Final Report for the Lake Tahoe Asian clam Pilot Project

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I. Executive Summary

The increasing dispersal and establishment of aquatic invasive species in natural freshwater ecosystems has led to efforts to remove non-native taxa and/or restore native species. A widely distributed invasive bivalve Asian clam (*Corbicula fluminea*) recently established in Lake Tahoe, CA-NV and in 2008 was observed in large abundances (up to 6000 individuals/m^2). As the population has to date been restricted to only a portion of the lake littoral zone, experimental efforts to remove and reduce the impacts of *C. fluminea* were undertaken through two nonchemical control methods: (1) harvesting live populations with suction dredging apparatus, and (2) reducing dissolved oxygen concentrations and food availability to fatal levels for *C. fluminea* through the application of bottom barriers in Marla Bay, NV and near Lakeside Marina, CA in Lake Tahoe. Prior to treatment both sites were sampled to define the benthic macroinvertebrate community in order to understand long term impacts of experimental management actions.

Diver assisted suction removal plots treated in March – April 2009 were monitored for a 450 day period post dredging to observe target species (*C. fluminea*) and non-target macrofauna recovery rates after the experimental management action. A BACIPs (paired Before After Control Impact) analysis was used to assess the short and long term impacts of suction dredging. The results show that while physically harvesting *C. fluminea* is effective in reducing the abundance of this invasive species, it also reduces associated invertebrate abundances and causes disruptions to the structure of the macrobenthos community. Full recovery of the diversity of the dredged sites did not occur within one reproductive season (i.e., 365 days from impact), whereas the effect (difference between control and treatment plots) to the target invasive species (*C. fluminea*) is absent after 240 days when recolonization is noticeable.

A secondary treatment strategy that was tested in 2009 involved the use of bottom barriers to reduce dissolved oxygen concentrations under the barriers to levels that cause *C. fluminea* mortality. Hypoxic or anoxic conditions often restrict the establishment of species in freshwater systems. Invasive bivalve species such as the Asian clam (*Corbicula fluminea*) or Dreissenid mussels are well known to be limited in distribution as a result of low dissolved oxygen concentrations that occur in hypolimnion during stratification periods, and are typically restricted to well or moderately oxygenated strata of lake and reservoir systems. The artificial creation of anoxic conditions in lakes or reservoirs can provide an effective control strategy for invasive mollusks. In Lake Tahoe, *C. fluminea* are widely distributed in the well oxygenated littoral zone and are impacting native biodiversity, nearshore aesthetic values and are associated with accelerated growth of filamentous algal species (*Cladophora glomerata, Zygnema sp.*) A non-chemical control method was tested to reduce dissolved oxygen concentrations available to *C. fluminea* populations. An initial application of 3600 ft^2 of polyethylene fabric bottom barrier (typically used to manage invasive aquatic macrophytes in freshwater systems) was installed in 2009. This fabric proved to be ineffective at reducing *C. fluminea* populations due to physical

properties of the material. In a second iteration of the experiment, 1160 ft^2 of ethylene propylene diene Monomer (EPDM rubber) pondliner was applied as a benthic barrier at a depth of approximately 22 ft in Marla Bay, NV and near the Lakeside Marina, CA in the southwestern region of Lake Tahoe. Dissolved oxygen concentrations reduced to zero after 36 hours and the application resulted in 100% *C. fluminea* and 70-95% benthic macroinvertebrate mortality after a 28-day period from August to September 2009. Prolonged exposure to extreme hypoxia can provide an effective control strategy for benthic dwelling invasive bivalves. In September 2010 the Tahoe Research Team (UCD and UNR) transferred the results of this experiment to researchers and stakeholders at Lake George, NY who are currently using this Lake Tahoe developed technique to eradicate Asian clams.

The financial and logistical costs associated with suction removal of *C. fluminea* such as specialized divers, disposal of sediment materials, and limited seasonal opportunity for removal preempts this strategy from qualifying as a cost effective means to reduce *C. fluminea* populations. Labor and materials costs associated with this management strategy are presented; the cost per square foot for treatment by diver assisted suction removal is $24.71 and the cost per square foot of EPDM bottom barrier treatment is $2.58. This total does not include costs associated with research and development of the management strategies.

Other experiments carried out in this management-directed scientific program included investigations of the impacts of *C. fluminea* on filamentous algal growth. Nuisance blooms of filamentous algal species *Cladophora glomerata* and *Zygnema spp.* occurred in 2008 – 2010, with different taxa dominating biomass on an annual basis. Laboratory experiments showed that the addition of *C. fluminea* nutrient-laden excrement caused an increase in algal growth. In addition, laboratory experiments studying the rate of *C. fluminea* feeding through filtering Lake Tahoe water showed that in summer temperature conditions (18-20°C) *C. fluminea* filter feed 20 liters (5 gallons) of Lake Tahoe water in a 24 hour period, suggesting that this species in high abundances can impact phytoplankton dynamics and associated food web processes.

The seasonality of the Asian clam reproductive cycle can have significant impacts on management efforts undertaken at Lake Tahoe. Optimal control methodologies should incorporate optimal periods in terms of the minimization of target species reproductive effort, which is seasonally based on temperature, food availability and other abiotic and biotic factors. Monitoring for water column presence of pelagic Asian clam juveniles was carried out during experimental management treatments as well as a seasonally based survey of reproductive status of adult Asian clam. No Asian clam juveniles were observed in the water column during the March 2009 treatment period and Asian clam adults release juveniles during the warmer summer period during August – October 2009.

This research represents a wholly collaborative effort between research institutions: University of California Davis Tahoe Environmental Research Center, and the Aquatic Ecosystems

Laboratory of the University of Nevada Reno, and federal, state, and regional agencies: Tahoe Regional Planning Agency, United States Fish and Wildlife Service, Lahontan Regional Water Quality Control Board, Nevada Division of State Lands, Nevada Department of Environmental Protection, Tahoe Water Suppliers Association, California Department of Fish and Game, California State Parks and Recreation, Nevada Department of Wildlife, Tahoe Resource Conservation District, and the California State Lands Commission. Coordination between the research team and the agency representatives occurred through the Asian Clam Working Group (ACWG). This research has been presented to local stakeholders at Lake Tahoe, and at regulatory and academic meetings both nationally and internationally. This research was funded by the United States Fish and Wildlife Service, the Nevada Division of State Lands and the Lahontan Regional Water Quality Control Board.

Acknowledgments to participatory agencies and institutions:

II. Introduction

a. General Background

Asian clam (*Corbicula fluminea*) is the first molluscan aquatic invasive species (AIS) to have established in Lake Tahoe. The invasive bivalve, Asian clam (*Corbicula fluminea*) is established and spreading in Lake Tahoe. In 2002, low density populations (2-20 individuals per m^2) were observed in the south eastern portion of the lake, and in 2008 densities up to 6000 individuals per m^2 have been measured. Where populations are dense, Asian clam dominates native benthic macroinvertebrates, impacts water quality through concentrated nutrient excretion and associated algal blooms, and alters water chemistry as well as the aesthetic values of nearshore regions through shell deposition. In rapid response to this nearshore invasion, federal and state agencies collaborated with UC Davis and UN Reno to develop a short term Asian clam management plan and implement a series of studies to understand the distribution, life history and reproductive strategies of this species in relation to population control. Fortunately, Asian clam is in the early invasion stage, i.e., it has not fully dispersed to all available suitable habitat in Lake Tahoe. Asian clam is distributed mostly in south eastern portion of the lake, with some low density satellite populations in Glenbrook Bay, Camp Richardson and Emerald Bay. Asian clam are located at depths greater than 70 m, which is deeper than any published literature has described. A biological invasion in its early stages allows for the study of dispersal mechanisms, impacts to native communities and also provides an opportunity for the control and management of a species before it has completely invaded an ecosystem. In collaboration with federal and state agencies and organizations (TRPA, TRCD, USFWS, CSP, LRWQCB, NDEP, NDOW, NDSL, TWSA) and funding from sources including SNPLMA science and capital funds, LRWQCB Clean up and Abatement, and NDSL license plate funds our researchers from UC Davis and UN Reno have spent the last 18 months intensively researching the lakewide distribution, life history, and impacts to water quality and native ecosystems of Asian clam.

Asian clam (*Corbicula fluminea*) is a sediment dwelling, rapidly reproducing species that incurs ecological and economic damages where it establishes. *C. fluminea*, and invasive bivalves in general, have been observed to alter organic matter cycling in sediments (Hakenkamp and Palmer 1999), decrease phytoplankton abundance (Cohen 1984; Qualls et al. 2007), increase available substrate for other species (Werner and Rothhaupt 2007), dominate benthic invertebrate communities and alter biodiversity (Dermott and Kerec 1997; Karatayev et al. 2003), change porewater chemistry to toxic levels for native bivalve species (Cooper et al. 2005), impact industrial activities by macrofouling (Goss and Cain 1977; Isom 1986, Johnson et al. 1986; McMahon 1982) and affect governmental ecosystem assessment practices (Arndt et al. 2009). Because of the far-reaching impacts of this aggressive invasive species, there is a need to develop techniques to manage and reduce populations.

Hypoxic or anoxic conditions have been observed to play an important role in the prevention of invasive bivalve establishment in fresh waters. Low dissolved oxygen concentrations have been

cited as explanatory variables in the absence of zebra mussel (*Dreissena polymorpha*) colonization in deep hypolimnetic waters (Mackie et al. 1989; McMahon 1996; Stanczykowska 1977; Walz 1973). In Hargus Lake (OH), high *D. polymorpha* mortality rates (100%) have also been observed in anoxic shallow waters (5 – 12 m), and the combination of high ammonia and low dissolved oxygen concentrations have been observed with a 0% survival rate and significantly reduced growth rates (Yu and Culver 1999). *C. fluminea* are typically not found in profundal zones of lakes, usually due to hypoxic conditions (McMahon 1996), and the promotion of stratification and subsequent development of anoxia within the hypolimnion to kill adult quagga mussels (*D. bugensis*) in southern Californian impoundments is currently under development (De Leon 2008).

The physical removal of invasive species has promise as a non-chemical means to reduce negative economic and ecological impacts to native biodiversity, water quality and commercial and recreational use of ecosystems (Simberloff 1997, Mack et al. 2000). Efforts to remove invasive species have been attempted for a number of taxa (Zipkin et al. 2009) including rusty crayfish (Hein et al. 2006), dreissenid mussels (Wimbush et al. 2009), aquatic macrophytes (Tobiessen et al. 1992, Eichler et al. 1993), smallmouth bass (Weidel et al. 2007), birds (Brooks and Lebreton 2001, Frederiksen et al. 2001), nutria and other mammals (Gosling 1989, Campbell and Donlan 2005, Howald et al. 2007) and insects (Faccoli and Stergulc 2008). The successful reduction of invasive species, as well as the recovery of native communities in these managed systems is variable, and analyses have suggested that the magnitude of the management disturbance, fecundity, dispersal, rates of juvenile survivorship and maturation, and biological community composition can determine community succession patterns (Keller et al. 2007, Zipkin et al. 2008, Tobiessen et al. 1992, Levine and D'Antonio, Mack et al. 2000).

Disturbance of benthic habitats in aquatic systems disrupts the population structure of taxa and is widely documented as an important factor in the structuring of soft sediment macroinvertebrate communities (Grassle and Sanders 1973, McCall 1977, Dernie et al. 2003). A number of disturbance types that may impact benthic macroinvertebrate populations include point or nonpoint source pollutants (Beketov et al. 2008, Snucins 2003), water withdrawal or diversion (Otermin et al. 2002), modification of benthic geomorphology (Guerra-Garcia et al. 2003), habitat disruption because of upstream of watershed disturbances or land uses (Lewis et al. 2002), or the introduction of invasive species (Wittmann et al. 2011). Such disturbances can cause changes to the biodiversity and abundance of macroinvertebrate populations and may allow successional colonization by opportunistic species on various temporal and spatial scales (Kenny and Rees 1994, MacArthur and Wilson 1967). Recolonization of macrofauna in these communities has been well studied in river and marine ecosystems (Thrush 1991, Hall et al. 1994) and less so in lacustrine systems.

In general, aquatic insect communities in both marine and freshwater aquatic environments recover rapidly from disturbance. Models of marine macroinvertebrate recolonization predict

that disturbance produces spatial heterogeneity and changes to species diversity and ecological characteristics such as species fecundity, feeding type and brooding strategy will determine successional patterns (Johnson 1970, Grassle and Sanders 1973, Van Blaricom 1982, Pearson and Rosenberg 1978, Rhoads et al. 1978). Studies of recolonization dynamics of freshwater aquatic insects in have been overwhelmingly focused on lotic systems (Sheldon 1984). Stream macroinvertebrates are characterized by mechanisms to deal with flow variability, such as specific life histories that minimize the presence of vulnerable stages during times of peak flows. There is a lesser body of research concerning the recolonization after disturbance in lentic systems with studies in small natural lakes (Niemi et al. 1990, Snucins 2003), freshwater ponds (Caquet et al. 2007) and in artificial or manipulated systems (Woin 1996, Beketov et al. 2008).

The benthic species diversity of large high elevation lakes is typically low because of the harsh physical environment and lack of habitat heterogeneity (Brinkhurst 1972). Deevey (1941) and Rawson (1930) found in a number of landscape scale surveys of lakes that benthic faunal production decreases with lake area and mean depth of lake. Low temperatures and nutrient concentrations combined with large size and light penetration means that the lake is oligotrophic and benthic production should be low. Combined with potential decreases to the diversity and abundance of macroinvertebrates in Tahoe (Caires pers comm), the recent introduction of passively transported aquatic invasive species such as Eurasian watermilfoil (*Myriophyllum spicatum*), curly leaf pondweed (*Potamogeton crispus*) and Asian clam (*Corbicula fluminea*) can potentially have impacts to the sensitive and unusual benthic invertebrate assemblage in Lake Tahoe (Frantz and Cordone 1996). Increases in global climate pressures, urban development and recreational and commercial activities and subsequent spread in invasive species pressure will likely further alter benthic communities. It is becoming common practice amongst resource managers to mitigate these impacts through regulatory, monitoring and lake control procedures such as invasive species removal or control.

Dredging, or the physical removal of sediments, is a human induced disturbance in all aquatic environments used for a variety of commercial, conservation or water conveyance purposes. Dredging in marine and river systems usually significantly reduces benthic populations and can change the environmental abiotic features of habitat (Pranovi et al., 1998, Lopez-Jamar and Mejuto 1988, Kenny and Rees 1996, Lewis et al. 2001). Dredging or suction removal has been used for the treatment and removal of invasive aquatic macrophytes and has shown to alter habitat, create long term reductions to exotic target species and in some cases increase diversity to the plant community (Eichler et al. 1993, Tobiessen et al. 1992, Nichols and Cottam 1972, Nichols 1984). Dredging or suction removal for the removal of invasive bivalve species has never been carried out or reported on.

There have been few developments since the 1990‘s in *C. fluminea* control. Most methods are developed to reduce biofouling at steam electric generating plants or other commercial areas particularly in the southern United States. These methods include the use of screens, strainers,

filters, physical removal (vacuuming clams from floors or horizontal surfaces of intake bays), thermal control, paints and coating, metals (copper and zinc), oxidizing compounds such as chlorine, bromine, ozone, and halogenation (Doherty et al. 1986). These treatments are applicable in a power plant setting because they are typically targeted at removing clams from structures, intakes or other discrete areas, and are likely not appropriate for use at a lake or reservoir scale, especially when these water bodies are designated for multiple uses. Physical methods include the use of manual water draw down (White and White 1977) or water level fluctuations such as those in Lake Constance (Baumgartner et al. 2008) which are effective where populations can be emerged for a period long enough to cause total mortality.

Extended periods of valve closure can protect *C. fluminea* from exposure to pesticides, anoxia or emersion. Valve closure has been observed in *C. fluminea* when it is exposed to metals (Doherty et al. 1987), chlorine (Mattice et al. 1982), and suspended solids (Aldridge et al. 1987) enabling them to survive for long periods under unfavorable conditions (Doherty et al. 1986). A more recent control approach, developed for Dreissenid mussels but with application for *C. fluminea*, uses the encapsulation of KCl in microscopic particles of edible material, which inhibits valve closure and increases exposure to pollutants or other pesticides (Aldridge et al. 2006). Despite wide variety of available options, most are not effective due to application limitations and/or *C. fluminea*‘s ability to withstand via valve closure and are only appropriate for water conveyance infrastructure or areas where water level can be manipulated. Thus, control in natural lakes or reservoirs present prohibitive logistical issues, and thus populations in these systems largely go untreated.

C. fluminea are unique in their ability to withstand a wide range of environmental conditions (largely due to valve closure), but research on the physiological tolerance of *C. fluminea* suggests a limitation in life history and growth of populations due to anaerobiosis. *C. fluminea* cannot maintain normal O_2 uptake under severely hypoxic conditions (McMahon 1999) and thus are typically restricted to shallow well oxygenated habitats. In contrast, some native clams (*Pisidium spp.*) are extreme O_2 regulators (Burky 1983) allowing them to inhabit highly hypoxic and hypolimnetic habitats. Depending on temperature conditions, *C. fluminea* may remain anaerobic with the valves shut for a minimum of 3 – 4 days at high temperatures and for several weeks at low temperatures (Mathews and McMahon 1999). Thus, through valve closure, *C. fluminea* can avoid temporary lethal effects of pollutants until the accumulation of toxic anaerobic end products (acetate, propionate, succinate) (Grieshaber et al., 1994) cause clams to open valves, resume aerobic gas exchange and are subsequently impacted by pesticides or other toxicants (Jenner 1990; Mattice et al., 1982; McMahon and Lutey 1988).

The use of bottom barriers has been a common management approach for controlling nuisance aquatic macrophytes since the 1960‘s (Nichols 1974), however to date there has been no published information on the use of this method for controlling invasive benthic invertebrates. There are many types of benthic covers applied in harbors, boat lanes, swimming areas of lakes

and reservoirs (Ussery et al. 1997) which include: sand, gravel, plastic, synthetic fabrics held with weights (polypropylene, polyethylene terphthalate, Typar, Hypalon (nonbuoyant synthetic rubber), PVC (polyvinyl chloride) coated fiberglass, Permealiner). Most of the barriers used in macrophyte control are fabricated with gas-permeable materials, yet some studies have shown that benthic macroinvertebrates can be eliminated underneath due to low dissolved oxygen concentrations and high BOD (Engel 1984; Ussery et al. 1997).

b. Lake Tahoe and the management of Asian clam

Two non-chemical control methods were considered for field experimentation on Asian clam populations in Lake Tahoe: bottom or benthic barriers and diver-assisted suction removal. The proposed mechanism to induce removal or mortality of Asian clams given the use of bottom barriers is the reduction of dissolved oxygen concentrations and food availability. The proposed mechanism to induce removal or mortality of Asian clams given the use of diver assisted suction removal is the physical removal of Asian clams using a suction mechanism combined with off-site disposal of the clam and lake bottom sediment materials.

The use of bottom barriers placed over Asian clam beds in Lake Tahoe shows some theoretical promise for the localized control of both high and low density beds since Asian clam are dependent on dissolved oxygen as well as food sources from the water column to survive (McMahon and Bogan 2001). Oxygen concentrations at the sediment-water interface are determined and maintained by multiple factors: 1) continual exposure to highly oxygenated lake water, 2) localized sources produced from benthic algal production, 3) solubility of oxygen based on water temperature, 4) respiration from organisms such as clams and other invertebrates in the water or nighttime metabolism changes in algae, and 5) losses due to decomposition of organic material on or in the sediments. The use of bottom barriers was selected as a management option for Asian clam because it is a relatively low-impact, non-chemical and previously experimented-with in Lake Tahoe technique. The hypothesized impacts for bottom barriers include reductions in dissolved oxygen levels and food availability.

Ussery et al. (1997) used an 800 ft^2 gas permeable bottom barrier to treat the aquatic plants *Ceratophyllum* sp. and *Potamogeton* spp. in a 62 hectare reservoir in Wisconsin. They found that oxygen levels below the barrier were at or near zero the entire time. Within four weeks of placement, macroinvertebrate density decreased by 69%, and one year after the barrier was removed the density at the barrier site was 86% less than the macroinvertebrate density at the reference (open) site. Also porewater ammonium-N, and phosphate-P decreased significantly compared to an adjacent reference site. They used three porewater samples to test for nutrient change and five benthic grabs to test for macroinvertebrate density changes. Gunnison and Barko (1992) found little to no gassing under barriers where no macrophytes were present. Matthews and McMahon (1998) found that at 25 °C water temperature in a laboratory, the mean period to mortality for Asian clam under an anoxic condition is 31 days. However, at water temperatures

less than 15 C, Asian clam are unlikely to show fatal response to anoxic conditions in less than a 12 week period (Matthews and McMahon 1999).

Diver assisted suction removal is a mechanical control technology often used for plant removal, in which divers use pump systems to suction plants and roots from the sediment. The pumps are generally mounted on barges or pontoon boats and the diver uses a long hose with a cutter head to remove plants. The plants are vacuumed through the hose to the support vessel where plants are retained in a basket and sediment and water are discharged to the water body—in this case the basket where the material is retained could possibly be fitted with an appropriate filter or sieve to retain Asian clam but could return sediment to the lake bottom, or possibly deliver all materials to a nearby shore site to aid in the reduction of turbidity created as a result of the suction. Experiments were carried out to examine these factors. Diver assisted suction removal was selected as a management option because of its previous use in Lake Tahoe for Eurasian watermilfoil control. It is intended to physically remove Asian clam from sediments as a non-chemical control technique.

c. Project Area

Lake Tahoe (Figure 1) is a large (maximum depth: 501 m, surface area: 495 km^2) oligo- to meso-trophic lake located in the Sierra Nevada mountain range at a subalpine elevation of 1898 m. The Tahoe basin‘s largely granitic geology, the lake‘s large volume ($1.5x10^{14}$ L) and relatively small drainage (800 km^2) watershed explain the low nutrient concentrations and primary productivity rates (Goldman 1988). Increased development has caused eutrophication and increased growth of periphyton in the littoral zone in recent decades (summarized by Reuter and others 2009). Water temperature ranges from 5 to 28 °C in the littoral zone and temperatures averages continue to rise (Ngai et al. accepted for publication). A number of aquatic invasive species have recently established in the nearshore of Lake Tahoe, including curly leaf pondweed (*Potamogeton crispus*), largemouth bass (*Micropterus salmoides*) and *C. fluminea*. Lake Tahoe is a federally protected waterway where the application of pesticides or other non-natural chemicals is restricted. Lake Tahoe supports an unusual assemblage of benthic invertebrates. The last lakewide benthic survey was carried out in 1962-1963 and the invertebrate community was characterized as dominated by oligocheates, amphipods, ostracods, dipteran larvae (Frantz and Cordone 1996). Two sites in the southern portion of Lake Tahoe where benthic suction dredging and benthic barriers were applied are called Marla Bay and Lakeside (near Lakeside marina), which both have recently established populations of the invasive bivalve *C. fluminea*. These portions of the lake have a heavily developed shoreline with residential and commercial structures, golf courses, public beaches as well as high recreational boater traffic.

The project area described herein is focused on the southeast shores of Lake Tahoe where Asian clam has been found in widespread abundance. The greatest densities of clams are found with heterogeneous distribution between Zephyr Cove and the Tahoe Keys East Channel with smaller

satellite populations present in the mouth of Emerald Bay. For the purposes of this study, Marla Bay and a region north of Lakeside marina (–Lakeside") were chosen as our study sites due to significant differences in habitat types and Asian clam densities as determined through 2008 field surveys (Hackley et al. 2008). Marla Bay is located in Nevada approximately 2 miles north of Stateline (Figure 1A). Marla Bay is surrounded by residential properties as well as Round Hill Pines Beach and Marina which sees heavy boat traffic and recreational usage during the summer months. A large shelf is present here and extends between approximately 300-800m at a fairly consistent depth of 5 m, before sharply dropping off. Marla Bay has the highest recorded population density of Asian clams in Lake Tahoe with a median density of 2000 individuals/m^2 and a maximum of 9,000 individuals/m². Water temperatures in Marla Bay range from a 5 to 22 ^{0}C (Figure 2) and sediment type primarily consists of coarse sand and very coarse sand (0.5mm-1.18mm) (Figure 3). The Marla Bay suction removal treatment plot is located at 38˚59.517 N, 119˚57.521 W and the polyethylene barriers were also placed at Marla Bay with the northern edge located at: 38˚59.492 N, 119˚57.577 W, and the southern edge of these plots located: 38˚59.464 N, 119˚57.575 W.

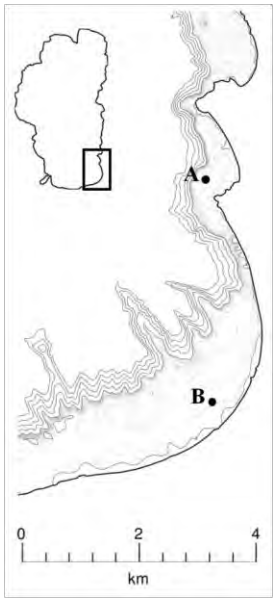


Figure 1. Location of Asian clam treatment plots in Lake Tahoe. A represents Marla Bay and B represents Lakeside. Both plot locations are at a 5 m water depth in the southeastern portion of Lake Tahoe where Asian clam populations are found.

Lakeside is located in California just west of the California-Nevada state line (Figure 1B) and offshore from multiple residential and commercial properties including the Edgewood Golf Course and the Lakeside Marina. Asian clam densities at Lakeside are lower than Marla Bay with a median concentration of approximately 200 individuals/m² and maximum density of 800 individuals/m². Water temperatures at Lakeside range 4˚C to 22˚C (Figure 2) and the sediment type Lakeside is finer than at Marla Bay and is comprised of medium sand to coarse sand (Figure 3). No polyethylene barriers were applied at Lakeside, however diver assisted suction removal was carried out here. The Lakeside suction removal treatment plot was located at 38˚57.572N, 119˚57.344 W.

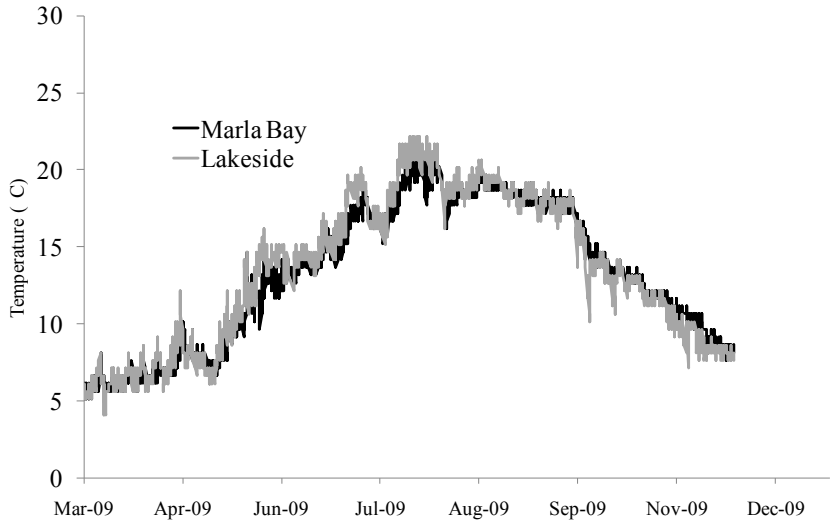


Figure 2. Temperature recorded at the Marla Bay and Lakeside treatment sites over the duration of the experimental period. Marla Bay is represented by the black line and Lakeside by the gray line. In general, Lakeside temperatures are slightly warmer during the summer period and Marla Bay temperatures are slightly warmer during the autumn period of 2009.

