Report 1

Report

Dissipation and Movement of Sonar, and Komeen Following Typical Applications for Control of *Egeria densa* in the Sacramento/San Joaquin Delta
and
Production and Viability of *E. densa* Fragments Following Mechanical Harvesting (1997/1998)

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I. Introduction.

A. Herbicides

There are only three active ingredients currently registered for control *of Egeria densa* in waters such as the Sacramento/San Joaquin Delta: Copper-based products (e.g. Komeen, Cutrine, copper sulfate), diquat (Reward), and fluridone (Sonar). Sonar, the only systemic herbicide among these, has been registered by EPA in more states for the past 10 years. It recently obtained California EPA registration (spring, 1997). Based upon testing in laboratory and outdoor cultures by ARS, *Egeria densa* can be controlled by long-term (4 to 6 week) exposures at 10 to 20 ppbw. The copper products and diquat are contact herbicides that generally require much shorter exposures with the egeria (e.g. a few hours).

Water movement in the Delta is complex due to effects of (1) diurnal tidal cycles; (2) seasonally variable net flows from snow melt; (3) highly variable bathymetric (physical bottom characteristics); and (4) presence of macrophyte populations. All these variables contribute to the flow-rates, channelizing effects (i.e. areas of higher flows adjacent to quiescent zones) and therefore the movement and dilution of herbicides applied to the water.

This study was aimed at determining if sufficient herbicide contact time/concentrations can be achieved in typical reaches and sloughs in the Delta where egeria is established. The protocol incorporates monitoring of fluridone and copper levels in the water to determine exposure. In addition, pre- and post-treatment copper levels in egeria tissues were determined. Komeen is a contact herbicide registered for use in California waters, and requires much shorter time than Sonar (ca. few hours vs. several weeks) at concentrations between 250 and 1,000 ppbw (o.25 and 1. 0 ppmw).

B. Dye studies

To characterize the water movement and estimate the residence time of herbicides in target sites, the fluorescent dye, Rhodamine WT, was injected and monitored closely at several sites deemed representative of different levels of water movement and water exchange. These studies helped determine what treatment frequency and formulation might be most likely to be effective, particularly for Sonar since it requires long-term contact time (several weeks). Although dyes such as Rhodamine WT are extremely useful in assessing water movement and dilution effects, they cannot be assumed to reflect precise dissipation and fate of a given aquatic herbicide. This is because each herbicide active ingredient is chemically unique and therefore will exhibit different reactions with the water constituents, target and non-target plants as well as light and temperature. However, by following dye movement and dilution, a good estimate can be made of where a herbicide may move and how fast it will be diluted. These studies also help determine destinations of floating egeria fragments.

C. Mechanical

Mechanical harvesting consists of physically cutting and removing some above-ground portion of the target plants and transferring the cut plants to an off-site point of disposal. Mechanical systems employ articulating cutter bars that can vary the cutting depth from the surface to ca. 8-10 feet. As a consequence of cutter and conveyance systems designs, fragments of the plants are produced and can escape collection and transport to the shore. Most fragments are produced during the initial cutting and collection operation; however, fragments are also lost during cutter-to-tender transport and upon off-loading of the tender to onshore trucks. This study focused on determining the numbers, sizes and viability of fragments produced and lost at the harvest site. Viable fragments are, in effect, dispersal agents since they float and are transported with water movement.

II. Herbicide Application Methods.

A. Sonar. Both pelleted (SRP) and liquid Sonar (4AS) were used. Liquid (4AS) formulation was applied via air-boat mounted booms from which weighted hoses allowed for the injection directly under water ca. 2ft. The SRP formulations was applied via an electric powered granular spreader mounted on the bow of an airboat. Applications were made twice per week (either Monday & Thursday or Tuesday and Friday) at each site. Application rates were used to produce nominal levels of ca. 20 ppbw for six weeks. Table 1 shows the amount of Sonar used at each site. Figures show the sites, sampling stations.

