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Examination of Water Quality in Clear Lake, California for Dreissenid Mussel Suitability

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Executive Summary

RNT Consulting Inc. examined environmental variables in Clear Lake, California to better understand how calcium in combination with pH may or may not limit the population size of dreissenid mussels in that water body. Data obtained from three water quality stations in Clear Lake (Upper Arm, Oaks Arm and Lower Arm) were examined to determine if the lake is susceptible to invasion by dreissenid mussels based on the combination of calcium and pH, two essential water quality parameters affecting shell formation.

From data collected between 2005 and 2011, it was concluded that all three sites in Clear Lake would be able to support long-term dreissenid populations based on pH and calcium levels. In addition to calcium and pH, dissolved oxygen, nutrient and metal concentrations were examined. Dissolved oxygen levels are above the minimum limit (3 mg/L) for supporting dreissenid mussels. Average nutrient and ammonia concentrations were outside the limits for supporting dreissenid mussels, suggesting that a dreissenid population may be affected by these variables, primarily due to the algal blooms these nutrients stimulate. However, we do not draw conclusions regarding the effects of nutrients in Clear Lake on a long-term dreissenid population. Equally, the presence of various heavy metals may or may not affect the success of a dreissenid population. Research has shown that dreissenid mussels bioaccumulate and regulate metals in their tissue, however it is unknown what effect the metal concentrations in Clear Lake would have on a dreissenid population.

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1 Introduction

When examining the suitability of the environment for dreissenid invasion, the level of calcium (Ca) present in the water is of primary concern. Without adequate calcium, introduced adults will not survive and veligers will not develop into reproducing adults. Calcium is essential for the production of shell material in dreissenids. For most mollusks, calcium is obtained from the water and their food. Increases in shell length occur by secretions of calcium carbonate by the outer fold of the mantle edge, and increases in shell thickness occur by secretions from the outer wall of the outer lamella of the mantle. Dreissenids have higher calcium requirements than most other fresh water mollusks. At low calcium levels, adult dreissenids will experience a negative shell growth due to loss of calcium from the shell to the surrounding water. Therefore, unless adequate calcium is present, all environmental parameters other than pH are irrelevant.

The shells of dreissenid mussels are comprised of approximately 40% (dry weight) calcium (Secor *et al.* 1993), primarily in the form of calcium carbonate. The examination of pH is important when determining the suitability of waters for dreissenid survival because the solubility of calcium carbonate increases as pH decreases. Regardless of the presence of adequate calcium for dreissenid growth, if pH is low then mussel shells will become thin and eroded.

Ranges of values for different parameters required for dreissenid survival are shown in Table 1. This table represents a compilation of data by various authors from Europe and North America. The minimum concentration of calcium required for long-term mussel survival is around 15 mg/L. Mackie and Claudi (2010) consider the range of 8 – 15 mg/L of calcium to have little potential for larval development of dreissenid mussels. The minimum pH required for long-term dreissenid survival is approximately 7.5. Claudi *et al.* (2012) found that adult mussels show evidence of calcium loss from their shells at pH levels of 7.3. Calcium loss was accelerated at pH levels of 7.1 and 6.9. Larval survival is unlikely under these low pH conditions.

The examination of the success of dreissenid mussels in different bodies of water has generally focused primarily on important parameters (i.e. calcium, pH, temperature) more-or-less independently of other parameters. Due to the connection between calcium and pH, in particular, we believe it is important to consider these two parameters in concert, primarily at marginal conditions.

This report examines calcium and pH data obtained from 3 water quality stations (Figure 1) located in Clear Lake to determine their suitability for supporting long-term dreissenid populations based on the criteria discussed above. Unlike other studies which have focused on calcium and pH independently, this study examines calcium and pH in combination and the suitability of locations to long-term mussel populations was determined based on a pairing of calcium and pH measurements collected at the same time.

Table 1. Criteria used in determining the levels of dreissenid infestation in the temperate zone of North America and Europe (Mackie and Claudi 2010).

