

# **PRELIMINARY RESEARCH ON *APHANIZOMENON FLOS-AQUAE* AT UPPER KLAMATH LAKE, OREGON**

Investigations to Set Direction for Research of Factors  
with Potential for Influencing *Aphanizomenon* Growth  
at Upper Klamath Lake

Funded by the United States Department of the Interior  
Fish and Wildlife Service  
Purchase Order Number 1448-10181-04-M360(KY)

**Prepared for**

**Klamath Basin Ecosystem Restoration Office**  
Klamath Falls Fish and Wildlife Office  
Klamath Falls, OR

**Prepared by**

***Aquatic Scientific Resources***  
***Wetland Research Consortium***  
Portland, OR

October, 2004

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**Prepared for**

**Mr. David A. Ross**  
Restoration Supervisor  
**Klamath Basin Ecosystem Restoration Office**  
Klamath Falls Fish and Wildlife Office  
6610 Washburn Way  
Klamath Falls, OR 97603

**Prepared by**

***Aquatic Scientific Resources***  
N. Stan Geiger, Project Manager  
12425 SW 57<sup>th</sup> Avenue  
Portland, OR 97219-7117  
**Project Number 20046-5**

**And**

Robert Gearheart, **HydroResources, International**, Arcata, CA  
Eric Henry, **BioScience Services**, Philomath, OR  
John Rueter, **Portland State University**, Portland, OR

October 29, 2004

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#### **A. (for Section 2.0)**

- Table of treatments and results (Miller and Tash 1967)- scanned GIF images.
- Miscellaneous Literature Searches of DOC, UV, *Daphnia*, AFA in MS Word files

#### **B. (for Section 3.0)**

- MS Excell spreadsheets of Klamath Tribes-Bureau of Reclamation Upper Klamath Lake field measurements and laboratory water chemistry results (UKL 1990-2003 includes list of all Klamath Tribes-BR sampling sites) zooplankton data (UKLZOOPS 1990-1996) and phytoplankton data (UKL PHYTO 1990-1996)
- Spatial Solutions 2000 satellite images of UKL
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- MS Word file of Station Locations for Section 4.0.

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- Water quality data for enclosure assessment August 3-September 1, 2004 (MS Excel spreadsheet Limnocorral WQ Data)
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- Pelican Butte wetland assessment water quality data: January-February 2000. (MS Word file)
- Results of literature searches on water quality functions of wetlands in or associated with lakes (two MS Word files)
- NWI Palustrine wetland data for UK Basin HUC's (source M. Neuman, BR, Klamath Falls.

## ACKNOWLEDGMENTS

Our Aquatic Scientific Resources Wetland Consortium would like to express our appreciation to Dave Ross, Restoration Supervisor for the Ecosystem Restoration Office, USFWS, Klamath Falls for his encouragement and support through this year which developed and grew from the Department of Interior Research Needs Workshop held in early February 2004. We also acknowledge the support of Tim Mayer, with the Engineering Division of the USFWS, Portland, whose research on the wetlands of the Lower Klamath Lake NWR demonstrated the value of knowing more about the water quality functions of wetlands in the Klamath Basin and underscored the need for similar future research on wetlands of the UKL Basin.

We also express our appreciation to the following for their support and their willingness to share information and discuss the feasibility of our hypothesis about why UKL changed during the past century.

Mark Buettner, USFWS-BR, Klamath Falls  
Dan Snyder, USGS, Portland  
Jake Kann, Aquatic Ecosystem Sciences, Ashland  
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Mike Neuman, Bureau of Reclamation, Klamath Falls  
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Steve Kirk, ODEQ, Bend  
Ken Rykbost, OSU Agriculture Experiment Station, Klamath Falls  
Rodney Todd, OSU Extension Service, Klamath Falls  
Kurt Carpenter, USGS, Portland

And finally, two of us (Geiger and Henry) who knew the late Harry Phinney, former professor and phycologist at Oregon State University, express our appreciation of him as a person but also call attention to the interest he aroused in the algae of Upper Klamath Lake, particularly the cyanobacteria *Aphanizomenon flos-aquae*, the reason for this project.

"The exploring party camped on a hilly promontory which projected into the lake, where the eastern shore contained a wide band of tule. The party was greeted by two squaws with lake fish. The water taken from the lake had a dark color and a disagreeable taste, occasioned apparently by decayed tule" (Anon. 1857).

"Williamson River, the principal stream flowing into Upper Klamath Lake, is of considerable size and much importance in its relations to the fishes of the lake...It flows before entering the lake through a low, flat, marshy plain covered with tules and swamp grass. On the banks of the river is a rich growth of willows. The current is very moderate. We rowed about 2 miles or more up the river and noticed no important change in it. The water in the river was very dark, evidently stained to some extent by vegetation." (Evermann and Meek, 1897).

"Cold night, nearly froze. Got started at 8:30 drove down a creek to Billy Balls' (about 3 miles) then across his meadow to a yellow pine ridge and to Kellogg's (Indian) about 8 miles (Indian said 2 1/2!!) where took lunch, then across his meadow and into a yellow pine forest, partially along edge of Klamath Marsh, then through woods, and down to the marsh again where we camped. Across the marsh we saw Mt. Scott and Mt. Theilson though the air was very hazy. Mt. Scott has good deal snow. Mt. Theilson none- too sharp. Klamath Marsh is a wonderful one, large, full of reeds, water-lilies, tules, etc. Waterbirds common...Our camp tonight is a splendid one in all regards except the swamp water." (B.W. Evermann, August 9, 1897).

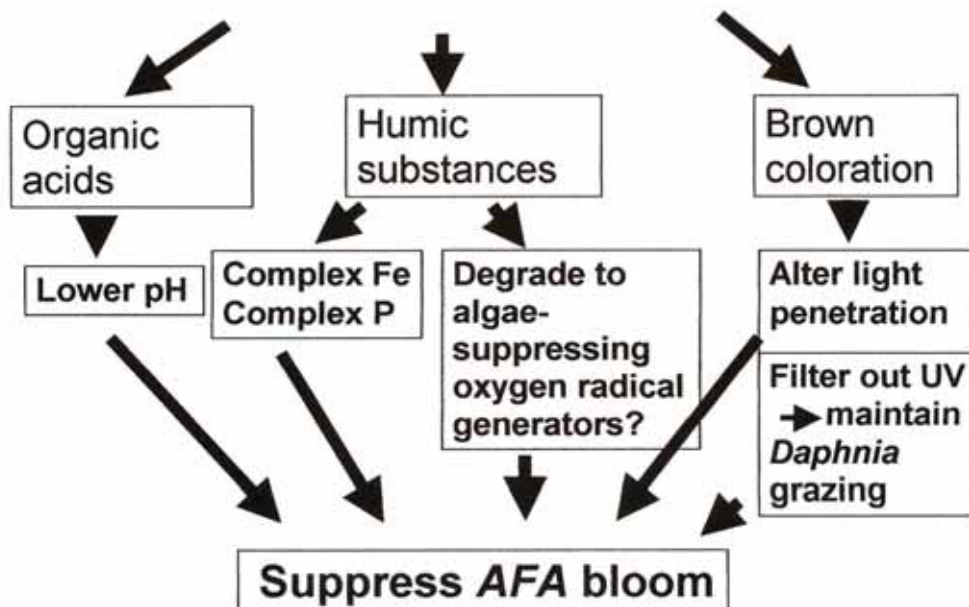
# 1.0 INTRODUCTION

## OVERVIEW OF REPORT

The organizing goal of the work completed and reviewed in this report is a thorough understanding of the cyanobacteria species *Aphanizomenon flos-aquae* (L) Ralfs (designated AFA in this report). At this point in time our reach, expressed in this goal, exceeds our grasp. The realistic objective of our work under this contract with the U. S. Fish and Wildlife Service (FWS), however, is not the thorough understanding, but preliminary analyses and development of techniques that will help move future research towards that thorough understanding.

The elements of the work are suggested in Figure 1 that notes factors with potential for suppressing the growth of AFA. Despite the complexity of the potential effects of wetland effluents on AFA growth illustrated in Figure 1, there is additional complexity not shown related to wetland habitat as sources and sinks of nutrients that limit or support the growth of AFA and other algae species. AFA is a very important algal species in Upper Klamath Lake.

Figure 1. Factors with potential for suppressing growth of *Aphanizomenon flos-aquae*.



It is our operating assumption about more complete research of these factors yet to be undertaken that unless we understand thoroughly the complexity of wetland loss effects at Upper Klamath Lake and the interaction of these effects we will continue to be handicapped in our approaches to changing the lake for improved fish habitat.

The elements of the preliminary research tasks completed under this U. S. Fish and Wildlife Service (FWS) purchase order included the following (Aquatic Scientific Resources 2004):

- Develop a preliminary analysis of algae-zooplankton-water chemistry relationships (Klamath Tribes-Bureau of Reclamation data base 1990-present);
- Conduct laboratory assay of the "potency" of multiple-source humic compounds originating from typical wetland plant vegetation at or near the lake, and from decomposing barley straw;
- Chemically characterize humic compounds from multiple sources;
- Establish laboratory cultures of *Aphanizomenon flos-aquae*;
- Specify what appear to be UKL-appropriate methods for field approaches to assaying effects of multiple-source humic compounds on growth of *Aphanizomenon flos-aquae*;
- Define an approach to in-lake research on wetland water quality functions, with summary of past research conducted on in-lake wetlands;
- Produce a more comprehensive literature search and review, with synopses of findings, focusing on humics-algae-zooplankton-UV and barley straw-humics-algae-UV.

## **TESTING A HYPOTHESIS FOR CHANGED LAKE CONDITIONS**

There are many reasons for supporting multiple approaches to improving the water quality of Upper Klamath Lake (UKL). There is general agreement that during the first half of the 1900's UKL changed from a eutrophic to a hypereutrophic lake. This change altered the habitat of algae, zooplankton, benthos and fish of this largest lake in Oregon. While two of the sucker species in the lake have been listed as endangered (Lost River sucker [*Deltistes luxatus*] and Shortnose sucker [*Chasmistes brevirostris*]), it is likely that many other species in the lake have been severely impacted by changes in the lake.

Seventeen years before the listing of the two sucker species in 1988, the last diking and draining of in-lake wetlands occurred at the site of what is now the Agency Lake Ranch of the Bureau of Reclamation. Between 1889 and 1971 approximately 35,000 acres of wetlands at various locations around UKL that were seasonally inundated by the lake were diked and drained. In the report that follows, we review results of a multi-strand investigation of approaches to a program of research that assay factors with potential for influencing growth of *Aphanizomenon flos-aquae*. Most of the approaches relate to lost wetland functions and their importance related to cyanobacteria growth: nutrient uptake and effects of wetland decomposition. Included in the mix however is the known influence of decomposing barley straw on cyanobacteria growth and the present barley production in Klamath County on 24,000 acres of land. There is the potential for the same or similar suppressing effect on algae growth from the decomposition products deriving from wetland plants *and* barley varieties grown in the county.

## **ASR WETLAND RESEARCH CONSORTIUM**

The principal investigators of the Consortium include:

- Stan Geiger, M.Sc., Project Manager, Professional Wetland Scientist, Aquatic Scientific Resources, Portland, OR
- Eric Henry, Ph.D., Bioscience Services, and courtesy appointment as Research Associate in the Dept. of Botany and Plant Pathology at Oregon State University, Corvallis, OR
- John Rueter, Ph.D., Professor, Environmental Sciences Program, Portland State University, Portland, OR
- Robert Gearheart, Ph.D., HydroResources International, Professor of Environmental Resources Engineering, Humboldt State University, Arcata, CA

Principal responsibility for authorship of report sections is as follows:

- S. Geiger (Sections 1.0, 3.0 and 5.0)
- E. Henry (Section 2.0)
- J. Rueter (Section 4.0)
- R. Gearheart and S. Geiger (Section 6.0); Section 6.0 combines recommendations from each of the principal investigators for future research.

The Consortium members have worked together since fall of 2001 to evaluate and analyze the role of wetlands in the Klamath Basin and the likely consequences of their loss from both the watershed of UKL and from within the lake. The primary aspects of this thought have been

presented in the MS Power Point presentation (*Rise of superabundant cyanobacteria [**Aphanizomenon**] populations at Upper Klamath Lake with progressive loss of wetlands*) made at the USGS-Bureau of Reclamation UKL Research Needs Workshop February 3-6, 2004 (included as Attachment A of this proposal).

The other collaborators on the work who include:

Yangdong Pan, Ph.D., Associate Professor, Environmental Sciences and Resources, Portland State University, Portland, OR

Allan Vogel, Ph.D., Life Sciences Instructor, Chemeketa Community College, Salem, OR

Further information on Principal Investigators and collaborators is have been provided in Aquatic Scientific Resources (2004)

## 2.0 CULTURING APHANIZOMENON FLOS-AQUAE

### INTRODUCTION

#### ***Taxonomy of *Aphanizomenon flos-aquae*: implications for usefulness of the literature***

The scientific name usually applied to the species of blue-green algae (cyanobacteria) that dominates the phytoplankton in Upper Klamath Lake (UKL) is *Aphanizomenon flos-aquae* (L) Ralfs. There exists a considerable literature of taxonomic, ecological, and physiological studies on "*Aphanizomenon flos-aquae*," so one would expect that much could be learned from this literature that would be helpful for understanding why this species is so successful in UKL. However, recent molecular genetic studies now show that several morphologically similar forms that have all been called *Aphanizomenon flos-aquae* are in reality genetically distinct, and in some cases are not closely related (Gugger *et al.* 2002, Iteaman *et al.* 2002, Laamanen *et al.* 2002, Li *et al.* 2000, 2003, Lyra *et al.* 2001). Therefore the literature on "*Aphanizomenon flos-aquae*" must be interpreted with caution, because often researchers have been studying organisms that are not in fact similar to the species found in UKL.

Fortunately, one of the most easily observed morphological characteristics of the UKL species is its occurrence in large colonies up to 1-2 cm in length, often described as "flakes." The molecular genetic evidence suggests that colony formation is a distinct character. Some of the *Aphanizomenon flos-aquae* literature clearly describes the organism being studied as either colonial or solitary, but much of it does not. So any studies of solitary species or those not characterized clearly as colonial should probably not be interpreted as informative about the UKL *Aphanizomenon flos-aquae*. It is very significant that only non-colonial forms have been found to produce toxins, so it can be concluded that the species in UKL is non-toxic.

It should be noted that when H.K. Phinney began to study *Aphanizomenon flos-aquae* in UKL, he was so impressed by the large size of the colonies (often significantly larger than had been described in the literature) that he assumed that this represented a new species. He referred to this putative new species as "*Aphanizomenon oregonensis*" (Phinney 1959) or "*Aphanizomenon* n. sp." (Phinney 1960) but he never published a description to formally establish a new name. As he gained more experience with populations in UKL and other locations in Oregon he realized that designation of a new species was not called for, and he

reverted to the use of *Aphanizomenon flos-aquae* (O'Flaherty and Phinney 1970).

## **LITERATURE REVIEW**

### **Culture and physiological studies of *Aphanizomenon flos-aquae***

Only a few of the published studies on the culture and physiology of "*Aphanizomenon flos-aquae*" from localities other than UKL clearly describe the alga studied as colonial (McLachlan *et al.* 1963, Rose 1934, Wildman *et al.* 1975). These studies focused on the colonial habit or the formation and germination of akinetes, specialized resistant cells ("spores" or "overwintering stage") produced by *Aphanizomenon flos-aquae*. These reports all noted the difficulty of obtaining vigorous vegetative growth, or consistent germination of the akinetes. In their study of *Aphanizomenon flos-aquae* from UKL, O'Flaherty and Phinney (1970) noted the difficulty of culturing and were pleased to be able to maintain isolates for over a year in the colonial form, even though growth rates were quite slow, at a maximum averaging only 0.25 doublings/day. Significantly, initially they were only able to maintain cultures at 15°C, and after improving the culture medium also at 20°C, but ostensibly not at higher temperatures even though temperatures in UKL during the summer *Aphanizomenon flos-aquae* bloom can easily reach 25-28°C, indicating that some aspect (medium, light) of their culture conditions was still very suboptimal. Other workers experienced in the culture of algae have also encountered difficulties in culturing isolates of *Aphanizomenon flos-aquae* (Lembi, pers. com. 2003).

While the author was employed at PuriPonics LLC (Portland, OR), in collaboration with J. Rueter (Portland State Univ.) he was engaged in development of mass-culture methodologies for *Aphanizomenon flos-aquae*. We found that we could successfully make culture isolates of *Aphanizomenon flos-aquae* from UKL and culture them at high densities (over 1g dry wt./L), with optimal productivity obtained at temperatures of 25-27°C. We found that the size of the colonial flakes decreased as the isolates were progressively rid of the associated bacterial flora, but the tendency to form colonies was never lost. We also found that even very high concentrations of nitrate did not eliminate the formation of heterocysts (specialized nitrogen-fixing cells), but that heterocysts disappeared in cultures grown in media containing ammonium. Akinetes were also not seen in cultures kept in phosphorous-replete conditions, but both heterocysts and akinetes re-appeared when nitrogen and phosphorus became exhausted. One other finding may have great significance for understanding the ecology of *Aphanizomenon flos-aquae* in UKL: we found that although the isolates tolerated a pH as high as 10,

the cultures would spontaneously lyse after a few hours at pH 6.5, a response not known for this species from any reports in the literature.

### ***Aphanizomenon flos-aquae* in Upper Klamath Lake**

Studies of *Aphanizomenon flos-aquae* in UKL have focused on the lake conditions associated with the development of nuisance blooms by this species. The earliest studies (Phinney 1959, Phinney and Peek 1960) quantified phytoplankton species composition throughout the year and attempted to associate the chemistry of the lake water with the development of the *Aphanizomenon flos-aquae* blooms.

Miller and Tash (1967) reported on UKL AFA primary productivity studies using the isotope  $^{14}\text{C}$  that were performed in late summer and fall of 1996 by C. Goldman of the University of California, Davis, CA. Studies were conducted in the field and in the laboratory. In the field, samples of lake water with the natural concentrations of algae present in lake in late August were cultured *in situ* with additions of various concentrations of forms of potential algae macronutrients (nitrogen, phosphorus and potassium) and ferric citrate. In each experiment, one sample was inoculated only with  $^{14}\text{C}$  as a control and other samples were inoculated with  $^{14}\text{C}$  and with varying concentrations of elements (see Appendix A of this report for the table of treatments and results). In the laboratory at Davis, in late October, experiments differed only in that water from the lake was incubated under cool-white fluorescent lights at 16.0°C with constant rotary movements. Samples in the field or in the laboratory were incubated for 4-hr periods. Counts of  $^{14}\text{C}$  were made from filters of the various samples. With the exception of the addition of sodium chloride and an ammonium salt ( $\text{CH}_3\text{COONH}_4$ ), there appeared to be no significant change relative to the control in “AFA” response (this was after all a natural assemblage of phytoplankton the composition of which was not reported).. The choice of late August and October for assaying AFA populations may have been a reason for generally non-responsive assays, although responses of controls in field studies, as  $^{14}\text{C}$  uptake, ranged from 74 to 1477, with responses of laboratory controls ranging from 75 to 1312. This would suggest varying physiological condition of natural samples assayed.

More recently the importance of akinete recruitment and zooplankton, particularly *Daphnia*, grazing effects on *Aphanizomenon flos-aquae* bloom development have been studied (Barbiero and Kann 1994, Kann 1998). It was established that akinetes do contribute to the growth of *Aphanizomenon flos-aquae* populations in the spring, but that they do not transport appreciable phosphorus from the sediments into the water column. Findings that high biomass of *Daphnia* corresponded to high

*Aphanizomenon flos-aquae* biomass, with *Daphnia* failing to keep *Aphanizomenon flos-aquae* in check, are consistent with other studies in other types of lakes. It has been shown that once *Aphanizomenon flos-aquae* colonies reach a critical size, *Daphnia* are not capable of effectively grazing on the alga, so their grazing pressure is directed toward smaller species, helping eliminate them as competitors and ensuring the dominance of *Aphanizomenon flos-aquae* (Ganf 1983, Holm *et al.* 1983, Kasprzak and Lathrop 1997). This confirms that colony-formation is indeed a character critically important for this species, ensuring its success in dominating the phytoplankton of UKL.

### **Effects of humic substances on algae**

Although humic substances are recognized as significant components of many natural waters, their effects on biota are complex (Steinberg and Muenster, 1985, Stewart and Wetzel 1982, Wetzel 2001). Some studies indicate that even though they can block transmission of harmful UV wavelengths in the water column, they can nevertheless result in the inhibition of algae due to binding of essential metals or promotion of the formation of damaging forms of oxygen (Gjessing and Kaellqvist 1991, Imai *et al.* 1999, Kaczmarska *et al.* 2000, West *et al.* 1999). Other effects of dissolved organic matter on algae have been reported including humics inhibiting alkaline phosphatase (Kim and Wetzel 1993); the effect of dissolved organic material on light availability (Havens *et al.* 1998); various interactions among dissolved iron, dissolved organic matter and phosphorus (Jones *et al.* 1993; Guildford *et al.* 1987, Jackson and Hecky 1980); and complexation of toxic metals by dissolved organic matter (Xue and Sigg 1999). See Section 4.0 of this report for a review of other effects of dissolved organics on algae.

Peek (1963) investigated the effects of humic marsh water from Klamath Marsh on a different filamentous blue-green alga (*Anabaena cylindrica*), and found that, using the same added mineral salts, media made with marsh water supported better growth than medium made with distilled water. However, the relevance of this study to the question of the effects of marsh effluents on *Aphanizomenon flos-aquae* in UKL is doubtful for several reasons: The test alga was a different species, in fact not even a strain isolated from UKL; media with marsh water were not compared to media made from UKL water with no marsh influence, only with medium made with distilled water; mineral salts were added to all the media, resulting in a very different chemical environment from that in UKL; the effects of other natural conditions in UKL, such as seasonal temperature variations and spectral quality under sunlight (which may be an important factor in the photochemical dynamics of humic substances) were not considered.

The results of literature searches on keywords including *Aphanizomenon*, UV, Daphnia, DOC, humics, etc. are provided in Appendix A of the Appendix CD accompanying this report.

## **NEW ISOLATES OF *Aphanizomenon flos-aquae* from UKL**

### **Source and cultivation conditions**

We established new isolates of *Aphanizomenon flos-aquae* from collections made by S. Geiger at UKL in August 2003 and July 2004. Cultures were initially isolated into a growth medium (the medium components are listed in Table 1) made up with UKL water collected at the same time the algae samples were taken. The water was autoclaved and initially supplemented with an EDTA-metals mix, sodium tripolyphosphate, and selenium; my earlier work has shown selenium allows UKL *Aphanizomenon flos-aquae* to tolerate bright light. Fixed nitrogen was omitted from the original isolation medium to suppress growth of contaminating species of algae, most of are not capable of nitrogen fixation.

Table 1. Concentrations of salts added to UKL water to make culture medium

disodium EDTA	Na <sub>2</sub> EDTA·2H <sub>2</sub> O	<b>20.2 <math>\mu</math>M</b>
ferric chloride	FeCl <sub>3</sub> ·6H <sub>2</sub> O	<b>4 <math>\mu</math>M</b>
manganese chloride	MnCl <sub>2</sub> ·4H <sub>2</sub> O	<b>7 <math>\mu</math>M</b>
zinc chloride	ZnCl <sub>2</sub>	<b>3.2 <math>\mu</math>M</b>
cobalt chloride	CoCl <sub>2</sub> ·6H <sub>2</sub> O	<b>0.08 <math>\mu</math>M</b>
sodium molybdate	NaMoO <sub>4</sub> ·2H <sub>2</sub> O	<b>0.1 <math>\mu</math>M</b> <b>0.0008</b>
cupric sulfate	CuSO <sub>4</sub> ·5H <sub>2</sub> O	<b><math>\mu</math>M</b>
Na tripolyphosphate	Na <sub>5</sub> P <sub>3</sub> O <sub>10</sub>	<b>100 <math>\mu</math>M</b>
Di-potassium phosphate	K <sub>2</sub> HPO <sub>4</sub>	<b>200 <math>\mu</math>M</b>
sodium selenite	NaSeO <sub>3</sub>	<b>0.17 <math>\mu</math>M</b>
sodium nitrate	NaNO <sub>3</sub>	<b>4 mM</b>

## Isolation methodology

Single filaments or small colonies of a few filaments were isolated under a dissecting microscope using fine forceps, and transferred by 50-microliter micropipet through a series of 6 brief (< 1 min) immersions in sterile medium or distilled water, in order to wash off and dilute away contaminating organisms. Each washed isolate was then transferred to a well in a 24- or 48-well polystyrene culture plate, containing medium.

A total of 120 isolates from August 2003 (two 48-well plates, one 24-well plate) and 144 isolates from July 2004 (two 48-well and two 24-well plates) were initially made. Isolates were cultured in a 17°C culture chamber or at room temperature (21-27°C) under continuous cool-white fluorescent light at 10-20 micromol/m<sup>2</sup>/sec.



Figure 2. 24-well plate with isolates.

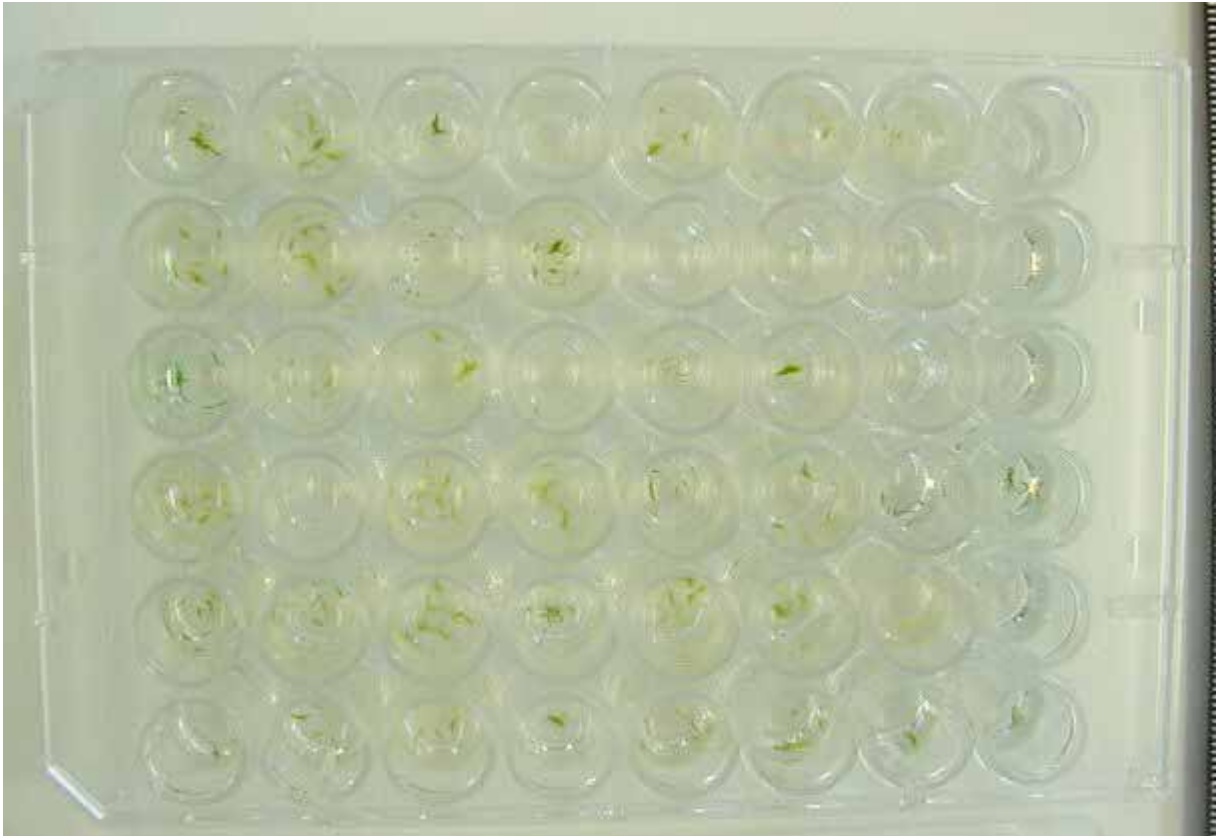


Figure 3. 48-well plate with isolates.

### **Amplification of isolates**

After 4-8 weeks it became clear whether a sample well contained viable *Aphanizomenon flos-aquae*, which by now had developed into small colonial flakes, and was free of contaminating algae. Approximately 25% of the sample wells contained apparently uni-algal isolates, but many consisted of more than one colony, so each was transferred to a new sample well. These uni-algal isolates were transferred to new well plates (one 48-well and one 24-well plate of the August 2003 samples; three 24-well plates of the July 2004 samples) containing medium with added nitrate and potassium phosphate to encourage faster growth of *Aphanizomenon flos-aquae* and permit any undetected contaminating algae to grow and become evident.

When isolates developed into colonies several mm long, they were transferred to 25 ml Erlenmeyer flasks on a rocker platform (~40 strokes/min) at 23-25°C and continuous light of 10-50 micromol/m<sup>2</sup>/sec, with higher light intensities used for denser cultures. As growth continued, each isolate was further amplified by sequential transfer to 50 ml, 250 ml, and 500 or 1000 ml flasks on a reciprocating

shaker. Although faster growth can usually be obtained by aerating the flask cultures with a stream of air bubbles, it is not practical to do this with a large number of flasks.

*[The number of isolates ultimately available will not be certain for a few weeks, when there has been time for more growth]*

Development of these isolates was rather uneven, with some growing much faster than others. The reasons for this are unknown; besides possible genetic differences, it is possible that the associated bacterial flora may differ, depending on how thoroughly each isolate was washed during the initial isolation. There also appears to be a density-dependence effect, with higher-density cultures growing faster; possible the alga secretes organic substances that complex with essential trace metals and make them more available.



Figure 4. Flask cultures



Figure 5. Flask cultures on rocker table.



Figure 6. Flask cultures on reciprocating shaker.

### **Use of isolates for assay of AFA inhibition by plant decomposition products**

These uni-algal isolates will be very useful in assays of the effects of plant decomposition products (marsh effluents, barley straw extracts) on *Aphanizomenon flos-aquae*. They will provide a ready supply of study material, eliminating the necessity of making new field collections, which is impractical or even impossible in the winter. The algae will be in a known physiological state, well-adapted to laboratory conditions, so there will be no confounding factor of physiological shock from the transfer from field conditions to the laboratory. Repeated experiments can be carried out on individual isolates, so that strain-to-strain variation will not be a confounding factor. The puzzling results of the 1966 <sup>14</sup>C studies of natural UKL August and October AFA populations reviewed above [Literature Review] led Miller and Tash (1967) to recommend for future work "...to study the requirements of the algae in question in pure cultures where known amounts of elements are present in the culture medium and can be more precisely manipulated."

Besides being ideal for study of short-term (seconds to hours) effects, uni-algal cultures are essential for assessing longer-term (days to weeks) effects, because in such experiments no other algal species, which could suppress or out-compete *Aphanizomenon flos-aquae* over these longer time periods, should be present.

### **Laboratory studies**

Longer-term (days to weeks) culture studies of the effects of plant decomposition products will be useful for more directly assessing the effects of these substances on the development of *Aphanizomenon flos-aquae* populations in UKL. Such phenomena as gradual adaptation to adverse conditions, or conversely, cumulative damage, can be observed. The significance of even fairly short-term effects such as recovery from photo-damage during the night can only be reliably assessed by experiments running several days, which is otherwise very difficult with *Aphanizomenon flos-aquae* because field material of this species typically declines very rapidly in condition with a few days of collection. Seemingly small effects on growth rates can be amplified by allowing a culture to grow for a number of days, to give a more direct representation of the significance of such effects for *Aphanizomenon flos-aquae* populations in UKL

### ***In situ* field studies**

The uni-algal isolates can be used in small-volume field experiments, such as bottle tests incubating the isolates in filtered UKL water suspended in the lake, or incubation in microporous chambers that allow lake water but not algae or grazers to enter, to study the effect of lake conditions on *Aphanizomenon flos-aquae* material that is well-defined and free of interferences by other organisms. Such chambers could be suspended directly in the lake or inside limnocorrals containing modified lake water (e.g. with added marsh effluents, barley straw, etc.). Large volumes (e.g. 20 L carboy cultures) could even be produced for inoculation of limnocorrals with known *Aphanizomenon flos-aquae* strains, or for assessment of growth potentials in early spring conditions before there is sufficient *Aphanizomenon flos-aquae* in the plankton of UKL to readily assess its growth within the relatively small volume of a limnocorral.

## **DISCUSSION**

### **Need for characterization of genetic diversity of AFA in UKL, and relationship to AFA found in other locations**

Although *Aphanizomenon flos-aquae* has dominated the summer plankton of UKL at nuisance levels for decades, as yet nothing is known about its population biology in the lake. It has been shown that morphologically indistinguishable, co-occurring strains of the sometimes toxic blue-green algae *Microcystis* can be distinguished by molecular genetic methods (Bittencourt-Oliveira and do Carmo 2003, Neilan *et al.* 1997, Pan *et al.* 2002), so these methods should be appropriate for distinguishing whether *Aphanizomenon flos-aquae* in UKL is genetically homogeneous. If strain heterogeneity is found, this suggests that findings derived from study of only a few isolates may not have general applicability, calling for physiological experimentation on different strains to determine whether some are seasonally or otherwise specialized for particular conditions.

Molecular genetic characterization of *Aphanizomenon flos-aquae* in UKL would also present the possibility of comparing similarity with *Aphanizomenon flos-aquae* strains from other locations, possibly justifying use of experimental results obtained on similar strains from other locations to understand UKL phenomena.

### **Further investigation of factors affecting the physiology of *Aphanizomenon flos-aquae***

Despite the success of *Aphanizomenon flos-aquae* in dominating the plankton of UKL, this species exhibits some physiological peculiarities that are not yet well understood. It is surprisingly sensitive to certain environmental conditions, and field samples deteriorate rapidly after collection, so that if a sample is placed in a culture incubator often after a few days only other algal species are detectable. Small isolates are very slow growing, and several investigators experienced in algal culture have failed to make successful isolates, yet it has proved possible to mass-culture UKL isolates at high densities in volumes as great as several thousand liters (author's unpublished observations). This species is surprisingly sensitive to low pH, 6.5 proving lethal after a few hours. Selenium appears to be necessary to provide tolerance to bright light, a requirement not reported for other algae. Finally, despite possessing specialized nitrogen-fixing cells (heterocysts), *Aphanizomenon flos-aquae* from UKL appears to be nitrogen-limited under otherwise optimum growing conditions even in the presence of high concentrations (8 mM) of nitrate, because heterocysts continue to be produced unless cultures are

supplied with ammonium. Better understanding of such critical physiological factors is important for correctly interpreting the findings of laboratory experiments, and how these results can be applied to understanding the performance of *Aphanizomenon flos-aquae* in the real-world conditions of UKL.

### **3.0 ALGAE-ZOOPLANKTON-WATER CHEMISTRY RELATIONSHIPS 1990 - 2003**

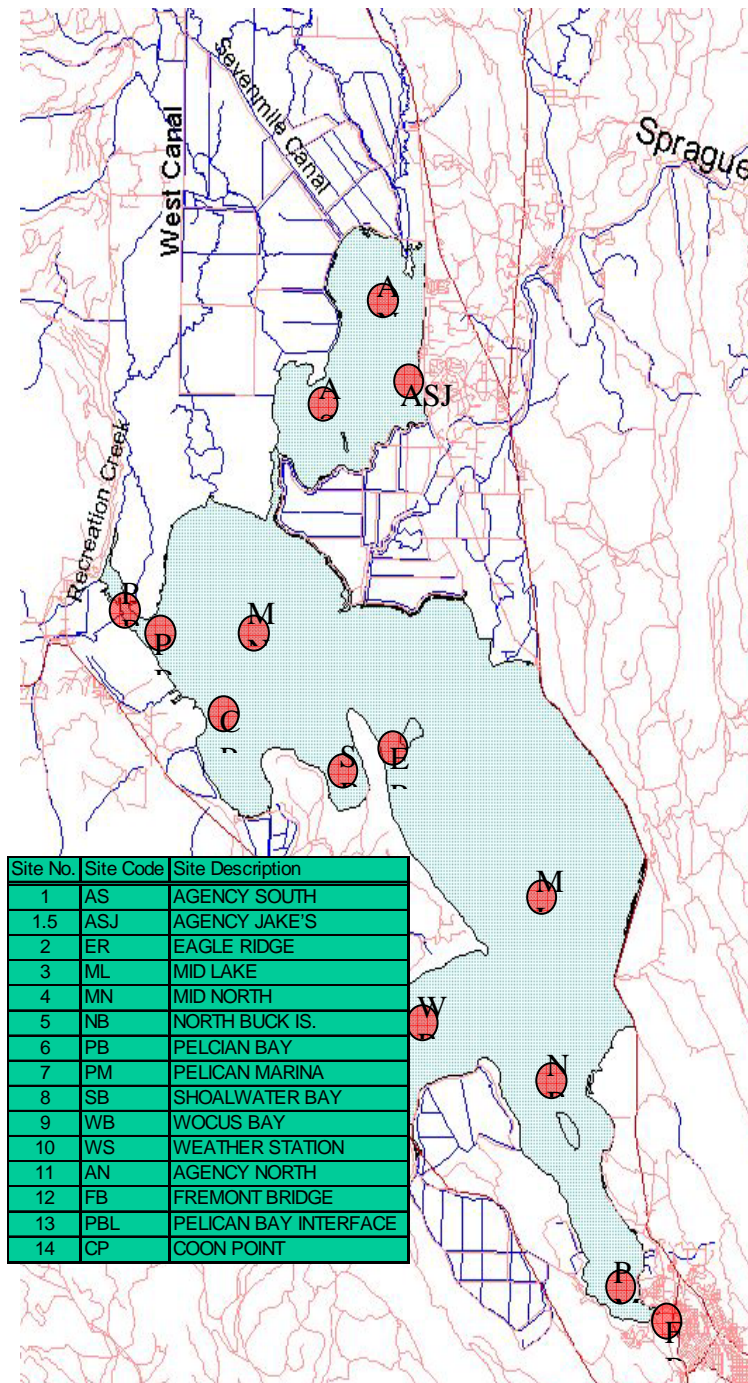
#### **INTRODUCTION**

Since 1990 the Klamath Tribes in work supported by the U. S. Environmental Protection Agency and the Bureau of Reclamation have sampled and monitored the quality of Upper Klamath Lake at many locations. They have also collected samples from inflowing tributaries to UKL and the waters discharged to the lake from pumps draining diked and drained former wetland areas.

