

Set up / background

Pond/culture type/strain: Lab SE60445 in 16NFL1-307 150/40

Date started: 8/17

Owner/labbook #: PL

Reason/Hypothesis: Propagation of pest crash for further study via TEM, archiving, pest isolation, and clean up of crash source

Conditions tested: Archived tube #31 from -80 was thawed at room temperature and added to 50ml culture at approx. 0.2 OD750nm via spectramax plate reader. Control flask uninfected. OD and FL data taken in triplicate on single flasks.

Suspected pests: Unknown pest from *Nannochloropsis* ponds 2015, suspected bacterial. Source details below.

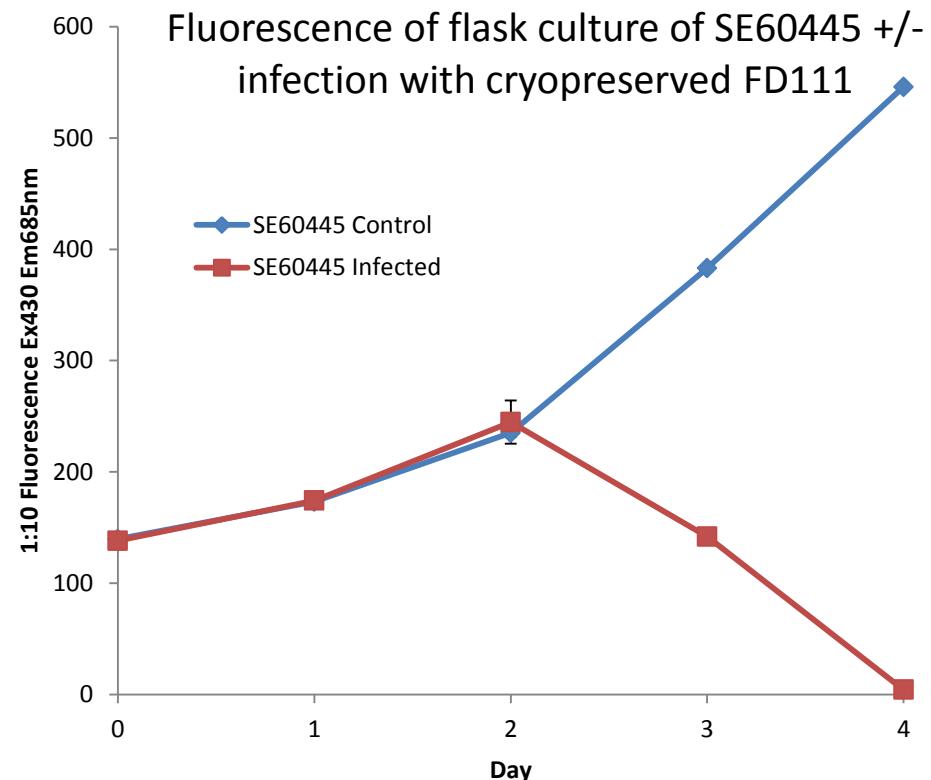
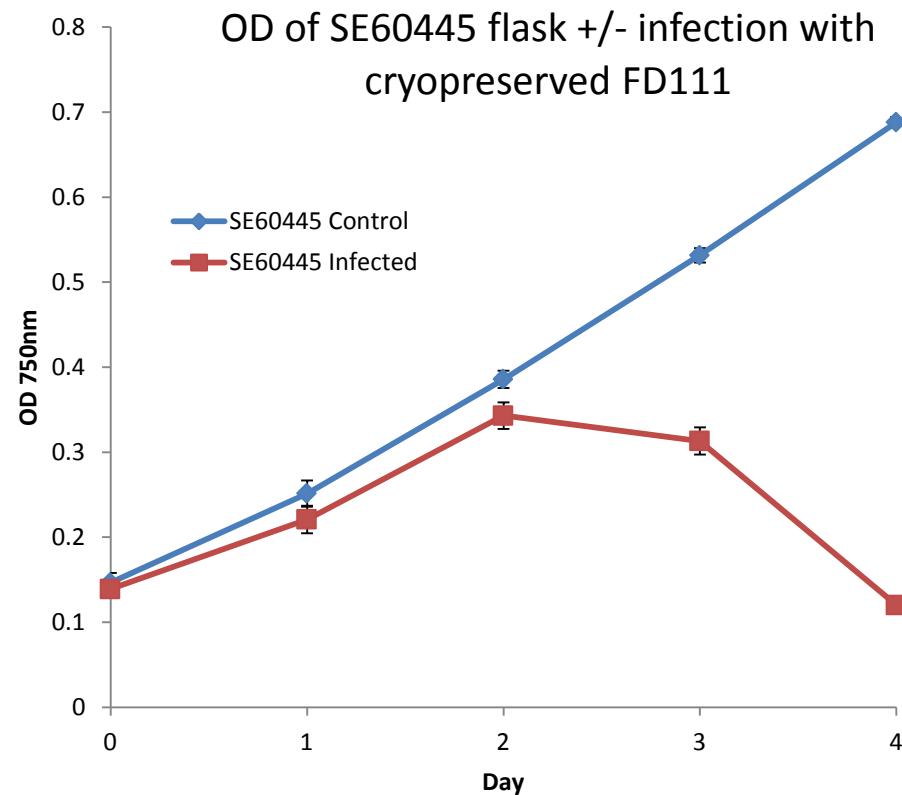
SOPs used: preview tubes, 24-well plates, microscope

File path for data folder: \\lcfs1\LasCruces\Crop Protection\Crop Protection\2015\Previews\150817 infection propagation PL KL

Freeze Date	Pest	Source	Original Source Culture ID	Strain	Media	Preservative	Total Volume
7/7/2015	Unknown	Pond 6	60445-150601-1006	60445	16NFL1-307 150/40	DMSO 7%	1 mL

