EXPERT REPORT

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Prepared for Cottonwood Environmental Law Center

In the case of *Cottonwood Envt'l. L. Ctr. v. Yellowstone Mountain Club*
2:23-cv-00026-BMM

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This report summarizes the nitrogen and carbon isotope analyses conducted for Cottonwood Environmental Law Center ("Cottonwood") in the South Fork/West Fork of the Gallatin River. The overarching concluding opinion is that this system is highly enriched with nitrogen from wastewater. **The Yellowstone Club's draining of treated sewage from its golf course water hazard into the South Fork West Fork of the Gallatin River and spraying treated sewage out of its snow guns into Second Yellow Mule and ultimately South Fork West Fork is causing irreparable harm to the aquatic ecosystem by further degrading the already water-quality impaired waterbody.**

1.0 Introduction

For more than a decade, concerns over the increases in algal growth and nitrogen pollution and the relationship between resort development have been expressed in the West Fork region of the Gallatin River. Since the 1970s, in addition to residential development, there has been development of three new ski resorts and golf courses in Big Sky, Montana. As documented by Gardner (2010, Gardner et al. 2011), the public wastewater and sewer receives secondary treated water that is retained in lined sewer retention ponds and stored until midspring when it its released as irrigation water onto the three golf courses in Big Sky.

Gardner et al. (2011) showed the relationship between residential development and annual average nitrate (NO_3^-) concentrations (Fig. 1):

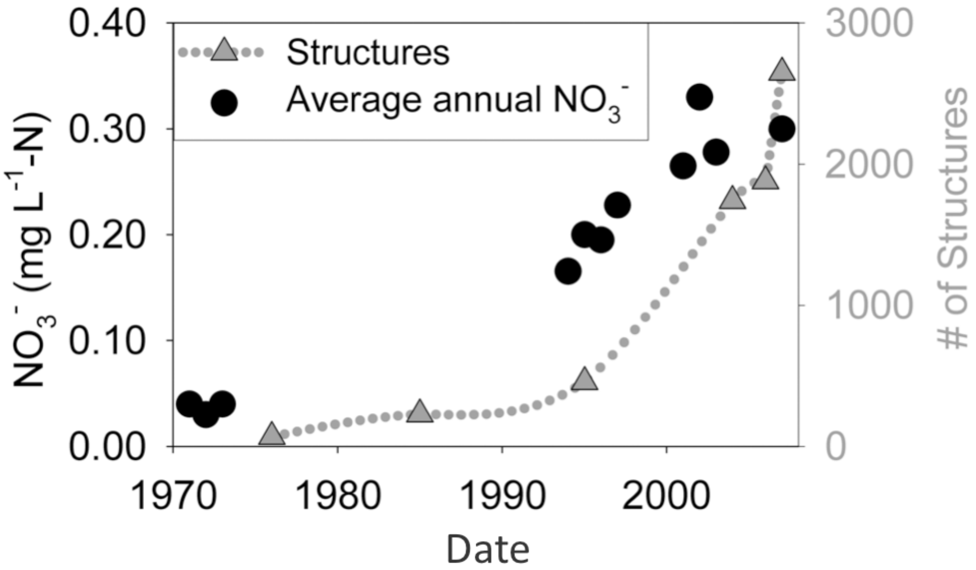

Figure 1. In the West Fork watershed, residential development and annual average stream water NO_3^- concentrations have followed a similar upward trend since resort development. [NSF, 1976; Blue Water Task Force, and Big Sky Water and Sewer District, unpublished data]. Reproduced from Gardner et al. (2011).

The South Fork West Fork Gallatin River is included on the Montana Department of Environmental Quality's list of impaired waters due to high nitrogen concentrations as well as other factors (DEQ Montana 2020). Higher exports of nitrogen (N) as nitrate +nitrite (NO_3^- +NO_2^-) and as organic forms of nitrogen have been observed in the developed regions of the West Fork watershed compared with undeveloped watersheds (Gardner (2010)). As an impaired water body, the nutrient loads to the West Fork are to be kept below threshold levels set by the DEQ of 0.3 mg N/l as total nitrogen (ΣN) and 0.03 mg/l as total phosphorus (ΣP) from July through September (Allen and Howell 2020). Exceedances of these levels have been previously associated with application of treated municipal water to the Yellowstone Club golf course.

Determination of nitrogen sources (e.g., wastewater treatment, septic systems, chemical fertilizers) is difficult with conventional water quality measurements. Stable isotopes provide sensitive indicators that can be used to distinguish between chemically synthesized agricultural fertilizers and human wastes.

Two types of samples were analyzed here. All samples were collected in the Yellowstone Club region by Cottonwood and its contractors. First, water samples were collected in August 2021 and September 2022 to determine ambient nitrogen concentrations. Second, algal samples were collected on September 19, 2023, to determine whether a signal related to nitrogen pollution could be detected in benthic algae because of activities related to the Yellowstone Club. The change in nitrogen and carbon isotope content of collected algal samples was used as the analytical technique.

2.0 Sampling

2.1. Water sampling for nutrient analyses

Cottonwood collected water samples in August 2021 and September 2022 in the South Fork/West Fork of the Gallatin River. Exhibit 1; Exhibit 2. The samples were analyzed for nutrient concentrations by Bridger Analytical Lab.

The August 2021 analysis included samples from 1) an unnamed tributary that appears to start on the Yellowstone Club golf course; 2) a sample from above the unnamed tributary that appears to start on the Yellowstone Club golf course; 3) A sample from above Second Yellow Creek. Cottonwood was unaware of spraying above Second Yellow Mule Creek when it collected samples in 2021 and did not collect a sample from Second Yellow Mule Creek.

The September 2022 analysis included samples from 1) the unnamed tributary that appears to start on the golf course: 2) a sample from above the unnamed tributary that appears to start on the Yellowstone Club golf course; 3) a sample from Second Yellow Mule Creek; 4) a sample from upstream of Second Yellow Mule Creek.

Photographic evidence suggests the Yellowstone Club is maintaining a water hazard on its golf course that is draining into the South Fork/West Fork of the Gallatin River (Figs. 2-4). Lab results (Section 7.0 below) suggest the Yellowstone Club is using the water hazard to dispose of treated sewage.

Figure 2. The top left portion of the photo above shows a Yellowstone Club water hazard draining towards the South Fork/West Fork of the Gallatin River.

Figure. 3. The confluence of the unnamed tributary that appears to be fed by the water hazard on the Yellowstone Club's golf course (left) and the South Fork/West Fork of the Gallatin River (right).

Figure 4. Spatial relationship of Yellowstone Golf Course, discharges, and sampling areas.

3.0 Isotope analysis preparations

Samples of benthic algae (*Cladophora*) were collected by a contractor for Cottonwood from below the confluence of Second Yellow Mule and the unnamed tributary that appears to begin on the golf course in 2023. These samples were accompanied with too much water which rendered them usable. They were not analyzed. A second set of benthic algae (*Cladophora*) samples were collected by a contractor for Cottonwood (Hank Healey) on September 19, 2023, at two site locations geographically located within the South Fork/West Fork of Gallatin River. The samples were taken at the "No Trespassing" sign that was posted by the Yellowstone Club after the first set of samples were collected. Healey did not go past the No Trespassing sign because a Cottonwood employee and a contractor were arrested in April 2023 for criminal trespass after the contractor collected water samples at the two locations in 2022.

Benthic algae samples were hand collected, placed in Ziploc bags, and shipped to the University of Maryland Center for Environmental Science laboratory overnight. Samples were cooled with a "blue ice" pack. Once samples were received at the receiving office, the package was immediately retrieved, unpacked, and refrigerated. Samples were identified as "1" and "2" with no further identifying markings. Within 48 hours, samples were removed from the refrigerator and dried in a laboratory drying oven. This drying step took 2-3 days.

Dried samples were transferred to a desiccator, and within 48 hours, subsamples of the algal material were transferred to tin capsules required for analysis. Each sample provided by Cottonwood gave enough material to subsample 2-4 aliquots or replicates of each sample for analysis. Once all subsamples were prepared for analysis, they were shipped to the University of California Davis Stable Isotope Facility for analysis. It is of note that UC Davis does not accept any samples for analysis until they confirm that the samples have been properly prepared.

4.0 Data reporting and analysis

All isotope samples were analyzed using an Elementar vario MICRO cube elemental analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany) interfaced to a Sercon Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, United Kingdom).

Each sample was simultaneously analyzed for carbon (C) and nitrogen (N) total mass and for its isotopic composition. The amount of mass of each sample was originally based on the ideal mass range for sample detection. As long as the amount of mass of material is within range of instrument detection, the absolute amount of mass does not affect the isotopic analysis.

Nitrogen isotopic composition (see background below) is reported using the convention delta notation:

$\delta^{13}C_{sample}$ or $\delta^{15}N_{sample}$ $[(R_{sample}/R_{standard}-1)]$ where R (ratio) = $^{13}C/^{12}C$ or $^{15}N/^{14}N$

$\delta^{13}C_{sample}$ or $\delta^{15}N_{sample}$ are expressed as a per mil deviation ($^{o}/_{oo}$) from international standards. The $R_{standard}$ for $^{13}C/^{12}C$ is international V-PDB (Vienna PeeDee Belemnite) and the $R_{standard}$ for $^{15}N/^{14}N$ is air. Most studies report δ * 1000 to amplify the small differences between samples and standards (e.g., Fry 2006). The unit (parts per thousand, ‰, per mil) may be implied rather than directly stated.

External and internal standards are run with each batch of samples by the UC Davis laboratory. During the isotopic analysis, the isotope laboratory used different certified reference materials for analytical control quality. Analytical uncertainties are given in Table 1:

Table 1.

