Arch. Environm. Contam. Toxicol. 9, 733–741 (1980)

Archives of Environmental
Contamination
and Toxicology

Lethality of a Suspended Clay to a Diverse Selection of Marine and Estuarine Macrofauna

V. A. McFarland and R. K. Peddicord
U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi

Abstract. In this investigation, an evaluation was made of the lethality of a suspended clay mineral texturally representative of the sediment-size fraction with which contaminants are most commonly associated. The study involved a phylogenetically diverse selection of marine and estuarine macrofauna. The time-concentration mortality response of 16 species of fish and invertebrates indicated widely differing sensitivities to high concentrations of clay suspended in the water. Organisms restricted to muddy bottoms were found to be very insensitive to high suspended clay concentrations. However, some open water fish, fouling organisms, and sandy bottom epifauna were found to be relatively sensitive. Tolerant species were also identified from these groups.

In a number of studies, various aquatic organisms have been exposed to suspended particulate materials including a variety of processed clay minerals, Fuller's earth, powdered chalk, incinerator ash, coal washings, and glass shards (Herbert and Merkins 1961, Rogers 1969, Sherk *et al.* 1974, Peddicord *et al.* 1975). Several investigators have used natural sediments (Wallen 1951, U.S. Fish and Wildlife Service 1970, Schubel and Wang 1973, Peddicord and McFarland 1978). In most cases, these sediments have been sized, dried, or otherwise altered with regard to their physical, chemical, and biological properties before using them in experiments. A comprehensive review of the literature on effects of turbidity and suspended material in relation to dredging in aquatic environments has been prepared by Stern and Stickle (1978).

The different methodologies employed in previous experiments make comparison of results difficult. A variety of techniques have been used to suspend particles, including periodic stirring with intermittent settling, continuous stirring, and mixing by aeration. Almost all laboratory work has been done in closed systems requiring frequent changing of the water with consequent handling of animals and limiting the duration of experimentation.

For the most part, previous studies have not related responses of animals to actual mass concentration of particles in suspension. Most studies relate responses to turbidity, an optical property of water containing suspended material of unknown absolute concentration. The turbidity produced by suspended

0090–4341/80/0009–0733 $01.80

particulate matter is influenced by many factors, including particle size, shape, mineralogy, and color. There is no predictable relationship between the turbidities produced by equal mass concentrations of different materials (Pickering 1976). This matter has also been discussed by Kunkle and Comer (1971), who showed that turbidity could be related to the mass concentration of particles only when the particles are of a uniform physical and chemical nature and instruments are calibrated against weighed samples. Turbidity is usually expressed in Jackson or Formazin Turbidity Units or, in earlier work, as equivalent to the turbidity produced by suspensions of the stated concentration of a standard silica flour. The latter is misleading, because statements like "turbidity of 1000 ppm" are easily misinterpreted as indicating a measured mass concentration of particles per unit volume.

Sediments dredged in this country span the range from all sand-gravel-shell down to all silt-clay and may be any combination in between (Boyd *et al.* 1972). The higher the content of fine particles the sediment may have, the greater will be its surface area, and consequently, the greater the number of sites for adsorption of contaminants. Organic material associated with natural sediments may also adsorb or complex contaminants, as will iron and manganese oxides and hydroxides that exist as discrete particles or fine coatings. Contaminants associated with natural sediments also are dissolved in the interstitial water (Brannon *et al.* 1976).

All of the above factors greatly complicate the question of the toxicity of sediment-associated pollutants to aquatic organisms. The effect of suspended fine mineral particles alone has not been adequately defined.

The clay fraction of natural sediments is of three general types: kaolinite, illite, and montmorillonite (Boyd *et al.* 1972). The present research evaluated the impact of suspensions of processed kaolinite, a common sheet-silicate clay mineral, on marine and estuarine macrofauna. Kaolinite occurs in large, pure beds usually as the product of chemical weathering of feldspars, and as such is presumably free of contaminants introduced by man. It was selected for its uniformity of particle size, and permitted study of the lethal effect of suspended particles themselves rather than the combined physical and chemical effects of sediments in various conditions of contamination.

