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## **Periphyton in Alpine Lakes and Streams along the John Muir Trail in the Sierra Nevada: Relationship to Human Impact**

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### **Abstract**

**OBJECTIVE:** Within the Sierra Nevada, the 335 km (210 mile) John Muir Trail (JMT) attracts thousands of backpackers each year who complete either segments or the entire trail from Yosemite Valley to MT. Whitney. This report is a follow up study to a research project done during summer 2008 along the JMT which analyzed the risk of acquiring waterborne disease by determining prevalence and changes in coliform bacteria, and *Escherichia coli* (*E. coli*). The aim of the current study is to assess the biomass of attached periphyton (attached algae) in the same lakes and streams and compare to indicator bacterial prevalence. **METHODS:** Water from 20 sites adjacent to the JMT and identical to the prior years study was analyzed. Water was collected on Millipore bacteria transport filters for coliform and E-coli, and transported to the lab for analysis. Chlorophyll-a was collected on glass fiber filters, preserved and transported. Attached algae biomass was measured using a modified California Dept of Fish and game method. Findings were correlated with environmental land use which might affect water quality. **RESULTS:** We analyzed surface water at 20 different sites in the summer of 2009. Coliforms prevalence was higher in Mixed-use sites (63%) compared with Backpack sites (28%), and Pristine sites (0%). Chlorophyll-a measurements were inconclusive. Periphyton biomass was highest at Mixed use sites, where mean maximum biomass readings were 59% compared to 42% at Backpack sites and 10% at Pristine sites ( $P < 0.05$ ). **CONCLUSION:** Our study provides confirmation for the findings of earlier studies, and presents evidence that lakes and stream impacted by pack and stock animal traffic have increased eutrophication levels compared to watersheds without pack animals. In addition higher levels of periphyton are associated with coliform presence.

## Introduction

Several remote trail systems have been constructed in the Sierra Nevada of California that are popular with summer backpackers. Most well known of these is the John Muir Trail (JMT) which serves as an internationally recognized high elevation mountain trail that begins in Yosemite Valley and traverses through multiple watersheds 335 km (210 miles) to the top of Mount Whitney at 4,418 m (14,495 ft) above sea level (Lowe and Lowe, 1982). It is one of the most popular and crowded long distance mountain trails in the United States. The northern portion of the trail is located within Yosemite National Park, the central portion between Yosemite and Kings Canyon (123 km), and the southern portion within Kings Canyon and Sequoia National Parks (Wenk and Morey, 2007). Water can only be obtained from trailside or nearby lakes and streams, and backpackers commonly collect water for drinking several times each day. The purity of water along the trail is important to increase the success of completing the entire trail, as well to the health of each individual backer. In addition, the JMT crosses the headwaters of Reverse Creek, the Merced, Tuolumne, San Joaquin, Kings and Kern Rivers watersheds that provide drinking water to the many water systems in California (Carle, 2004).

The high usage of the JMT and adjacent areas create a risk for contamination by many different species of microbes. Pathogenic microorganisms such as enterotoxic strains of *Escherichia coli* (*E. coli*), *Salmonella*, *Giardia*, or other harmful microorganisms may be introduced into wilderness lakes and streams from visiting humans, pack and stock animals, and to a lesser extent by endogenous wildlife (Ursem 2009, Derlet et al, 2008, Atwill 2004). In

addition, organic carbon, nitrogen, phosphorus and iron are added to an otherwise oligotrophic environment by various human activities (Horne and Goldman, 1994). These nutrients stimulate algae and aquatic growth, which in turn provide a supportive environment for sustaining exogenous microorganisms, which may have been introduced (Jasson et al., 2006). Furthermore, certain algae produce and secrete toxins, which are harmful and can be lethal to fish, wildlife, pets and humans. Introduction of algae toxins into domestic waters systems can be catastrophic.