	$\delta^{13}C$	$\delta^{15}N$
Mean standard deviation reference materials replicates in this project	+/- 0.15 $^{o}/_{oo}$	+/- 0.07 $^{o}/_{oo}$
Mean absolute accuracy for calibrated reference materials	+/- 0.07 $^{o}/_{oo}$	+/- 0.06 $^{o}/_{oo}$

5.0 Background

5.1 Effects and harms caused by *Cladophora*

Freshwater *Cladophora* outbreaks are among the most notorious harmful macroalgal blooms worldwide (Lapointe et al. 2018 and references therein). *Cladophora* is a genus of filamentous (hair-like) green macroalgae (recognizable to the human eye; Lapointe et al. 2018). The many species of *Cladophora* are commonly found attached to rocks or other hard surfaces in rivers, streams, and shallow lakes but can also be found as floating mats (Fig. 5). Although abundant in both fresh and marine waters, these algae are notorious noxious responders to sewage in freshwaters (Stevenson et al. 2012, Zulkifly et al. 2013). They are considered nuisance algae due to their increasing prevalence in many freshwaters and subsequent environmental effects. *Cladophora* is widely considered as the most important filamentous macroalgal genus of inland waters and the most abundant in alkaline streams throughout the world (Burkholder 2009). It is highly stimulated by phosphorus and ammonia pollution (Burkholder et al. 2020 and references therein). *Cladophora* has high nutrient optima (Leland and Porter 2000). Its relatively large cells can consume and store substantial phosphorus and nitrogen at 'luxury" levels, i.e., levels above their metabolic needs (Young 2010, Lohman and Priscu 1992).

Figure 5. Massive bloom of the noxious benthic macroalga, *Cladophora*, in the Gallatin River, MT, August 2020. From https://www.uppermissouriwaterkeeper.org/summer-2020-neon-green-algal-bloom-on-the-gallatin-river/

Excessive growth and biomass accumulation of *Cladophora* are considered a classic symptoms of **cultural eutrophication** (Dodds and Gudder 1992 and references therein). *Cladophora* spp. are considered nutrient opportunists that grow profusely when there is sufficient bedrock or substrate for attachment, optimal temperatures (considered in the range of 55-63ºF) and sufficient light and nutrients (Pitcairn and Hawkes 1973, Dodds and Gudder 1992, Lohman and Priscu 1992) (Table 1). They have rapid growth rates and proliferate because they can more quickly take advantage of the elevated nutrient levels and shade out other species. *Cladophora* is also able to withstand the shear stress of flowing waters (Bellis and McLarty 1967, Whitton 1970).

Cladophora provides habitat for the growth of enteric bacteria such as *Escherichia coli*, enterococci, *Shigella*, *Campylobacter*, and *Salmonella* in partially treated sewage. For example, in a two-year study of the Little Calumet River (Chicago - tributary of Lake Michigan), shiga toxin-producing *E. coli* and *Shigella* were detected in 100% and 25% of *Cladophora* samples, respectively, as well as *Campylobacter* in 60% and 100%, and *Salmonella* in 40% and 80%, of lake and ditch samples, respectively (Ishili et al. 2012). These bacteria have been routinely documented in *Cladophora* mats and may detach from the algae and infect surrounding waters (Ishii et al. 2006, Englebert et al. 2008, Beckinghausen et al. 2014, Verhougstaete et al. 2020). These pathogenic microbes are potential human health hazards. They are able to grow on the carbon and nutrients associated with the mats, and the biofilms that develop. When blooms die, the rotting algal material laden with fecal bacteria can accumulate in quiet backwater areas and along river shores and can threaten the health safety of recreationists. Stream banks and lake shorelines in various regions have been fouled by rotting *Cladophora* (e.g., Garrison and Greb 2005, Higgins et al. 2008). The sequestered fecal bacteria in these rotting *Cladophora* mats have led to warnings for recreationists and declines in property values (Lapointe et al. 2018).

Cladophora commonly clogs drainage canals, smother beneficial benthic stream animals, and cause other problems due to their sheer biomass accumulation (e.g., Bootsma et al. 2004). Their growth displaces beneficial aquatic plants and causes a reduction in species biodiversity. When blooms begin to die, the rotting biomass accumulates along shores and banks, impairing aesthetics and creating toxic hydrogen sulfide odors for recreationists.

As the *Cladophora* mats decay, oxygen is quickly consumed. Decaying mats are associated with kills of other aquatic life when oxygen is consumed (Burkholder et al. 2009). Decaying mats also lead to a further change in the microbial community, which transitions from an aerobic one to an anaerobic one. One such bacterium is *Clostridium botulinum*, the causal agent of botulism in humans, birds, and other wildlife. Thus, decaying mats of *Cladophora* have been associated with avian botulism and bird kills. Sulfate-reducing bacteria also proliferate. Subsequently, fermentation products, such as organic acids, sulfide compounds, and alcohols are produced in the oxygen–deprived algae (Peller et al. 2014).

Cladophora blooms often co-occur with abundant toxic cyanobacteria, as documented in various rivers and lakes (Bergey et al. 2010, Young et al. 2010, Lapointe et al. 2018, Bouma-Gregson et al. 2019). Recent detection of *Microcystis* and its microcystin toxins, in the Custer Gallatin National Forest and surrounding waters (https://bozemanmagazine.com/news/2023/07/31/118093-detection-of-harmful-algal-blooms-at-hyalite) raises the further issue of relationships between *Microcystis* and these macroalgae. *Microcystis* is a cyanobacterium that is promoted by eutrophication.

Cladophora itself is poorly grazed except when the filaments are small in early growth phase (Burkholder 2009). It is generally considered a poor, non-preferred food source, and its abundant growth shifts the food web to small grazers. Joniver et al. (2021) showed that macroalgal blooms have a negative influence on fish and molluscs. *Cladophora* provides micro-habitats for epiphytic smaller algae and small animals such as larval stages of macroinvertebrates. Epiphyte communities on *Cladophora* can vary seasonally and spatially. Variation in this epiphyte algal/macroinvertebrate community and density can affect fitness and survival of those that feed on them, i.e., via differences in the quality and quantity of food (Gresens 1997, Hessen et al. 2002). Thus, grazer–epiphyte interactions in rivers may have strong ecological consequences for foodweb dynamics and biogeochemical processes at reach and watershed scales (Furey et al. 2012). As summarized by Vadeboncoeur and Power (2017), the food web that emerges on submerged rocks that are colonized by diatoms, thick mats of benthic diatoms, and green macroalgal assemblages heavily colonized by epiphytes varies greatly. Overall, the food quality of *Cladophora* and its epiphytes tends to become lower over the growing period. In the Great Lakes, sport fishes such as walleye and yellow perch have been threatened by *Cladophora* blooms (Lapointe et al. 2018).

The massive *Cladophora* blooms fueled by nutrient pollution in Montana streams such as the South Fork West Fork of the Gallatin River are adversely impacting trout and other salmonids in more obvious ways as well, based on available research on streams and rivers in various regions: *Cladophora* is known to degrade the habitat needed for trout reproduction, especially the substrata (clear gravel/rock conditions) where eggs are deposited (Dorr et al. 1981). The filamentous algal overgrowth smothers affected areas and can restrict dissolved oxygen that is critically needed by the eggs and by beneficial macroinvertebrate animals used as preferred food by the fish.

5.2 Use of isotopic composition as a tool to trace sources of nitrogen and other elements

The use of isotopic composition of nitrogen and carbon to trace the source and fate of these elements in aquatic systems has a long history. Stable isotopes have been previously applied to assess sewage contributions to *Cladophora* growth in the Gallatin River. Gardner (2010).

The fundamental concept of isotope application begins with the molecular weight of each element. The Periodic Table tells us that the molecular weight of carbon is 12.011 and that of nitrogen is 14.07. However, these elements also have isotopes that are atoms with the same chemical properties but which differ in mass. Stable isotopes are those that do not emit radiation. Carbon has an isotope with a molecular weight of 13, and nitrogen has an isotope with a molecular weight of 15. These are natural forms of these elements, but which occur in very tiny amounts of the elements. Isotopes are specified by the name of the element (e.g., C or N), with a superscript indicating their weight. Thus, "normal" carbon is ^{12}C (its atomic weight is 12), but its stable isotope is ^{13}C. For nitrogen, its "normal" isotope is ^{14}N, but its stable isotope is ^{15}N. Isotopes with a higher molecular weight are referred to as "heavy". Heavy carbon, ^{13}C, makes up about 1.1% of all natural carbon. Heavy nitrogen, ^{15}N, makes up about 0.36% of natural nitrogen.

