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MASTER

INVESTIGATING THE IMPACT OF DRILLING MUD
AND ITS MAJOR COMPONENTS ON BIVALVE SPECIES
OF GEORGES BANK

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DEPARTMENT OF ENERGY

PROGRESS REPORT #2

FEBRUARY 28, 1979 to NOVEMBER 1, 1979

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PROGRESS REPORT
#2

DEPARTMENT OF ENERGY

INTRODUCTION

Results described in Progress Report #1 indicate that 0.3-1.0 grams of drilling mud/liter of seawater can stress bivalve molluscs and that metallic components of drilling muds are assimilated by these organisms. The results posed questions about the short- and long-term effects of stress on energy stores, reproduction, and metal assimilation; accordingly, this year's research program has focused on these three areas and on a continued chemical characterization of the drilling muds used. The results are summarized below; detailed procedures and data are given in the appendices.

1. Chemical Characterization of Trace Elements in Synthetic and Used Drilling Muds

Last year's report describes our attempt to synthesize representative drilling muds. The composition of these muds are given in Table 1. To evaluate the availability of such elements as barium, chromium and iron in these muds to the marine environment, dissolved concentrations of Ba, Cr and Fe in seawater suspensions of the muds were determined just after mixing, after two days suspension, and after portions of each were autoclaved for 2 hr at 120°C. The results (Tables 2 - 4) indicate that dissolved Cr and Fe concentrations in mud suspensions decrease with time of exposure to pH 8 seawater; they

TABLE 1

COMPOSITION OF NEA'S SYNTHETIC DRILLING MUD

<u>COMPONENT</u>	<u>WEIGHT ADDED</u>
BARITE	1.50 PARTS
ATTAPULGITE OF BENTONITE CLAY	1.00
"Q-BROXIN" (FCLS)	0.20
ALUMINUM STEARATE	0.01
PLANT STARCH	0.01

TABLE 2
DISSOLVED FE CONCENTRATIONS (MG/L)
IN SEAWATER SUSPENSIONS OF SYNTHETIC DRILLING MUD

	<u>BACKGROUND CONCENTRATION <0.004</u>			
	<u>UNHEATED</u>		<u>AUTOCLAVED</u>	
	<u>FRESH</u>	<u>2 DAYS OLD</u>	<u>FRESH</u>	<u>2 DAYS OLD</u>
<u>ATTAPULGITE MUD</u>				
0.1 g/L	0.019	<0.004	<0.004	<0.004
0.3	0.07	0.025	0.105	<0.004
1.0	0.34	0.16	0.073	<0.004
3.0	0.86	0.39	0.23	0.061
<u>BENTONITE MUD</u>				
0.1	<0.004	<0.004	<0.004	<0.004
0.3	0.045	0.022	<0.004	<0.004
1.0	0.27	0.021	<0.004	<0.004
3.0	0.63	0.057	0.17	0.17

TABLE 3
DISSOLVED CR CONCENTRATIONS (MG/L)
IN SEAWATER SUSPENSIONS OF SYNTHETIC DRILLING MUDS

<u>BACKGROUND CONCENTRATION <0.002</u>				
<u>ATTAPULGITE MUD</u>	<u>UNHEATED</u>		<u>AUTOCLAVED</u>	
	<u>FRESH</u>	<u>2 DAYS OLD</u>	<u>FRESH</u>	<u>2 DAYS OLD</u>
0.1 g/L	0.16	0.10	0.017	<0.002
0.3	0.45	0.30	0.09	0.041
1.0	1.36	0.92	0.09	0.09
3.0	3.56	2.22	1.16	0.27
 <u>BENTONITE MUD</u>				
0.1 g/L	0.19	0.13	0.018	0.023
0.3	0.49	0.37	0.032	0.026
1.0	1.59	1.24	0.10	0.13
3.0	4.36	3.43	1.96	1.95

TABLE 4
DISSOLVED BA CONCENTRATIONS (MG/L)
IN SEAWATER SUSPENSIONS OF SYNTHETIC DRILLING MUDS

<u>BACKGROUND CONCENTRATION = 0.006</u>				
<u>ATTAPULGITE MUD</u>	<u>UNHEATED</u>		<u>AUTOCLAVED</u>	
	<u>FRESH</u>	<u>2 DAYS OLD</u>	<u>FRESH</u>	<u>2 DAYS OLD</u>
0.1 g/L	0.022	0.021	0.077	0.063
0.3	0.037	0.040	0.040	0.043
1.0	0.034	0.050	0.047	0.025
3.0	0.039	0.037	0.056	0.040
<u>BENTONITE MUD</u>				
0.1 g/L	0.027	0.029	0.103	0.055
0.3	0.039	0.034	0.055	0.048
1.0	0.048	0.043	0.045	0.034
3.0	0.061	0.048	0.062	0.048

also decrease as a result of heating. It should be noted that the concentration of dissolved Cr in heated, aged muds reaches levels well in excess of theoretical solubility of $\text{Cr}(\text{OH})_3$ in pH 8 seawater. Clearly, trivalent Cr, if that is the valence in Q-Broxin brand ferrochrome lignosulfonate (FCLS), is being stabilized in solution by association with lignin derivatives or other complexing agents. Dissolved concentrations of Ba do not closely follow the total amount of Ba in suspension. They are likely limited by the insolubility of BaSO_4 , which, in seawater, would precipitate at Ba levels above about 0.05 mg/L. Some higher Ba values were measured in freshly autoclaved suspensions, but these probably reflect temporary supersaturation of the test media.

Total and dissolved concentrations of Ba, Cr, Fe and several elements were determined for three used drilling fluids (Tables 5 and 6). Total metal content was determined after an HF/HClO_4 digestion. Filtrate samples were prepared by centrifugation of the whole fluids and passage of the supernatant through a glass fiber prefilter and 0.45 μm porosity membrane.

A comparison of the results in the two tables shows that proportionately more Cr was present in the liquid phase than were Al, Ba or Fe, again reflecting the association of Cr with soluble organic substances. However, Ba and Fe, as well as Cr, were present in the filtrate samples at concentrations higher than would be expected based on the solubility of barite and chromic and ferric hydroxides. Apparently,

TABLE 5

COMPOSITION OF 3 USED DRILLING FLUIDS

<u>MATERIAL</u>	<u>% H₂O</u>	<u>% ORGANICS</u>	<u>METALS (MG/G OF SOLIDS)</u>					
			<u>AL</u>	<u>BA</u>	<u>CR</u>	<u>CU</u>	<u>FE</u>	<u>MN</u>
"EXXON" ¹	76.1	2.0	48	202	0.85	0.02	19	0.34
"BAROID" ²	47.2	0.9	20	133	0.20	0.28	16	0.34
"SHELL" ^{2,3}	51.9	2.0	20		0.45	0.15	20	0.15

¹FROM BALTIMORE CANYON²FROM GULF OF MEXICO³CONTAINED GILSONITE

TABLE 6

METALS IN FILTRATES OF USED DRILLING FLUID

<u>MATERIAL</u>	<u>CONCENTRATION (MG/L)</u>					
	<u>AL</u>	<u>BA</u>	<u>CR</u>	<u>FE</u>	<u>MN</u>	<u>PB</u>
"BAROID"	2.2	15	9	17	2.2	<0.4
"EXXON"	41	15	45	34	0.6	<0.4
"SHELL"	100	18	41	71	2.4	15

all three of these elements undergo some chelation or complexation with soluble organic additives such as the sulfonated lignin derivatives. Dissolved concentrations of Cr and Ba in 8°C seawater suspensions of these fluids also exceeded theoretical solubility limits (Table 7). Dissolved Ba levels declined to near the theoretical saturation limit in the more dilute suspensions.

It appears that the discharge of drilling fluids into a cold marine environment would produce elevated levels of such elements as Ba, Cr, Fe and perhaps Cu and Mn, in the water column. The elements will be associated principally with settling particles, however there is likely to be a significant increase in their dissolved concentrations as well. Association with soluble organic additives appears to enhance the solubility of Cr, Fe, Mn and, to a lesser degree, Ba.

2. Uptake and depuration of Ba and Cr in P. magellanicus exposed to drilling fluids

Populations of the sea scallop, Placopecten magellanicus, were exposed to suspensions of heated, attapulgite-based synthetic mud (Table 1) and of the "Exxon" drilling fluid and to untreated Q-Broxin. During the 28- to 42-day tests, media was changed every two days. The animals were fed a daily ration of Isochrysis galbana. For tests run at winter conditions (5-7°C) the daily feeding rate was 1.0 µg chlorophyll A/L of I. galbana. In tests run at higher temperatures the feeding rate was 10-15 µg chlorophyll A/L/day. At the end of each

TABLE 7

BARIUM AND CHROMIUM IN FILTRATES OF SEAWATER SUSPENSIONS
OF THREE DRILLING FLUIDS

<u>MATERIAL</u>	<u>LOADING (G. OF SOLIDS/L)</u>	<u>BA (μG/L)</u>	<u>CR (μG/L)</u>
"BAROID"	1.0	100	4
	0.7	110	2
	0.1	64	2
"EXXON"	1.0	140	56
	0.5	82	25
	0.1	30	6
"SHELL"	1.0	74	17
	0.5	61	8
	0.1	40	3

¹FLUID SAMPLES WERE SUSPENDED FOR 2 DAYS AT 8°C
BEFORE SUBSAMPLES WERE TAKEN FOR ANALYSIS.

experiment, the test organisms were sacrificed and samples of adductor muscle and kidney were taken for analysis. The tissues were freeze-dried and then digested in redistilled nitric acid at reflux temperature. The concentrations of Al, Ba, Cr and Fe in the digestates were determined by plasma emission spectrometry.

