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# An Integrated Pest Management Tactic for Quagga Mussels: Site-Specific Application of Fish Biological Control Agents

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**Running title**

Site-specific biological control of quagga mussels using Centrarchid sunfishes

Abstract

The quagga mussel, *Dreissena bugensis*, is a harmful aquatic pest that invaded the Southwestern United States in 2007. Challenges with managing this pest have been encountered because the invaded systems are primarily open water sources used for human consumption and/or are connected to freshwater habitats containing threatened and endangered species. Existing chemical and physical control methods are undesirable, with use of some methods restricted or prohibited, because they pose risks to humans and ecosystems more broadly. To address this problem, we investigated the efficacy of using resident fishes as biocontrol agents for managing different life stages of quagga mussels on different spatial scales in a site-specific manner. We conducted field experiments to test whether planktivorous Bluegill, *Lepomis macrochirus*, reduced mussel infestations on substrates of varying orientations in small and large pens through predation on larval mussels. We also conducted an experiment to evaluate whether the carnivorous Redear Sunfish, *Lepomis microlophus*, reduced mussel infestations established on substrates of varying orientations in small pens through predation on juvenile and adult mussels. Bluegills significantly reduced mussel infestations on all substrates in the pens through predation on larvae and small, juvenile mussels. Redear Sunfishes reduced existing juvenile and adult mussel populations in some cases, with consumption varying among individuals and substrate orientation. Our results indicate that fishes, specifically Bluegill, may represent effective site-specific biocontrol agents for quagga mussels, reducing impacts on targeted infrastructure (e.g., water towers, docks, pipes) and habitats having different surface orientations by controlling more than one life stage of the pest. Development of an integrated pest management strategy, that considers application of this tactic in combination with others, would undoubtedly improve management of quagga mussels, and potentially congeneric zebra mussels, within lake and reservoir ecosystems.

## Introduction

The quagga mussel, *Dreissena bugensis*, and its congener the zebra mussel, *D. polymorpha*, are two of the most devastating aquatic pests in the United States (U.S.) (U.S. Congress 1993; Glassner-Shwayder 2000; Western Regional Panel 2010). Native to Eurasia, these small (< 50 mm) freshwater mussels cause significant economic and ecological impacts (reviewed in Nalepa and Schloesser 1993; Mackie and Claudi 2010; Van der Velde et al. 2010; Nalepa 2010). They attach to hard surfaces, often obstructing water delivery systems such as intakes, dams, and irrigation pipes, substantially increasing infrastructure maintenance costs. They also filter large quantities of water while feeding on microscopic plants and animals which has been linked to declines in freshwater fisheries and other environmental impacts. Despite vigorous public education and boat interdiction programs (Mangin 2001; Western Regional Panel 2010), distributions of quagga and zebra mussels expanded from the East and Midwest to the Western U.S. where they now infest a diversity of waterbodies (rivers, ponds, lakes and reservoirs), including the Colorado River system and its associated aqueduct. Abundant populations of quagga mussels exist throughout the Southwestern U.S., particularly in California, Nevada, and Arizona, whereas zebra mussel populations are scattered only in a few locations in Colorado, Utah and California (U.S. Geological Survey 2019). The cost to manage these dreissenid mussel populations is estimated at millions of dollars annually, with total costs surpassing billions of dollars since their introduction to the Southwestern U.S. (De Leon 2008) and U.S. nationwide (Pimentel et al. 2005;).

Several tactics for controlling dreissenid mussels have been long used in Europe and much of the U.S. Primarily designed for large water conveyance facilities and power plants, control is accomplished through a combination of chemical applications to the infested water within a defined system, toxic coatings to infrastructure, and mechanical removal of existing mussels through the use of large machines or by divers using hand-held tools with or without suction pumps (Mackie and Claudi 2010; Van der Velde et al. 2010). However, mechanical and chemical control strategies in many Southwestern

waterbodies that serve as drinking water sources are problematic because human contact is limited and treatment with pesticides or other biocides is restricted (e.g., California Code of Regulations, Title 17, 7626; Clean Water Act, Section 301(a)). Chemical treatments also have been prohibited in aquatic systems that are not sources of drinking water until further research has been conducted (United Water Conservation District 2017) to enable the evaluation of potential impacts to endangered and threatened species, *e.g*., Lake Piru, California, where the oceangoing Rainbow Trout (steelhead) *Oncorhynchus mykiss*, inhabit downstream areas.

Scientists and managers identified a critical need for development and implementation of additional control tactics for Southwestern U.S. lake and reservoir systems, including the use of biological control (Southern Nevada Water Authority and Metropolitan Water District of Southern California 2008; Western Regional Panel 2010). In addition, managers of waterbodies in California expressed to us a specific interest in using predatory resident fishes as biological control agents, as many had witnessed or heard about fishes consuming quagga and/or zebra mussels. Indeed, many European and North American fishes have been reported to prey upon larval (i.e., veliger), juvenile and adult dreissenid mussels (see review Molloy et al. 1997) in the laboratory and infested waterbodies around the world. Notably, these mussels were found to be a prey item for several freshwater fishes commonly found in the U.S., including Bluegill (*Lepomis macrochirus)* and Redear Sunfish (*Lepomis microlophus)*. In fact, these sunfishes contributed to the reduction of zebra mussel biomass on experimental substrates placed on a river bottom (Magoulick and Lewis 2002; Bartsch et al. 2005). Despite evidence that fishes consume these invasive mussels, dense mussel populations continue to occur in waterbodies containing the predatory fishes, suggesting augmentation or manipulation of predatory fishes may be required for effective mussel control.

We evaluated the efficacy of fish predators as site-specific biological control agents for quagga mussels in open water systems. Our principal objectives were to 1) determine the potential for planktivorous Bluegill to minimize mussel infestations on substrates of varying orientations through predation on larval quagga mussels, and 2) evaluate whether the carnivorous Redear Sunfish are capable of reducing mussel infestations on substrates of varying orientations through predation on juvenile and adult quagga mussels. Rather than employing traditional tactics of increasing the number of biological control agents in a system to reduce the pest population *system-wide*, we evaluated the potential of two biological control agents for *site-specific* pest control. The targeted application of effective biological control agents would presumably reduce impacts to specific structures and habitats, such as water towers, docks, and rock habitats that represent preferred mussel settlement sites. Furthermore, by targeting the preferred mussel habitats that may serve as the primary larval sources, the targeted application could potentially reduce the overall mussel population of the waterbody.

We further considered and discussed our findings as a component of an integrated pest management (IPM) strategy for quagga mussel infestations within open waterbodies (not in water delivery facilities/power plants). Application of the IPM framework provides an opportunity to improve pest management as it entails combining control tactics that target all life stages, an approach that generally is not considered in the management of aquatic invasive species. The lack of systematic control of quagga mussel populations in the Southwestern U.S., the likelihood of continued mussel transport, and the current and anticipated long term impacts posed by this aquatic invasive species (AIS) supports the need to develop an IPM program.

[A]Methods

We evaluated the efficacy of two potential biological control agents: Bluegill and Redear Sunfish. Both species of sunfish are known to consume dreissenid mussels (Molloy et al. 1997) and they occur in many Southwestern U.S. waterbodies (Moyle 1976). These non-

native species were intentionally introduced into these waterbodies in the early- to mid-1900s, and are now abundant and support valuable recreational fisheries (Dill and Cordone 1997) in a region with few natural lakes and associated native species.

Our field experiments were conducted at one of two locations, depending on the species being evaluated. Experiments evaluating Bluegill were conducted within the western arm of El Capitan Reservoir, San Diego County, California, U.S.A (32° 53' 0.2034" N, 116° 48' 23.904" W). The Redear Sunfish experiment was conducted on the eastern side of Lake Havasu, Arizona, U.S.A. (34° 26' 35.1780" N, 114° 18' 59.5512" W). Both reservoirs are warm monomictic, mesotrophic lakes where quagga mussels have persisted at high infestation levels since they were first detected in January 2007 at Lake Havasu and January 2008 at El Capitan Reservoir.