\\lcfs1\LasCruces\Crop Protection\Pest Storage\Crashed culture and pest storage

Data



- Error bars represent standard deviation of three analytical replicate samples from one flask

Pictures – Flasks



Day 2



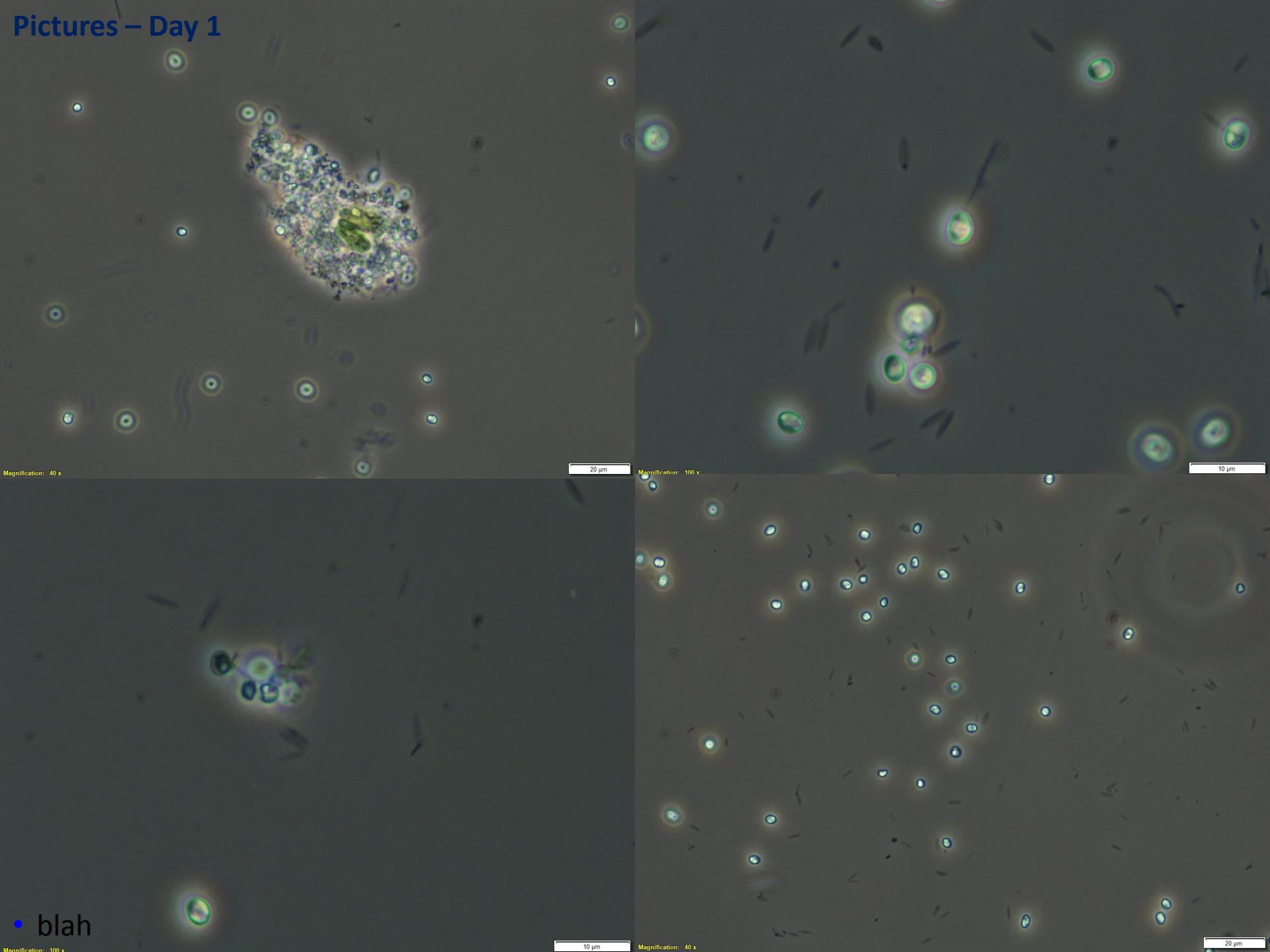
Day 3



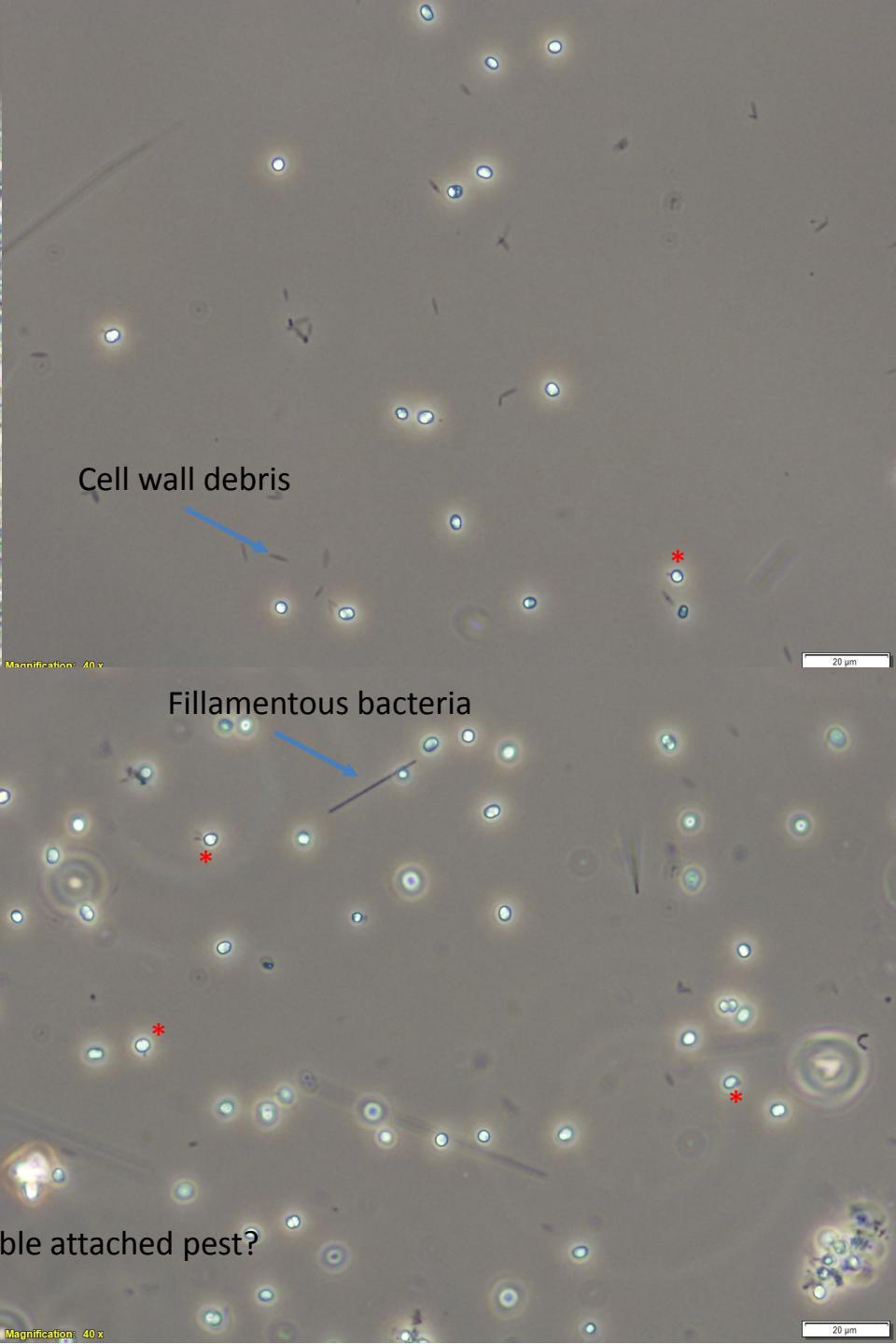
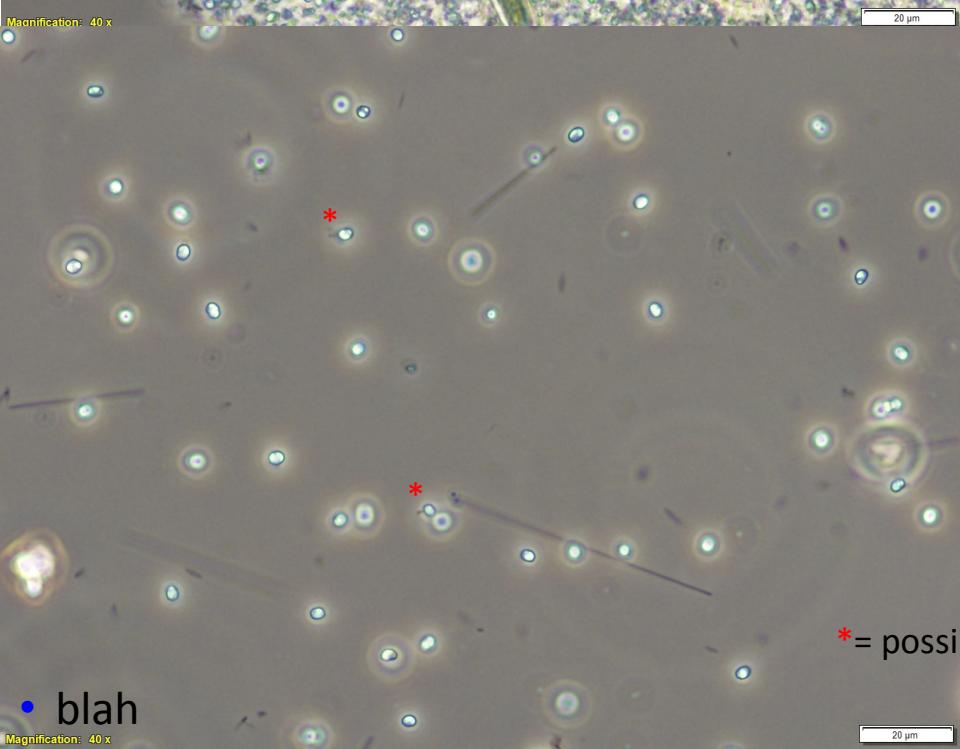
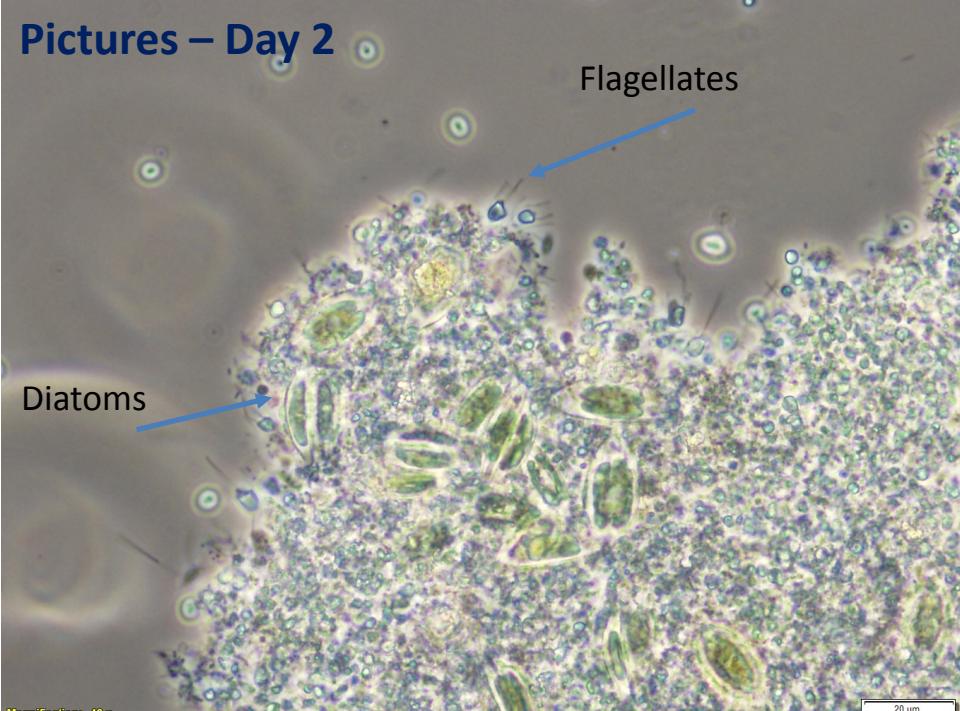
Day 4

