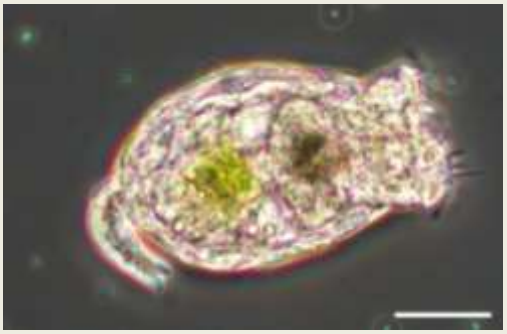
Rotifers are considered a pest if they consume the target culture. They are beneficial in cultures of SE50359 and other *Spirulina* strains as they control green algae invaders. Common signs of infection include:

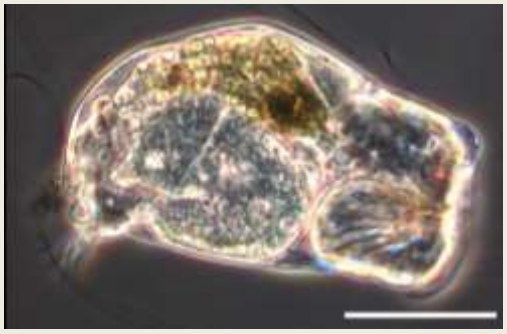
- Cell aggregation
- Flocculation (cultures settles quickly)
- A reduction in biomass not associated with harvest or another pest

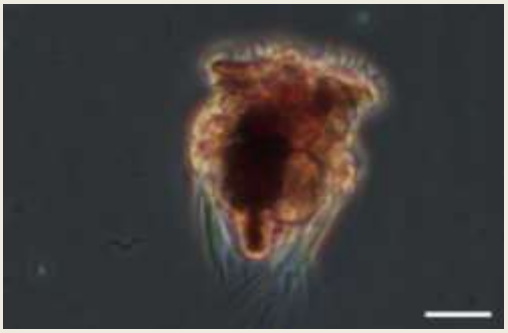
Identification and Treatment

ITS 1 & 2 sequencing exists for a single native rotifer identified as the genus *Brachionus*. No qPCR primers are available for this pest. Omega 500F, Bravo, Calcium Hypochlorite and Thiram 42S can effectively treat an infection. Ammonium sulfate and bleach have also been used to control populations but results can vary. Rotifers should be treated as soon as they are found, as treatment at larger numbers is challenging.









Scale bar = 50 μM

Amoeba

Amoeba are cells or organisms that move by extending projections of protoplasm. Many small amoebas are ubiquitous in outdoor algae cultures and present no threat to production strains. Other amoebas are indiscriminate grazers which can hurt algal culture productivity, but are rarely identified as the initial cause of a crash. Presence of high numbers of amoeba is often a secondary effect of an unrelated culture health issue.

Size
5-100 µm

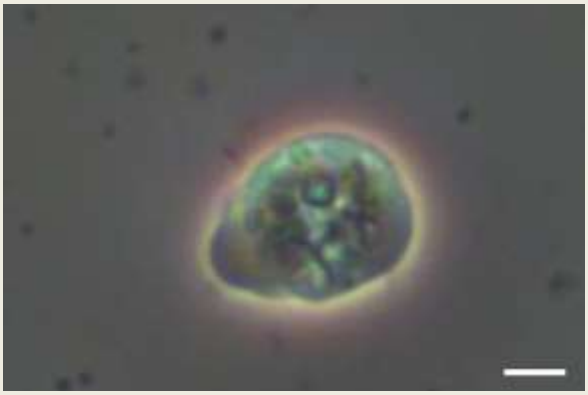
Morphology
Irregular, shape constantly changing. Semi-transparent. Contain the contents of the organisms they are consuming.

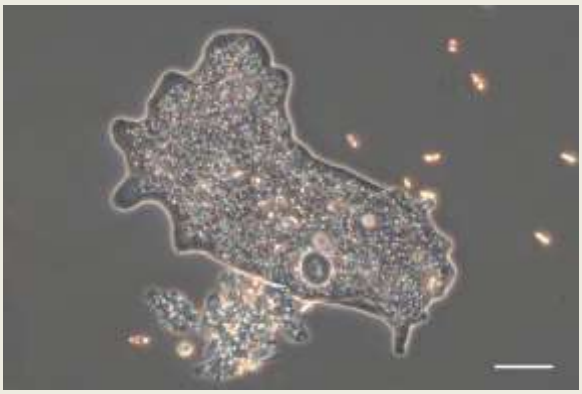
Symptoms of Infection
Amoeba are considered a pest if they reduce culture productivity. They tend to consume filamentous cyanobacteria (e.g. *Leptolyngbya*) and smaller green algae. They also consume dead organic matter.