[C]*Phase I: Small Pen Experiments.*— For both sunfishes, we first evaluated their ability to reduce mussel infestations within small experimental pens deployed in the field. Our null hypothesis was mussel infestations would not be affected by the presence of the biological control agent on a small spatial scale. Specifically, mussel recruitment to experimental substrates would not be affected by planktivorous Bluegill, and existing juvenile and adult mussels on experimental substrates would not be affected by carnivorous Redear Sunfish. Each pen consisted of a 1 $m^3$ PVC pipe frame covered by 12 mm vexar plastic mesh (Fig. 1A). Inside of the pen was a vertical PVC bar that supported four thin (3 mm), flat plastic experimental substrates (413 $cm^2$) made from grey PVC. Experimental substrates were attached to the arms extending perpendicular to the vertical bar such that two randomly chosen substrates were oriented vertically and the remaining two oriented horizontally (Fig. 1B). Substrate orientations were designed to mimic orientations of reservoir infrastructure, including vertical water towers, horizontal docks and pipelines, and vertical and horizontal benthic habitats. The substrates were sanded thereby producing fine scratches (grooves) that increase surface area for mussel byssal thread attachment, and thus strength of adhesion by the

mussels. Sanding also resulted in a more rugose texture preferred by settling quagga mussels and characteristic of reservoir infrastructure. Substrates were free of mussels at the start of the Bluegill experiment, thereby providing a surface for mussels to recruit if they were not consumed as veligers (i.e., larvae) by the planktivorous Bluegill. For the Redear Sunfish experiment, half of the substrates were free of mussels (i.e., blank substrates) and the other half of the substrates contained artificially seeded juvenile and adult quagga mussels (i.e., mussel substrates). This design enabled us to evaluate the ability of Redear Sunfish to consume newly settled mussels on the blank substrates and preexisting juvenile and adult quagga mussels on the mussel substrates. To seed the substrates, we placed unattached mussels uniformly over a substrate, covering it with a mesh screen and then placing them in water allowing mussels to attach naturally to the substrates via their byssal threads. The screen material was removed after one week at which time mussels had firmly attached.

We used a paired design with two test groups: 1) absence of fish (control) and 2) presence of fish (treatment) (Fig. 1C). There were 10 pens (5 replicate pairs) and 12 pens (6 replicate pairs) in the Bluegill and Redear Sunfish experiments, respectively. One pen in each pair received no fish and represented a paired control for each treatment. Fish for the treatment pens were collected using standard electrofishing methods (Reynolds 1983) within the waterbody where the experiment was being conducted and were randomly allocated to the fish (treatment) pens soon after being collected. For the Bluegill experiment, two pens were stocked with 35 fish and three pens were stocked with 20 fish. Bluegill density varied due to restrictions on the number of fish that we were able to collect. Also, because effective stocking densities were unknown, using variable densities provided insight into the potential role fish density might play in the effectiveness of the biological control agent without impacting our experimental design (effect of fish presence/absence, not fish densities, on mussel infestations). Collected fish ranged in size from 6.1 to 10.0 cm total length (TL), with a mean TL of 8.3 cm (SE, 2.0). We used Bluegill that were approximately 8 cm TL because they selectively feed on

plankton at that size (Mittelbach 1984; Werner and Hall 1988) and thus are likely to feed on planktonic mussel larvae. For the Redear Sunfish experiment, two adult sunfish ranging in size and weight from 25.2 to 28.5 cm and 312 to 440 g, with a mean TL and weight of 27.2 cm (SE, 0.35) and 370.5 g (SE, 12.7), were randomly put into each of the treatment pens. We used this size of Redear Sunfish because they can feed on juvenile and adult mussels, mussel life stages that attach to lake infrastructure. Low Redear Sunfish stocking densities were used because, unlike the planktivorous Bluegill that continually received food (plankton) naturally, the carnivorous Redear Sunfish were limited to the mussels that we seeded onto the substrates.

Our deployment schedules and operations differed for each experiment. We deployed the pens at a time and water depth that coincided with expected mussel recruitment for each waterbody (D. Daft and K. Carp pers. observation; Culver et al. 2015), and the optimum feeding strategy (pelagic or benthic) of the candidate control agent. The Bluegill pens were deployed in mid-December 2012, suspended from a surface longline to a water depth of 6 m (Fig. 1C). Redear Sunfish pens were deployed in mid-February approximately 50 cm above the benthos at a water depth of approximately 4.5 m. Legs attached to each of the paired pens in the Redear Sunfish experiment kept the floor of the pen above the bottom of the lake, preventing the stocked Redear Sunfish from feeding directly on the benthos. Prior to deployment, the seeded mussel substrates in the Redear Sunfish pens were photographed to allow later assessment of percent cover of mussels on each substrate at the start of the experiment.

Our pen retrieval activities also varied with each experiment. We left the Bluegill pens undisturbed until mussel settlement was detected. Mussel settlement was determined through weekly observations of two experimental substrates that were deployed from the surface longline at the same depth as the experimental pens. Following confirmation of mussel settlement in mid-April 2013 (18 weeks after deployment), each pair of pens was brought to the surface, the substrate bars were removed, both sides of

each horizontal and vertical substrate were photographed, and the experiment was terminated. For the Redear Sunfish experiment, we retrieved pens monthly to evaluate the health of the fish and the need for additional food (mussels). If few seeded mussels remained on the experimental substrate, the substrate was exchanged with a new substrate containing seeded mussels (described previously). We took photographs of both sides of each horizontal and vertical substrate to evaluate the feeding activities of the fish on the retrieved panels and to assess the starting density of mussels on the replacement substrates. We terminated the Redear Sunfish experiment in early summer (July 2013), 21 weeks after deployment, when water temperatures at the experimental depth were approaching sub-optimal levels for the fish.

To determine percent cover of mussels and other organisms on the substrates, we analyzed the photographs using Coral Point Count with Excel extensions software (CPCe v3.6) (Kohler and Gill 2006). One hundred randomly generated points were superimposed on each photograph. For the Bluegill experiments, we determined the percent cover of large mussels (> 3mm shell length (SL)), algae, other fouling organisms, silt and bare space by assigning the object under each point to the appropriate category. In cases where a point overlaid multiple objects falling into more than one of our categories, the category of the object most covered by the point was recorded. To estimate the number of new mussel recruits (≤ 3 mm SL), we used a dissecting microscope and enumerated subsamples of mussels from four control and four treatment substrates. This method provided more accurate estimates of newly recruited mussels than the CPCe software which often missed these mussels because they were too small to accurately resolve in the photographs and they were difficult to distinguish when they settled on top of larger mussels. Similar procedures were used to determine percent cover in the Redear Sunfish experiment, except we used an additional category for new recruits (small mussels). Unlike the Bluegill experiment, new recruits were readily identifiable in this experiment because the substrates were analyzed monthly as opposed to after many months of exposure and recruitment was extremely limited.

Upon termination of the experiment, we also collected, enumerated and measured (TL) the fish from each treatment pen. A subsample of Bluegill (n = 10) taken from each of the five replicate pens and all Redear Sunfish (n = 11) were euthanized by immersion in the liquid anesthetic MS-222 at high doses (>250mg/l). We then removed mussels and mussel shell fragments from the stomach and intestines of these individual fishes. We weighed recovered mussel material to the nearest 0.0005 g for the Bluegill experiment, with presence and absence of material recorded for the Redear Sunfish experiment.

[C]*Phase II: Large Pen Experiment.*— Based on the results of our Phase I experiments, we conducted an additional experiment to assess the effect of promising control agents on mussel recruitment at a larger spatial scale; the null hypothesis being that mussel recruitment would not be as affected by the presence of the biological control agent at larger spatial scales. For this experiment, we used nylon netting to construct 1 x 1 x 6 m collapsible pens (Fig. 2). The 6 m length of the pen was based on the depth range of the water column where mussels were frequently observed to occur in the El Capitan Reservoir system (water depths of 3 to 9 m), and thus where the removal of mussel infestations on structures such as water towers and other lake infrastructure is most critical. Substrate bars like those in the other experiments were interspersed at 2 m intervals within the pen to enable assessment of mussel recruitment near the top, middle and bottom of the large pens. Our three-substrate location design enabled us to assess mussel recruitment throughout the large pens, thereby enabling evaluation of whether fish effectively consumed mussels throughout the water column within a pen. Four replicate control pens (fish absent) and four replicate treatment pens (fish present) were constructed and attached to a surface longline using 3 m mooring lines. This configuration placed each pen at depths ranging from 3 to 9 m below the reservoir surface (Fig. 2). Control and treatment pens were alternated every 8 m to minimize the potential effect of adjacent pens. The bottom of each pen was above the observed thermocline and hypolimnion, below which mussels cannot survive (Culver et al. 2015).