The location of stations where phytoplankton algae and zooplankton samples have been obtained is shown in Figure 7. Water chemistry samples have been collected and field measurements have also been made at these locations. Some stations have been discontinued (e.g. Pelican Bay [PB]), and other stations have been added over time (e.g. Coon Point and Freemont Bridge). Generally, the lake monitoring program has begun in early spring and ended in late fall with each year startup determined by lake conditions (e.g. presence or absence of ice cover).

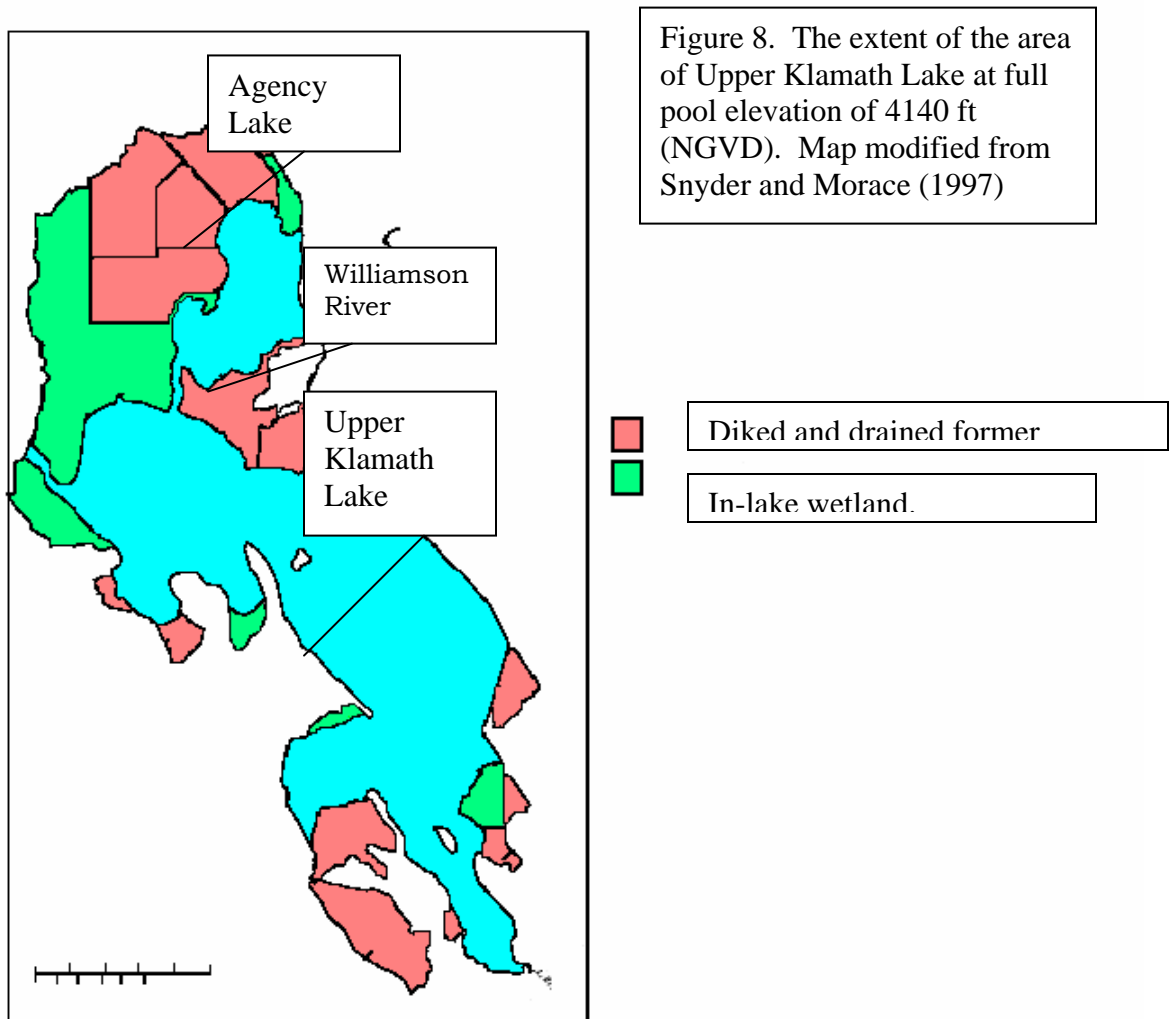
MS Excel spreadsheet files with Klamath Tribes-Bureau of Reclamation data related to this report section is provided in Appendix B of the Appendix CD, accompanying this report. These files are the UKL water chemistry data, which includes chlorophyll  $\alpha$  and phaeophytin  $\alpha$  analyses [with file name UKL 1990-2003], and UKL phytoplankton [file name UKL PHYTO 1990-1996] and zooplankton [file name UKLZOOPS 1990-1996] data. The phytoplankton and zooplankton data from 1997 to the present was not available to ASR at the time of writing this report.

Figure 7. Locations of Klamath Tribes monitoring stations on Agency and Upper Klamath Lake (1990-2004). Some stations have been discontinued. Source of map and table is Klamath Tribes, data reviewed by J. Kann (2003) available as MS Excel spreadsheet files (see MSEXcel file in Appendix B named UKL 1990-2003).



## CHOICE OF LAKE MONITORING STATIONS

The map that has accompanied the Klamath Tribes data sets depicts the “lake” as the open water areas of UKL outside emergent vegetated areas. When this map of the “lake” is compared with the map produced by Snyder and Morace (1997) reflecting the extent of open water wetland and diked and drained former wetlands (Figure 8), the extent of the “lake” becomes an important consideration in directing investigations that will characterize the biota of UKL. For the past fourteen years lake monitoring has been conducted in the limnetic areas of UKL



ignoring the extensive littoral areas of the lake of approximately 17,000 acres (Snyder and Morace 1997) that comprise 21.1% of the area of the lake at full pool (see Section 6.0 of this report; also Geiger 2001a).

The prominence of AFA during late spring, summer and fall in UKL and the role of AFA in influencing lake water quality has resulted in the primary focus of past work on the planktonic algae of the open waters of the lake. This focus has, however, diverted attention from other algae that are present in the wetland areas and areas of the lake influenced by these wetlands during and after the times of the year when AFA is typically dominant. Bureau of Reclamation funding for the Klamath Tribes' data collection supports examination of limnetic samples. A sole exception to the limnetic location of sample sites, has been the Pelican Bay site monitored from 1990-1997. The potential influence of wetlands in the lake on the growth of AFA is suggested by the data on this species' biomass at the Klamath Tribes Pelican Bay station (in the vicinity of the Upper Klamath Lake NWR) compared with its biomass at other locations (mid-north and mid lake stations of the Klamath Tribes monitoring program) (see Appendix A file UKL PHYTO 1990-1996). Figure 9 shows the very significant reduction in AFA biomass in the vicinity of the marsh at Pelican Bay compared with the other open lake stations.

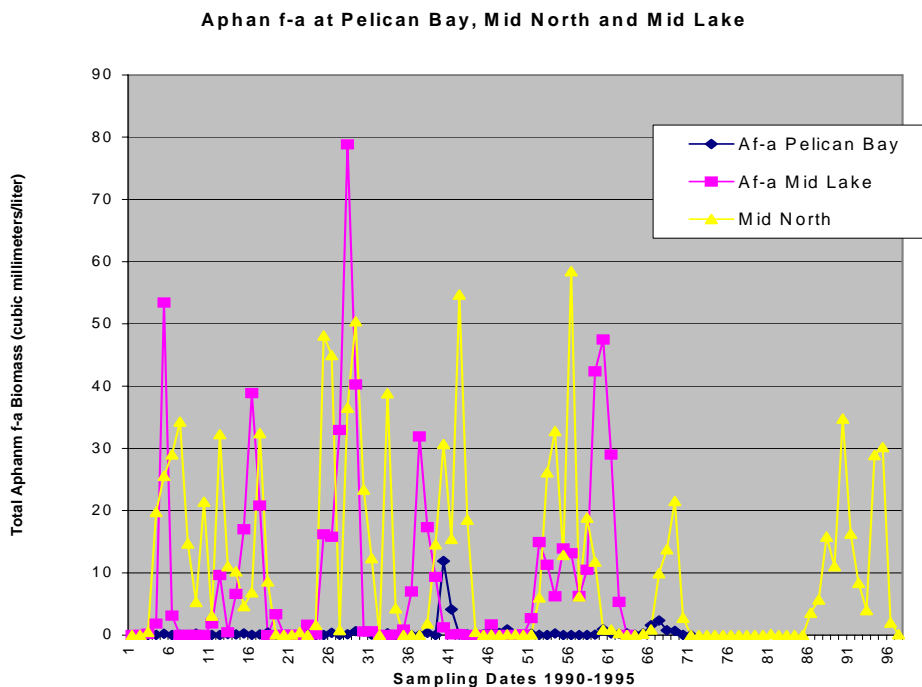


Figure 9. Seasonal occurrence of *Aphanizomenon flos-aquae* biomass at the Klamath Tribes-Bureau of Reclamation Pelican Bay monitoring station adjacent to Upper Klamath Lake NWR marsh as compared with its biomass at two open water stations in Upper Klamath Lake (Mid-Lake and Mid North). Numbers on the x axis are the numbers of sampling occasions from start of sampling in 1990 through 1995.

Areas *in the lake* where AFA is not dominant or is absent are of obvious interest since environmental factors in these areas may provide clues

that could lead to AFA control. Recent observations of water quality conditions by the author approximately one mile south of the mouth of Thompson Creek (UKL NWR) in north UKL were of the absence of AFA, presence of brown water, and pH in mid-day considerably lower than southern lake sites. The absence or reduction of AFA just downstream, at or within marsh environments has been noted by those few scientists who have spent time obtaining data on marsh character. Forbes (1997) noted the absence of AFA except at the waters off the edge of Hanks Marsh, and Sartoris, Sisneros and Campbell (1993) noted the same in their report on characteristics of Upper Klamath Marsh National Wildlife Refuge. Perdue *et al.* (1981) noted the absence of AFA in Upper Klamath Lake at a location heavily influenced by the Williamson River that drains extensive wetland areas upstream. The difference of the marsh influenced areas of the lake was also suggested by a note on zooplankton differences in the lake where four species of zooplankton were typical here but not in the open waters of the lake (USCOE 1982).

## **FIELD AND LABORATORY METHODS**

The description of field and laboratory methods for collection and analysis of water chemistry and biological samples has been provided in Klamath Tribe Natural Resources Department *Laboratory and Field Quality Assurance and Quality Control Plan* (1991), Kann (1998, 1993a, 1993b), and Kann and Walker (1999). The Klamath Tribes water chemistry data set in the MS Excel file UKL 1990 – 2003 (Appendix B) contains only data obtained by the Klamath Tribes at time of field collection of samples (Hydrolab measurements) and resulting from analysis of samples by their chosen analytical laboratory (Aquatic Resources, Inc. [ARI], Seattle, WA). All laboratory water chemistry analyses for the 1990-2003 data set have been performed by ARI. Analytical methods used by ARI, excluding chlorophyll analyses, are available at [www.ecy.wa.gov/apps/acclabs](http://www.ecy.wa.gov/apps/acclabs). Analyses of zooplankton samples have all been done by BSA Environmental Services, Inc., Beachwood, OH. Phytoplankton samples collected from 1990-1996 were analyzed by M. Falter. Phytoplankton samples collected from 1997 to the present have been analyzed by BSA Environmental Services, Inc.

### **Zooplankton methods**

The following description of the methods for collection and analysis of zooplankton samples is taken from Kann (1998). Species abundance and biovolume are estimated in the laboratory analyses. Crustacean zooplankton and rotifers were sampled at each location using a 12 L Schindler-Patalas trap with 64 micrometer mesh. A depth-integrated sample was collected by taking one sample from between the surface to 1

m depth interval, one from between the 1 m above bottom to bottom interval, and then combining the two samples for a total of 24 L filtered. The exception to this was the deeper Eagle Ridge site (ER) where a third collection was made from the midpoint of the water column (approximately 3 m) for a total of 36 L filtered. Due to very shallow nature of Upper Klamath and Agency Lakes (mean depth = ~2.4 m), the collection method provided a representative water column sample. Zooplankton samples were narcotized with carbonated water prior to preservation in an ethanol-glycerin solution (Pennak 1989). Individual species abundance was determined (at 100x) from the average of three subsamples taken from the depth-integrated sample at each site and date. Measurements of individual species (at least 10% of the total count) were made on a site-composited sample for each date, and dry weight biomass conversions were made according to Dumont *et al.* (1975), McCauley (1984), Culver *et al.* (1985), and Lawrence *et al.* (1987).

Additional detail on zooplankton laboratory methods was provided [as follows] to S. Geiger by J. Beaver of BSA Environmental Services, Inc., Beachwood, OH (2001a). The volume of sample concentrate was measured and three 1-ml aliquots were examined at 100x on a Wilovert inverted microscope equipped with phase contrast. If the total tally was less than 200, additional aliquots up to a maximum of 20ml were examined. Taxonomic identification followed Ruttner-Kolisko (1974), Edmundson (1959), and Pennak (1989).

Biomass estimates were based on established length/width relationships (Dumont *et al.* 1975; McCauley, 1984; Lawrence *et al.* 1987). The lengths or the lengths and widths of each species encountered were measured from a composite sample formed by pooling approximately 5ml from each sample for that date. The number of specimens examined was equal to 25 for common species and lesser for more rare taxa. For cladocerans, the length was measured from the tip of the head to the end of the body (shell spines excluded). For copepods, the length was determined from the tip of the head to the insertion of the caudal ramus. The length of rotifers was measured from the tip of the head to the end of the body (spines, toes, etc. excluded). In accordance with McCauley (1984), biomass was computed for the appropriate number of individuals for each sample location and the arithmetic mean biomass was multiplied times the species abundance to produce a species biomass for each sample.

### **Phytoplankton Methods**

The analysis of chlorophyll a, and its breakdown product phaeophytin a, has been done since 1990 in the ARI laboratory in Seattle, WA. The method of analysis was described by ARI principal Steve Lazoff (S. Geiger pers. com. Jul 26, 2004a) as follows. Chlorophyll and phaeophytin

analyses were performed on a subsample of the composited weighted 5 cm diameter plastic tube samples (three replicates composited). Water samples for chlorophyll analysis, preserved in the field with MgCO<sub>3</sub>, were shipped to Seattle following collection. Upon receipt at the laboratory, within 24-48 hours after collection, samples were filtered with Millipore HAWP 0.5 micrometer membranes. The filters were then sonicated in acetone in the centrifuge tube for 20 to 30 seconds. The equipment used for this has been a Sonicor U-400, operated at about 30% power. The spectrophotometric analysis then followed APHA standard methods 10200h. The supporting reference for this method is Nusch (1980). The method for estimating phytoplankton species abundance and biovolume have been described in the Klamath Tribe laboratory and field quality assurance/quality control plan (Klamath Tribe Natural Resources Department 1991), and in Kann (1998) as follows. A final subsample from a depth-integrated sample (a minimum of three replicate hauls from a weighted 5 cm diameter plastic tube at each sample site – p. 15] was placed in 500 ml opaque polyethylene bottles and preserved with 5 ml Acid-Lugol's solution (APHA 1985). Algal species abundance was then determined using the inverted microscope/settling chamber technique (Lund *et al.* 1958). Depending on algal density, from 5-50 ml were settled for a minimum of 10 hr per cm of chamber height. After settling, the slide was scanned at 100x and 320 magnification to ensure random distribution of organisms. Enough cells were then counted in haphazardly chosen fields until the standard error as a percent of the mean was 10% for dominant species. Cell volume was then determined by measuring cell dimensions of 10 individuals per species in each sample (generally 6-10 samples per date), and averaging among the samples to obtain an average size per species per date. Volumetric equations for shapes most resembling species form were used to determine total cell volume (e.g., Willen 1976). The total biovolume of each species was then computed as cell abundance (individuals/L) multiplied by average individual volume (cubic micrometer). Final biovolume estimates are reported as cubic millimeter/L, which approximates wet weight biomass in mg/L. Due to high variability in flake size of *Aphanizomenon flos-aquae*, all counts and volume measurements were made on individual cells within a filament (flakes tended to break apart into filaments during preservation and transport). Heterocysts and akinetes were enumerated separately.

The above method of estimating phytoplankton abundance and biovolume was followed by M. Falter from 1990-1996. Additional details on the phytoplankton methods have been provided S. Geiger by J. Beaver at BSA Environmental Services, Inc. related to the analyses they have performed on samples collected since 1996 (S. Geiger pers. com. Jan. 2001b). Cell numbers of all identified phytoplankton taxa were be quantified on a per milliliter basis using the Utermohl method (Lund *et al.*

1958). The abundance of common taxa were estimated by random field counts. Rarer taxa were quantified by scanning a strip of the Utermohl chamber. Counts were performed on a Wilovert inverted microscope equipped with phase-contrast at a magnification of 600x. Recently viable cells were included in the analyses as determined by the respective physiological characteristics of eukaryotes (the presence of chloroplasts and other organelles) and prokaryotes (pigmented cells). At least 400 units (colonies, filaments, unicells) were enumerated from each sample. For samples with common colonies or filaments, the counts likely will include several thousand cells since total cell numbers of multi-cell units (colonies, filaments) will be quantified. In accord with Lund *et al.* (1958), the data will be accurate within 90% confidence limits.

In colonies with extremely small cells (e.g., *Microcystis*), cells were enumerated from a small representative area of the colony containing at least 100 cells. For common filamentous taxa, the total cells per filament were determined by first quantifying the cell number within a known length (e.g., 100  $\mu\text{m}$ ). This process was repeated for 25 filaments of each abundant filamentous taxon, and subsequently used to calculate the mean number of cells per filament length for that taxon. This quantity was applied to measurements of the length and width of each filament encountered so that the total number of cells per filament could be estimated.

Cell biovolumes of all identified phytoplankton taxa were quantified on a per milliliter basis using the Utermohl method (Lund *et al.* 1958). Biovolumes were estimated using formulae for solid geometric shapes that most closely match the cell shape (Burkholder and Wetzel 1989). Subaliquots were examined at 400x under phase contrast with a Will inverted microscope. Cells were settled for at least 20 hours in darkness within an enclosure protected from vibration and temperature alteration (Burkholder and Wetzel 1989). Homogeneity in settling was checked to determine the minimum number of fields necessary to obtain consistency in enumerating abundant taxa (>5% of the total biovolume) per unit chamber area.

For taxa with substantial size variation (such as diatoms), size classes were designated arbitrarily to determine average cell size (biovolume). For each taxon, 25 cells were measured from each size class (assuming that sufficient numbers are available). Mean biovolumes within each size class were used to calculate the total biovolume contributed by the taxon to its representative sample (Burkholder and Wetzel 1989).

## **COMPLICATIONS FOR ESTIMATING AFA DENSITY AND BIOMASS**

The typical form of *Aphanizomenon flos-aquae* (AFA) in Upper Klamath Lake produces complications for those who would quantify its seasonal presence in the lake, that is, estimate its density and biovolume among other species present. Chlorophyll a provides a measure of biomass indirectly but of the entire assemblage of algae present at time of sampling. As noted in Section 2.0, AFA populations in the lake are visible not just as a certain lime-green color to the water, but as flakes visible to the observer without magnification that look like grass clippings. These flakes are comprised of hundreds or thousands of cylindrical (stiff, spaghetti-like) filaments (also called trichomes) generally lying parallel to each other. The individual filaments are comprised of cells varying in length but typically ~4.8 micrometers. The flake is typically a shortened ribbon-like structure, width to thickness ratio large and a length sometimes recurving to form a near circle. Occasionally flakes are less ordered with filaments both parallel and vertical to each other, and, flakes are in sizes ranging from dimensions of a few filaments coming together to flakes that are 1.5+ cm x 1.0+ mm (no estimate of thickness).

A cursory examination of these flakes was made on UKL water near the Skillet Handle of Running Y just west of the North Buck Island station (see Figure 2) September 1, 2004. A gently shaken sample from the surface (~0.25 m depth) at 1015 in the morning was poured into a 9 cm diameter petri dish and viewed with a dissecting scope (15.3x – 30.6 x). All sizes of visible flakes of AFA were counted in the entire dish (separate counts of 64, 81, and 60), with an average count of 68 flakes for the 50 ml of sample. The estimate of AFA density based on this cursory examination was 1,360 flakes/liter. A sample of flake sizes in the dish was also obtained: 0.5 x 0.1 mm; 0.25 x 2 mm; 1.1 cm x 0.75 mm; 1.6 cm x 1.2 mm; 1.5 cm x 1.0 mm; 0.5 m x 0.1 mm. Some flakes visible at powers observed were smaller than these but still comprised “flakes” that were visible when magnified.

Considering that a single filament of AFA typically ranges from 3.9-4.4 in width (average of 10 filaments was 4.3 micrometers) to 59-335 micrometers in length (average of 25 filaments was 128 micrometers), there is a large (and difficult to measure) number of filaments in any of the above measured flakes. There are 1000 micrometers in 1 mm, so side-by-side, as AFA arranges itself in flakes, there could be around 230 filaments lying parallel with each other just in one plane (this is not to say that they indeed lie in planes or layers but said to estimate a crude number of filaments per flake). In a flake that is 1.6 cm in length (16000

micrometers) there could be ~29,000 filaments lying in this one plane of the flake. There are multiple (and difficult to discriminate) planes or layers in a flake but flake thickness could be 1/10<sup>th</sup> of the width, or 100 micrometers in this example, which could accommodate ~20 layers, affording a flake or “colony” size of 580,000 filaments. Now this is a very gross estimate for a large flake but the calculation suggests a complex typically multifilament lifestyle for AFA in UKL. This flake formation was very significant to Phinney *et al.* (1959) in their attempts to culture this alga (see Section 2.0), and it appears to be significant with respect to toxic properties of this species.

The complications for estimating density and biovolume are only suggested by characterizing the unusual colonial form (flake form) of this alga. Once samples are obtained for microscopic analysis according to the sampling procedure noted above, they are preserved as a 1% Lugol’s solution (a preparation of iodine, potassium-iodide and acetic acid). Following preservation, the samples in recent years have been shipped to Ohio for analysis by BSA Environmental, Inc. It has been this author’s experience with AFA from UKL that flakes devolve into separate filaments when preserved and may over time become only separate filaments what with occasional shaking for settling chamber preparation and movement in the laboratory. This observation has been corroborated to S. Geiger both by M. Falter (Geiger pers. com. 2004c) and by J. Beaver (Geiger pers. com. 2004b). There may be an ionic attraction of filaments in the flake mass that is ended by preservation allowing filaments to disengage from each other. The implications of this for the fourteen years of data generated through 2003 is that there is no information about flakes and filaments. Considering flakes appear to be the primary physiological form of AFA in UKL, this is information that would appear to be important for its bearing on the physiology of AFA within layers, e.g. N<sub>2</sub> fixation, uptake of nitrogen and phosphorus and micronutrients, the range of the size of flakes and possible seasonality of flake size versus food preferences of zooplankton like *Daphnia*, and the relationship of flake form and size to buoyancy of AFA.

Why are there no counts of filaments (trichomes) in the Klamath Tribes data set (see Appendix B CD file for UKL PHYTO 1990-1996)? Past reports of attempts to estimate densities of AFA in UKL elicit reader confusion when compared with each other due to different metrics used to describe results. For example, Bonnell and Mote (1942) reported observations of AFA as *filaments*/cubic meter; Phinney (1959) reported many estimates from 1956-57 as *individuals*/liter; and the US Army Corps of Engineers (1982) reported their estimates from 1981-82 as *number*/ml. Considering the reported *numbers* per milliliter, the counts were of filaments, not cells, because densities were comparable to

Phinney *et al.* (1959) estimates of *filament* density. Adding to the confusion, the Corps of Engineers (1982) refer to estimates by Phinney *et al.* (1959) as *cell* counts, which they were not (see Phinney and Peek 1960). Miller and Tash (1967) plotted Phinney *et al.*'s (1959) data on AFA density as "cell counts" in cells/liter and compare the rise and fall of densities with their data on phytoplankton dry weight. A doubly confusing reference to the work of Phinney *et al.* (1959) is that of Klamath Consulting Service, Inc. (1983): "During major blooms in July/August in Upper Klamath Lake, *cell* counts of the algae have reached almost 200,000 *filaments* per milliliter (ml) of water, with average *counts* ranging from 10,000 to 30,000 per ml" [ASR italics]. The lack of information on filaments of AFA in the Klamath Tribes-Bureau of Reclamation data set may seem strange since filaments are the primary form of AFA in preserved samples with residual flakes a minor component. This requires some attention because it relates to the choice by the Klamath Tribes of a sample analysis method, described in its 1991 quality control plan, that has focused on "cell" densities rather than algal "unit" densities.

Standard methods for the estimation of phytoplankton densities are available in *Standard Methods for the Examination of Water and Wastewater* (APHA 1998) and U. S. Geological Survey's (1989) *Techniques of Water-Resources Investigations*. *Standard Methods* notes that doing total cell counts for samples is tedious and time-consuming when colonies consist of thousands of cells, as, for example, with AFA and *Microcystis*. It also notes that the *natural unit* or clump is the most easily used system. It recommends it be made clear which method is used. It further notes that if the distribution of plankton organisms is random and the population fits a Poisson distribution, the counting error may be estimated. The approximate 95% confidence limits, as a percentage of the number of units counted (N), equals:

$$\frac{2}{\sqrt{N}} \quad (100\%)$$

If 100 units are counted, the 95% confidence limits approximate  $\pm 20\%$ . For a count of 400 units, the limits are about 10%. Note that the entity to count is a *unit* rather than a cell to estimate a counting error.

The USGS methods section (USGS 1989) for phytoplankton analysis comprises three subsections that describe methods of analysis. The introductory section again refers to the need for a random distribution of phytoplankton *cells* to estimate the precision of *cell* counts. In fact the

same outcome as above for counting 100 randomly distributed cells or 400 cells is stated: “the true count does not vary from the mean estimated count” by more than 20% or 10%. The key assumption here is the randomness of what is counted, but instead of calling these *units* they are called *cells*. Obviously, a unit can be a cell (e.g. *Euglena*, *Stephanodiscus*) or as with AFA a colonial form of multicellular filaments. Therefore counting 360-440 *units* makes it possible to estimate the relative *accuracy* of your density estimate ( $\pm 10\%$  of the actual density in the water you sampled).

The USGS (1989) inverted-microscope method (B-1520-85), based on information provided by Lund *et al.* (1958) and others, which has been the method used for the analysis of all Klamath Tribes phytoplankton samples, notes that samples require use of two different magnifications to include species with widely differing sizes. This has been done according to methods stated above in the report section *Phytoplankton Methods*. The method then notes that random microscopic fields need to be counted until a sufficient number of “units (cells, filaments, chains, or colonies) have been enumerated for the desired level of precision.” The object to count therefore is a discrete particle that settled to the chamber bottom independent of other particles. The particle could be a single cell, multiple-celled organism, or a colony of multi-celled organisms such as AFA, but the unit must have settled independently of other units. Counting individual cells in a filament of AFA would not be counting *units* in the sense of this method because the cells of a particular filament were only an aspect of a randomly distributed filament, i.e. the filaments and residual flakes as *units* are randomly distributed but not the *cells* of these units. These counting instructions are completed with the comment about estimating cell densities: “If a large number of colonies or filaments appear within the field, determine the average number of cells in an average-size colony or filament and multiply by the number of colonies or filaments present. Count only viable cells, those having protoplasm or pigments.” This approach was not taken in the analysis of, it appears, all of phytoplankton samples analyzed since 1990.

The author has discussed the methods detailed above in this report’s *Phytoplankton Method’s* section with the first analyst Meg (Falters) Findley (1990-1996) and with John Beaver of BSA Environmental, Inc. (1997-present) to confirm the application of the proposed methods. Counts by the analysts have always been of *cells* in settling chamber bottom areas rather than randomly settled and distributed *units*. The difference in approaches is illustrated in the following figure (Figure 10)

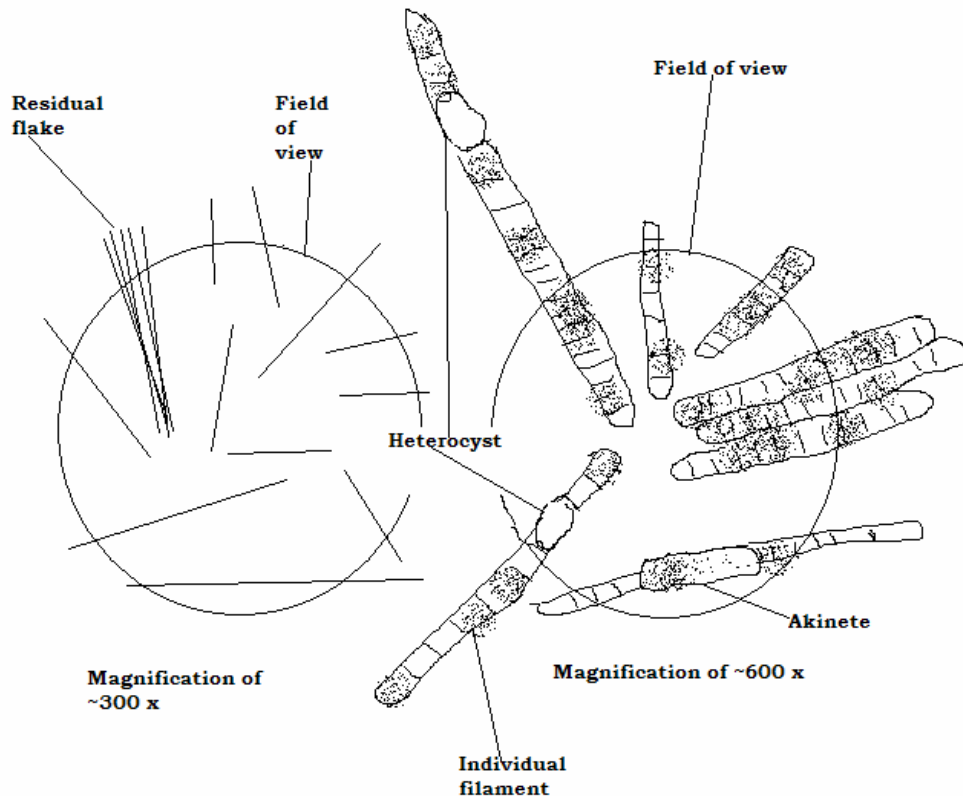


Figure 10. Representation of *Aphanizomenon flos-aquae* observed with a microscope at magnifications of ~300 x and ~600 x. The circles represent the field of view of the analyst.

In this example, the units that have settled independently and randomly on the settling chamber bottom are the separate particles. In the left field of view there are 12 units (one of the units is a residual flake of 5 filaments). In the right field of view there are 6 units (one unit is a residual flake of 3 filaments). The approach taken by the Klamath Tribes' analysts has been to count the cells *within the field of view* at ~600 x magnification and have this be the type of tally for each of the 10-20 fields counted. In the right field of view there are approximately 55 cells including the akinete and the heterocyst (which have also been tallied). The tally, then for the field of view would be 53 cells, 1 akinete and 1 heterocyst. Using appropriate calculations that factor in settling chamber bottom, area of bottom counted, settling chamber volume, perhaps amount of Lugol's solution added to sample as preservative, and total cells counted, an estimate of density (cells/liter) is obtained. The individual cell volume has also been estimated by the analysts. A sufficient number of cells have been measured to arrive at an average cell

volume which on average can be ~70 cubic micrometers. Total biovolume for the AFA in the sample is then the cell density estimate multiplied by the biovolume per cell. Only tallies of cells, heterocysts and akinetes are provided in the data with no tally of AFA *units*.

The approach described in the standard methods of *Standard Methods* and the USGS *Techniques* is to first obtain a *unit* density estimate for the sample. Since AFA is predominantly what is observed in summer and fall samples from UKL, virtually all *units* are AFA, however other rare or sparse species are also observed including colonial *Microcystis* with its hundreds and even thousands of individual cells within a gelatinous matrix and *Anabaena* a twisted and contorted filamentous form of Cyanophyta posing a similar counting challenge. In the example of Figure 10, the tally of the right field of view would be six *units*. The next field would be counted and so on until a total unit number of 360-440 would be observed. To obtain estimates of cells/filament a sufficient number of cells would be counted per set filament length (say in a 100 micrometer long length of a filament) and an average filament length would be determined. The cell density (number of cells/liter) would then be derived on the back, so to speak, of the estimate of unit density. The estimate of cell volume would be made in a similar way, or, perhaps more useful, the estimate of an average filament or AFA unit volume, could be made by measuring a sufficient number of units to arrive at an average volume. The initial tally with successive estimates of cells/filament and cell or AFA unit biovolume is grounded in a Poisson distribution with the ability then to estimate confidence limits around these estimates. All such tallies would be available in data sets for further evaluations.

To examine outcomes of each approach, a sample of UKL water August 3, 2004 off the Skillet Handle of Running Y Ranch Resort, west of Buck Island, was obtained from 0.5 m depth and analyzed in these two ways to examine differences in results. Duplicate analyses were performed on a 5 ml settled sample. Fields of view at 1 mm intervals across 2 transects were examined for a total of 20-21 fields. For the duplicate analysis the location of transects across the chamber bottom was shifted to observe a different area of the bottom. Counts of at least 100 units were made to achieve an estimate of the unit density that was  $\pm 20\%$  of the actual unit density in the UKL water. Average numbers of cells/121 micrometer length segments were estimated (25.24), in addition to average cell width (4.3 micrometers), as well as the average AFA unit lengths (128 micrometers). Taxa encountered included the dominant AFA, 2 small, spherical Chlorophytes, 1 2-celled diatom unit, a few-celled colony of *Microcystis*, and 1 segment of the Cyanophyte *Lyngbya*. An initial scan of one-half of the chamber bottom prior to field counts resulted in observation of 68 residual flakes of 3-20 filaments. Counts of each field

of view were tallied in such a way as to provide numbers for calculations of the two different density estimates. Table 2 provides the results of the analyses.

Table 2. Results of unit and cell count approaches to determining AFA densities in UKL derived from analysis of shallow water sample near Skillet Handle August 3, 2004.

<b>ESTIMATE TYPE</b>	<b>ESTIMATE</b>	<b>CONFIDENCE LIMIT <math>\pm</math> 20%</b>
Unit Density A	$1.46 \times 10^7$ units/liter	$2.92 \times 10^6$ units/liter
Unit Density B	$1.93 \times 10^7$ units/liter	$3.86 \times 10^6$ units/liter
Unit Cell Density A	$3.83 \times 10^8$ unit cells/liter	$7.66 \times 10^7$ unit cells/liter
Unit Cell Density B	$5.39 \times 10^8$ unit cells/liter	$1.08 \times 10^8$ unit cells/liter
Cell Density A	$2.29 \times 10^8$ cells/liter	$4.58 \times 10^7$ cells/liter
Cell Density B	$4.02 \times 10^8$ cells/liter	$8.04 \times 10^7$ cells/liter
Average Unit Density (A and B)	$1.70 \times 10^7$ units/liter	$3.40 \times 10^6$ units/liter
Average Unit Cell Density (A and B)	$4.60 \times 10^8$ unit cells/liter	$9.20 \times 10^7$ unit cells/liter
Average Cell Density (A and B)	$3.16 \times 10^8$ cells/liter	$6.32 \times 10^7$ cells/liter

Based on the analysis suggested in Lund *et al* (1958), and the determination of significant difference between estimates based on presence or absence of  $\pm 20\%$  overlap of confidence limits, the duplicate unit density and unit cell density estimates are not significantly different. The duplicate cell density estimates are significantly different. The average unit cell density estimate and the average cell density estimate are not significantly different.