Parameter	Adults do not survive long-term	Uncertainty of veliger survival	Moderate Infestation Level	High Infestation Level
Calcium (mg/L)	<8 to <10	<15	16-24	≥24
Alkalinity (mg $CaCO_3$/L)	< 30	30-55	45-100	>90
Total Hardness (mg $CaCO_3$/L)	<30	30-55	45-100	≥90
pH	<7.0 or >9.5	7.1-7.5 or 9.0-9.5	7.5-8.0 or 8.8-9.0	8.2-8.8
Mean Summer Temperature (°F)	<64	64-68 or >83	68-72 or 77-83	72-75
Dissolved Oxygen mg/L (% saturation)	<3 (25%)	5-7 (25-50%)	7-8 (50-75%)	≥8 (>75%)
Conductivity (µS/cm)	<30	<30-60	60-110	≥100
Salinity (mg/L) (ppt)	>10	8-10 (<0.01)	5-10 (0.005-0.01)	<5 (<0.005)
Secchi depth (m)	<0.1 >8	0.1-0.2 or >2.5	0.2-0.4	0.4-2.5
Chlorophyll a (µ/L)	<2.5 or >25	2.0-2.5 or 20-25	8-20	2.5-8
Total nitrogen (µg/L)	<75 or >750	75-150 or 525-750	150-375	375-525
Total phosphorous (µg/L)	<5 or >50	5-10 or 30-50	15-25	25-35

Figure 1. The location of sites in Clear Lake that were analyzed in this study.

2 Data Analysis

Grab data for three sites (i.e. Upper Arm, Oaks Arm, Lower Arm) in Clear Lake were obtained from the California Department of Water Resources' (DWR) Water Data Library (WDL). Calcium and pH measurements were available at all sites examined for the period of time between March 2005 and July 2011.

2.1 Calcium-pH Time Series Plots

Calcium and pH for the three Clear Lake sites were first plotted against time. On the calcium-pH plots, 12 mg/L of calcium is given as a threshold for possible long-term survival of adult dreissenid mussels, provided the average pH is above 7.8. This conservative calcium value is based on experience from Lake Superior where the pH ranges from 7.9-8.2 but the average calcium level is 13 mg/l (Mackie 2010). Lake Superior has only isolated pockets of dreissenid mussels along the shoreline despite the fact that the first mussels were documented in Duluth Harbor more than 20 years ago.

Furthermore, studies by Nierzwicki-Bauer et al. (2000) documented that adult zebra mussels were able to survive in Lake George water (Ca=12 mg/L, pH=7.15), but the development of veligers failed unless both calcium and pH levels were raised. Lake George has had a minimal level of dreissenid infestation for the last ten years. It is hypothesized that the reason dreissenid mussels survive at all in Lake George is due to limestone outcroppings in various parts of the lake which provide microzones with higher calcium levels.

Hinks and Mackie (1997) tested adult survival, juvenile growth rates and veliger production against different concentrations of calcium, alkalinity, total hardness, chlorophyll and pH by rearing adults and newly settled juveniles collected from Lake St. Clair in water from 16 Ontario lakes. Six of these lakes had mean calcium levels below 8.5 mg/L and mean pH of 8.4 or less. In these low calcium waters all adults died within 35 days, juvenile growth rates were near zero or negative, and no veligers were produced.

The threshold pH on the graphs is given as 7.3. This value is based on experimental data from a study conducted by RNT Consulting Inc. in 2009 (Claudi *et al.* 2012). In the study, it was found that even at high calcium levels (40mg/L) if the pH is depressed to 7.3 mussels begin to show evidence of calcium loss from their shells. At pH levels of 7.1 and 6.9 the calcium loss is accelerated. After 12 weeks, the mortality of adult mussels was greater than 7% at a pH of 7.3, just under 15% at a pH of 7.1, and almost 40% at a pH of 6.9. The mortality in the control group was less than 3%. This experimental evidence is further supported by observations from Lough Allen which has an average calcium concentration of 25 mg/L and an average pH of 7.4 (Lucy *et al.* 2010). Although this lake is connected to lakes which support established zebra mussel populations, mussels have only casually been observed in Lough Allen suggesting this body of water may be unable to support a long-term population of dreissenids due to its low pH.

2.2 Pie charts

To quantify the ability of sites with marginal calcium and/or marginal pH to support dreissenids, both calcium and pH values were examined in combination to determine if the interaction of the two parameters was important for creating favorable conditions for established mussel populations. The calcium and pH results for each sample collected were examined and then assigned to one of nine categories based on their paired calcium and pH values.

The minimum limits of calcium and pH were set at 12 mg/L and 7.3, respectively. Samples having low calcium and/or low pH were deemed to represent conditions unsuitable for supporting dreissenids. Samples with calcium concentrations above 15 mg/L and pH levels above 7.8 were considered favorable for dreissenid mussels. Samples with marginal calcium (12 mg/L < Ca ≤ 15 mg/L) and/or marginal pH (7.3 < pH ≤ 7.8) were considered potentially able to support mussels. Following is a description of each combination of calcium and pH values and their corresponding suitability for dreissenid survival.

Low Calcium/Low pH: Samples with calcium below 12 mg/L and pH below 7.3 have conditions that are unsuitable for dreissenid mussels.

