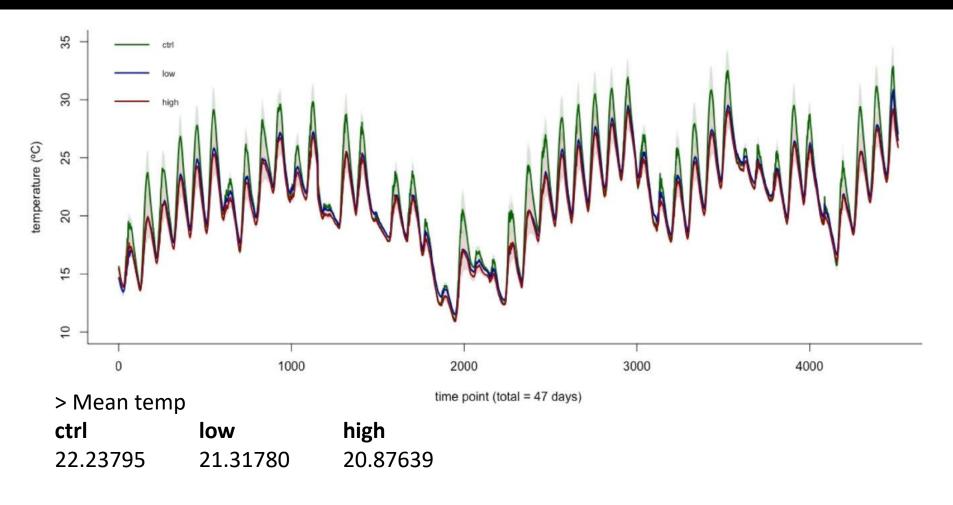
Temperature from mid May – mid July



>	Range	tem	p
---	-------	-----	---

	ctrl	low	high
[min]	11.0250	11.49567	10.89483
[max]	32.8805	30.86183	29.20117

Covering had small negative effect on chl. a

Design – MS experiment

Stressor 1: Nutrient enrichment

Stressor 2: Herbicide Glyphosate

(Roundup)

Stressor 3: Insecticide Neonicotinoid

Imidacloprid

Nutrient enrichment | 2 levels (N:P = 33)

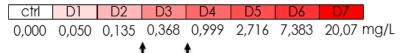
Low nutrient level: lake water (naturally mesotrophic; ~15µgP/L, ~225µgN/L)

High nutrient level: water from Lac Hertel spiked so as to reach a <u>4x</u> <u>higher</u> P concentration (i.e. eutrophic conditions ~60µgP/L, ~900µgN/L)

Nutrient background (press treatment) Mesotrophic **Eutrophic** $\sim [15 \mu gP/L]$ ~ [60 µgP/L] N:P ratio ~ 33 N:P ratio ~ 33 Glyphosate only ctrl + 7 doses [0.05 - 20.07 mg/L]two pulse treatments) Pesticide addition Imidacloprid only ctrl + 7 doses $[0.15 - 60.3 \,\mu\text{g/L}]$ Control (no pesticide) **Both pesticides** ctrl + 7 pairs of doses Max dose of pesticide

Glyphosate addition | 7 doses

Glyphosate Gradient

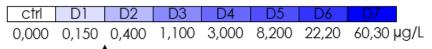


0,8 mg/L Maximum concentration considered safe for <u>aquatic environments</u> (CWQG treshold²)

0,2 mg/L Maximum concentration allowed in <u>drinking water</u> (CWQG treshold²)

