Appendix A:

Large scale deployment of gas impermeable benthic barriers to control invasive Asian clams in Emerald Bay, Lake Tahoe

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Introduction

The introduction of invasive species to aquatic ecosystems is often detrimental to the natural ecology of lakes. For example, the Laurentian Great Lakes have suffered plant invasions by Eurasian water milfoil (*Myriophyllum spicatum*) and zooplankton such as the Spiny waterflea (*Bythotrephes longimanus)*, both have altered natural ecosystem function, and displaced native species (Mills et al. 1994, Ricciardi and MacIsaac 2000). More recently, zebra (*Dreissena polymorpha*) and quagga (*Dreissena bugensis*) mussels have entered the Great Lakes (Riccardi and MacIsaac 2000), and have continued to spread throughout the United States (Ludyanskiy et al. 1993, Stokstad 2007) causing ecosystem wide consequences (Ludyanski et al. 1993), and economic loss (Pimentel et al. 2000, Leung et al. 2002). As invasive species continue to spread throughout North America, it is critical that we further our understanding of their ecology and develop novel approaches for management.

In 2002 the Asian clam (*Corbicula fluminea*) was discovered in Lake Tahoe, this species is considered an aquatic invasive species (AIS) and generally considered an aquatic nuisance as well. The clam population was first discovered in the southeast portion of Lake Tahoe, but has since spread to other parts of lake and has established in "satellite" populations. One of these populations is located in the sandy sill that separates Emerald Bay and Lake Tahoe, SCUBA surveys have indicated that the population spans approximately 5.5 acres. Clams are visual nuisances but more importantly have been linked to increased algal blooms (Sousa et al. 2008), can out-compete native benthic invertebrates, and can cause ecological changes at the ecosystem level (Strayer et al. 1999). To reduce the spread and help control the populations of clams in Lake Tahoe, rubber barriers have been deployed to kill off portions of the population (Wittmann et al. 2012). The objective is to mechanistically reduce dissolved oxygen (DO) levels underneath the barriers, literally suffocating the clams to cause mortality, and potentially reducing their pelagically derived food supply. Success with this technique in the southeast area of Lake Tahoe (Wittmann et al. 2012) led lake managers to the decision to treat 5 acres of clam infestation on the sill between Emerald Bay and Lake Tahoe with a similar method.

In fall of 2012 (November/December) barriers were deployed over the infested area. Barrier deployment occurred under the direction of resource and management agencies. The Southern Nevada Public Lands Management Act (SNPLMA) funded the University of California - Davis and the University of Nevada - Reno to document the effectiveness of the project. This included pre and post sampling of the area for clam density, bi-monthly monitoring of mortality rates inside and outside of the plots, quantification of the reproductive capacity of Asian clams, measurement of any changes in percent carbon in the sediment, and documentation of physicochemical conditions (reported separately by UC Davis).

Methods

Barrier deployment:

Barrier deployment was coordinated by local agency partners and with the consulting partner which was chosen to do the deployment.

Population density, size structure and mortality rates (UNR Tasks 1 and 2):

Control samples:

Control samples were collected from outside of the barriers, before deployment (October/November 2012), during deployment (January, March, June, July, August, and October 2013) and again after the barriers were removed (November/December 2014). Because the barriers were left in place for one year longer than originally proposed, funding was not included to sample during the majority of 2014. Control samples were collected from the UNR research vessel using a petit-Ponar grab sampler (0.023 m^2). The sampler was slowly lowered to the sediment, triggered and retrieved to the boat. GPS points were collected for each sample taken before and after barrier deployment to be compiled into a clam density map for pre and post treatment. Approximately 500 samples were collected before and after barrier deployment, and approximately 30 samples were collected during the barrier deployment for each sampling period. After collection, samples were sieved through a 500 µm mesh sieve and transported back to the UNR laboratory for detailed analysis. At the laboratory, each sample was elutriated, rescreened through a 250 µm mesh sieve, and placed into a white bottomed sorting chamber. Each sample was carefully examined for the presence of young-of-year clams. After clams were picked they were identified as live or dead by the presences of live clam tissue within the shell. Each clam (live or dead) was measured to the nearest 0.01 mm at its widest point to estimate the size structure of clams during the treatment.

Treatment samples:

Treatment samples were collected by UC Davis research divers from underneath pre-cut ports in the barriers during similar sampling periods as the control samples. Sediment was sieved from a 0.5 m^2 quadrat, and clams were collected. Samples were then sent to UNR laboratory for detailed analysis. Clams were identified as live or dead and measured using the same methods used for the control samples. Young-of-year clams were identified as either *Corbicula fluminea* (invasive) or as the native genus, *Pisdium* (Thorp and Covich 2010).

Data analysis:

Clam density was calculated for samples collected by petit-Ponars before and after the deployment. Because we assumed that clam density would not change underneath the barriers we did not include the samples from underneath barriers in our analysis. Density was calculated as the number of live clams collected in a sample divided by the area of the petit-Ponar (0.023 m^2). Density of clams before and after barrier deployment was determined using a two sample t-test. All statistics were done in the statistical software R (R-project.org).

Coordinates from each sample were paired with the calculated density of live clams collected in that sample and input in to ArcGIS. A clam density contour map was built using the Inverse Weighted Distance tool in ArcGIS for samples collected before and after barrier deployment.

Percent mortality was calculated in all samples to determine the effectiveness of the barriers. Mortality was calculated as the number of dead clams divided by total clams in a sample and multiplied by 100. All samples from each area (treatment, control) were averaged together for each sampling time period and a standard error was computed. Average mortality rates were compared using a two sample t-test between the before and after barrier deployment samples, and during barrier deployment between treatment and control collections for each sampling period.