Low Calcium/Marginal pH: Samples with low calcium (<12 mg/L) and marginal pH (7.3<pH≤7.8) would be unable to support dreissenids due to insufficient calcium, regardless of pH levels above the minimum limit.

Low Calcium/High pH: Samples with low calcium (<12 mg/L) and high pH (>7.8) would be unable to support dreissenids due to insufficient calcium, regardless of pH levels above that deemed favorable for mussels.

Marginal Calcium/Low pH: Although marginal calcium levels (12 mg/L < Ca ≤15 mg/L) may be sufficient for adult mussels to survive, the low pH is expected to prevent established dreissenid populations. Samples with marginal calcium and low pH are unlikely to support mussels.

High Calcium/Low pH: Regardless of high calcium levels (>15 mg/L), a low pH would not support dreissenid mussels. Therefore samples with high calcium and low pH are considered unable to support long-term dreissenid populations.

Marginal Calcium/Marginal pH: Samples with marginal calcium (12 mg/L < Ca ≤15 mg/L) and marginal pH (7.3 < pH ≤7.8) may be able to support adult mussels. These conditions, however, are unsuitable for veligers.

Marginal Calcium/High pH: Since dreissenid success in marginal calcium levels (12 mg/L < Ca ≤15 mg/L) may be enhanced by high pH (>7.8), samples with marginal calcium and high pH are considered potentially able to support mussels.

High Calcium/Marginal pH: Adult dreissenids and veligers are believed to favor high calcium concentrations (>15 mg/L) however their success may be reduced under marginal pH conditions (7.3 < pH ≤7.8). Samples with high calcium and marginal pH are potentially able to support dreissenid populations.

High Calcium/High pH: Samples with high calcium (>15 mg/L) and high pH (>7.8) are considered favorable for dreissenid mussels and therefore able to support long-term populations.

At each site, the frequency of the above categories was plotted on pie charts to visually express the potential the site has for supporting mussels. The paired samples with calcium and/or pH below the required limits are presented in green, the samples with conditions potentially able to support mussels are presented in orange, and samples with both high calcium and high pH are presented in red (Table 2). The high calcium, high pH range was further divided into favorable conditions occurring during the height of the breeding season and those occurring during the remainder of the year. Based on data for San Justo Reservoir, the height of the dreissenid breeding season (maximum number of veligers present) in California is between May and June (Janik 2010). As such, samples with high calcium and high pH during May and June were separated from those samples with high calcium and high pH during July to April as representing the maximum potential for establishment of dreissenids.

Table 2. Conditions of low, marginal, and high calcium and pH that are unable (green), potentially able (orange) and able (red) to support dreissenid mussels.

	Calcium Concentration		
pH Level	**Ca ≤ 12 mg/L**	**12 mg/L < Ca ≤ 15 mg/L**	**Ca > 15 mg/L**
pH ≤ 7.3	**unable**	**unable**	**unable**
7.3 < pH ≤ 7.8	**unable**	**potentially able**	**potentially able**
pH > 7.8	**unable**	**potentially able**	**able**

3 Results

Calcium and pH data were examined in combination for sites in Clear Lake. All three sites have high calcium concentrations (i.e. ≥ 18 mg/L) and the pH is generally above limiting levels. The Clear Lake sites are deemed to have conditions suitable for supporting long-term dreissenid populations.

3.1 Upper Arm

The average calcium concentration at the Upper Arm site in Clear Lake is 22.6 mg/L and the average pH is 8.1. All samples collected between March 2005 and July 2011 had calcium concentrations above the minimum limit required for dreissenid mussels and all but one sample had pH values above the minimum limit (Figure 2). Only 3.0% of samples collected had conditions that would be unable to support mussels whereas 97.0% had high calcium and high pH (Figure 3). Based on these results, the Upper Arm site of Clear Lake is likely to be able to support a long-term dreissenid population.

