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Effects of porewater hydrogen sulfide on the feeding activity of the subsurface deposit-feeding polychaete, *Clymenella torquata,* Leidy

by Charlotte M. Fuller[1,2]

ABSTRACT

The effects of porewater hydrogen sulfide concentrations on the feeding and tube-building activity of the subsurface, deposit-feeding polychaete, *Clymenella torquata* were experimentally determined in the laboratory. Porewater hydrogen sulfide concentrations were manipulated by injecting a buffered, isotonic sodium sulfide solution into the experimental chambers. Fecal material was collected and weighed and tube-building activity was monitored daily. Fecal production was negatively correlated with porewater hydrogen sulfide concentration during experiments conducted in June and July. Hydrogen sulfide concentrations greater than 1000 μM in June and 700 μM in July resulted in reduced fecal production. Fecal production was not correlated with porewater hydrogen sulfide concentration in experiments conducted in September and October, and some worms in the September experiment were surprisingly tolerant of porewater hydrogen sulfide concentrations as high as 3.8 mM. The lower tolerance of *C. torquata* to porewater hydrogen sulfide concentrations during the June and July experiments compared to the September and October experiments indicates that worms in the field became acclimated to increasing porewater hydrogen sulfide levels as temperatures increased. Tube-building frequencies were higher in worms in the June and July experiments than in the September and October experiments. In addition, experiments demonstrated that *Clymenella* is capable of modifying the porewater hydrogen sulfide concentration at depth in the sediment around its tube possibly by irrigation activities.

These results suggest that porewater hydrogen sulfide concentrations may have significant non-lethal effects on the ecology of this species. Acquired tolerance to increased hydrogen sulfide concentrations may enable organisms to survive and feed under conditions in which other organisms cannot, making possible the exploitation of a niche used by relatively few benthic polychaetes. Porewater chemistry should be monitored and regulated during experiments on feeding rates and bioturbation in soft-bottom infaunal organisms.

1. Introduction

Previous studies have established that the stress of low oxygen conditions coupled with μM concentrations (as low as 2–200 μM) of hydrogen sulfide (H_2S and HS^{-1}) decrease the survival of many infaunal organisms (Henriksson, 1969; Theede *et al.*,

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1969; Theede, 1973; Shick, 1976; Jorgensen, 1980; Groenendaal, 1980; Vismann, 1990; Llanso, 1991; Rosenberg *et al.,* 1991) and fish (Torrans and Clemens, 1982; Bagarinao and Vetter, 1989). Although hydrogen sulfide is known to be toxic to most organisms at μM concentrations (Evans, 1967; National Research Council, 1979), high abundances of some benthic organisms are often found in sulfide-rich sediments (Pearson and Rosenberg, 1978). Changes in the activity of infaunal organisms due to low oxygen and the presence of hydrogen sulfide have also been observed where organisms emerge from the sediment and lie on the sediment surface (Jorgensen, 1980; Vismann, 1990; Llanso, 1991). To date only qualitative observations of the effects of hydrogen sulfide on feeding behavior have been reported in two studies involving species capable of both surface deposit-feeding and filter-feeding (Vismann, 1990; Llanso, 1991). However, the effect of porewater hydrogen sulfide on the feeding rate of subsurface deposit feeders, which are often exposed to sulfide-rich sediments, has not been quantified.

Recently, H_2S has been shown to be more toxic than HS^{-1} by the stronger inhibition of the cytochrome c oxidase system at the pH where H_2S will dominate (Somero *et al.,* 1989). The dissolved species of sulfide (H_2S, HS^{-1} and S^{-2}) present in porewaters is dependent upon pH. At a pH of 6.6, 75% of the soluble species will be H_2S and the other 25% will be HS^{-1}. At a pH of 7.0, 50% of each species will be present, while at a pH of 8.0, 90% of the species will be HS^{-1} and 10% will be H_2S. Typical values from the literature for porewater pH in the field for sites on the Connecticut shore of Long Island Sound range between 6.6 and 7.2 (Lyons, 1979). At this range of pH, both H_2S and HS^{-1} will be present and negligible amounts of S^{-2}.

The impact of the physiological stress of porewater hydrogen sulfide on the survival and activity of organisms that inhabit sulfide-rich sediments is important in studies of growth and reproduction of infaunal organisms (Sanders, 1968), bioturbation activity (Huettel, 1990), sediment-nutrient flux (Huettel, 1990), free-ion activity of heavy metals (Emerson *et al.,* 1983) and community ecology (Sanders, 1968; 1969; Pearson and Rosenberg, 1978). In particular, porewater hydrogen sulfide may alter feeding rates and behavior of infaunal organisms. The presence of such behavioral responses to changes in porewater chemistry have relevance to studies that examine feeding and behavior both in the field and in laboratory experiments where the porewater chemistry may be very different.

The subsurface deposit-feeding maldanid polychaete, *Clymenella torquata* Leidy, is widely distributed on the east coast of North America (Sanders *et al.,* 1962; Mangum, 1964; Rhoads and Stanley, 1965; Dobbs, 1981). *C. torquata* is often gregarious, occurring in densities as high as 700 individuals m^{-2} on sandflats of southern New England (Dobbs, 1981). *C. torquata* builds a semi-permanent tube perpendicular to the sediment surface that typically extends as deep as 10–20 cm. A characteristic conveyor-belt feeder (*sensu* Rhoads, 1974), *C. torquata* primarily feeds head-down at depth in the sediment and defecates on the sediment surface. *C. torquata* reworks a

considerable amount of sediment, between 0.4 and 1.25 ml feces · day^{-1} (Mangum, 1964; Rhoads and Stanley, 1965; Dobbs, 1981). Because *C. torquata* feeds at depths of 10–20 cm in the sediment, it is likely to encounter high levels of hydrogen sulfide. It's fecal matter is often dark black when fresh in comparison to the sediment surface. This dark fecal matter is indicative of the presence of iron monosulfides (which are precursors to pyrite, FeS_2) and porewater hydrogen sulfide. Concentrations of porewater dissolved hydrogen sulfide range from 0–1.6 mM in Long Island Sound (Lyons, 1979), and 0–2.6 mM in sandy, intertidal sediments (where organisms were collected for this study, Fuller, 1992).

This study examined the effect of porewater dissolved hydrogen sulfide (H_2S and HS^{-1}) on the feeding and burrowing activity of the subsurface, deposit-feeding polychaete, *Clymenella torquata.* The null hypothesis, that there will be no change in feeding or tube-building activity as dissolved hydrogen sulfide concentration in the porewater changes, was tested in the laboratory.

2. Materials and methods

a. Feeding experiments

i. Experimental organism. Porewater dissolved hydrogen sulfide concentrations were experimentally manipulated in the laboratory between June and October, 1991. Sediment and *Clymenella torquata* were collected from an intertidal sand flat near the mouth of the Poquonock River Estuary, Long Island Sound, CT. The sediment was 98% fine to coarse sand with an organic carbon content of 0.5% by weight for all experiments. Organic carbon content was determined by weight loss upon ignition at 550°C for 4 hours (Gross, 1971).

Two supporting experiments (i.e., the Color and Profile experiments) and four feeding experiments (i.e., June, July, September and October) were conducted. Typically, sediment and worms for each experiment were collected approximately three weeks prior to the experiment in which they were used (except in the Color experiment, where sediment and worms were collected two months earlier and the September experiment where the sediment was obtained approximately three months before the experiment, and was from the same collection batch as sediment for the July experiment). Sediment was frozen until the day chambers were set up and the experiment was begun. Worms were maintained in mass culture (approximately 30 worms per 1375 cm^3 of sediment) in mesh (150 μm) chambers containing natural sediment in a running seawater table with a slow water exchange at approximately 20°C until they were used in the experiments.

Prior to addition to the experimental chambers, worms were gently sieved from the stock cultures and teased from their tubes. Because worm size is often connected to feeding rate (e.g., Nichols, 1974; Cammen, 1980; Forbes and Lopez, 1987), only whole worms of similar volume (approximately 0.2 ml) were used within and between

all of the feeding experiments. Reproductive state of the worms was not known. The total time elapsed that worms were in seawater without sediment during cleaning and measurement prior to experiments was 1-2 hours.

