Identifying the limiting factor(s) of *Pithophora* growth in the Thornapple River

A report shared with the Thornapple River Board

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Introduction

Results from the report by Harmer et al. (2025) indicated light levels play a critical role in the growth of Pithophora in the Thornapple River. These findings suggested there may be important differences in growth dynamics between Pithophora filaments growing at the surface of the mat versus those growing at the bottom of the mat. In particular, the nutrient content may be different in the surface and bottom portions of the mat; if the light-limited mat bottom is senescing, it may be releasing nutrients which then get cycled within the mat, and help maintain the mat's growth and development.

Freshwater ecosystems and water quality are threatened by algal overgrowth; an understanding of the resource(s) that limit the growth of these species is crucial for management. In the Laurentian Great Lakes, algal blooms indicate the early phases of eutrophication (Higgins et al. 2008). Excessive nutrient loading leads to eutrophication, which in turn can result in algal overgrowth, leading to hypoxia as respiration by heterotrophic organisms mineralizes the decaying algae, and possible mortality of aquatic organisms (Yang et al. 2024). Non-point source inputs of nutrients that cause eutrophication include poorly maintained septic tanks, shallow injection wells, agricultural pesticides and fertilizers, and runoff from urban areas and yards (Lapointe et al. 2018, Lor et al. 2021).

Pithophora is a genus of filamentous algae in the family *Cladophoraceae* found in freshwater ecosystems across the state of Michigan (Vadeboncoeur et al. 2021; Integrated Taxonomic Information System 2025). *Pithophora* and *Cladophora* are both macroscopic green, filamentous algae that are often misidentified as one another; *Pithophora*, is described as "horsehair," as it grows coarse, more rigid, narrow filaments (Lor et al. 2021). *Pithophora* reproduces by akinetes—these reproductive structures are non-motile, perennating, and spore-like, and have thick cell walls that vary in shape and size (Lor et al. 2021).

Overgrowth of filamentous algae can create mats covering the water surface, degrading recreational and aesthetic values as well as threatening water quality and aquatic life (Yang et al. 2024). Thick growths shade the water column and senescing cells release nutrients that maintain high densities of bacteria; hence, ecosystem structure can be altered by algal overgrowth (Lor et al. 2021). In the 1950s, filamentous algal blooms (FAB) in Lakes Erie, Ontario, and Michigan were linked to point source (coming from discrete conveyances, such as pipes) phosphorus loadings; nutrient abatement programs implemented in the 1970s were successful at reducing blooms of *Cladophora* (Vadeboncoeur et al. 2021). By the mid-1990s FABs in the lower Great Lakes occurred again, despite continued lower phosphorus concentrations, suggesting other resources (such as increased sunlight) due to filtration from zebra mussels were stimulating their growth (Vadeboncoeur et al. 2021). Identification of the limiting resource(s) in algae can be done by assessing enzymatic activities, physiological responses, elemental composition of biomass, and nutrient enrichment bioassays (Steinman and Duhamel 2017).

Algal productivity and growth are influenced by physical, chemical, and biological factors including light, temperature, nutrient availability, salinity, water motion, grazing, water residence time, depth, and desiccation (Lapointe et al. 2018). Laboratory studies found algae in

the genus *Pithophora* use light most efficiently at 15 °C, and that it is tolerant of temperatures above 30 °C. This alga is also adapted to low light conditions, and can withstand warmer temperatures, allowing it to thrive in shallow littoral areas and within and under densely vegetated areas (O'Neal et al. 1985). During spring and autumn months when water temperatures were under 15 °C, growth rates of *Phithophora oedogonia* were significantly inhibited (O'Neal and Lembi 1995). Shallow waters allow benthic biomass to intercept light and increase water temperature; these conditions may benefit filamentous algae, as they thrive under high-light conditions, especially when attached to a shallow bottom (Vadeboncoeur et al. 2021).

This study was designed to examine the effect of nutrients and light on the growth of *Pithophora*. The goal was to determine if one of these factors was limiting *Pithophora*'s growth, which could then be manipulated in situ to control its abundance in the Thornapple River.