B. Komeen. Komeen (a liquid, organic-chelated copper product) was applied via the same air-boat mounted boom systems as Sonar. Application rates were adjusted to provide ca. 0.75 ppmw Cu based upon high-tide water volumes.

Injections were started at the incoming tide. Table 1 shows the amount of Komeen applied at each site.

C. Sites. Table 1 summarizes the treatment sites, herbicide applied and amount of each herbicide and frequency of applications. Site maps are shown in figures 35, 38, 41, 44, 47 and 50.

Table 1. Description of herbicide sites and herbicides applied.

Site:	Plot Size (acres)	Volume (ac.ft.)	Herbicide	Amount Applied	Frequency of Application	Target Concentration
White Slough			Komeen		Once	
6/15/98	4.13	41.3		102.5gal.		0.75ppm
8/4/98	4.13	41.3		95 gal.		0.75ppm
Seven Mile Slough			Komeen		Once	
6/17/98	3.44	30		105gal.		0.75ppm
8/5/98	3.44	30		95gal.		0.75
Sandmound Slough			Komeen		Once	
6/1998	5.0	50		170gal.		0.75
8/5/98	5.0	50		115gal.		0.75
Big Break Marina	8.0	64	Sonar 4AS	0.875gal per treatment (total=10.5 gal.)	Twice per week for 6 weeks	10 to 20 ppb
Supplemental treatment	1.3	13	SRP	13.3 lbs. per treatment	Twice per week for 3 weeks	10 to 20 ppb
Venice Isl.	10	50	SRP	54lb per treatment (Total=648 lbs)	Twice per week for 6 weeks	10 to 20 ppb
Franks Tract	8.25	74	SRP	80lb per treatment (total=960 lbs)	Twice per week for 6 weeks	10 to 20 ppb

III. Herbicide Monitoring and Characterization of Water Movements

A. Dye studies.
The water-soluble dye Rhodamine WT was applied at sites listed in Table in the same manner as Komeen and Sonar 4AS via airboat mounted boom to achieve initial concentrations of approximately 30 ppb. Immediately after applications, fluorescence was continuously monitored with a Turner Model 10 AU Field Fluorometer. A submersible pump provided continuous flow through the fluorometer cuvette. Fluorescence data was stored on-board the instrument and later down-loaded for presentation. Various transects were run through each application site, upstream and downstream according to tidal flows. In sloughs where flows diverged (e.g. White Slough), transects were continued in the direction of tidal flow to determine the extent of excursion into those diversions. Average fluorescence values were summarized over time to obtain the general "washout" rate for the sites. Also, by following the movement of the dye, probably routes of plant fragment dispersal could be determined. This may be important if mechanical harvesting or dredging operations were incorporated into the implementation plans for egeria management.

B. Copper (from Komeen).
Sampling stations were established to determine dissipation and movement of copper within and outside the treated areas. Anchored buoys provided fixed points at which samples were taken in duplicate at 20 cm. below the water surface and 20cm above the bottom. The sampling systems consisted of a battery powered, submersible pump (Rule 360 GPH, 12 VDC) that was mounted on a 3/4" pvc pipe marked in 20 cm intervals. The pump transferred water via tygon tubing to the boat where samples were collected in pre-labeled 20 ml, screw-capped glass vials. Vials were rinsed three times with water from the desired depth before collection. Pump effluent was flushed for ca. 1 minute between each depth to ensure that the collection represented the water form the desired depth. Sampling was conducted pre-application and at the following posttreatment times: 3h, 6h, 9h, 24h. Collected samples were returned to the AQWL for copper analysis. It should be noted that since the sample regime encompassed four tidal cycles, actual depths of the upper (surface) 20 cm varied. Thus at low tide, the collection depth for the 20-cm below surface sample may have been 0.8m depth, at high tide (ca. 6 hours later) the same sample may have been taken at 1.4m depth.

For analysis, each sample was acidified with 20 µl concentrated nitric acid, filtered through glass fiber filter (Whatman 13mm 1.0µm GF/B filter) and brought to standard volume. Copper levels were determined by atomic absorbance spectrometry (Thermo Jarrell Ash Smith-Hieftje 1000) using standard methods and calibrations with known Cu solutions.