Eutrophication of lakes and streams is a complicated multistep process that occurs over an extended time. One of the first effects of pollutants containing phosphorus (P) or Nitrogen (N) is to stimulate growth of attached algae. Small amounts of spot localized growth over time becomes confluent, and ultimately a flume extends progressively downstream. In lakes and stagnant streams, increases in suspended algal species populations, such as diatoms can greatly degrade water clarity. With time and continuing nutrient support, blue green algae may have a competitive advantage and become dominant. The blue green algae, once established are difficult to remove, and secrete harmful toxins.

Water in the Sierra, along the JMT is highly sensitive to eutrophication due to the environmental and geographic features of the Sierra Nevada Mountains. The high elevation portions of the watershed consists of surface or subsurface granite and metamorphic rock, with little topsoil (Moore, 2000). Therefore total soil adsorption and buffering capacity is low, providing minor or no biogeochemical

retention or transformation of nutrients such as nitrogen (N) and phosphorus (P) (Horne and Goldman, 1994). Therefore relatively small amounts of nutrient or microbial additions or habitat disturbances can lead to significant impacts on nutrient flux and subsequently degrade water quality and aquatic ecosystems (Goldman, 2000). For example, sunscreen, soaps, detergents, skin oils, food particles, may be leach into lakes and streams by passing through thin porous soil. Pack animal and Cattle manure may be deposited directly into waterways, or be swept into the water, instead of being absorbed and fixed in deep soil. Snowmelt and precipitation, especially episodic summer thunderstorms bring surface pollutants into creeks, lakes, and downstream reservoirs.

A previous study conducted along the JMT in 2008 found that Coliforms and *E-coli* prevalence were associated with mixed use areas defined as those watershed areas used both by packers and backpackers (Ursem 2009). These increased temporally over the summer. At mixed use sites, suspended algae as measured by chlorophyll-a levels increased and were correlated with *E-coli* and Coliforms prevalence. That study did not document the biomass of periphyton, which was observed but not quantified or recorded. The purpose of this current study is to determine 1) the biomass of attached algae in lakes and streams, and 2) to confirm the results of the 2008 study that measured coliform and *E-coli* prevalence, and Chlorophyll-a levels.

**Methods**

Field site selection

Water was sampled from sites in the John Muir Wilderness along a 72 km portion

of the JMT between Duck Creek just south of the Mammoth Lakes region, to the JMT entrance of Kings Canyon National Park at Piute Creek (2,438 m), as shown on figure 1. As a designated wilderness area, both logging and motorized vehicle use are prohibited within the John Muir Wilderness. In addition, wilderness permits are required for overnight use. John Muir Trail hikers collect drinking water both from water sources crossing the trail as well as nearby off trail campsites. Therefore we sampled water at selected off trail sites, up to three km from the trail. Sampling sites were categorized into three different types based on land usage and activity patterns that could impact the water quality. Classification categories and criteria are described in the following table.

**Sampling Site Classification Criteria**

1) Wildlife/Pristine	watersheds trampled only by wildlife: rarely traversed by humans and inaccessible to pack and stock animals
2) Backpack	watersheds used lightly by backpackers and rarely by pack and stock animal impact
3) Mixed Use	watersheds traversed by both humans and domesticated animals

At each sampling the sites were studied for evidence of humans and/or pack animal impact by inspecting campsites, terrain changes, and residual manure. Table 1 describes the 20 sites, with a specific description and location of each sampling site. Site characteristics were also stratified during previous years with the assistance of the United States Department of Agriculture Forest Service. Some sites are officially

recognized as geographic milestones and therefore are not reported in metric units.

### Field Sampling and Measurements

Sites were studied during August 2009. Lakes were sampled at the outlet or as close as possible to the outlet. When the water source was a river or creek it was sampled in the one most representative location. A modified California Dept of Fish and Game method of estimating attached algal biomass was applied (California Fish and Game 2006). A 1m grid was placed at two separate locations within 10m of the established sampling site: where the largest area of periphyton biomass was observed, and where the least was observed. The percent of green, brown or orange algae bottom cover within the 1m grid was estimated, photographed, and recorded. Bacteria samples were 16 ml in volume and collected in Millipore total coliform count samplers (Millipore, Bedford, Massachusetts). Samples were then kept cool for the remainder of the seven day sampling trip and then refrigerated before transport to the laboratory.