Clams were grouped in 1 mm categories (i.e. 5-6mm, 6-7mm, etc). Size frequencies were plotted as histograms, and visually examined to determine the number of cohorts present.

Clam reproductive analysis (UNR Task 2):

Reproductive output of each live clam was determined by dissecting the clam and counting eggs and veligers (Britton and Morton 1982). Clams were carefully opened using a scalpel, gills of the clam were gently laid on a glass slide and wet mounted with a glass coverslip (Britton and Morton 1982). Eggs and veligers in the gill of clams were counted with aid of a compound microscope. Because not all eggs and veligers are attached to the gills, we rinsed the shell and remaining clam tissue with deionized water onto a petri dish. Any eggs and veligers on the petri dish were counted with aid of a dissecting microscope. All eggs and veligers counted were summed to be the reproductive output of each clam. The mean reproductive output from the treatment was compared to the control for each month sampled using two-sample t-test's performed in R (r-project.org).

Analysis of Sediment Carbon Content (UNR Task 6):

To determine the amount of food available to clams we measured carbon content in the sediment. Five or more sediment samples were collected from the treatment (under the barriers) and the control (outside barriers) each month that clams were sampled. Samples were first dried at 70°C to a constant mass for 24hrs and weighed to the nearest 1.0 mg. After, each sample was combusted at 500°C for 2 hrs and re-weighed, the difference between the two masses was the amount of organic carbon in the sediment. The amount of carbon (mg) was then divided by the total mass of the sample (g) to determine the concentration of organic carbon in the sediment (mg/g). Samples collected before and after barrier deployment, and control and treatment samples for each month were compared using a two sample t-test in R (r-project.org).

Results

Population density, size structure and mortality rates (UNR Tasks 1 and 2):

Pre –treatment sampling (October/November 2012):

During fall 2012, before rubber mats were deployed in Emerald Bay we collected 485 petit-Ponar grab samples from the clam infested area of Emerald Bay to establish a baseline understanding of the population. In these 485 samples, 667 total live clams were collected, with an average of 1.38 ± 0.07 individuals/sample collected (Table 1). During this period clams were estimated to have an approximate 11% natural mortality (Figure 1). Live clams were identified in 63% of all the samples collected, and the average density was approximately 60 ± 3 individuals/m^2 (Table 1). The majority of clam sizes were < 6mm or between 13 and 20 mm, indicating one small sized cohort (age 1) and 1 or 2 older cohorts (age 2 and 3+; Figure 2). Clam density varied between 0 and 510 individuals/m^2 on the sill in Emerald Bay before barrier deployment. The highest densities occurred in the north central portion of the sill (Figure 3).

During treatment sampling (January – October 2013):

Mortality increased underneath the mats over time while the small population outside of the mats appeared to be unaffected and natural mortality remained approximately 10-15% (Figure 1, 3). The mats became the most effective later in the summer (July, August, October) achieving 100% mortality under several specific barriers (Figure 4).

During barrier deployment, small sample size made it difficult to determine specific number of cohorts in the control area; however similar size classes were collected (Figure 2). Later in the summer (July, August, October) larger individuals were collected more frequently, indicating the maturation of the previous year's recruitment (Figure 2). In the treatment area clam size frequencies did not change over time (Figure 2).

Post-treatment sampling (November 2014):

Dive teams began removing barriers on November 3 2014, and we began post-treatment sampling on November 6 2014. We collected 512 petit-Ponar grab samples from the area, collecting 77 total live clams (Table 1). An average of 0.15 ± 0.02 clams/sample was collected, which was significantly (t-test, $p < 0.001$) less than the density estimated before treatment (Figure 4; Table 1). Live clam density decreased throughout the entire treatment area and few areas had densities > 100 individuals/m^2 (Figure 4). Clam mortality after barrier removal was estimated to be 79% (Table 1, Figure 1). The chance of collecting a sample with live clams in it reduced from 63% (pre-sampling) to 10% (post-sampling), while density of clams was almost an order of magnitude less and significantly lower (t-test , $p < 0.001$) than what was recorded before barrier deployment (Table 1). In addition, the density of native clams (*Pisidium* sp.) decreased significantly (t-test, $p > 0.001$) from 8 ± 1 individuals/m^2 before barrier deployment to 1 ± <1 individuals/m^2 after barrier deployment (Table 1).

Clam reproductive analysis (UNR Task 2):

Clam reproductive output remained low (83 ± 89 eggs/clam) throughout the year in live clams collected from underneath the barrier, while those collected from the control area increased from 593 ± 221 eggs/clam during spring to 1529 ± 155 eggs/clam in late summer (Figure 5). Clam reproductive output was significantly higher in the control area during June, July, August and October compared to the treatment area (t-test, $p > 0.001$). The reproductive potential of live clams that were collected post-barrier removal was similar to levels recorded from clams collected under the barriers while treatment was in progress (Figure 5).

Analysis of Sediment Carbon Content (UNR Task 6):

To simplify the analysis sediment carbon samples were placed into 5 groups, fall 2012 (pre-deployment), Spring, Summer, Fall 2013 (during deployment, and winter 2014 (post-deployment). Sediment carbon content ranged from 2.8 ± 0.2 (Spring 2013) to 3.6 ± 0.7 mg/g (Fall 2013) in the control and from 2.8 ± 0.3 (Fall 2013) to 3.9 ± 0.9 mg/g (Summer 2013) in the treatment area (Figure 6). There were no seasonal trends of sediment carbon content. Control sites were statistically similar to treatment sites during all sampling periods (t-test, $p < 0.001$; Figure 7).