Open water disposal of dredged material frequently produces intermittent, and sometimes sustained, high particulate concentrations in the form of suspended fluid mud on the bottom in the vicinity of disposal operations (May 1973, Barnard 1978, Nichols *et al.* 1978). Fluid muds are dense suspensions of fine-grained mineral and organic particles ranging in concentration from about 10 to approximately 480 g/L. They occasionally flow outside the boundaries of disposal areas (Nichols *et al.* 1978) and thus have the potential for ecological harm through invasion of previously unimpacted areas. Concentrations of the suspended clay used in this study were maintained within the range of concentrations of fluid mud observed in the field. Many of the organisms included here may be subjected to such conditions in their natural habitat.

Materials and Methods

The laboratory system used in this study has been described in detail elsewhere (Peddicord in press, Peddicord and McFarland 1978). It was a flow-through system of 16 hemispherical 75-L aquaria supplied with a suspension of kaolin and a complementary volume of dilution water at estuarine or oceanic salinity. A gradient of concentrations of kaolin was maintained in the aquaria at a replacement rate of 90 percent in 12 hr. The highest concentration tested was 117 g/L. Salinity was controlled at estuarine levels by mixing seawater with freshwater from a spring-fed pond. The water and suspended kaolin were metered into the aquaria using two sets of a modified version of the serial dilution apparatus of Mount and Brungs (1967).

The kaolin used in all tests was hydrite Flat D,[1] obtained in a single batch. The dry material had a median particle size of 4.5 μm with 10% of the particles finer than 0.5 μm equivalent spherical diameter and 10% coarser than 15 μ.

Suspension of kaolin in the test aquaria was maintained by individual circulation pumps which continuously withdrew water from the side of a tank and returned it through a disperser head in the tank bottom. Heat introduced by this method was removed by passing the circulating aquarium water through an electronically controlled heat-exchanger system. The system produced stable temperatures in all aquaria at a chosen set-point, maintained homogeneous suspensions of kaolin near the desired concentrations, and allowed continual atmospheric exchange at the air-water interface. Thus, dissolved oxygen concentrations in the aquaria were maintained at or near saturation.

For all experiments except those involving *Nassarius obsoletus* and small *Mytilus edulis,* the 16 aquaria were arranged in 2 replicate sets of 8, each set consisting of 2 clear-water controls and 6 concentrations of kaolin progressing in approximately logarithmic increases over a one order of magnitude span. The first two experiments were with *Nassarius obsoletus* and small *Mytilus edulis.* In these tests, the 16 aquaria were arranged in 4 replicates each of a control and 3 kaolin concentrations. The design was changed to improve statistical reliability of the results.

Water-quality parameters (pH, D.O., salinity, temperature, and suspended solids) were continuously monitored by a remote sensing apparatus that stored data on magnetic tape for later retrieval and provided performance information on which daily adjustments to the system were based. The suspended solids sensor was a Densitrol,[2] which was calibrated against gravimetrically analyzed samples pipetted directly from the test aquaria. The other instruments used were adapted to the system from a Martek shipboard water-quality monitoring package.[3]

The experimental marine organisms were collected at Bodega Bay, along the north coast of California, U.S.A., in an area with a seasonal water temperature range of 8 to 14°C, salinities of 30 to 33 ‰, high dissolved oxygen, and low background suspended solids levels. Estuarine organisms came from nearby San Francisco Bay, where temperatures range between 10 and 20°C seasonally. Variable freshwater inputs cause wide fluctuations in salinity and background suspended solids concentrations in San Francisco Bay.

All experiments were conducted within ±2°C of the temperature and ±3 ‰ of the salinity at which the animals were collected. The animals were maintained under these conditions in the laboratory holding tanks for 3 to 12 days before the experiments began. In some cases, two nonantagonistic species, isolated in separate baskets so no direct contact could occur, were tested simultaneously in the same aquaria.

The experiments were conducted over a period of one year, and the duration of each varied up to 16 days. Fish and crustaceans were fed a diet of adult brine shrimp or chopped squid. Feeding and removal of uneaten food was done once daily throughout the holding period and during tests which lasted more than four days. Invertebrates other than the crustaceans were not fed.