Uptake of copper in egeria shoots. The level of copper accumulated in susceptible target weeds such as egeria can be related to the efficacy of control. To help assess the effectiveness of the Komeen applications, intact shoots of egeria were removed at each sampling station 24 h posttreatment. Three to five 5 cm tips were collected and returned to the AQWL for analysis. Some of the tips were rinsed 30sec in 0.01 N HCL, followed by a distilled water rinse before they were digested in 4 N nitric acid. The tips were oven dried (48 h at 70C), and extracted in 4 N nitric acid for 48 h at room temperature. Samples were filtered through glass fiber filters, brought to standard volume and analyzed via AA as described above for water samples.

C. Fluridone (Sonar).

The sampling systems for fluridone was identical to that described for copper. However, sampling intervals were weekly for 4 to 5 weeks depending upon the site, and sampling depths were at mid-depth and 10cm from the bottom for the SRP formulations. Sampling depths for the 4AS applications were the same as for copper (20cm above the bottom and 20 cm below the water surface). After collecting samples, vials were placed on ice in coolers and returned to the laboratory where they were refrigerated at ca. 4C.

For analysis, samples were shipped to SePro Corp. where the level of fluridone was determined with their proprietary immunoassay (FasTest) system. The minimum detection is 0.5 ppb for most water samples.

IV. Egeria fragment production and viability

A. Collection of fragments. Floating fragments of *E. densa* were collected pre- and post-harvest with nets having a mesh of 0.25 in. and an opening of 13 in. by 13 in. and 17in. by 15 in. openings, attached to a four ft. handle. While the collection boat was positioned 300 to 500 ft. downstream of the mechanical harvester, all fragments within reach of one net on each side of the boat were retrieved and placed in plastic bags. Collections were made along linear transects and the fragments were composited in bags representing from 15min to 30 min collections intervals. Collections continued until the harvest was completed. From these collections, the total numbers of fragments, size distribution and weights were determined after the samples had been brought to the AQWL facility.

B. Fragment viability. After fragments had been counted and sizes determined, their ability to produce new lateral shoots and roots was assessed by placing subsamples in 1 liter glass vessels containing water taken from the collection sites and maintaining in a growth chamber for four weeks at 24C under 200 μmol /m2/sec cool-white fluorescent light on an LD 14:10 schedule. To determine effects of fragment lengths on initiation of new growth, three size classes were either assayed together or in separate containers (replicates) for each size class: 9cm, 16cm and 23cm. All assays were replicated five times.

Thus, in one set of bioassays, a single replicate vessel contained three fragments of a single size; in a separate assay, each replicate contained all three size classes. The purpose for this was to determine if fragments of different lengths had any affect on root and lateral shoot initiation when maintained in the same medium.

In other bioassays, fragments were place on top of sediments collected from the harvest sites and maintained in outdoor cultures with well water. The fragments were held on the surface of the sediments by plastic mesh netting (l0.25 in mesh) so that they were in contact with the sediment, but not inserted into the sediments. Water level was maintained a few cm above the surface of the sediments. This configuration simulated the lodging of free-floating fragments on shoreline areas in the Delta. With all bioassays, original fragment length was recorded as well as number and lengths of any new lateral shoots and adventitious roots 37 to 55 days after starting the assays.

V. Results.

A. Rhodamine WT Dye Studies. Table two summarizes the sites and approximate dilution of the dye.

Table 2. Rhodamine WT Sites and Estimated Half-life and Wash-out Times.