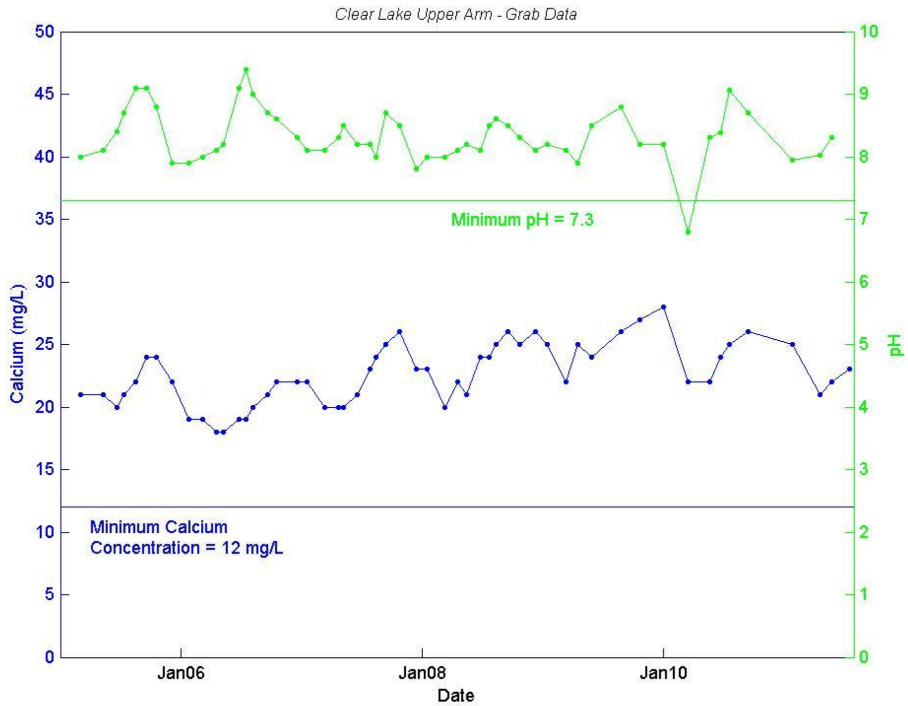

Figure 2. Changes in calcium and pH at the Upper Arm site in Clear Lake, California.

Figure 3. Distribution of samples from Upper Arm, Clear Lake into zones of low, marginal, and high calcium and pH. Samples are separated into conditions that are unable (green) and able to support dreissenid mussels (red).

3.2 Oaks Arm

Oaks Arm has high calcium and pH levels (Figure 4). The average calcium concentration at this site is 23.0 mg/L and the average pH is 8.0. Figure 5 shows that the majority of samples (84.8%) collected between March 2005 and July 2011 have high calcium and high pH values. The remaining samples had either high calcium and marginal pH (9.1%) or low calcium and/or low pH (6.1%). Under these conditions, this site could support a long-term mussel population.

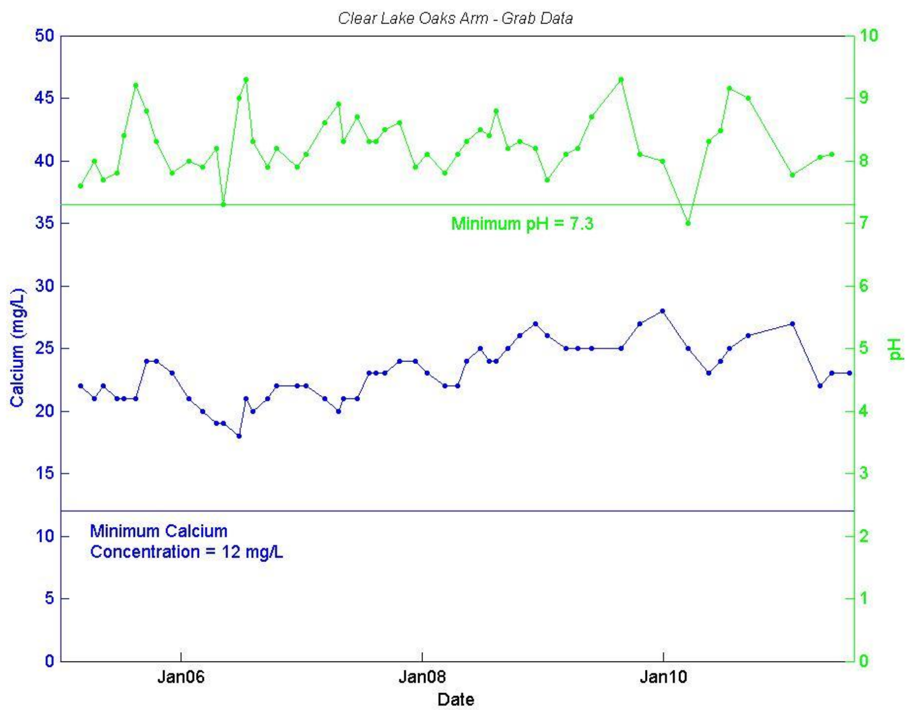

Figure 4. Changes in calcium concentrations and pH levels at the Upper Arm site in Clear Lake.