The formation and behavior of isotopes is well known and these principles are used in interpreting differences between sites or between samples taken at different times. The most basic concept is that in any chemical or biological reaction, the tendency is for the "lighter" isotope, that is ^{12}C or ^{14}N, to move through the reaction faster. Thus, in any biological or chemical reaction, if both isotopes of the same element are present (and different isotopes are always present), the lighter isotope will react faster, leaving the heavier isotope behind. With multiple cycles of such a reaction, the product will become lighter with respect to its isotopic composition and the residual left behind will become heavier over time (Fig. 6)

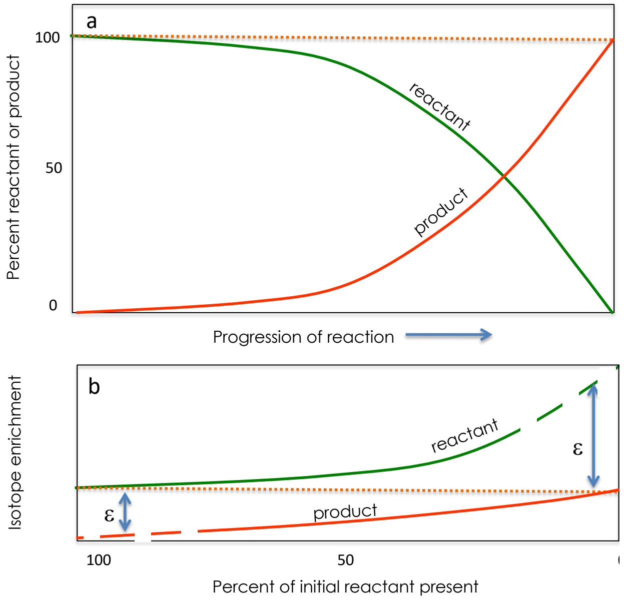

Figure 6. Relationship between isotope fractionation of reactant and product and their consumption, Rayleigh distillation kinetics. The term ε denotes the difference in isotope enrichment between reactant and product. Note that at the initiation of the reaction and near completion of the reaction this value is difficult to determine as there is either virtually no product at the start and if the reaction has gone to completion, no reactant at the end. In the figure, the ends of these curves have dashed lines. Reproduced from Glibert et al. (2019).

An important aspect in interpreting all such isotopic changes over time is that there must be sufficient "reactant" in the system to be reacted upon. Here, the "reactant" is the dissolved nitrogen or carbon in the water. If there is no reactant there can be no chemical or biological reaction, and if there is no reaction, there can be no isotopic change. Also, if a reaction has gone to completion (all reactant has been used up), the isotopic composition of the product will match that of the original reactants.

Differences in δ values between two substances are expressed with an uppercase delta, Δ. Thus:

$$\Delta_{A-B} = \delta_A - \delta_B$$

Differences in δ, or Δ, for example $\Delta\delta^{15}$Nor $\Delta\delta^{13}$C, may be between reactant and product, food source and consumer, or any other comparison between a measured value and a baseline, however that is defined. Values of Δ may reflect changes in isotope ratios associated with isolated processes or net effects of multiple factors influencing differences in isotope values between any two pools of interest. Due to the sensitivity of analyses (see above), very small differences in reactant and products can be determined.

6.0 Nitrogen reactions

Nitrogen exists in aquatic systems in many forms and these forms are transformed from one to another by bacterial-mediated reactions or by uptake of nitrogen by aquatic plants (micro- or macroscopic), or by other chemical reactions (Fig. 7).

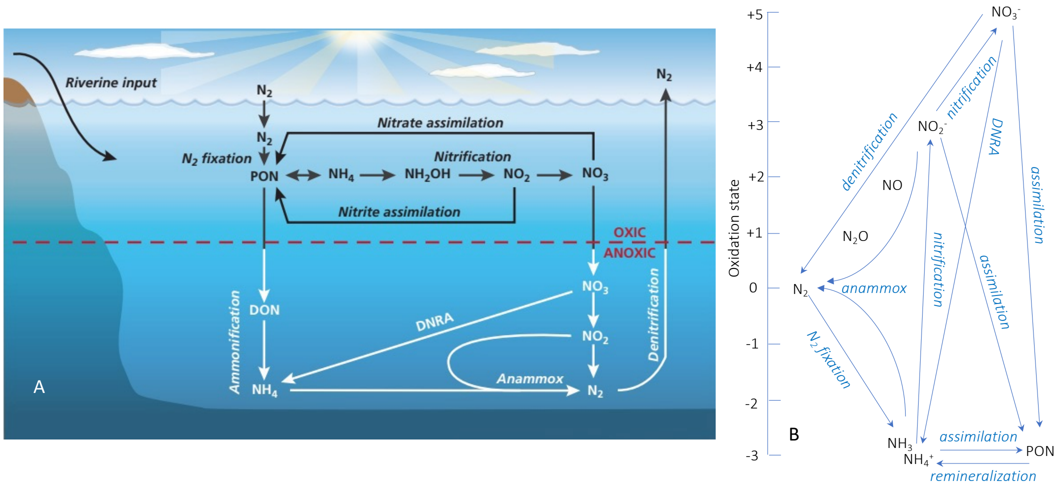

Figure 7. Panel A- The nitrogen cycle, depicting where in the water column the dominant processes occur. Panel B- the processes of the nitrogen cycle and chemical forms of nitrogen relative to their oxidation state. Images modified based on Arrigo (2005) and Hutchins et al. (2009).

One of the important reactions is denitrification, defined as the process by which nitrogen in the form of nitrate (NO_3^-) is converted to atmospheric nitrogen, N_2. The overall reaction is:

$$4HNO_3^- + 5\ CH_2O \longrightarrow 5CO_2 + 7H_2O + 2N_2\uparrow$$

in which CH_2O represents organic matter. Denitrification is actually a summed series of reactions (Fig. 7), each of which is involves different enzymes and different organisms and different degree of fractionation,

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2\uparrow$$

The end product of denitrification is release of N_2, a harmless gas to the atmosphere (indicated by the up arrow in the equations above). It is considered a favorable reaction to rid a system of excess nitrogen. It is therefore a reaction that is carried out in sewage treatment plants, and it is also carried out naturally when there is available NO_3^- and associated bacteria. As can be seen from the equations above, the conversion of NO_3^- to N_2 is a multi-step reaction. The steps in this reaction each favor the lighter isotope–and thus the reactant (the nitrogen pool left behind) becomes heavier over time. Denitrification is a process with strong isotopic discrimination, and the NO_3 in the dissolved pool can become substantially enriched with ^{15}N. Isotope discrimination factors are on the order of 20-30 $^{o}/_{oo}$ (Cline and Kaplan 1975, Altabet et al. 1999, Voss et al. 2001). Denitrification depends on availability of NO_3^- and increases under conditions of low oxygen.

Another process that can contribute to isotopic fractionation of nitrogen is ammonia (NH_3) volatilization. This process occurs when the concentration of ammonia in water is high. Again, the lighter isotope moves through the reaction faster, leaving behind NH_3 that would be proportionately heavier. This process has been well studied in soils (where NH_3 is applied as a fertilizer), and also in hot springs, where it is shown that factors such as temperature and pH play important roles in the extent of volatilization and fractionation. As pH increases, so does volatilization. In waste stabilization ponds, ammonia volatilization can be a major removal process, especially in warm periods of the year. Volatilization may increase in spray irrigation.

Nitrification of NH_3 to NO_2^- and then to NO_3^- (Fig. 7B) in oxygenated surface waters is another process that can fractionate nitrogen and leave a residual ammonia pool which would be highly enriched in ^{15}N. Moving downstream, as this NH_3 is further transformed, the remaining pool decreases in concentration and increases in ^{15}N content. Thus, over time and distance, the available nitrogen pool for biological uptake differs in isotopic composition; it gets heavier. In this study (see results below), these different processes in the nitrogen cycle cannot be distinguished but they clearly show that discrimination did occur.

Both nitrate (NO_3^-) and ammonia (NH_3) are important nitrogen sources for primary producers, that is, algae and aquatic plants. Just as fertilizer nitrogen is used to grow agricultural crops, aquatic primary producers also use nitrogen for their metabolism and growth. Nitrogen is a building block of protein and without protein, a cell–any cell–cannot carry out metabolism and ultimately cannot survive. When more nitrogen is available (along with other required elements), growth is faster, and biomass can accumulate. When NO_3^- or NH_3 is taken up by the microscopic or macroscopic algae, its nitrogen isotopic composition reflects its source. The process of uptake of nitrogen by the plant also fractionates nitrogen, but fractionation by macroalgae is slight (0.2 – 1.4 $^{o}/_{oo}$; Umezawa et al. 2002, Lapointe et al. 2018). It is generally thought that the uptake of NO_3^- leads to more discrimination than the uptake of NH_3 due to their different transport mechanisms (Evans 2001).

Benthic algae (those that are attached to bottom materials like rocks or shells) are ideal for tracing the changes in isotopic composition (and therefore nitrogen processes) in space or time in aquatic systems. They sit and incorporate the dissolved nitrogen from their environment, and therefore integrate and reflect any changes that occur in that nitrogen (Lapointe et al. 2005, 2018). Thus, if nitrate changes in isotopic composition as it flows from upriver to downstream, and as bacteria denitrify this nitrate, or as ammonia volatilization occurs, the isotopic composition of the nitrogen available to be used changes. The difference in the resulting isotopic composition of the benthic algae informs us that nitrogen processing occurred. As a reminder, such a change only occurs if there is enough reactant or substrate (NO_3^- or NH_3) in the water to undergo such reactions. If there is no substrate, there can be no isotopic change.

Natural abundance stable isotope ratios are widely used to help identify and track biogeochemical sources in the environment (Kendall 1998; Kendall et al. 2008). Stable isotopes are frequently used to track anthropogenic nitrogen in aquatic systems (e.g., Owens 1987; Tucker et al. 1999; Costanzo et al. 2001; Lapointe et al. 2011; Loomer et al. 2014). In particular, increases in $\delta^{15}N$ (relative to a defined baseline or reference site) are often associated with contributions of sewage-derived N (Kendall 1998). Different sources of inorganic nutrients or organic matter often have distinct isotopic signatures, and various biological and/or physical processes alter isotope ratios in expected ways (Kendall et al. 2008; Fig. 8). Fertilizer has a $\delta^{15}N$ around zero, as it is formed using a process that fixed atmospheric nitrogen into ammonia. Atmospheric nitrogen has a $\delta^{15}N$ of zero. The $\delta^{15}N$

of NO_3^- can distinguish a wastewater signal from other sources of nitrogen, including precipitation, fertilizer, and mineral weathering (Kaushal et al. 2006).

