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Technical Memorandum No. MERL-2011-46

Natural Biocides for Zebra and Quagga Mussel Control

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Bureau of Reclamation
Technical Service Center
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Natural Biocides for Zebra and Quagga Mussel Control

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Introduction

Zebra and Quagga mussels are freshwater fouling organisms which began appearing in western US waters in 2007. Multifaceted research is being undertaken by USBR and others to mitigate the known and potential impacts of these mussels on hydraulic equipment. One aspect of this research involves a literature review of natural biocides that may potentially kill or disrupt attachment of zebra and quagga mussels to infrastructure. This paper is a review of literature on natural chemical biocides for biofouling of mussels.

There are many naturally occurring compounds that are useful biocides for preventing marine growth; however most of the research has been done for marine organisms only.[1, 2, 3, 4, 5] Many different marine organisms have natural defense mechanisms to prevent fouling species from attaching to the surfaces. Many natural biocide products have been isolated from sponges, seaweeds, coral, and ascidians.[2] Current antifouling coatings use cuprous oxide and possibly eighteen different organic biocide boosters that have a broad range of toxicity.[1] However, there is much concern over the bioaccumulation of these different biocides and more environmentally friendly biocides are desired.[1] There are only a few articles that discuss the effectiveness of the naturally occurring biocides for zebra and quagga mussels.

By definition, antifouling means "inhibiting the growth of barnacles and other marine organisms on a ship's bottom"[6] or "intended to prevent fouling of underwater structures."[7] There are a few different approaches to making an antifouling coating. One method is for the chemical (biocide) to be toxic and kill the targeted organisms. Rarely are the compounds only toxic to the targeted organisms. They are also usually toxic to non-targeted species. Another approach is to develop or find a non-toxic chemical that disrupts the bonding mechanism. Both approaches will be discussed in this review.

Background on Marine Natural Product Antifoulants

There have been hundreds of different chemicals that have been isolated, extracted, and evaluated for antifouling properties. The majority of these compounds falls under certain classes or categories of chemicals. Terpenoids, steroids, carotenoids, saponins, phenolics, fatty acids, furanones, bromotyrosine, bromopyrrole, amino acids, alkaloids, peptides, lactones, and benzenoids are classes of chemicals that have been shown to deter bacterial films, algae, barnacles, and bryozoans attachment at varying levels of antifouling activity.[2, 3, 4] There are a few commercially available natural antifoulant products on the market, and they include Sea Nine 211, Netsafe, and Pearlsafe.[4] Sea Nine 211, manufactured by Rohm and Haas, is the most popular natural product antifoulant and is effective against bacterial slime, algae, barnacles, tubeworms, hydroids, bryozoa, tunicates, and diatoms.[8] MERL tested a coating containing Sea Nine 211 in the first year of the study on coatings for mussel control, but it fouled heavily with mussels in the first 7 months of exposure.[9] Figure 1 shows that there was little algae attached to the coated surface, we determined the biocide worked on freshwater algae which was a targeted

species. However, Sea Nine 211 was not effective against zebra mussels. One nice characteristic of Sea Nine 211 is that it breaks down very fast and does not bioaccumulate.[10]

Figure 1. E-Paint SN-1 at 7 months, mussels attached to the surface, however very little algae.

Since the main goal of this project was to investigate and review natural products for zebra and quagga mussel control, we will focus on natural products that have been identified for inhibiting marine mussel byssus formation or attachment or are toxic to marine mussels.

A Brazilian brown seaweed (*Canistrocarpus cervicornis*) extract showed strong inhibition signs of mussel (*Perna Perna*) byssus formation.[11] Three diterpene compounds were isolated that did not kill the mussels, but reduced the number of byssal threads up to 82%. The compounds are shown in Figure 2 chemicals A, B, and C.[11] These studies were relatively short and did not explain long term effects and were not evaluated for toxicity to other marine species. Another study investigated brown algae (*Cystoseira baccata*) and isolated a two chemicals called

meroditerpene and terpenoid that cause inhibition of mussel phenoloxidase in blue mussels (Mytilus edulis).[12] The chemicals are shown in Figure 2 D, E, and F. Phenoloxidase is a key component in oxidation of the mussel adhesive to form a stronger bond to the substrate.