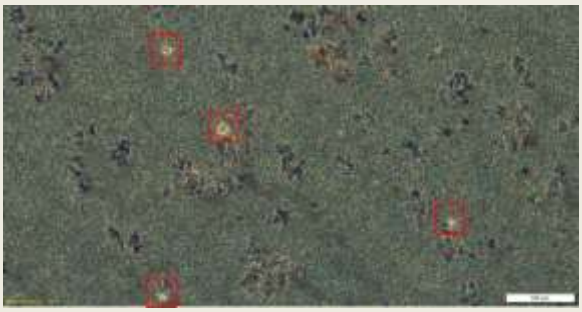
Common signs of infection include:

- Presence of amoeba cysts
- Severe aggregation, with amoeba embedded in aggregates
- Rapid settling of culture

Identification and Treatment
ITS 1 & 2 sequencing and qPCR primers are available for one amoeba (*Naegleria*). Omega 500F and ammonium sulfate can effectively treat an infection. Amoeba cysts can remain in cultures after treatment.







Scale bar = 5µM (top), 20µM (other images)

Ciliates

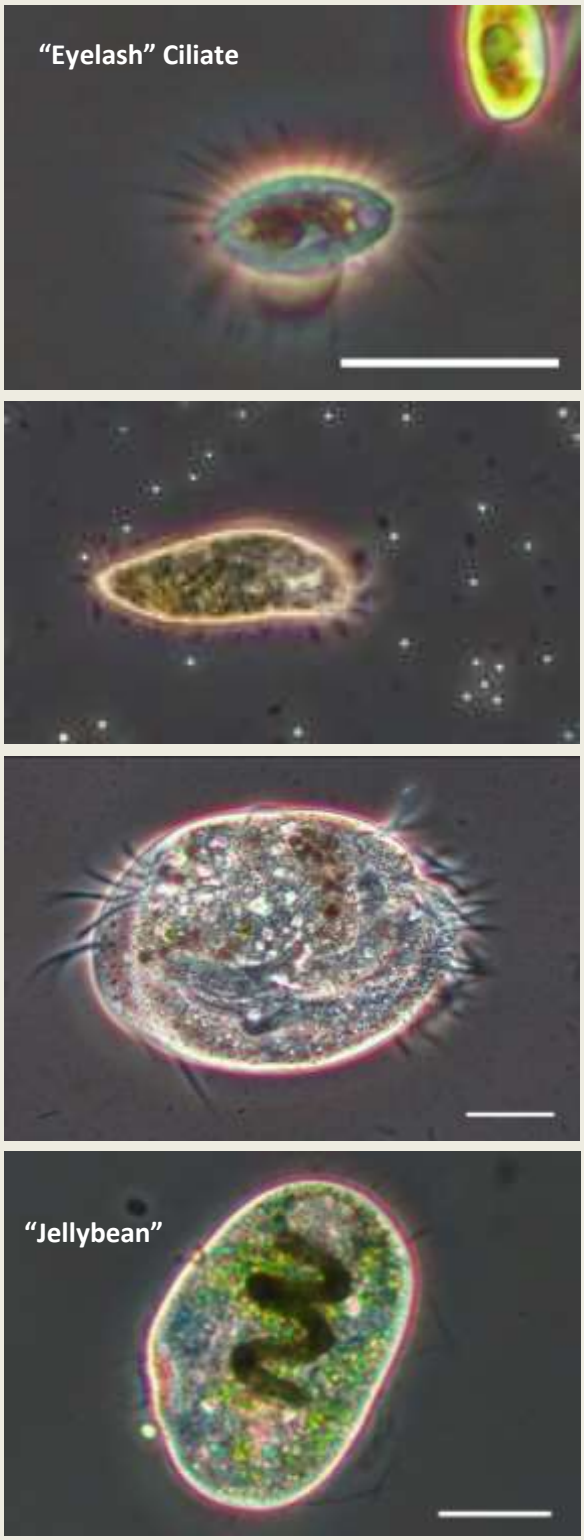
Ciliates are a diverse group of protozoans that move by hair-like projections on the outer surface of the cell. Ciliates are often indiscriminate grazers which consume any microbe of sufficiently small size. Most ciliates are capable of consuming small algae cells, but have not been implicated as the primary cause of a crash in outdoor culture.