Materials and Methods

Study Site

The Thornapple River is in Michigan's lower peninsula. The headwaters are in Eaton County, near Lansing, flowing northwest towards Ada where it connects to the Grand River (Lower Grand River 2025, Barry Conservation District 2025). The river has two watersheds: the upper Thornapple River watershed is within Eaton and Barry counties, and the lower Thornapple River watershed is within Barry, Eaton, and Kent counties.

The location of the study site is within the lower Thornapple River watershed, more specifically the stretch between the Ada and Cascade dams. This watershed is characterized mainly by agricultural and forested land use, at 52.1% and 25.8%, respectively (Lower Grand River 2025). Remaining land use includes 12.9% non-production vegetation, 3.3% paved roads, 2.1% urban, and 1.5% for both open water and wetlands (Lower Grand River 2025). Between the dams, there are no wetlands and few natural areas adjacent to the Thornapple River; the shoreline is almost entirely residential (U.S. Fish and Wildlife Service 2025). Many older homes in this area have septic tanks; the lack of regular septic tank maintenance poses a risk of additional nutrients entering the water (Lower Grand River Organization of Watersheds 2015).

Figure 1. (left) Shaker table holding 4 flasks containing *Pithophora* samples in unamended river water. (right) Dried *Pithophora* sample on a weigh boat inside a microbalance.

Preliminary Experiment: Pithophora growth in the Lab

Samples of *Pithophora* were collected on 5/6/25 from the Thornapple River, downstream from the Ada dam. Samples were collected with river water and stored in plastic zip seal bags, which were stored in a cooler and refrigerator until algal mass was measured. The *Pithophora* samples were then split into smaller segments, patted dry, and weighed on 5/8/25 (Fig. 1). Four samples were then put into 125mL flasks with 100mL of unfiltered river water. Flasks were numbered, covered loosely with tin foil, and randomly placed on a Thermo MaxQ 2000 analog orbital shaker table set to 50 rpm the first day, and then increased to 75 rpm on the second day and kept at that speed for the duration of the experiment. The table was then placed into an incubation chamber programmed at a constant temperature of 17ºC and a 18:6 light: dark cycle, at the intensity of 60 µmol/m²/s. Flasks were incubated for eight days, after which algae were removed from the flasks by forceps, patted dry, and weighed on 5/16/25. An average increase of 23.88 mg (65.55%) in biomass was observed after incubation, as shown below in Table 1.

Table 1. Change in *Pithophora* wet mass (n = 4) following 8-day incubation.

Initial Weight (mg)	Final Weight (mg)	Growth Increase (mg)	Growth Increase (%)
28.5	50.4	21.9	76.8
48	75.2	27.2	56.7
25.4	43	17.6	69.3
48.5	77.3	28.8	59.4
Mean		23.88	65.55
SD		5.12	9.25

<u>Treatment Experiment: Effect of nutrients and light on *Pithophora* growth</u>

Based on the results from the preliminary experiment, we felt confident that Pithophora would grow in river water under our lab conditions. As a consequence, we undertook the treatment experiment, where we tested the effect of both light level and nutrients (phosphate and ammonia [NH_3]) on *Pithophora* growth in the lab.

<u>Field and Laboratory Methods:</u>

For the treatment experiment, *Pithophora* samples were collected from the Thornapple River on 6/11/25; rakes were used to collect algae growing above the sediment. Algae samples and a carboy of unfiltered river water were collected; algae were stored in plastic zip seal bags and stored in a cooler until returned to the laboratory. Macroinvertebrates were removed from the algae using forceps, algae were patted dry, and then weighed on a microbalance. Immediately after the weight was recorded, samples were put into 125 mL flasks with 100 mL of ambient river water. The experimental design was a 2x2 factorial design with four levels of nutrient treatments (NH_3, phosphate, NH_3 and phosphate together, control) and two levels of irradiance (light, dark), with four replicates each.