Site	Date	Half-life (hours)	Wash-out (hours)
White Slough	6/1/98	8	35
Owl Harbor	6/3/98	2-4	12-14
Sandmound slough	6/5/98	18-20	30-35
Franks Tract	6/4/98	6-7	30-32
Big Break Marina	6/9/98	20-24	40-45
Venice Island	6/11/98	8-10	15-20
Pixley Slough	5/5/98	20 to 24	90

From Table 2, it is clear that the longest residence time based upon dye dilution occurs in Big Break Marina, Sandmound Slough and Pixley Slough. The half-life in the other sites represent less than two tidal cycles and indicate that only rapidly acting herbicides would have the potential for use if a one-time application were made. Secondly, these sites represent a wide range of estimated wash-out times, i.e. estimated time required for a complete dilution and/or off-site removal of a water-soluble herbicide. Although these sites differ somewhat in depths and widths, the primary feature affecting wash-out would appear to be a combination of tidal exchange and net flow-through. For example, Big Break Marina is a "dead end" appendix adjacent to the San Joaquin River at Antioch. Although tidal exchange occurs, complete dilution takes about two days. Pixley Slough, though not a complete dead end systems, has the unique feature of a mid-channel through which water actually moves both out of Pixley Slough and into Pixley Slough. Therefore, soluble materials do not become diluted as much on each tidal cycle; rather there is a temporary off-site transport followed by a return of the same water to the mid-channel of the slough. This was clear from detection of Rhodamine WT levels than decreased and later increased at reverse flows at the point where water exited Pixley Slough proper and entered the side channel.

The other sites having rapid wash out rates are characterized by having generally unimpeded flows and in-filtration of new "flood-tide" waters on each incoming cycle. Venice Island and Franks Track exhibited the most rapid and complete wash-outs of all the sites. These areas are extremely open with no "back-water" impediments (i.e. end-levees or dams or other constrictions).

The dye studies have shown that the test sites used for initial herbicide trials represent a large range of tidal exchange and wash out rates. These sits also are typical of those infested with problematic populations of *Egeria densa*.

Figures 1 to 7 provide details of dye concentrations over time for the several sampling areas (transects) run at each site. Note that the upper panel for each figure ("A") represents the mean of fluorescence levels at the times indicated. These are quite variable since multiple flow-through samples were taken at different depths at a given sample time. However, the overall trend for each site is clearly seen. The lower panel for each figure ("B) shows the fluorescence values for the different positions within and adjacent to the area initially injected with dye. From these data, it is clear that tidal flows moved dye in both directions from the original application sites as one would expect. For example, in Owl Harbor, dye was confined to the applications site during the first 30 to 60 minutes; however, by five hours after application, the dye had not only been diluted, it had also moved ca. 500 feet away from the injection site (Fig. 2B).

B. Fragment production.

Table 3 summarizes the fragment collection sites, times, total fragments collected and fresh weights. Prior to harvesting, the number of fragments ranged from none to several hundred depending upon the site and date of harvest. However, collection periods during harvest operations resulted in up to over 18,000 for a 4 hour period at White Slough. When fragment collection was normalized for duration of collection, the mean "baseline" (i.e. pre-harvest) was 8.6 per minute compared to 53.5 per minute during harvest operations, or over a six-fold increase per unit time. Therefore, with the "collection zone" around the boat, the average production exceeded 3,000 fragments per hour. Since there was no feasible method to collect all fragments generated by the harvest operations, these numbers are an estimate of the relative increase in fragment production caused by the operation of the harvester at the harvest site. Based upon the scale of actual collection zone around the boat compared to the harvest-swath, total fragment generation could easily exceed 30,000 per hour of harvest operation.

Table 3 A, B, C *Egeria densa* fragment collections from three sites in the Sacramento-San Joaquin Delta following mechanical harvesting operations.

3A:Sandmound Slough

Date:	Start of Harvest	Duration of collections (minutes)	Length (ft) of collect-ion zone	Number fragments	Frags. per min.	Fresh wt(kg)
10/21/97 (pre)	NA	NA	NA	NA	NA	NA
10/21/97	0830	180	300	10,444	58	44.49
5/12/98 (pre)	0730	60	stationary (17 ft.)	136	2.3	0.60
5/12/98	0945	180	stationary (17ft.)	3972	32	13.89
7/28/98(pre)	0745	60	200	119	2.0	3.07
7/28/98	0910	60	200	5685	94.7	30.23