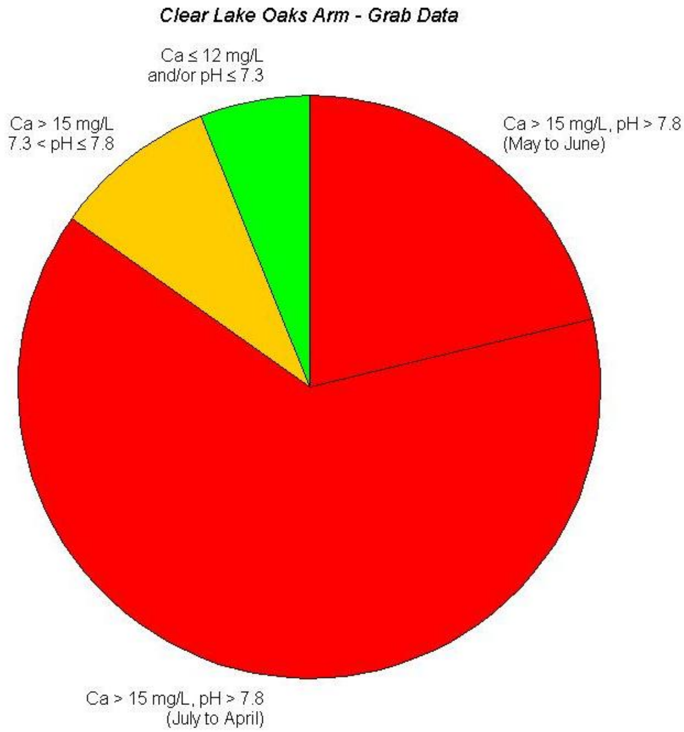

Figure 5. A large percentage (84.8%) of samples collected at the Oaks Arm site in Clear Lake had high levels of both calcium and pH (red). The remaining samples had either low calcium and/or low pH (green) or were marginal (orange).

3.3 Lower Arm

The calcium concentration in samples from the Lower Arm at Clear Lake between March 2005 and July 2011 are all well above the minimum of 12 mg/L (Figure 6). The average calcium concentration during this time is 23.5 mg/L. The pH is also above the minimum limit in all samples with an average of 8.0. In 82.4% of samples collected, both the calcium and pH are high enough to support mussels (Figure 7). The remaining 17.6% of samples have high calcium levels and marginal pH. It is believed that a long-term dreissenid population could be supported at this site.

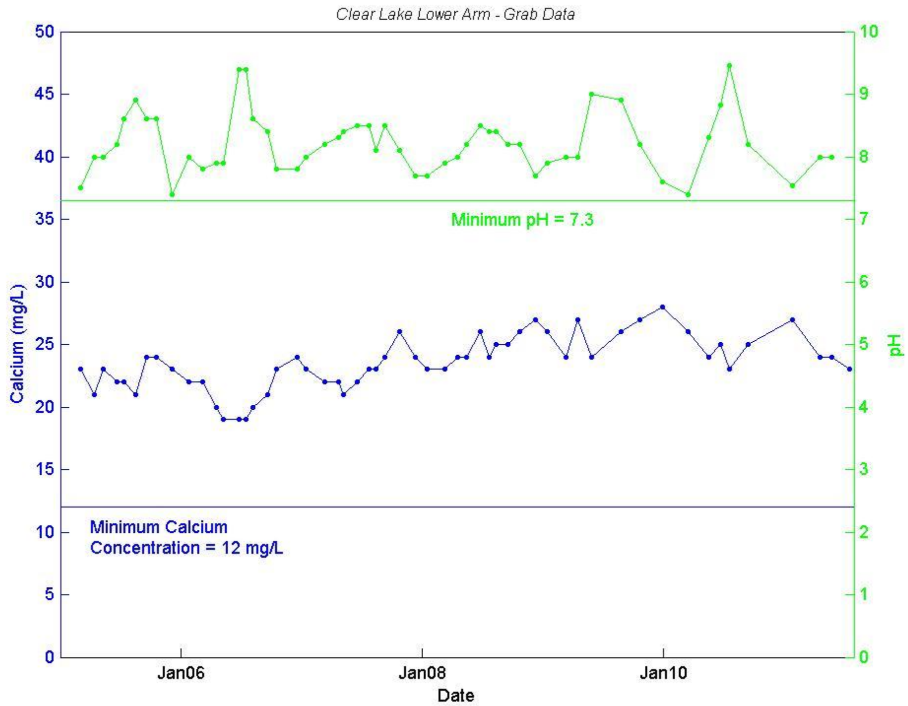

Figure 6. Changes in calcium and pH in samples from Lower Arm at Clear Lake.

