RECLAMATION
Managing Water in the West

Impact of Freshwater Sponge on Invasive Mussel Settlement

Research and Development Office
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Mission Statements

The U.S. Department of the Interior protects America's natural resources and heritage, honors our cultures and tribal communities, and supplies the energy to power our future.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

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Executive Summary

This scoping project is a literature review designed to examine the variety of sponge predatory defenses and their potential effects on mussel settlement. Additionally, a small scale field study was designed to visually examine if sponges exclude mussel settlement. This study is based on field observations indicating that invasive quagga mussels (*Dreissena bugensis*) were not settled on or near freshwater sponges. Sponges may produce mechanical or chemical defenses that prevent mussel settlement. It is possible that these natural defenses may be used to develop settlement prevention treatments that could be used to prevent bio-fouling in Reclamation facilities. This scoping study will help determine if there is potential for freshwater sponges to prevent mussel settlement. The next step would be to determine what the specific defense mechanism is and if it can be utilized, or artificially created for use as a settlement prevention technique. Reclamation facilities may benefit from this research as it may provide a low maintenance and passive protection from mussel settlement.

Zebra (*Dreissena polymorpha*) and quagga mussels are some of the most aggressive freshwater invasive species in recent history (Karatayv et al. 2006). Quagga mussels are established and have spread throughout the Lower Colorado River system, including Lake Mead, Lake Mohave, Lake Havasu, and Lake Powell (Wong et al. 2012). Quagga mussels impact Reclamation facilities by clogging water pipelines, dam gates, trash racks, and other water conveyance structures (Wong et al. 2012). Nelson and Nibling (2013) observed the presence of freshwater sponges may have altered the abundance of mussel settlement in the Central Arizona Project (CAP), as mussels settlement was less frequently observed around sponges.

Little is known about freshwater sponges and their natural defenses against competing organisms like mussels. Only 32 species of freshwater sponges in 12 genera exist in North America (Pennak 1991). The majority of the research reviewed for this literature review was based on the behavior of marine sponges. However, the research conducted by Ricciardi et al. (1995) suggests that freshwater sponges possess a chemical defense that inhibits mussels from settling. When Ricciardi et al. introduced mussels to already established sponge colonies, in a lab setting, the mussels failed to attach and settle. Additionally, young, mobile mussels preferentially attached to sponge free substrates.

Sponges are sessile organisms that defend themselves by chemical or mechanical/ structural means. Marine sponges have been found to produce chemical compounds to protect themselves from predator attacks and overgrowth from competing organisms (McClintock and Baker 2001). Chemical defense is typically thought to be a sponge’s most important anti-predator strategy (Pawlik et al. 1995). The sponge structure itself is also a good defense against predators. Nearly 75% of a freshwater sponge’s skeletal structure is made up of inorganic compounds called spicules (Rutzler and Macintyre 1978). Researchers have

found that these spicules are usually arranged with their sharp end pointed out towards the surface which is used to protect against predators (Uriz et al. 2003). Researchers have found evidence of abrasions on animals that feed on sponges, as a result of spicule contact (Meylan 1988). Since both sponges and mussels are sessile filter feeders, it is not likely that spicules alone will prevent mussels from settling. Studies have shown that both, chemical and mechanical defensed may work in corporation to establish predatory defense (Paul and Puglisi 2004).

Table 1 is a summary of the types of defenses observed among multiple sponge species. Most of the articles indicate that chemical defenses against predators are the most common type of protection. However Ricciardi et al. (1995) found that sponges were also able to grow over the top of mussels preventing the mussels from functioning and eventually leading to death (Ricciardi 1995). The results of this literature review indicate that there is potential for sponges to exclude mussel settlement as a result of overgrowth and physical or chemical defense. Sponge chemical defense compounds will likely be of most interest for development of mussel settlement prevention techniques.