Discussion

Population density, size structure and mortality rates (UNR Tasks 1 and 2):

The control of invasive aquatic species is extremely difficult once a system has been invaded. Previously, Asian clams (*Corbicula fluminea*) were treated in Lake Tahoe with success on a smaller scale (≈ 2000m^2; Wittmann et al., 2012). However, the treatment in Emerald Bay is the largest deployment of gas impermeable benthic barriers to control an invasive bi-valve.

The deployment of 5 acres of gas impermeable benthic barriers in Emerald Bay resulted in approximately 80% mortality of Asian clams in the treated area (Table 1, Figure 1) and drastically reduced the density throughout the area (Figure 3). However the mortality rate may be an underestimate because many of the dead clams likely washed away to outside of the sampling area and were not taken into consideration for this calculation. We attempted to reduce the probability of this happening by sampling immediately after the barriers were removed allowing minimal time for waves to move the dead shells.

Treatments in southeastern Lake Tahoe by Wittmann et al. (2012) reduced clam density from 4103 to 37 individuals/m^2 or over two orders of magnitude, while the Emerald Bay project only reduced clam density by one order of magnitude. The Emerald Bay barrier deployment lasted approximately 2 years, while the barriers deployed by Wittmann et al. (2012) were on the Lake bottom for ≈ 3 months (120 days). Given the results of our interim sampling periods (Figure 1 and 2) it is unlikely that we would have seen significant declines or mortality of the clam population in that short of a period. This may be because of upwelling, porosity, and high wind/wave activity associated with the Emerald Bay site. These variables may cause increased water flow underneath the barriers resulting in dissolved oxygen levels to be variable as opposed to consistently 0 mg/L (see companion UC Davis Report for this project). Additionally, Emerald Bay mats were deployed at ≈ 1.5-3 m, while barriers from Wittmann et al. (2012) were approximately 5 m deep, and were likely exposed to much less wave action.

In general, the treatment reduced the Asian clam population in Emerald Bay greatly. However, 100% mortality was only achieved in several discreet samples. The treatment effectiveness was similar to those which have been deployed before in Lake Tahoe before (Wittmann et al. 2012), however this treatment took considerably longer (2 years compared to 3 months) to achieve that level of effectiveness.

Clam reproductive analysis (UNR Task 2):

Invasive species commonly produce high levels of offspring and provide little to no parental care to them. However having such a high fertility, typically ensures the survival of some individuals, this is commonly referred to as an *r* reproductive strategy. Similar to other invasive species, Asian clams are *r* strategists (McMahon 2002, Denton et al. 2012). Denton et al. (2012) analyzed veliger counts in Asian clams in the southeast of Lake Tahoe and found a univoltine reproductive pattern triggered by temperature. Our results are similar to that of Denton et al. (2012), with the largest reproductive potential found during (July, August and October) in the treatment area. The amount of reproductive output we observed was similar to that of other studies in Lake Tahoe (Denton et al. 2012), but was less than some other, more productive systems (Aldridge and McMahon 1978).

Interestingly, reproductive output underneath the barriers was significantly less than those in the control area, suggesting that the stress of the barriers caused clams to put more energy into survival and metabolism instead of reproduction. To our knowledge this has not been documented before in Asian clams, however it is a commonly observed hypothesis in many organisms (Allen et al. 2008).

Based on reproductive analysis the working group made the decision to leave the barriers in place for more time, we believed that if the mats were removed in late fall of 2013, that high reproductive output from the untreated clams may have resulted in rapid recolonization of the treated area.

Analysis of Sediment Carbon Content (UNR Task 6):

Sediment carbon content did not change seasonally and was not statistically different between control and treatment sites. This suggests that food supply did not play a role in mortality or survival of clams and that hypoxia is the likely mechanism, but we cannot rule out the possibility of ammonium build up contributing to clam mortality as well (see UC Davis physicochemical Report). Additionally, it suggests that the presences of the barriers did not significantly alter the sediment biogeochemistry. Denton et al. (2012) also measured the amount of carbon content in the sediment in clam infestations in the southeast of Lake Tahoe and did not observe any changes over time, thus our results are similar to theirs.

Conclusions

The large scale deployment of gas impermeable benthic barriers in Emerald Bay, Lake Tahoe significantly reduced the density and caused significant mortality in the population of Asian clams. However, complete eradication from the area was not achieved and is highly improbable. Our data suggest that the stress caused from the benthic barriers forced clams to put less energy into reproduction and more into survival and metabolism. Finally, the presence of benthic barriers did not affect the sediment carbon content. These results suggest that gas impermeable barriers can be used to control for Asian clams, however many variables, such as upwelling, wave action, and temperature contribute to the success of these barriers. Future deployments should be considered on a site by site basis to determine external variables that may affect the success of barriers.

Tables and Figures

Table 1: Pre-treatment and post-treatment metrics of the Asian clam (*Corbicula fluminea*) in Emerald Bay, Lake Tahoe.