The results of these measurements indicate that the concentrations of Al and Fe in the adductor muscle and kidney were not significantly affected by the exposure of sea scallops to the drilling muds tested. Cr and Ba in the kidney were enriched, but there was little change in the concentrations of these two metals in adductor muscles. Chromium concentration in the kidneys and slow adductor of scallops exposed to synthetic mud are plotted in Fig. 1. Data points represent the mean value $\pm$ standard deviation for four animals unless otherwise noted. Chromium concentration in the kidneys may be seen to increase during the four week exposure period, the initial rate of increase being slightly greater than during the remainder of the test. Mean chromium concentration rose from 1.74 $\mu\text{g/g}$ dry tissue to 4.35 $\mu\text{g/g}$ dry tissue; control concentrations dropped to 1.50 $\mu\text{g/g}$ during the same period. The difference between concentrations in control and exposed kidney tissue is significantly at the 95% confidence level at both 14 and 28 days. During the same period chromium concentrations in the slow adductor of test animals did not differ significantly from those of the controls.

CHROMIUM CONCENTRATIONS IN KIDNEY AND ADDUCTOR MUSCLE
OF P. magellanicus EXPOSED TO 1.0 PPT SYNTHETIC MUD AT
5°C

FIGURE 1

Similar results were obtained in the "Exxon" test (Fig. 2). After 27 days of exposure, chromium concentration in the kidneys of exposed animals was 2.94 $\mu\text{g/g}$, while that of controls was 1.40 $\mu\text{g/g}$, a significant difference, but not as marked an increase as the synthetic mud test. Following the discontinuation of exposure, chromium levels continued to increase in the test animals and also in the control population. Concentrations of chromium were below the limit of detection in many of the slow adductors analyzed. Those groups which included one or more such samples are reported as a median value and are indicated on the graph by a carat ($\vee$) above the data point.

Chromium from both muds gained entry into the organism, but it appears that P. magellanicus has the ability to accumulate the metal in kidney tissue while maintaining a constant and relatively low concentration in the slow adductor. The continued rise in the kidneys of exposed animals after the "Exxon" mud exposure stopped may indicate that the animals continue to purge organs other than the muscle of chromium by transporting it to the kidney for excretion.

In the synthetic mud test, the chromium concentration of control kidneys decreased slightly over four weeks, while in the "Exxon" test chromium concentrations increased. These different trends may reflect seasonal differences such as those observed by Bryan (1973), who hypothesized that variations in the concentrations of several elements in Chlamys opercularis and Pecten maximus were due to changes in the availability of

CHROMIUM CONCENTRATIONS IN KIDNEY AND ADDUCTOR MUSCLE OF *P. MAGELLANICUS*
EXPOSED TO 1.0 PPT "EXXON" DRILLING FLUID AT 8°C

FIGURE 2

food. Food as a route of entry for metals may well account for the changes in the control populations during the two tests, since the "Exxon" test conditions included a warm weather food ration that was 10 times that of the synthetic mud test.

Barium concentrations in the kidneys of sea scallops exposed to drilling muds exhibit rather high variability. Figure 3 shows this trend for the synthetic mud test. Mean concentration in the kidneys increased from less than 1.0 $\mu\text{g/g}$ to 55 $\mu\text{g/g}$ after only one day of exposure; over the balance of the test the mean increased to near 100 $\mu\text{g/g}$ and remained at that level, while controls remained below 100 $\mu\text{g/g}$ with one exception. Barium levels in the slow adductor increased as well, but not by a significant amount at the 95% confidence level. In the "Exxon" test barium concentrations varied over three orders of magnitude and so were not plotted.

This variability may be ascribed to ingestion of solid barium sulfate. Assuming that the solubility of barium in seawater is limited by sulfate activity, Chow and Goldberg (1960) have estimated the upper limit of dissolved barium to be 49 $\mu\text{g/L}$. Measured concentrations are generally between 5 and 15 $\mu\text{g/L}$ in the Atlantic Ocean (Chan, et al., 1977). In the suspensions used in the bioassay, as much as 140 $\mu\text{g/L}$ passed through a 0.45 μm filter (see Table 7). Organisms exposed to these suspensions experience a saturated barium solution and perhaps a colloidal barite suspension representing

BARIUM CONCENTRATIONS IN KIDNEY AND ADDUCTOR MUSCLES OF
P. MAGELLANICUS EXPOSED TO 1.0 PPT SYNTHETIC MUD AT 5°C

FIGURE 3

about a tenfold increase over the "normal concentration" in seawater. This increase in non-filterable barium does not by itself adequately explain the observed increases in barium in the kidneys of exposed animals. It is possible that some organisms ingest solid BaSO_4 , either because it is too fine for them to sort out on their gills, or because the stress of turbid water disables the sorting function to some degree.

To further characterize the availability of Cr in lignosulfonate-muds, sea scallops were exposed to suspensions of "Q-Broxin" alone. Uptake and depuration rates were evaluated by analyses of kidney tissues. The results (Fig. 4-6) show that as little as 0.03 g FCLS/L produced an increase of 15 g Cr/g of dry tissue after 14 days exposure. Higher loadings produce somewhat greater Cr concentrations in the kidneys; however, the differences in these values for dosages of 0.03, 0.1 and 0.3 g FCLS/L are not significant. Higher and more variable values were produced by exposure to 0.5 and 1.0 g FCLS/L (Fig. 7). The latter dosage also produced significant mortality in the test population, so some of the observed variability was probably due to severe stress. That the kidneys of organisms exposed to FCLS loadings of 0.03 to 0.3 contain similar amounts of Cr suggests that there is a limit to the rate at which a relatively unstressed sea scallop can excrete the Cr it has ingested. This suggestion is supported by the depuration data which show a significant decrease in

CHROMIUM CONCENTRATION IN *P. MAGELLANICUS*
KIDNEY EXPOSED TO 03PPT FCLS AT 5°C

FIGURE 4

MEAN CHROMIUM CONCENTRATION IN P. MAGELLANICUS
KIDNEY EXPOSED TO 0.1 PPT FCLS AT 5°C

FIGURE 5

MEAN CHROMIUM CONCENTRATION IN P. MAGELLANICUS
KIDNEY EXPOSED TO 0.03 PPT FCLS AT 5°C

FIGURE 6

MEAN CHROMIUM IN P. MAGELLANICUS KIDNEY AFTER 14
DAYS EXPOSURE TO 5 FCLS CONCENTRATIONS

FIGURE 7

Cr only in the kidneys of sea scallops that had been exposed to the smallest loading of FCLS (see Fig. 6).

A comparison of the results plotted in Fig. 1 with those in Fig. 6 discloses that exposure to .03 g FCLS/L (or to 0.9 mg of soluble Cr/L; previous analyses showed that FCLS is 3% w/w Cr) resulted in an increase in Cr concentration 10 times that produced by the 1.0 g/L suspension of synthetic mud. The latter contained 0.074 g FCLS/L (see Table 1), but only 0.09 mg soluble Cr/L (Table 3). Thus, while the total FCLS loading in the 0.03 g/L suspension was 2-1/2 times lower than in the synthetic mud suspension, the dissolved Cr concentration was 10 times higher. One may conclude that the extent to which sea scallops concentrate Cr in their kidneys is a function of the dissolved rather than total Cr concentration in suspensions of drilling fluids.

3. Histological changes in the kidneys of P. magellanicus exposed to ferrochrome lignosulfonate

General Structure

The kidney of Placopecten magellanicus is a paired structure of glandular nature. They lie closely attached to the anterior side of the large adductor muscle, ventral to the pericardium and communicate with the pericardium via ciliated reno-pericardial canals. Each kidney has a valve-like pore through which the contents of the organ are emptied into the mantle cavity. In P. magellanicus the healthy kidney is normally reddish-brown in color, but can vary from red to almost black.

The external side of the kidney is covered by a layer of single columnar epithelial cells (Fig. 8). Short cilia with distinct basal bodies can be seen on these cells and in a few areas tufts of longer cilia can be seen at irregular intervals. Mucus-secreting goblet cells occupy some space between the epithelial cells, and the surface of the organ is covered with mucus. These surface epithelial cells appear folded in cross section and are separated from the internal part of the organ by muscle bundles which run parallel and perpendicular to the kidney. The folded cells and muscle layer appear to be elastic and may provide the elasticity needed to accommodate the larger volume of the kidney when it is full of excretory products.

The inner surface of the saccular section of the scallop kidney is made up of folds and tubules which are covered by a single ciliated columnar epithelium (Figs. 9, 10). Underlying the epithelium is connective tissue containing blood sinuses and capillaries and some muscle bundles. The epithelial cells are mostly ciliated, some with much longer cilia that border the main lumen. Each cell is approximately twice as tall as wide and range in size from $\sim 25\mu$ to $\sim 40\mu$ wide at the base. A distinct nucleus is observed in each cell. Along with some chromatin granules, a few nucleoli, "droplets" and many mitochondrial-like organelles at the basal end of the cell can be seen (Fig. 10). All internal cells have a distal vacuole which occupies up to two-thirds ($2/3$) of the

volume of the entire cell. The vacuole is situated between the cytoplasm and the lumen side of the cell. Many times, within this apical vacuole, there is found a particle of dark coloration of various sizes. These particles are referred to as urinary concretions. Some "free" concretions can also be observed in the lumen (see Fig. 9).