Size
5 – 100 µm

Morphology
Shapes can range from spherical to ovoid to cylindrical. Most ciliates tend to move very quickly. Cilia on the cell membrane can range in size and placement.

Symptoms of Infection
Ciliates are considered a pest if they reduce culture productivity. Currently, the only sign of infection is microscopically observing pond samples for the physical presence of ciliates.

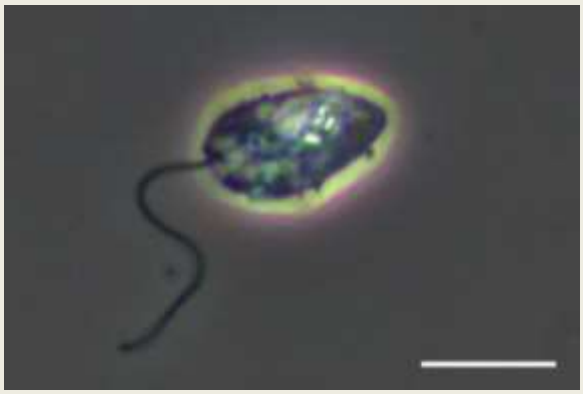
Identification and Treatment
ITS sequencing and qPCR primers aren't available for ciliates. Since ciliates seen in our cultures have not been implicated in health issues, there is currently no need to treat for them and crop protection strategies have not been evaluated.

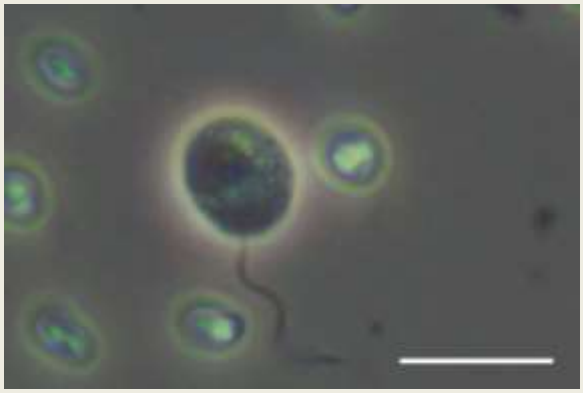


Scale bar = 20µM

Flagellates

Flagellates are single-celled grazers that move with a single or double whip-like appendage. Like ciliates, flagellates typically graze on any microbe small enough to consume. Flagellate species are often observed to consume small algae, although are more commonly observed consuming bacteria. High numbers of flagellates are often associated with cultures experiencing bacterial blooms.





Size
3-15 µm

Morphology
Variable. Flagellates can be pigmented or unpigmented and may be observed with either bacteria or algae within their bodies.

Symptoms of Infection
Currently, the only sign of infection is microscopically observing pond samples for the physical presence of flagellates.

Identification and Treatment
ITS sequencing and qPCR primers aren't available for ciliates. Preliminary crop protection work suggests that Omega 500F is a successful deterrent.

Appendix: Chytrids

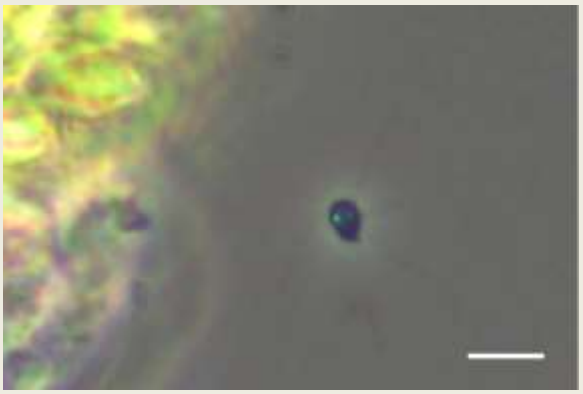
On the following pages, we list fungal pests that were isolated from ponds of non-*Nannochloropsis* taxa at Sapphire. To date, no fungal infections have been observed in *Nannochloropsis* cultures. Yet, they are included here for informational purposes and to illustrate Sapphire's abilities to isolate these pests, develop pest models, and evaluate control agents.

