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Keywords
Corbicula fluminea, Medetomidine, burrowing speed, antifouling, toxicology

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Abstract:

A new anti-fouling drug, medetomidine, was tested to determine if it reduced the burying speed of a freshwater alien-invasive bivalve species, *Corbicula fluminea*. *Corbicula* are known to damage underwater structures and must be managed with chemical paints. The burying speeds of *Corbicula* were measured both before and after exposure to two different concentrations of medetomidine. The burying speed of *Corbicula* before exposure to a $1x10^{-6}$ M medetomidine solution was not significantly different from the burying speed after exposure ($t=.55$, $df=21$, $p=.588$). The burying speed of *Corbicula* was significantly slower after exposure to a $1x10^{-5}$ M medetomidine solution than before exposure ($t=4.08$, $df=8$, $p<.01$). The results of this study indicated that medetomidine could be effective against *Corbicula* at concentrations higher than $1x10^{-5}$ M due to sedation of the foot muscles involved with burying. If so, medetomidine could be a superior chemical for anti-fouling applications compared to older, more toxic compounds.

Introduction:

Corbicula fluminea is an Asian species of freshwater bivalve that has invaded North American waters. This species is dangerous because they have no native predators in North America, can outcompete other native invertebrates, and can foul underwater structures (Hilvarsson *et al.*, 2009). Commonly harmed structures include boat hulls, pipes, drains, marinas, underwater cables, buoys, and commercial fishing equipment. *Corbicula*, and many other related species, pose a nuisance to humans because the damage they can cause is very costly. In the past, toxic biocides laced with heavy metals

like Tributyltin have been used to prevent fouling, but were very harmful to the environment (Bellas *et al.*, 2006). These chemicals, although incorporated into anti-fouling paints, managed to dissolve into the water in significant quantities and dispersed throughout the ecosystem. Bioaccumulation of these chemicals was found in a diverse array of wildlife and caused noticeable and lasting harm to them (Bellas *et al.*, 2006). Most were so terrible that they have been banned in recent decades by International Maritime Organization legislation (Bellas *et al.*, 2006). New restrictions on biocides have stipulated research and development on new compounds for use in underwater structural protection. This study was done to investigate a new, more environmentally friendly chemical to resolve the problems associated with fouling.

A drug called medetomidine was examined to see if it had any effect towards the reduction of burying speed in *Corbicula fluminea*. Medetomidine was originally created as a synthetic alpha-2 adrenegeric-agonist sedative for use in veterinary medicine (Bellas *et al.*, 2006). In addition to its effectiveness in that role, medetomidine has been found to be comparable in anti-fouling applications against barnacles (Ulrika *et al.*, 2010). After extensive testing, medetomidine was determined to be more eco-friendly than its toxic predecessors and worth continuing to study (Hilvarsson *et al.*, 2009). I hypothesized that exposing *Corbicula* to dilute concentrations of medetomidine would significantly slow down their burying speed. Since medetomidine is a sedative to many species, it should sedate the foot muscles in the *Corbicula*, which is their main organ involved with burying (Olsson and Phalen, 2012). As a result, the foot would not be able to work as quickly or efficiently, and a decrease in burying speed would be observed. By seeing a reduction in the rate of burying, a reduction in the efficiency of other biological processes integral to

fouling could also be expected. This would reinforce other pieces of evidence agreeing that medetomidine is an effective and desirable anti-fouling biocide.

