Three Special Studies on Nitrogen Offsets in semi-desert Lake Elsinore in 2006-08 as part of the nutrient TMDL for reclaimed water added to stabilize lake levels

For the Lake Elsinore and San Jacinto Watershed Authority (LESJWA)

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30 June 2009

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1.0 Summary

A primary management goal for Lake Elsinore, Riverside County, California is to stabilize the water level. Without additional water supplies semi-desert Lake Elsinore becomes much smaller and shallower and even dried up completely in the 1960s drought. Since 2004 nutrient-stripped reclaimed wastewater has been added to the lake each year by Elsinore Valley Municipal Water District (EVMWD). Although treated to remove most nutrients, some nitrogen and phosphorus compounds are still present and must be balanced by nutrient offsets to comply with the Total Maximum Daily Load (TMDL) developed for Lake Elsinore. In principle, the improvement in the beneficial uses of the higher lake levels could indirectly offset the nutrients added in the reclaimed water, but no approved mechanism has yet been established for this kind of trade-off. There are several accepted ways to make direct nutrient offsets that will satisfy the TMDL but partial lake aeration was chosen. In other lakes and reservoirs aeration-oxygenation removed or sequestered an average of 50% of P and N. Aeration in Lake Elsinore became operational in 2008. Laboratory experiments using Lake Elsinore water showed that aeration removed about 30% of the free soluble phosphate, mostly as a result of the formation of an insoluble precipitate. Less is known about the mechanism through which aeration reduces nitrogen in water. In particular, the specific amount or mechanism of N-reduction for Lake Elsinore is not known because demonstration of N-reduction in laboratory microcosms is technically difficult. Therefore the Regional Water Quality Control Board staff requested further studies to elucidate the possibly ways in which aeration could offset added N. The three N-offset studies for Lake Elsinore were: (i) a calculation of denitrification rates based on information available in the literature, (ii) a lake study of changes in the microbial benthic felt, and (iii) a preliminary study of N_2-fixation in Lake Elsinore.

Denitrification ($NO_3 \rightarrow N_2$; conversion of soluble nitrate in lake water to nitrogen gas that is vented to the atmosphere). The hypothesis for Lake Elsinore was that aeration will reduce internal loading of N from the sediments by increasing the rate of denitrification. Denitrification can only occur when several conditions are suitable [enough nitrate, suitable bacteria, areas or times of both high and low dissolved oxygen (< 0.5 mg/L), warm temperature (> 15-25^{o}C), a source of organic carbon energy (decaying algae,) a solid surface (sand grains, mud, algae), low rates of competing reactions, and low grazing on the bacteria that carry out denitrification]. Under pre-aeration conditions low concentrations of nitrate in Lake Elsinore limited denitrification to an estimated 0.35 tonnes/y (3.4 mg/m^2/d for only 15 days). Aeration was estimated to increase denitrification rates about 4.4 fold to 15 mg/m^2/d (effect of increasing nitrate level) and for a longer period (120 days) to give a net increase of 11.6 tonnes or about 33 fold increase over pre-aeration conditions. *If the estimates made in this report are correct, denitrification of 11.6 tonnes due to aeration more than balances the input of N from EVMWD's reclaimed water permit goal of 7.4 tonnes (as a 5-year running average). However, it was not possible to achieve this goal in 2008 where TN addition was 19 tonnes. Thus, in 2008 at least, the estimated denitrification offset only 61% of added TN in makeup water. The TMDL goal for the N in the current reclaimed water additions therefore was not fully offset by denitrification alone.*

Benthic felt (the microbial film that forms on the sediments in deeper water). The hypothesis was that increased oxygen in deep water will make the growth of microbial felt more efficient and that the resulting greater microbial biomass will lock up and offset ammonia-N released from the sediments. A fourfold increase in benthic felt coverage from 25% of the area of sediment to 100% was found when comparing zones close to the aeration pipes with those

distant from the aeration pipes in 2008. This observation was supported by the finding of a similar fourfold increase in ATP (adenosine triphosphate, an indicator of living biomass) in the sediments where bottom water DO was moderate (~ 2.5 mg/L; ATP 196,000 Relative Light Units (RLU)) when compared with samples collected when the bottom water showed low DO (~ 0.4 mg/L; ATP 49,000 RLU). Microscopic examination of the benthic felt showed it to consist of a distinct layer about 1 mm thick with two parts; a thin whitish surface layer of the large gliding filamentous bacterium *Beggiatoa* underlain by a thicker secondary brownish layer of amorphous material. A preliminary estimate of the mat area under moderate DO conditions (2-3 mg/L) was 1,000 acres (29% of the lake bed). In addition, *Beggiatoa* is capable of denitrification, further reducing nitrogen loads. *Based on the average N-content of living matter, the preliminary conclusion was that the increased Beggiatoa benthic felt grown under aerated conditions provided an N-offset of 19.3 US tons or almost the amount of 19 tonnes of TN from EVMWD's reclaimed water in 2008.*

N_2-fixation (the process where atmospheric N_2-gas is fixed into blue-green algal biomass). The hypothesis was that aeration could change the lake conditions so that N is added to the lake via N_2-fixation. Any increase in N_2-fixation would work against the other two N-offsets so is not desirable. *Preliminary results showed that organisms capable of fixing N_2 (heterocyte-bearing blue-green algae) were present but uncommon (~ 5% of filament numbers) compared with the dominant blue-green algae.* Further work is needed to collect more representative samples of algae.

Provisional conclusion. Past experience has shown better lake water quality occurs at higher water levels. The recent additions of reclaimed water have raised the level of Lake Elsinore a few feet higher than it would be in the current drought conditions. The first year of lake aeration in 2008 improved the dissolved oxygen in much of the bottom water and was estimated to have increased both denitrification and the growth of benthic microbial felt. Aeration did not promote high rates of N_2-fixation. The net result of the aeration was estimated to offset approximately four times the annual average goal of N to be added in the reclaimed makeup water but offset only 1.5 times as much TN as was actually added in 2008. Thus an overall net offset of TN was achieved. Nonetheless, the offsets would allow only a limited amount of additional water in future, probably less than would be ideal to achieve the long-term management goal of year-round stable water levels. However, improvement in TN removal methodology at the wastewater treatment plant or elsewhere and possibly lessons learnt from the operation of the aeration system in the lake during 2008 may allow more TN to be offset in the future. In particular, the current aeration system does not elevate DO in the bottom waters to anywhere near theoretical saturation. In some other lakes and reservoirs it has taken a few years before the bottom sediment oxygen demand has been fully saturated by artificial aeration and this may be the case in Lake Elsinore. Modification of operation as experience is gained over the first few year and future upgrading of the aeration system is also possible if needed.

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3.0 INTRODUCTION

3.1 THE NEED FOR ADDITIONAL WATER IN LAKE ELSINORE

Water flow into Lake Elsinore comes only as occasional pulses after heavy rains and there is no year-round inflow as in most temperate lakes. For thousands of years, the original lake of almost 7,000 acres presumably dried out completely or became very shallow during dry spells. The lake is located in a semi-desert region with an average rainfall on the lake of only 12 inches (30 cm). For comparison the average rainfall in the US is 37 inches (93 cm), in San Francisco 25 inches (63 cm), and Las Vegas 4.5 inches (11 cm). Evaporation from Lake Elsinore is 15,000 acre-feet/y (af/y) or about 4.5 feet of lake level each year. Since the average water depth is only 25 feet the lake soon becomes much shallower and smaller following several drier years. In the last 75 years the lake has received an inflow of 15,000 af/y or greater only 20% of the time (15 out of 75 years). The lake was often only half full and dried out in the 1960's. The ratio of lake surface area (3,400 acres when full) to drainage basin area (~ half a million acres) is ~ 1:147 – much larger than for many lakes where 1:10 to 1:40 is more common. Even though the lake often is often only half-full, flooding around the lake occurs easily in occasional wet years such as 1980.

Unfortunately, these frequent flood-dry conditions with large exposed mud flats in dry periods and floods in wet ones are not compatible with human recreation, lakeside homes, or aesthetic appearances. To improve conditions for people, a levee was constructed across the middle of the lake in the mid 1990s. The levee cut off the southern portion which now remains dry and halved evaporation losses to the current rate of 15,000 af/y. The northern half (now 3,400 acres) became artificially deeper for longer periods and some beneficial uses were enhanced. To prevent flooding with the loss of half of the original lake storage, a new overflow structure was constructed. The modifications may have assisted in preventing the lake from totally drying out, but it still becomes very shallow during droughts. Also, the lake still shows very large water level fluctuations, for example, a maximum depth of 12 feet in fall 2004 rose to 35 feet by spring 2005. Unfortunately, the weather since 2005 has been dry and the lake has received only limited inputs from direct precipitation, some pumping from island wells, and from the San Joaquin River below Canyon Reservoir Dam. As a result, the water level in Lake Elsinore has again fallen drastically and had a maximum depth of about 26 feet in 2008. In addition, water quality is often lower than desirable for recreation and fish kills have occurred from time to time. The lake appears on the 303(d) list for nutrients, organic enrichment/low dissolved oxygen, sedimentation and unknown toxicity.

Increases in water in Lake Elsinore bring an obvious benefit, as can be seen from the dramatic improvement in water quality following the 2004-05 wet winter storms. For example chlorophyll *a* in surface water reached 300 ug/L in dry years such as 1993 and 2003 but reached only about 100 ug/L following the wet winter of 2005-06. These improvements came despite the fact that the storm water increased the nutrient loads to the lake washed in from the large drainage basin. Higher water level itself is apparently more important to reduce beneficial use impairment than a simple reduction in nutrient loading.

For Lake Elsinore, the only non-storm water source available in sufficient and increasing quantities in the future is reclaimed wastewater from local and more distant sewage treatment plants. As the population of the Lake Elsinore area increases, this valuable resource will become increasingly available. Like Lake Elsinore's stormwater, reclaimed wastewater also contains nutrients. The N and P in reclaimed wastewater added to the lake have been substantially decreased but cannot economically be further reduced at present. However, as the beneficial effect of the 2004-05 storm inflows suggests further reduction in N and P loading may not be essential. Permitted concentrations under the current TMDL are 1 mg/L TN and 0.5 mg/L TP.

The apparently simple management of a lake to produce a stable water level brings many benefits, including easier launching of boats and less exposed muddy shoreline. In addition, a truly stable water level induces "biomanipulation," the only low-cost, long-term solution to remove beneficial use impairments. The results of successful biomanipulation in Europe and the U.S. have been clearer water, lower nuisance algae, and satisfaction for lake users. It is not clear if Lake Elsinore can be made more suitable for human use by biomanipulation but the potential benefits are large enough for the method to be proposed at this time.

Biomanipulation is a method of modifying the lake ecosystem by encouraging natural organisms that improve water quality. For biomanipulation, the exact depth of the water is less important than the stability of the water level. Under ideal conditions, an annual variation of only a foot in water level would be ideal. Since Lake Elsinore naturally drops 4.5 feet each summer due to evaporation and may rise 16 feet in a wet winter, lake stability will not be easy to accomplish. However, for now, some moderation of the large shoreline water level changes will be good.

Biomanipulation requires the presence of submerged aquatic plants which are not now able to grow in Lake Elsinore. These plants require a fairly stable water level because if the water level drops they dry out and die. Biomanipulation has three components:

- <u>A zooplankton refuge</u>. Submerged plants provide a refuge from fish predation for small animal zooplankton like *Daphnia* that filter the lake water and eat the algae that cause so many recreational and water quality problems. In Lake Elsinore, Professor Anderson and his students have shown that *Daphnia* requires the kind of lower TDS water found in storm runoff or reclaimed water, and dies in the high TDS water that occurs when the lake evaporates and salt concentrates, as in the early 2000s.
- <u>Stable sediments</u>. Submerged and emergent plants form dense root mats that stabilize shallow water sediments. At present the sediments in the one third of the lake area around the edges are regularly wind-mixed. The root mat has the effect of reducing wind mixing and nutrient recycling from the mud in summer. In addition, the plant roots prevent the wind from mixing oxygen to the surface mud which has the indirect effect of encouraging denitrification and reducing nitrate. In turn lower nitrate may reduce algae if other sources of nitrate are small.
- <u>Inter-algal competition.</u> Submerged plants become partially covered with diatoms that compete successfully with floating algae for nutrients, again decreasing the algae in the lake water.

3.2 Method of water additions

There are numerous ways in which water can be added to lakes but few are available for Lake Elsinore. These are:

- Restoring historical inflows. At present Canyon Lake Reservoir on the San Jacinto River, which is the main inflow, intercepts smaller flows leaving only the larger storms to feed Lake Elsinore. It is unlikely that this situation will change in the near future.
- Adding well water. Addition of potable water is of questionable legality since Lake Elsinore is not a drinking water source. Some well water is high in nitrates which stimulate eutrophication in the lake. Also there is only a limited supply and depleting the groundwater is not a good idea since subsidence and other problems such as increased salinity in other wells may occur.
- Adding Metropolitan Water District Water. This supply is potable and cannot legally be used in drought conditions in a lake that is not a drinking water supply. It is also expensive and likely to become more limited and costly in future as upstream users of the Colorado River demand their historical allocations.
- Adding recycled (reclaimed) water. This renewable supply comes from local wastewater treatment plants and can be further treated to remove most nutrients as needed. It is less costly and more sustainable than the other sources. Reclaimed water is likely to become available in larger amounts in the future as the local population expands. It is thus the best available choice at this time and probably for the foreseeable future.

3.3. Concerns with the use of reclaimed water to stabilize the level of Lake Elsinore: nutrient additions

It is inevitable that even with advanced nutrient-stripping treatment there will be some nutrients still present in reclaimed water added to Lake Elsinore. When reclaimed water is to be added to maintain the desirable more stable lake level, the TMDL process requires N and P offsets if the waste loads allocated to the Regional Plant discharges are exceeded. Few other lakes in the U.S. have a substantial need for additional water although some lakes in the West such as Lake Tahoe would benefit from additional supplies. Thus most of the lake cleanup work in the US has been to remove wastewater discharges from lakes. For Lake Elsinore, the Santa Ana Regional Water Quality Control Board has had to be innovative while still conforming to the TMDL rules.

In many areas of the U.S. and Europe, offsets are made via reductions in watershed input (external loading) but it is not clear how to do this in Lake Elsinore's very large drainage basin where floods may move most inputs to the lake in a few hours. For example, there are cattle and dairies in the drainage basin but holding and treating the occasional very large volumes of storm water is logistically challenging. The Regional Board has therefore allowed as an offset the reduction of release of nutrient from sediments (summer internal loading). Internal loading supplies N and P to the nuisance algae in summer.

The nutrients added via reclaimed water can be offset in various ways:

- Additional treatment of inflow to Lake Elsinore. Natural water supplies could have nutrients reduced by various BMPs in the watershed. These could include nutrient reduction retention basins &/or wetlands for storm &/or summer nuisance flows, reduced lawn and agricultural fertilizer applications, subsidy of "rain gardens" and other actions such as changes in agricultural practices.