Two experimental substrates were attached to the longline at a similar depth as the experimental pens to monitor mussel settlement weekly without disturbing the pens.

Bluegills were stocked in the experimental treatment pens when the pens were deployed. Two of the treatment pens were stocked with 50 fish, one pen with 40 fish and one pen with 30 fish. As with the small pen Bluegill experiment, fish density varied due to collection restrictions. Also, it enabled us to explore the potential role of fish density on the effectiveness of the biological control agent without impacting our experimental design. We again targeted small, planktivorous sunfish ranging in size from 6.5 to 10.6 cm TL, with an average TL of 8.9 cm (SE, 0.16). The pens were stocked and deployed in late April 2013 and retrieved in early November 2013 (27 weeks after deployment) when mussel recruitment was detected on the two monitoring substrates. Experimental substrates and fish were processed as described previously.

[C]*Data analyses*.— All statistical analyses were performed using SAS Software procedures (SAS v. 9.4, SAS Institute Inc. 2012). A cut-off value of $\alpha <= 0.05$ was used to determine P-values indicating statistically significant results.

*Phase I small pen Bluegill experiment*. We evaluated whether percent cover of quagga mussels recruiting to the experimental substrates in the small pens was significantly different between pairs of control (no fish) and treatment (fish added) pens using SAS PROC TTEST®. To avoid pseudo-replication, values for percent cover were averaged across all substrate orientations within a pen, resulting in a single value of mussel percent cover for each pen (5 each for controls and treatment). A two-tailed t-test was then used to test the null hypothesis that the difference in mussel recruitment between treatments was zero. We arcsin, square root transformed the data prior to analysis to more adequately meet assumptions of normality and homogeneity of variances (Sokal and Rohlf 1994).

We also used a paired t-test to further evaluate the effect of fish on mussel recruitment for each substrate orientation; bottomside, topside, vertical side. Our null hypothesis was that the difference in percent cover of mussels in pens with fish and without fish was equal to zero for each substrate orientation. Data from both sides of the vertical substrates were combined as the sides were not consistently oriented in a specified direction (right or left) and a similar foraging behavior provided access to either vertical side. Values for mussel recruitment onto the topside and underside of horizontal substrates were not pooled because the two surface orientations could be readily distinguished from one another and different foraging behavior was required for fish to access mussels on each side. Percent cover data were arcsin, square root transformed prior to analysis to more adequately meet assumptions of normality and homogeneity of variances (Sokal and Rohlf 1994)

*Phase I small pen Redear experiment*. We again used SAS PROC TTEST® to test for the fixed effect of substrate orientation (topside, underside, vertical side) on the change in the percent cover of existing quagga mussels deployed on our experimental substrates between pairs of control (fish absent) and treatment (fish present) pens. First, we calculated the change in percent cover for each seeded experimental substrate by subtracting the percent cover of mussels on a given substrate at the end of each month from the percent cover of mussels on that same substrate at the beginning of each month. Data from both sides of the vertical substrates again were combined. To avoid pseudo-replication, values for the percent change in existing mussel cover were then averaged for each experimental substrate orientation within a given pen over the duration (five months) of the experiment. This averaging process resulted in a single measure for the three substrate orientations (topside, underside and vertical side) within each of the 12 pens.

Finally, our paired design allowed us to calculate the average monthly change in quagga mussel percent cover for each substrate orientation between each pair of pens. We did

this by subtracting the value observed for each orientation in the treatment (Redear Sunfish present) pen from the value observed for each orientation in the corresponding paired control (Redear Sunfish absent) pen. A t-test was then used to compare the resulting six differences in the average monthly change in quagga mussel percent cover for each substrate orientation to a value of zero representing the null hypothesis of no difference in the average monthly change in quagga mussel percent cover between pens with and without fish. Differences in the average monthly change in quagga mussel percent cover were arcsin, square root transformed prior to analysis to more adequately meet assumptions of normality and homogeneity of variances (Sokal and Rohlf 1994).

*Phase II large pen Bluegill experiment.* As done for the Phase I Bluegill experiment, we began by using a two-tailed t-test (SAS PROC TTEST®) to evaluate whether to reject our null hypothesis : there was no difference in the percent cover of quagga mussels recruiting to the experimental substrates between control (fish absent) and treatment (fish present) large pens. The data were averaged and transformed and the statistics reported as previously described. If cases where variances were unequal following the transformation, we report the Cochran t-statistic (Sokal and Rohlf 1994). Prior to analysis, data from the substrates located at the bottom of one of the pens were removed from the data set because the lower 2 m of the pen became twisted during the experiment, blocking access by the fish to the bottom substrates in that pen. We noted substantially more mussels and algae occurred on these substrates than on any of the substrates deployed at the same depth in the other replicate pens.

We used SAS PROC GLM® to run a 3-factor ANOVA to examine the effects of substrate orientation (topside, underside, vertical side), substrate location (top, middle, bottom of the pen), and the presence or absence of Bluegill on mussel recruitment. Because there were significant interactions among the three variables, we analyzed the control and treatment pens separately. Evaluating the distribution of mussels among substrate

orientations and locations within the control pens enabled us to identify natural mussel recruitment patterns. Our evaluation of the same factors in the treatment pens allowed us to understand whether the fish effectively prevented the settlement and recruitment of mussels and foraged throughout the water column within the large pens. We used SAS PROC GLM® to conduct a 2-factor ANOVA to test for each of these assessments. If significant interactions occurred, we used a one-way ANOVA to evaluate for significant differences in mussel recruitment among substrate orientations at each location within the pen, and among substrate locations for each substrate orientation. The Ryan-Einot-Gabriel-Welsch Q (REGWQ) test was used for post-hoc comparisons in cases where significant treatment effects were found. Data were grouped and averaged by location, substrate orientation and pen, and arc-sin, square-root transformed prior to analysis (Sokal and Rohlf 1994)

[A]Results

[B]Phase I: Small Pen Experiments - Bluegill

Bluegill survivorship was nearly 100%, with only a single mortality from one of the five experimental treatment pens. At the end of the experiment, fish ranged in size from 6.4 to 11.9 cm TL, with a mean TL of 9.1 cm (SE, 0.1). Three Bluegills recruited into two of the control pens. One largemouth bass (*Micropterus salmoides*), measuring 10.4 cm TL, also recruited to one of the treatment pens.

Mussels were virtually the only organism recruiting to the experimental substrates within the small pens. Algae averaged less than 4.5% cover in all cases. Silt and/or detritus averaged less than 2% in control pens, whereas it ranged from 4% to 30% in pens with fish and was the highest on the topsides of the experimental substrates.

Mussels recruited to experimental substrates within all the pens. The percent cover of mussels was significantly reduced by fish ($t_4 = 14.13$, $P < 0.0001$; Fig. 3). In pens containing Bluegill, we found a significant 7-fold reduction in mussel infestations for the

underside of substrates ($t_4$ = 9.94, $P$ < 0.001) and a significant 4-fold reduction in mussel percent cover for the vertical substrates ($t_4$ = 5.33, $P$ = 0.006). The percent cover of quagga mussels on the topsides of substrates, however, were not significantly different in either pens with and without fish ($t_4$ = -0.43, $P$ = 0.691) (Fig. 3). Overall, the underside of substrates represented the substrates with the highest mussel recruitment, and substantially lower recruitment was observed on vertical substrates. Additionally, recruited to the topside of substrates was extremely limited regardless of whether fish were present or not.

We observed a negative relationship between Bluegill fish density and percent cover of mussels on the underside of the substrates in some pens. In pens with a higher initial number of fish (35 fish) we observed an average mussel percent cover of 7.2% (SE, 1.45) as compared to 15.6% (SE, 3.01) for two of the three pens with initial fish stocking density of 20 fish per pen. In the third pen with a low initial stocking density of 20 fish per pen we observed the lowest cover of mussels on experimental substrates; mean percent cover was 2.7% (SE, 1.6). This pen contained the largest Bluegills at the end of the experiment, and fish size was a good predictor of quagga mussel percent cover ($r^2$ = 0.66) (Fig. 4).