For suspended algae, a sample was taken in order to measure chlorophyll-a concentration. 200 ml ambient surface water was filtered on a 25 mm GF/C following standard methods after fixing with  $MgCl_2$  (Whatman, Cambridge UK) (American Public Health Association, 1998). Chlorophyll-a samples were fixed in 8ml tubes containing 100% ethanol, then protected from light by wrapping in aluminum foil. Preservation of chlorophyll-a through utilization of this technique has been shown to retain >98% of chlorophyll at temperatures less than 22C for up to a week prior to freezing (Derlet, unpublished data). Air and water temperature, as well as altitude was also measured at each site.

### Laboratory Analysis

Prevalence of coliforms and *E. coli* from total bacterial counts was calculated following standardized laboratory procedures, and as described in a prior study (American Public Health Association, 1998, Ursem 2009). In summary, the Millipore tubes were incubated at 35° C for 24 hours; colonies were then counted and sub-plated in duplicate onto sheep blood and MacConkey agar plates (Remel, Lenexa, Kansas). These plates were incubated for 48-72 hours at 35° C, followed by counting of lactose-fermenting colonies. These colonies were then sub-plated in duplicate onto MacConkey agar plates and incubated at 42° C for 24 hours. This was done to identify thermo tolerant coliform species. After the samples containing thermo tolerant coliforms had been identified, further sub-plating was done onto *E. coli*-specific media following standardized laboratory procedures. As one ml of water was filtered, any positive samples contained at least one CFU/ml, equivalent to 100 CFU/100ml. Samples containing growth were scored as positive and those without growth were scored as negative. No attempt was made to quantify the number of colonies on each sub-plate.

### Chlorophyll-a

After being fixed in the field, the samples of suspended algae were frozen upon arrival at the laboratory then analyzed using standard methods (American Public Health Association 1998). Filters were banked, and analyzed several weeks later. At that time extracted chlorophyll was diluted with an additional 8ml of 100% ethanol (total 16ml) and analyzed with a spectrophotometer comparing absorbance before and after addition of 0.1 N HCl at wavelengths of 750, 665, and 664 nm. The

resulting absorbance was used to calculate chlorophyll-a levels following standardized methods (American Public Health Association, 1998).

### Data Analysis

Potential correlations between factors such as sample site location, water temperature, and altitude were examined using multivariate logistic regression. Differences between the chlorophyll-a concentration of each site was analyzed with repeated measures ANOVA. Univariate analysis of coliform and *E. coli* prevalence for the three collection periods and site categories was performed using the Chi square test. Data is reported with standard deviation and 95% confidence intervals where indicated. Results with p-values equal to or less than 0.05 were considered to be statistically significant.

## **Results**

A total of 20 lakes or streams were studied. These sites were categorized by land usage pattern as: 7 Wildlife, 5 Backpack, and 8 Multiuse sites. Elevations ranged from 2,100 m to 3,200 m. All sites were within three km of the main trail. Locations of the sites sampled are shown in Table One.

### Qualitative Field Observations

A surprising biomass of fresh manure was noted along the entire study length of the JMT. However the observed frequency of pack animal traffic was limited. Along Duck Pass Trail large group of approximately 8 stock were passed on trail on the first day of the journey. This was only sighting of actual stock during

sampling period of 6 days. This portion of the trail from Duck Pass to the JMT Junction featured highest frequency of manure, and the trail above Duck Lake (Site 1) had very high occurrence of manure. Additionally at Duck Lake high concentrations of periphyton were observed (Figure 2.1). Site along creek in Reds Meadow, North of JMT/ Duck Pass Trail Junction had large camping site directly next to creek with large amount of stock manure. This indicated that multiple stock had been tied up overnight approximately 10 m from creek. JMT portion south of JMT/ Duck Pass Trail junction to Florence Lake Trail junction contained distinctly less manure than previous section.