Single celled fungi were some of the worst and most destructive pests identified at LCTS. Fungal algae parasites are typically referred to as 'chytrids' although species inside and outside of that taxonomic group have been identified. Chytrids have been observed parasitizing strains of *Scenadesmus, Desmodesmus*, *Chlorella, and Selenastraceae.*

FD01

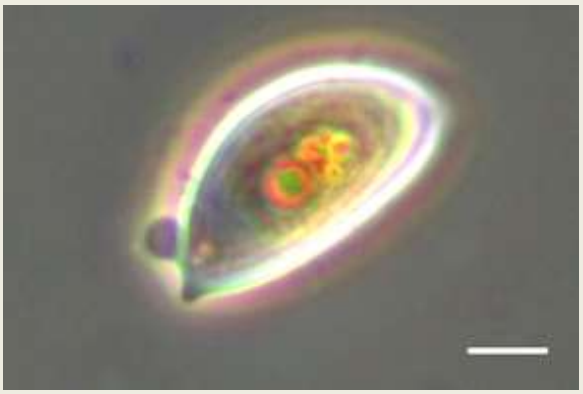
Aplanospore
Size: 2-3 µm

Morphology: Irregular. Motile, amoeboid. Center refracts light, revealing a greenish hue. Move slowly, using their extended filopodia. Best seen with 1000X magnification, phase III contrast.



Prosporangia
Size: 2-4 µm

Morphology: Spherical. Non-motile and opaque. Protoplast is injected into the host, which can sometimes be seen inside the cell. Best seen at 100X objective, phase III contrast.

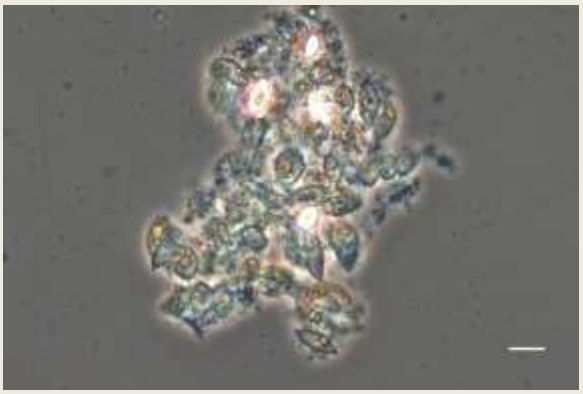


Symptoms of Infection

To date, FD01 is known to infect SE0004 and its descendants. Usually, 1 -2 zoospores will attach and infect a single cell.

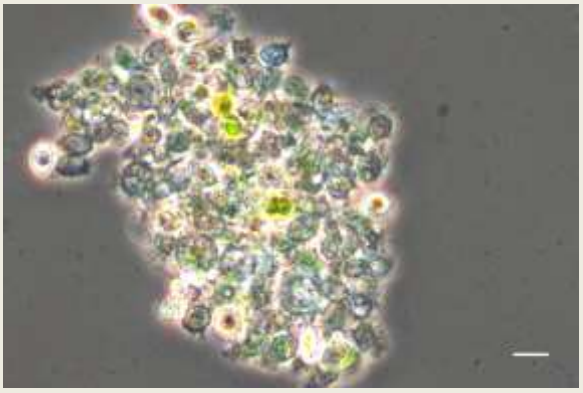
Common signs of infection include:

- Cell aggregation
- Pigment loss
- Hollow cells with residual organelles that appear orange, red, and/or black



Identification and Treatment

18S, ITS 1 & 2, and 28S sequencing and qPCR primers are available for this pest. Headline, Omega 500F, Thiram 42S, and Bravo can effectively treat an infection. Dead sporangia can remain on cells after treatment.



Scale bar = 5 µM

FD61

Aplanospore
Size: 4-6 µm

Morphology: Irregular. Motile, amoeboid. Refract light, revealing a greenish hue. Move slowly, using their pseudopodia. Best seen with 1000X magnification, phase III contrast.

Sporangia
Size: 5-7 µm

Morphology: Spherical. Non-motile, semi-translucent. Houses zoospores, which can sometimes be seen inside the body. Best seen at 100X objective, phase III contrast.