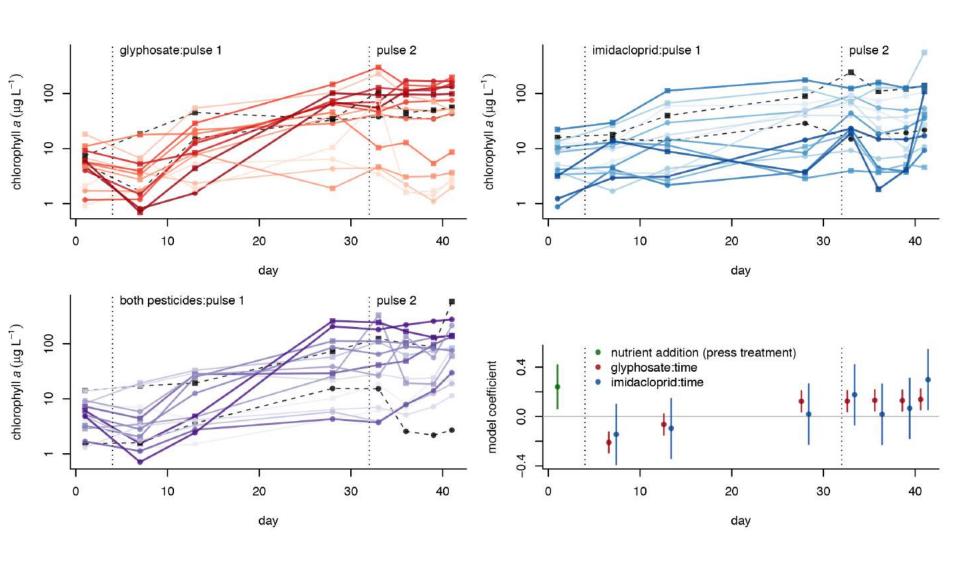
Imidacloprid addition | 7 doses

Imidacloprid Gradient

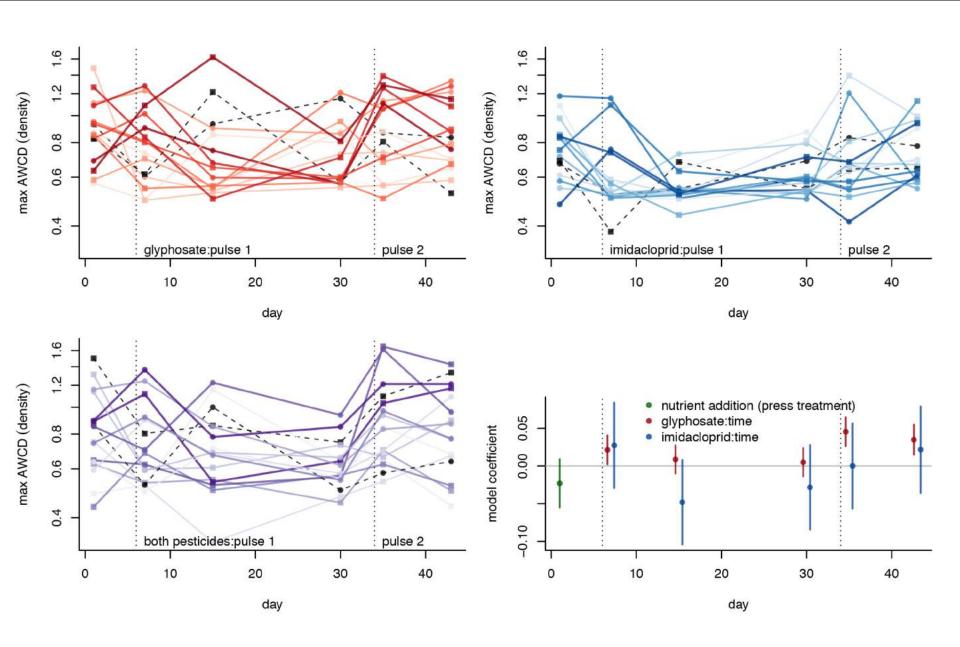


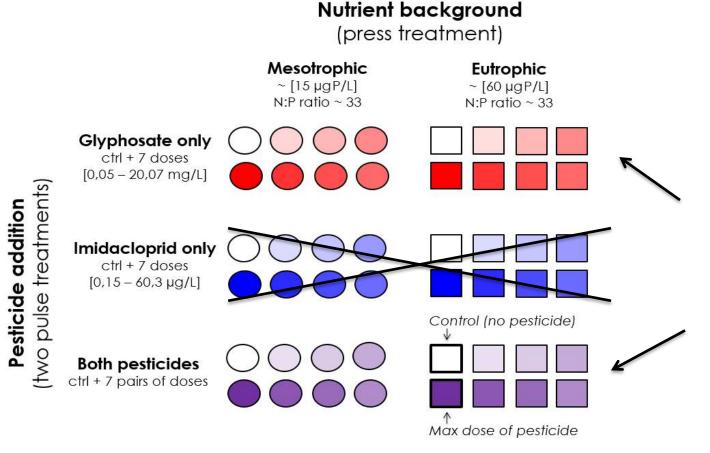
0,23 µg/L Maximum concentration considered safe for <u>aquatic environments</u> (CWQG treshold²)

