According to our findings, the water content of these worms appears to be independent of the presence or absence of the cerebral ganglia and the cephalic gland during a 1 to 24 hour exposure to 70% seawater. This may be linked with the fact that, to our knowledge, neuroendocrine control has only been demonstrated for mechanisms of extracellular (e.g., Grimm-Jørgensen, J. Exp. Zool. 212: 471-473, 1980) rather than intracellular volume regulation in invertebrates. In nemerteans, extracellular volume regulation may be obscured because such a small fraction of the total water is extracellular fluid. However, since neuroendocrine influences on intracellular volume have not been well studied (Gilles, 1979. In: Mechanisms of Osmoregulation in Animals, Gilles (ed.), Wiley, N.Y.), and since the intracellular compartment in this species is of significant magnitude in the intact animal, mechanisms involving the control of intracellular volume lend themselves to study in this species. Support was provided by NIH awards GM 07047, AM 15972 and AM 15973.

DRILLING FLUID EFFECTS ON TELEOST AND ECHINODERM DEVELOPMENT

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As a consequence of petroleum drilling offshore, large quantities of drilling fluids (muds) are introduced into the marine environment. These drilling fluids are aqueous suspensions of a variety of components pumped down the center of the drill bit. The fluid composition is varied as needs of the drilling operation are encountered, such as lubrication, cooling, antibacterial action, suspension of drill cuttings, prevention of intrusion of seawater into the bore hold, and capture of H_2S. Portions or all of the drilling fluid may be discharged into the surrounding waters during the drilling program and especially at its completion. This discharge can amount to significantly large quantities (e.g., 2,000 tons per hole) in the vicinity of a drilling platform.

The impact of drilling fluids on marine and estuarine environments is unknown due to the paucity of toxicological studies which have been conducted. In this report we describe the effects of five different drilling fluids on fertilization and early embryo development in a teleost and an echinoderm.

Five drilling fluid samples were tested, all obtained from a Mobile Bay drilling rig and supplied by the U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Fla. These samples were lignosulfonate-mud types containing barium sulfate. They are identified by the date the sample was taken in 1979: March 12, April 24, May 29, June 26, and October 11. Stock materials were kept at 4° and solutions or suspensions prepared from the stock were kept at the temperature of the developing embryos. Standardization of the drilling fluid solutions was based on analyses of dry weight. For example, the dry weight of one of the stock solutions was 262 mg/ml which was then indicated as 262 ppt. The test solutions were dilutions of this to make 10 ppt, 1 ppt, 100 ppm, 10 ppm and 1 ppm. Upon making these solutions with seawater, copious precipitates developed. Therefore following dilution the fluids were filtered through Whatman #1 paper. Therefore the drilling fluid components tested were those which remain soluble in sea water.

Embryos of Fundulus heteroclitus were used as the model for teleost development studies. Adult fish were obtained from estuarine waters of Frenchman Bay and kept in floating live cars in seawater. Eggs were stripped from females and fertilization was initiated by adding a sperm suspension obtained by mincing dissected testes. Filtered seawater kept at 18° was the normal incubation medium.

For these studies, embryos were placed in the drilling fluid solutions 1 min after fertilization and they were maintained at those concentrations for the duration of their development. The incubation dishes contained 10 ml of medium, changed daily, and from 50 to 100 embryos. The particularly susceptible events to environmental toxins observed with the dissecting microscope included early cleavage, blastulation, gastrulation, formation of the body

axis, appearance of somites, yolk and body pigmentation, heart development and circulation, eye pigmentation, formation of organs and various fins, jaw and body movements, and hatching. At 18° the time from fertilization to hatching is approximately 25 days.

The results from these studies showed the considerable variability of the drilling fluids tested. Embryos in 10 ppt of the October 11 sample showed developmental delay during early stages of gastrulation. These embryos continued development, although slowly, until late stages of organogenesis at which time they were arrested and died. Their appearance and movements were normal except for a reduction in pigmentation. The June 26 sample produced another effect at the 10 ppt and 1 ppt levels. At the onset of organogenesis development was delayed and the rate of heartbeat was reduced (inhibited 70% in 10 ppt, 20% in 1 ppt). The 10 ppt embryos all died without further development while the 1 ppt group seemed to recover by the time hatching stages were reached.

