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EFFECTS OF WHOLE DRILLING MUD AND SELECTED COMPONENTS ON THE SHELL MOVEMENTS OF THE BAY SCALLOP, *Argopecten irradians*

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ABSTRACT: The shell movements of bay scallops (*Argopecten irradians*) were electronically monitored before and after different amounts of whole drilling mud, barite, lignosulfonate, and calcium carbonate were added to their tanks. Movements were compared with those made by scallops exposed to seawater for the same duration using six response measures. For whole drilling mud, a graded dose-response relationship existed for two response measures: change in the number of major Rapid Valve Closures (RVCs) and change in the cumulative magnitude of all RVCs. Ejection of pseudofeces is frequently associated with RVCs. Scallops tested simultaneously with barite, lignosulfonate, and calcium carbonate showed irregular but similar dose-response relationships for these two response measures. Three other measures (changes in gape width, RVC magnitude, and number of all RVCs) were not reliable indicators of responsiveness for any materials. None of the materials caused significant changes in the number of swimming attempts, but only one-third of the animals ever attempted to swim. Scallops exposed to seawater showed no significant change for any response measure.

Drilling mud and drill cuttings may be discharged into the surrounding water when drilling for oil in the marine environment. Ambient water currents distribute this material in the water column and on the bottom around the drilling rig (Monaghan *et al.*, 1976; Richards, 1979). Measurements of actual concentrations discharged into the water are not reported. Drilling muds are chemically complex and contain water-soluble and water-insoluble components.

Relatively few critical studies have evaluated the influence of whole mud or its components on the marine environment. Effects of whole drilling mud and components have been documented on the species composition of benthic communities (Tagatz *et al.*, 1977, 1978; Tagatz and Tobia, 1978; Cantelmo and Rao, 1978; Cantelmo *et al.*, 1979). Thompson and Bright (1977) found that hermatypic coral polyps could not remove whole drilling mud settling onto their epidermis, but could remove equal doses of barite, aquagel, and calcium carbonate. Studies on other species and other behaviors are lacking.

The present study was designed to measure the effects of whole drilling mud and two of its major particulate components (barite and lignosulfonate) on the shell movements and swimming responses of the bay scallop, *Argopecten irradians.* A chemically innocuous particulate, powdered calcium carbonate, was also tested for comparison. Bay scallops were chosen because they are commercially important filter feeders and occur in areas of the Gulf of Mexico where drilling occurs. Also, because swimming is a common behavior for scallops, it was anticipated that they might display an escape response when exposed to drilling mud contaminants. Because the shell movements of bivalves have not been analyzed in detail before, it was not known which parameters would most clearly reveal responses to particulate matter. Therefore, this study also comprises an analysis of the relative reliability of various shell movement parameters as indicators of response to pollutant materials.

MATERIALS AND METHODS

Apparatus

Each scallop was cleared of encrusting organisms and attached by its lower valve to an individual monitoring frame. The movable upper valve was mechanically linked to an inductance transducer which was connected by waterproof cable to external circuitry. This method required the scallop's hinge ligament to lift 2 g (dry weight) in the process of opening the valves. This was not considered an unusual detriment because we regularly encountered scallops with more than 5 g (dry weight) of encrusting organisms on the upper valve. The external circuitry consisted of an oscillator to drive the transducer and a rectifier to sample the voltage drop across it. Each rectifier's output was plotted on a chart recorder operating at 2.5 cm/min. Complete details on the circuitry and calibration procedures can be obtained from the first author. Figure 1 shows sample traces for swimming attempts and a period of normal feeding activity.

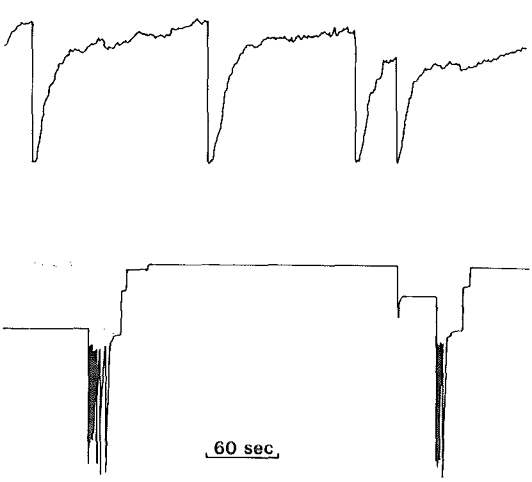

Figure 1. Sample traces for a scallop exhibiting four Rapid Valve Closures (RVCs) during a period of normal activity (top) and a scallop exhibiting two swimming attempts (bottom).

