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# ***Pseudomonas fluorescens* CL145A (Zequanox®) for Zebra Mussel Control: A Synopsis of Peer-reviewed References**

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Beginning in the early 1990s, researchers at the New York State Museum began investigating bacteria and their natural metabolic products as selective agents for the biological control of zebra and quagga mussels (*Dreissena polymorpha* and *D. rostriformis bugensis*, respectively). This research team discovered that one specific strain (CL145A) of the soil bacterium *Pseudomonas fluorescens* can cause mortality in dreissenid mussels. When ingested via a mussel's filter feeding, a metabolite associated with the bacterium's cell wall destroys the mussel's digestive system and leads to death. Exposure to either live or dead *P. fluorescens* CL145A cells results in mussel mortality. Recently, dead *P. fluorescens* cells have been formulated under the trade name Zequanox®, which has been commercially available since 2012 when the U.S. Environmental Protection Agency (EPA) approved its use for mussel control in enclosed systems (e.g., infra-structures for energy producers and manufacturing companies). The EPA approved Zequanox for open water use to combat invasive mussels in lakes, rivers, and other waterbodies in 2014. To better understand the possible benefits and effects of this product, this report summarizes the available peer-reviewed, scientific literature on *P. fluorescens* CL145A and its effects on non-target organisms.

## **2014**

Meehan, S., B. Gruber, and F.E. Lucy.

2014. Zebra mussel control using Zequanox® in an Irish waterway. *Management of Biological Invasions* 5(3):279-286.

**Synopsis:** This paper presents results from an enclosure experiment that assessed the effectiveness of Zequanox® as a control method for zebra mussels in an open water setting. The pilot demonstration was conducted in an Irish canal that was infested heavily with zebra mussels. Enclosures were treated with a target concentration of 150 mg active ingredient/L water that was maintained for 8 hours. The investigators monitored water quality, observed product dispersion, and measured effects on settled juvenile mussels, seeded adult mussels, and naturally settled adult mussels. Zebra mussel mortality ranged from 46% to 75% in the treatment areas. Temperature and pH ranges appeared unaffected by Zequanox®, but turbidity and total organic carbon in the treated areas increased temporarily and dissolved oxygen levels within the enclosures dropped following treatment. These changes were anticipated as Zequanox® is made up of organic material, which degrades in the natural environment. Water quality parameters returned to background levels within 24 hours following treatment.

Meehan, S., A. Shannon, B. Gruber, et al.

2014. Ecotoxicological impact of Zequanox®, a novel biocide, on selected non-target Irish aquatic species. *Ecotoxicology and Environmental Safety* 107: 148-153.

**Synopsis:** This paper presents results from ecotoxicology tests conducted on three freshwater species that fit within different functional feeding groups: a mussel (*Anodonta*, filter feeder), a non-biting midge (*Chironomus plumosus*, decomposer), and a crayfish (*Autropotamobius pallipes*, omnivore). These organisms were exposed to Zequanox® at concentrations of 100-750 mg active ingredient/L water in 72-hour static renewal toxicity tests. The results suggest that Zequanox® does not negatively affect these organisms at concentrations required for >80% zebra mussel mortality.

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## 2013

Meehan, S., F.E. Lucy, B. Gruber, and S. Rackl.

2013. Comparing a microbial biocide and chlorine as zebra mussel control strategies in an Irish drinking water treatment plant. *Management of Biological Invasions* 4(2):113-122.

**Synopsis:** This paper presents results from a study that compared the use of a developmental formulation of Zequanox® and chlorine treatments at a zebra mussel-infested drinking water treatment plant in Ireland. Zebra mussels were exposed to Zequanox® at concentrations of 200 mg active ingredient/L water for 8 hours. Chlorine treatments used a concentration of 2 mg/L and lasted 7 days. The authors report comparable zebra mussel control results from the two treatments. Both resulted in 100% mortality of juvenile mussels and as much as 80% mortality in adult mussels.

Molloy, D.P.; D.A. Mayer, M.J. Gaylo, et al.

2013. *Pseudomonas fluorescens* strain CL145A – a biopesticide for the control of zebra and quagga mussels (Bivalvia: Dreissenidae). *Journal of Invertebrate Pathology* 113(1):104-114.

**Synopsis:** This paper presents results from a study that used molecular methods to reconfirm that CL145A is a strain of *Pseudomonas fluorescens* and provide a phylogenetic analysis of its relation to other *Pseudomonas* species. The authors report a close relationship between the C145A strain and other *P. fluorescens* strains. The authors provide evidence that the cause of the bacteria's lethal action is a heat-labile (i.e. susceptible to alteration or destruction at high temperatures), secondary metabolite associated with the bacterium's cell wall. They report that this metabolite has a degradable toxicity within 24 hours when applied to water. Their experiments resulted in high levels of mortality for both zebra mussels and quagga mussels and demonstrated that similar mussel mortality could be achieved with treatments lasting 1.5 to 12 hours (as long as the total quantity of bacterial cells applied during the treatment period was the same).

Molloy, D.P., D.A. Mayer, M.J. Gaylo, et al.

2013. Non-target trials with *Pseudomonas fluorescens* strain CL145A, a lethal control agent of dreissenid mussels (Bivalvia: Dreissenidae). *Management of Biological Invasions* 4(1):71-79.