Table 1. **Summary of literature review on sponge defense mechanisms.**

Literature Cited	**Sponge Defense to Predators**			**No Defense Observed**	**Marine/ Fresh Water Sponge**	**Comments**
	Chemical	**Structural**	**Other**			
Agell et al. (2001)	X	_	_	_	Marine	Sponges in the western Mediterranean have chemical defenses that may be induced by environmental stressors.
Bartik et al. (1987)	X	_	_	_	Marine	Noxious compounds may act to minimize predation or to reduce settlement and overgrowth of fouling organisms.
Carsten and Schupp (2007)	X	X	_	_	Marine	Both chemical and structural defense were observed. Predicted that they may work together defend a wider range of predators.

Ricciardi et al. (1995)	–	–	X	–	Fresh water	Sponges grew and covered the mussel siphons causing them to suffocate and die.
Walters and Pawlik (2005)	X	–	–	X	Marine	Sponges with chemical defenses tended to have slow wound healing capabilities when compared to sponges without a chemical defense.
Bobzin and Faulkner (1992)	X	–	–	–	Marine	Secondary metabolites may provide a competitive advantage for a sponge by deterring predation, deterring settlement or overgrowth by other benthic organisms competing for substrate space.
Jones et al. (2005)	X	X	–	–	Marine	Sponge spicules may be defensive in isolation, or may enhance chemical defenses against consumers, but the lack of synergisms for individuals in 4 of 7 species with intermediate levels of chemical defense suggests that defensive synergy is not the general rule and, when present, may be an example of an exaptation.

Hills et al. (2005)	X	X	_	_	Marine	Spicules and crude extracts interacted to enhance deterrence in experiments involving *Microciona prolifera* because combining spicules and crude extract in artificial food significantly reduced crab feeding rates by 53%.

Field Study

The goal of this field study was to observe mussel exclusion by freshwater sponges. This field experiment was conducted on the lower Colorado River at Imperial Dam, CA, because both quagga mussels and freshwater sponges have been identified at the site. Three stringers of plates were deployed at Imperial Dam, CA on 2/11/2015. Each stringer contained six, black PVC plates, situated between 3-7 meters below the surface. The goal was to submerge the plates until they were colonized by sponges. The plates were monitored monthly for sponge growth. Once the plates were colonized, they would be transported a few miles upstream to Senators Wash, where mussel settlement is more intense. Three additional stringers of settlement plates without sponge colonization would be deployed at Senators Wash and mussel settlement would be compared on plates with and without sponge presence.

Field Study Results

The plates at Imperial Dam were observed monthly and only a small number of sponges were observed colonizing by July. There was not enough sponge colonization to continue the project in the allotted time frame. It is likely that the plates were not deployed at the right time of year for best sponge colonization. This field study was limited because field work was conducted along-side another study to save on travel costs.

Mussel settlement has been found to be significantly less at Imperial Dam in comparison to sites, such as Parker and Davis Dams, upstream in the Colorado River. Another Reclamation project is being conducted to determine the causes of this reduced settlement. Sponge settlement has been observed on settlement plates associated with this other project, but it has not been determined if the sponge presence is causal of reduced settlement. More sponge was noticed on Imperial Dam settlement plates in 2014 compared to 2013, but there were more mussels collected from plates in 2014 (40 adults) than in 2013 (25 adults). Fewer sponges were observed on plates in the first two meters of water, while thicker sponge growth started at about 3 meters. Plates closer to the trash rack had fewer sponges, possibly because of high flow and more sediment on the plates. Sponges were found growing on top of some adult mussels (Figure 1). In February 2015, a significant amount of sponge gemmules (Figure 2) were found on the plates and wire stringers deployed at Imperial Dam, indicating there was a large sponge population that year. Gemmules are internal buds involved in asexual reproduction, that are resistant to harsh environmental conditions and can lay dormant until less hostile conditions appear.

Figure 1. Adult quagga mussels collected at Imperial Dam, CA with sponge overgrowth.

