SEDIMENTATION CHARACTERISTICS OF

Giardia intestinalis

CYSTS--RELEVANCE TO STANDARD

METHODS OF DETECTION

A Thesis

Presented to

The Graduate Faculty

Central Washington University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

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May, 1993

Approved for the graduate faculty

Committee Chair

SEDIMENTATION CHARACTERISTICS OF *Giardia intestinalis* CYSTS--RELEVANCE TO STANDARD METHODS OF DETECTION

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I investigated the sedimentation time of *Giardia intestinalis* cysts in water. Four to five month-old cysts, and less than two week-old cysts were purified and sedimented in 17 cm columns for periods of 0 to 48 hours. After 18 hours (the current standard time), 45% of the cysts remained in the supernatant, but after 48 hours, only 5% to 10% of the cysts remained in the supernatant. I recommend that the standard sedimentation time be increased to 48 hours, or eliminated in favor of centrifugation.

ACKNOWLEDGMENTS

I would like to thank my graduate committee, and the biology department faculty and staff for their instruction, guidance and assistance. I would also like to thank my wife, Jannette, for her patience and support.

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INTRODUCTION

Since 1980, *Giardia* has been the most often identified cause of water-borne enteritis (CDC, 1990). *Giardia* spp. infect humans and other animals. Presumably, *Giardia intestinalis* (formerly *G. lamblia*, *G. duodenalis*) is the species which infects humans (Schmidt and Roberts, 1989). *Giardia* is transmitted by the fecal-oral route, or by the ingestion of cysts in contaminated water. Inside the duodenum, the parasite excysts, divides, and attaches to the intestinal mucosa where it multiplies. Symptoms of diarrhea and abdominal cramps in the host are thought to be due to the parasite's interference with normal intestinal absorption of food materials.

Giardiasis is a cosmopolitan disease. Many outbreaks occur in the West, Northwest, and Northeast United States, presumably because communities in these areas rely on mountain drainages for their water supplies (Jakubowski, 1988). Many wild animals, primarily aquatic rodents, serve as reservoirs for *Giardia* and may contaminate water supplies downstream from their habitats (Pacha *et al.*, 1985, 1987). Routine chlorination of water supplies does not always kill the parasite (Hoff, 1986). New water quality regulations require public water supplies to incorporate filter systems which are capable of reducing the *Giardia* cyst concentration in water by three orders of magnitude (*Federal Register*, 1989). In order to test new filtration systems, and to test existing water supplies, a reliable method of detecting *Giardia* cysts in drinking water is required.

The present standard method for detecting *Giardia* (Franson, 1985, 1989) is outlined in Figure 1. The method is still considered to be experimental. In this study, I was concerned with the sedimentation step of the process (highlighted in Figure 1). Although not directly cited in the standard method, the procedure is supported by research on the sedimentation of *G. muris* (Sauch, 1984). Sauch reported that *G. muris* had a sedimentation rate of one cm/hour in Percoll. The authors of the standard method indicate that *G. intestinalis* has the same sedimentation rate in water. However, Edtl-Hampton's (1988) study using unpurified *G. intestinalis* cysts indicates that the sedimentation rate for this species is much lower than one cm/hour. Preliminary studies of sedimentation of cysts (Pacha and Clark, 1993), and research on cyst density (Kramar, 1992) indicate that the densities of individual cysts may change over time, thus changing their sedimentation rates. Since *G. intestinalis*, not *G. muris*, is the parasite of interest when testing water supplies, the suitability of the standard method to sediment the cysts needs to be verified.

My goal for this study was to improve the standard method of detecting *G. intestinalis* cysts in water by determining the reliability of overnight (18 hour) sedimentation of purified *G. intestinalis* cysts; determining the sedimentation rate of purified *G. intestinalis* cysts in water; determining the constancy of the aforementioned sedimentation rate; and determining the effect of cyst age on sedimentation.

Figure 1. Standard method flowchart for the detection of *Giardia* in water. The bold, italicized portions are the primary concerns of this study.

MATERIALS AND METHODS

For this study, I used human source *Giardia intestinalis* strain CH3 cysts which were propagated in Mongolian gerbils (*Meriones uguiculatus*). Stock cysts were obtained by suspending gerbil fecal pellets in distilled water, and mixing them to form a loose slurry. The slurry was filtered through two layers of cheesecloth, and then purified by zinc flotation (Hibler, 1991) as follows. Two to four milliliters of the filtrate was dispensed into a 50 mL conical centrifuge tube, and diluted to 20 mL with distilled water. The suspension was underlaid with 25 mL of zinc sulfate solution with a specific gravity of 1.20, and centrifuged for five minutes at 600 x g. The supernatant containing the cysts was drawn off at the water-zinc sulfate interface with a pipette, and filtered through a five micron Nucleopore membrane. The cysts were rinsed from the membrane and stored at $4^{o}C$.

To determine sedimentation times of the cysts, two to five milliliters of the purified cyst suspension was added to four liters of distilled water. After thoroughly mixing the suspension, three one milliliter aliquots were taken to determine cyst concentration and percent recovery. One liter of the cyst suspension was dispensed into each of four one liter Fleakers. This provided a sedimentation column approximately 17 cm high. At 0, 18, 24 and 48 hours, four 250 mL subsamples were drawn off from one of the Fleakers (Figure 2) by vacuuming from the surface of the suspension with the apparatus shown in Figure 3. I performed three replicates of this process with

4-5 month-old cysts and with <2 week-old cysts in order to determine any differences in sedimentation between the two age groups.

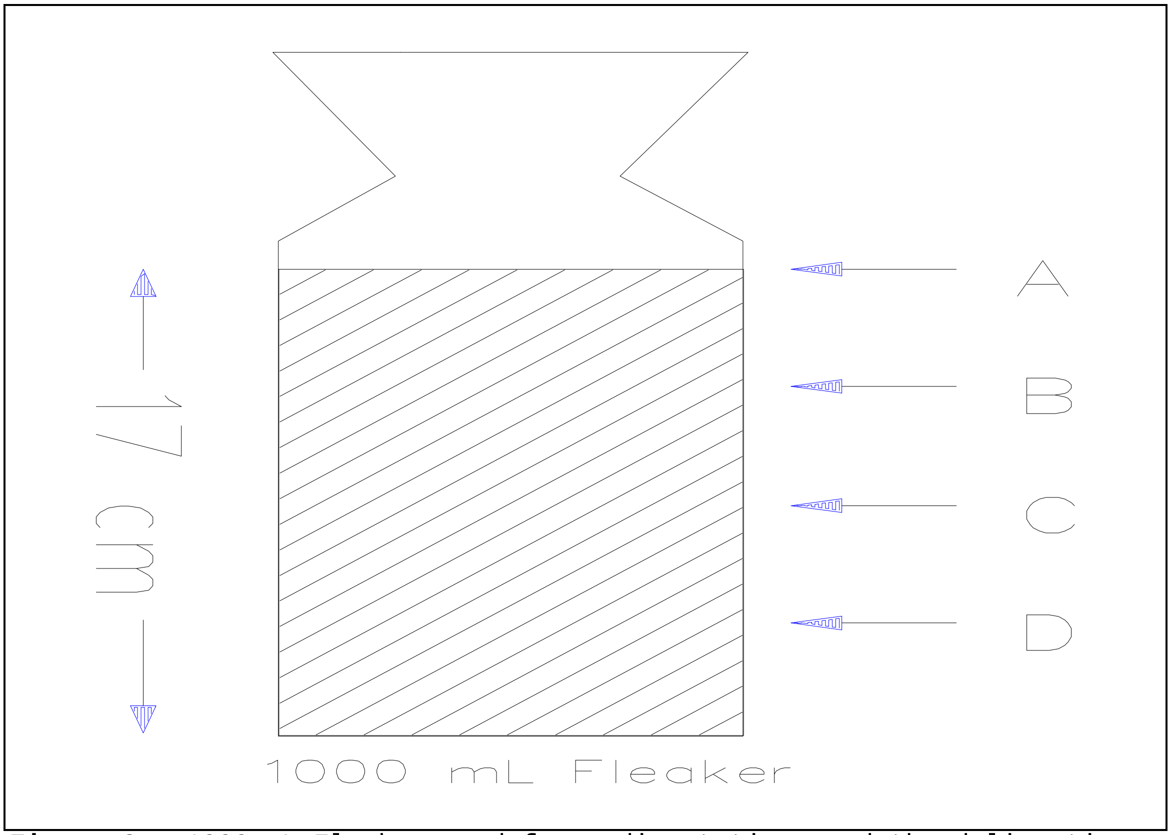


Figure 2. 1000 mL Fleaker used for sedimentation, and the delineations for the subsamples.

I quantified the number of cysts in the original suspensions and in each subsample by membrane filtration and staining (Spaulding, *et al.*, 1983). Three 0.1-50 mL aliquots of each subsample were filtered through five micron cellulose ester Millipore membrane filters. The volumes of the aliquots used depended on the original number of cysts in the suspension. The membranes were then trichrome stained (Figure 4). After the filter was cleared, it was mounted on a glass slide and a cover slip was applied with Coverbond. The number of cysts on each entire membrane was counted under a bright-field microscope, and the number of cysts in each sample was calculated (Figure 5).

In order to determine the constancy of cyst sedimentation, I increased the volume of the 48 hour "A" fractions ("floaters") and "D" fractions ("sinkers") to one liter each in one liter Fleakers. The Fleakers were shaken to resuspend the cysts. Three aliquots were taken from each Fleaker, and the cysts were enumerated to determine the cyst concentration and subsequent percent recovery. The cysts were then allowed to settle for 48 hours. Afterward, the suspensions were subsampled into "A" through "D" fractions (Figures 2 and 3), and quantified using the cyst quantification procedure described for the original sedimentation studies (Figure 4).

I evaluated the differences in settling time between the samples with the student's t-test. Alpha was set at 0.10. When $n = 6$, the results were significant when $t > 1.533$.