3B: Owl Harbor

Date:	Start of Harvest	Duration of collections (minutes)	Length (ft) of collect-ion zone	Number fragmens	Frags. per min.	Fresh wt(kg)
10/23/97(pre)	0730	25	300	353	14.1	2.98
10/23/97	0855	190	300	16,813	88.5	45.36
5/13/98(pre)		30	stationary (17ft.)	724	24.1	2.63
5/13/98	1100	65	stationary (17ft.)	1323	20.3	4.21
7/29/98(pre)	0740	15	300	1	0.07	0.002
7/29/98	0820	30	300	991	33	5.18

3C: **White Slough**

Date:	Start of Harvest	Duration of collections (minutes)	Length (ft) of collection zone	Number fragments	Frags. per min.	Fresh wt(kg)
10/24/97(pre)	0830	7	300	117	16.7	3.01
10/24/97	0900	240	300	18,791	78.3	50.36
5/11/98(pre)	1432		200	0	0	0
5/14/98	0840	112	stationary (17ft.)	4826	43.1	13.22
7/30/98(pre)	0730	30	300	295	9.8	0.44
7/30/98	0810	45	300	1517	33.7	8.52

Figure 8 provides a summary of the size distribution of all fragments collected at all sites at the three harvest dates. Although there are some differences in the total number of fragments, at most sites, fragments from 1 to 20 cm in length comprised 70 to 80 % of the total fragments produced. Also, harvest in October of 1997 resulted in the largest number and greatest diversity (range) in size classes. In particular, the Owl Harbor harvest on 10/23/97 produced a large number of fragments exceeding 1 meter in length. The high fragment biomass (Table 3) associated with the fall harvests probably reflects the typical late-summer/fall maximum standing crop often observed in rooted macrophytes.

C. Fragment Viability.

The large proportion of fragments in the range from a few to 20 cm prompted the use of the three size classes selected for viability bioassays (9, 16 and 23 cm). Results from the fall, 1997 harvest are shown in figures 9-12. New lateral shoots were produced on all fragments regardless of initial size or harvested site, and most new lateral shoots attained lengths of from 2 to 4 cm within 21 days of their starting in the growth chamber (Figs. 9, 10). Likewise, cut fragments generated many new adventitious roots and during the same period. However, it appeared that when the different size-classes of fragments were cultured together (i.e. in the same vessel), overall root production was significantly lower compared to fragments grown only with same-size classes (Fig. 12). Two potential explanations for these difference are (1) presence of the larger fragments led to more rapid uptake of available nutrients and limited light within the vessel; and (2) indirect effects of "leaked" plant growth regulators from the larger fragments may have inhibited root production. At this time, these or other causes cannot be clearly identified.

The spring (May) harvest results are shown in figures 13 to 16 for fragments cultured in the growth chamber. As with the fall samples, fragments produced numerous lateral shoots and adventitious roots. However, it is clear that there were fewer lateral shoots and roots on these fragments at the time they were cultured compared to the fragments started from the fall harvest (e.g. compare figures 11 and 14). Average lengths of laterals at the end of the 45 day incubation were similar to those observed for the fall cuttings. Only the fragments from Owl Harbor exhibited reduced root production in fragments that were cultured with all size classes present (Fig. 15), although there appeared to be fewer lateral shoots formed in the Owl Harbor fragments as well (Fig. 13, Compare "separate" vs. "together" cultures).

Both outdoor and growth chamber systems were used to assess regrowth of fragments from the July 1998 harvests (Figs. 17-22 and Figs. 23-28, respectively). Figures 17 and 18 show that the original fragments' fresh weight and length either remained unchanged or actually declined over the 37 day culture period. All fragments produced some new lateral shoots regardless of initial starting length, though the new production was lower than observed for either of the other harvest times. It is also clear that in general, fragments collected at the July harvest had more existing lateral shoots than those collected from the May harvest. Adventitious roots were produced in all size classes cultured.