Symptoms of Infection

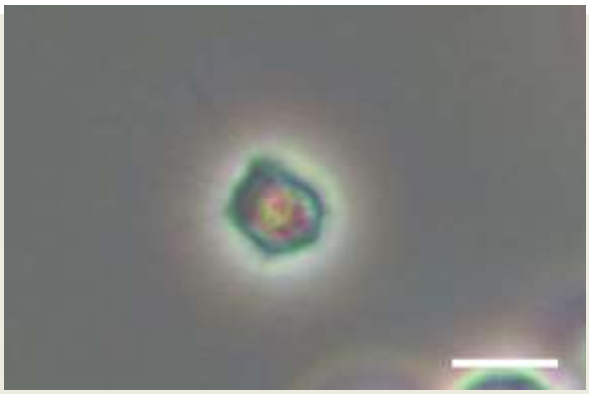
To date, FD61 is known to infect SE0004 and occasionally (under stressful conditions) SE0107. Usually, 1 -2 zoospores will attach and infect a single cell.

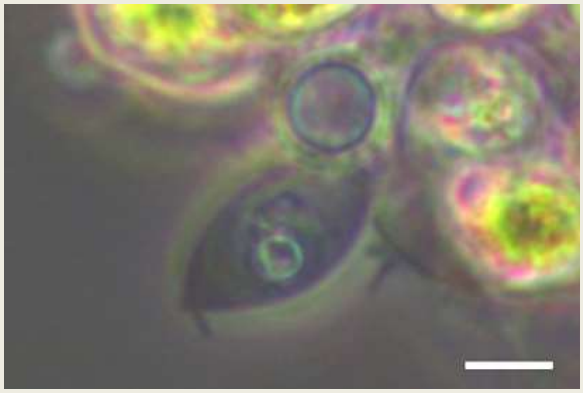
Common signs of infection include:

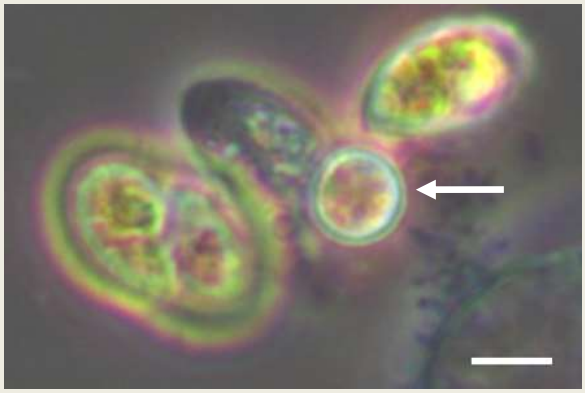
- Cell aggregation
- Pigment loss
- Hollow cells with residual organelles that appear orange, red, and/or black
- Cells with elongated horns

Identification and Treatment

18S, ITS 1 & 2, and 28S sequencing and qPCR primers are available for this pest. Headline, Omega 500F, Thiram 42S, and Bravo can effectively treat an infection. Dead sporangia can remain on cells after treatment.







Scale bar = 5 µM

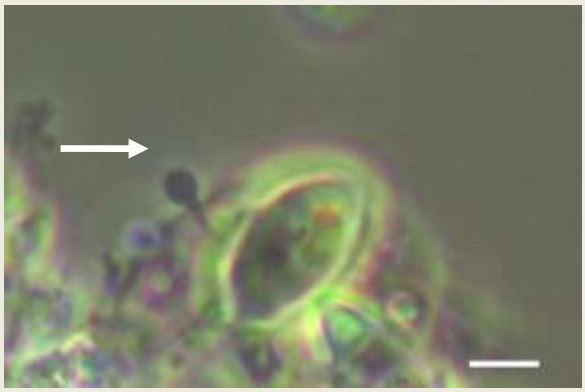
FD95

Aplanospore
Size: 2-3 µm

Morphology: Irregular. Motile, amoeboid. Center refracts light, revealing a greenish hue. Move slowly, using their pseudopodia. Best seen with 1000X magnification, phase III contrast.

Sporangia
Size: 2-4 µm

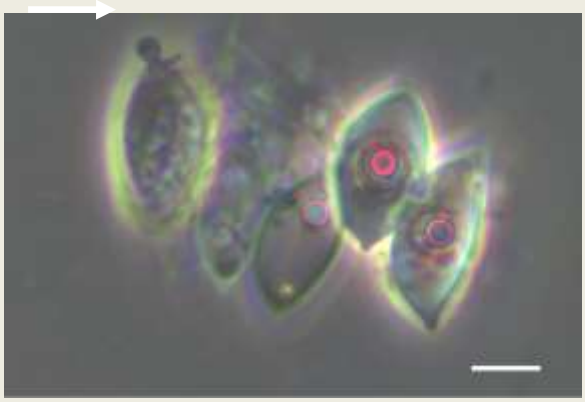
Morphology: Spherical. Non-motile and opaque. Protoplast is injected into the host, which can sometimes be seen inside the cell. Best seen at 100X objective, phase III contrast.



