Nearshore Science Review: Final Report

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1.1 Introduction

The nearshore environment of many of the world's clear water lakes are perceived to be changing, often in ways that are detrimental to human uses such as recreation. In particular, the littoral (nearshore) zones of clear, low-nutrient lakes may be 'greening', or supporting higher growth and biomass of algae than historically present (Vadeboncoeur et al., 2021). In Lake Tahoe, both attached algae (periphyton) and unattached algae (metaphyton) growing in nearshore habitats may be changing in response to drivers such as climate warming, nutrient inputs, and shifts in invertebrate species (ex. crayfish, Asian clams). Recent research funded by agencies has focused on evaluating the condition of the nearshore of Lake Tahoe, in particular the amount and forms of algae, and on understanding drivers of algal growth.

The goal of this review is to evaluate the methods, results, and implications of the following five recent reports on the conditions and environmental drivers of Tahoe's nearshore ecosystem, to inform future research and monitoring:

1. Determining the seasonal sensitivity of periphyton metabolism to climate warming (Lead author Dr. Steven Sadro, University of California Davis)

2. The Role of Crayfish and Invertebrate Food Web Dynamics in Controlling Algae in Lake Tahoe (Lead author Dr. Sudeep Chandra, University of Nevada Reno)

3. Using a multi-isotopic approach to identify nitrogen sources in groundwater and periphyton along the nearshore of Lake Tahoe (Lead author Dr. Ramon Naranjo, U.S. Geological Survey)

4. Characterization of algal community composition and structure from the nearshore environment, Lake Tahoe (United States). (Lead author Dr. Paula Noble, University of Nevada Reno)

5. Interim Report: Integrated Nearshore Algal Monitoring (in-situ and aerial surveys). (Lead author Dr. Adrianne Smits, University of California Davis). Report evaluated by Dr. John Melack.

For each report, the main results and the methods pertaining to them were critically evaluated (Task 1). Given the project budget and timeline, ancillary methods or results that were not critical to the main study questions (ex. data or sample collection in support of other contracts or grants) were not evaluated. The strength of evidence for the major results was assessed if possible, or deficiencies in methods (or descriptions thereof) that limited the ability to evaluate results or their implications were identified. The main findings of each report are discussed in relation to relevant, recent research. It is important to note that "knowledge gaps" identified with specific research findings do not represent deficiency in a given report; rather, they identify broad gaps in our conceptual understanding as informed by the result or finding.

1.1 Report Structure

In section 2, we synthesize what was learned from all the reports and what questions remain partially or completely unanswered.

In section 3, we provide recommendations for future research and monitoring, both in terms of research topics as well as potential approaches.

In section 4, individual summaries are provided for each report. For each report summary, we include (1) a summary of each report's objectives and methods, (2) a list of conclusive findings and supportive evidence, and (3) a list of inconclusive findings and remaining knowledge gaps warranting additional research.

Detailed assessments of methods and findings in each report (e.g., Task 1) are included as Appendices.

1.2 Feedback from TSAC Water Quality Working Group

On 10-21-2024 Dr. Smits presented this report and its findings for discussion to TSAC scientists and agency management personnel. The following points capture the highlights from the discussion and subsequent comments provided by participants:

- There was consensus that there are many interesting results from the research done in the nearshore of Tahoe, but overall, more questions are raised than answered. The discussion touched on several areas where more research is necessary, most notably to determine attribution of nitrogen sources for periphyton and metaphyton.
- There was consensus that section 3.2 of this report, which identifies key areas for future research, should be a starting point for a discussion about setting research priorities
- There was consensus that a working group meeting focused on updating existing nearshore conceptual models for periphyton and expanding them to include metaphyton using the findings summarized in this report should be prioritized.
- Dr. Naranjo suggested and Dr. Sadro agrees that a synthesis and analysis of isotopic data from across all available sources (i.e., rather than the one report reviewed here) might support mixing model results that would inform a broader working group discussion. Any formal recommendation of N source attribution that influences management actions within the basin needs to be well verified and demonstrated quantitatively.
- There was discussion of variation in the structure among the reports that were reviewed and how a template might help facilitate identifying key findings and the support for them; alternatively, guidelines for how such findings are reported can be incorporated into RFPs and work orders with consultation from scientists. Overall, a report structure that focuses on linking recommendations clearly to results and quantitative support is recommended over one that links reporting to effort associated with specific tasks.

2. Synthesis: What was learned across studies? What are the most important knowledge gaps?

2.1 What we learned from the five reports that were reviewed

1. Warming water temperatures enhance periphyton growth in Lake Tahoe, particularly in colder months (Report 1; Sadro et al.). Quantifying thermal regimes and warming in nearshore waters, and how those affect different components of Tahoe's algal communities (ex. diatom, cyanobacteria, green algae), is therefore an important research area.

2. Nearshore periphyton algae at Tahoe are N-limited, though evidence is from only two locations. Direct evidence for this is demonstrated from the nutrient-diffusing substrate deployments in Report 2 (Chandra et al.), and indirect evidence is provided in Report 4 (Noble et al.) showing that west shore periphyton contain *Epithemia* diatom assemblages with N-fixing symbionts as well as N-fixing cyanobacterial species. These findings reinforce the importance of understanding N sources to nearshore algae in Tahoe.

3. There are distinct periphyton assemblages across depths (eulittoral versus sublittoral) and on different substrates (rocks versus sediment), but our knowledge is still limited in terms of spatial variability (depth, along shoreline, across substrates).

4. There is high algal biomass in the sublittoral zone (> 2 m depth), where little research or monitoring is done. Even if the public is less likely to interact directly with algae at depth, it may affect the dynamics in shallower areas (or in the case of metaphyton, drift on-shore), and should be incorporated into future research and monitoring.

5. Groundwater nitrate isotopic signatures assessed with 15N – 18O biplots and literature values suggest multiple possible sources across time and space, but were not indicative of a high contribution of manure/septic or atmospheric nitrate sources. Nitrification of ammonium from fertilizer is likely to be an important source for some locations and during certain time periods, but overlap among endmember values is high and source attribution between nitrified ammonium from fertilizer, snowpack or soil sources is unclear. Given frequent N-limitation in the nearshore algae, N fluxes and cycling to nearshore algae should continue to be a focus of research.

6. Periphyton isotopic signatures were far less variable in time and space than groundwater values, suggesting that while groundwater may be a source in some locations/times, we are still not entirely accounting for potential N sources to periphyton (or at least that $\delta^{15}N$ is not sufficient as an N tracer in periphyton given the number of potential sources).

2.2 Important future research topics/knowledge gaps:

1. Though recent work has solidified our understanding of periphyton growing on rock substrate, we know almost nothing about algae growing on other substrates (ex. macrophytes) or

unattached algae (metaphyton), including its spatial, temporal variation or main drivers of growth. Given recent detections of large metaphyton patches and algae associated with plants (Smits et al. Report 5), this is a major knowledge gap in our understanding of Tahoe's nearshore.

2. The recent reports begin to show significant spatial variation in algal biomass and community composition, as well as spatial variation in drivers of algal growth (ex. groundwater nutrients), but so far spatial patterns in algal biomass have not been explicitly linked to these drivers, either statistically or otherwise, nor has data collection been sufficient to do so. Characterizing spatial variation in algae and its main drivers (nutrients, temperature, light, maybe grazers) would enable understanding of why certain areas are algae hotspots.

3. Interannual variation in algal biomass, algal community composition, and their drivers (nutrients, light, temperature, grazers) are poorly constrained due to the generally short duration of recent studies. Therefore, it is somewhat unclear if the findings of short term studies are generalizable to years with very different conditions (ex. a high snow year with cold water temperatures and high lake levels).

3. The taxonomic variation of nearshore algae and how that interacts with environmental drivers is unclear as monitoring and experimental work to date has focused on diatom-dominated periphyton (but see Report 5 for preliminary data on metaphyton). For example, do diatoms respond differently than green algae or cyanobacteria to warming, light, nutrients, etc.? What about free-living taxa or taxa growing on aquatic plants? As Tahoe continues to change due to a warming climate, will taxonomic shifts occur, or have they already occurred?

4. Warming temperatures clearly have the potential to enhance periphyton growth, but can we identify shoreline areas where warming is greatest? Do we understand nearshore thermal conditions at scales relevant to nearshore algae? There may be existing data from the TERC nearshore network to quantify thermal regimes and warming rates in a few shoreline locations, but this would require synthesis/analysis.

5. How do light availability, nutrient availability, and temperature interact to structure algal growth/metabolism? Can we start to build/parameterize a periphyton growth model for Lake Tahoe, that would enable predictions based on hydroclimatic conditions and/or management actions?

6. Nutrient sources fueling algal growth are still somewhat unclear. Given the degree of spatial and temporal variability in groundwater nitrate isotopic signatures and overlap in end member values, additional sampling is necessary for attribution and may require validation of end member values. When and where is fertilizer an important N source to groundwater or algae? What are non-groundwater N sources to nearshore algae (ex. N-fixation, excretion)? What is the contribution of N-fixation by cyanobacteria/symbionts to growth of periphyton and metaphyton? Warming water temperatures should enhance N-fixation, as should availability of P. What are P sources to nearshore algae? Do algae attached to or in close proximity to aquatic plants derive nutrients from different sources than do periphyton or metaphyton (may be especially relevant of aquatic plants are increasing in the nearshore)? A conceptual nitrogen budget for periphyton

linked to isotopic signatures of all end members and potential cycling transformations would be useful for supporting and communicating decision-making.