Other effects of drilling fluids on Fundulus development were not seen until hatching (Table 1). The March 12

TABLE 1

EFFECTS OF DRILLING FLUIDS ON HATCHING AND FRY VIABILITY OF FUNDULUS

Drilling Fluid	Concentrations	% Hatched[a]	% Fry Alive[a]
March 12	0	60	87
	1 ppm	72	94
	10 ppm	70	93
	100 ppm	79	96
	1 ppt	72	75
	10 ppt	10	10
April 24	0	97	95
	1 ppm	62	100
	10 ppm	53	93
	100 ppm	83	91
	1 ppt	71	83
	10 ppt	55	96
May 29	0	78	95
	1 ppm	80	94
	10 ppm	68	89
	100 ppm	97	87
	1 ppt	76	63
	10 ppt	61	74
June 26	0	34	93
	1 ppm	27	93
	10 ppm	38	98
	100 ppm	17	96
	1 ppt	9	83
	10 ppt	0	0
October 11	0	64	100
	1 ppm	69	87
	10 ppm	67	94
	100 ppm	66	95
	1 ppt	44	95
	10 ppt	0	0

[a]At 30 days post fertilization.

sample prevented nearly all hatching at the 10 ppt concentration while lower concentrations appeared to have no effect. The April 24 and May 29 samples had little or no significant effect on development and hatching at all concentrations tested. The June 26 sample was the most toxic, causing a reduction of hatching rate at 100 ppm while the October 11 sample significantly reduced hatching at 1 ppt. It should also be noted that those fry which hatched survived for several days except for those in high concentrations of the March 12 and May 29 drilling fluids.

Embryos of the sand dollar Echinarachnius parma were used as the model for echinoderm development studies. Adults were collected in Frenchman Bay, Maine and were kept at 10° to minimize spontaneous spawning. Gametes were obtained by coelomic injection of about 1 ml of 0.5 M KCl. Fertilization was begun by adding a few drops of a 1% sperm suspension to a suspension of eggs in about 100 ml of filtered seawater. The normal incubation medium was filtered seawater kept at 16°. The progress of development was observed with a compound microscope, particularly noting any effects on early cleavage, blastulation, hatching of the blastula to a spinning form, gastrulation, development of the prism and formation of the pluteus. When noting the progress of pluteus development, attention was directed to motility and to the symmetry and lengths of the "arms". Approximately 10,000 embryos were placed in 30 ml of each incubation medium 10 to 15 minutes after fertilization and they remained in these solutions for the duration of the experiment.

Table 2 summarizes the effects of the drilling fluids on sand dollar development when embryos are placed in

TABLE 2

EFFECTS OF DRILLING FLUIDS ON DEVELOPMENT OF SAND DOLLAR EMBRYOS

Drilling Fluid	Concentration[a]	Stage of Arrest[b]
March 12	10 ppt	Blastula
April 24	1 ppt	Early pluteus
	10 ppt	Blastula
May 29	1 ppt	Early pluteus
	10 ppt	Blastula
June 26	1 ppt	Early pluteus
	10 ppt	Blastula
October 11	1 ppt	Early pluteus
	10 ppt	1st cleavage

[a]At all lower concentrations development was normal.

[b]In all cases, development to the stage of arrest was retarded and in a variety of ways abnormal.

the fluid 15 min. after fertilization. Embryos were able to develop normally in all of the fluids up to concentrations of 100 ppm, and in the case of the March 12 sample at 1 ppt. At higher concentrations arrest of development may be seen occurring from prevention of first cleavage using the October 11 fluid to inhibition of post-blastula development in others. With most drilling fluids, concentrations of 1 ppt allow for slow and abnormal development to a late prism or early pluteus stage.

Effects of drilling fluids on fertilization of sand dollar eggs was also tested. Gametes were pre-incubated for 15 min in the drilling fluid solutions prior to mixing. Fertilization was initiated by adding 5 drops of the 1% sperm

suspension to 20 ml of egg suspension. Within two minutes the fertilized eggs may be identified microscopically as those with raised fertilization membranes. In each experiment, percent fertilization was determined by observing 100 eggs.