Materials and Methods:

I collected *Corbicula fluminea* near Dick's Dam in the Conewago Creek, PA. Roughly 110 *Corbicula*, averaging 1.0 cm in width (+/- 0.3 cm), were collected and transported in river water back to the laboratory at Gettysburg College. The contents of the bag were placed into an aerated aquarium with sand in the bottom. Burying apparatus were made using labeled, plastic 100ml beakers filled with 40ml of KolorScape green label all-purpose sand (manufacturer part number 40105120) and 40ml of dechlorinated tap water. The *Corbicula* were transferred from the common aquarium into the burying apparatus and were allowed to acclimate. The time it took each *Corbicula* to bury was recorded with stopwatches to establish control times. The apparatus were allowed to rest for 18 hours after the control timings. The samples were then drugged with a $1x10^{-6}$ M or $1x10^{-5}$ M medetomidine solution for 24 hours following the resting period. The $1x10^{-6}$ M was the first concentration chronologically tested. The burying times of the *Corbicula* were recorded again following an acclimation period, and correlated sample two-tailed t-tests tests were used to analyze the data for any differences. A total of 69 replicates were exposed to the $1x10^{-6}$ M medetomidine, and 37 replicates were exposed to the $1x10^{-5}$ M medetomidine. The maximum time limit that the *Corbicula* were given to bury was 30 minutes after acclimation. Usable data from this study was referred to as viable, and came only from the specimens with recordable burying times (shorter than the maximum time limit) for both before and after drug exposure. Any specimen that had at least one

maximum burying time for either before or after exposure was considered non-viable and was statistically unusable.

The original protocol of this study involved placing the *Corbicula* in the center of the burying apparatus, with their shell openings facing up, and were allowed to acclimate until the shells began to open. A buried *Corbicula* was defined as one that's uppermost point of the shell was below the plane of the sand, one that was at least halfway buried by surface area and had not moved in the last two minutes, or one that was at least halfway buried by surface area and closed its shell completely for at least ten seconds. Satisfaction of any one of these three criteria would stop the timing of that specimen. This protocol had to be modified partway through the experiment based on the resulting data. The modification was made after the first 31 samples were completed with the $1x10^{-6}$ M medetomidine concentration. The shell openings were then placed downwards toward the sand, the *Corbicula* were given only two minutes to acclimate, but were still considered buried when they fell under the same circumstances as before. The remaining $1x10^{-6}$ M concentration samples, and all of the $1x10^{-5}$ M concentration samples were done following the modified protocol. Any samples that were not viable after the control timing under the original protocol did not receive any further drug treatment. After the modification in protocol, all samples received drug treatment regardless of viability in the control timing.

Results:

The null hypothesis that medetomidine exposure does not significantly slow down the burying speed of *Corbicula* was not rejected with the $1x10^{-6}$ M concentration. There

was no significant difference between the mean burrowing times of *Corbicula* after exposure to a $1x10^{-6}$ M medetomidine solution than before exposure (Figure 1)(t=0.55, df=21, p=0.588). The null hypothesis was rejected with the $1x10^{-5}$ M medetomidine data. The mean burrowing speed of the *Corbicula* was significantly slower after exposure to a $1x10^{-5}$ M medetomidine solution than before exposure (Figure 2)(t=4.08, df=8, p<.01). Out of the 106 total specimens tested, only 31 were found to be viable. Twenty-two of the 31 were exposed to the $1x10^{-6}$ M medetomidine solution, and the remaining nine were exposed to the $1x10^{-5}$ M. Combined from both concentrations, seven samples also recorded a time below 30 minutes before exposure and a time over 30 minutes after exposure. Twenty-three samples recorded a time higher than 30 minutes before exposure, but below 30 minutes after exposure. No significant difference was found due to the protocol modification partway through experiment, so all $1x10^{-6}$ M concentration data was pooled together (t=1.09, df=20, p=0.289).