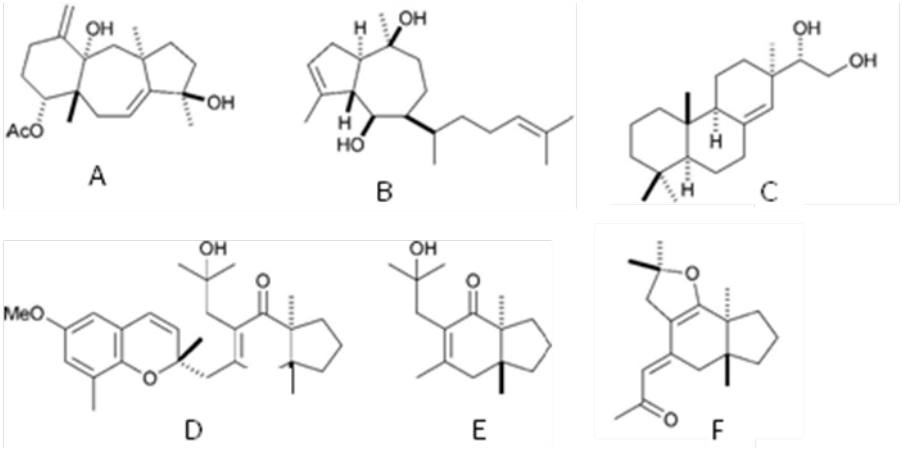

Figure 2. A, B, and C show chemical structures of extracts from Brazilian Brown Seaweed. D, E, and F show chemical structures of extracts from brown algae.[3]

A marine sponge *Geodia berretti* extract named Barettin was shown to inhibit the reattachment of two different marine mussels. The extract showed 89% inhibition for the *Balanus improvises* and 81% inhibition in *Mytilis edulis*. The chemical structure of Barettin is shown in Figure 3.[13]

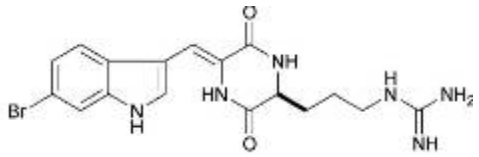

Figure 3 Chemical structure of Barettin, extracted from a marine sponge *Geodia berretti*.[3]

A brominated diphenyl ether extracted from a marine sponge in the *Dysidea* genus showed excellent inhibition of marine mussel (*Mytilus edulis*) attachment at very low concentrations (0.66 µM) while showing very low toxicity.[14] The chemical structure is shown in Figure 4.[14]

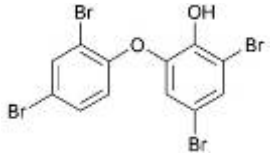

Figure 4 Brominated diphenyl ether extracted from a marine sponge in the *Dysidea* genus.[3]

A group of chemicals were extracted from a marine bacteria (*Streptomyces albidoflavus*) that contain furanone rings.[15] These chemical structures are shown in Figure 5. These chemicals inhibited the attachment of barnacle *Balanus amphitrute* at low concentrations. It was essential that the furanone ring was present for the antifouling activity, and the alkyl chain influenced the effectiveness of the antifoulant.[15]

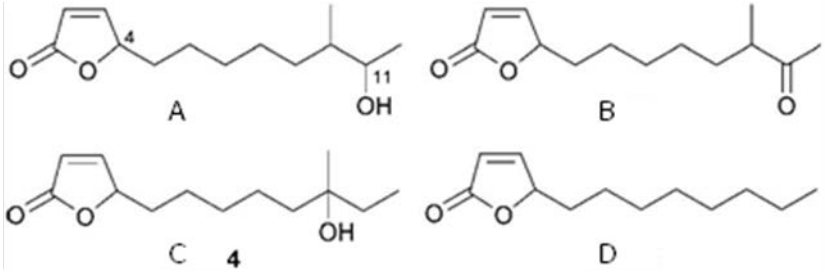

Figure 5 Chemical structures of furanones extracted from *Streptomyces albidoflavus*[3]

Maculalactone, which is a tribenzyl lactone extracted from a marine cyanobacteria (*Kyrtuthrix maculans*) and was lethal to barnacles had specific antifouling activity towards bivalves.[16] The chemical structure of Maculalactone is shown in Figure 6.