Metric	Pre-Treatment (Nov 2012)	Post-Treatment (Nov 2015)
Total Samples Collected	485	512
Total Live Clams Collected	667	80
Average Clam Density (Adult and YOY) ± SE (ind/m2)	60 ± 3	7 ± 1*
Average Adult Live Clam Density ± SE (ind/m^2)	47 ± 3	4 ± 1*
Average Live YOY Clam Density ± SE (ind/m^2)	13 ± 2	2 ± 1*
Average Dead Clam Density ± SE (ind/m^2)	7 ± 1	24 ± 3*
# of Live Clams/Sample	1.38 ± 0.07	0.15 ± 0.02*
% Chance of Live Clam Detection of all Samples	63%	10%
% Mortality (Dead Clams/Total Collected)	11%	79%
% Survival (Live Clams/Total Collected)	89%	21%
# of Native (*Pisidium* sp.) Clams/Sample	0.17 ± 0.02	0.03 ± 0.008*
Average Density of Native (*Pisidium* sp.) clams (ind/m^2)	8 ± 1	1 ± <1*

*Statistically significant differences between pre-treatment and post-treatment metrics ($p < 0.05$).

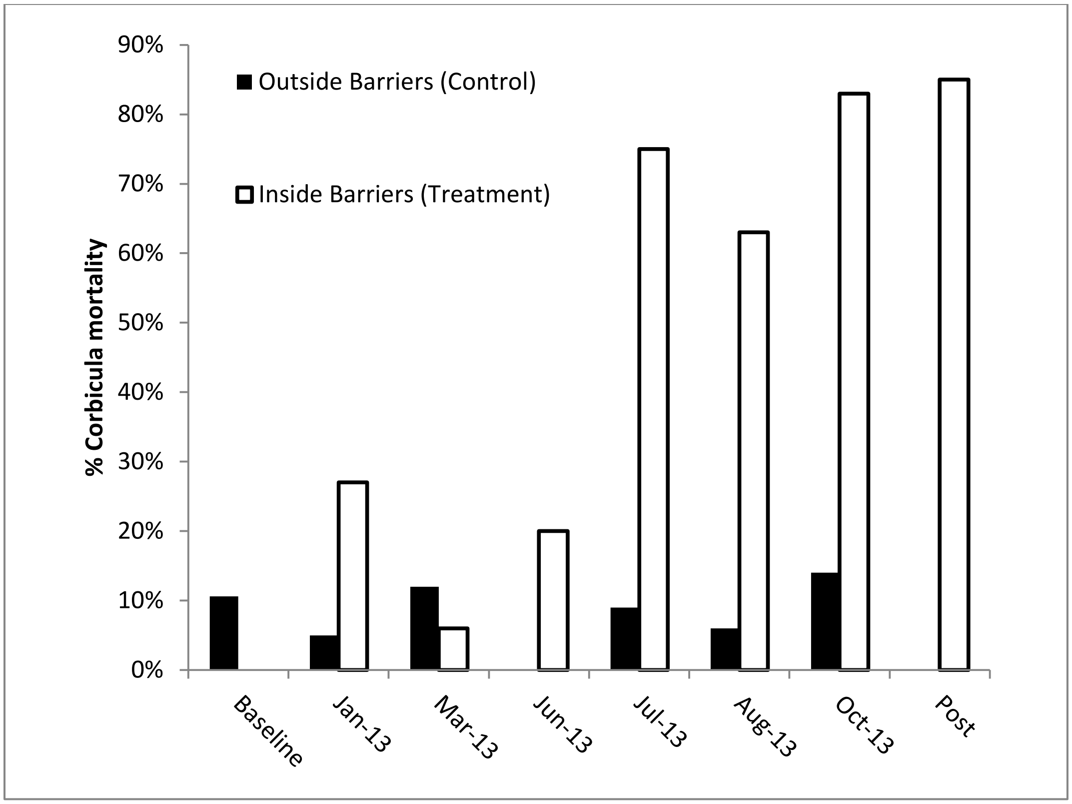
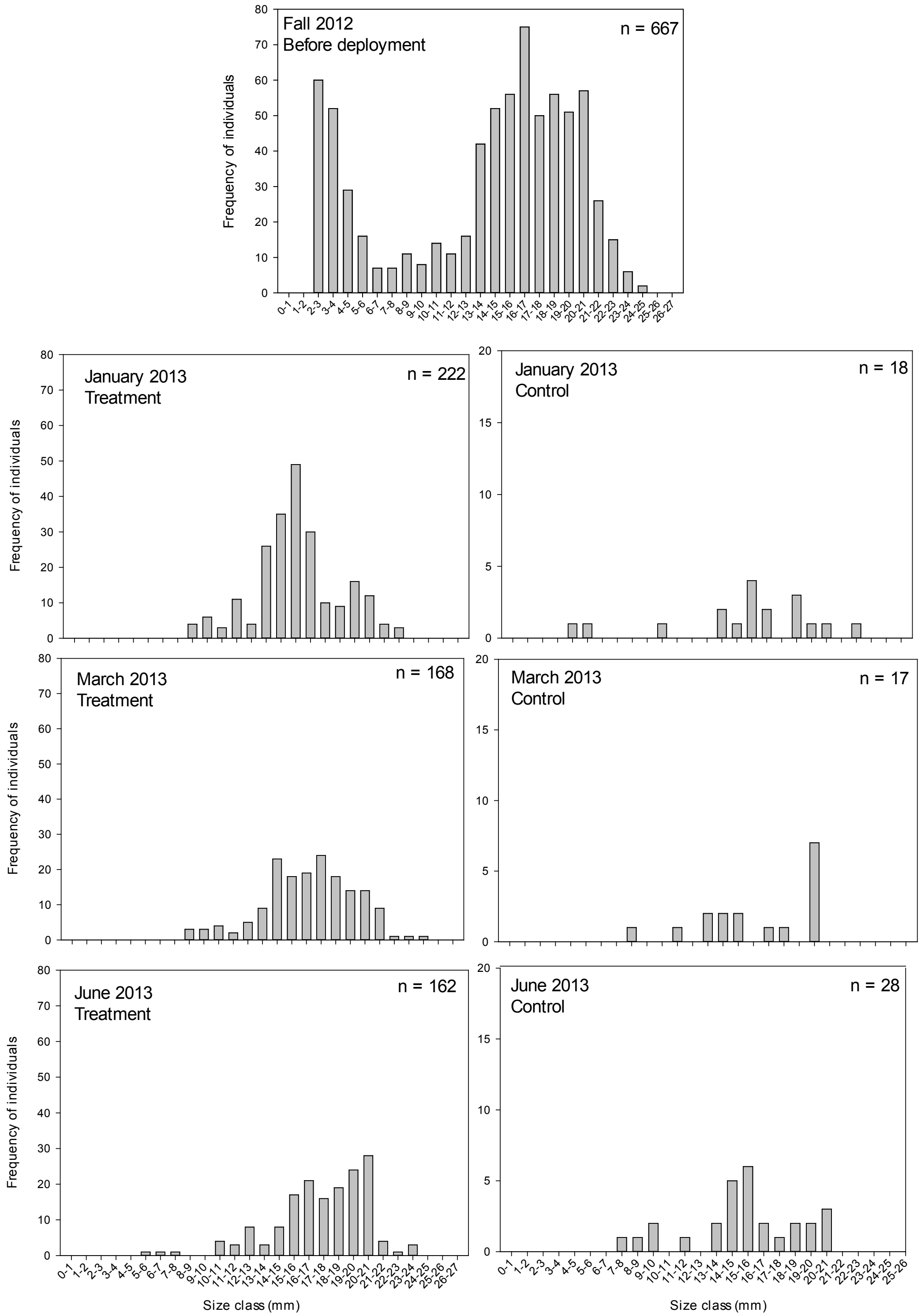