Figure 2. Sponge gemmules collected from settlement plates at Imperial Dam, CA in February 2015

In February 2015 a sponge sample along with sponge gemmules were collected from plates at Imperial Dam and were sent to BSA Environmental Services Inc. for taxonomic identification. The sample was identified as *Trochospongilla leidii.*

An additional sample was sent to the Reclamation Detection Laboratory for Exotic Species (RDLES) for DNA barcoding analysis. DNA barcoding is a molecular test that uses a conserved DNA sequence, such as cytochrome oxidase 1 (COI), for the identification of organisms. For this assay, DNA was isolated from three sponge subsamples using the Qiagen DNA extraction kit (Keele et al. 2014). The PCR assay and primers (dgLCOI1490 and dgHCO2198) used by Vargas et al. (2012) were used to amplify part of the COI gene. The resulting PCR products were sent to a commercial laboratory for DNA sequencing.

The forward and reverse DNA sequences from each sample were aligned to each other and to on-line databases (DNA Bold and NCBI Blast) of DNA sequences to identify the organism (Tables 2, 3, 4, and 5). The DNA sequencing analysis of the second sample did not yield DNA sequences that could be analyzed with any confidence.

The databases matched the sequences with several sponge species (Table 2), including *Pachydictyum globosum* and *Pachydictyum incrustans*, which are sponges from Indonesia and not likely the species collected in CA. The data base also matched the sequences to *Trochospongilla pennsylvanica*, which is a species in the same genus identified by the taxonomist. Based on the alignment results, it is possible that the sponge analyzed here is not present in either the DNA BOLD or NCBI databases. There are only a few nucleotide differences between the sequence of the sample DNA and the three sponge species to which it was matched (Figures 4 and 5).

Table 2. BOLD and BLAST analysis results for the forward and reverse sequences obtained from the sponge collected at Imperial Dam, CA.

Sample	BOLD Analysis	BLAST Analysis
Sequence 1 Forward	*Pachydictyum incrustans* (99% similarity)	*Pachydictyum globosum* (94% ID, 99% coverage)
Sequence 1 Reverse	Species level match could not be made, highest ranked species was *Trochospongilla pennsylvanica* (99.2% similarity)	*Trochospongilla pennsylvanica* (98% ID, 100% coverage)
Sequence 3 Forward	Species level match could not be made, highest ranked species was *Trochospongilla pennsylvanica* (99.1% similarity)	*Pachydictyum globosum* (97% ID, 100% coverage)
Sequence 3 Reverse	Species level match could not be made, highest ranked species was *Pachydictyum globosum* (99.29% similarity)	*Pachydictyum globosum* (98% ID, 99% coverage)

Search Result:

A species level match could not be made, the queried specimen is likely to be one of the following:

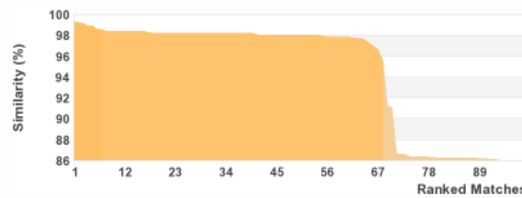
Pachydictyum globosum
Pachydictyum incrustans
Trochospongilla pennsylvanica
Spongilla vastus
Spongilla lacustris
Trochospongilla horrida
Echinospongilla brichardi

For a heirarchical placement - a neighbor-joining tree is provided: Tree Based Identification

Identification Summary:

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Porifera	100
Class	Demospongiae	100
Order	Haplosclerida	100
Family	Malawispongiidae	100
Genus	Pachydictyum	100

Similarity Scores of Top 99 Matches:



TOP 20 Matches :

Display option: Top 20

Phylum	Class	Order	Family	Genus	Species	Similarity (%)	Status
Porifera	Demospongiae	Haplosclerida	Malawispongiidae	Pachydictyum	*globosum*	99.29	Published
Porifera	Demospongiae	Haplosclerida	Malawispongiidae	Pachydictyum	*incrustans*	99.22	Published
Porifera	Demospongiae	Haplosclerida	Spongillidae	Trochospongilla	*pennsylvanica*	99.11	Published
Porifera	Demospongiae	Haplosclerida	Spongillidae	Spongilla	*vastus*	98.91	Published
Porifera	Demospongiae	Haplosclerida	Spongillidae	Spongilla	*vastus*	98.91	Published
Porifera	Demospongiae	Haplosclerida	Spongillidae	Spongilla	*lacustris*	98.57	Published
Porifera	Demospongiae	Haplosclerida	Spongillidae	Trochospongilla	*horrida*	98.53	Published
Porifera	Demospongiae	Haplosclerida	Potamolepiidae	Echinospongilla	*brichardi*	98.4	Published
Porifera	Demospongiae	Haplosclerida	Spongillidae	Spongilla	*lacustris*	98.4	Published
Porifera	Demospongiae	Haplosclerida	Spongillidae	Spongilla	*lacustris*	98.4	Published
Porifera	Demospongiae	Haplosclerida	Lubomirskiidae	Baikalospongia	*intermedia*	98.4	Private