**Synopsis:** This paper reports results from acute toxicity trials (primarily single-dose, short-term exposures of unformulated, laboratory-cultured cells under aerated conditions) of Zequanox® at concentrations lethal to mussels (100-200 mg active ingredient/L water). The authors report no mortality to a ciliate protozoan (*Colpidium colpoda*), a water flea (*Daphnia magna*), three fishes (fathead minnow, brown trout, and bluegill), and seven bivalve mollusk species (blue or common mussel, giant floater, eastern floater, creek heelsplitter, strange floater, lamp mussel or fat mucket, and eastern elliptio). They report low (3%-27%) mortality to an amphipod (scud, *Hyalella axteca*), but believe this to be due to factors other than the Zequanox® exposure. The authors suggest a high host specificity of the product, but recommend additional testing of other susceptible organisms to better define the margin of safety associated with Zequanox® (especially with C145A cells cultured, killed, and formulated using industrial-scale protocols). The authors also note that the use of dead-cell formulations reduces the risk of any non-target infection.

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Molloy, D.P., D.A. Mayer, L. Giamberini, et al.

2013. Mode of action of *Pseudomonas fluorescens* strain CL145A, a lethal control agent of dreissenid mussels (Bivalvia: Dreissenidae). *Journal of Invertebrate Pathology* 113(1): 115-121.

**Synopsis:** This paper reports results from an investigation of the mode of action of *Pseudomonas fluorescens* against zebra mussels. Laboratory trials compared the relative efficacy of live versus dead *P. fluorescens* cells in causing mussel mortality. The authors report that exposure to live and dead bacteria cells resulted in the same temporal patterns and percentage of mussel mortality. Based on these observations, they conclude that the mode of action is intoxication rather than infection. Histological examination after 24 hours of exposure showed hemocyte (immune cell) infiltration into the lumina (central cavity) of the digestive gland and stomach. Mussel deaths occurred following lysis and necrosis of the digestive gland and sloughing of the stomach epithelium. The authors hypothesize that natural products are released from the bacterial cells during the digestive process within the stomach, but note that the specific disruptive mechanisms that occur at the cell level are unknown.

Polanski-Cordovano, G., L. Romano, L.L.C. Marotta, et al.

2013. Nutritional studies on production of antibacterial activity by the zebra mussel antagonist, *Pseudomonas fluorescens* CL0145A. *Journal of Microbiology and Biotechnology* 23(5): 656-660.

**Synopsis:** This paper presents findings from an investigation of the anti-microbial properties of *Pseudomonas fluorescens* CL145A. The authors report that *P. fluorescens* CL145A produces and excretes antibiotic activity against the bacteria *Bacillus subtilis*. It is unclear if this antibiotic production relates to the toxic effect *P. fluorescens* has on zebra mussels and the authors report that they were not yet able to identify the antibacterial compounds produced. The authors discuss culturing the bacteria in a complex corn meal medium to produce the antibiotic, describe a more defined (less complex) medium, and review the effects of carbon and nitrogen sources on production of the antibiotic.

## 2011

Rackl, S.M.

2011. Evaluating the efficacy of a naturally derived invasive mussel control technology, Zequanox™. *Journal of Shellfish Research* 30(2): 546-546.

**Synopsis:** This abstract of a technical paper presented at the annual meeting of the National Shellfisheries Association provides an overview of the commercialization of Zequanox®. The author comments that Zequanox® shows potential for use by fish hatcheries to prevent the spread of zebra mussel veligers during fish stocking.

Allen D. Skaja, A.D.

2011. Natural biocides for zebra and quagga mussel control. Technical Memorandum No. MERL-2011-46. Materials Engineering and Research Laboratory, Bureau of Reclamation, Denver, CO.

**Synopsis:** This report from the U.S. Bureau of Reclamation reviews literature on natural chemical biocides that may potentially kill or disrupt attachment of zebra and quagga mussels to infrastructure. The summary regarding *Pseudomonas fluorescens* is based exclusively on Marrone Bio Innovations' patent of the commercial formulation.

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## 1998

Molloy, D.P.

1998. The potential for using biological control technologies in the management of *Dreissena* spp. *Journal of Shellfish Research* 17(1): 177-183.

**Synopsis:** This paper reviews the potential for using selectively toxic microbes and natural enemies (parasites, predators, benthic competitors) to control zebra mussels. This paper includes one of the first published reports that treatment with *Pseudomonas fluorescens* CL0145A kills zebra mussels. The author reports 80% to 100% mortality in laboratory experiments, without mortality to unionid mussels. The author also reports results from a small-scale trial conducted under once-through conditions within a hydroelectric station on the Mohawk River in New York. The trial achieved a 94% kill rate. The article indicates that a patent application for the use of the bacterium had been filed.

## 1991

Molloy, D.P.

1991. Biological control of zebra mussels: use of parasites and toxic microorganisms. *Journal of Shellfish Research* 10:260.

**Synopsis:** This abstract of a technical paper presented at the annual meeting of the National Shellfisheries Association describes the New York State Museum's initiation of two research projects focused on zebra mussel control. One study focused on the laboratory screening of microorganisms. The article points out that the candidate control microorganisms "would not be 'natural' parasites of zebra mussels, but rather naturally occurring soil and water microbes, which just by 'chance' happen to be highly toxic to zebra mussels when the mussels are exposed to artificially high densities of the microbe." The abstract notes how this approach was used to identify *Bacillus thuringiensis* var. *israelensis*, a widely used and effective control of black flies and mosquitoes.

Synopsis compiled by Dreux J. Watermolen, Bureau of Science Services

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