Nutrient additions were based on concentrations measured by Progressive AE collected two months prior to algae sampling and water collection. Our goal was to add nutrients to reach a a concentration in the flasks that was 10 times the river concentration. Ammonia (NH_3) was added as ammonium chloride (NH_4Cl), hereafter referred to as N, and phosphate as potassium phosphate monobasic (KH_2PO_4), hereafter referred to as P. Controls received no nutrient additions.

All flasks were covered loosely with tin foil and placed onto two Thermo MaxQ 2000 analog orbital shaker tables set to 75 rpm. Four replicates of each treatment (+N, +P, +N + P, and control) were placed under high irradiance ("light" condition received 136 µmol/m²/s), with the others under low irradiance ("dark" condition received 16 µmol/m²/s); otherwise, flasks were randomly arranged onto the shaker tables (Figure 2). The shaker tables were placed into a growth chamber programmed to 18:6 light: dark cycle, at a constant temperature of 17ºC.

Figure 2. Left: Single shaker table shows *Pithophora* under shade cloth (low light) and exposed (high light). Right. *Pithophora* samples on two orbital shaker tables, with each table having flasks exposed to low or high light, situated in a temperature-controlled growth chamber.

Because ash-free dry mass (AFDM) is a destructive measurement, we derived a calibration curve between blotted wet mass and AFDM from excess *Pithophora* samples (Appendix A). We then used this curve to estimate starting AFDM of *Pithophora* samples used in the experiment. AFDM measurement followed the procedures in Steinman et al. (2017).

Following the incubation period, 20 mL of water was removed from each flasks for analysis of nitrate (NO_3), ammonia (NH_3), and soluble reactive phosphorus (SRP). *Pithophora* was then removed from the flasks, blotted dry, and measured for AFDM as described above. After weighing, pairs of the 4 replicates from each nutrient/light treatment were pooled and homogenized into two crucibles for analysis. These 16 crucibles were analyzed for tissue phosphorus content.

Nutrient Analysis

Chemical analyses of water samples were conducted on a Bran and Luebbe Autoanalyzer, measuring ammonia (NH_3) and soluble reactive phosphorus (SRP); ion chromatography was used to analyze NO_3 (Su et al. 2019, USGS 2025). For nutrient analysis, values below detection limit are reported as half the detection limit.

Statistical Analysis

Algal biomass, water column nutrient concentrations, and algal P content were used to assess the effect of nutrient amendments and irradiance on *Pithophora* growth. Changes in biomass and nutrients were calculated as the difference between pre- and post-incubation measurements; changes in nutrients were normalized by biomass and time and are presented as rates of change. A two-factor analysis of variance (ANOVA) test or, where appropriate, a nonparametric Aligned Rank Transform (ART) ANOVA test was conducted on each parameter (AFDM, NO_3^-, NH_3, SRP, and algal TP) to determine the limiting nutrient and how light affects growth of *Pithophora* (Wobbrock et al. 2011; Tank and Dodds 2003).

Results

The nutrient additions, designed to increase the N and P concentrations by ~10-fold resulted in lower concentrations than anticipated for N, with ammonia increasing ~2.5x and phosphorus ~10-fold (Table 2). While lower than anticipated, these increases in absolute concentration are substantial and large enough to stimulate growth if the algae are limited by ammonia and/or phosphorus.

Table 2. Ambient (Thornapple River) and amended (lab) ammonia and phosphorus concentrations.

Nutrient	Ambient Concentration	Amended Concentration
NH_3-N (N)	50 µg/L	120 µg/L
SRP (P)	<10 µg/L	160 µg/L

Change in Biomass

Pithophora growth, measured as AFDM, was significantly greater under light than dark conditions (Figure 3). There was considerable variance among the four replicates in each treatment, which may be related more to the blotted dry estimates of wet mass than actual differences in growth. The addition of nutrients had no statistically significant effect on *Pithophora* growth in either the light or the dark. Mean increase in growth in the light was very similar among all treatments including the control (Figure 3). Mean negative growth (decrease in biomass) was observed in the control and +N+P treatments, but due to high variance among treatments, these treatments were not significantly different than the ammonia or phosphate additions (Figure 3).