The $\delta^{15}N$ of inorganic N derived from manure or sewage is often enriched (>10 $^{o}/_{oo}$) due to isotopic fractionation that occurs at either the sewage treatment facility or downstream thereof. Human septic waste has a $\delta^{15}N$ value around 4-5 (Kreitler 1975). The $\delta^{15}N$ values of N in sewage vary with amount of processing at the facility; processes such as NH_3 volatilization and denitrification drive the $\delta^{15}N$ values of the residual DIN up during treatment and/or processing within the environment. This, in turn, imparts a ^{15}N-enriched signal to primary producers that take up the sewage-derived N (McClelland et al. 1997, McClelland and Valiela 1998, Lapointe et al. 2005). In one classic example, Savage and Elmgren (2004) used $\delta^{15}N$ values in benthic macroalgae to track sewage-derived N in an embayment of the Baltic Sea and quantify effects of reductions in N inputs following implementation of tertiary sewage treatment. They sampled the algae along a 36 km transect and documented a gradient of elevated $\delta^{15}N$ that extended from peak values near the sewage outfall to ~25 km downstream of the outfall. Studies of the isotopic signatures of macroalgae in Florida have been used to distinguish agricultural nitrogen sources from those of sewage (Lapointe and Bedford 2007, Lapointe et al. 2015) and sewage pollution in macroalgae was traced using isotopes in Negril, Jamaica (Lapointe et al. 2011). A variation of this approach for N source tracking is the deployment of specific organisms for a set length of time over which the isotopic signature of their biomass will change, reflecting the local environment. Costanzo et al. (2001) deployed macroalgae in porous containers for several days, during which time their biomass incorporated the $\delta^{15}N$signature of dissolved N and were thus able to map a sewage plume in Moreton Bay, Australia. Fertig et al. (2009) were able to identify human and animal waste signatures based on $\delta^{15}N$ in macroalgae in coastal lagoons in Maryland. Their data were interpreted in conjunction with land use data, and indeed the macroalgal $\delta^{15}N$ signal was highest in residentially developed areas.

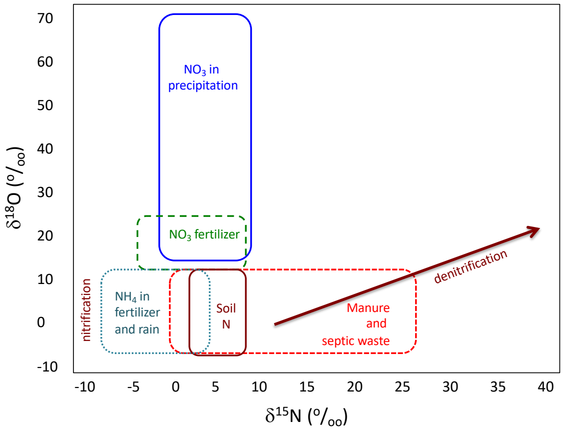

Figure 8. Typical $\delta^{18}O$-NO_3 and $\delta^{15}N$-NO_3 ranges for nitrate sources and the processes that alter these values. Modified and redrawn from Kendall (1998; Kendall et al. 2008).

Changes in carbon isotopic composition are more complicated than those of nitrogen. Most of the variability on algal $\delta^{13}C$ is due to changes in the concentrations of CO_2 in the water. CO_2 is fixed into biomass during photosynthesis and the enzymes involved discriminate against ^{13}C, but the degree to which this happens depends on availability of CO_2. These concentrations, in turn are affected by temperature, pH and the productivity of the water (Finlay 2004). In a study of a wide range of macroalgae from the Gulf of California (which used the same isotope analysis facility as used herein), values lower than -30°/₀₀ denoted uptake of CO_2 by diffusion, as opposed to uptake of carbon as HCO_3^- (Velázquez-Ochoa et al. 2022). Notable is the fact that the macroalga *Cladophora* takes up CO_2 via diffusion. Studies that have reported $\delta^{13}C$ discrimination by benthic algae also have reported that light availability also causes some discrimination (MacLeod and Barton 1998). Hill et al. (2008) reported that light effects depended also to some degree on the phosphorus content of the water. Where both light and phosphorus levels were relatively high, the highest $\delta^{13}C$values were found. In contrast, when phosphorus was somewhat lower even with available light, the lowest $\delta^{13}C$ values were observed. Algal cells growing in thick stands are likely to experience more CO_2 depletion and therefore may have a more positive $\delta^{13}C$, which those in thinner stands are likely to have more negative $\delta^{13}C$ values (Fig. 9).

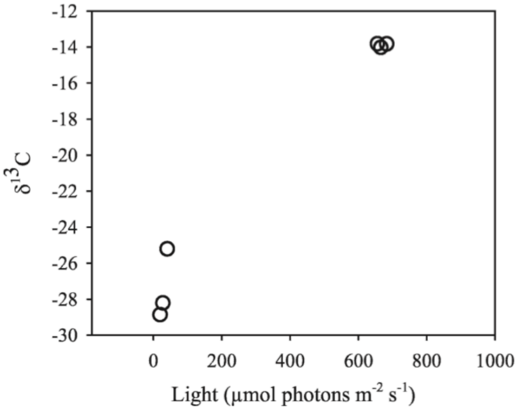

Figure 9. Algal $\delta^{13}C$ vs light in the study site reported by Hill et al. (2008).

7.0 Results

7.1 Nutrient analyses

Samples analyzed for ambient nitrogen showed higher concentrations of both NO_3+NO_2 and ΣN from the golf course tributary than from the sample above the golf course tributary. (Table 2). Concentrations impacted by the Golf Course were 15–25-fold higher than concentrations upstream of the golf course tributary. Concentrations in Yellow Mule Creek and above second Yellow Mule Creek were 2–3-fold higher than upstream of the golf course tributary.

Table 2.

Concentrations of nitrogen from 2021 water samples.

	Nitrate+nitrite	Total nitrogen
Golf course tributary 45.23901, -111.38288	0.974 mg/L	0.974 mg/L
Upstream of golf course tributary 45.24095, -111.38833	0.106 mg/L	0.106 mg/L
Above Second Yellow Creek	0.124 mg/L	0.124 mg/L

Concentrations of nitrogen from 2022 water samples.

	Nitrate+nitrite	Total nitrogen
Golf course tributary 45.23900, -111.38286	1.28 mg/L	1.87 mg/L
Upstream of golf course tributary 45.23909, -111.38295	0.073 mg/L	0.073 mg/L
Second Yellow Mule Creek 45.23839, -111.37542	0.217 mg/L	0.217 mg/L
Above Second Yellow Mule	0.137 mg/L	0.137 mg/L

7.2. Isotope enrichments

Results from the UC Davis laboratory are included at the end of this report. The laboratory completed analyses for *Cladophora* collected from the Spanish Peaks Mountain Club and the Yellowstone Club reported the results together. The results of the *Cladophora* analysis from the Yellowstone Club's discharges into the South Fork West Fork are identified as samples 3 a-d and 4 a-b in the attached report.

The subsamples of each of the two samples had excellent replication of both ^{15}N and ^{13}C isotopic composition (Table 3).

Table 3. Mean and standard deviation of isotope analyses.

Site number	Mean δ^{13}C ($^{o}/_{oo}$)	Standard deviation δ^{13}C	Mean δ^{15}N ($^{o}/_{oo}$)	Standard deviation δ^{15}N	No. of replicates
Above golf course	-40.80	2.00	3.49	0.33	4
Golf course	-14.50	0.31	7.78	0.12	2

Values of $\delta^{15}N$ doubled from above the golf course to the stream within the golf course. Such trends would be consistent with in-water nitrogen processing via denitrification or volatilization. Such trends would also require sufficient nitrogen (as NO_3^- or NH_3) in the water column for such discrimination effects to be observed. The golf course values are not consistent with fertilizer nitrogen as the source. Differences in $\delta^{13}C$ were also substantial, possibly reflecting a change in the light regime as described above.

7.3 Nutrient concentrations in relation in Total Maximum Daily Loads (TMDLs)

All concentrations upstream of the golf course tributary, above second Yellow Creek and Second Mule Creek (both years of sampling) were below the DEQ designated TMDL of 0.3 mg/L for TN. The golf course tributaries in both years of sampling were well above this designated TMDL.

AS defined in the West Fork TMDL (West Fork TMDL at 113), “A Total Maximum Daily Load (TMDL) is a calculation of the maximum pollutant load a water body can receive while maintaining water quality standards.” The TMDL is comprised of the sum of all point sources and nonpoint sources (natural and anthropogenic), plus a margin of safety that accounts for uncertainties in loading and receiving water analyses. *Id.* at 114.

TMDLs are allocated to point (wasteload) and nonpoint (load) NO_3+NO_2 sources. *Id.* In addition to pollutant load allocations, the TMDL must also take into account the seasonal variability of pollutant loads and adaptive management strategies in order to address uncertainties inherent in environmental analyses. *Id.* These elements are combined in the following equation:

$$\text{TMDL} = \Sigma\text{WLA} + \Sigma\text{LA} + \text{MOS}$$

where:

> WLA = Waste Load Allocation or the portion of the TMDL allocated to point sources. Since there are no individual permitted point sources in the West Fork Gallatin watershed, the WLA=0.
>
> LA = Load Allocation or the portion of the TMDL allocated to nonpoint recreational/residential sources and natural background.
>
> MOS = Margin of Safety or an accounting of uncertainty about the relationship between pollutant loads and receiving water quality. Where the MOS is implicit an additional numeric MOS is unnecessary; therefore the ―explicit MOS is set equal to 0 here.