- Crash appeared visually evident between day 2 and 3

Pictures – Day 1



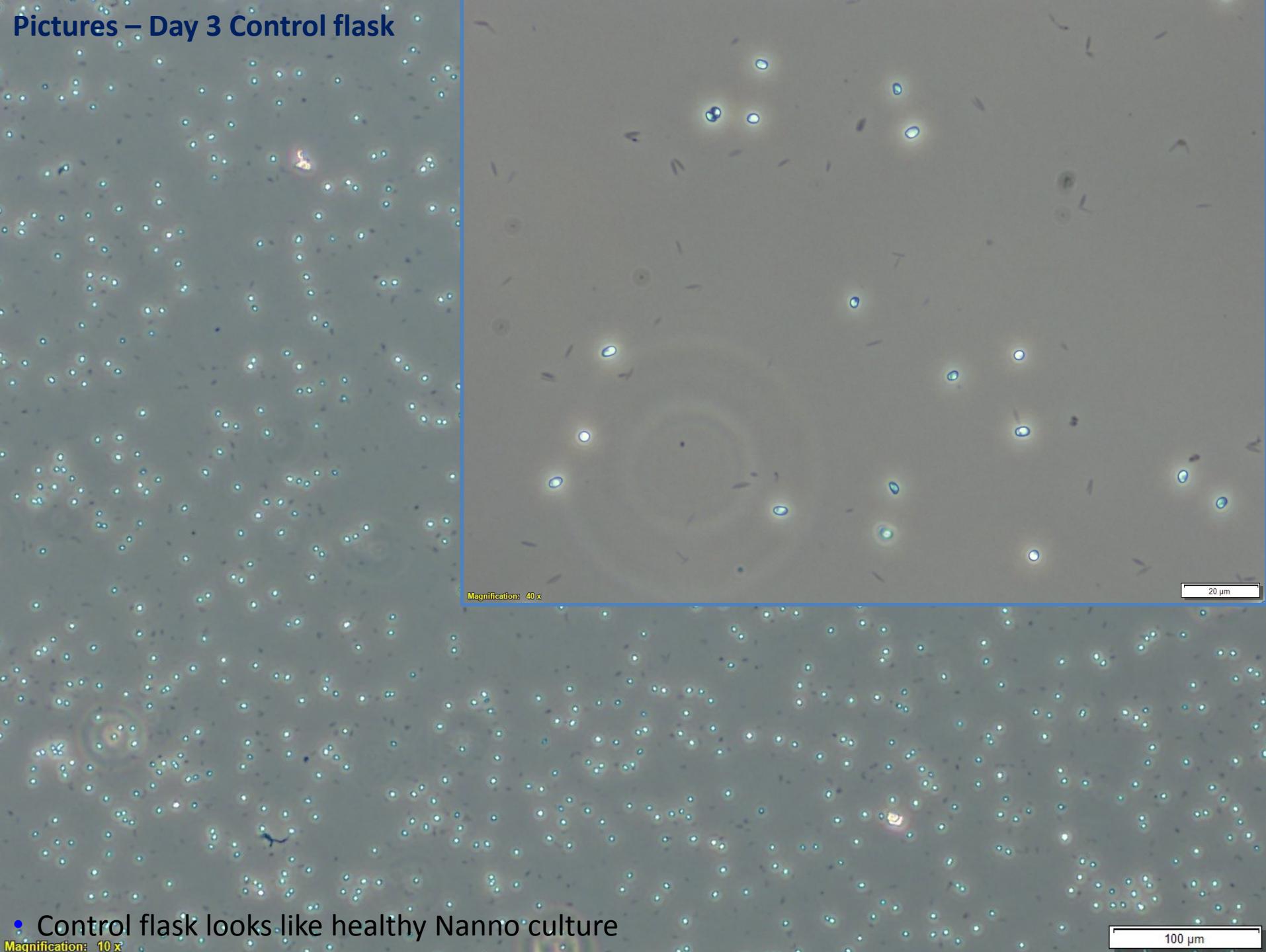
Pictures – Day 2



* = possible attached pest?