All experiments were conducted in the laboratory in a rectangular tank 1.17-m wide by 2.39-m long. Approximately 5 cm of seawater covered the top of the chambers. Temperatures in the June, July and September feeding experiments were similar, ranging between 19.5°C to 21.5°C. In the October feeding experiment, temperatures were slightly lower (16.5°C to 17°C). During the June feeding experiment and the Color experiment, the seawater was warmed to 20°C and aerated in an adjacent tank then recirculated through the experimental tank. In the other feeding experiments, the water flowed through the tank at ambient temperature and dissolved oxygen content. Dissolved oxygen content (YSI oxygen meter, model 57) was measured to be near saturation in all experiments. For all experiments, the rate of seawater flow through the tank was about 4 l min^{-1}.

ii. Experimental chambers. In all experiments, chambers were placed in the tank and one worm was assigned to each chamber in random order. The chambers (except in the Color experiment) were 1150-ml capacity polypropylene beakers (Fig. 1). Two types of chambers were used, solid and mesh. Solid chambers were as described above while mesh chambers had four panels (approximately 7 by 11 cm) cut out of the sides leaving four supporting strips from top to bottom each 1- to 2-cm wide, to which 150 μm Nitex nylon mesh was glued with silicon aquarium sealer. In all experiments, worms were initially placed in mesh chambers with sediment, then, for those chambers where hydrogen sulfide concentration was artificially increased, the mesh chambers were inserted into solid chambers to eliminate contact with oxygenated sea water thereby reducing the potential for oxidation of the injected sulfide.

All chambers were divided into four layers and each layer was filled with a different color of freeze-thawed sediment (Fig. 1). The freeze-thawed sediment was mixed with inert fluorescent paint particles, (Blue, Chartreuse and Red, stock number R105 made by Radiant Color, Richmond, CA). A ratio of approximately 1:1000 of tracer to sediment was used and all sediment-tracer mixtures were thoroughly mixed by hand. The color of the defecated material indicated the depth at which the worms were feeding, and thus, dictated the appropriate depth at which to sample the total dissolved hydrogen sulfide concentration. There was no evidence of craters on the sediment surface which might indicate that feeding voids had collapsed. The three bottom layers (Fig. 1), each about 4.5-cm deep, were filled with red-, chartreuse- or blue-colored sediment, respectively. A 1-cm deep surface layer, consisted of uncolored sediment alone which facilitated differentiation of the colored fecal material from the colored sediment. Determination of the fluorescent color of the fecal material was facilitated with a UV light.

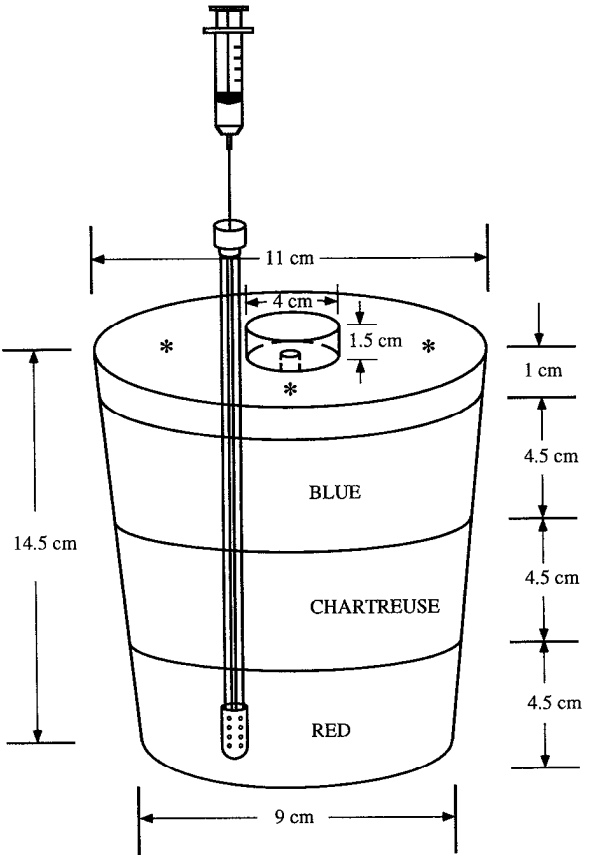

Figure 1. Schematic drawing of an experimental chamber, porewater sipper with a syringe and fecal material collection dish. The fecal collection dish is shown in the center for diagrammatic purposes only; the dish was placed over the worm tube wherever it was built. The position where the sipper was inserted to sample the porewater is indicated relative to the fecal collection dish. The three approximate locations where the sipper was inserted to inject sulfides are indicated by an asterisk.

iii. Sampling regimes. The sampling protocol for the June, July, September and October feeding experiments was similar. However, each experiment had a different duration (i.e., 6-, 16-, 15-, 12-days, respectively). Following an initial period of three days to allow worms to establish a tube and a constant defecation rate (see results of Color experiment, Fig. 2), fecal material was collected daily and dissolved hydrogen sulfide concentration at the feeding depth was sampled every other day. Porewater samples were taken within 2-3 hours after fecal material was collected. Worms were often seen defecating while porewaters were being sampled. After the initial three-day collection period at ambient hydrogen sulfide concentrations, porewater hydrogen sulfide concentrations were increased in half the number of chambers.

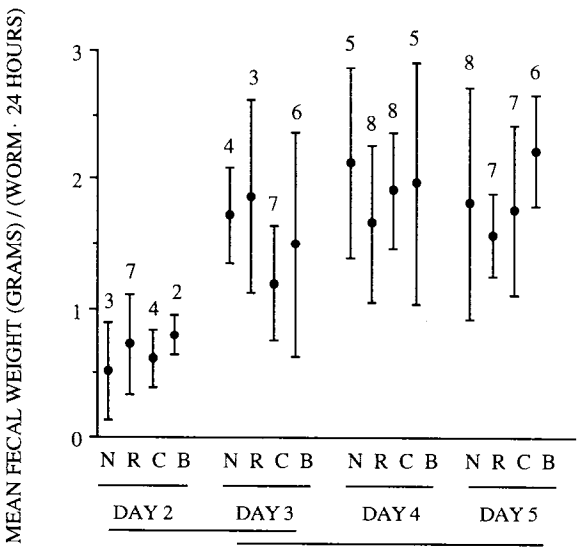

Figure 2. Fecal production in the Color experiment comparing differences between uncolored (N), red (R), chartreuse (C) and blue (B) sediments and changes in fecal production with time. Numbers indicate the number of worms in each color treatment sampled for the full 24 hours on each day. Symbols indicate the mean weight of feces; the error bars represent ± 1 standard deviation. Bars under the color treatment symbols and days represent treatments that are not significantly different at $\alpha = 0.05$.

iv. Hydrogen sulfide analysis. Total dissolved porewater hydrogen sulfide concentration was sampled using a modified porewater sampler (Howes *et al.*, 1985), hereafter referred to as a "sipper" (modifications to the sampling tip were made by Howes, personal communication). At the sampling end, holes made with a 23-gauge needle began 1 cm from the bottom of the sipper and extended over 2 cm at about 0.6-cm intervals (Fig. 1).

Sippers were filled with seawater and all air bubbles removed. A sipper was then inserted into the sediment next to the fecal collection dish (Fig. 1), approximately 2 cm from a worm's tube (the fecal collection dish radius was 1.7 cm). A 3-cm^3 syringe inserted through the serum stopper was used to withdraw about a 0.5-cm^3 porewater sample to purge the contents of the sipper. An additional 1.0-1.5 cm^3 was withdrawn as the actual porewater sample and 0.8 cm^3 transferred without exposure to air through tubing to a 1-cm^3 syringe containing 0.2 cm^3 of Cline's reagent (Cline, 1969) modified for the range of hydrogen sulfide concentration expected in the experiments. Absorbance was determined at 670 nm with a spectrophotometer. A standard curve to convert absorbance to hydrogen sulfide concentration was made each time porewater hydrogen sulfides were sampled. The coefficient of variation for analytical error of hydrogen sulfide concentration measurements for the standard

curves was 5%. Interference by the fluorescent pigment in the porewater samples was found to be negligible at 670 nm.