7. What is the role of nearshore biota (ex. crayfish, other invertebrates) in either enhancing or controlling algal growth? Answering this question requires better spatial/temporal surveys of nearshore biota, as well as experimental work.

3. Recommendations for future research and monitoring of Tahoe's nearshore

3.1 Recommendations for research and monitoring priorities

1. We know almost nothing about algae growing on non-rock substrates (ex. macrophytes) or unattached algae (metaphyton), including its spatial, temporal variation or main drivers of growth. Given recent detections of large metaphyton growth areas and algae associated with plants, often dominated by the green algae *Zygnema* (Smits et al. Report 5), this is a major knowledge gap in our understanding of Tahoe's nearshore. We recommend expanding both monitoring and mechanistic studies to account for the diverse growth forms of algae in Tahoe's nearshore. We need to update our conceptual model of Tahoe's nearshore to account for non-periphyton, non-diatom algae.

2. We haven't captured spatial variability in Tahoe's nearshore ecosystems well enough to scale study findings to the whole lake shore. Recent studies still mostly reflect shallow depths (eulittoral zone; but see Chandra and Noble reports), the west shore, and rock substrates, e.g. the eulittoral diatom periphyton assemblage. In a lake as large as Tahoe, characterizing spatial variability in key aspects of the nearshore (algal community composition and biomass, substrates, nutrient sources/fluxes, other environmental drivers) will be critical for management.

3. It was not clear if the findings from the reviewed reports are sensitive to interannual differences in lake level and hydrology (especially as this affects what part of periphyton assemblage is sampled). Interannual variation is not accounted for in any of the studies (given scope of sampling/budgets very understandable), but may be a major limitation to our understanding of the nearshore. We don't know how much algal growth varies year to year in response to variable environmental conditions. For example, the Atkins et al. (2021) study findings were confounded by lake level variation (sampling at fixed depth resulted in different algal communities being sampled in different seasons and years), limiting interpretation of the periphyton biomass trends (or potentially obscuring real trends). Addressing this would require continued regular monitoring and synthesis of existing datasets to quantify the scale interannual variation in key variables.

4. Most of what we know about nearshore algae in Tahoe is based on studies of diatom-dominated periphyton, and as Lake Tahoe continues to change, other taxa may become more important (ex. filamentous green algae in metaphyton or attached to plants). More focus is needed on algal community composition, and how that affects spatial and seasonal variation in observed algal biomass/metabolic rates/nutrient limitation. We need more understanding of what drives or limits metaphyton, and filamentous green algae + cyanobacterial growth

5. Report 1 (Sadro et al.) demonstrated that warming, particularly during winter, enhances periphyton growth. We still need to quantify if there are spatial trends in nearshore water temperature/thermal regimes that may drive spatial and temporal patterns in algal growth. Are some nearshore areas warming more than others? Do these areas support higher algal growth?

We also need to understand thermal dynamics as they extend deeper within the littoral zone, where algal biomass can be high.

6. N and P sources to periphyton (or metaphyton) are not yet clear. More work is needed to understand N and P sources to algae, especially 'internal' sources such as N-fixers, recycling within algal mats, excretion by invertebrates. Deployment of nutrient-diffusing substrates would be a good approach to continue as a monitoring tool, or in targeted studies to understand seasonal/interannual/spatial variation in periphyton nutrient limitation. Though $\delta^{15}N$ and $\delta^{13}C$ signatures of periphyton and metaphyton have a limited ability to pinpoint specific N sources, some focused analysis of existing isotopic datasets may be useful for guiding future research. For example, the $\delta^{15}N$ of periphyton in the current reports suggest N-fixation could provide N to periphyton, but the isotopic data needs more rigorous analysis to determine whether this is likely (ex. integrating data from Naranjo et al. and Smits et al., some basic modelling, statistical analysis of spatial and temporal patterns).

7. There is much yet to be learned regarding the role of nearshore biota (ex. crayfish, other invertebrates) on algal dynamics. This topic needs more experimental work and assessment of spatial, seasonal variation in invertebrate density/community composition, and how that compares to spatial, seasonal variation in algal biomass.

3.2 Recommendations for improving information generation and transfer

1. The organizational structure of the reports is currently not conducive to evaluation of the main findings/conclusions. Linking the statements in the executive summaries to evidence (figures, tables, statistics) is very difficult given the length and structure of reports. One suggestion is to move away from 'task-oriented' report structures, and towards a 'question-oriented' structure, where study questions and the methods/results/interpretations associated with them are found together. Another possibility might be for agency funders to formulate a template for executive summaries attached to reports, that makes linkages between conclusions/interpretations and supportive evidence easier to verify.

2. Sampling/analysis/experimental methods, as well as interpretations of results, were not always described in enough detail or with sufficient clarity to evaluate them. Results/figures were not always as informative as they could be. Allocating more funding or resources to report writing could improve information transfer from scientists to managers. More frequent feedback from funders and managers during the report-writing process could also help avoid poor knowledge transfer.

3. Allocate funding to support more data analysis in reports when appropriate (rather than only descriptive results). These projects involved a huge amount of data collection and sample analysis effort, but their utility is undercut by lack of rigorous data analysis that would enable assessment of significance and uncertainty.

4. Allocate funding to support data syntheses and analyses: for some aspects of the nearshore environment, we do have a lot of data, but resources are needed to integrate datasets and rigorously analyze them to answer questions. Another round of support to update our conceptual periphyton model for Tahoe is recommended.

4. Report summaries and assessments

4.1 Determining the seasonal sensitivity of periphyton metabolism to climate warming

4.1.1 Summary of study objectives and methods

The main objectives of this report were to understand the effects of warming water temperatures on rates of periphyton metabolism (gross primary production-GPP, respiration-R, and net ecosystem production-NEP) at the scale of individual rocks collected from Lake Tahoe's nearshore environment, and whether warming effects might vary across seasons and nutrient availability.

The study asked three questions: 1) How do periphyton metabolic rates vary seasonally? 2) To what extent do nutrients and warming water temperatures individually or interactively affect periphyton metabolic rates, and 3) how do temperature and nutrient effects on metabolism vary seasonally?

To answer these questions, the authors conducted 7 short-term laboratory incubation experiments across multiple seasons in 2021 and 2022 (only the last 5 experiments are discussed further, first two were exploratory). In each experiment, periphyton were subjected to different water temperatures and nutrient levels. For each experiment, 16 periphyton-covered rocks were collected at 0.5 depth from Pineland on Tahoe's west shore. Rocks were then randomly assigned to 4 temperature treatments (control, +3°C, +6°C, +9°C) at ambient lake nutrient levels, and then re-assigned to temperature treatments at elevated nutrient levels (~6.5x above typical groundwater concentrations of NH_4, NO_3, and SRP measured at Lake Tahoe). Warming treatments were always relative to in-situ lake temperatures at the time of rock collections (e.g., warming treatments had colder absolute temperatures in winter than in summer). Periphyton responses measured after warming and nutrient treatments included metabolic rates (GPP, R, NEP) normalized either to biomass (ash-free dry weight; AFDW) or to habitat area, nutrient uptake rates, and nutrient-use efficiency. The significance of warming effects, nutrient effects, and interactive effects of warming and nutrients on periphyton metabolism were determined using linear mixed effects models.

4.1.2 Significant findings and supportive evidence

1. <u>Finding</u>: Warming water temperatures stimulate periphyton metabolic rates (both GPP and R). <u>Evidence</u>: Statistical significance of temperature effects in linear mixed models for GPP, R, NEP.

2. <u>Finding</u>: Warming stimulates periphyton metabolic rates the most in winter (when periphyton growth is often highest and temperatures are coldest) and least in summer. <u>Evidence</u>: Statistically significant month-specific temperature effects in linear mixed models.

4.1.3 Inconclusive findings warranting additional research/knowledge gaps

1. Finding: Periphyton are net autotrophic (GPP > R) year-round. Likely an artifact of experimental design, this does not match seasonal patterns measured in-situ in Lake Tahoe. Important to compare lab incubations of isolated components of the ecosystem (ex. periphyton on a rock) with measurements from the 'whole' ecosystem (rocks + surrounding sediments, other substrates).

2. Finding: Nutrients did not stimulate periphyton primary production. Artifact of short-term incubation experiments.

3. Finding: Lack of interactive effects between warming and nutrients on periphyton metabolism. Likely related to short duration of nutrient exposure during incubations.

4. Knowledge gap: What is the role of light availability/depth on periphyton metabolism? Not assessed, all periphyton were collected at 0.5 m depth and incubated at the same light level.

5. Knowledge gap: What is the role of periphyton community composition on the temperature sensitivity of metabolism? Not assessed. What would the temperature-sensitivity results look like if periphyton were collected from greater depths with different diatom/cyanobacterial assemblage? How sensitive to warming are green algae or cyanobacteria? How sensitive are metaphyton assemblages to warming?