These results seem to be consistent with the idea that an analysis procedure that is based on a random distribution of the units counted will produce more consistent results that are more accurate. Hence the significant difference between the duplicate cell density estimates but not between the unit density or the unit cell density estimates. Averaging

the duplicates resulted in eliminating the significant difference between the unit cell and the cell density estimates. Figure 10 suggests what can happen with counts when the *units* are not only multi-cellular but multi-filament particles. Randomly encountering these units can result in a field-of-view count that has an extraordinary number of cells because of the occurrence of residual flakes or inclusion of longer segments of AFA filaments within fields.

The practical concern from a comparison of counting approaches is there are no replicate sample analysis results for sampling stations in the Klamath Tribes' data set to smooth out what appears to be a wider variance in results from single sample cell density counts. Averaging all of the lake station density estimates for a particular date may help improve the accuracy of some "lake cell density" estimate. This averaging may then reflect and address the complication of any lake density average for AFA suggested by the seasonally repeated visual pattern of AFA distribution in the lake where there are visual differences in the spatial distribution of AFA as seen in photographs taken by high altitude airplanes or by satellites (e.g. satellite imagery included in Appendix B from 2000 that shows the succession of AFA blooms and the 1995 imagery and estimation of chlorophyll a produced by the Bureau of Reclamation).

In conclusion, then, the phytoplankton data set appears to:

- Lack information on the typical natural occurrence and size of flakes;
- Lack information on the number of filaments and residual flakes that are the form of AFA in preserved samples;
- Lack information on the number of akinetes or heterocysts per filament or flake;
- Lack data on the biovolume of filaments or flakes;
- Have single estimates of density (and biovolume) that are not based on a random distribution of units, which makes it impossible to estimate the relative accuracy of the counts;
- Does have information on when AFA becomes present in the lake and shows the relative increase and decrease in cells including akinetes and heterocysts.

The importance of clear understanding of methods used in the past analysis of AFA is related directly to the use of the resulting data to draw conclusions about its growth in relation to changes in lake water chemistry or associated biota such as zooplankton. The extensive work by Ehinger in 1990-1991 (1992, 1993a, 1993b) assaying effects of zooplankton and nutrient (N and P) additions to mesocosms and cubitainers included the estimation of phytoplankton (and AFA) density

and biovolume but description of methods used were not specific enough to evaluate the methods. Section 5.0 of this report provides information on tests of in-lake enclosures that could be used to assay effects of nutrient additions, humics or barley straw on growth of AFA. The growth form of AFA and its changes with preservation are the challenges to quantification of both density and biovolume, as examined in the above paragraphs. Using a method of quantifying density and biovolume that is based on the method described in APHA *et al.* (1998), USGS (1989) and Lund *et al.* (1958) will set expectations for confidence limits related to counts. This will improve the usefulness of the data in drawing conclusions about results of any such assays.

## **PHYTOPLANKTON AND ZOOPLANKTON QUALITY ASSURANCE ANALYSES**

Recently an analysis of duplicate samples for chlorophyll a was done along with an analysis of other duplicate samples related to water chemistry characterization. This analysis is provided in the Appendix A CD (see UKL 1990-2003 file). None of the 24 duplicate chlorophyll a sample analyses performed during the 1990-2003 period were significantly different based on a measure of Relative Percent Difference (if chlorophyll a value >5x Reporting Limit then Relative Percent Difference within  $\pm 20\%$  or if value <5x RL then Difference within  $\pm RL$ ). The differences between duplicate phaeophytin a values indicated six exceeded the RPD standard. Phaeophytin a is a chlorophyll a degradation product that has been used to provide a measure of the relative viability of chlorophyll a present.

A review of the USACOE (1982) zooplankton analysis results and the experimental work of Ehinger 1990-1991 on zooplankton effects on AFA growth indicated a difference in the identification or naming of the dominant UKL zooplankton taxa. The dominant *Daphnia* taxon, called *Daphnia schodleri* by the USACOE (1982), and identified as such in the Klamath Tribes' zooplankton data (see Appendix B CD UKLZOOPS 1990-1996), but called *Daphnia pulicaria* by Ehinger suggests the need for a clarification of the taxonomy for naming UKL zooplankters.

Inquiries have been made of analysts and their clients to locate quality assurance analyses related to the analysis of phytoplankton or zooplankton samples (e.g. Geiger 2004b, 2004c). This could include verification of identifications, duplicate analyses, duplicate analyses of samples by other analysts, data used for determining cell/filament length averages or average cell biovolume estimates, or bench sheets with notes on individual sample analyses. No quality assurance information of this

kind has been provided the author in response to inquiries. There has been no archiving of zooplankton or phytoplankton samples, other than possibly retaining samples for the present year's contract (Geiger, pers. com. 2004b). The absence of a report, or periodic data-review reports, on the significance of the phytoplankton and zooplankton data in relation to the water quality data set for the 1990-2003 period, excepting the work of Kann (1993a, 1998), may have lessened the need for a more thorough review of how the data were generated.

## **MULTIVARIATE APPROACH TO ANALYSIS OF DATA SETS**

We intended to characterize changes of phytoplankton assemblages, especially *Aphanizomenon*, with relation to zooplankton and water quality in the Upper Klamath Lake over 13 years. Our approach was to first summarize changes of phytoplankton assemblages over time using multivariate analyses such as ordination. The ordination method called non-metric multidimensional scaling (NMDS) was to be used to summarize algal species distributional patterns (both spatial and temporal patterns). NMDS is a 'mapping' technique that would order all sites in the low dimensional space (2-3 axes). The distance among the sites is proportional to the species similarity among the sites. This analysis would allow detection of algal distributional patterns. If the patterns emerge, we would have related the patterns with environmental variables to identify potential 'predictors'. We would attempt to relate the patterns with zooplankton species. Together we would summarize species patterns and generate hypotheses of what may be 'responsible' for the patterns. Several software packages would be used in the analysis (PRIMER, CANOCO, and PC-ORD).

We have not been able to undertake the proposed preliminary multivariate analysis of the K Tribes' 1990-2003 data set because we lack the phytoplankton and zooplankton data from 1997-2003. The Tribes' and their consultant J. Kann are working through a variety of issues related to this data and may produce the complete and reviewed set in the near future. We are prepared to work on the data set as proposed if data is made available to our Consortium within a reasonable time.

## **DISCUSSION**

The affects of wetlands in Klamath Lake on lake water quality is potentially large with current conditions of reduced wetland acreage. Since they are in the lake it would seem that monitoring of UKL would include stations that are located in these littoral areas. The existing, but

seemingly unacknowledged, stratification of the “lake” into littoral and limnetic zones based on the presence of emergent wetlands has also been done by the U. S. Fish and Wildlife Service in its National Wetland Inventory mapping of Klamath County (1989-1994; see Section 6.0 for additional details on NWI wetland mapping). This 21.1%/78.9% areal stratification should be the basis for future “lake” monitoring station allocations, and allocations of funding to generate data on each of these lake areas. We suggest that the location of lake monitoring stations be revisited and appropriate stations established for long-term monitoring that will provide a more complete characterization of UKL water quality.

Two known attempts have been made to quantify the spatial distribution of phytoplankton dominated by AFA in summer in the lake using measurements of chlorophyll a (Bureau of Reclamation 1995 and the Klamath Tribes 2000). Imagery used to produce estimations of spatial distribution is provided in Appendix B. While there is a brief description of the BR work, there is yet no draft report on the Klamath Tribes’ 2000 field investigations. We feel that progress in using satellite imagery to quantify phytoplankton in UKL should be made to provide better perspective on factors influencing the distribution of AFA in UKL such as wind and water movement.

The importance of clear understanding of methods used in the past analysis of AFA is related directly to the use of the resulting data to draw conclusions about its growth in relation to changes in lake water chemistry or associated biota such as zooplankton. The extensive work by Ehinger in 1990-1991 (1992, 1993a, 1993b) assaying effects of zooplankton and nutrient (N and P) additions to mesocosms and cubitainers included the estimation of phytoplankton (and AFA) density and biovolume but description of methods used were not specific enough to evaluate the methods. Section 5.0 of this report provides information on tests of in-lake enclosures that could be used to assay effects of nutrient additions, humics or barley straw on growth of AFA. The growth form of AFA and its changes with preservation are the challenges to quantification of both density and biovolume, as examined in the above paragraphs. Using a method of quantifying density and biovolume that is based on the method described in APHA *et al.* (1998), USGS (1989) and Lund *et al.* (1958) will set expectations for confidence limits related to counts. This will improve the usefulness of the data in drawing conclusions about results of any such assays. We suggest sample analysis methods for UKL phytoplankton be revisited and changes made in the analysis methods to provide a more complete characterization of AFA.

## **4.0 DISSOLVED ORGANIC MATTER AND *APHANIZOMENON FLOS-AQUAE***

### **INTRODUCTION**

#### **Potential inhibition of AFA by wetland humics**

The primary purpose of this work was to provide initial evidence that addition of humic water to lake water would inhibit the growth of AFA. Inhibition can be measured over a wide range of time scales from photosynthetic electron transport (seconds to minutes), gross photosynthesis (minutes to tens of minutes), net photosynthesis (hours), and cell growth (tens of hours to days). Each of these time scales and processes has available techniques; electron transport - Pulse Amplitude Modulated (PAM) Fluorometry, gross photosynthesis - O<sub>2</sub> electrode, net photosynthesis - <sup>14</sup>C fixation, and population growth - cell counts.

Our starting hypothesized mechanism for inhibition of AFA entails photoreactive dissolved organic compounds causing damage, directly or indirectly, under high light. Several of us on this project (EH and JR) developed this hypothesis from our experience growing AFA in mass culture for a commercial enterprise. This hypothesis can be applied to Upper Klamath Lake; the combination of high UV radiation at UKL and fresh dissolved organic carbon coming from the marshes would retard the growth of AFA. The proposed inhibition doesn't have to kill the cells but could result in photoinhibition that would slow the growth. The photoinhibition response to light and media conditions can be measured with a PAM fluorometer. PAM fluorometer techniques also allow us to differentiate between photoprotection mechanisms (that decrease photosynthesis to avoid damage to the cell) and photoinhibition (which results in decreases in photosynthesis because of damage to the cell). PAM Fluorometry has been a method of choice in similar studies between allelopathic compounds from macrophytes (Korner and Nicklisch 2002) in part because inhibition of photosynthesis seems to be a common mechanism of allelochemicals (Gross 1999).

#### **Marsh water characteristics**

From previous studies on humic acids in the marshes in the UKL basin (Barber *et al.* 1999; Lytle and Perdue, 1981; Peek 1963; Perdue *et al.* 1981) we know that there are a wide variety of materials coming out of different types of marshes. We have no *a priori* basis for choosing any particular marsh type for producing potentially algistatic or algicidal organics. We do, however, have an end constraint in the context of

management; only certain types of marshes could be included in the lake in sufficient areas to be a viable strategy. For this reason we focused our exploration of the humic components coming from the major marshes represented in the lake already.

The main features that we needed to know about the water coming out of marshes are the total dissolved organic content, the photoreactivity of that material, and the biological activity. All of these factors are multi-dimensional. For our purposes we would like to have easily measured indicators of amount and reactivity that relate to the inhibitory potency. For this reason, our analysis of total organic carbon, and absorbance or fluorescence characteristics focused on indices rather than full characterization. For the amount of work that it would take to do a full chemical characterization of the water, we could directly measure inhibition potential using bioassays with algae.

### **Multiple possible models for marsh water effects**

One of the complicating aspects of this type of work is that there are so many possible inhibitory mechanisms. These mechanisms could be acting individually or in combinations to produce the net end-effect. Below is a list of mechanisms that have been demonstrated or proposed by others for the effects of humics on algae:

- a. inhibition of cell membrane processes;
- b. indirect inhibition of photosynthesis through the production of hydroxyl-radicals from hydrogen peroxide (photo-Fenton reaction) (Van der Zee *et al.* 1993, Scully *et al.* 1996);
- c. inhibition of alkaline phosphatase and other surface enzymes (Stewart and Wetzel 1982, Kim and Wetzel 1993);
- d. impacts on phosphorus and iron availability (Stewart and Wetzel 1982, Francko 1986, Purdue *et al.* 1981, Klug 2002, Peek 1963, Jones 1998);
- e. a large number of possible community structure changes associated with organic carbon availability (for example heterotrophic bacteria competing with autotrophic algae for nutrients) (Williamson *et al.* 1999, Vahatalo *et al.* 2003).

In a complex system, the interactions between these mechanisms would be expected rather than be a surprise. The high reactivity of fresh dissolved organic compounds should allow them to be involved in many reactions. In such a system, the reductionist approach of isolating a single cause is likely to fail. For this reason we designed our experiments to maintain as much of the structure of the original community as possible.

## **METHODS**

### **Sampling sites**

A list of all of the sampling sites is provided in Appendix C in separate CD. These sites were used for collection of samples for dissolved organic carbon analysis, fresh algae and for humic water that was used in the inhibition experiments.

### **Criteria for AFA health**

Sometimes it is obvious when AFA populations are unhealthy. Unhealthy conditions usually include a surface scum of AFA, broken colonies, and an earthy smell. These accumulations often occur at the shore or in bays and may be caused as much by hydrodynamic concentration mechanisms as by growth of the AFA. Algae for our assay were not collected from these conditions.

After collection of algal samples it was necessary to use the PAM fluorometer to examine their photosynthetic characteristics. Measuring the photosynthetic efficiency and a light curve will immediately indicate whether the population is healthy enough for subsequent inhibition studies. The description of how these parameters are measured is described below.

### **Photosynthesis parameters measured by PAM F**

Photosynthetic productivity (the amount of energy that is used to fix carbon) is very generally correlated to the amount of energy that is absorbed and inversely related to how much of the absorbed energy is re-emitted as fluorescence. When algal cells are exposed to light, there three fates for the absorbed light energy; 1) the energy can be used for carbon fixation, 2) the energy can be re-emitted from the cell as longer wave light (fluorescence), or 3) the energy can be re-emitted as heat. When cells are left in the dark for a short time (10 minutes) the molecules in the photosynthetic membrane that would initially receive electrons from the

light trapping reactions are all in the oxidized state, ready to pick up electrons. In this case the amount of fluorescence is at a minimum. After exposure to light the electron transport molecules are partially reduced. In this case, more of the light energy that is absorbed by the light trapping pigments cannot be transferred to carbon fixation and is re-emitted as fluorescence. There also conditions that change the amount of energy that is lost as heat, but those conditions will not be discussed here.

The PAM fluorometer is a very flexible instrument that makes rapid measurements of the fluorescence before and after pulses of different strengths of light. After at least 5 minutes in the dark, the fluorescence should be at a minimum (called F<sub>0</sub>). This is because all the electron acceptors are oxidized and readily accept electrons. After a saturating light pulse the fluorescence should be at a maximum (F<sub>m</sub>). This high fluorescence is because there are few electron acceptors left in the available state to absorb electrons, and the energy is dissipated as fluorescence. The difference between the minimum and maximum fluorescence values (F<sub>v</sub>) is an indicator of the number of photons that were absorbed and utilized in the pre-flash condition. F<sub>v</sub>/F<sub>m</sub> is a robust measurement of potential photosynthetic efficiency of cells. Because this is a ratio of the almost instantaneous measurement, this parameter is particularly useful when studying flakes of AFA. Individual readings of F<sub>0</sub> and F<sub>m</sub> may differ because of the geometry of the flakes in the cuvette, but the ratio is very robust.

The PAM fluorometer also has the capacity to expose the cells to an internally generated (and calibrated) range of light. This is used to generate a relationship between light energy and electron transport rate (ETR). Many studies with different algae and higher plants have shown that ETR is a suitable proxy for short-term photosynthesis under non-stressed conditions (Gilbert *et al.* 2000). In our work, the light exposures were usually chosen to be light values of 0, 50, 100, 200, 400, 800, 1200 and 2000  $\mu\text{E m}^{-2} \text{s}^{-1}$ .

A crucial piece of our starting hypothesis is that under certain conditions, AFA is sensitive to photoinhibition. We think these conditions are the combination of high light exposure and the presence of photo-reactive. There are protective mechanisms that allow algae to respond to high light with a temporary decrease in photosynthesis. Our preliminary work is not concerned with these photoprotective mechanisms except as they dictate the time scale of our measurements of photoinhibition. The decrease in photosynthesis that persists after an hour is what we call "photoinhibition". Photoinhibition of AFA by humics and light exposure should only be reversible by the synthesis of new

cellular material. This means that a 50% photoinhibition should set the cells back one division time.

### **Inhibition and recovery studies with AFA**

Two protocols were used to explore humic and light exposure. In both protocols lake water containing AFA was mixed with filtered water containing (or not containing) high concentrations of humic material. In the first protocol these mixtures were exposed to artificial light sources that have only a PAR spectrum or a PAR spectrum plus 8% higher UV light than a sun light. This system is easy to control but, unfortunately the maximum PAR is in the range of  $100 \mu\text{E m}^{-2} \text{ s}^{-1}$  and the UVR is only around  $5 \mu\text{E m}^{-2} \text{ s}^{-1}$ . The second protocol used natural light for exposure. This allowed us to give PAR dose rates up to around  $1600 \mu\text{E m}^{-2} \text{ s}^{-1}$  and UVR doses up to  $106 \mu\text{E m}^{-2} \text{ s}^{-1}$ . In the future, this second protocol should be modified to provide multiple light levels (using neutral screening), UVA + PAR (using a mylar filter) and just PAR (using a Plexiglas filter).

### **Analysis of DOC characteristics**

The dissolved organic carbon (DOC) fraction of the water was analyzed using three methods; spectrophotometer scans from 250 to 700 nm, spectrofluorometer readings at selected combinations of excitation and emission, and total DOC as analyzed in a total carbon analyzer. The analysis and choice of indicator ratios is described in the results section. A Shimadzu total organic carbon analyzer, model TOC-V CSH was used. Forty mL samples were acidified and placed in the automatic sampler for injection. Comparative absorption spectra were determined in 1 cm path length cells in a spectrophotometer with a 6 cell chamber. Fluorescence response spectra were determined in 1 cm path length cells in a Jasco spectrofluorometer. Excitation/emission pairs suggested by Marhaba *et al.* (2000) were used as a rapid screening process for different DOC components. The components and wavelength pairs are given in Table 3. Other wavelengths were selected as proxies for dissolved organic carbon (McKnight *et al.* 2001) and to explore the effects of photobleaching (Morris and Hargreaves 1997, Osburn *et al.* 2001). We were able to verify these relationships by comparing these spectrophotometer and spectrofluorometer readings to filtered TOC values (see the results section).

Table 3. Dissolved organic components that should be indicated by fluorescence peaks using the rapid screening method of Marhaba *et al.* (2000).

Fraction	excitation	emission
hydrophobic neutral	231	315
hydrophilic acidic	231	351
hydrophilic basic	231	363
hydrophobic basic	231	375
hydrophilic basic	231	615
hydrophobic acidic	243	423

The potential to support the photo-Fenton reaction was not studied even though it was originally proposed. The photo-Fenton reaction is just one mechanism that could lead to photodamage and inhibition of the cells. The specific conditions that are necessary for the photo-Fenton reaction to occur (photoreactive humics, dissolved iron and H<sub>2</sub>O<sub>2</sub>) may or may not be present in the lake. Instead of studying the photo-Fenton reaction, we studied the more general effect of photoinhibition (using PAM fluorometry) and worked on developing more robust measures of photoreactivity of the humic compounds in the water (such as using the "slope" method described in the results.) These techniques are more directly related to the inhibition of AFA and they can be implemented in the context of either limno-corrals work or transects of the lake.

### **Pattern of photosynthesis potential in the limno-corrals**

The photosynthetic potential of phytoplankton in the limno-corrals was compared to the potential of cells sampled from right outside the bags in the morning and in the afternoon when wind mixing was starting to occur. Duplicate samples from in and out of the bags were run for F<sub>v</sub>/F<sub>m</sub> and light curves using the PAM fluorometer. Results of this work are reported in Section 5.0

## **RESULTS**

### **Inhibition and recovery studies**

Two protocols were used to examine the effects of humics and light on AFA. The first protocol used low light doses and resulted in minimal

inhibition. The second protocol used 10 to 30 minute exposures to the natural sunlight spectrum and resulted in dramatic inhibition.

An example of the low exposure protocol is given in Figure 11. In this experiment fresh colonies from the dock in the west bay at Moore Park Marina were diluted with pure drinking water or filtrate from the Running Y golf course channel. These samples were incubated under the Reptisun8, enhanced UV lamp and measured for photosynthetic characteristics. The efficiency of the cells (measured as the Fv/Fm ratio) is lower immediately after exposure to the marsh water extract. This clear inhibition by marsh water doesn't last long, and after 20 minutes of recovery in the dark both cultures demonstrate responses that are overlapping. Such observations underscore the importance of being able to take rapid measurements on the highly dynamic responses of algae to light and humics.

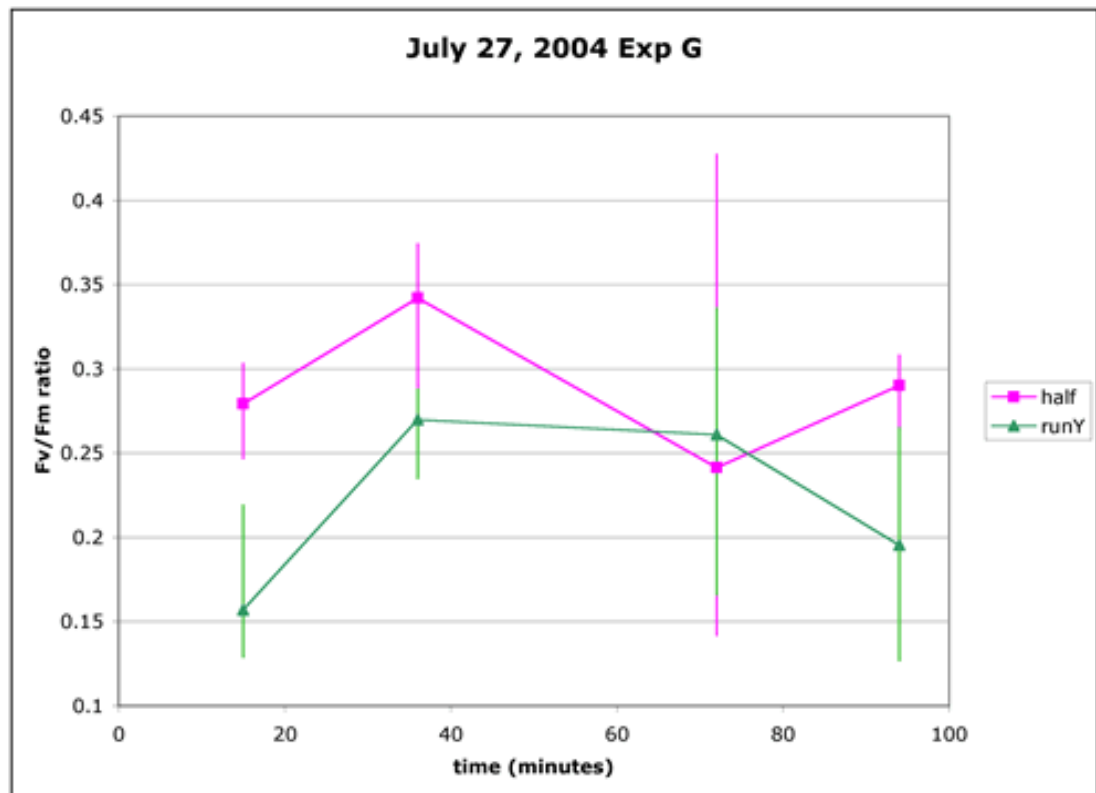


Figure 11. Moore Park colonies mixed with RunningY brown water. After a 20-minute exposure to a Reptisun8 light source the cells were put in the dark. Each point

represents 5 readings on one sample, the point is the mean and the bars show the range (not the standard error).

The second protocol inhibition experiments all show definite inhibition of cells by light treatment, dilution and mixture with filtered brown water. Without further work, we can't be definitive about the statistical significance of these data, however it is clear that the mixture of algae with humic rich water had a detrimental effect. The results from just one of the three experiments are shown because they all had similar general behavior. In "run 2" (Figure 12), all exposed cells had much lower Fv/Fm ratios but the humic addition caused the most severe limitation. Two other runs had slight differences that should be examined more fully. Run 1 was similar except that the humic mixture "F" had a dramatic and unrecoverable inhibition. In run 3 the humic addition had the lowest average Fv/Fm ratio and, importantly showed the least sign of recovery. In run 3 (and to a lesser extent in the other two runs) the addition of humics had an inhibitory effect even on the samples kept in the dark. This effect may be particularly important in the application of this to real mixing scenarios where the PAR and UV doses would be very small except for at the very top of the water column.

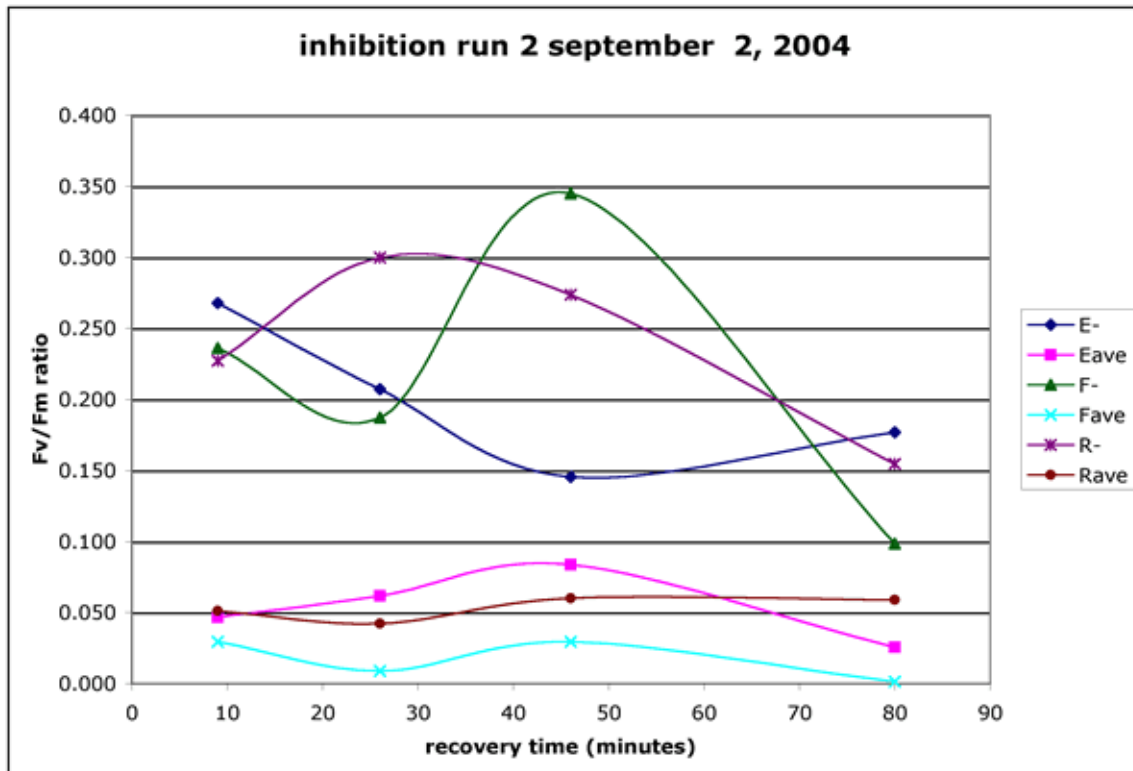


Figure 12. Inhibition experiment run2, Sept. 02, 2004. Algae were collected from the limno-corrall site. The brown water was collected from the canoe dock on the Running Y resort. The different conditions are: E was 1/2 water with algae and 1/2 filtered water both from the limnocorrall site, F is 1/2 algae from the limnocorrall site and 1/2 water from the Running Y canoe dock, and R was full strength water and algae from the limnocorrall site. The “-“ treatment is in the dark. The “ave” treatment is the average of two samples that were in the same light. The samples were exposed to light for 21 minutes from 14:29 to 14:50. The PAR dose averaged  $1609 \mu\text{E m}^{-2} \text{ s}^{-1}$ . The UV dose was  $106 \mu\text{E m}^{-2} \text{ s}^{-1}$  of UV (UVA and UVB). The non-exposed points are the mean of four readings on a single sample at each time point. The light exposed points represent the mean of two separate samples with four readings on each sample.

### **Comparison of photoinhibition results with different DOM sources**

Three sources of humic rich marsh water were used in this study. A short description of the sampling location and the exact GPS coordinates are given in Appendix C. The three sites were the BLM Wood River wetland, the canal near the fourth tee on the Running Y golf course and the restored wetlands water at the canoe dock on the Running Y resort. All of these showed inhibitory impact on AFA. In addition, a commercial barley straw extract was used to inhibit photosynthesis. It is difficult to compare the potency of the individual sources because the live algal cells that were used were collected at separate times.

The promising result in this study was that all four sources had some inhibitory effect even though they came from different environments. The Wood River Marsh is a holding area for water that should have very "old" humic rich water. The Running Y golf course channel water is a small area that is rich in algae and nutrients. When we sampled the Running Y canoe dock location the water was not very brown and didn't have a high microalgae concentration but it was very potent as an inhibitor. The Running Y canoe dock is in a restored wetland that was constructed in 1997.

### **Analysis of different water sources**

Humic rich water was collected from many sources and analyzed with several approaches. Filtrates of the all water samples show strong absorption in the near UV. Samples from Running Y golf course canal and from the Wood River wetland show a sharp decrease in the specific absorbance as wavelength is increased (Figure 13). Samples collected

from the canoe trail near Crystal Springs boat ramp show the same pattern but have lower starting absorbances.

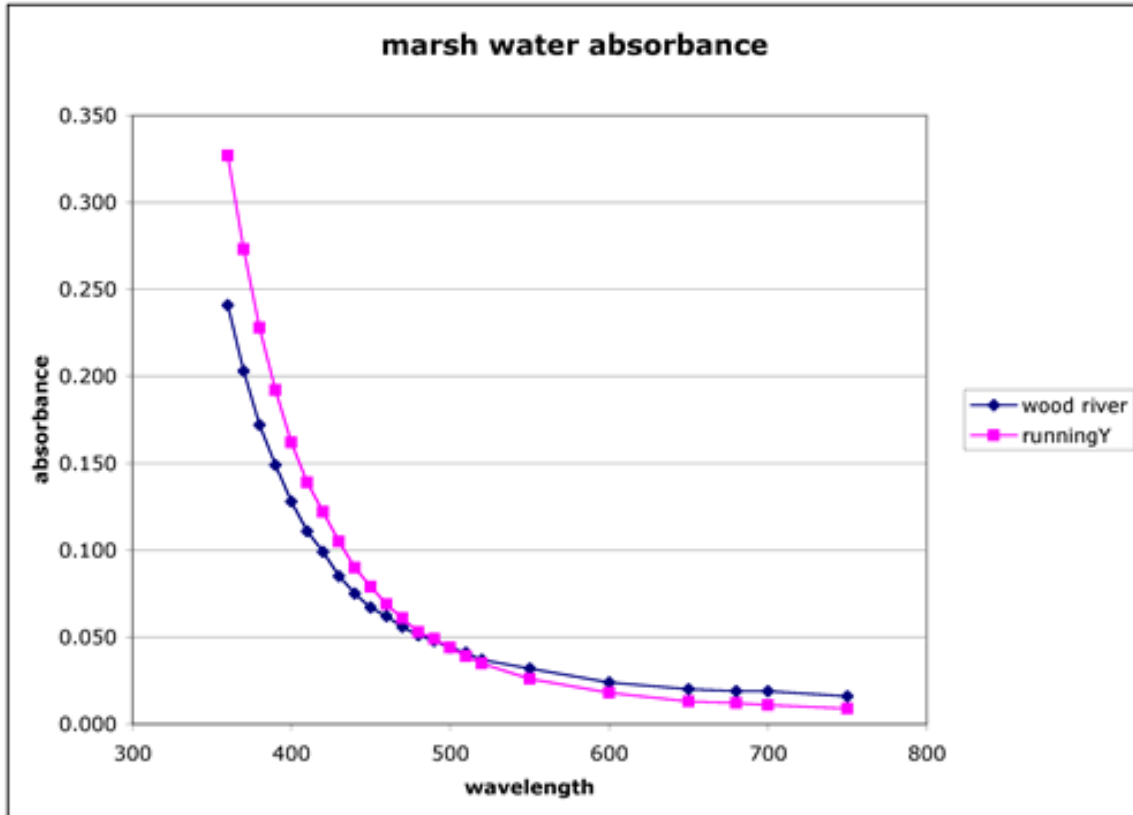


Figure 13. Absorbance of filtrates of Wood River Wetland and Running Y golf course channel.

As part of an attempt to chemically characterize the humic content of these samples, we used a rapid screening fluorescent method developed by Marhaba *et al.* 2000. This method looks for relative peaks in fluorescence that are supposed to correlate to six fractions of acid, neutral, base with hydrophobic and hydrophilic conditions for each. The results that we obtained from this screening method show that the samples have higher fluorescence in the basic fractions and that acidic hydrophobic fraction does not show a relative peak. This is in contrast to Barber *et al.* 1999 work on the humic fractions in UKL, which all contained a prominent acidic hydrophobic component. There are major differences in the collection and analysis between the methods used by Barber *et al.* (1999) and Marhaba *et al.* (2000). It would take a study using both methods on the same sample to resolve the differences. For the purposes of this report however, it is a useful finding that the rapid

screening method cannot be used on the samples from this lake without more research.

In Tables 4 through 6 the relative fluorescence for different excitation/emission pairs from the method of Marhaba *et al.* 2000. are compared. A "+" means a relatively high fluorescence signal and the "-" is the lowest. The absolute fluorescence signals on the Running Y samples were lower than the Wood River Marsh even though the absorbance (see Figure 13) was higher.

Table 4. Relative fluorescence peaks in BLM Wood River Wetland.

	hydro-phobic	hydro-philic
Acid	.	.
Base	+	+
Neutral	.	-

Table 5. Relative fluorescence peaks in Canoe Trail sample 1.

	hydro-phobic	hydro-philic
Acid	.	+
Base	+	.
Neutral	.	-

Table 6. Relative fluorescence peaks in Running Y golf course channel.

	hydro-phobic	hydro-philic
Acid	.	.
Base	+	.
Neutral	-	-

Samples were collected for dissolved organic carbon content on trips on July 27 and 28 and on August 23. These samples were kept on ice and then refrigerated. About 50 mL was filtered through Whatman GF/A filters using a syringe filtration system. A volume of just over 40 mL was used for TOC analysis and about 6 mL was used for absorbance and fluorescence measurements. Thirteen samples were analyzed for DOC using a Shimadzu total carbon analyzer. The samples were acidified prior to analysis. For a complete description of the sampling sites and the Lat-Long coordinates please see Appendix C. These samples fall in the expected range of total organic carbon, however our samples for the BLM Wood River wetland had lower TOC than previous measurements (Kurt Carpenter, pers. com. 2004).

#	Sample site	date	comment	TOC mg/mL	slope*
1	Moore Park Marina	2004.08.23		26.31	- 0.0133
2	Lower Klamath Marsh channel	2004.08.23		33.42	- 0.0133
3	UKL at Running Y Apple Tree	2004.08.23		24.05	- 0.0124
4	Running Y golf course channel	2004.08.23		137.10	- 0.0154
5	UKL at Shoal Water Bay	2004.08.23		28.23	- 0.0109
6	Thompson Creek	2004.08.23	not sure if this creek leads into Thompson Creek	26.62	- 0.0147
7	BLM Wood River Wetland	2004.08.23		52.15	- 0.0140
8	BLM Wood River Wetland	2004.07.27	looks more muddy than brown	45.20	- 0.0149
9	UKL Pelican Bay Canoe Trail 1	2004.07.27	from under the Wocus plants	24.40	- 0.0139
10	UKL Pelican Bay Canoe Trail 2	2004.07.27	from open water in the trail	24.60	- 0.0141
11	Running Y golf course channel	2004.07.27		139.10	- 0.0183
12	Commercial Barley extract 1/1000	2004.07.27		7.39	- 0.0133
13	Commercial Barley extract 1/100	2004.07.27		7.88	- 0.0133

\* The "slope" is calculated as the decrease in the natural log of the absorbance against the wavelength between 250 and 500 nm.

These samples were examined with both spectrophotometer and spectrofluorometer analysis. We were attempting to relate the total organic content and the little we know about each of these water sources with absorbance or fluorescence characteristics that have been shown to indicate particular processes. The slope of the absorbance curve should indicate the photoreactivity. This is because the most photoreactive compounds would absorb strongly in the shorter UV wavelengths. A lower slope indicates that relatively less of the higher shorter wavelengths are being absorbed. We found a relationship between the total organic carbon and the slope of the decrease in absorbance (Figure 14a.). This relationship implies that sources of water with higher organic content would probably contain a smaller proportion of UV absorbing compounds, and thus are likely to be less photoreactive. The highest point (DOC = 28.23, decrease in absorbance = -.109) is from Shoalwater Bay. The sample came from the outflow of a large marsh area and was highly turbid with mud. The high turbidity could have protected the organics from degradation.

The UV absorbance at 250 nm (Figure 14b) and the fluorescence emission at 450 nm with excitation at 370 nm (Figure 14c) were both related to the dissolved organic carbon. These indicators could be used as rapid estimates for the amount of dissolved organic carbon. It should be an advantage in future work to have a spectral or fluorescence signature that could be used to track or identify biologically active humic water.