The radius around a sipper from which the porewater was sampled was estimated from the porosity of the sediment. Porewater content was determined by difference in wet and dry weight of sediment plugs taken from 16 experimental chambers. The porewater content by weight was converted to porewater content by volume (32%) using the density of quartz (2.65 $g \cdot cm^{-3}$). Each porewater sample had a volume of 2-cm^3 and was calculated to draw porewater from a cylindrical area which is 2-cm high and has a 2.2-cm diameter.

v. Procedures for increasing hydrogen sulfide concentration. In all experiments, except the Color and June experiments, after an initial monitoring period, hydrogen sulfide levels in roughly half the number of chambers were increased. A solution of 10 mM Na_2S dissolved in 40 mM Tris-HCl buffer made isotonic in seawater was injected through the sipper. A total of 10 ml of 10 mM buffered sulfide solution was injected at each of three positions around the chamber (denoted by an asterisk in Fig. 1) and three depths (12-, 8- and 4-cm) at each of those positions with one injection about 2-3 cm from the worm's tube (see Fig. 1). Approximately 4 ml were injected at 12 cm and 3 ml at the 8- and 4-cm depths. The greater volume (4 ml) of sulfide was injected into the deeper layer of the chamber in an effort to insure that the hydrogen sulfide concentration was increased in the bottom of the chamber because that was where most of the worms were feeding. The other chambers were injected in the same manner with 30 ml of 40 mM Tris-HCl buffer to control for effects due to the injecting technique and buffer. Porewater pH was measured on several days in each experiment with approximately 0.5 ml of the sample using a semi-micro electrode to monitor that there was sufficient buffering of the added Na_2S solution. Buffering of the injected sulfide solution was sufficient (no significant difference, multiple *t*-tests) between pH in the Tris-HCl and the sulfide-Tris-HCl injected chambers (Fuller, 1992).

vi. Worm fecal material measurements. A positive correlation between worm feeding and defecation rates was assumed. Because a periodicity in fecal production over a 24-hour period has been found in some deposit feeders (Fuller *et al.,* 1988), feces were collected daily during an experiment. If a worm moved during a 24-hour interval, feces for that individual were removed but the weight was not determined. The fecal material was collected in a dish placed over the worm's tube. Feces were rinsed with distilled water, dried at approximately 65°C, cooled in a desiccator and weighed. Fecal weights were corrected for daily humidity changes by the mean weight change between pre-weighing and final weighing of 5 empty dishes. Corrections for humidity changes ranged from 0.1-0.3 mg and were $<0.05\%$ of the feces weight.

vii. Tube-building activity. In addition to sampling fecal material, worm tube-building activity was noted daily for each individual. Here, tube-building activity applies only to building a new tube in a different location or a new branch extending to the sediment surface. Not included as tube-building activity is adding to the top of the existing tube above the sediment surface. Tube-building activity is defined as the number of days a worm engaged in building a new tube expressed as a percent of the total number of active days (activity here is defined as producing fecal material or building new tubes) in an experiment. Not all worms were active for the same number of days during any one experiment. To account for between-worm differences in duration of activity within an experiment, tube-building activity for each worm was normalized to the number of days each worm was active. For example, if a worm was active 11 days in an experiment and built a new tube 1 of those days, then it spent 9% of the time building new tubes. Tube-building activity (%) was divided into 11 bins, each 10% in size. In all experiments, worms were excluded from tube-building calculations if any of the following criteria were met: (1) if the worm built a curved tube (determined at the end of the experiment when the chambers were emptied; a tube with a curved end or one that extended diagonally across the chamber was excluded because porewater hydrogen sulfide concentrations were determined based on the tube location at the surface and worms in a curved tube would be feeding and exposed to hydrogen sulfides at a different location than the one sampled); (2) if a worm broke (e.g., a dead tail became apparent at the surface) at any time during the experiment because the effect of regenerating a new tail on activity was unknown; (3) if no worm was found in the chamber; (4) if worms were never active; or (5) if worms were active less than 4 days in the experiment (4 days was chosen as the cutoff based on results of the Color experiment (Fig. 2) which determined that 3 days were necessary for worms to establish a steady feeding rate).

viii. Experiment termination. At the end of an experiment, each chamber was dismantled over a 1-mm mesh screen and the worms were retrieved. In some cases, worms were not found (indicating that the worms had died or might have emigrated from the chamber), or were found dead. In addition, worm tubes were examined and any curved or transverse tubes were recorded. This information was important because sippers were always inserted next to the fecal collection dishes, and therefore, if the tube did not extend perpendicular to the sediment surface, the porewater was not sampled where the worm was actually feeding.

b. Supporting experiments

i. Color experiment. Because worms might feed at different depths in the experimental chambers, it was necessary to determine whether the fluorescent-colored particles affected defecation rates. Forty (ten for each treatment: uncolored, red, chartreuse and blue) chambers (plastic cups measuring 9-cm in diameter at the top,

6-cm in diameter at the bottom and 11-cm deep) were set up. A thin layer of uncolored sediment was layered on the sediment surface of the chambers. Chambers were randomly placed into a seawater table and one worm was placed into each chamber on the following day. Fecal material was collected daily from day 2 through day 5, dried and weighed as in the other experiments.

This experiment addressed two questions: (1) Did the amount of fecal material produced differ between the four sediment treatments (i.e., uncolored, red, chartreuse and blue)? (2) Did defecation rate change over time (between days) in the experiment?

ii. Profile experiment. Total dissolved hydrogen sulfide concentration profiles were determined in six experimental chambers (three control chambers without worms and three chambers each containing one worm) to determine if the injection technique increased the hydrogen sulfide concentration. Chambers were set up as described for the feeding experiments. After four days (hereafter referred to as the "initial" date), the porewater was sampled for total hydrogen sulfide concentration at three positions across the center of the chamber and at each of three sediment depths, 4-, 8- and 12-cm. One of these positions was next to the worm's tube (see Figs. 3A-C). For the smallest chamber diameter (9 cm, Fig. 1), three sips would mean a 3-cm diameter was allotted for each sip, however, each sip was calculated to draw from a cylindrical area with a height of 2-cm and a diameter of 2.2-cm, thus, the probability of any overlap in the sampled areas would be small. On the following day (day 5), all chambers were injected with 10 mM Na_2S buffered with 40 mM Tris-HCl at three positions around the chamber (denoted by * in Fig. 1) and at three depths below the sediment-water interface (4-, 8- and 12-cm) as for the feeding experiments. After 24-hours (hereafter referred to as "injection"), chamber porewaters were sampled again. Care was taken to insure that the sipping positions did not coincide with previous injection positions. Finally, chambers were sipped one week later (hereafter referred to as "week"). For all sampling dates, the location of worm burrows and the color of the fecal material was noted to indicate the depth where the worm was feeding.

Three questions were addressed in the Profile experiment: (1) Did the injection technique increase porewater dissolved hydrogen sulfide concentrations? (2) Were hydrogen sulfide concentrations significantly different at the three depths within the chambers in both treatments? (3) Did *Clymenella torquata* significantly affect the dissolved hydrogen sulfide concentration at the feeding location?

3. Results

a. Supporting experiments and measurements

i. Color experiment. Fecal production by worms that did not build a new burrow during the 24-hour intervals in the Color experiment are shown in Figure 2. The data

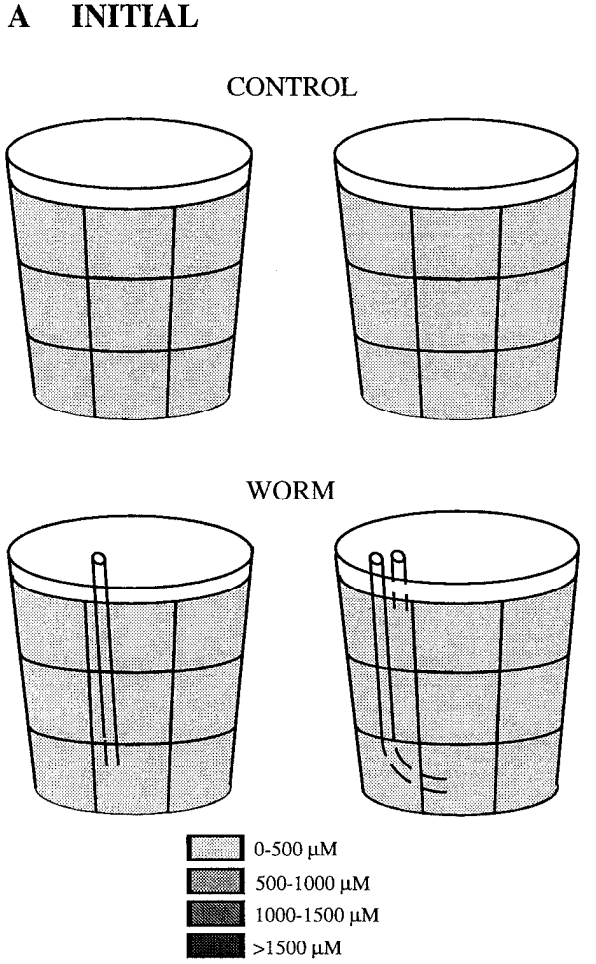

Figure 3. Sulfide concentration profiles in experimental chambers for the "initial" (A), "injection" (B) and "week" (C) measurements for the control and worm treatments in the Profile experiment. In the initial measurements, only two chambers were mapped for each treatment. The location of the worm's tubes have been drawn in. Because it was not possible to determine how far into a particular sediment layer the tube extended, the bottom of the tube is shown with a dashed line. The shaded areas represent different concentrations (see legend) of dissolved hydrogen sulfide.

