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Effect of Eight Outer Continental Shelf Drilling Muds on the Calcification Rate and Free Amino Acid Pool of the Coral *Acropora cervicornis*

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During most offshore drilling operations, drilling muds are routinely discharged into surrounding waters. Because corals are relatively sensitive to many environmental perturbations (e.g. Jaap, 1979; Loya & Rinkevich, 1980; Egana & DiSalvo, 1982) and can be adversely affected by offshore drilling operations (Hudson et al., 1982; Choi, 1982), the effects of drilling muds on corals have received considerable attention (Thompson & Bright, 1977; Thompson et al., 1980; Hudson & Robbin, 1980). Discharged drilling muds potentially can affect corals in three ways: direct burial, increased turbidity and direct toxicity. Different muds can vary considerably in relative toxicity to marine organisms (e.g. Conklin et al., 1980).

Because drilling muds are discharged intermittently, only periodic exposures of short duration should impact nearby coral reefs. To fully assess the impact of a drilling mud discharge on corals requires an assessment of the capacity for corals to recover from short-term exposure. Kendall et al (in press) and Powell et al (in press) investigated recovery capacity by monitoring protein composition, calcification rate and the free amino acid (FAA) pool in Acropora cervicornis 48 hr after the termination of exposure. Recovery was defined as at least a partial return to control levels of those parameters significantly different from control levels after a 24-hr exposure. At the highest exposure levels, a clear deterioration in health occurred during the recovery period in spite of the fact that drilling mud exposure had ceased. Both protein and amino acid loss and a reduction in calcification rate were observed. Calcification rate, which normally was reduced after a 24-hr exposure at most exposure concentrations, recovered rapidly to control levels over the 48-hr recovery period only at exposure concentrations where protein loss was not observed; however, recovery of the FAA pool to control levels was never observed during the recovery period at any exposure concentration used. Thus, the FAA pool recovered much less rapidly than calcification rate and significant differences after 48-hr recovery were produced by much lower exposure concentrations than for calcification rate. Unfortunately, the observed toxicity of the muds after the 48-hr recovery period could not be predicted from the coral's response during the exposure. Both Kendall et al (in press) and Powell et al (in press) concluded that a correct assessment of toxicity

required data on recovery capacity. Few such data are available on corals; those that do exist are from muds which were slated for land disposal and would not have impacted coral reefs. The purpose of this study was to assess the relative toxicity of a number of muds that were slated for marine disposal for the coral Acropora cervicornis after a 48-hr recovery period.

MATERIALS AND METHODS

The methods used for field exposure and recovery and laboratory analyses are described in Kendall et al (1983), Kendall et al (in press) and Powell et al (in press). Briefly, the corals were placed in a grid mounted on a plexiglass base which was attached to a PVC holding frame. Plexiglass domes were affixed to the bases using silicone coated gaskets to seal the chambers. Gravity flow ($32\ l \cdot hr^{-1}$) was used to circulate solutions through these STACH (short term aerated coral habitat) units of which there were four per STACH experiment, 3 exposure groups and one control. Four STACH experiments were run; STACH VIII, IX, and X with drilling muds diluted to 25 ppm (v/v) by clean seawater, STACH XI with drilling muds diluted to 50 ppm (v/v). An exposure concentration of 25 ppm was chosen because this concentration of a Mobile Bay mud tested by Kendall et al. (in press) and Powell et al (in press), which was considered by Neff (1981) to be among the more toxic of the drilling muds previously tested, produced only changes in the FAA pool in Acropora cervicornis after 48-hr recovery. Neither calcification rate nor protein concentration varied from control levels 48 hr after exposure to this Mobile Bay mud nor was zooxanthellae loss observed at this exposure concentration.

Exposures were for 24-hr periods, after which the domes were removed and replaced by domes with numerous holes which allowed continuous circulation of clean seawater past the corals, but prevented access to corals by other large organisms. Recovery periods were 48 hr. Nine muds were tested; eight from outer continental shelf (OCS) drilling operations, one was the NBS (National Bureau of Standards) standard mud. Chemical analyses of the 8 OCS muds are given by SCIENCE APPLICATIONS, INC. (1983). Weight to volume conversions for the muds are as follows (in g dry $wt \cdot ml^{-1}$): P1, 0.92; P2, 1.32; P3, 1.36; P4, 0.94; P5, 1.30; AN, 0.71; SV, 0.75; MIB, 0.40; NBS, 0.48.

