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**Edmund G. Brown Jr.**  
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**MEMORANDUM**

Date: March 23, 2012

To: File: Laguna de Santa Rosa; TMDL Development and Planning

From: Steve Butkus

Subject: Laguna de Santa Rosa TMDL Linkage Analysis and Loading Capacity Assessment for Total Nitrogen and Ammonia Nitrogen Toxicity

The development of the Laguna de Santa Rosa (Laguna) Total Maximum Daily Load (TMDL) for impairment of beneficial uses due to low dissolved oxygen and high nutrients requires a "linkage analysis" (CSWRCB 2005). A linkage analysis describes the method used to establish the relationship between pollutant loading and instream water quality response. Linkage analyses can vary widely across TMDLs. The basic goal is to describe the process for establishing a linkage between total nitrogen (TN) loads and the instream toxicity due to ammonia nitrogen (NH<sub>3</sub>-N). This memorandum serves to document the relationship between TN and NH<sub>3</sub>-N concentrations and to estimate the loading capacity required to meet the water quality objective for instream toxicity due to NH<sub>3</sub>-N concentrations.

**BACKGROUND**

In 1995, a TMDL addressing nitrogen, ammonia, and dissolved oxygen in the Laguna was completed by the North Coast Regional Water Quality Control Board (Regional Water Board) (Morris 1995). The TMDL took the form of the *Waste Reduction Strategy for the Laguna de Santa Rosa* (Waste Reduction Strategy) and it addressed the reduction of nitrogen loading from point and nonpoint sources.

The Laguna was removed from the 1998 Section 303(d) List for ammonia/nitrogen. The ammonia and nitrogen delisting was due to the approval of the TMDL (i.e., Waste Reduction Strategy) by the U.S. Environmental Protection Agency (USEPA) in May 1995. Because of continued water quality problems due to eutrophication, the USEPA placed the Laguna on the 2002 Section 303(d) List as impaired for excess phosphorus and nitrogen. The USEPA concluded that the nitrogen and phosphorus levels found in the Laguna far exceed the levels associated with excessive aquatic plant growth, that

the water quality standard for biostimulatory substances was violated, and thus it would be necessary to develop nitrogen and phosphorus specific TMDLs.

During the development of the Laguna TMDL for biostimulatory substances, Regional Water Board staff have determined that although both nitrogen and phosphorus contribute to short-term algal productivity, the control of phosphorus is required to reduce algal biomass (Butkus 2012). However, excessive nitrogen loading can contribute to instream toxicity through conversion to the ammonia form of nitrogen. The term total nitrogen refers to all forms of nitrogen: nitrate, nitrite, ammonia, and organic. Ammonia-nitrogen, a major component of the nitrogen cycle, is formed by chemical and bacterial decomposition or breakdown of animal wastes, principally urea and other protein-bearing materials.

## **LINKAGE ANALYSIS**

In water, ammonia is measured as total ammonia-nitrogen and exists in either an ionic state or unionized state. It is the unionized form that is toxic to fish and aquatic life. The percentage of measured total ammonia-nitrogen which exists in the toxic unionized ammonia form is increased when the pH or water temperature increase. Since total nitrogen may contribute to ammonia nitrogen, high nitrogen concentrations provide the potential for high ammonia concentrations. High ammonia concentrations provide the potential for high unionized ammonia concentrations. Because of the nature of the nitrogen sources, we expect that reductions in total nitrogen will also result in reductions in unionized ammonia and instream toxicity.

The TMDL developed in 1995 applied the criterion for toxicity that existed at the time. The USEPA criterion of 0.025 mg NH<sub>3</sub>-N/L for the unionized form of ammonia was used as the basis establishing the nitrogen loading capacities. A loading capacity was established for both TN and total NH<sub>3</sub>-N. The USEPA criterion was converted to total NH<sub>3</sub>-N based on a formula that used critical condition values of temperature and pH. The TMDL established a total NH<sub>3</sub>-N concentration limit of 0.5 mg NH<sub>3</sub>-N/L. The TN concentration limits were based on an estimate of the percent of total NH<sub>3</sub>-N in TN. The TMDL estimated that 13% of TN was in the form of total NH<sub>3</sub>-N. Therefore, the TMDL established a TN concentration limit of 3.7 mg-N/L. Estimates of flow were applied to the concentration-based limits to derive seasonal TN and NH<sub>3</sub>-N loading capacities at four (4) Laguna locations: Stony Point Road, Occidental Road, Guerneville Road, Trenton-Healdsburg road.

Water quality data collected over time from numerous agencies were combined for an assessment of trends in NH<sub>3</sub>-N and TN concentrations and loads. Water quality data was compiled for the four (4) locations in the Laguna identified with TMDL goals from the following studies: Otis (1990), NCRWQCB (1992), Church and Zabinsky (2005), Sloop et al. (2007), and NCRQWCB (2008). Figures 1-3 presented plots of TN concentration, total NH<sub>3</sub>-N concentrations, and the percent of total NH<sub>3</sub>-N to TN. Visual observation of these time series plots suggested that concentrations of both TN and total NH<sub>3</sub>-N are trending down over time since the early 1990's. However, the

percentage of total  $\text{NH}_3\text{-N}$  to TN does not appear the change over time. Most of the measurements appear to be below the 13% assumed in the 1995 TMDL (Figure 3).