Two scallops and their monitoring frames were maintained in each of two tanks for testing. The 60-cm diameter tanks were constructed following the basic design of Creutzberg (1963). A centered drain in the cone-shaped bottom of each tank led to an air-lift pipe which ascended the tank's outer wall and emptied into its periphery. The inside diameter of the paddle-containing cylinder centered in each tank was 30 cm. The paddles in both tanks were driven with a single motor. The current speed in the peripheral compartment of each tank (where the scallops were located) was 6 cm/sec during all tests. The tank's shape, the current circulation, and the air-lift insured continuous recycling of the water, thus helping to keep particulate materials in suspension. Two fluorescent bulbs were centered over each tank. The test tanks and all apparatus were isolated in a small room, with an investigator present only when test substances were added.

Test Substances

A drilling mud sample was obtained from a Mobil Oil Co. rig located in Mobile Bay, Alabama, on August 7, 1979. The mud had a smell resembling diesel fuel, a substance sometimes added to mud on drilling rigs. The mud sample was transported to the U.S. Environmental Protection Agency Laboratory in Gulf Breeze, Florida, where it was stored temporarily at 13° C, and later at 3° C. The mud was thoroughly mixed before testing. Independent analysis (Science Applications, Inc., La Jolla, CA) of a sample of this mud for heavy metals found it to contain (on a weight basis) 56% water, 18.6% Ba, 2.5% Al, 1.87% Fe, 0.23% Cr, 0.0083% Zn, and 0.00047% Pb; the remaining components (20.8%) were not identified but should have included lignosulfonate, rock particles, and seawater salts. Barite (IMCO-BAR) and lignosulfonate (IMCO RD-111) were obtained from IMCO Services in Mobile, AL the distributor that supplied the Mobil Oil rig. Powdered calcium carbonate (analytical grade) was obtained from Mallinckrodt Chemical Works.

When aliquots of the mud sample were dried by heating, the average residue concentration was 440 g/l. This figure was used to determine the volume of whole drilling mud needed for standard concentrations in the test tanks. Concentrations of barite, lignosulfonate, and calcium carbonate were computed on a weight basis.

Test Procedures

Adult scallops (shell height ≥ 45 mm) were collected periodically from shallow grassflats in East Bay, Panama City, Florida. They were held in 10-cm deep outdoor tanks exposed to natural photoperiod and unfiltered seawater from Santa Rosa Sound (flowthrough system). This same water, to which the animals were already acclimated, was also used in the two tanks during tests. Water temperature and salinity were always ambient.

Tests were conducted from August 15, 1979, to February 20, 1980. During that period, salinity ranged form 18 to 26 ppt and temperature ranged from 9 to 28° C. For a given concentration, 9 to 12 scallops were tested with each of the four substances, and with ambient seawater (the control), following a rotating sequence. Once all scallops were tested at one concentration, testing at a new concentration was begun. Hence, all control and experimental scallops tested at one concentration experienced approximately the same temperature and salinity.

Preliminary observations revealed considerable variation in shell movement patterns among animals, with much less variation for individuals over time. For this reason we compared the responses of individuals before and after exposure to a test substance. Thirty minutes after the four scallops were introduced to tanks containing undosed seawater (a period of acclimation), a 54-min pre-dose period of monitoring was begun. Measured amounts of whole mud, barite, lignosulfonate, or calcium carbonate were then added to appropriate tanks; nothing was added during seawater control tests, but those scallops were equally disturbed by the investigator's movement in the room. Preliminary observations indicated that the scallops' behavior stabilized within 5 min after introduction of test substances and departure of the investigator from the room. Therefore, after 5 min had elapsed, a 54-min post-dose period of monitoring was begun. Both tanks were flushed with clean seawater and scrubbed clean between tests.

Data Analysis

The following data were obtained from recorder charts for both the pre- and post-dose periods for each scallop:

1. The instantaneous shell gape distance (mm) at 2-min intervals. This yielded 27 measurements for each period.

2. The change in gape distance (mm) resulting from each Rapid Valve Closure (RVC) and the number of RVCs. An RVC was defined as a change in gape width completed within 2 sec. In practice, most closures were completed in less than 1 sec and we could detect valve closures as small as 0.3 mm. Those occasional scallops exhibiting fewer than three RVCs during the pre-dose period were excluded from the analysis as these were assumed to be unhealthy.