Throughout the experiments, counts were made of living animals at approximately 8-hr intervals. At these times, dead animals and molts were removed and suspended solids concentrations were determined. The observed mortalities in each test aquarium were adjusted for any deaths in the control aquaria by the method of Bliss (1935). The LC50, LC20, and LC10 values, the lethal concentration of suspended solids to the stated percent of the sample population, were calculated for every observation time by the logit method of Berkson (1953). These LCX values were regressed on exposure time to estimate the time-concentration mortality response.

---

[1] Georgia Kaolin Co., 433 North Broad Street, Elizabeth, NJ 07203

[2] Princo Instruments, Inc., Southampton Industrial Park, Southampton, PA 18996

[3] Martek Instruments, Inc., 879 West 16th Street, Newport Beach, CA 92660

Table 1. Observed mortalities of species relatively insensitive to suspended kaolin

Species[a]	Exposure time in days	% Mortality at 100 g/L
Strongylocentrotus purpuratus (sea urchin)	9	0
Tapes japonica (Japanese clam)	10	0
Pagurus hirsutiusculus (hermit crab)	12	0
Sphaeroma pentodon (isopod)	12	0
Nassarius obsoletus (mud snail)	5	0
Mytilus edulis (blue mussel) (2.5 cm)	5	10
Mytilus edulis (blue mussel) (10 cm)[b]	11	10
Molgula manhattensis (tunicate)	12	9
Styela montereyensis (tunicate)	12	10

[a] Species grouped together were tested simultaneously in the same aquaria

[b] Tested simultaneously in the same aquaria with *Mytilus californianus* (Table 2)

Results and Discussion

The laboratory facility kept the major physical parameters stable at the desired levels. Mean salinities remained at the collection and holding values with a standard deviation of <1 ‰ in all experiments. Mean temperature in each aquarium remained at or near the set point value with a standard deviation of 0.5°C. Dissolved oxygen was maintained near saturation, with standard deviations of 1 ppm or less in all aquaria. Suspended solids control was somewhat less precise, but means remained close to the desired concentrations in each aquarium with standard deviations of about 10% of the mean values. The pH decreased from about 7.8 in the clear-water control aquaria to about 6.8 in 117 g/L of suspended kaolin.

A very wide range of sensitivities to suspended kaolin was observed among the 16 species studied. Eight had <10% mortality in the length of time during which they were exposed, and no LCX estimates could be made. These species are listed in Table 1 with their length of exposure and the percent mortality observed in suspended kaolin concentrations of 100 g/L. Blue mussels *Mytilus edulis* measuring 2.5 cm in length reached 10% mortality after 5 days, while 10-cm-long specimens had 0% mortality after the same time and did not reach 10% mortality until 11 days of exposure.

A variety of species were found to be more sensitive than those in Table 1. For these species, Table 2 presents the equations for the time-concentration mortality curves, the r^2 values for those equations, and the estimated 200-hr LCX values. The tests with *Ascidia ceratodes, Anisogammarus confervicolus,* and *Cymatogaster aggregata* were all terminated in less than 200 hr, because of high mortality or inability to determine the shape of the curve beyond that time due to spacing of the test concentrations. For these organisms Table 3 presents the equations used for time-concentration mortality curve estimations, r^2 values, and the estimated 100-hour LCX values.

The LC50, LC20, and LC10 curves for 10-cm-long coast mussels *Mytilus californianus* exposed to suspended kaolin are shown in Fig. 1A. This species,

Table 2. 200-hr LCX estimates, equations, and coefficients of determination for those species tested longer than 200 hr