were analyzed by six multiple one-way, repeated measures ANOVAs and corrected for multiple comparisons by Bonferroni's inequality (α_{exp} divided by number of tests, $\alpha_{exp} = 0.05$, $n = 6$). Variability in fecal production between worms was high. No significant difference existed in fecal production between the four sediment treatments for all days in the experiment (Fig. 2, $p = 0.05$). However, fecal production increased significantly (Fig. 2, $p = 0.05$) with time. Fecal production rates were

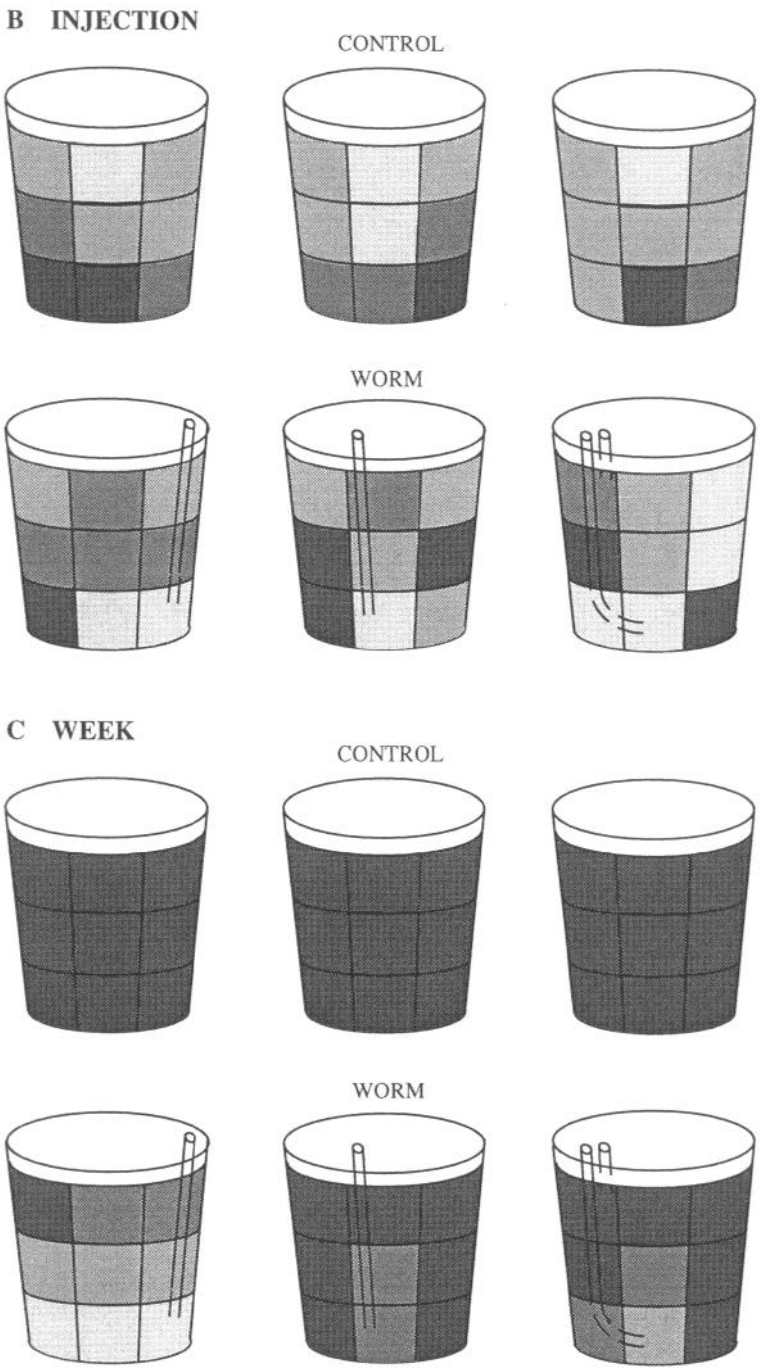

Figure 3. (Continued)

significantly lower on day 2 than on days 4 and 5. These data indicated that a 3-day period from introduction into the sediment is necessary for worms to establish a constant feeding rate.

ii. Profile experiment. Porewater hydrogen sulfide concentrations in control and worm chambers for the profile experiment are shown in Figures 3A-C. The worm's burrow location has been drawn based on: (1) observation of the tube opening on the surface, (2) the shape of the tube when the chamber was dismantled at the end of the experiment and (3) the color of fecal material produced. For the "initial" and "injection" measurements, this experiment addressed three aspects of the experimental methodology developed in this study.

In chambers with worms, the feeding location of the worm (e.g., Fig. 3B) corresponded to the minimum hydrogen sulfide concentration at that depth. The concentration at the feeding location was relevant to studies of how porewater hydrogen sulfide concentration affects the feeding activity of the worm. In control chambers, the minimum hydrogen sulfide value at a particular depth was also used. Comparison of the concentration at the feeding location with the minimum concentration at a particular depth in the control chambers is a conservative comparison of the effect that *Clymenella torquata* has on the hydrogen sulfide concentration at its feeding depth.

First, these data indicate that hydrogen sulfide concentrations changed considerably from the initial sampling time to the injection date (Figs. 3A, B and 4) indicating that hydrogen sulfide concentration increased when 10 mM buffered sodium sulfide solution was injected into the chamber through the sipper.

Secondly, the hydrogen sulfide concentration was also different between depths in the chambers. The mean minimum hydrogen sulfide concentrations were highest in the bottom of the chamber in the control treatment for both the initial and injection dates (Fig. 4). The occurrence of the highest concentrations in the bottom of the chambers was probably a function of the injecting technique where 4 ml of sulfide were injected into the bottom layer and only 3 ml were injected in the middle and top layers. In contrast to the control chambers, the mean minimum hydrogen sulfide concentration in the bottom layer of the worm chambers (Fig. 4) was lower than the concentrations in the middle and top layers. The lower concentrations in the bottom of the chambers were most likely a function of the worm's feeding and irrigation activity (Figs 3A-C, note that concentrations were lower at depth in the area of the tube opening) and resulted in oxidation, flushing or displacement of hydrogen sulfide away from the feeding area. On the initial date, porewater hydrogen sulfide concentrations were low at all depths and for all chambers (Figs. 3A and 4A). The change in hydrogen sulfide concentration between the control and worm chambers on the initial date was negligible at the 4- and 8-cm depths and decreased slightly in the worm treatment (a mean decrease of 80 μM) at the 12-cm depth (Fig. 4A). For the