• blah

Pictures – Day 3 Control flask



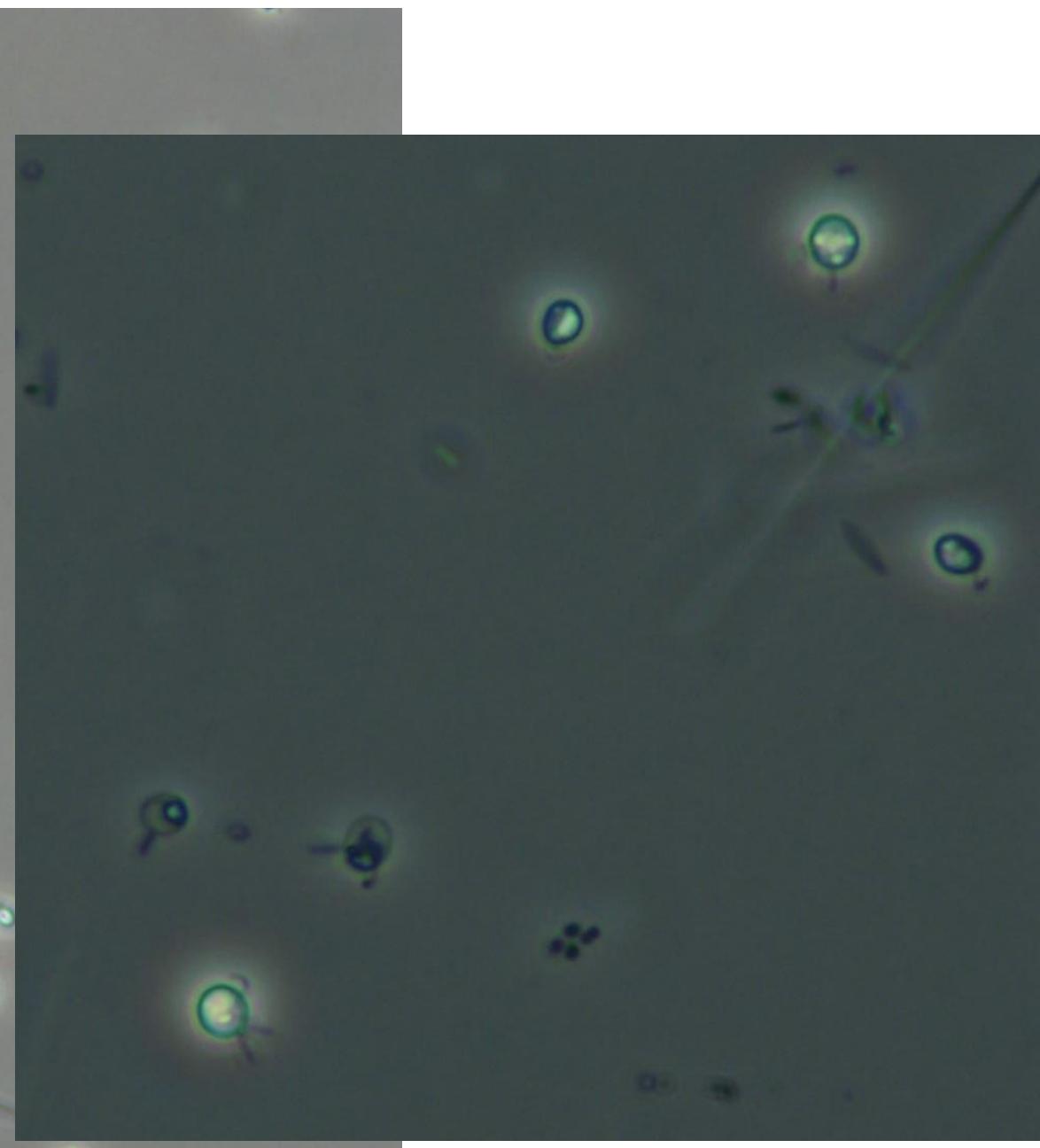
- Control flask looks like healthy Nanno culture