3. The number of swimming attempts. A swimming attempt consisted of a series of RVCs occurring in rapid sequence, with the series usually not lasting longer than 5 sec.

It became apparent from observing the shell movement traces of individual scallops that the frequency distribution of the magnitude of RVCs was often bimodal, even during pre-dose periods; over a period of time, scallops tended to exhibit either large magnitude or low magnitude RVCs. Examples for two scallops are shown in Figure 2. Because large magnitude RVCs were probably associated with ejection of pseudofeces, we felt it was desirable to analyze responses to particulates on the basis of changes in large magnitude RVCs, or as we term them here, 'major' RVCs.

This approach required defining what constituted a major RVC for each individual. As illustrated in Figure 2, most major RVCs for one scallop were in the 8 to 12-mm range, while most major RVCs for the other scallop were in the 5 to 9-mm range. Our solution was to define a major RVC for a scallop as any RVC greater than or equal in magnitude to the average RVC exhibited by that scallop during the pre-dose period. This criterion was then applied to both the pre- and post-dose RVCs of a scallop to identify those RVCs which were major for that animal.

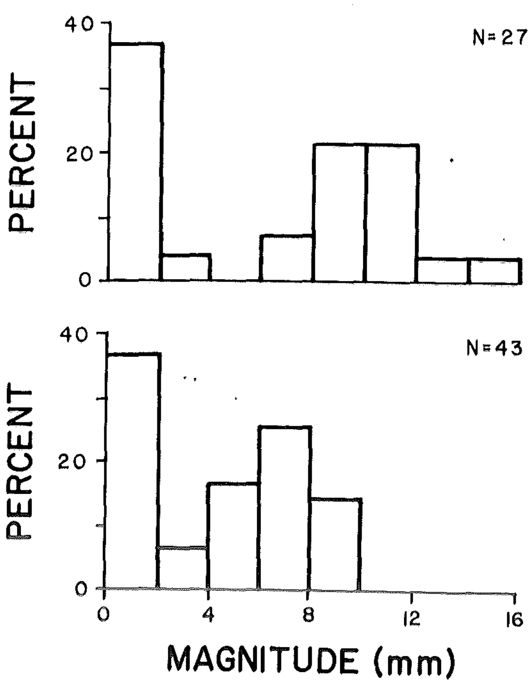

Figure 2 Frequency distributions for the magnitude of all RVCs exhibited by two scallops during 54-min pre-dose monitoring periods.

It was also apparent from observing the shell movement traces of individual scallops during post-dose periods that some individuals responded by making few large-magnitude RVCs while others responded by making many small-magnitude RVCs. Hence it was also desirable to analyze responses on the basis of cumulative magnitude of all RVCs.

From these data, the following statistics were examined as possible measures of responsiveness of scallops to the test substances. Each change refers to a comparison between the pre- and post-dose period.

1. The change in average instantaneous gape distance
2. The change in average magnitude of all RVCs
3. The change in number of all RVCs
4. The change in number of all major RVCs
5. The change in cumulative magnitude of all RVCs
6. The change in number of swimming attempts

RESULTS

In order to compare the usefulness of the first five response measures, the dose-response relationship was examined visually for each substance-response measure combination, and a one-way ANOVA was performed on the response data for the five concentrations tested, for each substance-response measure combination (Table 1). Using the criteria of which response measures showed graded dose-response relationships for test substances, and which response measures showed significant differences among the concentration test groups for the most test substances, two response measures were selected for closer scruti-

Table 1. Values of the F-statistic from one-way ANOVA performed on response data for the five concentrations tested, for each substance-response measure combination. Denominator degrees of freedom are indicated in parentheses after each substance.

	SUBSTANCE TESTED				
Response Measures	Mud (47)	Lign. (46)	Barite (49)	Calc. (47)	Water (43)
Gape Distance	3.53*	.85	1.14	2.33	1.03
Magnitude of All RVCs	2.02	1.11	3.19*	1.20	1.03
Number of All RVCs	2.23	2.77*	1.21	1.68	.24
Number of Major RVCs	.98+	3.04*	2.24	2.27	.93
Cum. Magnitude of All RVCs	3.44* +	3.38*	.71	1.35	.31

*Significant difference among concentrations at P<.05.
+Graded dose-response relationship.

ny. Figure 3 shows the dose-response relationships for all four test substances for the change in number of major RVCs and Figure 4 shows these relationships for the change in cumulative magnitude of all RVCs. Because no significant differences existed among the concentration test groups in any of the response measures for scallops which were only exposed to seawater (Table 1), data from these "control" animals were subsequently pooled to obtain the 95% confidence interval for their responses shown in each figure.