Purple Lake (Site 2) was off limits to camping due to United States Forest Service (USFS) restoration project to improve water quality. However, it appeared backpackers were still camping at this site. Purple lake had very high level of periphyton growth and substrate coverage (Figure 2.2).

Virginia Lake (Site 3) had no evidence of periphyton growth in sampling location (Figure 3.1). Warrior creek (site 5) had high degree of periphyton growth (Figure 2.3), though no evidence of pack animal manure was discovered. North Fork Mono Creek (site 7) had no evidence of periphyton growth (Figure 3.4); small quantity of manure was located above sampling site (Mott Lake Trail) indicating infrequent use of trail by stock. Four subsequent sites (sites 9-12) lacked evidence of any stock use, however JMT Side Creek mile 86.6 (Site 9) contained high amount of periphyton while at sites 10-12 no periphyton was observed (Figure 3.3).

Hilgard Branch (Site 13) had high periphyton level, though Hilgard Branch Trail was inspected for stock animal

presence and none was found. This trail is popular with backpackers leaving the JMT to ascend Italy Pass. At Lake Beverly (Site 16, 1.1 miles from JMT) no evidence of stock animals was found even though this historically sees heavy pack animal traffic; however this site had high periphyton levels (Figure 2.4). JMT Side Creek mile 95 (Site 17) was classified as “pristine” and no evidence of stock animals was observed, though some periphyton was found at this site

Observation of backpackers along JMT indicated high degree of awareness in respect to adherence of Wilderness area guidelines for waste disposal, campsite selection, fire regulations, etc. In terms of stock animals, frequency of stock animal waste and waste location indicate high usage, and some cases of noncompliance with Wilderness regulations.

### Periphyton

Periphyton (attached and benthic algae) was noted to be a significant problem in many lakes and streams. Figure 2 presents selected photos of the maximum algae density for 4 of the 20 sites sorted by wilderness land usage. Figure 3 depicts photos from 4 of the sites with the smallest periphyton biomass. Maximum density measurements of periphyton ranged from 0 to 90%, and minimums from 0 to 50% (table 4). Differences in attached algae biomass were found when comparing mean maximal periphyton coverage (%) with usage categories: Pack animals; 59%, Backpack only; 42%, and Wildlife 10%. The difference between wildlife and pack animals sites was statistically significant ( $p < 0.05$ ).

Chlorophyll-a concentration ranged between 0.9 to 10.7 mcg/L . Only 4 sites

were analyzed for chlorophyll as collecting equipment was damaged early in the expedition as a result of alpine weather conditions. Results were: Duck Ck 0.9, Purple Lake 3.3, Virginia Lake 2.2 and Squaw lake 10.7 mcg/L respectively. Comparisons using ANOVA was performed on the data collected from the 4 sites based on land use patterns. The collection numbers were too small to make statistical comparisons.

### Coliforms and *E. Coli*

The prevalence of coliforms and *E-coli* are depicted in Table 2. Overall, the highest prevalence of coliforms was found in Multiuse areas, where 5 of the 8 sites (62%) exceeded the threshold of 100 CFU/100ml. This compares to an overall prevalence of 0 of 5 (0%) in Wild areas, and 2 of 7 (28%) in Backpack sites. Comparison of Mixed use area coliform prevalence to Wild was statistically significant ( $p < 0.05$ ). Comparison of Periphyton levels and Coliform prevalence among the land usage categories showed a correlation of high levels of periphyton with Mixed use areas. Multivariate logistic regression analysis was performed for water temperature, altitude, sampling sites. No significant association between the finding of coliforms and water temperature, altitude, Wild, Backpack, and Mixed sites. *E-coli*  $> 100$ CFU/100ml was detected at only one mixed use site, Purple Lake, and at no other sites.