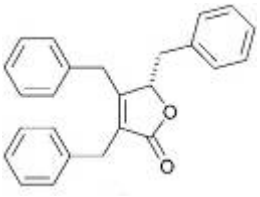

Figure 6 Chemical structure of Maculalactone.[3]

All of these natural biocides have been shown to have some antifouling activity towards marine mussels. However, all of these studies were fairly short term, typically less than one week. In addition many of these studies only evaluated the targeted species to determine if the chemicals had effective antifouling activity. Further studies would be required to evaluate the toxicity affects towards other marine organisms.

Natural Biocides That Kill Zebra Mussels

The University of Mississippi has evaluated a number of different extracts from marine sponges looking for a compound that will be selectively toxic to zebra mussels or inhibit byssal attachment.[17] The first extracts evaluated were from the marine sponge *Verongida*.[17] The extract that showed the most antifouling activity was Moloka'iamine. Its chemical structure is shown in Figure 7. Moloka'iamine was extremely effective at low concentrations (15 µM) at

inhibiting byssal attachment. It also killed the mussels at slightly higher concentrations. Moloka'iamine was also evaluated for toxicity toward the duck weed *L. pausicostata* and showed low toxicity up to concentrations of 200 µM.[17]

The second marine sponge the University of Mississippi investigated was *Aaptos*.[18] There were three effective extracts that inhibited mussels from attaching to surfaces. However, all showed some toxicity towards the zebra mussel. Two of the most effective chemicals against the mussels also showed some toxicity affects toward duck weed *L. pausicostata* below concentrations of 100 µM.[18] Figure 8 shows the chemical structure for Aaptamine which had the least toxicity toward the duck weed *L. pausicostata*.[18] Much more research needs to be conducted to determine toxicity towards other freshwater species before either of these products could be used as a biocide in a coating.

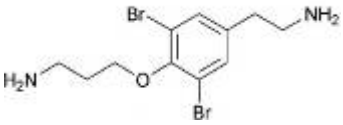

Figure 7 Chemical structure of Moloka'iamine.[3]

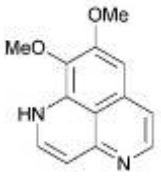

Figure 8 Chemical structure of Aaptamine.[3]

Marrone Bio Innovations has been able to isolate many chemicals from the bacterial strain CL145A of *Pseudomonas fluorescens*, which is known to be lethal to zebra and quagga mussels.[19] Marrone was able to isolate 44 different compounds.[19] Due to the limited amount of sample material, Marrone purchased some commercially available chemicals.[19] There were a few chemicals that had a very high mortality rate of mussels within 24 hrs of treatment.[19] The most effective chemical tested was γ-dodecalactone shown in Figure 9.[19] In their patent, they claim that the extract from *Erwinia carotovora* was effective at killing mussels; however, it took longer exposure times in order to kill mussels.[19] They evaluated other compounds that also killed mussels effectively; however, it is unknown if the compounds were from natural products.[19] Currently, Marrone Bio Innovations has not conducted studies to see the effects on fresh water organisms with any of the chemicals isolated from *Pseudomonas fluorescens*.

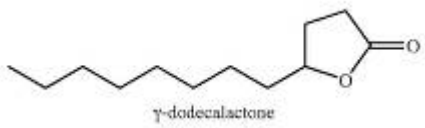

Figure 9 Chemical structure of molluscicide extracted from Pseudomonas fluorescens.[19]

Natural Biocides That Disrupt Zebra Mussel Attachment

The University of Texas at Arlington has been studying capsaicin for many years with great laboratory results at inhibition of the zebra mussel byssal attachment.[20, 22] However, field studies showed significantly different results. The laboratory studies are normally for a short duration of time (48 hrs to 96 hrs) and the capsaicin is at known concentration.[20, 22] The field tests were conducted with an antifouling coating containing capsaicin, but the leach rate is not constant and diminishes over time. The chemical structure of capsaicin isomers are shown in Figure 10.[20] According to the scientists the capsaicin inhibits the attachment of byssal threads. However, at slightly higher concentrations than needed for mussel inhibition, the mortality rate of the water flea, *Daphnia Magna*, increased.[20, 22]