Changes from exposure to FCLS

Exposure to 0.3 g FCLS/L and higher concentrations seems to alter the structural morphology of the kidney. Histological examination of the kidney tissue has shown a breakdown of the cells after being exposed to concentrations of FCLS. Exposure at a temperature of 15°C appeared to have a more drastic effect than did similar tests at 5°C.

In scallops exposed to 1.0 ppt FCLS for 2 weeks, the kidney had lost some of its dark coloration and was less firm. The exposed kidney contains broken epithelial cells and has lost most of its cilia (Fig. 11). Cell debris is evident in the lumen. The nucleus and cytoplasm are not clearly defined as in the control organisms. The connective tissue of the exposed kidney has separated in many areas and the vascular sinuses are occupied by an increased number of blood cells and amoebocytes. The epithelial cells that comprise the outer tissue layer covering the kidney do not appear to be affected.

Observations on kidney tissue after a 14 day depuration period indicated that less than 25% of the epithelial cells and connective tissue showed the effects of the FCLS exposure.

More cells had intact cell membranes and apical cilia. The blood sinuses were not as packed with blood cells and amoebocytes and the connective tissue appeared less separated than the recently exposed kidney. Even after 2 weeks of depuration the effects of the FCLS were still evident. The once affected kidney appears to start the repair process when put in clean water, but recovery is a long-term phenomenon.

FIG. 3 CROSS SECTION OF *P. MAGELLANICUS* KIDNEY SHOWING EXTERNAL EPITHELIAL CELLS WITH DIFFERENT LENGTH CILIA AND GOBLET CELLS ALL COVERED BY A MUCOUS COAT. MUSCLE BUNDLES ARE BETWEEN THE INTERNAL EPITHELIAL CELLS AND THE EXTERNAL EPITHELIAL CELLS.

FIG. 9 CROSS SECTIONAL VIEW OF *P. MAGELLANICUS* KIDNEY SHOWING INTERNAL COLUMNAR EPITHELIAL CELLS AND URINARY CONCRETIONS.

FIG. 10 CROSS SECTIONAL VIEW OF COLUMNAR EPITHELIAL CELLS WITH THE VASCULAR SINUS OCCUPIED BY SOME BLOOD CELLS AND AMOEBOCYTES.

FIG. 11

CROSS SECTION OF *P. MAGELLANICUS* KIDNEY AFTER EXPOSURE TO 1.0 PPT FCLS FOR 14 DAYS. NOTE THE LYSED CELLS AND SEPARATION OF THE CONNECTIVE TISSUE WHICH IS FILLED WITH BLOOD CELLS AND AMOEBOCYTES.

4. The Effects of Drilling Mud Suspensions on the
Digestive Efficiency of Spisula solidissima

APPROACH

Feeding experiments were undertaken in June-July 1979. Surf clams, Spisula solidissima, were exposed to various concentrations of drilling mud suspensions (0.00, 0.05, 0.50, 1.00 g/L⁻¹) for either 2-day or 17-day periods and consequently tested for differences in algal food ingestion and digestive efficiencies.

Two questions were posed:

- 1) Are feeding and digestive processes affected by various concentrations of drilling mud suspensions?
- 2) Is the efficiency of digestion affected by various concentrations of drilling mud suspensions?

METHODS

Spisula solidissima were hydraulically dredged from Rhode Island Sound by commercial fishermen on 19 June 1979, and transported to the Marine Science Institute, Northeastern University, Nahant, Massachusetts, the following day. Healthy, normally-pumping individuals were selected and separated into two groups for either short-term (2 days) or long-term (17 days)

exposures to spent "Baroid" drilling mud suspensions. Each group was further divided into four subgroups of 12-15 clams for treatment with different concentrations of the spent mud (control or 0.0, 0.05, 0.5 and 1.0 g/L⁻¹).

Each subgroup of clams was placed in one of the 80-L recirculating aquaria of the Suspended Sediment Aquarium System (SSAS). All animals were allowed to acclimate for three days prior to treatment with drilling mud suspensions. Temperature was maintained at $8 \pm 0.3^{\circ}\text{C}$. throughout the acclimation and treatment period. Water was changed every two days to preclude the build-up of metabolites. Animals from the first group (3 days acclimation and 2 days treatment) were not fed, but those of the second (3 day acclimation and 17 days treatment) were maintained on a low food ration using the brown flagellate Isochrysis galbana (10 μg chlorophyll/L⁻¹/aquarium⁻¹/day⁻¹). Feeding was discontinued five days prior to the end of treatment to allow clearance of material from the gut.

Feeding experiments were conducted after the 2-day or 17-day treatment period. All animals from a mud-treated subgroup (0.05, 0.5 or 1.0 g/L⁻¹) plus 5-6 control animals (0.00 g/L⁻¹) were tested simultaneously, resulting in a total of six separate experimental runs (three for the 2-day and three for the 17-day groups). Clams were removed from the SSAS and placed in individual aquaria containing 4.38 L of filtered seawater (Vortex Products, Diatom Filter Apparatus), along with the concentration of drilling mud to which they were

previously treated. A volume of Isochrysis galbana culture was added to each experimental aquarium to bring the concentration of algal chlorophyll up to approximately $25 \mu\text{g/L}^{-1}$. After a one-hour feeding period, animals were removed from the experimental tanks and placed in individual aquaria containing only filtered seawater. Pseudofeces and two 100 mL water samples were collected from each experimental aquarium for determination of chlorophyll and phaeopigment concentrations. Feces were collected daily over a 4-day period for similar pigment concentration determinations.

The amount of chlorophyll and phaeopigment added to the 4.38-L experimental aquaria at the start of each of the six runs was determined by standard methods (Yentsch & Menzel, 1963; Holm-Hansen, et al., 1965; Strickland and Parsons, 1972). The same volume of algal culture which was added to each of the experimental aquaria was placed in a blank aquarium containing 4.38 L of filtered seawater. Five 100-mL water samples were taken and filtered through Millipore HAWG 047S1 (0.45 μm pore size) acetate filters. The filters were extracted overnight in 10 mL of 85% (analytical grade) acetone containing approximately 0.1 g of magnesium carbonate. The samples were then centrifuged for 5 min. at 4750 rpm, decanted into Bausch & Lomb Spectronic 20 colorimeter tubes, and read on a Turner Model 110 fluorimeter (10x scale) equipped with a Corning CS-5-60 excitation filter and Corning CS-2-60 emission filter. Samples were then acidified with three drops of 1N HCl and

reread after 5 mins. standing. The amount of chlorophyll and phaeopigment contained in the 4.38-L aquarium was then calculated based on a standard curve of chlorophyll a and the equations presented by Strickland & Parsons (1972). An acid factor of 1.8 was determined for pure chlorophyll a. The amount of chlorophyll and phaeopigment calculated for the blank aquarium was used as the initial level of these pigments for each of the experimental aquaria for the respective runs.

Similar procedures were used for the determination of chlorophyll and phaeopigment in the water at the end of the 1 hour feeding period and in the pseudofeces and feces. Water samples were extracted in 10 mL of 85% acetone and read on the 10x scale. Both pseudofeces and feces were extracted in 25 mL of acetone, although the pseudofecal sample was subsequently read on the 3x scale whereas the 10x scale was used for the feces. The contribution of fluorescing pigment by the diatom filtered seawater and the drilling mud itself was similarly measured. In addition, the dry weight of the algae used for feeding was determined.

From the measurements of pigments thus obtained, the amount of chlorophyll consumed and present in the feces can be compared for animals in each experimental group. In addition, "digestive efficiency" can be calculated. This index is based on the principle that chlorophyll from algal foods is degraded to phaeopigment as it passes through the gut of invertebrates where it is acted upon by digestive processes (Gorham, 1960;

Yentsch & Menzel, 1963; Shuman & Lorenzen, 1975). By knowing the amount of chlorophyll which is ingested and the amount which goes through the gut undegraded, the efficiency of digestion can be calculated:

$$\text{digestive efficiency(\%)} = 1 - \frac{\text{fecal chlorophyll}}{\text{ingested chlorophyll}} (100)$$

Although phaeopigment levels are not used in this calculation, they aid in the interpretation of results.

Differences in ingested chlorophyll, fecal chlorophyll and digestive efficiency between treatments as well as length of treatment were analyzed using One Way Analysis of Variance, Student's t-test between group means, and Duncan a posteriori contrast (SPSS computer statistical packages).

RESULTS

Surf clams ranging from 105.2 - 147.8 mm in length (longest anterior-posterior axis) were tested. Total wet weights (shell and soft tissue) ranged from 212.4 - 604.4 gm (length = 0.081 total wet wt. + 99.85; N = 41; r = 0.87). Soft tissue wet wt./length ratio of 0.788 (SD $\pm$ 0.851, N = 20) and ash free dry wt./length ratio of 0.062 (SD $\pm$ 0.025, N = 19) were calculated using animals from the 0.00 and 0.05 g/L⁻¹ subgroups at the end of runs 1 - 3.

The calculated total amount of chlorophyll per aquarium at the start of each experimental run is presented in Table 8. The maximum reading of the five water samples from the blank aquarium of each run was chosen as indicative of the initial chlorophyll level. Chlorophyll input from the diatom filtered

seawater was negligible. The drilling mud, however, did exhibit a fluorescence which resulted in a low calculated chlorophyll value. Although not significant for the 0.05 g/L⁻¹ treatment subgroup, a mud correction value was added to the initial amount of chlorophyll for the 0.5 and 1.0 g/L⁻¹ subgroups.