6. Knowledge gap: What is the role of substrate (ex. sediment, boulder, macrophytes?) on periphyton metabolic rates and temperature sensitivity? Would require either more incubations, or in-situ metabolism measurements

4.2 The Role of Crayfish and Invertebrate Food Web Dynamics in Controlling Algae in Lake Tahoe

4.2.1 Summary of study objectives and methods

This study had two main objectives associated with in-situ periphyton growth in Lake Tahoe: 1) to understand role of crayfish in periphyton dynamics, 2) to assess nutrient limitation of periphyton.

To achieve objective 1, five crayfish exclusion experiments were conducted at a west shore location (Sunnyside) across multiple seasons (Nov. 2019 – Aug. 2021). Crayfish were excluded from lake bottom areas using either fencing (four 10 by 10 m areas) or cages (20 small cages, separated by > 2 m). Bottom substrates in the experimental areas were fist-sized rocks placed on soft sediments. The following response variables were measured in the control areas (with crayfish) and exclusion areas (no crayfish): periphyton biomass (chlorophyll-a, ash-free dry weight-AFDW) on rocks and sediment, invertebrate density, and algal community composition (on rocks and sediment). Differences in periphyton biomass between control and exclusion areas were assessed using t-tests (separately for each experiment, rather than in a single mixed effects model).

In-situ exclusion experiments are exceptionally challenging for a variety of reasons and a certain amount of trial and error in their development is to be expected. However, certain methodological details of the crayfish exclusion experiments were not clear from the report text, hindering interpretation of the results. The duration of crayfish exclusions was not reported. Not clear how deep the experimental areas were. Not clear how large the control areas were, or if they were paired with each fenced area? Not clear which exclusion method was used for each of the five experiments. Crayfish density in the control areas was not reported. It was not clear why the sample sizes for variables such as periphyton chlorophyll-a and AFDW were usually much smaller for the control areas than the exclusion areas.

In addition to the exclusion experiments, excretion measurements were made on 27 crayfish in the laboratory, to determine the rate and stoichiometry (N, P, C) of crayfish excretion, to test the possibility that crayfish excretion could stimulate periphyton production by providing limiting nutrients. After incubation, water from tanks containing crayfish, as well as water from a control tank, was analyzed for nutrient concentrations.

To achieve objective 2, nutrient-diffusing substrates (NDS) were deployed at 5 depths along one west shore transect (Sunnyside) and one east shore transect (Glenbrook). NDS were deployed on 4 dates at Sunnyside (09/2020 – 09/2021) and 3 dates at Glenbrook (05/2020-08/2020). The following nutrient treatments were added to agar substrates: none (control), NH_4, NO_3, PO_4, PO_4 + NO_3, PO_4+NH_4. Differences in periphyton biomass (chlorophyll-a) on substrates (relative to the control) were assessed after ~ 3 weeks time using Dunnett's tests (separately for each experiment).

In addition to the experiments conducted for objectives 1-2, a suite of ancillary sampling was done to quantify spatial (across depths) and seasonal variation in the following variables: crayfish density (using traps), periphyton biomass, periphyton community composition, and near-sediment water chemistry.

Note: Periphyton community composition methods and results presented in this report were not evaluated since these are presented in more detail in Report 4 (PI Dr. Paula Noble). Free-water ecosystem metabolic rates were measured near the crayfish exclusion experimental areas, but the results are ancillary to the main study objectives and this component was not reviewed further.

4.2.2 Significant findings and supportive evidence

1. Finding: Tahoe's periphyton are N-limited across depths and seasons. Evidence: Deployment of nutrient diffusing substrates (NDS) showed N-limitation of periphyton across depths and seasons in Tahoe (at one west shore and one east shore location). Dunnett's tests showed statistically significant increases in periphyton biomass on NDS containing nitrate (NO_3)

amendments relative to the control substrates lacking nutrient amendments. Effects of NO_3 amendment were significant across multiple depths and multiple experiment dates.

2. Finding: Crayfish excrete bioavailable N, which theoretically could stimulate periphyton. Evidence: Increased N concentration in water containing crayfish relative to control water lacking crayfish. No statistics reported but responses were large.

4.2.3 Inconclusive findings warranting additional research/knowledge gaps

1. Finding: Crayfish have variable or non-significant impacts on periphyton biomass. Much of value remains to be learned in this area. Do crayfish stimulate or reduce periphyton biomass in Lake Tahoe (or neither)? By what mechanism? These questions remain unanswered due to the lack of significant results from the crayfish exclusion experiments, and partly form lack of methodological details needed to assess the results of the experiments. It was not clear which exclusion method was used for each experiment and how that might have affected the results (were cages or fencing equally effective exclusion methods?). Since crayfish could affect periphyton through at least three pathways (directly via excretion of nutrients, indirectly by reducing invertebrate grazer density, and directly by grazing on algae), more/different experimental approaches are needed to understand their potential role in periphyton dynamics. In addition to experimental approaches, it may be informative to do spatial surveys to compare algal biomass in low versus high crayfish density areas, as was done in a study at ultra-oligotrophic Crater Lake by Scordo et al. (2023). Compound-specific isotopic analyses (amino acids, fatty acids) of crayfish could be used to quantify direct grazing on periphyton (not completed in this report due to pandemic-related challenges).

2. Knowledge gap: How prevalent are crayfish and other littoral invertebrates around the Tahoe's shoreline? Is there spatial variation in crayfish density, and does this correspond to spatial variation in periphyton biomass (approach used in Scordo et al. (2023) Crater Lake study)?

3. Knowledge gap: Do the $\delta^{15}N$ signatures of periphyton presented in Report 3 (PI Dr. Ramon Naranjo) and Report 4 (PI Dr. Adrianne Smits) support excreted N from crayfish as a major nutrient source to periphyton?

4. Knowledge gap: How much spatial variation is there in nutrient limitation of Tahoe's nearshore algae (periphyton and metaphyton)? In this study, NDS were deployed at only two locations (1 west shore, 1 east shore), and nutrient limitation might vary around the lake depending on variation in sources and fluxes.

5. Knowledge gap: How might nutrient limitation vary across algal assemblages? How much would N loading need to occur before periphyton became co-limited by N and P? Are metaphyton nutrient-limited, and at what point in their growth cycle? What about periphyton growing on different substrates such as macrophytes? Do diatoms and green algae differ in nutrient limitation?

6 Knowledge gap: Is there interannual variation in nutrient limitation of Tahoe's periphyton? This study was done in a single water year, would we find the same patterns in a different year?

4.3 Using a multi-isotopic approach to identify nitrogen sources in groundwater and periphyton along the nearshore of Lake Tahoe

4.3.1 Summary of study objectives and methods

The objectives of this study were to (1) identify sources of nitrogen (N) and phosphorus (P) in groundwater around Lake Tahoe, and (2) to determine if these nutrient sources were identifiable in nearshore periphyton.

To identify sources of N in groundwater inputs to Lake Tahoe (Objective 1), this study quantified seasonal variation in stream discharge and nutrient concentrations, groundwater discharge and nutrient concentrations, and nitrogen and oxygen isotopes of stream and groundwater nitrate, near the outlet of Ward Creek, on the west shore of Lake Tahoe. Nitrate samples collected from Ward Creek and groundwater in two nearby transects (NS3, NS5) were analyzed for the following isotopes: $\delta^{15}N$, $\delta^{18}O$, $\delta^{17}O$, and $\Delta^{17}O$. Isotope ratios of stream water and groundwater nitrate were then plotted and visually compared to published literature ranges for 6 potential N sources (atmospheric NO_3, NO_3 fertilizer, NH_4 fertilizer, soil NH_4, nitrification of snow NH_4, & manure/septic). N sources were not sampled or measured directly (ex. snow, rain). Mixing models were not used to quantify proportional contributions of N sources to stream water or groundwater nitrate. N source contributions to nitrate in groundwater or stream water samples were attributed based on visual overlap between published literature values and measured sample values.

In addition to the more frequent seasonal measurements made near Ward Creek, spatial variation in groundwater discharge, N and P concentrations, and isotopic composition was quantified by sampling once in winter and spring at 20 synoptic sites around Lake Tahoe (sites were historically sampled by TERC periphyton monitoring program). All sampling occurred during water year 2020 (Nov. 2019 – July 2020).

To identify N sources contributing to growth of nearshore periphyton (objective 2), periphyton biomass, C:N ratio, and $\delta^{15}N$ were measured ~ biweekly from boulders and rocks in Ward Creek and near groundwater transects NS3 and NS5. Spatial variation in periphyton biomass and isotopic composition ($\delta^{15}N$, $\delta^{13}C$) was captured by sampling periphyton on rocks at the 20 synoptic sites around Lake Tahoe in winter and spring/summer.

Note: Analytical methods related to P sources in groundwater were unsuccessful and were not discussed at length in the report, so I did not evaluate these methods or results. I am not an expert in groundwater sampling or modeling, so methods related to estimating groundwater discharge and sampling were not critically evaluated. I assumed these were done appropriately and have focused the review on the overall sampling design (spatial and temporal components, constituents measured) and on interpretations of the isotope data.