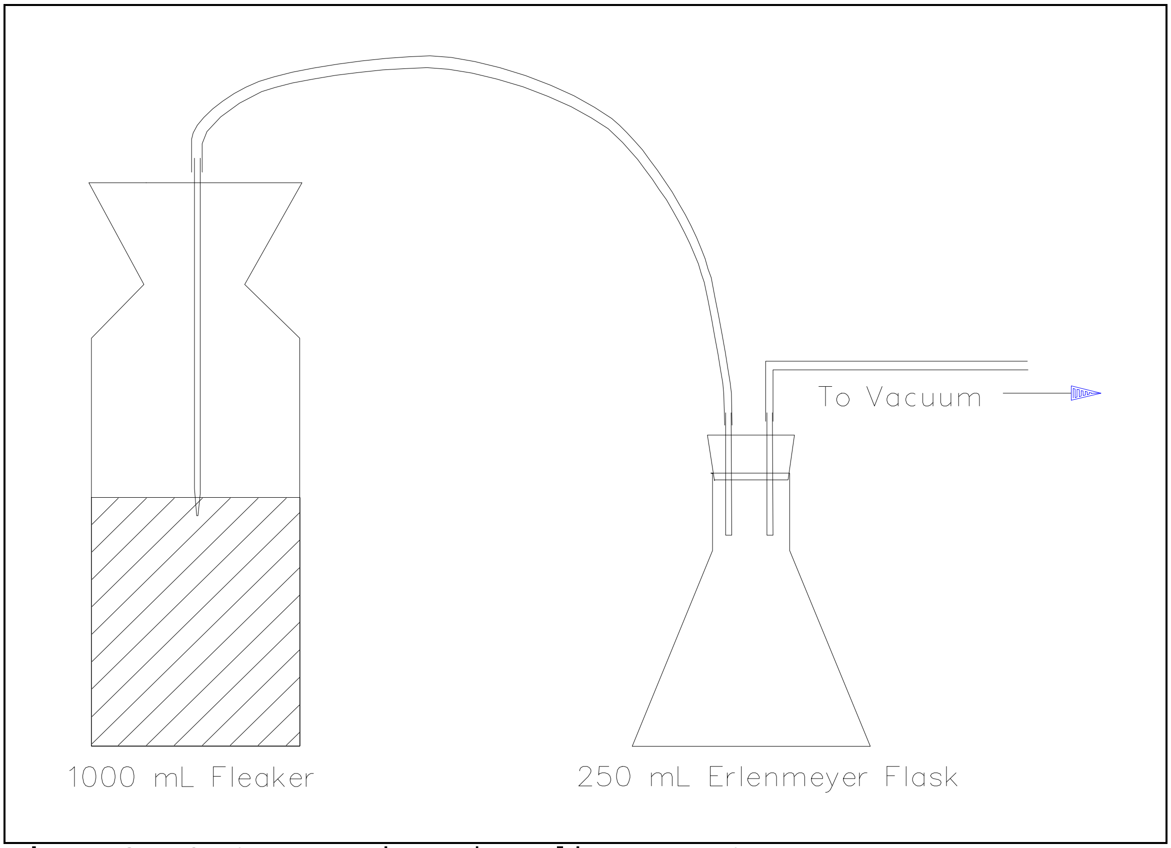


Figure 3. Cyst suspension subsampling apparatus.

Dispense a given aliquot (0.1 to 50 mL) of cyst suspension into the Millipore apparatus with 5 micron type HA polycarbonate filter installed. Apply vacuum to remove excess water.

Add 1 mL 1:5 Lugol's iodine in 70% EtOH. After 1 minute, apply vacuum to remove excess.

Add 2-3 mL 70% EtOH. After 1 minute, apply vacuum to remove excess.

Add 2-3 mL 70% EtOH. After 1 minute, apply vacuum to remove excess.

Remove filter from the Millipore apparatus.

Place filter in trichrome stain for 5 minutes.

Place filter in 90% EtOH with acetic acid (6 drops in 50 mL) for 2 minutes.

Dip filter twice in 95% EtOH. Repeat with fresh EtOH.

Place filter in 100% EtOH for 1 minute.

Place filter in xylene for 1 minute.

Apply filter to a glass slide with Coverbond or similar adhesive. Cover with a coverslip.

Figure 4. Membrane filtration and cyst staining procedure.

Figure 5. Stained *G. intestinalis* cysts on a membrane.

RESULTS AND DISCUSSION

The data from three trials each of old cysts (4-5 month-old) and fresh cysts (<2 week-old) are shown in Tables I and II, respectively. The data are graphically represented in Figures 6 through 13. After 18 hours, an average of 45% of the old cysts and 46% of the fresh cysts remained in the supernatant ("A"-"C" layers, total of 750 mL). The difference between the supernatant fractions of the old and fresh cysts was not significant ($n = 6$, $t = 0.045$). In comparison, 70% of unpurified cysts (less than one month old) remained in the upper 800 mL of a 1000 mL sedimentation column (Edtl-Hampton, 1988). Edtl-Hampton used initial cyst concentrations of 47 cysts/mL to 380 cysts/mL with very little variation in sedimentation. The initial cyst concentrations used in this study ranged from 60 cysts/mL to 95 cysts/mL, except for trial 1 with the old cysts (Table III). If the cysts settled at one cm/hour as previously postulated, I would expect virtually all of the cysts to be in the sediment ("D") layer. In the case of unpurified cysts, the slow sedimentation rate might be attributed to adhesion of the cysts to less dense particulate matter. However, this does not adequately explain the slow sedimentation rate in the purified cysts. Therefore, there must be some quality of the cysts themselves which results in the slow sedimentation rate.

At 24 hours, an average of 21% of the old cysts and 13% of the fresh cysts remained in the supernatant. This was not a significant difference ($n = 6$, $t = 1.290$). By 48 hours, an average of 10% of the old cysts and 5% of the fresh cysts remained in the supernatant. This difference was also not significant (n = 6, t = 1.329) (Figure 14).

During the period between the 18 and 24 hour samples, the amount of old cysts in the "D" layer increased from 55% to 79%, and the amount of fresh cysts in the "D" layer increased from 54% to 87%. This was not a significant difference with the old cysts (n = 6, t = 1.136), but it was significant with the fresh cysts (n = 6, t = 2.274). During the time between the 18 hour samples and the 48 hour samples, the amount of cysts in the "D" layer increased from 55% to 90% with the old cysts, and from 54% to 95% with the fresh cysts. The differences were significant in both cases (old: n = 6, t = 1.658; fresh: n = 6, t = 3.020). In the period between the 24 hour samples and the 48 hour samples, the amount of cysts in the "D" layer increased from 79% to 90% with the old cysts, and from 87% to 95% with the fresh cysts. This was a significant change with the old cysts (n = 6, t = 2.005), but not with the fresh cysts (n = 6, t = 1.519). Overall, there were no significant differences between the "D" fractions of old and fresh cysts at any given time. This indicates that cyst age, within the parameters of this experiment, has no effect on gravity sedimentation.

The 0 and 18 hour old cyst samples in trial 1 appear to be appreciably different from the respective samples in trials 2 and 3. Since trial 1 was the first attempt at sedimentation in this study, there may have been some experimental error. However, if the results of trial 1 are ommitted from the t-test calculations, the results of the previous comparison do not change with respect to their significance. The decrease in variance due to the omission of the trial 1 data is offset by the decrease in degrees of freedom. Thus, the significance or lack thereof, remains the same.

Table IV lists the recovery data for the initial sedimentation experiments. The mean recovery rate was 95% with a low of 45% and a high of 170%. The standard deviation was 29%. In comparison, the mean recovery rate for all samples of unpurified cysts was 92% (Edtl-Hampton, 1988). Indications of the variability of the mean recovery rate for unpurified cysts were not provided. The recovery rates for these experiments were calculated by dividing the total number of cysts accounted for in the "A"-"D" subsamples of a sample by the initial cyst concentration of the sample. The variation seen in cyst recovery is probably due to errors in enumerating the stock cyst suspensions. Three replicate slides were counted for each subsample for a total of twelve slides, but only three slides were counted for four liters of stock cyst suspension. Each one liter sample column was assumed to have the same cyst concentration as the original four liters of stock suspension.

Evaluation of the resuspended cyst data (Tables V and VI) reveals that among the old cysts, 67% of the "floaters" and 93% of the "sinkers" were found in the sediment ("D") layer after resuspension and sedimentation for 48 hours (Figures 15-18). Among the fresh cysts, 83% of the "floaters" and 98% of the "sinkers" were found in the sediment layer after the same treatment. The differences between the "floaters" and "sinkers" were not significant in either case (old: $t = 1.460$; fresh: $t = 1.229$). The difference between the number of old "floaters" and fresh "floaters" in the sediment layer (67% and 83%, respectively) was also not significant ($n = 6$, $t = 0.523$). However, the difference between the amount of old "sinkers" and fresh "sinkers" in the sediment layer (93% and 98%, respectively) was significant (n = 6, t = 2.475). This indicates that the fresh "sinkers" have a faster sedimentation rate than the old "sinkers." However, I cannot provide an adequate explanation as to why this occurred. There was no discernible difference in cyst morphology between any of the groups tested.

Cyst recovery in the resuspension experiments was much more erratic than in the initial sedimentation experiments (Table VII). The mean recovery was 99%, but recovery ranged from a low of 28% to a high of 220% with a standard deviation of 58%. Part of the variation may be due to the very low numbers of cysts involved in the resuspension and sedimentation of the "floater" cysts ("A" layer). In many instances, the initial number of cysts was less than 100 per 250 mL. A miscount of only a few cysts could result in a relatively large error in recovery. I cannot provide an adequate explanation for the variations in recovery with the "sinker" ("D" layer) cysts. However, the purpose of the resuspension experiments was to show the variability in cyst sedimentation. The recovery rate variability in these experiments is not of consequence to the analysis of water samples, if they are allowed to sediment for at least 48 hours.

Table I. Results of sedimentation of 4-5 month-old *G. intestinalis* CH3 cysts.

Time Hours	Layer	Trial 1 Cysts/mL	Trial 2 Cysts/mL	Trial 3 Cysts/mL	Mean % Cysts	Std. Dev.
0	A	530 (27%)	62 (15%)	40 (23%)	21.7	6.2
0	B	926 (47%)	139 (34%)	50 (29%)	36.5	9.6
0	C	374 (19%)	97 (23%)	33 (19%)	20.6	2.4
0	D	130 (7%)	117 (28%)	50 (29%)	21.2	12.6
18	A	236 (16%)	20 (7%)	13 (5%)	9.3	6.2
18	B	619 (43%)	28 (10%)	18 (6%)	19.6	20.1
18	C	354 (25%)	45 (16%)	20 (7%)	15.7	8.8
18	D	238 (16%)	192 (67%)	237 (82%)	55.4	34.5
24	A	93 (2%)	32 (14%)	30 (8%)	8.1	6.3
24	B	216 (4%)	17 (8%)	28 (8%)	6.4	2.2
24	C	572 (10%)	17 (8%)	13 (4%)	7.1	3.2
24	D	4794 (85%)	160 (71%)	287 (80%)	78.5	7.0
48	A	197 (4%)	27 (8%)	3 (1%)	4.5	3.8
48	B	153 (4%)	12 (4%)	3 (1%)	2.7	1.6
48	C	130 (3%)	15 (5%)	8 (2%)	3.2	1.3
48	D	3950 (89%)	268 (83%)	353 (96%)	89.6	6.5

Table II. Results of sedimentation of <2 week-old *G. intestinalis* CH3 cysts.