Calculated values for the total amount of chlorophyll consumed (Con), total fecal chlorophyll (Feces), and digestive efficiency (Dgeff) are presented in Table 9 for the 50 surf clams used in the subsequent data analyses. Since the number of control animals per run was generally low, testing for differences between mean fecal chlorophyll, chlorophyll consumption and digestive efficiency between the controls of each run could not be done. Control animals were nevertheless lumped together for runs 1-3 and 4-6 for the purpose of the data analyses.

Although 100 surf clams were tested, statistical analysis could only be done on 50 of them (Table 9). A few died during either the treatment or feeding experiment itself and were discarded. The remainder (Table 10), however, demonstrated negative consumption values following the 1-hour feeding period (i.e., the amount of chlorophyll in the experimental aquarium was higher at the end of the feeding period than it was calculated to be at the start). Some negative values were collected for control as well as mud treated animals. In view of these negative consumption values, digestive efficiencies could

not be calculated for these individuals. For example, none of the data from the experimental animals of run 5 (17 day, 0.5 g/L^{-1}) could be used.

Of the remaining animals (Table 9), the mean value of chlorophyll consumption (Con), fecal chlorophyll (Feces), and digestive efficiency (Dgeff) for each subgroup is presented in Table 11. One-way Analysis of Variance on the combined data (disregarding the 17 day, 0.5 g/L^{-1} treatment subgroup null set) indicates that the mean level of chlorophyll consumption is not significantly different in each subgroup ($F_{6,43} = 1.937$). Significant differences between mean values for fecal chlorophyll ($F_{6,43} = 9.743$) are present at the 95% confidence level. In both of these latter groups, there was more variability of individual values between the subgroups than within the subgroups.

T-test analyses between treatment subgroups with respect to mean fecal chlorophyll and digestive efficiency are presented in Table 12. Treatment subgroups with mean values which are not significantly different from each other at the 95% confidence level are listed together as subsets. The interrelationships between the mud treated groups and the length of exposure (2 day vs 17 day) can be seen. For fecal chlorophyll, treatment with drilling mud suspensions (2 day, 0.5 g/L^{-1} ; 17 day, 0.05 g/L^{-1} ; 17 day, 1.0 g/L^{-1}) produce elevated levels of fecal chlorophyll. The significance of the 17 day, 0.05 g/L^{-1} subgroup should be taken with caution, however, since it represents only 2 animals. Overall, the 17-day exposure appears to result in greater fecal

chlorophyll than the 2-day exposure. Since fecal chlorophyll is increased, an inverse relationship is shown for digestive efficiency. At high concentrations of drilling mud, and at the longer exposure time (17 day), lower digestive efficiencies are found.

DISCUSSION

Using only 50% of the experimental animals for data analysis because of the negative consumption values obtained for the remainder is, at the very best, disconcerting. The significance of the results obtained may be questioned because of this. One reason for the negative consumption values may be in the method of determining the initial amount of chlorophyll in the aquaria for each run. It is assumed that since all aquaria were given identical volumes of the same algal culture as the blank aquarium, the chlorophyll value obtained for the blank aquarium could be used as representative of each aquarium for that run. As an added insurance, the maximum value for the blank aquarium was used instead of the slightly lower mean value.

The major cause of the negative consumption values, however, is probably due to the retention of large amounts of material within the intestines of the clams. This material could either be treatment mud, algal food, or else natural sand, mud and rubble introduced into the mantle cavity and ultimately the digestive tract when the clam was dredged. In a number of cases it was quite evident that more mud was present in the experimental

aquarium after the one-hour feeding period than was initially added. These negative consumption values would not be so disturbing if they were given only by mud-treated clams. Their presence in the control groups (39.3% of all control animals) is surprising, since preliminary experiments had show that the majority of feces from a single meal passed through the surf clam within 24 hours. This retention of material is thought to reflect the general poor condition of the experimental animals which showed negative consumption values, as well as of the entire sample of surf clams. This poor condition is also reflected by the large standard deviation obtained for the soft tissue wet wt./length ratio. Of the more than 225 animals which were dredged, over 100 died within the first few days in the aquarium system. Only 100 survived to the end of the 2-day or 17-day treatment periods (>56% died). Of these, 50% demonstrated negative consumption values.

Assuming that the results obtained using the remaining animals can be accepted with some confidence, these experiments have shown that drilling muds adversely affect digestive efficiencies. Both an increased concentration of drilling mud suspensions and an extended exposure period to the same concentration of suspension result in a decrease in digestive efficiency, even though a similar amount of chlorophyll is ingested. Algal food is probably bound up in the mass of ingested material, protecting it somewhat from the digestive processes.

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The relationship between the total dry weight of the algae added to each experimental aquarium and the dry weight of each concentration of drilling mud used gives an indication of the "dilution" of the algal food for each of the treatment subgroups. The diatom filtered seawater contributes a mean value of 0.0040 gm dry wt. of material ($\pm$ SD = 0.0019, N = 7) per 4.38-L aquarium. Similarly, the algae itself adds only 0.0141 gm dry wt. ($\pm$ SD = 0.0083, N = 5) per aquarium. The dry wt. of the drilling mud is defined by the treatment concentration. The ratio of mud dry wt. plus dry wt. of the material in the filtered seawater divided by algal dry wt. is as follows:

Treatment	Calculation	"Dilution" Ratio
0.00 g/L ⁻¹	0 + 0.0040/0.0141	0.3
0.05 g/L ⁻¹	0.2190 + 0.0040/0.0141	15.8
0.50 g/L ⁻¹	2.1900 + 0.0040/0.0141	155.6
1.00 g/L ⁻¹	4.3800 + 0.0040/0.0141	310.0

Assuming a uniform distribution of algae and mud particles, the 1.0 g/L^{-1} treatment subgroup were exposed to 310.9 times more mud particles by weight than algal food. As stated earlier, significant differences in the quantity of chlorophyll consumed were not found between the treatment subgroups. Since bivalves are not able to qualitatively select algal food from inert materials, but rather filter-feed and process material solely on the basis of particle size (Foster-Smith, 1975, 1978; Widdows et al., 1979), the surf clams subjected to drilling mud suspensions had to process an enormous amount of material just to ingest the same quantity of chlorophyll as the control subgroups. Since more material was processed by the mud-treated subgroups than the controls to yield the same amount of chlorophyll, it can be agreed that more energy is required by the mud-treated animals to perform this task. Since the efficiency of digestion is simultaneously reduced, the surf clam gains fewer calories in return. This may have a marked effect upon a population of surf clams subjected to a chronic turbidity stress.

TABLE 8. Total amount of chlorophyll (μgm) in the 4.38-L aquaria at the start of each run attributable to diatom filtered seawater, spent Baroid mud and the algal food (both $\bar{x}$ and max. amount).

	Diatom Water	Baroid Mud	$\bar{x}$ algae	max. algae
Run #1 (0.00 & 0.05 g L^{-1})	<0.01	0	221.5	226.4
Run #2 (0.00 & 0.5 g L^{-1})	<0.01	0.90	177.6	183.8
Run #3 (0.00 & 1.0 g L^{-1})	<0.01	1.81	111.6	114.5
Run #4 (0.00 & 0.05 g L^{-1})	<0.01	0	77.2	88.5
Run #5 (0.00 & 0.5 g L^{-1})	0.04	0.90	81.7	94.8
Run #6 (0.00 & 1.0 g L^{-1})	0.04	1.81	118.1	135.7

TABLE 9. Summary Table of calculated chlorophyll values (μgms) for Spisula solidissima feeding experiments. (R, run number; Conc, concentration of drilling mud; No, animal number; Delta, change in total amount of chlorophyll in the experimental aquarium water after the 1-hour feeding period; Pseudo, amount of chlorophyll in pseudofaeces; Con, amount of chlorophyll consumed; Faeces, amount of chlorophyll in the faeces; Dgeff, digestive efficiency.)

R	CONC	No	Delta	Pseudo	Con	Faeces	Dgeff
1	0.	1	37.41	21.72	15.69	.14	99.09
1	0.	2	36.81	20.82	15.99	4.59	71.29
1	0.	3	65.35	2.76	62.60	0.	100.00
1	0.	4	52.36	15.56	43.80	.88	97.99
1	0.	5	33.80	23.88	9.92	0.	100.00
1	.05	3	31.28	13.60	17.68	1.11	93.72
1	.05	4	64.48	23.80	40.68	8.75	78.48
1	.05	5	93.69	33.15	60.54	0.	100.00
1	.05	6	12.01	7.41	11.60	1.78	89.64
1	.05	7	32.96	8.89	31.07	1.58	94.90
1	.05	8	32.27	13.18	19.09	1.56	92.06
1	.05	9	31.12	26.82	4.30	0.	100.00
1	.05	10	35.14	6.55	28.60	.80	97.21
1	.05	11	84.91	36.79	48.12	.84	98.25
2	0.	12	6.73	0.	6.73	1.44	79.59
2	.50	1	66.82	7.14	52.14	20.47	65.38
2	.50	2	13.27	0	13.27	5.65	57.41
2	.50	3	25.39	13.49	11.90	8.40	21.03
2	.50	7	22.81	20.38	4.05	3.60	23.04
2	.50	11	36.24	0.	36.24	.10	99.73
3	0.	13	15.79	.85	14.94	0.	100.00
3	0.	17	7.50	6.72	66.55	9.76	85.40
3	1.00	3	14.08	1.66	11.42	3.03	73.45
3	1.00	5	23.45	9.21	14.23	3.20	77.51
3	1.00	6	27.06	5.32	21.74	2.82	87.05
3	1.00	7	22.25	1.32	21.42	3.40	88.13
3	1.00	9	23.54	2.77	20.57	3.51	82.92
3	1.00	10	22.28	3.60	18.68	3.46	81.48
3	1.00	11	13.29	.13	13.16	0.	100.00
3	1.00	12	33.11	.54	32.17	2.87	91.07

TABLE 9, cont.

