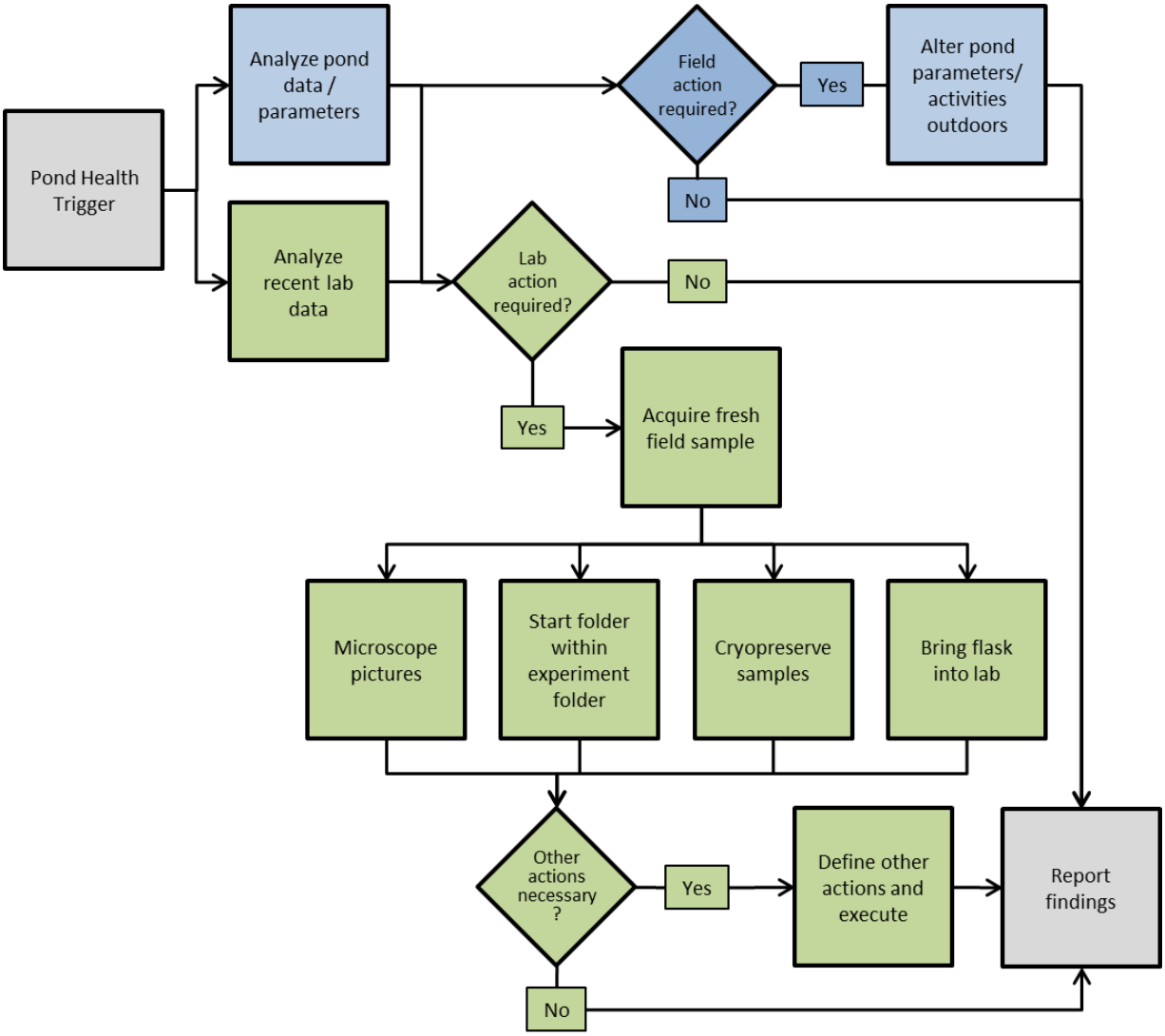
Pond Scene Investigation

This procedure describes the steps that should be taken upon observation of a pond helath trigger. The objective is to determine what the potential cause of the trigger was and if any further action should be taken such as Biotic review, Pest capture and ID, Prevent and control.



Pond health trigger

1. If any member of any team notices something unusual or concerning about the pond/culture/data, this would constitute a “health trigger”. Examples are shown below.
2. Bring this observation to the attention of a member or leader of Crop Protection or Production teams or line manager/Director of field testing if none available.
3. Once a pond health trigger observation is made, at least one member from each of the Crop protection and Production teams should be assigned to investigate further. These individuals are

responsible for tracking and communicating any information discovered managing agreed actions through completion.