In the laboratory, four contiguous sections were cut from each coral branch starting from the growing tip and proceeding toward the base. Sections were 1 cm long and were labelled from the tip down, A through D, respectively. Calcification rate, protein concentration, polyp number and skeletal weight were measured as described by Kendall et al (1983). For determination of the FAA pool, combined samples from sections A&B and sections C&D were brought up to 10% trichloroacetic acid. After centrifugation, the supernatant was desalted using AG50W-X4 resin and the amino acids eluted using 0.5 N LiOH. Analysis was performed using an amino acid analyzer with a lithium citrate buffer system and ninhydrin as the detecting compound. Data for the six amino acids composing the bulk of the FAA

pool are reported here.

The rationale for the choice of the normalizing parameters, methods for statistical analyses and the extent to which stresses caused by collection, handling, and enclosure in the domes limit the interpretation of recovery data have been discussed in detail by Kendall et al (1983, in press) and Powell et al (in press). Data were normalized to both skeletal weight and polyp number. The polyp number to skeletal weight ratio was very similar within each experiment; it differed significantly between an exposure group and its control only for STACH X, mud P5, section D (Table 1). Kendall et al (1983, in press) suggested that skeletal weight was the better normalizing parameter for calcification rate and polyp number for protein concentration, however no preference could be determined for amino acid concentration (Powell et al., in press). Statistical analyses used Duncan's multiple range test (α = .05) performed on log-transformed and ranked data for each normalizing parameter. This yielded four sets of comparisons for each data set. On occasion the four comparisons disagreed as to whether differences were significant (Tables 2, 3). Such cases will be termed equivocal results. Except where noted, we base all conclusions for amino acids only on data for which the results of all four comparisons agreed; for calcification rate and protein concentration, we follow the recommendations of Kendall et al (1983). Glycine was unusual in that equivocal results occurred much more frequently than in any other amino acid (Powell et al., in press). Because glycine is the amino acid present in highest concentration, the data are reported, but no inferences on toxicity are based on these data.

TABLE 1

Results of Duncan's multiple range test on the polyp number to skeletal weight ratio of the corals used for the data analyses tabulated in Tables 2 and 3. Each test utilized data from one of the 8 columns only. Different letter designations within columns designate significant difference (α=.05). Valid comparisons are limited to single columns only. Same (different) letter designations between columns do not imply any statistical similarity (difference). L, log-transformed data; R, ranked data.

		Polyp/wt. ratio							
	Section	A		B		C		D	
		L	R	L	R	L	R	L	R
STACH VIII	Control	a	ab	ab	ab	a	a	a	ab
	MIB	a	a	ab	ab	abc	a	ab	a
	AN	a	ab	ab	a	ab	a	ab	a
	SV	ab	bcd	ab	ab	bc	ab	abc	ab
STACH IX	Control	a	ab	ab	a	ab	a	ab	a
	P3	ab	abc	ab	a	abc	a	abc	ab
	P1	ab	abcd	ab	ab	bc	ab	bc	ab
	P5	ab	abcd	ab	ab	bc	ab	c	b
STACH X	Control	ab	abcd	ab	ab	bc	ab	bc	ab
	P4	ab	abcd	ab	ab	abc	a	abc	a
	P2	a	ab	ab	ab	abc	ab	bc	ab
	NBS	ab	cd	b	b	c	b	bc	b
STACH XI	Control	ab	abcd	ab	ab	abc	a	abc	ab
	SV	ab	abc	a	a	ab	a	ab	a
	P4	a	abcd	ab	ab	abc	a	bc	ab
	P3	b	d	b	ab	bc	ab	bc	ab

TABLE 2

Calcification rate and soluble protein concentration in Acropora cervicornis after 48 hr recovery from drilling mud exposure. n = 5; upper value is the mean; in parentheses, the standard deviation; A-D, 1 cm sections from the growing tip (A) down the stalk respectively; brackets, results of statistical tests ($\alpha = .05$) of comparisons between controls and the indicated exposure group in which calcification rate or protein concentration was significantly lower than controls; left brackets, log-transformed data; right brackets, ranked data. Units are: $CaCO_3$, mg $CaCO_3 \cdot min^{-1}$; weight, mg skeletal weight; protein, µg protein; polyp, per single polyp.