4 0.	1	32.83	4.41	28.42	5.17	81.79
4 0.	2	31.75	5.53	31.21	2.54	91.86
4 0.	3	26.79	12.11	13.69	2.16	84.19
4 0.	4	31.80	0.	31.80	9.29	76.78
4 1.00	1	10.01	5.54	17.51	3.26	81.41
4 1.00	2	19.47	4.07	15.40	8.86	42.44
5 0.	8	67.82	3.26	64.56	3.77	94.16
5 0.	11	17.87	.01	17.86	2.86	83.99
5 0.	12	26.92	7.44	19.48	6.27	67.84
6 0.	13	79.73	1.33	78.40	12.09	84.58
6 0.	14	49.00	8.06	30.94	5.11	83.50
6 0.	15	83.12	13.98	69.14	5.58	21.93
6 0.	16	69.79	0.	69.79	.46	99.34
6 0.	17	81.04	5.53	80.51	3.90	95.16
6 1.00	2	22.66	4.34	18.32	12.15	33.66
6 1.00	8	45.66	1.07	34.59	15.44	55.35
6 1.00	10	28.46	4.64	23.82	11.87	50.17
6 1.00	11	11.42	3.10	8.29	6.33	23.22
6 1.00	12	19.03	1.10	17.93	5.39	70.01
6 1.00	13	14.80	5.21	11.59	4.14	64.24

TABLE 10. Summary of Spisula solidissima specimens omitted from data analysis because of negative consumption values.

Run	Conc	# Omitted	# Analysed
1	0.00	0	5
	0.05	1	10
2	0.00	5	6
	0.50	4	9
3	0.00	3	5
	1.00	4	12
4	0.00	2	6
	0.05	11	13
5	0.00	3	6
	0.50	10	10
6	0.00	0	5
	1.00	7	13
Control		13 (39.3%)	33
Mud-treated		37 (55.2%)	67

TABLE 11. Mean values ($\pm$ SD) for the total amount of chlorophyll consumed (Con), extracted from the faeces over a subsequent 4-day period (Faeces) and the calculated digestive efficiency (Dgeff) for groups of Spisula solidissima treated with spent Baroid drilling mud suspensions.

Mud Conc (g l ⁻¹)	N	$\bar{x}$ Con $\pm$ SD	$\bar{x}$ Faeces $\pm$ SD	$\bar{x}$ Dgeff $\pm$ SD
Run 1, 2, 3 (2 day exposure)				
0.00 (control)	8	29.6 $\pm$ 24.4	2.1 $\pm$ 3.4	91.2 $\pm$ 12.0
0.05	9	29.1 $\pm$ 18.1	1.8 $\pm$ 2.7	93.2 $\pm$ 7.3
0.50	5	25.1 $\pm$ 22.7	7.7 $\pm$ 7.9	53.4 $\pm$ 32.5
1.00	8	20.1 $\pm$ 7.4	2.8 $\pm$ 1.1	85.2 $\pm$ 8.3
Run 4, 5, 6 (17 day exposure)				
0.00 (control)	12	44.6 $\pm$ 25.5	4.9 $\pm$ 3.2	85.8 $\pm$ 9.5
0.05	2	16.4 $\pm$ 1.5	6.1 $\pm$ 4.0	61.9 $\pm$ 27.6
0.50	0	_____	_____	_____
1.00	6	19.1 $\pm$ 9.4	9.2 $\pm$ 4.5	49.4 $\pm$ 18.0
	50	29.4 $\pm$ 20.9	4.4 $\pm$ 4.4	79.3 $\pm$ 21.4

TABLE 12. Groups of homogeneous subsets of mean faecal chlorophyll and digestive efficiencies for Spisula solidissima, where the mean values are not significantly different at the 95% confidence level (based on Least Square Difference Student's t-test and Duncan a posteriori contrast).

Group 1 = 2 day exposure, 0.00 g L⁻¹ (control)

2 = 2 day exposure, 0.05 g L⁻¹

3 = 2 day exposure, 0.50 g L⁻¹

4 = 2 day exposure, 1.00 g L⁻¹

5 = 17 day exposure, 0.00 g L⁻¹ (control)

6 = 17 day exposure, 0.05 g L⁻¹

8 = 17 day exposure, 1.00 g L⁻¹

Faecal chlorophyll:

SUBSET 1

GROUP	GRP 2	GRP 1	GRP 4	GRP 5	GRP 6
MEAN	1.8244	2.1267	2.7862	4.9333	6.0600

SUBSET 2

GROUP	GRP 5	GRP 6	GRP 8
MEAN	4.9333	6.0600	7.7420

SUBSET 3

GROUP	GRP 6	GRP 8	GRP 8
MEAN	6.0600	7.7420	9.2183

TABLE 12, cont.

Digestive efficiency:

SUBSET 1

GROUP	GRP 8	GRP 3	GRP 6
MEAN	49.4417	53.4380	61.9250

SUBSET 2

GROUP	GRP 6	GRP 4
MEAN	61.9250	85.2012

SUBSET 3

GROUP	GRP 4	GRP 5	GRP 1	GRP 2
MEAN	85.2012	85.7600	91.1700	93.2511

5. Body Component Index Changes in Placopecten Field Samples

APPROACH

Indices were determined on selected tissues in order to (1) identify seasonal changes in tissues which could be correlated with energy store data; (2) provide baseline index values for comparison with drilling mud-exposed specimens.

METHODS

Field samples of 20 to 25 specimens were collected at intervals of about 6 weeks from 3/1/79 to 9/14/79. Index values were determined on gonadal mass (GMI), digestive gland (DGI) and fast and slow adductor muscles (FAI, SAI) by the formula:

$$\text{index} = \left(\frac{\text{wet weight of tissue}}{\text{wet weight of total soft tissue}} \right) (100)$$

and a soft tissue index (STI) was determined by:

$$\frac{(\text{wet weight of total soft tissue}) (100)}{\text{wet weight of organism}}$$

Individual index values were either arcsin transformed* or left unaltered prior to statistical analysis. Mean sample values of each index in the 5 samples are presented in Table 13, in both transformed and unaltered forms. Mean sample values within each index type were compared by pairwise Student's t-test (transformed only) and by single factor analysis of

* Arcsin transformation has the effect of significantly lowering apparent variation in grouped data.

variance (ANOVA) followed by the Student-Newman-Keuls (SNK) multiple range test in order to identify statistically significant (at the 95% confidence level) differences. The indices are discussed separately below.

RESULTS

GMI (Figure 12). The basic trend in GMI values is of relatively constant increase in samples 1-4, followed by a sharp drop in sample 5. Mean male values are consistently larger than female in samples 1-4, but drop very sharply in sample 5, while females continue to increase. The trend is assumed to reflect the development and accumulation of sex products from spring to late summer (samples 1-4), followed by the loss of these products in spawning (sample 5). The data suggest that males may achieve reproductive maturity earlier than females, or may spawn earlier.

The results of ANOVA/SNK testing on untransformed GMI values can be illustrated as: sample 1 = 2 = 3 = 4 = 5, with no significant differences occurring in the series. However, $4 > 1, 2; 3 > 1; 5 > 1$. Pairwise t-tests on arcsin transformed values are somewhat different: $1 < 2 < 3 < 4 > 5$, all differences being significant. It is likely that the differences are a result of considerably lower relative variance in the transformed values.

DGI (Figure 13). Mean sample DGI values increase in samples 1-3, decrease sharply in sample 4, and may recover slightly in sample 5. Mean sample male and female values appear

relatively similar in size throughout. A substantive explanation is not offered for the increase-drop + recover pattern. The timing of the drop and recovery suggest, however, that these may be related to changes in reproductive condition. The results of ANOVA/SNK are summarized: sample $1 < 2 = 3 > 4 = 5$. The relatively low variance of arcsin transformed DGI values is evident in that pairwise t-test results show only a single difference: sample $4 < 5$.

TAI (Figure 14). The adductor indices (FAI and SAI) are presented as a cumulative total adductor index (TAI). The pattern of change in the TAI is similar to that of the DGI (and to the FAI and SAI), but with a more dramatic drop in sample 4 and a substantial recovery in sample 5. While it is difficult to imagine a direct relationship between the GMI and TAI, it is noteworthy that the major shifts in sample 3-5 are reciprocal in the two indices (as between the DGI and GMI), and a relationship of some kind must exist. Sex differences in the TAI are large in samples 2-4 and small in samples 1 and 5. In contrast to the GMI, females appear to have larger TAI values than males early in the year. ANOVA and SNK tests yield the following pattern of significant TAI changes: sample $1 = 2 = 3 > 4 < 5$, where $1 > 4$ and $2 > 4$. This pattern is reproduced by pairwise t-tests of arcsin transformed values.