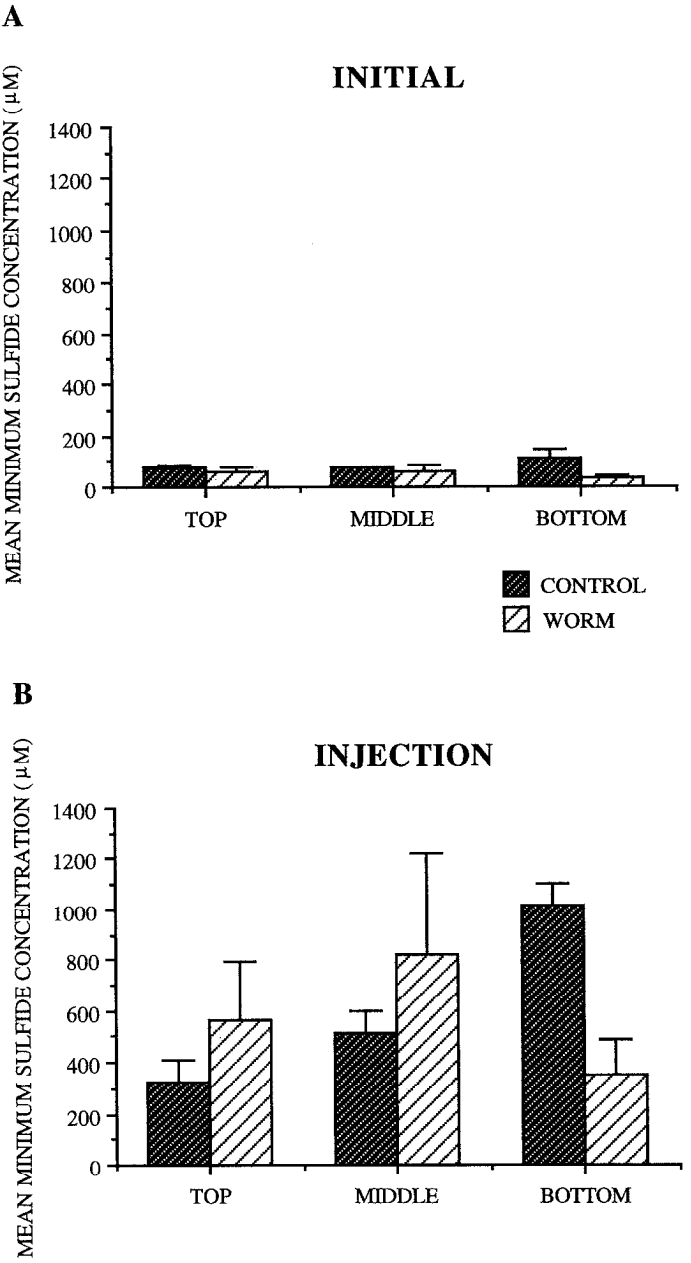

Figure 4. Mean minimum sulfide concentrations ± 1 standard deviation for the three depths in the worm and control chambers for "initial" (A) and "injection" (B) dates in the Profile experiment.

injection date when porewater hydrogen sulfide concentrations were higher (Fig. 3B and 4B), the presence of the worm lowered the concentration considerably at 12 cm which corresponded to where the worm was feeding. Hydrogen sulfide concentration at the shallower depths, 4- and 8-cm, increased in the chambers with worms on the injection date. This increase may be due to the upward advection of porewater surrounding the worm's tube once all the oxygen has been used up by either the worm's respiration or hydrogen sulfide oxidation, resulting from irrigation activity by the worm, thus, displacing the porewater hydrogen sulfides to shallower depths in the chamber. Alternatively, this increase in hydrogen sulfide concentration above the feeding depth may be due to the worm using up the porewater dissolved oxygen, thus, enhancing an anoxic environment and increasing hydrogen sulfide production. These data also indicate that the worm's tube is relatively impermeable to oxygen.

Finally, in chambers with a worm, hydrogen sulfide concentrations at the worm's feeding location (determined by the position of the tube and the color of the fecal material produced) were significantly different from the corresponding minimum hydrogen sulfide concentration at the same depth in the control chambers for each date (*t*-tests for equal variances corrected for multiple comparisons by Bonferroni's inequality, $p = 0.05$, initial, $t = 4.017$ and injection, $t = 8.565$, $df = 4$). Hydrogen sulfide concentrations in the bottom of the chambers were lower in chambers with worms than in control chambers for both dates (Figs. 3A, B and 4).

A week after sulfides were increased through injection (Fig. 3C), the hydrogen sulfide concentrations in the control chambers were uniformly high (above 1500 μM), possibly due to continued organic carbon oxidation in an anoxic sedimentary environment and an increase in sulfur-reducing bacteria. After one week, in chambers with worms, hydrogen sulfide concentration at the feeding location ranged from less than 500 μM to between 1000 and 1500 μM. Three possible explanations for this are: (1) different between-worm abilities for lowering or maintaining a lower porewater hydrogen sulfide concentration, (2) between-worm variability in tolerance to elevated hydrogen sulfide concentrations which may affect their ability to lower hydrogen sulfide concentrations (3) differences between chambers in the amount of hydrogen sulfide produced.

b. Feeding experiments

i. Effect of hydrogen sulfides on feeding rate. The relationship between porewater hydrogen sulfide concentration and fecal production in *Clymenella torquata* varied among worms. Some worms were capable of modifying the hydrogen sulfide environment around the opening of their tube while others were not (e.g., Figs. 3B and C, worm chambers). The specific mechanisms by which the hydrogen sulfide concentration was modified was beyond the scope of this study. However, some possible mechanisms which *C. torquata* may employ to lower the hydrogen sulfide concentration are irrigation activity (Mangum, 1964; Dobbs, 1981), defecation activity (which

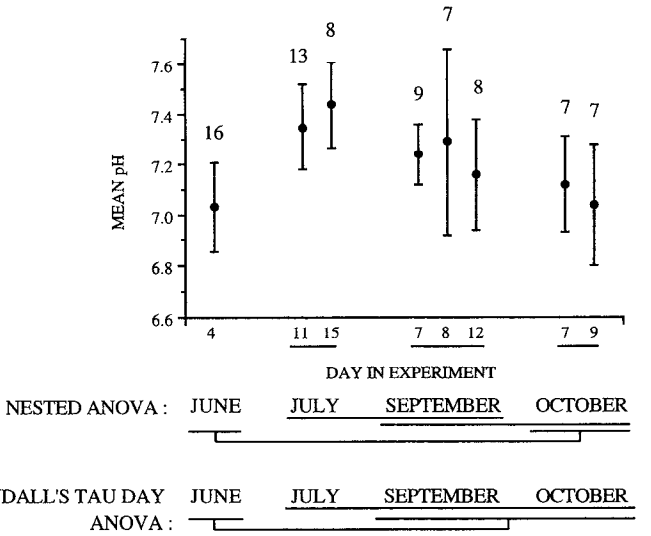

Figure 5. Mean pH ± 1 standard deviation for different days in the four feeding experiments. Numbers indicate the number of chambers sampled. Bars under the days and the experiments represent treatments that are not significantly different at $\alpha = 0.05$.

Mangum (1964) showed is not exclusive of irrigation but moves a smaller volume of water), or some combination of these activities.

The effect of hydrogen sulfide concentration on fecal production for feeding experiments conducted in June, July, September and October of 1991 is shown in Figures 6A-D, respectively. In all four experiments, there was between-worm variability in the amount of fecal material produced. At the lower hydrogen sulfide concentrations (i.e., less than 1000 μM in June and 700 μM in July), variability in the weight of feces produced per day between individuals was large, ranging from 0-4 grams. Possibly, low hydrogen sulfide concentrations did not influence fecal production. However, under higher hydrogen sulfide concentrations (i.e., greater than approximately 1000 μM in June and 700 μM in July, Figures 6A and B), fecal production was reduced to about 1.5-2 grams or less.

Kendall's Tau correlation coefficient (Kendall, 1962) comparing total hydrogen sulfide concentration and fecal production was determined for one day during each experiment (exact test, 2-tailed probability, StatView SE + Graphics Macintosh software). The day after sulfides were injected was chosen to be tested *a priori* because this was when the highest hydrogen sulfide concentrations were expected. For the July, September, and October experiments, days 11, 8 and 9, respectively, were used because these were the days after sulfides were artificially increased by injection (Figs. 6B-D). For the June experiment, where only ambient hydrogen sulfide was measured, day 6 was used because more worms were active (Fig. 6A).