Time Hours	Layer	Trial 1 Cysts/mL	Trial 2 Cysts/mL	Trial 3 Cysts/mL	Mean % Cysts	Std. Dev.
0	A	102 (25%)	61 (22%)	42 (24%)	23.5	1.8
0	B	59 (14%)	65 (23%)	52 (30%)	22.4	7.8
0	C	128 (31%)	62 (22%)	39 (22%)	25.2	5.3
0	D	121 (30%)	96 (34%)	41 (24%)	28.9	5.1
18	A	30 (9%)	23 (24%)	11 (8%)	13.8	8.8
18	B	32 (10%)	21 (22%)	25 (18%)	16.7	6.2
18	C	28 (9%)	25 (26%)	15 (11%)	15.2	9.5
18	D	236 (72%)	27 (28%)	85 (63%)	54.3	23.2
24	A	7 (2%)	3 (2%)	10 (6%)	3.2	2.2
24	B	12 (3%)	2 (1%)	18 (10%)	4.8	4.8
24	C	19 (4%)	4 (4%)	13 (7%)	5.1	2.0
24	D	400 (91%)	107 (93%)	135 (77%)	86.9	8.8
48	A	3 (1%)	4 (2%)	4 (4%)	2.2	1.3
48	B	3 (1%)	1 (1%)	3 (2%)	1.3	0.9
48	C	5 (2%)	1 (1%)	3 (2%)	1.5	1.0
48	D	330 (97%)	193 (97%)	108 (92%)	95.0	2.9

Table III. Initial cyst concentration in sedimentation experiments. N/R = not recorded.

	Cyst concentration (cysts/mL)		
Cyst type	Trial 1	Trial 2	Trial 3
Old original	N/R	60	95
Old "floaters"	N/R	1.9	2.3
Old "sinkers"	N/R	75	33
Fresh original	93	61	46
Fresh "floaters"	0.27	0.03	0.90
Fresh "sinkers"	77	58	27

Table IV. Cyst recovery data for the initial sedimentation experiments. Initial cyst numbers were calculated from cyst counts from four liter stock suspensions. N/R = not recorded.

Age Group	Trial	Hours	Initial Cysts	Recovered Cysts	% Recovery
Old	1	0	N/R	4.9×10^5	N/R
		18	N/R	3.6×10^5	N/R
		24	N/R	1.4×10^6	N/R
		48	N/R	1.1×10^6	N/R
		Total	N/R	3.4×10^6	N/R
	2	0	6.0×10^4	1.0×10^5	170
		18	6.0×10^4	7.1×10^4	120
		24	6.0×10^4	5.7×10^4	95
		48	6.0×10^4	8.1×10^4	130
		Total	2.4×10^5	3.1×10^5	130
	3	0	9.5×10^4	4.3×10^5	45
		18	9.5×10^4	7.2×10^5	76
		24	9.5×10^4	9.0×10^5	95
		48	9.5×10^4	9.2×10^5	97
		Total	3.8×10^5	3.0×10^6	80
Fresh	1	0	9.3×10^4	1.0×10^5	110
		18	9.3×10^4	8.2×10^4	88
		24	9.3×10^4	1.1×10^5	120
		48	9.3×10^4	8.5×10^4	91
		Total	3.7×10^5	3.8×10^5	100
	2	0	6.1×10^4	7.1×10^4	120
		18	6.1×10^4	4.6×10^4	75
		24	6.1×10^4	2.9×10^4	48
		48	6.1×10^4	5.0×10^4	82
		Total	2.4×10^5	2.0×10^5	83
	3	0	4.6×10^4	4.4×10^4	96
		18	4.6×10^4	3.4×10^4	74
		24	4.6×10^4	4.4×10^4	96
		48	4.6×10^4	3.0×10^4	65
		Total	1.8×10^5	1.5×10^5	83

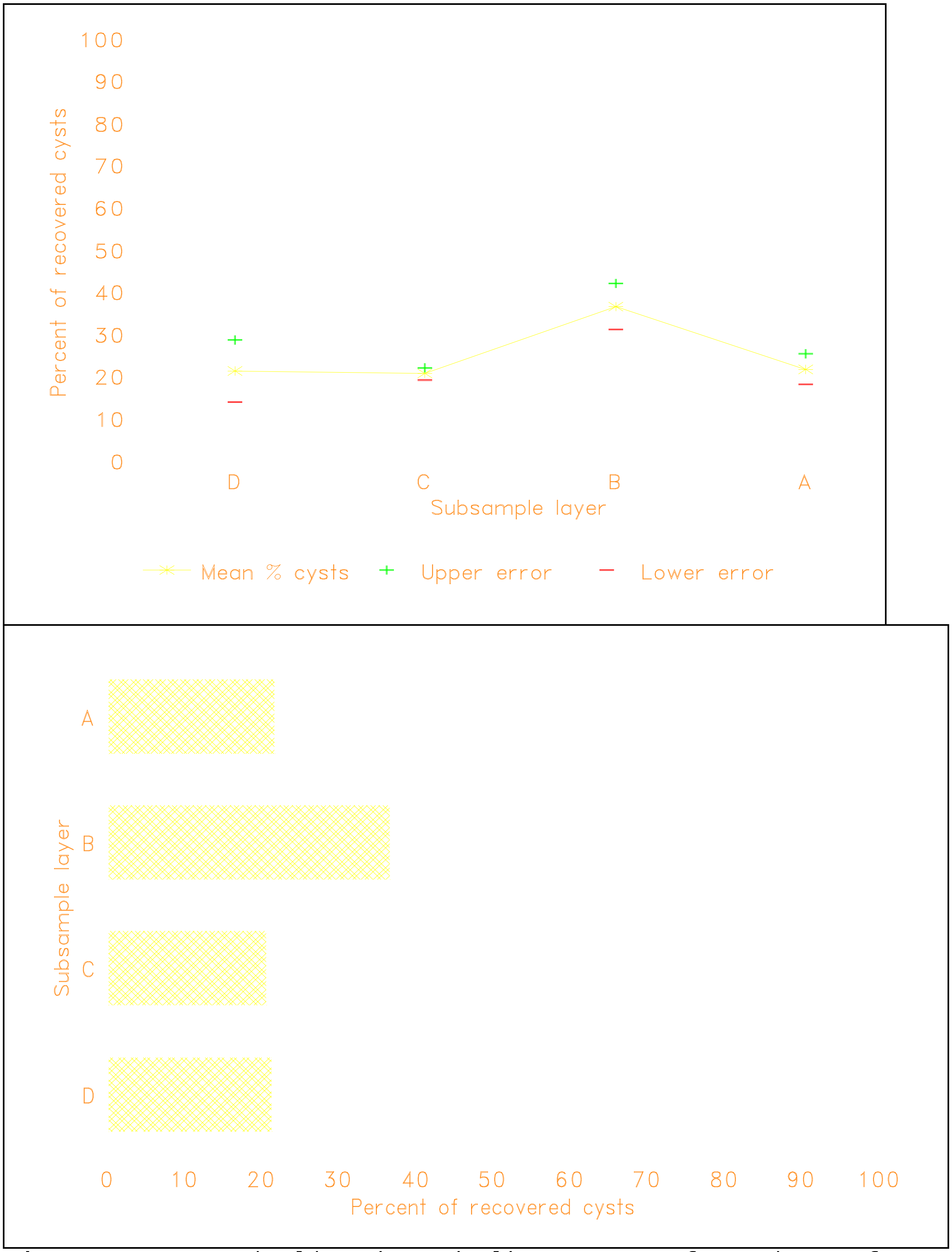


Figure 6. 4-5 month-old *G. intestinalis* CH3 cysts after 0 hours of sedimentation.

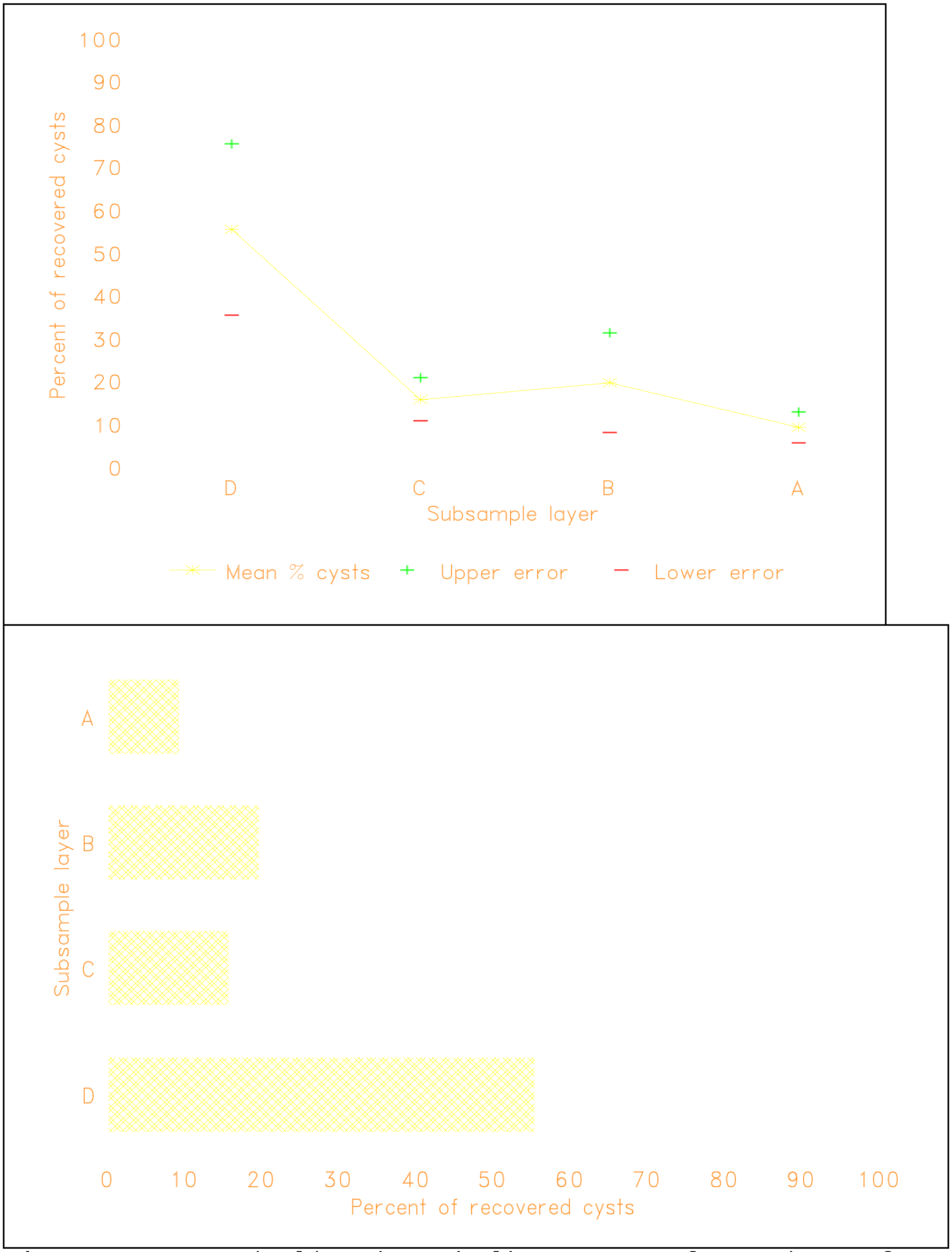


Figure 7. 4-5 month-old *G. intestinalis* CH3 cysts after 18 hours of sedimentation.