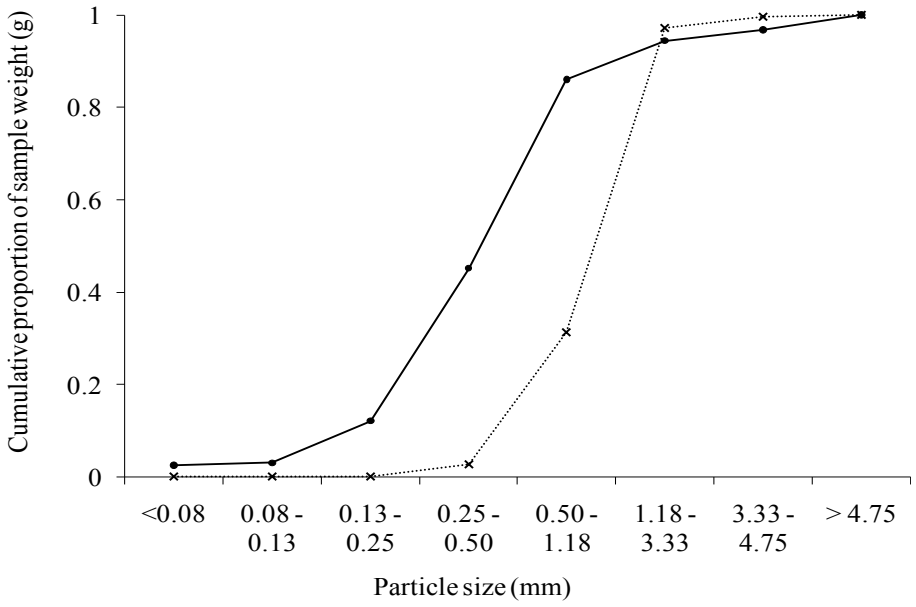


Figure 3. Sediment particle size distribution for the Marla Bay (dashed line) and Lakeside (solid line) sites. Sediment samples (N = 6) were wet sieved using methods described in Gordon et al. (1992). Marla Bay median particle size M_e = 1.18 and Lakeside M_e = 0.38.

III. Asian clam removal efficacy evaluation and experimental research design: bottom barriers and diver assisted suction removal

The following is a description of the field methodology, data processing, quantitative analysis and conclusions related to the application of polyethylene fabric bottom barriers and diver assisted suction removal experiments carried out in Lake Tahoe.

a. Baseline assessment of benthic community prior to experimental treatment

i. Field collection and laboratory processing

To characterize the macroinvertebrate community (including Asian clams) in both the bottom barrier plots and diver assisted suction removal plots prior to treatment, sediment grab sampling was employed. A total of three sediment grabs per bottom barrier treatment plot (N = 36) and three sediment grabs per control plot (N = 9) were collected in March 2009. Upon removal of bottom barriers and immediately after suction removal, this sampling was repeated. A total of three sediment grabs in each diver assisted suction removal plot (N=18) and three in each control plot (N = 6) were also collected in March 2009. Monitoring of the benthic barrier and suction removal plots using this sampling design was repeated every 6 – 8 weeks until January 2010 to observe recolonization rates.

A petite Ponar grab sampler (Wildco, 2.4 L volume, 231 cm^2) was used to collect the sediment grab samples. The Ponar was deployed from a small boat over the plots and grabs were taken from the inside, middle and outside portions of the plots in order to be able to differentiate between potential recolonization types (i.e., movement of individual Asian clams from adjacent plots or juvenile release and water column based transport). Once the sample was collected, it was placed in a 500-µm mesh sieve bucket and sieved immediately to remove silt. Samples were then placed in a labeled Ziploc bag and stored on ice in a cooler until they can be further processed at the lab. The sieve chamber is visually inspected after each sample has been removed to ensure no invertebrates are left in bucket. If the Ponar misfires or only a half sample is collected, it is discarded and resampled. Samples are then returned to the laboratory for elutriation, processing and identification.

Elutriation

In the laboratory sediment samples are elutriated to separate invertebrates from the sediment matrix. To carry out the elutriation process, contents of one sample bag are emptied into a 20 Liter (5-gallon) container. The bag is rinsed over the bucket to ensure no materials or invertebrates are lost. A solution of sugar water is then added to the sample until the bucket is approximately 1/8 full with water. The bucket is picked up and swirled numerous times in order to float invertebrates to the top of the solution. While the water is still in motion, it is poured into

a 500-µm sieve. This process is repeated 5 to 10 times. Invertebrates and sediment caught by the sieve are then transferred to a container for invertebrate removal from sediments.

Invertebrate removal from sediments

Each container is visually inspected for invertebrates. If invertebrates are present, they are gently removed from the sample with forceps or pipette and preserved in 70 % ethanol. Asian clams and the native pea clams (*Pisidium spp.*) are stored in small Nalgene bottles and all other invertebrates are kept in a separate glass scintillation vial. Each sample must be inspected by at least three people to ensure the removal of all invertebrates from the sediment substrate. Sediments are then discarded and the processed samples are stored in a cool place until the invertebrates can be counted and identified. Invertebrates must be removed from the sediment samples the same day of collection in order to minimize fatality and/or damage to biota.

Invertebrate identification and measurement

Two measurements are taken for each clam using digital calipers. Measurement 1 is the longest distance from the umbo, or shell hinge, to the edge of the shell opening. Measurement 2 is the longest distance from the anterior to posterior edge (Figure 4). These measurements are carried out for both Asian clam and the native pea clam (*Pisidium spp.*). After the measurements have been recorded, the clams were returned to the Nalgene bottle with 70 % ethanol solution and stored in a cool dry place. Invertebrates are identified to family, separated, counted and noted on datasheet. After identification of the sample is complete, invertebrates are return to scintillation vial with 70 % ethanol solution and stored indefinitely in a cool dry place.

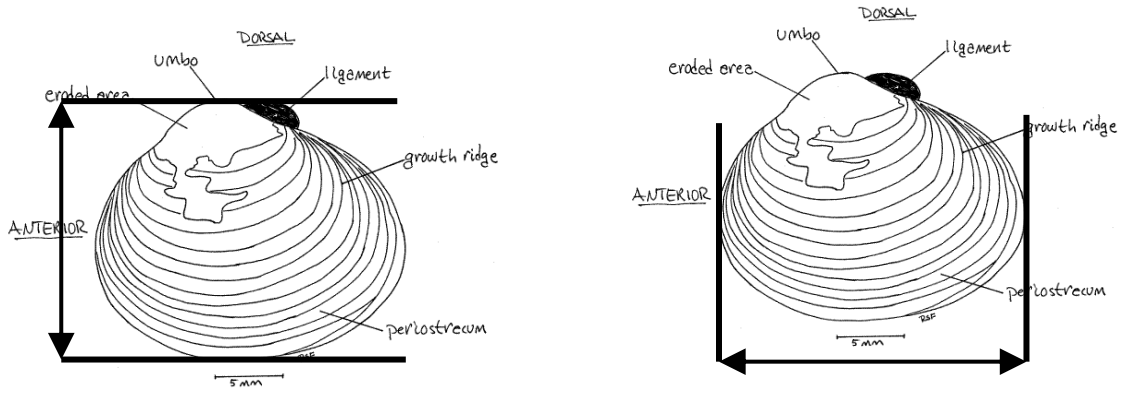


Figure 4: Measurements recorded for Asian clams during processing and data collection. 4a represents Measurement 1 and 4b represents Measurement 2.

ii. Results

In Marla Bay, the total average density of macroinvertebrates was 6,400 individuals/m² in the suction removal plots prior to treatment and 6,800 individuals/m² in the benthic barrier plots prior to application (Figure 5). In Marla Bay, Asian clam densities accounted for one third of the total invertebrate community while worms and midges made up 40 % of the community. In Lakeside, the total density of invertebrates was 3,600 individuals/m² prior to suction removal (barrier treatment was not tested at this location). One third of the community was comprised of amphipods (scuds) and one third was made up of worms and midges. Thirteen percent of the invertebrate community at Lakeside was *Pisidium* spp. and Asian clam accounted for 8 % of the total community prior to suction treatment.

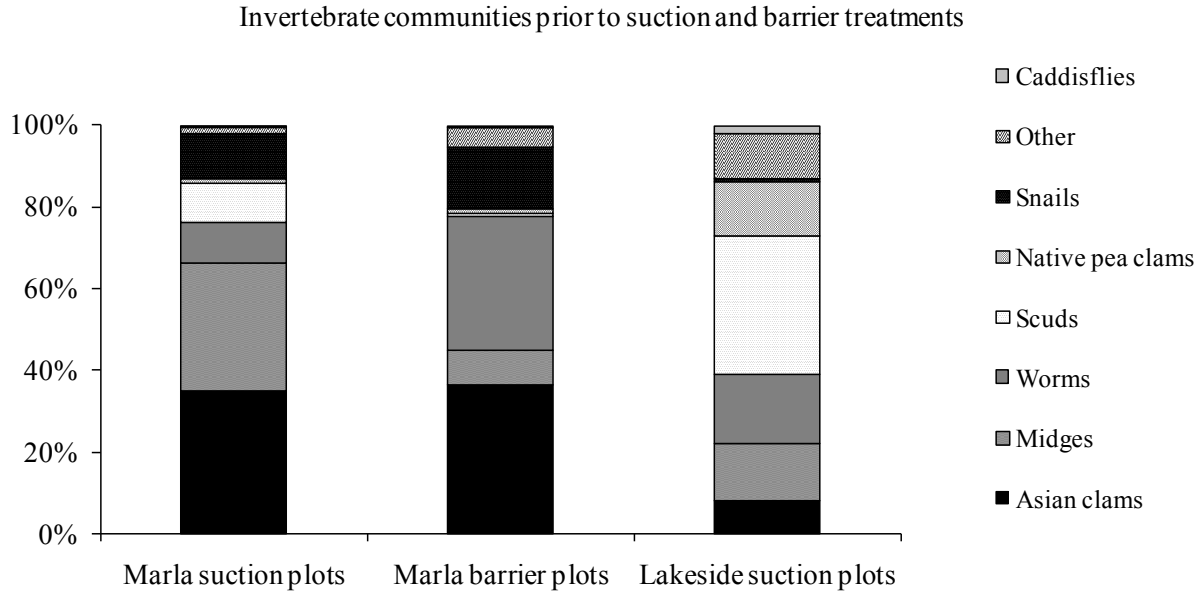


Figure 5. Baseline densities of benthic macroinvertebrates prior to suction and polyethylene barrier treatments. Barrier treatment was not conducted at the Lakeside site.

b. Polyethylene bottom barrier application

i. Experimental design and methodology

In March 2009, 12 polyethylene fabric barrier plots and 3 control plots were installed in Marla Bay within a checkerboard grid treatment area (Figure 6). The black squares in Figure 6 represent 20 ft x 20 ft barrier plots (created by overlapping four 10 ft x 10 ft barriers—requiring a total of 48 10 ft x10 ft barriers), and the white areas represent uncovered control plots. Of the 12 barrier plots, one set of nine (i.e., plot numbers 1 – 9) was intended to be applied in the following configuration: three will be installed for a 1 month period, three for a 2 month period, and three for a 4 month period in order to observe variable amounts of time in which the

requirements for significant Asian clam mortality rates could be observed. Three barrier plots (i.e., plot numbers 10 - 12) were to be applied in this area as well, also to be removed after either 1, 2, or 4 month period—determined by an observation of barrier-induced clam mortality in other plots—and treated with suction removal to evaluate the efficacy of a combination bottom barrier and suction removal treatment. *In situ* dissolved oxygen probes (Zebra-Tech, Ltd. D-Opto Logger, Accuracy ± 0.1 °C for temperature, 1% of reading or 0.02 mg L^{-1} for DO) were deployed in the treatment area: one underneath a bottom barrier and one adjacent to this to measure background lake condition. Because of the failure of the polyethylene fabric barriers to reduce dissolved oxygen concentrations or impact Asian clam mortality rates (see results section), the combination of barrier and suction removal plot experiment was not carried out, as agreed to by the Asian Clam Working Group in 2009.

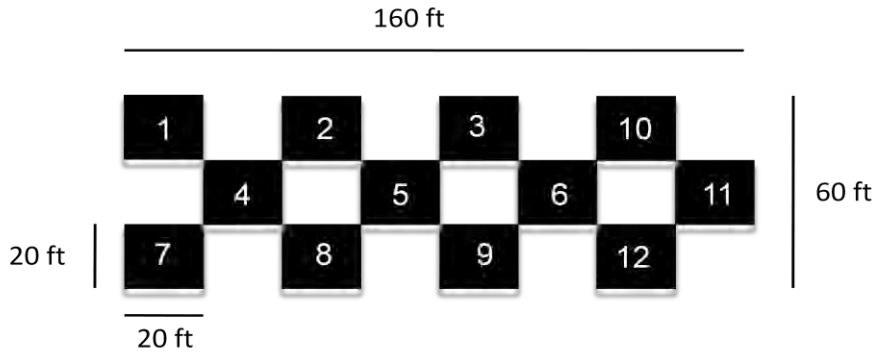


Figure 6. Lake Tahoe bottom barrier experimental configuration. Installed in Marla Bay on March 1, 2009. Each 20 ft by 20 ft plot was created by overlapping four 10 ft by 10 ft bottom barriers. Barriers were installed on plots 10-12 for use in combination treatment (bottom barrier + suction removal).

ii. Results

Upon removal of the first set of polyethylene bottom barriers it was found that there was no impact to Asian clam or other macroinvertebrate communities nor any impact to the dissolved oxygen concentrations under the barrier in comparison to background lake condition (Figure 7). Because of this the polyethylene bottom barriers were removed from the lake and the experiment concluded. Please see Section IV for the amendment to the bottom barrier experiment using ethylene propylene diene monomer (EPDM) sheeting.

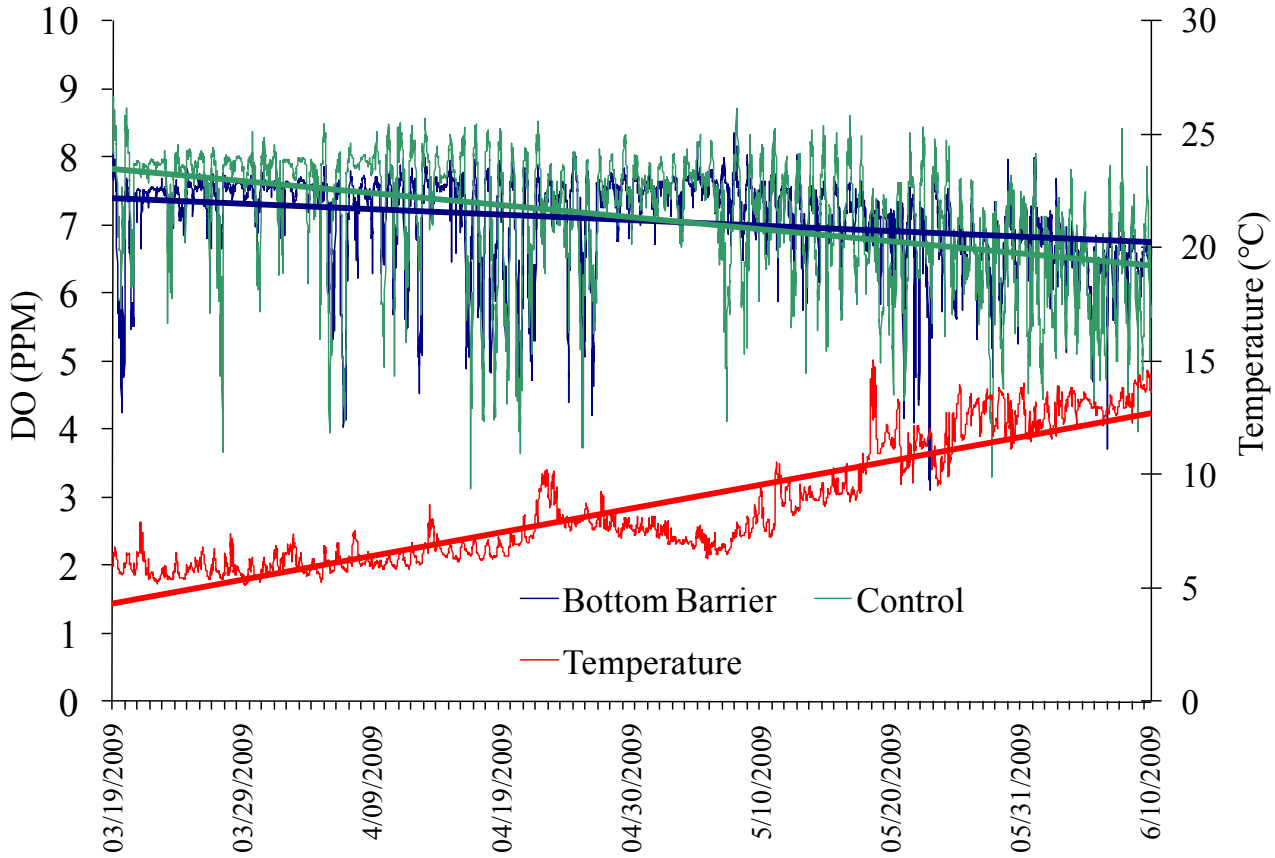


Figure 7. Dissolved oxygen concentrations under polyethylene bottom barrier and in control (uncovered) plots at Marla Bay from March 19 to June 11 2009. Note the insignificant reduction of dissolved oxygen under the polyethylene barriers (blue line) compared to the control (open lake) condition (green lines). Red line represents temperature (C).

The following figures (8 – 16) show the impact to various macroinvertebrate populations (including Asian clam) after treatment with polyethylene barriers at Marla Bay. Error bars represent one standard error. All taxa do not have a significant difference between barrier and control plots except for amphipoda (a highly mobile taxa) and trichopetera (highly variable taxa in space).

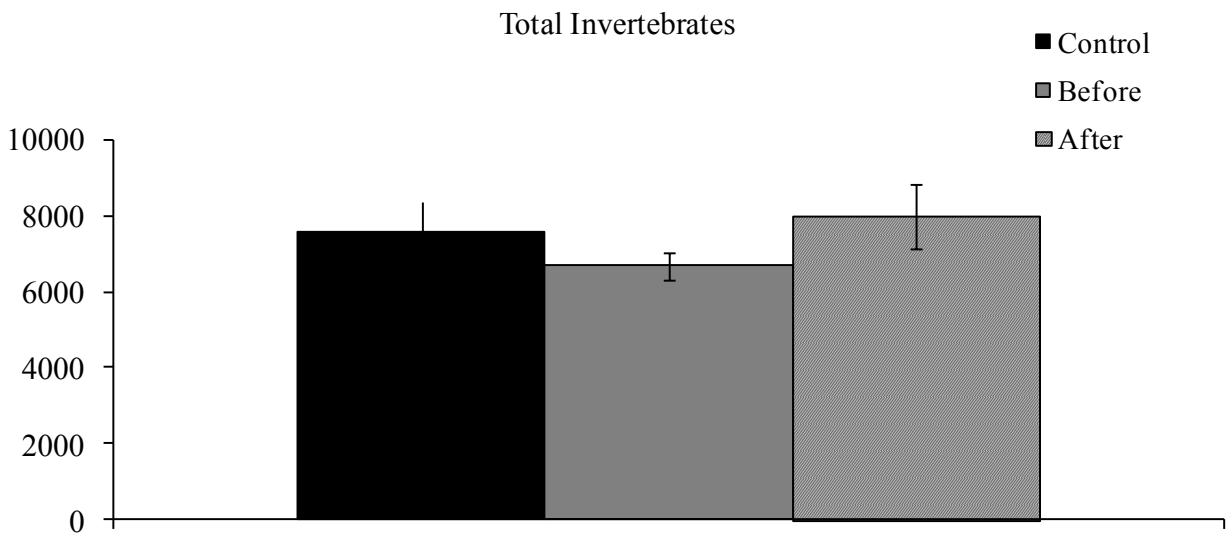


Figure 8 Mean density of all benthic macroinvertebrates collected in Marla Bay before and after polyethylene barrier treatment; controls are plots where barriers were not applied. Error bars represent standard error.

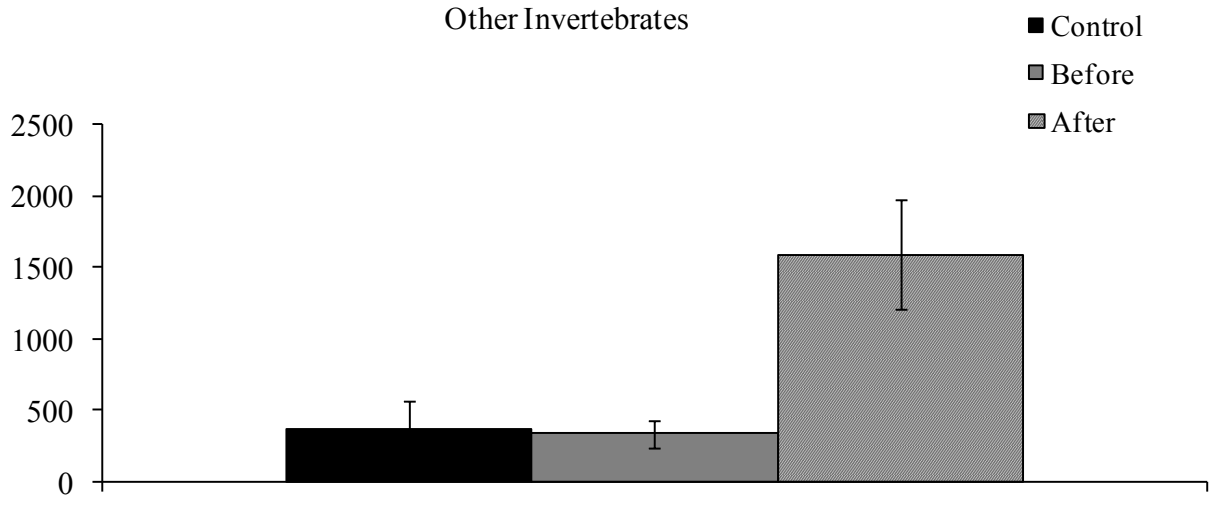


Figure 9. Mean density of non-dominant macroinvertebrates collected in Marla Bay before and after polyethylene barrier treatment; controls are plots where barriers were not applied. Error bars represent standard error. Non-dominant taxa include ceratopogonidae, non-chironomid diptera, ostracoda, copepoda, hydracarinidae, cladocera, nematoda, turbellaria, and hirudinea.

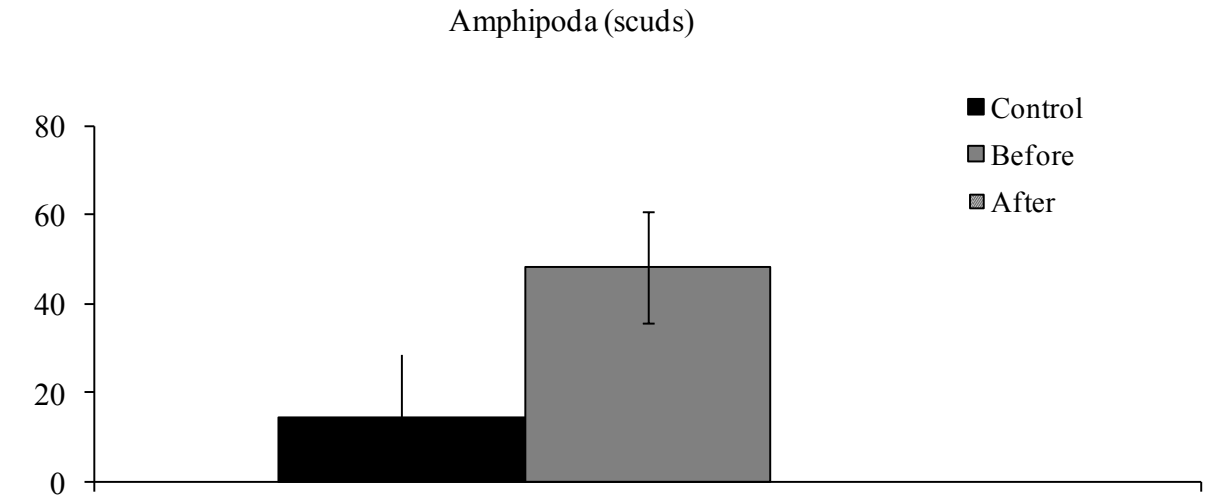


Figure 10. Mean density of all amphipods collected in Marla Bay before and after polyethylene barrier treatment; controls are plots where barriers were not applied. Error bars represent standard error.

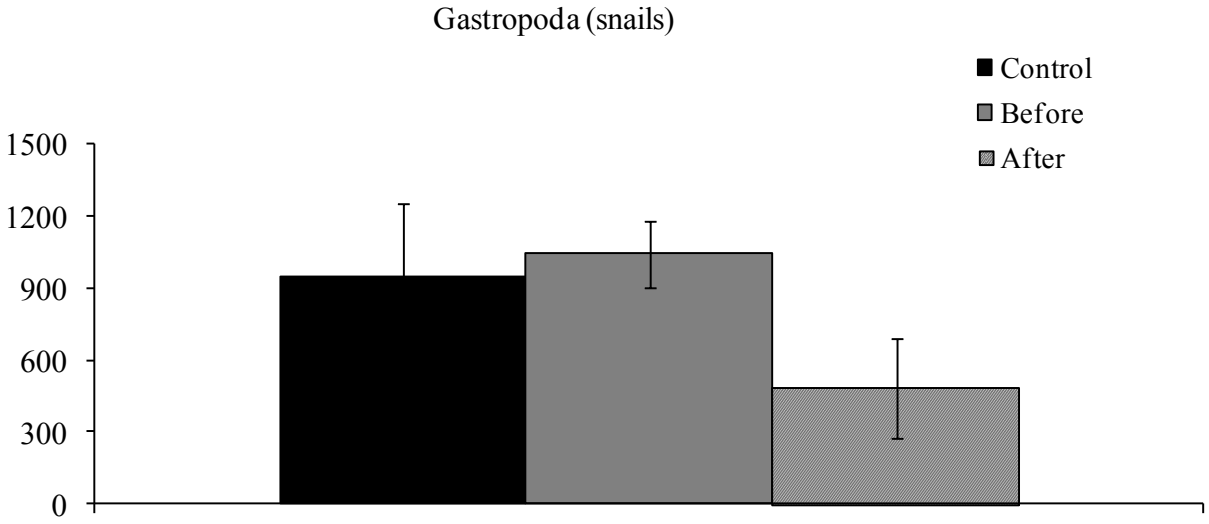


Figure 11. Mean density of all gastropods collected in Marla Bay before and after polyethylene barrier treatment; controls are plots where barriers were not applied. Error bars represent standard error.

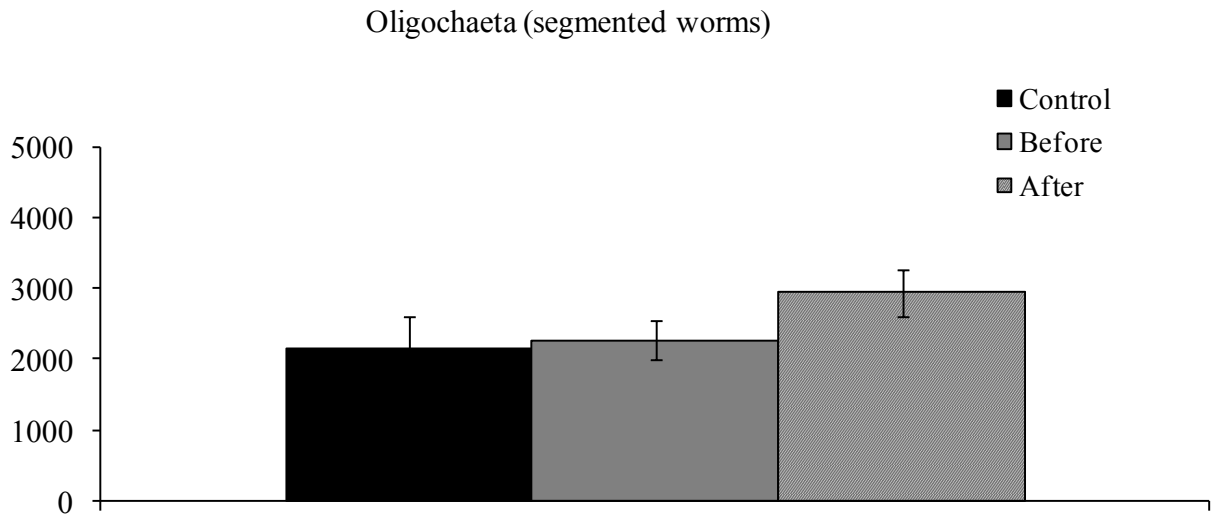


Figure 12. Mean density of all oligochaetes collected in Marla Bay before and after polyethylene barrier treatment; controls are plots where barriers were not applied. Error bars represent standard error.