In June and July (Figs. 6A and B, respectively), fecal production and hydrogen

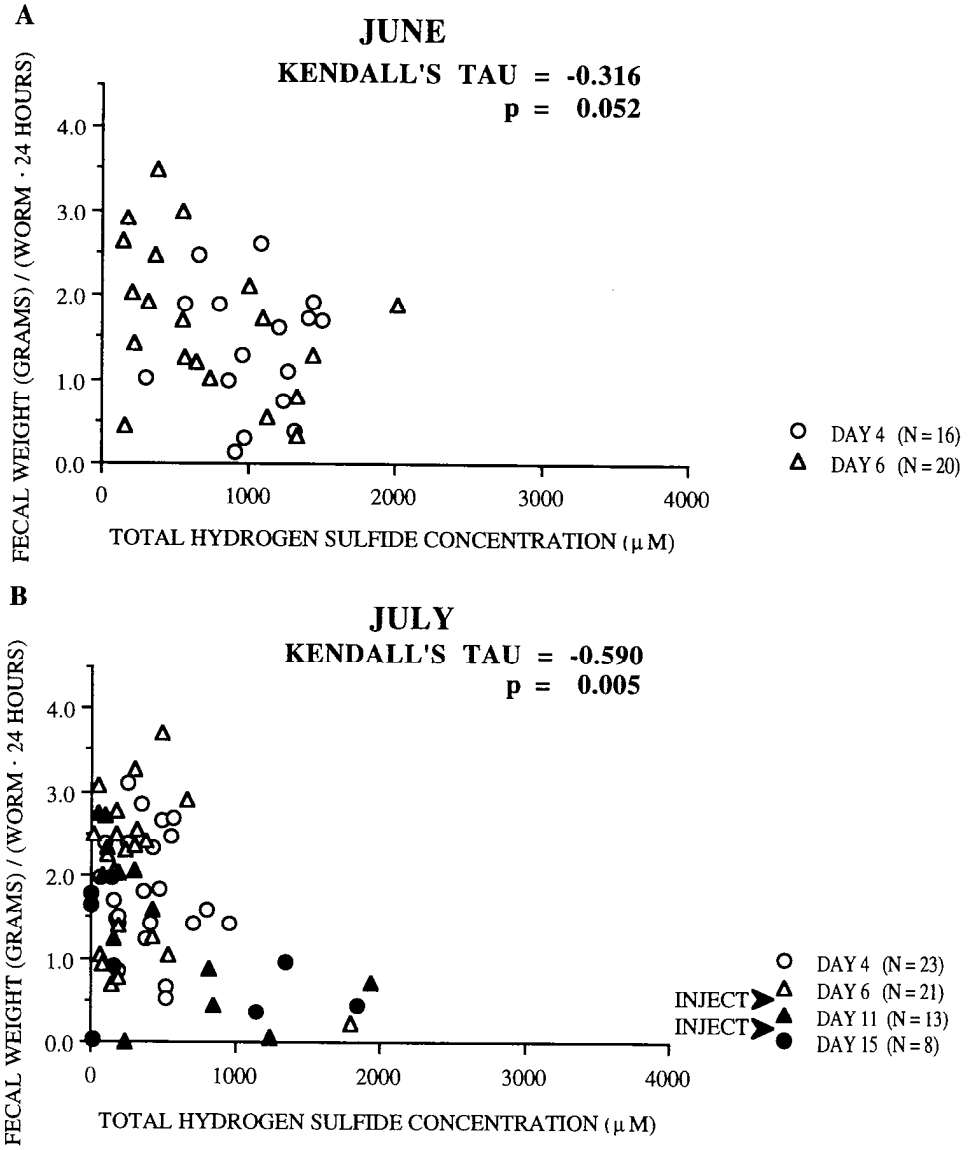

Figure 6. Effects of porewater sulfide concentration on fecal production in the June (A), July (B), September (C), and October (D) experiments. Each type of symbol (see legends) represents a different day, each point represents a worm and the numbers in parentheses indicate the number of individuals for that day. Shaded symbols represent days after sulfides were increased by injecting sulfides. The reported Kendall's Tau values were calculated for one day in each experiment (day 6 in June, 11 in July, 8 in September and 9 in October) chosen *a priori* (see text).

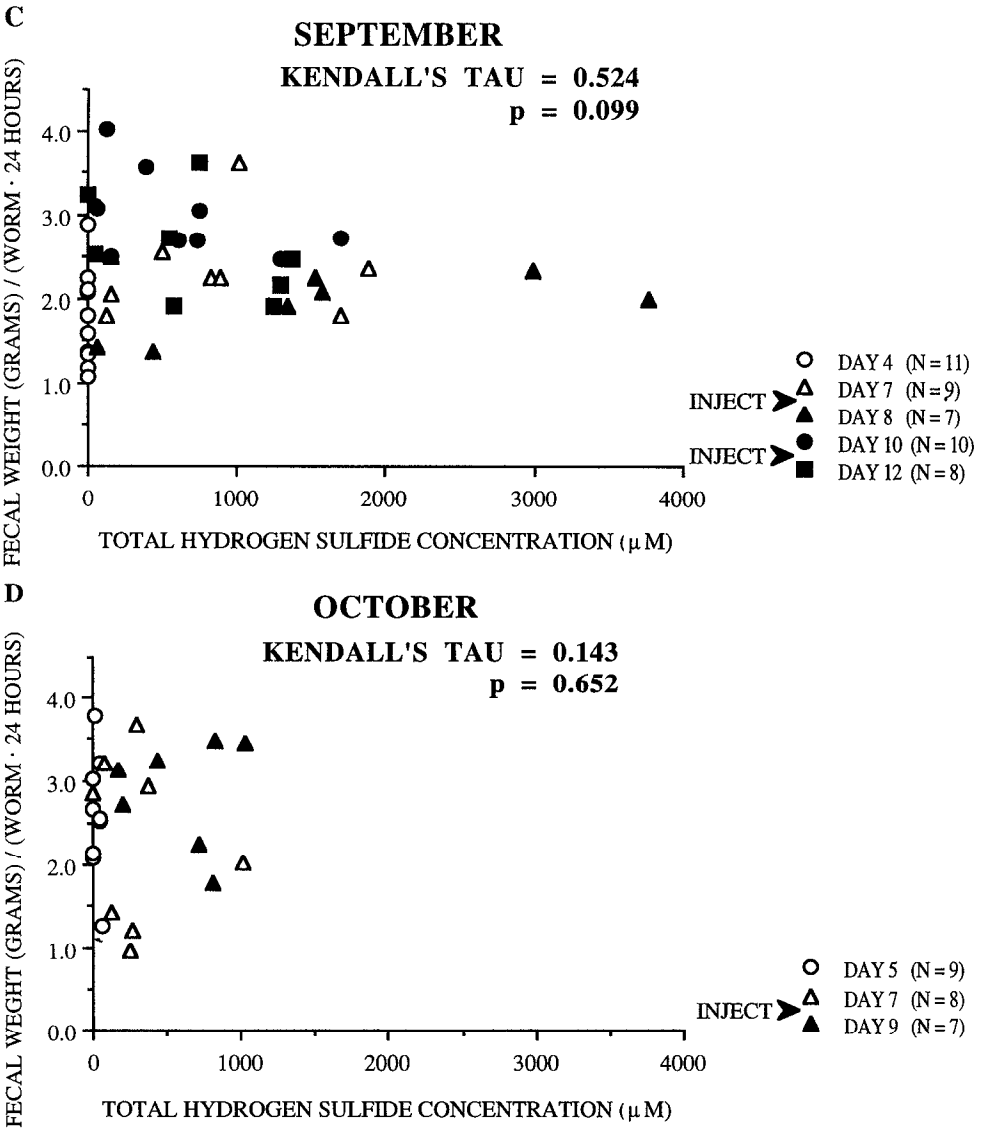

Figure 6. (Continued)

sulfide concentration were negatively correlated by Kendall's Tau test for the one day during the experiment chosen *a priori* (day 6 in June, tau = -0.316, $p = 0.052$; day 11 in July, tau = -0.590, $p = 0.005$). This negative relationship existed even in the presence of high between-worm variability in fecal production. It is possible that the effect of hydrogen sulfide concentration on fecal production in June was not as severe as in July because hydrogen sulfide levels in June were not artificially

increased. Therefore, the concentration probably increased more slowly than when sulfides were injected into the chambers.

In contrast, the significant negative correlation between hydrogen sulfide concentration and fecal production did not exist in the September and October experiments (Fig. 6C, day 8 in September, tau = 0.524, p = 0.099; and Fig. 6D, day 9 in October, tau = 0.143, p = 0.652). In fact, some individuals in September (Fig. 6C) were strikingly tolerant to hydrogen sulfide levels as high as 3 to 3.8 mM, maintaining fecal production of approximately 2 grams of sediment in a day. Although no hydrogen sulfide concentrations above 1 mM occurred in the October experiment, fecal production at 1 mM hydrogen sulfide concentration was about 2 to 3.5 grams · day^{-1} which was similar to values found in September and a generally higher fecal production than in June (0-2.5 grams) and July (0-1.5 grams) for that hydrogen sulfide concentration.

The length of time the hydrogen sulfide concentration remained at a particular level is unknown. This is due to several factors: the changeable nature of hydrogen sulfides, differences due to the presence and activity of *Clymenella torquata* and technical limitations of frequency of measuring porewater hydrogen sulfide concentrations. However, porewater hydrogen sulfide concentrations were compared with fecal production from the 24-hour interval immediately preceding the porewater measurements, and therefore, are assumed to be representative of the hydrogen sulfide exposure during that feeding interval.