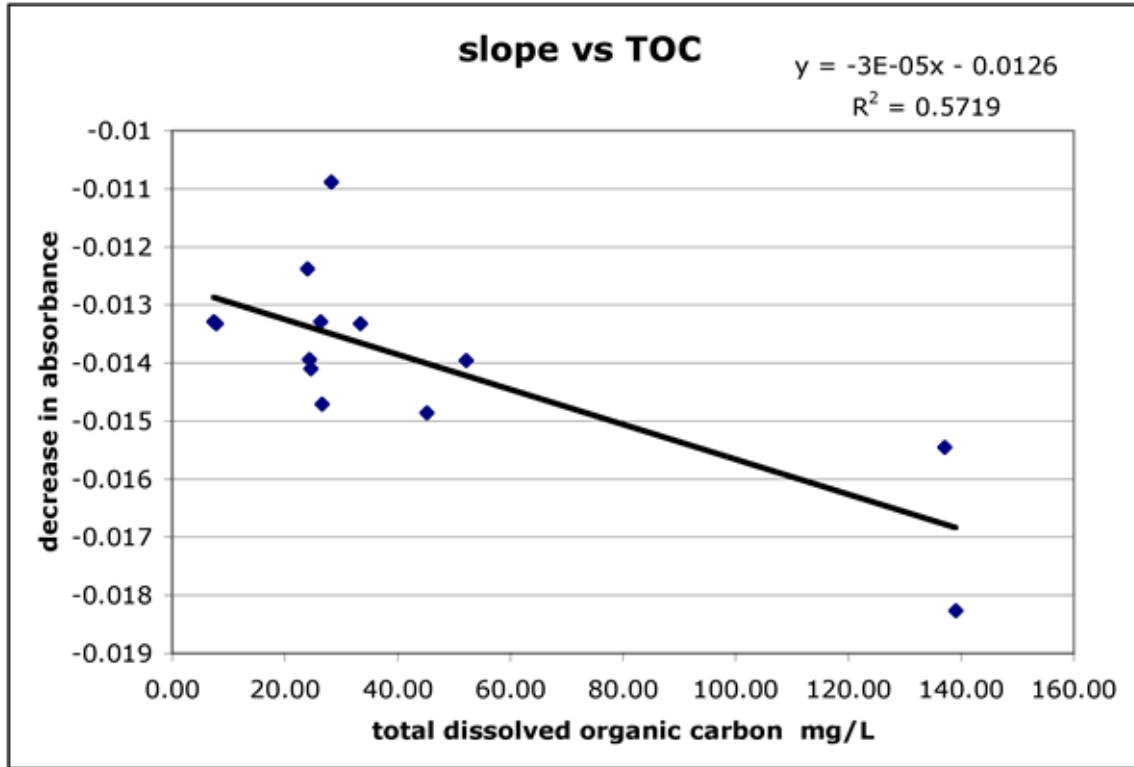


Figure 14a. The slope of the decrease in absorbance with wavelength plotted in relation to the total organic carbon concentrations.

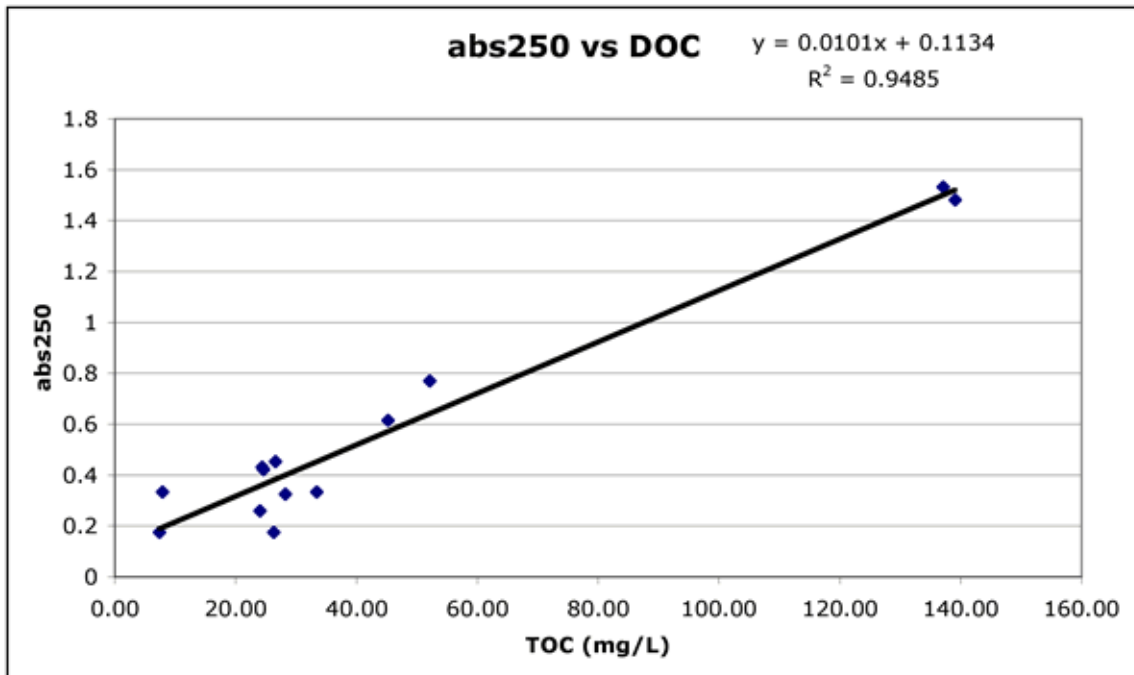


Figure 14b. Absorbance of the filtered water at 250 nm.

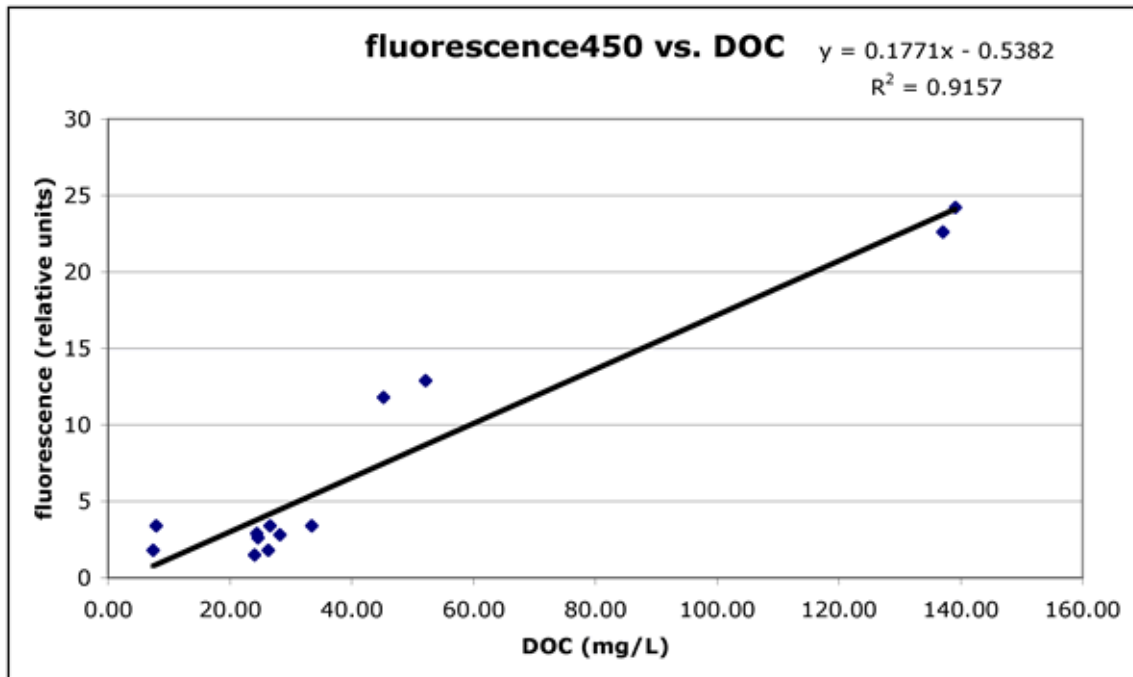


Figure 14c. Fluorescence for excitation at 370 nm and emission at 450 nm.

## DISCUSSION

All of the evidence collected during this work supports the idea that the AFA population in the Upper Klamath Lake could be reduced by introduction of humic substances from marshes. All of the high organic sources tested (BLM Wood River wetland, a drainage canal at Running Y, and a restored marsh at Running Y) had inhibitory effect on AFA. This means that natural marshes, restored marshes and agricultural field type drainage related to barley production could be effective components of an overall strategy for lake restoration.

If in-lake marshes are used as a restoration measure, the complex nature of the problem will require flexible assessment methods. With the investment of more time in experiments, the PAM fluorometer could be a valuable tool for rapid assessment of the inhibition of AFA. Multiple samples can be run quickly on the current PAM fluorometer and there are other PAM fluorometer configurations (such as a fiber optic detector in combination with a microplate reader) that would be ideally suited to the type of monitoring that this project would require. The PAM

fluorometer has limitations. In particular it is difficult to compare light curves from one sample to another because of the sensitivity of the PAM fluorometer to the number of flakes and their orientation in the chamber. An attempt at overcoming this limitation by homogenizing the flakes prior to reading showed that the cells would show near normal fluorescence for several minutes and then totally crash. There are alternatives methods for measuring primary productivity that could be considered. The O<sub>2</sub> electrode methods would be accurate enough for this project but it would be impossible to scan multiple samples rapidly. <sup>14</sup>C fixation is always a regulatory issue in field work and in this case, the changes in photosynthetic rate over the day would complicate this type of analysis. Growth rate estimates from net increases in flakes, filaments and cells should be done on the limno-corrals but can't be used as a rapid screening tool for the short-term effect of humic addition.

Continued studies and in particular the transects into natural marshes will require the ability to characterize the humic content and photoreactivity rapidly. Although future studies will probably include concentration of humic samples and more sophisticated analysis of that material, we will still need a field method. There are several absorbance ratios and fluorescence characteristics that have been shown by other investigators to be useful. Some of these were tested here and found to be workable.

Future work should focus on the effect of humic additions to the lake during the spring. During this time the AFA should be establishing a population and setting the trajectory for either a large bloom or possibly, a greatly reduced population of AFA during the summer. This second trajectory is still highly speculative. We didn't collect any direct evidence that would prove that the population of AFA would stay lower. A demonstration project that uses limno-corrals to examine the effects of humics, light, and temperature on the community structure is a crucial next step.

Our project should also expand its theoretical basis. The current demonstration project is based on a single descriptive model. Although it is important that the results so far have supported our hypothesis, it will be difficult to increase the sophistication and depth of the project without considering multiple models. In particular, we need multiple models for how the humic input to the lake may effect AFA populations. We can generate specific hypotheses from these models and test them against the data. For example, humics may be playing several roles such as direct inhibition of AFA photosynthesis and the indirect effect as a carbon source for competing bacteria. Given the applied nature of the overall project the theoretical development should keep in mind the constraints of possible reconstruction strategies.

This preliminary study demonstrated the feasibility of the general experimental approach but there are still specific details that need to be worked out during the continuing work. Below are six major points that should be addressed as part of future work.

1. These results are promising but very mixed. The simplest and usually most robust measurement of photosynthetic efficiency ( $F_v/F_m$ ) did not provide consistent results in these incubations. More detailed examinations of photosynthetic parameters show that the marsh water and UV treatments usually had an effect on non-photosynthetic quenching. The parameters that describe the non-photosynthetic quenching have to be determined in samples that are exposed to ambient light, making this a much more complicated and difficult measurement to make. It would be very interesting if the marsh water effect is to reduce the effectiveness of the cells to deal with photoinhibition. If this is the case, it could have maximum impact during the spring when the water is clearer and colder.
2. The commercial barley straw extract served as a nice comparison treatment. We should consider using this as one of the treatments that we employ in the limno-corrals (see Section 5.0; see information on barley straw decomposition and potential effects on cyanobacteria in Appendix D).
3. We need to understand how we might modify the method of Marhaba *et al.* (2000) to achieve a correlation with the results of Barber *et al.* (1999). This might require a more sophisticated spectrofluorometer that can perform an entire matrix scan. The new diode array spectrofluorometers can capture all the emissions as it scans through a range of excitation wavelengths.
4. It would be difficult to identify reactive humic waters given the absorbance and fluorescence measurements that we have been able to take. Perhaps concentrated humics samples could be used in inhibition tests (see Perdue 2003).
5. The work over the last month helped clarify the value of the PAM fluorometer in these studies. It turned out that the dilution and light exposure regimes used were too "heavy handed". More work should be done that explores lower humic contributions (in the range up to 10% of the total rather than 50%) and lower light exposures. Dilutions and light exposures in these ranges are much more realistic for humic inputs during the spring.



## **5.0 IN-LAKE ASSAYS**

### **INTRODUCTION**

Being able to test, in the lake, hypotheses of what may influence the growth of *Aphanizomenon flos-aquae* has been very attractive for many years (e.g. Miller and Tash 1967, U. S. Army Corps of Engineers 1979, 1982, and Ehinger 1992). The advantages of having a volume of water, which for a short while is a mesocosm of large Upper Klamath Lake is attractive because the important variables of light, temperature, drifting algae population composition and dynamics, and zooplankton, may, for a short time, be similar to that of adjacent water in UKL. Mesocosm, which means middle-sized world, is distinguished from laboratory environments (microcosms) and the lake ecosystem (macrocosm). It is valuable to have laboratory assays or experiments, i.e. microcosms, that control variables with known AFA in cultures under well defined conditions (as reviewed in report Section 2.0), but the next step in experimentation or testing to confirm laboratory results and introduce natural variables is something done in the lake, *in situ*.

### **REVIEW OF PAST UKL IN-LAKE ASSAYS**

#### **Ehinger 1990-1991 Mesocosm and Microcosm Experiments**

In 1990, the Bureau of Reclamation prepared plans and designated funding for a long-term assessment that would focus on the Agency Lake subbasins of the Upper Klamath Basin, particularly the Wood River and Sevenmile Canal drainage areas. The objectives and plans for this study were reviewed by Campbell in 1992 and 1993 (Campbell 1993a and 1993b). Campbell noted in 1993b, "The immediate objective of the studies is to develop a management plan for the Agency Lake sub-basin to improve lake water quality and habitat as well as stream and watershed habitat, which will maintain and enhance the endangered sucker populations." The project did not result in a management plan, but it did initiate a cooperative arrangement with the Klamath Tribes that has resulted in a long-term water quality monitoring program and database reviewed in Section 2.0 of this report. The project also initiated some interesting in-lake assay work by W. J. Ehinger reported in Campbell (1993a, 1993b).

The Ehinger research performed in 1990-1991 at Agency Lake is described in Ehinger (1993a, 1993b, and 1992). The Ehinger 1993b

report is a summary of 1990-1991 in-lake cubitainer research findings that were described in more detail in Ehinger's Ph.D. dissertation (1992).

Before reviewing what he called "microcosm" experiments using small cubitainers, we note results of his limited work with "mesocosms" described in Ehinger (1993a). He described three experiments performed using 380 liter plastic livestock watering tanks that were located on the shores of Agency Lake. Tanks, generally 12 per experiment with three replicates per treatment, were filled with Agency Lake water.

Experiments were conducted in June 1991 (21 days), July 1991 (13 days) and July-August 1991 (13 days). The objective of this work was to examine trophic level interactions under nutrient enriched and unenriched conditions in the mesocosms. On-site culture of *Daphnia pulicaria* (*D. schodleri*? [see report section 2.0]) provided zooplankton treatments, and blue chub (*Gila coerulea*) from mouth of Williamson provided fish treatments. Phytoplankton and zooplankton composition, density and biovolume estimates were made at the start, during and at the end of the experiments. Measurements of water quality were also made (NH<sub>3</sub>-N, TN, NO<sub>3</sub>+NO<sub>2</sub>-N, SRP, TP, Chlorophyll a, phaeophytin a, DO, pH, SPC, temperature). Tanks were also screened to prevent insects from entering and to lower intensity of sunlight in tanks (because previous work with *Aphanizomenon flos-aquae* showed that shallow, 0.6 m depth, tanks "...did not provide a refuge from excessive ultraviolet radiation.") At the time of providing preliminary results, some phytoplankton analyses had not been completed. Our observations on this work are:

- There appeared to be no difference between the high or low TN:TP mass supply ratios with respect to changes in total phytoplankton biomass in the 21-day June experiment and some evidence of the influence of fish on reducing zooplankton and thereby allowing greater phytoplankton biomass. June phytoplankton was not yet dominated by AFA, with diatom biomass greater than AFA biomass.
- In the two 13-day experiments (one unenriched, one enriched) where fish were used (or not) to change zooplankton density, phytoplankton biomass (presumably dominated by AFA in July and August) peaked at midcourse, with high fish predation on zooplankton resulting in highest phytoplankton biomass in unenriched experiment. The enriched experiment had chlorophyll a values similar among replicates and 2-3 times higher than unenriched. Chlorophyll a values in both experiments declined drastically (the "crash") by the 13<sup>th</sup> day.
- No observations of concurrent water quality conditions in Agency Lake were provided by Ehinger for perspective on how different

conditions in the mesocosms were (pH, DO, temperature) from lake conditions.

- Estimates of AFA biomass are conversion of density and biovolume estimates to biomass where  $1 \text{ mm}^3 = 1 \text{ mg}$  fresh weight (previous caveats noted in this report in Section 2.0 apply to these estimates of AFA density and biovolume).

Six cubitainer (cube-shaped plastic containers holding 4 liters) experiments were conducted by Ehinger from September 1990 – October 1991. Each experiment consisted of a nutrient (nitrogen and phosphorus) bioassay and a gradient in the population density of *Daphnia pulicaria* in cubitainers. The replicated (n=2-4) bioassay treatments consisted of: 1) no nutrient addition; 2)  $1000 \mu\text{g KNO}_3\text{-N L}^{-1}$ ; 3)  $100 \mu\text{g K}_2\text{HPO}_4\text{-P L}^{-1}$ ; and 4)  $1000 \mu\text{g KNO}_3\text{-N L}^{-1}$  plus  $100 \mu\text{g K}_2\text{HPO}_4\text{-P L}^{-1}$ . In separate cubitainers (7-14 [Ehinger 1993a] a gradient of *Daphnia* densities (5 to 125 individuals/liter) was set from on-site *Daphnia* cultures. Decreased *Daphnia* in cubitainers was produced by removing *Daphnia* (and AFA) with a net, killing *Daphnia* with carbonated water, then adding net AFA back to cubitainer. Water quality analyses, like those performed for mesocosm experiments above, were performed on water in the 380 liter livestock tank, one-half full, at the time water was taken from the tank to fill the 4-liter cubitainers. Nutrients were analyzed at the end of the September-October 1991 experiment only. The cubitainers were suspended or anchored just below the water surface and incubated 3-4 days in Agency Lake.

Results of these cubitainer assays are summarized below in Table 7.

Table 7. Summary results of six cubitainer experiments conducted in Agency Lake, OR 1990-1991 (Ehinger 1992, 1993a, 1993b). TPB=total phytoplankton biomass; AFA=*Aphanizomenon flos-aquae*; GR=growth rate; REPS.=replicates.

<b>DATE</b>	<b>REPS.</b>	<b>SUMMARY RESULTS</b>
<b>September 28 - October 1, 1990</b>	2	TPB sig. positive response to <i>Daphnia</i> TPB N-limited
<b>October 15-18, 1990</b>	3	TPB no sig. effect from <i>Daphnia</i> AFA GR not affected by <i>Daphnia</i> TPB P-limited
<b>December 2-5, 1990</b>	3	TPB neg. affected by <i>Daphnia</i> AFA no response to <i>Daphnia</i> TPB P limited
<b>August 15-18, 1991</b>	3	TPB not sig. affected by <i>Daphnia</i> AFA not affected by <i>Daphnia</i> TPB N and P-limited AFA P-limited
<b>September 20-23, 1991</b>	3	TPB not sig. affected by <i>Daphnia</i> AFA not affected by <i>Daphnia</i> TPB N-limited AFA N-limited
<b>September 28-October 1, 1991</b>	4	TPB positively affected by <i>Daphnia</i> AFA marginally sig. Response to <i>Daphnia</i> TPB N-limited AFA responded to P-enrichment

Ehinger (1993b) noted in a summary discussion of results that of the six times the experiment was conducted, chlorophyll a concentration increased in response to nitrogen addition three times, to phosphorus addition twice, and to both one time and that these results were unexpected for a hypereutrophic lake. He noted that the phosphorus limitation in August 1991 and October 1990 experiments occurred when soluble reactive phosphorus concentration with which cubitainers were filled was >150 µg-P/l, which suggested to him that SRP may not always be in a form readily available to phytoplankton. He also noted that the combination of zooplankton grazing resistance (i.e. by AFA) and zooplankton regenerated nutrients was responsible for increase in observed growth rates. He concluded that his experiments showed the relative importance of nutrient limitation and zooplankton grazing varied seasonally in Agency Lake (1993b):

“During summer and fall, N-limitation is strong and *Daphnia* have no effect or a positive impact on total phytoplankton biomass. In late fall and winter, P-limitation was evident and increased susceptibility to *Daphnia* grazing was seen as the phytoplankton community shifted to dominance by diatoms and flagellates. This variability in the relative strength of nutrient and zooplankton effects on phytoplankton biomass and community structure reflects the interdependence of zooplankton and phytoplankton biomass and community structure. Species that are susceptible to grazing tend to be dominant in the winter and spring when *Daphnia* biomass is low, and large, colonial blue-green algae tend to dominate when *Daphnia* biomass is high....The study indicates that P-limitation, although transitory, may be important in determining algal growth rates.”

After completing his dissertation and reflecting on results of his experiments, he wondered (1993b) whether P-limitation existed in the lake itself when phosphorus flux from the sediments could have satisfied algal demand, or, could phosphorus limitation in the experimental containers have been an artifact of isolating the algae from the phosphorus source.

Our observations on the cubitainer experiments are:

- In spite of the small volume of the cubitainers (4 liters), the in-lake microcosms provided a lot of valuable information both on zooplankton and phytoplankton resulting in feasible hypotheses about trophic interactions and seasonal nutrient limitation;
- There is no information in Ehinger 1993a and 1993b on water quality conditions in the cubitainers versus conditions in water surrounding the cubitainers or why 4 liters was chosen as the assay volume rather than, say, ~19 liters;
- There is no information on why the 3-4 day incubation period was chosen.
- There is not enough information provided to evaluate the counting procedure and the method of estimating biovolumes, although it is mentioned that counts were performed using a Sedgewick-Rafter cell (which limits magnification to <~250x), and, that “biovolume calculations were based on measurements of at least ten *cells* of each dominant taxon from each sample” (italics ours).

## **U. S. Army Corps of Engineers 1977-1982**

The U. S. Army Corps of Engineers (the Corps) was directed in 1977 to undertake a comprehensive study of the water resources of the Klamath River Basin in Oregon. The work was conducted out of the Corps San Francisco District office. The first report (1979) on the effort provided an overview of the natural resources of the area, identification of problems related to water quality and water resource development and

management and concluded with a program and budget to address the problems. In the proposed work program for Stage 2 work to reduce uncertainty in choosing among alternatives for restoring UKL, was a proposal to estimate the effectiveness and impacts and to determine the costs of various restoration programs. Included in the list of restoration programs were: increase lake depth, aerate and circulate, introduce biological controls, chemical controls, phosphorus control, combined alternatives and utilization of dredge materials. The proposed experimental approach was to construct a system of 25 ponds located adjacent to the lake that would be filled by water from UKL for tests of the various alternatives. A biological and a water quality laboratory were to be built adjacent to these test ponds.

Funds were allocated for follow-on assessments of UKL that entailed field and laboratory investigations to improve understanding of the limnology of UKL, and, the development of an experimental design and operations plan for restoration technique evaluation. Findings on phytoplankton from this study were noted in this report in Section 2.0. Of particular interest for this section on in-lake UKL assays is the 1982 report section on the development of an experimental design. Further considerations of an appropriate approach for testing restoration alternatives eliminated the lakeside ponds proposed in the Corps' 1979 report, primarily because they would likely not simulate lake characteristics. What was proposed as an alternative experimental design was the use of large in-lake enclosures.

The enclosures were modeled after those used at Blelham Tarn in the English Lakes District (Lack and Lund 1974; Lund and Reynolds 1982; see also review in Lundgren 1985). The proposed enclosures were to be 150 ft in diameter, and 12 or 20 ft deep. The larger enclosure size was chosen to avoid the effects of sidewalls in smaller enclosures that would detract from ability to simulate ambient lake biological and chemical characteristics. Enclosures were to be open to bottom sediments with a top flotation ring and bottom concrete-filled pipes that would connect the reinforced plastic enclosures to the bottom. The flexible walled enclosures were seen to be valuable because wave action outside the enclosures would produce water movement, and therefore sediment entrainment in the enclosures similar to lake conditions. The enclosures would be ringed by wood piles to which the enclosures would be anchored. Sliding fasteners to the piles were to accommodate a six-foot change in lake water surface elevation. Further anchoring of pilings would be accomplished with concrete gravity anchors and pilings would be surrounded by floating breakwaters to attenuate large waves and reduce dilution of enclosure volumes.

Enclosures (four deep and two shallow) were proposed to be sited just north of Ball Bay. The proposed experimental design for the deep enclosures included a control enclosure, a zooplankton enhancement, an aluminum sulfate treatment and an aeration/circulation test. The plan for the shallow enclosures was to have one control and a test of the aluminum sulfate treatment. Details were provided for the evaluation of performance during treatments concluding with a note on the need for comparing conditions in the enclosures with ambient lake conditions: “The critical parameter will be the ability of the enclosures to sustain the presence of *Aphanizomenon flos aquae*, the bluegreen algae indigenous to the lake. This characterization will be absolutely necessary to successfully evaluate management techniques which do not affect nutrient concentrations (deepening, aeration/circulation, zooplankton enhancement)” (USACE 1982).

To our knowledge the proposed future work in the 1982 report was never undertaken. In some respects this is similar to the fate of the 1990 Bureau of Reclamation Agency Lake watershed project (see above introduction to report Section 2.0).

## **AUGUST 2004 ENCLOSURE TESTS**

### **Design, Construction and Location**

In reflection on a test of the use of 18.9-liter cubitainers for observing effects of marsh water on the growth of AFA August 2001 (unpublished data), we (Henry and Geiger) concluded:

- A tubular enclosure open to sediments and atmosphere would be preferable because AFA could move vertically during the day as it does naturally;
- Opening to sediment containing akinetes would allow seasonal recruitment by AFA to overlying water column;
- A larger volume of water for the assay would perhaps allow AFA movement without forcing an ‘agglutination’ of AFA flakes that was observed in the cubitainers as early as the second day of the three-day assay (however, very large agglutinations of AFA were observed at the surface of the mouth of Howard Bay August 2001);
- Ambient atmospheric conditions above the assay may reduce concerns about interfering with AFA N<sub>2</sub> fixation;
- The relatively sheltered location for the cubitainer assays on the eastern side of the northern end of the geographic feature called

the Skillet Handle at the Running Y Caledonia Marsh was suitable for servicing an assay array; and,

- That an anchored array of ropes and tubes may provide sufficient stability for short-term testing and provide good access to enclosures for sampling.

The availability of two sizes of polyethylene tubing led to the development of an enclosure design that was constructed in July 2004 for deployment and testing during August 2004. Rolls of these two continuous tube 6-mil thickness translucent polyethylene (2 3-mil bonded layers food grade) materials were cut to the appropriate lengths for use at the selected assay site. The width of the narrower flattened tube was 82 cm and the wider tube 212 cm. The need for a spreader device associated with the plastic tube to maintain approximate starting enclosure volume led to the development of an internal structure rather than a tubular exoskeleton (e.g. chosen by Spencer 1988). The internal spreader could float independently of the tube that would be anchored to the bottom by chain in tube at the bottom of the cylinder and float arrangement at the surface. Figure 15 is an illustration of the two-part test enclosure.

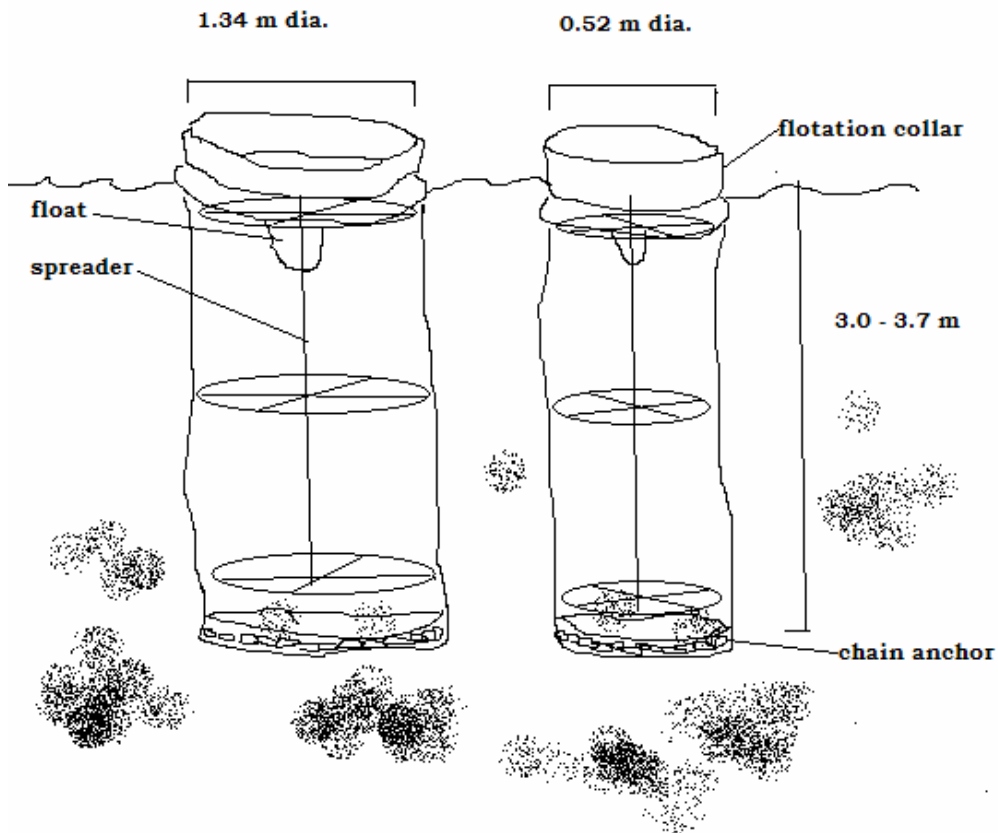


Figure 15. Illustration of two sizes of enclosures tested at Skillet Handle site August 3 – September 1, 2004.

The float material for the collars were “noodle” water toys, the flat (2 x 17 cm cross-section) “noodle” boards and the more popular “noodle” cylinders (6 cm diameter with hollow center of 1.5 cm). This polyolefin float material was enclosed within a section of the polyethylene tube that was folded back and down the outside of the tube. A hot glue gun was used to tack the inside and outside tube sheets together to enclose first the top board section and then tacked again to enclose the cylindrical noodle material. The 17 cm vertical board at the top of the cylindrical noodle was to reduce wave contribution to the enclosure volume. Reinforced holes at three equally spaced locations around the circumference of the tube were made immediately above and below the cylindrical noodle using grommets through six thicknesses of the 6 mil tubing. This provided opportunity for connection to the rope array. A chain, with a weight of ~28 grams/link, was enclosed in a pocket at the bottom of the tube in the same way that the float material was enclosed at the top of the tube.

The internal spreader structures consisted of three circular spreaders connected vertically with sections of 7/8-inch OD cold water PVC pipe. The circular spreaders in the small enclosures were made with central axis of PVC pipe and end fittings that received flexible sections of Sanitech 7/8 inch OD clear tubing. Neither the tubing nor the PVC pipe floats. One-half of a crabpot float was used for flotation of the internal spreader structure as shown in the illustration. The circular spreaders or the larger enclosures were constructed in a similar way to the smaller spreaders with an axis of 7/8 inch OD PVC pipe with end fittings large enough 1 5/16 ID to receive 1 3/8 inch curved sections of black plastic service pipe. Ends of the black plastic pipe were reduced slightly by rasping to mate tightly. The black plastic pipe did float and these sections of the spreader laterals were drilled to reduce air trapping at installation. All connections of circular spreader materials and of the vertical stack of spreaders were made with plastic cable ties. This allowed compact transportation of the entire enclosure materials and assembly of pieces of the spreaders and the enclosure units on site. Tubes were each rolled flat and assembled with spreaders on site as shown in Figures 16-18.



Figure 16. Assembly of larger enclosure. Note drilling of hole in central fitting and vertical connector pipe for cable tie. Circular spreaders were preassembled.



Figure 17. Assembly of spreader system for smaller enclosures on shore at Skillet Handle assay site. Note location of sliding small floats.



Figure 18. Assembled small and large enclosures ready for deployment with boat.

The enclosures were transported by boat a short distance to the location of the test within the small embayment on the east side of the northern end of the Skillet Handle (see location Figure 19).



Figure 19. Enclosure test site. Base map Winema National Forest, Forest Service, USDA, 1994.

The array of ropes, corner floats and anchors was installed prior to deployment of the enclosures. Figure 20 is a schematic drawing of the array with enclosures shown attached by cable ties to the ropes.

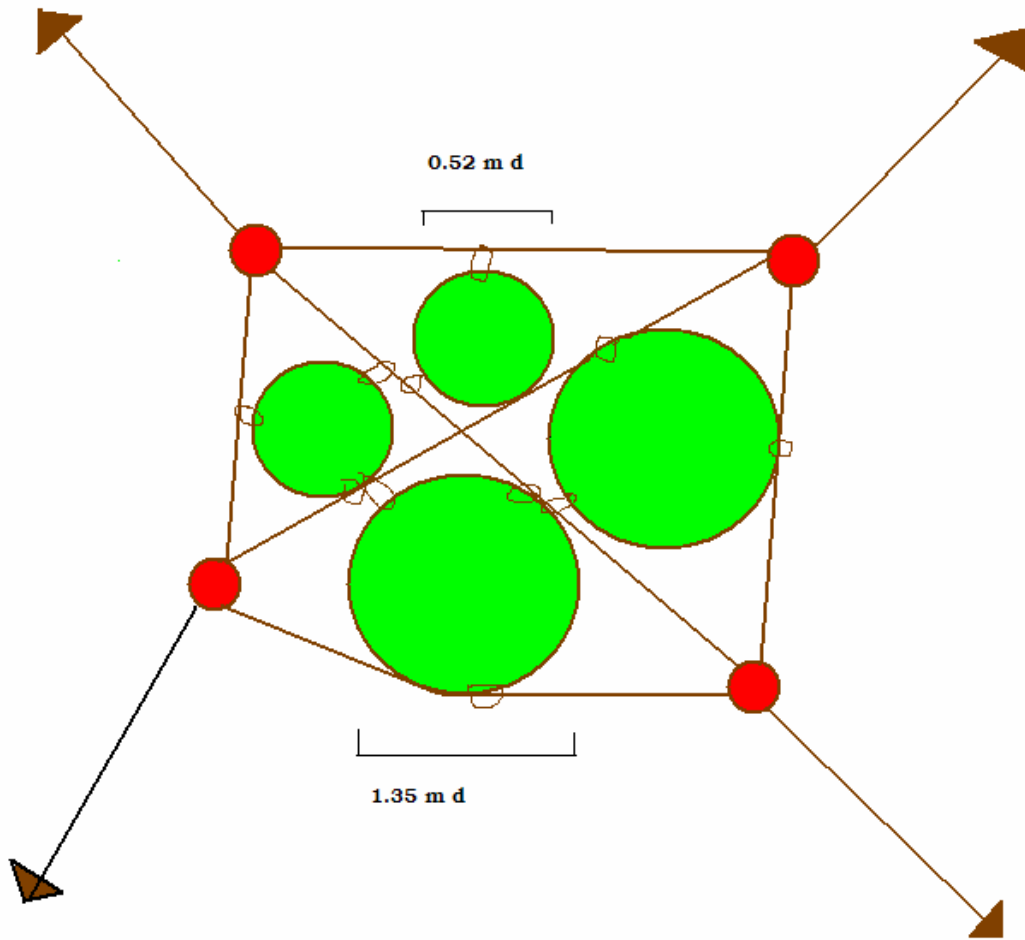


Figure 20. Schematic illustration of enclosure array with anchors shown as arrow points, corner floats and large and small enclosures attached to adjacent ropes with cable ties.

The enclosure array was anchored at ~12 ft depth of water at deployment August 3, 2004. By the end of the test September 1, 2004 water depths had dropped and depth was 10 ft. The bottom contour in the small embayment slopes gradually to the east to a depth of ~18 ft then drops rapidly to form the side of the deep trough (>35 ft) that runs parallel to the Skillet Handle. Sediment at the test site was sampled with an Ekman grab. Sediment was light brown in color, apparently oxic, with oligochaetes and leeches typical of other locations (e.g. Howard Bay) sampled by the author. A subsample (~3 cm diameter x 4 cm deep) was obtained from the sediment surface in the Ekman grab for presence of AFA akinetes. AFA akinetes were present in a sediment mixture that was primarily comprised of centric diatoms (*Stephanodiscus*) and scattered

pinnate diatom frustules (e.g. *Pinnularia* spp.) and occasional sponge spicules.