Results: chlorophyll

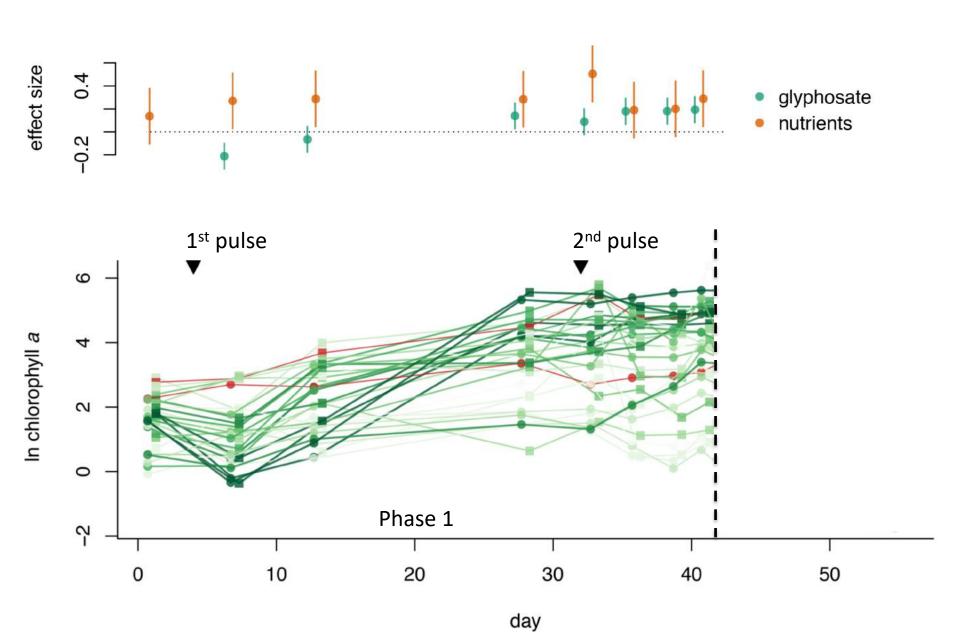


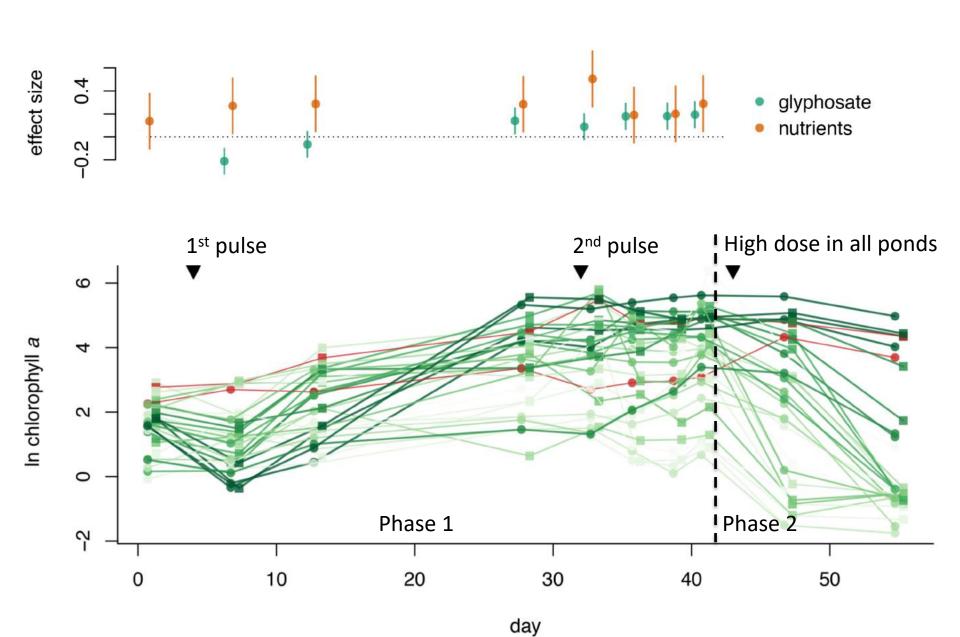
Results: ecoplates



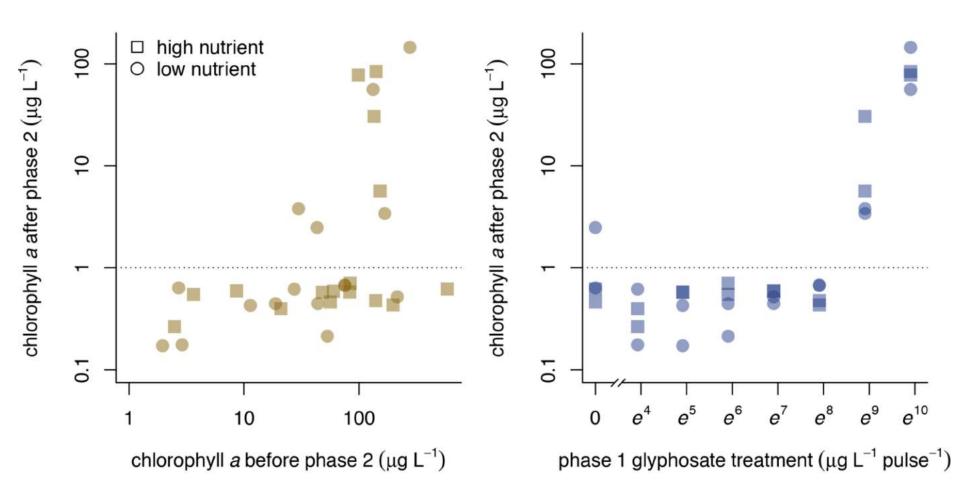


- 35 mg L⁻¹ Roundup
- Previously established as lethal (chl. a < 1)
- Which communities persist? (chl. a > 1)





Rescue from severe stress as a function of overall abundance and history of stress:



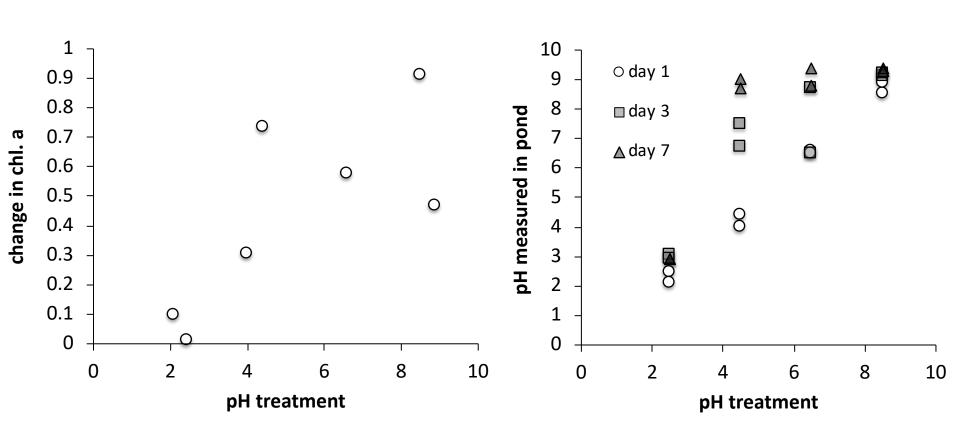
Gamma GLM:

p = 0.6691

p = 0.0000000258

2017 experiment

pH not Roundup



2017 experiment

- pH not Roundup
- Metacommunity rescue: if pre-exposure to sublethal stress improves likelihood of rescue, can such evolutionary resilience spread across a metacommunity via dispersal?

2017 field team

• So far, me + 3 undergraduate students:



Nathalie Chehab



Charles Bazerghi



Ilke Geladi

- Most likely a new PhD student of Graham and Andy (Kristina Krebs) arriving in June
- Charles Cong Xu (Barrett lab)? Naila Barbosa da Costa (Shapiro lab)?
- Interns at Gault can probably help occasionally if need be

Discussion

- Thoughts on design and choice of stressor? Additional response variables? (LOPC, PAM fluorometery? **DNA**?)
- Do you want to send student from your lab? If so, to work on what, and to measure what response variables? Housing and transport has to be planned soon.
- Pilot in prevision of 2018? In my nserc appl.: dilution-toextinction with well water

NOTES

Summary of 2016

1. May-mid August:

- Built piping system & sampling gear, equipped lab, developed methods. FC, Ecoplates, FlowCam, FLP + basic limno procedures (filtrations, probes, loggers, zoops sampling, cleaning of glassware, etc.). HPLC-MS to measure contaminants (V. Yargeau in chemical engineering).
- Basic monitoring of environmental parameters and communities: No spatial (but strong temporal) variation in temperature or any parameters measured with YSI. Large diel temperature changes that would be hard to control. Subset of lake species persisting (eg. greens over diatoms for phytoplankton, scapholeberis dominates zoops). Still, a MINIMUM of 10 species of zoops and 20 genera of algae, as much FD of HB as the lake, and apparently a lot of un-id' protists. I.e., a pretty complex food web.
- Graham tested various duckweed corrals and monitored population growth.
- Several pilots: nutrient addition, shading, acidification, salt: salt is good for phytoplankton, shading had little effect and was apparently more beneficial to periphyton, nutrient addition works (but a lot of variation), and severe acidification (pH of 3) seems to kill most algae, duckweed, and zoops

