BURROWING RESPONSE OF THE HARD CLAM, *MERCENARIA MERCENARIA* UPON EXPOSURE TO TOXIGENIC MICROALGAE

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ABSTRACT

We experimentally compared burrowing behavior of hard clams (*Mercenaria mercenaria*, Lamarck 1819) when exposed to bloom densities of several toxigenic microalgae. Sediment burial is a major mechanism used by clams to reduce predation, and to isolate themselves from unfavorable conditions in the water-column. Inhibition of clam burrowing has been used to assay for heavy metal contamination in estuarine sediments (e.g. Cd, Cu) and narcotic and/or neurotoxic substances. We hypothesized that infaunal clams would exhibit a rapid burrowing response to toxic algae. In these experiments, the most pronounced burrowing response was documented for clams exposed to *Karlodinium micrum*. Relative to filtered seawater (0.2 µm-porosity) and nontoxic algal controls (*Storeatula major*, HP9101 isolate), after 40 minutes a significant burrowing response had occurred, and a notable reduction in siphon activity was also observed. A similar but less dramatic burrowing response was shown by clams exposed to *Pfiesteria* spp. In contrast, clams exposed to *Alexandrium monilatum* and *Karenia* spp. apparently were narcotized and their burrowing response was less than that of control animals. Lack of a burrowing response could render clams more vulnerable to predators, whereas extended burial could restrict access to food resources.

Figure 2. (A) Side view of the experimental setup, (B) overhead view of the experimental setup as imaged through a digital video recorder.

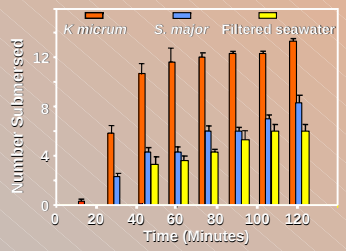

Figure 3. Burrowing response of *M. mercenaria* with *K. micrum* at 3,000 cells mL^{-1} (30 ppt, n=3, number submersed as means ± 1 SE of a total of 15 animals).

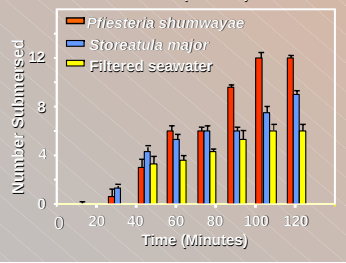

Figure 4. Burrowing response of *M. mercenaria* with *P. shumwayae* (1048C Isolate) at 3,000 cells mL^{-1} (30 ppt, n=3, number submersed as means ± 1 SE of a total of 15 animals).

Figure 1. Representative burrowing progression observed for *M. Mercenaria* exposed to 3 * 10^3 cells mL^{-1} of *Karlodinium micrum* (time given in minutes)

MATERIALS AND METHODS

Acclimated *M. mercenaria* were held in a recirculating seawater system prior to experimentation and maintained on a commercially available, mixed algal assemblage (Shell 1800©, Reed Mariculture, Inc.). Toxigenic dinoflagellate cultures (*Karlodinium micrum, Pfiesteria* spp., *Karenia brevis, Alexandrium monilatum,* or *Prorocentrum minimum*) were grown in seawater media enriched with Guillard's f/2 media and maintained in a temperature-controlled incubator (30 ppt, 22°C, 100 µmol photons m^{-2} s^{-1}; the heterotrophic, *P. shumwayae*, was previously fed fish prey).

All experiments were run in autoclaved 1-L plastic containers provided with gentle aeration (0.22-µm-filtered, 2 bubbles s^{-1}) (Figure 2). Shellfish (n=15) were exposed to treatment concentrations (< 1.0 x 10^4 cells mL^{-1}) for 24 hours. Phytoplankton samples were collected at 15-minute intervals and preserved with acidic Lugol's solution, as well as any feces produced by the bivalves. These samples were later analyzed with light microscopy (Model AX-70; Olympus Corp., Melville NY) to verify grazing activity and qualitatively examine pseudofecal material or fecal strands.

During experiments, a digital video recorder (Model MV1; Canon U.S.A., Lake Success NY) was used to document the number of clams that had burrowed (defined here as ≥ 80% of the shell below the sediment surface). General behavior (e.g. foot and/or siphon extension) was also noted.

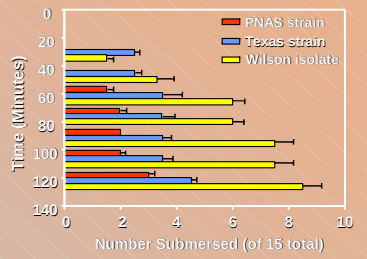

Figure 5. Burrowing activity of *M. mercenaria* exposed to three strains of *K. brevis* at densities of 3,000 cells mL^{-1} (30 ppt, n=2; means ± 1 SE).

RESULTS AND DISCUSSION

The burrowing activity of *M. mercenaria* differed substantially among the four species of toxigenic microalgae tested. The most pronounced burrowing response was observed for *M. mercenaria* exposed to *Karlodinium micrum* (Figure 1). Most *M. mercenaria* individuals burrowed within 45 minutes of exposure to elevated densities (3,000 cells mL^{-1}) of *K. micrum* (Figure 3). By comparison, *M. mercenaria* exposed to the nontoxic cryptomonad *S. major* or filtered seawater typically required 2-4 hours for a similar number of burrowed individuals (data not shown). A similar but less pronounced burrowing response was observed for clams exposed to a toxic clonal isolate of *P. shumwayae* at similar cell densities (Figure 4). The observed behavior may have represented an attempt at isolation from unfavorable water-column conditions created by the toxigenic organisms.

Variations in burrowing activity were also observed for clams exposed to different strains of *K. brevis*. Normalizing for cell densities of the three *K. brevis* strains, we found that *M. mercenaria* exposed to the PNAS Isolate were least likely to burrow, with an intermediate response to the Texas strain and highest burrowing activity when exposed to the Wilson strain (Wilson>Texas>PNAS; Figure 5). Clams exposed to *A. monilatum* (AMO3 strain) and *K. brevis* (PNAS strain) appeared somewhat lethargic relative to their activity in other treatments.

Burrowing activity also varied depending on the initial concentration of algal cells inoculated into the treatment vessels (Table 1). At the highest cell densities, *M. mercenaria* exposed to *A. monilatum* burrowed significantly more then at lower cell densities ($P < 0.05$), whereas *M. mercenaria* burrowed at higher densities of *P. minimum*. Such behavior may have been influenced by feeding processes or bioactive substances associated with the toxigenic algal cultures.

Table 1. Cell density-dependent variation in burrowing activity among *M. mercenaria* exposed to three microalgal species (30 ppt, n=2, number submersed as means ± 1 SE of a total of 15 animals).

Algal Cell Density (Cells mL^{-1})	*A. monilatum* (AMO3 isolate)	*Prorocentrum minimum* (NEU-1 isolate)	*S .major* (HP9101 isolate)
100	7.0 ± 0.44	8.5 ± 0.22	7.0 ± 0.44
1,000	4.0 ± 0.44	10.0 ± 0.44	8.0 ± 0.44
3,000	5.0 ± 0.89	10.5 ± 1.10	6.5 ± 0.67
7,500	11.5 ± 0.67	7.5 ± 0.22	5.5 ± 0.22

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