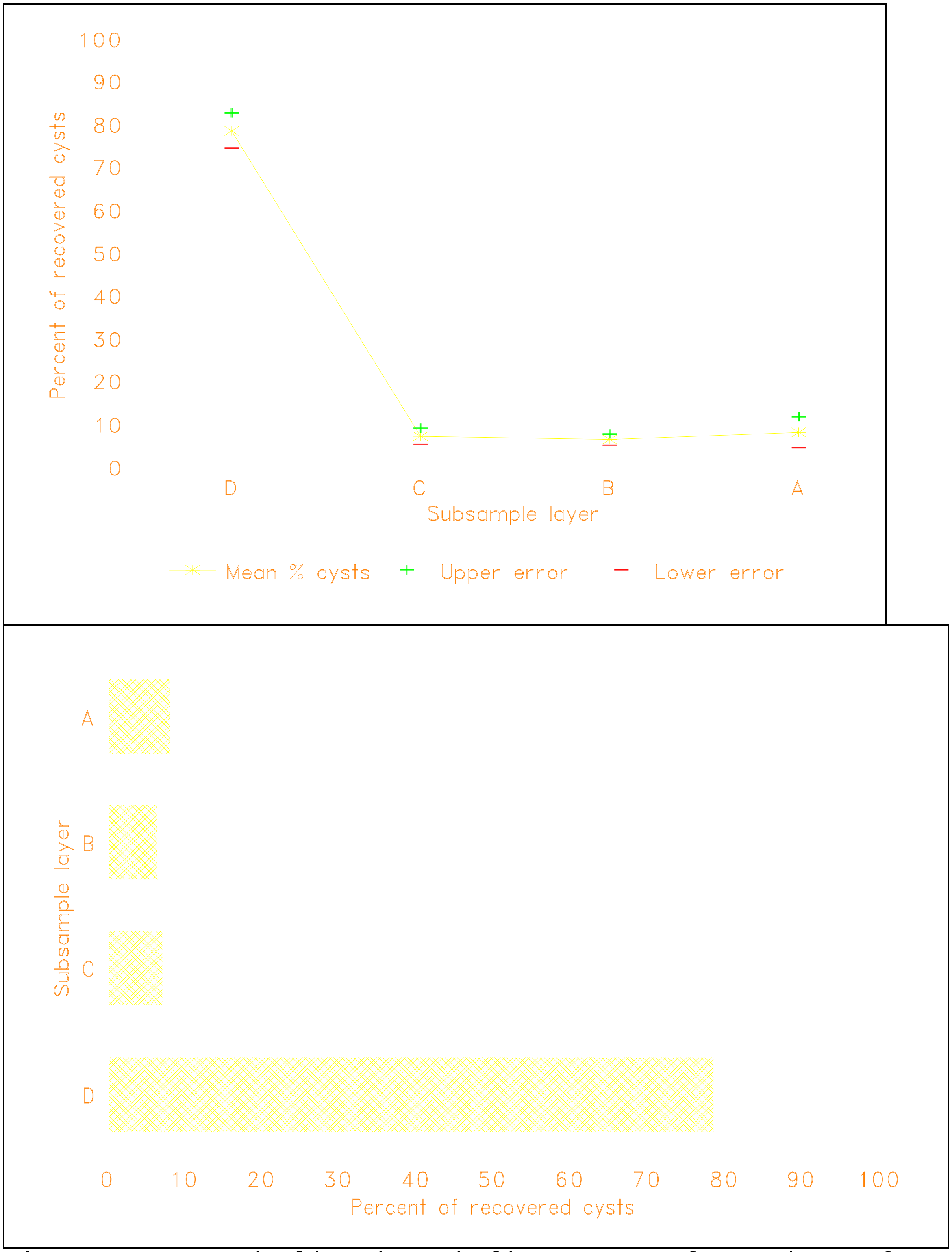


Figure 8. 4-5 month-old *G. intestinalis* CH3 cysts after 24 hours of sedimentation.

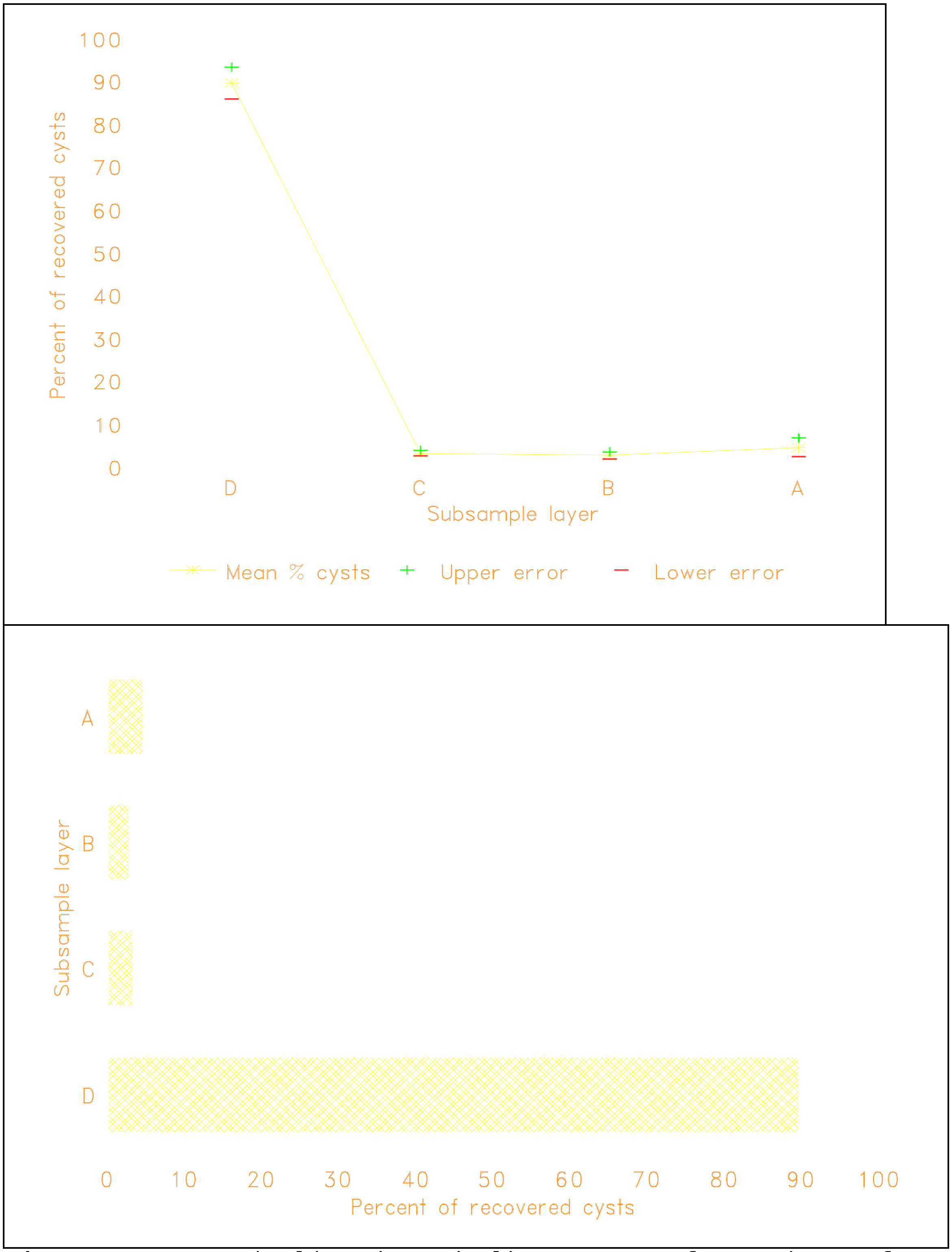


Figure 9. 4-5 month-old *G. intestinalis* CH3 cysts after 48 hours of sedimentation.

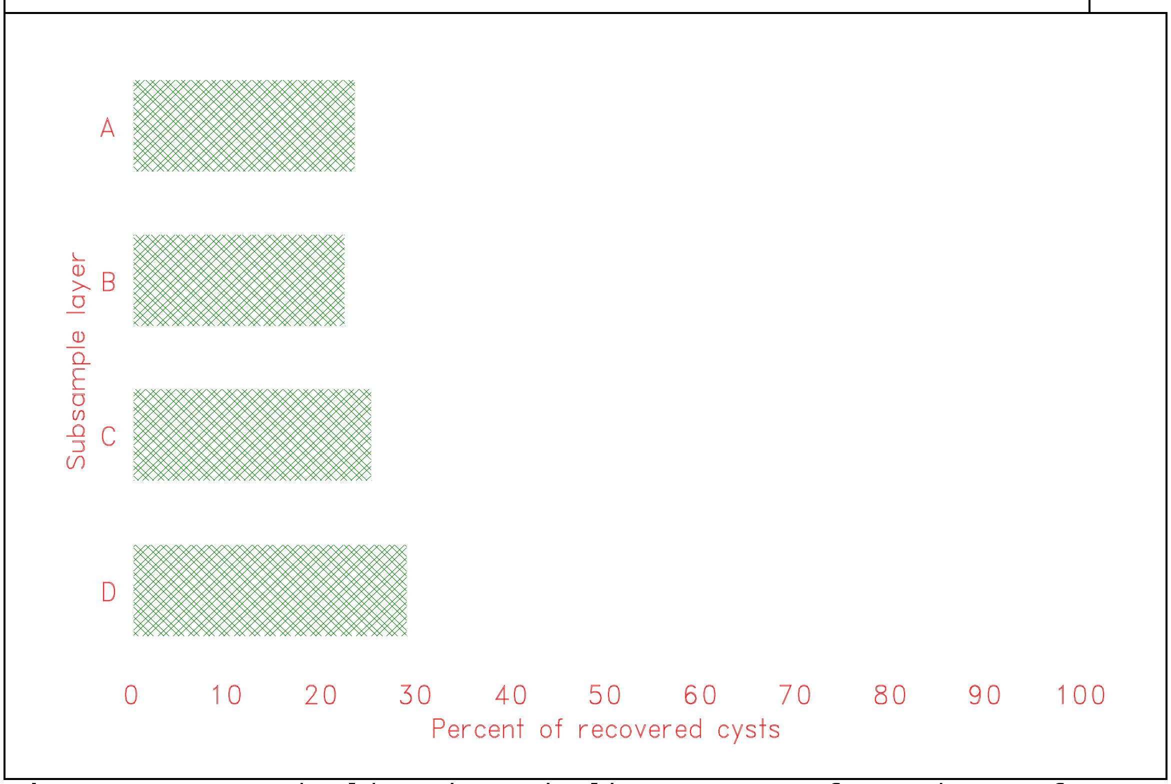


Figure 10. <2 week-old *G. intestinalis* CH3 cysts after 0 hours of sedimentation.