Species	200-hr LCX in g/L	Equations used for estimates	Coefficient of determination r^2
Mytilus californianus[a]	LC10 = 26	$\ln Y = 22.3 - 3.59 \ln X$	0.93
(coast mussel)	LC20 = 42	$Y = 11s - 0.349X$	0.89
	LC50 = 96	$1/Y = 0.020 + 1.93(1/X)$	0.75
Crangon nigromaculata	LC10 = 16	$\ln Y - 5.01 - 0.0113X$	0.76
(spot tailed sand shrimp)	LC20 = 28	$\ln Y = 5.04 - 0.00850X$	0.87
	LC50 = 50	$\ln Y = 7.96 - 0.756 \ln X$	0.98
Palaemon macrodactylus[b]	LC10 = 24	$\ln Y = 10.3 - 1.34 \ln X$	0.71
(grass shrimp)	LC20 = 77	$\ln Y = 4.94 - 0.00300X$	0.96
	LC50[c]		
Cancer magister	LC10 = 10	$\ln Y = 6.37 - 0.766 \ln X$	0.72
(dungeness crab)	LC20 = 18	$\ln Y = 7.01 - 0.766 \ln X$	0.96
	LC50 = 32	$\ln Y = 3.05 + 83.1(1/X)$	0.99
Neanthes succinea	LC10 = 9	$Y = 58.6 = 0.246X$	0.88
(polychaete)	LC20 = 22	$\ln Y = 4.51 - 0.00700X$	0.92
	LC50 = 48	$\ln Y = 5.91 - 0.386 \ln X$	0.91

[a] Tested simultaneously in the same aquaria with 10-cm *Mytilus edulus* (Table 1)

[b] Tested simultaneously in the same aquaria with *Anisogammarus confervicolus* (Table 3)

[c] Fifty % mortality was not reached

Table 3. 100-hr LCX estimates, equations, and coefficients of determination for those species tested 200 hr or less

Species	100-hr LCX in g/L	Equations used for estimates	Coefficient of determination r^2
Ascidia ceratodes	LC10 = 7	$\ln Y = 3.88 - 0.0192X$	0.88
(tunicate)	LC20 = 13	$\ln Y = 4.61 - 0.0203X$	0.94
	LC50 = 38	$\ln Y = 5.71 - 0.0207X$	0.94
Anisogammarus confervicolus[a]	LC10 = 38	$\ln Y = 9.02 - 1.17 \ln X$	0.90
(amphipod)	LC20 = 51	$1/Y = 0.0377 - 1.79(1/X)$	0.97
	LC50 = 78	$\ln Y = 3.68 + 67.7(1/X)$	0.73
Cymatogaster aggregata	LC10 = 1	$Y = -0.523 + 154(1/X)$	0.91
(shiner perch—specimens	LC20 = 3.6	$Y = 6.34 - 0.0270X$	0.82
from San Francisco Bay)	LC50 = 6	$\ln Y = 0.398 + 138(1/X)$	0.79

[a] Tested simultaneously in the same aquaria with *Palaemon macrodactylus* (Table 2)

found along the open coastline, was more sensitive than the closely related blue mussel *M. edulis* (Table 1), usually found in bays and harbors, which may be more turbid. The estimated 200-hr LC50 for 10-cm *M. californianus* was 96 g/L.

The time-concentration mortality curves for the tunicate *Ascidia ceratodes* collected at Bodega Bay (Fig. 1B.) show it to be one of the most sensitive species tested. The experiment was terminated at 136 hr because of the high mortality reached by that time. This response was very different from that of

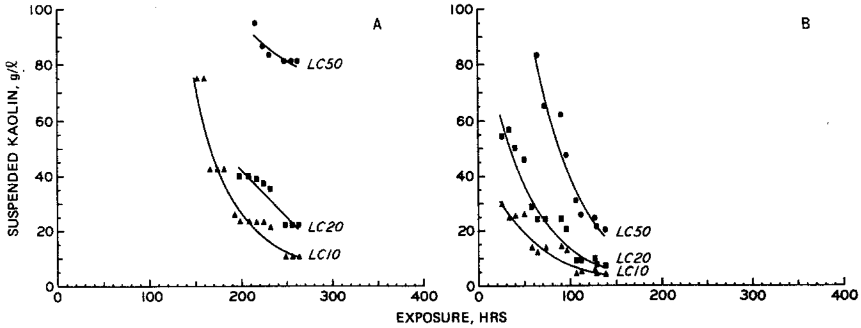

Fig. 1. Suspended kaolin time-concentration mortality curves: A. *M. californianus* (coast mussel), 31 ⁰/oo salinity, 12°C B. *A. ceratodes* (tunicate), 33 ⁰/oo salinity, 9°C.