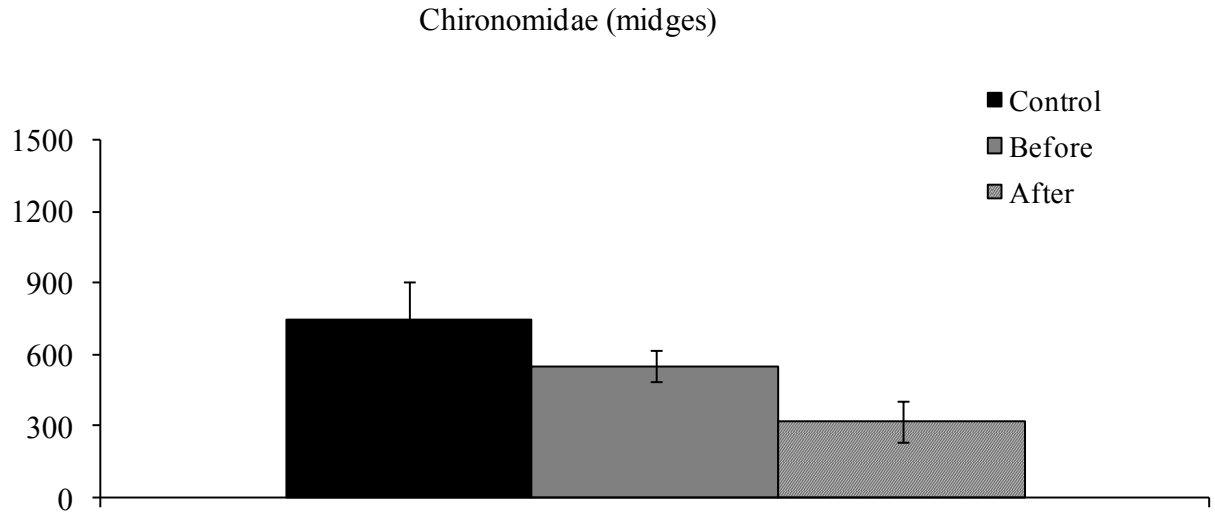


Figure 13. Mean density of chironomids collected in Marla Bay before and after polyethylene barrier treatment; controls are plots where barriers were not applied. Error bars represent standard error.

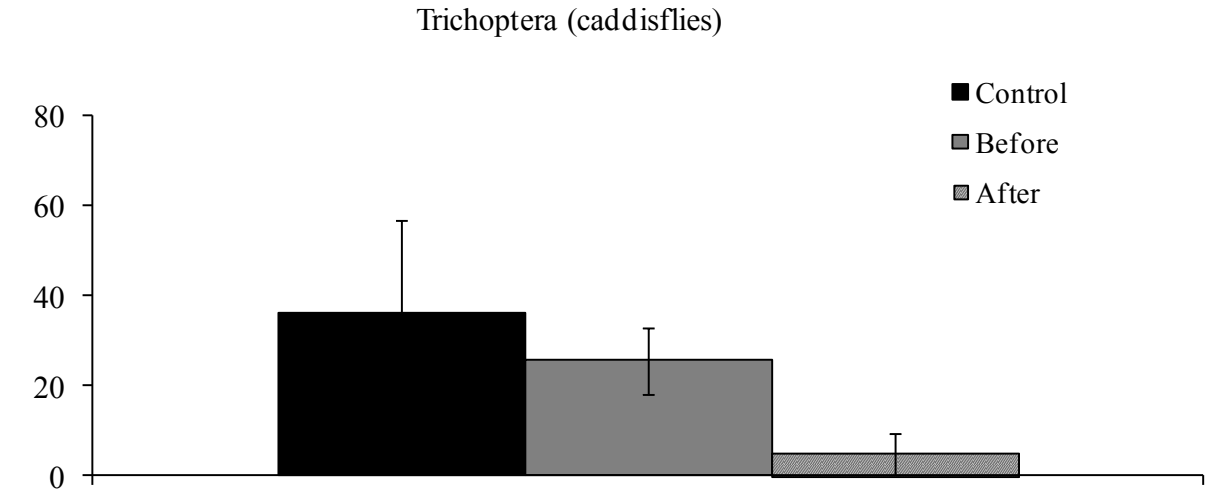


Figure 14. Mean density of all trichopterans collected in Marla Bay before and after polyethylene barrier treatment; controls are plots where barriers were not applied. Error bars represent standard error.

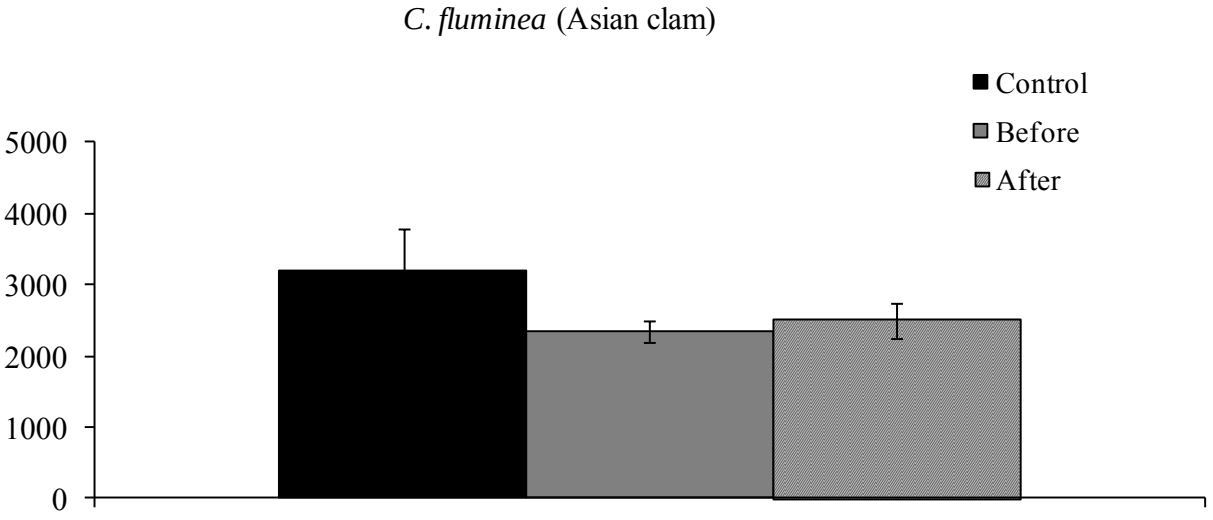


Figure 15. Mean density of all C. *fluminea* collected in Marla Bay before and after polyethylene barrier treatment; controls are plots where barriers were not applied. Error bars represent standard error.

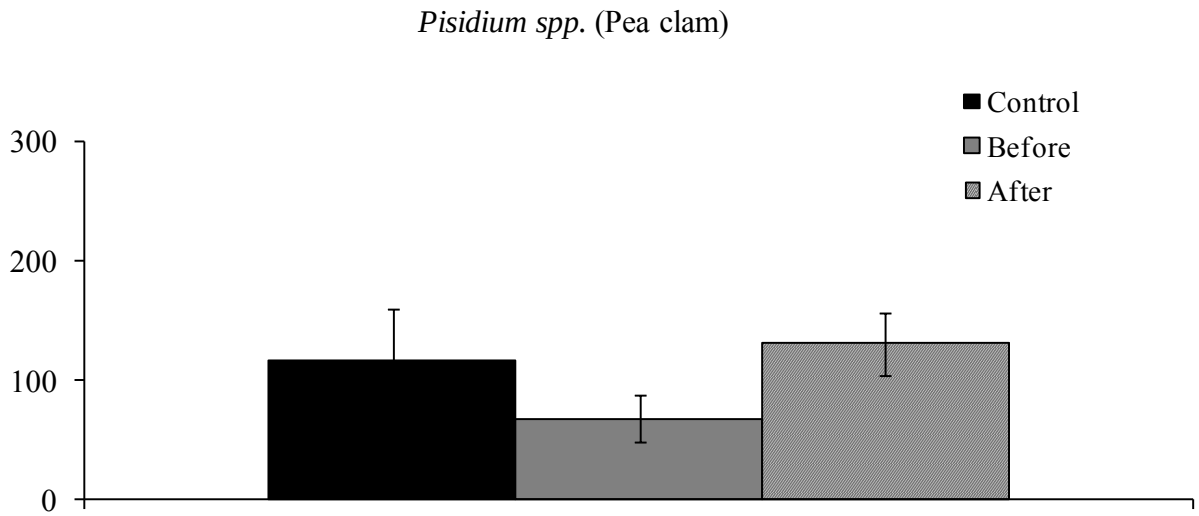


Figure 16. Mean density of all *pisidium spp*. collected in Marla Bay before and after polyethylene barrier treatment; controls are plots where barriers were not applied. Error bars represent standard error.

iii. Discussion

The use of polyethylene sheeting as a benthic barrier to control aquatic macrophyte populations is successful due to its light impermeability and gas permeability (to allow the release of gasses generated as a result of biomass degradation). Because the treatment of Asian clam relies on the reduction of dissolved oxygen, the use of a gas permeable fabric such as polyethylene is not suitable for Asian clam treatment using the mechanism of anoxia-induced mortality. There was no significant impact to dissolved oxygen concentrations underneath polyethylene bottom barriers and little to no impact on the benthic macroinvertebrate community as a result of this treatment methodology.

c. Diver assisted suction removal

i. Experimental design and methodology

Prior to diver assisted suction removal of *C. fluminea* infested sediments, macrobenthic sampling plots (surface area: 36 m^2) were created using SCUBA in two locations (Marla Bay and Lakeside) at Lake Tahoe. At each site, three suction removal plots and one control plot were delineated on the lake bottom to guide the dredging diver and to demarcate sampling areas for monitoring purposes (Figure 17). In March 2009 sediments were dredged to a 13 cm depth at

Marla Bay (9.5 m^3 removed) and to an 8 cm depth (until clay hard pan boundary) at Lakeside (8.5 m^3 removed). Because of field resource limitations, only two plots were suctioned at Marla Bay and three at Lakeside.

Because of the risk entailed and expertise required for under water AIS removal, specialized divers were retained to carry out the diver assisted suction removal in Marla Bay and Lakeside (Figure 18). A diver manipulated sediment removal apparatus was configured using a combination of a suction dredge system, a sluice box (to deposit materials), and 5 inch diameter tubing. The suction apparatus and divers were deployed off of on-site barge. Each of the three plots at Marla Bay and Lakeside (N= 6) were to have sediments containing Asian clams and other native macroinvertebrates removed for eventual disposal in an out-of-basin landfill. Rates of removal and depth of removal were recorded.

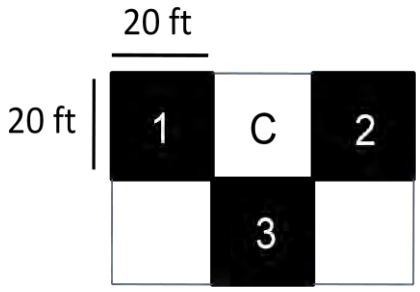


Figure 17. Plot design for the diver assisted suction removal experiment. Three plots (i.e., plot 1, 2, 3) each 400 ft^2 in size, were installed at Marla Bay and at Lakeside, with one control plot amidst the treatment plots.

Basic efficiency of removal techniques was also evaluated, including a short scale experiment to look at suction dredge mesh size required to retain the most clam material and diver time per unit removal area and the time required to dispose of sediment and clam material. To investigate the potential for reducing the amount of sediment material (and associated costs) requiring disposal, a small-scale experiment was carried out to look at the difference in retained Asian clams after sieving sediment materials. Two ¼ m^2 plots were delineated on the lake bottom in order to create a known area from which to time the rate of sediment removal. One plot was sieved through a ¼ inch mesh and the other was sieved through a ½ inch mesh. Sediment materials were weighed and remaining Asian clams found in the substrate were measured and counted.

Sediment sampling included a combination of particle size analysis, macrobenthos collection, and resource quantity measurement. Sediment particle size distribution was analyzed using a wet sieve method following methods of Gordon et al. (1992) and described using a Wentworth scale. A petite Ponar grab sampler (Wildlife Supply Company, 2.4 L volume, 231 cm^2 sample area)

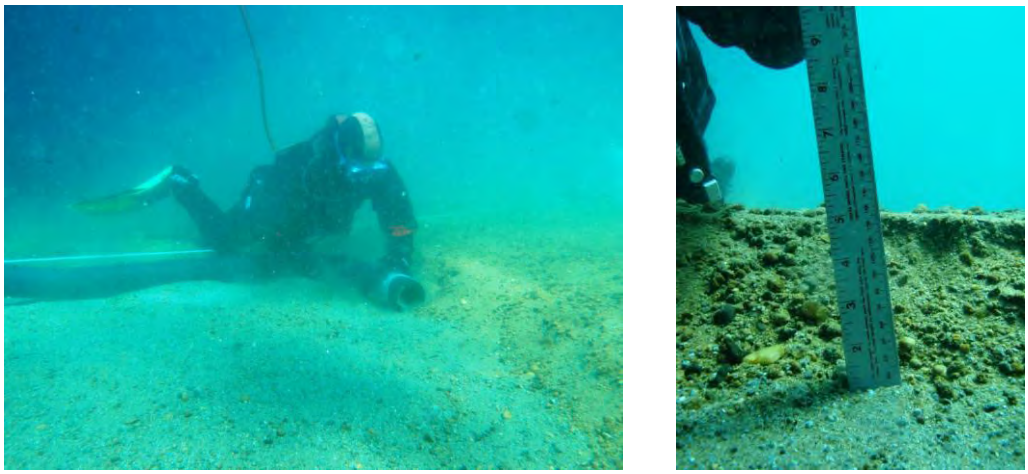


Figure 18. (Left) Diver assisted suction removal shown at Marla Bay, NV. In total, five 400 sq ft. plots had sediments removed for the purpose of Asian clam harvest. At Marla Bay, sediments were removed to a depth of 5 – 9 inches (right). *Photo credit: Brant Allen, UCD*

was used to collect 3 grab samples per treatment and control plot at inner, middle and edge positions relative to the perimeter of the plot. Upon collection all samples were screened (500 µm mesh) and the retained sediment then placed in a super-saturated sugar solution to separate invertebrates. All visible invertebrates were hand-picked and preserved in 70% ethanol until identification (Thorp and Covich 1991, Merritt and Cummins 1996). Environmental parameters collected included measurements of total organic carbon (TOC) and particulate organic matter (POM) from water column and sediment samples. Water collected from the water-substrate interface was processed to measure total organic carbon (mg TOC per 1 L water) with the elemental analyzer, Shimadzu TNPC-4110C. To measure particulate organic matter (POM), a thin scrapping of substrate gathered in the Ponar was processed within 24 hours of collection. Samples were dried at 90°C for 48 hours, weighed, combusted at 475°C for 8 hours and reweighed. POM is expressed as the amount of organic matter ashed off during the combustion, calculated as the mean of the difference between pre- and post-combustion weights (mg POM per mg substrate). Temperature samples were collected using iButton® temperature loggers (Model #DS1922L, accuracy of ±0.5°C).

Total macroinvertebrate abundance, abundance by taxonomic grouping, Simpson Diversity Index (Simpson 1949), and Shannon-Weiner evenness index (Shannon and Weaver 1964) were represented for each sampling. The influence of sediment grain size, POM, TOC, temperature and time on these parameters was analyzed using multivariate analysis of variance and most parsimonious models are selected based on Akaike Information Criterion (AIC) (Bozdogan 1987). Because benthic samples were collected in multiple treatment sites prior to dredging we adopt a BACIPs (Paired Before-After Control-Impact) analysis to describe the sediment removal

effect on *C. fluminea* and benthic macroinvertebrate community dynamics (Stewart-Oaten et al. 1986, Underwood 1991, 1994, Guerra-Garcia et al. 2003). Effect size is calculated by forming differences between pairs:

Equation 1

Where X represents taxonomic abundance or diversity and evenness index values, μ is the mean difference between control and impact, is the change in difference from before to after and ε_{ik} is the error associated with the differences (Stewart-Oaten et al. 1986). Differences (D_{ik}) are then compared for the before and after period using a two-sample t-test. A significant change in the mean difference (μ) between populations after the onset of the perturbation is strong evidence of an effect of the environmental impact. That is, control and impact sites respond differently to the disturbance regardless of their initial relationship. All statistical analysis is carried out using R 2.12.1.

ii. Results

Sediment removal efficiency

The Lakeside site is characterized coarse to medium sand with a median sediment particle size M_e = 0.375 mm and very coarse sand at Marla Bay (M_e = 1.180 mm) (Figure 3). A total of 5 400 ft^2 plots were suctioned over a two week period (delays due to weather and professional diver availability). This extrapolates into a production of 16,567 kg of wet sediments produced per plot, at a total of over 81,000 kg of sediments to be disposed of. It took approximately 3 days (10 dive hours per day, 2 divers) to suction 2 plots at Marla Bay and 3 days (5 diver hours per day, 2 divers) to suction 3 plots at Lakeside. The difference in timing is due to sediment substrate differences (coarse sand with large sediment column length (>20 cm) at Marla Bay in comparison to a finer sand and shorter sediment column length (~8 cm) at Lakeside) and logistical errors at Marla Bay. Results from the screening experiment are shown in Figure 19. In total, there were 27.9 kg of wet sediments recovered from each ¼ m^2 square plot.

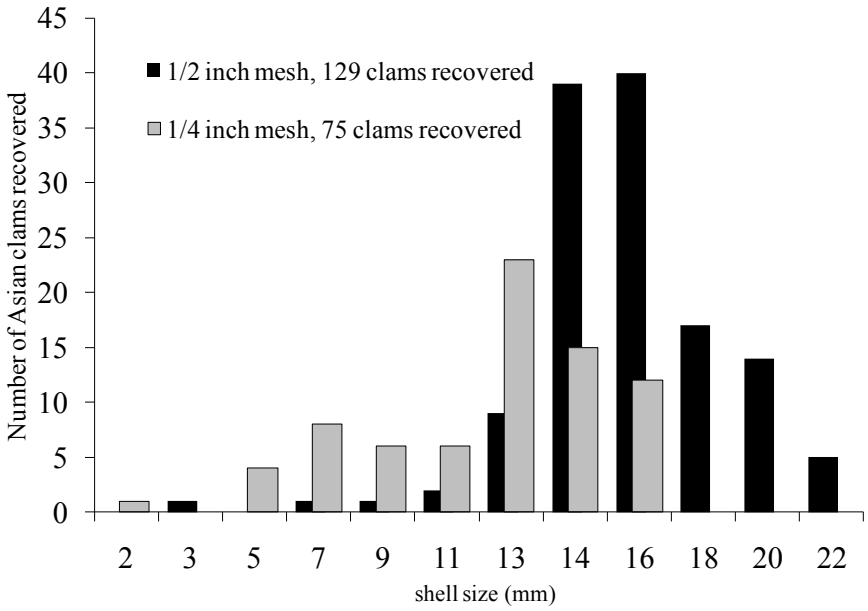


Figure 19. Two ¼ m^2 plots were sieved through different mesh size (¼ inch mesh and ½ inch mesh) to determine which is most effective capturing clams. Neither of the mesh sizes were able to capture all adult Asian clams but the ¼ inch mesh size resulted fewer Asian clams being returned into the treatment area.

As expected, using a smaller mesh size reduces the number of Asian clam recovered from the dredge materials compared to the larger mesh size. However, both mesh sizes permit the retention of adult (reproductive, > 7 mm shell size) Asian clam in the treatment plots which permits the immediate recolonization of the suction plot.

Invertebrate community composition and recolonization

Macrofaunal assemblages are similar in both sediment substrate types but with lesser overall abundances at the Lakeside site (Figure 20). Common taxonomic groupings observed included order Amphipoda (*Hyalella sp., Gammarus sp.*), family Chironomidae (multiple species), Oligocheata (multiple species), class Gastropoda (families Planorbidae and Physidae), order Trichoptera (*Hydroptila sp., Limnephilus sp.*), and non-native and native bivalves, family Corbiculidae (*Corbicula fluminea*) and family Sphaeriidae (*Pisidium spp.*), respectively. Other less common taxonomic groups included non-dominant taxa include family Ceratopogonidae (*Palpomyia sp.*), class ostracoda, subclass copepoda, suborder Hydracarinidae, order Cladocera, class Hirudinea *Helobdella stagnalis*, *Erpobdella punctata*) and phylum Nematoda and are considered as ―other‖ in this analysis. For a complete historical listing of macroinvertebrate taxa found in Lake Tahoe please refer to Frantz and Cordone (1996).

All control and impact plot taxonomic abundances and diversity and evenness indices were not significantly different from each other prior to suction removal (Table 1). Immediate impacts of suction removal were variable for different taxa (Figure 20 a-d). At 14 days after suction removal treatment, average total invertebrate abundance was reduced from 7934 to 837 ind/m^2 (89% reduction) in Marla Bay and from 3980 to 125 ind/m^2 at Lakeside (97% reduction). The impact to the harvesting target (*C. fluminea*) was equally significant with a reduction from 3177 to 149 ind/m^2 (95%) at Marla Bay and 271 to 0 ind/m^2 (100%) at Lakeside. BACIPs analysis results show that overall, chironomids, gastropods and trichopterans were not significantly impacted in treatment sites as a result of suction removal. However, by looking at the difference in taxonomic abundance by site, Lakeside shows significant reductions in these taxa whereas Marla Bay does not (Figure 20 a-d). This is due to the differential sediment type at both locations and the ability for the suction dredge to remove finer sediments more efficiently than coarse sediments. Both diversity and evenness are significantly impacted immediately after suction removal, with significant differences in effect size compared to pre-treatment values (Table 1).

Temporal changes in effect size show that total invertebrate abundance effect size is non-significant at 240 d (Table 1). However, diversity and evenness indices remain significantly different from pre-impact conditions throughout the entirety of the sampling period (450 d) suggesting that community dynamics are being altered by the harvesting action. Chironomids and ~~O~~ther" taxa have a positive effect size at 240 d, indicating that abundances in treatment plots at this time surpass abundances in control and impact plots prior to treatment. All other taxonomic groupings have negative, but reducing, effect sizes at the end of the sampling period. Figure 21 shows the proportional change in effect size for total invertebrate abundance, *C. fluminea* abundance, native clam (*Pisidium spp.*) abundance and Simpson diversity index. By the end of the sampling period, both the native clam and total invertebrate abundance show the greatest reductions in effect size, while *C. fluminea* populations and species diversity remains impacted.

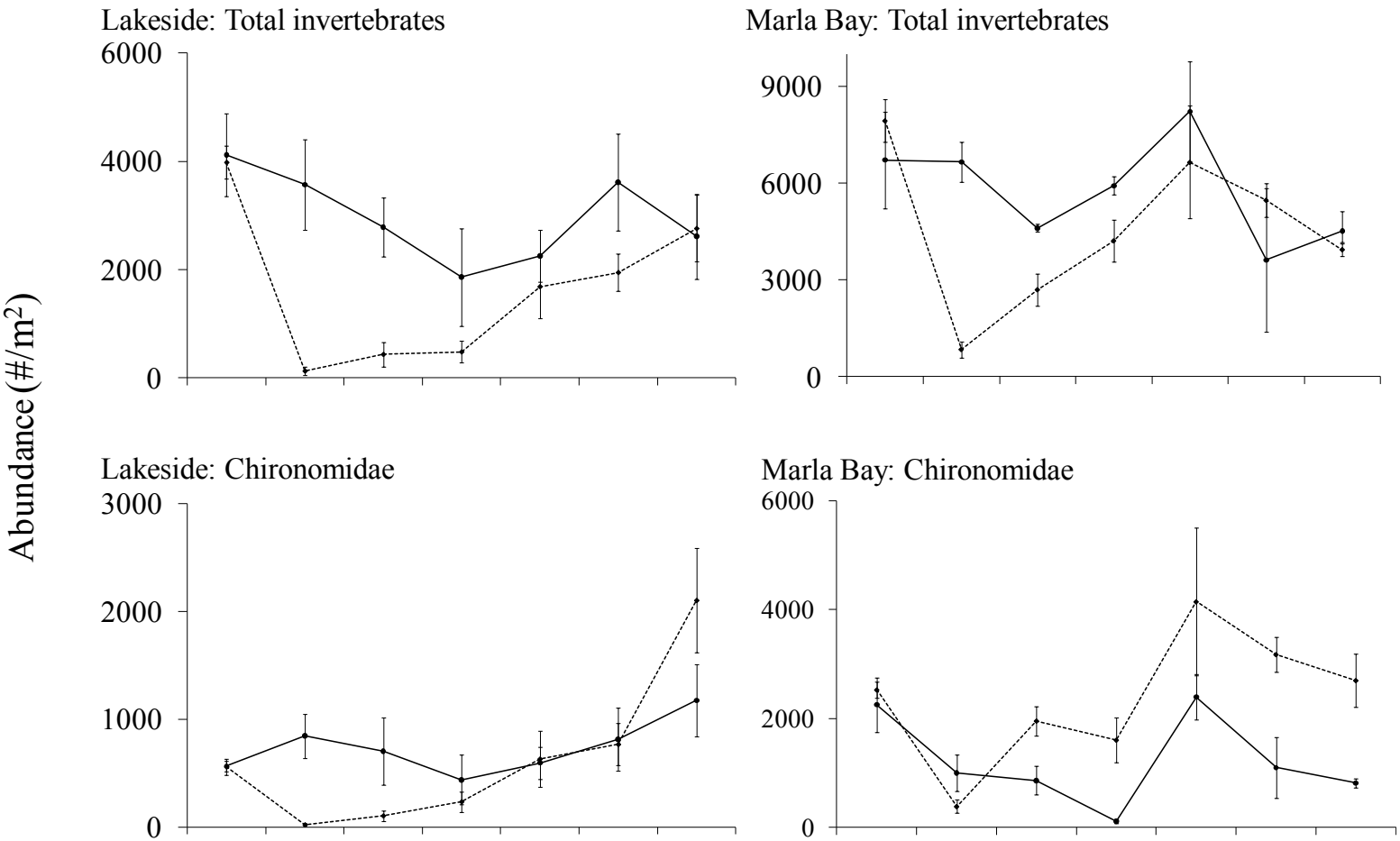
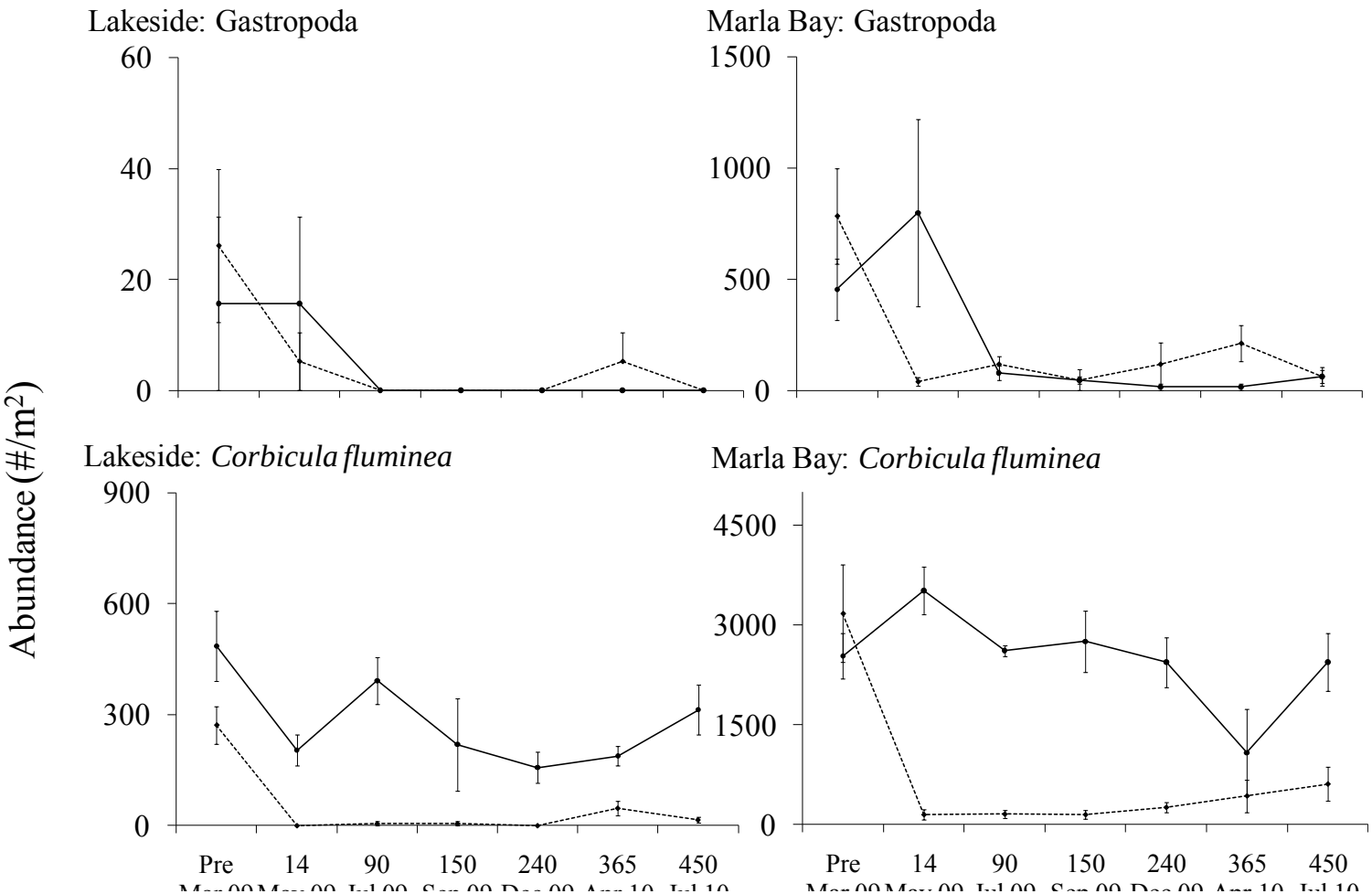


Figure 20a - d. Temporal changes of the total abundance (ind/m^2) of each taxonomic grouping in suction (dashed line) and control (solid line) plots at Lakeside and Marla Bay from March 2009 (prior to suction treatment) through July 2010 (post suction treatment). Error bars represent standard error. Time of sampling is indicated by days after treatment and month/year in which the sampling event occurred.