4.3.2 Significant findings and supportive evidence

1. Finding: Substantial seasonal and spatial variation in groundwater nitrate isotopic signatures are suggestive of either heterogeneous N sources to groundwater at small spatial scales, or N cycling that alters the isotopic signature at small spatial scales (or both). An implication of this finding is that snapshot sampling (ex. 1-2 time per year) is not sufficient to understand N sources and fluxes to Lake Tahoe, higher frequency sampling is necessary (as was done at Ward Creek and nearby groundwater transects). Evidence: Large range in $\delta^{15}N$, $\delta^{18}O$ of groundwater nitrate sampled from NS3, NS5 transects.

2. Finding: Manure/septic is probably not a substantial N source to groundwater or periphyton in Lake Tahoe. Evidence: $\delta^{15}N$ of groundwater nitrate and periphyton was never enriched far above 0‰. This finding does not rule out some contribution of manure/septic to groundwater, but it is unlikely to be the main source.

3. Finding: Atmospheric nitrate is unlikely to be the predominant N source to stream water or groundwater around Lake Tahoe. Evidence: $\Delta^{17}O$, a conservative tracer for atmospherically oxidized nitrate (either natural or anthropogenic in origin), was near zero for all nitrate samples.

4. Finding: Nitrified ammonium is likely a primary source to groundwater nitrate in many locations. The study results ruled out major contributions of atmospheric nitrate and manure/septic to nitrate in groundwater, however, it remains somewhat unclear the relative contributions from ammonium fertilizer, snow ammonium, or soil ammonium pools. Likewise, how processes such as nitrification, denitrification might change the isotopic signature of nitrate as it is transported to the lake nearshore environment is unclear. Identification of N sources to groundwater may require further research/more explanation of findings in the report. Measuring isotopic signatures of potential N sources to groundwater and algae (snowmelt, rain, hypolimnetic lake water etc.) may help constrain contributions or seasonal variation associated with cycling transformations, and potentially reduce uncertainty in mixing models estimate of relative contribution of different N sources.

4.3.3 Inconclusive findings warranting additional research/knowledge gaps

1. Knowledge gap: Sources of P to groundwater remain unclear (no results presented due to issues with analytical methods).

2. Finding: While stream water nitrate and groundwater nitrate near Ward Creek had somewhat distinct signatures, it is not clear if the differences are related to a) processes such as denitrification occurring in low oxygen environments in groundwater, or b) contribution of distinct N sources to groundwater that were absent from stream water.

3. Finding: Based on the $\delta^{15}N$ of periphyton measured in this study, it is not clear what N sources are fueling periphyton growth. Although $\delta^{15}N$ mean values in periphyton were often slightly

negative, suggesting fertilizer as a possible source, the large range in fertilizer end member values in groundwater, and lack of sampling of other potential sources (ex. lake water) make attributing relative importance of these possible sources impossible. It is clear, however, that high contributions of manure/septic are unlikely. Periphyton $\delta^{15}N$ was <u>not</u> highly variable either spatially or through time, and was usually near 0‰, which was a bit surprising given the large spatial and seasonal variation in $\delta^{15}N$ of groundwater nitrate. The periphyton-groundwater linkage was not apparent in this study. The disparity between groundwater nitrate isotopic signatures and periphyton signatures (both in terms of their actual values and their degree of variability) suggests we either a) are not measuring major sources of N to periphyton (ex. N-fixation by cyanobacteria/symbionts in periphyton mats, excretion by invertebrates such as crayfish or clams, internal cycling in algal mats, lake water), or b) integrating the $\delta^{15}N$ signatures of variable groundwater N yields an average value near 0‰. Given the findings in Report 2 (Chandra et al.) of N-limitation of periphyton, the finding in Report 4 (Noble et al.) that Tahoe's sublittoral diatom assemblage is often dominated by species with N-fixing symbionts, and the finding in Report 5 (Smits et al.) of similar periphyton $\delta^{15}N$ near 0‰ this is an important area for further research.

4. <u>Finding</u>: The significant correlations between periphyton biomass (chlorophyll-a) and periphyton $\delta^{15}N$ and $\delta^{13}C$ were interesting, suggesting either 1) a decrease in fractionation effects against heavy C and N isotopes during periods of high growth/biomass accumulation (e.g., C or N limitation), or 2) different N source contributions to periphyton in higher growth areas.

5. <u>Knowledge gap</u>: What role does interannual variation in lake level play in determining linkages between groundwater N and periphyton growth? How might higher water levels in water year 2020 have affected the study's findings, especially relating to groundwater fluxes or groundwater contributions to N used by periphyton? Would periphyton $\delta^{15}N$ be correlated with groundwater $\delta^{15}N$ in a dry year where low lake levels enhanced groundwater fluxes to the lake?

6. <u>Finding</u>: West shore areas (synoptic sites) showed higher groundwater N and P concentrations than other shoreline areas, but shoreline areas were not sampled equally (less on east shore). The report did not assess potential causes/correlates for spatial (around shoreline) variation in nutrient concentrations or isotopic signatures of groundwater. This variation around the shoreline is interesting and should be investigated in future research, potentially some version of the Ward Creek/transect sampling could be replicated in a few other areas.

7. <u>Knowledge gap</u>: All the periphyton samples in this study were collected from shallow depths (presumably, as depth of sampling was not described in report). How might the periphyton results differ if deeper algal communities were sampled (greater contribution of cyanobacteria/N-fixers to community)?

4.4 Characterization of algal community composition and structure from the nearshore environment, Lake Tahoe (United States)

4.4.1 Summary of study objectives and methods

The objectives of this study were (1) to characterize the community composition of Lake Tahoe's periphyton across depths (eulittoral 0- 2 m depth, sublittoral >2 m depth), substrate types (rock versus sediment), and seasons. The emphasis of sampling and community analysis was on diatoms. (2) A diatom voucher flora was created for Lake Tahoe to enable better reconciliation in species identification across research and monitoring efforts.

A total of 29 periphyton samples were collected to create the diatom voucher flora, and for analysis of the diatom component of the periphyton assemblage. Diatom samples were collected from rock scrapes and sediment. Diatom samples were collected from three sites on Tahoe's west shore (Sunnyside, Pineland, Dollar Point), and from multiple depths (eulittoral (0-2 m) versus sublittoral (8 m)). Diatom samples were collected on multiple dates during water year 2020, though seasonal sampling was not consistent for all sites/depths/substrates.

Of the 29 diatom samples collected, 19 samples were enumerated (valves were counted) and used to determine relative species abundance and identify groupings within the diatom community using principal components analysis (PCA). 15 of the 19 enumerated diatom samples were from the Sunnyside sampling location. 16 of the 19 diatom community samples were from rock scrapes, and three were from sediment substrates. 7 diatom samples were collected from the eulittoral zone, and 12 were collected from the sublittoral zone.

In addition to the samples collected for diatom analysis, 12 samples were collected to enumerate all soft-bodied algal species (including diatoms, cyanobacteria, green algae). These were collected from a single location on the west shore (Sunnyside), from both the eulittoral zone (n=6) and the sublittoral (n=6), and from both rock (n=8) and sediment substrates (n=4). Samples for soft-bodied algae were collected on multiple dates, but seasonal sampling was not consistent across depths or substrates (ex. sediment samples were only collected in autumn). Samples were preserved in Lugol's solution and were then enumerated using 'natural counting units'. For diatoms, a natural counting unit was an individual frustule, whereas for taxa that form chains or filaments, a natural counting unit would include multiple individual cells. No statistical tests or multivariate ordination techniques were used to assess the significance of community differences between depths, substrates, or seasons.

Note: I am not an algal taxonomist, so the methods used to preserve/identify/enumerate algal samples or to develop the diatom voucher flora were not critically evaluated. I assumed that those steps were done appropriately and focused my review on the sampling design (spatial + temporal aspects, samples sizes), and its implications for interpreting the findings.

4.4.2 Significant findings and supportive evidence

1. Finding: Periphyton assemblage at Sunnyside (west shore) is dominated by diatoms across depths and seasons, with cyanobacteria the second-most abundant group. Evidence: Enumeration of the soft-bodied algal species from 12 samples.

2. Finding: Diatom species found on rocks and sediment substrate are different (at Sunnyside). Evidence: Diatom valve counts, principal components analysis.

3. Finding: Diatom species in the eulittoral zone (0-2 m) and sublittoral zone (8 m depth) are different (at Sunnyside). Evidence: Diatom valve counts, principal components analysis.

4. Finding: Epilithic (rock) diatom species in the sublittoral zone (>2 m depth) dominated by *Epithemia* species, a low-N specialist. Evidence: Valve counts.

4.4.3 Inconclusive findings warranting additional research/knowledge gaps

1. Knowledge gap: Although this taxonomic analysis yielded valuable insights, scaling such studies up is inherently challenging. Applicability of the taxonomic/community composition results to other shoreline areas at Tahoe remains uncertain. The overall low sample sizes and limited spatial scale of sampling means it is hard to generalize these results to other shoreline areas around Lake Tahoe. What's happening away from the west shore, or even away from Sunnyside? Given how different the diatom assemblages were between rocks and sediment in the same location, spatial variation/heterogeneity is likely substantial.