	STACH VIII				STACH IX				STACH X				STACH XI			
Section	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	CONTROL				25 ppm SV				25 ppm M1B				25 ppm AN			
$\frac{CaCO_3}{polyp}$	184.4 (46.2)	179.1 (45.0)	134.5 (30.3)	114.9 (8.5)	284.6 (83.0)	188.2 (63.4)	172.1 (60.4)	125.9 (46.9)	293.1 (83.5)	278.6 (82.2)	226.9 (53.8)	202.9 (62.2)	225.5 (80.8)	205.6 (63.4)	186.7 (73.9)	177.0 (77.6)
$\frac{CaCO_3}{weight}$	44.6 (12.1)	16.8 (4.0)	9.5 (3.1)	6.3 (2.7)	51.1 (11.3)	16.1 (5.2)	8.1 (2.5)	4.8 (1.7)	68.8 (17.8)	24.9 (5.3)	13.2 (2.9)	8.4 (1.8)	51.7 (6.3)	20.7 (2.8)	10.9 (1.6)	7.3 (1.7)
$\frac{Protein}{polyp}$	92.5 (36.7)	183.3 (86.9)	209.8 (119.3)	235.8 (139.3)	103.9 (14.1)	177.4 (31.8)	255.6 (55.6)	258.0 (35.6)	85.9 (17.1)	163.3 (36.6)	209.6 (41.3)	247.8 (60.0)	87.8 (30.4)	154.0 (17.5)	209.3 (40.9)	235.9 (49.2)
$\frac{Protein}{weight}$	21.2 (1.6)	15.9 (1.2)	12.8 (1.5)	10.4 (1.6)	18.8 (1.5)	14.9 (1.4)	11.9 (1.7)	10.0 (1.3)	20.6 (5.7)	14.8 (3.3)	12.3 (2.7)	10.3 (1.9)	20.6 (3.9)	16.0 (2.1)	12.7 (1.7)	10.2 (1.6)
Section	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	CONTROL				25 ppm P3				25 ppm P5				25 ppm P1			
$\frac{CaCO_3}{polyp}$	246.0 (49.0)	201.8 (22.7)	165.8 (24.5)	152.1 (32.5)	273.6 (38.7)	199.3 (34.2)	198.6 (52.6)	154.4 (32.0)	219.0 (54.3)	175.5 (40.8)	135.9 (20.3)	115.5 (32.3)	184.7 (51.4)	131.5 (38.4)	121.0 (56.1)	101.4 (56.4)
$\frac{CaCO_3}{weight}$	56.0 (6.7)	20.2 (4.6)	10.7 (3.5)	7.2 (3.1)	55.9 (13.4)	21.2 (4.9)	11.4 (3.9)	6.1 (2.0)	40.7 (8.3)	13.4 (2.6)	6.2 (1.5)	3.5 (1.1)	37.6 (13.9)	11.7 (6.1)	6.3 (4.5)	3.9 (2.8)
$\frac{Protein}{polyp}$	102.6 (11.9)	177.0 (33.6)	218.1 (85.3)	284.4 (81.4)	104.5 (14.4)	143.3 (13.2)	213.5 (22.0)	244.2 (27.4)	135.0 (33.8)	180.9 (72.7)	240.2 (59.9)	274.2 (66.4)	113.6 (21.7)	136.7 (45.7)	205.5 (58.8)	269.9 (86.3)
$\frac{Protein}{weight}$	23.7 (2.9)	17.2 (1.4)	12.9 (1.3)	11.5 (1.3)	21.1 (3.5)	15.2 (2.9)	12.1 (2.5)	9.5 (2.3)	25.0 (3.7)	13.2 (2.2)	10.5 (1.0)	8.1 (0.5)	22.9 (7.8)	11.2 (3.5)	9.9 (4.0)	9.7 (3.1)