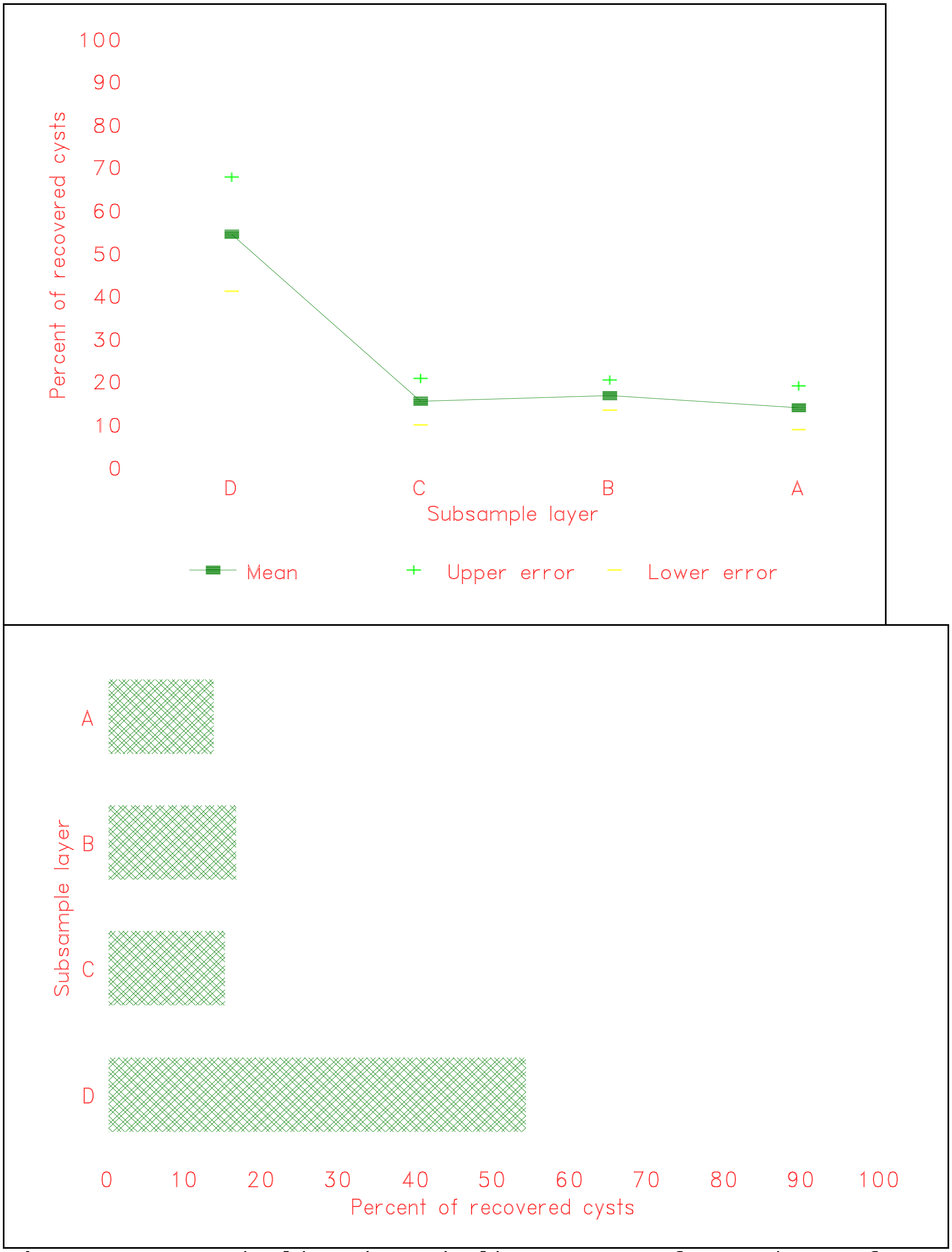
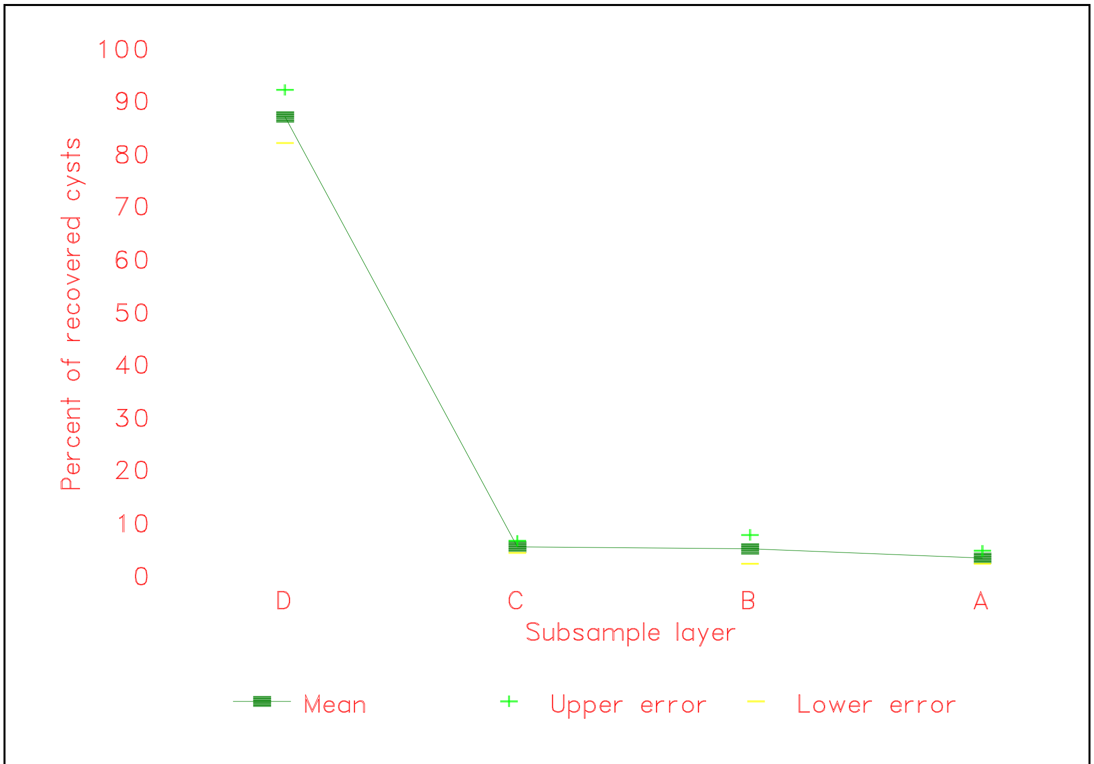


Figure 11. <2 week-old *G. intestinalis* CH3 cysts after 18 hours of sedimentation.



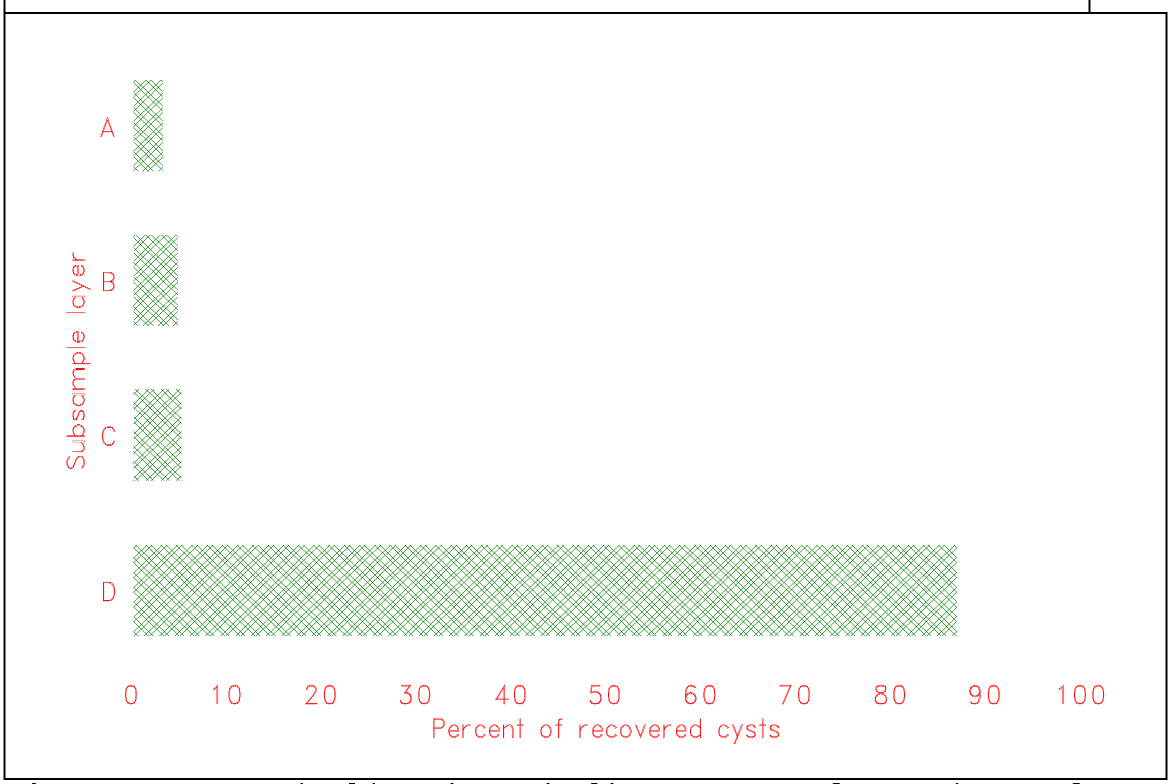


Figure 12. <2 week-old *G. intestinalis* CH3 cysts after 24 hours of sedimentation.