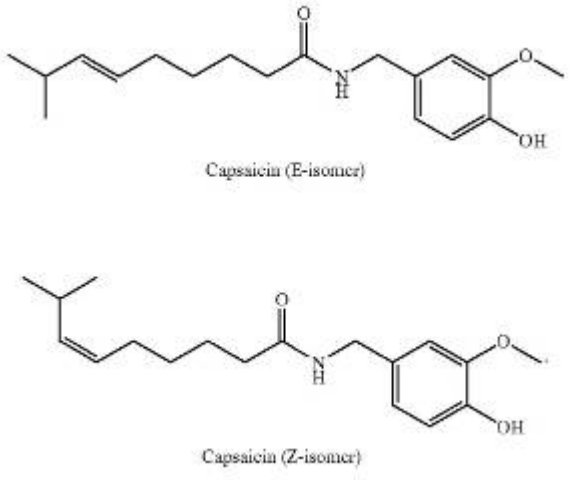

Figure 10 Chemical structure of capsaicin isomers.[20]

In addition to the studies of capsaicin, the University of Texas at Arlington has been evaluating many other natural products for the antifouling activity towards zebra mussel attachment, cannabinoids and lipophilic amide spacer electronegative pharmacophore (LASEN) with great laboratory results at inhibiting of the zebra mussel byssal attachment.[21, 22] The chemical structures that inhibit the mussel attachment are shown in Figure 11.[21, 22] All of these

chemical structures showed signs of inhibiting byssal attachment while having low toxicity towards the mussels. Again all of these compounds showed an increase in the mortality rate of the water flea, *Daphnia Magna*, at slightly higher concentrations than that needed for inhibiting byssal attachment.