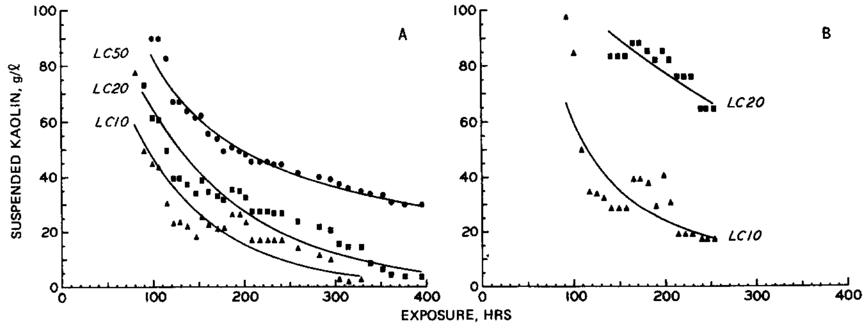

Fig. 2. Suspended kaolin time-concentration mortality curves: A. *C. nigromaculata* (spot-tailed sand shrimp), 31 ⁰/oo salinity, 10°C B. *P. macrodactylus* (grass shrimp), 13 ⁰/oo salinity, 11°C

Molgula manhattensis and *Styela montereyensis*, two tunicates from San Francisco Bay, whose 288-hr (12-day) LC10 may be estimated at 100 g/L (Table 1).

The 200-hr LC50 estimate for the spot-tailed sand shrimp *Crangon nigromaculata* was 50 g/L. The experiment was continued for over 400 hr, or 16 days. At the end of the exposure period, the LC50 estimate for this organism had dropped to slightly less than 40 g/L, indicating a high tolerance to suspended clay (Fig. 2A.).

The euryhaline grass shrimp *Palaemon macrodactylus* (Fig. 2B.) was even less sensitive to suspended kaolin than *C. nigromaculata*. Fifty % mortality was not reached during the 250-hr experiment and the 200-hr LC20 for *P. macrodactylus* was 77 g/L, higher than the estimated 200-hr LC50 for *C. nigromaculata*.

The other decapod crustacean tested was the dungeness crab *Cancer magister*. These crabs, about 5-cm carapace width, were tested for 10 days and found to be more sensitive than any of the shrimp species, with a 200-hr LC50 of 32 g/L (Fig. 3A.). Their tolerance to suspended kaolin decreased more rapidly with time than any other species except the tunicate *A. ceratodes*.

Due to the spacing of the experimental concentrations, the amphipod *Anisogammarus confervicolus* remained alive after 157 hr in only 3 of the 12 aquaria. This resulted in erratic LCX estimates and the analysis was halted at

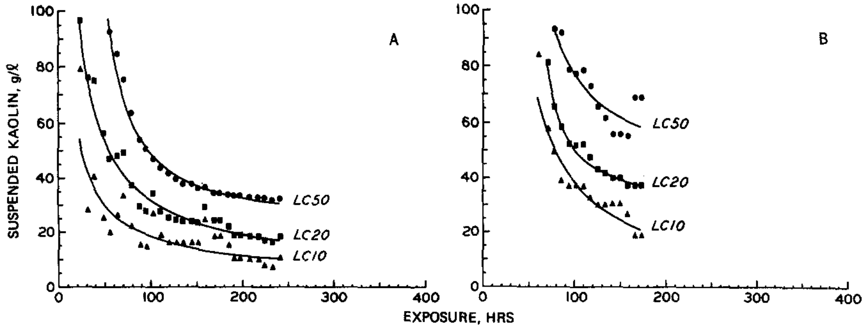

Fig. 3. Suspended kaolin time-concentration mortality curves: A. *C. magister* (dungeness crab), 32 ‰ salinity, 10°C B. *A. confervicolus* (amphipod), 13 ‰ salinity, 11°C