STI (Figure 15). The pattern of STI change is similar to that of the GMI: increasing in samples 1-4 and decreasing in sample 5. This similarity is not coincidental; it is suggested that a considerable portion of the change in STI

can be attributed directly to changes in the gonadal mass weight, though other factors no doubt exist.

Mean male STI values appear to be consistently larger than female values, although the difference appears to narrow to insignificance by sample 5. It is suggested that the opposite dominances of male and female values in GMI and TAI produce the relationship seen in the STI.

The pattern of significant STI differences defined by ANOVA and SNK tests is similar to that of GMI values: sample 1 = 2 = 3 = 4 = 5, but 4,5,3 > 1 and 4,5 > 2. Pairwise t-tests of arcsin transformed values reproduce this pattern with the single difference 4>3.

SUMMARY

1) Two major patterns of change are seen among the four indices:

- a) GMI and STI increase in size in samples 1-4 and decrease in sample 5
- b) DGI and TAI increase in samples 1-3, decrease in sample 4, and at least partially recover in sample 5

2) The majority of the changes, especially in samples 3-5, are statistically significant in all indices.

3) Change in the GMI is assumed to be due to the development and accumulation of sex products, followed by their loss in spawning.

4) It is suggested that the changes in STI can be largely attributed to changes in gonadal mass weight.

5) The reciprocal pattern of DGI and TAI compared to GMI and STI cannot be directly explained with the data available.

6) It appears that the period from sample 3 to sample 5 (and perhaps beyond) is one of dramatic change in Placopecten and should be an ideal period for drilling mud experiments.

7) Sex differences in index values are pronounced at different times in the GMI, TAI and STI. These differences may correlate with sex-specific differences in energy stores.

8) Comparison of analyses of arcsin transformed and unaltered values in the four indices reveals little difference except in the GMI, where arcsin transformation of index data appears to increase the sensitivity of the comparative methods.

TABLE 13

Body component indices, field samples 1 - 5. Mean arcsin transformed sample values with 95% confidence interval. Non-transformed values are enclosed parenthetically.

	1	2	3	4	5
	3/1/79	4/13/79	5/30/79	7/13/79	9/14/79
GMI	10.70 $\pm$ 0.76 (3.51 $\pm$ 0.48)	14.01 $\pm$ 0.98 (5.98 $\pm$ 0.82)	16.58 $\pm$ 1.66 (8.44 $\pm$ 1.65)	18.99 $\pm$ 1.37 (10.78 $\pm$ 4.62)	15.22 $\pm$ 3.56 (8.15 $\pm$ 4.04)
DGI	11.70 $\pm$ 0.33 (4.17 $\pm$ 0.24)	12.84 $\pm$ 0.59 (4.98 $\pm$ 0.45)	13.51 $\pm$ 0.56 (5.49 $\pm$ 0.45)	10.95 $\pm$ 0.22 (3.61 $\pm$ 0.14)	11.70 $\pm$ 0.50 (4.15 $\pm$ 0.35)
FAI	8.16 $\pm$ 0.31 (2.03 $\pm$ 0.16)	8.98 $\pm$ 0.38 (2.45 $\pm$ 0.20)	8.58 $\pm$ 0.40 (2.25 $\pm$ 0.21)	7.39 $\pm$ 0.41 (1.68 $\pm$ 0.17)	8.29 $\pm$ 0.34 (2.10 $\pm$ 0.16)
SAI	28.57 $\pm$ 0.78 (23.13 $\pm$ 1.21)	28.80 $\pm$ 0.95 (23.27 $\pm$ 1.36)	29.49 $\pm$ 0.81 (24.28 $\pm$ 2.09)	26.64 $\pm$ 0.60 (20.12 $\pm$ 0.84)	28.99 $\pm$ 1.29 (23.60 $\pm$ 1.96)
TAI	30.03 $\pm$ 0.78 (25.19 $\pm$ 1.24)	30.42 $\pm$ 1.00 (25.73 $\pm$ 1.50)	31.06 $\pm$ 0.89 (26.52 $\pm$ 1.32)	27.85 $\pm$ 0.64 (21.85 $\pm$ 0.87)	30.39 $\pm$ 1.31 (25.69 $\pm$ 2.03)
STI	39.98 $\pm$ 1.01 (41.31 $\pm$ 1.73)	40.94 $\pm$ 1.63 (42.98 $\pm$ 2.81)	41.85 $\pm$ 0.74 (44.53 $\pm$ 1.29)	43.37 $\pm$ 0.72 (47.17 $\pm$ 1.25)	42.72 $\pm$ 0.68 (46.02 $\pm$ 1.18)
n	25	20	20	20	20

FIGURE 12. Field sample Gonadal Mass Index (untransformed values).

Figure 13. Field sample Digestive Gland Index
(untransformed values).

Figure 14. Field sample Total Adductor Index
(untransformed values).

Figure 15. Field sample Soft Tissue Index
(untransformed values).

6. The effects of mud exposure and Suspended Sediment Aquarium System (SSAS) confinement on Plactopecten in Experiments 12, 14 and 16 are summarized as follows:

GMI (Figure 16). Significant effects of mud exposure and SSAS confinement have not been observed in these experiments. There is some evidence that mud exposure may have an effect of accelerating spawning (Experiments 12 and 14), but the evidence is tenuous at the present time.

DGI (Figure 17). Experimental DGI values were significantly lower than field values in all cases in these experiments. In only one case, however, has a mud-exposed group been found significantly lower than the relevant control (1.0 ppt Baroid in Experiment 12). As 1.0 ppt Baroid did not measure lower than control in Experiment 14, the single value in Experiment 12 should be viewed cautiously.

TAI (Figure 18). Experimental TAI values significantly lower than corresponding field values were found in Experiment 12 (1.0 and 0.3 ppt Baroid) and Experiment 14 (1.0 ppt Baroid and 1.0 ppt Exxon). However, in both cases the mud-exposed groups did not differ from control groups, so that the relative lowering of TAI values should be regarded as an effect of SSAS-confinement rather than of mud exposure.

STI (Figure 19). A consistent pattern of STI response to experimental conditions has not emerged. In no case is there a clear, significant effect of either mud exposure or of SSAS

confinement on the STI. It is suggested that some of the observed changes are due to the influence of the gonadal mass weight, especially during the time just prior to and following spawning.

Body Weight Changes

Body weights are consistently depressed in mud-exposed groups relative to controls in all three experiments. Body weight changes significantly different from controls were measured in Experiments 12 (1.0 ppt Baroid) and 14 (1.0 ppt Exxon). The accurate measurement of total body weight is difficult due to entrapment of water in the mantle cavity, and variation in groups is large; however, the consistency of relative weight loss in mud-exposed groups suggests that the effect is both real and attributable at least in part to mud exposure.

FIGURE 16. Effects of drilling mud on Gonadal Mass Index.

FIGURE 17. Effects of drilling mud
on Digestive Gland Index.

FIGURE 18. Effects of drilling mud on Total Adductor Index on Total Adductor Index

FIGURE 19. Effects of drilling mud on Soft Tissue Index.

7. Energy Stores in Placopecten magellanicus

Rationale

Our purpose was to develop a complete picture of the interactions between the energy storage compartments and the organism's reproductive cycle. The one year study of nutrient stores on both freshly collected and experimentally stressed specimens was designed to measure the effect of such stress on the extant field population and determine whether potential survival is compromised. The reproductive cycle is of importance in two major respects: first, intense gametogenic activity places demands on stored nutrients and thus may reduce the viability of externally stressed individuals; second, the long-term survival of stressed populations may be endangered if external stress reduces reproductive activity either by direct effect on the gonads or by reducing nutrient stores to a level insufficient for successful reproduction.

Various tissues of the sea scallop were investigated histochemically for the presence of lipid and glycogen. The study was conducted in order to 1) localize sites of lipid and glycogen deposition; 2) rank the tissues studied as to the relative amounts of these stores; and 3) recommend target tissues for quantitative biochemical assay of lipid and glycogen.

All tests were conducted on specimens of P. magellanicus which had been maintained for 1-2 months in running seawater at the Marine Science Institute, Nahant, Massachusetts. The results reported should reflect energy store levels under winter conditions.

The following tissues were examined:

1. digestive gland
2. gonad
3. kidney
4. mantle
5. mantle edge
6. slow muscle
7. fast muscle
8. foot

I. Histochemical localization of lipid

A. Materials and Methods

Tissues were fixed overnight in 10% formalin (0.2 M phosphate buffer, pH 7.5). Frozen sections (8 μ m) were cut, air dried, and stained according to either the Chiffelle and Putt's propylene glycol method using Sudan Black B as a stain (Lillie & Fullmer, 1976), or the Lillie and Ashburn supersaturated isopropanol method using an Oil Red O staining solution (Lillie & Fullmer, 1976). In addition, tissues were fixed in 10% formalin (0.2 M phosphate buffer, pH 7.5) for two hours, blackened for 7 days with 2% osmium tetroxide (Galigher & Kozloff, 1971), dehydrated, embedded in polyester wax (Steedman, 1960) and sectioned at 5 μ m. Following clearing and mounting, these blackened sections were examined for lipid deposits. Tissues fixed in Rossman's solution which resulted in the extraction of all lipid served as a control in this instance.

B. Results

Results are presented in Table 14. Interpretation of the results from all three methods was extremely difficult. With

Sudan Black B and Oil Red O staining, the drying of the cryostat tissue sections alters the structure of the tissue, but should not effect the actual lipid store. Exact localization of the lipid is, however, sometimes difficult within the tissue section, although presence/absence could generally be determined.