Figure 1. Percent mortality of Asian clam (*Corbicula fluminea*) populations in Emerald Bay, Lake Tahoe during sampling periods in both control (dark bars) and treatment (open bars).


Fall 2012
Before deployment
n = 667
January 2013
Treatment
n = 222
January 2013
Control
n = 18
March 2013
Treatment
n = 168
March 2013
Control
n = 17
June 2013
Treatment
n = 162
June 2013
Control
n = 28
Frequency of individuals
Size class (mm)

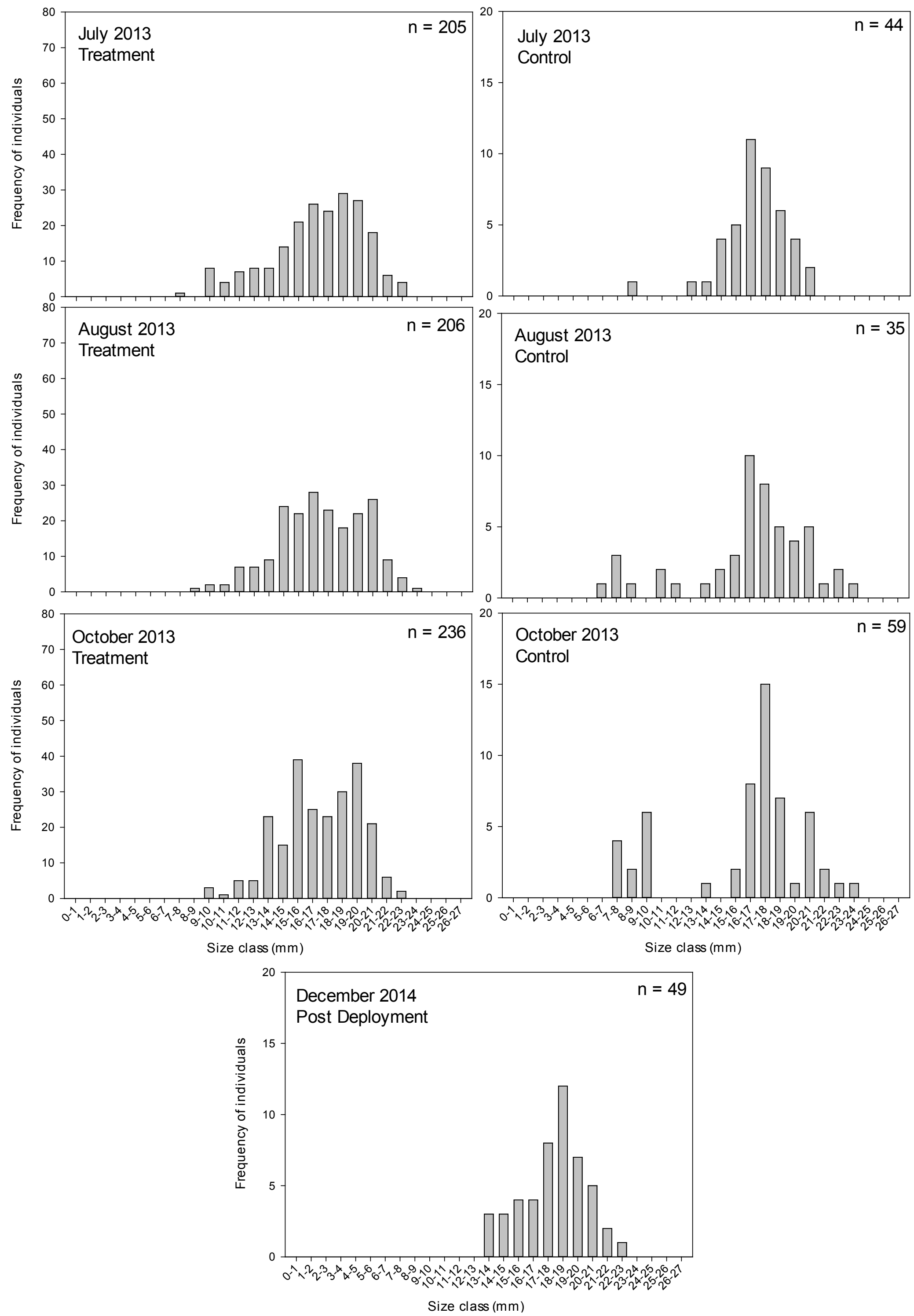


Figure 2. Size frequencies of Asian clam *(Corbicula fluminea)* in Emerald Bay, Lake Tahoe. Month and location of each size frequency are detailed in each sub-figure in the top left corner.

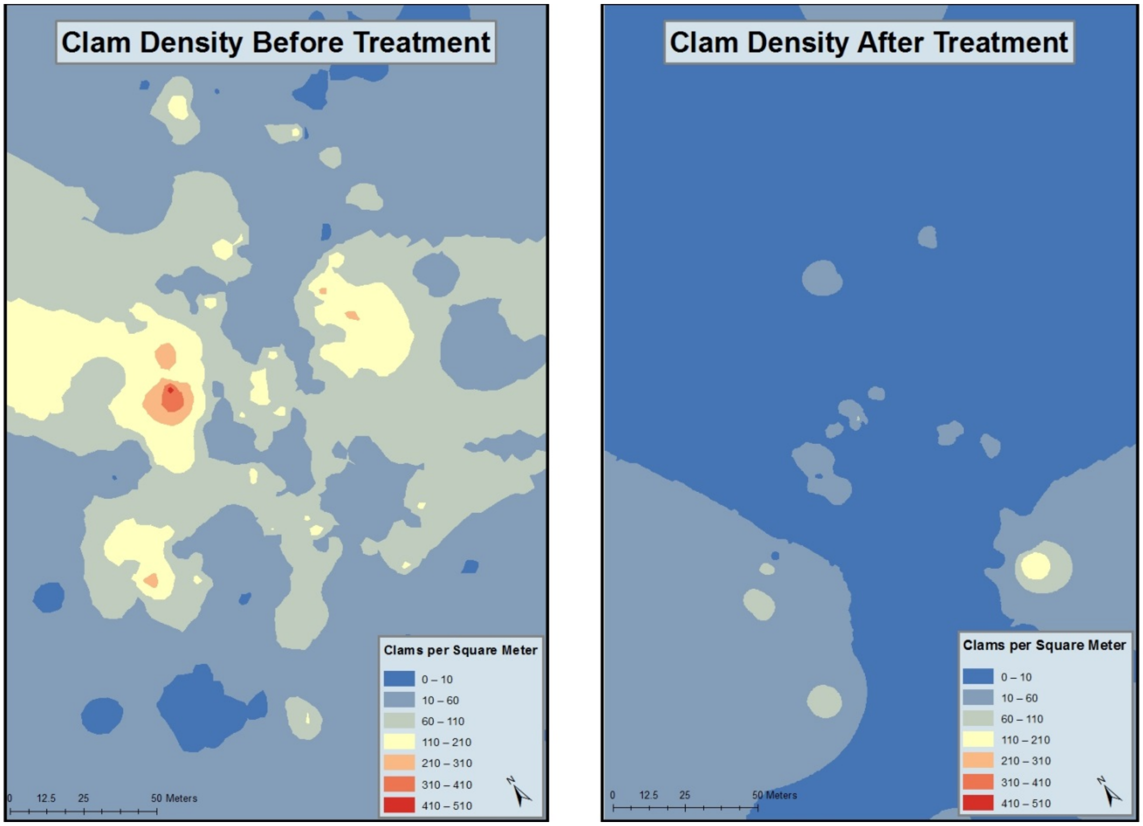


Figure 3. Map of the distribution of Asian clam (*Corbicula fluminea*) density on the sill in Emerald Bay, Lake Tahoe, before and after barrier treatment.