- In-Lake treatment. Alternatively, in-lake techniques can be used to reduce internal loading or recycling of nutrients from the sediments in summer. In Lake Elsinore, internal loading of N and P occurs each summer when the sediments run out of oxygen and become anoxic. Since anoxia causes the internal loading, the addition of oxygen (directly or via aeration-mixing) can reduce internal loading. Alum, calcium and iron additions could remove some soluble phosphate as a precipitate. Biomanipulation could retain N & P in the sediments or in plant rhizospheres. Finally, surface sediment dredging could remove accumulated nutrients in the sediments.

3.4 Expected benefits of aeration and Possible TMDL offsets

For Lake Elsinore, the decision was made to remove as much N and P as economically possible in the reclaimed water to be added to the lake and then to use in-lake aeration to reduce N and P internal cycling and offset any remaining added nutrients. Aeration will bring many benefits, primarily the reduction or prevention of fish kills caused by low oxygen and suppression of internal loading of the nutrients N and P.

The decrease in internal loading is anticipated to give some in-lake nitrogen and phosphorus offsets as required by the Total Maximum Daily Load (TMDL) for nutrients present in reclaimed water added to Lake Elsinore. The question is how much offset will occur following successful aeration? Water added by Elsinore Valley Municipal Water District (EVMWD) is currently permitted to contain 7.4 tonnes per year of Total Nitrogen (TN) and 3.7 tonnes of total phosphorus (TP). The amounts are calculated on a 5-year running average. Unlike the inflow from stormwater but like the inflow from internal loading from the sediments most of the EVMWD nitrogen and phosphorus is mostly in the bioavailable form (mostly as nitrate, ammonia and phosphate).

The Regional Board has not specified how nutrient offsets should be made. However, over the years of initiation, design and installation of the aeration device there was regular interaction of Board staff as the offset methods were proposed.

4.0 MEASUREMENT OF THE OFFSET EFFECT OF AERATION

4.1 BACKGROUND

It is proposed to accomplish the N and P offsets by adding oxygen to the lake bed by aeration of the water. Oxygenation or aeration of sediments have been shown in worldwide studies to approximately halve N and P in the water. The mechanisms of reduction of phosphorus by aeration-oxygenation are well known and have been measured in laboratory studies for Lake Elsinore (Anderson, 2001, 2003). The research by Professor Anderson and his students showed that at least 30% of the phosphate in the water column of Lake Elsinore would be precipitated by aeration. However, the mechanisms for reduction of nitrogen by aeration are less well known and harder to estimate in the laboratory. The California Regional Water Quality Control Board, Santa Ana Region staff requested additional information to assure them that aeration/oxygenation would reduce N loads to offset the added N.

It was determined that the main demonstration of nutrient offsets due to aeration would be an N and P mass balance. The regular compliance monitoring program is carried out by California State University San Bernardino and measures total-N and total-P as well as dissolved oxygen, nutrients, and chlorophyll. From this monitoring a mass balance of N and P will be made and the years before and after aeration compared. However, it is expected that measuring a statistically significant change in the mass balance of N and P will be difficult, especially since the makeup water has been added in a period of two multi-year droughts and one very wet year (2004-05). For the first quinquennium (five year period), the change imposed by aeration will almost certainly be dwarfed by the large natural variations over the last few years. Thus, statistically significant reductions over time calculated by mass balance will be harder to distinguish than in other kinds of lakes where the climate is more uniform. The Regional Board staff may be more likely to accept any declines in the mass balance of N and P as real changes if it they are supported by knowledge of how N-declines following aeration could occur in Lake Elsinore. Three additional TMDL N-offset special studies were therefore proposed. They were:

- (i) Theoretical estimation of sediment microbial denitrification as an atmospheric sink for N
- (ii) In-lake measurements of the microbial benthic felt as a living sink for N
- (iii) In-lake estimates of the nitrogen-fixing potential of the lake as a source for N that would negate the anticipated declines.

4.1.1 Special Study 1. Evaluation of the hypothesis that denitrification will increase under aerated conditions offsetting N-inflows

After half a century of debate, some limnologists have concluded that a management plan that removes both N & P is perhaps the safest solution to reduce eutrophication (Conley et al., 2009). A decrease in N due to an increase in denitrification is thus a desirable result when lakes are oxygenated or aerated. A literature survey of aeration-oxygenation-mixing projects world-wide shows an average of 50% reduction in N and P but with a wide variation between individual lakes or reservoirs (Horne & Commins, 1997; Beutel & Horne, 1999). Addition of pure oxygen in the large and thermally stratified Camanche Reservoir on the Feather River, California has been shown to decrease internal loading of both N and P (Horne, 1995 & in prep.). In particular, the availability of soluble iron is a key variable for reductions in P. The mechanisms whereby oxygenated water causes decreases in P are well understood and primarily depend on the precipitation of ferric phosphate. However, the mechanisms for N reduction are less well understood. One possible mechanism is denitrification. As its name suggests, denitrification is the process whereby nitrate (but not ammonia or organic-N) is converted to nitrogen gas (N_2). Recently it has been found that ammonia can be converted directly to nitrogen gas in the anammox reaction but only in the presence of nitrite as the primary electron receptor. Although possibly important in the very N-depleted ocean environment (Dalsgaard et al., 2003), the anammox reaction in more N-rich lakes is not known and may make only a small contribution to the N-budget in Lake Elsinore. In addition, since nitrite is a reduced form of nitrate and is an unstable ion, nitrate is still required in relatively large amounts in the anammox reaction. Thus by any process, nitrate or nitrate is needed for the release of N_2-gas.

The nitrogen gas produced by denitrification dissolves in the water at the sediment-water interface or, when denitrification rates are high, is released as bubbles – often accompanied by methane gas. Either way the N_2-gas is eventually vented to the atmosphere at the lake surface. In this way N is removed from the lake and cannot directly cycle back in again. Since the atmosphere is 81% nitrogen gas and the amounts added by the denitrification that might occur in Lake Elsinore are minute, no effects akin to global warming can occur. In fact, the world atmosphere is currently in a slight deficit of N_2 gas due to the increase in N_2-fixation (the reverse of denitrification) attributable to human activities (soybean and similar plant cultivation and the industrial Harbor-Bosh process that makes nitrogen fertilizer). At present human-caused N_2-fixation is two or three times the natural level (Thomas & Weller. 1996; Vitousek *et al.*, 1997a-b, Townsend, 2004). Thus denitrification is probably beneficial for the global N-cycle since there are few offsetting human-amplified denitrification sources. These include an increasing number of sewage treatment plants using nitrification-denitrification and the also increasing numbers of treatment wetlands specifically designed to remove nitrate from rivers, storms water and agricultural runoff (Horne, 2000; Kadlec & Wallace, 2009).

Prior to oxygenation, ammonia was the dominant soluble inorganic N-compound rather than nitrate (compare Figs.1 & 2). Under oxygenated conditions ammonia in the sediments is soon converted to nitrate because bacteria can gain energy from nitrification (oxidation of ammonia to nitrate). Despite the general presence of oxygen in the water, there will be times during the night, just under the sediment surface, and in less well-mixed bottom water pockets when oxygen will be low enough that denitrification is favored. A spatially and temporarily variable mosaic of oxygenated areas producing nitrate and low oxygen areas denitrifying it is needed for high rates of denitrification. At least in 2008 the partial lake aeration system installed in Lake Elsinore provided this mosaic.

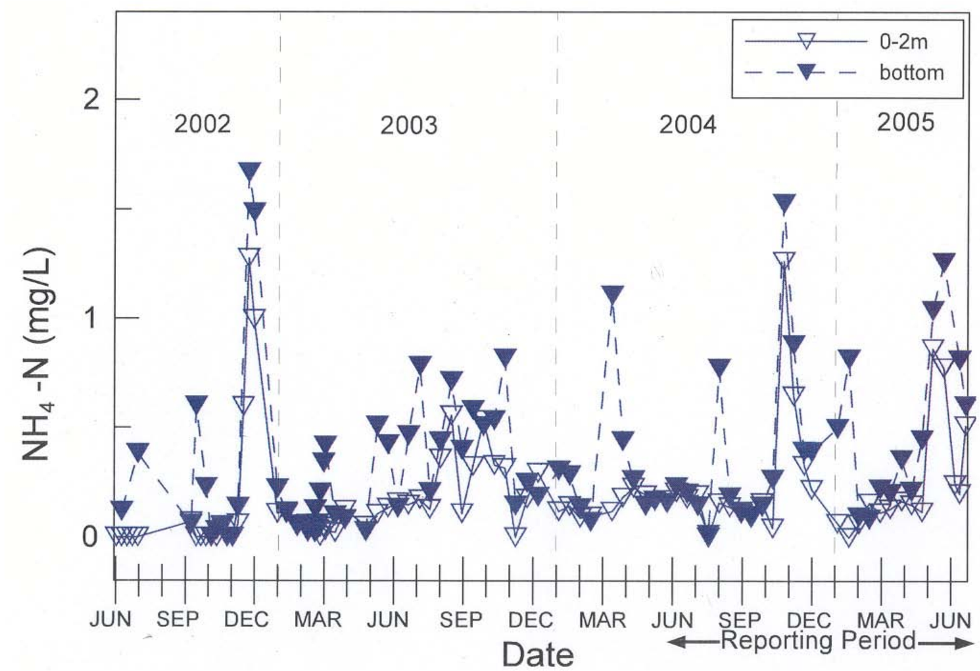

Figure 1. Ammonia in eutrophic Lake Elsinore 2002-05 prior to aeration. Ammonia is the dominant bioavailable N-source for algae. From Anderson & Lawson, 2005.

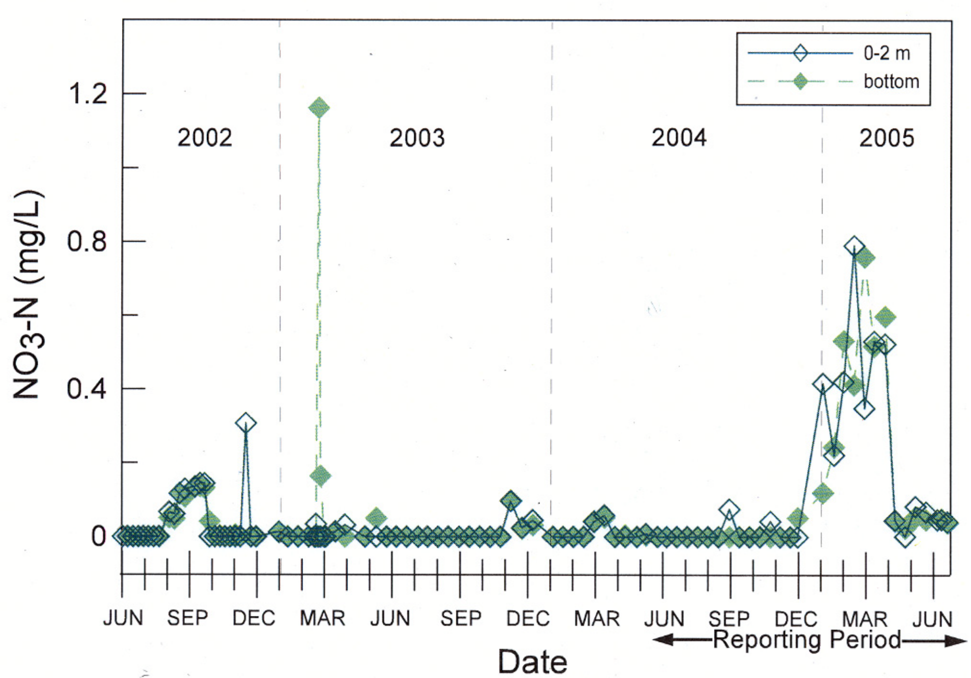

Figure 2. Nitrate in the top and bottom water of Lake Elsinore 2002-05. High winter-spring 2005 values are due to nitrate carried in from the watershed following unusual storms. Many eutrophic lakes would show nitrate values > 500 ug/L, for example even oligotrophic Lake Superior NO_3-N is ~ 300 ug/L so Lake Elsinore is generally lower in nitrate than most other waters. From Anderson & Lawson, 2005.

Although it seems as if denitrification would be an important way in which nitrate in lakes would move to the atmosphere as N_2-gas, the rate of the reaction is low in most eutrophic lakes. The specific conditions required for denitrification do occur in some wetlands and estuaries but they are unlikely to be widespread in lakes. In particular, the level of redox (**red**uction-**ox**ygenation) needed by denitrifying bacteria is quite specific (+ 100-200 mV, Table 1). Correct redox is needed to provide the thermodynamic gradient to favor the reaction series $NO_3 \rightarrow NO_2 \rightarrow N_2$ and is equivalent to about 0 to 0.5 mg/L of dissolved oxygen. In Lake Elsinore, a constant level of this concentration of oxygen above the sediments was present prior to aeration but, as will be described later, was not accompanied by very much nitrate. If the lake is stirred artificially by aeration there is then a balance between more available nitrate formed under oxygenated conditions and some low oxygen in the sediment-water microzone. If so the balance could favor increased rates of denitrification.

The Redox table

Redox Potential Eh (mV)	**Chemical state or change**	**Names**
+500 to +300	Fe^{3+}, NO3', SO4'' ,Mn^{4+}	Ferric, nitrate, sulfate, manganous
Denitrification		
~ +200	NO3' to NO2 to N2 gas or NH4	Nitrate to nitrite to nitrogen gas or ammonia
~ 0 to +100	Fe^{+++} (precipitate to Fe^{++} (soluble)	Ferric to ferrous
Sulfate reduction & hydrogen sulfide gas production		
-100 to -200	$SO^{4''}$ to S to H2S (gas)	Sulfate to sulfur (solid) to hydrogen sulfide (gas)
Methanogenesis		
-300 to -400	CO_2 to CH_4 gas	Carbon dioxide to methane

Table 1. Redox table for common terminal electron acceptors in water showing the relatively high Redox that allows denitrification. In lakes oxygen, nitrate, sulfate and carbon dioxide are the common species that are reduced under anoxic conditions.

4.1.2 Special Study 2. An evaluation of the hypothesis that a thicker benthic felt will be present and sequester more N when there is more oxygen present

In lakes and reservoirs, the most active part of the sediments is the uppermost few millimeters called the sediment water microzone. The zone is populated by many bacteria but bacterial growth is only about one-third as efficient when growing under anoxic or low oxygen conditions compared to growth where oxygen is present. Thus anoxic benthic organisms make less biomass than similar organisms growing with oxygen present. The hypothesis is thus that a thicker mat of benthic organisms will grow following aeration in Lake Elsinore than in past times. In turn the greater mass of microbes will sequester and hold ammonia or nitrate that would be released to the lake if aeration was not provided.