West Fork TMDL at 114. Potential wastewater NO_3+NO_2 loads derived from land-applied effluent are not permitted and were given a zero load allocation. West Fork TMDL at 115.

“The Middle Fork West Fork Gallatin River is listed on the 2008 303(d) List as impaired due to nitrate/nitrite.” THE WEST FORK GALLATIN RIVER WATERSHED TOTAL MAXIMUM DAILY LOADS (TMDLS) AND FRAMEWORK WATERSHED QUALITY IMPROVEMENT PLAN at 68 (hereinafter “West Fork TMDL”). “[W]astewater-sourced NO_3+NO_2 loads are the primary factor causing impairment conditions in the West Fork Gallatin River and is driving high TN concentrations[.]” West Fork TMDL at 109.

Nitrogen sources affecting algal growth include nitrogen derived from development activity as well as wastewater inputs." West Fork TMDL at 111. Gardner et al. (2011) determined approximately 28% of the summer baseflow load in the lower South Fork was attributed to wastewater sources, indicating potential discrete or localized nutrient inputs not accounted for in modeling assumptions. West Fork TMDL at 113.

7.4. Comparison with previous isotope analyses in the region

Gardner (2010), in a more extensive study of spatial and seasonal isotopes of NO_3^- (compared to the algae analyzed herein) in the West Fork watershed showed that the wastewater influence was most evident in the summer and winter baseflow and that a substantial biological cycling of N loading occurred prior to watershed export. She suggested that the more enriched values of $\delta^{15}N$ during summer were caused by direct nitrogen loading of wastewater irrigation into streams or quick transport if nitrogen from areas hydrographically connected to the stream. Her values ruled out fertilizer nitrogen as an important source. Moreover, her isotope analyses of NO_3^- in the West Fork watershed provided essential evidence for establishment of Total Maximum Daily Loads (TMDL) in two areas of the watershed.

<u>Summary Opinion</u>

The Yellowstone Club's draining of treated sewage from its golf course water hazard into the South Fork West Fork of the Gallatin River and spraying treated sewage out of its irrigation guns into Second Yellow Mule and ultimately South Fork West Fork is causing irreparable harm to the aquatic ecosystem by further degrading the already water-quality impaired waterbody. The green macroalga *Cladophora* is a notorious ecosystem engineer that causes adverse impacts in many surface waters across geographic regions, especially when stimulated by nutrient pollution from sewage. As summarized by the West Fork TMDL (West Fork TMDL at 109), "wastewater-sourced NO_3+NO_2 loads are the primary factor causing impairment conditions in the West Fork Gallatin River and is driving high TN concentrations[.]". The data presented here support the Montana Department of Environmental Quality listing of the South Fork/West Fork of the Gallatin River, as water-quality impaired. The isotopic signals of nitrogen in the collected algal samples of the South Fork West Fork of the Gallatin River were consistent with that of wastewater. The spraying of treated sewage above Second Yellow Mule and the discharge of treated sewage into the golf course tributary are responsible for the algae growth in the South Fork/West Fork of the Gallatin River and the Gallatin River. "[E]limination of wastewater NO_3+NO_2 loading will result in attainment of TN TMDLs and source allocations." West Fork TMDL at 109.

The Yellowstone Club's unpermitted discharges cause irreparable harm by degrading the chemical, physical, and biological integrity of the South Fork West Fork and preclude the restoration and maintenance of the water quality of the River. Unpermitted discharges of treated sewage will not just "wash away." The Yellowstone Club's unpermitted discharges are responsible for the growth of *Cladophora* that is causing irreparable harm to the aquatic ecosystem. The unpermitted discharges are causing algae blooms, which in turn cause irreparable harm to the aquatic ecosystem, including aquatic insects and valued fish species.

Continued development in the Yellowstone Club will only increase the volume of treated sewage that needs to be disposed. Such development should end until it can ensure its disposal methods do not cause or contribute to additional nitrogen loading and *Cladophora* growth in the West Fork of the Gallatin River and its tributaries. The Yellowstone Club should be stopped from discharging its treated sewage into the South Fork West Fork of the Gallatin River to maintain and restore the chemical, physical, and biological integrity of the Gallatin River and its tributaries.

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Stable Isotope Facility Data Report

Principal Investigator:	Pat Glibert	Email:	glibert@umces.edu
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Institution: Horn Point Lab - UMCES

Project: Glibert-plant 10/23 (SIF Order P156428)

Submission Date: October 18, 2023
Completion Date: November 3, 2023
Report Date: November 14, 2023

Analysis: ^{13}C & ^{15}N Analysis of Natural Abundance Solid Samples

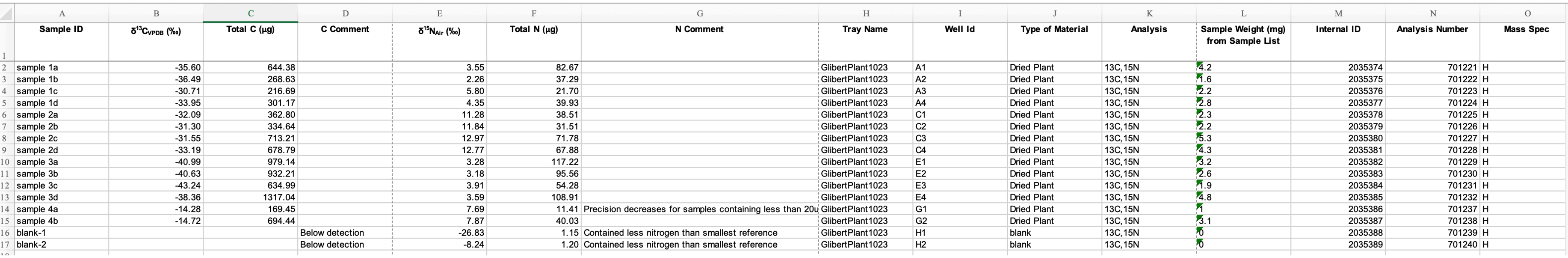
	$\delta^{13}C$	$\delta^{15}N$
Mean SD for reference materials replicates in this project:	±0.15 ‰	±0.07 ‰
Mean absolute accuracy for calibrated reference materials within:	±0.07 ‰	±0.06 ‰

Notes:

Sample count to be charged: 16 Normal combustion
Additional charges:

Reported by: Emily Ngo Schick
ekngo@ucdavis.edu

Please review your data in a timely fashion, so that we may fully address any questions or concerns.



Sample ID	$\delta^{13}C_{VPDB}$ (‰)	Total C (µg)	C Comment	$\delta^{15}N_{Air}$ (‰)	Total N (µg)	N Comment	Tray Name	Well Id	Type of Material	Analysis	Sample Weight (mg) from Sample List	Internal ID	Analysis Number	Mass Spec
sample 1a	-35.60	644.38		3.55	82.67		GlibertPlant1023	A1	Dried Plant	13C,15N	4.2	2035374	701221	H
sample 1b	-36.49	268.63		2.26	37.29		GlibertPlant1023	A2	Dried Plant	13C,15N	1.6	2035375	701222	H
sample 1c	-30.71	216.69		5.80	21.70		GlibertPlant1023	A3	Dried Plant	13C,15N	2.2	2035376	701223	H
sample 1d	-33.95	301.17		4.35	39.93		GlibertPlant1023	A4	Dried Plant	13C,15N	2.8	2035377	701224	H
sample 2a	-32.09	362.80		11.28	38.51		GlibertPlant1023	C1	Dried Plant	13C,15N	2.3	2035378	701225	H
sample 2b	-31.30	334.64		11.84	31.51		GlibertPlant1023	C2	Dried Plant	13C,15N	2.2	2035379	701226	H
sample 2c	-31.55	713.21		12.97	71.78		GlibertPlant1023	C3	Dried Plant	13C,15N	5.3	2035380	701227	H
sample 2d	-33.19	678.79		12.77	67.88		GlibertPlant1023	C4	Dried Plant	13C,15N	4.3	2035381	701228	H
sample 3a	-40.99	979.14		3.28	117.22		GlibertPlant1023	E1	Dried Plant	13C,15N	3.2	2035382	701229	H
sample 3b	-40.63	932.21		3.18	95.56		GlibertPlant1023	E2	Dried Plant	13C,15N	2.6	2035383	701230	H
sample 3c	-43.24	634.99		3.91	54.28		GlibertPlant1023	E3	Dried Plant	13C,15N	1.9	2035384	701231	H
sample 3d	-38.36	1317.04		3.59	108.91		GlibertPlant1023	E4	Dried Plant	13C,15N	4.8	2035385	701232	H
sample 4a	-14.28	169.45		7.69	11.41	Precision decreases for samples containing less than 20u	GlibertPlant1023	G1	Dried Plant	13C,15N	1	2035386	701237	H
sample 4b	-14.72	694.44		7.87	40.03		GlibertPlant1023	G2	Dried Plant	13C,15N	3.1	2035387	701238	H
blank-1			Below detection	-26.83	1.15	Contained less nitrogen than smallest reference	GlibertPlant1023	H1	blank	13C,15N	0	2035388	701239	H
blank-2			Below detection	-8.24	1.20	Contained less nitrogen than smallest reference	GlibertPlant1023	H2	blank	13C,15N	0	2035389	701240	H