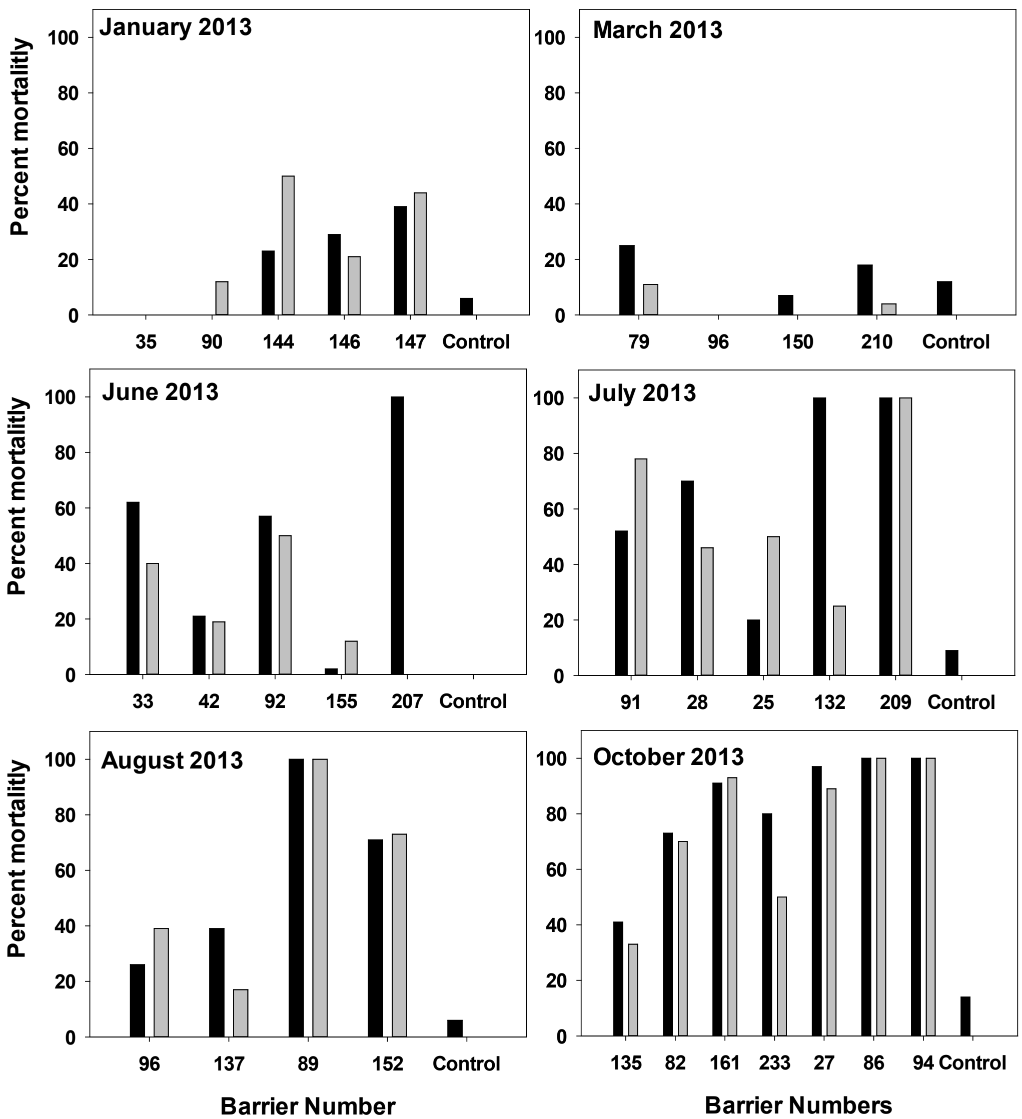


Figure 4. Percent mortality of Asian clams (*Corbicula fluminea*) by each barrier sampled during the barrier deployment in Emerald Bay, Lake Tahoe. Gray and dark barks are replicates 1 and 2 respectively from each barrier sampled. Because so few clams were collected in the control site ponars, all clams collected were used to calculate percent mortality in the control site. Thus, only one replicate is presented.

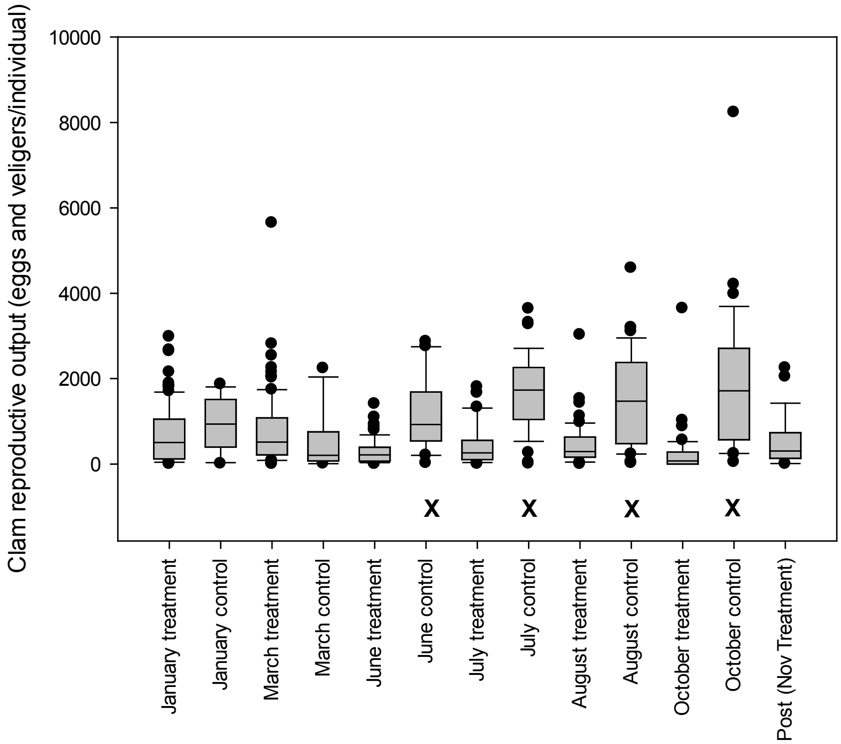


Figure 5. Asian clam (*Corbicula fluminea*) reproductive output during barrier deployment and at both treatment and control sites in Emerald Bay, Lake Tahoe. Statistical differences between control and treatment clams are designated by X's underneath the control boxplot. In all instances the reproductive potential from control clams was significantly higher than the treatment clams.