Figure 21 shows the array August 3, 2004 at the time of the first monitoring of water quality in and outside of the enclosures. In addition to the red corner floats, identification markers were placed at two locations on the array. Location within the small embayment out of what might have been a N-S trafficway over the deep N-S trench would also have reduced potential hazard of the array to boat traffic.



Figure 21. Array of large and small enclosures showing the freeboard created by the “noodle boards”.

### **Performance and Site Conditions**

***Change in enclosure volume.*** Based on measurements of water depth at the enclosure array August 3 and September 1, the changes in volume for the small enclosure were from 786 l to 637 l. The large enclosures changed volume from 5218 l to 4231 l. The numbers for volume are good approximations but hardly exact as the sides would deform slightly as the tube settled two feet with water level decrease.

***The stability of the array*** The array stability was maintained through the month of August. Examination of the enclosures August 20 with submersible video camera showed bottoms resting on the sediment.

Wind conditions during the month of August as recorded at the Oregon State University Agriculture Experiment Station (part of the Bureau of Reclamation Hydromet System) are provided in Appendix D. Wind conditions during August were generally mild with daily average wind speeds ranging from 1.67 to 4.88 mph. Daily wind runs, which would produce increasing swells on the lake, ranged from 40 to 117 miles. Winds were generally from the W and NW on 23 of the 31 days and from the S and SW on the remainder of the days. Four days of higher-than-average daily average wind speeds occurred August 25-28 (4.63-4.88 mph) with associated daily wind run of 85-117 miles. When the enclosures were revisited September 1 the array was intact and seemingly at the same location (no GPS coordinates were determined for the array). Wind intensity built during the day and by noon the next day the array had been disrupted with enclosure bottoms angled in a NE direction. Wind data provided in Appendix D shows maximum daily average wind speed increased September 1 and reached a high of 6.21 mph on September 2. Swells of 3-4 feet were encountered on a visit to the array the evening of September 1 with decreasing swells the next day by noon for the last visit. The daily wind run for September 2 was the highest for the period of the test (31 days) at 149 miles. These increases likely occurred early morning of September 2.

**Water quality in and outside of enclosures.** Appendix D provides the data on water quality obtained with Hydrolab measurements at the start of the test and at the end. A Hydrolab® minisonde and Surveyor 4 or 4a water quality data display were used to obtain field measurements of temperature, pH, conductivity, and dissolved oxygen (Hydrolab, 1999a, 1999b). Calibration of the Hydrolab MiniSonde multiprobe was conducted near to the test site according to manufacturer's recommendations. A Hydrolab calibration log was maintained to document instrument calibration during the project.

Results of measurements of the lake water immediately adjacent to the enclosures, at three depths within each of the two small and two large enclosures are provided for morning, noon (2) and afternoon during August 3-5 and for morning and noon September 1. We have chosen not to discuss the data from the small enclosures, which due to their size and to the shading produced by the surface collars to be unacceptable for use as bioassay apparatus. They were, however, useful as prototypes for developing the larger enclosures. Data on water quality within these small enclosures is provided in Appendix D.

The water quality data on temperature and specific conductivity within the enclosures is unremarkable showing what appear to be insignificant differences vertically among enclosures or between enclosures and the ambient lake conditions (see Appendix D).

The data on dissolved oxygen (DO) and pH is of interest because it reflects movement of AFA within the enclosures and in the lake. Figures 22 and 23 show results of DO and pH for August 3-5 for enclosures LC 2/1 and LC 2/2.

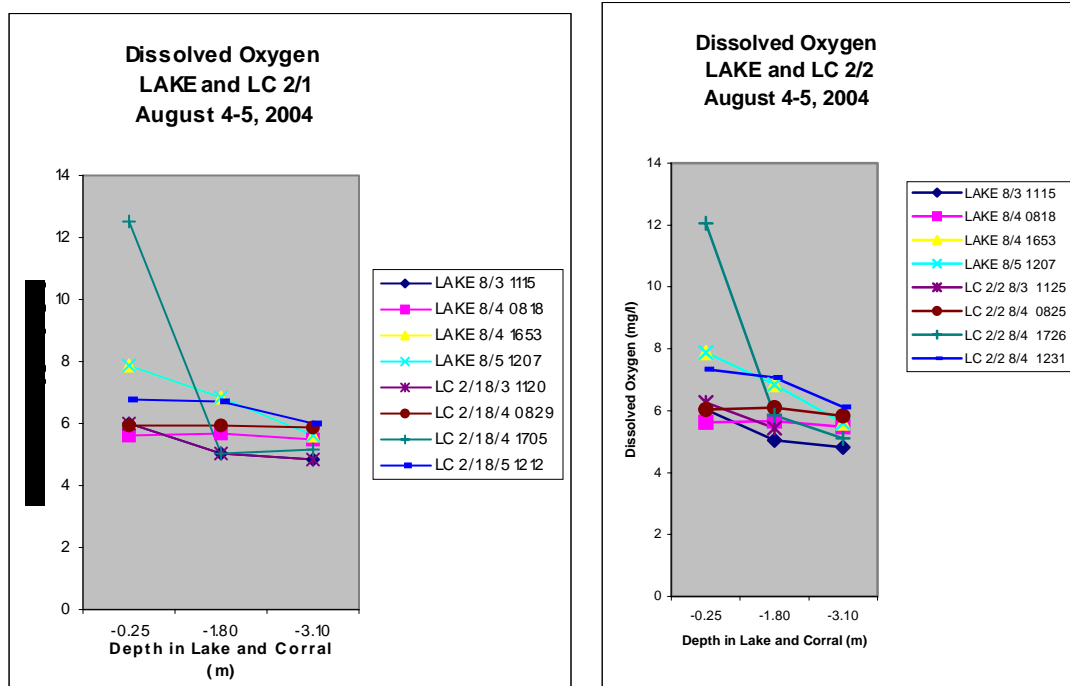


Figure 22. Dissolved oxygen in large enclosures August 3-5.

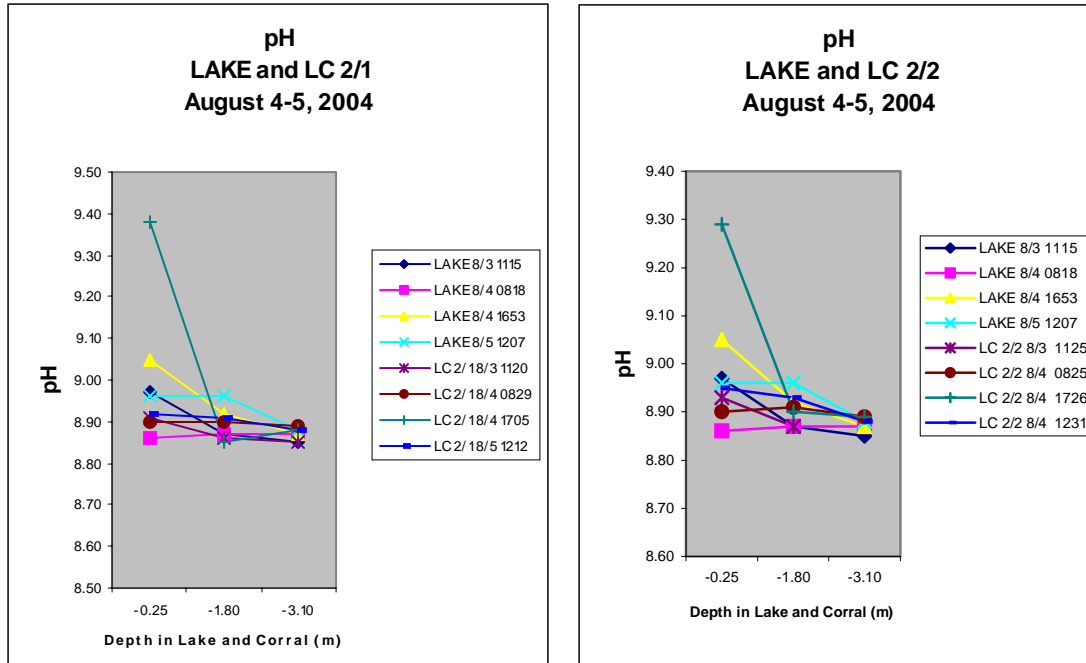


Figure 23. pH in large enclosures August 3-5.

From morning to late afternoon both dissolved oxygen and pH increased in the lake and in the enclosures. Within the enclosures by late afternoon dissolved oxygen and pH increased above conditions in the surrounding lake water. This difference between the lake and the enclosures was also noted at the end of the test. Figures 24 and 25 provide data from September 1 on DO and pH. However, the surrounding lake water also had increases by late afternoon in DO and pH relative to early morning values as shown for early August and September 1.

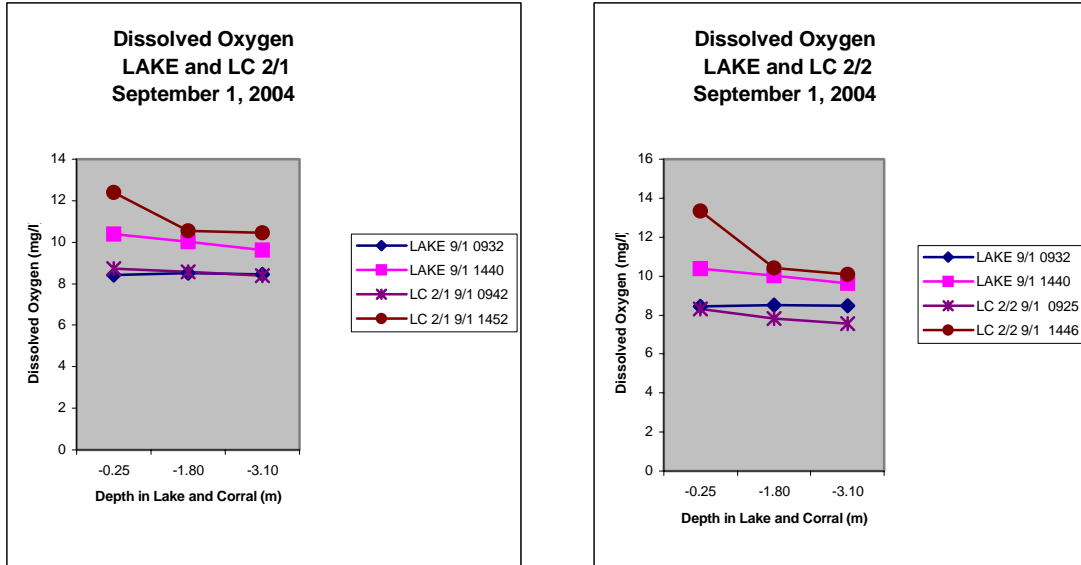


Figure 24. Dissolved oxygen in the lake and in enclosures LC 2/1 and LC 2/2 September 1.

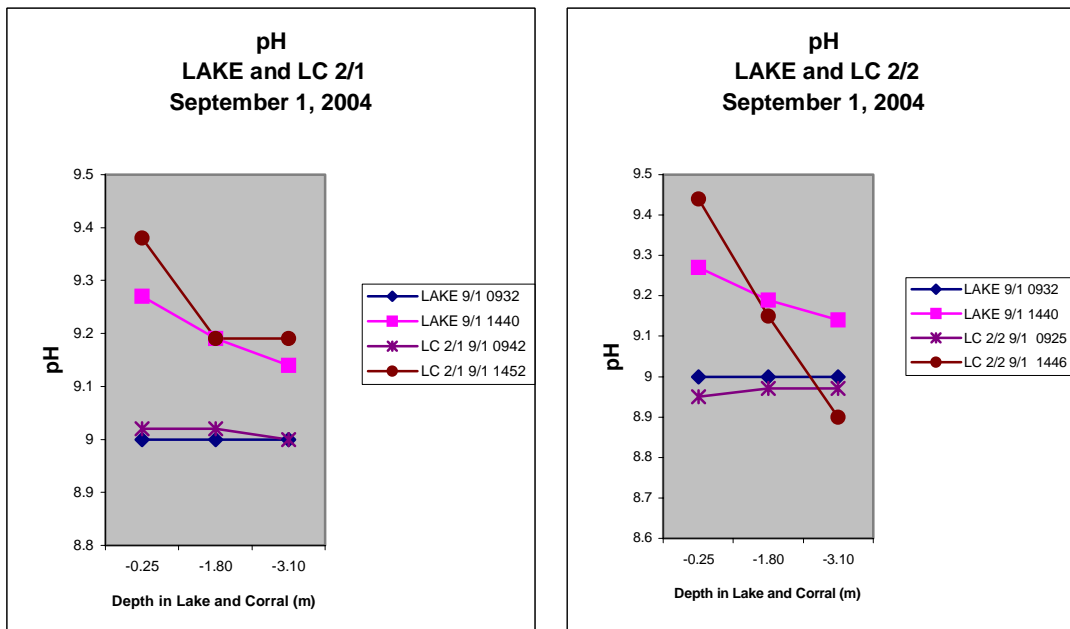


Figure 25. Lake and enclosure pH LC 2/1 and 2/2 September 1.

Enclosure LC 2/1 performed similarly to LC 2/2 but there were differences (e.g. pH) that were greater than in early August..

**Growth of flora and fauna on enclosure walls and spreaders** One of the concerns noted earlier in the Corps' analysis of enclosure size related to the effects of attached materials on enclosure walls related to

water quality and light conditions within the enclosures. The larger the enclosure the less influence these growths would have on enclosure water quality. An inspection of rocks at the lake's edge near the test site that are seasonally submerged would suggest that adding surfaces within the photic zone of the lake would provide an opportunity for attachment of similar colonizers (e.g. the green, branched alga *Cladophora*, which is common on shoreline rocks). This growth on the polyethylene walls of the enclosure occurred gradually but steadily over the 30 day test period. Figures 26 and 27 show the condition of spreader floats August 20 and September 1.

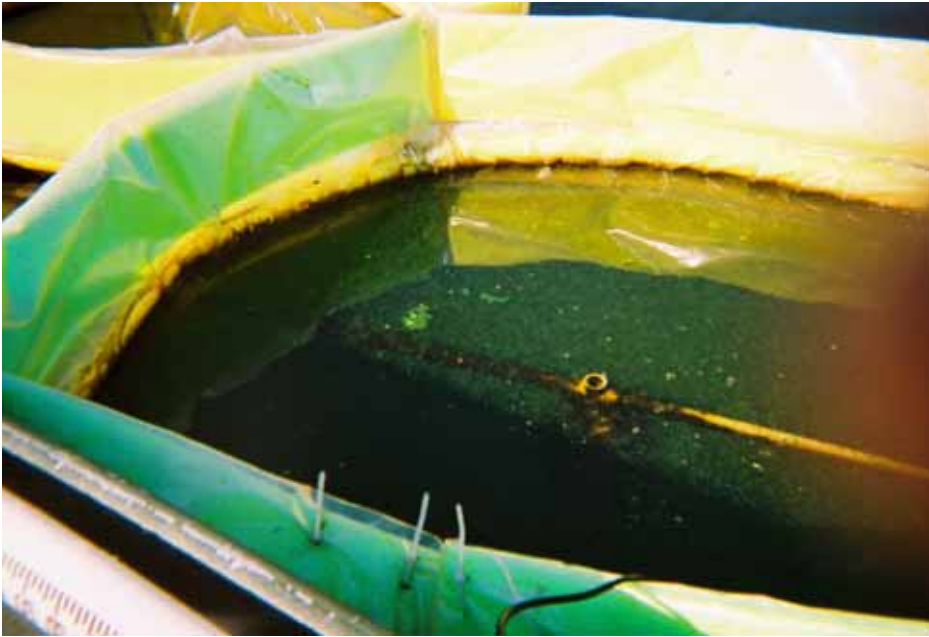


Figure 26. Large enclosure with float below central fitting with attached algae growth. Note growth on side of enclosure. Flakes of AFA are accumulating in top of enclosure near noon when photo was taken. The top spreader pipe and white float of an adjacent small enclosure had no visible surface accumulation of algae growth, due, presumably to the shading effects of the “noodle” wave boards.



Figure 27. Condition of spreader axis and supporting float September 1. Note floating attached algae that had been dislodged from spreader assembly.

On September 2, the enclosures were dismantled and pulled to shore. At the array each of the large enclosures was slit vertically from top of wave boards to the bottom chain pocket. The wire connecting the two ends of the chain was removed and the chain was freed from the enclosure. The spreader assembly was disassembled as it was raised from the water. The enclosures were attached to the side of the boat, along with the entire small enclosures, and moved to shore. An inspection was made of the walls of the enclosures on shore. Figures 28, 29, and 30 show attached flora and fauna.



Figure 28. Growth on enclosure walls. Small enclosures were removed with spreaders seen through plastic walls. The spreaders for the large enclosures were removed and enclosure is displayed with external wall up.



Figure 29. The zone of algae growth shown in relation to a 5-ft length of “noodle” board.

Growth on the enclosures, comprised primarily of brown diatomaceous material and scuds, extended below typical water level 1.3 m on each of the enclosures, large and small. The top spreader element had this aggregated brown material but the two lower spreaders did not. Extending down the outside of the enclosure to the bottom, with highest density from the water surface to a depth of around 2.6 m were invertebrate cases (Figure 30) and an occasional leech (Figure 31).

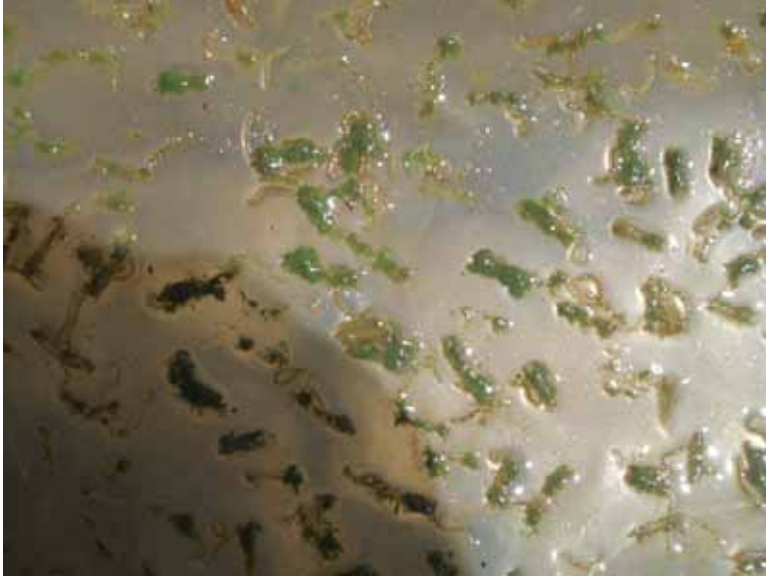


Figure 30. Cases of invertebrates on outside surface of enclosures.



Figure 31. Leech on surface of enclosure.

In addition to these accreting growths of algae and attached invertebrates, there were spiders moving around the wave boards and inner above-water surfaces of the enclosures.

***Condition of AFA within enclosures.*** We note again the comment made by the author of the Corps' 1982 section on enclosure performance: "The critical parameter will be the ability of the enclosures to sustain the presence of *Aphanizomenon flos aquae*, the bluegreen

algae indigenous to the lake.” In the mesocosm work of Ehinger, reviewed in this section of our report, AFA ‘crashed’ at 13 days into the experiments. There was no indication of changes in AFA flake size or condition throughout the duration of the enclosure tests near the Skillet Handle. While there was no indication of water exchange between the enclosures and the surrounding water, based on mid-course video surveillance, this cannot be entirely discounted since daily monitoring of enclosures was not done. There was wave splashing into at least the large enclosure LC2/1 as judged by accumulation of surface debris including feathers.

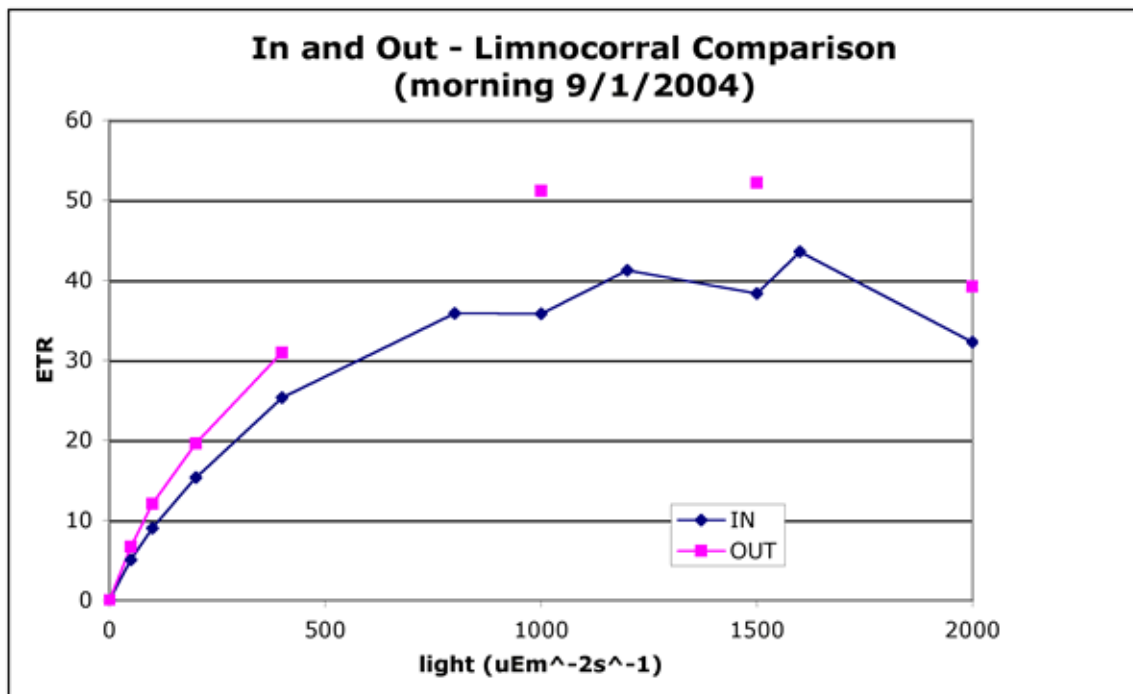
On the first and third days of the enclosure test, at noon, samples of water at three depths (0.5, 1.8 and 3.0 m) were obtained from within and immediately outside the enclosures for further inspection. It was clear that this method of sampling enclosures to detect differences between ambient and enclosures or between ‘control’ and treatment enclosures would not be satisfactory. At the sampling of enclosures September 1, a clear plastic flexible tube (ID ~1.5 cm) weighted at the end was lowered to near bottom to sample drifting algae. The upper end above water was clamped shut and the intact sample within the tube could be raised to where the bottom end of the tube could be clamped below the water and the tube retrieved and then emptied into a sample bottle. The sampler can see immediately the distribution in the column of AFA. The value of this method, which has been used by the Klamath Tribes to sample UKL phytoplankton (see this report Section 2.0), is being able to obtain an integrated sample of the water column since AFA changes position vertically as the day progresses. Sampling at the same time near noon or early afternoon in this manner would provide a defensible basis for estimating AFA biomass and biovolume in enclosures.

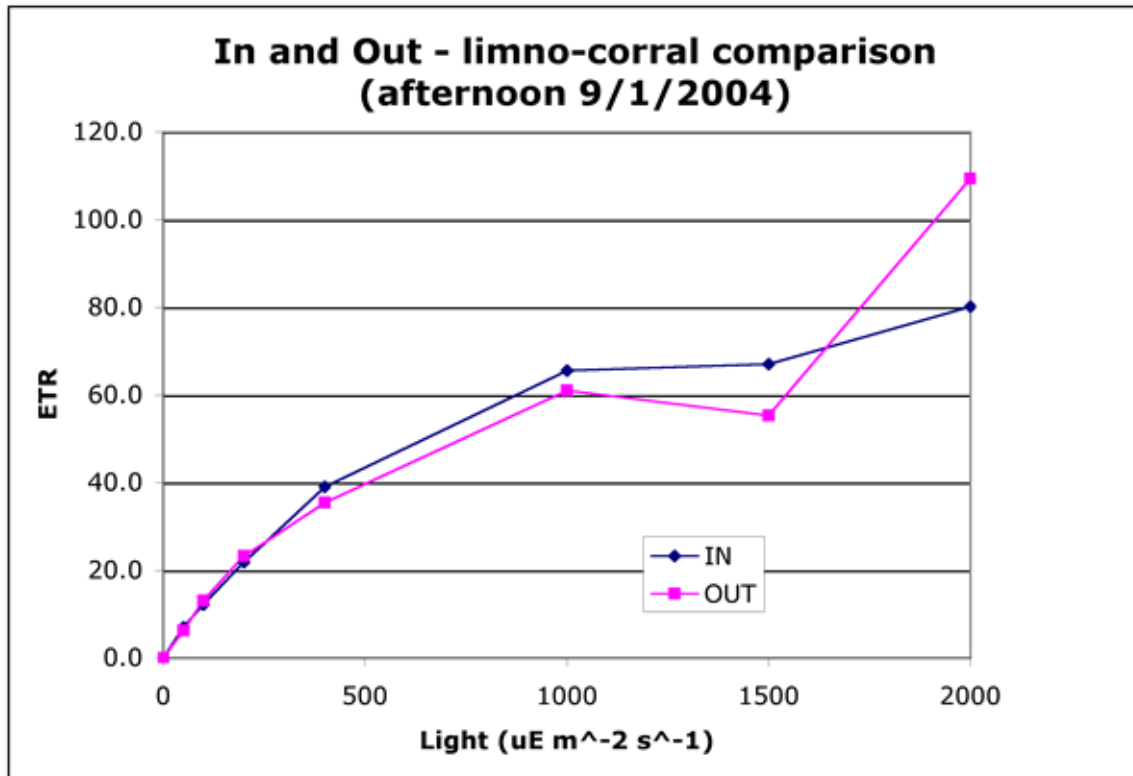
The elevated DO and pH values in the enclosures at noon and late afternoon reflect the movement of AFA vertically in the lake and in the enclosures. Even by the third day of the test, August 5, it was apparent that AFA was accumulating at the surface in the enclosures in a way that it was not in the adjacent water. As the charts of DO and pH values suggest, AFA had also risen to the surface in adjacent lake waters, but was likely being dispersed and circulated shallowly, whereas the reduction of surface waves within the enclosures allowed AFA to accumulate closer to the surface. The occurrence of AFA flakes in the enclosures August 20 and September 1 (Figures 32 and 33) apparently similar to those at the beginning of August suggest, but does not prove, that this enclosure feature of enhancing surface accumulation of AFA harmed the alga.

While there were no measurements made through the test of algae nutrient concentrations (i.e. forms of N and P), the growth of algae on the

inner surfaces of the enclosures and associated grazers (scuds) with their uptake of N and P in the process, did not appear to reduce the density of AFA in the enclosures by competition for nutrients. In some respects the test was an intensifying limiting nutrient bioassay (both N and P being reduced, presumably, in the enclosures by the increased wall growth of algae, however cropping by invertebrates may have put some of the nutrients back into circulation).

Photosynthesis response curves of light vs. electron transport rate (ETR) (see Section 3.0 of this report for information on methods) were run on samples collected from inside and outside the large enclosures September 1. Samples were taken in the morning and in the mid-afternoon just as the wind had built up. The afternoon sample was timed to determine if the AFA in ambient water was more mixed than those in the bags.





Figures 32 and 33. ETR light curves measured by PAM fluorometry. ETR is related to other photosynthetic measurements such as oxygen production or <sup>14</sup>C fixation. All but four points on these graphs represents the mean of two readings on two separate samples taken from either the lake or the limno-corrals. For the Figure 32 “IN” data series, there are duplicate readings on single samples a range of six light levels. For the Figure 32 “OUT” data series there are duplicate readings on two samples from on 3 of those light levels. This is what leads to the gaps in the line for the “OUT” series.

These differences in photosynthetic efficiency are minor and could be explained by a small (20%) difference in the cell count. The important result is that the cells in the enclosures were healthy and had high photosynthetic efficiencies. This indicates that there was no severe limitation by light or nutrients.

## DISCUSSION

The large enclosures of ~5000 liters used in the August test appear to be a feasible alternative for examining the response of AFA to treatments

ranging from concentrations of typical local marsh waters, to use of barley straw (see Appendix D for project paper on potential use of barley in cyanobacteria suppression and results of a literature search on the use of barley for this purpose), to reexamination of nutrient limitation hypotheses. AFA appeared to persist in its typical flake form through the duration of the test and at densities visually, and physiologically, comparable with surrounding lake water. The logistics of implementing treatments will need to be addressed for future work, e.g. the volume of marsh water needed to achieve target concentrations of humics in the enclosures.

The embayment used for the tests near the northern end of the Skillet Handle at Caledonia Marsh is not an area where AFA accumulates as it does e.g. on the north shore of Howard Bay or along Eagle Ridge or in Ball or Shoalwater Bays. The area is also sheltered from predominant N, W, NNW winds of May through August (see location map Figure 19; see annual wind direction data in Appendix D from the Klamath Falls Airport). Increased protection from strong N winds could be gained, perhaps, by moving the test array further south in the embayment to be further from what may be eddies at the north end produced by waves developed from strong N winds, and, or constructing a temporary breakwater of tires. Other sites were considered for the assays (e.g. near Cove Point, immediately south of Hanks Marsh, north of the Yacht Club, the embayment south of McCornack Point, but each of these sites had different drawbacks related either to shallow depths, being too close to marsh water, more likely encountered by boaters, or restricted land access.

The duration of future tests, suggested from the August test of the enclosures, may be 2-3 weeks. Increasing accumulation of enclosure wall growth of algae and invertebrates may interfere or at least appear to compromise assays. The attraction of the particular enclosure design developed is the ability to re-deploy the relatively low-cost enclosures without entirely rebuilding them. The spreader assembly could be cleaned thoroughly, stored and reassembled quickly. The polyethylene walls can be discarded and new lengths of tubes made to accommodate reusable wave boards, floats and bottom anchors. The low cost of these enclosures and the flexibility of the anchor, rope and float array for stabilizing them during the test, affords the opportunity of having replicate enclosures for estimating the significance of the results. The design of the array allows for simple extension in a north or south direction vertical to the gradual site gradient to provide for easy access to enclosures for monitoring. Assays could be run seasonally starting as early as May and continuing through August or September. A more robust anchoring system for the array and additional warning signage at the array would allow the array to be used repeatedly without having to reinstall it with each new assay.

The limited replication available in the Corps 1982 experimental design due to the cost of the large enclosures was a negative feature of that design. This was also a negative feature of the limnocorral that was purchased by the Forest Service for use at Diamond Lake 2002-2003, where the well-constructed 3 m diameter x 15 m long corral made of clear PVC cost \$3,000 not including cost of external spreaders, top floats, bottom anchors, etc. The cost of enclosures must be low enough to encourage replication of treatments and thereby improve the credibility of the assays.

The AFA flakes and filaments in the enclosures seemed to be as healthy (in terms of photosynthetic response) as the cells in the surrounding lake. The similarity between the corrals and the lake also would indicate that the cells in the bag were not light or nutrient limited. Future work should address the potential of water in the bag exchanging with the water in the lake. In addition to submersed video or SCUBA inspection one could use a non-toxic conservative tracer in the bags.

A more extensive and robust array of assay enclosures will require various federal and state approvals. Inquiries were made preceding the August 2004 enclosure tests of required approvals for such an installation. A special use permit would be required from the Oregon Division of State Lands with a one-time and an annual fee. A fill permit would be required by the U. S. Army Corps of Engineers related to the use of concrete anchors for the assay array. Both the ODSL and the COE permit would likely involve review by the Oregon Department of Environmental Quality. The Oregon Department of Agriculture, which is responsible for overseeing the permitting of materials used to suppress algae growth, would need to issue a special use permit to conduct research involving the use of barley straw. Finally, an ESA Section 7 Consultation by the U. S. Fish and Wildlife Service would be required for approval of the final design of the array and plan for seasonal testing. It would appear that securing these approvals would require at least three months. Approval from the adjacent landowner (Running Y Ranch) was obtained and will be required for future assays.

# 6.0 MEASURING IN-LAKE UKL WETLAND WATER QUALITY FUNCTIONS

## INTRODUCTION

Like a lot of losses in life, you seldom know what you've really lost until it's gone. Sometimes it takes awhile to know what it was. The loss of over 35,000 acres of wetlands from within UKL between 1889 and 1971 (Figure 34; Snyder and Morace 1997) and the transformation and loss of UKL watershed wetlands above the lake occurred during a period when our country had a different perspective on wetlands than it does today. Today there is conflict over wetland protection. Until the enactment of the Federal Water Pollution Control Act amendments of 1972 (PL 92-500), specifically Section 404, also known as the Clean Water Act (Mitsch and Gosselink 2000), wetlands had very little protection in the United States and there was little conflict within the country over decisions to drain and fill wetlands. Wetland loss and conversion to agricultural use was supported by various swampland acts and ambitious drainage projects (National Research Council 1995).

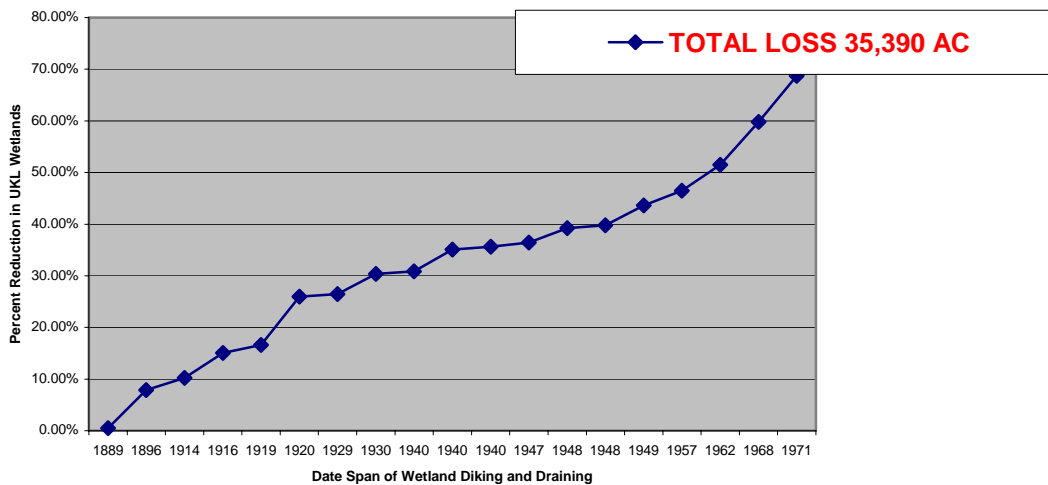


Figure 34. Cumulative wetland loss from original 51,510 acres within Upper Klamath Lake 1889-1971 (Snyder and Morace 1997).

The extensive wetland loss in the Klamath Basin came to an end as a new awareness of wetland importance gained political majority in the United States. Wetland transformation, however, continued as it did

elsewhere, along with permitted wetland fill. Extensive regulatory jurisdiction over wetlands in Oregon was eventually centered in the U. S. Army Corps of Engineers starting in 1978 and the Oregon Department of State Lands in 1985. Support for protection of wetlands in the 1960's and 1970's appeared to derive more from what they did for waterfowl than what they did for water quality. Support has since diversified.

What has been learned from the extensive research on wetlands in the relatively short time since the 1970's has been enough to convince wetland scientists at least that the hydrologic and water quality functions of wetlands are complex and extremely valuable to society. In addition, associated wetland research on the use of wetlands to treat storm and wastewater from the 1980's until the present has deepened the understanding of these functions and provided the design and construction expertise that has led to the use of wetland treatment systems by towns and cities in the United States and around the world. It is on the basis of this knowledge about wetlands gained so relatively recently that the Society of Wetland Scientists was formed in 1980 and has grown to a current membership of over 4000 members ([www.sws.org](http://www.sws.org)).

Our perspective as wetland scientists is that the elimination of over 35,000 acres of wetland *from within the lake*, without environmental impact assessments and without mitigation, and the transformation and loss of other UKL basin wetlands, was the primary factor in changing the lake from eutrophic to hypereutrophic and altering algae populations to dominance by the cyanobacteria *Aphanizomenon flos-aquae*. Our operating hypothesis related to the general goal of our work stated in the report introduction is:

*The isolation of former wetlands from UKL by diking and draining has resulted in degraded lake water quality through severing important lake-wetland functional connections.*

This is, of course, a hypothesis. Recent reports of sediment coring results in UKL supported by the Bureau of Reclamation and the U. S. Geological Survey do not conflict with this hypothesis (Bradbury *et al.* 2004, Colman *et al.* 2004, and Eilers *et al.* 2001). A recent National Research Council report (2003) supported the feasibility of this hypothesis. There were other factors that contributed to changes in the lake including changes in upland forest and wetland habitats that would also have influenced the lake but whose relative influence in the change cannot yet be assessed.

This section of our report first reviews what is known of the water quality functions of wetlands in UKL, suggests what knowledge of wetlands gained at UKL and elsewhere can be applied to these wetlands to project wetland function back to presettlement times, then concludes with some proposed elements of a wetland research program.