Section	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	CONTROL				25 ppm P2				25 ppm P4				25 ppm NBS			
$\frac{CaCO_3}{polyp}$	241.6 (26.8)	203.4 (40.9)	187.1 (31.3)	167.5 (36.8)	266.7 (70.1)	228.5 (30.3)	217.6 (26.3)	212.0 (34.0)	148.3 (54.5)	111.2 (48.4)	93.9 (40.4)	72.0 (34.2)	276.4 (100.0)	193.1 (71.8)	164.2 (58.7)	126.6 (50.2)
$\frac{CaCO_3}{weight}$	43.8 (10.0)	15.7 (3.5)	9.0 (1.9)	5.7 (0.9)	55.6 (12.8)	20.0 (2.9)	11.3 (1.7)	7.7 (1.1)	28.8 (9.9)	10.4 (4.7)	4.9 (2.2)	3.0 (1.8)	45.4 (19.4)	14.4 (5.7)	7.0 (3.0)	4.2 (1.9)
$\frac{Protein}{polyp}$	127.3 (17.1)	209.9 (43.1)	268.9 (37.4)	298.7 (28.3)	125.1 (22.2)	180.8 (17.1)	221.3 (19.8)	268.1 (42.2)	95.9 (31.3)	126.6 (20.6)	200.1 (25.7)	217.9 (10.3)	110.6 (55.3)	170.2 (28.8)	234.1 (39.7)	238.4 (40.0)
$\frac{Protein}{weight}$	22.8 (3.3)	15.9 (1.7)	12.7 (0.8)	10.3 (1.4)	26.3 (5.0)	15.8 (2.0)	11.5 (1.4)	9.7 (1.1)	18.9 (6.9)	11.9 (1.6)	10.5 (1.1)	8.8 (1.2)	17.9 (9.9)	12.7 (4.5)	9.7 (2.6)	8.0 (2.6)
Section	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	CONTROL				50 ppm P3				50 ppm P4				50 ppm SV			
$\frac{CaCO_3}{polyp}$	165.7 (46.7)	133.3 (23.7)	120.4 (24.6)	110.5 (26.2)	211.7 (98.3)	154.7 (39.0)	136.8 (37.4)	115.4 (36.6)	143.8 (22.7)	133.5 (46.7)	106.4 (31.3)	94.2 (43.9)	208.7 (55.0)	140.9 (28.6)	150.6 (32.6)	141.1 (33.4)
$\frac{CaCO_3}{weight}$	31.1 (10.1)	12.4 (3.1)	6.8 (2.3)	4.4 (1.6)	33.5 (16.6)	12.2 (5.2)	6.6 (2.7)	4.0 (1.6)	29.9 (8.8)	11.5 (4.0)	5.8 (1.7)	3.4 (1.1)	39.4 (6.6)	16.0 (3.2)	9.3 (2.2)	6.1 (1.7)
$\frac{Protein}{polyp}$	111.4 (13.6)	144.9 (21.4)	178.5 (23.0)	223.7 (21.0)	114.1 (24.8)	167.5 (49.0)	216.2 (45.0)	237.0 (87.0)	83.7 (12.3)	141.9 (49.8)	164.1 (26.9)	201.4 (48.0)	105.8 (41.0)	122.1 (21.3)	180.5 (35.6)	228.8 (75.1)
$\frac{Protein}{weight}$	20.9 (4.4)	13.3 (1.7)	9.8 (1.4)	8.7 (1.4)	17.6 (4.9)	12.3 (3.9)	10.0 (2.9)	8.0 (2.9)	17.2 (3.4)	11.9 (2.7)	9.0 (2.0)	7.3 (1.5)	19.6 (3.3)	14.0 (2.5)	11.3 (2.8)	9.8 (2.8)