Reference Materials

Summary	%C	$\delta^{13}C_{VPDB}$ (‰) Expected	$\delta^{13}C_{VPDB}$ (‰) Mean*	$\delta^{13}C_{VPDB}$ (‰) Standard Deviation*	%N	$\delta^{15}N_{Air}$ (‰) Expected	$\delta^{15}N_{Air}$ (‰) Mean*	$\delta^{15}N_{Air}$ (‰) Standard Deviation*
Alfalfa Flour	42.62	-29.67	-29.59	0.14	4.68	1.81	1.75	0.06
Amaranth Flour	42.11	-12.89	-12.84	0.31	2.45	2.45	2.47	0.16
Caffeine	47.64	-35.05	-35.05	0.14	28.35	-2.81	-2.81	0.02
Chitin	42.46	-20.45	N/A	N/A	6.19	-0.92	N/A	N/A
Enriched Alanine	[redacted]	[redacted]	[redacted]	[redacted]	15.72	41.13	41.13	0.04
Glutamic Acid (GLAC)	40.82	-11.07	-11.07	0.08	9.52	-8.53	-8.53	0.07
Keratin	49.31	-24.46	N/A	N/A	14.91	4.87	N/A	N/A
Scallop	41.03	-16.89	N/A	N/A	11.25	9.46	N/A	N/A
Nylon Powder (NYLOW)	70.10	-25.23	-25.02	0.09	10.61	-0.72	-0.99	0.06

* Mean and standard deviation excludes references below limit of quantification (LOQ)

Reference	Weight (µg)	Total C (µg)	$\delta^{13}C_{VPDB}$ (‰)	Total N (µg)	$\delta^{15}N_{Air}$ (‰)	Analysis	Comments
Alfalfa Flour	214	94.68	-29.29	10.25	2.02	701200	Below LOQ, excluded from calculations
Alfalfa Flour	398	170.70	-29.59	18.06	1.79	701323	
Alfalfa Flour	519	223.58	-29.81	24.42	1.78	701242	
Alfalfa Flour	961	412.86	-29.63	45.32	1.64	701305	
Alfalfa Flour	2152	910.31	-29.37	101.25	1.80	701274	
Alfalfa Flour	3629	1539.17	-29.56	168.55	1.79	701216	
Alfalfa Flour	4911	2086.68	-29.62	231.37	1.70	701322	
Alfalfa Flour Average			-29.59		1.75		
Alfalfa Flour StdDev			0.14		0.06		
Amaranth Flour		1633.03	-12.62	101.87	2.59	701234	
Amaranth Flour		1720.63	-13.06	103.73	2.36	701289	
Amaranth Flour Average			-12.84		2.47		
Amaranth Flour StdDev			0.31		0.16		
Caffeine		251.42	-35.03	143.31	-2.83	701218	
Caffeine		242.66	-34.92	137.03	-2.82	701258	
Caffeine		237.03	-35.19	133.90	-2.79	701307	
Caffeine Average			-35.05		-2.81		
Caffeine StdDev			0.14		0.02		
Enriched Alanine				122.42	41.13	701217	
Enriched Alanine				127.63	41.10	701257	
Enriched Alanine				152.76	41.17	701306	
Enriched Alanine Average					41.13		
Enriched Alanine StdDev					0.04		
Glutamic Acid (GLAC)		378.44	-11.16	87.10	-8.51	701219	
Glutamic Acid (GLAC)		500.46	-11.01	116.18	-8.47	701259	
Glutamic Acid (GLAC)		494.20	-11.05	114.10	-8.60	701308	
Glutamic Acid (GLAC) Average			-11.07		-8.53		
Glutamic Acid (GLAC) StdDev			0.08		0.07		
Nylon Powder (NYLOW)		506.72	-25.12	48.78	-0.97	701195	
Nylon Powder (NYLOW)		641.25	-24.89	61.64	-0.94	701196	
Nylon Powder (NYLOW)		538.01	-25.16	51.63	-0.95	701197	
Nylon Powder (NYLOW)		519.23	-25.11	49.70	-0.92	701198	
Nylon Powder (NYLOW)		503.59	-25.07	48.47	-1.13	701201	
Nylon Powder (NYLOW)		731.98	-24.87	70.45	-1.03	701202	
Nylon Powder (NYLOW)		691.31	-24.96	66.24	-0.98	701215	
Nylon Powder (NYLOW)		528.62	-25.01	50.61	-1.07	701220	
Nylon Powder (NYLOW)		528.62	-25.05	50.31	-0.98	701233	
Nylon Powder (NYLOW)		588.06	-24.92	56.22	-0.94	701236	
Nylon Powder (NYLOW)		744.50	-25.05	71.06	-0.97	701241	
Nylon Powder (NYLOW)		700.70	-25.02	66.96	-0.99	701243	
Nylon Powder (NYLOW)		534.88	-25.10	50.61	-1.02	701256	
Nylon Powder (NYLOW)		656.89	-24.95	62.66	-0.90	701260	
Nylon Powder (NYLOW)		719.47	-25.06	68.81	-0.96	701273	
Nylon Powder (NYLOW)		603.71	-25.14	57.76	-1.14	701275	
Nylon Powder (NYLOW)		663.15	-24.91	63.38	-0.97	701288	
Nylon Powder (NYLOW)		609.96	-25.01	58.27	-0.97	701291	
Nylon Powder (NYLOW)		697.57	-25.04	66.35	-1.02	701304	
Nylon Powder (NYLOW)		525.49	-25.04	49.80	-1.00	701309	
Nylon Powder (NYLOW)		703.82	-25.11	66.65	-1.01	701324	
Nylon Powder (NYLOW)		566.16	-24.91	53.77	-0.96	701325	
Nylon Powder (NYLOW) Average			-25.02		-0.99		
Nylon Powder (NYLOW) StdDev			0.09		0.06		

UC Davis Stable Isotope Facility

Sample Submission Form - Solid Sample Analysis

ast Name: Vidyarathna
irst Name: Nayani
Email: nvidyarathna@umces.edu

Institution: Horn Point Lab-UMCES
Purchase Order#:
Project Name: Glibert-plant 10/23
PPMS Order#
Data Deadline: asap Nov 10
Irreplaceable: (y/n) y

Sample Submission Instructions: Complete all yellow fields as applicable. Email the completed file to sif@ucdavis.edu.
A hardcopy should also be included in your shipment.
Tip: Set Print Area to "Fit All Columns on One Page"
Enriched samples, including their natural abundance controls, should be roughly organized from lowest to highest expected enrichment.

LIMS for Light Stable Isotopes
Version 8.1

Counter	Sample ID	Amount (mg)	Tray Name	Well Id	Type of Material	Analysis	Enriched ?	Estimated Enrichment	Comment
	max 20 alphanumeric characters - no identical		maximum 16 alphanumeric char		maximum 20 characters				(SIF Internal Use Only)
Example 1	Rice 1	0.4	Oxygen1	A1	rice in silver	18O	No		
Example 2	Del 080415 B29	N/A	Tray1-LastName	G2	seston on filter	13C, 15N	Yes	1 at-%	
Example 3	empty 1			H11					
1	sample 1a	4.2	GlibertPlant1023	A1	Dried Plant	13C,15N	No		
2	sample 1b	1.6	GlibertPlant1023	A2	Dried Plant	13C,15N	No		
3	sample 1c	2.2	GlibertPlant1023	A3	Dried Plant	13C,15N	No		
4	sample 1d	2.8	GlibertPlant1023	A4	Dried Plant	13C,15N	No		
5	sample 2a	2.3	GlibertPlant1023	C1	Dried Plant	13C,15N	No		
6	sample 2b	2.2	GlibertPlant1023	C2	Dried Plant	13C,15N	No		
7	sample 2c	5.3	GlibertPlant1023	C3	Dried Plant	13C,15N	No		
8	sample 2d	4.3	GlibertPlant1023	C4	Dried Plant	13C,15N	No		
9	sample 3a	3.2	GlibertPlant1023	E1	Dried Plant	13C,15N	No		
10	sample 3b	2.6	GlibertPlant1023	E2	Dried Plant	13C,15N	No		
11	sample 3c	1.9	GlibertPlant1023	E3	Dried Plant	13C,15N	No		
12	sample 3d	4.8	GlibertPlant1023	E4	Dried Plant	13C,15N	No		
13	sample 4a	1	GlibertPlant1023	G1	Dried Plant	13C,15N	No		
14	sample 4b	3.1	GlibertPlant1023	G2	Dried Plant	13C,15N	No		
15	blank-1	0	GlibertPlant1023	H1	blank	13C,15N			
16	blank-2	0	GlibertPlant1023	H2	blank	13C,15N			
17	empty 1			H3					
18	empty 2			H4					
19	empty 3			H5					
20	empty 4			H6					
21	empty 5			H7					
22	empty 6			H8					
23	empty 7			H9					
24	empty 8			H10					
25				H11					
26				H12					
27									

BRIDGER ANALYTICAL LAB

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US EPA ID# MT00953 MT Certification Number CERT0094

Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
09/15/2021 12:56

Project Name: South Fork Gallatin

Client Sample ID: 45.24095-111.38833
Lab Sample ID: 2108586-01

Collection Date: 08/30/2021 12:45
Collected By: Eric Berry

Date Received: 08/30/2021

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
Inorganic							
Nitrate + Nitrite as N	0.106	mg/L	0.05		10	EPA 300.1	09/01/21 06:59/FAF
Nitrogen, Total (TKN+N+N)	0.106	mg/L	0.05			Calculation	09/14/21 11:35/DJA
Service							
Filtration fee	1.00		0.22			0.22 um	08/31/21 12:51/FAF

Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
09/15/2021 12:56

Project Name: South Fork Gallatin

Client Sample ID: 45.23901-111.38288
Lab Sample ID: 2108586-02

Collection Date: 08/30/2021 12:45
Collected By: Eric Berry

Date Received: 08/30/2021

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
Inorganic							
Nitrate + Nitrite as N	0.974	mg/L	0.05		10	EPA 300.1	09/01/21 08:50/FAF
Nitrogen, Total (TKN+N+N)	0.974	mg/L	0.05			Calculation	09/14/21 11:35/DJA
Service							
Filtration fee	1.00		0.22			0.22 um	08/31/21 12:51/FAF

Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
09/15/2021 12:56

Project Name: South Fork Gallatin

Client Sample ID: 45.23836-111.37546
Lab Sample ID: 2108586-03

Collection Date: 08/30/2021 12:45
Collected By: Eric Berry

Date Received: 08/30/2021

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
Inorganic							
Nitrate + Nitrite as N	0.124	mg/L	0.05		10	EPA 300.1	09/01/21 10:04/FAF
Nitrogen, Total (TKN+N+N)	0.124	mg/L	0.05			Calculation	09/14/21 11:35/DJA
Service							
Filtration fee	1.00		0.22			0.22 um	08/31/21 12:51/FAF

Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
09/15/2021 12:56

Project Name: South Fork Gallatin

Data Analyzed by: Pace Analytical Services, LLC -

Client Sample ID: 45.24095-111.38833
Lab Sample ID: 2108586-01

Collection Date: 08/30/2021 12:45
Collected By: Eric Berry

Date Received: 08/30/2021

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
Inorganic							
Total Kjeldahl Nitrogen as N	ND	mg/L	0.50	U		EPA 351.2	09/11/21 22:26/AP2

Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
09/15/2021 12:56

Project Name: South Fork Gallatin

Data Analyzed by: Pace Analytical Services, LLC -

Client Sample ID: 45.23901-111.38288
Lab Sample ID: 2108586-02

Collection Date: 08/30/2021 12:45
Collected By: Eric Berry

Date Received: 08/30/2021

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
Inorganic							
Total Kjeldahl Nitrogen as N	ND	mg/L	0.50	U		EPA 351.2	09/11/21 22:30/AP2

Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
09/15/2021 12:56

Project Name: South Fork Gallatin

Data Analyzed by: Pace Analytical Services, LLC -

Client Sample ID: 45.23836-111.37546
Lab Sample ID: 2108586-03

Collection Date: 08/30/2021 12:45
Collected By: Eric Berry

Date Received: 08/30/2021

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
Inorganic							
Total Kjeldahl Nitrogen as N	ND	mg/L	0.50	U		EPA 351.2	09/11/21 22:31/AP2

Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
09/15/2021 12:56

Notes and Definitions

Item	Definition
U	[Undefined]
cfu	Colony Forming Unit
MCL	Maximum Contaminant Level
mg/L	milligrams per liter (ppm)
mL	milliliter
MPN	Most Probable Number
ND	Not Detected
NTU	Nephelometric Turbidity Units
ppb	parts per billion (µg/L)
ppm	parts per million (mg/L)
RL	Reporting Limit
S.U.	Standard Units
µg/L	micrograms per liter (ppb)
µS/cm	microsiemens per centimeter

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Bozeman, MT 59771

Reported:
10/04/2022 07:58

Project Name: Yellowstone Club 9-19-2022

Client Sample ID: #1 2nd YM Upstream
Lab Sample ID: 2209373-01

Collection Date: 09/19/2022 9:58
Collected By: Isaac Cheek

Date Received: 09/19/2022

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
Inorganic							
Nitrate + Nitrite as N	0.137	mg/L	0.05		10	EPA 300.1	09/19/22 20:13/DJA
Nitrogen, Total (TKN+N+N)	0.137	mg/L	0.05			Calculation	10/03/22 11:34/DJA

Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
10/04/2022 07:58

Project Name: Yellowstone Club 9-19-2022

Client Sample ID: #2 2nd YM Stream
Lab Sample ID: 2209373-02

Collection Date: 09/19/2022 10:07
Collected By: Isaac Cheek

Date Received: 09/19/2022

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
Inorganic							
Nitrate + Nitrite as N	0.217	mg/L	0.05		10	EPA 300.1	09/19/22 20:32/DJA
Nitrogen, Total (TKN+N+N)	0.217	mg/L	0.05			Calculation	10/03/22 11:34/DJA

Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
10/04/2022 07:58

Project Name: Yellowstone Club 9-19-2022

Client Sample ID: #3 Golf Course Stream
Lab Sample ID: 2209373-03

Collection Date: 09/19/2022 10:52
Collected By: Isaac Cheek

Date Received: 09/19/2022

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
Inorganic							
Nitrate + Nitrite as N	1.28	mg/L	0.05		10	EPA 300.1	09/19/22 20:51/DJA
Nitrogen, Total (TKN+N+N)	1.87	mg/L	0.05			Calculation	10/03/22 11:34/DJA

Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
10/04/2022 07:58

Project Name: Yellowstone Club 9-19-2022

Client Sample ID: #4 Upstream of Golf Course
Lab Sample ID: 2209373-04

Collection Date: 09/19/2022 10:59
Collected By: Isaac Cheek

Date Received: 09/19/2022

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
Inorganic							
Nitrate + Nitrite as N	0.0732	mg/L	0.05		10	EPA 300.1	09/19/22 21:30/DJA
Nitrogen, Total (TKN+N+N)	0.0732	mg/L	0.05			Calculation	10/03/22 11:34/DJA

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Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
10/04/2022 07:58

Project Name: Yellowstone Club 9-19-2022

Data Analyzed by: Pace Analytical Services, LLC -

Client Sample ID: #1 2nd YM Upstream
Lab Sample ID: 2209373-01

Collection Date: 09/19/2022 9:58
Collected By: Isaac Cheek

Date Received: 09/19/2022

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
EPA 351.2							
Nitrogen, Kjeldahl, Total	ND	mg/L	0.50	U		3512 WDU	09/30/22 10:15/AP2

Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
10/04/2022 07:58

Project Name: Yellowstone Club 9-19-2022

Data Analyzed by: Pace Analytical Services, LLC -

Client Sample ID: #2 2nd YM Stream
Lab Sample ID: 2209373-02

Collection Date: 09/19/2022 10:07
Collected By: Isaac Cheek

Date Received: 09/19/2022

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
EPA 351.2							
Nitrogen, Kjeldahl, Total	ND	mg/L	0.50	U		3512 WDU	09/30/22 10:16/AP2

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US EPA ID# MT00953 MT Certification Number CERT0094

Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
10/04/2022 07:58

Project Name: Yellowstone Club 9-19-2022

Data Analyzed by: Pace Analytical Services, LLC -

Client Sample ID: #3 Golf Course Stream
Lab Sample ID: 2209373-03

Collection Date: 09/19/2022 10:52
Collected By: Isaac Cheek

Date Received: 09/19/2022

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
EPA 351.2							
Nitrogen, Kjeldahl, Total	0.59	mg/L	0.50			3512 WDU	09/30/22 10:18/AP2

BRIDGER ANALYTICAL LAB

7539 Pioneer Way Suite B, Bozeman, MT 59718 Phone: (406) 582-0822
US EPA ID# MT00953 MT Certification Number CERT0094

Cottonwood Environmental Law Center
P.O. Box 412
Bozeman, MT 59771

Reported:
10/04/2022 07:58

Project Name: Yellowstone Club 9-19-2022

Data Analyzed by: Pace Analytical Services, LLC -

Client Sample ID: #4 Upstream of Golf Course
Lab Sample ID: 2209373-04

Collection Date: 09/19/2022 10:59
Collected By: Isaac Cheek

Date Received: 09/19/2022

Analyte	Result	Units	RL	Qual	MCL	Method	Analysis Date/By
EPA 351.2							
Nitrogen, Kjeldahl, Total	ND	mg/L	0.50	U		3512 WDU	09/30/22 10:19/AP2

CURRICULUM VITAE

PATRICIA M. GLIBERT

Horn Point Laboratory
University of Maryland Center for Environmental Science
P.O. Box 775
Cambridge, MD 21613
Phone 410-221-8422
Email: glibert@umces.edu

I. Education

1974	BA	Skidmore College, Saratoga Springs, NY, Biology [*Phi Beta Kappa*]
1976	MS	University of New Hampshire, Earth Sciences
1982	PhD	Harvard University, Organismal and Evolutionary Biology

II. Professional Background

1993 - present	Professor, University of Maryland Center for Environmental Science (UMCES), Horn Point Laboratory (HPL)
2014 - 2017	Visiting Professor, Zhejiang University, Hangzhou and Zhoushan, China
1989 - 1993	Associate Professor, UMCES, HPL
1986 - 1989	Assistant Research Scientist, UMCES, HPL
1982 - 1986	Assistant Scientist, Woods Hole Oceanographic Institution
1981 - 1982	Postdoctoral Scholar, Woods Hole Oceanographic Institution

III. Significant Honors and Awards

2001	Environment Expert Award bestowed by the Minister of Health, Kuwait.
2006	University of Maryland Board of Regents Award for Excellence in Research, Scholarship and Creative Activity.
2011	HPL Director's award for outstanding productivity.
2011	Honorary Doctorate, conferred by Linnaeus University, Sweden.
2012	Distinguished Service Award, Kuwait University
2012	Elected Fellow, AAAS
2013	Named one of the top 25 women professors in the State of Maryland (www.statestat.org)
2015	Named Sustaining Fellow, Association for the Sciences of Limnology and Oceanography
2018	Invited Distinguished Scientist, Marine Biological Laboratory, Woods Hole MA
2019	Named Sawyer Visiting Professor, Maine Maritime Academy
2020	Visiting Professor, Shanghai Jiao Tong University, Shanghai, China
2022-2024	President, Association for the Sciences of Limnology and Oceanography (ASLO)

IV. Research

A. *Research Interests*

Transformations and fate of inorganic and organic nitrogen in marine and estuarine systems; global changes in the nitrogen cycle by anthropogenic activities; eutrophication; ecology and physiology of phytoplankton in estuarine and oceanic environments; harmful algal blooms; stable isotope techniques; ecological stoichiometry; effects of ocean fertilization for carbon sequestration.