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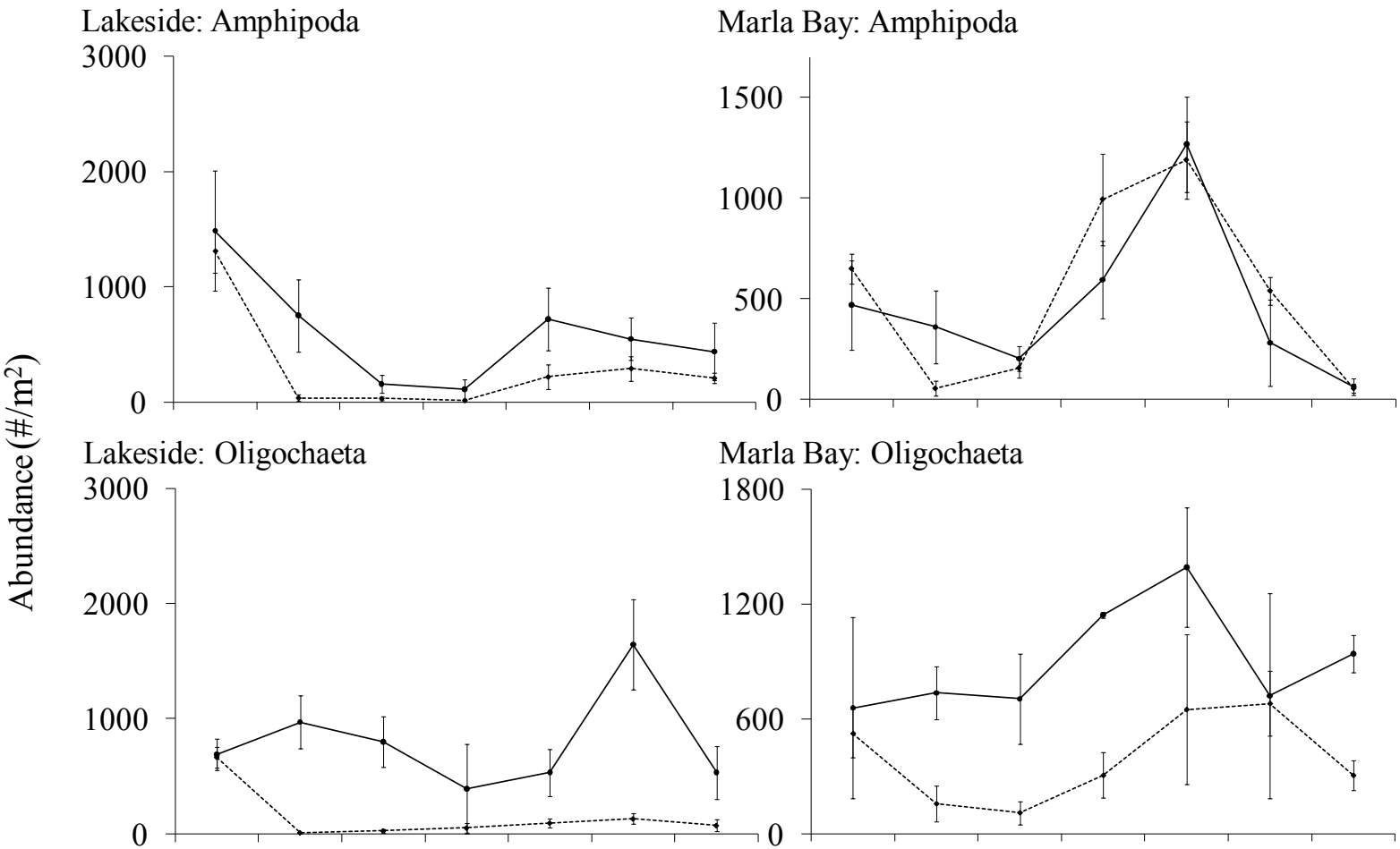

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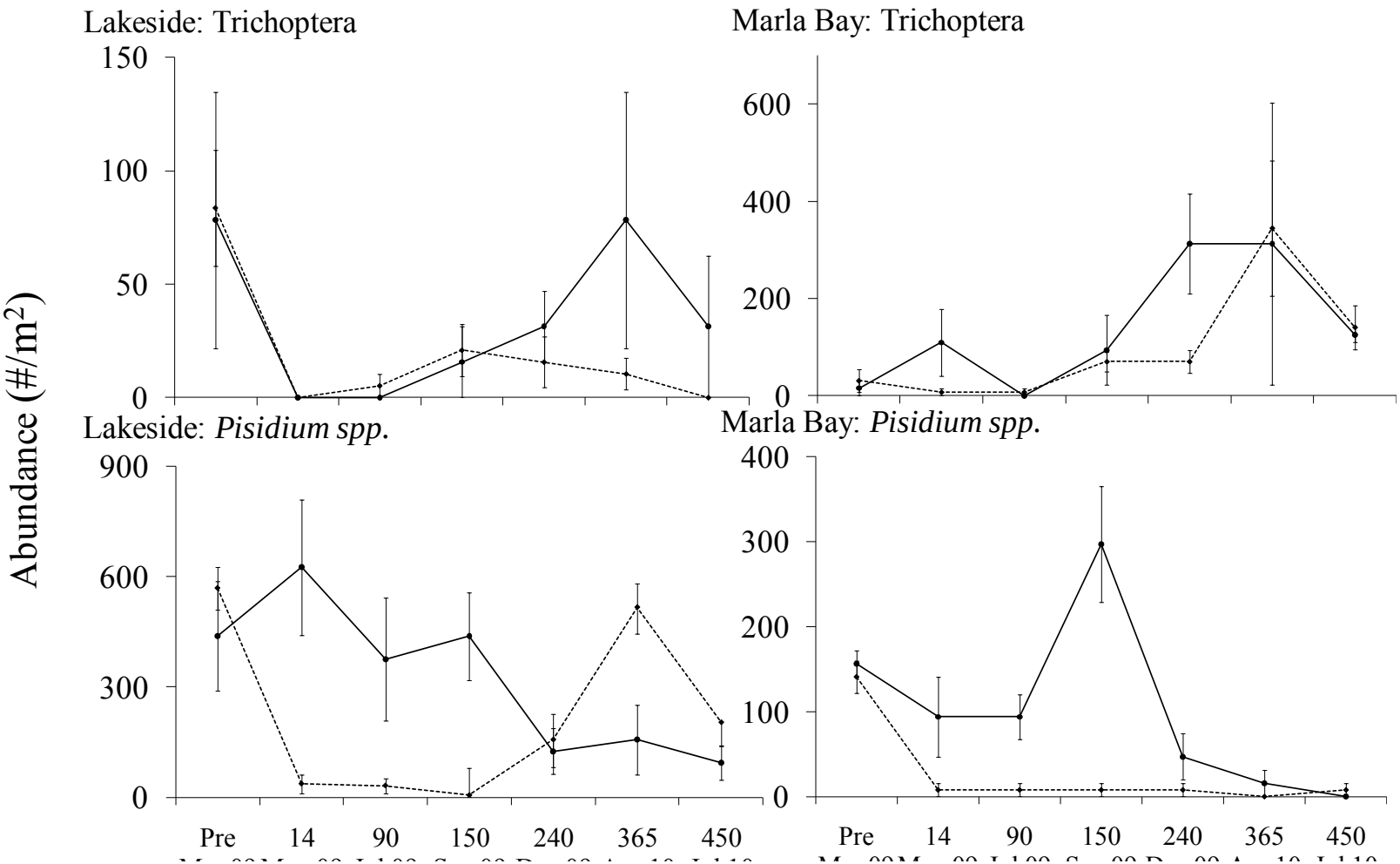

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	Before	14	90	150	240	365	450
Total invertebrates	-1230 -0.06	-4372 5.30***	-3255 4.11*	-2674 3.29*	-2248 2.54	-1850 1.94	-1565 1.54
Amphipoda	34 -0.33	-551 2.11**	-322 1.37	-180 0.83	-218 0.98	-185 0.86	-177 0.84
Chironomidae	-651 0.13	-742 0.31	-332 -1.06	-63 -1.97*	134 -2.43*	268 -2.95**	442 -3.59***
Oligocheata	69 0.99	-808 4.69***	-754 4.75***	-682 4.42***	-652 4.15***	-706 4.38***	-677 4.33***
Gastropoda	-138 0.87	-310 1.11	-147 0.09	-98 -0.45	-63 -0.89	-34 -1.28	-29 -1.39
Trichoptera	-9 -0.94	-41 1.02	-17 0.32	-14 0.18	-37 1.09	-35 0.89	-31 0.81
Corbicula fluminea	-453 -0.22	-1471 1.82*	-1343 2.01*	-1285 2.00*	-1206 1.88*	-1033 1.48	-1013 1.45
Pisidium spp.	-31 -1.26	-369 3.22**	-286 3.07**	-316 3.64***	-236 2.94**	-147 1.67	-111 1.24
Other	-50 -1.27	-81 0.46	-53 0.04	-35 -0.16	31 -0.92	23 -0.92	31 -1.10
Simpson Index (diversity)	-0.007 -0.02	-0.405 3.77***	-0.355 5.16***	-0.336 6.00***	-0.310 6.69***	-0.256 6.26***	-0.258 7.13***
Shannon-Wiener (evenness)	-0.019 -0.63	-1.691 4.02***	-1.051 2.34**	-0.867 1.99*	-0.571 1.17	-0.120 0.02	-0.128 -0.21

Table 1. CI and BACI analysis results for taxonomic groups collected prior to treatment (Before) and after treatment (days 14 - 450). The first value for each taxa indicates the mean effect size: between control and impact sites (CI) in the Before column, and in subsequent columns (days 14 – 450) before/after and control/impact (BACI). The second entry shows the t-statistic (H0: EffectSizeBefore = EffectSizeAfter) with significance level indicated: *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.1$

Sediment type, particulate organic matter (POM) and temperature were significant explanatory variables for the following response variables (as effect size): Simpson Index, Shannon-Weaver index Total invertebrate abundance and *C. fluminea* abundance (Table 2). Analysis of variance results showed that recovery rates of total invertebrate abundance is determined by time since treatment. Changes in effect size for *C. fluminea* are based on additive modeling of sediment type and temperature. Effect size reductions for the Simpson Index are determined by an interaction of sediment type and temperature and Shannon-Weaver index by POM and time since treatment. Total organic carbon (TOC) was not a significant term for any of the models.

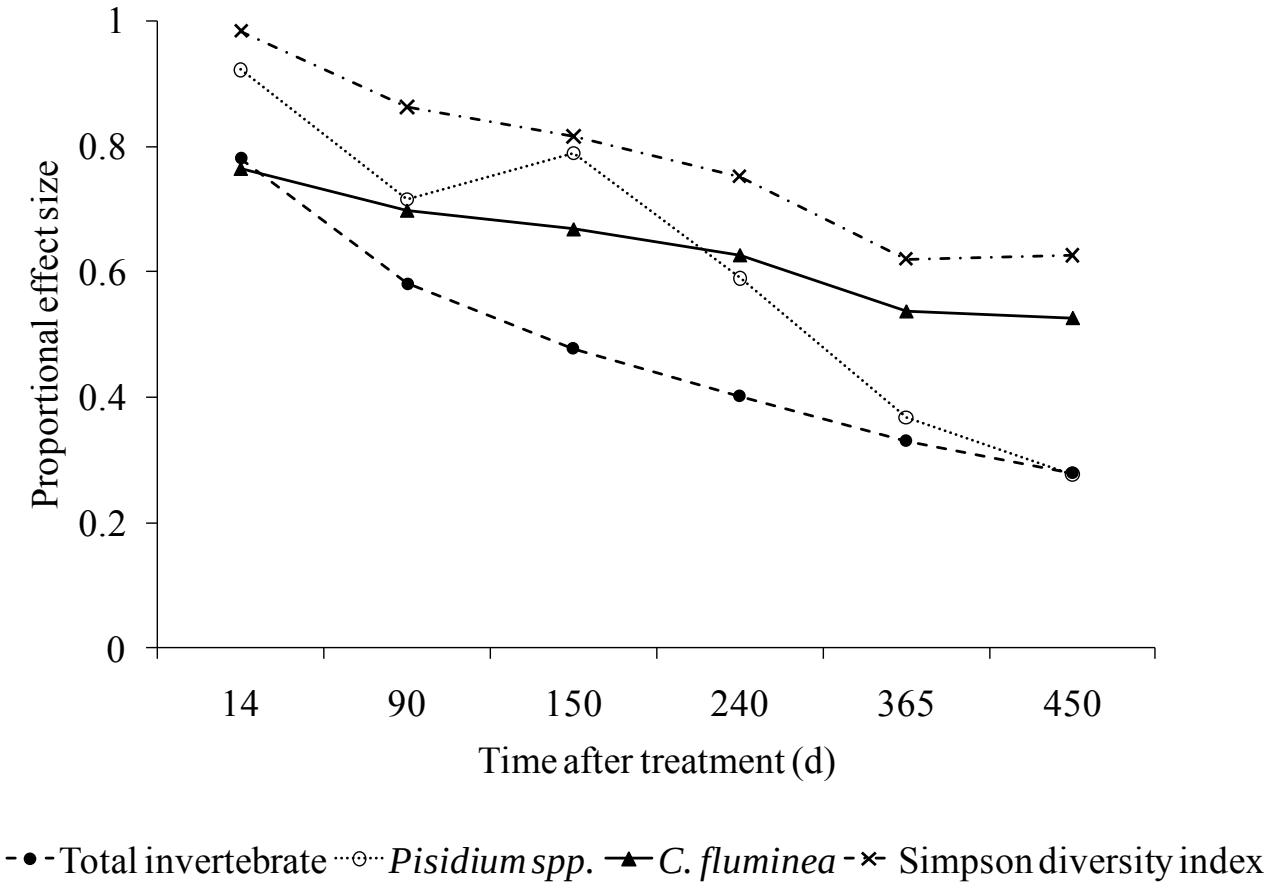

Figure 21. Temporal changes in effect size for three taxonomic groupings (Total invertebrate, Native clam species (*Pisidium spp.*), and invasive clam species (*Corbicula fluminea* i.e., treatment target)) and the Simpson Diversity Index. Changes in effect size standardized by differences in effect size prior to treatment.

a. Simpson Index ~ sediment * temperature

	df	*SS*	F	Pr(>F)
Sediment	1	0.76	10.04	0.002 **
Temperature	1	0.76	9.98	0.002 **
Sediment*Temperature	1	0.32	4.17	0.044 *
Residuals	101	7.67		

b. Total invertebrate abundance ~ time

	df	*SS*	F	Pr(>F)
Time	1	103991813	14.93	0.000 ***
Residuals	103	717346525		

c. Shannon ~ POM + time

	df	*SS*	F	Pr(>F)
POM	1	17.99	6.79	0.011 *
Time	1	36.79	13.89	0.000 ***
Residuals	102	270.17		

d. *Corbicula fluminea* ~ sediment + temperature

	df	*SS*	F	Pr(>F)

Table 2. Analysis of Variance Tables for BACI analysis with effect size as response variable. Stepwise model selection based on Akaike Information Criterion (AIC) was used to identify the most parsimonious model for each response variable: (a) Simpson Diversity Index, (b) Total Invertebrate Abundance, (c) Shannon-Weiner Evenness Index, (d) *Corbicula fluminea*.

iii. Discussion

Physical harvesting of invasive species causes disruption to soft-sediment benthic communities by reducing diversity and abundance. Zajac and Whitlatch (1985) suggested that the factors controlling soft-sediment succession should be considered as a combination of three interacting levels: environmental conditions, species life histories and biotic interactions (Zajac et al. 1998). Dredging to remove an invasive species in Lake Tahoe has reduced all benthic macroinvertebrate communities and has had variable recolonization patterns.

Dominance of a few groups is a common feature of macroinvertebrate communities in the early stages of the recolonization process (Ladle et al. 1980, Otermin et al. 2002) because they are able to persist in sediments in viable life stages (Yount and Niemi 1990). Migration strategies such as water column transfer of adults or juveniles will depend on the life history of particular taxa. As suction dredging was carried out in early April in Lake Tahoe, the immediate recolonization of taxa will depend on three main factors: (1) which taxa remain after the disturbance event, (2) taxonomic specific temporal patterns of reproduction for neighboring communities, and (3) environmental parameters. Suction dredging to remove *C. fluminea* reduced the abundance of all taxa except for chironomids, gastropods, trichopterans and other taxa. Chironomids have been described as ubiquitous and resilient organisms (Palmer et al. 1995) and observed as early colonizers in other systems (Otermin et al. 2002) in part because of their production of resistant eggs, high developmental rates and multivoltine reproduction (Gray 1981, Malmqvist et. al., 1991, Katano et al. 1998). Additionally, it is possible that chironomids simply did not get removed during the suction procedure, or that individuals migrated from adjacent untreated plots given their high abundances in this system. Mobile taxa such as the gastropod, trichopteran and other taxa are generally of low abundance in this system, and population density heterogeneity with sampling these taxa is observed prior to treatment. In general, other dominant taxa in plots (i.e., *C. fluminea,* oligocheates, amphipoda) were significantly reduced immediately after dredging and show increased abundances after warmer temperatures are observed during the summer months.

Long term monitoring of the treatment plots revealed important factors of recolonization dynamics to this system. While total invertebrate abundance recovers to pre-disturbance levels, species diversity remains significantly impacted throughout the entirety of the post-dredging monitoring period (14-450 d). This suggests change to community structure which is most strikingly observed as the relative shift to a chironomid dominated community in treatment plots at 450 d. Continued monitoring is required to understand whether species diversity indices return to pre-treatment conditions and whether further community shifts occur.

At the Lakeside site, native bivalve *Pisidium spp.* abundances also increase to levels greater than or equal to those observed in the treatment plots at 365 d compared to control plot conditions whereas the invasive target species, *C. fluminea* does not. *C. fluminea* is well known for its high reproductive rates (Williams and McMahon 1989), ability to both filter and pedal feed (Hakenkamp and Palmer 1999), and is also a generalist in terms of feeding preference (Way et al. 1990, Cataldo and Boltovskoy 1998) and tolerance to a wide variety of environmental conditions (Aguirre and Poss 1999, Williams and

McMahon 1989, Ortmann and Grieshaber 2003). The relative success of recolonization of the native *Pisidium* abundances compared to the opportunist traits of *C. fluminea* suggests that *C. fluminea* is perhaps not as well adapted to the low water temperatures and food availability of this high elevation oligotrophic lake. Further research on species competition between functional groups in Lake Tahoe is needed to refine this topic. Programs to control or maintain invasive species can fail without a component for impacts to communities other than the target removal species (Davidson and Stone 1989, Simberloff 1999). Control of a biological invasion is most effective when it employs a long-term, ecosystem wide strategy rather than directed approach focusing on removing individual invaders (Moody and Mack 1988, Mack et al. 2000). Our results show that while physically harvesting *C. fluminea* is effectivein reducing the abundance of this invasive species, it also reduces associated invertebrate abundances and causes disruptions to the community structure of the macrobenthos. Full recovery of the diversity levels of the dredged sites does not occur within one reproductive season (i.e., 365 days from impact), whereas the effect to the target invasive species is absent after 240 days. The maintenance of population abundances is highly dependent on density-dependent processes (Zipkin et al. 2009), however stochastic elements (or random variation) can also play a role in recovery.

IV. Project Amendment: Use of EPDM sheeting

Because of the failure of the polyethylene benthic barrier experiment due to the gas nature of the fabric (i.e., gas permeability and density of material), research and management teams agreed to experiment with other fabrics. It was decided that EPDM (rubber sheeting) was an appropriate and cost effective material to experiment with in the field. In this study we present (1) results of a physical field manipulation to control populations of a recent *C. fluminea* invasion in a large natural lake using gas impermeable benthic barriers, (2) collateral impacts of these field manipulations to co-occurring native benthic macroinvertebrates, (3) impacts to nutrient concentrations as a result of barrier application, and (4) the recolonization rate of *C. fluminea* approximately 1 year after removal of the bottom barriers.

i. Experimental Design and methodology

Two sites in Lake Tahoe where the EPDM benthic barrier treatments were applied are Marla Bay and Lakeside (Figure 1). Both sites have a heavily developed shoreline with residential and commercial structures, golf courses, public beaches as well as high recreational boater traffic. Marla Bay has a shallow shelf that extends approximately 0.75 km from shore with an average water depth of 5 m. The sediment type in Marla Bay is comprised mostly of a very coarse to coarse sand on the Wentworth class grade scale (Gordon et al. 1992) with a relatively diverse and benthic macroinvertebrate community

comprised of Corbicula, Gastropoda, Oligocheata, Ostracoda, Diptera, Amphipoda, Pisidium, Nematoda, and other. Annual average (standard deviation in parentheses) *C. fluminea* density in 2009 in Marla Bay was 2270 (1217) m^{-2} and average density for all macroinvertebrates is 8841(805) m^{-2}. The Lakeside site has a similar shallow shelf structure to Marla Bay although has a finer sediment type than Marla Bay, with a fine to very fine sand with a hard clay pan at about 5 cm below the sandy sediment top layer. The macroinvertebrate community diversity is similar to Marla Bay, but has much lesser abundances with an average density for all macroinvertebrates at 2778 (1997) m^{-2} and 556 (397) m^{-2} average *C. fluminea* density.

EPDM Benthic barriers

In order to understand the variability of treatments between habitats with differing benthic sediment composition (see above) we placed six barriers in two locations (total of 12) in the lake (Lakeside and Marla Bay). The 9 m^2 ethylene propylene diene monomer (EPDM) barriers (1.14 mm thick) were weighted with 3 m long, 1 inch thick rebar rods placed on the edges of each sheet (Figure 22). Dissolved oxygen probes (Zebra-Tech, Ltd. D-Opto Logger, Accuracy ± 0.1 °C for temperature, 1% of reading or 0.02 mg L^{-1} for DO) were deployed at each of the two locations and one probe was placed in open water adjacent to the barriers as a control measurement. The experiment was carried out for a 56 days from August 5 to September 30 2009. Barriers are installed on Day 0, and one barrier each was removed after 4, 7, 14, 21, 28 and 56 days. On each removal date, three 45 cm length by 7 cm diameter PVC hand cores were collected from the uncovered plot and processed in the laboratory for *C. fluminea* number, mortality and depth distribution within the sediment column in 1 cm increments (Figure 23).

Figure 22. The application of 10 x 10 foot sheet of EPDM rubber benthic barrier in Lake Tahoe. Sheets were lowered to the bottom of the lake and rolled out underwater, using rebar as a weighting mechanism to keep water from exchanging beneath the barriers. *Photo credit: UC Davis.*

Water samples for nutrient (nitrogen and phosphorus) concentrations were collected under benthic barriers and also in the water column adjacent to the barriers using SCUBA. Prior to removal from the field, the benthic barriers were lifted at the end of the experiment and sample bottles were opened and held underneath to collect each water sample. Control samples were collected from a 5 m depth in the same manner before any of the benthic barriers were lifted. Total nitrogen and total phosphorus were processed without filtering and all other analytes were filtered through a 0.7 µm GF/F filter.

Figure 23. Left: A scuba diver takes hand cores using PVC pipe in order to measure the abundance and viability of Asian clams in the sediment column after treatment with EPDM benthic barrier sheeting. Right: The sediment hand core, buried to a 20 cm depth ready for collection. Photo credit: Brant Allen, UC Davis

Macroinvertebrate sampling and sediment particle size analysis

To characterize the benthic macroinvertebrate community at the end of the treatment (Day 56), we used a petite Ponar grab sampler (Wildco, 2.4 L volume, 231 cm^2) to collect 3 sediment grabs per 9 m^2 plot. Using methods employed in Vander Zanden et al. 2006, all invertebrates were removed from the sediment sample after screening through 500 µm mesh to separate detritus from coarse sediment. Preserved samples in 70% ethanol were identified using Thorp and Covich (2001) and Merritt and Cummins (1996). A student‘s t-test was used to determine the significance of the difference of nutrient concentrations (above) and invertebrate densities between control and treatment plots. All statistical analyses were carried out using the program language R version 2.9.0. Sediment particle size analysis was carried out using a dry sieving method as described by Gordon et al. (1992) using US standard testing sieves (ASTME-11 specification) and grade classes for particle size were determined using the Wentworth scale.

ii. Results

Dissolved oxygen (DO) concentrations under the EPDM barrier declined dramatically within the first 12 hours and with the lake temperature in the range of 15 – 20 °C (normal diel fluctuation) it took approximately 36 hours for the DO concentration to reduce to zero (Fig 24a). Conditions remained anoxic for the entirety of the experiment, suggesting that the integrity of the bottom barriers was not compromised. DO concentrations adjacent to the bottom barriers in open lake water showed a well-oxygenated environment with a diurnal pattern (Fig 24b). Average temperature under the barrier was 18.83°C and average temperature in the open lake was 18.33°C, a difference of 0.5 °C.

Initial conditions (Day 0) at the Marla Bay site show most *C. fluminea* inhabiting the top 5-6 cm of the sediment column, with a few individuals occurring at depths down to 15-19 cm (Figure 25; Day 0). In the Lakeside site, which is characterized by 3-4 cm of sediment with a hard clay pan underneath, all *C. fluminea* are restricted to the top sediment layers with none occurring in the clay layer (Figure 25). After four and seven days of treatment there is a slight migration to the top of the sediment column at both sites, with about 50% mortality at the seven day mark in Marla Bay. At day 14 in Marla Bay > 90% of *C. fluminea* found in the sediment column are dead, and there are no observed mortalities in Lakeside. After 21 days of barrier treatment at the Lakeside site there is 100% mortality of *C. fluminea* and after 28 days at the Marla Bay site 100% mortality is also achieved. The experimental period was carried out for 56 days, at which point 100% mortality of *C. fluminea* is also observed in both sites.