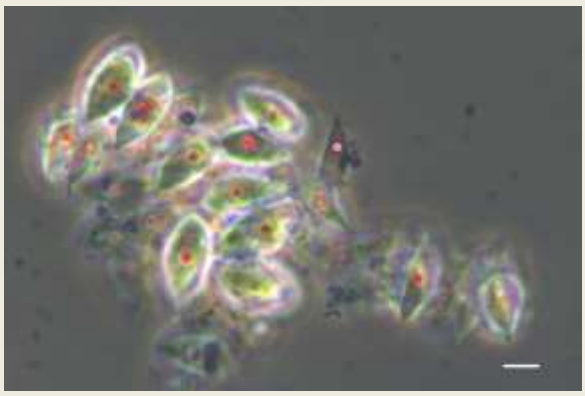
Symptoms of Infection
To date, FD95 is known to infect primarily SE0004 and its descendents. Usually, 1 -2 zoospores will attach and infect a single cell.

Common signs of infection include:

- Cell aggregation
- Pigment loss
- Hollow cells with residual organelles that appear orange, red, and/or black



Identification and Treatment
18S, ITS 1 & 2, and 28S sequencing and qPCR primers are available for this pest. Headline, Omega 500F, Thiram 42S, and Bravo can effectively treat an infection. Dead sporangia can remain on cells after treatment.

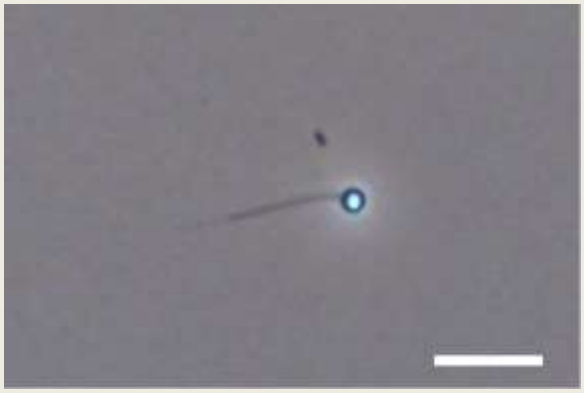


Scale bar = 5 µM

FD100

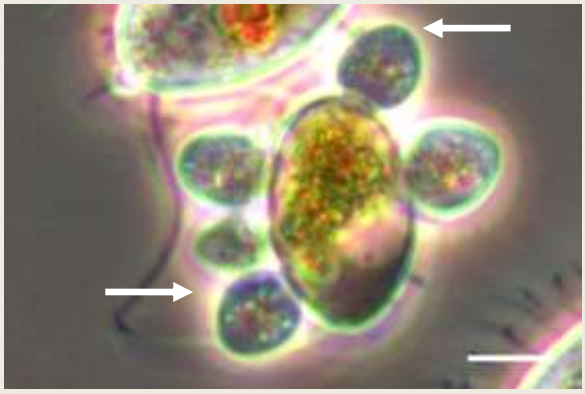
Zoospore
Size: 1-2 µm

Morphology: Spherical. Motile, uniflagellate. Refract light, revealing a greenish hue. Move quickly in a circular path. Best seen with 100X objective, phase III contrast or with 400X magnification, dark field contrast.



Sporangia
Size: 5-7 µm

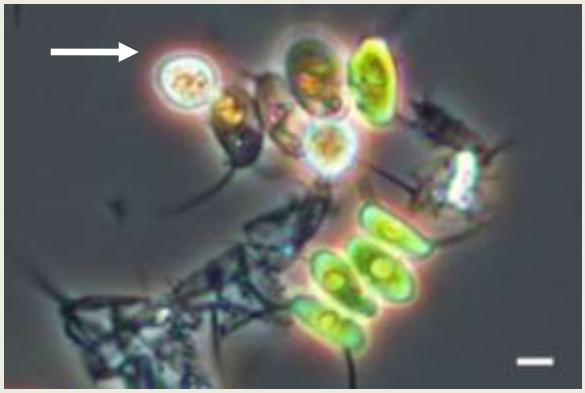
Morphology: Pear shaped. Non-motile. Semi-translucent. Houses zoospores, which can sometimes be seen inside the body. Best seen at 100X objective, phase III contrast.