Following addition of pure oxygen, increases in the amount of benthic microbes have been demonstrated in Upper San Leandro Reservoir (East Bay MUD, Oakland; Horne et al., 2003). Studies made before oxygenation used remotely guided video cameras equipped with magnifying lens, SCUBA divers and remote sample collections. The videos showed a carpet-like growth covered most of the deep water. The direct collections found that a thin film of sulfur bacteria and blue-green algae (cyanobacteria) covered the lake bed. Following oxygenation, the composition of the lake bed film changed with more microbial filaments than in the previous years of a fully anoxic hypolimnion (Horne et al., 2003a). Some benthic mats are common in eutrophic lakes (Jones & Jones, 1985).

However, less is known about the effects of aeration (rather than oxygenation) on benthic mats and the how it would affect a shallow lake such as Lake Elsinore (rather than deeper reservoirs). The most important difference is that in shallower waters, mixing by wind waves becomes more important. In very shallow water (0-2 m) it is unlikely that a stable mat could form and remain following windy days. However, the mixing effect of waves drops almost exponentially with depth and wavelength so that in waters > 2 m the water movement should be sufficiently gentle to allow stable mat formation. The maximum depth in Lake Elsinore has recently ranged from 5 m (2002-5) to 11 m (2005-7) so there is a large amount of sediment surface below 2 m where mats could be potentially stable in windy conditions.

4.1.3 Special Study 3. An evaluation of the hypothesis that aeration will promote nitrogen fixation (N_2-fixation)

Nitrogen fixation is the opposite of denitrification and also requires anoxic conditions. It is the process whereby atmospheric N_2-gas is fixed into ammonia and amino-compounds inside tiny cells of blue-green algae or other bacteria. Most of the blue-green algae that fix N_2 in lakes occur as long chains of individual vegetative cells. Under normal lake conditions N_2-fixation can only occur inside special cells called heterocytes (formerly heterocysts). Heterocytes are formed from ordinary vegetative cells when N is in short supply. Heterocytes can easily be identified under the microscope. In lakes, heterocytes have been shown to be well correlated with the amount of N_2-fixed measured with the ^{15}N isotope or the acetylene reduction process (Horne & Fogg, 1970; Horne et al., 1972; Horne & Galat, 1985). The heterocyte provides the anoxic site that allows the nitrogenase enzyme to fix N_2. In contrast, the rest of the vegetative cells in the algal filament carry out photosynthesis and their cells are rich in oxygen. Oxygen irreversibly denatures the nitrogenase enzyme (Fogg et al., 1973; Murray et al., 1984). Ammonia also suppresses N_2-fixation and acts on the cells that lie adjacent to the heterocyte. When supplied with this alternative N-source, the adjacent cells no long need the N fixed by the heterocytes. They cease supplying the heterocyte with sugar energy so respiration inside the heterocyte stops and oxygen invades the cell and denatures the nitrogenase enzyme. The heterocyte then stops fixing N_2.

Even small quantities of ammonia irreversibly suppress N_2-fixation so it was probably quantitatively insignificant in Lake Elsinore prior to aeration. Large fluxes of ammonia reach surface water from the anoxic sediments (Fig. 1). Evidence for the lack of N_2-fixation in Lake Elsinore would be that few blue-green algae present possess heterocytes. Little systematic work has been done on the algal species in Lake Elsinore let alone the presence of heterocytes.

Aeration may reduce biologically available-N (nitrate + ammonia) to low levels and stimulate N_2-fixation. New blue-green algae with heterocytes could then invade Lake Elsinore and the N-fixed would act as a counter offset in the TMDL process. Thus the third N-offset special study was to examine the kinds of blue-green algae in Lake Elsinore and look for heterocytes.

4.2 EXPERIMENTAL METHODS & DESIGN OF THE THREE PROPOSED N-OFFSET METHODS

4.2.1 Denitrification

Denitrification can be measured directly in lakes but the several measurement techniques used tend to give different answers since they manipulate the sediments or water in different ways (Steingruber et al., 2001). Fortunately, there is considerable recent literature on the topic so as a first step calculations can be made with some degree of confidence. The method employed was to examine the denitrification literature for lakes and some wetlands to determine if the rates of denitrification measured elsewhere could account for the projected declines in N in the lake following the installation of aeration.

4.2.2 N in benthic microbial felts

The amount of nitrogen stored in the benthic felt from various parts of the lake with different benthic oxygen levels was measured in two ways. The percentage cover of the felt was estimated on the lake from examination of the mud sample immediately after collection. Modern sediment samplers can be modified to allow easy visual access to the sample within seconds of collection from the bottom. The thickness of the benthic mat was measured later using live samples examined under the dissecting microscope. Making assumptions of bacterial cell density and N-content, the amount of N-stored in the mats can be estimated.

The amount of living matter in the benthic mats was also measured using Adenosine Tri-Phosphate (ATP). The ATP method is a common method to estimate living biomass since ATP is the normal way energy is transmitted in cells (ATP→ ADP + energy). The method combines luciferin and the enzyme luciferase. When ATP is added to a fresh mixture of these substances, they emit light. Most people are used to this reaction as the glow of a firefly or glow worm.

The reaction catalyzed by firefly luciferase takes place in two steps:

$$\text{luciferin} + \text{ATP} \xrightarrow{\text{Luciferase enzyme}} \text{luciferyl adenylate} + \text{PP}_i$$

$$\text{luciferyl adenylate} + O_2 \rightarrow \text{oxyluciferin} + \text{AMP} + \text{light}$$

(PPi = inorganic phosphate; AMP = adenosine mono-phosphate)

Under controlled conditions, the amount of ATP is proportional to the light emitted and thus ATP can be quantified. Although useful in the laboratory, the method is not easy to use directly in the field and thus has not been widely used in lakes or oceans. In most occasions, extraction of ATP from cells requires considerable analytical effort and use of organic solvents. However, an ingenious method of ATP assay that does not require extraction and is a direct measure was invented by Dr. Charm (Charm Sciences Inc., Lawrence, MA; www/charm.com. 978-687-9200). The method was designed to test if carpets were clean of waste or water coolers free of bacteria. The trick to the method is that is measures free ATP which is always present from broken or damaged cells. For example, clean tap water has an ATP value in the Charm device of < 5 Relative Light Units (RLU). Swipes from a clean hand give values of 80,000 to 100,000 RLU, a hand freshly washed with soap and water gives about a quarter of these values. Undiluted surface water from Lake Elsinore in summer has an ATP value of about 400,000 RLU. I adapted the method for Lake Elsinore to provide a relative measure of the living biomass in the benthic felt. Initial tests showed the method to be very sensitive.

Since ATP is not extracted, the ATP associated with the benthic felts (if present) was measured directly. In these experiments is not the absolute amount of ATP but the gradient that can be assessed. For example a gradient from low to high dissolved oxygen should show a difference in free ATP. The other measurements of bacterial cells and felt thickness were used for the absolute N-offsets.

5.0 RESULTS SPECIAL STUDY 1. DENITRIFICATION

5.1 Denitrification: Calculation based on literature data

5.1.1 Requirements for denitrification

Denitrification is a two-step bacterial process that can only occur in the almost total absence of oxygen. Normal lake denitrification occurs as follows:

$\mathbf{NO_3}$ (nítrate) → $\mathbf{NO_2}$ (nitrite) → $\mathbf{N_2}$ (nitrogen gas)

Each stage must be thermodynamically favored by environmental conditions such as redox, oxygen, and temperature but the rate of reaction is primarily controlled in lakes by bacteria. Technically these microbes use the nitrate or nitrite as a terminal electron acceptor. More colloquially bacteria respire or breathe using the oxygen in nitrate (NO_3) as a terminal electron source just as animals breathe using oxygen (O_2).

Eight conditions are needed for substantial rates of bacterial denitrification as follows:

1. Presence of denitrifying bacteria
2. Low or no oxygen also shown as suitable redox potential (not necessarily for low oxygen to be continually present, see nitrate)
3. Warm temperature
4. High nitrate levels (these are normally supplied intermittently since continual high nitrate levels would inhibit denitrification)
5. Availability of labile organic carbon energy source
6. Suitable solid surfaces such as sand grains
7. Low rates of competing reactions
8. Low predation on the bacteria that carry out denitrification

All of these 8 conditions are rarely present in terrestrial soils, groundwater, lakes, rivers or oceans but are found in some wetlands and shallow coastal oceans (Sietzinger et al., 2006). This is understandable, because if high denitrification rates were common there would be no ocean "dead zones" caused by nitrate pollution in the rivers flowing into the coastal zones of the world, no groundwater problems for drinking water and none of the almost ubiquitous nitrate pollution of freshwater lakes. For example, the nitrate-N in the River Thames in England more than doubled from about 3 mg/L in the 1930s to 8 mg/L in the 1980s. The much larger Rhine River also rose from ~ 3 to 4 mg/L over a similar period. Nitrate in Lake Superior has risen to over 0.3 mg/L from original values that were probably < 0.1 mg/L or even < 0.01 mg/L. In more oligotrophic lakes the rise was as dramatic but from very low levels. Nitrite-N in Lake Tahoe doubled from about 0.005 to 0.010 mg/L between 1960 and 2000 and changed the growth-limiting nutrient for algae from nitrogen to phosphorus. Lake Superior probably had summer nitrate concentrations of 10-30 ug/L prior to western farming techniques but now has concentrations of over 300 ug/L (Horne & Goldman, 1994).

Where the process has been quantified, denitrification rates in lakes are relatively low (Table 2). Nonetheless, since lake sediments can provide the rare conditions where denitrification is possible, large lakes have recently been estimated to contribute 20% of the world's denitrification (Seitzinger et al. 2006). Unfortunately, small lakes such as Lake Elsinore could not be included in Seitzinger's estimations because the hydraulic residence times she needed to make estimates were not fully available. Even in nitrate polluted estuaries such as the Tejo in Portugal (Table 2), denitrification rates were not very high relative to those than can be produced in sewage plants or treatment wetlands such as OCWD's Prado wetland or the IRWD's San Joaquin Marsh (Table 3).

Table 2. Rates of denitrification in natural and polluted open water systems in the world.

Lake, estuary or wetland	**Rate of denitrification** mg NO_3-N/m^2/d	**Comment & source**
Natural lakes: averages		
Low nitrate	**4.3**	Mean from Appendix Table A1
Moderate nitrate	**15**	Mean from Appendix Table A1
High nitrate	**130**	Mean from Appendix Table A1
Lowest	3.9	Setzinger et al., 2006[a]
Middle	19	Setzinger et al., 2006[a]
Highest	62	Setzinger et al., 2006[a]
Individual Streams		
Swift Brook	103	See Horne, 1995
Drainage ditch, Netherlands	160	See Horne, 1995
Agricultural streams	100-1,000 (500)	See Horne, 1995
Mean for Streams	**254**	
Individual Estuaries		
Potomac, US	79	Measurement made on sediments
Tejo, Portugal	170-359 (265)	Average of measurement made on sediments
MERL mesocosms	203-301 (252)	Large containers simulating estuarine conditions
Means for Estuaries	**199**	

[a] Calculated from original values in mmoles/m^2/y assuming a denitrification season of 180 days/yr.

Table 3. Rates of denitrification in constructed treatment wetlands.

Lake, estuary or wetland	**Rate of denitrification** mg NO_3-N/m^2/d	**Nitrate** N mg/L	**Area ha**	**Mean depth m**	**Comment & source**
Des Planes, IL	11-63 (37)	~ 20?	72?	~ 0.5	Exact area of treatment wetlands not known
Kelly Ponds, Santa Rosa CA	625	~ 20	4.6	~ 0.5	High nitrate from treated sewage
Arcata, CA	800	~ 20	3.0	~ 0.5	High nitrate from treated sewage
Prado, Riverside Co. CA	1-1,000 (522)	8-11	200	~ 0.5	High nitrate from treated sewage
Experimental mesocosms, Prado	> 2,000 (1,000)	8-11	~ 1	~ 0.5	Very rapid flow-thru wetlands
San Joaquin Sanctuary marsh Irvine CA	175-500 (300)	8-16	~ 40	0.1-2.0	High nitrate from farm-polluted river, higher rates in warm weather
Danish stream	Summer max 140	0-11	na	~ 0.1	Shallow, macrophyte-dominated (Pind et al., 1997)
Mean[1]	**547**				

[1] Excludes stream values.

Most of the lakes where denitrification has been measured (Table 2) were eutrophic, shallow and thus often well-stirred by the wind. During mixing the nitrate present in the oxygenated surface water would be mixed into the surface sediments along with oxygen. When the wind dropped for a few days, the stagnant water would promote anoxic conditions in the sediments – a condition favorable to denitrification. However, once this supply of nitrate was exhausted, then no more denitrification could occur until the next mixing and subsequent settling under calm conditions. These lakes had similarities with Lake Elsinore but also some differences.

5.1.2. Conditions controlling denitrification in Lake Elsinore

Most of the 8 conditions needed for denitrification in lakes were estimated to have normally been present in Lake Elsinore from late spring to late autumn in the years prior to the first successful test of aeration in the summer of 2008 (Tables 4-5). The most important conditions are discussed below.

Presence of denitrifying bacteria. Many kinds of common bacteria can catalyze one or other of the two denitrification steps. Potential denitrification bacteria are usually present in lake sediments but most are in an inactive resting stage. They remain inactive awaiting suitable conditions for increased metabolism. Some lake sediment bacteria are also facultative, that is they can switch between respiring with oxygen or nitrate/nitrite as conditions dictate. The most common bacterium found in the benthic felt in Lake Elsinore in 2008 was the white mat of *Beggiatoa*. This genus can perform denitrification (Jean-Pierre et al., 1990). The conclusion is that denitrifying bacteria are present in Lake Elsinore.

Oxygen & Redox Potential. **Dissolved oxygen/ redox potential.** The level of redox (or DO) needed by denitrifying bacteria is quite specific but fortunately also rather broad (Table 1). The required redox potential is + 100- 200 mV and is equivalent to about 0.1- 0.5 mg/L of dissolved oxygen in the water immediately over the sediments. These conditions favor the thermodynamic gradient for the reaction series $NO_3 \rightarrow NO_2 \rightarrow N_2$. Past studies (e.g., Anderson & Lawson, 2005; MWH quarterly monitoring reports) showed low DO (< 0.5 mg/L) and moderately low redox potential (minus 90 to + 100 mV) as long-term summer features of the deep waters of Lake Elsinore. The conclusion is that Lake Elsinore can supply the redox potential needed for denitrification.

Temperature. Denitrification is a bacteria process and rates increase with temperature. In most systems, the greatest denitrification is found at temperatures over 25^{o}C and in some California studies rates increase substantially above 16^{o}C (Reilly, 1998). Denitrification does occur at low temperatures but rates are very low (Christensen & Sorensen, 1986). Recent monitoring in Lake Elsinore (2002-2005, Anderson & Nascimento, 2004; MWH, 2006) shows that lake-wide average deep water temperatures in the shallower lake (z ~ 5 m) at this time were 20-26^{o}C from April-October. Following the 2005 rains the lake level rose to 11 m but temperatures in summer were still above 22^{o}C. The conclusion is that temperature was suitable for high denitrification in Lake Elsinore.