Figure 24. Temperature (gray line) and dissolved oxygen concentrations (black line) as measured underneath the (a) EPDM barrier plot and (b) adjacent to the plot in a control (open lake) condition. Both measurements occur at a 5 m water depth and show diurnal patterns for the first 11 days of the experiment. Dissolved oxygen concentrations reduce to zero after approximately 36 hours in the treatment plot (a).

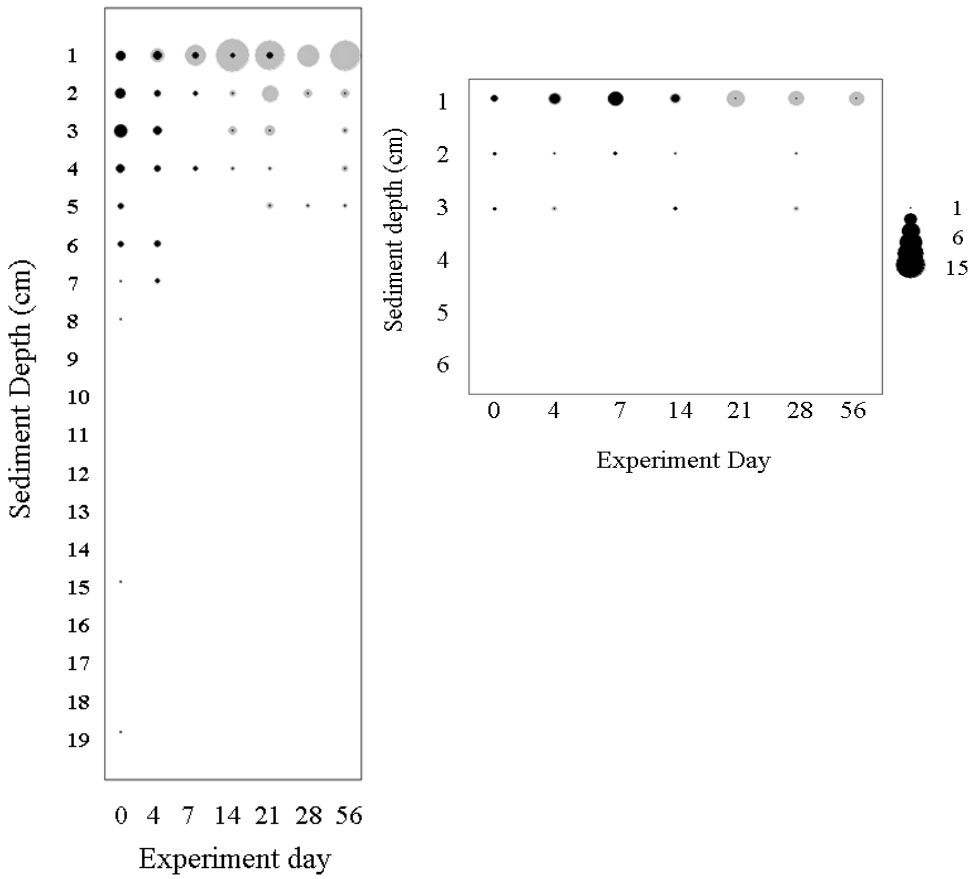

Figure 25**.** Asian clam behavioral response to EPDM benthic barriers during the 56 day e xperimental period. Black circles indicate average number of live clams per hand core (N = 5) and gray circles indicate average number of dead clams per hand core. Figure 42a represents Marla Bay (note deeper sediment depth) and 42b represents the Lakeside site (3 cm of sediment upon hard clay pan). Note 100% mortality at Marla Bay on experimental day 28 and on day 21 at Lakeside.

After 56 days of treatment, there was a 72 – 95% reduction in overall macroinvertebrate density at Lakeside and Marla Bay respectively (Table 3). In general, macroinvertebrate densities are higher in Marla Bay with an average total invertebrate abundance of 8841 m^{-2} compared to Lakeside at 2778 m^{-2}. No live *C. fluminea*, Ostracoda, Amphipoda, Trichoptera, Ephemeroptera, Nematoda or other (comprised of Trombidiformes, Cladocerans, Collembella) were found in the Marla Bay treatment site following the nearly two month treatment period. The native pea clam (*Pisidium spp.*), Gastropoda, Oligocheata, Diptera and Hydracarina did not experience total defaunation but had significant population reductions ranging from 64% to 99% under barriers compared to control sites. At Lakeside, no live *C. fluminea*, Gastropoda, Ostracoda, Hydracarina,

Ephemeroptera or other were found in the barrier site. Oligocheata, Diptera and Amphipods were not eradicated, but experienced significant population declines. *Pisidium spp.* were not significantly different between control and barrier sites in Lakeside. No Nematoda were found at Lakeside in control or barrier sites. Relative to the other taxa, *Pisidium spp.* were affected the least by the anoxic conditions.

	MARLA BAY			LAKESIDE		
	Control	Barrier	Average Reduction	Control	Barrier	Average Reduction
C. fluminea	2270 (382)	0	100%	556 (463)	0	100%
Pisidium spp.	175 (99)	63 (73)	64%	365 (397)	444 (385)	0%
Gastropoda	1984 (572)	16 (27)	99%	32 (55)	0	100%
Oligocheata	1317 (858)	16 (27)	99%	397 (220)	111 (99)	72%
Diptera	857 (459)	16 (27)	98%	317 (310)	63 (110)	80%
Ostracoda	1349 (1437)	0	100%	127 (73)	0	100%
Amphipoda	429 (454)	0	100%	365 (317)	16 (27)	96%
Hydracarina	95 (165)	16 (27)	83%	63 (55)	0	100%
Trichoptera	111 (99)	0	100%	16 (27)	16 (27)	0%
Ephemeroptera	48 (82)	0	100%	508 (334)	0	100%
Nematoda	48 (48)	0	100%	0	0	N/A
Other	159 (73)	0	100%	32 (27)	0	100%

Table 3. Benthic macroinvertebrate average densities with standard deviation in parentheses in control and barrier plots plots after 56 days in Lake Tahoe (August – September 2009). Average reduction column reflects the average difference between the control and benthic barrier treatment plots per tax.

Nutrient concentrations measured under the barriers after 56 days of application show large departures from control plot concentrations (Figure 26). Differences in dissolved

inorganic nitrogen concentrations ranged from one to two orders of magnitude in difference with the barrier plots at 1771 µg L^{-1} TNH4-N compared to 5 in the control plot and 1711 µg L^{-1} NH4-N compared to 4 µg L^{-1} in the control plot and 10 µg L^{-1} TNO3-N in the barrier compared to 1 µg L^{-1} in the control plot. Phosphorus concentrations also differed significantly, average total phosphorus under barrier and control plots were 56 and 11 µg L^{-1} respectively and average dissolved phosphorus was 14 and 10 µg L^{-1}.

Sampling after the barriers were removed allowed for recolonization measurement following the defaunation of *C. fluminea* under the bottom barriers. In August 2010, eleven months after barrier removal in Marla Bay, *C. fluminea* populations were approximately one third of the population density in the barrier sites compared to the control sites (Figure 27).

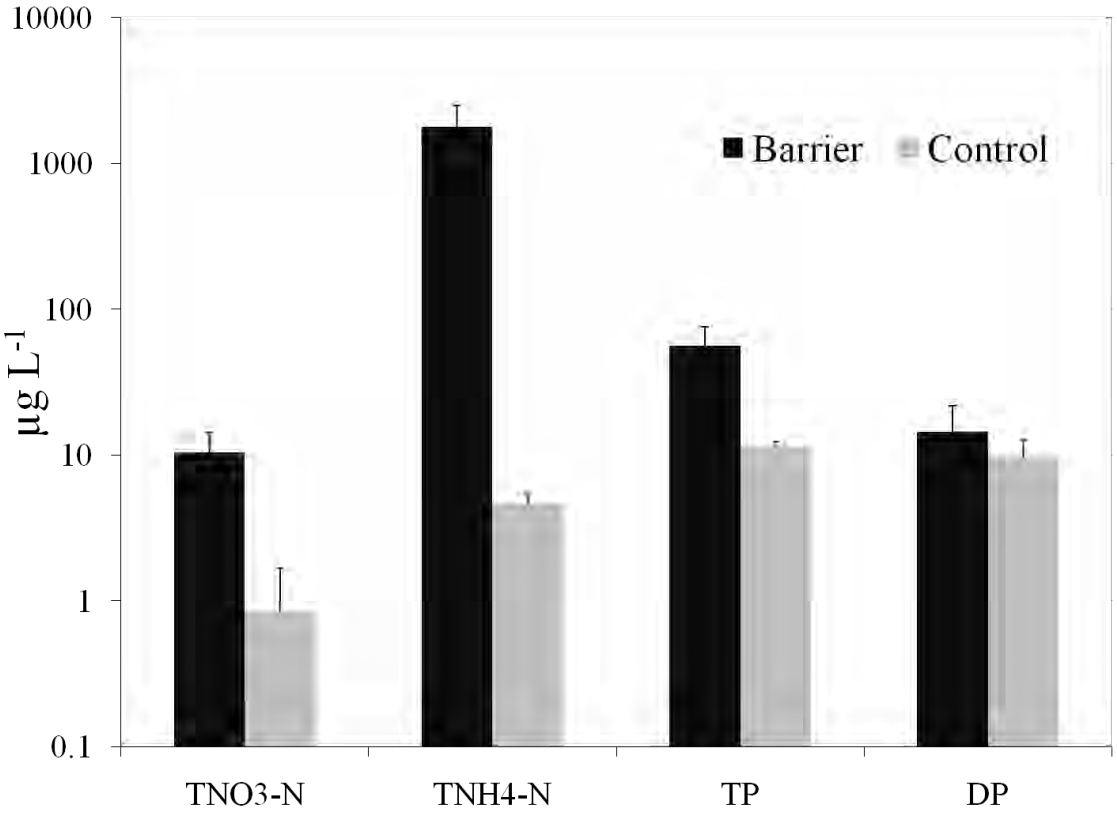

Figure 26. Water nutrient concentrations under EPDM benthic barriers (black) and in open water lake condition adjacent to benthic barrier plots (gray), N = 2. Error bars represent one standard deviation. Note the logarithmic scale on y axis showing the multiple orders of magnitude difference between treatment and control condition

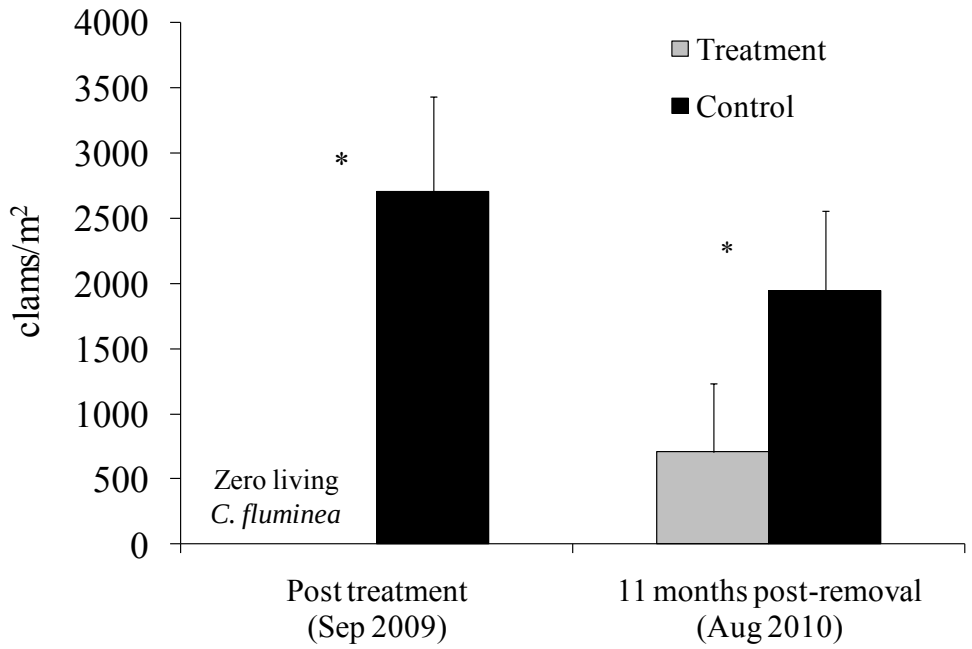

Figure 27. *C. fluminea* recolonization of Marla Bay benthic barrier plot (gray) compared to control plot (black) 11 months after removal (N = 5); Error bar represents one standard deviation and * indicates significant difference ($p < 0.01$) between treatment and control plots. Post treatment refers to measurements taken on the day of barrier removal.

iii. Discussion

Benthic bottom barriers made of gas impermeable fabric such as EPDM is successful in reducing dissolved oxygen concentrations and causing the mortality of the invasive bivalve *C. fluminea*, other non-target benthic macroinvertebrates, and has the potential for use in the treatment of dreissenid mussels, specifically the soft-sediment dwelling quagga mussel (*Dreissena bugensis*). After a 28 day period 100% of *C. fluminea* populations are eradicated under benthic barriers and 70 to 95% of total macroinvertebrate diversity is reduced. This is not the first use of benthic barriers for control of aquatic invasive species, but the first application of this technology for *C. fluminea*, and first published experimentation for control of this species in a natural lentic system.

The proposed mechanism behind laying out the bottom barriers as a control strategy is to reduce available dissolved oxygen in an attempt to target *C. fluminea* metabolic process during anaerobiosis. Bivalves with opened valves rely on a glycogen fuelled aerobic metabolism (Ortmann and Grieshaber 2003). Many authors suggest that after closing valves, most enclosed oxygen is spent within a few minutes (Davenport and Woolmington

1982; Taylor 1976; Widdows 1987). However, Ortmann and Grieshaber (2003) observed that with the onset of valve closure, *C. fluminea* reduced its metabolic rate to 10% of the standard metabolic rate, and this depressed metabolism remained aerobic for several hours. Only during extended periods of valve closure (more than 5-10 h), did the clams become anaerobic and accumulate succinate within tissues. At any rate, after some period of time (dependent on temperature, rate of ATP replenishment), anaerobiosis in *C. fluminea* will stimulate valve opening (Mathews and McMahon 1999). *C. fluminea*, and all bivalve species then circulate water over gills to excrete anaerobiotic end products (succinate, proprionate) and resume exchange with overlying waters and potentially harmful ambient stressors (Jenner 1990; Mattice et al., 1982; McMahon and Lutey 1988). While this mechanism is specific to filter feeding species, the use of benthic barriers to reduce dissolved oxygen concentrations to lethal levels for an extended period of time can have applicability to any invasive invertebrate species that are not tolerant to hypoxic or anoxic conditions.

The overall macroinvertebrate response to barrier treatment at both sites (Marla Bay and Lakeside) was generally similar. Most notable is the less severe impact to the native Pisiid clams in both sites—experiencing a 64% reduction in Marla Bay and no significant reduction at the Lakeside site. *Pisidium spp.* have been cited for their ability for extreme oxygen regulation (Burky 1983), and *Sphaerium corneum* and *Pisidium amnicum* have also been observed to be tolerant of long periods of valve closure (Holopainen and Penttinen 1993). After the 56 day exposure to hypoxia, Oligocheates and Dipterans experience 72 – 80% population reduction in Lakeside yet almost complete eradication at the Marla Bay site. This variability could be attributed to the difference in macroinvertebrate abundances between the sites. Both temperature (~0.5 C warmer) and densities of *C. fluminea* and other macroinvertebrates are much higher (4- 5 times total invertebrate abundance) at Marla Bay than at Lakeside, potentially increasing BOD or other contributions to under barrier nitrogen production.

C. fluminea die offs are known to produce high concentrations of unionized ammonia (NH3-N) which are well above concentrations that cause acute mortality of macroinvertebrate species (Cherry et al. 2005). In addition to mortality, unionized ammonia is observed to impact benthic macroinvertebrates by impairing filtering, growth, byssal thread secretion and deplete energy stores (Chetty and Indira 1995; Epifanio and Srna 1975; Yu and Culver 1999). Cherry et al. (2005) reported a 96-h median lethal concentration (LC_{50}) for NH3-N was 0.78 mg/L for adult *C. fluminea* and 0.28 mg/L for juveniles in *C. fluminea* laboratory die-off conditions at 10,000 m^{-2} density. Unionized ammonia concentrations observed under barriers in our experiment are 0.004 – 0.005 mg/L, pH = 6.92, temp = 20.5 C, compared to 0 mg/L in the control condition (calculated after Thurston et al. 1979) which is lower than the LC_{50} reported in the Cherry et al.

(2005) experiment. However, their experiment occurs in a well oxygenated environment (DO of overlying water ranging from 5.02 – 8.05 mg/L) and over a short period of time. The combination of elevated unionized ammonia concentrations and anaerobic conditions, plus extended exposure likely contributes to our observed mortality rates.

Benthic barriers made of gas permeable fabrics (for use in nuisance aquatic plant control) such as polyethylene have been shown in other studies to reduce macroinvertebrates (Ussery et al. 1997) and decrease dissolved oxygen concentrations (Engel 1984; Ussery et al. 1997). First iterations of this experiment were carried out with polyethylene fabrics (because of low cost and ease of handling), with poor results. There were no depletions of dissolved oxygen concentrations under barrier plots, and no difference in invertebrate or *C. fluminea* abundance between control and barrier plots observed. Lake Tahoe's oligotrophic conditions in the littoral zone harbors little plant, algal or benthic macroinvertebrate biomass compared to other systems in which polyethylene barriers have been applied. Relatively, BOD in Lake Tahoe is low compared to plant benthic barrier studies mentioned above.

The control of invasive species is an important concern for preserving ecosystem health, particularly in recent decades were the introduction and establishment of harmful aquatic invaders has increased exponentially. One common control method of aquatic invasive species is to harvest, or to permanently remove individuals from a population which can be implemented through a variety of techniques such as pesticide application, physical removal (hand pulling or dredging), or system manipulation (i.e., thermal changes or water drawdown). In most cases, 100% removal or harvest of the target invasive species is not likely, or where it does occur, reintroduction or recolonization from adjacent regions is highly probable. Several studies have demonstrated that increased mortality of an invasive species can not only lead to greater variability in abundance and population instability, but can also lead to increases in total population (Buckley et al. 2001; Zipkin et al. 2008). Predicting the long term response of a population to a management strategy can be challenging because of demographic stochasticity and density dependent processes (Zipkin et al. 2009). A current unknown in this study is the competitive ability of *C. fluminea* to recolonize, given influences from native benthic macroinvertebrate community structure, density dependence within *C. fluminea* populations and impacts of environmental conditions in Lake Tahoe. Long term monitoring of treatment plots is imperative to understand the impacts of any control methodology.

With increases of connectivity between aquatic systems as a result of human pressures such as recreational boating, water conveyance and commercial uses of fresh waterways, the need for invasive species control in lake, reservoir and river systems has increased. The use of non gas permeable fabrics is effective at reducing dissolved oxygen concentrations and contributing to the mortality of *C. fluminea* and other benthic

macroinvertebrate populations on a small scale. In Lake Tahoe, 1 acre of EPDM barriers have been installed in 2010 with similar results to those presented here. While this is an effective control technique at a small scale in the short term, it is important to monitor the recolonization of *C. fluminea* and other benthic macroinvertebrates over time to understand the efficiency and feasibility of this treatment and control strategy.

V. Asian clam vertical profiling and sediment porewater nutrient analysis

In order to understand the potential impacts of Asian clam to the sediment porewater chemistry as a result of nutrient cycling associated with filter feeding and concentrated nutrient excretion, as well as to observe the distribution of Asian clam within the sediment column to inform diver assisted suction removal efforts, columnar sediment samples from known Asian clam beds in Marla Bay and Lakeside were collected and analyzed in 2009.

i. Experimental design and methodology

To measure the vertical distribution of Asian clam with depth, sediment hand cores were taken during the pre-installation baseline establishment period. Sediment hand cores were collected from Marla Bay and Lakeside by pounding 18-inch lengths of 2-inch diameter PVC pipe into the sediment. Hand cores were immediately plugged with stoppers underwater to preserve the stratigraphy of the sample and placed in a -80°C freezer the same day. After thoroughly frozen, the hand cores were cut into 2 cm sediment segments with a table mitre saw. Porewater was extracted and filtered through a Whatman GF/C filter and immediately stored in the freezer until nutrient (ammonium (NH_4), nitrate (NO_3) and soluble reactive phosphorus (SRP)) analysis was carried out. All clams found at each 2 cm depth interval were counted and measured with digital calipers.

ii. Results

Sediment cores (N = 39) sampled in Marla Bay and Lakeside were processed to obtain clam distribution by depth (Figure 28). 84% of observed Asian clams were found in the top 6 cm of sediment, and remaining clams were found to a maximum depth of 16 cm in the Marla Bay site core samples. The greatest abundance of clams were found at 4 cm sediment depth; figure 21 shows the size distribution of clams found in the sediment cores. The smallest clams (1-5 mm) were most commonly found: 36.3% of all measured clams belonged to this size class followed by 32.5% (10-15 mm), 25% (5-10 mm), and 6.25% (>15 mm). Clams (1-5 mm) composed 50% and 58.6% of the clams in the 2 and 4 cm depths respectively. Only one clam of this size was found below 6 cm. The largest clams (>15cm) were exclusively found in the top 6 cm. Midrange clams, both 5-10 mm and 10-15 mm, were found at all depths with clams, except 12 cm which only had clams 10-15mm.

Characteristics of the sediment were observed and recorded. Clams were commonly found in somewhat uniform, fine-coarse brown sandy sediments. Grey/black sediments had fewer occurrences of clams. Also, as clay content increased in the Lakeside sediment cores, clam occurrence decreased, especially when clay was the primary particle size. Nutrient analysis data is summarized in Figure 29.

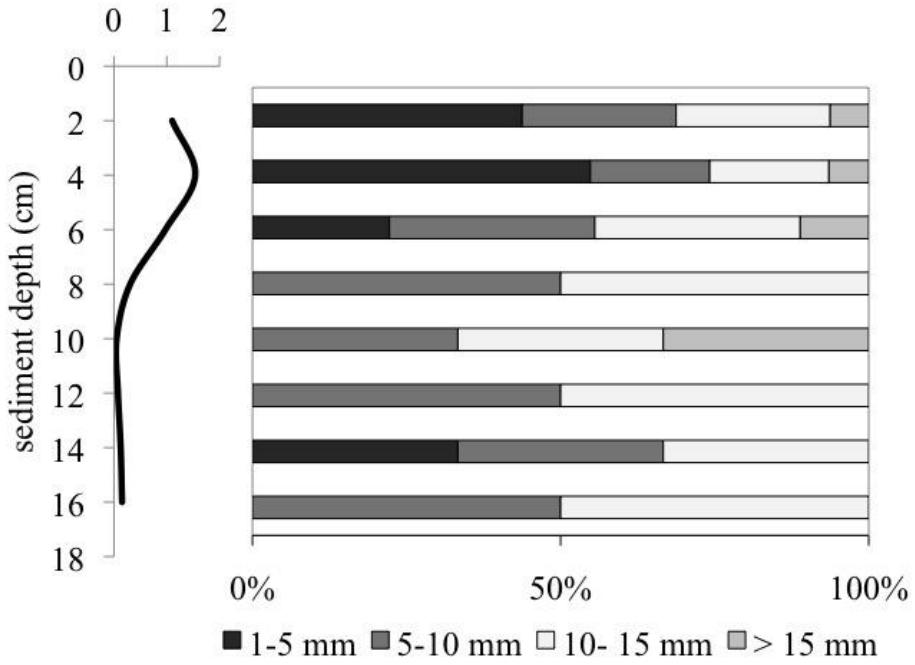

Figure 28. Average number of Asian clams found clams within sediment column per hand core. Asian clams were broken into four major size classes and shell length is shown in varying color strata. The x-axis represents the mean percentage of the sample that includes the particular size class. The greatest abundance of Asian clams occur in the top 4 - 6 cm of the sediment column, with individuals observed down to 16 cm below the sediment surface.

Figure 29 shows results from the sediment porewater nutrient analysis. Samples were averaged by site. In general, sediment porewater concentrations of SRP, NO3-N and NH4-N were higher than concentrations typically found in ambient Lake Tahoe water. This is attributable to the abundance of organic matter in sediment substrate such as seston, invertebrate communities and other organics that settles on benthic substrates. Marla Bay showed higher median concentrations of SRP and ammonia, and lower NO3-N concentrations compared to the Lakeside site. Further study is needed to assess the relative contribution of Asian clam populations to differing nutrient concentrations within the sediment porewaters.

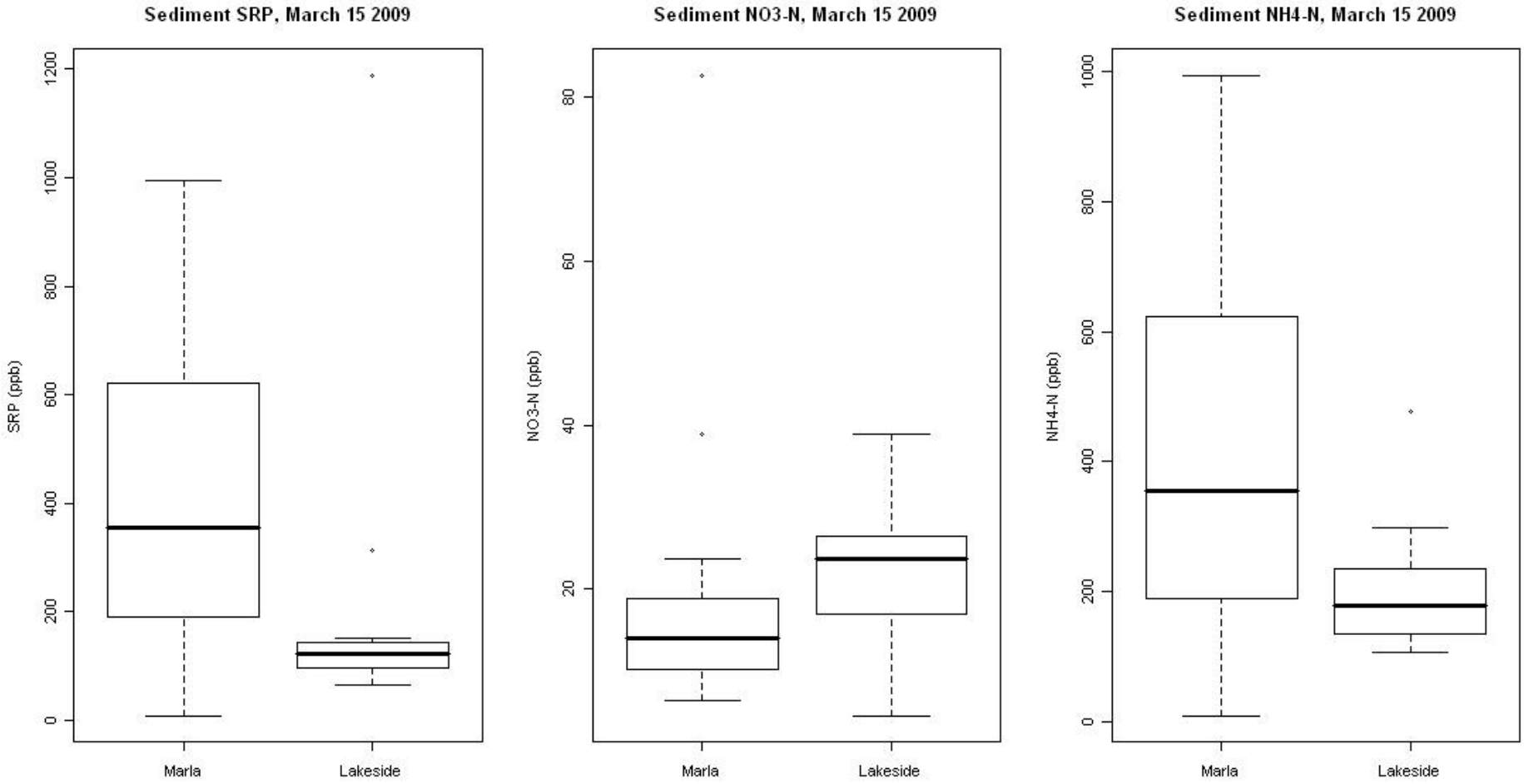

Figure 29. Box and whisker plots showing sediment porewater nutrient concentrations collected from Marla Bay and Lakeside (N=39). All nutrient concentrations are shown in ppb or µg L^{-1}

.

iii. Discussion

The distribution of clams in sediment has several implications. Density observations may be misleading, blinding our ability to assess potential ecosystem impacts, management will be more difficult, and there may be detrimental effects on the benthic community. Clams burrowing and pedal feeding decreases populations of bacteria and flagellates (Hakenkamp et. al., 2001). Clams burrow primarily in the top 6 cm, but were found up to 16 cm, therefore clams may affect the benthic community deeper than just the surface sediments. Considering the size distribution of clams by depth, medium sized clams (5-10 mm and 10-15 mm) were the only clams found deeper than 6 cm, for the exception of one small clam (1-5 mm). Medium clams are more likely to reach a large size class (>15mm) than the 1-5 mm clams and are less vulnerable burrowed at these depths. The largest clams have greater ecosystem impacts both through filter feeding and active burrowing and it may be possible that larger clams will become more prevalent in Tahoe with the abundance of deeply burrowed medium-sized clams.