Magnification: 10 x

100 µm

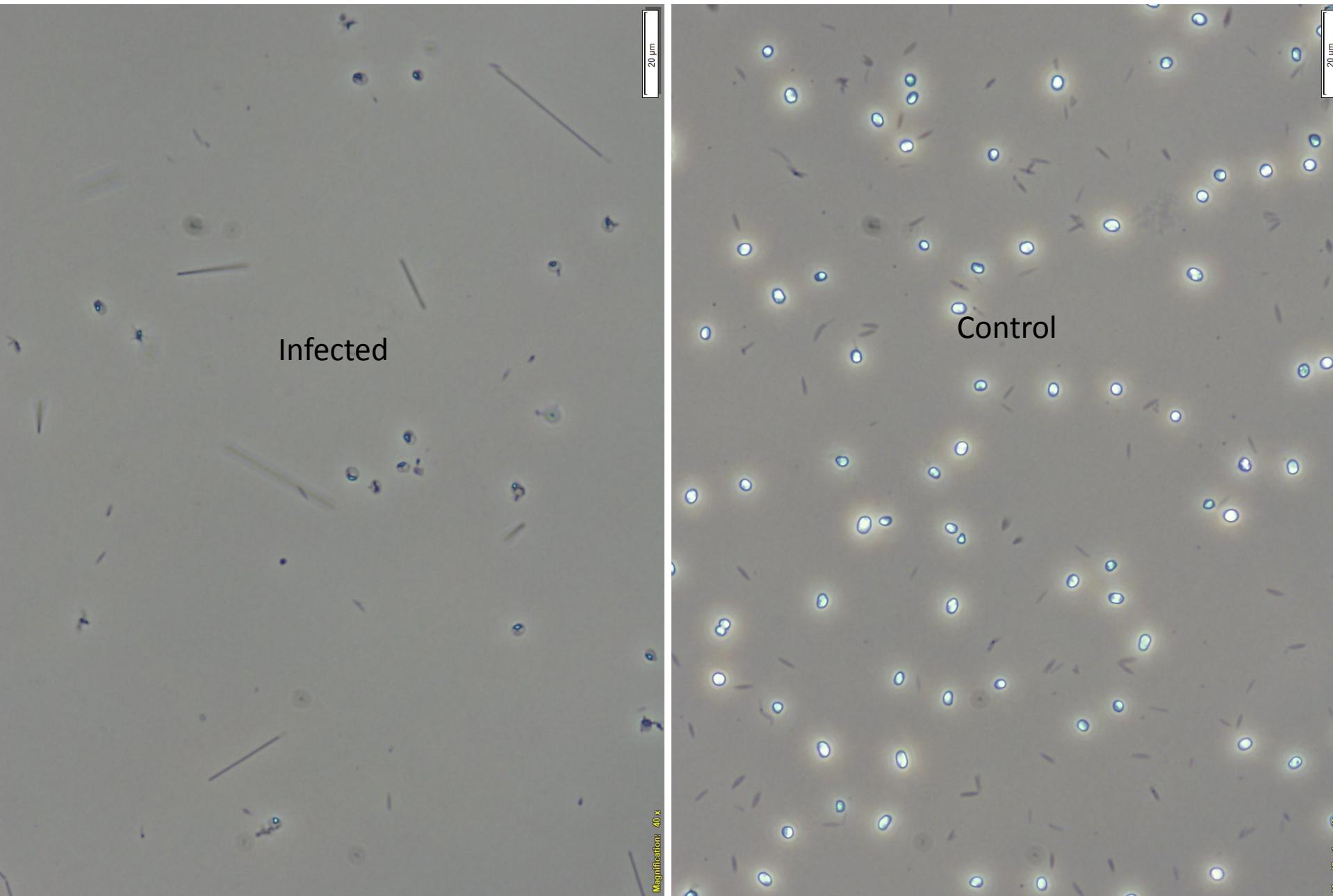
Pictures – Day 3 Infected flask

20 µm



- Numerous cells with attachments, hooked and straight.
- Some cells displaying hollowed death phenotype

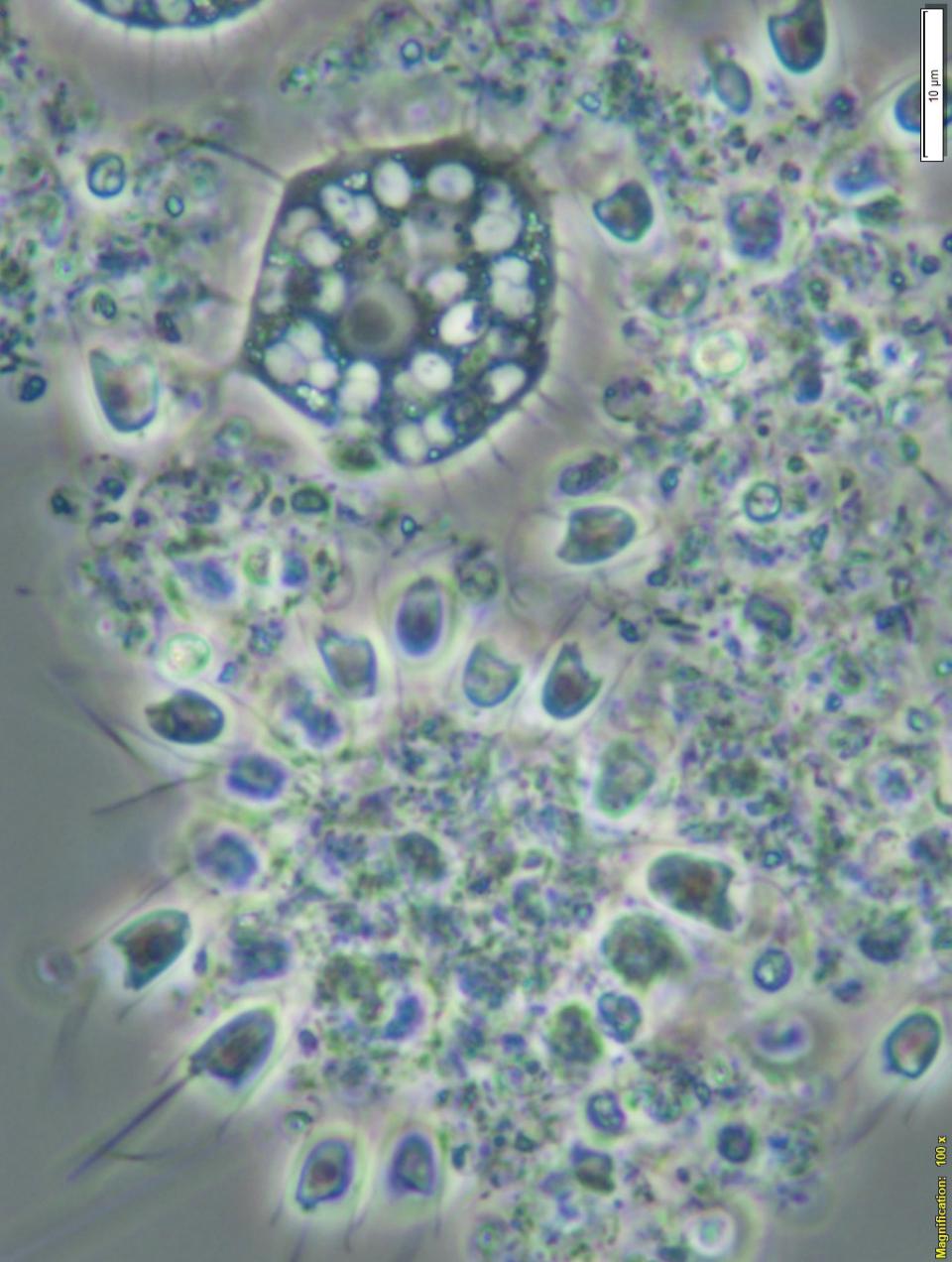
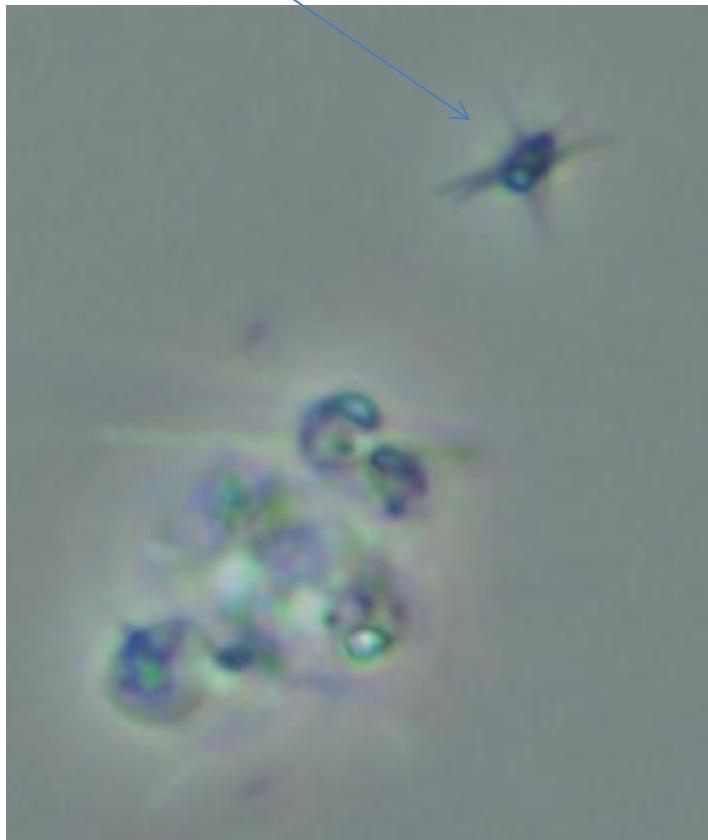
Pictures – Day 4