Seawater temperatures in the June, July and September feeding experiments were all approximately 20°C. Temperatures in the October feeding experiment were lower, at approximately 17°C. If this difference in temperature alone affected worm hydrogen sulfide tolerance, different fecal production values for the October experiment would be expected. However, this pattern was not observed (Figs. 6A-D). Alternatively, if both feeding activity and hydrogen sulfide toxicity are reduced at the lower temperature of the October experiment, then the fecal production in the October experiment may be a consequence of the interaction between temperature and hydrogen sulfide toxicity.

ii. PH data. Mean porewater pH values between the feeding experiments ranged from 7.0 to 7.4 (Fig. 5). PH values for days within experiments were not significantly different, however, pH significantly differed between experiments (Nested ANOVA, F = 10.77, p = 0.0001, df = 3, 67, Fig. 5). PH values in the June and July experiments, the June and September experiments and the July and October experiments were significantly different (Scheffè's multiple comparisons, α = 0.05, Fig. 5). PH values for the July and September experiments were not significantly different, nor were the September and October experiments or the June and October experiments (Scheffè's multiple comparisons, α = 0.05, Fig 5).

PH values for the same day tested for Kendall's tau correlation in each experiment

were significantly different between experiments (one-way ANOVA, $F = 6.27$, $p = 0.0014$, $df = 3, 39$, Fig. 5). In June, pH values for day 6 were not available, therefore, values for day 4 were used. It was assumed that pH values for the two days were similar because the chambers were not injected with sulfides or buffer. PH values for June were different from July and all other experiments were not significantly different (Scheffè's multiple comparisons, $\alpha = 0.05$, Fig. 5).

iii. Tube-building activity. The percentage of days each worm spent building new tubes (or branches) was calculated for the June, July, September and October experiments. Only worms in the September experiment built curved tubes and those worms made up less than 4% of the worms in all feeding experiments (Fuller, 1992). The occurrence of curved tubes was not related to the injection treatment (i.e., buffer or buffer plus sulfide). Curved tubes were not included in the data analysis because hydrogen sulfides would not be accurately determined for tubes that were not perpendicular to the sediment surface for their entire length.

Differences in duration of activity, where some individuals became inactive during the experiment while others did not, occurred primarily in the July experiment. Possibly, differences in the duration of activity were related to the negative effect of hydrogen sulfide concentration on fecal production found in the July experiment. In addition, the July experiment was sampled over the most days, however, examination of the number of tubes built and the number of days a worm was active revealed no relationship for all four experiments (Fuller, 1992).

Worm tube-building activity for the June, July, September and October experiments was compared using an unbalanced ANOVA and Scheffè's multiple comparisons. The data were transformed to meet conditions of homoscedasticity (Hartley's F_{max} test) by a $\log(x + 1)$ transformation. Tube-building activity differed between the four feeding experiments (ANOVA, $F = 3.84$, $p = 0.0011$, $df = 13, 30$). Tube-building activity did not differ between June and July experiments nor between June, September and October experiments (Scheffè's multiple comparisons, $\alpha = 0.05$). Worms in the June and July experiments built more tubes than worms in the September and October experiments (Figs. 7A-D).

4. Discussion

In laboratory experiments conducted in June and July 1991, hydrogen sulfide concentration above a threshold of 700-1000 μM had a negative effect on fecal production in recently collected worms. In other studies, Llanso (1991) found that *Streblospio benedicti,* a surface deposit-feeding polychaete, stopped feeding at hydrogen sulfide concentrations between 20 and 60 μM and Vismann (1990) observed that *Nereis diversicolor* and *N. virens,* both tube-dwelling polychaetes, stopped feeding at concentrations of 170 μM. These polychaetes stopped feeding at much lower hydrogen sulfide concentrations than *Clymenella torquata.* In contrast to *C. torquata,* the

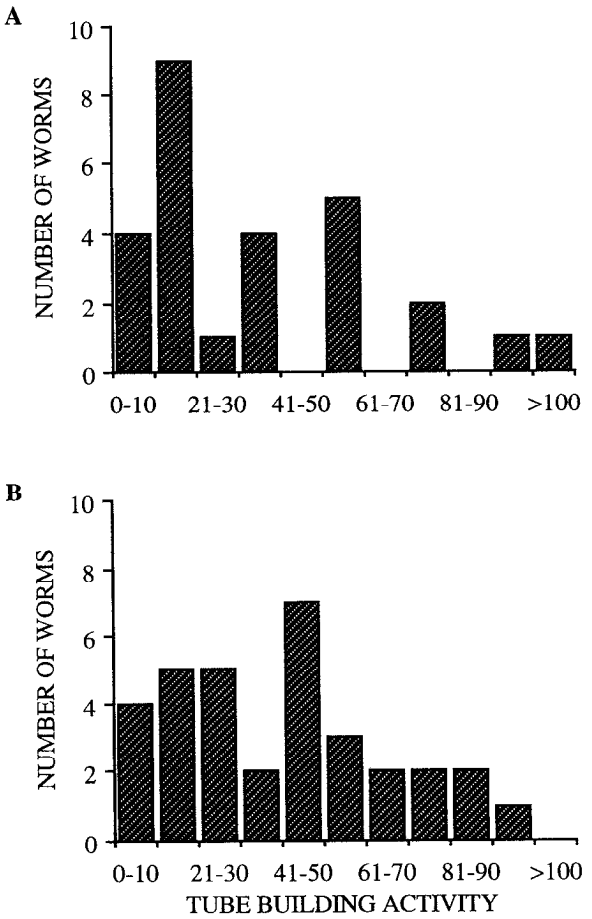

Figure 7. Tube-building activity (number of days worms built a new tube expressed as a percent of the days worms were active) for worms in the June (A) July (B) September (C) and October (D) feeding experiments.

other three polychaetes (*S. benedicti, N. diversicolor* and *N. virens*) are relatively motile and often feed and live near the sediment-water interface.

In the September and October 1991 experiments, hydrogen sulfide concentration did not negatively affect fecal production. Furthermore, *Clymenella torquata* was strikingly tolerant to hydrogen sulfide concentrations as high as 3 to 3.8 mM. In another laboratory experiment where hydrogen sulfide was bubbled into seawater and isolated from exposure to air, *Arenicola marina* also tolerated hydrogen sulfide even when it could not increase its irrigation activity (Patel and Spencer, 1963). Both *C. torquata* and *A. marina* are semi-sessile polychaetes and feed at depth in the sediment (Fauchald and Jumars, 1979). Groenendaal (1981) found that lower

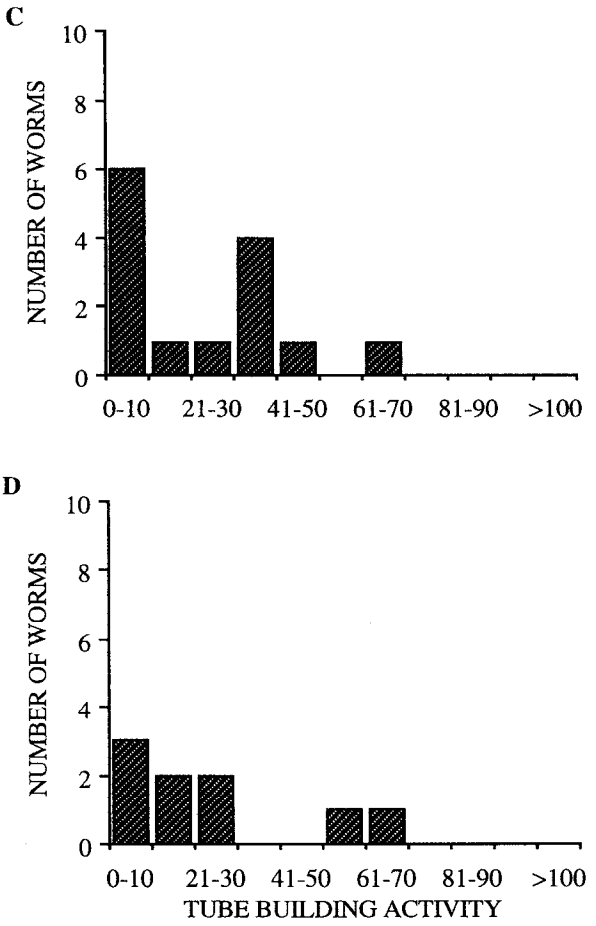

Figure 7. (Continued)

coelomic pH values in *A. marina* resulted in lower internal concentrations of the more toxic hydrogen sulfide species, H_2S.