B. Publications

1. Synthesis of publications and citations

Total peer reviewed journal papers (including in press but not in review): 205
Total book chapters/proceedings: 52
Other publications (peer review reports/articles for kids, public, etc): 14

Statistics as of Nov 2023	**Web of Science**	**Google Scholar**
Total number citations	18,698	29,878
Ave annual citations (2019-2022)	1,439	2,098
h' index	67	82

2. Publications

2.1 Books

2.1.1 Sole Authorship

Glibert, P.M. 2024. *Phytoplankton Whispering: An introduction to the physiology and ecology of microalgae.* Springer. In press

2.1.2 Books Edited

Glibert, P.M. and T.M. Kana (eds.). 2016. *Aquatic Microbial Ecology and Biogeochemistry: A Dual Perspective.* Springer.

Glibert, P.M., E. Berdalet, M. Burford, G. Pitcher and M. Zhou (eds.). 2018. *Ecology and Oceanography of Harmful Algal Blooms (GEOHAB).* Springer.

Glibert P.M., M. A. Altabet, J. Montoya and D. McGillicuddy (eds.). 2019. *The current and future ocean: Advancing science from plankton to whales–Celebrating the contributions of James J. McCarthy*. The Sea. Yale University Press.

2.2 Journal Papers and Other Articles

2023

Millette, N.C., R. J. Gast, J. Luo, H. Moeller, K. Stamieszkin, K. H. Andersen, E. Brownlee, N. Cohen, S. Duhamel, S. Dutkiewicz, **P. M. Glibert**, M. Johnson, S. Leles, A. Maloney, G. McManus, N. Poulton, S. Princiotta, R. Sanders, S. Wilken. 2023. Mixotrophs and mixotrophy: Future research priorities. *J. Plankt. Res.* doi.org/10.1093/plankt/fbad020

Li, J., Y. Gao, Y. Bao, X. Gao, and **P.M. Glibert**. 2023. Summer phytoplankton photosynthetic characteristics in the Changjiang River Estuary and the adjacent East China Sea. *Front. Mar. Sci.* doi.org/10.3389/fmars.2023.1111557

Vidyarathna, N., S. H. Ahn, **P. M. Glibert**. 2023. Thermal niche of the dinoflagellate *Karlodinium veneficum* across different salinity and light levels. *J. Plankt. Res.* doi.org/10.1093/plankt/fbad019

Glibert, P.M. and M. Li. Warming, wheezing, blooming waters: hypoxia and harmful algae. In: D. Baird (ed), Treatise on estuarine and coastal science, 2nd edition. Elsevier. In press.

Ahn, S., **P.M. Glibert** and C.A. Heil. In hot water: Interactions of temperature, nitrogen form and availability and photosynthetic and nitrogen uptake responses in natural *Karenia brevis* populations. *Harmful Algae.* doi.org/10.1016/j.hal.2023.102519

Chen, Y., M. Li, **P.M. Glibert** and C.A. Heil. Mu*r*Ky waters: Modeling the succession from *r* to K strategists (diatoms to dinoflagellates) following a nutrient spill from a mining facility in Florida. *Limnol. Oceanog.* doi.org/10.1002/lno.12420

Editorials and Non-reviewed Publications

Glibert, P.M. 2023. Message from the President: ASLO is global: Nurturing cross-cultural connections. *Limnol. Oceanog. Bull.* 32: 18-19

Glibert, P.M. 2023. Message from the President: Kudos to the people of ASLO. *Limnol. Oceanog. Bull.* 32: 61-62

Glibert, P.*M.* 2023. Message from the President: Trials and tribulations of transitions and transformations in publishing: what it means for you. *Limnol. Oceanogr. Bull.* 32: 110-112.

Glibert, P.M. 2023. Message from the President: Finding balance in a world of extremes. *Limnol. Oceanog. Bull.* 32: 139-140.

2022

Li, R., M. Li and **P.M. Glibert**. 2022. Coupled carbonate chemistry–harmful algal bloom models for studying effects of ocean acidification on *Prorocentrum minimum* blooms in an estuary. *Front. Mar. Sci.* doi.org/10.3389/fmars.2022.889233.

Glibert, P.M. and A. Mitra. 2022. From webs, loops, shunts and pumps to microbial multi-tasking: Evolving paradigms of marine microbial ecology, global mixoplankton importance and implications for a future ocean. *Limnol. Oceanogr.* 67: 585-597. doi.org/10.1002/lno.12018.

Ahn, S.H. and **P.M. Glibert**. 2022. Shining light on photosynthesis in the harmful dinoflagellate *Karenia mikimotoi*– Responses to short-term changes in temperature, nitrogen form and availability. *Phycology* 2:30-44. doi.org/10.3390/phycology2010002.

Li, M., Y. Chen, F. Zhang, Y. Song, **P.M. Glibert** and D.K. Stoecker. 2022. A three-dimensional mixotrophic model of *Karlodinium veneficum* blooms in a eutrophic estuary: seasonal and spatial dynamics and effects of nutrient ratios, prey concentration and temperature. *Harmful Algae.* 113:102203. doi.org/10.1016/j.hal.2022.102203.

Glibert, P.M., F. Wilkerson, R.C. Dugdale, A.E. Parker. 2022. Ecosystem recovery in progress? Initial nutrient and phytoplankton response to nitrogen reduction from sewage treatment upgrade in the San Francisco Bay Delta. *Nitrogen.* doi.org/10.3390/nitrogen3040037.

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[2] Invited review, Web of Science highly cited paper; one of the top cited papers of the journal.

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[4] Invited review, one of the top downloaded papers for *Limnol. Oceanogr.* from 2018-2020

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[5] Web of Science highly cited paper

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Glibert, P.M., and G. Pitcher (Guest Editors), 2005. Special section of *Oceanography* on *Harmful algal blooms.* Vol, 18(2).

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Burkholder, J.M. and **P.M. Glibert** (Guest Editors). 2009. Special section of *Harmful Algae* on *Strain Differences in Harmful Algae*. Vol 8(5)

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Glibert, P.M., M.J. Zhou, M.Y. Zhu, and M.A. Burford (Guest Editors). 2011. Special issue of *Chinese Journal of Oceanology and Limnology* on *Eutrophication and HABs: The GEOHAB Approach.* Vol. 29(4).

Chen, J., W.-J. Cai, **P.M. Glibert** and D. Huang (Guest editors). 2022, 2023. Eutrophication, algal blooms, hypoxia, and ocean acidification in large river systems. *Front. Mar. Sci.* Vols I, II

C. Membership in Professional Societies

American Association for the Advancement of Science (*Fellow*)
Association for the Sciences of Limnology and Oceanography (*Sustaining Fellow*, ***President-July 2022-2024***)
American Geophysical Union
The Oceanography Society
Estuarine Research Federation
International Society for the Study of Harmful Algae

V. Teaching and Training

1986- present	Member, UMCES Graduate Faculty
1986- present	Member, USM Graduate Faculty
2014-2017	Zhejiang University, Hangzhou and Zhouzhan, China

VI. Outreach and Service

A. Editorships and Journal Reviewing

Member of Editorial Board, *Harmful Algae*, 2001-2019
Member of the Editorial Board, *Limnology and Oceanography Letters* 2015-2019
Subject Editor, *Aquatic Microbial Ecology*, 1995-2001, 2007-2013
Member of Editorial board of *Estuaries and Coasts*, 2004- 2013

B. Federal, State, Local Government

Co-Chair, US National HAB Committee, 2006-2012, ex-officio member 2013-present
Member, Maryland Harmful Algal Technical Advisory Committee, 1999- present
Member, Scientific and Technical Advisory Committee, Coastal Bays, 2006-present
Expert Reviewer, EPA, Florida nutrient criteria development, 2009
Consultant on nutrient issues, California State Water Contractors and Bay Delta Conservation Plan, 2009-2015

C. National/International Working Groups and Advising

GEOHAB Scientific Steering Committee (1999-2015) and chair of the core research project on Eutrophication (1999-2017)
Co-chair, SCOR/LOICZ Working Group 132, Land based nutrient pollution and HABs, 2008-2013
Consultant to the Ministry of Oman on harmful algal blooms, 2010, 2015
Member, GEOHAB Working Group on HABs and Ocean Colour, 2010-2015
Member, working group on developing models for mixotrophy, Leverhulme Foundation, 2011-2016
Member, working group on Mixotrophs and Mixotrophy, OCB, Woods Hole

D. Testimony

Expert report for District Court: Natural Resources Defense Council vs Metropolitan Water Reclamation District of Greater Chicago

Expert report and witness testimony in US Supreme Court: Florida vs Georgia

E. Service to the Broader Community

Member and Secretary, Estuarine Research Federation Governing Board, 2007-2009; Representative CERF Policy Committee 2012-2015

Representative, Council of Aquatic Science Societies (CASS), 2011-2014

Member, Gunston School (Centreville, MD) advisory board on Chesapeake Watershed Semester Program, 2018-2021

President, Association for the Sciences of Limnology and Oceanography, July 2022- July 2024