Of the subsample of fish that were dissected (n=61), 19.7% had mussel shells and shell fragments in their stomach and/or intestines, albeit minuscule amounts (< 0.002 g). Fish that contained shell material ranged in size from 8.3 to 12.0 cm TL, with all but one fish larger than 11.0 cm containing mussel shell fragments. A single whole mussel shell was found in each of seven fish. The recovered shells ranged in size from 1.4 to 2.5 mm SL, and a mean SL of 1.8 mm (SE, 0.32). No veligers were detected during dissections.

On experimental substrates in the control pens, we observed natural recruitment of many small (< 3 mm SL) mussels. The underside of the substrates had the highest natural recruitment with 1012 mussel recruits (SE, 156), followed by substantially less

recruitment to the vertical and topsides of the substrates with 172 (SE, 33) and 152 (SE, 31) mussels, respectively.

[B]Phase I: Small Pen Experiments - Redear Sunfish

Redear Sunfish survivorship was nearly 100%, with only a single mortality observed during the 21 week experiment. Total biomass of Redear Sunfish in the pens decreased over the course of the experiment; average biomass ranged from 350 - 413 g at the beginning and 251 - 300 g at the end. Both pen types (control and treatment) successfully enclosed Redear Sunfish and excluded other fishes throughout the experiment.

Changes in the percent cover of mussels was nearly significant for the topsides of the substrates, with a decrease in mussels when fish were present versus virtually no change when fish were absent ($t_5$= 2.10, $P$ = 0.09; Fig. 5). There was a high degree of variation among treatment pens, with the average change in percent cover of mussels ranging from an increase of 2.3 % to a decrease of 28.7 %. There was no significant effect of Redear Sunfish on percent cover of mussels on the vertical sides of substrates ($t_5$= 0.38, $P$ = 0.72) or the underside of substrates ($t_5$= 1.28, $P$ = 0.26; Fig. 5). All Redear Sunfishes had mussel shell material in their stomach and/or intestines, but the amount of shell material varied from a few shell fragments in only the stomach to many shell fragments and a few whole shells in both the stomach and intestine. We did not observe a relationship between fish size and the amount of shell material recovered from the fish.

During the last trial of our Redear Sunfish experiment, we observed a mean percent cover of 1.8% (SE, 0.2) of small mussel recruits on the underside of substrates that did not contain transplanted mussels in three sets of the six replicate paired pens, with mussels occurring in both control and treatment pens. While we observed one or two

mussel recruits on our gear in one earlier trial, their presence was not detected through CPCe analysis on any of our substrates during the other trials.

[B]Phase II: Large Pen Experiment - Bluegill

Mean survivorship of Bluegill was 77.8 % (SE, 4.0) among the four replicate treatment pens. At the end of the experiment, Bluegill mean TL was 12.1 cm (SE, 0.15), and ranged from 6.5 - 17.5 cm. Twenty Bluegills recruited into the four control pens (3 - 6 per pen), as well as2 largemouth bass (1 in each of 2 control pens).

The diversity of organisms that recruited to the substrates was limited. Mussels were the most common organism that recruited onto experimental substrates. Algae also were observed, with a mean percent cover of 24.1 % (SE, 3.0) overall, 24.2 % (SE, 4.2) in control pens and 24.0 % (SE, 4.4) in pens containing Bluegill. Algae were particularly present on experimental substrates located near the top and middle of the pens. Silt and/or detritus also was present: 16.6 % (SE, 2.3) overall, 15.2 % (SE, 2.8) in control pens and 18.1 % (SE, 3.8) in pens with fish.

Mussels recruited to experimental substrates within all pens. Fish dramatically and significantly reduced percent cover of mussels (Cochran: $t_3 = 10.36$, $P = 0.002$; Figs. 6, 7) to extremely low levels. Such did not occur in pens without fish. A significant interaction among the three variables (treatment × substrate location × substrate orientation: $F_{4,124} = 3.23$, $P = 0.015$) precluded the comparison of the main effect of pen type, thus, the influence of these variables was examined for the control and treatment pens separately.

Among the control pens, natural recruitment of mussels varied for substrate orientation and substrate locations (Fig. 7A). The significant interaction between these two variables ($F_{4,27} = 2.93$, $P = 0.04$) required us to compare percent cover of mussels among the three substrate orientations for each of the three locations separately, and among

substrate locations for each of the three substrate orientations separately. Recruitment of mussels significantly differed among substrate orientations for each of the three substrate locations (top, $F_{2,9}$ = 15.15, $P$ = 0.001; middle, $F_{2,9}$ = 7.88, $P$ = 0.011, bottom, $F_{2,9}$ = 31.06, $P$ = < 0.0001); it was highest on the undersides of substrates (Fig. 7A). Mussel recruitment also was significantly different across locations for the underside ($F_{2,9}$ = 5.39, $P$ = 0.029) and topside of substrates ($F_{2,11}$ = 43.69, $P$ < 0.0001), but not for vertical side ($F_{2,9}$ = 0.46, $P$ = 0.645). In general, mussel recruitment decreased on substrates that were shallower inside the pen (Fig. 7A).

In pens containing Bluegill, mussel recruitment was significantly different among substrate locations ($F_{2,30}$ = 5.49, $P$ = 0.009) (Fig. 7B), but not substrate orientations ($F_{2,30}$ = 1.58, $P$ = 0.223) (Fig. 7B). Although mussel recruitment was low throughout the pens, it was highest on substrates near the bottom of the pen, followed by the middle and top. For all locations, mussel recruitment was low and similar among the three substrate orientations (Fig. 7B).

Percent cover of mussels on the underside of the experimental substrates was lower in pens with intermediate and high densities of fish; the undersides of substrates had the highest mussel recruitment. The percent cover of mussels was extremely low in the two pens with the highest fish densities (n = 40 and 41 fish pen$^{-1}$) at the end of the experiment; 0.73 % (SE, 0.41) and 0.72 % (SE, 0.36), respectively. The pen with the lowest fish density (n=25 fish pen$^{-1}$) had a higher percent cover of mussels, albeit still quite low at 2.2 % (SE, 0.83).

The majority (71%) of dissected Bluegill (n=45) had mussel shell material in their stomach and/or intestines. Shell material was found in fish that averaged 12.4 cm TL (SE, 0.23) and ranged in size from 10.1 - 15.2 cm TL. Additionally, 36% of Bluegill dissected contained one or more whole mussel shell. Shells averaged 4.95 mm (SE, 0.36)

in SL and ranged in size from 2.1 - 7.5 mm SL. No veligers were detected in the digestive tracts of dissected fish.

[A]Discussion

The use of fish predators as site-specific biological control agents for dreissenid mussels is promising. Our study demonstrated the utility of Bluegill for controlling mussel infestations on a variety of infrastructure and habitats where mussels typically settle. Bluegill greatly reduced mussel recruitment on the underside of the substrates, where most mussels recruited, at all locations within the pens suggesting their usefulness for controlling mussels on docks, pipelines, and floating pump barges and restrooms. Bluegill also reduced mussel recruitment on vertically oriented substrates, supporting their utility for controlling mussels on vertical structures, such as water towers, rock drop-offs and sloped habitat. Mussel infestations on the topside of the substrates showed a similar trend of being lower when fish were present, indicating the potential application of Bluegill for controlling mussels on the top of pipelines and benthic habitat. This finding was not as striking, but mussel recruitment was generally quite low for the topside of the substrates. Taken together, application of penned Bluegills as biocontrol agents at specific sites could be an effective way to control mussel infestations on and around infrastructure and habitats within lacustrine and reservoir systems.

Although not quantified, the noticeable reduction in mussel recruitment on the surfaces of the pens with fish has implications for use of this tactic. It suggests that fish readily foraged throughout the pen as we concluded from the analysis of mussel settlement on our small experimental substrates. It also provided proof that Bluegill can reduce mussel settlement over larger surface areas than provided by the substrates, such as would be encountered with lake infrastructure and habitats. The fish's ability to prevent significant mussel recruitment on the surfaces of the pen itself validates that the method of containment – where the pen itself provides substantially more surface area

for settlement – does not interfere with the ability of the fish to reduce mussel percent cover on the desired substrates (e.g., docks, water tower, rock habitat).