Table 3 shows a typical experimental series, in this case on the June 26 sample. In no experiments was treat-

TABLE 3

EFFECT OF JUNE 26 DRILLING FLUID ON FERTILIZATION OF SAND DOLLAR EGGS

Drilling fluid Treatment medium	Sperm-Egg Treatment Combinations	% Fertilization
1 ppm	Normal sperm + Normal eggs	90
	Treated sperm + Normal eggs	90
	Normal sperm + Treated eggs	89
	Treated sperm + Treated eggs	90
10 ppm	Normal sperm + Normal eggs	90
	Treated sperm + Normal eggs	90
	Normal sperm + Treated eggs	90
	Treated sperm + Treated eggs	90
100 ppm	Normal sperm + Normal eggs	90
	Treated sperm + Normal eggs	83
	Normal sperm + Treated eggs	88
	Treated sperm + Treated eggs	88
1 ppt	Normal sperm + Normal eggs	90
	Treated sperm + Normal eggs	77
	Normal sperm + Treated eggs	6
	Treated sperm + Treated eggs	4
10 ppt	Normal sperm + Normal eggs	93
	Treated sperm + Normal eggs	84
	Normal sperm + Treated eggs	1
	Treated sperm + Treated eggs	1

ment of sperm effective in significantly reducing the percentage of fertilization. In Table 4 the results of all drilling

TABLE 4

SUMMARY OF DRILLING FLUID EFFECTS ON SAND DOLLAR EGG FERTILIZABILITY

Drilling Fluid	Concentration[a]	% Fertilization of Treated Eggs[b]
March 12	10 ppt	48, 58, 82, 92, 97
April 24	10 ppt	11, 8
May 29	10 ppt	3, 6, 0, 8, 16
June 26	1 ppt	6, 63, 93, 95
	10 ppt	1, 0, 0, 0
October 11	1 ppt	3, 1, 0
	10 ppt	1, 1, 0

[a]At all lower concentrations fertilization was not affected.

[b]Each figure represents a separate experiment.

fluids are summarized. The effects on eggs of the 10 ppt March 12 sample and the 1 ppt June 26 sample are not clear from the data which show a wide range of toxicity. It would seem plausible that these fluids are on the borderline of toxicity and therefore gametes from different animals demonstrate various susceptibilities.

In an effort to identify the toxic components in the drilling fluids some ions present in large quantities in the most toxic fluids were tested for their effects on sand dollar fertilization. These results are seen in Table 5.

TABLE 5

EFFECTS OF SOME DRILLING FLUID COMPONENTS ON FERTILIZATION OF SAND DOLLAR EGGS

Component[a]	Results
$Na_2Cr_2O_7$	10 mM, Highest concentration tested, no effect
Na_2CrO_4	10 mM, Highest concentration tested, no effect
Ba^{++}	Approx. 10^{-5}M, level of saturated solution of $BaSO_4$, no effect
$ZnCl_2$	0.1 mM or higher concentration, completely inhibits

[a]All solutions were made in seawater and the pH was adjusted to 7.9.

In the case of barium, note that the large quantity of sulfate in seawater limits the quantity of dissolved barium in the medium, whether from the drilling fluid or $BaCl_2$. Zinc is the substance most toxic in these tests and is also present in concentrations which are highest in the most toxic drilling fluids. Values of 606 μg/g and 163 μg/g zinc have been determined for the October 11 and June 26 samples respectively (R.F. Shokes, Science Applications, Inc., reported to E.P.A. April 4, 1980). Zinc levels in the other fluids are less than 100 μg/g. This work was supported by U.S.E.P.A. Grant No. CR-807102010.

JUNCTIONAL COMPLEX STRUCTURE OF SMALL INTESTINAL EPITHELIAL CELLS IN WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS)

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INTRODUCTION

Epithelial cells of the small intestine are interconnected at their apices by junctional complexes. The most apical portion of this junctional zone consists of the tight junction which is characterized by localized sites of fusion of adjacent epithelial cell membranes. It has been suggested that the tight junction may, in part, regulate the flow of small molecules through the paracellular pathway (Schultz et al., Ann. Rev. Physiol. 36:51-91, 1974). Moreover, the complexity of the structure of tight junctions as revealed by freeze fracture may correlate with their ability to regulate paracellular flow (Claude and Goodenough, J. Cell. Biol., 58:390-400, 1973). In mammalian small intestine these structural features vary along the crypt-villus axis and with cell type (Madara et al., Gastroenterology, 78: 963-975, 1980) and may in part be determined by local events within the epithelium such as cell division (Tice et al., Tissue and Cell 11:293-316, 1979). Since cell proliferation in flounder small intestine differs markedly from that in