- Most cells now hollowed shells with attachments

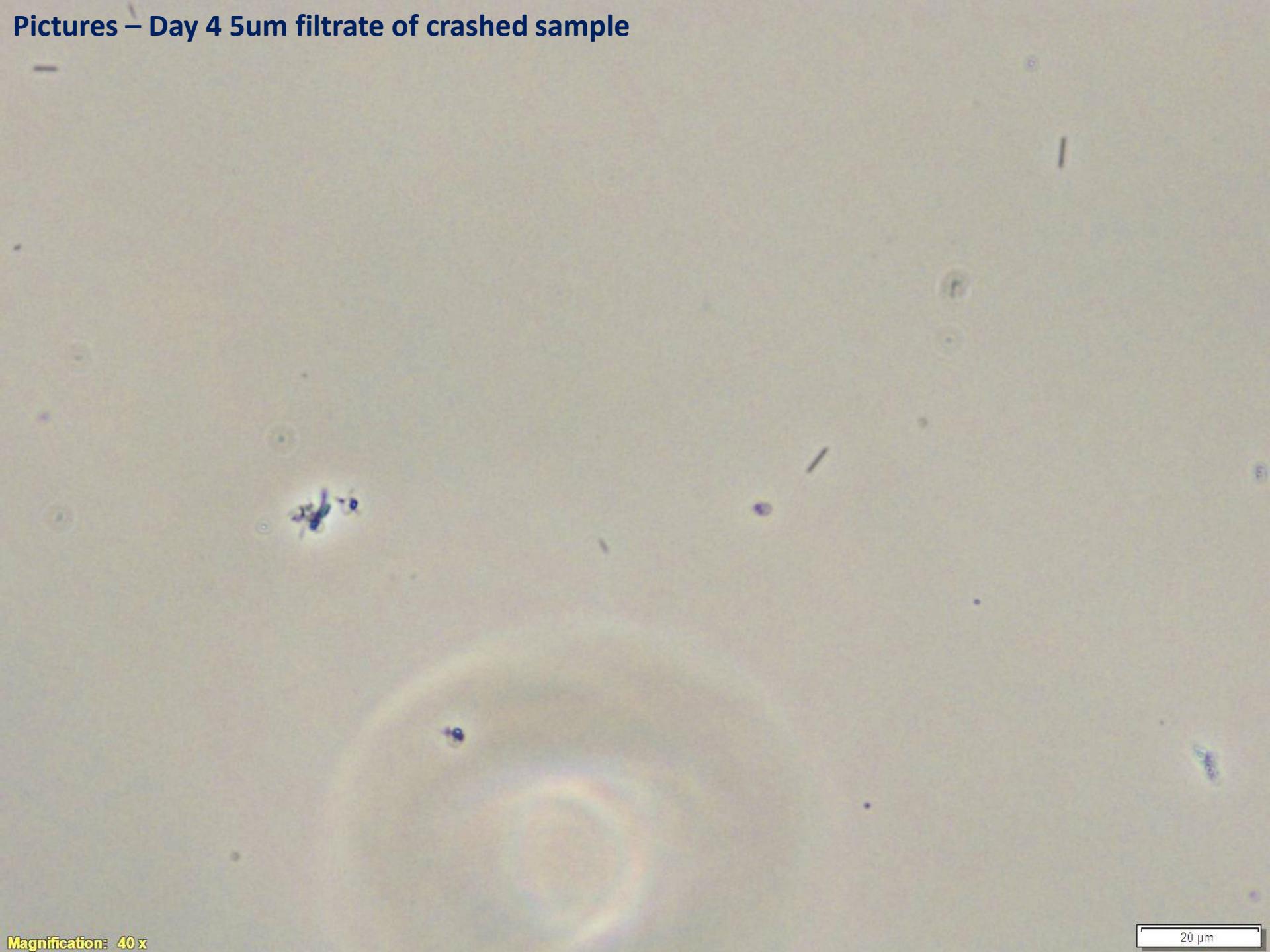
Pictures – Day 4

What is this?



- Flagellates swarm around flocks of dead/dying algae – are they cleaning up the bacteria or one of many causal agents? They seem to be most present after the crash