2. Knowledge gap: The statistical significance of species groupings across depths and substrates were not provided, and may require greater sample sizes (e.g., more sample collection and analysis).

3. Knowledge gap: Seasonal patterns in community composition are important for our understanding of algal growth at Tahoe because they should reflect changes in environmental drivers (light/UV, temperature, nutrient availability, disturbance).The scale of seasonal variation in community composition of periphyton is not clear from this study due to inconsistent seasonal sampling across depths and substrates (though some indication that variation can be large at least in the eulittoral zone).

4. Knowledge gap: Because sample collection occurred within a single year, we don't know how much periphyton community composition might vary year to year (or how that might alter the seasonal patterns). This study provides a nice starting point against which to make future comparisons.

5. Finding/Knowledge gap: Cyanobacteria and green algae were not dominant in any soft-bodied algae samples collected in this study, but could this differ across substrates, or shoreline areas? What about periphyton growing on large boulders? On macrophytes?

4.5 Interim Report: Integrated Nearshore Algal Monitoring (in-situ and aerial surveys)

Year 1: April 2023 – December 2023
Submitted to Tahoe Regional Planning Agency
Contract #23C00042
Submitted by
UC Davis, Tahoe Environmental Research Center (Adrianne Smits, PI).

4.5.1 Summary of study objectives and methods

The work reported by Smits et al. (2024) was done as a contract with TRPA awarded in response to an RFP that requested a monitoring program. The objective is to quantify the extent and distribution of attached and suspended algal communities in Tahoe's nearshore to provide the information necessary to assess the status and trends in nearshore algal conditions.

The report presents data obtained during the first 9 months of the contract and includes all the proposed tasks. The nested set of measurements entailed direct measurements of algae attached to hard substrata and metaphyton, algae suspended above the bottom, in nearshore waters of Lake Tahoe complemented by remotely sensed imagery that extended the spatial coverage. The work included measurements of areal cover, biomass, and nutrient concentrations and isotopic content of metaphyton and attached algae at multiple stations and times, identification of taxonomic composition of nearshore algae, and use of a citizen science app to obtain public perception of nearshore algae. A challenge of monitoring nearshore algae is the considerable spatial heterogeneity and seasonal changes. The nested design provided a reasonable approach to deal with these complexities and is a significant improvement compared to a previous monitoring program summarized in Atkins et al. (2021).

Eight nearshore sites were surveyed for attached algae by divers, and by aerial surveys using a camera mounted on a drone. Eight sites were surveyed for metaphyton by divers and aerial surveys. Observations and collection of algae were made at 0.5 m and 1.5 m depths. Eight helicopter flights were conducted along the entire shoreline within a week of in-situ sampling events. The timing of the sampling was selected to capture the expected periods with higher algal abundance (winter and spring) and lower abundance (summer).

Given this is a first-year project report, there are no major findings to assess and interpretations of the results are not provided. Recommendations for slight modifications to the design are offered and seem reasonable.

4.5.2 Significant findings and supportive evidence

1. <u>Assessment</u>: Smits et al. (2024) provide a thorough description of sampling sites, times and analytical methods, and graphical and tabular data.

2. Assessment: Though the sampling sites are distributed around the lake, their representativeness with respect to available substrata or factors influencing algal growth is not offered. The justification for selecting sites for metaphyton sampling with Asian clams present is not provided. Though collections below 1.5 m are difficult, given the clarity of Lake Tahoe, algal growth at deeper depths is expected.

3. Assessment: Analytical assays for algal abundance and composition include ash free dry weight, chlorophyll *a,* nutrient content (C, N, P), stable isotope ratios (δ13C and δ15N), and taxonomic identification. The methods are appropriate. Were chlorophyll values corrected for phaeophytin? The assays for nutrient content and isotope ratios are amenable to ecological interpretation, to be expected in future reports. More information about the taxonomic composition, including species and relative abundances, would be helpful.

4. Assessment: The methods developed by TERC for efficient, high-resolution, quantitative collection and processing of remotely sensed data from aerial imagery to provide measures of change in areal extent and biomass of algal growth in the nearshore are a significant advance and should lead to improved spatial and temporal information.

5. Assessment: Further comparisons with in situ data and advances in data processing will strengthen the approach. Though the aerial imagery of the entire nearshore of Lake Tahoe obtained by helicopter is non- georeferenced and of lower resolution than that from the drone, it provides information on the status of the total nearshore of Lake Tahoe. Whether the cost and frequency of these flights is justified should be evaluated.

6. Assessment: The algae watch survey in TERC's citizen science Tahoe app provides a useful link to public perceptions of nearshore algae in comparison to the quantitative sampling and aerial imagery.

7. Assessment: The plans to expand use and evaluation of these results are reasonable, though to obtain statistically meaningful information will require careful design.

4.5.3 Recommendations for future research and monitoring

Looking ahead to further work and reporting on TERC's contract with TRPA with a focus on the design of an affordable, scientifically sound, and practical monitoring program, suggestions and questions for consideration include:

1) Provide costs per task to allow evaluation of the proportion of effort required for each major activity.
2) Conduct statistical analyses of results to determine if the number of sites and frequency of sampling is sufficient to detect status and trends in nearshore algae.
3) Provide a justification for site selection in the context of the lake-wide distribution of substrata and likely factors influencing algal growth or accumulation.
4) Offer suggestions for sampling depths deeper than 1.5 m. For example, consider suspension of substrata and/or collections by divers.
5) Evaluate the need for regular helicopter overflights.

6) Could a combination of in situ sampling and drone overflights be applied with a stratified random design that could provide statistically robust results?
7) To provide more frequent data could in situ sensors track algal cover?
8) Could the combination of in situ sensors and models lead to forecasts of likely increases in algal abundance?

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Appendix 1. Detailed Assessment of Report 1: Determining the seasonal sensitivity of periphyton metabolism to climate warming

Summary of study findings

Baseline seasonal variation in periphyton metabolism and nutrient uptake:

-periphyton biomass (AFDW, chla) varied seasonally, highest in winter and spring, lowest in summer (June, August), with increases in autumn. Similar patterns as seen in long-term TERC monitoring dataset published by Atkins et al. (2021)

-Areal rates of GPP and NEP of periphyton (e.g. ecosystem-level production rates) were highest in October (when periphyton biomass was increasing after the summer minimum) and lowest in June (when periphyton biomass was lowest).

-Biomass-normalized metabolic rates (AFDW, representing organism-level rates) were greatest in June and August (when periphyton biomass was lowest). Authors suggested this was due to high in-situ water temperatures in June, but water temperatures were actually highest in August and just as high in October, so other factors may be more important, such as PAR/UV levels or differences in algal species composition.

-Periphyton metabolism in incubations was always net autotrophic (GPP > R) across seasons

-At in-situ water temperatures, nutrient (NH4, SRP) uptake rates (per unit biomass; AFDW) were highest in June and August, and lower the rest of year. NH4 and SRP uptake rates are greater at higher temperatures, though interestingly, rates in October were much lower than summer rates despite similar water temperatures.

-N-use efficiency was similar across all experiments except June. Most N uptake was NH_4 (interesting contrast to Chandra crayfish study, where nitrate-N stimulated periphyton growth on NDS but NH_4 did not).

Overall warming and nutrient effects on periphyton metabolism:

-Warming treatments stimulated rates of GPP, ER, and NEP- significant relationships/parameter estimates from the linear mixed model (though unclear which model)

-Elevated nutrients significantly stimulated ER, but not GPP or NEP. -lack of significance in mixed models

-No significant interactive effects were found between warming and nutrients (mixed model coefficients non-significant). In other words, adding nutrients to the incubations did not significantly change the effects of warming water temperatures on periphyton metabolic rates.

-Higher nutrient use efficiency (NUE) at higher temps (moles O2 as NEP/moles N or P taken up), but warming treatments during summer did not improve NUE (not statistically assessed)

Seasonal variation in warming and nutrient effects:

-Warming treatments caused the largest increases in periphyton metabolic rates in winter (February). Warming treatments did not stimulate metabolism at all in summer (June).

-N-use efficiency did not vary with temperature across seasons. SRP-use efficiency was stimulated more by warming in cold months relative to warm months. Not assessed with statistics.

Assessment of strength of conclusions given methodology and significance of results

Baseline seasonal variation in periphyton metabolism and nutrient uptake

-seasonal patterns in baseline metabolism and nutrient uptake rates were plotted but no statistical tests were used to assess significance. Plots did show large differences in baseline rates.

-nutrient uptake results…supports idea that N is important, but lack of NO3 uptake somewhat conflicts with Chandra report results (where NO3 substrates had higher algal growth than NH4 substrates). May be related to different time scales of investigation. Short-term incubations were not appropriate method for determining nutrient limitation.

-net autotrophy of periphyton metabolism across seasons- Rocks were incubated for 10-23 hours in the dark, 1 hour in the light, oxygen mass balance used to calculate metabolic rates (standard approach). However, the finding of net autotrophy may be an artifact of the short duration of light treatment, or due to isolation of a small subset of the nearshore community (single rock with periphyton). Impossible to assess true metabolic state of nearshore ecosystem from short-term lab incubations of individual components. Lab findings of year-round net autotrophy do not fit with published in-situ metabolism measurements that show shorter seasonal duration of net autotrophy (metabolism results in Chandra report, Smits et al. 2024 Comm. Earth & Env.)