The compiled water quality data were used to evaluate the relationship between TN and total  $\text{NH}_3\text{-N}$  concentrations measured in the Laguna. The data were fitted to equations using simple least squares linear regression constraining the intercept to zero. By constraining the intercept the resulting coefficients represent the percent of total  $\text{NH}_3\text{-N}$  to TN. Table 1 shows the results of the linear regressions. All regressions were statistically significant but explained only about half of the variance overall. The fitted equations show the percent of total  $\text{NH}_3\text{-N}$  to TN ranges from 8.1% to 20%. The 95<sup>th</sup> percentile is also presented in Table 1 for use in developing the TMDL margin of safety. Figure 4 presents the nitrogen concentration data with the regression equation lines. The 1995 TMDL assumption of 13% is also shown for comparison to the equations derived from instream measurements. The overall Best Fit equation represents a percent of total  $\text{NH}_3\text{-N}$  to TN of 16.8%.

### **Changes in Nitrogen Concentration over Time**

The measured Laguna TN and total  $\text{NH}_3\text{-N}$  concentration data were compiled for all four TMDL attainment locations into three time periods.

- 1985 to 1994: This period represents the Laguna prior to the implementation of the Waste Reduction Strategy
- 1995 to 2000: Monitoring during this period captures the effect of operational improvements at the City of Santa Rosa's Laguna Wastewater Treatment Plant and improvements in waste storage and disposal activities at local dairies
- 2001 to 2010: During this period implementation of the Waste Reduction Strategy continued in a scaled-down fashion.

Figures 5 - 7 presents box plots depicting the distribution of these data for each time period. Table 2 presents descriptive statistics of the data depicted in the box plots. Inspection of these distributions shows apparent declines in both TN and total  $\text{NH}_3\text{-N}$  concentrations during the TMDL implementation years (i.e., 1995-2000). However, the concentrations appear to have increased recently. The measured percentage of total  $\text{NH}_3\text{-N}$  to TN also apparently increased as well.

Two statistical tests were applied to the measured Laguna TN and total  $\text{NH}_3\text{-N}$  concentration data to evaluate the apparent changes over time observed in the data plots. Statistical hypothesis tests were conducted to evaluate the difference between concentrations measured between sampling dates. Hypothesis tests were conducted at a 95% confidence level (i.e. the probability of *incorrectly* rejecting the null hypothesis with  $\alpha = 0.05$ ).

Non-parametric statistical methods were used for the hypothesis tests. Nonparametric methods are often referred to as *distribution free* methods as they do not rely on assumptions that the data are drawn from a given probability distribution. Commonly

used statistics like mean and standard deviation assume the data set follows a Gaussian (i.e., normal) distribution. Water quality data rarely meet that assumption. Non-parametric methods are more robust due to reliance on fewer assumptions.

The *Mann-Whitney U Test* is a non-parametric test for assessing whether two samples of observations come from the same distribution. The method is also known as the Wilcoxon Rank-Sum test. This statistical test is a nonparametric (i.e., distribution-free) inferential statistical method. The test null hypothesis is that the two samples are drawn from a single population. The test is similar to performing an ordinary parametric two-sample *t* test, but is based on ranking the data set.

The *Mann-Whitney U Test* was used to assess if there was a measureable difference in concentrations between identified time periods (Table 3). The results showed that there was a significant decline in TN and total NH<sub>3</sub>-N concentrations between the pre-TMDL and TMDL implementation periods. However, those gains in improved water quality may have been lost as there has been a significant increase in TN and total NH<sub>3</sub>-N concentrations in the last decade. The percent of total NH<sub>3</sub>-N to TN was much lower than was assumed in the 1995 TMDL (i.e., 13%) for the pre-TMDL and TMDL implementation periods. There was no significant difference found in the percentage between these periods. However, the percentage has increased significantly in the last decade to near the original assumption of 13 percent.

The *Seasonal Kendall Test* for trend was developed by the U.S. Geological Survey and has become the most frequently used non-parametric test for trend. The Seasonal Kendall test addresses trends for individual seasons of the year. The test then combines the individual seasonal results into one overall test that detects monotonic trends over time (Helsel et al. 2005). The test accounts for serial correlation in the time series. Serial correlation violates the assumption of independence of data (i.e., there is no short-term correlation between subsequent measurements). When serial correlation occurs, trends may be observed that do not actually exist (Hirsch and Slack 1984).

The *Seasonal Kendall Test* for trend was applied to the data collected at the Laguna 1995 attainment locations over time (Table 4). The trend test showed that TN concentrations declined significantly over time at all locations except at Stony Point Road. However, the NH<sub>3</sub>-N concentrations showed no significant trend with time. These results support the conclusion derived from the *Mann-Whitney U Test* that gains in improved water quality may have been lost as there has been a significant increase in total NH<sub>3</sub>-N concentrations during the last decade. The percentage of total NH<sub>3</sub>-N to TN showed an increasing trend at the two downstream locations. However, the two upstream locations showed no significant change over time. Therefore, overall no significant trend was detected in the percentage with time.

These results demonstrate the linkage between instream total NH<sub>3</sub>-N concentrations and TN concentrations. The 1995 TMDL approach of limiting both TN and total NH<sub>3</sub>-N concentrations to control ammonia toxicity resulted in significant reductions in both TN and total NH<sub>3</sub>-N concentrations initially. However, a significant increase in total NH<sub>3</sub>-N concentrations during the last decade has increased the relative percentage of total

NH<sub>3</sub>-N to TN concentrations. Therefore, the application of the same linkage approach used in the 1995 TMDL to derive a loading capacity of TN would require a different percentage.