Labile carbon sources. Labile or bioavailable carbon used as an energy source for the bacteria that carry out denitrification is considered a major controlling factor in both water and sediments (Brettar & Rheinheimer, 1992; Torbjorn & Stahl, 2000). Lake Elsinore is very eutrophic with chlorophyll a values as high as 300 ug/L. The amount of labile carbon available when this mass of algae dies and sinks to the bottom is large. The conclusion is that there should be an ample supply of organic carbon for bacterial denitrification in Lake Elsinore.

Nitrate. Denitrification obviously requires nitrate (> 0.1 mg/L, better > 1-5 mg/L, and best > 10 mg/L). There is some evidence that denitrification can occur in shallow water wetlands even when nitrate-N is very low (~ 0.05 mg/L; Horne et al., 2003). Unpolluted waters contain little nitrate (~ 0.01-0.1 mg/L) but runoff from agriculture and sewage disposal increases natural concentrations in the polluted rivers and lakes many developed countries. Nitrate concentrations of over 0.3 mg N/L are common and some US and European lakes and rivers contain much more. A measure of the affinity of bacteria for nitrate is the Monod half-saturation coefficient. It is defined as the concentration of nitrate that produces half the maximum rate of denitrification. The Monod half saturation coefficient (K_s) for denitrification varies since a wide range of bacteria carry out the process. Some literature values are 1.2 mg/L (Oremland et al., 1988) and 3-10 mg/L (Oh et al., 2000). With such a high K_s, denitrification proceeds best at values of > 1 mg/L or possibly > 10 mg/L. Lakes rarely show values above 1 mg/L. Thus the potential nitrate substrate for denitrification in lakes is present but normally at concentrations that result in low rates of reaction.

Prior to aeration, oxidized soluble inorganic-N (Nitrate, NO_3 + nitrite, NO_2) in Lake Elsinore was normally low compared with many lakes in temperate zones. During the summers if 2002-2005 nitrate was always below detection (0.05 mg/L) at all depths and times. In particular, nitrate was normally low and often undetectable (Fig. 2) in the deep waters where denitrification is mostly likely to be favored. The conclusion is that lack of nitrate probably kept denitrification at low levels in Lake Elsinore prior to aeration.

Physical substrate for the bacteria. Most denitrification occurs in biofilms since most bacteria prefer to grow attached to a solid surface rather than suspended in free water. Suitable surfaces are leaves, soil fragments, sand, organic matter or stones. The sediments of Lake Elsinore contain organic debris (dead algae, zooplankton pellets, zooplankton carapaces, dead fish) and inorganic matter (sand, mud). The conclusion is that solid surfaces suitable for denitrifying bacteria are present in Lake Elsinore.

Reactions competing with denitrification. Nitrate can undergo other reactions in lakes. These are principally ammonification ($NO_3 \rightarrow NH_4$) and uptake for growth ($NO_3 \rightarrow$ algal or bacterial proteins). Unlike denitrification, ammonification provides energy for the bacteria so it is favored if other conditions are suitable. The rates of these reactions vary but both will compete with denitrification for nitrate. However, lack of oxygen for oxidation of ammonia to nitrate will automatically prevent any denitrification leaving ammonia as the main decay product. In Lake Elsinore before aeration, ammonia was the dominant soluble nitrogen species at all depths and times. The conclusion is that prior to aeration, any denitrification was reduced by competing processes for nitrate.

Animals feeding on denitrifying bacteria. Surface biofilms of bacteria are excellent food for many small grazing animals such as small fish, snails, mayflies, worms and protozoa. However, the absence of dissolved oxygen deters such grazing since almost all animals require DO levels above 2 mg/L and most prefer above 5 mg/L. In contrast, denitrification needs < 0.5 mg/L DO. Most grazing animals will therefore not be present during denitrification although they could move down and graze during windy periods when oxygen is mixed to the sediments. The conclusion is that the potential for intensive predation on denitrifying bacteria was thus small in Lake Elsinore prior to aeration. The 2008 results show that more DO was present in the deeper waters following aeration. Nonetheless, the concentration of DO was only 2-3 mg/L which will still deter many grazing species.

5.1.3. Estimated annual denitrification pre-aeration rate in Lake Elsinore

Using the mean value for denitrification calculated from the daily rates shown in Table 2, the list of required conditions discussed above, and for only that part of the year when favorable conditions occur (Tables 4-5), the expected denitrification rate in the lake can be estimated. The calculation is shown below:

Likely annual denitrification pre-aeration rate in Lake Elsinore = (area of sediments suitable for denitrification in Lake Elsinore) x (mean denitrification rate) x (the time suitable conditions for denitrification persist).

The area of sediments and low oxygen suitable for denitrification in Lake Elsinore is that area that becomes anoxic during calm periods and that has fine organic sediments rather than coarse sands. The natural water motion in any lake winnows the fines to the deeper parts and leaves the coarse sediments along the shorelines. In Lake Elsinore, Professor Anderson has measured fine organic sediments and determined that they cover over about 1/3 of the lake. Since the lake area varies with water years, the area of sediments suitable for denitrification will also vary. For simplicity a value more representative of a more full lake was be used since the addition of wastewater should keep the lake deeper. According to Anderson (2005) almost 50% of the lake sediments had < 1 mg/L DO between July and October (~ 120 days). The area of Lake Elsinore when full is about 3,400 acres and the sediments suitable for denitrification can be estimated to be about half of the lake area (1,700 acres or ~ 6,900,000 m^2).

The mean denitrification rate for other lakes low in nitrate such as Lake Elsinore has been estimated at 4.3 mg NO_3-N/m^2/d (Table 2) or as 2.5 mg NO_3-N/m^2/d (see Monod K_s estimates below). The mean of these two estimates (3.4 mg NO_3-N/m^2/d) was used for the pre-aeration estimates. A recent low estimate for lakes is given by Seitzinger et al., 2006 as 3.9 mg N/m^2/d which is close to the 3.4 mg N/m^2/y used in this calculation. Low denitrification rates, including low denitrification efficiency are found in other lakes and estuaries (eg, Palmer et al., 2000; Blevins, 1998). Work by Liikanen et al., 2003 stated "Low availability of nitrate did severely limit N_2O production" in a boreal eutrophic lake in Finland (N_2O release is one of the methods used to measure denitrification). Similar work by the same author indicated that "the highest N_2O flux rates were measured with 300 umoles of nitrate" (Liikanen et al., 2002). 300 umoles is about 4.2 mg/L of nitrate-N so much more than was found in the past (< 0.05 mg N/L) or is predicted following aeration (0.3 mg N/L). Stream studies by Mulholland et al., 2006 showed that "denitrification rates were also positively related to stream water nitrate concentrations."

The time during which denitrification is favored. Lake Elsinore is warmer than many lakes due to its southerly latitude and hot semi-desert climate. The warm period (> 20^oC) is June-November (180 days, Table 5). However, the time of low bottom water DO is somewhat shorter at 120 days, so the warm water in early summer and late autumn will not be accompanied by suitable redox conditions for denitrification.

Effect of low amounts of nitrate in Lake Elsinore. Lake Elsinore is unusually low in nitrate. Occasionally, when large storms provide enough runoff to reach the lake they carry nitrate in from the watershed. On these occasions, nitrate in the lake will rise to 0.4 to 0.8 mg/L in the later winter and spring. One such occasion was spring 2005 (Fig. 2). However, by summer the nitrate has all been used for algal growth and the normal conditions return. Since the brief high nitrate periods occur only when other conditions (temperature, DO) are not favorable for denitrification, little effect on the N-balance is likely to occur.

As can be seen from Figure 2, in the period June 2002-04, nitrate was often not detectable (< 0.05 mg/l) and was above 0.1 mg/L for only a few days during each of the 180 days when it was warm enough for denitrification to occur or the 120 days when redox potential-low DO was favorable. Nitrate never exceeded 0.16 mg N/L at any time. The Monod coefficient (K_s) for denitrification for sulfur bacteria is 3-10 mg/L and an apparent K_s for denitrification in Soda Lake Nevada was ~ 1.2 mg/L. Using the lowest values from these published studies; denitrification will proceed at half its full rate at 1.2 mg N/L. The Monod curve of activity versus concentration is usually an inverse exponential curve so that with a K_s of 1.2 mg/L, the amount of denitrification occurring at 0.05 mg/L NO_3-N (the detection limit for Lake Elsinore) can be approximated as < 1% of the maximum rate. If the maximum reported rate of denitrification under very favorable conditions was about 500 mg $N/m^2/d$ then 1% would be 5 mg $N/m^2/d$ which corresponds well with the values in Table 2. For values less than 0.05 mg/L nitrate it can be assumed that denitrification will be between 0 and 1% of maximum (assume a mid level of 2.5 mg $N/m^2/d$). This amount is normally insignificant for the mass balance of most lakes. However, possibly not for Lake Elsinore since in dry years there are limited inflows and nitrogen arrives primarily as dry or wet fallout directly on the water surface.

Table 4. Periods when temperatures were favorable for denitrification in Lake Elsinore 2002-04 during the 180 day warm summer-fall period (June-November). Samples were taken from deep water above sediments.

Period	**No of days > 0.1 mg/L nitrate-N**	**No day > 0.5 mg/L**
2002	30	0
2003	0	0
2004	14	0
Mean for 2002-04	**15**	**0**

Table 5. Regulatory factors for denitrification in Lake Elsinore before and after successful aeration.

Denitrification requirement	**Before successful aeration**	**After successful aeration**
Presence of denitrifying bacteria	Yes	Yes
Low or no oxygen also shown as suitable redox potential	Yes: Redox – 82 to + 100 in bottom waters	Yes: in places
Warm temperature	Yes (20-25°C)	Yes (20-25°C)
High nitrate levels	**No (usually undetectable)**	**Some (low) but supply increased**
Availability of labile organic carbon energy source	Yes (dead algae)	Yes (dead algae)
Suitable solid substrate	Yes	Yes
Low rates of competing reactions	Unknown	As before
Low predation on denitrifying bacteria	Probably	Probably

The estimated time during which the levels of denitrification shown in Table 2 will occur in Lake Elsinore was 15 days/year on average (Table 4). The 15 days occur when low DO and redox potential are favorable for denitrification. No higher rates are likely to occur due to a shortage of nitrate substrate. The expected regulatory factors for denitrification before and after successful aeration are summarized in Table 5 and differences are discussed in detail following the summary table.

5.1.4. Summary of Estimated Denitrification in Lake Elsinore <u>prior</u> to lake aeration

Denitrification = (area of sediments suitable for denitrification in Lake Elsinore) x (mean denitrification rate) x (the time of suitable conditions for denitrification persist).

= 6.9×10^6 m^2 x 3.4 mg NO_3-$N/m^2/d$ x 15 days = 0.35×10^9 mg NO_3-N/y
= <u>0.35 tonnes/y (metric units)</u>
= 0.39 US short tons

The importance of this amount of denitrification can be ascertained by a comparison with other N-sources in the lake (Table 6). The likely denitrification rate in Lake Elsinore prior to aeration of 0.35 tonnes/y is trivial for the N-budget compared with the total loading of over 380 tonnes/y or the actual 2008 EVMWD input of reclaimed water of 19 tonnes/y.

Table 6. Various annual fluxes of nitrogen in Lake Elsinore. Metric tonnes (1,000 kg) are similar to US tons and can be converted to US short tons by multiplying by 1.1. Most data from Rebecca Veiga Nascimento & Michael Anderson & SARWQCB, TMDL

Kind of N-flux	Amount (tonnes/y)
Estimated denitrification	0.4
SARWQCB permit requirement for 5-year running average mass TMDL limit	7.4
External N-load & internal N-cycling 2003-04	
EVMWD reclaimed water	7.4 (2.0 %)
EMWD	26.2 (7.0 %)
Canyon Lake	45.6 (12.1 %)
Internal loading	296 (78.9%)
Total	**375.2**

5.2. CONDITIONS CONTROLLING DENITRIFICATION IN LAKE ELSINORE AFTER AERATION

5.2.1. Changes likely to occur

The changes in denitrification due to aeration are not straightforward. The literature surveyed contains no direct measurements of denitrification before and after aeration-oxygenation-mixing partially due to difficulties in making unambiguous denitrification measurements in lakes. The most recent comprehensive review on denitrification (Seitzinger et al., 2006) states, "Comparative studies of the magnitude and controlling factors for denitrification across freshwater and marine systems with periodic sub-oxic waters are needed." Lake Elsinore is a freshwater system with periodic (summer-fall) sub-oxic conditions. In addition, the estimate of potential nitrate (i.e., that nitrate that would exist with aeration and without denitrification) is not direct. That is, if denitrification occurs in a closely coupled nitrification-denitrification reaction in the sediment-water microzone, then nitrate will not be released to the deep lake waters where it can be measured. Instead, the nitrate will be denitrified as fast as it is released from the sediment surface. Nevertheless, using the existing and expected changes in nitrogen species in Lake Elsinore, some estimate of the changes in denitrification can be made for the aeration system which was installed in 2007 but only became fully operational in summer 2008.

The changes in Lake Elsinore that affect denitrification and can be expected from successful aeration are an increase in deep water oxygen levels and either actual or transient higher nitrate concentrations. Measurements in summer 2008 showed that the deeper water was not very often anoxic and that dissolved oxygen concentrations of 2-3 mg/L were often present in the water layer just above the sediments in large areas of the deeper sections of the lake (Table 7).

Table 7. Summary of temperature-DO profiles in Lake Elsinore on 23rd September 2008. Deepest measurements were ~ 10 cm above the bottom.

Site	**Time**	**Depth (m)**	**Temperature (^oC)**	**Dissolved oxygen (mg/L)**	**Difference Top v. bottom**
Station A (moderate DO)	8:30	0	24.5	8.2	5.4
		6.9	24.0	2.8	
Station A (moderate DO)	11:50	0	26.1	11.5	8.9
		6.8	24.1	2.6	
Station B1 (low DO)	10:00	0	25.0	9.9	9.4
		7.7	24.05	0.5	
Station B1 (low DO)	11:10	0	25.9	9.5	9.2
		7.6	24.1	0.3	
Station B2 (intermediate DO)	10:40	0	25.8	8.9	7.3
		7.5	24.1	1.6	
Station C (moderate DO)	11:30	0	25.6	12.1	9.7
		6.8	24.1	2.4	

No major change occurred in temperature, amount of labile carbon on the lake bed or in solid substrates for bacteria as a result of aeration. Nitrate should replace ammonia as the main soluble inorganic-N species. This change occurs often when lakes are oxygenated or aerated (e. g., the bubble oxygenation of Upper San Leandro Reservoir in Oakland, Horne et al., 2003).