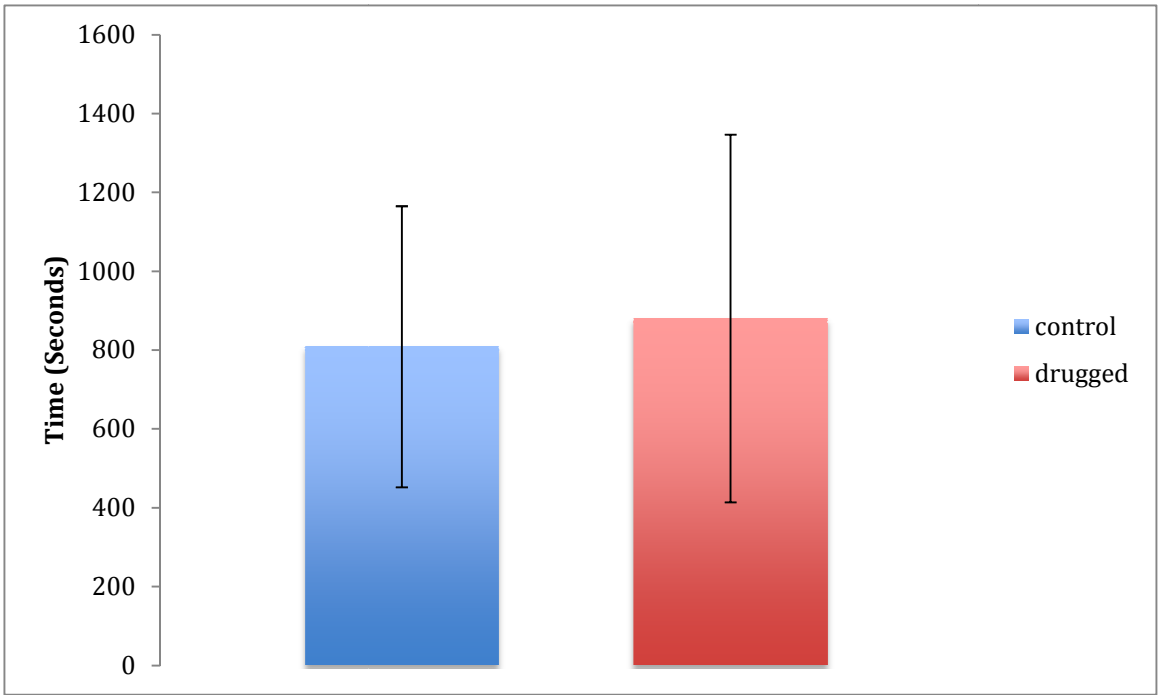

Figure 1: A comparison of the mean burying times of *Corbicula fluminea* before and after exposure to a $1x10^{-6}$ M medetomidine in laboratory beaker apparatus (+/- stddev).