-Autumn of 2021 included heavy smoke cover/possibly deposition of ash to Lake Tahoe from Caldor Fire, may have influenced the autumn experimental results (interesting that periphyton biomass was quite high by October)

Effects of warming and nutrients on periphyton metabolic rates

-warming significantly stimulated both GPP and R of periphyton on rocks: high confidence (highly significant temperature effects in mixed effects models)

-role of nutrients on periphyton metabolism: less clear than role of temperature, not possible to assess nutrient limitation or interactions with short time scale incubations, inconclusive.

-No significant temperature-nutrient interactive effects on periphyton metabolism. Authors use this result to hypothesize that periphyton growth in the nearshore is not currently nutrient-limited. But lack of nutrient effects or interactions is very likely an artifact of experimental design (short term exposure, only rocks collected from shallow depths).

Seasonal variation in warming and nutrient effects on periphyton metabolism

-Strong support for higher sensitivity of periphyton to temperature increases during winter (significance of effects derived from p-values; unclear if a single linear mixed model was fit, or multiple models to separately get at seasonal variation in warming effects, nutrient effects)

Comment on the overall importance and defensibleness of the results

The study shows clear, interpretable seasonal patterns in baseline periphyton metabolic rates, as well as increases in metabolic rates with warming temperatures. Warming stimulated periphyton metabolism more during winter than in other seasons. Overall the methods were appropriate, with the exception that the short-term nutrient incubations were not appropriate for determining nutrient limitation of periphyton, which may account for the lack of significant nutrient effects on GPP or NEP, or lack of significant nutrient-temperature interactive effects. Likewise, the finding of year-round net autotrophy (GPP > R) of periphyton metabolism may be an artifact of a) short term incubations, and b) isolating a small component of the ecosystem (rocks). The presentation of the linear mixed model results and the statistics associated with seasonal variation in warming and nutrient effects were somewhat confusing (did they fit a single, mixed effects model to all the metabolism data, or did they fit separate models to get estimates of warming effects, nutrient effects across experiments?).

There were a few important drivers of periphyton metabolism that were not included in the experiment design (understandably given scope/budget). The role of algal taxonomy is a major unresolved question. The community composition of periphyton shifted from diatoms to cyanobacteria across seasons (what if these experiments had been performed in a wetter year without dramatic lake level fluctuation, and periphyton community had been diatom-dominated all year?). The community composition data were not reported or shown in figures in this report, so the importance of this factor was not possible to assess. The role of light/UV is another major knowledge gap. Algae growing on substrates other than rocks may have different responses to warming and nutrients. All rocks were collected from the shallow eulittoral zone, so it is possible that algae growing at deeper depths may have different relationships with water temperature and nutrients (perhaps very likely given different community composition and light environment at depth).

Synthesize the findings and their implications in the context of the recent published literature

The finding that warming increases periphyton metabolic rates generally agrees with prior work showing that rates of ecosystem respiration tend to be more temperature-sensitive than rates of gross primary production in aquatic environments, but that both are temperature-dependent (Yvon-Durocher et al., 2010).

Seasonal periphyton biomass/chlorophyll measurements in this report closely matched historic patterns in Atkins et al. (2021), given collection was done from the same site/depth, this is not surprising. It was interesting that uptake rates were highest for NH4 in this experiment (not nitrate), but periphyton biomass was highest on NO3 diffusing substrates in Report 2 (Chandra et al.).

Appendix 2. Detailed assessment of Report 2: The Role of Crayfish and Invertebrate Food Web Dynamics in Controlling Algae in Lake Tahoe

Summary of findings

<u>Role of crayfish in periphyton dynamics</u>

-crayfish excretion measurements: Crayfish excrete lots of N (compared to P), larger crayfish excrete more. No differences in excretion rate or stoichiometry between male and female crayfish.

-crayfish density transects: crayfish density higher at east shore site (Glenbrook) than at west shore transect (Sunnyside). Seasonal increases in density at shallower littoral depths in summer.

- Of the 5 crayfish exclusion experiments conducted, three showed no changes in algal biomass or composition, two showed changes in periphyton biomass (chlorophyll-a) on rocks (not sediment) but in opposite directions, and some change in community composition (mostly diatom species). Not clear why in the control versus exclusion t-tests, sample sizes for the control treatment are always much smaller than the exclusion treatment. Why is there a sample size imbalance? Only one experiment showed a significant change in AFDM. No difference in density of non-crayfish invertebrates between control and exclusion areas. Overall, the crayfish exclusion results are inconclusive due to lack of methodological detail as well as lack of statistical significance. Potential problem with different exclusion methods across experiments, potentially cages not large enough to cause/see changes? How localized should we expect crayfish excretion to be?

Nutrient limitation of Tahoe's periphyton

-Periphyton growth was N-limited across two depth transects. Biomass on NO3 substrates was usually higher than the control across seasons, depths, whereas biomass was almost never higher than control substrates on the NH4 or PO4 substrates. Nitrate-N appears to be preferred form for algae, in contrast to finding from Sadro report (where NH4 uptake rate was greater than NO3 uptake). The PO4 + NO3 substrates sometimes did not show higher algal biomass than the control.

Depth distribution of periphyton biomass and water chemistry

- spatial and seasonal patterns in periphyton biomass were hard to evaluate because in Figure 8 - 9 the extent of y and x axes differ between panels. Significance of spatial and seasonal differences in biomass was not evaluated with statistical tests.

- high spatial (site and depth) and seasonal variation in near-sediment water chemistry. Significance of differences were not evaluated with statistical tests. Values from Sunnyside were similar to those measured by Naranjo et al. (2019).

Assessment of strength of conclusions given methodology and significance of results

The effect of crayfish on periphyton biomass and community composition was inconclusive due to 1) lack of certain methodological details provided in report (for example, which exclusion method was used in each experiment? How long was each experiment duration? How deep were the experimental areas? What was the crayfish density in 'control' area?, and 2) mostly non-significant or variable experimental results. It was difficult to assess aspects of the experimental design given lack of relevant details, and difficult to assess the results due to the presentation of figures (typically separate figures for each experiment/transect, often with different axis extents). Given that there are at least three pathways (that could occur simultaneously) by which crayfish could affect periphyton biomass (stimulation via excretion, stimulation via release from grazing pressure by invertebrates, reduction via direct grazing by crayfish), a lack of clear results from the experiments is potentially not surprising.

Excretion measurements demonstrated that crayfish excrete bioavailable N.

Periphyton nutrient limitation results were more conclusive (consistent results across experiments/sites, significant differences in algal biomass relative to control substrates)—periphyton appears to be N-limited across most depths and seasons. Nitrate stimulated algal growth on substrates, whereas NH4 and PO4 did not. This finding contrasts with finding in Sadro report, where NH4 uptake was much higher than NO3 uptake (though methods are not directly comparable).

Depth transect results (crayfish density, algal biomass etc.) were difficult to assess for spatial and seasonal shifts due to inconsistent axes in the figures. No statistics were used to determine if depth or seasonal differences were significant, but visually it was apparent that substantial algal biomass occurs below the eulittoral zone (> 2 m depth).

Comment on the overall importance and defensibleness of the results? Were interpretations supported by findings?

The results related to nutrient-limitation of Tahoe's periphyton, and the crayfish excretion results, were informative and defensible. A compelling finding was that algal biomass was consistently highest on NO3-diffusing substrates across depths, dates, and locations (west shore versus east shore). Caveat: only deployed NDS at two locations (Glenbrook and Sunnyside), potentially larger spatial variation in nutrient limitation around the entire shoreline. Interesting that NO3 (and not NH4) stimulated the highest growth.

The role of crayfish in either stimulating or reducing periphyton biomass (or neither) was not clear given the ambiguous experimental results from this study, and lack of methodological details provided. In part, some of the lack of clarity on the defensibleness of results was caused by the report length and structure, how figures were presented, and lack of methods description, which made assessing evidence for interpretations more difficult.

The exclusion experimental results were mostly non-significant and of mixed direction, so while the study did not rule out the *potential* for crayfish excretion to stimulate periphyton biomass (through nutrient limitation experiments and excretion experiments), there was no clear evidence for this mechanism (or other mechanisms, such as release from grazing or direct grazing). Many important details regarding the exclusion experiments were missing, such as which method of exclusion (fencing versus cages) were used in each experiment, the depth of the experimental area, the duration of the exclusion experiments etc.

The various transect samplings (for crayfish, algal biomass, water chemistry) showed intriguing patterns with depth that deserve greater consideration, but these results were not presented in a format that made comparisons across sites/seasons/constituents straightforward. That said, clearly depth-related variation is substantial and merits consideration in further nearshore research.