Ammonia levels in the pre-aeration Lake Elsinore at 5 m just above the sediments in 2002-05 were 0.31 mg/L. Values in 2006 were similar. Assuming no other losses to growth, nitrate should potentially rise from < 0.05 to 0.31 mg/L. The change in nitrate in Lake Elsinore has not yet been analyzed but the information has been collected as part of the monitoring plan and should be analyzed in 2009. Such a rise would move the denitrification rate into the class of moderate nitrate lakes (15 mg NO_3-N/m^2/d, Table 2). In practice, the lake will not show such higher levels since some of the new nitrate will be consumed by denitrification. The process of coupled nitrification-denitrification is discussed further below. In addition, the conditions suitable for denitrification (adequate nitrate, warm temperature, and low DO/redox potential) will increase from 0-15 days to 120 days.

This time period assumes that the pre-aeration DO and redox values in the immediate sediment-water interface have not changed very much with aeration. Aeration in 2008 was only able to raise some bottom water DO values to 2-3 mg/L and other bottom water regions remained < 1 mg/L. Given these DO values, it is assumed that the sediments below the very surface of the mud will remain anoxic allowing denitrification to proceed as nitrate diffuses down from the deep water. This seems reasonable since even in very well aerated sediment cores, anoxic conditions persist close (0.5 to 3 cm) to the sediment-water interface. The water physics of successful aeration, as distinct from some forms of no-bubble oxygenation, produce a well-mixed water column except for a layer above the sediments. The layer forms as a consequence of the air stream mixing of low-oxygen cooler bottom water up to the warm well-oxygenated surface layers producing a water mass with both intermediate temperatures and oxygen. The intermediate temperature water layer is also of intermediate density and thus floats at an intermediate level, leaving a less mixed, less oxygenated layer over the sediments. Oxygen will diffuse or possibly mix occasionally between the layers providing a source of nitrate to the sediments while still keeping sufficient anoxia for denitrification. More intensive synoptic surveys of the bottom water DO in Lake Elsinore could be done to characterize this process further.

An independent way to estimate the increase in denitrification following successful aeration is to consider other literature data. Because the process of nitrification can be so closely coupled with denitrification, actual nitrate concentrations in Lake Elsinore may not change much even though nitrate is being formed at higher rates than without aeration. The review and analysis of a large amount of data by Seitzinger et al., (2006) has provided a general guide to when coupled nitrification-denitrification is important. In lakes with low bottom water nitrate concentrations (< 140 ug/L) the process of denitrification is normally coupled with the nitrification of any ammonia present. Under these low nitrate conditions the coupled reaction will account for ~90% of the denitrification so long as the bottom waters are oxygenated (Seitzinger et al., 2006). Based on the pre-aeration ammonia concentrations a bottom water nitrate or equivalent nitrate potential was about 0.3 mg/L (300 ug/L). This is somewhat higher than the guide of < 140 ug/L) given by Seitzinger et al., (2006) so less than 90% of the nitrate produced by nitrification of ammonia may be denitrified. However, in Lake Elsinore, much of the past ammonia produced in the sediments under highly anoxic condition may not be ever formed with the new aeration regime so tightly coupled decay of algae amino acids to ammonia to nitrate may follow other paths including direct uptake of ammonia in the enhanced benthic felts.

There is an almost 1:1 relationship between increased N-loading and denitrification (Seitzinger et al., 2006) so some increase in N-loss can be expected as the makeup water to Lake Elsinore is increased. However, since the natural inflow is so variable in Lake Elsinore, this relationship is not very useful for predictions, especially as the sediment-water interface DO conditions will be changed by aeration.

The rates of denitrification in lakes are also related to hydraulic residence time. For all kinds of aquatic ecosystems the most recent data (Seitzinger et al., 2006 using a large variety of previous studies) suggest that denitrification will remove almost 100% of nitrate in shallow lakes with long water residence times. Of course most lakes and almost all reservoirs have short hydraulic residence times measured in months or a few years so nitrate flows in and out of such lakes. Also in lakes with very long residence times such as Lake Tahoe (HRT ~ 700 years), nitrate has accumulated since at least 1960 so the effect of residence time may be complicated. Nonetheless, in many ways Lake Elsinore can be said to have a long water residence time since it rarely overflows. The prediction of Seitzinger et al. (2006) makes some sense since with a long water residence time the cycle of algae→ sediments & decay → release of sediment ammonia will repeat and no ammonia (or nitrate if nitrification occurs in winter) will be washed downstream. However, if this simple scenario were true for Lake Elsinore algae would decline during dry periods since nitrate (or ammonia) would become growth-limiting. Recent observations have shown that the opposite is true since flood periods with inflows of nitrate and phosphate show strong declines in algae. Seitzinger et al. (2006) do point out that exceptions will occur to their general model and Lake Elsinore appears to be so unusual that it may not conform.

5.2.2 Summary of Estimated Denitrification in Lake Elsinore after successful lake aeration

Denitrification = (area of sediments suitable for denitrification in Lake Elsinore) x (mean denitrification rate) x (the time of suitable conditions for denitrification persist).

= 6.9×10^6 m^2 x 15 mg NO_3-N/m^2/d x 120 days = 1.2×10^9 mg NO_3-N/y

= 12 tonnes/y

Net increase in denitrification due to lake aeration = new estimate with aeration minus old estimate prior to aeration.

= 12.0 - 0.35 tonnes/y = ~ **11.6 tonnes/y**

5.2.3 Comparison of estimated Denitrification in Lake Elsinore after successful lake aeration with required N-offsets

The estimated value of 11.6 tons/y can be related to the N added in the reclaimed water (19 tonnes in 2008 and likely similar amounts for the next few years). If the denitrification estimates in this report are correct, then the increase in nitrate alone due to aeration will counteract or offset about 61% of the TN added in the EVMWD makeup water for Lake Elsinore (Table 6).

Thus the current (2008) N-additions from recycled water will slightly increase the overall N-budget of the lake by about 7.4 tonnes/yr. Given that on average the inflow from Canyon Lake is about 46 tonnes/yr and the internal N-loading was estimated by Professor Anderson as 296 tonnes/yr it is unlikely that the change of 7.4 tonnes could ever be detected. However, because it may be desirable to increase the lake levels more than is now being done, the offsets provided by other methods may be needed. Such offsets could include balancing the eutrophication-reducing effect of higher water levels, estimation of the contribution of increased benthic microbial felt (if any), increased N-removal at the sewage treatment plants, or construction of an in-line nitrate-removing wetland prior to the addition of the reclaimed water to the lake. This report is limited to consideration of the direct effects of aeration which have been limited to denitrification, benthic felt enhancement, and N_2-fixation.

6.0 RESULTS SPECIAL STUDY 2. CHANGES IN BENTHIC MICROBIAL FELT RELATIVE TO BENTHIC DISSOLVED OXYGEN 2006-08

6.1. METHOD DEVELOPMENT (COLLECTIONS # 1 & 2: 2006-07)

The first field work for Special Study 2 was conducted in September, 2006 and included the collection of mud samples from offshore but relatively shallow sites in Lake Elsinore. These mud samples were used for initial testing of the ATP method. The ATP measurements were made with equipment provided on a test basis by the equipment manufacturers. The test was a qualified success in that it established that the method was easily sensitive enough to measure ATP in the interstitial water of the sediments. Following this test the equipment was purchased and field tested in aquatic sediments in experimental ponds in El Cerrito prior to use in Lake Elsinore. The first intact sediment collections in Lake Elsinore were made in August 2007 when it was assumed that the aeration system was working. However, due to problems with the size of the holes in the aeration pipe only very small amounts of air were actually added to Lake Elsinore in 2007. However, the August 2007 campaign was productive in that samples were taken to field test the sampling grab device and the new field ATP meter. Like most meters that measure emitted light ATP meters are sensitive to direct sunlight and a protocol was devised to overcome this limitation. These tests were successful as far as they went but in the absence of an aeration system, the remaining or the summer 2007 tests were postponed until 2008.

6.2. BENTHIC FELT COLLECTIONS DURING AERATION OPERATION IN 2008 (COLLECTIONS # 3-5)

Mats or layers of microbial organisms are ubiquitous on submerged surfaces. To most people they are familiar because of the slimy feel they impart. In shallow sunlit waters they are often green or brown due to algal growths along with bacteria and small protozoans, rotifers, nematodes and midge larvae. Where light is dim the algae cannot thrive and the color of the benthic felt usually becomes mostly a mat of bacteria with a grey or white cast to the film. It was this layer that was anticipated to change in some fashion when Lake Elsinore was aerated.

6.2.1 Method development

Collection # 3 (23-4 July 2008) was made at the three regular compliance monitoring index station sites at the same time as the compliance monitoring measurements were made by Professor James Noblet and his students from California State University at San Bernardino. At this time the bottom waters were uniformly partially oxygenated and no obvious DO gradient was observed. For collections # 4-5 in September and October 2008 a new spatial design was used to include both oxygenated and low oxygen sites.

<u>Benthic felt collections.</u> Problems were encountered in taking quantitative samples of the benthic felt in Lake Elsinore because it was much more delicate and friable than found at other lakes such as Upper San Leandro Reservoir (Oakland) or Pyramid Lake (Nevada). The benthic felt collected as an intact layer from Lake Elsinore with the Ekman Grab was disturbed by even the

slight rocking motion of the boat and soon broke into small fragments. It was unfortunate that unusual activities on the lake attract curious lake users who bring with them boat wakes that add to the rocking and sample collection problems. To reduce the rocking problem, for the September 2008 collection a stainless steel Ekman Grab was purchased. It had upper doors that could be fully opened to allow better collection of the fragile mats. The new sampler was successful in collecting undisturbed sediment samples but boat rocking still tended to break up the film. A more rapid sampling of the mat provided a partial solution to the rocking problem. Collection # 5 was carried out during windy, cool weather in early October 2008. Waves as high as 3 feet were reported on 11th October although the lake was sufficiently settled for benthic mat collections to be made on the following day.

Once a more or less undisturbed benthic felt sample had been collected in the Ekman Grab and hauled to the surface, it was sampled directly. A quantitative sample of the benthic felt was obtained using either a metal loop sampler of known area (23 mm^2) or samples were scooped out with a small spoon and a 1 mm^2 core taken using a cut-off plastic tube. Triplicate or quadruplicate cores were taken at each site. Samples of the whole integrated surface mat and associated pore water were collected with a pipette. For all the 2008 collections, samples were placed in small glass vials and kept on ice until measurement of ATP could be made.

ATP measurements. The ATP probe (Water Genie) and its associated light-measuring device (novaLUM) were developed for measuring bacterial contamination of carpets, furniture and drinking water fountains. For example, after floods there is the possibility that sewage or other bacterial contamination present in the flood water will have remained viable on the carpet or other flooded structures. It is also possible that the crust that forms at the tap of a drinking water fountain can be contaminated by bacteria that have grown there over time. After cleaning the carpets, furniture and water fountains can be tested for ATP concentrations. If the ATP is very low, then the cleaning was satisfactory. However, the ATP method is very sensitive so poor cleaning can be detected. The technique was modified and adapted for ATP analysis of Lake Elsinore sediments.

In the benthic mats ATP is present in free soluble form due to leaks and releases from damaged cells. Free ATP in the benthic mat in Lake Elsinore was assumed to be proportional to the living bacterial biomass in the felt since this is the principle on which the test is used commercially for proof of cleanness in carpets or drinking fountains. The amount of free ATP in the mats was high so the samples were diluted to reduce the ATP values to a more manageable number. A sub-sample of the supernatant water was taken, diluted with various volumes of tap water as the situation required following initial test measurements and then shaken gently. Tap water, which is disinfected, has a very low ATP (ATP < 1 RLU) while the sediment extractions after dilution normally exceed 100,000 RLU so there was no effect of the tap water.

Free ATP dissolved in the inter-filament spaces was extracted into the water and measured by dipping the absorptive cotton tip of the Water Genie probe into the water for 5 seconds. The probe tip was then placed in the sampler, screwed down to mix the luciferin and luciferase with the felt extract, and the device shaken twice. The black tip of the Water Genie probe was then removed and the probe slipped into the novaLUM portable ATP analyzer. The device reads the ATP concentration automatically. Individual replicates from each lake benthic mat extraction were tested between 2 and 4 times for ATP. The samples were iced and an hour later the entire process was repeated.

6.2.2 Qualitative observations and estimates of percent cover of the benthic mat

In July 2008 the whitish mat characteristic of the benthic felt in Lake Elsinore was found at two out of the three regular compliance monitoring stations. There was no visible benthic felt at regular monitoring station #1 (~ 18 feet deep) but a felt was seen at #2 (23 feet deep) and was more obvious at #3 (also ~ 18 feet deep). The DO at all these stations was > 2 mg/L (see regular monitoring data).

In September 2008 more extensive surveys showed large patches of an obvious whitish felt at the more oxygenated sites. When first observed it appeared that the entire mud surface in the oxygenated sites was covered with a continuous film of felt. In contrast, samples taken from the low DO areas showed only small discontinuous patches of felt which did not cover the entire surface. After many samples were taken in the two kinds of sites it was estimated that the oxygenated areas had 100% cover while the low DO sites had 25% cover

In the October 2008 collections the cool, windy weather caused the lake water to mix top-to-bottom and thus the water overlaying the sediments was fully oxygenated. The sediment surface consisted of small mat fragments and other particles such as mud and sand. The distribution of the white benthic felt was similar to that found after the gentle wave-induced rocking that occurred inside the Ekman Grab that was described earlier and was presumably due to wind mixing from the storm. Mat samples were collected for ATP and microscopic examination.

6.2.3. Benthic felt ATP results

The benthic felt-ATP samples for the July 23, 2008 collections were taken at the same sites and times as the aforementioned regular index measurements. The results of these ATP measurements are shown below in summary (Table 8) and in detail in Appendix Table B-1. There was considerable variability between replicate samples from the grab samples and also between repeated tests of the same sample. Nonetheless, the amount of ATP associated with the sites with visible benthic felt was higher than that at the sites with no visible felt (Table 8).

Table 8. Summary of ATP associated with benthic felt in the three standard TMDL compliance sampling stations Lake Elsinore, 23 July 2008. ATP data rounded, replicates averaged. RLU = Relative Light Units read directly from the novaLUM meter.

Site (depth)	Felt observations	Mean ATP (RLU)/ mL water
# 1 (5.5 m) North site	No white crust	5,900
#2 (7.0 m) Central site closest to aeration pipes	Some white crust	23,900
#3 (5.5 m) South site	White crust obvious	9,100

Based on DO measurements from the previous day, the 24 July, 2008, samples were collected from the two most promising sites (#2 and 3 of the EVMWD standard TMDL compliance sampling stations). Both of these sites showed the white mat on the previous day (Table 8). A quantitative sample of the felt was collected with a wire loop. The results are shown in summary in Table 9. Once again site # 2 contained the most ATP associated with the felt crust (Table 8). The amounts of ATP/ml were much greater in this test since the loop contained 100% felt while the integrated surface sample had a mixture of water, other sediments in addition to the felt fragments. The ratio of ATP associated with the felt in the two stations 2 and 3 was 2.5:1 (23,000: 9,100) on 23 July and 2.4:1 (251,000: 104,000) the next day. Thus the method seems to show some consistent mean differences despite the high variation in individual replicates and ATP tests. However, as can be seen in Table 8 there was not a good relationship between visible felt and ATP on this occasion (but see September study where more samples were taken).