## **LOADING CAPACITY ASSESSMENT**

The loading capacity is based on the linkage demonstrated between total ammonia-nitrogen and total nitrogen concentrations. Higher ammonia concentrations provide the potential for higher unionized ammonia concentrations and instream toxicity. Since total nitrogen may contribute to ammonia-nitrogen, higher nitrogen concentrations provide the potential for higher ammonia concentrations. Therefore, reductions in total nitrogen are likely to result in reductions in unionized ammonia and instream toxicity.

### **Margin of Safety**

One of the most important elements of a Total Maximum Daily Load (TMDL) is defining a reasonable margin of safety (MOS). The MOS is required by statute to “take into account any lack of knowledge.” It serves to protect beneficial uses from uncertainties in the data or calculations used to link pollutant sources to water quality impairment. Published guidance identifies two approaches for defining a MOS. An *explicit* MOS quantifies an allocation amount separate from other load and wasteload allocations. With an explicit MOS, numeric TMDL targets are established at more protective levels than the analysis results indicate. Explicit MOS can also incorporate reserve capacity for unknown population growth or effects of climate change on water quality. An *implicit* MOS is not specifically quantified but consists of statements of the conservative assumptions used in the analysis approach. A wide range of approaches are used to define an implicit MOS.

The relative percentage of total NH<sub>3</sub>-N to TN concentrations measured at the TMDL attainment locations was used to estimate a loading capacity for TN concentrations. The regression equation derived from all available measurements showed that 16.8% best represented the percentage of total NH<sub>3</sub>-N to TN concentrations. This value is just slightly higher than the 13% assumed for the 1995 TMDL loading capacity. However, these data show high variability with a coefficient of variations ranging from 73% to 301% (Table 2). The regression equation showed that 18.5% represents the upper 95<sup>th</sup> percentile. As an inherent margin of safety, the upper 95<sup>th</sup> confidence level of the regression equation was used to estimate a loading capacity for TN. Therefore, the value of 18.5% of total NH<sub>3</sub>-N to TN concentration was applied to derive the loading capacity of TN concentrations from instream toxicity due to unionized ammonia-N.

The percentage of measured total ammonia-nitrogen which exists in the toxic unionized ammonia form is increased when the pH increases. One must identify a pH value to derive the ammonia-N criterion for waters with salmonids. The USEPA (1999) criterion for acute ammonia toxicity (i.e., Criterion Maximum Concentration) is based on pH and is calculated as follows:  $CMC = 0.275 / (1 + 10^{(7.204 - pH)}) + 39.0 / (1 + 10^{(pH - 7.204)})$ . The

units are in mg-N/L. The CMC is lower for higher pH values. As an inherent margin of safety, the pH value of 9.0 was used to calculate the acute ammonia-N criterion for the Laguna. This value represents the highest pH allowed from the Basin Plan to support beneficial uses. This pH value represents the 99.6<sup>th</sup> percentile value measured from available data collected from the TMDL attainment locations.

### **Target Nitrogen Concentrations**

The acute total ammonia-N criterion was calculated for the Laguna using the maximum measured pH value of 9.0. The resulting instream total NH<sub>3</sub>-N concentration that would protect salmonid beneficial uses against acute toxicity was derived to be 0.885 mg-N/L. This criterion is larger than the total NH<sub>3</sub>-N concentration-based limits in the 1995 TMDL (i.e., 0.500 mg-N/L).

The relative percentage of total NH<sub>3</sub>-N to TN concentrations measured at the TMDL attainment locations was used to estimate a loading capacity for TN concentrations. The percentage of total NH<sub>3</sub>-N to TN concentrations used to estimate a loading capacity for TN was 18.5%. This value represented the upper 95<sup>th</sup> confidence level of the regression equation between total NH<sub>3</sub>-N to TN concentration. The TN concentration that would protect salmonid beneficial uses against acute toxicity was derived to be 4.782 mg-N/L. This criterion is 30% higher than the TN concentration-based limits in the 1995 TMDL (i.e., 3.7 mg-N/L). The criterion is also above the 0.400 mg-N/L criterion that the U.S. Environmental Protection Agency recommends for prevention of eutrophication in lakes (USEPA 2001). Figure 8 shows the concentration-based loading capacities for total nitrogen and ammonia-N across a range of pH values.

### **Target Nitrogen Loads**

The loading capacity for this TMDL will be concentration-based. However, for illustrative purposes, the mass based load allocations were derived to indicate the level of mass reduction needed. The 1995 TMDL derived target loads for both total NH<sub>3</sub>-N and TN using seasonal estimates of the mean stream flow at each attainment location. Applying seasonal estimates of flow addresses the seasonal change in instream ammonia toxicity. Instream toxicity due to ammonia is increased when the water temperature increases. Water temperatures are directly related to the season and amount of stream flow available to disperse thermal loading. However, the application of mean seasonal stream flow is not protective of beneficial uses. The application of mean stream flow does not account for stream flows lower than average. Instream loading capacity has to account for the critical conditions that occur during periods with low stream flow.