Summary of 2016

- 1. mid August mid October:
 - 48-ponds multiple stressor experiment with imidacloprid, Roundup, and nutrient enrichment: food web & B-EF story
 - First evolutionary rescue experiment with Roundup-treated ponds: expose all ponds to severe stress, see which ones recover
 - Key findings

2017: why not roundup

- 1. Doses required to kill phytoplankton are very high...
- 2. Risk of contamination of equipment, people, lab
- 3. Hard to monitor: high cost of HPLC-MS in V. Yargeau lab
- 4. Don't know if toxic effects are due to glyphosate or POEA (surfactant). Applying glyphosate salt would be crazy expensive.
- 5. Negative effects on phytoplankton only? Zoops? Duckweed: no apparent effect. Bacteria on ecoplates: positive effect.
- 6. Glyphosate contains phosphorus. After a few days, chl-a increases to very high concentrations. To compensate with nutrient addition, would need to maintain all ponds > 200 ug/L chl.a..

2017: why pH

- 1. Less conservation-relevant since acid rains have been controlled, but still possible via pollution and mining spills.
- 2. Well known, severe stressor in limnology: lots of work at ELA, mesocosm work in lake hertel by Bea. Can we rescue communities from this 'classic' stressor? If so, would be a convincing case for the relevance of ER theory for real-world conservation.
- 3. Targets most groups & TL (zoops, phytops, duckweed some but not all bacteria). Yet we know some genotypes are resistant from Beatrix and E. Low-Decarie's work.
- 4. Very cheap (couple gallons of sulphuric acid) + can be measured with a probe for free
- 5. No risk of contamination of equipment
- 6. pH buffering system: intermediate stress (5-6) recovers to 7-8 shortly. Can transfer organisms without trasnfering stressor. Severe stress (pH 3) will not bounce back.

design

- 1. 6 types of 2 pond metacommunities during phase 1: manipulate landscape degradation (0, 50, 100%) and dispersal (0 vs 10%). Degradation = pH 5. Stressor + dispersal treatment applied weekly. 8 replicate metacommunities per treatment combination.
- 2. Alternative: Landscape degradation as a factor with 3 levels (0, 50, 100%), but continuous dispersal gradient instead (48/3 = 16 dispersal treatments possible). Could cover a larger ranger of dispersal rate (safer) but no replication (riskier)
- 3. Phase 2: all ponds severely degraded (pH 3).
- 4. Like last year, phase 1 is itself an interesting ecological experiment that can produce many potential stories. e.g., do dispersal rate and local disturbance influence metacommunity beta diversity? Can priority effects influence community assembly and response to stressor, and are those effects dampened by dispersal?
- 5. Timing: fill ponds in mid May. Add nutrient spike (also silica spike?) then let settle for 2 weeks. Phase 1 during June and July. Phase 2 in August (allow 1 month for recovery).

measurements

- Continuously (loggers): temp, light
- Weekly (quick and free): fluoroprobe, YSI, depth, maybe flowcam if ready. Graham: duckweed counts?
- Beginning and end of phase 1 and phase 2 (4TP): zooplankton (cheap but time consuming), bacterial abundance with flow cytometry (need to bring liquid nitrogen, need -80 freezer space), ecoplates (expensive), lugol-preserved phytoplankton samples (time-consuming)
- At 3 TPs (beginning and end of P1, end of P2): in-pond 1L microcosms. Incubate phytoplankton for 24hrs in control vs. ph 3.5 conditions, measure chl.a with FLP, then fix for FlowCam analysis. Quantify stress response/performance of all species within all ponds over time as they experience stressor. Evolution of reaction norm over the course of the experiment? Influences by dispersal?
- Do you want to add anything? Filtrations for DNA? If so, how many ponds and TPs?
- Do we need nutrients? No nut treatment. Time-consuming + need more tubes, but should maybe be done at least once or twice during experiment
- Additional functions? Respiration, production, nutrient cycling?

If someone asks about salt