Table 9. Fixed area benthic felt APT concentrations in the two EVMWD standard TMDL compliance sampling stations in Lake Elsinore, 24 July 2008. Test of samples of "pure" felt collected from the sediment surface using a loop of known area. ATP data were rounded, tests of replicates averaged from 2 to 3 replicate tests for each extraction, and ATP data in these tables were normalized per mL of extracted water. In this table the value for lake water (used for field dilution) was subtracted. RLU = Relative Light Units read directly from the novaLUM meter.

Site (depth)	**Felt observations**	**ATP (RLU)/mL**	**ATP (RLU)/mm^2 of felt**
Closest to aeration #2 (7 m)	white crust collected in wire loop	251,000	161,000
Distant from aeration #3 (5.5 m)	white crust collected in wire loop	104,000	66,000

For the September 2008 collections the results are summarized in Table 10 and details are shown in Appendix Table B-2. As was found earlier in the season, there was an obvious difference in ATP that reflected the visual observations made in the grab on the lake (Table 10). The mean for the sample with the higher DO (2.6 to 2.8 mg/L) showed about four times the ATP (196,000 RTU to 49,000 RTU) as the samples with low DO. As was found earlier the ATP from replicate samples was highly variable with the same water sample showing considerable range (Appendix Table B-2).

Table 10. Summary of ATP measurements in Lake Elsinore benthic felt on 23rd September 2008. The ~ 1 mm thick felt covered the more oxygenated sites and was thinner or patchy on those sites with low DO. These stations were selected after a preliminary survey of DO and are not at the TMDL compliance sites. The second test was a repeat but after an interval of 2 hours.

Site	**DO in bottom water (mg/L)**	**ATP (mean RLU) 1st test**	**ATP (mean RLU) 2nd test**
Station A (higher DO)			
Early collection mean (8:30 am)	2.8	84,000	31,000
Later collection mean (11:50 am)	2.6	365,000	Not detected
All higher DO **Sta. A averages (am + pm)**		**198,000**	**31,000**
Station B1 (low DO)			
All low DO ***Sta B1 averages)***	*0.3 to 0.5*	***49,000***	***29,000***
Station B2 mean (intermediate DO	1.6	43,000	8,000
Station C mean (higher DO)	2.4	13,000	Not detected

The ATP concentration of 43,000 RLU in the felt at the intermediate DO sample station (station B2 located about 100 m north of B1 and with a DO of 1.6 mg/L) showed a similar result to the lower DO station (B1; 49,000 RLU). This finding indicates that DO > 2 mg/L may be critical for the maintenance of a healthy benthic felt. An attempt to replicate the higher DO site was made at another station, C (DO = 2.4 mg/L). Of the two cores, only one sample showed no ATP and the other one was quite low (~ 13,000 RLU). Since a zero ATP is highly unlikely, the results indicated that some field error may have occurred.

6.3. TEMPERATURE AND DISSOLVED OXYGEN

The aeration system had been operational for a few months by 2008 but for reasons of cost is not run continually as in smaller aeration systems. The aeration system was not actually bubbling air during the time of any of the 2008 collections. However, the system was operated within a few days before the summer tests (July-September) were made. Thus the collections were made under what was then normal operations. The effect of one operation of the aeration device for several hours lasts for a considerable time (days) so it is not necessary to take measurements during actual bubbling. For the September 2008 N-offset work samples were taken based on the DO measured immediately above the sediments. Several sites were tested and these were generally different from the routine index stations (Table 11). Those sites with the higher DO (2.6-2.8 mg/L) and a single area which showed the lowest DO (0.3-0.5) were selected for mat and ATP measurements. The DO ranged from 8-9 mg/L in the surface waters which rose to 11.5 – 12.1 as photosynthesis increased towards noon (Tables 7 & 11). The weather was hot and calm with no wind and the air temperatures rose to over 40^{o}C by mid day. The deepest water over the sediments showed a constant temperature of 24.0 to 24.2 ^{o}C all through the morning while surface temperatures rose from 24.1 to 26.1 ^{o}C between 8:30 am and noon. In general, the greater the temperature difference between surface and bottom water, the lower the DO was near the bottom (Table 7).

Also shown on Table 11 is a comparison with the temperatures and DO between 2006 (prior to aeration) and the first aeration year of 2008. The lower DO in the pre-aeration year of 2006 is obvious in the deeper water.

In mid October 2008 following holomixis (top-to-bottom stirring) the DO throughout the bottom waters of the lake had risen to 6-7 mg/L and no anoxic or low DO areas were found. Water temperature at this time had also fallen to about 19.5 to 19.6 ^{o}C at the water surface and 19.1 to 19.4 ^{o}C at the bottom (Table 11). This compares with 24-26 ^{o}C for surface temperatures measured only a month earlier.

Table 11. Comparison of temperature-DO profiles in Lake Elsinore on 23rd September 2008 compared to pre-aeration profiles collected on September 28th 2006 at compliance station # 2. The deepest measurements were made about 10 cm above the bottom. Air temperature rose to about 40°C by mid morning.

Site	**Time**	**Depth (m)**	**Temperature (°C)**	**Dissolved oxygen (mg/L)**
Station A (moderate DO)	8:30		2008 (2006)	2008 (2006)
		0	24.5 (24.7)	8.2 (12.2)
		2	24.3 (24.0)	7.8 (9.0)
		4	24.1 (23.5)	4.0 (3.9)
		5	24.1 (23.5)	4.0 (2.5)
		6	24.0 (23.5)	3.0 (1.5)
		6.9	24.0 (23.3)	2.8 (0.9)
Station A (moderate DO)	11:50	0	26.1	11.5
		2	24.6	8.0
		4	24.1	4.5
		5	24.1	3.6
		6	24.1	2.6
		6.8	24.1	2.6
Station B1 (low DO)	10:00	0	25.0	9.9
		2	24.8	7.7
		4	24.6	6.5
		5	24.3	2.5
		6	24.1	2.0
		7	24.1	2.0
Station B1 (low DO)	11:10	0	25.9	9.5
		7.6	24.1	0.3
Station B2 (intermediate DO)	10:40	0	25.8	8.9
		7.5	24.1	1.6
Station C (moderate DO)	11:30	0	25.6	12.1
		2	24.9	8.8
		4	24.3	5.2
		5	24.2	3.3
		6	24.1	2.6
		6.8	24.1	2.4
Station 2		0	24.7	12.2
		0.5	24.5	12.3
		1	24.2	10.9
		1.5	24.1	10.0
		2	24.0	9.0
		3	23.6	6.1
		4	23.5	3.9
		5	23.5	2.5
		6	23.4	1.5
		7	23.3	0.91
		8	23.2	0.23
		8.7	23.2	-
		9	-	0.13

6.4. MICROSCOPIC EXAMINATION OF THE MICROBIAL BENTHIC FELT

6.4.1. Visual appearance and identification of the living felt

The whitish benthic felt was easily separated from the underlying sediments. All benthic felt samples were examined in live form. They were kept in ice and transferred to the El Cerrito Laboratory where they were examined under a dissecting microscope and also a higher powered compound microscope. The composition and thickness of the mat was determined under the dissecting microscope

Under the lower power of the dissecting microscope (x 10 – 70), the bacterial filaments were a wavy layer similar to spaghetti. The cores consisted of a thin white layer of multiple filaments of bacteria underlain with a thicker amorphous light brown layer (Fig. 3). As mentioned before no sediment grains were visible in the felt. The white bacterial threads were inter-twined only with the upper amorphous layer but did not penetrate far. The thin upper white layer could not be peeled off the lower browner mat. However, unlike the large areas of mat in the lake which were easily broken into smaller fragments by wave action, the very small samples taken with the corer or wire loop formed a strong unit. Even when rinsed and shaken in water the unit stayed together. The mat is thus defined as the entire felt of living organic matter.

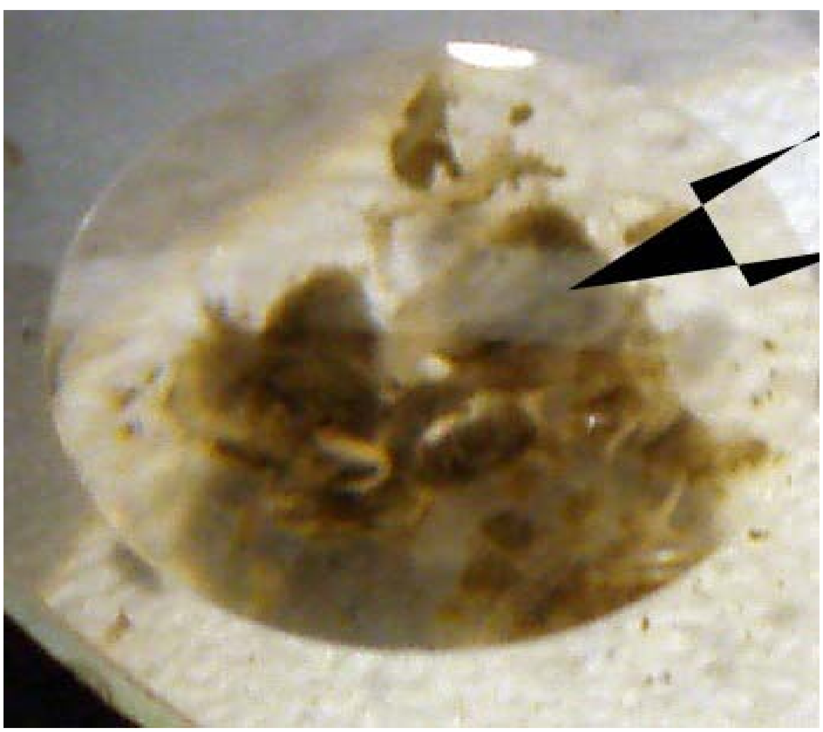

Figure 3. Macroscopic appearance of the benthic felt from Lake Elsinore in 2008. The whitish film in the upper part of the picture indicated with an arrow is the bacteria *Beggiatoa.* The brown material is the underside of the bacterial mat. The magnification is ~ x 6.

Under the higher power of the compound microscope, the bacterial filaments from both the September and October 2008 samples were un-branched, uniformly 7.8 um wide and several examined were 0.7 – 1.0 mm long (710 – 1,000 um) with rounded ends and curved tips. There was no visible sheath and no visible cross walls under x 400 magnification. Small dark inclusion ~ 1 um across (possibly sulfur granules) were scattered in the filament. The filaments exhibited substantial gliding motion.

Based on the macroscopic white mat appearance, location on the bottom sediments, massive quantities, lack of branches, very large size, presence of possible sulfur granules, single filamentous form, and vigorous gliding motion, the benthic felt in Lake Elsinore was tentatively identified as *Beggiatoa* (Fig. 4). As described by Jones and Jones (1986) "Populations of *Beggiatoa* are, on occasions, so large at the surface of profundal sediments of eutrophic lakes that a white mat develops." In the CRC Manual of Microbiology, Romano states "*Beggiatoa* occurs widely in lake, pond, and river muds …" (Romano, 2000). The 2006 web site of the Environmental Leverage Inc, a company that provides microbial training, states "This (*Beggiatoa*) is the only actively motile filamentous bacteria with gliding and flexing action."

The only feature that does not track with the samples from Lake Elsinore is the lack of cross cell walls which are present in *Beggiatoa* but were not observed in 2008. However, sometimes the cross walls are hard to see even under a x 400 magnification. Romano (2008) states "Cross walls are not easily seen … only the inner (cell) wall participates in the formation of cross walls…" If further examination does not find cross walls the most likely alternative to *Beggiatoa* is *Flexibacter* but this genus is not normally nearly as long (5-100 um) as those filaments found in Lake Elsinore (700-1,000 um). *Beggiatoa* is stated as being 50 to 10,000 um (Jones & Jones, 1986) and 80 to 1,500 um (Romano, 2000), and so nicely encompasses the filaments found in the benthic felt in Lake Elsinore. In addition, the literature gives the width of *Beggiatoa* as 1-3 um rather than the ~ 8 um measured in Lake Elsinore. However, other authors (M. Dudley, *Beggiatoa* notes web site) give a cell width of 2-10 um encompassing the 7.8 um width found in Lake Elsinore.

Beggiatoa grows readily on a variety of organic substrates with and without the presence of oxygen. No doubt organic compounds are present in abundance in the decaying algae that sink to the sediments in Lake Elsinore. The bacteria can also use hydrogen sulfide (H_2S), the characteristic sulfur compound in anoxic sediments. Even under oxygenated conditions the sediments in a eutrophic lake like Lake Elsinore will be anoxic under the microzone that is the sediment-water interface. Thus it may be that *Beggiatoa* gains some energy from the oxidation of H_2S but lacks the capacity to fix CO_2 so utilizes the energy from the organic matter in sunken algae. It thrives best in a gradient with oxygen diffusing in from above and H_2S diffusing up from below. This condition is well provided by an aerated Lake Elsinore. The fairly small numbers of possible sulfur granules in the samples from Lake Elsinore indicate that H_2S metabolism was not dominant in 2008. However, in 2008 the Lake Elsinore samples from anoxic areas (~ 0.4 mg/L DO) were not specifically compared with those from areas with higher DO (2-3 mg/L) with regard to the abundance of sulfur granules. In addition, poly-β-hydroxybutyric acid inclusions are also found in *Beggiatoa*. As stated earlier, the basic hypothesis is that aeration will allow the benthic microbes to grow more efficiently than under anoxic conditions and *Beggiatoa* is well equipped to be able to adapt to the change in Lake Elsinore.

Measurements of the thickness of several of the stronger cores from the oxygenated site A were made and the white bacterial filament cover including the firmly attached amorphous layer averaged 1.05 mm. The literature gives a typical thickness of 0.6 mm for *Beggiatoa* (Microbe Wiki, 2006) but the thickness depends on water movement. For calculation purposes a mean width of 1 mm was used.