Figure 3: DNA Bold output for the sequence analysis of sample 3 reverse sequence. Note that a species level match could not be made.

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Trochospongilla pennsylvanica.str from 1 to 579
Alignment to
Sponge 1 forward.str--  Matches:493; Mismatches:29; Gaps:106; Unattempted:0
Sponge 1 reverse.str--  Matches:520; Mismatches:9; Gaps:146; Unattempted:0
Sponge 3 forward.str--  Matches:504; Mismatches:14; Gaps:133; Unattempted:0
Sponge 3 reverse.str--  Matches:488; Mismatches:10; Gaps:155; Unattempted:0

                *         *         *         *         *         *         *         *         *         *
  1>GATCAATTATATAATGTTATAGTTACAGCCCATGCTTTTCTAATGATATTTTTCTTAGTTATGCCAGTAATGATTGGGGGATTTGGAAATTGATTCGTGC>100
 50>GATCAATTATATAATGNTATAGTTACAGCCCATGCTTTTCTAATGATATTTTTCTTAGTTATGCCANNNATGATTGNNGNNNNTGNAAATTGATTCGTGC>149
 97>GATCAATTATATAATGTTATAGTTACAGCCCATGCTTTTCTAATGATATTTTTCTTAGTTATGCCAGTAATGATTGGGGGATTTGGAAATTGATTCGTGC>196
 73>GATCAATTATATAATGTTATAGTTACAGCCCATGCTTTTCTAATGATATTTTTCTTAGTTATGCCAGTAATGATTGGGGGANTTGGAAATTGATTCGTGC>172
 75>GATCAATTATATAATGTNATAGTTACAGCNCATGCTTTTCTAATGATATTTTTCTTAGTTATGCCAGTAATGATTGGGGGATTTGGAAATTGATTCGTGC>174

                *         *         *         *         *         *         *         *         *         *
101>CACTATATATTGGTGCACCCGATATGGCTTTTCCAAGATTAAATAATATTAGTTTTTGATTATTACCTCCGGCTTTAACTCTATTATTAGGATCTGCTTT>200
150>CACTATATATTGGTGCACCCGATATGGCTTTTCCAAGATTAAATAATATTAGTTTTTGATTATTACCTCCGGNTTNANCNCNNTTATTAGGATCTGCTTT>249
197>CACTATATATTGGTGCACCCGATATGGCTTTTCCAAGATTAAATAATATTAGTTTTTGATTATTACCTCCGGCTTTAACTCTATTATTAGGATCTGCTTT>296
173>CACTATATATTGGTGCACCCGATATGGCTTTTCCAAGATTAAATAATATTAGTTTTTGATTATTACCTCCGGCTTTAACTCTATTATTAGGATCTGCTTT>272
175>CACTATATATTGGTGCACCCGATATGGCTTTTCCAAGATTAAATAATATTAGTTTTTGATTATTACCTCCGGCTTTAACTCTATTATTAGGATCTGCTTT>274