The lengths of initial fragments from the July harvest cultured in the growth chamber did not change significantly during the culture period (Figs. 23, 24). However, fresh weights did increase slightly from about 2 g to 4-8 g. Generally, the fragments that were in the largest size classes increased the most over the observation period (Figs. 25, 26). New lateral shoot production (i.e. numbers of new shoots formed) increased with time at about the same rate regardless of initial shoot length or whether the fragment size classes were cultured together or separately (Figs. 27, 28). However, the lengths of new lateral shoots were much more variable when the different size classes were cultured together compared to those cultured in separate vessels (Figs. 29, 30). For roots, both frequency of production (numbers) and lengths were much more variable in the combined size-class cultures than in same-size class cultures (Figs. 31-34). These differences also suggest that plant growth regulators may have affected root initiation in the combined cultures. However, regardless of these interesting effects, all size classes from harvest sites formed abundant new roots which elongated dramatically during the observation period. In fact, all size classes of fragments were able to initiate both new lateral shoots as well as roots from all sites and at all harvest times sampled from spring to mid-summer to fall.

D. Herbicide Applications

1. Sonar

Big Break Marina is depicted in Figure 35 which shows that this site does not have a "flow-through" capacity. It is actually a kind of enlarged "appendix" from the San Joaquin River that is subject to tidal rise and fall and therefore partial exchange. As the dye dissipation study showed, once would expect a two-fold dilution of dissolved herbicide to occur in about two days (Table 2). By applying Sonar twice-weekly, the intent was to maintain a relatively constant exposure of egeria to fluridone during a 6 week period. Figure 36 shows that, although there was considerable variation between stations, levels were ca. 15 to 20 ppb for most of the period. Also, there appeared to be sufficient vertical mixing to result in relatively uniform concentrations between mid-water samples and those taken near the bottom. Between day 7 and 14 there was a decrease to ca. 7 to 10 ppb. These levels resulted in typical fluridone-symptoms: chlorosis of new shoot tips within in the treated site starting ca. 10 to 14 DAT.

After the 6 week application was finished, we noticed that some newly emerging shoots (mostly on the bottom) showed no symptoms by 50 DAT. Therefore, a second round of twice-weekly applications were made for three weeks to a part of the marina and monitoring was continued to 77DAT. However, the data (Fig. 37) show that these final treatment did not result in adequate levels of fluridone since the highest concentration achieved was ca. 0.5 ppb. Generally, at least 10 ppb exposure is required for responses in egeria. The lower levels probably resulted from the higher proportion of water exchange relative to the total volume of treated water compared to the first 6 weeks of applications. In addition, the second round of applications were with the pelleted SRP formulation, which sinks to the bottom and often does not result in measurable levels in the water column. The first application was to 8 acres; the second application was to only 1.3 acres.

Franks Tract (Figure 38) represents a relatively high-flow site where dilution rates are expected to be high (Table 2). However, the site chosen has a partially protected aspect due to low-lying, vegetated islands. The SRP formulation was used for 6 weeks. Figure 39 shows that within the treated site, fluridone levels were generally higher in the bottom-layer samples, ranging from ca. 1 ppb to 10 ppb for four weeks (see Fig. 40). These data also show that essentially no fluridone was detected in the stations 600 to 900 ft. outside the treated plot (i.e. stations 4 and 5 on Fig. 38). Within three weeks after the start of applications, symptoms (chlorosis) were observed sporadically within the treated plot. There appeared to be areas within the treated site where plants were little affected by Sonar in close proximity to patches were obvious symptoms occurred. This suggests that the combination of high water-volume exchange and flows and non-uniform granular distribution may have led to zones or areas where Sonar was not at sufficient concentration long enough to affect the plants.

