1. Procedure summary

This procedure details the procedure to monitor invading pests and other organisms in pond samples.

Related Procedures

Pond Sampling and Data Collection	LC-01-003-001
Microscope Operation	LC-06-001-003

Procedure impacts and concerns

Safety	Proper PPE for this procedure: safety glasses, safety toe shoes and gloves. Nitrile gloves should be worn when handling pond samples. The MSDS/SDS for chemicals used in this SOP should be reviewed.
Quality	Consistency across observers is necessary for data quality.
Delivery	Samples should be counted the same day as collection to ensure sample integrity.
Environmental	Pond samples are properly disposed of in the evaporation basins.
Cost	NA
Compliance	Compliance with OSHA's Hazardous Waste Operations and Response, and Hazardous Communication Standard in addition to the Sapphire Energy, Inc. Chemical Hygiene Plan is required (see 29 CFR 1910.120 and 1200).

Responsibilities and owners

Document Owner	Manage content and distribution	Alina Corcoran/Chris Meenach
Process Owner	Responsible for content and process validation	Alina Corcoran/Chris Meenach
Site Manager	Responsible for implementation and conformance	Becky Ryan

2. Process

2.1 Process description

To protect crops, it is necessary to monitor invading organisms. Consistency across observers is critical to ensure that data can be compared longitudinally. This procedure standardizes observations. This protocol can be used to count observe and count organisms in both pond and lab samples, although it is written specifically for pond monitoring.

2.2 Process steps

1. Open an observation log for all routine monitoring. Log templates are located at: N:\Production\Scope Observations\Mini Pond Scope Observation Logs and N:\Production\Scope Observations\Raceway Scope Observation Logs. Ensure that the template is blank before filling it out.
2. For each pond sample, pipette 10 μL onto a microscope slide and cover with a coverslip.
3. Scan the entire sample at 100X for large predators/invaders and flocculation.

Score observations on the log sheet. It is important that scores only are listed in columns. List comments in the comments column.

4. At 100X, move to the middle of the slide and take a picture, ensuring that is representative of the sample. Name this file YYMMDD_pond#_100X. Save this in the experiment folder either N:\Production\Scope Observations\Minipond Pics or N:\Production\Scope Observations\Raceway Pics.

5. Move objective to 400X and examine 10 fields of view to look for other invaders/predators, pigment recession, discoloration, % target culture. Score observations in the log.

6. At 400X, move to the middle of the slide and take a picture, ensuring that is representative of the sample. Name this file YYMMDD_pond#_400X. Save this in the experiment folder either N:\Production\Scope Observations\Minipond Pics or N:\Production\Scope Observations\Raceway Pics.

7. Move objective to 1000X, using immersion oil, and examine at least 100 cells to characterize patterns of pigment recession, discoloration, predatory mechanisms. Score observations in the log.

8. Take pictures throughout the observation process of any invaders/predators/unique processes. Name this file YYMMDD_magnificationX_pond#_YYYY where YYYY is a descriptor of the invader/predator/unique observation. It should be saved into the pond folder within the experiment folder.

3.   **Input documents**

NA
Output documents
NA

Document control

4   Revision history
R0 – Alina Corcoran

Document approval

5/20/16

Document reviewers