## **REVIEW OF RESEARCH ON UKL WETLANDS**

### **PSU Assessment of the Impact of Klamath Marsh on the Williamson River 1977-1981**

Perdue, Lytle, Sweet and Sweet of Portland State University carried out the first comprehensive assessment of Klamath Marsh during 1976-1979 (Perdue *et al.* 1981). Their work was a specific program for exploring the observations of previous investigators such as Phinney *et al.* (1959) regarding the suspected influence of UKL marshes on lake water quality. Figure 7 shows the location of monitoring stations for the 1977-1979 data collection.

Klamath Marsh is a complex aggregation of wetlands that extends north above Kirk Bridge Rd, at the north end of the Williamson River canyon, to a location on the Williamson River approximately 15 miles from its source. The approximate size of this total wetland complex is 82,000 acres. This is the largest aggregation of wetland area above the lake of the three subbasin areas draining to UKL

This PSU work provides a very valuable foundation for this work element for the following reasons:

- One of the unrealized values of this work is the opportunity for relating continuous flow data collected at the Kirks Bridge gaging station (#11493500) to the chemical concentration data collected during the study, providing a basis for calculating phosphorus and nitrogen loads, and dissolved or total organic carbon loads out of the marsh;
- The PSU study was the first known study in the UKL basin to characterize the marsh decomposition products; results were reported in Perdue *et al.* (1981) and in Sweet and Perdue (1980) and Lytle and Perdue (1981); and,
- The study provides a framework for developing a hydrologic and nutrient budget for the wetland complex through its two-year characterization of both flow and chemical composition of spring and stream inflow to the marsh and outflow into the Williamson River.

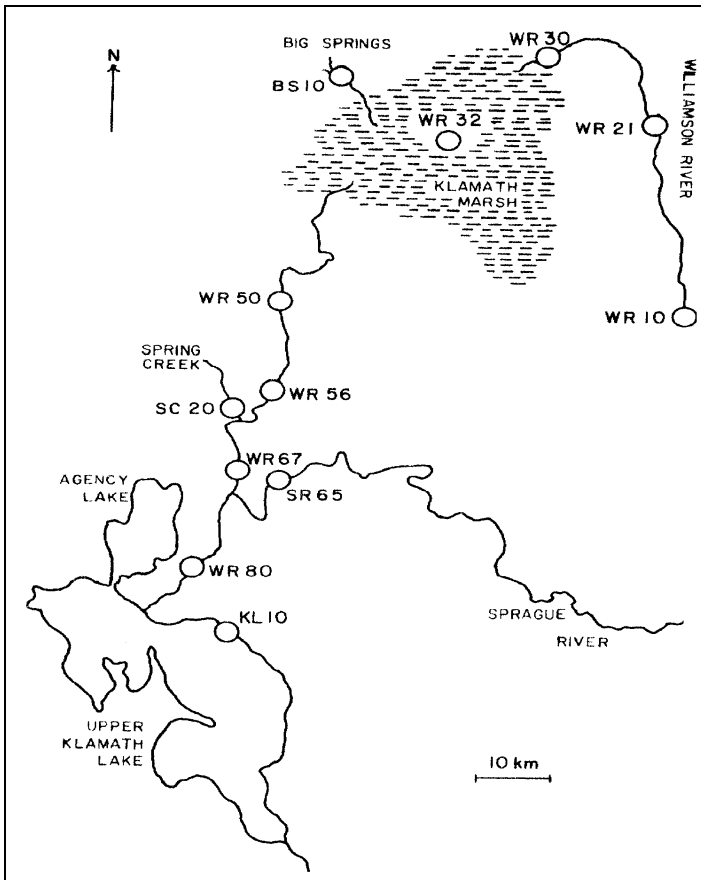


Figure 35. Portland State University monitoring sites (1977-1070) (Perdue *et al.* 1981)

### **Bureau of Reclamation Research 1991-1994**

There were two Bureau of Reclamation research projects focused on Upper Klamath Marsh and Hanks Marsh that sampled the water and biota of UKL marsh areas in a preliminary reconnaissance mode. The studies resulted in some information that can be used as the basis for additional and more focused sampling.

The initiative by the Bureau of Reclamation in 1990 to develop a water quality management plan for the Sevenmile Canal-Wood River watershed (noted in this report Section 5.0; see Campbell 1993a, 1993b) was associated with an assessment of the Upper Klamath Marsh National Wildlife Refuge (NWR) (Sartoris and Sisneros 1993). The intent of the Bureau of Reclamation investigation of the lake-associated Upper Klamath NWR marsh and the diked marsh areas was to “assess and quantify the relative effects of restored and undisturbed lacustrine wetlands on the eutrophication and water quality of Upper Klamath Lake” (Sartoris, Sisneros, and Campbell, 1993). Preliminary fieldwork

was conducted July-September 1991 in the northern unit of the UKLNWR at Crystal Creek and Thomason Creek. In June 1991 water quality measurements were made in the Wood River wetland below and immediately adjacent on the east side to Wood River (see Table in Appendix 5.1, Sartoris and Sisneros 1993).

In 1992 monthly monitoring of stations in wetland channels (Crystal Creek and Thomason Creek and two adjacent drainages (Four-mile and Sevenmile Creeks) was conducted January-December 1992 concurrent with other Bureau of Reclamation studies in the Sevenmile Canal-Wood River watershed (see Section 5.0 of this report for additional information on the project).

Only preliminary and summary reports were produced for this work (Sartoris and Sisneros, 1993; Sartoris, Sisneros, and Campbell, 1993). Flow measurements were only made for Sevenmile Canal at its mouth and for Fourmile (West Canal) locations. The summer of 1992 was a period of severe drought in the Klamath Basin. This likely influenced the findings. In addition, benthic macroinvertebrates, zooplankton, and limited periphyton analyses were performed. The field investigations were valuable in providing the only known “in-wetlands” water quality data collection for the 12,000-acre marsh.

Charts were produced in Sartoris, Sisneros, and Campbell, 1993 that show fluxes in the Crystal Creek Channel site with decreasing DO in summer (<6.0) and increases in NH<sub>3</sub> in summer and fall (~0.02-0.06 mg/l), possibly reflecting decomposition processes in the marsh. The Bureau of Reclamation investigation, in spite of only preliminary reporting of findings, does illustrate clearly the complex chemical transformations that are typical of marshes (see, for example, Vymazal, 1995). No attempt was made to develop a water or nutrient budget for the marsh. The authors commented that the phytoplankton collections made for each of the marsh sites from 1991 and 1992 were dominated by diatoms or green algae:

“The dominance of algal groups other than blue-greens supports previous studies that report the absence of blue-green algae in marsh areas. The waters in both Crystal and Thomason Creeks exhibit a “peaty” color and speculation has indicated that the tannins and lignins dissolved in marshy waters are not conducive to blue-green algal growth.”

It is unfortunate that some sample collection sites were not sited within the marsh vegetation rather than in easily accessible channels draining the marsh. The Thomason Creek site may be more representative of wetland water quality conditions but it too may have been influenced by flow down the West or Fourmile Canal on the west side of what is now Agency Lake Ranch. The authors noted that both marsh tributaries had dense populations of aquatic macrophytes in the stream channels (*Potamogeton*

*natans*, *P. crispus*, *P. foliosus*, and *Utricularia vulgaris*, and *Elodea canadensis*). Crystal Creek, in addition to marsh water influx would have been influenced by numerous springs at the western periphery of the marsh, not the least of which is Crystal Springs at Crystal Creek's northern terminus.

Reclamation also funded an assessment of Hanks Marsh (Forbes 1997, Forbes, Sartoris and Sisneros 1993 and Barber *et al.* 1999). There are ~17,000 acres of wetland in the lake during the seasonal fluctuations of lake water levels. Approximately 14,000 acres of this total is the Upper Klamath Marsh NWR (north and south units) and 1,000-1,200 of this total is Hanks Marsh. The remaining wetland areas are generally in the north end of UKL, including portions in the vicinity of Agency Lake such as the Wood River Marsh. Figure 35 is a GIS map of Hanks Marsh with a sampling design for marsh water quality studies.

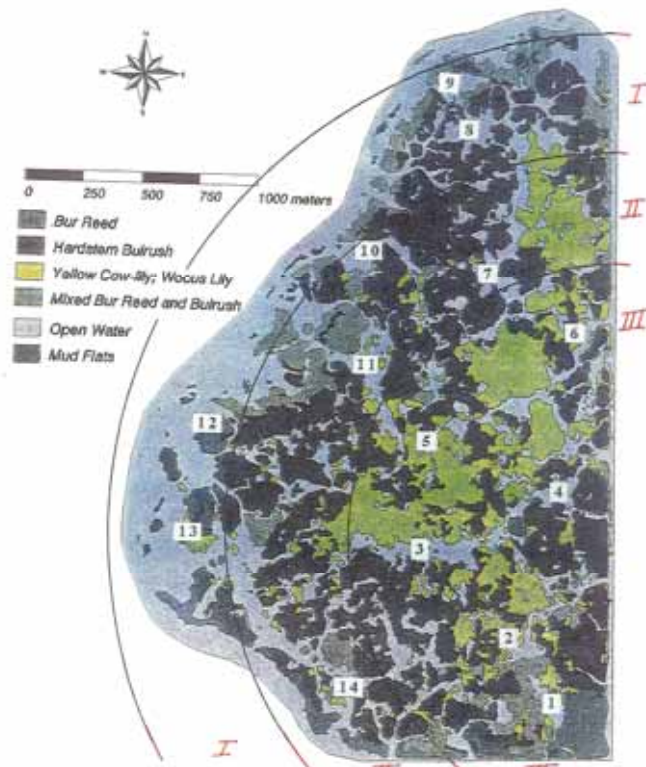


Figure 2. Hanks Marsh Sampling Zones and 14 sampling stations.

Figure 36. Hanks Marsh on the east side of UKL northeast of Buck Island (from Forbes, Sartoris and Sisneros 1998).

Concentric arcs were used to define three zones extending away from the interior of the marsh on the east side to the open waters of UKL on the west side. Figure 35 shows location of the sampling stations in each zone and the vegetative composition of the marsh. Water samples were collected on six two-day trips: May 20-21, 1992, June 9-10, August 17-18, and October 6-7, 1993; May 24-25 and July 12-13, 1994. Stations were visited in numerical order: 1-6 on day 1 and 7-14 on day 2. An airboat equipped with GPS was used to locate each sampling station in the marsh (Forbes 1997). Forbes notes in the introduction to her MS Thesis: “The purpose of this study was to investigate the role of lacustrine wetlands in the eutrophication of Upper Klamath Lake and determine whether these littoral areas function as nutrient sources or sinks.”

Forbes’ primary interest in the study was to characterize periphyton (attached algae, fungi, bacteria, animals, inorganic and organic detritus) communities on the three common vegetative constituents of UKL marshes: *Nuphar*, *Scirpus* and *Typha*. She concluded that a high variety and abundance of aquatic plants and animals in zones II and III were

associated with abundant mineral nutrients in these zones. For example mean ortho-phosphate-P concentrations for the study were 0.023 for zone I, 0.050 for zone II and 0.078 mg/l for zone III. The decrease in chlorophyll a in periphyton, but increase in periphyton biomass as distance from the marsh edges increased, supported her assertion that heterotrophy increased in Hanks Marsh as distance from the open water increased. She also noted the absence of cyanobacteria in zones II and III and attributed this absence to the likely higher concentration of humates based mainly on the research of R. Wetzel (e.g. Kim and Wetzel 1993 and Wetzel 1993 cited in her reports).

An associated study (Barber *et al.* 1999), referenced in Section 3.0 of this report, provides detailed results of humic acid analyses performed on waters from seven stations within Hanks Marsh and one station in the middle of UKL. Samples were obtained July 11, 1994, the last sampling trip for the marsh study. The results of that work are also reported in Forbes, Sartoris and Sisneros 1998. The DOC concentration in lake water was 10 mg/l while those from the marsh ranged from 13 to 20 mg/l. Analysis of the dissolved organic material showed that humic and fulvic acids constituted the majority of the DOC in the marsh samples, in contrast with the lake sample which contained significant amounts of carbohydrates, proteins, and lipids. They also note that lower pH values in the wetland (7.8-9.6) as contrasted with the lake value of 10.3 were likely the result of humic and fulvic acid production in the marsh environment.

In the end this study of Hanks Marsh did not address one of the prime purposes of the work, namely, to characterize the flux of materials between the pelagic and littoral zones. This was because the hydrologic regime was not characterized (Forbes, Sartoris and Sisneros 1993). That flux does occur is suggested by the gradients of phosphorus and the implication of dilution of internal high concentrations by rising lake water levels during late fall, winter and spring. Water depth measurements were made at the time of each of the six sampling trips but no attempt was made to develop a water or nutrient budget for the marsh. The study provides data on timing of the senescence of marsh vegetation and associated changes in water quality (e.g. increases in ammonium-N and decreases in DO). It also provided the second and last characterization of algae in the wetlands of UKL.

### **United States Geological Survey Drained Wetlands Study**

As the Bureau of Reclamation studies concluded, the U. S. Geological Survey initiated a study of drained marshland around UKL. The purpose of this study was to quantify the contribution of nutrients from drained wetlands to UKL relative to other sources of nutrients in the lake. The

approach taken was to obtain soil cores from drained wetlands and cores from undrained wetlands, compare concentrations of phosphorus and nitrogen in cores, and estimate the loss of N and P to UKL as pump discharge from seasonal draining. In addition to sampling a variety of former wetland soils, measurements of pump discharge flow and water quality were made, and an estimate was developed of where wetlands once were below 4140 ft elevation (NGVD; full UKL pool equivalent to the BR datum of 4143 ft). This estimate included the dates when diking and draining occurred from 1889-1971.

Figure 36 is a map from the report (Snyder and Morace 1997) that shows the location of soil sampling sites and the location of drained and undrained wetlands below the full pool elevation of UKL .

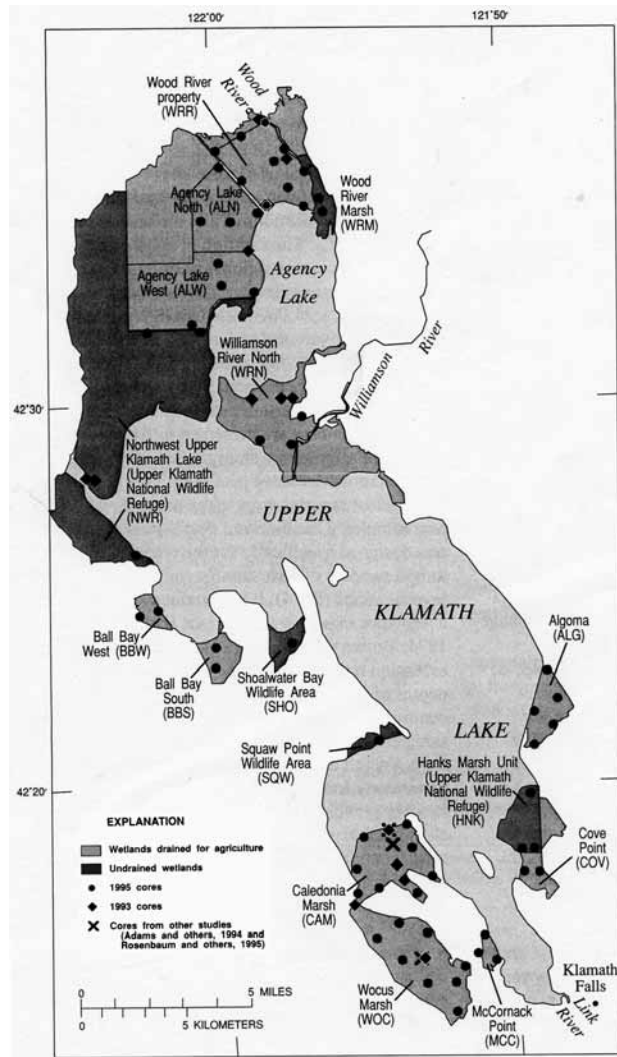


Figure 37. Snyder and Morace (1997) project map showing soil sampling sites and the extent of drained and undrained wetlands below the elevation of full UKL pool.

The study was a significant step in providing definition of the extent of Upper Klamath Lake before diking and draining began in the late 1800's. The Snyder and Morace analysis of wetland loss from within UKL was built on the work of Carlson (1993). The study findings made it clear where and when wetlands had been lost from the lake, resulting in a smaller and changed lake. The Bureau of Reclamation in its 1997 estimate of what constitutes the area and volume of Upper Klamath Lake (UKL), including Agency Lake, has included wetlands at lake level which are seasonally inundated by fluctuating lake water levels as part of the lake area and volume (Basdekas 1997). Table 8 provides an overview of how the lake has changed in area through the isolation of 35,390 acres of marsh wetland from the lake through diking and draining.

Table 8. The area of littoral marsh and limnetic open water of Upper Klamath Lake (including Agency Lake) before commencement of diking (~1889) and after diking, draining and UKL outlet (Link River) dam.

Condition of Lake	Lake Surface Elevation	Lake Area (Ac)		
		Littoral	Limnetic	Total
Before Diking	Minimum (BR datum, ft)			
	4140.0	20,320 (30%)	47,400 (70%)	67,720
Before Diking	Maximum (BR datum, ft)			
	4143.0	51,510 (46.2%)	60,000 (53.8%)	111,510
After Diking and Dam	Minimum (BR datum, ft)			
	4136.0	0.0 (0.0%)	55,800 (100%)	55,800
After Diking and Dam	Maximum (BR datum, ft)			
	4143.3	16,120 (21.1%)	60,223 (78.9%)	76,343

The littoral wetland area of the lake once comprised 51,510 acres (46.2%) of the total lake area of 111,510 acres at maximum pool surface elevation. The historical records of lake fluctuation prior to construction of the Link River Dam in 1921 document the lake fluctuating between a maximum of 4143.0 ft (U. S. Bureau of Reclamation datum [~1.18 – 2.03 ft above the USGS NAVD 88 datum]) and a minimum of 4140.0 ft. Following dam construction and after the last diking and draining in 1968 (Snyder and Morace 1997), the lake area at maximum pool elevation of 4143.5 ft had decreased to 76,343 acres, with littoral marsh area decreased to 16,120 acres (21.1% of the total lake area). In other words, the lake lost 31.5% of its area, and the associated lake volume associated with this area, through diking and draining. The in-lake wetland area was reduced by 68.7%. In addition, there was a reduction of littoral lake volume from 82,000 ac-ft to 28,000 ac-ft; a reduction of 65.9%. Further, the wetland area at minimum lake storage volume (4136.0 ft versus the pre-dike, pre-dam minimum of 4140.0 ft, had shrunk from 20, 320 acres to 0.0 acres (Basdekas 1997), which represents an additional seasonal loss of function. The lowering of the

reef at the Link River Dam would allow more water to be withdrawn from the lake. These are significant implications of the work of Snyder and Morace.

In an attempt to compensate for these wetland losses, both the federal government and privately funded organizations have, with support of some wetland scientists (e.g. Gearheart *et al.* 1995), purchased former farmed and ranched wetlands areas and are reclaiming these areas as wetland. The present total of this intended reclaimed wetland area is 17,239 acres, a combination of Agency Lake Ranch (Bureau of Reclamation, 7,159 ac), the Wood River Ranch (Bureau of Land Management, 2,880 ac), the Williamson River Delta Preserve (The Nature Conservancy, 6,960 ac), and portions of Caledonia Marsh (Running Y Ranch/Resort, 240 ac). The land surfaces of these areas to be reclaimed are at elevations six or more feet below maximum lake elevations. The total investment to acquire these former wetland areas has been \$19-20 million. The cost of reclaiming and developing wetlands is additional. The reassociation of these reclaimed wetlands with the lake is proceeding very slowly, however, the recent release of the DEIS for the restoration of The Nature Conservancy Williamson River Preserve (David Evans and Associates, Inc. 2004), which proposes dike breaching as the preferred alternative, suggests progress is being made.

In addition to the value of clarifying the extent of wetland loss from within the lake, the Snyder and Morace project team report has continuing usefulness in providing data on the consequences of diking and draining ranging from a better understanding of peat composition and accumulation rates in drained and undrained marshes, challenges for wetland restoration with subsidence of drained peat ranging from 1-13 ft, to focusing attention on the quality of seasonally discharged drain water from drained wetland areas used for agriculture. The latter primary objective of the study produced estimates that indicate the significance of both N and P losses, primarily from peat oxidation, from wetland areas as a result of diking and draining:

“For all the drained wetlands sampled, the cumulative nitrogen and phosphorus loss since drainage totaled 250,000 tons and 4,300 tons, respectively. This represents about 30 percent and 22 percent of the mass of nitrogen and phosphorus, respectively, that initially existed in the organic soils. The loss of nutrients from the drained wetlands is considered to be a maximum estimate of the possible contribution of nutrients to Upper Klamath Lake from the peat soils of the drained wetlands sampled. However, not all the nutrients released by the soils are discharged to the lake.”

The Oregon Department of Environmental Quality (ODEQ) used the work of Snyder and Morace to supplement its estimates of N and P load to UKL

in developing its Total Maximum Daily Load estimates for P (Boyd *et al.* 2002). The estimate of the P load to the lake in the ODEQ TMDL is ~29% of the total load to the lake.

The work of the USGS Snyder and Morace project team focused on two consequences of diking and draining in-lake wetlands:

- subsequent export of N and P from these areas resulting from peat oxidation, much but not all of which was transported via continued draining and pump discharge to UKL; and
- variable subsidence of peat in former wetlands now behind dikes.

Due to the logistics and expense it was not possible to compare and contrast export of N and P from undrained functioning wetlands (Snyder pers. com. 2001), although cores were obtained from many of the undrained UKL wetlands (see Figure 36). There is no caveat regarding the likely annual contribution N and P from these former wetland areas to the lake prior to diking and draining. The analysis of discharge data from the BLM Wood River Ranch (east and west side discharges) before and after the 1995 change of land use indicates that seasonal N and P loads from mature wetland areas through and following periods of wetland plant decomposition were very high (Geiger *et al.* 2001).

The Snyder and Morace work was an imaginative and thorough analysis of selected consequences of wetland loss from UKL. However, use of their data for estimates of current N and P loads to the lake require statement of caveats that were not included in Boyd *et al.* 2002. For example, there is no caveat associated with the findings of Snyder and Morace (1997) incorporated into the TMDL regarding the absence of a water and nutrient budget for any of the former diked and drained wetland areas that discharge to the lake each year, primarily in late winter and spring, from field flooding. There is a need to estimate the annual *net* export of P and N from these former wetland areas based on inflow and outflow flow and water quality measurements. This is not to discount the work of Snyder and Morace, but rather to indicate what should be done next to refine estimates for current conditions.

### **SAI P and N loading analysis of Upper Klamath Lake NWR NW Unit**

In 1999 Shapiro and Associates, Inc. (SAI) and Cogan Owens Cogan were contracted to perform an analysis of potential water quality impacts from a proposed skiing facility on Pelican Butte located due west of the Upper Klamath National Wildlife Refuge (UKNWR). The proposal for the facility included options for treating stormwater runoff and sewage from the facility. An option for sewage treatment was to use a leach field on the lower slopes of Pelican Butte with potential of seepage via ground water flow into the western side of UKNWR. The analysis is reported in Geiger

*et al.* 2000. The project was terminated by its sponsor, Jeld-Wen, in 2000. A selected part of this analysis is provided here because the DEIS for the project was never completed for public review.

The analysis is an example of how data collected by SAI and many other entities (BR, USGS, K Tribes, FS, etc.) can be used to develop a conceptual framework for assessing project impacts on a complex wetland system. The resulting loading analysis is an example of what one-half of an annual hydrologic budget might look like (a preliminary estimate of N and P loads into, but not out of, the wetland). At the time of this work SAI had nearly completed its loading assessment for the Sevenmile Canal-Wood River watershed (Geiger *et al.* 2001) and had data tabled and analyzed for flow and water quality in drainages that influenced the east side of the northern 12,000 acre unit (the area north of Pelican Bay). To gain at least preliminary information about inflow to the marsh on its west side SAI measured flow and water quality during January and February 2000 of springs and creeks on the west side of this unit. These measurements were made of Crystal, Malone, Harriman and Fourmile, and flow and water quality of the west side creeks Rock and Fourmile, and the channels within the marsh Crystal Creek and Recreation Creek. Figures 37 and 38 show the location of Westside named and observed unnamed springs, and a graphic illustration of presumed flow directions in the marsh.

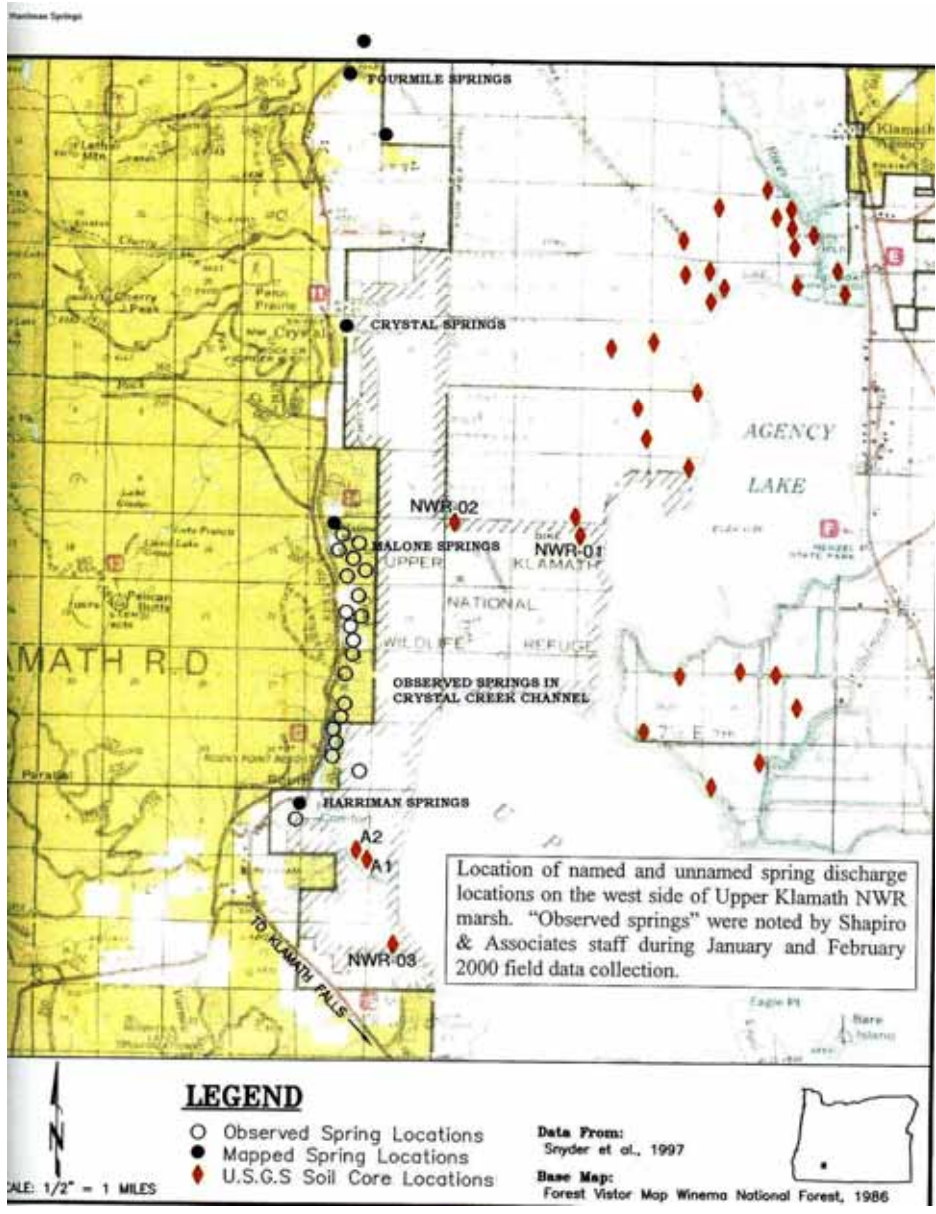


Figure 38. The location of named and unnamed springs on the west side of the UK NWR (January-February 2000). Map D. J. Newton Assoc

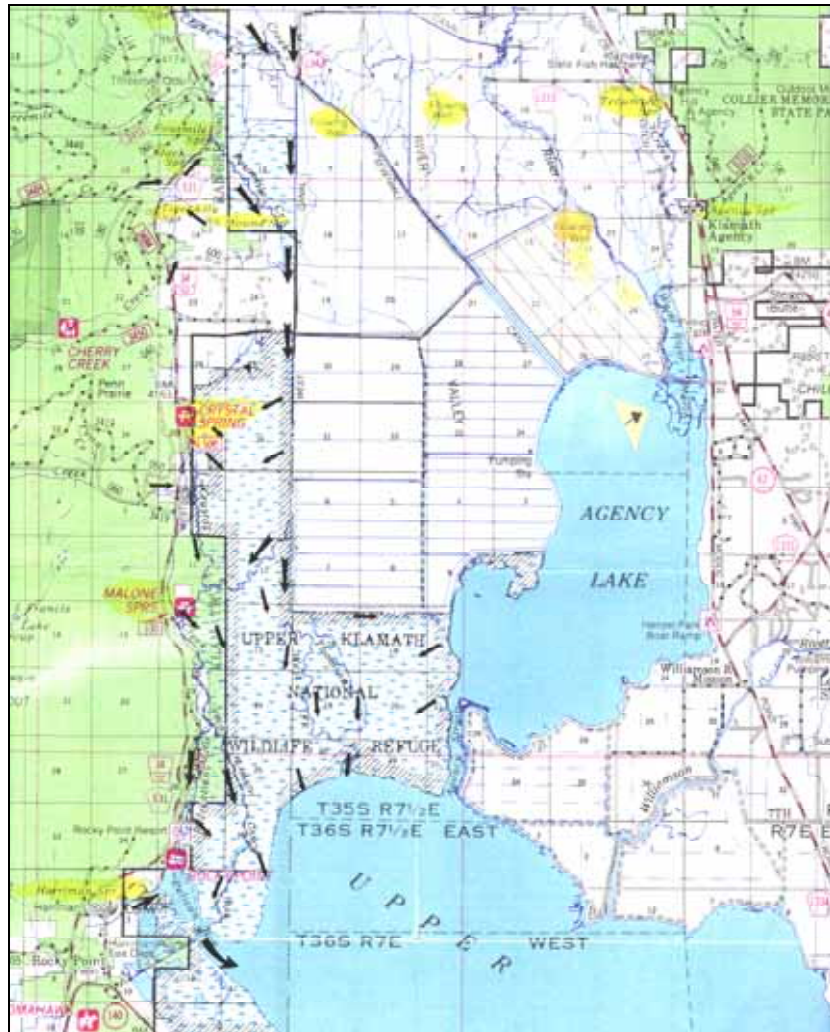


Figure 39. Simplified characterization of flow direction in the UK NWR marsh (assumes typical declining lake levels May-September). Winema National Forest map 1994.

Table 9. Measurements of flow (cubic feet/second) for Creeks, Springs, and Marsh Channels associated with Upper Klamath NWR marsh, January – February 2000

<b>Stations</b>	<b>Station No.</b>	<b>Jan 5-6</b>	<b>Jan 18-19</b>	<b>Feb 8-9</b>	<b>Average</b>
Crystal Springs	Site # 1	40.3		30.3	35.30
Rock Creek	Site # 2	0.5	1.51		1.01
Crystal Creek #1	Site # 5			5.73	5.73
Malone Springs	Site # 7		15.06	9.46	12.26
Crystal Creek #2	Site # 8		25.93	27.29	26.61
Recreation Creek	Site # 9			22.13	22.13
Crystal Creek #3	Site # 10			8.05	8.05
Harriman Springs	Site # 12		88.62	56.73	72.68
Fourmile Creek	Site # 13		1.44	0.73	1.09
Fourmile Springs	Site # 14	11.67	9.63	10.7	10.67

On the basis of information available to estimate elements of a water budget for the marsh, Table 10 provides perspective on the relative magnitude of inputs and outputs. This preliminary budget is just for the north unit of the marsh, which comprises approximately 12,000 acres. Note that numbers in the table are in millions of cubic meters/year. Volumes associated with fluctuations in lake elevation, totaling over 127 million cubic meters, constitute the largest and the most dynamic element of the lake budget. Next is the input from the West Canal, which seasonally conveys water from a variety of tributaries and irrigation return flows. The unnamed west side spring discharge is simply a guess. Given the number of presumed spring locations observed during winter, and the fact that most of these various locations have not been so obvious as named spring discharge locations, the volume of the unnamed component was set approximately equal to the combined total of Crystal and Malone springs. The budget also includes an estimate of present (1998) quantity of septic system discharge from the Rocky Point (RPt) area (see Section 3.6 for more details on this estimate). The last component of the output part of the water budget is diffuse and channeled outflow from the marsh, estimated by difference between the summary inputs and the two output units. Decreasing lake elevations will move water out of the marsh via diffuse flow and the marsh channels, but the other inputs to the marsh produce an additional output of large magnitude. This preliminary budget ignores the small contributions, such as diverted irrigation water, to the northern side of the marsh.

Table 10. Elements of a Preliminary Annual Water Budget for Upper Klamath NWR Marsh – North Pelican Bay Unit\*

<b><i>Input</i></b>	<b>Cubic Meters (x 10<sup>6</sup>)</b>
Precipitation	27.13
West Canal	43.47
Rock Creek	0.96
<i>Named West Side Springs</i>	
Crystal Spring	25.22
Malone Spring	8.49
Unnamed West Side Spring Discharge	30.00
Diffuse Groundwater Inflow	42.90
Septic System Discharge	0.02
Lake Elevation Increase Inflow	64.70
<b><i>Output</i></b>	
Evapotranspiration	30.66
Lake Elevation Decrease Outflow	63.60
Diffuse and Channeled Outflow	~140.00

\*Estimates are in millions of cubic meters per year

The development of an N and P budget for the northern marsh unit on the back of this preliminary water budget was based on water quality data from four sources: 1) Klamath Tribes-Bureau of Reclamation nutrient inflow and limnology data (1991-1992); 2) Bureau of Reclamation 1991-1992 data deriving from Campbell, Sartoris and Sisneros 1993; 3) SAI January-February 2000 data; and 4) the Klamath Tribes-Bureau of Reclamation 1990-1997 limnology data. A quality assurance (QA) plan was prepared to define data objectives and was reviewed and approved by the Oregon Department of Environmental Quality (Shapiro and Associates, Inc., 2000a). An analysis of the field and laboratory data with respect to criteria in the QA plan indicated that only one sample had a relative percent difference (RPD) exceeding the criterion in the QA plan, and no samples fell outside the criterion for accuracy. Table 11 contains the values for the forms of N and P measured by SAI. The complete data set for stations sampled is provided in Appendix E.

Table 11. Summary of laboratory results for forms of nitrogen and phosphorus in streams, springs, marsh channels and Pelican Bay from sampling January 5-6, January 18-19 and February 8-9, 2000.

<b>Sample Type</b>	<b>NH4-N (mg/l)</b>	<b>N03+N02- N (mg/l)</b>	<b>TPN (mg/l)</b>	<b>ORGANIC N (mg/l)**</b>	<b>SRP (mg/l)</b>	<b>TOTAL-P (mg/l)</b>
<b>STREAMS</b>						
<b>Rock Creek</b>						
<b>Mean*</b>	<b>0.005</b>	<b>0.016</b>	<b>0.025</b>	<b>0.004</b>	<b>0.001</b>	<b>0.002</b>
<b>Records</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>0</b>	<b>3</b>	<b>3</b>
<b>Four Mile Creek</b>						
<b>Mean*</b>	<b>0.033</b>	<b>0.020</b>	<b>0.633</b>	<b>0.580</b>	<b>0.007</b>	<b>0.047</b>
<b>Records</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>0</b>	<b>3</b>	<b>3</b>
<b>SPRINGS</b>						
<b>Fourmile Spring</b>						
<b>Mean*</b>	<b>0.005</b>	<b>0.020</b>	<b>0.068</b>	<b>0.043</b>	<b>0.083</b>	<b>0.074</b>
<b>Records</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>0</b>	<b>4</b>	<b>4</b>
<b>Crystal Spring</b>						
<b>Mean*</b>	<b>0.007</b>	<b>0.025</b>	<b>0.076</b>	<b>0.044</b>	<b>0.061</b>	<b>0.062</b>
<b>Records</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>0</b>	<b>4</b>	<b>4</b>
<b>Malone Spring</b>						
<b>Mean*</b>	<b>0.011</b>	<b>0.022</b>	<b>0.109</b>	<b>0.076</b>	<b>0.063</b>	<b>0.068</b>
<b>Records</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>0</b>	<b>4</b>	<b>4</b>
<b>Harriman Spring</b>						
<b>Mean*</b>	<b>0.005</b>	<b>0.042</b>	<b>0.097</b>	<b>0.050</b>	<b>0.034</b>	<b>0.035</b>
<b>Records</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>0</b>	<b>4</b>	<b>4</b>
<b>MARSH CHANNELS</b>						
<b>Crystal Creek #1</b>						
<b>Mean*</b>	<b>0.014</b>	<b>0.013</b>	<b>0.161</b>	<b>0.133</b>	<b>0.058</b>	<b>0.074</b>
<b>Records</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>0</b>	<b>3</b>	<b>3</b>
<b>Crystal Creek #2</b>						
<b>Mean*</b>	<b>0.026</b>	<b>0.013</b>	<b>0.164</b>	<b>0.125</b>	<b>0.042</b>	<b>0.047</b>
<b>Records</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>0</b>	<b>3</b>	<b>3</b>
<b>Crystal Creek #3</b>						
<b>Mean*</b>	<b>0.009</b>	<b>0.013</b>	<b>0.177</b>	<b>0.155</b>	<b>0.029</b>	<b>0.039</b>
<b>Records</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>0</b>	<b>3</b>	<b>3</b>
<b>Recreation Creek</b>						
<b>Mean*</b>	<b>0.013</b>	<b>0.014</b>	<b>0.198</b>	<b>0.171</b>	<b>0.031</b>	<b>0.042</b>
<b>Records</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>0</b>	<b>3</b>	<b>3</b>
<b>PELICAN BAY</b>						
<b>Mean*</b>	<b>0.009</b>	<b>0.012</b>	<b>0.159</b>	<b>0.138</b>	<b>0.023</b>	<b>0.033</b>
<b>Records</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>0</b>	<b>4</b>	<b>4</b>

Table 12 is the estimation of annual loads of total phosphorus (TP) and inorganic nitrogen in the principal hydrologic inputs to the marsh that incorporate data from the four sources noted above. Values of P and N used for estimating annual loads from atmospheric inputs are from Kann and Walker (1999); West Canal Complex data from Campbell 1999 and Walker 1995; Crystal and Malone Springs (Shapiro and Assoc, Inc., 2000); Unnamed spring discharge and diffuse groundwater inflow estimates of phosphorus and nitrogen concentrations based on average of Malone and Crystal Spring values (Shapiro and Assoc, Inc.); estimates of diffuse spring discharge volume based on area proportion of the “springs” value in Upper Klamath Lake water budgets derived from Kann and Walker (1999); septic system discharge contribution is based on Cogan Owens Cogan 2000; lake elevation changes based on 1996 and 1997 Upper Klamath Lake hydrographs (Bureau of Reclamation).