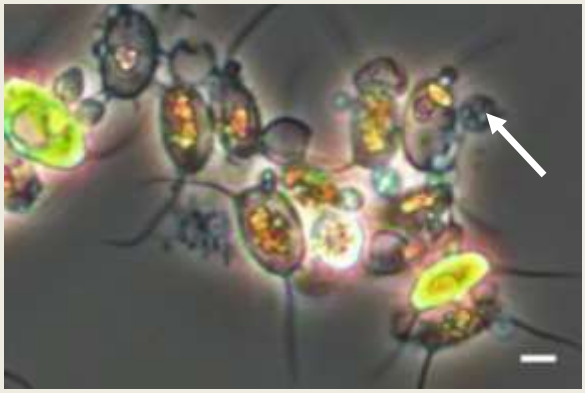
Symptoms of Infection
To date, FD100 is known to infect only SE0107. Multiple zoospores can attach and infect a single cell.

Common signs of infection include:

- Cell aggregation
- Pigment loss
- Hollow cells with residual organelles that appear orange, red, and/or black
- Cells with elongated horns



Identification and Treatment
18S, ITS 1 & 2, and 28S sequencing and qPCR primers are available for this pest. Headline and Omega 500F can effectively treat an infection. Dead sporangia can remain on cells after treatment.



Scale bar = 5 µM

FD101

Zoospore
Size: 2-3 µm

Morphology: Spherical. Motile, uniflagellate. Refract light, revealing a greenish hue. Move quickly in an erratic pattern. Best seen with 100X objective, phase III contrast or with 400X magnification, phase II contrast.

Sporangia
Size: 4-5 µm

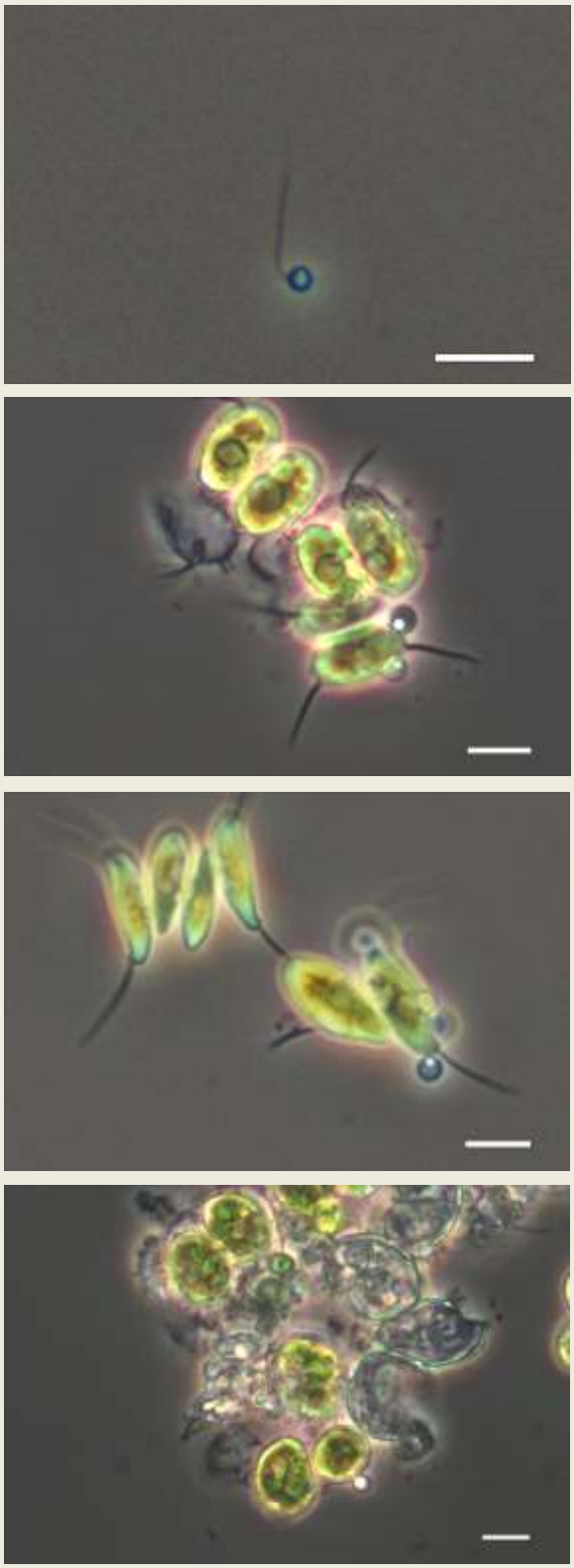
Morphology: Spherical. Non-motile. Semi-translucent. Houses zoospores, which can sometimes be seen inside the body. Has a characteristic spot that refracts light. Best seen at 100X objective, phase III contrast.