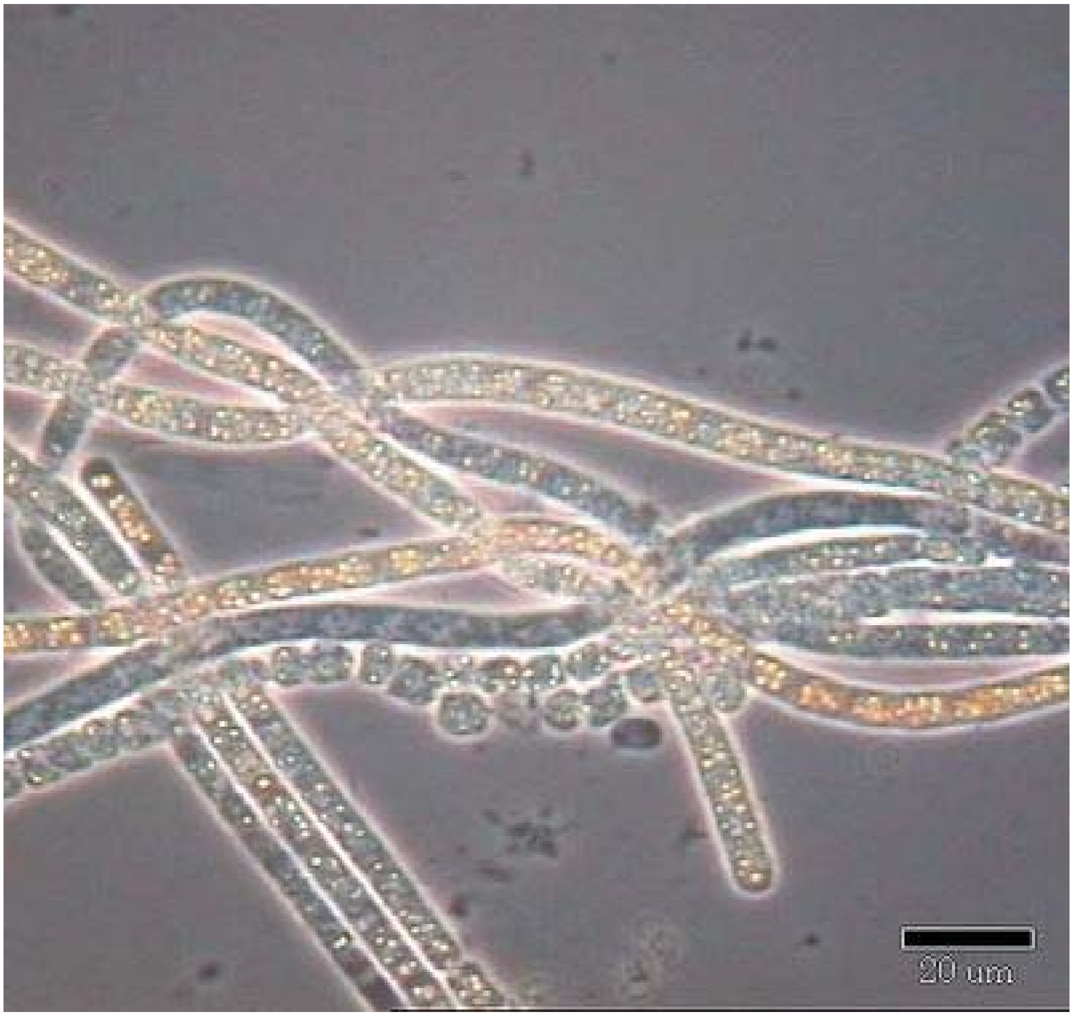

Figure 4. Higher power photomicrograph of *Beggiatoa* from a sulfur-rich site (photograph by Rolf Schauder, 1997 from web site).

6.4.2. Provisional calculations of N-offset in the benthic felt

Only one set of good samples of the felt that could be used to measure the size of the mat was obtained in September 2008. Thus any calculations must be provisional until more data are obtained.

The amount of N-sequestered in the increase benthic felt can be estimated by determining the amount of felt and its relationship to oxygen in the deep water. The data from the summer 2008 season indicate that increasing the dissolved oxygen (DO) from close to zero to ~ 2.6 mg/L increases the felt coverage by ~ 75%.

The felt was estimated to be 1 mm thick and the area of bottom water that was formerly anoxic was set at 1,000 acres (4 x 10^6 m^2) based on earlier work by Dr. Michael Anderson. The amount of N sequestered in the benthic mat was thus:

Mat volume = 4 x 10^6 m^2 (former anoxic area) x 1 x 10^{-3} m (mat thickness)
= 4 x 10^3 m^3 x 10^6 ($m^3 \rightarrow cm^3$) = 4 x 10^9 cm^3

Mat weight = 4 x 10^9 cm^3 x 1 g/cm^2 (assuming a mat density of 1 g/cm^2)
= 4 x 10^9 g or 4,000 tonnes (1 tonne = 1 metric tonne or 1.1 short US tons)

Weight of dry organic matter in mat = 4,000 tonnes x 0.1 (assuming 90% water in mat) = 400 tonnes dry weight

Nitrogen content of mat = 400 tonnes x 0.05 (assuming 5% of dry wt. is N)
= 20 tonnes of N in the mat

In lake survey in September 2008 mats in oxygenated water = 100% coverage; those in low oxygen areas = 25% and mat was half as thick = (0.25 x 0.5) x 20 = 2.5 tonnes

Thus remaining mat N increase attributable to aeration = 17.5 metric tonnes or
~ 19.3 US short tons of N

7.0 NITROGEN FIXATION ESTIMATES: BLUE-GREEN ALGAE AND HETEROCYTES

7.1. RATIONALE

It is possible that N_2-fixation may increase if nitrogen in the lake declines. If N_2-fixation becomes a major source of N for Lake Elsinore, any beneficial aeration-induced declines in N-flux from the sediments (denitrification & benthic felt increases) might be negated. Aeration would then produce a lower or perhaps no net N-offset. This poses an intractable problem for lake management. If, because of N_2-fixation, no net N-offset occurs due to aeration then other offsets are needed (e.g., a denitrifying constructed wetland) or a makeup water with a lower N-level than the lake (e.g., nitrate-free well water, if available).

Typically algae in lakes in semi-arid climates are growth limited by nitrogen and this may be the case at Lake Elsinore, despite the alterations in the lake and watershed due to farming and other developments. Certainly, nitrate in the Elsinore water is low in the spring-fall growth season (below detection, ~ < 50 ug/L, Fig. 1) while total phosphate is abundant (~100-800 ug/L), especially considering that 10 times more N than P is required for algae growth. Studies by the Professor Michael Anderson at the University of California at Riverside before the aeration project show that large amounts of ammonia (100-1,500 ug/L) were released from the sediments when oxygen was depleted (Fig 1). This amount of ammonia would often supply the needs of algae in summer and suppress N_2-fixation. The reduction in this sediment ammonia release is part of the N-offset for the proposed lake aeration system.

Nitrogen fixation is the opposite of denitrification and also requires anoxic conditions. It is the process whereby atmospheric N_2-gas is fixed into ammonia and amino-compounds inside tiny cells of blue-green algae. In lakes and reservoirs N_2-fixation is restricted to blue-green algae that produced special cells called heterocytes (formerly heterocysts) when the cellular N:C ratio falls to critical levels because nitrogen is in short supply. The heterocyte is formed from a normal vegetative cell and forms thicker cell walls that reduced the diffusion of oxygen into the heterocyte. In addition the heterocyte loses the photosystem I part of photosynthesis so is not longer able to split water and release oxygen inside the cell. Finally, the rate of respiration increases dramatically inside the heterocyte mopping up any oxygen that does enter the cell. The increased respiration requires more energy and the process of N_2-fixation requires enormous amounts of energy compared with all other cell processes so adjacent vegetative cells cooperate with the heterocyte by sending simple sugars. In return the nearby vegetative cells receive nitrogen in the form of amino-sugars from the heterocyte.

The net result is that the heterocyte provides the anoxic site in the algae that allows nitrogenase to fix N_2 in the otherwise well-oxygenated algal cell which carries out photosynthesis. Oxygen irreversibly denatures the nitrogenase enzyme (Murray et al., 1984). Ammonia acts on the cells that lie adjacent to the heterocyte and supplies them with N. At this point they cease supplying the heterocyte with sugar energy so respiration stops and oxygen invades the cell. The heterocyte then stops fixing N_2. Heterocytes can easily be identified under the microscope and have been shown to be well correlated with the amount of N_2-fixed measured with the 15N isotope or the acetylene reduction process (Horne & Fogg, 1970; Horne et al., 1972; Horne & Galat, 1985).

Even small quantities of ammonia irreversibly suppress N_2-fixation so as indicated in the last paragraph it was probably quantitatively insignificant in Lake Elsinore prior to aeration due to the large fluxes of ammonia from the anoxic sediments. Evidence for the lack of N_2-fixation is that few blue-green algae that possess heterocytes have been reported. However, little systematic work has been done on the algal species in Lake Elsinore let alone the presence of heterocytes.

Over the last decade the kind of dense blue-green algal scums that are characteristic of eutrophic lakes have not been common. Such scums often are due to algae with heterocytes so few algae capable of fixing N_2 have grown in Lake Elsinore in recent years. This is not surprising since the algae in Lake Elsinore are only occasionally growth-limited by nutrients. The lake sediments release large amounts of phosphate and ammonia during the summer growth season and these nutrients rapidly reach the surface waters and eliminate any need for N_2-fixation. In Lake Elsinore, the main blue-green alga, *Oscillatoria*, has no heterocysts and surface water dissolved oxygen is high. Since heterocytes are few overall, substantial amounts of N_2-fixation in Lake Elsinore seem unlikely. However, aeration may decrease the amounts of ammonia and nitrate in the surface waters. In that case some N_2-fixation may occur. The third N-offset project enquires into that possibility.

7.2. METHOD DEVELOPMENT

N_2-fixation can be measured in several ways including direct assay with samples of water where the normal nitrogen molecules $^{14}N_2$ (^{14}N-^{14}N = atomic mass 28) are replaced with the stable but uncommon and slightly heavier isotope $^{15}N_2$ (^{15}N-^{15}N = atomic mass 30). After incubation the samples are analyzed for the mixed molecule (^{15}N-^{14}N = atomic mass 29) using an isotope mass spectrometer. Although an ideal method it is technically difficult and requires a large amount of costly equipment (Horne & Fogg, 1970). A simpler, somewhat indirect method replaces $^{15}N_2$ with acetylene (C_2H_2) which is similar in size and charge to N_2. The nitrogenase enzyme mistakes acetylene for N_2 and produces ethylene which is rather easier to measure but still requires a gas chromatogram (Horne et al., 1972, 1985). Because heterocytes are almost always associated with N_2-fixation in lakes the measurement of heterocytes provides an easier way of estimating if N_2-fixation is important in Lake Elsinore now that aeration has been established.

Heterocyte counts. Samples were collected in two ways; with a net and with a whole surface water sample. Nets sample large volumes of water so can detect quite rare algae. However, the net method only collects larger forms or colonies. Normally whole water samples range from 100 mL to a liter and so may miss uncommon algae. Different nets were used ranging from 64 to 130 um mesh to collect larger blue-green algae colonies and large filaments characteristic of the main potential N_2-fixers in freshwater. Samples were taken at each station with an ~ 100 m long surface horizontal tow. Sub-samples of ~ 100 mL were collected of the concentrate, taken to the lab and iodine added as a preservative. Whole water samples of 1 L were collected from the surface water and preserved in the same way.

Algal identification. Even though it is very unlikely that any planktonic blue-green algae that do not possess heterocytes will show N_2-fixation in fresh water, there is a possibility that some

N_2-fixation could occur at night or within thick felts of algae (as on seashores). Thus the main blue-green algae collected were examined and species identification attempted.

7.3. Results

7.3.1 Algal identification: dominant algae

The water in Lake Elsinore in summer-fall 2008 was, as usual, green in color with low water clarity (Secchi disc depth ~ 0.5 m). Since the lake is not muddy or stained with humic matter (dystrophic), the low clarity must be due to a large amount of algae. Under such conditions the algae is said to form an algae "bloom." No obvious scums or individual large colonies of blue-green algae were observed despite the fact that such species are common in many eutrophic lakes. It should be noted that the closest water to Lake Elsinore is a small reservoir supplied with treated effluent. This reservoir is a popular fishing spot. When examined in 2006 this reservoir had a similar green color with low water transparency and also does not show scums or large colonial blue-green algae.

The alga most common in Lake Elsinore in summer 2008 was tentatively identified to genus as *Pseudanabaena*. Similar forms that could be present included *Limnothrix* and *Planktothrix* (formerly *Oscillatoria*) which commonly form blooms in lakes. Nowadays the thinner planktonic forms such as *Limnothrix* and *Planktothrix* have been separated from the larger, often attached or benthic species which remain as *Oscillatoria*. None of these genera have heterocytes and despite its name *Pseudanabaena* which resembles the name *Anabaena* (a common N_2-fixing genus) *Pseudanabaena* is not normally able to fix N_2 in the planktonic environment.

7.3.2. Heterocyte-bearing blue-green algae

Two other blue-green algal genera were present in Lake Elsinore in July 2008. They were both much less common than *Pseudanabaena* with a ratio of about 1 filament of each type to 30 filaments of *Pseudanabaena*. They were tentatively identified as *Anabaenopsis* (flat curved form with fat cells and round terminal heterocytes at both ends of the filament) and *Cylindrospermopsis* (spiral coiled with pointed heterocytes at both ends of filaments 80 um long if stretched out).

Most importantly for this project, the presence of heterocytes indicates that active N_2-fixation was occurring at some time during the summer. The heterocyte-bearing blue-green algae were sub-dominants and relatively uncommon (~ 5 % of the total blue-green algae) so the magnitude of the N_2-fixed will be relatively small.

7.4. Toxicity of the blue-green algae identified.

Pseudanabaena is a native species and is known to form blooms in freshwaters of various water quality levels throughout the world. *Cylindrospermopsis* may be an introduced species; at least it has shown great increases in abundance in some lakes and reservoirs over the past few years. However, often these increases are due to changes in conditions that make a formerly rare species more common. *Anabaenopsis* is apparently a native form. *Pseudanabaena* and

Anabaenopsis can produce a dermal irritant and a hepatotoxin (liver damage compounds, normally cyclic peptides) but not neurotoxins (NALMS, 2007). *Cylindrospermopsis* can produce dermal irritants, hepatotoxins and neurotoxins. In general, hepatotoxins cause chronic illness and it is often similar in symptoms to alcohol. Neurotoxins are much more toxic and can kill within minutes. It should be noted that the presence of these blue-green algae and the detection of some toxins does not necessarily mean that the water is dangerous for human contact. There is only one case of neurotoxin fatality in the US and that was in a golf course pond following copper sulfate treatment which releases the toxin from the algal cells. However, thousands of hogs, cows, dogs and horse die each year from ingesting scummy water that generally contains *Anabaena*. Several immuno-compromised people have been killed by hepatotoxins in hospitals where the water supply became contaminated with blue-green alga products but there is little evidence of acute effects for the general public. Caution is thus needed in interpreting the results of the findings made in this report. The amount of toxin per algal cell apparently varies greatly. The USEPA provides no guidelines for the use of lakes with blue-green algae but the World Health Organization has provided guidelines for the cell numbers of some genera of blue-green algae and for toxins (if measured). Some countries (UK, Australia, and Canada) also have issued guidelines but enforcement is generally voluntary as it is with swimming beaches that show elevated levels of bacteria or protozoa.