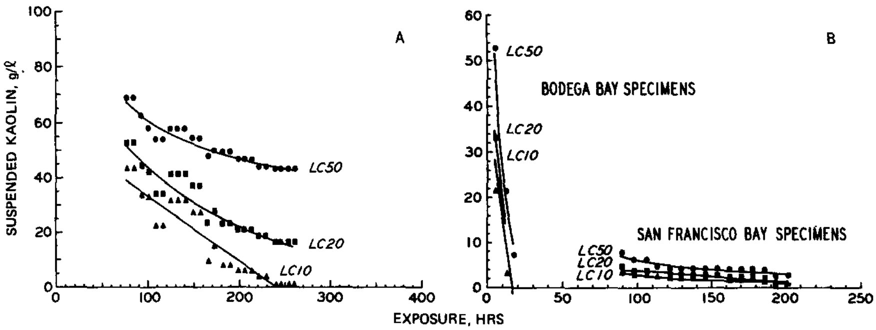

Fig. 4. Suspended kaolin time-concentration mortality curves: A. *N. succinea* (polychaete), 13 ‰ salinity, 11°C B. *C. aggregata* (shiner perch), Bodega Bay specimens: 14 to 89 g/L at 31 ‰ salinity and 9°C. San Francisco Bay specimens: 2 to 16 g/L at 16 ‰ salinity and 13°C

that time (Fig. 3B.). The 100-hr LC50 for this amphipod was 78 g/L, indicating an intermediate sensitivity to suspended kaolin.

The mortality curves for the polychaete *Neanthes succinea* in suspended kaolin are presented in Fig. 4A. In reality, the LC10 curve should not cross 0 g/L, but due to the choice of test concentrations the shape could not be determined below 10 g/L. The concentration causing 50% mortality of *N. succinea* in 200 hr was estimated to be 48 g/L.

Experiments with juvenile English sole *Parophrys vetulus* were conducted for 10 days at concentrations of 10 to 117 g/L. No mortalities were observed at 70 g/L or less, but 80% mortality had occurred after 10 days at 117 g/L. Since deaths occurred in only one concentration, no mortality curves could be calculated for these data. However, the results did indicate that the lethal concentration of suspended kaolin for *P. vetulus* is relatively high.

The shiner perch *Cymatogaster aggregata* was the most sensitive species studied. The first test of this species was with organisms collected at Bodega Bay and tested over a concentration range of 14 to 89 g/L. Mortality was rapid and complete, with only one fish alive after 26 hr in 14 g/L suspended kaolin. A second abbreviated test of San Francisco Bay specimens was conducted at three lower concentrations to determine the most suitable range of concentrations over which to test this species. This pilot test was continued for eight days and gave a 200-hr LC50 estimate of 3 g/L (Fig. 4B.). This is an order of magnitude lower than the corresponding value for any other organism from San Francisco Bay and indicates a relatively high sensitivity to suspended kaolin for *C. aggregata*.

In none of the experiments with any species could the magnitude of the LC50 value be predicted from the LC20 or LC10 values. Sherk *et al.* (1974) studied the effects of suspended solids on a variety of estuarine fish and found that the range of concentration between the LC10 and LC90 values did not necessarily indicate the magnitude of the LC50 value. These results indicate the necessity for studying the tolerance of the most sensitive members of the population and for comparing only those results based on the same LCX values.

There seemed to be some correlation between normal habitat of the species studied and sensitivity to suspended kaolin, although no phylogenetic correlations were apparent. No species that is restricted to soft muddy bottoms was found to be sensitive. While many species occupying other habitats were also highly tolerant, all species shown to be sensitive to high suspended kaolin concentrations were either invertebrates occurring predominantly on sandy bottoms or in fouling communities, or fish not intimately associated with the bottom. Such species are probably subjected to high suspended solids concentrations in their environment less often than those living in or on soft muddy bottoms and may have less well-developed mechanisms for dealing with such occurrences. It should be emphasized that this research did not consider sublethal responses and that lack of mortality in 10 days does not necessarily imply the absence of important effects.

Acknowledgments. This research was supported by Contract No. 033-3-022-42 to Dr. Cadet Hand, Director of the University of California Bodega Marine Lab, Bodega Bay, CA, from the National Oceanic and Atmospheric Administration.