Figures 3 and 4 both show the same basic response patterns for the same test substances. Whole drilling mud produced a graded dose-response relationship in both measures, with a greater number and larger cumulative magnitude of RVCs at 600 ppm and a lower number and lower cumulative magnitude of RVCs at 50 ppm, as compared to control scallops. Although lignosulfonate, barite, and

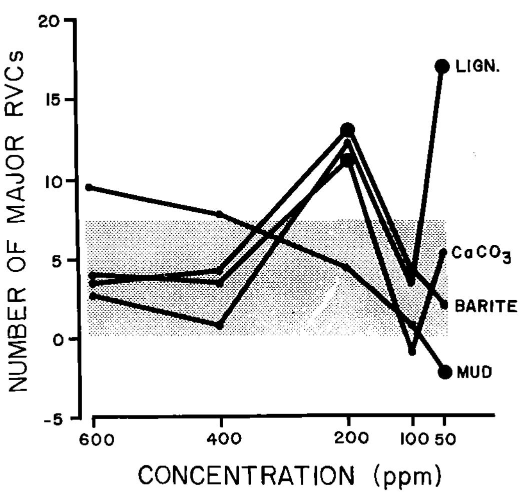

Figure 3. Average change in number of major RVCs caused by different concentrations of drilling mud, lignosulfonate, barite, and calcium carbonate. Nine to 12 scallops were tested at each concentration of each test substance. The shaded area indicates the 95% confidence interval for all control scallops tested. The larger dots indicate points significantly different from the controls at P<.05 using the Mann-Whitney *U*-test.

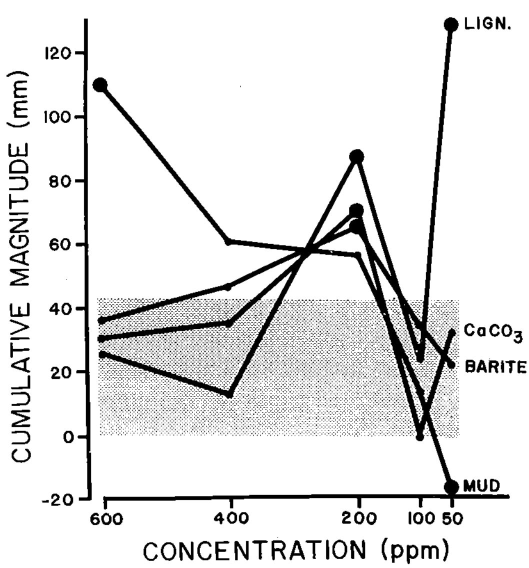

Figure 4. Average change in cumulative magnitude of all RVCs caused by the same four test substances. Shaded area and larger dots as in Figure 3.

calcium carbonate did not produce graded dose-response relationships for either measure, they all exhibited strikingly similar response patterns for both measures. All three substances produced a greater number and larger cumulative magnitude of RVCs at 200 ppm, as compared to controls, but lower values of these responses at 400 and 100 ppm. Of these three substances, the response patterns for barite and calcium carbonate appear more similar to each other than either's response pattern does to that for lignosulfonate. This is especially evident at 50 ppm where lignosulfonate produced significantly higher values of both response measures, as compared to controls.

Of the 250 animals studied, only 83 (33.2%) attempted to swim at any time during pre- or post-dose monitoring periods. If at least two scallops tested with the same material at the same concentration attempted to swim, a paired t-test was conducted. None of the 22 (out of a possible 25) test groups revealed a significant change in swimming frequency between pre- and post-dose periods.

DISCUSSION

Of the six shell movement parameters studied, three were not reliable indicators of response according to the criteria indicated in Table 1 (change in valve gape, change in RVC magnitude, and change in number of all RVCs). There were no significant changes in a fourth parameter, number of swimming attemps, but swimming attempts were relatively uncommon. Gruffydd (1976) found that only 4 to 11% of scallops (*Chlamys islandica*) swam at current speeds of 5 cm/sec, and that the percent swimming increased with current speed. The current speed during our tests (6 cm/sec) may have been too low to observe frequent swimming.

The final two shell movement parameters (change in number of major RVCs and change in cumulative RVC magnitude) were more reliable indicators of responsiveness, presumably because these parameters were least influenced by the natural variations exhibited by scallops. The major RVC parameter permits defining what constitutes a 'major' or large closure for each scallop while the cumulative RVC magnitude parameter ignores individual differences in response pattern. Using these last two shell movement parameters, certain conclusions can be drawn about the effects of whole drilling mud and the other materials on scallop behavior.