TABLE 3

Amino acid concentration in Acropora cervicornis after 48 hr recovery from drilling mud exposure. n = 5; upper value is the mean; in parentheses, the standard deviation; AB, CD, coral sections as explained in the text; brackets indicate significant results (α = .05) of statistical tests of comparisons between controls and the indicated exposure group; left brackets, log-transformed data; right brackets, ranked data.

		μmol·g skeletal wt^{-1}								$n$mol·polyp^{-1}							
		Control		25 ppm SV		25 ppm MIB		25 ppm AN		Control		25 ppm SV		25 ppm MIB		25 ppm AN	
		AB	CD	AB	CD	AB	CD	AB	CD	AB	CD	AB	CD	AB	CD	AB	CD
STACH VIII	Aspartate	1.05 (.91)	.42 (.42)	.48 (.04)	.26 (.04)	[.41 (.06)	.25 (.10)	[.44 (.10)	.23 (.05)	10.0 (10.0)	6.1 (3.6)	4.3 (0.5)	6.1 (0.7)	3.2 (0.6)	5.3 (2.1)	3.1 (0.1)	4.4 (0.6)
	Threonine	.56 (.28)	.28 (.23)	[.33 (.05)	.19 (.03)	[.28 (.08)]	.17 (.05)	[.27 (.03)]	.15 (.06)	5.2 (4.2)	4.3 (1.8)	2.9 (0.2)	4.5 (0.1)	[2.1 (0.5)	3.5 (0.9)	[1.9 (0.3)]	2.9 (0.8)
	Serine	2.70 (1.35)	1.29 (.68)	1.83 (.15)	1.05 (.16)	[1.61 (.27)	1.28 (.51)	[1.61 (.18)	.98 (.24)	26.1 (24.7)	20.9 (6.0)	16.5 (1.7)	24.9 (3.7)	12.3 (2.2)	26.4 (11.6)	11.6 (1.7)	19.2 (5.7)
	Glutamate & Glutamine	1.07 (.83)	.57 (.51)	.52 (.03)	.38 (.07)	.59 (.13)	.46 (.12)	.58 (.12)	.42 (.08)	10.0 (9.3)	8.9 (4.7)	4.7 (0.6)	8.9 (1.4)	4.5 (1.0)	9.5 (3.0)	4.1 (0.9)	8.2 (0.8)
	Glycine	7.79 (7.05)	4.10 (5.04)	3.75 (.47)	1.98 (.27)	3.45 (.79)	2.28 (.49)	3.32 (.39)	1.92 (.46)	73.6 (74.6)	56.9 (46.8)	33.6 (2.2)	46.5 (3.3)	26.2 (5.6)	47.5 (13.7)	24.0 (4.2)]	37.0 (6.6)
	Alanine	.88 (.62)	.44 (.39)	[.41 (.02)	.28 (.07)	[.46 (.08)	.26 (.05)	[.45 (.06)	.28 (.06)	8.1 (7.1)	6.5 (3.3)	3.7 (0.6)	6.7 (1.2)	3.5 (0.3)	5.4 (1.0)	3.2 (0.3)	5.5 (0.7)
		Control		25 ppm P3		25 ppm P5		25 ppm P1		Control		25 ppm P3		25 ppm P5		25 ppm P1	
		AB	CD	AB	CD	AB	CD	AB	CD	AB	CD	AB	CD	AB	CD	AB	CD
STACH IX	Aspartate	.38 (.07)	.20 (.03)	.35 (.08)	.15 (.06)	.24 (.08)	.15 (.03)	[.33 (.12)]	.19 (.05)]	2.8 (0.7)	4.0 (0.9)	2.6 (0.4)	3.3 (0.7)	2.4 (0.9)	4.2 (1.0)	3.0 (1.2)	4.6 (1.4)
	Threonine	.26 (.09)	.14 (.01)	.22 (.05)	.11 (.03)	[.14 (.05)]	[.09 (.03)]	[.16 (.05)]	.11 (.03)	1.9 (0.5)	2.8 (0.7)	1.6 (0.2)	2.5 (0.4)	1.4 (0.4)	2.4 (0.5)	1.4 (0.3)	2.6 (0.7)
	Serine	1.65 (.21)	.91 (.11)	1.52 (.28)	.70 (.22)	[.80 (.30)]	.50 (.19)	[.77 (.20)]	[.55 (.15)]	12.4 (2.1)	18.7 (8.1)	11.3 (1.1)	15.5 (2.2)	[7.9 (2.9)]	13.7 (4.7)	[7.0 (1.0)]	13.5 (3.0)
	Glutamate & Glutamine	.44 (.11)	.35 (.02)	.36 (.03)	[.25 (.05)]	.44 (.11)	.35 (.06)	.60 (.24)	.55 (.18)]	3.3 (0.6)	7.7 (2.0)	2.8 (0.7)	5.6 (0.7)	4.5 (1.9)	9.9 (2.8)	[5.4 (1.7)]	[13.6 (4.7)]
	Glycine	2.61 (.25)	1.42 (.14)	2.25 (.40)	[1.06 (.30)]	1.95 (.33)]	1.17 (.17)	2.93 (1.48)	1.52 (.45)	19.6 (4.0)	28.9 (11.9)	16.8 (1.3)	23.6 (2.8)	19.7 (6.4)	32.5 (5.4)	27.7 (17.0)	37.5 (12.2)
	Alanine	.35 (.02)	.21 (.02)	.33 (.05)	[.14 (.04)]	.27 (.08)	.19 (.03)	.33 (.12)	.25 (.07)	2.7 (0.5)	4.2 (1.5)	2.5 (0.5)	3.3 (0.5)	2.8 (1.3)	5.2 (0.9)	3.0 (1.1)	[6.3 (2.0)]