                *         *         *         *         *         *         *         *         *         *
201>TGTAGAGCAAGGGGTTGGTACAGGATGGACAGTATATCCCCCTTTAGCAGGGATACAAGCACATTCTGGGGGATCAGTTGATATGGCAATATTTAGTCTT>300
250>TGTAGAGCAAGGGGTTGGTACAGGATGGACAGTATATCCCCCTTTAGCAGGCATACAAGCCCATTCTGGGGGATCAGTTGATATGNCCATATTTAGTCTT>349
297>TGTAGAGCAAGGGGTTGGTACAGGATGGACAGTATATCCCCCTTTAGCAGGCATACAAGCCCATTCTGGGGGATCAGTTGATATGGCCATATTTAGTCTT>396
273>TGTAGAGCAAGGGGTTGGTACAGGATGGACAGTATATCCCCCTTTAGCAGGCATACAAGCCCATTCTGGGGGATCAGTTGATATGGCCATATTTAGTCTT>372
275>TGTAGAGCAAGGGGTTGGTACAGGATGGACAGTATATCCCCCTTTAGCAGGCATACAAGCCCATTCTGGGGGATCAGTTGATATGGCCATATTTAGTCTT>374

                *         *         *         *         *         *         *         *         *         *
301>CACTTGGCGGGTATTTCTTCGATATTAGGGGCTATGAATTTTATCACTACAATCTTTAATATGAGAGCGCCCGGTATTACAATGGATAGAATGCCATTAT>400
350>CACTTGGCGGGTATTTCTTCGATATTAGGGGCTATGAATTTTATCACTACAATCTTTAATATGAGAGCGCCCGGTATTACNATGGATAGAATGCCATTAT>449
397>CACTTGGCGGGTATTTCTTCGATATTAGGGGCTATGAATTTTATCACTACAATCTTTAATATGAGAGCGCCCGGTATTACAATGGATAGAATGCCATTAT>496
373>CACTTGGCGGGTATTTCTTCGATATTAGGGGCTATGAATTTTATCACTACAATCTTTAATATGAGAGCGCCCGGTATTACAATGGATAGAATGCCATTAT>472
375>CACTTGGCGGGTATTTCTTCGATATTAGGGGCTATGAATTTTATCACTACAATCTTTAATATGAGAGCGCCCGGTATTACAATGGATAGAATGCCATTAT>474

                *         *         *         *         *         *         *         *         *         *
401>TTGTATGATCTATTTTAATAACAGCCTTTTTATTATTATTATCTTTACCTGTATTAGCTGGTGGTATAACAATGCTTTTAACAGATAGAAATTTTAATAC>500
450>TTGTATGATCTATTTTAATAACAGCTTTTTTATTATTATTATCNTTACCTGNATNAGCTGGGGGTATAACAATGCTTTTAACAGANAGAAATTTTAATAC>549
497>TTGTATGATCTATTTTAATAACAGCTTTTTTATTATTATTATCTTTACCTGTATTAGCTGGGGGTATAACAATNCNNNTAACAGATAGAAATTTTAATAC>596
473>TTGTATGATCTATTNNAATAACAGCTTTTTTATTATTATTATCTTTACCTGTATNAGCTGGGGGTATAACAATGCTTTTAACAGANAGAAATTTTAATAC>572
475>TTGTATGATCTATTTTAATAACAGCTTTTTTATTATTATTATCTTTACCTGTATTAGCTGGGGGTATNNCAATGCTTNTAACAGATAGAAATTTTAAT~~>572

                *         *
501>AACATTTTTTGATCCTGCTGGAGGAGGAG>529
550>AACATTTTTTGATCCTGCTGNA~~~~~~~>571
597>AACATTTTTTGATCCTGCTGGAGGAGGAG>625
573>NNCNNTTTTTGATCCTGC~~~~~~~~~~~>590
572>~~~~~~~~~~~~~~~~~~~~~~~~~~~~~>572
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Figure 4. Sequence alignment of *T. pennsylvanica* and four sequences obtained from barcoding analysis of the sponge sample. There are only five nucleotides that are different between the sponge samples and the known sequence for *T. pennsylvanica*. An N in the DNA sequence indicated a nucleotide that could not be resolved in the sequencing reaction.