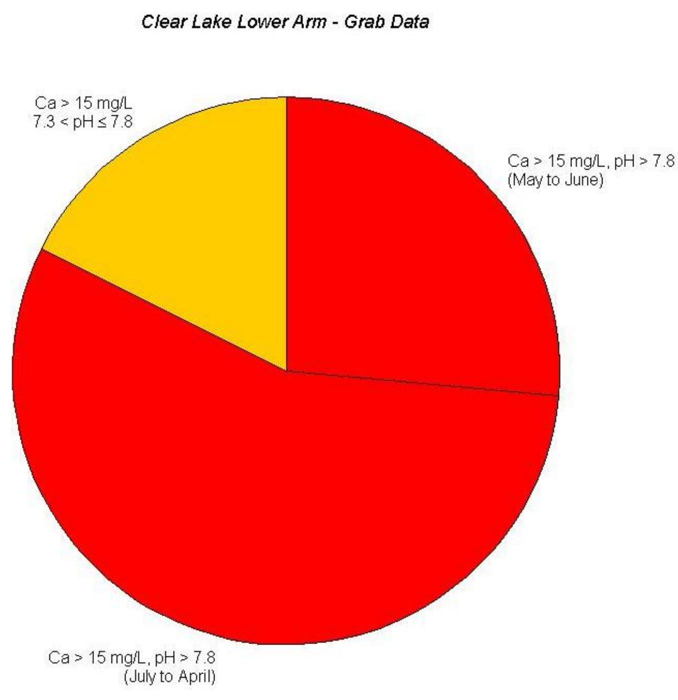

Figure 7. In samples from Lower Arm at Clear Lake, 82.4% had high calcium and high pH (red) whereas the remaining samples had high calcium with marginal pH (orange).

3.4 Other Variables

Based on calcium and pH data alone, the Clear Lake sites are likely to be able to support long-term dreissenid mussel populations. There may be other variables, however, that influence the ability of mussels to survive in Clear Lake. In addition to calcium and pH, we examined dissolved oxygen, nutrient and metal concentrations at the Upper Arm, Oaks Arm and Lower Arm of Clear Lake.

3.4.1 Dissolved Oxygen

Surface dissolved oxygen concentrations are generally above limiting levels due to oxygen exchange at the air-water interface, whereas bottom dissolved oxygen concentrations may become limiting due to decomposition of settled organic material and reduced mixing at depth. The surface dissolved oxygen concentrations in Clear Lake were above the minimum limit of 3 mg/L at all three sites. Bottom dissolved oxygen concentrations were also above the minimum limit in the majority of samples collected for the Clear Lake sites (Figure 8).

Based on discrete sampling results, the average number of consecutive days with bottom dissolved oxygen levels below 3 mg/L at the Lower Arm, Oaks Arm and Upper Arm sites is 81, 52, and 52, respectively. Bottom dissolved oxygen levels below 1 mg/L occur on an average of 42 consecutive days at the Lower Arm and Oaks Arm sites and 54 consecutive days at the Upper Arm site. Although these time periods of low dissolved oxygen may be long enough to inhibit dreissenid survival on the bottom of Clear Lake this is not the case in the surface zone where dissolved oxygen is plentiful. Furthermore, since sampling was not continuous, the average number of consecutive days at low dissolved oxygen may be shorter or longer than reported above. As such, dissolved oxygen is unlikely to limit dreissenid mussel survival should mussels be introduced to Clear Lake except at the bottom of the lake.