STACH X

	Control		25 ppm P2		25 ppm P4		25 ppm NBS	
	AB	CD	AB	CD	AB	CD	AB	CD
Aspartate	.53 (.50)	.16 (.05)	.29 (.09)	.19 (.04)	.43 (.13)	.23 (.06)	.34 (.10)	.19 (.06)
Threonine	.33 (.40)	.09 (.03)	.14 (.04)	.10 (.02)	.16 (.05)	.08 (.01)	.14 (.06)	.09 (.02)
Serine	2.31 (2.85)	.53 (.14)	.88 (.22)	.60 (.12)	.93 (.21)	.48 (.09)	.90 (.31)	.50 (.18)
Glutamate & Glutamine	.49 (.19)	.35 (.17)	.48 (.15)	.40 (.04)	.68 (.27)	.47 (.08)	.53 (.16)	.39 (.09)
Glycine	3.94 (2.64)	1.49 (.50)	2.33 (.62)	1.48 (.14)	3.15 (.46)	1.69 (.16)	3.02 (.81)	1.51 (.35)
Alanine	.59 (.77)	.13 (.03)	.25 (.09)	.19 (.04)	.42 (.13)	.22 (.03)	.29 (.08)	.17 (.05)

	Control		25 ppm P2		25 ppm P4		25 ppm NBS	
	AB	CD	AB	CD	AB	CD	AB	CD
Aspartate	4.5 (3.5)	4.0 (0.9)	2.4 (0.6)	4.3 (0.8)	3.4 (0.7)	5.0 (1.2)	3.4 (0.4)	5.2 (0.7)
Threonine	2.8 (2.9)	2.1 (0.7)	1.1 (0.3)	2.3 (0.5)	1.3 (0.2)	1.7 (0.1)	1.4 (0.1)	2.4 (0.7)
Serine	19.3 (20.7)	13.0 (2.8)	7.4 (1.2)	13.8 (2.2)	7.4 (1.2)	10.5 (1.6)	9.1 (0.7)	13.2 (2.6)
Glutamate & Glutamine	4.4 (1.0)	9.1 (5.9)	4.0 (1.1)	9.4 (1.5)	5.4 (1.5)	10.3 (1.7)	5.5 (0.6)	10.9 (2.8)
Glycine	34.2 (17.2)	36.9 (11.2)	19.3 (3.6)	34.3 (1.9)	25.5 (3.2)	37.5 (4.9)	31.2 (3.8)	42.5 (15.5)
Alanine	4.9 (5.6)	3.4 (1.2)	2.0 (0.7)	4.3 (0.7)	3.3 (0.8)	4.8 (0.9)	3.0 (0.4)	4.8 (1.2)

STACH XI

	Control		50 ppm P3		50 ppm P4		50 ppm SV	
	AB	CD	AB	CD	AB	CD	AB	CD
Aspartate	.26 (.09)	.60 (.06)	.25 (.08)	.60 (.22)	.71 (.17)	.34 (.16)	.44 (.08)	.41 (.18)
Threonine	.11 (.05)	.20 (.05)	.08 (.02)	.18 (.06)	.22 (.05)	.11 (.04)	.17 (.03)	.13 (.05)
Serine	.68 (.24)	1.22 (.09)	.63 (.19)	1.20 (.34)	1.59 (.36)	.80 (.27)	1.11 (.10)	.96 (.35)
Glutamate & Glutamine	.33 (.10)	.87 (.11)	.34 (.08)	.91 (.25)	.69 (.14)	.51 (.08)	.50 (.09)	.39 (.22)
Glycine	2.00 (.75)	4.37 (1.48)	1.99 (.69)	5.47 (1.79)	4.84 (1.02)	2.28 (1.06)	3.55 (.60)	2.66 (.59)
Alanine	.20 (.06)	.36 (.06)	.15 (.05)	.31 (.07)	.46 (.13)	.22 (.08)	.33 (.07)	.25 (.08)

	Control		50 ppm P3		50 ppm P4		50 ppm SV	
	AB	CD	AB	CD	AB	CD	AB	CD
Aspartate	2.4 (0.3)	12.8 (2.4)	2.6 (0.5)	14.9 (4.2)	6.1 (1.3)	7.6 (3.0)	3.3 (0.5)	7.7 (2.1)
Threonine	1.0 (0.2)	4.4 (1.4)	0.9 (0.2)	4.6 (1.1)	1.9 (0.5)	2.5 (0.9)	1.2 (0.2)	2.5 (0.6)
Serine	6.3 (0.6)	26.0 (3.7)	6.6 (1.4)	30.6 (7.3)	13.9 (3.8)	17.8 (4.7)	8.3 (0.8)	18.4 (4.2)
Glutamate & Glutamine	3.1 (0.3)	18.5 (3.3)	3.9 (1.3)	23.4 (4.5)	6.0 (1.2)	11.7 (2.5)	3.7 (0.4)	8.0 (4.7)
Glycine	18.4 (3.0)	90.0 (20.1)	20.5 (3.8)	137.6 (37.9)	42.5 (9.9)	50.9 (20.4)	26.3 (2.5)	52.9 (13.8)
Alanine	1.9 (0.2)	7.7 (2.1)	1.5 (0.6)	7.9 (1.2)	4.0 (1.0)	5.0 (1.7)	2.4 (0.3)	4.8 (1.6)