Table 12. Estimates of Annual loads of Phosphorus (TP) and Inorganic Nitrogen (Ammonia-N and Nitrate+Nitrite-N = TIN) in Principal Hydrologic Inputs to the Upper Klamath NWR Marsh North Unit

<b>Marsh Hydrologic Input Components</b>	<b>Cubic Meters/yr (x 10<sup>6</sup>)</b>	<b>Annual Total P Load (kg)</b>	<b>Annual TIN Load (kg)</b>
Precipitation (wetfall+dryfall)	27.13	874	52,448
West Canal	43.47	3,999	3,825
Rock Creek	0.96	2	20
<i>Named West Side Springs</i>			
Crystal Spring	25.22	1,574	807
Malone Spring	8.49	662	408
Unnamed West Side Springs	~30.00	2,100	1,200
Diffuse Groundwater Inflow	42.90	2,788	1,888
Septic System Discharge RPt	0.02	5	896
Lake Elevation Increase	64.70	5,286	1,747
<b>Total Loads</b>	<b>242.89</b>	<b>17,290</b>	<b>63,239</b>

Estimating annual hydraulic loads, and nitrogen and phosphorus loads to water bodies such as lakes has been done to provide perspective on the relative magnitude of the hydrologic influences and associated algae nutrient loads. Empirical relationships have been established between these loads and expected algae biomass as represented by concentrations of algae pigments (e.g., chlorophyll a). There is no widely accepted framework for assessing the trophic status of wetlands, in the

same way that the trophic state of lakes can be assessed (Walker, 1995; Kann and Walker, 1999).

Since Upper Klamath Lake is considered a eutrophic lake, even hypereutrophic by some (Walker 1995), by implication since the Upper Klamath NWR marsh is “within” the lake, it could be considered a eutrophic marsh. This is not necessarily the case. Empirical trophic predictors are poorly developed for marshes, and are marsh-specific (cf. work using algae as indicators of environmental conditions in Florida Everglades in McCormick and Stevenson, 1998). In other words, estimating the marsh inputs, as is provided in Table 12, is useful for determining the relative magnitude of a possible new influence on the marsh, such as input from the Pelican Butte project, but there is yet no widely accepted analytical framework that defines what constitutes a “eutrophic” marsh. It was estimated that the septic system contribution to the marsh (0.00984 cubic meters of flow/yr; 2 kg of total phosphorus/yr; and a total inorganic nitrogen load of 597 kg/yr) would be dwarfed by the other inputs to the marsh as defined in N and P budget of Table 12. It seems unlikely that this added load would change the trophic condition of the marsh, but then lacking the empirical framework there is no way to estimate what the impact would likely be. In addition, it would seem difficult, not impossible, to measure effects of this impact on such a complex marsh.

The term eutrophic is used to characterize an overly nutrient-rich lake reflected in excessive plant biomass, but in a typical marsh where chlorophyll is present in a wide variety of plant materials with substantial total biomass, the relevancy of the term as it has been used is questionable. A marsh’s capacity to provide additional uptake of nutrients without degrading its diversity of habitats appears to be greater than that of a lake. This interpretation is based on a variety of assessments of wetlands that function as treatment systems for diverse upland runoff types around the world (Kadlec and Knight, 1996; Moshiri, 1993; Reed *et al.* 1995).

It is likely there are differences in trophic (enrichment) status among wetlands in the Upper Klamath Basin (e.g., the 40,000 acres draining into the Williamson River compared with the NWR marsh) that would be based on differences in water chemistry and associated organisms. Studies have not been done in the basin to characterize wetlands in this way. The long association of the NWR marsh and Hanks Marsh with the enriched waters of Upper Klamath Lake would be expected to be reflected in the kinds of organisms living in the marsh. Data on the kinds of algae identified in Crystal Creek in the 1991-1992 Bureau of Reclamation assessment of this marsh (see earlier review in this section) indicate the presence of many algae taxa that are tolerant of increased concentrations

of nitrogen and phosphorus. The algae associated with springs at the periphery of the marsh may have a different composition than the algae within the marsh itself, but the relatively high concentrations of phosphorus in spring flow (see Table 12) may also select for species that have greater tolerance for higher nutrient concentrations. This is suggested by the presence in February 2000 (SHAPIRO observations of material collected during February 8-9 water quality sampling) of *Cladophora glomerata* in Crystal and Malone springs and *Spirogyra* sp. in Harriman spring. *Cladophora* is a dominant green algae species on stable substrate at the perimeter of UKL. These are green algae species that are tolerant of elevated concentrations of nitrogen and phosphorus (e.g. Taft and Kishler, 1973).

Before 1948, a large wetland complex of approximately 27,000 acres occupied land along the lower reaches of Sevenmile Creek on the valley floor. The northern residual wetland acreage, the subject of the preceding analysis, is shown adjacent to the recently diked and drained (1970) eastern portions of that wetland in Figure 39. The diversity of vegetation and its resulting structuring of the marsh is suggested in this photo, which is a 1974 color infrared aerial photograph of the marsh and adjacent Upper Klamath Lake. This photograph was obtained by means of a U2 aircraft overflight at 65,000 ft in July 3 1974 during the growing period at a time when vegetative development in the marsh was at its peak. Visible also in the photograph are lake surface drifts of phytoplankton algae along the southeast edge of the marsh, most likely *Aphanizomenon flos-aquae*, the filamentous blue-green algae typical of summer lake conditions.



Figure 40. 1974 color infrared aerial photograph of the ~27,000 marsh area that had been diked and drained and isolated from the ~12,000 acre residual marsh (now the UK NWR), and adjacent Upper Klamath Lake. This photograph was obtained by means of a U2 aircraft overflight at 65,000 ft in July 3 1974 (source D. .W. Larson, U. S. Army Corps of Engineers, Portland, OR).

The different tonal values of the July 3, 1974 aerial photograph suggest a variety of plant species in the marsh. On the basis of SAI field reconnaissance (Geiger *et al.* 2000), the basic emergent components are hardstem bulrush (*Scirpus acutus*) and cattail (*Typha latifolia*), with scattered growth of the yellow water-lily *Nuphar* (*Nuphar polypetalum*) in deeper water areas. Assemblages of willow (*Salix* spp.) are on slightly elevated hummocks within the marsh. Darker areas at the margin and within the marsh are open water areas that are populated with a diverse assemblage of submergent macrophytes, including species of pondweed (*Potamogeton*), tapegrass (*Vallisneria*), water-lily (*Nuphar*), and mare's-tail (*Hippuris*).

Prior to diking and draining, Sevenmile Creek meandered toward Agency Lake and drained into the lake at what is now the southeastern embayment of the Bureau of Reclamation Agency Lake Ranch. From 1948 through 1971, private landowners within the Meadows Drainage District diked and drained approximately 13,000 acres of the Sevenmile Creek wetland complex. The first project was the diking and draining of the 2,900 acres of what has been called the Wood River Ranch. Associated with this project was the dredging and realigning of Sevenmile Creek into what became the Sevenmile Canal. The second project, begun in 1961, was the diking of an approximately 5,000-acre area, including the northern end of the Agency Lake Ranch. The lowest and wettest portion of the wetland within the Agency Lake Ranch (in the Meadows Drainage District) was diked and drained from 1968 to 1971. This sequence of marsh conversions is illustrated and discussed in Carlson (1993) and Snyder and Morace (1997).

It may be obvious to the reader after reviewing this conceptual approach to wetland impact assessment that a variety of assumptions were required to perform the assessment. Addressing the details of these assumptions and providing more complete data sets, and additional data on marsh biota, would improve the quality and value of the water and N and P budgets for the marsh. Even for a rather minor projected impact to the marsh we now expect an analysis that includes many kinds of quantitative information about the marsh (see e.g. David Evans and Associates, Inc. 2004). There is no known assessment report of the impact of diking and draining this most extensive and complex marsh within UKL that resulted in the reduction of its area from 27,000 acres to 12,000 acres from 1948-1971. The projected effects of the Pelican Butte skiing facility on this residual marsh complex seems rather trivial compared to the loss in 22 years of 15,000 acres of this wetland.

## **INFORMATION TRANSFER FROM NON-UKL WETLAND RESEARCH**

### **Research on the effect on lake water quality of wetland loss**

We have been disappointed by our inability to locate assessments of the effects of wetland loss on lake water quality. There are a variety of studies that allude to effects and by implication suggest there would be an effect, but research projects with the specific objective of assessing effects from the loss of extensive wetlands either from the watershed or from within the lake have been scarce to non-existent. We have provided a review of literature in Appendix E which includes studies that have a bearing on this question, such as studies from the Laurentian Great Lakes, but there are none among the research reviewed which provided an assessment that would be analogous to the situation at UKL where a known amount of wetland was lost through isolation or transformation during a known time with an associated change in lake quality occurring during the period of wetland loss. There has been extensive research, however, on wetland water quality functions that have a direct bearing on the relative significance of wetland loss and transformation for UKL water quality.

### **Estimates of increases in N and P from wetland loss**

As is apparent from the previous review of research on wetland water quality functions:

- There has also been no characterization of the types of UKL wetlands as defined by their hydroperiods and hydraulic settings. These factors would be expected to affect the rate of nutrient uptake. A closer inspection of presettlement or pre-diking wetlands at lake surface suggests the different roles and functions of these wetlands.
- There has been no investigation at Upper Klamath Lake of nutrient uptake and release by wetlands positioned at these different hydraulic settings at the lake surface, and, through seasonal cycles of growth and decomposition. Only preliminary assessments have been made by Sartoris, Sisneros and Campbell (1993), Forbes (1997), Forbes, Sartoris and Sisneros (1998), and by Geiger, Caldwell and Hollen (2000). Snyder and Morace (1997) did not assess the functions of in-lake, undrained, wetlands.

In the absence of basin studies addressing the function of marsh wetlands in the littoral zone under specific hydrologic conditions, it is still possible to provide estimates of annual nitrogen and phosphorus uptake to suggest the relative magnitude of influence on lake quality of wetlands that have been lost and of wetlands remaining in the lake. Until the type of research that will provide this information for UKL

wetlands (and wetlands in the upper basin) can be done information on research performed outside of the UKL basin can be very useful.

As an example of this kind of information transfer, measurements of nitrogen and phosphorus annual net retention rates are readily available in wetland treatment literature. The following average to low-end estimates were selected from recent literature (Table 13).

Table 13. Nitrogen (N) and phosphorus (P) emergent wetland net retention rates (kg/ha/yr) and estimated quantities of nitrogen and phosphorus retained in pre-diking wetlands (51,510 ac/20,846 ha) and post-diking wetlands (16,120 ac/6,524 ha) at Upper Klamath Lake.

<b>N and P Forms</b>	<b>Retention Rate (kg/ha/yr)</b>	<b>Pre-Diking (metric tons)</b>	<b>Post-Diking (metric tons)</b>	<b>Rate References</b>
Ammonia-N	576.7	12,022	3,762	Kadlec and Knight 1996
Nitrate-N	401.5	8,370	2,619	Kadlec and Knight 1996
Total N	689.9	14,382	4,500	Kadlec and Knight 1996
Total P	10.0	208	65	Mitsch, Horne and Nairn 2000

Isolating ~35,390 ac (14,322 ha) of in-lake UKL wetlands through diking and draining, and severing their connection with UKL, would have meant a loss of nitrogen and phosphorus uptake capacity of 9,882 metric tons of total nitrogen and 143 metric tons of total phosphorus per year. The implications of these estimates are that if loading from the basin would have remained constant through the period of diking, draining and dam construction, the loss of wetland and the associated loss of nutrient uptake capacity would have resulted in increasing amounts of phosphorus and nitrogen becoming available to plants within the undiked portions of the lake, e.g. phytoplankton and particularly AFA.

The seasonality of wetland uptake or retention of nitrogen and phosphorus, the plant nutrients of primary interest to wetland treatment scientists, is well documented (e.g. Kadlec and Knight 1996, U. S. Environmental Protection Agency 2000, Mitsch, Horne and Nairn 2000). The seasonal uptake of phosphorus by the extensive precolonial wetland plants would have desynchronized the movement of dissolved phosphorus into the lake. This seasonal requirement and uptake of phosphorus by

aquatic plants would occur at approximately the same season as the need for phosphorus by the phytoplankton in the lake. Uptake of nitrogen and phosphorus in winter that appears associated with wetland detritus also occurs (Kadlec and Knight 1996).

Since phosphorus and wetland water quality dynamics may be of most interest in view of the recent ODEQ TMDL for total phosphorus, an overview of what is known from wetland research about phosphorus and nitrogen cycling in wetlands may help set direction for elements of future research.

## **Phosphorus cycling in wetlands**

**Wetlands as Sources and Sinks** It is well established that natural freshwater wetlands often function as sinks for nutrients, especially for nitrogen and phosphorus (see e.g. reviews by Nixon and Lee 1986; Johnston 1991). Nutrient removal in wetlands is facilitated by shallow water (which maximizes sediment-water exchange), waters of low velocity that allow sedimentation, high productivity of vegetation, the presence of both aerobic and anaerobic sediments in proximity, and the accumulation of litter and eventually peat (Mitsch and Gosselink, 2000). Due to the demonstrated success of natural and constructed wetlands in water quality improvement for high concentrations of nutrients (e.g., wastewater wetland studies such as Odum et al. 1977; Boyt *et al.* 1977; Kadlec and Kadlec 1978; Dierberg and Brezonik 1984; Knight *et al.* 1987; Brodrick *et al.* 1988, Knight 1990), there has been interest in the construction of wetlands to improve water quality by controlling nonpoint source pollution (Olson 1992). Over half of the sources of water quality degradation in rivers and streams are nonpoint sources, predominantly agricultural runoff (Baker 1992).

Wetlands operate as nutrient sinks, sources, and/or transformers. An example of a nutrient transformation is the change from a dissolved to a particulate form, where the mass balance has not changed. Within the wetland nutrient cycling involves the conversion of P from an inorganic to an organic form and back to the inorganic form. This cycle of materials involves changes from the particulate state to the dissolved state, and back again to the particulate state. Between the wetland and its surroundings, there may be exchanges between the terrestrial environment and the aquatic, as well as exchanges between the sediment and the overlying water (Bott 1976). A wetland can be both a source for one form of phosphorus and a sink for another (Mitsch and Gosselink 2000). According to Williams (1985), wetlands are superior natural nutrient sinks:

“The ability of wetlands to act as a buffer may be higher per unit area than that of other aquatic systems such as open

streams, ponds, lakes because: the storage compartments are large in relation to through flow volume; the system is complex with many storage compartments which have different uptake and release mechanisms; retention times of water are long relative to open channel streams; and surface areas for nutrient exchange per unit volume of water are high.”

The ecosystem mass balance for P represents inputs minus exports plus intrasystem cycling from litter production, remineralization, and translocation of nutrients through stems and leaves. Few investigations have developed a complete mass balance for wetlands with measurements of all the pathways such as stemflow, root sloughing, detrital-water exchanges, and sediment-water exchanges, due to the complexity of such a model (Mitsch and Gosselink 2000). A nutrient budget for an ecosystem requires sampling over several seasons and a study of biological uptake and soil adsorption capacity. The process of nutrient cycling, which in turn dictates the nutrient budget, is dependent on the hydrology, water chemistry, wetland species, and management practices.

The source/sink relationship depends on hydrologic conditions, the oxygen status of sediments, and activity of the biota, particularly emergent macrophytes. Rooted emergent macrophytes take up substantial quantities of phosphate from the sediments, but after tissue death, release 35 to 75% of the plant phosphorus (Richardson 1985; Nichols 1983). A secondary sink of phosphorus in wetlands is the epiphytes living both on the living plants and the detrital material. Little is known about the phosphorus content of the epiphytes in wetlands. Epiphytes do serve to translocate phosphorus by grazing communities of zooplankton, microcrustacea, and fish. A significant portion of the P assimilated during the growing season is flushed out during the non-growing season.

According to Williams (1985), P exchange equilibria is based on the simple model developed by Hayes and Phillips in 1958 which views P in aquatic systems as being distributed in a state of dynamic equilibrium between the water and the solids (plants, sediments and detritus). Each time new P enters the system, equilibrium is re-established, usually by solids taking up soluble P, so that the new equilibrium does not necessarily have increased soluble P. Over time, the exchange equilibrium can be viewed as having three phases, where  $x$  is the initial mass of dissolved P,  $y$  is the resultant dissolved P in solution, and  $y/x$  is plotted against time. In Phase A, the curve is a negative-slope straight line which flattens out when the system equilibrates and the output is constant. In Phase B, the output remains constant (flat line) until one of the storage components reaches capacity and the  $y/x$  ratio begins to

increase. The increasing ratio continues in Phase C, until eventually  $y=x$  (the system is overloaded by the input). The  $y/x$  ratio can be changed by altering the rate of throughflow (changing the residence time). This model is not adequate for nitrogen when gaseous exchanges such as denitrification occur.

Another example of this adsorb-release cycling process comes from a study of Wisconsin marshes receiving agricultural runoff and secondarily treated effluent from the village of Brillion (Spangler 1976). Phosphorus removal was recorded in the marsh system during the summer and fall. During spring warming, OP which is released by decomposition is flushed during flooding events. Thus, over an annual cycle, little OP was permanently removed by the marsh. The net removal of OP by plant uptake is the amount retained in non-degradable plant tissue. Some benefit is still obtained by keeping OP out of the lakes during the summer growing season.

In flowing water systems where there is a continuous downstream movement of water and particulate materials, the nutrient cycle becomes a spiral. An S-shaped path can be described in which nutrients move between the water and the particulate form. The spiral length index (S), is the sum of the distance traveled downstream during the soluble and particulate form of the nutrient, and is a measure of the utilization of nutrients provided to an aquatic system with a flowthrough. If nutrient utilization in a system increases, then S will decrease. Decreasing water velocity also decreases S. An increase, should there be one, of the regeneration rate of a nutrient will decrease the uptake rate and thus increase S (Williams 1985).

**The Role of Macrophytes in P Cycling** Wetland vegetation serves a number of important functions in purifying nutrient-laden water. The initial mechanisms for removing some of the nutrient load is by filtering and settling of inorganic and organic particulate matter. This occurs both by the reduction of flow velocities in the wetland, and by the interception of the particles with emergent vegetation. The vegetation also provides a substrate for the attachment of decomposer microorganisms behaving somewhat like a trickling filter in breaking down dissolved organic matter (Nichols 1983).

A major difference between nutrient cycling of wetlands and lakes is that many wetland plants obtain their nutrients from the sediments, while phytoplankton depend on nutrients dissolved in the water column. Wetland plants have often been described as “nutrient pumps” that bring nutrients from the anaerobic sediments to the above ground strata. Phytoplankton in lakes and estuaries can be regarded as “nutrient dump” that take nutrients in the aerobic zone and, through settling and

death, deposit the nutrients in the anaerobic sediments. Thus, the plants in these two environments can be viewed as having different functions in nutrient cycling (Mitsch and Gosselink 2000). An efficient wastewater treatment system for example would then utilize both of these natural strategies for nutrient uptake by providing open water for phytoplankton growth and vegetated areas for settling and filtering particulates.

The extent to which aquatic macrophytes take their nutrients from the sediments by root uptake, rather than from the water by foliar uptake, has been discussed by the scientific community (Nichols 1983; Allen 1974). Recent literature supports the view that roots are the main site of P uptake (Gabrielson 1984). It had previously been thought that the roots functioned primarily as attachment organs. But it is now agreed that the soil is the principle source of P under natural conditions, depending on the relative P concentrations of the water and the soil. In other words, if there is more P in the water, foliar uptake predominates; if there is more in the sediments, root uptake predominates. Some species can change absorption sites depending on the nutrient levels in the soil and the water (Nichols 1983). It is the net effect of plants on the water column that is ultimately important to the analysis of phosphorus budgets.

The net effect of rooted emergent vegetation is to transfer nutrients from the soil to the water. For example, in a Des Plaines River wetland study, the calculated uptake of P by macrophytes was higher than the amount supplied by the influent, indicated that some other source of P was utilized. Non-rooted plants such as algae, duckweed, and sphagnum obtain nutrients directly from the water, and the incorporation of their detritus into the soil is a net transfer of nutrients from the water to the soil. Both submergent and emergent vegetation release P when they die. This leaching of P is rapid after plant tissue death (Darkow 1988).

It has been shown that P stocks in the roots and rhizomes of *Typha latifolia*, a species typical of UKL wetlands, are mobilized into the shoots early in the growing season. The total stocks can increase to over 4g/m<sup>2</sup> during the summer. In the fall, some P in the shoots is mobilized into below ground organs before the shoots die, but most is lost by leaching and decomposition. By calculating the below ground deficit, this shift in P storage can be determined (Mitsch and Gosselink 2000).

In a Des Plaines River wetland study, the uptake of P by periphyton, phytoplankton, and submersed aquatic vegetation (collectively called water column uptake) was estimated to be 4 to 6 mg.P/m<sup>2</sup>week (approximately 0.2-0.3g.P/m<sup>2</sup>yr), well below the sedimentation rate or

macrophyte uptake rate. However, much of the uptake by periphyton remains in insoluble form and falls to the sediments in the wetlands.

The normal plant root is part of a system involving micro-organisms both on the root surface and inside the cells. Mycorrhizae give rise to significant increases in the rate of absorption, primarily as a result of increased functional surface area. Less readily-available forms of P can be exploited by the microbes as well. Mineralization of insoluble phosphates such as apatite by bacteria and fungi is well known. The other major aspect of biological mineralization is that of hydrolysis of ester forms released during the breakdown of organic materials from both plant and animal origin (Williams 1977).

**The Role of Detritus in Phosphorus Cycling** Detritus (annual deposition of litter) plays a central role in the ability of rooted wetlands to retain nutrients (Davis and Van der Valk, 1983; Atkinson and Cairns 2001). Extensive work by Howarth and Fisher in 1976 showed that when nutrients were plentiful, the decomposing tissue acted as a locus for nutrient accumulation by micro-organisms, but when nutrients were in short supply regeneration to the water occurred (Williams 1985).

The roles of fauna as nutrient cyclers are poorly understood. Howard-Williams and Allanson (1981) found that 17% of added <sup>32</sup>P ended up in invertebrates (a high-rate filtering mussel may have contributed an exceptional amount). Waterfowl, herbivorous fish, and other animals may play a significant role in removing and contributing nutrients. Wetland fungi and bacteria create a large amount of vitamin B12 which is necessary for the growth of plant uptake or by flushing or dilution with low phosphate water, will cause the release of some P from the sediment into the overlying water.

**Permanent Removal of Phosphorus by Peat Formulation** The process of nutrient uptake in wetlands is distinct from other natural systems. One of the major ways wetlands differ from drier upland ecosystems is that more nutrients are tied up in organic deposits such as peat and are lost from ecosystem cycling. The characterization of peat deposits in drained and undrained wetlands of UKL from peat cores show this clearly (Snyder and Morace 1997). Wetlands are similar to deep aquatic systems in that most of the nutrients are often permanently tied up in sediments and peat. However, aquatic system nutrient cycling in the autotrophic zone of lakes and coastal waters is more rapid than it is in the autotrophic zone of most wetlands (Mitsch and Gosselink 2000).

Estimates from the literature indicate that P accumulation in undisturbed organic soils is about 0.1 to 0.2 g/m<sup>2</sup>.yr. The first one or two years of application, a higher P retention efficiency can be expected,

with 90-95% retention at low loading rates of 2 to 5 g of wastewater P/m<sup>2</sup>-yr. and up to 70% retention at loadings as high as 10 to 15 g/m<sup>2</sup>-yr. This efficiency will not be maintained, however.

**Additional Factors Affecting P Removal in Wetlands** Freshwater wetlands are often formed and expanded by advanced eutrophication of lakes, ponds, river deltas, and shallow basins with poor drainage. At low loading rates, wetlands have a capacity to remove much of the phosphorus applied and will continue to do so for many years. As the loading rate is increased, however, the efficiency of phosphorus removal can decline rapidly as the system becomes saturated (Nichols 1983).

In a 3-year study of P retention in constructed wetlands along the Des Plaines River, wetlands treated river water under both high and low flow conditions (EPA 1993a). Despite a variable pattern of P concentrations in the river water delivered to the wetlands over the three-year period, the average inflow concentration of TP was substantially greater than the average outflow concentration in all wetlands in all three years ( $p < 0.01$ ). Very little difference was noted, however, in the percent decrease in TP between the high flow and low flow wetlands for the first two years. For example in the two mineral soil wetlands with the most complete data base (No 4 and No. 5), the average outflow concentrations decreased by 63, 92, and 87% respectively for 1990, 1991, and 1992 in low-flow wetland No. 4 and by 62, 90, 78% in high-flow wetland No. 5. Third year data from both routine and intensive sampling suggest, as had been originally hypothesized, that the low flow wetland reduced the concentration of TP more effectively than the high flow system.

The water balance of wetlands affects their structure and function by controlling the input and output of nutrients. Thus, nutrient budgets provide the basic framework for analyses of wetland nutrient dynamics. Where productivity and decomposition are rapid, so is the nutrient cycling. Organic accumulation in wetlands is controlled by the hydrology through its influence on primary productivity, decomposition rates, and export of particulate organic matter (Mitsch and Gosselink 2000).

A wetland's hydrology leads to its unique vegetative composition. Primary production in wetlands is enhanced by flowing conditions and a pulsing hydroperiod, and is often depressed by stagnant conditions. Primary production is greater with flowing water, less in sluggish flow, and least in still water. Flowing water can create a very uniform surface that might cause monospecific stands to dominate.

## **Nitrogen Cycling in Wetlands**

Nitrogen is a key element in biogeochemical cycles and occurs in a number of different oxidation states in natural and constructed treatment wetlands. For example, the dominant nitrogen species entering a free water surface (FWS) treatment wetland depends on the level and type of wastewater pretreatment, but may include organic, ammonia, nitrate, and nitrite nitrogen, and nitrogen gases (di-nitrogen gas [N<sub>2</sub>] and di-nitrogen oxide [N<sub>2</sub>O]).

A fraction of the organic nitrogen is readily mineralized to ammonia nitrogen in aquatic and wetland environments. Ammonia nitrogen is distributed between the ionized form (ammonium, NH<sub>4</sub>) and a smaller percentage of un-ionized ammonia (NH<sub>3</sub>). The distribution of total ammonia between NH<sub>4</sub><sup>+</sup> and NH<sub>3</sub> depends on water temperature and pH. Un-ionized ammonia is volatile and may be lost directly to the atmosphere.

Ammonium nitrogen can be oxidized in open, aerobic zones to nitrite and nitrate nitrogen through an aerobic microbial process called nitrification. Free dissolved oxygen and carbonate alkalinity are consumed in this process. Ammonium nitrogen may also be biologically assimilated and reduced back to organic nitrogen in the plants, or may be removed from the water column by adsorption to solid surfaces, such as wetland sediments. Adsorbed ammonium is readily released back to the dissolved ammonia state under anaerobic conditions.

Nitrate nitrogen is readily transformed to di-nitrogen gas in wetlands by the microbiologically mediated anaerobic process, denitrification. Denitrification occurs most readily in wetland sediments and in the water column utilizing carbon from the decomposition of the detrital matter.

The total nitrogen (sum of all nitrogen species) and total Kjeldahl nitrogen (organic plus ammonia nitrogen) in natural systems is a function of edaphic factors in the watershed and biological activity. Background levels of nitrates, for example, are usually a function of groundwater contribution to surface water. Ammonia nitrogen levels are a function of biological activity and the proximity of these activities to the point of entry into the watershed. Wetlands serve as both sources and sinks of nitrogen species, which, in turn, requires a speciated nitrogen balance for a specific system. Distribution of various species of nitrogen within a wetland indicates that the nitrogen dynamics are effective by external nitrogen loads, the extent of plant coverage, and the maturity of the emergent vegetation. Wetlands and wetland soils have the potential to remove nitrogen to the atmosphere via denitrification processes.

Given the condition of anoxic conditions sufficient dissolved organic carbon and temperate water temperature nitrate nitrogen can be removed by microbial denitrification processes. Nitrates have to be available for denitrification to occur which are formed under oxic conditions. In natural wetlands these distinctly differed redox conditions does not usually occur in the same location. In most cases in natural wetland nitrification processes occurs in the water column and denitrification process occurs in the soil/detritus complex. Wetland communities serve as a seasonal temporary sink for nitrogen species, ammonia preferentially as they retain nitrogen during the growing season. Ammonia nitrogen and particulate organic nitrogen are released upon decomposition of senescent aquatic plants.

### **Dissolved Organic Carbon Production from Wetlands**

Previous sections have noted water quality measurements that likely reflected senescence of wetland vegetation during late summer and fall (see e.g. BR UK NWR wetland studies). The release of nutrients that occurs is associated with the other plant components such as lignins that are more resistant to microbial or chemical reduction. Dissolved organic matter (DOM) is a chemical constituent in both natural and constructed wetlands of importance. Little is known about the molecular configuration and concentrations of DOM's. A study by the U.S. Bureau of Reclamation and Geologic Survey 1999 (Barber *et al.* 1999), investigated the occurrence, character, and transformation of DOM in six wetland systems in the Western United State. One of the six sample locations used in this study was Hank's Marsh, an in-lake wetland on Upper Klamath Lake. This study was intensive and exhaustive in terms of characterizing the nature of the DOM. Some of the data from that study has been noted in previous sections of this report. Dissolved organic carbon (DOC), which is a term derived from analytic methods used in characterizing DOM, is distinguished from total organic carbon (TOC) in water samples by filter pore size (the filtrate DOC component) and organic carbon in an unfiltered sample. DOC components range from lipids and carbohydrates to humic and fulvic acids. The section of this report (Section 4.0) provides additional information on humic composition and measurement.

The signature brownish coloration of marsh water reflects higher concentrations of DOC than are observed in the lake. Notes on DOC analyses at the end of the Forbes-BR study of Hanks Marsh indicated the difference between concentrations of DOC in UKL (typically 6-12 mg/l) and those in the marsh that were (13-20 mg/l). Measurements of DOC in open waters of restored wetlands at Caledonia Marsh at Running Y Ranch Resort ranged from 32-93 mg/l (Geiger 2001a).

Dissolved organic matter (DOC) in the Klamath Lake watershed is formed primarily from the decay of the aquatic plants and secondarily from the decay and transport of the litter fall of terrestrial plants. Some DOC is also contributed as pump discharge from the diked and drained agricultural lands around the lake and in the contributing watersheds. The aquatic communities are by far the more productive primary production systems in the watershed and thus contribute proportionately more DOC.

Since humic carbon or humates can comprise over 50% of the DOC concentrations (Perdue *et al.* 1981), both wetlands in the lake, reclaimed wetlands behind the dikes, and winter flooded farm fields are large reservoirs of what may be a valuable cyanobacteria suppressant (see Section 4.0). The loss of in-lake wetlands, diffusing these humic compounds differently and at different times depending on hydrologic setting, would have resulted in lower lake concentrations of dissolved humic substances.

## **Hydrogen Ion**

Natural wetlands exhibit pH values ranging from basic in prairie wetlands (8-9), to slightly basic in alkaline fens (pH=7 to 8), and to quite acidic in sphagnum bogs (pH=3 to 4) Mitsch and Gosselink 2000). Natural freshwater marsh pH values are generally slightly acidic (pH=6 to 7). Treatment wetland effluent hydrogen ion concentrations are typically neutral to slightly acidic. Data from an open water, unvegetated treatment “wetland”, displayed high pH during some summer periods (pH > 9), with circumneutral influent (7.0 < pH < 7.4) (Bavor *et al.* 1988). Algal photosynthetic processes peak during the daytime hours, reducing dissolved CO<sub>2</sub> concentrations, creating high pH during the day, followed by a night-time sag with low pH as respiration replaces photosynthesis.

Organic substances generated within a wetland via growth, death, and decomposition cycles are a source of natural acidity. The resulting humic substances are large complex molecules with multiple carboxylate and phenolate groups. The protonated forms have a tendency to be less soluble in water and precipitate under acidic conditions. As a consequence, wetland soil/water systems are buffered against incoming basic substances but not as well buffered against incoming acidic substances.

Background (non-growing season) Upper Klamath Lake pH is near neutrality. During photosynthetic activity, pH in pelagic areas frequently reaches levels above 10.0 (Kann and Smith 1993; Gearheart *et al.* 1995). Hazel (1969) determined that lake-wide pH was correlated to temperature and intensity of *Aphanizomenon* blooms. Inside littoral marshes

however, pH ranged between 6.5 and 7.5. Hazel observed a pH gradient between the marsh and the lake: “Inside marsh areas, pH varied from about 6.5 to 7.5 throughout the year. At Hanks Marsh on July 23, pH was 7.4 seventy-five yards inside the marsh, 9.4 at its edge and 9.8 twenty-five yards into the lake”.

## **Hydrologic Factors Affect Wetland Water Quality Functions**

**Water Budgets for Wetlands** The preliminary water budget or water balance for UK NWR developed to estimate impacts of the proposed Pelican Butte skiing facility on the marsh demonstrated the large role the annual fluctuations in the elevation of UKL surface water play in nutrient cycling in that marsh. Though water depth measurements were made during sampling of Hanks Marsh, the other in-lake marsh unit of the UK NWR, there was no water budget developed that would further understanding of what influence the marsh has on the water quality of UKL. The multi-year commitment and the complexity of data gathering required to develop a well-supported water budget for a marsh is the likely reason why this has not yet been done for UKL or upland basin marshes.

Determining the water balance through a littoral wetland is essential for any model that estimates the export of humics into the lake. There have been no attempts to develop water balances for inlake wetlands at Upper Klamath Lake. For that matter there have been few attempts to model inlake water balances for any wetland systems. The difficulty lies in the complexity of the inputs and outputs of water and the hydraulic considerations. Factors that are difficult to parametrize in lake wetlands are listed below:

- groundwater addition via the bottom of the wetland
- diffused shallow groundwater and surface flows into the wetland
- groundwater recharge
- outflows from the extensive wetland margin/s with the lake
- inflows from the extensive wetland margin/s with the lake
- condensation and ET from wetland plant community (micro-meteorological factors)
- hydroperiod of wetland compared to lake-energy gradient for mass transfer
- seasonal wetland plant moisture content
- moisture content (storage) in the detrital material and shallow soil layer

An important factor in understanding the advective forces that move humic laden water into Agency and Klamath lakes is the differential hydroperiod (energy gradient) through the wetland from watershed to lake. This energy gradient is affected by the rate and volume of inflow, the slope of the wetland ground surface, the lake water surface, and the resistance

afforded by the plants and detrital material. The scope of work for this study identified a work item which would locate sites within the Klamath National Wildlife Refuge for placing data logging water elevation, specific conductance, and temperature measuring devices which can be used to determine head loss through the wetlands and potentially estimate mixing volumes and rates. Initial attempts have been made to identify potential transects for placing these instruments in the wetlands. A reconnaissance trip was made in early August, 2004 to attempt to locate sites for this work item. In August the lake level was too low to allow the use of the available water craft equipment to penetrate the wetland. Several sites at the margin of the wetland and Upper Klamath Lake were assessed. Remote sensing information made available from the Bureau of Reclamation in form of aerial photographs of the in-lake wetland was reviewed for purposes of determining transect locations. There was not sufficient information afforded by either of these activities to specifically locate these transects. This is a critical task to be accomplished if humic rate and transport into the lake is understood.