The lack of veligers and presence of shell material we observed in the Bluegill digestive systems suggests that the observed reduction in mussels could have been due to the consumption of small, juvenile mussels, and not from consumption of the larvae. We do not believe that this is the case. Other studies (Mittelbach 1981, 1984; Werner and Hall 1988) have found Bluegill to be efficient planktivores, but detecting mussel veligers in fish stomach content can be problematic. Loomis et al. (2011) suggested that immediate examination is critical; something that we did not do. Nonetheless, the majority (80%) of the subsampled fish lacked shell material in their digestive tract at the end of the first experiment. Furthermore, nearly all (93%) of these penned fish were smaller than the size of fish (11 cm) that we found consistently contained shell material in their digestive system. We also observed very small, recently recruited mussels in the pens with fish, which suggests 1) veligers were present in the water column within the pens, and 2) veligers were capable of settling if they avoided predation by the Bluegill. Based on the presence of recent recruits and the dramatic difference (~15 fold) in the percent cover of mussels settling on the experimental substrates in the pens with fish compared to control pens, we conclude that the Bluegill were consuming veligers and veligers may have not been recognizable because of our processing techniques or because they already had been digested. As a few Bluegills recruited into the control pens and presumably consumed some mussels thus lowering the percent cover in those pens, the difference in percent cover of mussels in pens with and without fish is likely underestimated. An assessment of C and N stable isotopes of Bluegill fed dreissenids vs non-dreissenids and differences in length-weight relationships of the Bluegill fed different diets could provide additional understanding of the importance of mussel veligers in their diet.

Our use of varying Bluegill abundances within pens provided useful insight into the effective densities and sizes of fish necessary for controlling mussels. Based on our initial Bluegill experiment, densities of 20 to 35 small (6 to 11 cm TL) fish per $m^3$ were adequate for greatly reducing mussel recruitment. Results from our second Bluegill experiment suggested that if a larger size range of fish (6 to 16 cm TL) is used, stocking densities can be much lower (2 fish per $m^3$) and still be effective. Although fish of similar sizes were stocked in all pens at the start of each experiment, fish stocked in the pens at lower densities typically grew larger during the second experiment. This was presumably due to density-dependent effects on growth, i.e., fish reach larger sizes at lower densities (Osenberg et al. 1988; Bowen et al. 1991; Anderson et al. 2002). This variation in fish size was not seen in the first experiment, but that experiment occurred during the winter and early spring when Bluegill growth is limited (Wohlschlag and Juliano 1959). Notably, dissections revealed that all of the larger Bluegill (> 11 cm), and a few slightly smaller Bluegill (8 - 10 cm TL), consumed attached juvenile mussels. Thus, the consumption of both larval and juvenile mussels may be facilitated by an assemblage of small and medium sized Bluegill at a lower density (4 fish $m^{-3}$). However, additional studies that explicitly evaluate density- and size-dependent relationships between fish and reduction of quagga mussel infestations are needed to determine optimal fish stocking parameters. These further studies are warranted due to the unknown impact of fish that may have recruited into the treatment pens during the experiment. Nonetheless, the occurrence of additional fish that recruited into the pens does not impact the overall conclusion of this research, *i.e.* that mussel infestations were significantly reduced in the presence of Bluegill.

High survivorship and growth of penned Bluegills indicates that the fish were able to feed and obtain adequate nutrition over the duration of each experiment (4.5 and 7 months for the small and large pen experiments, respectively). Plankton of all types was available within the pens, including quagga mussel veligers as evident from their high settlement on the experimental substrates in the control pens. Although we did not

654 quantify the percent contribution of quagga mussels to the fish's diet, the occurrence of
655 crushed mussel shells in the digestive tracts of the fish suggest that they may have
656 received some nutritional benefit from the consumption of quagga mussels. The
657 Bluegill's ability to consume both larval and juvenile mussels suggests it alone can be
658 used to target and reduce two of the mussels' three life stages. This increases its
659 desirability as a biological control agent for quagga mussels.
660
661 The Redear Sunfish is far less encouraging as a biological control agent for mussels. Our
662 results are counter to those of Hatcher and McClelland (2015) who reported favorable
663 results based on field observations of a reduced mussel infestation in an area containing
664 Redear Sunfish. However, along with Karp and Thomas (2014), we found high variability
665 in consumption of mussels among Redear Sunfish within replicate pens, with minimal
666 reduction in mussel densities. In fact, Karp and Thomas (2014) reported no change in
667 mussel density in half of their experimental pens stocked with Redear Sunfish. In our
668 study, Redear Sunfish weighed less at the end of the experiment then the start,
669 signifying that at least some fish were not meeting 100% of their nutritional needs
670 through the consumption of mussels alone. We also noted that during laboratory
671 studies where mussels were the only prey available, some Redear Sunfish never fed
672 (Karp and Thomas 2014; Culver et al. unpublished data). The high variability in mussel
673 consumption among Redear Sunfish suggests that individuals of this species would need
674 to be prescreened to assess whether they would readily consume mussels and
675 potentially represent an adequate biological control agent. This would be a very time
676 consuming task. We also observed that Redear Sunfish tended to consume mussels
677 primarily on the top surface of our experimental substrates. This would limit the utility
678 of Redear Sunfish to controlling infestations found on benthic habitats or the top of
679 pipelines or other structures. The lack of mussel recruitment during our study indicates
680 that it is unlikely that we underestimated consumption of new mussel recruits, and thus
681 overall consumption, in the cages containing fish. Given the 1) small (10%) average
682 reduction in mussel infestations when Redear Sunfish were isolated from virtually all

683 other prey, 2) reduction in the weight of Redear Sunfish despite mussel prey being
684 available, 3) limitation in the application of penned Redear Sunfish to horizontal benthic
685 habitat/structures that will undoubtedly include their preferred prey (clams and snails in
686 benthos) (Moyle 1976; French and Morgan 1995) and 4) the need to prescreen Redear
687 Sunfish to select individuals that will consume mussels, the usefulness of Redear Sunfish
688 for biological control of mussels is likely minimal at best.
689
690 [B]Fish Predators as an IPM Tactic for Mussels
691 Our results support the use of Bluegill as a site-specific, predatory biological control
692 agent to be included in an IPM strategy for quagga mussels. This tactic would also likely
693 be useful for controlling zebra mussels, given their similarity to quagga mussels. Site-
694 specific application of control agents offers some advantages over the traditional
695 system-wide application of this tactic. First, encounter rates of the predator and prey
696 can be greatly increased by containing the predator in an area where the pest occurs,
697 regardless of whether it is a common foraging area for the predator. Typically, small
698 Bluegills (< 10 cm) forage in dense vegetation where they are provided refuge from
699 predators (Hall and Werner 1977; Savino and Stein 1982), whereas larger Bluegills
700 forage in open water habitats (Werner et al. 1983). These areas are not preferred
701 habitat for quagga mussels. Thus, penning Bluegill in areas where the mussels occur is
702 required to effectively reduce localized mussel populations. Second, by confining the
703 fish within a pen, their food source is purposefully limited. Fish are placed directly in
704 areas where veligers aggregate and high numbers of mussels settle. Subsequently, the
705 mussels are a dominant prey item for the penned fish. Third, maintaining fish in pens
706 provides protection from predators. A shift in diet from vegetation-dwelling prey (non-
707 gastropod) to plankton has been documented for Bluegill in the absence of largemouth
708 bass (Werner et al. 1983; Mittelbach 1984), a common Bluegill predator. Thus, penned
709 Bluegills that are protected from their predators are likely to forage uninterrupted on
710 mussel larvae and juveniles compared to unconfined individuals.
711