Saturated Oil Red O in isopropanol proved to be the best stain, both from the point of view of best localization and ease of use. For the osmium treated tissues, other tissue components beside lipid blacken as well (e.g., lipoproteins, some carbohydrates and some proteins), resulting in a great deal of "background" coloration. This method was, however, the most useful in visualizing the large lipid droplets found in the digestive gland.

Lipid appeared to be present in a variety of forms:

1. As small droplets (<1-2 μm in diameter), either singly or in clusters (present to some degree in all tissues)
2. As large spherical or irregularly shaped droplets (up to 25 μm in diameter) (digestive gland only)
3. As aggregates of small droplets apparently lying in membrane-bound oval or irregularly shaped vacuoles (4-10 μm long) (mantle, kidney and gonad).

TABLE 14

Relative concentrations of lipid identified within various tissues of Placopecten magellanicus using three histochemical techniques. Total lipid stores depend on concentrations and the size of the respective organ/tissue.

	<u>Sudan Black B</u>	<u>Oil Red O</u>	<u>Osmium Blackened</u>
Digestive Gland	NA	(5+) ¹	5+
Gonad	5+	4+ ²	3+ ²
Kidney	5+	4+	2+
Mantle	NA	3+	2+
Mantle Edge	2+	2+	2+
Foot	1+	2+	2+
Fast Muscle	2+	1+	1+
Slow Muscle	1+	1+	1+

NA = not available for observation

¹= intense background staining, not localized to specific droplets

²= attributable to style sac epithelium; gonad itself
2+ or 3+

The following observations are included for each tissue:

1. Digestive gland:

Numerous round to irregular shaped droplets, ranging up to 25 μm in diameter, were visualized with osmium blackened tissue. These largest droplets were present in the basal region of the digestive cells, although numerous smaller droplets ($<5 \mu\text{m}$) were also present there. In alcohol-extracted tissue, large empty "vacuoles" were all that remained where these droplets had been, indicating the lipid nature of these droplets. Lipid droplets were also present in the connective tissue between the tubules, within the lumen of the tubules and ducts (droplets up to 13 μm in diameter), and within the epithelium of the primary ducts (droplets $<3 \mu\text{m}$ in diameter). The majority of the droplets were less than 5 μm in diameter, but by far the most conspicuous were the large droplets (up to 25 μm) in the digestive cells. Oil Red O treatment of dried cryostat sections produced an intense staining of connective tissue and digestive tubules. In most cases, droplets were not visible, however. A few small droplets ($<2 \mu\text{m}$ in diameter) were found in the connective tissue and digestive cells, but the majority of the stain was evenly dispersed throughout these tissues. This artefact may be due to the drying of the tissue. Of all the tissues studied, digestive gland exhibited the most drastic change upon drying. This effect may have disrupted the normal spherical lipid droplets and redistributed the lipid evenly throughout the tissue, accounting for the general intense staining with Oil Red O.

2. Gonad:

Numerous small droplets ($\sim 1 \mu\text{m}$ diameter) were present in the epithelium of the ducts and the surrounding connective tissue. "Membrane-bound" aggregates of droplets ($\sim 10 \mu\text{m}$ long) were also present in these tissues. In addition, numerous droplets up to $5 \mu\text{m}$ in diameter were found in the epithelium and connective tissue of the style sac which extends through the center of the gonad. The lipid associated with the style sac accounts for a significant proportion of the total lipid of the gonad.

3. Kidney:

Droplets ($< 10 \mu\text{m}$ in diameter) and "membrane-bound" aggregates ($4-10 \mu\text{m}$ long) of droplets were present within the epithelium of the kidney tubules and the scant connective tissue between the tubules. Slightly more of the "membrane-bound" aggregates were observed in the kidney than in the gonad.

4. Mantle:

Small droplets and "membrane-bound" aggregates of droplets were located in the connective tissue overlying various muscle bands between the inner and outer epithelial layers.

5. Mantle edge:

Lipid droplets ($< 10 \mu\text{m}$ in diameter) were associated with the muscle bands within the inner mantle fold and in the mantle proper between the mantle edge and the pallial line. Some Oil Red O staining was also observed in the mantle epithelium of this latter region.

6. Foot:

Droplets were small ($\sim 1 \mu\text{m}$ in diameter) and mostly associated with the connective tissue between the various muscle bands of the foot. Some droplets were more directly associated with the muscle bands.

7. Fast and Slow Muscle:

Single droplets or small clusters of droplets were directly associated with the muscle bands. Slightly more droplets were found in the fast muscle stained with Sudan Black B.

II. Histochemical Localization of Glycogen

A. Materials and Methods

Tissues were fixed overnight in Rossman's fluid (Galigher & Kozloff, 1971), washed in 3 changes of 95% ethanol to remove excess picric acid, and embedded in polyester wax (Steedman, 1960). Sections were cut at $7 \mu\text{m}$ and stained in either a freshly made working solution of Best Carmine (Humason, 1972) or Periodic Acid-Schiff's solutions (PAS) (Galigher & Kozloff, 1971). In both cases, adjacent control sections were incubated at 40°C for 30 min. in a 0.1% α -amylase solution to remove all glycogen. Both control and untreated sections were then stained simultaneously. Glycogen could then be identified by comparison of the digested and untreated sections.

B. Results

Results presented in Table 15 show that both staining

TABLE 15

Relative concentrations of glycogen identified within various tissues of Placopecten magellanicus using two histochemical techniques. As in the case with lipid, total glycogen stores can be estimated by considering concentration and size of each organ/tissue.

	<u>Best Carmine</u>	<u>PAS</u>
Slow Muscle	+5	+5
Fast Muscle	+4	+4
Gonad	+3 ¹	+3
Mantle Edge	+2	+1 or +2
Digestive Gland	+1	+1
Foot	+1	+1
Mantle	+1	+1
Kidney	+1	+1

¹= gonoducts packed with glycogen (+5); follicles, however, contained little or no glycogen.

procedures yield identical results. Interpretation was more difficult with PAS staining, however, due to the greater number of substances which react positively to the staining procedure.

As with lipid, glycogen appeared to be present in a variety of forms:

1. Mostly as numerous small (1-2 μm) round to irregularly shaped bodies, scattered or in clusters (in all tissues studied)
2. As an evenly dispersed, agranular (at the light microscope level) form, identified by a general background staining reaction which is absent in α -amylase treated controls (slow muscle, fast muscle, foot muscle bands, and especially in digestive gland stained with PAS)
3. As a few large (up to 35 μm long) oval to irregularly shaped bodies (slow muscle)

The following observations were made on the glycogen localization in each of the following tissues:

1. Slow muscle:

Numerous small bodies ($\sim 1 \mu\text{m}$ in diameter) were scattered along the muscle fibers. Additionally, a few large oval bodies up to 35 μm long (average 7.5 μm) were similarly situated and a general background staining around the muscle fibers was apparent.

2. Fast Muscle:

A similar appearance to the slow muscle was evident, but glycogen bodies were not as abundant. The largest bodies were $\sim 5 \mu\text{m}$ in diameter.

3. Gonad:

The ciliated gonoducts were packed with small glycogen bodies (1-2 μm in diameter). Only a few glycogen bodies were seen in the pithelium and lumen of the follicles.

4. Mantle Edge:

Most of the glycogen appeared as small bodies (1-2 μm in diameter) associated with the external radial muscles (i.e., external retractors of the mantle edge). A little glycogen was associated with other muscle systems of the mantle edge (e.g., circumpallial muscles, internal radial muscle, etc.)

5. Digestive Gland:

Small bodies (1-2 μm) were scattered in the cytoplasm of the digestive cells. Sometimes a slight basal aggregation of 3-4 glycogen bodies was seen in these cells. Slightly larger bodies (1-3 μm in diameter) were infrequently seen in the connective tissue between the tubules. No glycogen was observed in the basophil cells, tubule lumen, primary ducts, secondary ducts, or around the blood vessels. An intense positive background stain was observed with PAS but not with Best Carmine. This may indicate a high glucose level within the digestive cells.

6. Foot:

Most glycogen appeared as the evenly dispersed, agranular form, surrounding the various muscle bundles of the foot. A few small glycogen bodies (1-2 μ m in diameter) were associated with these muscle bundles as well.

7. Mantle:

Little glycogen was associated with the various muscle fibers of the mantle proper. None was evident in the epithelial layers. A high concentration (+5) was present in the thick pallial line muscle in the form of small (1-2 μ m in diameter) bodies.

8. Kidney:

A few glycogen bodies (4-5 μ m in diameter) were present of the epithelial cells of the kidney tubules. In general, one body was present in the distal third of each tubule cell.

Discussion

In general, lipid appears to be associated with connective tissue (digestive gland, gonad, kidney, mantle, foot) and epithelial tissue (digestive gland, gonad, kidney, mantle edge) whereas glycogen is associated with muscle (slow muscle, fast muscle, mantle edge, mantle, foot). By considering the relative concentrations of each in conjunction with the size of the respective tissue/organ, it appears that the largest store of lipid is in the digestive gland. The slow and fast muscle clearly comprise the major storage area for glycogen. Once

actual quantities of each of these stores is biochemically determined, the importance of each of these stores can be assessed. It must be emphasized, however, that the relative concentrations of lipid and glycogen could (and, in fact, are expected to) drastically change seasonally. It is expected that the processes of gametogenesis and spawning will cause major shifts and fluctuations in each energy storage compartment.