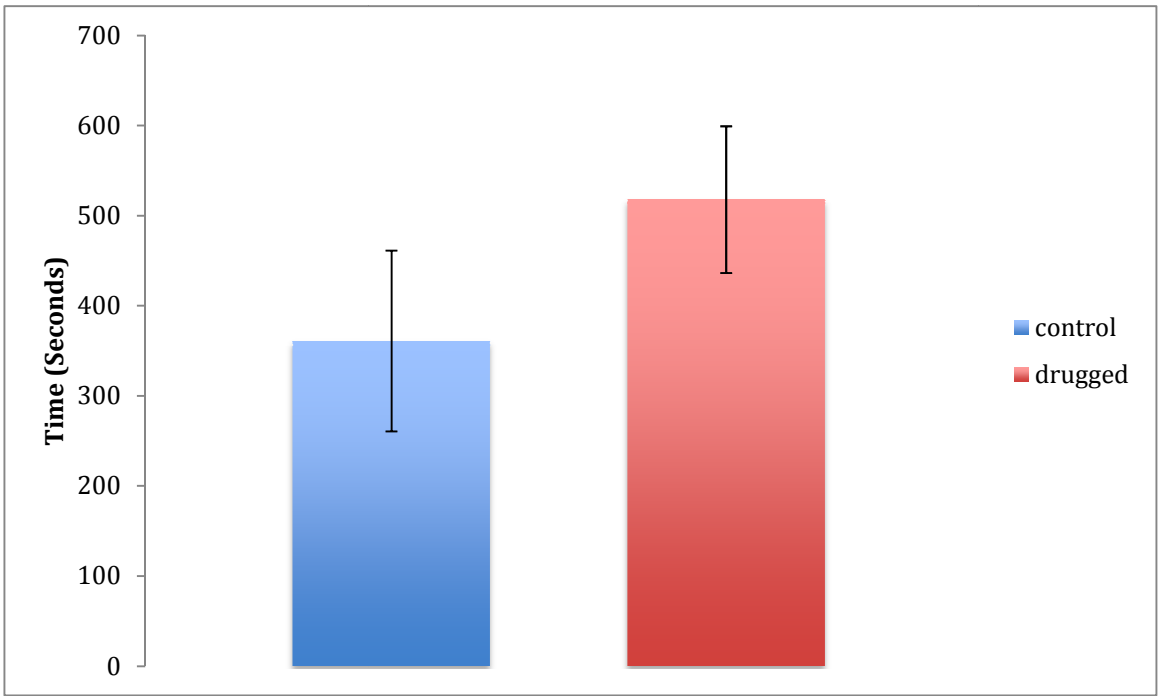

Figure 2: A comparison of the mean burying times of *Corbicula fluminea* before and after exposure to a $1x10^{-5}$ M medetomidine in laboratory beaker apparatus (+/- stddev).

Discussion:

The medetomidine had an effect on the burying speed of *Corbicula*. Almost every specimen's burying speed changed after being exposed to the medetomidine. Correlated sample two-tailed t-tests could not conclude any substantial differences in the $1x10^{-6}$ M data, but the mean post-exposure burying speed was still slower than before exposure. A true reduction in burying speed in *Corbicula* was observed after exposure to the $1x10^{-5}$ M medetomidine solution; all nine of the usable replicates slowed down after being exposed. A few specimens appeared to have an increased burying speed, but were being compared to non-viable times greater than 30 minutes. Most specimens that had an increased burying speed had similar post-exposure burying times to the specimens that slowed down, so the control tests were misrepresentative. This can indicate that medetomidine could speed up the burying process of *Corbicula*, however this data was not precise, was not statistically tested or proven, and was most likely erroneous. It was observed that most *Corbicula* could bury themselves in less than 10 minutes if they were motivated to do so immediately after acclimation. If this held true in real life, then there would have been a better indication of a difference in burying speed at $1x10^{-6}$ M. Only a few samples' burying times remained very similar for both before and after exposure, and more should have been very similar regardless of concentration if the chemical truly did not have any effect on the *Corbicula* regardless of concentration.

Related studies have been conducted on the effectiveness of medetomidine. A marine bivalve, *Abra nitida*, was drugged with dilute concentrations of medetomidine. The bivalves' burrowing behavior, sediment reworking capability, and feces production were all investigated (Bellas *et al.*, 2006). It was concluded that the medetomidine

significantly slowed down all of the biological processes except for feces production (Bellas *et al.*, 2006). This study was structurally similar to mine and indicated that medetomidine would be useful as an anti-fouling chemical. Another positive conclusion about medetomidine, found in another study done on toxicity to several aquatic species, was that most of the effects from the chemical were reversible after the specimens were removed from the drugged environment (Hilvarsson *et al.*, 2009). This would be a valuable environmental characteristic for a new anti-fouling chemical to possess because the older biocides severely harmed other aquatic flora and fauna (Hilvarsson *et al.*, 2009). A downside to this characteristic would be that it could indicate that medetomidine was less effective at permanently removing fouling organisms compared to other alternatives, or could fail to meet an effectiveness expectation.