RESULTS AND DISCUSSION

At the 25-ppm exposure level, none of the nine muds tested by us produced observable zooxanthellae loss, however substantial protein loss occurred in P4-exposed corals. Loss in P1- and P5-exposed corals could only be demonstrated statistically using skeletal weight as the normalizing parameter. Similarly, reduced calcification rates occurred in P4- and P1-exposed corals. Rates were reduced in P5-exposed corals as well, however most statistical comparisons were equivocal. The three muds, P4, P5, and P1, also were the only muds to cause significant changes in the relative composition of the FAA pool (Table 4). Serine-threonine levels decreased relative to aspartate-alanine-glutamate in all three cases. The remaining muds produced slight, occasionally significant reductions in the FAA pool at the 25-ppm exposure level, but most significant results were equivocal. In all cases, although only certain amino acids showed significant changes, the effect could not be distinquished from a general, moderate decrease in pool size.

Experiment to experiment variability in the effect produced by exposure to the same mud, probably produced by day-to-day variability in such extrinsic environmental factors as cloud cover, sea state and turbidity, can substantially affect the muds perceived toxicity relative to other muds. Kendall et al (1983, in press) observed substantial variability in the extrapolated no-effect level of a Mobile Bay mud, for example. In one set of experiments, Kendall et al (1983) found that a no-effect level of 5 ppm derived from data on calcification rate was associated with significant protein and amino acid loss at exposure levels of about 100 ppm. In a second set of experiments, a higher no-effect level of 16 ppm was found (Kendall et al, in press), and no protein loss was observed at the 100-ppm exposure level; however significantly increased amino acid levels suggested that some protein breakdown had occurred. In our experiments, P4 produced similar results. Calcification rate and protein concentration were affected significantly at the 25-ppm exposure level, yet a few days later, a 50-ppm exposure produced neither effect. In the latter case, however, significantly increased amino acid levels in section A&B and significantly decreased levels in section C&D were observed. In both cases, the observed effects probably resulted from the process that ultimately causes zooxanthellae loss, but extrinsic environmental factors modified the time course of the response. This suggests that experiment to experiment comparisons of mud toxicity must be treated with caution.

The relative degree of toxicity among muds in experiments run simultaneously are more consistent however. P4 produced the most toxic effects in both STACH X and XI for example. P3 was consistently low in toxicity relative to the other two muds tested simultaneously (STACH IX, XI). Thus a comparison of two experiments designed so that one or more muds are common to both can be used to examine the range of toxicities present even though some variability in the absolute response may be present.

TABLE 4

Results of Duncan's multiple range tests (α = .05) on the percent change between the amino acid concentrations in each pair (n = 5). Different letter designations within any one row for any one section denote significant differences. Valid comparisons are limited to single rows only. Same (different) letter designations between rows do not imply any statistical similarity (difference). Within each pair for each amino acid, values for the upper member of the pair were randomly paired with values from the lower member and the quotients calculated by dividing the upper by the lower. These sets of quotients were then compared for all six amino acids within a section (AB or CD) for any pair. If all six amino acids were affected equivalently (i.e. a general change in pool size occurred), then no significant differences should be present and all amino acids in a section would have the same letter designations. Conversely, where significant differences do exist as indicated by a change in letter designation, some amino acids changed significantly relative to others. Glutamate includes glutamine.

Ratios	Section	-Aspartate	-Threonine	-Serine	-Glutamate	-Glycine	-Alanine
STACH VIII SV (vs.)	AB	a	a	a	a	a	a
STACH VIII Control	CD	a	a	a	a	a	a
STACH VIII MIB (vs.)	AB	a	a	a	a	a	a
STACH VIII Control	CD	a	a	a	a	a	a
STACH VIII AN (vs.)	AB	a	a	a	a	a	a
STACH VIII Control	CD	a	a	a	a	a	a
STACH IX P3 (vs.)	AB	a	a	a	a	a	a
STACH IX Control	CD	a	a	a	a	a	a
STACH IX P5 (vs.)	AB	b	b	b	a	ab	ab
STACH IX Control	CD	abc	bc	c	a	ab	a
STACH IX P1 (vs.)	AB	ab	b	b	a	ab	ab
STACH IX Control	CD	bcd	cd	d	a	bc	ab
STACH X P2 (vs.)	AB	a	a	a	a	a	a
STACH X Control	CD	a	a	a	a	a	a
STACH X P4 (vs.)	AB	a	a	a	a	a	a
STACH X Control	CD	ab	b	b	ab	ab	a
STACH X NBS (vs.)	AB	a	a	a	a	a	a
STACH X Control	CD	a	a	a	a	a	a
STACH XI P3 (vs.)	AB	a	a	a	a	a	a
STACH XI Control	CD	a	a	a	a	a	a
STACH XI P4 (vs.)	AB	a	a	a	a	a	a
STACH XI Control	CD	a	a	a	a	a	a
STACH XI SV (vs.)	AB	a	a	a	a	a	a
STACH XI Control	CD	a	a	a	a	a	a