712 Relying on existing resident fish populations within a waterbody, as in this study, can be
713 beneficial as it limits the need to capture, transport and stock fish obtained from an
714 outside source. Bluegill were commonly stocked into lakes and reservoirs in the
715 Southwestern U.S. (Dill and Cordone 1997) and they were present in the system used in
716 this study. However, as this species is not native to California or other areas in the
717 Southwestern U.S., we do not support its introduction to waterbodies where it does not
718 already occur. Furthermore, sufficient numbers and sizes of fish must be available to
719 supply the required stocking densities and predatory diet for controlling mussel
720 infestations.
721
722 Given the high likelihood of success in reducing mussel infestation through site-specific
723 application of Bluegill biological control agents, additional steps may be warranted to
724 implement this tactic in waterbodies where existing Bluegill populations are limited. In
725 such circumstances, one might consider obtaining Bluegill of the appropriate size and
726 number from facilities that propagate the species for stocking. As the fish will be
727 contained within a pen, they can be removed from the lake following peak periods of
728 mussel settlement. Sterile Bluegills also might be considered if there are concerns that
729 the added Bluegill would contribute reproductively to the existing Bluegill population.
730 Sterile Grass Carp (*Ctenopharyngodon idella*) have been used to successfully control
731 aquatic weeds in the U.S. (Leslie et al. 1987; Mitchell and Kelly 2006). Sterile Black Carp
732 (*Mylopharyngodon piceus*) also have been used to control parasite-carrying snails in fish
733 farm ponds in the Southern U.S. (Nico and Jelks 2011). This species is now listed as an
734 injurious species though, highlighting the need to carefully consider and test biological
735 control agents.
736
737 Bluegill are widely distributed throughout many regions of the U.S. Where endemic, this
738 species is an excellent candidate for the biological control of dreissenid mussels. Clearly,
739 the use of a native fish would be a preferred alternative in the Southwestern U.S. to the
740 non-native Bluegill. However, despite concerted efforts we were unable to identify a

native fish that could have served as a potential biological control of quagga mussels in the man-made reservoirs where we were working. The Sacramento perch, *Archoplites interruptus,* has been proposed as a potential biological control agent for mussel infestations in central and northern California where it is native, should quagga mussels become established there. This species is ecologically similar to Bluegill and may have been excluded from areas within its native range via interspecific competition with Bluegills (Moyle et al. 1974). In other parts of the U.S., the Freshwater Drum, *Aplodinotus grunniens*, may be a good biological control agent candidate for dreissenid mussels. It is native to much of North America, including the Great Lakes and Mississippi River Basin where mussels are abundant, and undergoes an ontogentic shift in prey, consuming zooplankton when young and molluscs, including zebra mussels, as an adult (Daiber 1952; Morrison et al. 1997). Identifying other suitable native fish species that could be collected and/or propagated (sterile or not) and penned into appropriate waterbodies for mussel control warrants further investigation and would help to broaden the application of this tactic in other states and countries. Invertebrate species, such as crayfish and crabs, also may prove to be useful biological agents (Molloy et al. 1997; Boles and Lipcius 1997; Goncalves et al. 2016).

[C]*Application*.—Systems to contain the selected fish biological control agents would need to be engineered before this IPM tactic could be successfully implemented on a large scale. In some cases, pens could be incorporated around certain infrastructure (e.g., docks), with fish added to those pens during appropriate times of the year (e.g., when veligers are present and/or settling). We have successfully designed and pilot-tested plastic mesh pens for docks, floating barges and restrooms, and small benthic rock habitat. For larger infrastructure (e.g., water towers) and expansive benthic habitat, large pens would be needed to keep the fish in the desired area around the infrastructure. Systems used for culturing fish and/or in scientific caging experiments may be adapted for this use. As the types of infrastructure that would be targeted are

769 similar among many waterbodies, containment designs could be readily applied across
770 infrastructure types.
771
772 Determining the appropriate size(s) of containment systems for fishes requires an
773 understanding of the scale at which each system would be effective. This, in turn,
774 requires identifying optimum stocking densities that result in the largest reduction in
775 the mussel population possible. While such studies are still needed, our data provide a
776 starting point for evaluating the scale at which penned fish may be able to control
777 mussels in lakes/reservoirs. For example, most docks at Lake Piru, California, are the
778 same size as our pens; 6 m in length and 1 m wide. This suggests that our current net
779 pens stocked with 4 fish would be effective at greatly reducing mussel infestations on
780 individual docks. For large marinas with many docks, it may be difficult to develop and
781 maintain pens on each one. However, this might not be needed if fish stocked at
782 primary locations – such as under the main, long walkways of the marina – can
783 effectively reduce the mussel infestations throughout the area. The effectiveness of this
784 application would depend on the distance outside a pen the fish could reduce mussel
785 settlement through planktivory, an avenue of research that merits more exploration.
786
787 For water towers and large benthic habitats, a few hundred fish may be needed within a
788 pen to control mussel infestations. Based on the most effective stocking density used in
789 our study (4 fish $m^{-3}$) and a surrounding net pen that is 6.67 m in diameter (2 m greater
790 than the diameter of the water tower at El Capitan Reservoir) and 6 m high (based on
791 the segment of the water column where mussels settle; water depths of 3 to 9 m), we
792 estimate 425 fish would be needed to control mussel infestations on the water tower at
793 our study site. This estimate is likely high as we have yet to investigate optimum fish
794 stocking density. As much of the infested infrastructure (e.g., docks, floating restrooms,
795 pipelines), including other water towers, have smaller dimensions than those we've
796 used in our estimates, fewer fish presumably would be needed to scale up this method
797 for use in California, and elsewhere. Additional studies evaluating the extent to which

mussel infestations are reduced outside of the pens and associated optimum stocking densities would enable better assessments for scaling up this tactic.

Unlike other commonly used removal tactics, this approach requires limited human contact with the waterbody, a consideration in some areas. It also could prove to be more cost-effective for reducing mussel infestations in some areas of a waterbody. For example, penning Bluegills under or around mussel-infested infrastructure (water towers, floating restrooms, pump barges, docks) would likely eliminate the need to clean the structures frequently during periods of high mussel recruitment, a costly, labor-intensive tactic. This could be especially useful in systems where mussels reach maturity within a couple of months and reproduce for many months throughout the year, as occurs in the warm, productive reservoirs in the Southwestern U.S. (e.g., Gerstenberger et al. 2011; Culver et al. 2015). In such systems, mussels would need to be physically removed approximately every six to eight weeks for several months to prevent maturation and reproduction. Instead penned fish could be used requiring less manual labor; installation and stocking of fish only once during the mussel reproductive season as compared to numerous physical removal efforts over several months.

Site-specific application of fish as biocontrol agents in locations where mussels are most abundant may not only help maintain low mussel infestations at such sites, but it also may reduce the overall mussel population. These sites likely serve as major sources of larvae that contribute to the overall mussel population in the waterbody (Pulliam 1988; Dias 1996; Dauphinais et al. 2018). For waterbodies that receive mussel-infested water from other sources (e.g., Colorado Aqueduct), tactics should also be applied in areas where larvae initially enter and accumulate. Research on distribution patterns and `sources and sinks' of larvae (e.g., Pulliam 1988; Dias 1996) would be useful for planning larval source reduction strategies and for understanding the potential for reducing the mussel population system-wide.

[B]Summary

Applying an IPM strategy undoubtedly would enhance control efforts for invasive dreissenid mussels and AIS more broadly. Once infestations become established, and even when infestations are low due to control activities or a change in environmental conditions, aggressive actions are still critically important for minimizing the chance of a high-level infestation occurring (or re-occurring). This will minimize impacts and the potential for the infestation to spread to other waterbodies. We have provided information on a new management tactic – fish predators as site-specific biological controls – that shows promise. While more research is warranted to refine application of this tactic, our results support its use in IPM strategies for quagga and zebra mussels in the Southwestern U.S. and elsewhere.

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Figure 1. Small experimental pens used to assess the effect of Bluegill and Redear Sunfish on recruitment of quagga mussels to substrates with varying horizontal and vertical orientations (topside, underside, vertical side). (A) Vexar mesh pen with experimental substrates inside. (B) Experimental substrate bar containing four flat PVC substrates in horizontal or vertical orientations. (C) Illustration of paired set of experimental pens attached together and connected to a surface longline.

Figure 2. Large experimental pen used to assess the effect of Bluegill on recruitment of quagga mussels to substrates with varying horizontal and vertical orientations (topside, underside, vertical side) and at three locations (top, middle and bottom) within the net. (A) Illustration of nylon net experimental design. Substrate bars consist of four substrates (single substrate shown). (B) Nylon net experimental pen extended into water; substrate bars in pen not shown.

Figure 3. Recruitment of quagga mussels (≥ 3 mm shell length) to experimental substrates of three orientations in the absence (control) and presence (treatment) of Bluegill during small pen experiment. All orientations pooled (`All') also are indicated.

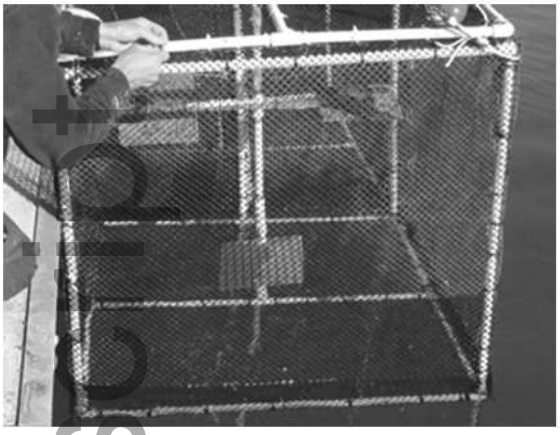
Figure 4. The relationship between Bluegill size (total length) and recruitment of quagga mussels (≥ 3 mm shell length) to the underside of experimental substrates in small treatment pens

Figure 5. The effect of Redear Sunfish on juvenile and adult quagga mussels (≥ 3mm shell length) seeded on experimental substrates of various orientations in the absence (control) and presence (treatment) of fish during the small pen experiment.