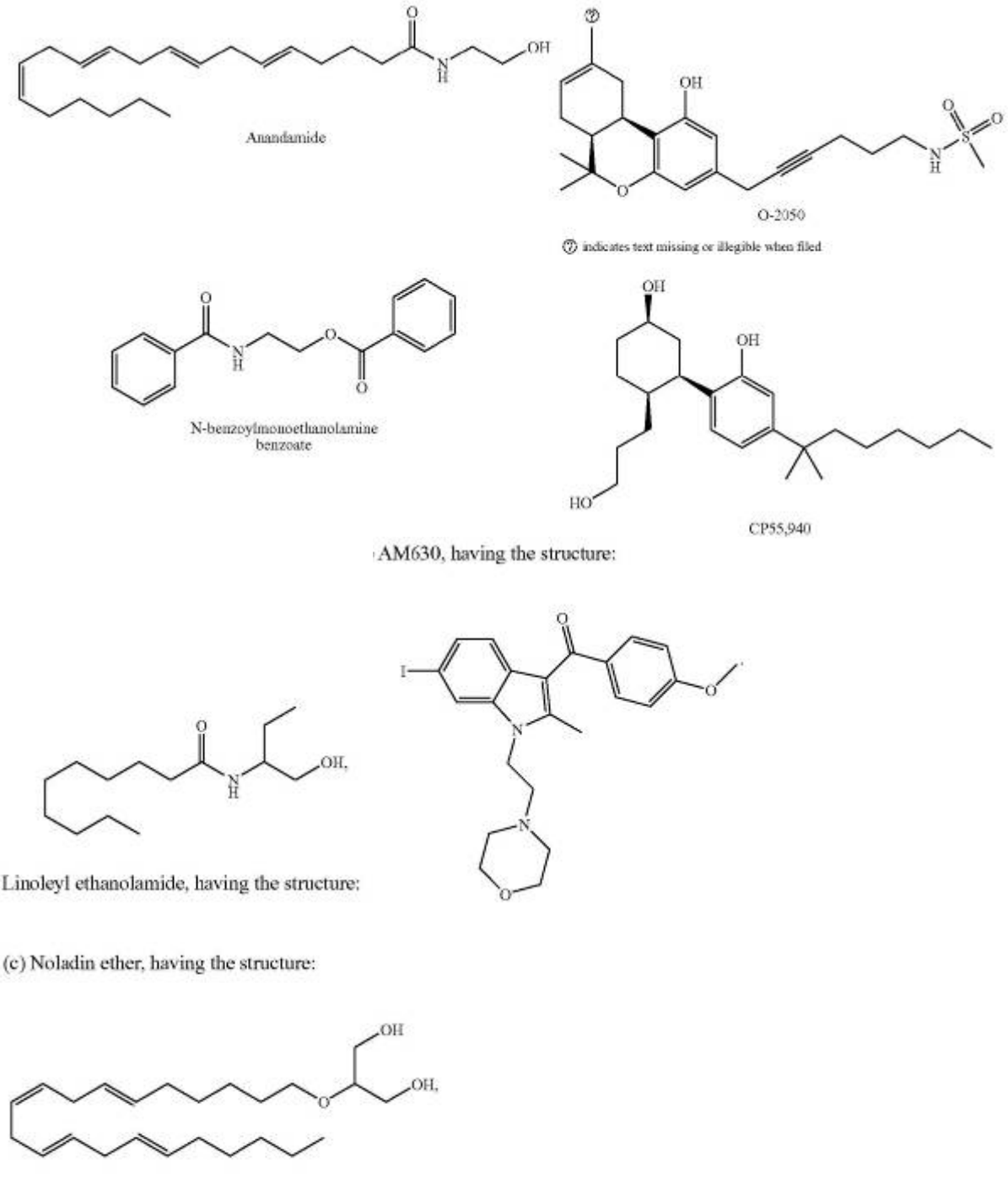

Figure 2 Chemical structures of LASEN and Cannabinoids.[22]

At the University of Akron, zosteric acid has been evaluated for inhibiting the byssal attachment shown in Figure 12.[23] Zosteric acid is extracted from a marine seagrass *Zostera marina*.[23] The zosteric acid has been able to prevent mussel byssus attachment for up to 3 days, with only 20% of the mussels attached after the 4th day.[23]

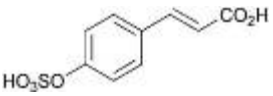

Figure 3 Chemical structure of zosteric acid.[3]

Practical Issues with Natural Biocides

Currently there are only a few commercially available antifouling coatings using natural biocides. Many natural biocides work on only select group of fouling species. Some natural biocides affect non-targeted species which raise environmental concerns. Environmental regulations dictate that any biocide go through rigorous testing to determine half life, chemical breakdown, environmental fate, toxicity, and other potential negative impacts on the environment. With the increased requirements, cost, and risks natural biocide coatings have huge challenges to make it to commercialization.

The protocol of testing various natural biocides for less than one week is too short to determine effectiveness unless the biocide is killing the targeted species. The majority of the natural biocides that effectively inhibit byssal attachment were only exposed to the targeted species for 48 hrs. The possibility exists that the biocides may not have long term performance, as shown with zosteric acid. If long term testing was required then the probability of finding a chemical that works would increase. With current practices it is unlikely that a coating manufacture or investor would take a chance on a compound that has shown only a few days of performance.

Future Considerations

The most promising natural biocides for zebra and quagga mussel control appears to be the compounds extracted from *Pseudomonas fluorescens*. Marrone Bio Innovations needs to evaluate the chemicals for fresh water species before developing natural biocides into an antifouling coating for fresh water use. In order for Marrone to be 100% successful, their formulation should also control the algae, slime, and bryozoans.

Sea Nine 211 should be included in the antifouling coating since that natural biocide targets the subject species. The concentrations of the natural biocides would have to be calculated to provide enough biocide to be effective at preventing all fouling.

Finally, Marrone may need to conduct research on using the correct binder. Most binders are formulated for hydrolysis in seawater. Very few studies have been done for fresh water and the hydrolysis rate might change depending upon the degradation mechanism of the coating. MERL would be willing to set up a cooperative research and development agreement (CRADA) to work with Marrone to develop an antifouling coating fresh water use.

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