Based on the results of the histochemical scans, the digestive gland, gonad, slow and fast muscles were assayed biochemically for lipid, glucose, and glucose + glycogen. Table 16 summarizes the results of biochemical analyses of digestive gland, gonad, and slow adductor tissues from test, control, and two field samples. There was no significant difference between the controls and test populations exposed to four concentrations of Baroid drilling mud. In all cases, laboratory specimens energy stores are lower than those of bracketing natural populations, indicating a stress condition produced by captivity in the test system.

Table 17 presents data for field samples, control, and experimental samples exposed to 1.0 ppt Baroid and Exxon drilling muds, but with a threefold increase in the feeding regime. During this experiment energy stores of the controls and bracketing populations are equivalent, indicating the stress condition of feeding has been removed. The specimens exposed to drilling muds have begun to consume energy stores as a result of the stress.

TABLE 16

EFFECTS OF "BAROID" DRILLING MUD ON THE ENERGY STORES OF PLACOPECTEN MAGELLANICUS
(Conc. in mg/gram $\pm$ SD)

TISSUE	Conc. + SD Field Sample Before Test	1.00 ppt	0.30 ppt	0.10 ppt	0.03 ppt	Control	Field Sample After Test		Sex
	4/13/79						5/31/79		
DIGESTIVE GLAND	3.63 $\pm$ 2	2.4	2.65	2.8	1.3	3.3	2.93 $\pm$.4	Glucose	M
	9.53 $\pm$ 1.2	3.3 $\pm$.7	4.8	5.1 $\pm$ 2.8	3.8 $\pm$ 1.4	4.55 $\pm$.77	4.33 $\pm$.3	(mg/g)	F
	6.97 $\pm$ 1.8	3.5	4.5	4.75	2.2	5.4	7.53 $\pm$.55	Glucose+	M
	14.67 $\pm$ 3.7	4.65 $\pm$.91	8.4	8.61 $\pm$ 6.1	6.05 $\pm$ 1.9	7.6 $\pm$.84	12.33 $\pm$.57	Glycogen	F
	283.0 $\pm$ 32	163.0 $\pm$ 14.1	187.0	246.5 $\pm$ 67	198.0	211.0	236.3 $\pm$ 60	Lipids	M
	357.0 $\pm$ 52	157.0 $\pm$ 38.1	226.0	205.5 $\pm$ 45	153.0 $\pm$ 5.6	180.0 $\pm$ 5.6	195.67 $\pm$ 84	(mg/g)	F
GONADAL MASS	1.6 $\pm$.43	0.1 $\pm$ 0	0.6 $\pm$ 0	0.55	0.1	0.3	<0.13 $\pm$ 0	Glucose	M
	5.1 $\pm$.6	1.8 $\pm$ 0	1.8 $\pm$ 0	1.7 $\pm$.14	1.55 $\pm$.35	1.6 $\pm$.28	1.03 $\pm$.41	(mg/g)	F
	4.47 $\pm$ 2.3	1.7 $\pm$ 0	4.4 $\pm$ 1.9	4.0 $\pm$.28	2.55 $\pm$.21	4.4	1.47 $\pm$.3	Glucose+	M
	16.67 $\pm$ 1.15	13.0 $\pm$ 1.4	18.0 $\pm$ 0	18.5 $\pm$ 0	19.0 $\pm$ 0	17.0	17.0	Glycogen	F
	52.0 $\pm$ 2.6	53.0 $\pm$ 0	58.0 $\pm$ 0	57.5 $\pm$ 24	57.5 $\pm$.70	54.0	62.67 $\pm$ 4.1	Lipids	M
	96.67 $\pm$ 9.0	135.0 $\pm$ 7.0	145.0 $\pm$ 0	146.5 $\pm$ 12	144.0 $\pm$ 12	134.0	135.67 $\pm$ 8	(mg/g)	F
SLOW ADDUCTOR	2.93 $\pm$.20	0.5	0.55 $\pm$.49	0.55 $\pm$.35	0.6	0.8	0.7 $\pm$.45	Glucose	M
	2.73 $\pm$.23	0.75	1.1	0.55	0.55 $\pm$.07	0.85 $\pm$.07	0.6 $\pm$ 0.1	(mg/g)	F
	95.0 $\pm$ 36	18.0	47.0 $\pm$ 46	38.5	35.0 $\pm$ 1.4	40.0	95.67 $\pm$ 28	Glucose+	M
	97.0 $\pm$ 6.9	36.0 $\pm$ 32	77.0	45.5 $\pm$ 46	41.0 $\pm$ 8.4	59.0 $\pm$ 7	110.67 $\pm$ 8.3	Glycogen	F
	30.67 $\pm$.57	42.0	38.5 $\pm$ 2.1	41.5	40.0	40.0	37.3 $\pm$ 2.3	Lipids	M
	30.35 $\pm$ 2.5	41.5 $\pm$ 2.1	40.0	40.5 $\pm$.7	42.0	38.0 $\pm$ 1.4	36.3 $\pm$ 1.15	(mg/g)	F

TABLE 17

EFFECTS OF "BAROID" AND "EXXON" DRILLING MUDS ON THE ENERGY STORES OF PLACOPECTEN MAGELLANICUS
(Conc. in mg/gram $\pm$ SD)

TISSUE	Field Sample Before Test 7/13/79	1.00 ppt "Baroid"	1.00 ppt "Exxon"	Control	Field Sample After Test 8/27/79		Sex
DIGESTIVE GLAND	6.66	0.12	1.1	3.6	7.3	Glucose	M
	6.20	1.4	1.1	1.0	6.3	(mg/g)	F
	10.9	0.6	1.2	4.5	10.1	Glucose+	M
	11.7	0.6	1.7	2.0	10.1	Glycogen	F
	159	39	76	94	103	Lipid	M
	105	57	61	48	101	(mg/g)	F
GONADAL MASS	<0.1	0.4	1.1	0.7	0.14	Glucose	M
	<0.2	0.5	0.04	2.3	1.0	(mg/g)	F
	2.9	4.6	4.5	6.8	4.5	Glucose+	M
	13	8.0	20	19	18	Glycogen	F
	68	43	63	68	51	Lipid	M
	158	63	85	147	141	(mg/g)	F
SLOW ADDUCTOR	0.9	<0.1	0.4	1.1	1.3	Glucose	M
	1.0	1.1	0.8	1.5	2.3	(mg/g)	F
	30	20	130	28	44	Glucose+	M
	73	13	251	17	40	Glycogen	F
	41	44	38	42	32	Lipid	M
	37	41	38	41	32	(mg/g)	F

8. Conclusions

The discharge of drilling fluids into a cold marine environment will produce elevated levels of Ba, Cr, Fe, and perhaps Cu and Mn, in the water column. The elements are associated with settling particles although there will be an increase in the dissolved concentrations. Association with soluble organic additives enhances the solubility of Cr, Fe, Mn, and, to a lesser degree, Ba.

The concentrations of Al and Fe in the adductor muscle and kidney were not significantly affected by exposure of sea scallops to the drilling muds tested. Cr and Ba in the kidney were enriched, but there was little change in the concentration of these two metals in the adductor muscles. There is a limit to the rate at which an unstressed sea scallop can excrete the Cr ingested. This suggestion is supported by depuration data which show a decrease in Cr only in the kidneys of sea scallops that had been exposed to the smallest loading of FCLS. Finally, the extent to which sea scallops concentrate Cr in their kidneys is a function of the dissolved rather than total Cr concentration in suspensions of drilling fluids.

Exposed kidneys show histological changes during exposure, but some repair processes go on once the specimens are removed from the stress of drilling fluids.

Histochemical studies showed that the largest store of lipid is in the digestive gland. The slow and fast muscles are the major storage areas for glycogen. In all cases,

laboratory specimens energy stores were lower than those of natural populations, indicating a stressed condition.

Indices were determined on selected tissues in order to (1) identify seasonal changes in tissues which could be correlated with energy store data; (2) provide baseline index values for comparison with drilling mud-exposed specimens. Two major patterns of change are seen among the four indices: a) gonad mass index and soft tissue increase in size in field samples 1-4 and decrease in field sample 5; b) digestive gland index and fast muscle index increase in field samples 1-3, decrease in sample 4, and partially recover in sample 5.

Body weights are depressed in mud-exposed specimens relative to control specimens, suggesting that the effect is attributable to mud exposure.

Two questions were posed in experiments on drilling mud suspensions on the digestive efficiency of Spisula solidissima. First, are feeding and digestive processes affected by various concentrations of drilling mud suspensions? Second, is the efficiency of digestion affected by various concentrations of drilling mud suspensions? The surf clam subjected to drilling mud suspensions had to process an enormous amount of material just to ingest the same quantity of chlorophyll as the control subgroups. More energy is required by the mud treated animals to perform this task, and the efficiency of digestions is reduced. This may have a marked effect upon a population of surf clams subjected to a chronic turbidity stress.

Energy stores measured showed that there is a depletion in experimental specimens compared to field populations. When feeding was increased the energy stores in control specimens were equivalent to field populations, and there was a decrease of energy stores of experimental populations indicating a stress due to drilling muds. Further analysis of these data will have to await the completion of the field and experimental programs.

Specimens do recover from brief exposures to drilling muds. There is good evidence of depuration and some puzzling histological evidence of change and then recovery. The presence of metals in various organs does not affect the physiological state and leads to the conclusion that at concentrations of 1 ppt of the muds used, the principal effect may be a turbidity effect that does stress the specimens.

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