Figure 6. Photographs showing quagga mussel recruitment on the underside of experimental substrates at three locations within the pen in the (A) absence (control) or (B) presence (treatment) of Bluegill during the large pen experiment.

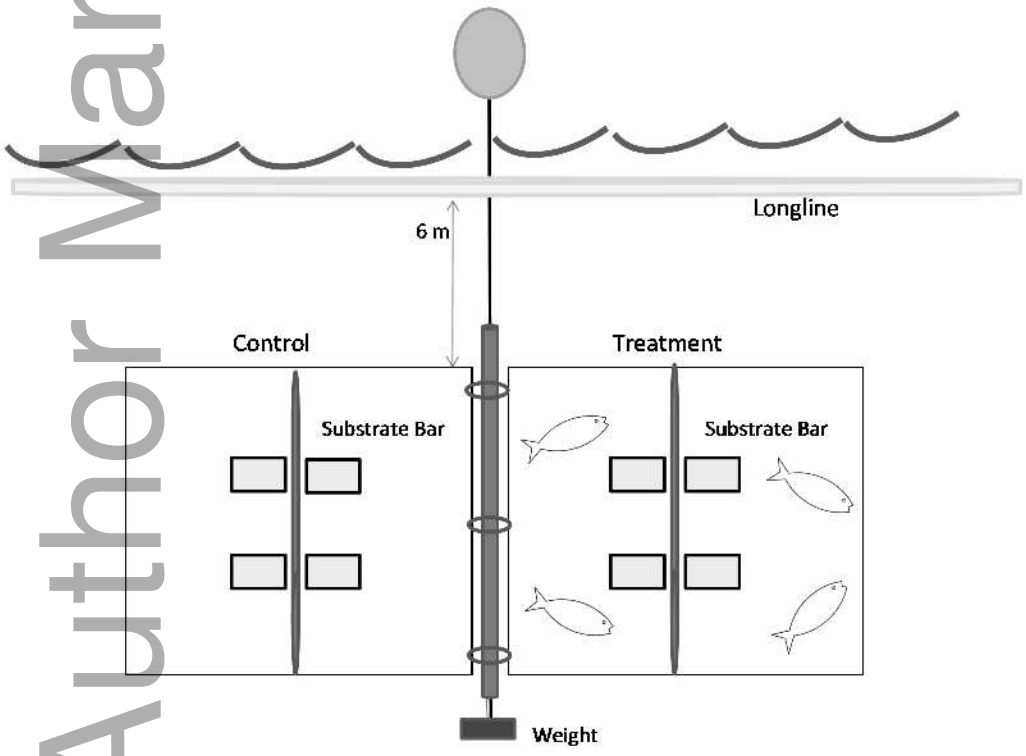
Figure 7. The effect of Bluegill on quagga mussels (≥ 3 mm shell length) recruiting to substrates of various orientations and locations within the pen in the (A) absence (control) and (B) presence (treatment) of Bluegill during the large pen experiment. Scales vary.

(A) Small experimental pen



(B) Experimental substrate bar

(C) Small experimental pen design

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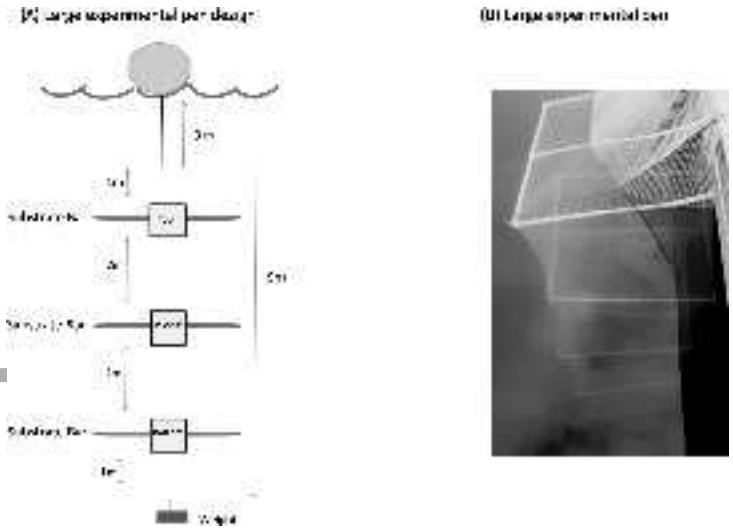

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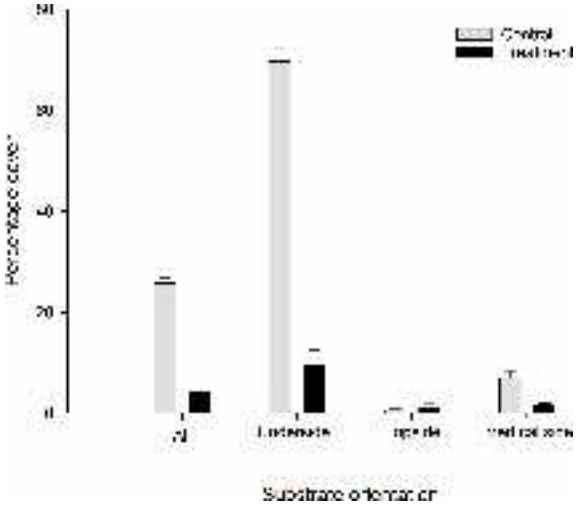

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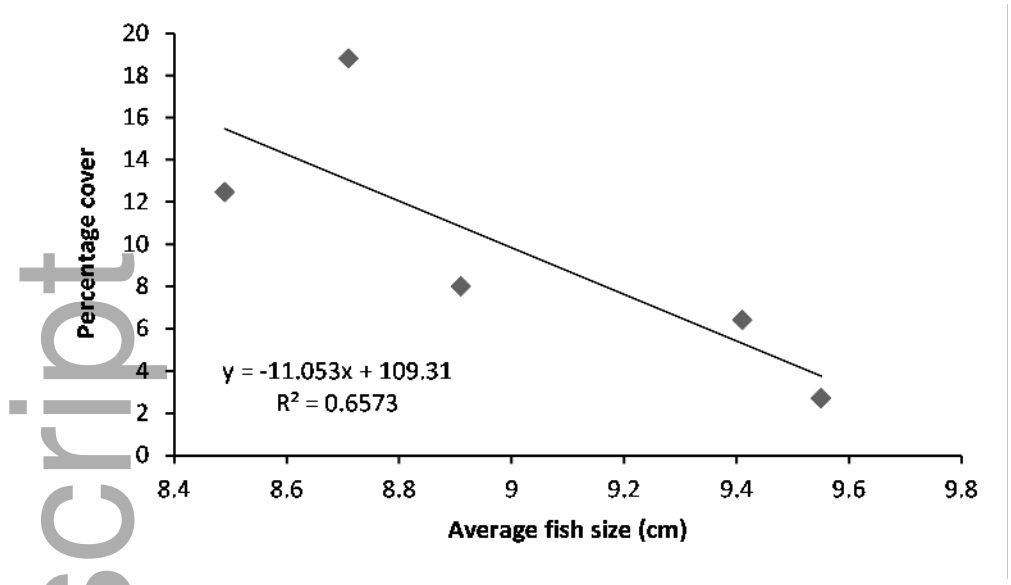