**The Location of Lost Wetlands Matters** It was noted in this section that the loss of wetland occurred at a variety of locations within UKL (see Figure 36 above from Snyder and Morace 1997). Some wetlands such as that of Wocus Marsh at Running Y were isolated but large wetlands with a very narrow connection to the lake. Other wetlands such as those at Caledonia Marsh and across the lake at the Algoma Marsh area were out in the lake with no distinct upland drainage moving through the marsh to the lake. Other wetlands such as those in the UK NWR, the wetland on the east side of the lower end of Wood River, and the large wetland area of the Williamson River Preserve on the north and south sides of the delta of the Williamson River were yet in a different setting with fluctuating seasonal flows of creeks and rivers moving through these extensive wetland areas. One would expect different water quality influences on UKL with respect to the timing and intensity of that influence. Table 14 suggests the various hydrologic influences on and provided by wetlands.

Table 14. Hydrological and Water Quality Role of Wetlands in Upper Klamath Basin.

	<b>Wetlands in upper watershed at the headwaters of streams and rivers</b>	<b>Large wetlands located on the margin of the UKL</b>	<b>Undrained wetlands within UKL</b>
Storage detention	Significant storage-ET loss similar to agricultural demand-Upper Klamath Marsh, Sycan, and Sprague	Significant storage Desynchronized wetland seepage release to lake/ET loss similar to agricultural	No storage increase
Peak flow Desynchronization	Desynchronized release	Limited effect except in high runoff periods	No effect
Regional Groundwater recharge	Significant groundwater recharge-release-Upper Klamath Marsh/Sycan/Middle Sprague	No effect	No effect
Fisheries habitat Patch	No effect	Limited effect	Significant addition i.e., Hanks Marsh
Fisheries Habitat Corridor	Upper Klamath Marsh-Williamson River,Sycan Marsh-Sprague	Significant addition Mouth of the Williamson, Wood, and Seven-mile	Potential Corridor with springs on East Side of UKL
Water Quality Treatment	Sediment trapping and associated particulate phosphorus-seasonal uptake of dissolved phosphorus	Sediment trapping and associated particulate phosphorus-seasonal uptake of dissolved phosphorus	Localized lower pH's, stable DO's.reduced algal densities
Humic Acid Production	Significant potential humic acid production delivered via Williamson and Sprague Rivers	Significant potential humic acid production with relatively fast addition to UKL	Minimal Lake effect, potential local effect

**Estimating Evaporation Rates** Annual wetland evapotranspiration (ET), a controversial element of water budgets and wetland restoration in the Upper Klamath Basin, is influenced by wetland setting, as suggested by Table 14. The estimation of ET for specific wetland areas is complex, for a variety of reasons.

Climate and weather, vegetative characteristics, and wetland physical characteristics all combine to affect evapotranspiration from wetlands. General trends have been identified as to how heterogeneity in these various factors affect ET in wetlands. The primary meteorological factor driving ET is the difference in the vapor pressure of water at the

evapotranspiring surface and the air above it (i.e., the VPD). The ability of wind to move air out of the wetland system and replace it with air of different quality will depend on the amount of sheltering provided by site vegetative and surrounding features.

The wetland physical characteristics of primary importance to wetland ET are the plant canopy areal extent, the geometry of the stand (i.e., long and narrow versus extensive surface area in all directions), and degree of peripheral surface area exposure in directions of fetch. The characteristics of the area surrounding the stand will influence the fetch and degree of shade and shelter from the sun and wind that the stand experiences. Stand geometry and peripheral surface exposure will have the greatest affect on ET in sparsely vegetated, arid regions where advective energy exchange is dominant.

The issue of whether wetland plants increase or decrease water loss relative to open water has been the most thoroughly and successfully researched aspect of evapotranspiration from wetlands. It has been well established that aquatic macrophytes with little to no peripheral surface area reduce water loss compared to open water from water bodies of any size. Phytoplankton has been shown to increase surface water temperatures enough to cause short term increases in daytime VPD over the water to of approximately 25% (Idso and Foster 1974) The effect of aquatic plants of various heights on water loss is less clear. Available data indicate that over extensive water bodies, the presence of tall aquatic vegetation (2.1 to 5.9 ft.) will increase water loss compared to open water by approximately 40% (Anderson and Idso 1985 and 1987a, Van der Weert and Kamerling 1974, Young and Blaney 1942.

Short aquatic vegetation (0.2 to 1.2 ft.) over extensive water bodies has been shown to result in water loss rates much lower than that from tall vegetation and in some cases less than open water evaporation (Anderson and Idso 1985 and 1987a). The effects on ET rates of plant height and stand areal extent in combination with stomatal conductance to water vapor are the least quantifiable based on existing research findings. These factors along with meteorological parameters appear to be the primary factors governing differences in ET rates between species and wetland locations.

The primary vegetative characteristics of importance are the stomatal conductance of water vapor, albedo, plant cover type, and height. Stomatal conductance and plant height have been addressed above. Plant cover type will affect the degree of shading and shelter from the sun and wind the plant canopy and underlying surface will experience. This will have a direct affect by influencing surface and air temperatures, the net radiative flux reaching the surface, and the degree of air mixing

provided by the wind. Reduced water loss rates for the broad, round leafed floating aquatic plant species are attributed in part to these effects (Gutenspergen et al. 1989, Otis 1914, Anderson and Idso 1985). The species of most interest to the Bureau of Reclamation, *Scirpus* and *Typha* spp., are emergent plants with long, erect, linear leaves that reduce self-shading while creating a large stomatal area.

Values for albedo (fraction of incident radiation reflected) from various aquatic plant species (*Typha* spp., *Calamagrostis canescens*, *Carex gracilis*, *Eichhornia crassipes*, *Salvinia molesta*, *Phragmites communis*, and *Nymphaea lotus*) have been found to range from 12 to 22% (Bray 1962, Priban and Ondok 1985, Rao 1988, Smid 1975). Albedo values during the growing season for these species are all approximately 20%. Bray (1962) found that albedo values for *Typha* spp. during the non-growing season were 50%. Non-growing season albedo values for the other species mentioned above ranged from 9 to 14%. The high value for *Typha* spp. should be confirmed if energy budget type equations will be used to estimate ET. Albedo data for other species including *Scirpus* spp. was not available from the literature reviewed and should be generated if energy budget type equations will be used to estimate ET.

The relationships between the above meteorological parameters and ET and E<sub>o</sub> rates have been well established in wetland evapotranspiration and general evaporation and evapotranspiration studies. The empirical and Penman type equations that account for these factors appear to give reliable ET estimates in all cases except where advective energy exchange is dominant. General trends regarding the effects of vegetative and wetland physical characteristics on ET rates and ET/E<sub>o</sub> ratios have been well documented. Yet, there are no firmly established methods for quantifying these effects for various species and canopy areal extents, geometry's, and degrees of canopy exposure and shelter provided by site and surrounding features.

Kann (2001) reported results of ET estimation by Bidlake (2000) of the USGS for a site in UK NWR in May 28 – October 12, 1997. ET loss for the *Scirpus-Typha* wetland was 1.6-1.8 ft (PM and PT model results respectively). During the same period water loss from the lake surface based on pan evaporation rates at the OSU Agriculture Experiment Station near the Klamath Falls airport ranged from 2.06 to 2.12 (using 0.74-0.76 pan evaporation rates to estimate evaporation from UKL lake surface).

It is likely that considering the heterogenous surface texture of the in-lake wetlands that have been discussed in this report, ET rates will vary across expansive wetland areas. The dispersion of open water channels within Hanks Marsh in the GIS picture of that marsh suggests there would be

variations of ET rates across the marsh suggesting choices for placement of stations that reflect this diversity.

## **ESTIMATING PRECOLONIAL WETLAND AND LAKE WATER QUALITY CONDITIONS**

### **Estimating the Extent of Precolonial Wetland in the UKL Basin**

This section of our report (Section 6.0) was introduced with a review of how much wetland was lost in the last century from within UKL. The 35,350 ac loss of the various wetland processes described above that lead to N and P uptake, and decompositional processes that result in the export of N and P and dissolved organic compounds from wetlands was very large. If humic materials in the brown water that is characteristic of wetland environments is something that suppresses the growth of AFA, as our preliminary findings suggest (see Section 4.0), this in-lake loss might have been enough to change the lake. But, what if other wetland losses in the watershed above UKL were even larger, and the loss of this other source of dissolved organic materials together with the loss of in-lake wetlands was what changed the lake? The implication of this is that it may not be enough to restore the former wetlands behind dikes around UKL and reconnect them with the lake. These restorations, such as is proposed for the Williamson River Preserve by TNC (David Evans and Associates, Inc. 2004), may not produce sufficient humic materials to the lake to mimic precolonial lake conditions. We chose to address this issue directly in the work on this project through the development of a model that would estimate precolonial dissolved organic material and humate production. We substituted the work on this model for resources in the project budget on limited wetland edge monitoring due to its importance for developing a significant caveat about the real opportunity for changing UKL water quality. Data on wetland edge water quality has been included in this section from more extensive reviews of previous wetland research on UKL wetlands (see second section of this report section).

Assertions about the amount of wetland lost in the Upper Klamath Lake Basin vary widely. Millstein reported the loss of 75% of the original ~350,000 acres of wetland in the Klamath Basin from government 'reclamation' programs (Millstein 2001). A slight variation on this wetland loss estimate was provided by Allen (1999) who noted that 80% of the basin's 350,000 acres of wetlands have disappeared. Was this the entire Klamath Basin or the UKL drainage basin? There are no references in these two articles about sources of information for the numbers. Campbell, in a recent book about Klamath marshes, noted

that wetlands cover only 25% of the land they did in the past (Campbell 2002). The source for the estimate of original wetlands number is apparently Atkins (1970) who concluded that there were approximately 350,000 acres of wetlands in the Upper Klamath Basin, which he defined as including wetlands associated with Lower Klamath Lake, Tule Lake and Clear Lake north to the upper reaches of the Sprague River and the Sycan Marsh and Klamath Marsh on the Williamson River. There is no table in Atkins that summarizes his estimates of loss, alteration, retention or re-flooding (terms that he used) to arrive at a net 'loss' of wetland. He did note that loss from actions of private landowners were ~50,000 acres whereas loss from government 'reclamation' actions was ~200,000 acres. His conclusions suggest there may have been a total loss of 63% of the original wetlands from the area he evaluated. He does provide a caveat for his work: "Records referring directly to changes in the extent and quality of wetlands in the Klamath Basin are not available."

An example of the change to a large wetland area in the UKL basin is the reduction in the area of Klamath Marsh on the upper Williamson River. Although quantitative data on the extent of modification of the Klamath Marsh is generally lacking, there is general agreement that significant alterations of the Marsh have occurred since the early 1900s. Some documents have indicated that the hydraulic control of the marsh was greatly altered around 1910 by blasting. One document (USFS 1996) suggests that this caused as much as 5 to 10 feet of change in the elevation of the control at Kirk Reef, although other accounts suggest that any blasting at Kirk Reef would not have had any impact. In any case, GLO maps and other historic descriptions of the area indicate a much more extensive marsh, with up to 10,000 acres of open water Wocus habitat (Colville 1902). The river channel in the portions of the marsh has been dredged and straightened, and drainage/irrigation canals have been built to modify the hydrology of the marsh and vicinity to better accommodate agriculture. It has been hypothesized that channel modifications on the east side of the marsh may have initiated a headcut that has migrated as far upstream as Rocky Ford, causing channel incision and instability (USFS 1998). Lack of quantitative data, such as surveyed long profiles of the river and its floodplain precludes definitive evaluation of such theories.

The Klamath Marsh was established as a National Wildlife Refuge in 1958 when approximately 16,400 acres was purchased from the Klamath Tribes. An additional acquisition of 25,000 acres in 1990 and 1998 increased the size of the KMNWR to 40,646 acres. There is a private inholding of 4,000 acres within the KMNWR. There is approximately 31,000 acres of private wetlands adjoining KMNWR River. Another approximate 9,000 acres of private land exists along the Williamson

River. The inventory gives a potential past wetland coverage of 81,000 acres.

The large caveat in this evaluation of changes in Klamath Marsh is absence of on the ground wetland delineations and/or interpretation of aerial photography to determine where wetlands were and how they changed through time. It is likely that much of what was formerly a different kind of wetland than exists today with respect to dominant vegetation types and hydroperiod is still wetland today but with less Wocus habitat and generally less vegetative biomass per unit area. This is a change that would not be reflected in an estimate of wetland loss but in the estimate of how a wetland area was transformed into a different kind of wetland area. There would still be evidence from wetland soils with their persisting chemical signatures that would indicate where wetland once was, but without hydrology data or aerial photography it would be more difficult to document and characterize wetland transformation.

The methodology for conducting wetland inventories has been developed since the Clean Water Act gave responsibility nationally to the U. S. Army Corps of Engineers to regulate fill of wetlands. Successive drafts of methodology development, reviewed in NRC 1995 and Mitsch and Gosselink 2000) resulted in the widespread acceptance of the *Corps of Engineers (COE) Wetlands Delineation Manual, Technical Report Y-87-1* (Environmental Laboratory, 1987). The U.S. Army Corps of Engineers (COE) and Oregon Division of State Lands (DSL) recognize the use of the 1987 Manual for delineation of wetlands. The Manual requires that three technical criteria be met in undisturbed situations before areas can be considered wetland under federal or state jurisdiction. These criteria are the presence of hydric soils, hydrophytic vegetation (Reed 1988), and wetland hydrology under normal circumstances. If one of these criteria cannot be determined because of disturbance caused by recent natural events or human activities, an alternative method must be employed in making wetland determinations.

The U. S. Fish and Wildlife Service National Wetland Inventory (NWI) unit inventoried wetlands within the Atkins study area, specifically within the Klamath Lake, Williamson, Sprague and Lost River hydrologic unit classes. The Klamath Lake HUC includes the Sevenmile-Wood River drainage area as well as drainage areas on the west side of UKL. The work was done using August 1982 B&W aerial photography, photographs which are still available at the USFWS NWI office in Portland. 'Ground truthing' of photographic interpretation was a part of the NWI mapping effort. Mylar overlays of USGS 7.5 minute quadrangle topographic maps were prepared for the each of the maps covering all of the area in the hydrologic units. Publication of these mylar overlays

occurred from 1989-1994. In addition, the inventory was digitized and is available as GIS files through the Bureau of Reclamation office in Klamath Falls. Paper copies of the mylars are available through the Oregon Department of State Lands Wetlands Division. The definition of “wetland” used by the NWI is grounded in the delineation methodology of the Corps’ manual, but where generally each of the three indicators of wetland must be present for a “wetland” to be considered under the jurisdiction of the Corps or the Department of State Lands, NWI would identify wetland if only two of the three indicators were present. Further NWI mapped “wetland” systems include riverine wetlands, which are waters of the state and if navigable under the jurisdiction of the Corps but not jurisdictional wetlands. Palustrine wetlands, which is the dominant wetland system in these hydrologic units e.g. wetlands within UKL, of Klamath and Sycan marshes (wetlands of marshes, bogs, wet meadows, swamp land with shrubs or trees, etc.) which presumably would be jurisdictional wetlands were mapped. The description of the NWI classification that appears on each of the NWI maps is provided in (Cowardin *et al.* 1979)

In view of the numbers of precolonial wetland acres estimated by Atkins (1970) in the northern end of the Klamath Basin (350,000), it is of interest to note there was total of 290,517 Palustrine wetland acres in the Klamath Lake, Williamson, Sprague and Lost River hydrologic units, essentially the same area evaluated by Atkins. Figure 40 provides a comparison of the wetland acreage and the acreage of the hydrologic unit classes. The total wetland acreage for each of the hydrologic unit classes in Figure 40 was: Klamath Lake (74,482 ac), Williamson (82,039 ac), and Sprague (68,040). The combined wetland acreage was 224,561 ac. These estimates of wetland do not include open waters of UKL, including Agency Lake, which the NWI classified as a lacustrine wetland system). The statistical summary of the FWS NWI GIS data, from which these estimates have been derived, was provided by M. Neuman (Geiger pers. com. 1999). An MS Excel file of this data is provided in Appendix E.

### Palustrine Wetlands Within UKL Basin HUCs

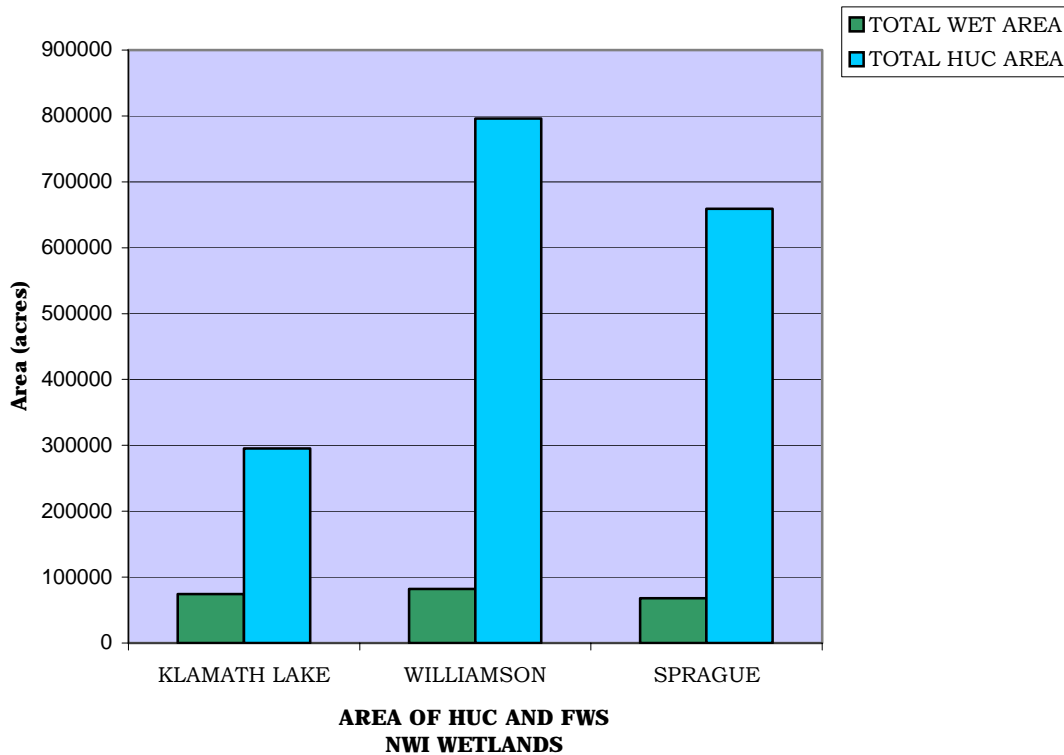


Figure 41. The area of the hydrologic unit classes (Klamath Lake, Williamson and Sprague Rivers) and the extent of the Palustrine system of wetlands (NWI August 1982 B&W aerial photography).

So, we conclude that estimates of wetland loss (such as are reported in the press and popular journals, perhaps based on Atkins 1970) might not reflect what may be a more important factor: wetland transformation, rather than wetland loss. One can see wetland transformation as a loss of a kind of wetland that was much more valuable than the wetland that remained after human alterations. However, discussion of changes in UKL Basin or Klamath Basin wetlands needs to be qualified carefully in view of the increase in knowledge of the location and kinds of wetlands remaining in the Basins. It does not seem that the large numbers of asserted wetland loss apply to jurisdictional or NWI wetland loss. Now that a baseline has been established through the NWI for wetlands in the UKL basin as of 1989-1994, it would be possible to estimate wetland loss and change as has been done recently by the FWS for the Reno/Carson City area (Hall *et al.* 2004). As for our interest in estimating changes in humic production from the watershed of UKL, what is important are the changes in vegetative biomass per unit area over time and the associated changes in the decomposition products over time. In the following report on the development of our model to estimate loss of humics from the watershed of

UKL, we have made assumptions about wetlands lost and remaining that are only loosely related to the actual changes in wetlands that have occurred in the basin. But assumptions can be improved through assessments that address the need for this kind of information.

### **Estimating the Precolonial and Present Amount of Humic DOC (Humic Acid) Production in the UKL Basin**

Transformation in UKL basin wetlands, or outright loss of wetlands through diking and draining, has occurred for many thousands of acres from precolonial times to the present. The magnitude of actual loss of wetland, as it is currently located and delineated by wetland scientists, is unknown, except for wetlands that were once in the lake below the maximum pool elevation. The transformation of wetland has been widespread occurring over thousands of acres in each of the hydrologic units of the UKL basin. This transformation has occurred for example in the Wood River valley where the entire area of approximately 52,700 ac (Geiger *et al.* 2001) remains wetland but changed considerably from precolonial conditions by seasonal cattle grazing. Changes in the Klamath Marsh have been noted above. Before purchase of much of the Sycan Marsh by TNC, marsh hydrology had been altered and cattle were grazing the marsh, but it was still wetland though considerably changed.

Reducing the amount of humic material created by reducing wetland type and acreage is not the only potential impact from converting and changing natural wetlands to agricultural land uses. It is hypothesized that the humic material created in wetlands is advected to the lake through hydrologic and hydraulic processes. By creating berms that separate and redirect runoff, the advecting forces that once moved surface water and groundwater flows through natural wetlands into the lake have been eliminated. For example the berms that separate the Tulana Farms property, located between Agency Lake, Klamath Lake, and the Williamsom River, have redirected flow directly to UKL without contacting wetlands which historically existed at this location. A similar condition existed on the west side of Agency and Upper Klamath Lake where Seven Mile creek has been channelized mainlining flows from the Northwest portion of the Wood River directly into Agency Lake. Another example exists on the Wood River where the BLM's Wood River Ranch restored wetland does not connect with the Wood River overflows. It is important to understand the role these natural wetlands played hydrologically and hydraulically in advecting the humic waters into the lake margins and lake volume.

We make the assumption for our model that since the beginning of the 20<sup>th</sup> century, wetland transformation and loss of 180,000 acres of wetlands

significantly diminished hydrological, ecological, and microbial functions that produce humic acids. These humic acids contribute to the presences of "brown water" in the lake as noted by Native Americans, early settlers and scientists. This study developed a one-dimensional tanks-in-series model that was used to predict the annual production of humic acids (dissolved organic carbon) from these wetlands under historic and existing wetland coverage conditions above UKL.

The conceptual model for these estimates was based upon annual dry weight production of wetland vegetations of 15,000 kg/ha of which approximately 10% would become detrital litter available for decomposition. Several studies have shown the relationship between liter fall and humic acid production. For purposes of this study a unit production of humic acid of 0.41 mg/l/acre/yr (Schlesinger 1977) was used. The litter fall and subsequent wetting of the detrital material occurs in the late fall and winter when the temperatures are low ( $\pm 5^{\circ}\text{C}$  from freezing) to more or less. These lower temperatures during late fall and winter tend to maintain microbial decomposition at relatively low rate. As spring begins the water temperature warms up and active decomposition begins. Based upon work at Humboldt State University, it takes about 10-20 days at  $15^{\circ}\text{C}$  for humic material to develop in this decaying vegetation. At the initial stages (5-10 days) the decaying material has a cloudy turbid appearance. These conditions are attributed to active biological decomposition that then clears to a true straw to dark brown color. It is hypothesized that this humic acid production process, along with the flux of inflow from the wetlands and the delivery of humic waters from upstream wetlands, pulses a "dark water plume" into the upper reaches of Upper Klamath Lake and around the edges of the UK National Wildlife Refuge. We can only assume that this dark water plume was once very much darker.

A simple mass balance dilution model was developed to demonstrate the potential level of humic material in the lake under two sets of conditions. The first condition was prior to draining and berming wetlands in the Upper Klamath Lake basin and the second condition was after these land use practices were introduced. The model assumes an annual set of conditions in the lake with the humic load being contributed by the average annual flow to the lake with their representative wetland areas and contributions. A lake volume of 700,000 acre-ft. was used for the historic condition and a volume of 500,000 acre-ft. was used as the present volume. While this is not a time varying hydrological model it does give some idea of what the humic levels might have been in the lake. Since the model assumes completely mixed conditions in each volume of the lake (ten volumes assumed) this could be considered the most conservative condition.

This model and its results are developed to give some idea of the significant of the loss of humic material as it was produced in the wetland and advected into and through the lake. There are several referenced humic levels in the lake and the input flows which were used to more or less see if the model is producing order of magnitude results. Figure 40 shows that the estimated level of humic acids entering the lake during historic conditions was on the order 175 to 200 mg/l and 50 to 60 mg/l for the existing wetland coverage conditions. The value for mid lake conditions about half way along the axis of the lake was predicted to be about 10 mg/l similar to one set of reported data (Forbes *et al.* 1998). Comparisons with data from Forbes, *et al.*(1998) and Perdue *et al* (1981) show that the model is within an order of magnitude in producing humic levels in the lake and in incoming flows on the Williamson River. At best this simple completely mixed model understates the humic level due to the fact that dispersion is not considered. Much more work needs to be done to develop a conjunction watershed/lake simulation model that can be used to determine humic loads and humic concentrations in the lake.

Table 15. Humic material production for two sets of wetland coverage conditions, historic and existing.

<b>BEFORE</b>	<b>Original Size</b>	<b>Depth</b>	<b>Volume</b>	<b>Humic FACTOR</b>	<b>Humic material generated</b>
	<b>Acres</b>	<b>FT</b>	<b>Acre-ft</b>	<b>lb/acre/year</b>	<b>DOC(lb/year)</b>
<b>Around Upper Klamath Lake</b>	35000	3	105000	3687	129054333
<b>Sycan Marsh</b>	25000	2	50000	3687	92181666
<b>Klamath Marsh</b>	80000	3	240000	3687	294981333
<b>Wood River Watershed</b>	60000	2	120000	3687	221236000
<b>Pelican Bay</b>	2800	3	8400	3687	10324346
<b>Williamson Delta(mouth)</b>	12000	3	36000	3687	44247200
<b>Wocus and caledonia</b>	7000	3	21000	3687	25810866
<b>Asquaw Point</b>	300	4	1200	3687	1106180
<b>Shoalwater and Ball Bays</b>	2300	4	9200	3687	8480713
<b>Algoma marsh</b>	1000	3	3000	3687	3687266
<b>Hanks Marsh</b>	2000	5	10000	3687	7374533
<b>Sprague Valley Marshes</b>	7500	2	15000	3687	27652500
	234900				
<b>AFTER</b>				Summation=	709430106.7
	<b>Existing Size</b>	<b>Depth</b>	<b>Volume</b>	<b>Humic FACTOR</b>	Humic material generated
	<b>Acres</b>	<b>FT</b>	<b>Acre-ft</b>	<b>lb/acre/year</b>	DOC(lb/year)
<b>Around Upper Klamath Lake</b>	26000	3	78000	3687	95868933
<b>Sycan Marsh</b>	24000	2	48000	3687	88494400
<b>Klamath Marsh</b>	25000	3	75000	3687	92181666
<b>Wood River Watershed</b>	1500	2	3000	3687	5530900
<b>Pelican Bay</b>	500	3	1500	3687	1843633
<b>Williamson Delta(mouth)</b>	1160	3	3480	3687	4277229
<b>Wocus and caledonia</b>	0	3	0	3687	0
<b>Asquaw Point</b>	300	4	1200	3687	1106180
<b>Shoalwater and Ball Bays</b>	500	4	2000	3687	1843633.
<b>Algoma marsh</b>	0	3	0	3687	0
<b>Hanks Marsh</b>	1050	5	5250	3687	3871630
<b>Sprague Valley Marshes</b>	500	2	1000	3687	1843500
<b>SUM</b>	80510				
				Summation=	199149272.7
	<b>Difference in Humic material generate</b>				<b>510280834</b>

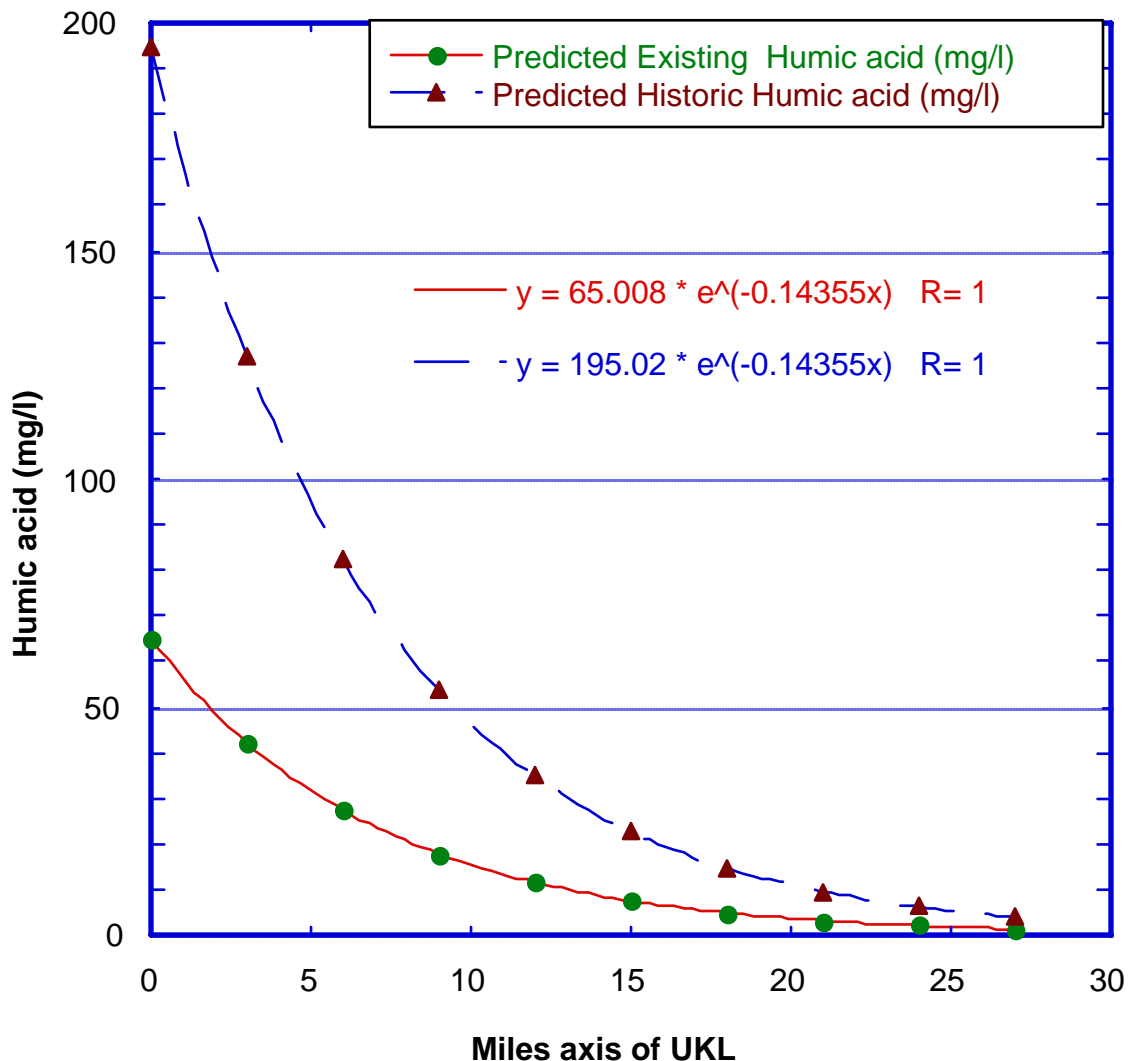


Figure 42. Simulation model demonstrating the extent of humic material through Upper Klamath Lake assuming average annual flow into the lake , a lake volume at 4141 ft elevation and historic and existing wetland coverage in the water shed.

Several factors need to be considered in continuing the development of a fate and transport model for humics in UKL and with supporting research activities to estimate the model parameters. The processes which produce the various types of humics in both in-lake and watershed wetlands need to be determined spatially and temporally. These “humic loading factors” needed to be localized for Klamath Basin conditions and either attenuated or accumulated ,as the case may be, as these wetland waters move into the lake system. The hydrodynamic factors which moves wetland waters into stream, rivers, and the lake

need to be understood to allow for characteristic hydrograph development to model the advective component of the model.

## **WETLAND-AFA RESEARCH PROGRAM ELEMENTS**

We conclude this section of our report with a list of wetland research program elements that are related to the growth of *Aphanizomenon flos-aquae* through our hypothesis that loss and transformation of wetlands in the UKL Basin have produced changes in the lake over the past century resulting in the dominance of AFA. These elements of a research program are preliminary, as is much of the work reported on in our report document. The list does reflect previous discussion in the report of past research and the direction suggested by our own research done in preparing this report.

### **Upper Basin Wetlands/ In-Lake Wetland Research**

- Estimate the precolonial coverage and function of wetlands in the UKL Basin with their associated hydroperiods and hydrological conditions (precipitation, evapotranspiration, infiltration, interception, etc..)

Obtain agency approvals for in-lake in-marsh wetland research (e.g. USFWS NWR unit and Section 7 agency consultation).

Determine the evapotranspiration from various wetland communities in the UKL (Sycan Marsh, Klamath Marsh, In-lake marsh communities, etc.)

Determine the water storage capacity for peat soils and sorbed detrital material in the precolonial and existing wetlands in the UKL basin

Estimate precolonial wetland coverage and associated hydroperiods and hydrological conditions for the Klamath Marsh, Sycan Marsh, and Sprague River flood plain wetlands .

Evaluate precolonial hydraulic settings of in-lake wetlands including their hydroperiods, seasonal outflow, and hydrologic contribution to UKL.

Estimate the detrital accumulation for wetland plant communities for precolonial conditions.

Estimate the loss of energy (head loss) for the wetland free water as the runoff and snowmelt water increases in the wetlands and/or when the lake elevation recedes.

For precolonial conditions determine the time of concentration for the free water and sorbed water in the historic wetland areas.

For precolonial conditions estimate the flow quantities and temporal variations for in-flows from rivers and springs into UKL

- Identify and quantify those water quality wetland functions that are hypothesized to contribute to phytoplankton populations in UKL.

For precolonial conditions determine the nutrient (nitrogen and phosphorus) cycling in aquatic macrophytes for in-lake and watershed wetland systems.

Couple nutrient cycling processes with fate and transport processes in watershed and in-lake wetlands.

For precolonial conditions estimate the nitrogen and phosphorus storage and release for watershed and in-lake wetlands. Specifically determine the mass reservoir for phosphorus and nitrogen during the growing season and the timing and amount of nutrient release upon decomposition of the aquatic macrophytes.

- For precolonial conditions determine the dissolved organic carbon (humic acid) contribution from the watershed and in-lake wetlands.

Develop a conjunctive watershed/lake two dimensional hydrodynamic-water quality model for Agency Lake and Upper Klamath Lake.

Develop a bathymetric survey of UKL for precolonial and existing conditions.

Determine the mixing effect of wind speed and direction on UKL .

Determine specifically the rate of decomposition of aquatic macrophytes and the microbial populations associated with this decomposition and chemical nature of the DOC by products.

Determine the unit area production of DOC (kg/ha/month) that can be used to estimated mass transfer of DOC contributions to UKL.

## **Wetland-Algae-Zooplankton Relationships**

- Determine the growth factors and autoecology of AFA

Base AFA humics physiological assays on uni-algal isolates to reduce the strain to strain variation and to provide a stable available assay organism

Conduct laboratory studies on the adaptation and cumulative effect of plant decomposition products on uni-algal AFA cells.

For AFA determine the laboratory physiological mechanisms for the limiting effects of low pH's (below 6.5), nitrogen forms (role and function of heterocysts under conditions of high nitrates, and selenium.

Utilize molecular genetic methods to determine if AFA is genetically homogeneous; separate strains into seasonally or otherwise specialized communities

Determine the parameters that describe non-photosynthetic quenching to samples exposed to ambient light.

Determine methods and equipment to better correlate with spectrofluorometric methods and results from other researchers

Analyze long-term algae-zooplankton data set developed by Klamath Tribes

Review and revise lake monitoring stations and include stations that will monitor quality of littoral areas that are likely to be heavily influenced by marshes with stations proportional to the area of the lake occupied by emergent-submergent wetlands.

Analyze results of the Klamath Tribes' and the BR's attempt to estimate biomass of *Aphanizomenon flos-aquae* by coordinated in-lake work and remote sensing.

Determine the algistatic property of DOC (humic acid) from decomposing native aquatic macrophytes and barley.

Determine the algistatic effect of commercial barley straw (DOC) on AFA populations in limnocorrals

Develop multiple models (hypotheses) for how humic inputs to the lake effect AFA; test the models against data collected from limnocorrals

Based on work this summer with PAM fluorometer, studies should be designed and performed exploring the use of lower humic contribution and lower light level (more realistic for conditions in the spring when humics move into the lake)

Utilize limno-corrals to study the effects of humics, temperature, light on the community structure in the lake specifically during the spring introduction of humics to the lake.

Characterize the seasonal differences in the sources of humics to the lake, inlake wetlands, barley field pump downs, grazing field pump downs, Williamsom River, and others.

- Given positive results from the work items above develop a strategy for the restoration of watershed and in-lake wetlands that optimizes the production, release, and lake distribution of DOC.

Make operational the hydrodynamic model/water quality model for use in prediction the distribution of DOC in the lake given the restoration of wetlands in the watershed and in the lake.

Develop and make operational a remote sensing procedure to determine the spatial and temporal distribution of DOC's in the lake

Develop and make operational a sampling and monitoring program to calibrate and validate the AFA community and DOC distribution model in the lake.

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