The first conclusion is that drilling mud produces opposite effects at different concentrations. This is most evident when using cumulative magnitude of all RVCs as the response measure (Figure 4). Mud at 600 ppm produced a significantly greater cumulative magnitude of RVCs than controls, while mud at 50 ppm produced a significantly lower cumulative magnitude of RVCs ($P<.05$, Mann-Whitney *U*-test); the response was graded at intermediate concentrations. This kind of dose-response relationship suggests the presence of substances in drilling mud which are antagonistic in their effect and have different relationships between dose and absolute magnitude of effect. The complex composition of whole drilling mud makes such a conclusion reasonable. For example, particulate substances such as barite, portions of lignosulfonate, and powdered rock may have a stimulatory effect on RVCs (at least up to some maximum "shut-down" concentration) while non-particulate substances such as the dissolved portions of lignosulfonate, diesel fuel, and chlorinated hydrocarbon biocides (e.g., pentachlorophenol) may have an inhibitory effect on RVCs.

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The second conclusion is that a clear dose-response relationship does not exist for the three 'pure' particulates tested (barite, lignosulfonate, calcium carbonate), although as a group they all exhibited approximately the same pattern of effect. A greater effect was observed for these three materials at 200 ppm than at either of the two higher concentrations, a situation not observed for whole mud, which was tested simultaneously. The basis of this irregular pattern of effect is unknown but the phenomenon appears to be real as all three particulates exhibited the same response pattern for both response measures.

Of the three 'pure' particulates whose patterns of effect are shown in Figures 3 and 4, barite and calcium carbonate have the most similar patterns. A possible explanation for this lies in the nature of the materials involved. Microscopic examination showed that the barite sample consisted of homogeneous approximately-spherical particles in the 3 to 5-μm size range. Powdered calcium carbonate consisted of homogeneous, approximately-spherical particles in the 5 to 13-μm size range. However, the lignosulfonate sample consisted of much larger particles of two types; approximately 10% of the particles were spheres from 17 to 38 μm in diameter while about 90% of the particles had irregular, oblong shapes with dimenstions ranging from 60 to 110 μm in width and 90 to 460 μm in length. Furthermore, while barite and powdered calcium carbonate are practically insoluble in water, a soluble component in the lignosulfonate colored the water yellow-brown.

The relative densities of these particulates must be considered when comparing their similar patterns of effect. Barite has a density between 4.2 and 4.7 (IMCO Services product data) while the density of calcium carbonate is about 2.75. Because concentrations of particulates were prepared on a weight basis, at a given concentration there were many more calcium carbonate particles than barite particles. Given the observed similarity in response patterns for these two particulates when concentrations were prepared on a weight basis, the density differences suggest that the triggering mechanism of a scallop's response to a particulate material involves discrimination of particle weight rather than particle number. The density of lignosulfonate was not obtained.

The third conclusion, alluded to above, is that whole drilling mud has a different effect than its major component in 'pure' form (barite), and both other particulates tested (lignosulfonate, calcium carbonate). This is not an unprecedented result and there are several possible explanations for it. As mentioned earlier, Thompson and Bright (1977) found that various corals were unable to clear whole mud settling on their epidermis but could clear equal amounts of barite, calcium carbonate, and aquagel. The actual concentrations they used were not stated. Such a difference could be due to synergistic interactions of the various constituents in whole mud, or to one or more of the constituents of whole mud that were not tested separately. There was no evidence that a biocide was being added to the mud at the time the test sample was obtained but, as mentioned earlier, diesel fuel had apparently been added. Finally, the greater effect of mud could be due to any chemical changes that might occur when it is heated to temperatures as high as those existing at a drill hole's bottom. When our mud sample was collected, the mud engineer estimated that the temperature at the bottom of the drill hole exceeded 175°C.

Several past studies have documented effects of particulate pollutants on bivalves. It is difficult to compare our dose-response results with those of Hopkins *et al.* (1931) and Odlaug (1948) for oysters, as these studies involved long-term exposures, in some cases up to 35 days. Loosanoff (1961) studied the short-term effects of several particulates on oysters and quahogs, but his methods are unclear. The length of time animals were observed before and after an exposure are only stated qualitatively, exactly what data were taken from the kymograph traces is unclear, and statements regarding significant effects are made without statistical support.

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