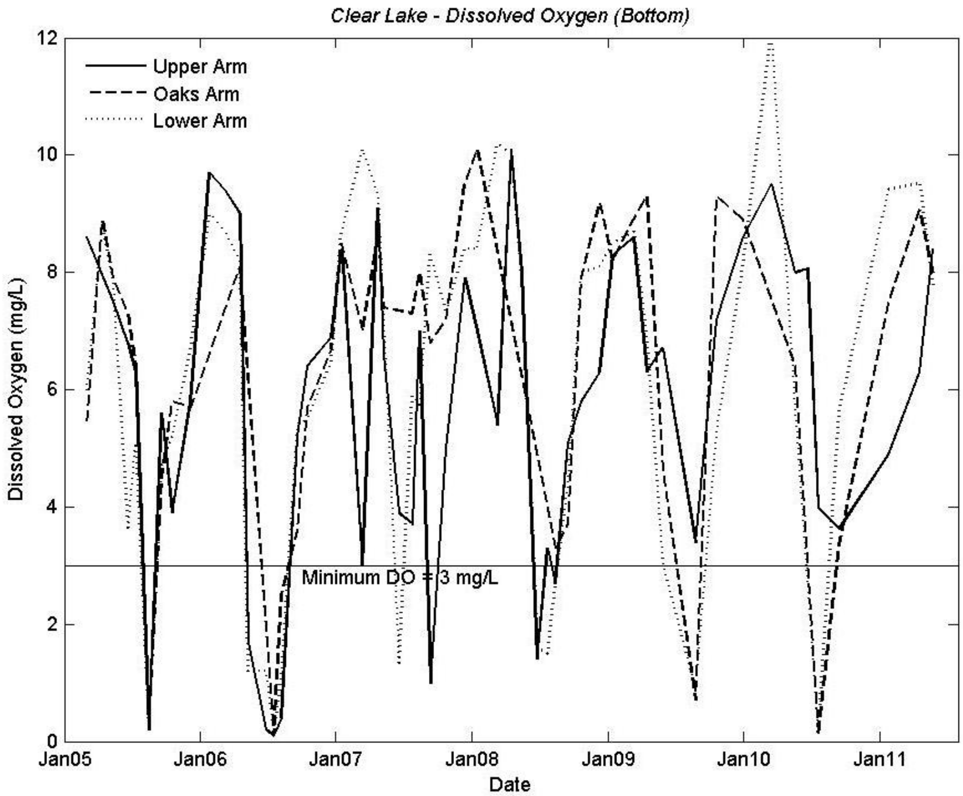

Figure 8. Seasonal variation in bottom measurements of dissolved oxygen in the Upper Arm, Oaks Arm and Lower Arm of Clear Lake.

3.4.2 Nutrients

Locations with total phosphorus concentrations <5 μg/L and >50 μg/L, and total nitrogen concentrations <75 μg/L and >750 μg/L were found to be unable to support mussels (Mackie and Claudi 2010). Excess nutrients lead to algal blooms which have been observed to interfere with mussel survival (Mackie and Claudi 2010; Stanczykowska and Lewandowski 1993). At all three sites examined, the average total phosphorus concentrations are >50 μg/L (Table 3). Total nitrogen is the sum of Total Kjeldahl Nitrogen (TKN) and nitrogen from nitrate and nitrite. Average TKN values for the Oaks Arm and Lower Arm are greater than the upper limit for total nitrogen (Table 3); adding nitrate+nitrite nitrogen would increase these values further. The Upper Arm site has an average TKN of 583 μg/L; dissolved nitrate+nitrite values could result in total nitrogen concentrations near or above the 750 μg/L limit at this site. A preliminary examination of nutrient values suggests that a long-term dreissenid mussel population is unlikely in Clear Lake, however a more detailed analysis of nutrient conditions is required.

Ammonia levels >20 μg/L are toxic to most aquatic organisms (Mackie and Claudi 2010). At all three Clear Lake sites, average ammonia concentrations exceed this limit. Survival of long-term dreissenid mussel populations may be hindered by high ammonia levels.

Table 3. A summary of results for ammonia, total Kjeldahl nitrogen (TKN), total phosphorus, and several metals at the Upper Arm, Oaks Arm, and Lower Arm in Clear Lake.

Variable	Average (Minimum, Maximum)		
	Upper Arm	**Oaks Arm**	**Lower Arm**
Ammonia (µg/L)	67.5 (0.0, 158.0)	194.9 (0.0, 596.0)	80.0 (0.0, 180.0)
Total Aluminum (µg/L)	68.2 (10.1, 626.0)	45.9 (7.1, 345.0)	31.9 (5.6, 308.0)
Total Arsenic (µg/L)	3.86 (0.65, 9.94)	3.32 (0.90, 8.61)	2.80 (1.01, 6.40)
Total Cadmium (µg/L)	0.15 (0.00, 0.40)	0.0 (0.0, 0.0)	0.19 (0.19, 0.19)
Total Chromium (µg/L)	0.54 (0.10, 2.97)	0.30 (0.07, 1.00)	0.30 (0.06, 1.15)
Total Copper (µg/L)	0.88 (0.38, 2.65)	0.66 (0.31, 0.96)	0.56 (0.29, 0.84)
Total Iron (mg/L)	60.2 (12.9, 158.0)	49.6 (12.3, 133.0)	58.2 (20.7, 132.0)
Total Kjeldahl Nitrogen (µg/L)	583 (180, 1800)	922 (410, 4400)	989 (220, 5000)
Total Lead (µg/L)	0.09 (0.02, 0.27)	0.04 (0.02, 0.07)	0.04 (0.02, 0.06)
Total Magnesium (mg/L)	16.5 (12.0, 21.0)	16.8 (12.0, 21.0)	17.3 (13.0, 21.0)
Total Manganese (µg/L)	36.0 (2.9, 179.0)	21.4 (4.3, 55.7)	12.0 (3.8, 32.1)
Total Mercury (ng/L)	1.42 (0.27, 3.70)	13.76 (0.84, 40.00)	2.77 (0.32, 9.30)
Total Nickel (µg/L)	1.95 (1.28, 2.58)	1.66 (1.11, 2.06)	1.51 (1.02, 2.03)
Total Phosphorus (µg/L)	133 (10, 490)	112 (20, 410)	91 (10, 350)
Total Selenium (µg/L)	0.29 (0.24, 0.38)	0.30 (0.21, 0.49)	0.25 (0.21, 0.32)
Total Silver (µg/L)	0.03 (0.01, 0.05)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
Total Zinc (µg/L)	1.78 (0.13, 4.41)	1.33 (0.16, 3.69)	1.26 (0.12, 4.02)