Medetomidine affects more than just the targeted invertebrates in an aqueous environment. A study was done on amphipods to determine if medetomidine had any effect on them (Krang and Dahlström. 2006). It was found that dilute concentrations of medetomidine influenced production, sensitivity, and reaction of amphipod pheromones (Krang and Dahlström. 2006). A decreased reliance on pheromones for mate attraction was observed, which significantly reduced reproductivity and interactions between the sexes (Krang and Dahlström. 2006). Medetomidine exposure to fish species was tested because they would also be collaterally effected by anti-fouling chemicals. Turbot, rainbow trout, and lumpfish, were all observed to have a reduction in respiratory functioning and oxygen efficiency due to dilute medetomidine (Lennquist *et al.*, 2010). Pigment changes that effected visual appearance and active camouflage defense systems were also noticed (Lennquist *et al.*, 2010). The effects of medetomidine were found to be

reversible in all of these species, and were less severe when compared to older biocides, but may still be harmful to the greater aquatic ecosystem (Lennquist *et al.*, 2010).

The skeletal muscles of an estuarine species of crocodile were injected with dilute concentrations of medetomidine (Olsson and Phalen, 2012). It was found to act as a strong, rapid sedative and muscle relaxant, similar to examples seen in veterinary medicine, making it effective in tranquilizer applications (Olsson and Phalen, 2012). The medetomidine was observed to function in blocking muscle activity, but could be remediated through time or antidotes and left no permanent damage on the animal (Olsson and Phalen, 2012). The foot muscles in *Corbicula* involved with burying could have been affected in a similar manner as the crocodiles, and could explain the reduction in burying speed after medetomidine exposure.

In another study, the effects of medetomidine on a species of marine barnacle, *Balanus improvisus*, were examined. It was observed in dilute concentrations, that medetomidine interacted with octopamine receptors in the barnacles after exposure and increased the functioning of various biological pathways (Ulrika *et al.*, 2010). The resulting hyperactivity of the barnacles prevented their attachment and cementation systems from working for binding to structures (Ulrika *et al.*, 2010). Medetomidine proved to be an effective bio-foulant in this situation. Despite going against some presumptions of my hypothesis, a similar effect could be induced on *Corbicula* that might result in faster burying times, assuming the observed increases in burying speed in some specimens were not erroneous (Hilvarsson *et al.*, 2009).

My study was complicated because only about half of the control samples actually buried, and then only a fraction of those buried again after being drugged. This resulted

in a low amount of usable data with a high degree of variance. A difference in individual sensitivity to medetomidine within a population could explain some of the variability. A seasonal difference to sensitivity and biological functioning in *Corbicula* may also explain the variability, even though this was proven to not be true in marine mussels drugged with similar chemicals (Hall, 1999). A reduction in burying speed in most individuals was likely attributed to the sedative properties of medetomidine on the burying foot muscles of *Corbicula* (Olsson and Phalen, 2012). Any increases in burying speed could have been attributed to dilute medetomidine interactions with octopamine receptors, like they did in barnacles, if they were not erroneous (Ulrika *et al.*, 2010). The high amounts of data variance may be related to temperature, freshness of the samples, seasonal drug sensitivity, natural variability within a population, and from the cooperation of the *Corbicula* themselves. The position of the *Corbicula* had little to do with the results, since the foot was placed in two different positions and little difference was observed in the burying times. Almost all of the *Corbicula* gave an indication of being alive, such as opening up or attempting to dig, so unusable data did not result from dead specimens.

Based on the results of my experiment, it was evident that further testing should be done before finally accepting or rejecting medetomidine as a solution to fouling. This study should be repeated with a larger sample size, and should also be done at different times of the year, at different temperatures, and with different substrates. *Corbicula* from different areas should also be tested, as well as different fouling species, and with different concentrations and methods of chemical exposure. The testing of medetomidine on many other aspects and components of the freshwater ecosystem should also be done

to determine any greater environmental effects. Additional obstacles to my study were the limited time span of it, the simple laboratory equipment, and the modifications in the experimental protocol. Seasonal weather, scheduling conflicts, and unexpected events also applied additional constrictions to my experiment and could not have been avoided. My experiment was successful as a preliminary study, as it lent some insight into solving the anti-fouling problem with medetomidine. Medetomidine is still a good candidate for anti-fouling purposes and is a better option for the environment compared to older chemicals; however, further studies should still be done to confirm or deny this alternative compound as a marketable anti-foulant.

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