Examples of pond health trigger:

1. Any photometric data out of normal ranges or not aligning with other parameters
2. Large declines in any PAM readings, fluorescence, OD, DW, or other date in the absence of (or unlikely to be caused by) a harvest or other normal operating procedure.
3. Pond is yellow/brown/unusual color
4. Pond physical characteristics show a change from normal e.g., unexpected flocking, color change, foaming, etc.
5. Any characteristic that is different from normal when working with the culture – e.g., it smells different, it flows differently in harvest return, it sticks to sample bottle during morning sample collection etc.

Initial investigation steps

Analyze pond data / parameters

1. Analyze recent scope observations and make fresh observations on today's sample.
2. A member from the production team is responsible for analyzing recent pond data and double-checking recent pond activities.
   Checklist
   a. Analyze recent pond data (today minus 2+ weeks) for any anomalies or signs of ill health.
   b. Analyze recent production activities and double check work done.
      i. Does work done match database entry?
      ii. Is math for chemical additions correct?
3. If an anomaly is found that can be resolved in the field, execute corrective action and report findings.
4. If no anomaly is found or the anomaly requires laboratory action/support, report to assigned representative and/or group leader of Crop Protection team with findings and discuss/decide further action.

Analyze recent lab data

A member/s of the crop protection team will be assigned to analyze any recent relevant data generated in the lab.

Checklist

1. Analyze recent scope observations and make fresh observations on today's sample. If there are signs of pest infection proceed immediately to pest capture activities.
2. Analyze recent preview tube data or any other experiments recently that used culture from pond of concern (or related pond e.g., same strain) for any signs of ill health that may have been missed or for which an alternative action could be taken than was taken previously.
3. Compile and/or analyze (or contact experiment team for such analysis) any minipond data for experiments that used the same source culture for any signs of similar concerns or possible remediate actions.

4. Any data corresponding to the culture of concern that may aid in choosing initial actions for pest capture type experiments.

Acquire a fresh sample

If further lab action is required, it is imperative that a fresh sample be taken in a clean sample bottle.

1. Collect and label a clean sample bottle or appropriately sized container.
2. Collect sample from pond as per SOP LC-01-003-001.

Start folder within experiment folder

1. Once further action in the lab has been decided, generate a folder for all related work to be stored or linked from.
2. Set up a regular meeting between at least the two representatives from CP and production to maintain communication on current activities until problem is resolved.
3. Start a checklist/action item list. And save within folder.

Water chemistry sample prep

If a water chemistry sample is required, prep and/or submit for analysis.

If water chemistry was tested within the two days and no modifications have been made to the media since that time it is not necessary to submit a sample.

If there is any likelihood that water chemistry played a role in the health concern, submit a sample for water chemistry.

Microscope pictures

1. As soon as possible after a fresh sample has been gathered, take many microscope pictures, and save in the folder associated with investigation.
2. Even if you do not knowingly see something obvious by eye at this time, take plenty of pictures at all magnifications of representative fields of view.
3. It is often the case that objects/morphologies relating to the pest/health concern are seen retrospectively in scope pictures.
4. Observe the sample under a dissecting scope to observe algal phenotype and the presence of larger organisms such as rotifers, vorticella, and other protists.

Photographs

1. Throughout the investigation, take photographs of samples and/or ponds and save in designated folder.
2. Save files with data taken in file name as well as an identifier of the subject of the photograph.

Some crash phenotypes can look quite different to others i.e., does the culture/media/biomass turn a different color? Does it clear out or remain opaque? How long does this take? How is settling affected? Photographs of culture in small test tubes or falcon tubes with backlighting tend to give good pictures.

Cryopreserve samples

1. Freeze multiple 1mL aliquots (at least 5 each) of culture at -80C under three conditions:
    a. 7% DMSO
    b. 25% Glycerol
    c. Untreated
2. Label vials and box appropriately and catalogue information in electronic or hard copy to include at least the following information:
    a. Crash Date/Time
    b. Freeze Date
    c. Source (e.g., pond, predose flask, lab crash etc.)
    d. Pond/culture ID
    e. Strain
    f. Media conditions
    g. Location in freezer
    h. Method of cryopreservation
    i. Suspected pest type or other phenotype observations
    j. Link to microscope pictures and other data
    k. Available/Used?

Bring flask into lab

1. Collect a clean flask and label appropriately.
2. Inoculate flask with pond sample in the flow hood.
3. Place in shaker box or other appropriate growth condition.
4. Monitor for crash or health trigger for at least 2-7 days. The level of monitoring required is to be determined by the assigned crop protection / production team members and may range from simple visual observations to full data collection.

This is the minimum lab action required after a health trigger and will allow you to observe what might happen to the pond culture if no action is taken. If the assigned team feel that the sample is already crashed or to the point of no return, further action must be taken (see below) to determine the cause, initiate further study, and to help determine a path of action in the pond.

Decide on further action

Biotic review, Pest capture and ID, Prevent and control