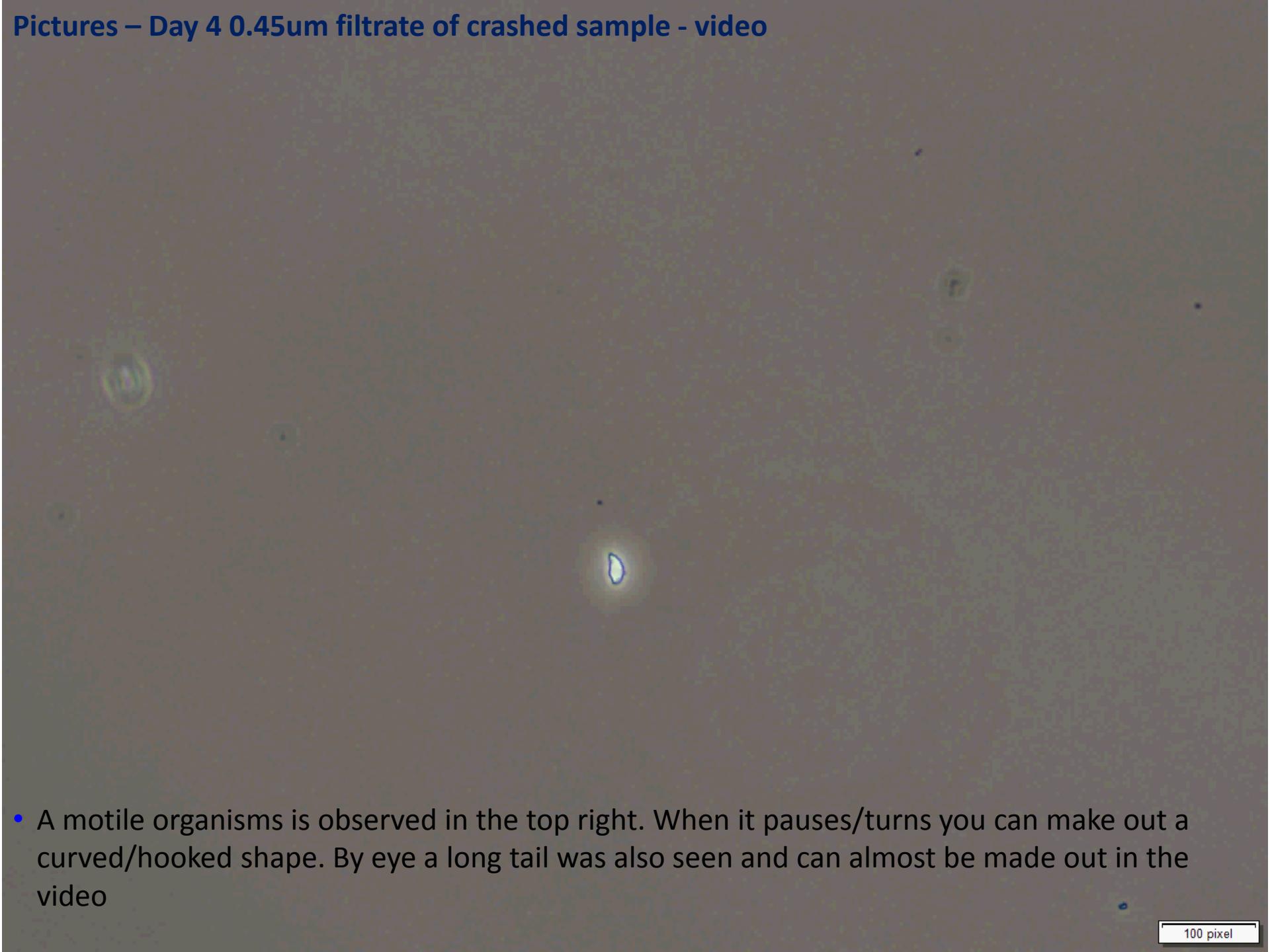
Pictures – Day 4 5um filtrate of crashed sample



Magnification: 40 x

20 μm

Pictures – Day 4 0.45um filtrate of crashed sample - video



- A motile organisms is observed in the top right. When it pauses/turns you can make out a curved/hooked shape. By eye a long tail was also seen and can almost be made out in the video

100 pixel

Conclusions/summary

- Crash from previous work with this source was replicated
- Again, attachments to cells are observed concurrently with crash timing/phenotype, one smaller hooked shaped and another longer rod shaped
- Crash may have been cleaner on day 3 than day 4 for filtering and propagation
- Other attachments are also observed e.g. “angler fish” likely to be caulobacter which we believe is unlikely to be a pest but at the same time may have effects on health.
- We suspect bacterial pest but high levels of bacteria are not observed post crash – is this due to other organisms e.g. flagellates cleaning up the bacteria? Or does this suggest pest is something else or have they just run out of food, or combinations of things...?
- On Day 4 further tubes were frozen with DMSO to store multiple sources of this infection
- Fixed samples will be sent to Pete for microscopy work
- On Day 4 reinfection of fresh culture was done at 0.1%v/v +/- 10ug/ml tetracycline after filtering the sample either through 0.45um or 5um syringe filters. This is in an effort to clean up the infection source for isolation. Flasks will be observed by eye and if a cleaner infection source is created it will be stocked.
- After filtering the crashed sample through 0.45um a small organism was observed in the filtrate. It was motile and difficult to capture a picture. It appeared to have a long tail and looked hook shaped when it turned. Videos were taken.
- On day 3 and Day 4 samples were plated onto various bacterial growth media for isolation efforts (\\\lcfslascruses\Crop Protection\PSI Pond Scene Investigation\150625 P5-6\180820 and 180821 bacteria plates)

Fixing procedure

Work in the hood and wear gloves/lab coat – solid and liquid waste is in hood too.

1. To falcon tube add: 1ml 10% glutaraldehyde and 2ml 0.2M sym-collidine buffer, then 1 ml infected culture
2. Incubate at room temp 1hr
3. Spin 3000 rpm 10min in eppendorf swing bucket centrifuge
4. Pour off sup' (liquid waste bottle in hood)
5. Resuspend 1ml 0.1M sym-collidine buffer and transfer to eppi tube
6. Spin 5min at 3000g/rcf (don't remember what rpm that was)
7. Pipette off sup
8. Resuspend pellet in 1ml 0.1M sym-collidine buffer
9. Spin again
10. Resuspend pellet in 1ml 0.1M sym-collidine buffer
11. Spin again
12. Add 1ml 0.1M sym-collidine buffer and store at 4 degrees

- Samples were fixed on Day 1, 3, and 4 for shipment to Pete Letcher for TEM studies