References

Barnard, W. D.: Prediction and control of dredged material dispersion around dredging and open-water pipeline disposal operations. Technical Report DS-78-13, U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, MS (1978).

Berkson, J.: A statistically precise and relatively simple method of estimating the bioassay with quantal response based on the logistic function. J. Amer. Stat. Assoc. 48, 565 (1953).

Bliss, C. I.: Calculation of the dosage-mortality curve. Ann. Appl. Biol. 23, 134 (1935).

Boyd, M. B., R. T. Saucier, J. W. Keeley, R. L. Montgomery, R. D. Brown, D. B. Mathis, C. J. Guice: Disposal of dredge spoil. Technical Report H-72-8, U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, MS (1972).

Brannon, J. M., R. M. Engler, J. R. Rose, P. G. Hunt, and I. Smith: Selective analytical partitioning of sediments to evaluate potential mobility of chemical constituents during dredging and disposal operations. Technical Report D-76-7, U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, MS (1976).

Herbert, D., and J. Merkins: The effect of suspended mineral solids on the survival of trout. Int. J. Air Water Poll. 5, 46 (1961).

Kunkle, S. H., and G. H. Comer: Estimating suspended sediment concentrations in streams by turbidity measurements. J. Soil and Water Cons. 26, 18 (1971).

May, E. B.: Environmental effects of hydraulic dredging in estuaries. Alabama Mar. Res. Bull. **9**, 1 (1973).

Mount, D. L., and W. A. Brungs: A simplified dosing apparatus for fish toxicology studies. Water Res. 1, 21 (1967).

Nichols, M. M., G. S. Thompson, and R. W. Fass: A field study of fluid mud dredged material: Its physical nature and dispersal. Technical Report D-78-40, U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, MS (1978).

Peddicord, R. K., V. A. McFarland, D. P. Belfiori, and T. E. Byrd: Effects of suspended solids on San Francisco Bay organisms. Report to U.S. Army Corps of Engineers, San Francisco District. Dredge Disposal Study, Appendix G. (1975).

Peddicord, R. K., and V. A. McFarland: Effects of suspended dredged material on aquatic animals. Technical Report D-78-29, U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, MS (1978).

Peddicord, R. K.: Direct effects of suspended sediments on aquatic organisms. Proceedings of American Chemical Society/Chemical Society of Japan Symposium on Processes Involving Contaminants and Sediments, Honolulu, HI, 1-6 April 1979. Ann Arbor Science Publishers, Inc., Ann Arbor, MI (in press).

Pickering, R. J.: Measurement of "turbidity" and related characteristics of natural waters. U.S. Geol. Survey, Open File Report 76-153, (1976).

Rogers, B. A.: Tolerance levels of four species of estuarine fishes to suspended mineral solids. M. S. Thesis, Kingston, RI, Univ. Rhode Island (1969).

Schubel, J. R., and J. C. S. Wang: The effects of suspended sediment on the hatching success of *Perca flavescens* (yellow perch), *Morone americana* (white perch), *Morone saxatilis* (striped bass), and *Alosa psuedoharengus* (alewife) eggs. Johns Hopkins Univ. Ches. Bay Inst. Spec. Rept. 10 (1973).

Sherk, J. A., J. M. O'Connor, D. A. Neumann, R. D. Prince, and K. V. Wood: Effects of suspended and deposited sediment on estuarine organisms. Phase II. Univ. Md., Nat. Res. Inst. Ref. No. 74-20 (1974).

Stern, E. M., and W. B. Stickle: Effects of turbidity and suspended material in aquatic environments; literature review. Technical Report D-78-1, U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, MS (1978).

U.S. Fish and Wildlife Service: Effects on fish resources of dredging and spoil disposal in San Francisco and San Pablo Bays, California. Unnumbered Spec. Rept., Nov. 1970 (1970).

Wallen, I. E.: The direct effect of turbidity on fishes. Bull. Oklahoma Ag. and Mech. College **48**, 1 (1951).

Wilber, C. G.: Turbidity. In O. Kinne (ed.): Marine Ecology. p. 1157. London: Wiley-Interscience (1971).

Manuscript received September 17, 1979; accepted January 14, 1980.