The California State Water Resources Control Board has adopted a policy that identified the 7Q10 stream flow as the "critical" condition for receiving water (CSWRCB 2005). The 7Q10 is the average low flow that occurs for seven consecutive days with a statistical frequency of once every 10 years. Critical low stream flows can be calculated for particular seasons. Point source wastewater discharges in the Laguna watershed

are limited by permit to the period from October 1 to May 14 of each year. Loading capacities were derived for two seasons: (1) the period of permitted discharge (October 1 to May 14), and (2) the period without permitted discharge (May 15 – September 30).

The critical low stream flow statistic of 7Q10 was derived from U.S. Geological Survey (USGS) stream gaging stations for each of these two seasonal periods to derive the loading capacities for total  $\text{NH}_3\text{-N}$  and TN (Table 5). Stream flow is not gaged at the Guerneville Road station, only stage. The stream flow at Guerneville Road was estimated by adding measured flows at Occidental Road and Santa Rosa Creek at Willowside Road. This approach assumed that the local drainage in the Laguna reach between Occidental and Guerneville roads is minimal. There are no large tributary inflows between these locations except Santa Rosa Creek. Therefore, the sum of measured stream flows at Occidental Road and Santa Rosa Creek at Willowside Road provided a good estimate of flow at Guerneville Road .

The critical low flow statistic of 7Q10 derived at Stony Pont Road and at Occidental road were both zero flow. The USGS gaging station rating curves have the lowest detectable flow of 0.01 cfs. One-half ( $\frac{1}{2}$ ) of the detectable stream flow (i.e., 0.005 cfs) was used to calculate loading capacities for these two locations (Table 5).

The seasonal critical flows were used with the nitrogen concentration targets to derive the loading capacity at each TMDL attainment location (Table 6). The loading capacities of the Laguna attainment locations at Stony Point and Occidental Road are very low due to the low critical stream flows in these reaches. The loading capacity is the greatest at the Guerneville Road location primarily due to the higher stream flow draining from Santa Rosa Creek just upstream of the bridge. The Laguna reach between Guerneville Road and Trenton-Healdsburg Road loses stream flow to ground water as suggested by the critical stream flow statistics (Table 5). The lower loading capacity at the Trenton-Healdsburg location was due to lower critical stream flows.

## **FINDINGS**

- A statistically significant decline in TN and total  $\text{NH}_3\text{-N}$  concentrations was observed between the pre-TMDL and TMDL implementation periods. However, those gains in improved water quality may have been lost as there has been a statistically significant increase in TN and total  $\text{NH}_3\text{-N}$  concentrations in the last decade.
- The percentage of total  $\text{NH}_3\text{-N}$  to TN showed no statistically significant change over time.
- The 1995 TMDL TN concentration limits were based on an estimate of the percent of total  $\text{NH}_3\text{-N}$  in TN. The 1995 TMDL estimated that 13% of TN was in the form of total  $\text{NH}_3\text{-N}$ . A regression of data subsequently collected at the TMDL Attainment locations shows the actual a percent of total  $\text{NH}_3\text{-N}$  to TN of 16.8%.

- As a margin of safety, a value of 18.5% of total  $\text{NH}_3\text{-N}$  to TN concentration was used to derive the loading capacity of TN concentration, which represents the upper 95<sup>th</sup> confidence level of the regression equation.
- As an inherent margin of safety, the pH value of 9.0 was used to calculate the acute ammonia-N criterion for the Laguna. This pH value represents the 99.6<sup>th</sup> percentile value measured from available data collected from the TMDL attainment locations.
- The loading capacity for this TMDL will be concentration-based. The resulting instream total  $\text{NH}_3\text{-N}$  concentration that would protect salmonid beneficial uses against acute toxicity was derived to be 0.885 mg-N/L. This criterion is larger than the total  $\text{NH}_3\text{-N}$  concentration-based limits in the 1995 TMDL (i.e., 0.500 mg-N/L). The TN concentration that would protect salmonid beneficial uses against acute toxicity was derived to be 4.782 mg-N/L. This criterion is 30% higher than the TN concentration-based limits in the 1995 TMDL (i.e., 3.7 mg-N/L).



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**TABLES**

Table 1. Results of Simple Linear Regressions between Measured Total Ammonia-N and Total Nitrogen.

<b>Attainment Location</b>	<b>Explained Variance (%)</b>	<b>Coefficient</b>	<b>Upper 95th Percentile Coefficient</b>
Stony Point Road	43%	0.125	0.152
Occidental Road	60%	0.200	0.230
Guerneville Road	18%	0.081	0.120
Trenton-Healdsburg Road	40%	0.059	0.076
All Locations Combined	51%	0.168	0.185

Table 2. Descriptive Statistics of Total Ammonia-N and Total Nitrogen during Three Time Periods.

<b>Variable</b>	<b>Statistic</b>	<b>Pre-TMDL 1985-1994</b>	<b>TMDL Implementation 1995-2000</b>	<b>Post-TMDL 2001-2010</b>
Total Nitrogen	Number of Measurements	85	253	42
	Median (mg-N/L)	2.78	1.46	3.24
	Coefficient of Variation (%)	173%	87%	58%
	Percent Above 1995 TMDL Goal (TN < 3.7 mg/L)	39%	12%	38%
Total Ammonia-N	Number of Measurements	139	504	43
	Median (mg-N/L)	0.130	0.100	0.300
	Coefficient of Variation (%)	189%	183%	64%
	Percent Above 1995 TMDL Goal (NH <sub>3</sub> -N < 0.5 mg/L)	24%	7%	26%
Total Ammonia-N to Total Nitrogen (%)	Number of Measurements	85	254	14
	Median (mg-N/L)	8.2%	6.9%	15.5%
	Coefficient of Variation (%)	102%	301%	73%
	Percent Above 1995 TMDL Assumption (NH <sub>3</sub> /TN = 13%)	35%	20%	57%

Table 3. Statistical Test Probability Values for a Difference in Concentrations between Time Periods. *Blue text* indicates a significant difference between the time periods.