3.4.3 Metals

A number of metals have been measured in Clear Lake (Table 3). It is known that dreissenid mussels are able to bioaccumulate and regulate metal concentrations in their tissues (Johns 2011; Richman and Somers 2010; Secor *et al.* 1993; Ware *et al.* 2011). Further research is needed to determine what effect metals in Clear Lake would have on a long-term dreissenid mussel population.

4 Conclusions

Based on calcium and pH data collected at the Upper Arm, Oaks Arm and Lower Arm between March 2005 and July 2011, Clear Lake may be able to support long-term dreissenid populations. Other variables such as dissolved oxygen, nutrients and metals were considered as possible limiters to dreissenid mussel survival.

Dissolved oxygen concentrations are unlikely to limit dreissenid populations except on the bottom of the lake as the majority of samples collected are above the lower limit of 3 mg/L. Average total phosphorus and ammonia concentrations exceed upper limits at the Upper Arm, Oaks Arm and the Lower Arm sites. Average TKN concentrations alone exceed the total nitrogen limit for the Oaks Arm and Lower Arm sites, however average TKN alone is not enough to exceed total nitrogen limits at the Upper Arm site. When adding dissolved nitrate+nitrite to TKN measurements for the Upper Arm site, total nitrogen values that are near or above the total nitrogen limit for dreissenid mussels may result. Various metals are present in Clear Lake. It is unknown what effect these metals may have individually and in combination in terms of limiting long-term dreissenid population survival.

5 References

Claudi, R., Graves, A., Taraborelli, A.C., Prescott, R.J., and Mastitsky, S.E. 2012. Impact of pH on survival and settlement of dreissenid mussels. Aquatic Invasions 7(1) doi:10.3391/ai.2011.ICAIS.

Hincks, S.S., and Mackie, G.L. 1997. Effects of pH, calcium, alkalinity, hardness, and chlorophyll on the survival, growth and reproductive success of zebra mussels. Canadian Journal of Fisheries and Aquatic Sciences 54: 2049-2057.

Janik, J. 2010. Personal communication.

Johns, C. 2011. Trends of total cadmium, copper, and zinc in the zebra mussel (*Dreissena polymorpha*) along the upper reach of the St. Lawrence River: 1994-2005. Environ Monit Assess [accessed 17 December 2011] doi:10.1007/s10661-011-2346-6.

Lucy, F., Mastitsky, S., and Claudi, R. 2010. pH and Calcium - Natural limits in zebra mussel spread. *17th International Conference of Aquatic Invasive Species*, San Diego, California, .

Mackie, G.L. 2010. Personal communication.

Mackie, G.L., and Claudi, R. 2010. Monitoring and Control of Macrofouling Mollusks in Fresh Water Systems. CRC Press, Taylor & Francis Group, Boca Raton, FL.

Nierzwicki-Bauer, S.A., McGrath, B.R., Hansen, A.A., and Wimbush, J. 2000. Bioassay for predicting survival of zebra mussel veligers. *10th International Aquatic Nuisance Species and Zebra Mussel Conference* , Toronto, Canada, .

Richman, L.A., and Somers, K. 2010. Monitoring metal and persistent organic contaminant trends through time using
quagga mussels (*Dreissena bugensis*) collected from the Niagara River. J. Great Lakes Res. 36(1): 28-36.

Secor, C.L., Mills, E.L., Harshbarger, J., Kuntz, H.T., Gutenmann, W.H., and Lisk, D.J. 1993. Bioaccumulation of toxicants, element and nutrient composition, and soft tissue histology of zebra mussels (*Dreissena polymorpha*) from New York state waters. Chemosphere 26(8): 1559-1575.

Stanczykowska, A., and Lewandowski, W. 1993. Thirty years of studies of *Dreissena polymorpha* ecology in Mazurian lakes of northeastern Poland. *In* Zebrea Mussels Biology, Impacts, and Control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton. pp. 3-38.

Ware, L.L., Petrie, S.A., Badzinski, S.S., and Bailey, R.C. 2011. Selenium concentrations in greater scaup and dreissenid mussels during winter on Western Lake Ontario. Arch. Enviorn. Contam. Toxicol. 61: 292-299.