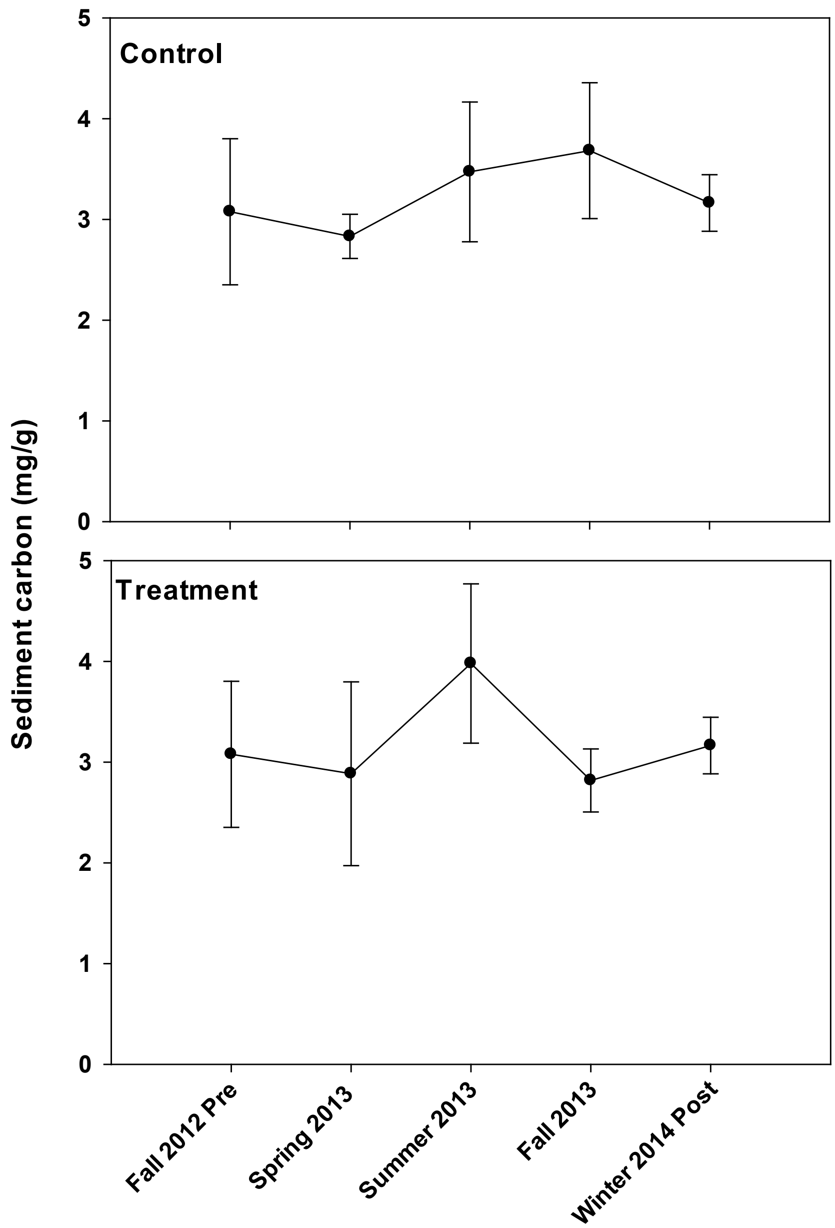


Figure 6. Seasonal concentration of sediment carbon in Emerald Bay, Lake Tahoe. Control plots are from outside of barriers, and treatment is from underneath barriers during deployment.

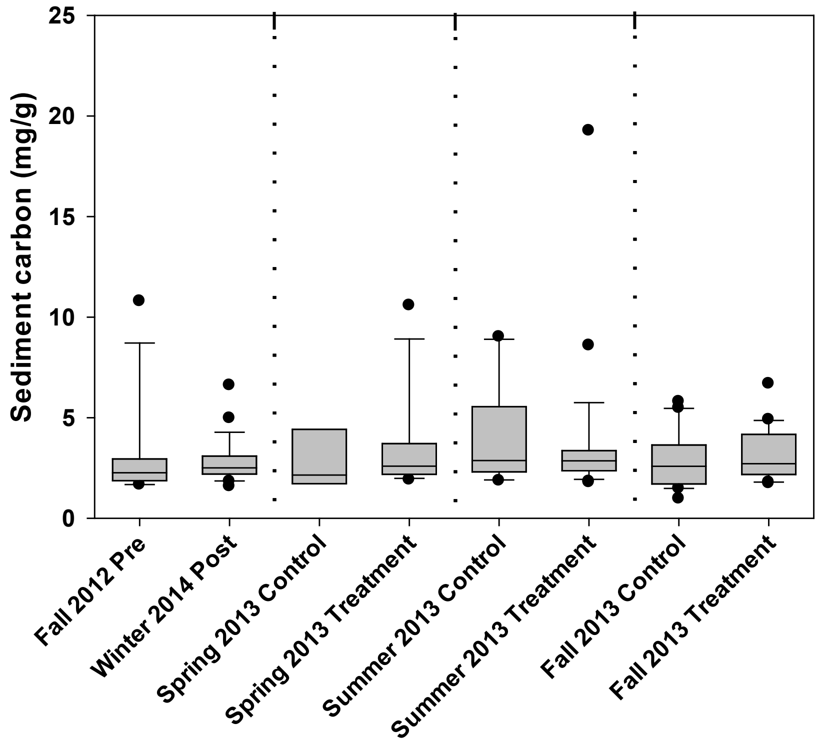


Figure 7. Boxplot of the concentration of sediment carbon in Emerald Bay, Lake Tahoe. No significant differences were detected between pre/post deployment sample and between seasonal control and treatment samples.

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