### **Discussion**

The chief objective of this study was analyze and document the extent of attached algae biomass in lakes and streams along the central JMT between Yosemite and Kings Canyon National Parks. In addition we

aimed to compare any patterns in algae biomass with usage patterns of the surrounding watershed among wildlife, humans and pack animals, and prevalence of coliforms. Our data shows that Mixed-use areas had the highest levels of attached algae biomass. It confirms the prior years study of this segment of trail as the prevalence of coliforms were elevated in mixed use areas when compared with backpack or wildlife watersheds.

Visible benthic algae and aquatic plants occur first after the addition of nutrients, as one of the first macro indicators of eutrophication (Horne and Goldman 1994). If the process continues, suspended algae levels also rise later in the process as nutrient bioavailability increase. Our finding of large amounts of visible periphyton biomass in nearly all Backpack and Mixed use sites shows that human activity is associated with a significant increase in visible periphyton, when compared to the natural wild watershed sites. This increase in eutrophication is concerning and threatens the water supply (Conley 2009). Our finding of lower periphyton biomass levels in wild sites implies low levels of phytoplankton and is not surprising. This may relate to the their natural oligotrophic state in the absence of added nutrients from the presence of humans or domesticated animals.

The finding that periphyton biomass is directly proportional to coliform prevalence is supports previous observations which associate pathogenic bacteria with algae (Islam 2007). Pathogenic bacteria have higher nutrient demands than environmental adapted bacteria. For example, over 95% of Vibrio species of bacteria in marine environments are not free

floating, but attached to various algae species. In this instance Manure from JMT pack animals has a 2 fold impact on water quality, first it provides large quantities of Nitrates and Phosphates which stimulate the algae growth, and secondly pack animals excrete huge numbers of fecal bacteria. (Miettinen et al, 1997, Tao et al, 2007, Yers et al, 2005). By one estimate, each 100 group of pack animals deposit 1 trillion bacteria into the environment, some directly into lakes and streams.

Chlorophyll-a concentrations have been noted to have increased in larger lakes in the Sierra Nevada as a result of pollution and deposition of growth stimulating compounds and elements (Winder et al, 2007). Algae biomass surveys are important, as water with higher algae levels also supports increased bacterial survival and growth (Tao et al, 2007). Conditions which support elevated algae biomass also support the growth of blue green algae, which can release several types of toxins harmful to humans and animals. For example in the Swiss Alps, a report described 100 mountain cattle deaths, as a result of the release of algae toxins, from blue green algae whose growth occurred from cattle manure pollution into alpine water (Mez et al, 1997). To our knowledge, no extensive survey of algae biomass has been undertaken in the High Sierra Nevada wilderness areas. Unfortunately we were able to obtain chlorophyll-a measurements from 4 sites, and unable to have the power for statistical comparisons. However the values we obtained from lakes are consistent with prior measurements in the sierra, including the central section of the JMT (Ursem 2009) and Humphrey's basin (Orlando and Knopp, 2005). The Tahoe Research Institute monitors Lake Tahoe frequently and surface

chlorophyll-a in the center of the lake is 1.0 mcg/L (Winder et al, 2008).

Coliforms prevalence provides estimation for the risk for acquiring pathogenic waterborne disease (American Association of Public Health, 1998). Coliform bacteria have been used as indicators of fecal contamination, including for other organisms such as *Giardia*, in water across the United States. In wilderness areas, coliforms may originate from one or a combination of sources including 1) wild animals endemic to the area; 2) humans visiting during daylight; 3) backpackers who camp overnight; 4) stock or pack animals, such as horses and mules; and 5) cattle or sheep grazing. Coliform pollution of wilderness areas by humans may occur through inadequate burial and disposal of fecal material. In addition, bathing or swimming in lakes may also result in microbial pollution (Blostein, 1991). Pack animals pollute by deposition of manure either directly into lakes and streams or indirectly by deposition of manure onto trails or meadows, and these animals have been documented to import pathogenic microorganisms into the Sierra wilderness (Yers et al, 2005, Atwill 2004). This manure may be washed into waterways by either summer storms or annual snowmelt (Gurber et al, 2006, Durkee 2008, unpublished data) Our findings are consistent with prior studies in the Sierra showing the highest prevalence of coliforms occur in areas used by domesticated animals (Derlet et al, 2008). The lower prevalence of coliforms in Wild areas and higher prevalence in mixed use and Backpack sites suggest a significant contribution from humans. Additionally, the Backpack sites were noted to have a similar density of campsites as the mixed use sites, suggesting increased coliform prevalence at mixed use sites is from the impact of pack