VI. Veliger monitoring and reproductive status of Asian clam

The seasonality of the Asian clam reproductive cycle can have significant impacts on management efforts undertaken at Lake Tahoe. Optimal control methodologies should incorporate optimal periods in terms of the minimization of target species reproductive effort, which is seasonally based on temperature, food availability and other abiotic and biotic factors. Monitoring for water column presence of pelagic Asian clam juveniles was carried out during experimental management treatments as well as a seasonally based survey of reproductive status of adult Asian clam.

i. Experimental design and methodology

Veliger tows

To assess the potential for gamete, trochophore, veliger or pediveliger release as a result of the applied control strategies, veliger tows in treatment areas occurred at the time of barrier plot removal and suction removal. These samples were sent to a sub-contracted histology lab to assess the amount of gamete or veliger release at treatment plots in relation to control plots. Materials and method for analysis: Each sample was collected through a 15 micron sieve. For each sample twenty 0.5 ml sub-samples were viewed with a compound microscope, 10x ocular. Before each sub-sample was taken, the sample was well mixed.

Reproductive status

Asian clams representing multiple size classes were collected during the winter and spring periods for laboratory dissection to quantify the amount of gamete developed in relation to body

mass and shell size over the duration of the pilot project period as an observation of reproductive potential with time. Sampling for *C. fluminea* at Lakeside and Marla Bay occurred approximately every two weeks from May through August 2010, and monthly September through November 2010.

C. fluminea unfertilized eggs are held in the inner demibranches of the ctenidia (gills) following release from the gonads and fertilized embryos are brooded in the same structure. To quantify unfertilized eggs and developing larval forms (hereafter referred to as late stage veligers), dissections of the demibranches occurred. Approximately forty clams with a shell length 13±1 mm per date per site were randomly selected from sample grabs and dissected; however, clams as small as 11 mm and as large as 19 mm were occasionally dissected. Each clam's shell length was measured with digital calipers to the nearest 0.01 (mm) and wet weighed to the nearest 0.01 (mg) prior to dissection. The demibranches were squash mounted and examined under 10X magnification light microscopy and abundances of unfertilized eggs and late stage veligers were assessed by visual count.

ii. Results

Veliger tows

Plankton samples taken March 2009, preserved in ethyl alcohol and viewed July 2009. Of primary interest was the presence of the bivalve *C. fluminea*. In all four samples received (1T, Marla Bay, Treatment Site; 1C, Marla Bay, North Bay; 2T, Marla Bay, Treatment Site; 2C, Marla Bay, North Bay) no presence of planktonic pedivelier *Corbicula* was viewed or any other bivalve veliger larvae. Invertebrates noted in samples were Copepods, both adult and nauplii, Cladocerans, and Ostracods. Present in greatest abundance were copepod nauplii. All samples had the same assortment and general abundance of plankton. Samples collected 3-20-09, 2T and 2C contained a number of adult male and female *Eurytemora*.

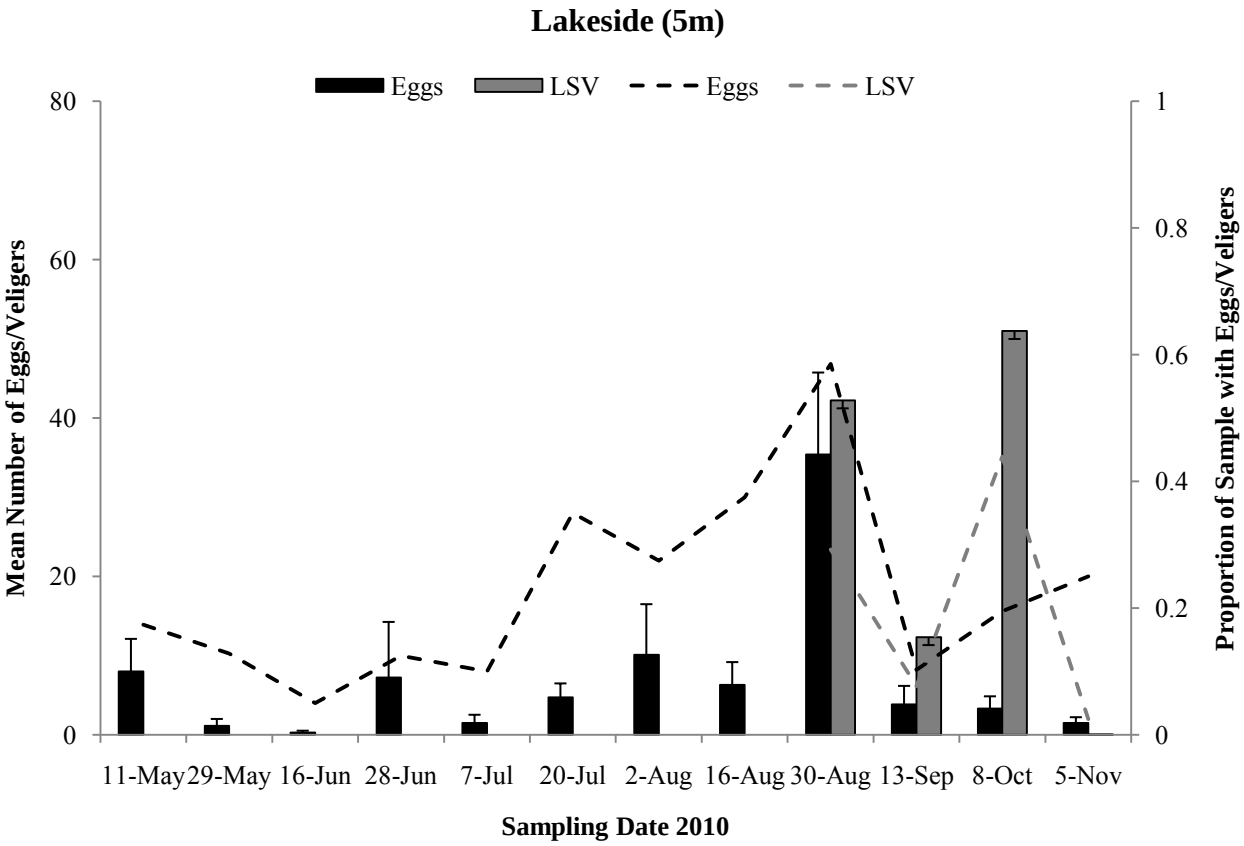

Figure 30. The primary vertical axis (solid bars) show the mean number of unfertilized eggs in the black and late stage veligers (LSV) in the grey. The secondary axis (dashed lines) indicates the proportion of unfertilized eggs and late stage veligers (LSV) present in the sample. Error bars represent standard error.

Reproductive analysis (Provided by Marianne Denton, University of Nevada Reno)

During all dissections, eggs were present in the demibranches, which is the typical year round condition for Asian clams. Veligers began to be observed in dissections on August 16, peaking on August 30 and September 30, with declines in abundance throughout the remainder of the sampling for the sites at 5 m (Figure 30, 31).

iii. Discussion

During low temperature periods in Lake Tahoe (prior to August in 2009) fertilization does not occur in Asian clams, nor are there veligers or other Asian clam gamete detected in the water column. To avoid treatment at a period of active fertilization and pediveliger release, management actions should be carried out during periods of low temperature.

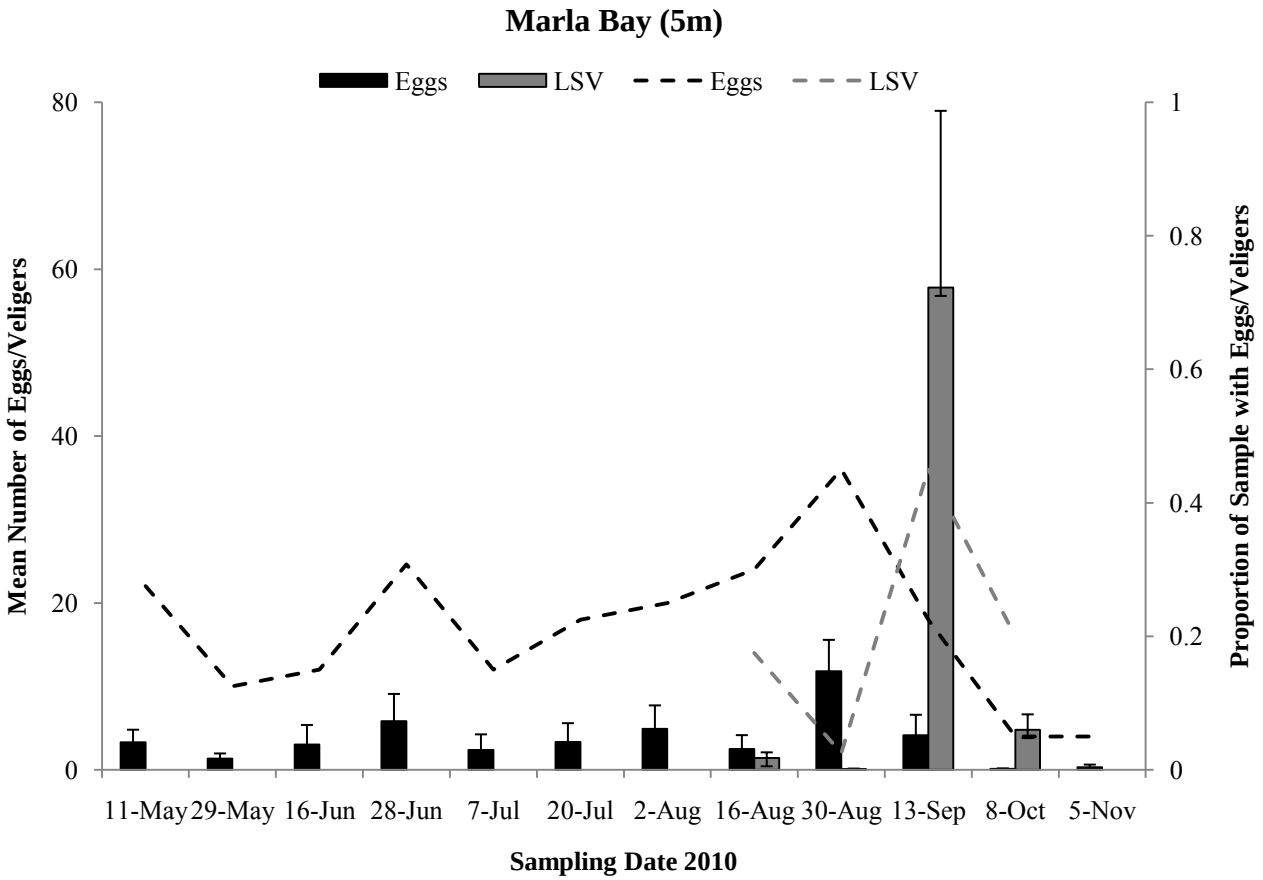

Figure 31. The primary vertical axis (solid bars) show the mean number of unfertilized eggs in the black and late stage veligers (LSV) in the grey. The secondary axis (dashed lines) indicates the proportion of unfertilized eggs and late stage veligers (LSV) present in the sample. Error bars represent standard error.

Asian clams are hermaphroditic, with adults able to carry eggs for the entirety of their life (Kraemer and Galloway 1986b). Thus, the production and release of sperm determines the reproductive period. In the Western United States spermatogenesis and fertilization generally begin when temperatures rise in spring and exceed 10 and 15°C respectively; temperatures in Lake Tahoe nearshore are above 10°C from early May to late October during the year and above 15°C between late May through early September (Ngai 2008). A majority of studies conclude the Asian clam reproduces twice a year (Sousa et al. 2008): once in the spring, continuing into the summer and again in late summer, continuing into the fall. However, some studies found only one reproductive event, while in others three were found with differences among years even at the same locations (Doherty et al. 1987, Darrigran 2002). The Asian clam can reproduce both by self-fertilization as well as by releasing sperm into the water that can be captured by other clams. The timing of reproduction is controlled by temperature, but also by food availability (Rajagopal et al. 2000, Cataldo et al. 2001). Individuals continue to fertilize their eggs and brood larvae until the temperature falls outside the acceptable range (13-30°C) or until food limitation occurs.

VII. Algal measurements (field)

Observations of filamentous algal blooms associated with Asian clam populations in 2008 prompted the need to monitor both pelagic and benthic algal dynamics associated with known high density infestations in Lake Tahoe. During April – August 2009, water column measurements of pelagic algae and field observations of filamentous algae through field survey and continuous monitoring with an *in situ* fluorometer were collected from 3 to 15 m water depths at Glenbrook Bay (control site) and Marla Bay (Asian clam infested site).

i. Experimental design and methodology

Phytoplankton

Asian clams can excrete elevated levels of nutrients into the water column, with a potential to stimulating algal growth. Chlorophyll is the key biochemical component in algae and phytoplankton that is responsible for photosynthesis. Chlorophyll *a* is the most abundant form of chlorophyll and when it degrades it loses the magnesium molecule resulting in formation of the pheophytin molecule. Measuring both these molecules is essential to understanding the health of a particular body of water as there is a direct relationship between the concentration of the growth-limiting nutrient (nitrogen or phosphorus) and phytoplankton abundance (Horne and Goldman 1994). Thus, chlorophyll measurement is an indicator of primary production and as an indirect indicator of nutrient levels. In order to assess this association, chlorophyll *a* and pheophytin concentrations were measured monthly at locations of high *C. fluminea* densities (Marla Bay), moderate densities (Lakeside), and low to no densities (Glenbrook) from spring (April) to late summer (September). Water samples were collected using a Van Dorn sampler at the water surface, 5, 15, and 20 m depths. Samples were filtered and chlorophyll readings were taken using a 10 AU fluorometer. See appendix A for the chlorophyll *a* extraction laboratory procedure.

Filamentous algae

Filamentous green algal (*Zygnema* sp. and *Spirogyra* sp.) blooms were visually monitored via field observations made over the course of a full year, February 2009 – February 2010. The amount of filamentous algae observed over an areal extent was estimated by randomly placing an aluminum chamber (radius = 10 inches) in high density Asian clam areas in Marla Bay and in Glenbrook Bay (Figure 30) and by quantifying the proportion of the aluminum chamber that was covered in filamentous algae through photography and laboratory processing of biomass. Due to the shift in dominant algal populations in the 2009 season (see results section), this sampling effort was discontinued after initial measurements were taken. Please see discussion for details. In addition, all filamentous algal samples were collected, preserved and sent to a phycologist on the UC Davis campus for photographic documentation and identification.

In addition, *in situ* field algal measurements were collected using a Turner C3 fluorometer. The submersible fluorometer was deployed at Marla Bay from April through August 2009. Prior to deployment, the C3 was and calibrated using deionized water and programmed to record fluorescence, CDOM and phycoerytherin concentrations at a ½ hour interval. Fluorescence is only reported here as CDOM and phycoerytherin concentrations were below detectable values. The unit was inserted into a weighted base stand so that all sensory probes were located 6 inches above the lake bottom to measure potential changes of filamentous metaphyton or periphyton species such as *Zygnema, Spirogyra,* or *Cladophora spp*. The fluorometer cannot distinguish between water column algae or filamentous algae and results presented include a sum of both taxonomic groupings.

ii. Results

In general pelagic phytoplankton concentrations are similar between all sites. Figure 32 shows chlorophyll concentrations from April through September 2009 at Glenbrook, Marla Bay and Lakeside with significant differences occurring during the warmer temperature periods of the year. Where there are no values for a particular date or site, no samples were collected. In April 2009 chlorophyll concentrations at Glenbrook and Marla Bay were not significantly different from each other but in June 2009 and August 2009 these sites showed significant differences at 5 m water depth in chlorophyll concentrations (Figure 32).

Phytoplankton

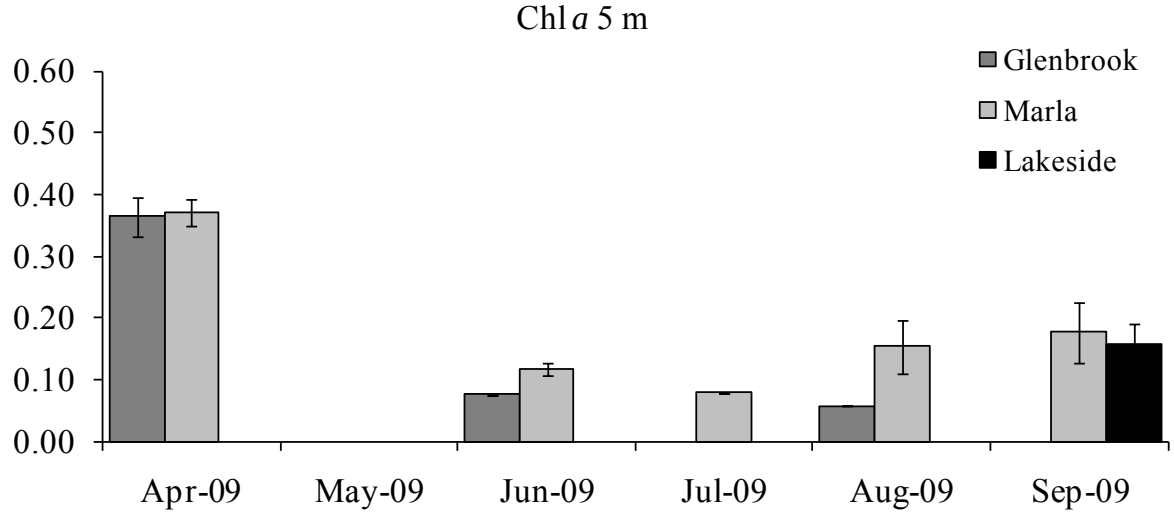

Figure 32. Mean concentration of chlorophyll *a* collected 5 meters below the surface at three locations (Glenbrook, Marla Bay, and Lakeside) from April to September 2009. Error bars represent standard error. The X-axis shown indicates the month in which samples were collected and the Y –axis indicates chlorophyll concentrations in µg/L.

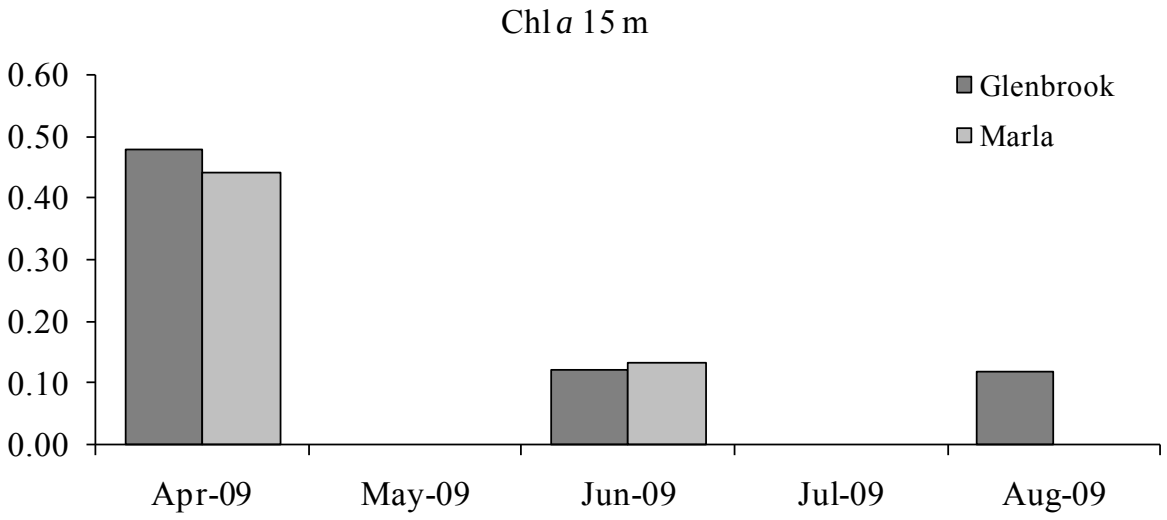

Figure 33. Mean concentration of chlorophyll a collected 15 meters below the surface at two locations (Glenbrook and Marla Bay) from April to August 2009. The X-axis shown indicates the month in which samples were collected and the Y –axis indicates chlorophyll concentrations in µg/L.

Comparing this to measurements collected at 15 m depth, differences between Glenbrook and Marla Bay are not significant and similar in both the spring (April) and summer (June) period (Figure 33). The difference between these depths can be attributable to differences in biological community composition, temperature, light or physical properties of the water column at those depths.

Differences in chlorophyll concentrations between Marla Bay (high density Asian clam populations) and Glenbrook Bay (no Asian clam presence) by depth are shown in Figures 34 - 36. Chlorophyll concentrations are generally low and similar at the 5 m and 15 m water depths in April period, with Glenbrook showing slightly higher values at the 20 m water depth. Samples collected in June 2009 show no major differences between sites at all depths (Figure 35) and it is not until August 2010 where differences are observed between Marla Bay and Glenbrook. At all depths at this time period, chlorophyll concentrations are higher in Marla Bay as compared to Glenbrook (Figure 36), yet all values are consistently low and within the typical range limit of chlorophyll concentrations observed at Lake Tahoe (Reuter et al. 2009).

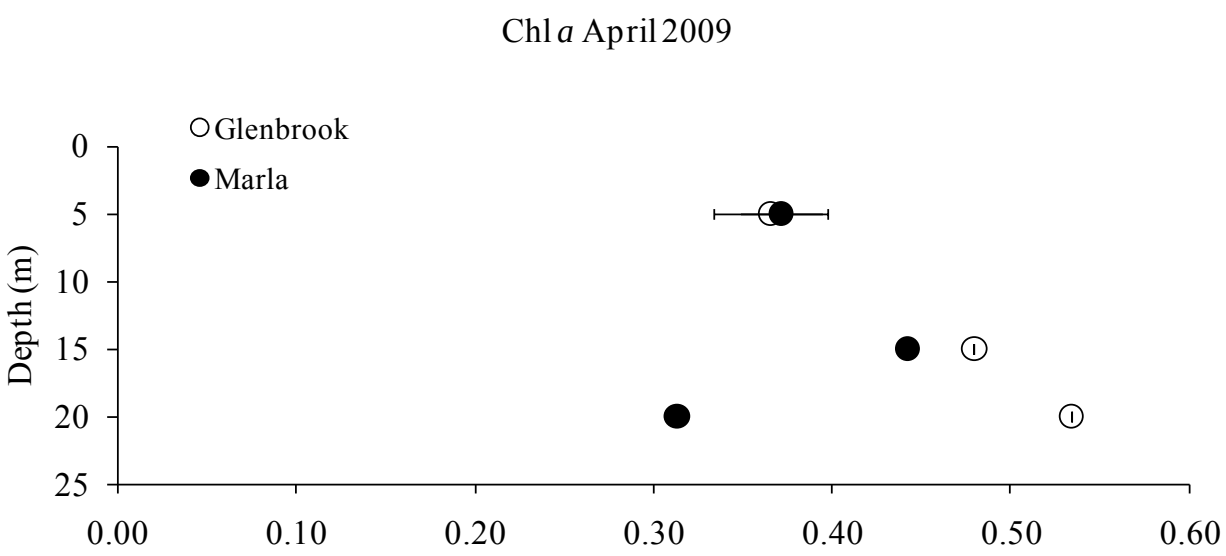

Figure 34. Mean concentration of chlorophyll *a* collected from the surface down to 20 meters at Glenbrook (open circles) and Marla Bay (closed circles) during April 2009. Error bars represent standard error. The x-axis shown indicates chlorophyll concentration (µg/L) and the y –axis indicates water depth.

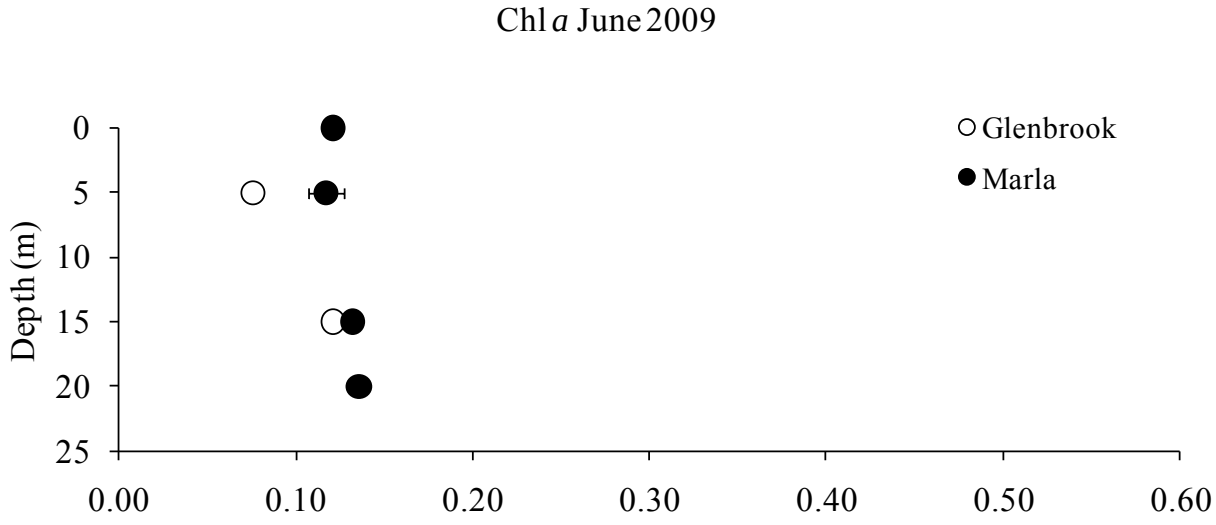

Figure 35. Mean concentration of chlorophyll *a* collected from the surface down to 20 meters at Glenbrook (open circles) and Marla Bay (closed circles) during June 2009. Error bars represent standard error. The x-axis shown indicates chlorophyll concentration (µg/L) and the y –axis indicates water depth.

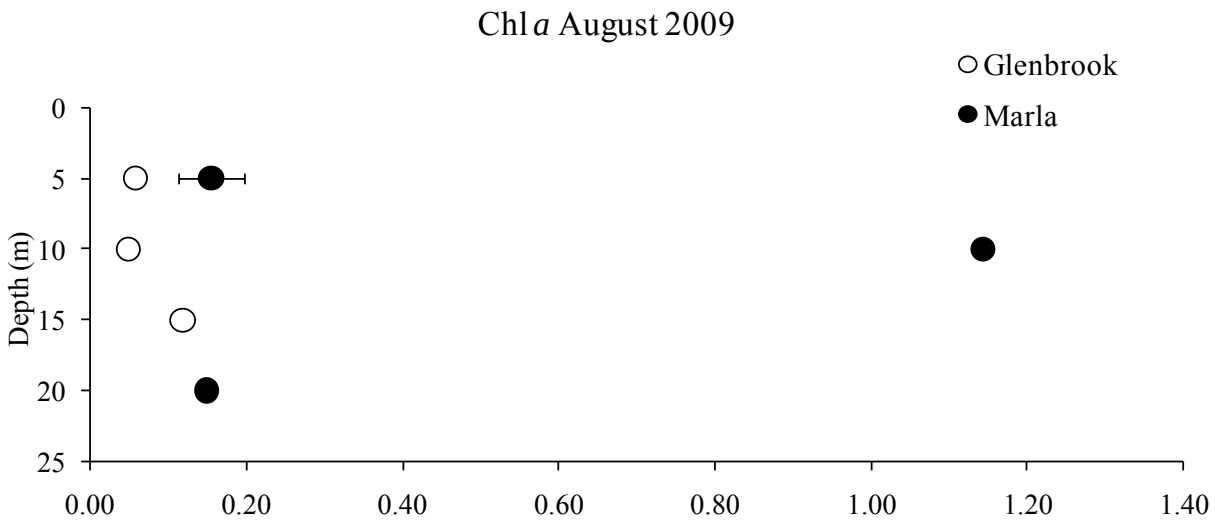

Figure 36. Mean concentration of chlorophyll *a* collected from the surface down to 20 meters at Glenbrook (open circles) and Marla Bay (closed circles) during August 2009. Error bars represent standard error. The x-axis shown indicates chlorophyll concentration (µg/L) and the y –axis indicates water depth.