Symptoms of Infection
To date, FD101 is commonly found in cultures of SE0107 and SE60239. It is unknown whether FD101 is an obligate parasite of these strains. Multiple sporangia are often seen on cells. Presence of FD101 may be an indicator of stress on the culture within effected ponds.

Common signs of infection include:

- Cell aggregation
- Pigment loss
- Hollow cells with receding pigmentation.
- Cells with elongated horns

Identification and Treatment
18S, ITS 1 & 2, and 28S sequencing and qPCR primers are available for this pest. Omega 500F, Bravo, and Thiram 42S can effectively treat an infection. Dead sporangia can remain on cells after treatment.

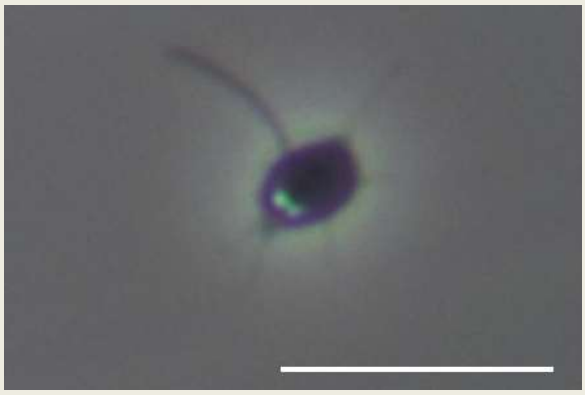


Scale bar = 10 µM

FD104

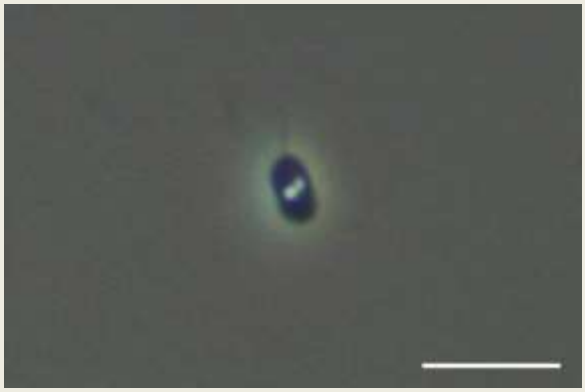
Aplanospore
Size: 4-5 µm

Morphology: Irregular. Motile, potentially both flagellated and amoeboid. Refract light, revealing a greenish hue centralized in one to two organelles. Move quickly in a wave like pattern when flagellated. Best seen with 1000X magnification, phase III contrast or with 400X magnification, phase II contrast.



Prosporangia
Size: 2-3 µm

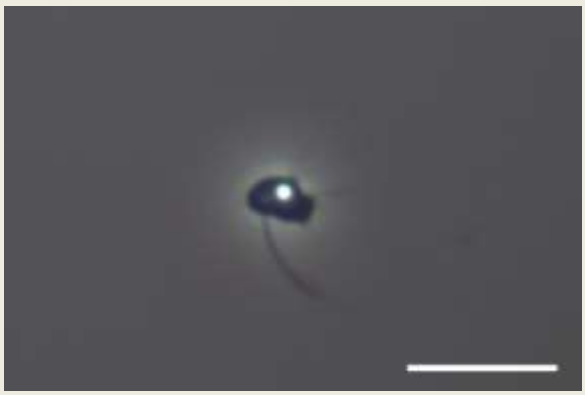
Morphology: Spherical. Non-motile and semi-translucent. Protoplast is injected into the host, which can sometimes be seen inside the cell. Best seen at 1000X magnification, phase III contrast.



Symptoms of Infection
To date, FD104 is known to infect only SE0107. Multiple zoospores can attach and infect a single cell.

Common signs of infection include:

- Cell aggregation
- Pigment loss
- Hollow cells with residual organelles that appear orange, red, and/or black
- Cells with elongated horns



Identification and Treatment
18S, ITS 1 & 2, and 28S sequencing and qPCR primers are available for this pest.

Scale bar = 10 µM