Variable	Difference Between Time Periods		
	Period 1985-1994 and Period 1995-2000	Period 1995-2000 and Period 2001-2010	Period 1985-1994 and Period 2001-2010
TN (mg/L)	<0.001	<0.001	0.678
NH3 (mg/L)	<0.001	<0.001	0.015
NH3/TN (%)	0.099	<0.001	0.013

Table 4. Statistical Test Probability Values for a Trend over Time at the 1995 TMDL Attainment Locations. *Blue text* indicates a significant monotonic trend with time.

Location	Total Nitrogen (mg-N/L)	Total Ammonia- (mg-N/L)	Percent Ammonia-N to Total N (%)
Stony Point Road	0.243	0.745	0.679
Occidental Road	0.009	0.202	0.163
Guerneville Road	0.018	0.411	0.048
Trenton-Healdsburg Road	0.027	0.301	0.047
All Locations Combined	0.010	0.479	0.146

Table 5. Critical Low Flow Statistics

Location	USGS Stream Gage ID	7Q10 Stream Flow (cfs)	
		Oct1-May14	May15-Sep30
Stony Point Road	11465680	0.005	0.005
Occidental Road	11455750	0.005	0.005
Guerneville Road	11466500	1.330	0.950
Trenton-Healdsburg Road	11466800	0.461	0.032

Table 6. Ammonia-N and Total Nitrogen Seasonal Loading Capacities

Location	Oct1-May14	Oct1-May14	May15-Sep30	May15-Sep30
	Ammonia-N (lbs/day)	Total Nitrogen (lbs/day)	Ammonia-N (lbs/day)	Total Nitrogen (lbs/day)
Stony Point Road	0.024	0.129	0.024	0.129
Occidental Road	0.024	0.129	0.024	0.129
Guerneville Road	6.349	34.305	4.535	24.503
Trenton-Healdsburg Road	2.201	11.891	0.153	0.825

**FIGURES**

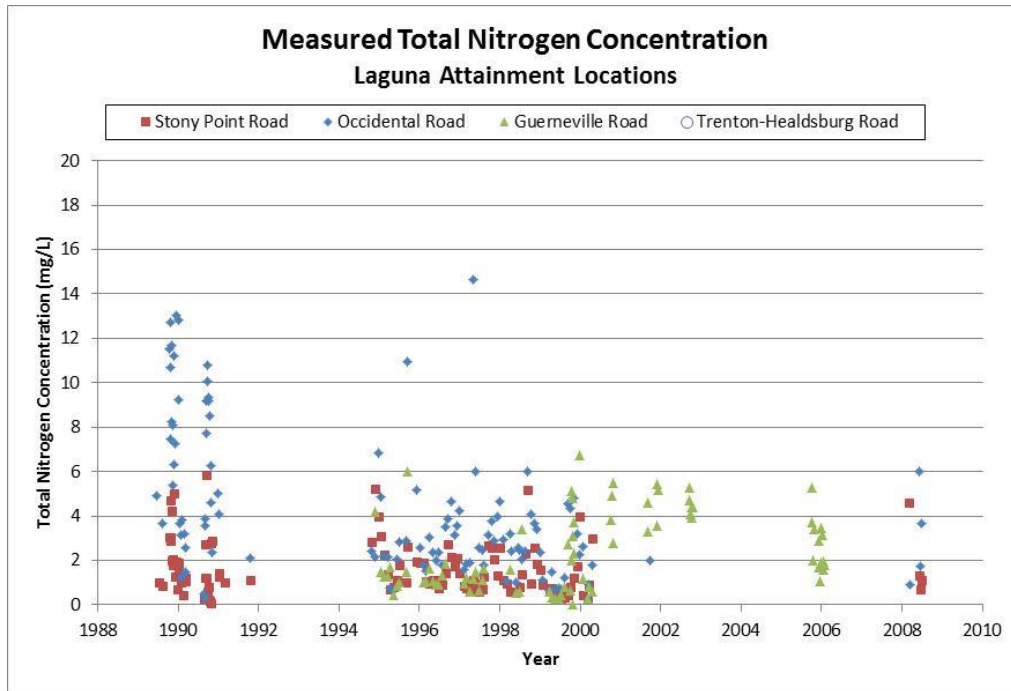


Figure 1. Total Nitrogen Concentrations Measured in the Laguna over time.