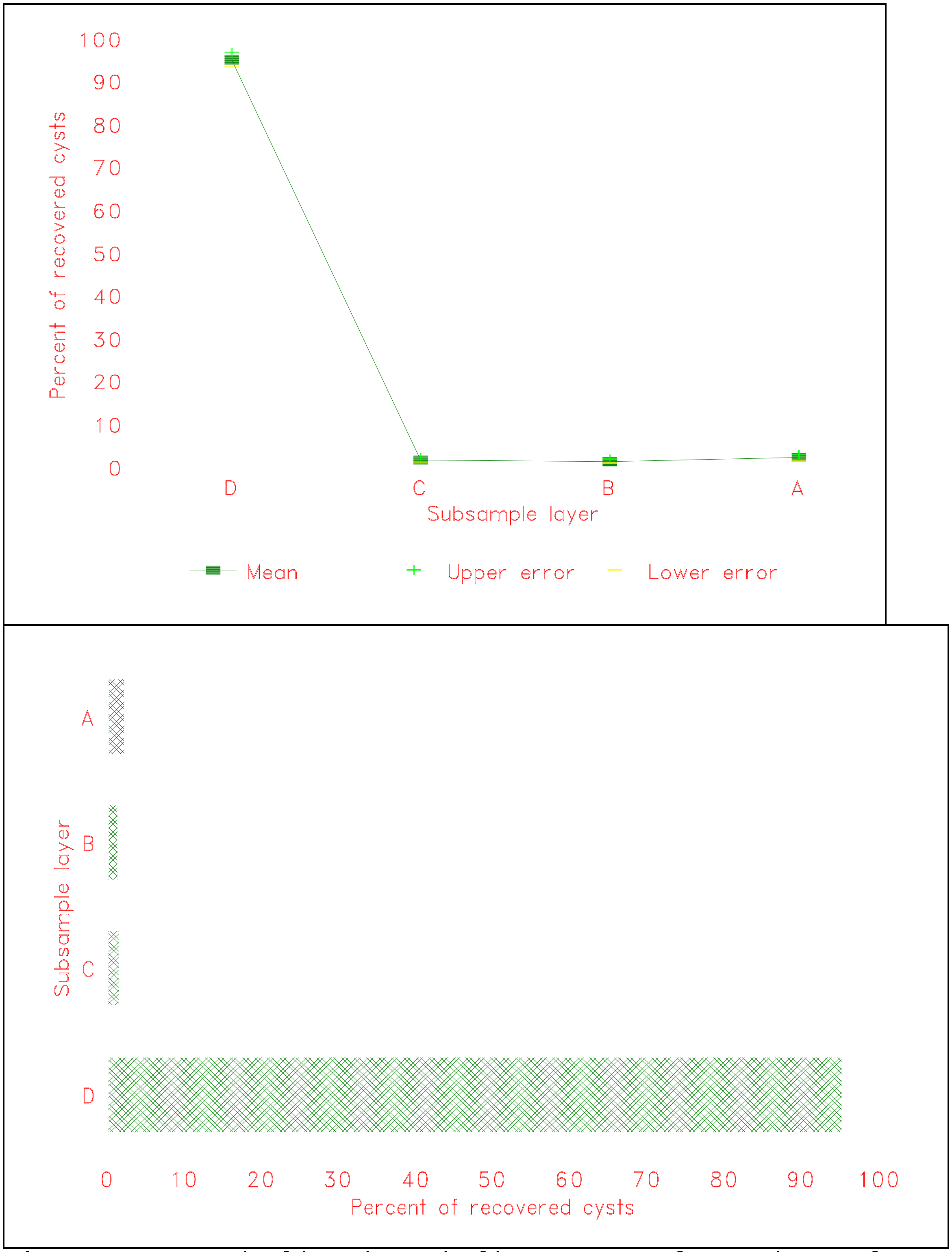


Figure 13. <2 week-old *G. intestinalis* CH3 cysts after 48 hours of sedimentation.

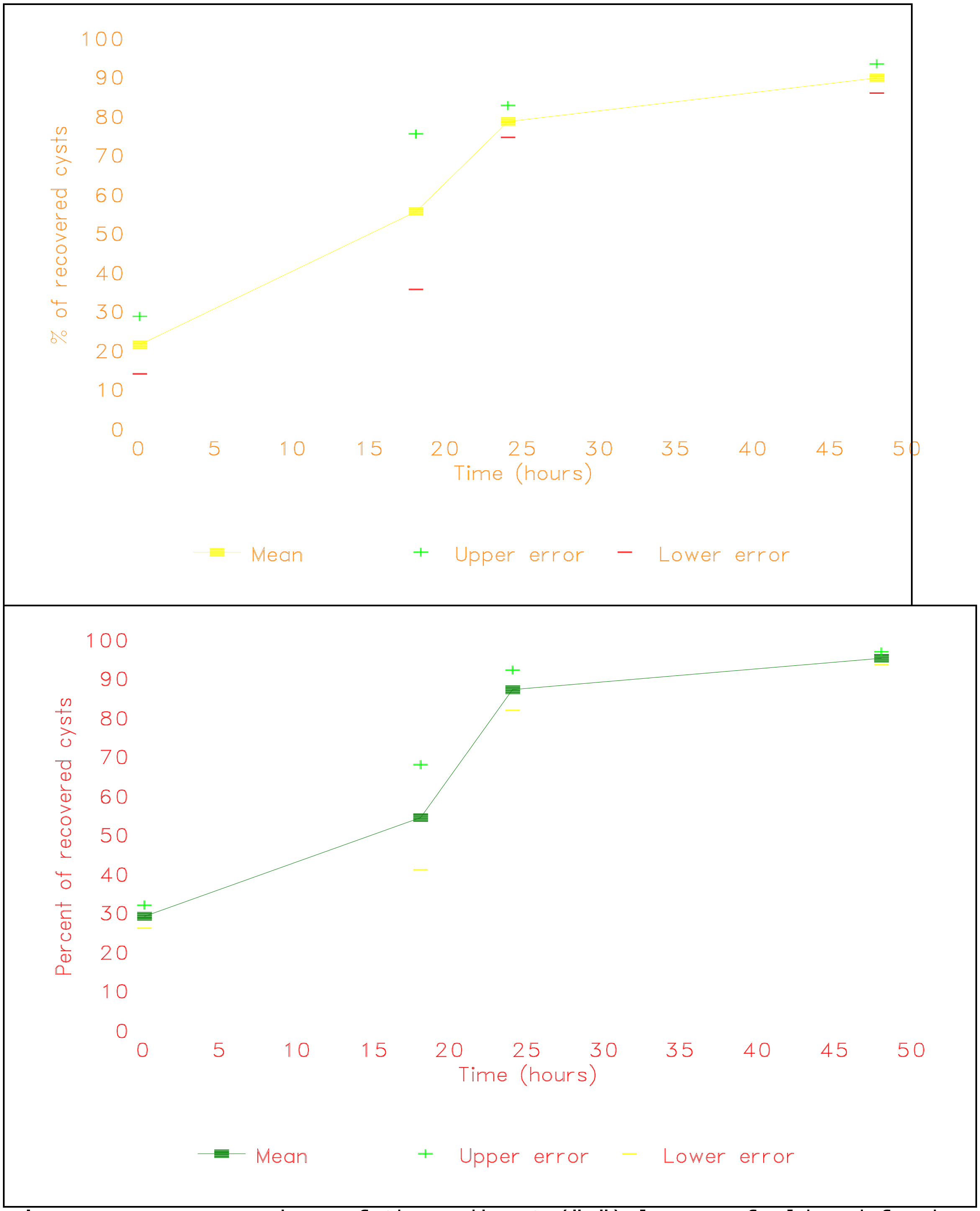


Figure 14. A comparison of the sediment ("D") layers of old and fresh cysts over time. There was no significant difference (alpha = 0.10) between the old cysts (top) and the fresh cysts (bottom).

Table V. Results of the resuspension and 48 hour sedimentation of 4-5 month-old *G. intestinalis* CH3 cysts.

Cyst Type	Layer	Trial 1 Cysts/mL	Trial 2 Cysts/mL	Trial 3 Cysts/mL	Mean % Cysts	Std. Dev.
floaters	A	3.0 (21%)	0.7 (11%)	0.1 (2%)	11.3	9.8
	B	5.0 (36%)	0.5 (8%)	<0.1 (1%)	14.7	18.5
	C	1.0 (7%)	0.7 (11%)	0.1 (2%)	6.6	4.4
	D	5.0 (36%)	4.7 (71%)	5.0 (95%)	67.4	29.9
sinkers	A	42 (2%)	9 (4%)	9 (3%)	2.8	0.9
	B	29 (1%)	2 (1%)	6 (2%)	1.4	0.7
	C	27 (1%)	11 (5%)	11 (4%)	3.1	1.8
	D	2260 (96%)	217 (91%)	264 (91%)	92.7	2.7

Table VI. Results of the resuspension and 48 hour sedimentation of <2 week-old *G. intestinalis* CH3 cysts. N/D = none detected.

Cyst Type	Layer	Trial 1 Cysts/mL	Trial 2 Cysts/mL	Trial 3 Cysts/mL	Mean % Cysts	Std. Dev.
floaters	A	0.06 (4%)	N/D (<1%)	0.03 (1%)	1.6	1.8
	B	0.04 (2%)	0.01 (21%)	0.01 (<1%)	7.8	11.1
	C	0.02 (1%)	0.01 (21%)	0.03 (1%)	7.7	11.2
	D	1.6 (93%)	0.02 (59%)	2.2 (97%)	82.9	21.0
sinkers	A	2 (<1%)	2 (2%)	1 (<1%)	0.7	0.7
	B	2 (<1%)	2 (2%)	<1 (<1%)	0.7	0.7
	C	<1 (<1%)	2 (2%)	<1 (<1%)	0.7	1.0
	D	420 (99%)	125 (95%)	173 (99%)	97.9	2.4

Table VII. Cyst recovery data for the resuspension and 48 hour sedimentation experiments. Initial cyst numbers were calculated from direct cyst counts immediately after resuspension; * indicates initial cyst numbers calculated from the cyst counts of the original subsample ("A" or "D" layer) before it was diluted to one liter. N/R = not recorded.