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Trochospongilla pennsylvanica.str from 1 to 579
Alignment to
Pachydictyum globosum.str--  Matches:526; Mismatches:2; Gaps:123; Unattempted:0
Sponge 3 forward.str--  Matches:504; Mismatches:14; Gaps:133; Unattempted:0

             *         *         *         *         *         *         *         *         *         *
  1>GATCAATTATATAATGTTATAGTTACAGCCCATGCTTTTCTAATGATATTTTTCTTAGTTATGCCAGTAATGATTGGGGGATTTGGAAATTGATTCGTGC>100
 73>gatcaattatataatgttatagttacagcccatgcttttctaatgatatttttcttagttatgccagtaatgattgggggatttggaaattgattcgtgc>172
 73>GATCAATTATATAATGTTATAGTTACAGCCCATGCTTTTCTAATGATATTTTTCTTAGTTATGCCAGTAATGATTGGGGGANTTGGAAATTGATTCGTGC>172

             *         *         *         *         *         *         *         *         *         *
101>CACTATATATTGGTGCACCCGATATGGCTTTTCCAAGATTAAATAATATTAGTTTTTGATTATTACCTCCGGCTTTAACTCTATTATTAGGATCTGCTTT>200
173>cactatatattggtgcacccgatatggcttttccaagattaaataatattagtttttgattattacctccggctttaactctattattaggatctgcttt>272
173>CACTATATATTGGTGCACCCGATATGGCTTTTCCAAGATTAAATAATATTAGTTTTTGATTATTACCTCCGGCTTTAACTCTATTATTAGGATCTGCTTT>272

             *         *         *         *         *         *         *         *         *         *
201>TGTAGAGCAAGGGGTTGGTACAGGATGGACAGTATATCCCCCTTTAGCAGGGATACAAGCACATTCTGGGGGATCAGTTGATATGGCAATATTTAGTCTT>300
273>tgtagagcaaggggttggtacaggatggacagtatatccccctttagcaggcatacaagcacattctgggggatcagttgatatggcaatatttagtctt>372
273>TGTAGAGCAAGGGGTTGGTACAGGATGGACAGTATATCCCCCTTTAGCAGGCATACAAGCCCATTCTGGGGGATCAGTTGATATGGCCATATTTAGTCTT>372

             *         *         *         *         *         *         *         *         *         *
301>CACTTGGCGGGTATTTCTTCGATATTAGGGGCTATGAATTTTATCACTACAATCTTTAATATGAGAGCGCCCGGTATTACAATGGATAGAATGCCATTAT>400
373>cacttggcgggtatttcttcgatattaggggctatgaattttatcactacaatctttaatatgagagcgcccggtattacaatggatagaatgccattat>472
373>CACTTGGCGGGTATTTCTTCGATATTAGGGGCTATGAATTTTATCACTACAATCTTTAATATGAGAGCGCCCGGTATTACAATGGATAGAATGCCATTAT>472

             *         *         *         *         *         *         *         *         *         *
401>TTGTATGATCTATTTTAATAACAGCCTTTTTATTATTATTATCTTTACCTGTATTAGCTGGTGGTATAACAATGCTTTTAACAGATAGAAATTTTAATAC>500
473>ttgtatgatctattttaataacagcctttttattattattatctttacctgtattagctggtggtataacaatgcttttaacagatagaaattttaatac>572
473>TTGTATGATCTATTNNAATAACAGCTTTTTTATTATTATTATCTTTACCTGTATNAGCTGGGGGTATAACAATGCTTTTAACAGANAGAAATTTTAATAC>572

             *         *         *
501>AACATTTTTTGATCCTGCTGGAGGAGGAGA>530
573>aacattctttgatcctgctggaggag-a-a>600
573>NNCNNTTTTTGATCCTGC~~~~~~~~~~~~>590
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Figure 5. Alignment of *T. pennsylvanica, P. globosum* and sponge 3 forward sequences. Note that there are only two differences between *T. pennsylvanica* and *P. globosum.*

Although this field study could not be completed the literature review and general field observations indicate that there is likely some interaction between sponges and mussels as they are in competition for similar resources. A more complete analysis of freshwater sponge chemical defense may provide insight into new methods for mussel settlement prevention.

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Data Sets that support the final report

- Share Drive folder name and path where data are stored: **ENVRES(//bor/do) (H:)/EnvRes Share/ MUSSEL SAMPLES/ 2015/2015 Prop C/Sponge Research**

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