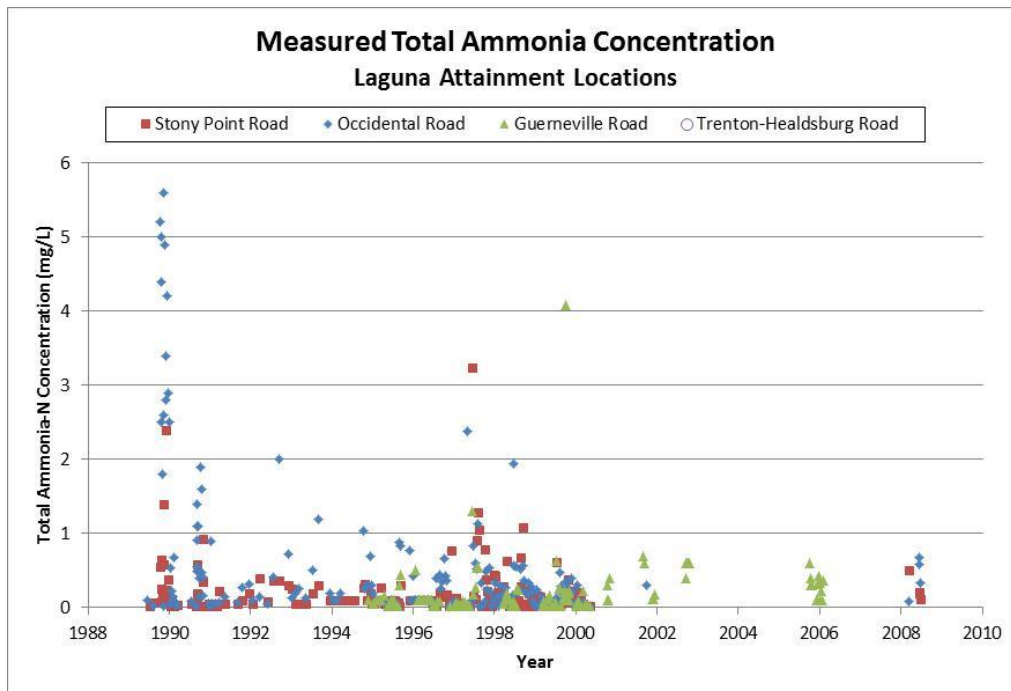


Figure 2. Total Ammonia-Nitrogen Concentrations Measured in the Laguna over time.

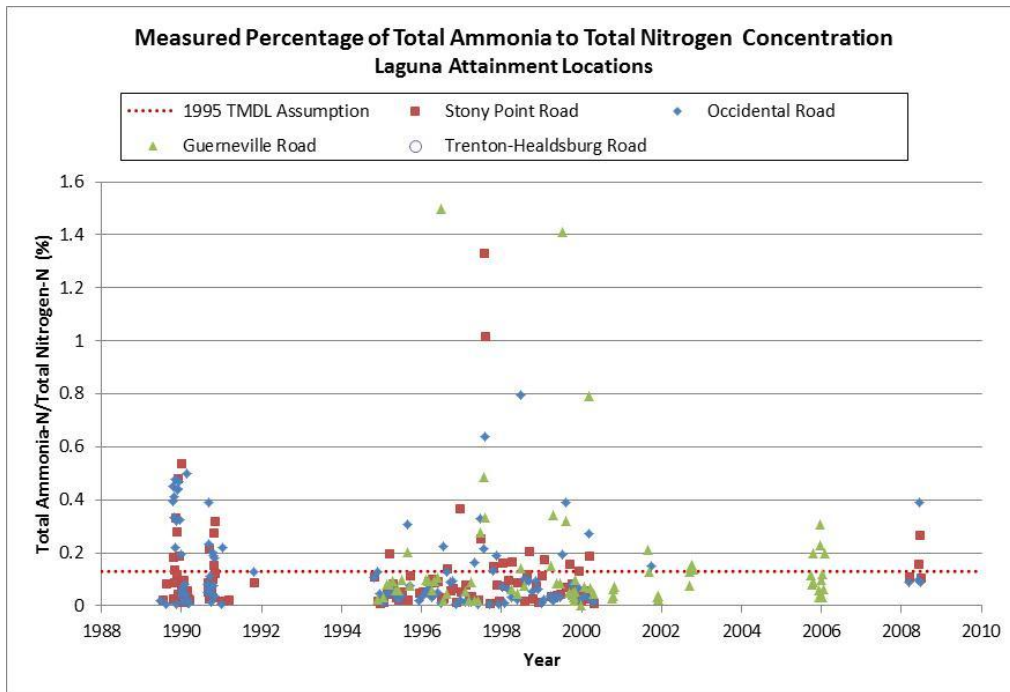


Figure 3. Percent of Total Nitrogen Concentrations present in the form of Total Ammonia-Nitrogen Concentrations Measured in the Laguna over time.