Age Group	Trial	Origin Layer	Initial Cysts	Recovered Cysts	% Recovery
Old	1	A	$4.9 \times 10^{4*}$	3.5×10^{3}	71
		D	$9.9 \times 10^{5*}$	5.9×10^{5}	60
	2	A	1.9×10^{3}	1.7×10^{3}	90
		D	7.5×10^{4}	6.0×10^{4}	80
	3	A	2.3×10^{3}	1.3×10^{3}	57
		D	3.3×10^{4}	7.3×10^{4}	220
Fresh	1	A	2.7×10^{2}	4.3×10^{2}	160
		D	7.7×10^{4}	1.1×10^{5}	140
	2	A	3.0×10^{1}	8.5×10^{0}	28
		D	5.8×10^{4}	3.3×10^{4}	57
	3	A	$9.0 \times 10^{2*}$	5.7×10^{2}	63
		D	$2.7 \times 10^{4*}$	4.4×10^{4}	160

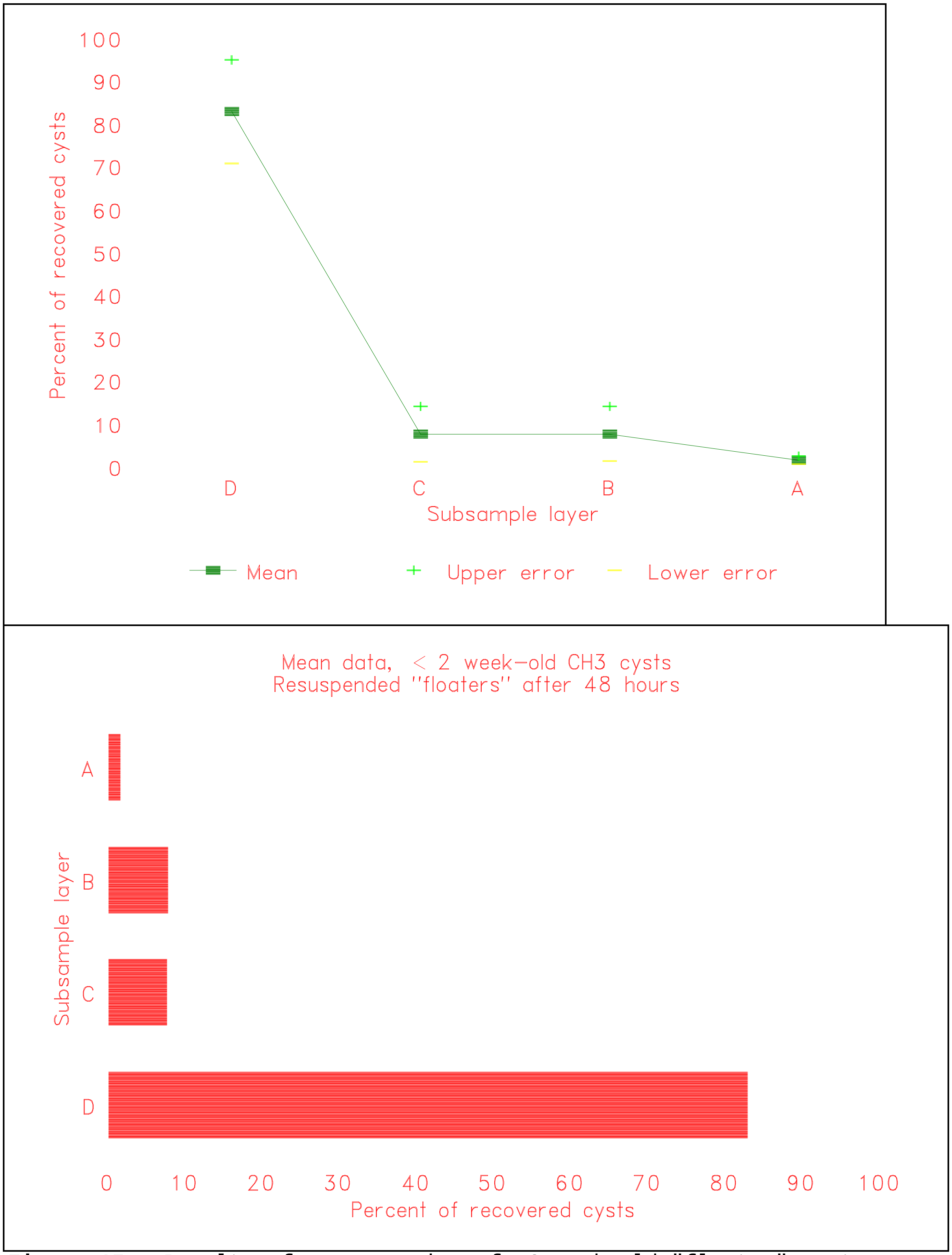
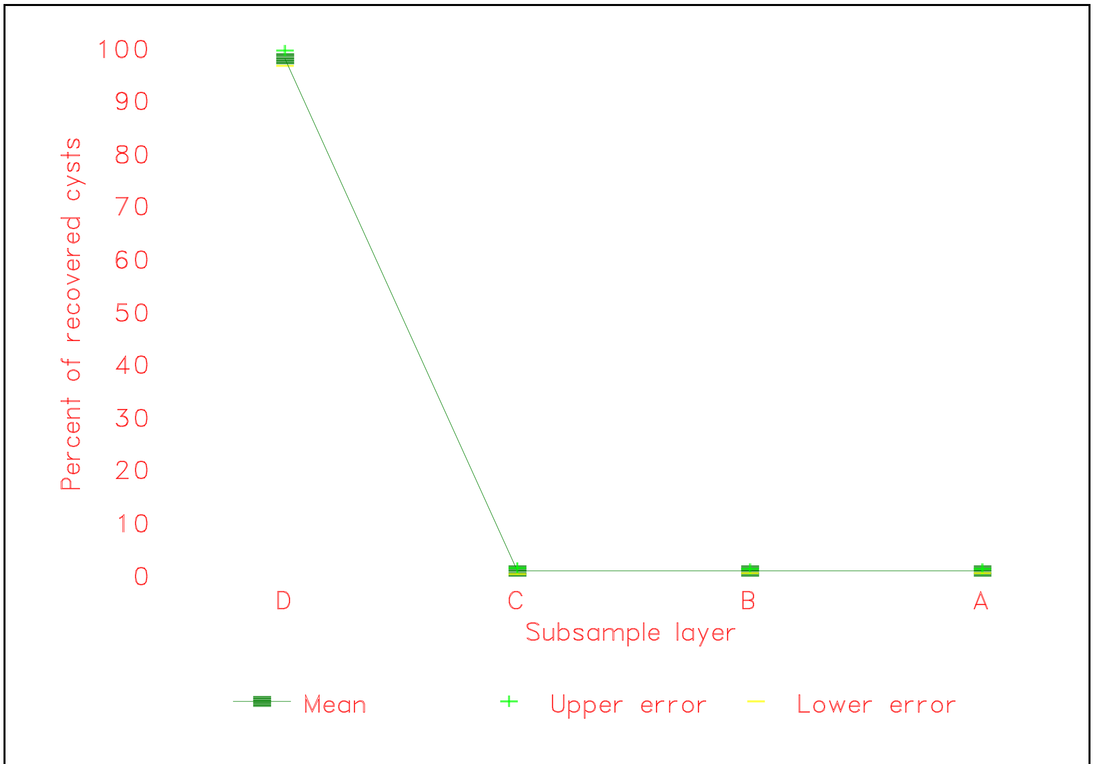


Figure 15. Results of resuspension of <2 week-old "floater" cysts followed by 48 hours of sedimentation.



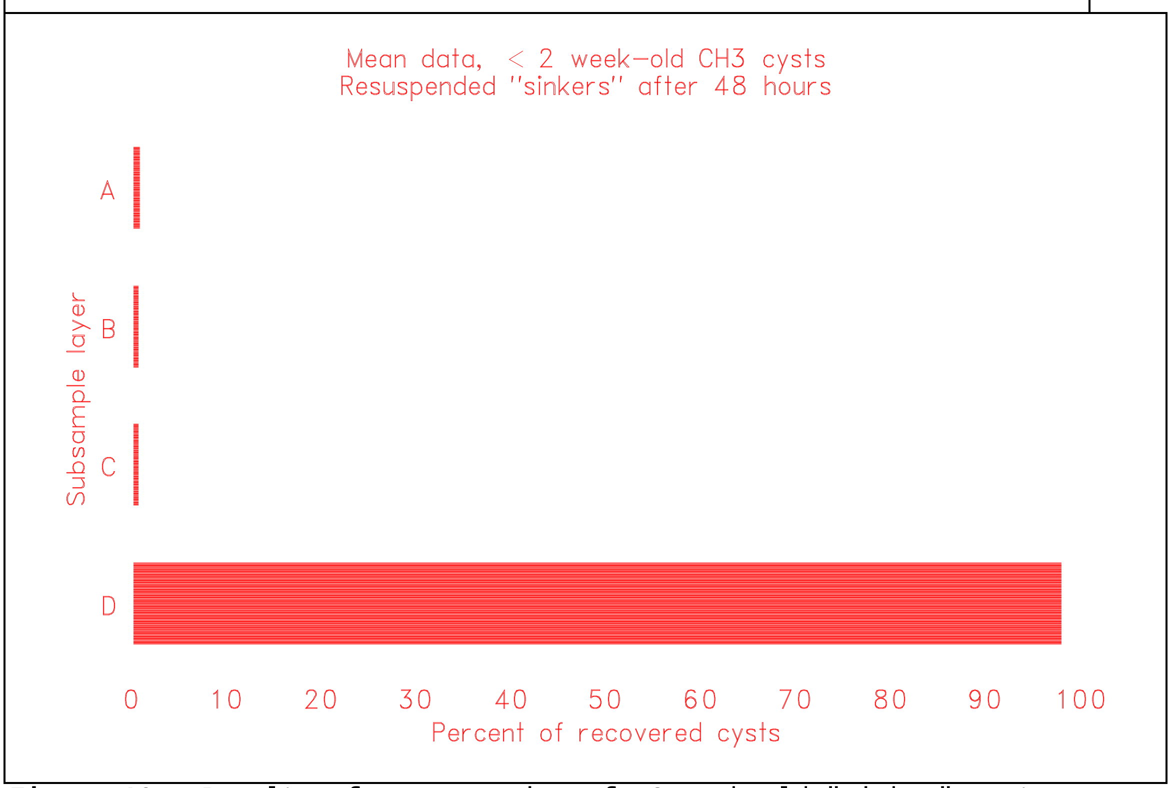
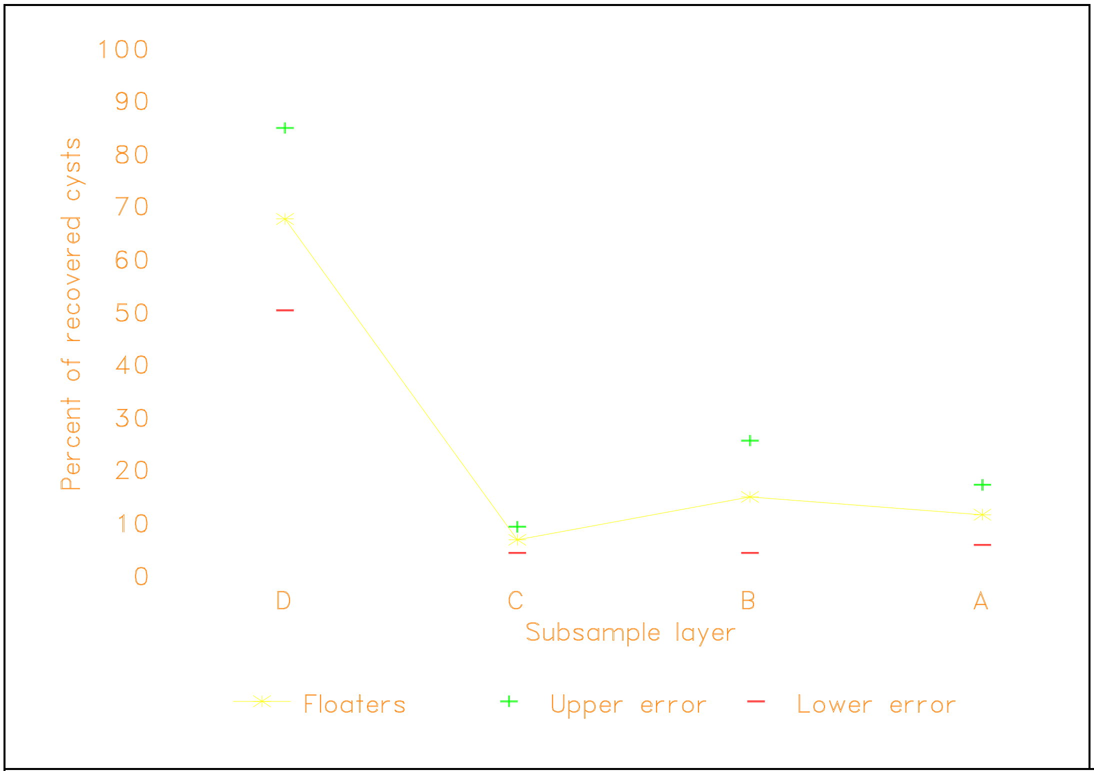