Filamentous algae

The filamentous algal bloom observed in 2009 was very much reduced in extent and concentration compared to that observed in 2008. The dominant taxa observed in 2009 was *Cladophora glomerata* compared to the dominance of *Zygnema spp.* in 2008. Other attached and unattached non-dominant taxa observed are included in Table 4.

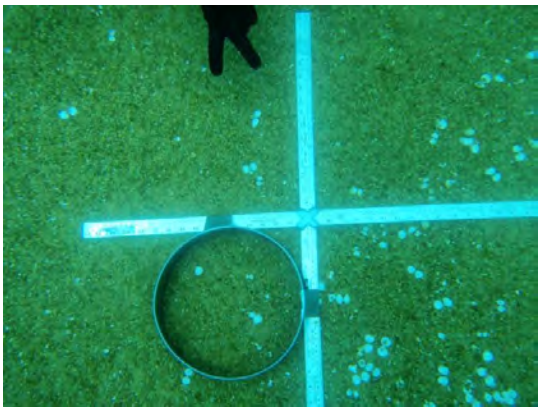

Figure 37. Filamentous algae sampling apparatus shown on the lake bottom at Marla Bay, 2009. The circular ring is the area from which any filamentous algae was collected and quantified, and measuring sticks are attached for scale. The number indicated by the hand in the upper frame shows the sample repetition (in this case, sample #2 collected).

Due to the reduction in extent and magnitude of the filamentous algal bloom, field researchers were unable to detect algal blooms for measurements using the above described methodology shown in Figure 37. As a result, the visual observation and biomass quantification sampling program was reduced to field observations of presence or absence of algal growth. In Marla Bay, filamentous algal blooms dominated by *Cladophora glomerata* peaked in abundance in August 2009 and by October most populations had senesced. At no point were filamentous algal populations observed in Glenbrook.

Table 4. Periphyton and metaphyton species detected at Lake Tahoe littoral zones, 2009. Note: Not a complete list of all taxa present. Samples collected by Scott Hackley, UCD

Species Name	Collection Date	Notes
Cymbella cistula	2009	Periphyton, collected from Lake Tahoe rock
Fragilaria construens	2009	Periphyton hard substrate scrape
Fragilaria capucina	2009	Lake Tahoe Periphyton
Cladophora glomerata	2009	Rock scrape
Epithemia zebra	2009	Rock scrape, cells epiphytic on Cladophora filaments
Rhoicosphenia curvata	2009	Lake Tahoe periphyton rock scrape
Epithemia sorex	2009	Lake Tahoe periphyton rock scrape
Cocconeis pediculus	2009	Epiphytic on Cladophora sp. filaments
Gomphonema longiceps var. subclavata	2009	Rock scrape, numerous thin striae and definite center area
Rhopalodia gibba	2009	Lake Tahoe periphyton rock scrape
Diatoma vulgare	2009	Lake Tahoe periphyton rock scrape
Synedra ulna var. spathulifera	2009	Lake Tahoe periphyton rock scrape
Gomphonema constrictum	2009	Rock scrape
Unknown	2009	Nodular algae found near Cladophora spp. at Elk Point
Cladophora sp.	2009	El Dorado Beach
Zygnema spp.	2009	Marla Bay, Lakeside
Spirogyra spp.	2009	Marla Bay, Lakeside

The *in situ* fluorometer provided a continuous monitoring of chlorophyll dynamics in Marla Bay. The first deployment of the instrument occurred on May 1, 2009 (Figure 38).

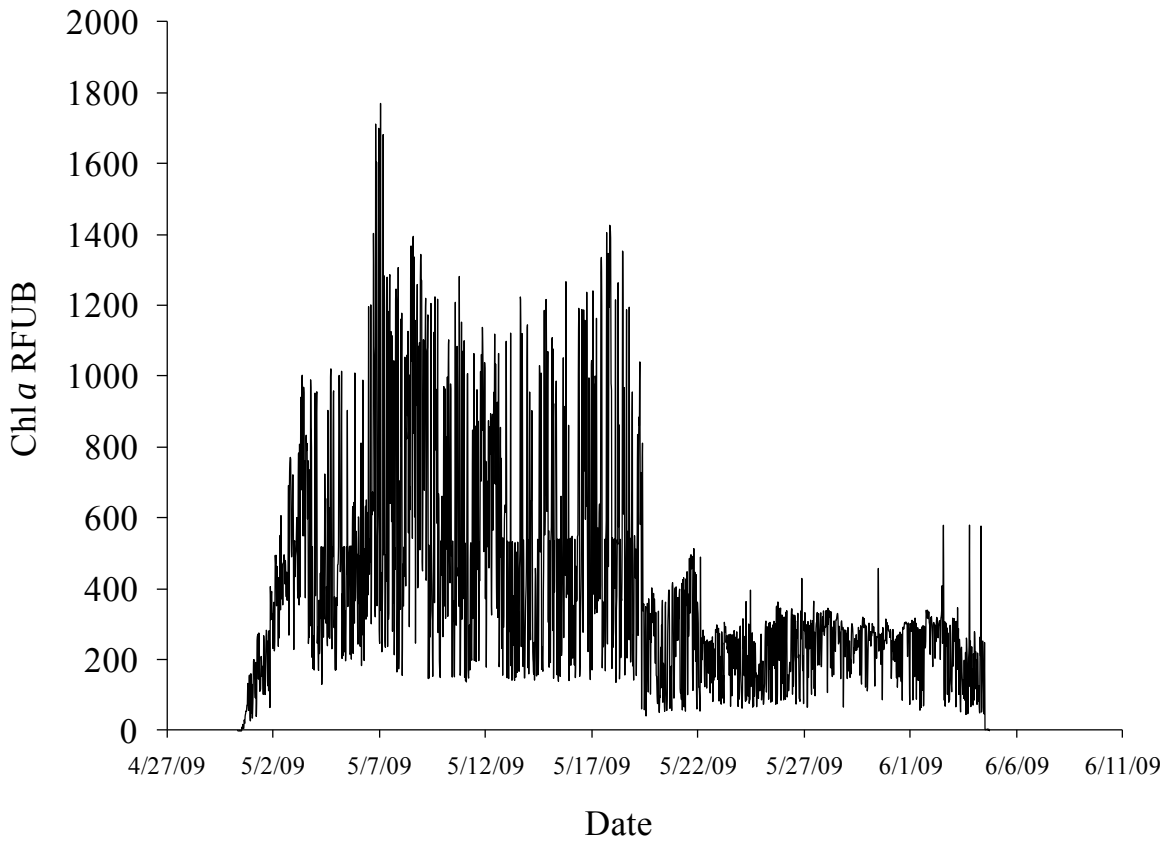

Figure 38. Observations from the first deployment of the *in situ* fluorometer; samples collected from May through June 2009.

There is daily variability in the relative chlorophyll *a* concentrations recorded. This variability is likely due to ambient water movements at the sediment water interface. However, on May 20 – 21 2009 (Figure 31) there is a dramatic reduction in relative chlorophyll *a* concentrations occurring. Wind data collected at South Lake Tahoe weather stations during this period suggest there was a major wind/storm even on May 20th, suggesting that physical processes such as water movement (and subsequent filamentous algal movement). The instrument was recovered on June 5 for battery replacement and redeployed on June 11, 2009 until July 26th 2009. During this period, relative chlorophyll *a* concentrations remained low with four increases in relative concentration on June 20, July 2, 7 and 14th (Figure 39). Field observations in Marla Bay agree with the low concentrations sampled during this period by the *in situ* fluorometer.

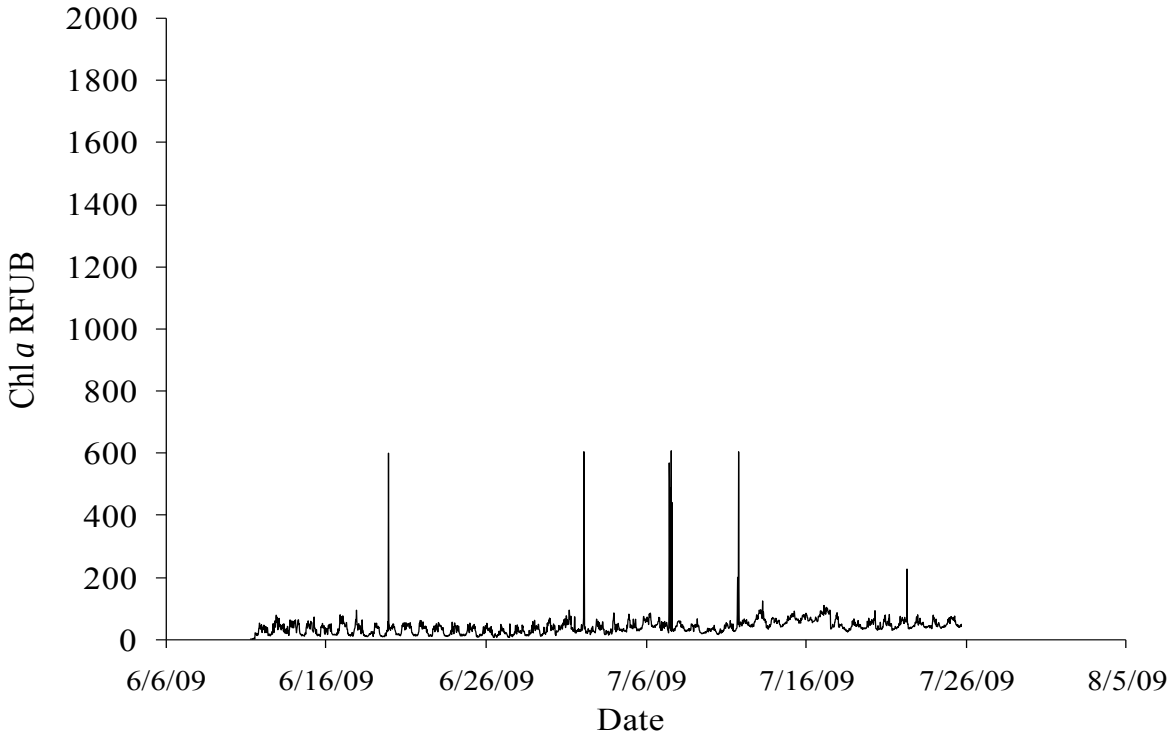

Figure 39. Observations from the second deployment of *in situ* fluorometer; samples collected from June through July 2009.

iii. Discussion

Results from algal monitoring indicate that pelagic chlorophyll *a* concentrations are generally similar in sites with Asian clam populations compared to those without established Asian clam populations. Departures from this similarity occur during the summer months (June through August period). Pelagic chlorophyll concentrations in Marla Bay are slightly higher than in Glenbrook Bay at this time, but compared to ambient lake concentrations, values sampled herein are within the range of concentrations observed in annual Lake Tahoe monitoring programs. However, the slight elevation of values observed in Marla Bay suggests the potential for physical or biological processes that may contribute to this subtle difference. Further research is necessary to understand the relationship between chlorophyll *a* concentrations and biotic or abiotic processes.

The deployments of the *in situ* fluorometer combined with visual field observations in both 2008 and 2009 show that filamentous algal blooms are occurring in Marla Bay and Lakeside but not at Glenbrook in Lake Tahoe. In two years these blooms have been dominated by different algal species (*Zygnema spp., and Cladophora glomerata*), and depending on the functional grouping (meta or periphyton), can be dispersed with physical processes such as wind events or ambient

water movement. The linkage between invasive bivalve filter feeding and excretion and associated impacts to both pelagic and filamentous algal species has been documented with variable results in the literature (Hakenkamp and Palmer 1999, Vaughn and Hakenkamp 2001). Results from experiments in Lake Tahoe suggest that there is potential for the relationship between this established species and alterations to algal concentrations. Further monitoring and experimentation is recommended.

VIII. Asian clam filter feeding experiment

Asian clams have demonstrated the ability to cause multiple ecosystem impacts on nutrient recycling and primary productivity (Hakenkamp and Palmer 1999). Nutrient cycling can be altered at the benthic-pelagic interface through Asian clam pedal feeding (from sediments), and nutrient excretion and active burrowing within sediments (Vaughn and Hakenkamp 2001). Pedal feeding may be more utilized than previously thought and it allows juvenile clams to grow faster than with filter feeding alone (Vaughn and Hakenkamp 2001). Primary productivity is thought to be an important aspect of aquatic ecosystem functioning which can be impacted by Asian clam filter feeding (Hakenkamp and Palmer 1999, Lauritsen 1986). The impacts of filter feeding are most severe when the filter feeding bivalve has a high relative biomass (Vaughn and Hakenkamp, 2001) or exists in high densities (Hakenkamp, 2001). Factors that influence the filtration rate are bivalve size, water temperature and food concentration. The impacts of Asian clams on such ecosystem processes have not been investigated in Lake Tahoe. The purpose of this study is to determine the filtration rates of the Asian clams found in Tahoe in order to understand the potential impact on Lake Tahoe phytoplankton communities.

i. Experimental design and methodology

All filtration rate experiments were carried out at the UC Davis Tahoe City Field Station. Water was collected from a depth of 1-1.5 meters at Tahoe City, CA and an 80 μm mesh was used to filter 2.5 gallons of water into each of the 44 20 L clam containment chambers used during the study. All chambers were acid washed with 0.1 N HCl and rinsed with deionized water prior to use to ensure the proper indication of algal presence in each chamber. Sediments and clams were collected from Marla Bay, NV and clams sorted into two size classes, large and small (large mean ~18mm: small mean ~10 mm). Clams were measured with a digital caliper and placed in a chamber, one clam per chamber. Each size class had 20-5 gallon chambers, 10 with 5 cm of sediment and 10 without sediment. Four chambers served as controls, 2 with sediment and 2 without. In addition, two chambers for each treatment (S-small clam without sediment, SS-small calm with sediment, L-large clam with without sediment, SL-large clam with sediment) and 1 control chamber (without sediment) were utilized for algae samples.

Chlorophyll readings were obtained with a 10 AU fluorometer. Three 5-mL samples of water were collected from each chamber after quickly stirring the water with the pipette tip and

measured for in vivo fluorescence. Dissolved oxygen (mg/L and %), conductivity, and temperature were measured with a YSI-85 prior to each time interval for 3 selected chambers in different treatments: S1, SL1 and C1. Samples of filtered (80 μm mesh) lake water preserved in lugol solution were saved for algae identification.

As in vivo samples were instantly measured for the selected chambers, 100 mL of was collected from each chamber and filtered with Whatman GF/C glass fiber filters. The Lake Tahoe chlorophyll *a* extraction procedure (Appendix 1) was followed to obtain values. Extractions were performed 3-6 days after sample collection. A linear regression comparing chlorophyll *a* concentrations to the mean fluorometer reading for the corresponding chamber/time point was used to convert all fluorescence measurements to chlorophyll a concentration ($R^2 = 0.32$).

Visual feeding/burrowing observations were recorded before and after each sampling interval. If the siphon of the clam was protruding from the shell or the clam was clearing feeding, it was recorded as feeding. If the siphon was not visible and the shell was completely closed it was recorded as not feeding. Also, it was noted if the clams in sediment were burrowed or not.

The experiment was conducted over a 60 h period measuring fluorescence at 12 hour intervals, at 7pm and 7am. Clams were collected the day of the experiment and allowed to acclimate 4-7 hours based on their treatment (sediment or no sediment) in fresh lake water. The natural light cycle was extended by 2 hours with artificial overhead lights from 8:30 pm-10:30 pm as measurements were taken. After the experiment, clams were collected and oven-dried at 60 C for 11 days. Dry flesh weight (grams) and total dry weight were measured after 11 days of drying.

ANCOVA (analysis of covariance) was used in S-Plus statistical software to determine if the difference in the filtration rate slopes of the control vs. large clams (with sediment) and control vs. small clams (with sediment) were statistically significant.

ii. Results

Pooling the DO, conductivity and temperature measurements from the chambers, DO (%) ranged from 62.7%-55.7%, DO (mg/L) 5.71-5.3, conductivity (uS/cm) 89.7-78.6 with measurements generally decreasing over time. Water temperature was initially measured at 22.5 °C immediately after distribution, but during the duration of the experiment, ranged from 20- 18.5 °C and followed daily temperature variation.

Figures 40-46 show the pooled filtration data (chlorophyll a concentration as a function of time) from the chambers in each treatment (S, SS, L, SL) and the two control groups. Large clams with sediment-SL (Figure 40), mean length = 17.9 mm, were able to reduce algal biomass by 56%, and filter 9.6 L (2.5 gal) of water, in 24 hours or 394 mL/hour. A line of best fit gives an R^2 of 0.52 for the SL group, indicating a fairly linear relationship. Large clams without sediment-L (Figure 40) reduced algal biomass by 81% and filtered the algae within the same amount of time

(9.6 L/24 hr). Chlorophyll *a* concentrations at each time point vary less in the L group when compared to SL group. Small clams with sediment-SS (Figure 41), mean length = 10.6 mm, decreased algal biomass by 54%, but took double the time (197 mL/hr) compared to large clams. The SS group data also decreases linearly, $R^2 = 0.62$.

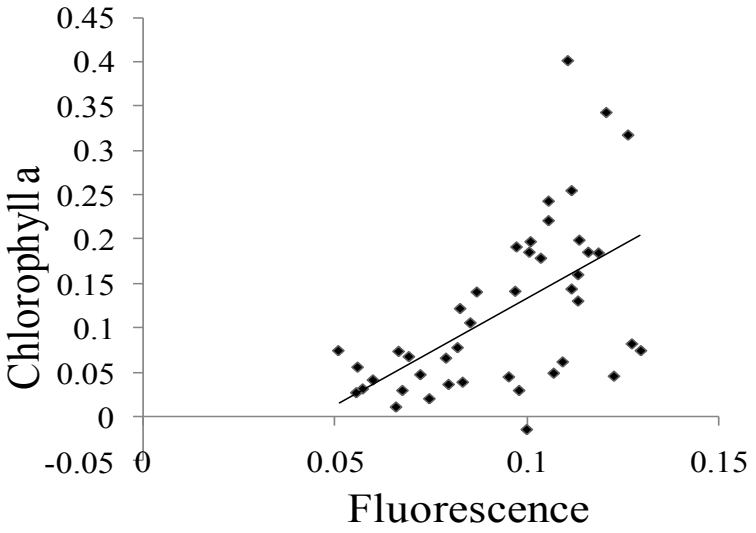

Figure 40. Linear regression of fluorescence readings and algal biomass (chl *a* concentration). The mean of the 3 fluorescence readings taken from the chamber at each time internal was compared to the algal biomass of sampled chambers. Two chambers from each treatment were used to extract chlorophyll and one control chamber, total 9 chambers.

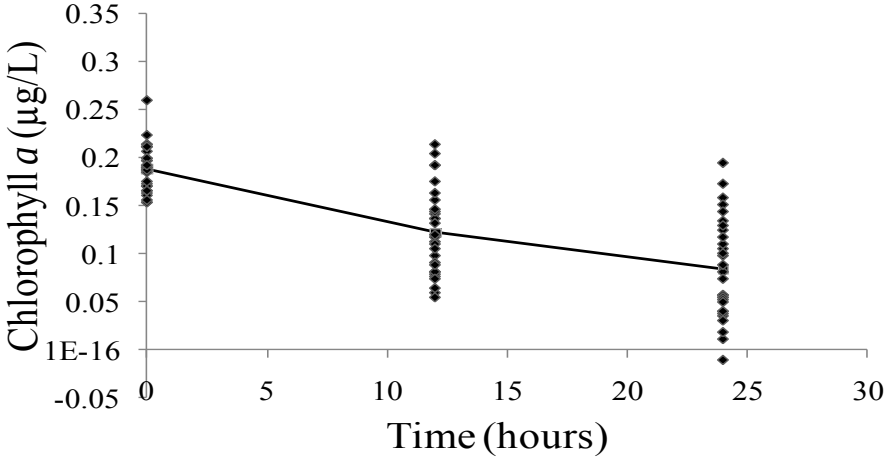

Figure 41. Mean chlorophyll *a* concentration as a function of time in a 20 L chamber with filtration by large clams (N=10) with sediment.

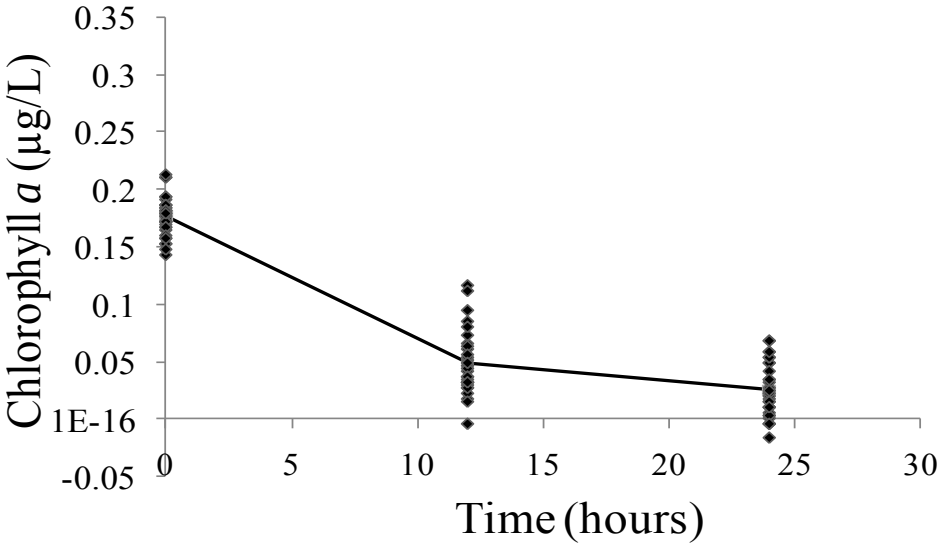

Figure 42. Mean chlorophyll a concentration as a function of time in a 20 L chamber with filtration by large clams (N=10) without sediment.

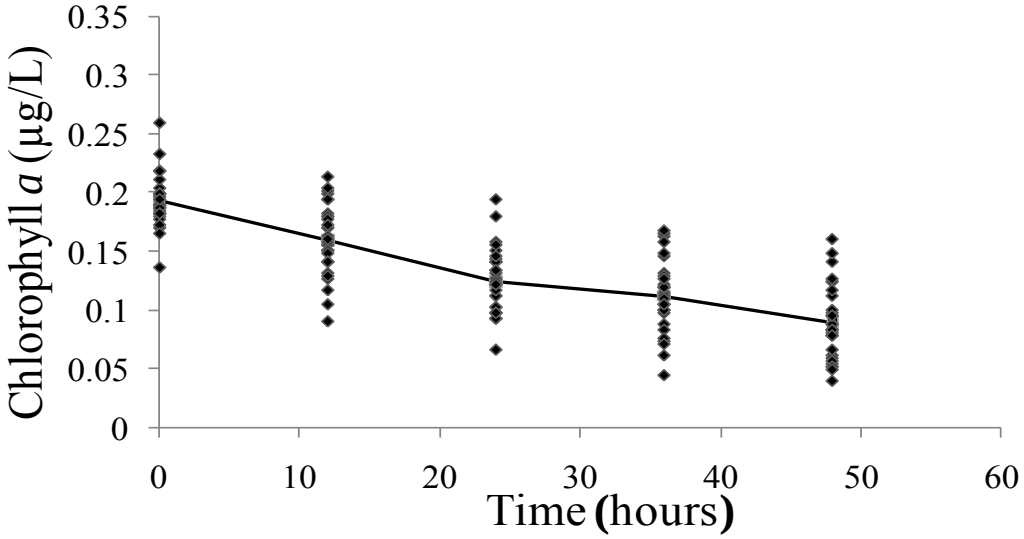

Figure 43. Mean chlorophyll a concentration as a function of time in a 20 L chamber with filtration by small clams (N=10) with sediment.

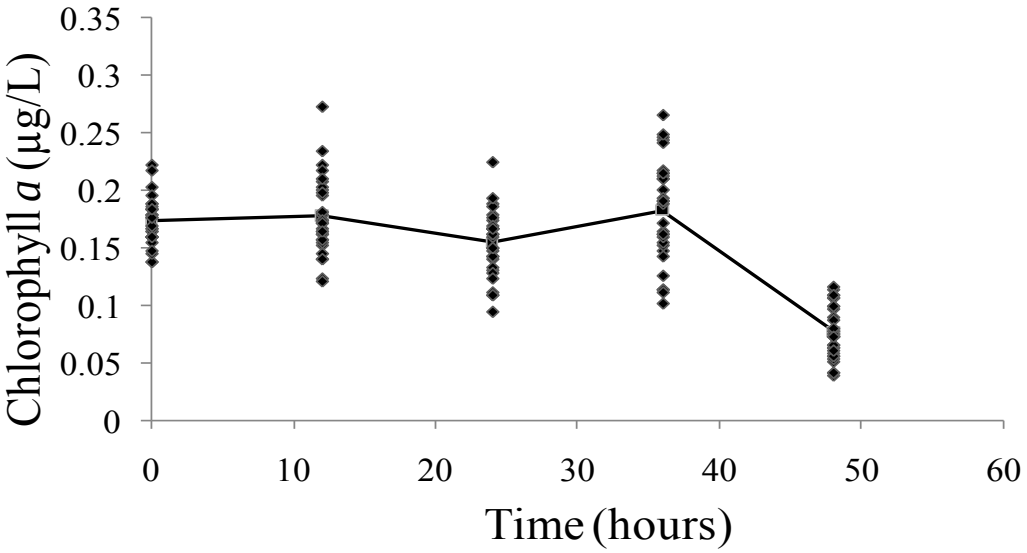

Figure 44. Mean chlorophyll *a* concentration as a function of time in a 20 L chamber with filtration by small clams without sediment.

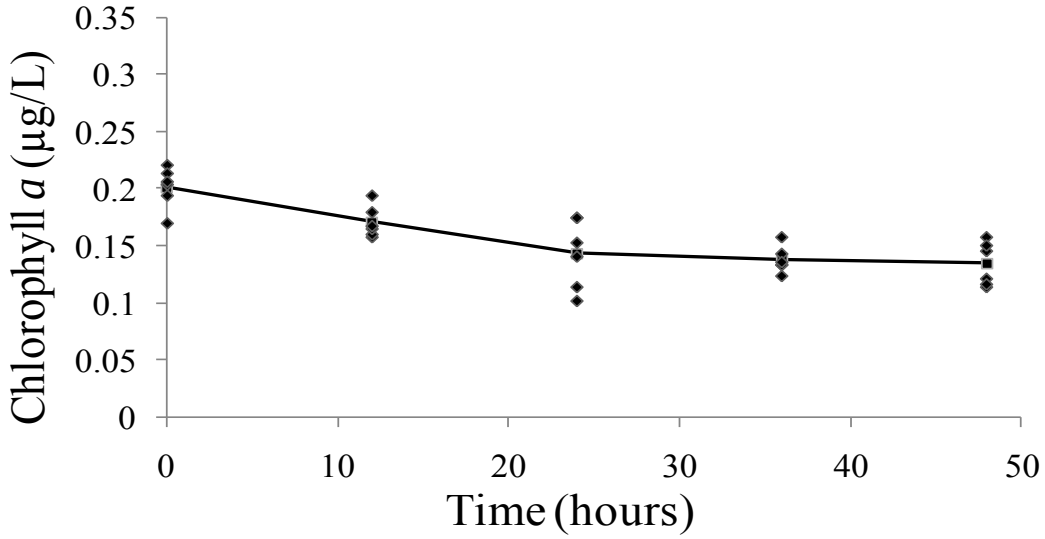

Figure 45. Mean chlorophyll a concentration as a function of time in a 20 L chamber for control with sediment.