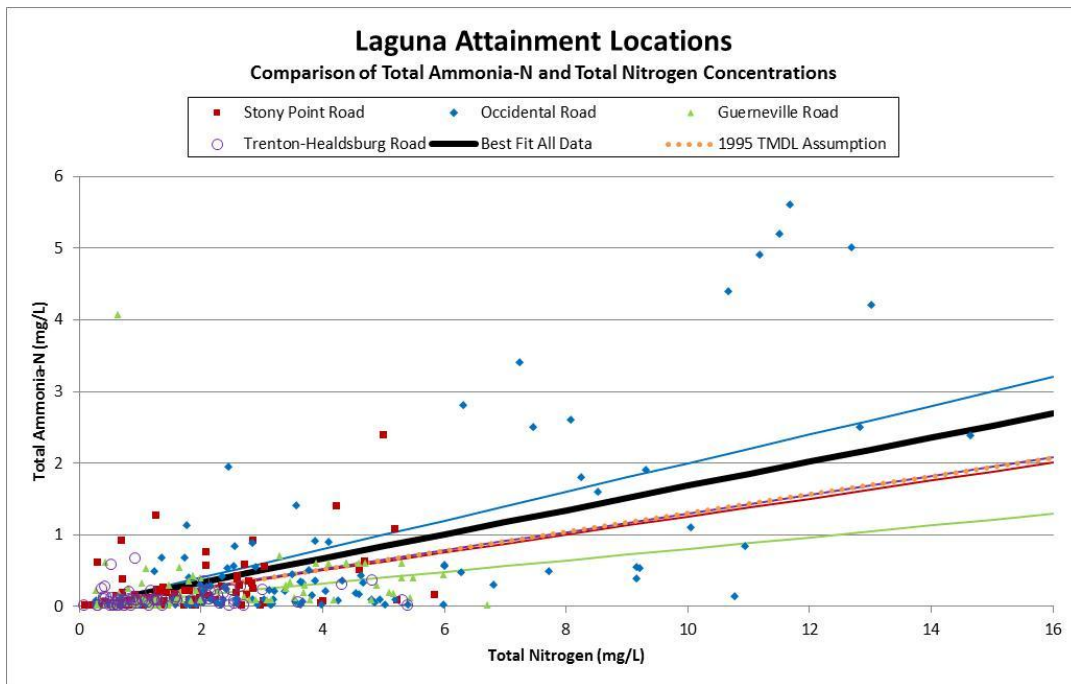


Figure 4. Relationship between Ammonia-Nitrogen and Total Nitrogen Concentrations.

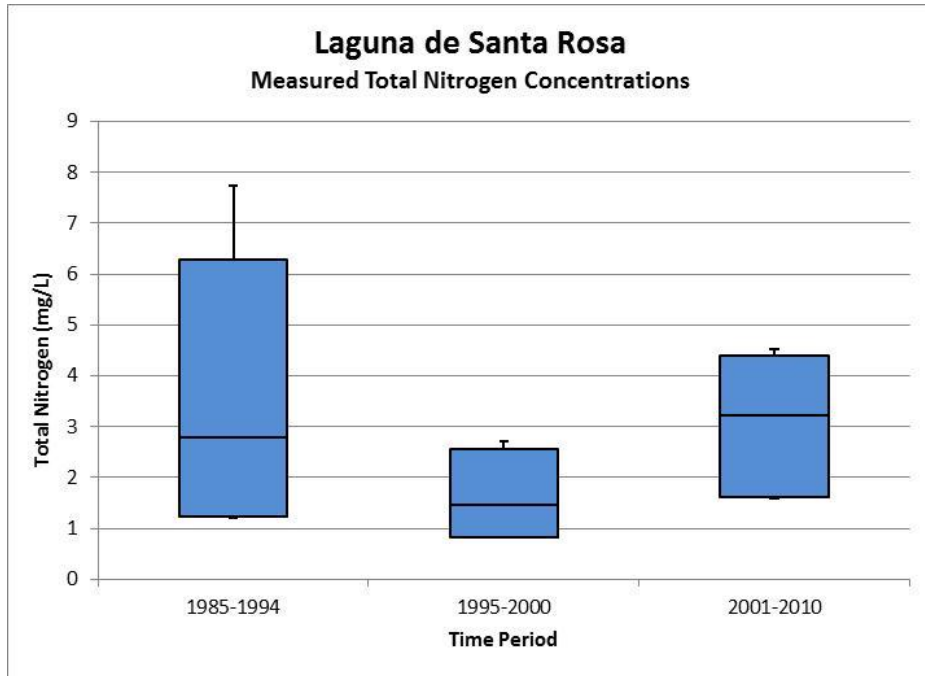


Figure 5. Distribution of Total Nitrogen Concentrations Measured in the Laguna during three time periods.

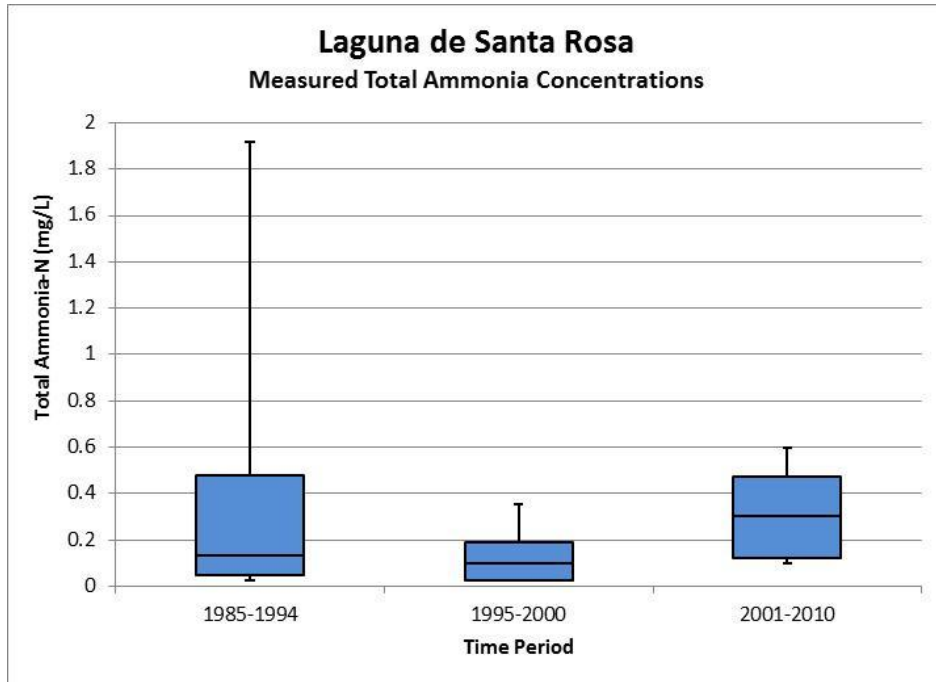


Figure 6. Distribution of Total Ammonia-Nitrogen Concentrations Measured in the Laguna during three time periods.