Figure 16. Results of resuspension of <2 week-old "sinker" cysts followed by sedimentation for 48 hours.



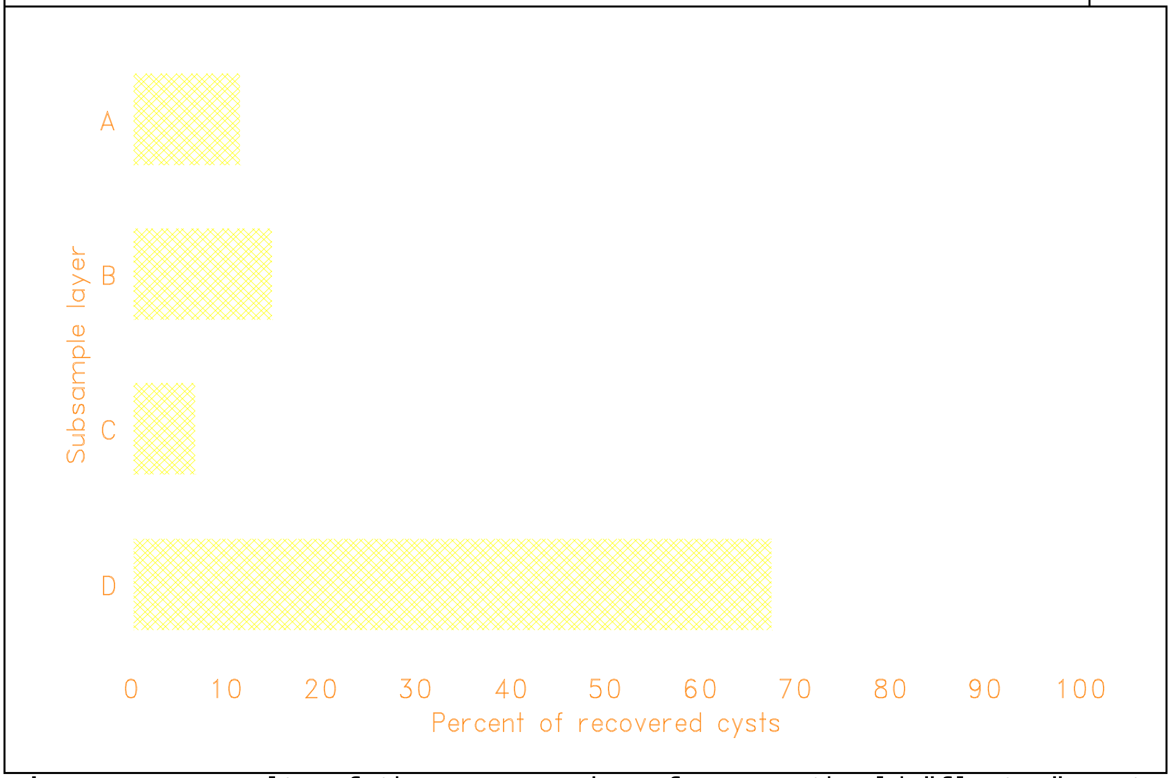


Figure 17. Results of the resuspension of 4-5 month-old "floater" cysts followed by 48 hours of sedimentation.

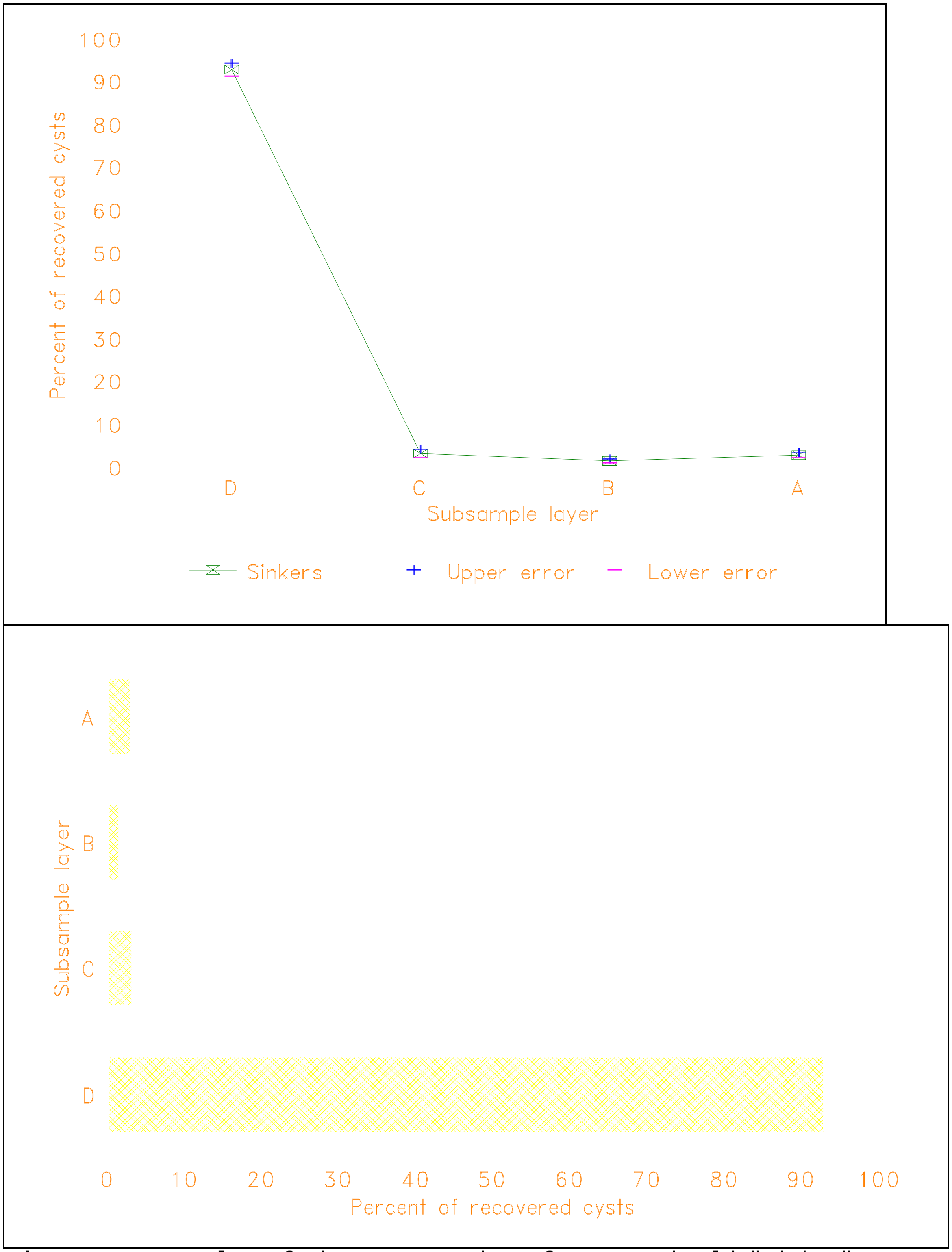


Figure 18. Results of the resuspension of 4-5 month-old "sinker" cysts followed by 48 hours of sedimentation.

The current standard method for the detection of *Giardia* cysts in water directs analysts to discard the supernatant after the overnight sedimentation period. The actual proportion of the supernatant to the entire sample is not specified. For this study, I assumed that the supernatant consisted of the upper 75% of the sedimentation column. If the experimental samples were actual drinking water samples, and the current standard method was used for analysis, approximately 45% of the cysts would have been discarded. Since the standard method does not specify a supernatant volume, it is quite likely that an even larger proportion of cysts could be discarded in the supernatant.

I have shown that a significant proportion of *G. intestinalis* CH3 cysts do not appear in the sediment within 18 hours. I have also shown that the sedimentation rate for a significant proportion of the cysts is much lower than one cm/hour. Since, in most of the trials, 48 hours were required for at least 90% of the cysts to accumulate in the sediment layer, the sedimentation rate may be as low as 0.35 cm/hour. Also, the fact that more than half of the "floater" cysts were found in the sediment layer after they were re-suspended indicates that the sedimentation rate is not always constant for a given population of cysts. Cyst age does not appear to have any significant effect on sedimentation.

Based on these findings, I recommend that the standard method for the detection of *Giardia* cysts in water be modified either to extend the sedimentation time to 48 hours or more, or to eliminate the sedimentation option in favor of centrifugation at 4300 x g.

Centrifugation at 4300 x g is faster, and it has a consistently high recovery rate of close to 97% with both fresh and aged cysts (Kramar, 1992). However, centrifugation does have one major drawback: large volumes of water must be centrifuged, thus a relatively large centrifuge is needed for efficient recovery of cysts. If sedimentation must be used, the supernatant, as well as the sediment, should be filtered and examined for cysts instead of being discarded.

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