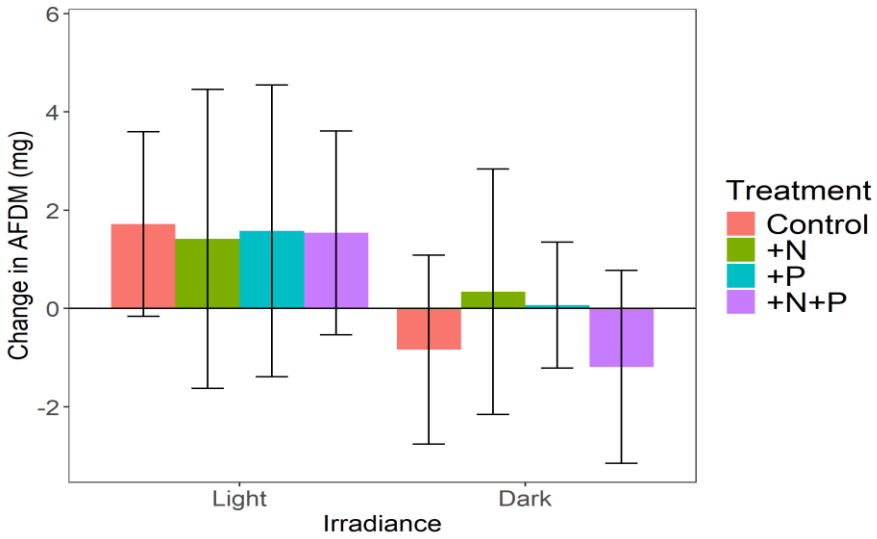

Figure 3. Change in Pithophora Ash Free Dry Mass (AFDM) over the experimental period. Flasks exposed to light on the left and flasks covered in shade cloth on the right. The different colored bars represent different nutrient treatments. Error bars represent one standard deviation from the mean.

Change in Nutrients

As *Pithophora* biomass either increases (light treatment) or possibly decreases (dark treatment), there may be concomitant changes in nutrient concentration in the flasks. With net increases, the alga will be assimilating nutrients, and therefore nutrient concentrations in the water column should decline over time. In contrast, decreasing growth over time should result in release of nutrients from decomposing algal tissue. We examined these changes for three different nutrients: nitrate; ammonia; and soluble reactive phosphorus.

Nitrate:

Although we did not include nitrate as a nutrient amendment, we did measure its change in concentration to see if it was influencing *Pithophora* growth (Figure 4). Nitrate uptake was positive (in other words, nitrate concentration declined over time) in all nutrient treatments in the light, consistent with net growth under light. Conversely, nitrate uptake was negative (net release) in the dark (Figure 4). Pithophora growth appears to be limited more by light than nitrate in our experiment.

Ammonia:

The effect of ammonia on *Pithophora* growth was different than that of nitrate. With ammonia, there was net uptake (that is, declines in concentration) in all treatments, but the uptake rates were significantly greater in those with added ammonia (both +N and +N+P treatments) than the control or +P (Figure 5). This was true in both the light and dark treatments. Unlike nitrate, these uptake rates suggest that ammonia was limiting *Pithophora* growth to a greater extent than either light or phosphorus.