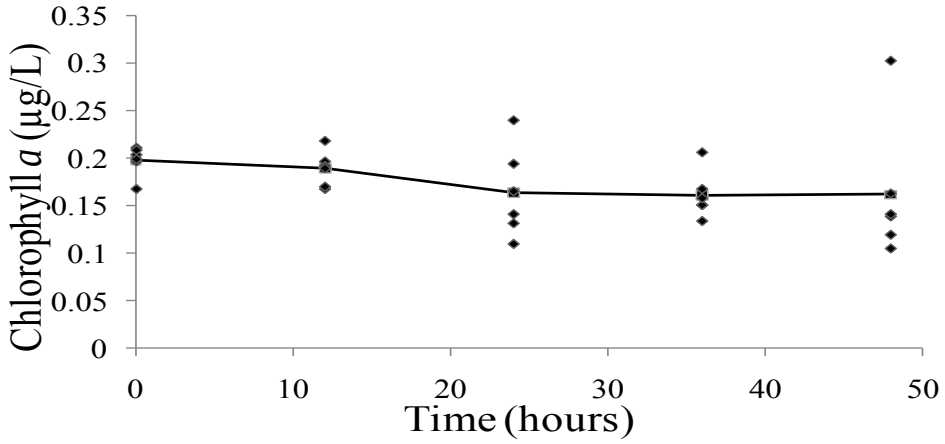

Figure 46. Mean chlorophyll *a* concentration as a function of time in a 20 L chamber for control with no sediment.

Algal biomass in the control with sediment (CS) reduced by 29% in 24 hours and 33% in 48 hours (Figure 42). In the control without sediment, algal biomass decreases were 17% and 18% for 24 and 48 hours respectively (Figure 44). Natural algal decay does occur, but ANCOVA results suggest that differences between treatments and control is significant (Tables 5 – 7).

Control-CS vs. large clams-SL had a p-value of 0.000 (Table 5). Control-CS vs. small clam-SS had a p-value of 0.049, using a 95% confidence interval (Table 6). Further, the slopes of large-SL vs. small clams-SS were compared (Table 7) and were also found to be statistically different (p=0.000). Statistical analyses of data from clams without sediment were not compared due to a lack of linearity in the data of large clams, and irregular data of small clams.

Control-CS vs. Large-SL Summary

	Value	Std. Error	T value	Pr(>|t|)
(Intercept)	0.1870	0.0068	27.3251	0.0000
Time	0.0029	0.0003	-9.9210	0.0000
Factor (treatment)	-0.0034	0.0068	-0.5017	0.6168
Time:factor (treatment)	-0.0015	0.0003	-5.1649	0.0000

Residual standard error: 0.0382 on 115 degrees of freedom
Multiple R-Squared: 0.54
F-statistic: 45.24 on 3 and 115 degrees of freedom, the p-value is 0

Table 5. ANCOVA results for Control with sediment (CS) and Large Clam treatment with clam size versus control as treatment factors.

Control-CS vs. Small-SS Summary

	Value	Std. Error	T value	Pr(>|t|)
(Intercept)	0.1904	0.0094	20.1798	0.0000
Time	-0.0014	0.0003	-4.3130	0.0000
Factor (treatment)	-0.0041	0.0103	-0.4012	0.6888
Time:factor (treatment)	-0.0007	0.0004	-2.0100	0.0460

Residual standard error: 0.02984 on 176 degrees of freedom
Multiple R-Squared: 0.58
F-statistic: 81.11 on 3 and 176 degrees of freedom, the p-value is 0.046

Table 6. ANCOVA results for Control with sediment and small clam treatment with clam size versus control as treatment factors.

Large-SL vs. Small-SS Summary

	Value	Std. Error	T value	Pr(>|t|)
(Intercept)	0.1863	0.0051	36.8200	0.0000
Time	-0.0021	0.0002	-12.1516	0.0000
Factor (treatment)	-0.0027	0.0078	-0.3473	0.7287
Time:factor (treatment)	-0.0023	0.0004	-5.4564	0.0000

Residual standard error: 0.03578 on 235 degrees of freedom
Multiple R-Squared: 0.54
F-statistic: 93.03 on 3 and 235 degrees of freedom, the p-value is 0

Table 7. ANCOVA results for comparison of large clams and small clams with size and time as treatment factors.

Clams were observed for feeding and/or burrowing before and after each sampling period, with a total of 11 observation records. Of the 10 replicates of small clams without sediment (S), percentage of positive feeding observations ranged from 36.4%-100% with and mean of 77.7%. Large clams without sediment (L) ranged from 9%-100% with a mean of 50% positive feeding. All replicates of small clams with sediment (SS) were burrowed 100% of the experiment, no feeding observations were possible. Three of the large clams with sediment (SL) were observed unburrowed on multiple occasions; the other 7 were burrowed 100% of the experiment.

iii. Discussion

Asian clam filtration rates observed in the literature vary considerably. Shell length correlates with filtration rate (Lauritsen 1986) which has also been observed in this experiment. Rates for clams 20 mm in length have been reported at a mean of 11 mL/h (chlorophyll *a*) and a maximum rate of 816 mL/h (Lauritsen 1986). Large clams (mean length = 17.9 mm) in this study filtered chlorophyll *a* at a rate of 394 mL/h which is within the range of clams observed in the Lauritsen (1986) study. Small clams (mean = 10.6) filtered at a rate of 197 mL/hr, which is still higher than studies using clams mean length of 20 mm.

While filter feeding is possible burrowed and unburrowed, Asian clam preference to burrow was demonstrated and the treatment with sediment reflected natural conditions and therefore more realistic filtration rates. There was greater variation in the data for large clams with sediment than without. This was expected as benthic food sources were made available. Sediments were not autoclaved, as in other studies, allowing both bacteria and nutrients to be present in the test chambers. The presence of bacteria in the sediments may explain the slight differences in the controls. The control with sediment had a greater decrease in chlorophyll *a* values than the control without sediment.

If Asian clams are able to continue spreading in Lake Tahoe, they may be able to impact the algal biomass in the lake littoral zone. Filtration rates are moderately high and clams are filter feeding burrowed and unburrowed. However, primary productivity is more likely affected by clams when clam biomass is large relative to water volume and if hydrologic residence time is long (Hakenkamp 2001). Lake Tahoe satisfies the latter, but the clams will likely not have a large biomass relative to water volume in Tahoe. However, there may be local decreases in algal biomass in near shore areas with dense clam populations. Primary productivity is an essential component for ecosystem integrity and declining productivity can have detrimental effects cascading through the trophic structure.

The following are the conclusions from this experiment:

- Declining algal biomass is due to the presence of clams and not natural decay, therefore clams may impact algal biomass in Lake Tahoe.
- Large clams filter feed at a rate faster than small clams.
- Clams filter algae from water column burrowed and unburrowed.
- Decreases in algal biomass may have cascading negative impacts through the trophic structure.
- Medium sized clams will burrow deeper than the large and small clams, which prefer 1-6 cm.

IX. Laboratory algal growth experiment

To test the effect of Asian clams, and in particular the effect of Asian clam excretion products (nitrogen, carbon and others) on filamentous algal growth, a 10-day laboratory bioassay was carried out at the TERC laboratory in Incline Village, NV in July – August 2009. This experiment included the use of Asian clam excretory materials (extracted from Asian clams collected from Lake Tahoe), a synthetic growth medium and filamentous algae also collected in Lake Tahoe (a combination of *Zygnema spp.* and *Cladophora glomerata*). This experiment was intended as a bioassay to investigate the impact of Asian clam nutrient excretion on filamentous algal growth in a laboratory setting.

i. Experimental design and methodology

Filamentous algal experiment design

A total of 4 treatments were used in this experiment: a 0, 2 and 50 % dilution of Asian clam excretion material with filamentous algal biomass, and one control treatment with no algal biomass or clam excretory material. Each treatment contained a combination of deionized water, a stock algal growth medium (see Appendix 2 for composition) and clam excretory material and 0.02 g of filamentous algal matter (Table 8). The stock algal growth medium was included to ensure the viability of algal material over the course of the experimental period and to observe whether Asian clam excretory material would provide a departure from background growth rates. Clam excrement solution was extracted in the laboratory using methods described in Lauritsen and Mozely (1989) with a known NH4+ concentration of 145 ppb and an SRP concentration of 21 ppb. The experimental period began on day 1, and algal biomass was sampled four times throughout a 10-day period to observe algal uptake of Asian clam excretory materials, temporal changes to algal nitrogen and carbon concentrations, and whether there was a departure in background levels of algal growth as a result of variable Asian clam excretory material additions. After each growth chamber has all components added, each was placed in an incubation chamber simulating Lake Tahoe field conditions (diel light schedule and 17 – 20°C temperature range).

Control (N=4)	**0% dilution (N = 4)**	**2% dilution (N = 4)**	**50% dilution (N = 4)**
25-mL GM	25-mL GM	25-mL GM	25-mL GM
25-mL DI	25-mL DI	24-mL DI	
		1-mL CM	25-mL CM
	0.02-g algal biomass	0.02-g algal biomass	0.02-g algal biomass

Table 8: Stock experimental concentration experimental design. A control with no Asian clam excretory materials and no algae was compared to treatments with 0, 2 and 50% Asian clam excretory material dilution, GM = WC Growth Medium (stock); DI = Deionized Water; CM = Clam excretory material; Algae = *Zygnema spp.* and *Cladophora sp.* collected from Lake Tahoe.

Carbon and nitrogen analysis

Algal materials were extracted, wet and dry weighed, and packed in aluminum foil packets for nitrogen and carbon analysis at the stable isotope facility on the UC Davis campus. Solid materials are analyzed for ^{13}C and ^{15}N isotopes using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Samples are combusted at 1000°C in a reactor packed with chromium oxide and silvered cobaltous/cobaltic oxide. Following combustion, oxides are removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flows through a water trap (magnesium perchlorate) and an optional CO_2 trap (for N-only analyses). N_2 and CO_2 are separated on a Carbosieve GC column (65°C, 65 mL/min) before entering the IRMS. During analysis, samples are interspersed with several replicates of at least two different laboratory standards. These laboratory standards, which are selected to be compositionally similar to the samples being analyzed, have been previously calibrated against NIST Standard Reference Materials (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, and USGS-41). A sample‘s preliminary isotope ratio is measured relative to reference gases analyzed with each sample. These preliminary values are finalized by correcting the values for the entire batch based on the known values of the included laboratory standards.

ii. Results

Algal uptake of carbon and nitrogen increased in the treatment with 50% dilution of Asian clam excretion material compared to the 0 and 2% dilution treatments (Figure 47, 48). At the initiation of the experiment, the algal biomass contained an average of 8914 ±354 (S.E.) µg/g carbon and 484 ±11 (S.E.) µg/g nitrogen. On experimental day 3, algal material in the growth chamber containing the 50% dilution of Asian clam excretion material showed a significant departure in both carbon and nitrogen composition, a relative carbon increase of 333% and 150% of nitrogen.

Carbon concentrations in the 0% dilution treatment showed a slight decrease in the initial period of the experiment, a small drop on experiment day 3, with gradually increasing carbon concentrations on day 3 and again on day 7. Carbon concentrations in the 2% treatment did not show the slight initial drop on day 3, suggesting that the extra nutrient from the Asian clam excretory material may have aided in the carbon uptake of the algal biomass in this solution. Carbon concentrations in the 0 and 2% treatment did not differ significantly from each other, while there was a clear departure from background growth rates in the 50% treatment. Nitrogen concentrations in algal biomass showed similar trends to carbon concentrations (Figure 48). The 50% dilution also showed a large departure from background growth rates (0%) dilution, with a 300% increase of nitrogen concentration in algal tissue matter.

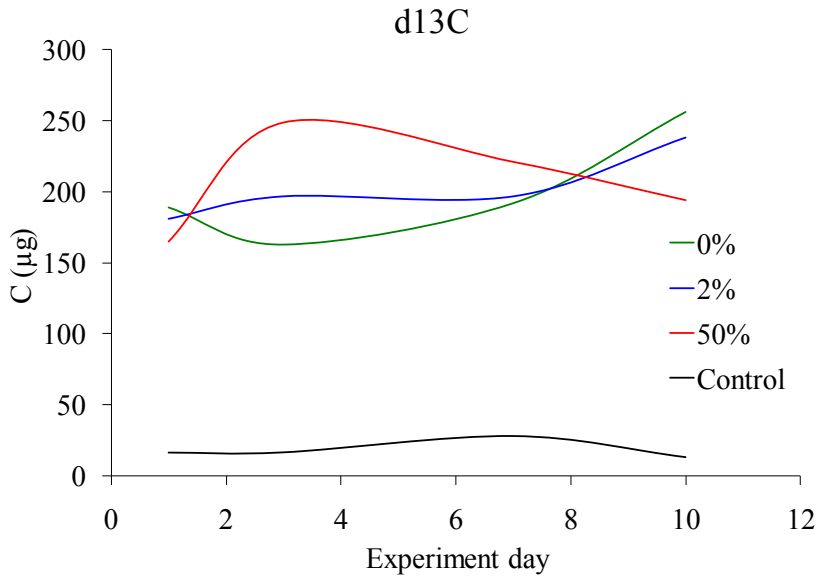

Figure 47. Temporal changes in carbon (d13C) amounts (µg) of algal biomass with exposure to variable dilutions of Asian clam excretory material. The three experimental permutations included a 0, 2, and 50% dilution compared to a control treatment (zero clam excretory material and zero growth medium). Samples were collected on the initiation day of the experiment (Day 1) and on day 3, 7 and 10.

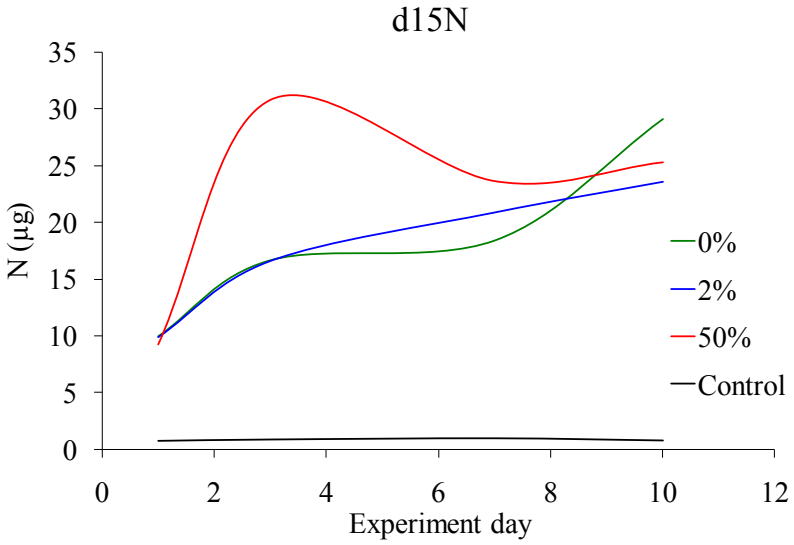

Figure 48. Temporal changes in nitrogen (d15N) amounts (µg) of algal biomass with exposure to variable dilutions of Asian clam excretory material. The three experimental permutations included a 0, 2, and 50% dilution compared to a control treatment (zero clam excretory material and zero growth medium). Samples were collected on the initiation day of the experiment (Day 1) and on day 3, 7 and 10.

iii. Discussion

Results from this experiment suggest that excess availability of nutrients produced through natural metabolic processes of Asian clams can stimulate algal uptake of carbon and nitrogen by filamentous algae species that are present in Lake Tahoe. Treatments containing a the greatest dilution of Asian clam excretory materials (50%) showed significant departures from treatments that contained solutions with only growth medium (0%) and a solution that had low concentrations of Asian clam excretory material (2%), suggesting that the magnitude of the contribution of Asian clam nutrient recycling plays an important role in nutrient availability for collocated algal populations. This is to say, that low density populations of Asian clam are likely not capable of altering nutrient cycling as extensively as high density populations.

We have shown in this experiment that direct nutrient inputs from Asian clams can stimulate the growth of filamentous algae present in Lake Tahoe. We have also shown that Asian clams can filter feed pelagic algae at a rapid rate (see previous section, g). Asian clams in Lake Tahoe are contributing to alterations in nutrient cycling through competitive uptake of phytoplankton and the localized concentration of these nutrients through excretion processes. Further research is necessary to understand the ecosystem level impacts of these processes to Lake Tahoe.

X. Costs

The following shows a summary of the combined cost totals for the labor and equipment involved for the application of polyethylene bottom barriers and diver assisted suction removal. 2000 sq ft of diver assisted suction removal plots and 3600 sq ft of polyethylene bottom barriers were applied for a total of $86,096. This total does not include costs associated with research and development of the management strategies.

These charges include construction of 48 bottom barriers ($125 each), dredging equipment use/diver assisted air (20 hrs at a rate of $200/hr), one master diver (to carry out suction removal and bottom barrier placement--40 hrs at a rate of $200/hr), one dive safety officer (40 hrs at a rate of $200/hr) and one senior diver (40 hrs at a rate of $200/hr). Additional charges include the fees for renting the barge and disposal of sediment materials (suction removal) (Table 9).

Cost per square foot estimation

To compare the costs per square foot of diver assisted suction removal and the successful implementation of EPDM pondliner on a large scale[1], the costs of labor and materials for the two methodologies are summarized using invoice-based estimation[2]. Because detailed information is not available on the actual number of hours allocated to the two different actions in 2009, the estimate of time spent is based on the barge cost ($4,600) for a sole barrier removal event, invoiced on July 30, 2009. The initial retainer of the barge and crew for a combination of barrier installation and suction removal implementation was $16,600, 27% of which is $4,600. While the barge and barge crew, and specialized divers carried out both tasks during the same 1-2 week period, it is estimated that barrier installation took approximately 27% of the time spent by divers and barge operations and suction removal took 73% of these resources.

As such, the cost of diver assisted suction removal includes 73% of the initial barge and excavator costs invoiced on March 13 – March 20 ($16,600) and April 2 – April 10 ($16,000), and Master Diver ($8000), Dive Safety Officer ($8000) and Senior Diver ($8000), miscellaneous dive costs ($65), and 100% of dredging equipment use ($4000) for a total of $49,414. With a diver assisted suction removal treatment area of 2000 sq. ft., the sq ft. cost for suction removal is $24.71.

Labor and materials costs for the installation of 1 acre of EPDM rubber benthic barrier includes $34,993 for materials (rubber, rebar weighting, fabrication, etc.) and labor for installation and

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[1] A large scale (1 acre) application of EPDM fabric benthic barrier occurred in Lake Tahoe during summer 2010, details not reported herein.

[2] Invoices provided by Tahoe Resource Conservation District on behalf of Tahoe Dive Conservancy.

removal was $33,642 and $43,642 respectively. The cost per square foot to implement bottom barriers in Lake Tahoe based on these estimates is $2.58, which is approximately an order of magnitude less than the costs associated with diver assisted suction removal.

The research teams are currently monitoring the recolonization rates of benthic communities including *C. fluminea* to understand the effectiveness of this lower cost, non-chemical control strategy. Monitoring will conclude in 2011, one and a half years after the application of the EPDM barriers.

Table 9. Implementation costs (labor and materials) for diver assisted suction removal and polyethylene barrier installation in Lake Tahoe CA-NV in 2009. Costs do not include research and development costs.

Item	Explanation	Date	Paid to	Cost
Labor	Loading dumpster and equipment also includes tug/barge/excavator costs and crew	March 13 - March 20, 2009	Tahoe Marine and Excavating	$16,600
Labor	Loading and unloading dumpster, tug/barge/excavator + crew and additional laborer at $50/hr	April 2 - April 10, 2009	Tahoe Marine and Excavating	$16,000
Labor	11.5 hrs @ $400/hr for mobilizing/demobilizing tug/barge/excavator and crew	July 30 - July 31, 2009	Tahoe Marine and Excavating	$4,600
Labor	Dredging and bottom barrier placement Master diver labor 40 hrs @ $200/hr	March 16, 2009	Tahoe Divers Conservancy	$8,000
Labor	Dredging and bottom barrier placement Dive Safety Officer 40 hrs @$200/hr	March 16, 2009	Tahoe Divers Conservancy	$8,000

Item	Explanation	Date	Paid to	Cost
Labor	Dredging and bottom barrier placement Senior diver 40 hrs @ $200/hr	March 16, 2009	Tahoe Divers Conservancy	$8,000
Labor	Bottom barrier removal Lead diver 6 hrs @ $200/hr	July 14, 2009	Tahoe Divers Conservancy	$1,200
Labor	Bottom barrier removal Dive Safety Officer 6 hrs @ $200/hr	July 14, 2009	Tahoe Divers Conservancy	$1,200
Labor	Placement of new barriers at Marla Lead diver 8 hrs @ $200/hr	July 28, 2009	Tahoe Divers Conservancy	$1,600
Labor	Placement of new barriers at Marla Dive Safety Officer 8 hrs @ $200/hr	July 28, 2009	Tahoe Divers Conservancy	$1,600
Labor	Bottom barrier removal Lead diver 16 hrs @ $200/hr	July 31, 2009	Tahoe Divers Conservancy	$3,200
Labor	Bottom barrier removal Dive Safety Officer 16 hrs @ $200/hr	July 31, 2009	Tahoe Divers Conservancy	$3,200

Item	Explanation	Date	Paid to	Cost
Materials	Construction of bottom barriers 48 barriers @ $125/each	March 16, 2009	Tahoe Divers Conservancy	$6,000
Materials	Dredging equipment use/diver assisted air 20 hrs @ $200/hr	March 16, 2009	Tahoe Divers Conservancy	$4,000
Materials	Misc dive equipment	April 2 - April 10, 2009	Tahoe Marine and Excavating	$90
Materials	Bottom barrier removal Boat transport	July 14, 2009	Tahoe Divers Conservancy	$800
Materials	Fabrication of new bottom barrier design at Marla 2 barriers @ $170	July 28, 2009	Tahoe Divers Conservancy	$340
Materials	Placement of new barriers at Marla Boat Transport	July 28, 2009	Tahoe Divers Conservancy	$1,600
Materials	Bottom barrier removal Dive tank refills	July 31, 2009	Tahoe Divers Conservancy	$66
Total Labor				$73,200
Total Materials				$12,896
TOTAL COST (MATERIALS AND LABOR)				$86,096

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XII. Appendix 1 -- Lake Tahoe Chlorophyll a Extraction Procedure

For pelagic algal monitoring, water samples collected in the field are returned to the lab and filtered through a 0.7 µm Whatman GF/C filter and frozen until ready for analysis. Samples are removed from the freezer and handled under a fume hood with no fluorescent light present. All glassware is cleaned using liquinox soap, rinsed well with tap water and then rinsed deionized water (x5). Dilute HCl is never used during washing as it will cause the chlorophyll molecule to degrade to pheophytin. Filters used to collect the samples are removed from the freezer and added to clean test tubes using forceps to minimize handling. Each test tube receives 5ml of methanol and is stopped with a cork before being covered with foil, double wrapped in plastic bags and stored at 4°C for 12 hours. In addition, three ~~"b~~lanks" (two Whatman GF/C filter blanks and one methanol only blank) were added to the analysis. These allowed turbidity blanks to be obtained which will eventually be subtracted from sample fluorescences in the calculations.

After 12 hours, the covered test tubes are allowed to equilibrate to room temperature. Fluorescence can be affected by temperature, so we aimed to measure fluorescence at a standard temperature of near 20°C. Tubes, with stoppers still in place, are vortexed 3-4 times at high speed to mix. Filters are then removed with clean glass rod and the stopper is replaced. The Turner Designs 10AU fluorometer is turn on and allow to warm up for 20-30 minutes before readings are taken. Samples are then re-vortexed and the fluorescence before acidification (F_b) is immediately read. Once this fluorescence value is obtained, the sample is acidified with 0.050ml of 0.3N HCl and allowed to sit for one minute before the fluorescence after acidification (F_a) can be measured. F_a fluorescence is read on the same range (Low, Medium, High) as F_b.

Chlorophyll *a* and pheophytin values were calculated using the following equations:

Formula for chlorophyll a:
Chl a (µg/l)=(r/(r-1)) x (R_b-R_a) x Vex/V_{fil}

Formula for pheophytin:
Pheo (µg/l)=(r/(r-1)) x (rR_b-R_a) x V_{ex}/V_{fil}

(Note the method gives only an approximation of pheophytin)

R_b = Fluorescence before acidification minus (-) avg filter blank fluor for range used
R_a = Fluorescence after acidification (-) avg filter blank fluor for range
Range = low/med/high measuring range on fluorometer
Vol $_{fil}$ = volume filtered (L)
Vol $_{ex}$ = volume used for extraction (L)
r = calibration factor determined from calibration with pure chlorophyll a where r = mean of R_b/R_a values for stds (r = 2.446 for current calibration of machine)

XIII. Appendix 2--Stock solution of growth media for laboratory algal growth experiment

Prepare stock solutions #1-#9 and WC growth medium in sterile acid washed glassware prepared by washing 1 time with 0.1 N HCl followed by rinsing 3 times with DI water.

Stock solution #1-#9 preparation

Stock solution #1: Add 1-L of DI water to 36.8g of $CaCl_2$

Stock solution #2: Add 37g of $MgSO_4$ to 1-L of DI water

Stock solution #3: Add 12.6-g of $NaHCO_3$ to 1-L of DI water

Stock solution #4: Add 11.4-g of K_2HPO_4 to 1-L of DI water

Stock solution #5: Add 85-g of $NaNO_3$ to 1-L of DI water

Stock solution #6: Add 4.36-g of Na_2EDTA to 1-L of DI water

Stock solution #7: Add 0.1-g of HCl Thiamine, 0.0005-g of Biotin, and 0.0005-g of Vitamin B_{12} to 1-L of DI water

Once all stock solutions have been made, autoclave them for 20 minutes (120°C) and store in refrigerator set at 4°C.

WC Growth Medium preparation

Add buffer solution of 0.23-g of TES; No. 53 to the 4-Liter Erlenmeyer flask, labeled WC Growth Medium. Make sure to rinse residual TES from weigh boat and into the 4-L Erlenmeyer flask. Next, add 2-mL of stock solutions #1-5 to the 4-L Erlenmeyer flask, each measured using its own acid washed 2-mL glass pipette. Once the five stock solutions are added, cover the top of the 4-L flask with aluminum foil and place into an autoclave for 40minutes at 120°C .Once autoclaved, add stock solution #7 to the 4-L flask. Finally, add enough DI water to the 4-L Erlenmeyer flask so that there is an exact 2-L of the final product of WC Growth Medium. Once finished, the WC Growth Medium is stored in a refrigerator set at 4°C.

High nutrient clam excretion material preparation

To obtain high nutrient clam excretion material, filter 750-mL of lake water though Whatman GF/C filter and place into nutrient washed 1-L container. With the use of brushes, 15-individual clams are scrubbed to remove organics. The 15-clams are then added to 750-mL of filtered Lake Tahoe water and left to sit for 12-hours. After the 12^{th} hour, the clams are removed and the 750-mL of clam excretion material is autoclaved for 15-minutes.

Filamentous algae stock solution preparation

Sterilize 32 Whatman GF/F filters by autoclaving in 100-mL beaker with enough 0.1N HCl to cover filters. Once autoclaved, rinse filters with DI water. The GF/F filters will serve to weigh out our 2-g of stock Lake Tahoe filamentous algae into each 250-mL beaker.

To prepare stock, collect filamentous algae from the lake and place in clean 1000-mL beaker. Using a hand blender, blend the stock filamentous algae until homogenized. Pipette the homogenized stock filamentous algae into a filtering apparatus and filter using a GF/F filter so that there is at least 64-g of wet weight of algae collected on filter. Place a sterilized GF/F filter on an electronic balance and tare. With sterile forceps, place 2-g wet weight of stock Tahoe filamentous algae which was collected on GF/F filter in previous step. Place the 2-g of filamentous algae in a sterile 250-mL flask by rinsing filter with 5-mL of DI water. Once filamentous algae stock is prepared, use immediately.