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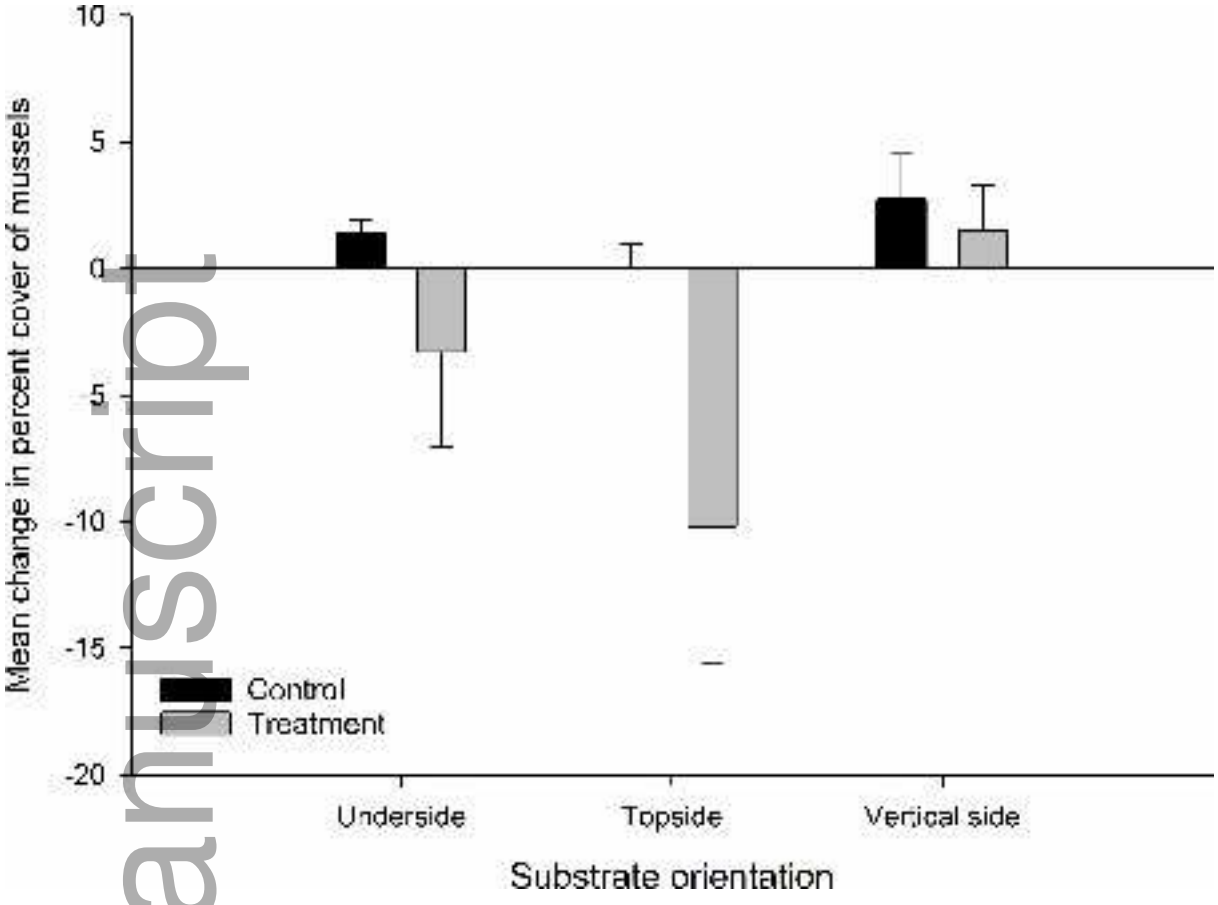


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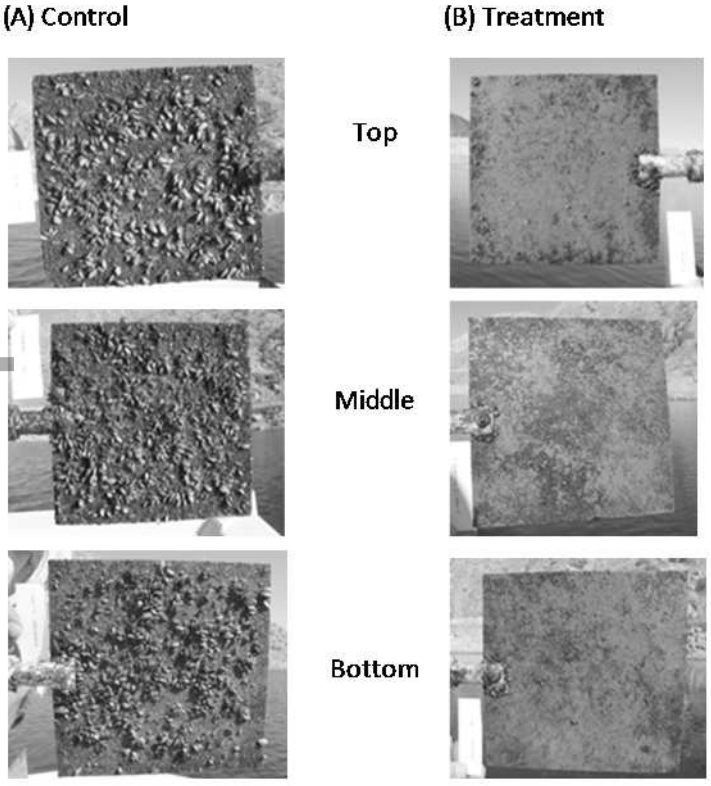


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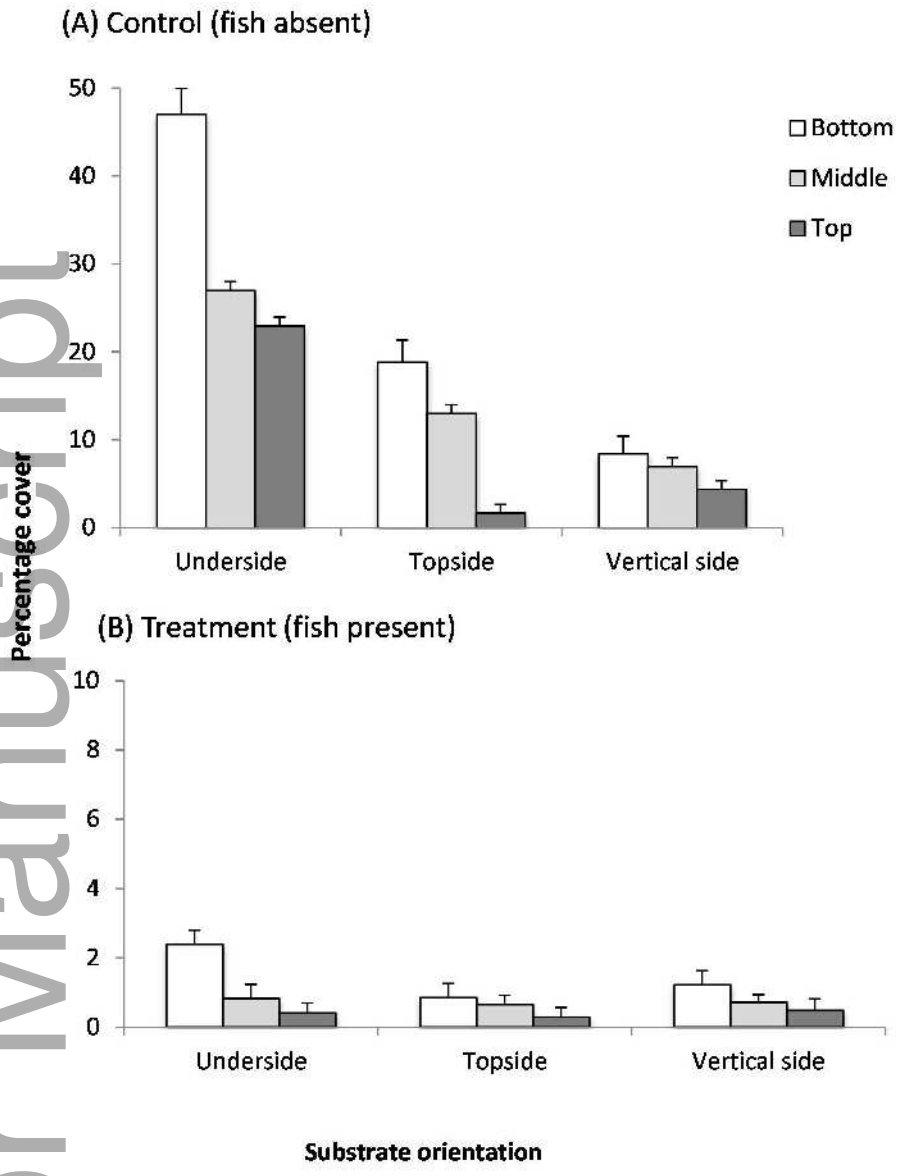


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