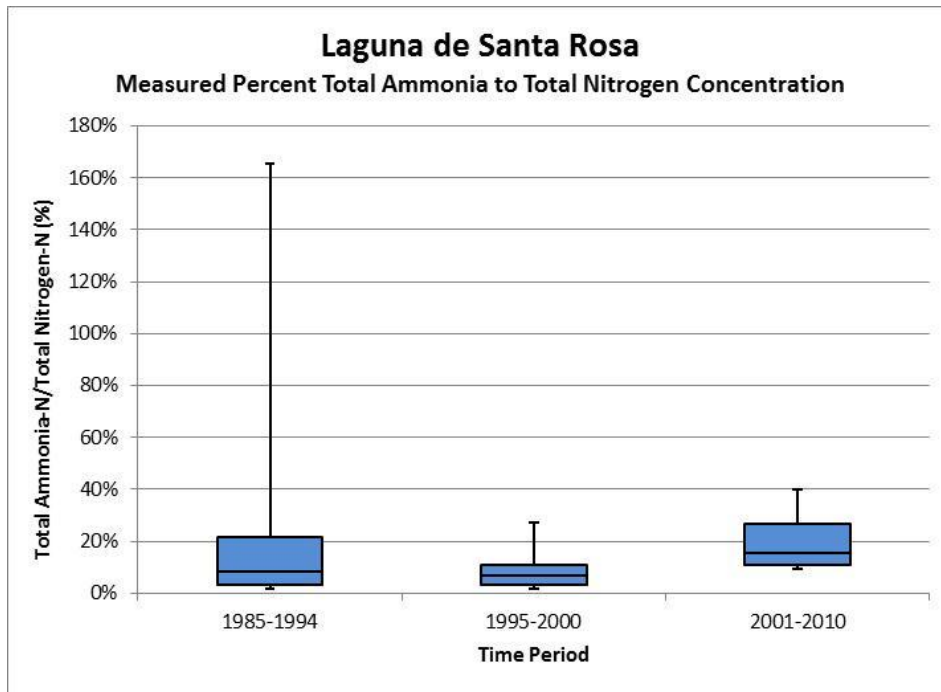


Figure 7. Distribution of Total Nitrogen Concentrations present in the form of Total Ammonia-Nitrogen Concentrations Measured in the Laguna during three time periods.

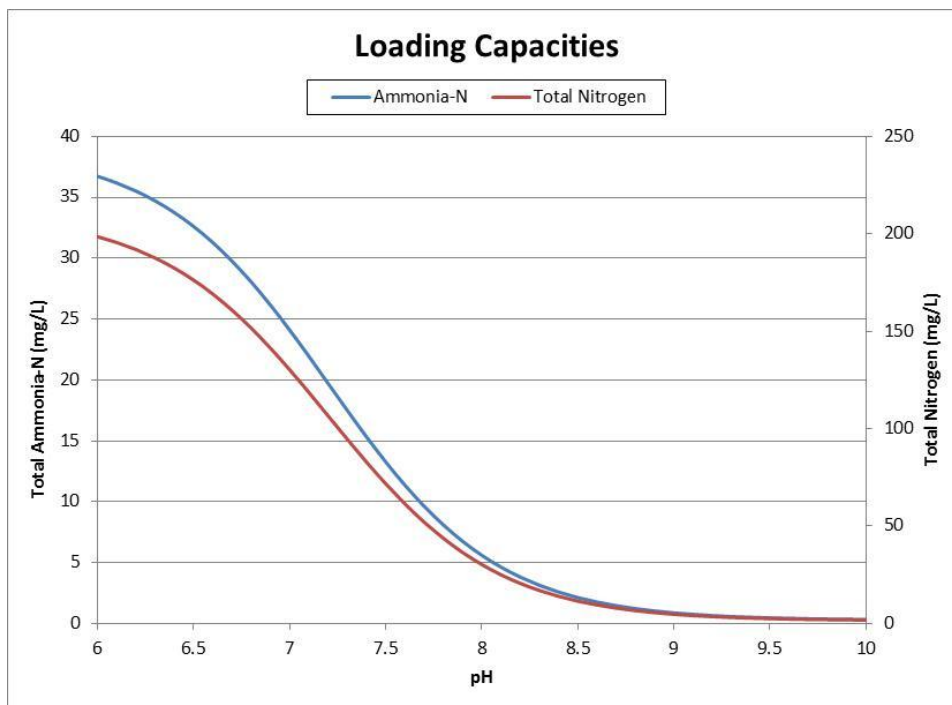


Figure 8. Total Nitrogen and Ammonia-N Concentration-based Loading Capacities across a Range of pH Values.