In these experiments total dissolved hydrogen sulfide (H_2S and HS^{-1}) concentration was measured. The dissolved species of sulfide present is dependent upon pH. At a pH of 7.0, equal proportions of the dissolved hydrogen sulfides, H_2S and HS^{-1}, will be present and negligible amounts of S^{-2}. When pH decreases, the percentage of H_2S, which has been shown to be the more toxic species (Somero *et al.,* 1989), will increase. This same species has been shown to penetrate biological membranes easier than the charged species, HS^{-1} and S^{-2} (Groenendaal, 1981). However, knowledge of the porewater pH and total hydrogen sulfide concentration is not enough to determine the toxic effect to the worm. At least one worm, *Arenicola marina* has been found to be capable of maintaining internal hydrogen sulfide concentrations lower than external concentrations. The internal hydrogen sulfide

concentration was maintained by a lower coelomic pH which increased the relative concentration of H_2S, a more permeable species, which inturn rapidly diffused out of the organism to an area of lower H_2S concentration (Groenendaal, 1981). Internal pH measurements were beyond the scope of this study, and therefore, it was not possible to know the proportion of each hydrogen sulfide species that the worms experienced.

Porewater pH and toxicity of the different hydrogen sulfide species cannot explain the decrease in fecal production with increased hydrogen sulfide concentrations in the June and July experiments and the absence of correlation in the September and October experiments. Furthermore, the decrease in fecal production occurred when pH values were the lowest (in June) and the highest (in July).

The lower tolerance of *Clymenella torquata* to porewater hydrogen sulfide during the June and July experiments compared to the September and October experiments suggests that worms obtained from the field may have acclimated to elevated levels of dissolved hydrogen sulfides as the summer progressed (see Appendix H, Fuller, 1992, for values from the field). Possibly, if hydrogen sulfide increased gradually in the field throughout the summer (high concentrations occurred in some areas in the field where worms were collected in September, 1991 (Appendix H, Fuller, 1992)), *C. torquata* may have acclimated to the higher hydrogen sulfide levels in the September and October experiments. A behavioral acclimation such as increased irrigation activity (Vismann, 1991) may increase the tolerance to hydrogen sulfide. If worms spent more time irrigating their tubes to reduce exposure to higher porewater hydrogen sulfides, then they might spend less time feeding, however, the amount of fecal material produced did not decrease indicating no trade-off between feeding and irrigation. Alternatively, a physiological acclimation such as an increase in the amount of an oxidizing compound like haemoglobin or hematin could be responsible for the increased tolerance to hydrogen sulfide (Patel and Spencer, 1963; Powell and Arp, 1989; Vismann, 1991). For example, the oxyhaemoglobin of *Arenicola marina* is capable of oxidizing sulfides (Patel and Spencer, 1963).

It is unlikely that these results are due to changes in sediment quality (e.g., food quality or organic carbon content) as the summer progressed because sediments for both the July and September experiments were collected at the same time and location. Furthermore, the organic carbon content of the sediment for all experiments was similar, 0.5% by weight.

This study also has implications for laboratory experiments in which feeding rates of subsurface deposit feeders are determined. Several laboratory studies (e.g., Mangum, 1964; Rhoads and Stanley, 1965; Rhoads, 1967; Nichols, 1974; Fuller *et al.*, 1988) measured fecal material production rates of subsurface deposit feeders. Feeding rates of subsurface deposit feeders are relevant to studies concerned with the role infaunal organisms play in sediment turnover rates, community structure and sediment transport properties. The porewater chemistry of sediments in which

infaunal organisms live and feed potentially impact their activities. This study indicates that the porewater chemistry of such potential toxins as hydrogen sulfide should be controlled and measured in experiments which quantify feeding and tube-building activity in order to compare rates between studies, or to assess their relevance in the field.

Clymenella torquata is not recognized as especially tolerant of low oxygen conditions (Mangum, 1964). However, data from the feeding experiments in September and October demonstrate that during some periods (perhaps after acclimation to gradually increasing hydrogen sulfide concentrations, *C. torquata* can be quite tolerant of high porewater hydrogen sulfide levels. Other studies have looked at survival of infaunal organisms in seawater with increased hydrogen sulfide concentrations (Theede *et al.,* 1969; Theede, 1973; Groenendaal, 1980) or in sediments with increased hydrogen sulfide concentrations in the overlying seawater and unknown porewater concentrations (Vismann, 1990; Llanso, 1991). In this study, porewater hydrogen sulfide concentrations were increased while the overlying seawater remained well-oxygenated (6.2-9.0 mg/l). Many of the worms in these experiments survived (between 40% and 100%, Fuller, 1992) for up to 16 days at elevated hydrogen sulfide concentrations. However, worms were exposed to a range of hydrogen sulfide values during an experiment so direct comparison of organism survival with previous experiments that report one value for hydrogen sulfide concentration is not appropriate. Two other studies observed changes in both survival and feeding activity in polychaetes capable of both surface deposit-feeding and filter-feeding in the presence of hydrogen sulfides. Vismann (1990) observed that feeding stopped at overlying seawater hydrogen sulfide concentrations of 170 μM in *Nereis diversicolor* and *N. virens.* Llanso (1991) also found a cessation in feeding between 20 and 60 μM in *Steblospio benedicti.* In this study, feeding rates in *C. torquata* decreased (less than 1.5-2 g feces · day^{-1}) at porewater hydrogen sulfide concentrations greater than 1000 μM in June and greater than 700 μM in July. However, in September, worms continued to feed (approximately 2 g feces · day^{-1}) at porewater hydrogen sulfide concentrations as high as 3.8 mM. Therefore, under some conditions (e.g., high hydrogen sulfides in June and July) porewater hydrogen sulfide concentrations may decrease the rates of sediment turn over by *C. torquata.*

Hydrogen sulfide concentration may also affect the tube-building activity of *Clymenella torquata.* If worms build new tubes in response to high porewater hydrogen sulfide concentrations (e.g., to escape areas high in hydrogen sulfide), then, it would be expected *a priori* that worms in the June and July experiments should have spent more time building tubes than worms in the September and October experiments. The data on tube-building activity support the hypothesis that worms may become increasingly tolerant of high sulfide levels as the summer progresses (i.e., they build fewer tubes). However, it is possible that this pattern in tube-building activity may be due to a normal variation over the course of the year.

Tolerance by either behavioral or physiological acclimation to hydrogen sulfide present in sediments enables some species to survive and continue to feed under conditions where other species cannot, and thus, extends their ecological niche. For example, Vismann (1990) found that *Nereis diversicolor,* which occurs in several different sediment types, was more tolerant to hydrogen sulfide than *Nereis virens,* which prefers oxidized sandy sediments. Organisms that are intolerant of increased hydrogen sulfide levels either die or emerge from the sediments (Henriksson, 1969; Theede *et al.,* 1969; Theede, 1973; Shick, 1976; Jorgensen, 1980; Groenendaal, 1980; Vismann, 1990; Llanso, 1991; Rosenberg *et al.,* 1991). Emergence from the sediments increases the risk of predation by epibenthic crustaceans and demersal fish. For these reasons, it is advantageous for organisms to have mechanisms which enable them to tolerate increasing hydrogen sulfide concentrations.

Finally, this study has implications for community structure. Bioturbation by infaunal organisms increases the distribution of sulfate-reducing bacteria (Hines and Jones, 1985), changes the geochemical environment around burrows (Rhoads, 1974; Aller, 1978; Aller and Yingst, 1978), increases microbial distributions and activity (Meyers *et al.,* 1987; 1988), and increases the flux of chemical species out of the sediments (Goldhaber *et al.,* 1977; Kristensen and Blackburn, 1987; Kristensen *et al.,* 1991). This study has demonstrated the ability of some *Clymenella torquata* individuals to significantly modify the porewater hydrogen sulfide concentrations (e.g., by irrigation activity), and thus, impact the community structure. Furthermore, changes in bioturbation activities of *C. torquata* due to the toxic effect of hydrogen sulfide decreasing its feeding activity, or resulting in death of the organism has implications for all these parameters.

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