animals. Therefore, our findings in this study confirms the association of pack and stock animal presence with poorer water quality. Increases in bacterial markers imply higher risk of waterborne disease to humans. In addition to pathogenic forms of *E. coli* and *Salmonella*, pack and stock animals in the Sierra have a 3 to 6% prevalence of *Giardia* (Atwill, 2004, Johnson et al, 1997). Although this number may seem small, according to a study conducted in Yosemite, each infected animal excretes over 10,000,000 cysts into the environment each day, placing wilderness hikers at risk if water is untreated (Atwill, 2004).

### Limitations

Many unforeseen obstacles were encountered during the course of this project. The aforementioned loss of chlorophyll-a filters presented a problem. Overall numbers of samples for coliforms were small, although consistent with prior studies. Estimation of periphyton biomass is visual, and although photographically archived, may be subject to different interpretations from various individuals.

### **Conclusion**

A high biomass of periphyton was found in lakes and streams adjacent to areas mixed-use areas those areas use by stock together with backpackers. In addition the prevalence of coliforms was highest in mixed use areas. In contrast, sites used only by backpackers had fewer coliforms and much less periphyton biomass. Wild sites showed little periphyton biomass. Taken together this study provides evidence that

pack and stock animals traffic are major contributors to the eutrophication of lakes and streams in the Sierra Nevada along the JMT.

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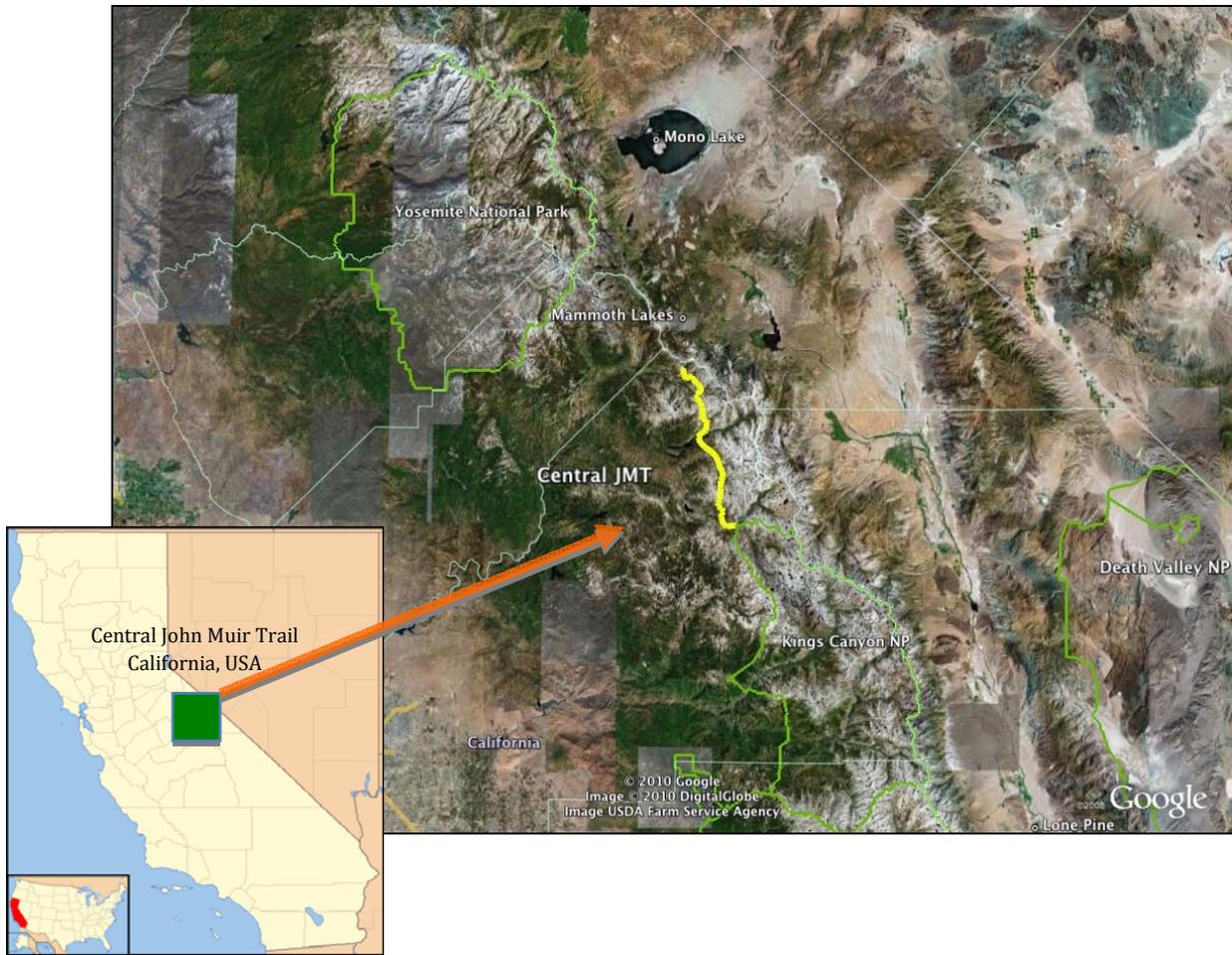
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**Figure 1.**