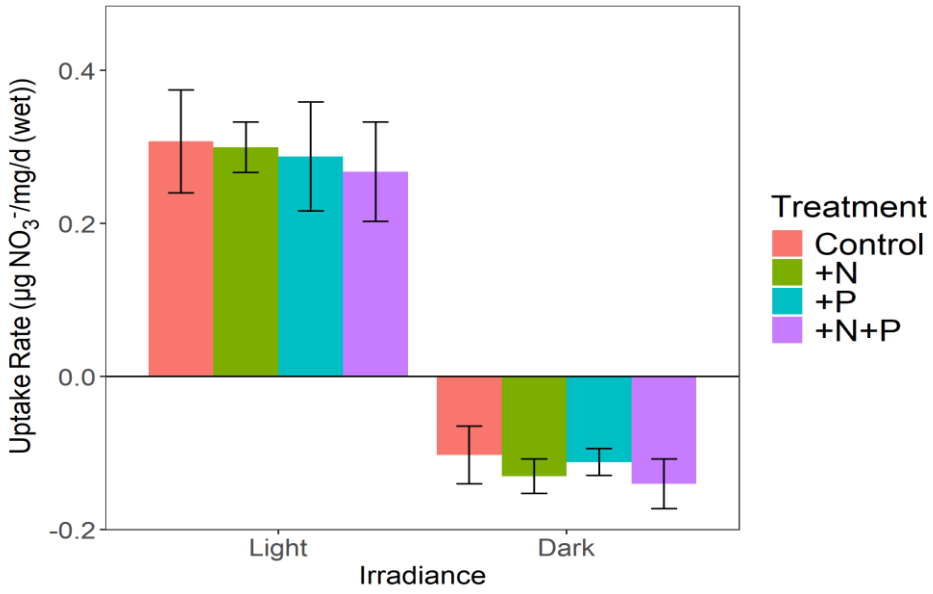

Figure 4. Change in nitrate over time. A positive uptake rate indicates a reduction in nitrate concentration whereas a negative uptake rate indicates an increase in nitrate concentration over time. The different colored bars represent different nutrient treatments. Error bars represent one standard deviation from the mean.

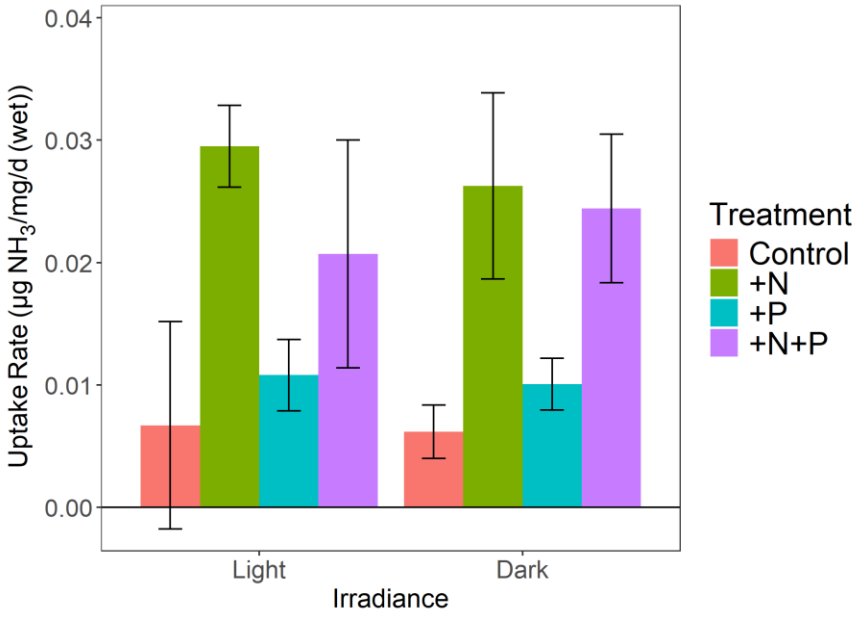

Figure 5. Change in ammonia. A positive uptake rate indicates a reduction in nitrate concentration whereas a negative uptake rate indicates an increase in nitrate concentration over time. The different colored bars represent different nutrient treatments. Error bars represent one standard deviation from the mean.

Phosphorus:

Uptake of soluble reactive phosphorus (SRP) was greater in the light than in the dark, but net uptake (reduction in SRP concentration) occurred only in treatments where P was added (as +P and +N+P), regardless of light or dark conditions (Figure 6). There was no net uptake at all in the control and +N treatments in the light, and slight net release of P in those treatments in the dark (Figure 6). This suggests P limited *Pithophora* growth to some degree, as well.