8.0 REFERENCES

Anderson, M. A. 200. Internal Loading and Nutrient Cycling in Lake Elsinore Final Report to Santa Ana Regional Water Quality Control Board, Riverside CA.

Anderson, M. A. 2003. Water Quality Model.

Anderson, M. A. & R. Lawson. 2005. Continuation of recycled water and aeration monitoring at Lake Elsinore. Report to LESJWA, Riverside CA. Sept. 2005.

Anderson, M. A. & V. Nascimento. 2004. Results from the Lake Elsinore recycled water monitoring project. Report to LESJWA, Riverside CA. Aug. 2004

Blevins, D. W. 1998. Hydrology and cycling of nitrogen and phosphorus in Little Bean Marsh: a remnant riparian wetland along the Mississippi River in Platte County, Missouri, 1996-97.

Beutel, M. W. & A. J. Horne. 1999. A review of the effects of hypolimnetic oxygenation on lake and reservoir water quality. *J. Lake & Reservoir Management.* **15**: 285-297.

Brettar I. & G. Rheinheimer. 1992. Influence of Carbon Availability on Denitrification in the Central Baltic Sea. *Limnology & Oceanography* **37**: 1146-1163.

Christensen, P. B. & J. Sorensen. 1986. Temporal variation of denitrification activity in plant-covered, littoral sediment from Lake Hampen, Denmark. *Applied & Environmental Microbiology* **51**: 1174-1179.

Conley, D. J., H. W. Paerl, R. W. Howarth, D. F. Boesch, S. P. Seitzinger, K. E. Havens, C. Lancelot and G. E. Likens. 2009. Controlling eutrophication: Nitrogen and phosphorus. *Science* **323**: 14-16.

Dalsgaard, T., D. E. Canfield, J. Petersen, B. Thamdrup & J Acuna-Gonzalez. 2003. N2-producton by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. Nature **422**: 606-608.

Fogg, G. W., W. D. P. Stewart, P. Fay & A. E. Walsby. 1973. *The Blue-Green Algae*. Academic Press, London. Pg 198.

Horne, A. J., & G. E. Fogg. 1970. Nitrogen fixation in some English lakes. *Proc. Roy. Soc. Lond. B.*, **175**: 351-366.

Horne, A. J. & C. R. Goldman. 1972. Nitrogen fixation in Clear Lake, California. I. Seasonal variation and the role of heterocysts. *Limnology & Oceanography* **17**: 678-692.

Horne, A. J. & D. L. Galat. 1985. Nitrogen fixation in an oligotrophic, saline desert lake: Pyramid Lake, Nevada. *Limnology & Oceanography* **30**:1229-1239.

Horne, A. J. 1995. The 1993-94 Camanche Reservoir oxygenation experiment: effects on oxygen, nutrients, heavy metals, and turbidity. In Brown & Caldwell Engineers, Walnut Creek "Report on Operation 1993/94" for East Bay Municipal Utilities District, Oakland, CA December 1995". approx. 200 pp. + Appendices.

Horne, A. J. 2000. Potential value of constructed wetlands for nitrate removal along some large and small rivers. *Ver. Int. Ver. Limnology.* **27**: 4057-4062.

Horne, A. J. & M. L. Commins. 1997. Hypolimnetic oxygenation and aeration: theoretical considerations of reduction of nuisance algae in Upper San Leandro Reservoir by reducing nutrients through hypolimnetic oxygenation or aeration. Report to East Bay Municipal Utilities District. November 1997. 19 p.

Horne, A. J., R. Roderick-Jones & C. Toms. 2003. The 2002 Oxygen Bubble Plume Hypolimnetic Oxygenation System in Upper San Leandro Reservoir: Effectiveness for Internal Nutrient Load Reduction, Effect on Benthic Blue-Green Algae and Potential to Reduce Taste and Odor Causing Blue Green Algae. Report to East Bay Municipal Utilities District, Oakland, CA, ~ 50 p. June 2003.

Horne, A. J., J. Kane, K. Elkins & S. Stoller. 2003. Beneficial use impairment in Bridgeport Reservoir: Final limnological report on nutrient loading, eutrophication, Twin Lakes, proposed numeric target and active reservoir management options. Rept. To Lahontan Regional Water Quality Control Board, S. Lake Tahoe, Summer 2003. 97 p.

Horne, A. J. and others (in prep.). Hypolimnetic oxygenation 1-V. A series of five papers on the effects of oxygenation on Camanche Reservoir. To be submitted to J. Lake and Reservoir Management.

Jacinthe, P-A. 2006. Spatio-temporal variability of nitrogen dynamics in a freshwater reservoir. Denitrification modeling across terrestrial, freshwater and marine systems. Workshop, Millbrook NY.

Jean-Pierre, R., A. Sweerts, D. De Beer, L. P. Nielsen, H. Verdouw, J. C. van den Heuvel, Y. Cohen & T. E. Cappenberg. 1990. Denitrification by sulfur-oxidizing Beggiatoa spp. Mats in freshwater sediments. *Nature* 344: 762-3.

Jones, J. G. & H. E. Jones. 1986. Benthic filamentous bacteria. pp. 375-382 in F. Megusar & M. Gantar (eds.), *Perspectives in Microbial Ecology*. Slovene Soc. Microbiol. Ljubljana, Yugoslavia.

Kadlec, R. H. & S. D. Wallace. 2009. *Treatment Wetlands* 2nd ed. CRC Press, Boca Raton, Florida. 1016 p.

Liikanaen, A., E. Ratilainen, S. Saarnio, J. Alm, P. J. Martikaninen & J. Silvola. 2003. Greenhouse gas dynamics in boreal, littoral sediments under raised CO_2 and nitrogen supply. *Freshwater Biology* **48**: 500-511.

Liikanen, A., L. Flojt & P. Martikainen. 2002. Gas dynamics in eutrophic lake sediments affected by oxygen, nitrate and sulfate. *J. Environmental Quality* 31: 338-349.

Mengis, M., R. Gachter & B. Wehrli. 1997. Nitrogen elimination in two deep eutrophic lakes. *Limnology & Oceanography* **42:** 1530-1543.

Miller, P. 2007. Lake Elsinore Proposed Monitoring Plan (Order No. R8-2005-0003, NPDES Np. CA 8000027). Responses to questions raised by the Regional Board regarding analysis of proposed Lake Elsinore monitoring program. Memo to Gerald Thibeault, Executive Officer, Santa Ana Regional Water Quality Control Board. Written by A. J. Horne & MWH Consultants for Phillip Miller, Director of Engineering at Elsinore Valley Municipal Water District

Mulholland, P., B. Hall, S. Hamilton, B. Peterson, J. Tank, L. Ashkenas, L. Cooper, C. Dahm, W. Dodds, S. Findlay, S. Gregory, N. Grimm, S. Johnson, B. McDowell, J. Meyer, B. Poole, M. Valett & J. Webster. 2006. Stream nitrate uptake and denitrification rates determined from 15N addition experiments: Preliminary results from the LINX II study. Denitrification modeling across terrestrial, freshwater and marine systems. Workshop, Millbrook NY.

Muller, B., M. Maerki, M. Schmidt, E. G. Vologina, B. Wehrli, A. Wuest & M. Sturm. 2005. Internal carbon and nutrient cycling in Lake Baikal: sedimentation, upwelling and early diagenesis. Global and Planetary Change **46**: 101-124.

Murry, M. A., J. R. Benemann, & A. J. Horne. 1984. Physiological studies of oxygen protection mechanisms in the heterocysts of *Anabaena cylindrica*. *Applied Environmental Microbiol*ogy **47**: 449-454.

MWH. 2006. Third quarter monitoring, 2006. MWH Americas consultants, Arcadia, CA.

Oh, S-E, K-S Kim, H-C Choi, J. Choi & IS Kim. 2000. Kinetics and physiological characteristics of autotrophic denitrification by denitrifying sulfur bacteria. Water Science and Technology 42: 59-68.& D. T. Heggie. 2000. Australian Geological Survey Organization Research Newsletter. December. No. 33.

Oremland, R. S., J. Cloern, Z. Sofer, R. L. Smith, C. W. Culbertson, J. Zwhr, L. Miller, B. Cole, R. Harvey, N. Iversen, M.Klug, D. J. Des Marais & G. Rau. 1988. pp. 59-75 in Kelts & Talbot (eds). Lacustrine Petroleum Source Rocks, Geographical Society Sp. Publ. No. 40.

Pind, A., N. Risgaard-Petersen & N. P. Revsbech. 1997. Denitrification and microphytobenthic consumption in a Danish lowland stream: diurnal and seasonal variation. *Aquatic Microbial Ecology* **12**: 275-286.

Reilly, J. 1998. Masters Thesis. California State University.

Romeno. A. H. 2000. pp. 147-151 in A. I. Ladkin & H. A. Lechevalier (eds.) Handbook of Microbiology 2nd ed. CRC Press Boca Raton Florida.

Sietzinger, S., J. A. Harrison, J. K. Bohlke, A. F. Bouman, R. Lawrance, B. Peterson, C. Tobias & G. Van Drecht. 2006. Denitrification across landscapes and waterscapes: a synthesis. *Ecological Applications* **16**: 2064-2090.

Strauss, E. A., W. B. Richardson, J. C. Cavanaugh, L. A. Bartsch, R. M. Kreiling & A. J. Standorf. 2006. Variability and regulation of denitrification in an upper Mississippi River backwater. *J. North Ameican Benthological Society* **25**: 596-606.

Steingruber, S. M., J. Friedrich & R. Gachter. 2001. Measurement of denitrification in sediments with the ^{15}N isotope technique. *Applied Environmental Microbiology* **67**: 3771-3778.

Thomas E. J. & D. E. Weller. 1996. Human Contributions to Terrestrial Nitrogen Flux: Assessing the Sources and Fates of Anthropogenic Fixed Nitrogen. *BioScience*, **46**: 665.

Torbjorn, E. D. & M. Stahl. 2000. The influence of carbon on nitrogen transformations in five wetland soils. *Soil Science America J*. **64**: 1129-1136.

UCMCES. 2007. Measurements of denitrification in aquatic ecosystems: literature review and data report. University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory. November 2005.

Vitousek, P. M., J. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, & G. D. Tilman. 1997a. Human Alteration of the Global Nitrogen Cycle: Causes and Consequences," *Issues In Ecology*: p. 2.

Vitousek, P. M. J. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, & G. D. Tilman. 1997b. *Ecological Applications* **7**: 737-750.

9.0 APPENDICES

APPENDIX A: FULL DETAILS OF DENITRIFICATION IN LAKES FROM LITERATURE

Table A1. Rates of denitrification in natural and polluted open water systems in the world. Mean value shown, values in parenthesis indicate range.

Lake, estuary or wetland	**Denitrification** mg NO_3-N/m^2/d	**Comment**	**Source**
	Mean		
Shallow lakes			
Lake St. George, Ontario	2		Loc lit. Horne, 1995
Soda Lake, NV	2	Eutrophic alkaline lake	Oremland et al,, 1998
Arreso, Denmark	4	Large, shallow & well mixed	
Okeechobee, Florida	9 (max)	Large, shallow & well mixed	
227 Canada	15	Shallow but stratified	Chan & Campbell, 1980
8 lakes in Ontario & New York	26		Rudd et al., 1986
Kvindso, Denmark	34		
Kasumiura-ura, Japan	(3-74)		Yoshida et al., 1979
Boyrup Langso, Denmark	58		Andersen, 1977
Smith, Alaska	90	Shallow but stratified	Goering & Dugdale, 1966
Norrviken, Sweden	100	Shallow but stratified	Tiren et al., 1976
Ramsjohn, Sweden	120		
Review of lakes	20 (3.8-60)		Seitzinger, 2006
Review of 90 lakes	30 (0-166)		UCMES, 2005
Shallow 10 lakes in N. Europe	35 (1-104)		Steingruber
Miss backwater	87		Strauss, 2006
Taihu Lake	172 (94-714)		Skleac
Mean (all)	40		
Mean of low nitrate	**4.3**	a	
Mean (moderate nitrate)	**15**		
Mean high nitrate	130		
Shallow reservoirs			
N. Europe	6.3 (5.6-7)		Steingruber
Eagle Creek res	72-120		Jacinthe,2006

Deep lakes			
2 in Europe	35 (5-60)		Steingruber
Deep Lake Baldegg	72	Deep, more productive Swiss lake	Mengis, 1997
Lake Bailkal	2	Deep oligotrophic lake, very large	Muller, 2005
Streams			
72 USA streams	0-210		Mulholland et at., 2006

[a]. Large difference between potential denitrification & actual measured rate

APPENDIX B: DETAILS OF BENTHIC FELT AND ATP MEASUREMENTS

Appendix Table B-1. Details of ATP associated with benthic felt in the three standard TMDL compliance sampling stations in Lake Elsinore, 23 July 2008. Test of samples from surface mixture of water, sediment and felt. The hypothesis being tested is that the white crust or film of bacteria may increase under oxygenated conditions. ATP data rounded, replicates A-D were averaged from 2 to 4 replicate tests on each extraction. RLU = Relative Light Units read directly from the novaLUM meter.

Site (depth)	Felt observations	Replicate	ATP (RLU)
# 1 (5.5 m)	No white crust	A	2,200
North site		B	9,000
		C	7,200
		D	5,200
		mean	**5,900**
#2 (7 m)	Some white crust	A	39,600
Closest to air		B	15,400
		C	14,500
		D	26,200
		mean	**23,900**
#3 (5.5m)	White crust obvious	A	6,500
South site		B	9,800
		C	10,800
		D	9,300
		mean	**9,100**

Table B-2. Details of ATP measurements in Lake Elsinore benthic felt on 23rd September 2008. The ~ 1 mm thick felt covered the more oxygenated sites and was thinner or patchy on those sites with low DO.

Site	**DO in bottom water (mg/L)**	**ATP (mean RLU) 1st test**	**ATP (mean RLU) 2nd test**
Station A (moderate DO)	2.8		
Core 1 (early collection)		165,414	41,111
Core 2 (early collection)		33,207	28,855
Core 3 (early collection)		52,442	28,418
Mean		*84,000*	*33,000*
Core 1 (later collection)	2.6	nd	
Core 2 (later collection)		184,843	
Core 3 (later collection)		554,550	
Mean		*370,000*	
All high DO (A) means (am + pm)		**198,000**	**31,000**
Station B1 (low DO)	0.3 to 0.5	36,488	16,126
		60,985	30,018
		nd	41,015
Mean		**49,000**	**29,000**
Station B2 (intermediate DO)	1.6	14,905	12,648
		6,383	4,065
		108,896	nd
Mean		**43,000**	**8,000**
Station C (moderate DO)	2.4	12,750	Not detected
Mean		**13,000**	