**Figure 2: Photos from 4 sites with the largest biomass of periphyton:**



**Fig 2.1-Duck Lake**



**Fig 2.3 - Warrior Creek**



**Fig 2.2 - Purple Lake**



**Fig 2.4 - Lou Beverly Lake**

**Figure 3: Representative photos from 4 sites with the least visible periphyton:**



**Fig 3.1 - Lake Virginia**



**Fig 3.2 - Silver Pass Creek**



**Fig 3.3 - JMT Side Ck mile 87**



**Fig 3.4 - North Fork Mono Creek**

**Table 1.** Specific site location by watershed category (Elevation above sea level in meters)

<b>Wildlife</b>	<b>Backpack</b>	<b>Multiuse</b>
JMT Side Creek mile 86.6 (2,780) by Aspens	Squaw Lake (3,133)	Duck Creek (3,072)
JMT Side Creek mile 86.9 (2,780) North side of Recess Peak	Warier Lake (3,188)	Purple Lake (3,026)
JMT Side Creek mile 87 (2,780)	North Fork Mono Creek above JMT (2,740)	Virginia Lake (3,151)
JMT Side Creek mile 88 (2,780) (South side of Recess peak)	Creek South of Crater Meadow (2,658)	Fish Creek crossing (2,804)
JMT Side Creek mile 95 (3,267) just South of Seldon Pass	Hilgard Branch (2,850)	Silver Pass Creek cross (2,926)
	Orchid Lake Outflow Stream (2,920)	Lou Beverly Lake (3,072)
	Senger Creek (2,975)	Marie Lake, Muir Wilderness (3,216)
		Sally Keys Lake (3,121)

**Table 2.** Coliform prevalence and observed Periphyton Biomass by watershed category. Data presented as prevalence of Coliforms (%), and Periphyton benthic coverage (%).

	<b>Coliforms</b>	<b>E-Coli</b>	<b>Periphyton</b>
<b>Wild</b>	0	0	10
<b>Backpack</b>	28	0	42
<b>Mixed-use</b>	63	13	59