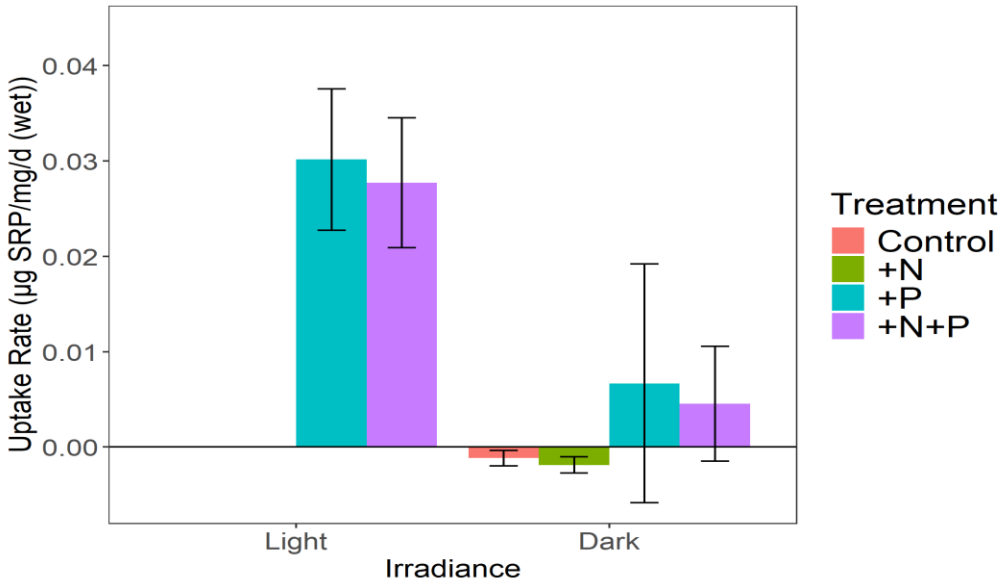

Figure 6. Change in SRP. The different colored bars represent different nutrient treatments. Error bars represent one standard deviation from the mean.

Tissue P Concentration in *Pithophora*:

Usually when an alga is nutrient limited, increasing the ambient concentration will result in net growth, a decline in nutrient concentrations in the growth medium, and an increase in the alga's tissue nutrient concentration. Our study showed that *Pithophora*'s phosphorus content was significantly greater in treatments with P in both the light and the dark (Figure 7). It is unknown in what form that phosphorus was stored within the alga, which may be an area of future investigation.

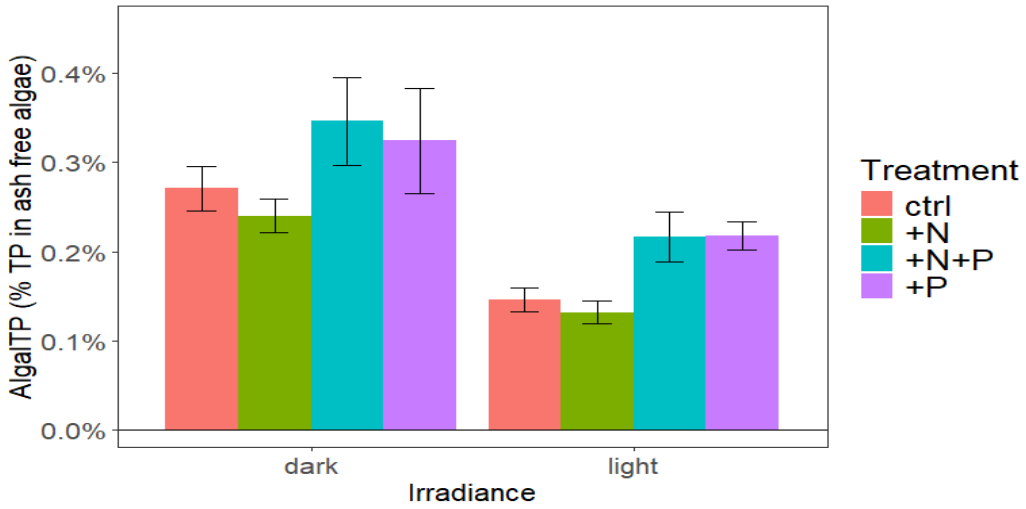

Figure 7. Total phosphorus concentration in *Pithophora*. The different colored bars represent different nutrient treatments. Error bars represent one standard deviation from the mean.