Synthesize the findings and their implications in the context of the recent published literature

Overall, this study demonstrates that there are plausible mechanisms (N excretion) by which crayfish could affect periphyton growth, but experiments did not demonstrate this definitively, potentially not possible with the experimental design used. A relevant study conducted recently

in ultra-oligotrophic Crater Lake, OR, and found significantly higher periphyton biomass in areas with high crayfish density (Scordo et al., 2023), suggesting a linkage between crayfish and periphyton biomass in Tahoe is a least somewhat plausible. However, much of the periphyton biomass in Crater Lake was comprised of filamentous green algae, in contrast to Tahoe's diatom-dominated periphyton. It is unclear how the crayfish densities at Crater Lake compare to those at Tahoe, and they used different methods for estimating crayfish density (snorkel surveys rather than traps).

Appendix 3. Detailed Assessment of Report 3: Using a multi-isotopic approach to identify nitrogen sources in groundwater and periphyton along the nearshore of Lake Tahoe

Summary of findings

<u>N sources in groundwater</u>

-Ward Creek nitrate isotope signatures were distinct from nearby groundwater signatures collected from transects NS3 and NS5 (more $\delta^{15}N$-depleted).

-The N source to Ward Creek nitrate was attributed to nitrification of snow ammonia. This determination was based on the biplot of $\delta^{15}N$ and $\delta^{18}O$, via comparison to published ranges for sources.

-Isotopic signatures ($\delta^{15}N$, $\delta^{18}O$) of groundwater nitrate collected from the Ward Creek transects were highly seasonally variable, and NS3 and NS5 differed substantially from each other. Seasonal and spatial variation in $\delta^{15}N$, $\delta^{18}O$ of groundwater nitrate from Ward Creek transects <u>exceeded</u> the variation seen across 20 synoptic sites during snapshot sampling. No statistical tests were used to assess significance of seasonal or spatial differences.

-$\Delta^{17}O$-nitrate was low (near zero) in all groundwater samples, indicating a low contribution of atmospheric nitrate. Authors attribute this low $\Delta^{17}O$ signature to contribution of nitrification.

- Authors attributed groundwater N sources to ammonium fertilizer, atmospheric nitrate, manure, and septic (and soil ammonia)? There is high overlap in isotopic composition across many of these sources (less so for atmospheric nitrate). No mixing models were used to estimate relative contributions of each source. Attributing relative contributions with mixing models that have overlapping sources generally results in estimates with a high degree of uncertainty, as many potential mixtures are equally likely.

-some spatial patterns in groundwater chemistry were apparent, for example, west shore had some hotspots for N and P in both winter and spring/summer. No statistical tests were done to assess significance of spatial patterns in groundwater chemistry.

<u>N sources for periphyton</u>

-Periphyton $\delta^{15}N$ was slightly negative on average but close to zero, occasionally > 0‰. Periphyton $\delta^{15}N$ did not vary much seasonally or spatially (at least in comparison with extremely high variation in the isotopic signatures of nitrate in groundwater),

-Periphyton biomass was significantly correlated with enriched $\delta^{15}N$ signatures and enriched $\delta^{13}C$ (spatially across synoptic sites). At the Ward Creek transect sites that were sampled more frequently, correlations between $\delta^{15}N$ and periphyton biomass were mostly not significant, not surprising given the low seasonal variation in $\delta^{15}N$.

-Authors concluded that the main N source to periphyton was synthetic fertilizer. Not clear how that was determined given overlapping $\delta^{15}N$ of various N sources (why was that specific source chosen?), and lack of sampling for other sources (ex. lake water).Wouldn't $\delta^{15}N$ of periphyton reflect the average value of all potential sources (groundwater, lake water, recycled N in biofilm, N-fixation)? Attributing relative contributions with mixing models that have overlapping sources generally results in estimates with a high degree of uncertainty.

Assessment of strength of conclusions given methodology and significance of results

<u>N sources in groundwater</u>

- A strength of the study was the high temporal frequency of sampling in Ward Creek and nearby groundwater transects, which revealed high seasonal and spatial variation in nitrate isotopic signatures. The seasonal and spatial variation in isotopic signatures of nitrate sampled from the groundwater transects exceed the variation in the snapshot samples collected at synoptic sites around the lake (though no statistical tests were done to assess significance). This suggests either that N sources to groundwater are highly localized and seasonally variable, and/or the N cycling within soils and groundwater alters the isotopic signature of nitrate. Regardless of the cause, however, this makes interpretation of snapshot sampling for groundwater nitrate isotopes challenging.

-The nitrate isotopic signature changed in Ward Creek during the ascending limb of the snowmelt hydrograph, towards more depleted $\delta^{15}N$, $\delta^{18}O$, and $\Delta^{17}O$, lending support to the interpretation that snowmelt-derived N is driving the nitrate isotopic signature ('snow ammonia';

not directly sampled). Nitrate isotopes in groundwater had more complex seasonal patterns that were harder to interpret.

-Ward Creek nitrate samples were isotopically distinct from nearby groundwater nitrate (based on visual examination of biplots, no statistics were used to assess significance of differences), suggesting alternate N sources or N processing is changing the signature within groundwater. Further justification is necessary to rule out other possible mechanisms

-The N source to Ward Creek nitrate was attributed to nitrification of snow ammonia. This determination was based on examination of the biplot of $\delta^{15}N$ and $\delta^{18}O$ for overlap with published isotopic ranges for snow ammonia and presumably on the basis of the changes in nitrate isotopic signature on the ascending limb of the snowmelt hydrograph. No mixing models were used to estimate proportional contribution.

- In this report, the following six potential N sources were considered: (1) snow ammonia, (2) atmospheric nitrate, (3) NO3 fertilizer, (4) NH4 fertilizer, (5) soil NH4, and (5) manure/septic. It was not explained in the report why the particular N sources (and their isotopic ranges) shown in the isotope biplots were chosen. A mixing model approach should consider more recent published ranges for endmembers and the possible benefits of sampling endmembers directly on a seasonal basis (e.g., Hundey et al. 2016, which looked at nitrate isotopes in snow, stream inflows, and lake water of mountain lakes in the Uinta Mountains of Utah used the following 4 source pools in a mixing model: (1) atmospherically oxidized nitrate (natural + anthropogenic), (2) NH4 +NO3 fertilizers and rain NH4 (isotopically indistinguishable), (3) soil nitrate, and (4) septic effluent/manure). Combining endmembers that are isotopically indistinguishable is a more conservative approach than trying to interpret mean mixing model estimates with high uncertainty.

-In the executive summary the authors attributed groundwater nitrate to 'nitrification of ammonia fertilizer'. This cannot be ruled out by the data, but neither is there definitive support for this source as the main contributor to groundwater N, since there are several other sources that have similar isotopic signatures (ex. nitrification of soil ammonia, snow ammonia), and these signatures can be highly altered by microbial processes within groundwater and soils. Since potential N sources were not sampled directly (ex. snowmelt, precipitation), the overlapping isotopic ranges of potential N sources preclude identifying contributions to groundwater nitrate samples with this degree of certainty.

<u>N sources for periphyton</u>

-we can rule out a dominant contribution of wastewater/manure/septic N sources to periphyton, since that would have resulted in more enriched $\delta^{15}N$ values than were observed (mean value ~ 0‰).

-the significant correlations between periphyton biomass and $\delta^{15}N$ and $\delta^{13}C$ (synoptic sites) were interesting, suggesting a decrease in fractionation effects against heavy C and N isotopes during periods of high growth/biomass accumulation.

-the authors attributed the $\delta^{15}N$ signature of periphyton samples to 'nitrification of ammonia from synthetic fertilizers', based on overlap between periphyton $\delta^{15}N$ values and published ranges in $\delta^{15}N$ of that N source. But $\delta^{15}N$ of periphyton also overlapped with the $\delta^{15}N$ of other sources (especially if you take into account fractionation during algal uptake). In order to justify this assertion, a discussion of potential non-groundwater N sources (periphyton should assimilate a mixture of available N sources and their signatures will reflect this) is necessary. Fractionation effects during N uptake by periphyton (which should cause depletion in $\delta^{15}N$ relative to N source) was not discussed but could influence periphyton $\delta^{15}N$. A mixing model or statistics should be used to quantify relative sourcing and uncertainty in those estimates. Overall low confidence in this conclusion.

-Determining attribution of periphyton N sources using only $\delta^{15}N$ in mixing models will be challenging because there are too many potential sources that overlap in $\delta^{15}N$. Besides groundwater, N sources to periphyton could include: lake water, localized excretion by invertebrates such as crayfish or Asian clam, internal recycling within algal mats, N-fixation by cyanobacteria/symbionts within algal mats.

- It was very interesting that the isotopic signature of lake periphyton near Ward Creek was so stable through time (given temporal variation in groundwater nitrate signatures seen at the transects or synoptic sites). Also interesting that spatial variation in periphyton $\delta^{15}N$ around the lake was also quite low. Is it because we have not measured the N sources used by periphyton? Is it because all these different N sources produce a mixture with relatively low variation in average $\delta^{15}N$?

-no correlations were presented between periphyton $\delta^{15}N$ and any predictors, such as groundwater N concentration, isotopic composition of groundwater nitrate, etc, so potential causes of spatial variation were not assessed.

Comment on the overall importance and defensibleness of the results? Were interpretations supported by findings?