Generally OCS muds have been considered less toxic than the two muds tested by Kendall et al (1983, in press) and Powell et al (in press), both of which were not slated for marine disposal. Neff (1981) considered one of the latter, from a Mobile Bay well, to be among the more toxic of the drilling muds previously used for effects assessment; the other, from Jay Oil Field, proved to be even more toxic to A. cervicornis (Kendall et al., in press). The results presented here do not support the belief that all OCS muds are substantially less toxic. Three, P4, P1 and P5, clearly are more toxic than the aforementioned Mobile Bay mud. All produced significant declines in calcification rate not observed in that mud even at the 132-ppm (v/v) exposure level. The remaining six muds produced effects similar to or less than those caused by the Mobile Bay mud at 13 ppm. Decreases in calcification rate were not observed. Effects on the FAA pool were very similar to those observed by Powell et al (in press). Significant effects were rarely unequivocal; however, in many cases, at least four of six amino acids were significantly different from controls in one or more of the four statistical comparisons used. Thus, the no-effect levels for a 24-hr exposure for these six muds should be in the range 5-15 ppm.

Clearly, the tested OCS muds have a wide range of toxicity; none could be considered completely non-toxic in the sense that no effects were observed at 25 ppm and a few were nearly as toxic as the Jay Oil Field mud tested by Powell et al (in press). Nevertheless, the exposure concentrations and exposure times used (25 and 50 ppm for the 24 hr) are above levels normally observed in discharge operations (e.g. Ray & Meek, 1980); thus, the data suggest that, under most discharge conditions, effects should be considerably below those reported here except, perhaps, within the immediate vicinity of a rig.

Considerable attention has been placed on the causative agent(s) for the observed toxicities of drilling muds. Heavy metals (especially chromium), hydrocarbons (especially diesel oil), various additives such as biocides; and particulate content (causing a turbidity increase) have been suggested as possible toxic agents (Tagatz et al., 1979; Loya & Rinkevich, 1980; Smith et al., 1982). Conklin et al. (1983), for example, demonstrated a correlation between toxicity and hydrocarbon content for the shrimp Palaemonetes pugio, but not for the sheepshead minnow and could find no relation between chromium content and toxicity for either organism. The 8 OCS muds used were analyzed in detail by SCIENCE APPLICATIONS, INC. (1983). As found by Kendall et al (1983), turbidity could not be implicated as the causative factor. The most toxic muds (P1, P4, P5) had particulate levels in the same range as the less toxic muds (0.9 - 1.3 g dry wt·ml^{-1}). P1 and P3 had much higher hydrocarbon levels than other muds (both >7000 mg·l^{-1} aliphatics and 600 mg·l^{-1} aromatics), yet P1 was quite toxic and P3 one of the least toxic of the muds tested. P4 and P5 had hydrocarbon levels below 1000 mg·l^{-1} (aliphatics) and 400 mg·l^{-1} (aromatics) yet were also quite toxic. It would appear that hydrocarbons are not the

primary toxic factor in all muds. The two muds with the highest dissolved metal content (Cr >40$\mu g \cdot g^{-1}$ wet st) were P4 and SV. P4 was among the most toxic of the eight muds. P1 and P5, however, were roughly as toxic but had a much lower dissolved metal content (Cr $\leq 3 \mu g \cdot g$ wet wt^{-1}). P5, in fact, was not unusual in any measured way save its toxicity. Thus, the cause for these mud's toxicity remains a mystery. Certainly, the data do not suggest that a single toxic agent present in all the tested muds was responsible for the observed effects in A. cervicornis but rather suggest that the toxic agents vary considerably among the muds and that, for corals, a priori predictions of toxicity from chemical analysis remains an elusive goal.

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