Discussion

Our study revealed several interesting points. First, and not very surprising, *Pithophora* growth was clearly limited by irradiance. Photosynthetic organisms, such as algae, need light to produce energy via photosynthesis. While we have not measured the light levels at the bottom of a Pithophora mat in the Thornapple River, it is likely to have very low light levels, even less than the 16 µmol/m^2/s we used on our incubations (cf. Gordon and McComb 1989). Future studies should measure light extinction through mats in the Thornapple River as well as the impact of harvesting upper layers on the lower layers of the mat once exposed to higher light levels (see future questions below).

Second, the very clear changes in nutrient uptake did not correspond directly to *Pithophora* growth. At this point, we can only speculate on possible reasons:

a) As noted earlier, the variance around algal growth was very high. This likely reflects the way we measured biomass in terms of blotted dry mass, which is a coarse measurement, but necessary to measure the same material from start to finish in the experiment. We also started with a small mass of Pithophora given the small volume of water in the flask (we did not want growth to be limited by the lack of nutrients), so any difference in retained water in the blotted mat would be magnified. In the future, using larger containers and larger mats would minimize the influence of retained water. It is possible that *Pithophora* growth did, in fact, correspond to changes in nutrients but the high variance masked our ability to detect a change.

b) The experimental conditions need to be adjusted. We focused on ammonia and phosphate because these nutrients were in the lowest concentrations in the Thornapple River. Data from PLM revealed that SRP concentrations were always below their detection limit of 10 µg/L while ammonia concentrations were about 50 µg/L—both of these concentrations are very low; in contrast, nitrate concentrations

were about 2100 µg/L and likely not to be limiting *Pithophora* growth. In theory our additions should have stimulated growth. It is possible that the experiment duration was too short to detect a significant growth spurt. We kept the experiment's duration short, again, to avoid potential confounding effects of nutrient limitation in the flasks.

The uptake of SRP in our study was significantly greater under light irradiance, but only in P-added treatments; it is unclear if change in SRP was due to adsorption or absorption. Understanding the fate of the phosphorus taken up would be helpful in determining possible P limitation in *Pithophora.* A study examining the influence of light and dark on phosphorus and ammonium uptake by epilithic biofilms in a Mediterranean river found ammonia and SRP uptake increased under higher light conditions; ammonia uptake was about twice as high in light than dark (Pineda-Morante et al. 2025).

It is well known that periphyton abundance is influenced by many factors, not only nutrients and light (Vadeboncoeur and Steinman 2002; Kemp et al. 2025; Pedro et al. 2025). Other factors that may be worth examining include grazing by macroinvertebrates, water temperature, and water velocity.

Controlling nutrients in the Thornapple River will not be trivial. A significant (albeit unknown) amount of nutrient load comes from the upstream river through the Cascade Dam, which is currently beyond the control of the Thornapple Association. A nutrient budget that measures the loads from the major sources may be worth calculating to help determine where the biggest return on investment would be. This might include the upstream river, stormwater runoff, nutrients from the sediment, atmospheric deposition, lawn runoff, and avian deposits. Another possibility is to control light levels, as it is apparent that *Pithophora* growth is hindered by low light; while difficult to scale up to the entire river, burlap barriers (or something analogous (Caffrey et al. 2010) placed in particularly problematic areas, may be worth investigating. They are currently being deployed on a trial basis in Lake Leelanau to control Eurasian watermilfoil.

Questions for future research on *Pithophora* growth:

- Light levels throughout the mat;
- How "lower level" *Pithophora* mat responds to high light levels;
- How important is "internal" nutrient cycling vs. "external" (river) nutrients for *Pithophora* growth; Gordon and McComb (1989) found that the nutrient concentrations of SRP and ammonia were much higher inside the mats of Cladophora (closely related to Pithophora) than in the surface water where they were growing;
- Is phosphorus being stored internally (and if so, where) or adsorbed to the *Pithophora* mat; and
- The influence of water velocity on *Pithophora* growth
- The influence of benthic barriers on *Pithophora* growth

A new graduate student is starting this fall and she is interested in studying the Pithophora issue; her thesis may address the above questions in the next two years.

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Appendix A. Calibration curve graph and equation. Results used to calculate estimates for initial AFDM data.