-Overall, it was difficult to assess the validity of some of the statements/conclusions in the executive summary due to lack of clear explanations linking the results/figures to the authors' interpretations.

-It was difficult for a reader who is not an expert in N cycling or nitrate isotopes to understand how a given pool (ex. snow ammonia) or process (ex. nitrification) should affect the isotopic signature of groundwater nitrate. Addition of explanatory sentences would be helpful, for example: nitrification should result in depleted $\delta^{15}N$-nitrate, denitrification should result in enriched d15N-nitrate. Brief explanations of N processes would have been very helpful, or some kind of conceptual diagram linking N pools and processes to expected changes in isotopic signature of nitrate, for each of the measured isotopes ($\delta^{15}N$, $\delta^{18}O$, $\delta^{17}O$).

- A clear justification for the interpretation of the isotope data in this report is needed. Interpretation relies on comparison with published isotope ranges for sources (no source sampling was done), thus this is an influential decision upon which most of the inference rests. More justification for the choice of N sources should be provided.

- We can probably rule out either atmospheric nitrate (though not atmospheric ammonia from agricultural fertilizer use) or manure/septic as dominant sources to groundwater, but we cannot distinguish among the other sources (snow ammonia (is that just atmospheric deposition onto snow?), soil NH4, NH4 fertilizer).The substantial overlap in multiple N source signatures (for $\delta^{15}N$, $\delta^{18}O$), coupled with a lack of any mixing models or direct sampling of N sources, means the results provide only very coarse constraints in N sources to groundwater.

-given that groundwater DO concentrations were consistently low (mean = 0.5 – 1.8 mg/L), it is possible that denitrification may have altered isotopic signatures of the residual nitrate pool in groundwater. Could that explain why $\delta^{15}N$ of groundwater nitrate was more enriched than stream water nitrate?

-The authors' interpretation of the periphyton $\delta^{15}N$ results (e.g. that the signatures reflect fertilizer) do not seem to be supported conclusively by the evidence given the scale of variation observed. There are many N sources which were not measured (internal N cycling in biofilms, N fixation by cyanobacteria, excretion, lake water, etc). N-fixation in particular would yield $\delta^{15}N$-periphyton with very similar isotopic composition to what was observed (e.g., ~ 0 ‰; Helmer et al. 2024). Moreover, there should be isotopic fractionation of N as it is assimilated by algae. More work is needed here. A conceptual mass balance model that characterizes all nitrogen transformations and their relative effects on the N signature would be useful.

Synthesize the findings and their implications in the context of the recent published literature

The $\delta^{15}N$ of periphyton in this study was less enriched than periphyton in a more eutrophic Great Lakes study, but they found a similar positive relationship between chlorophyll-a concentration and $\delta^{15}N$ (Camilleri & Ozersky, 2019). Periphyton $\delta^{15}N$ in this study were also less $\delta^{15}N$ - enriched than periphyton/filamentous algae samples collected in Crescent Lake, WA (Cox et al. 2016). This suggests N sources to Tahoe's periphyton are different from the typical 'eutrophication' sources in many other lakes, such as wastewater or septic sources.

Appendix 4: Detailed assessment of Report 4: Characterization of algal community composition and structure from the nearshore environment, Lake Tahoe (United States)

Summarize findings

- In the enumerated soft-bodied algae samples, diatoms dominated counts and biovolume across depths and seasons. Counts of community samples (all from Sunnyside) dominated by diatoms, followed by filamentous and coccoid cyanobacteria, and then green algae. Diatoms also dominated biovolume in all samples. Note that total sample size was only 12 samples across depths, substrates, and sampling dates.
- Greater algal biomass with depth (based on cell counts x biovolume), no figures were included to show this pattern, so it was difficult to verify. Not clear what the actual pattern is (besides that 0.5 m samples had the lowest biomass).
- Rocks and sediment substrates support distinct diatom assemblages within close spatial proximity. The principal components analysis showed separation of rock and sediment diatom samples and the diatom species driving separation. No statistics were provided for significance of groupings. Only three diatom samples were collected from sediment, so the diatom community dataset is highly skewed towards rock samples
- Eulittoral diatom species on rocks are distinct from sublittoral diatom species on rocks. The principal components analysis showed separation of eulittoral and sublittoral diatom samples and the diatom species driving separation. No statistics were provided for significance of groupings.
- Eulittoral epilithic diatoms were dominated by stalked gomphoneid, fragilarioid species. Some seasonal variation in eulittoral species.
- Sublittoral diatoms dominated by *Epithemia* species (a low-N specialist).
- Sediment diatom samples were dominated by colonial fragilarioid chains. More dead diatoms present than in rock scrape samples.
- Sublittoral flora dominated by diatoms, not cyanobacteria, in contrast what was reported in previous Goldman studies.

Assessment of strength of conclusions given methodology and significance of results

- Study is a big step forward for ability to compare diatom species/biodiversity at Tahoe in future studies.
- A limitation of this study is the spatial/seasonal scope of sampling (only west shore, most samples from Sunnyside), and lack of consistency in sampling (e.g., collecting from same depths, same substrates, on similar dates). No samples were taken during ‘peak’ biomass period (Mar-April). For taxa other than diatoms, all samples were taken from Sunnyside,

so we have a limited understanding of spatial variation in periphyton community- we don't know if those patterns apply to different areas of Tahoe's nearshore.

- Overall, seasonal patterns in diatom assemblages and the soft-bodied algal community were not clear due to inconsistent seasonal sampling, low samples size, and lack of statistical tests to support seasonal differences (descriptive text only).
- Some support for differences in the rock versus sediment diatom community due to the large compositional differences, though no statistics were used to assess significance, and total sample size is highly skewed towards rock scrape samples (16 of 19 total samples).
- Differences in the eulittoral versus sublittoral diatom community (on rocks) are somewhat apparent, but the significance is unclear because of substantial variation across seasons (for eulittoral diatoms). No statistics were used to assess significance of groupings, and sample sizes for the groups shown in the PCA are very small (ranging from 2 to 8 samples per group)
- Seasonal variation in sediment diatom assemblage is unknown since they were collected less often.

Comment on the overall importance and defensibleness of the results? Were interpretations supported by findings?

The community composition of Tahoe's periphyton assemblage is valuable information to better understand changes in algal biomass and productivity. The development of a diatom voucher flora will allow better reconciliation of diatom species lists/biodiversity metrics in future studies. This study demonstrates that there are likely differences in diatom community composition (as well as other taxa) across depths and substrates in Lake Tahoe. However, since all sampling was done on the west shore, and primarily at a single location (Sunnyside), it is not currently known how much variation in periphyton exists in different shoreline areas, especially where substrates may be different (ex., large boulders, macrophytes). This study is an important step in the right direction, but also highlights the current limitations of existing understanding regarding Tahoe's nearshore algal taxonomy.

The diatom community composition results are interesting, especially the variation across depth and between substrates, but the results are limited in scope regarding spatial variability around the lake (mostly reflecting a single west shore site, Sunnyside), and also regarding seasonal variation. Though no statistical tests were used to assess the significance of species groups across substrates, depths, or seasons, it is also likely that the samples sizes were too low to test for differences across so many variables. Sampling across seasons was not consistent enough to robustly assess seasonal variation in community composition. It seems likely that the community differences between substrates and across depths is real and would be verified with additional sampling. It is less clear how robust the seasonal differences shown here are, given the low sample size and potential for inter-annual variation to mediate seasonal patterns.

The community composition analysis for soft-bodied taxa (diatoms, cyanobacteria, green algae) is equally important as the diatom analysis, but even more limited in spatial scale (one site;

Sunnyside) and seasonal sampling. Given observations of filamentous green algae around the lake in recent years that may be highly patchy, additional work focused on more non-diatom taxa would be valuable.

Synthesize the findings and their implications in the context of the recent published literature

Comparison to earlier work in Lake Tahoe was done in the report, but comparisons were difficult to assess due to methodological differences, so not much inference was possible concerning community composition change from two snapshots across decades. There was some consistency with the taxonomic patterns found in Atkins et al. (2021) , for example a high prevalence of stalked diatoms in eulittoral areas, though Noble et al. found higher dominance of diatoms than found in other work (related to methods? Or to limited spatial scope of this study?). Other recent studies (ex. Report 5, Smits et al.) did not quantitatively enumerate periphyton samples (qualitative assessments of community composition, not relative abundance), so it is hard to directly compare across reports, though some common taxa were identified across studies.

This study found a lower abundance of green algae relative to diatoms or cyanobacteria than found by earlier Goldman studies (but again, limited comparability due to different methods and limited spatial scale of sampling). If this represents a real change, it would be highly interesting. However, given occurrences of filamentous green algae (both as metaphyton and periphyton) that have been documented by monitoring in recent years (Report 5), it seems premature to designate green algae as less important than diatoms, though they are clearly not the dominant periphyton taxa at the Sunnyside location.

Does the *Epithemia* diatom assemblage in sublittoral zones (which have N-fixing symbionts) indicate an N-limited periphyton community? This would support findings in Report 2 (Chandra et al. suggesting some N-limitation). Compare to taxonomy data in Report 5 (Smits et al.)? All periphyton samples in Smits collected from eulittoral zone (0-2 m).