Venice Island (Fig. 41) is probably in the most open, tidally-exposed area of all the sites used for Sonar. It not only is subject to typical changes in tidal elevations of 1 to 3 feet, it is also immediately adjacent to the rapidly flows of the San Joaquin River and has no "dead end" levee protection that could decrease net-flows. Dye studies also showed that flows generally go from the southeast corner of the site to the northwest corner and that complete wash out takes on three to 4 tidal cycles (i.e. less that 24 h). The SRP formulation was applied twice-weekly and resulted in fluridone concentrations of from 1 to 2 ppb in the mid-water samples and from 2 to 4 ppb in samples taken ca. 10 cm from the bottom (Figs. 42, 43). Within three weeks a few plants exhibited symptoms of fluridone along the northwest edge (i.e. the most prevalent "downstream" side of the plot), but these symptoms were non-uniform and did not persist. From the samples analyzed, it would appear that the application rate would have to be increased two-fold or more to achieve concentrations of at least 10 ppb.

Of the three sites treated with Sonar, only Big Break Marina had levels of fluridone sufficient to provide control of egeria based upon laboratory studies of exposure time and concentrations. It should be noted that Big Break Marina was treated primarily with the 4AS formulation, a liquid, that most readily disperses throughout the water column. It is also the site with the least dilution rate based upon the dye studies.

2. Copper (Komeen) Movement and Dissipation.

Sandmound Slough was treated on 6/19/98 and on 8/5/98; water sampling stations are shown in Figure 44. This site is particularly interesting since it contains a small tidal-gate dam separating it from Rock Slough. The dam has a tidal-flap that opens on the out-going tide and allows water from Rock Slough to pass into Sandmound Slough. Conversely, on the incoming tide, the gate closes in effect forming a dead-end basin that gradually fills with tidal flow water. Both applications were made on the incoming tide so that water pooled in the slough. Stations 2,3 and 4 were within the area treated with Komeen. From Figure 45, it is apparent that highest levels of Cu (from 0.5 to ca. 2 ppm) were observed at the three stations inside the site in the upper water column. Bottom water samples did not exceed ca. 0.2 ppm. However, all three stations located outside the immediate site had from none to 0.5ppmw Cu between 6 and 9 h posttreatment. Thus, Cu moved with the tidal flows up to 1000 feet from the treated plot, though levels were far below the maxima reached within the site. By 24 h posttreatment, Cu had declined to near-baseline levels at all stations and at both the upper and bottom water levels.

For the August applications, less Komeen was applied (see Table 2) and this is reflected in the lower maxima seen at station #4 within the site: ca. 1.0 ppm compared to ca. 2.0 ppm for the June applications (Fig. 46). Within 9 h posttreatment, Cu levels had fallen to ca. 0.1 ppmw, and gradually declined to near-baseline levels 24 h posttreatment. The August application resulted in more uniform vertical distribution of Cu compared to the June application.

Owl Harbor (Seven Mile Slough) sampling stations are shown in Figure 47. This site was 1000 ft. long and has a rapid wash-out rate (i.e. less that 14 h) due to high tidal exchange and relatively unimpeded flows. As with Sandmound Slough, stations within the site had the highest Cu levels ranging from 0.5 to 1.5 ppm between 3 and 6 h posttreaatment (Fig. 48). Off-site movement, on the incoming tide toward station #6 is apparent from the increase in Cu levels from baseline to ca. 0.5 ppm 9 h posttreatment. However, as with Sandmound Slough, all stations had baseline, or near-baseline Cu levels at the 24 h posttreatment sampling time. A similar pattern in Cu distribution and dissipation was observed for the August applications at this site (Fig. 49).

The third site, White Slough, shown in Figure 50, has an outlet at the east end that is subject to incursion of incoming tidal flows from the main slough. Dye studies showed that incoming water enters and goes north in this narrow channel. Therefore, a sampling station was established ca. 1000 ft. from the main slough (see station #1 for the June application and station #6 for the August applications, Fig. 50).

Stations 2,3 and 4 were located in the treated site for the June applications and exhibited the highest Cu levels (ca. 0.4 to 1.0 ppm) 3 to 6 h posttreatment (Fig. 51). By 9 h posttreatment, most stations had Cu levels near baseline. As in the other sites exposed to Komeen, Cu moved off-site for up to 1000 ft. (e.g. stations 1 and 6). As predicted from the dye movement Cu was found in the narrow channel (station #1) at ca. 0.5 ppm at the 6 h sampling time. By 24 h, all stations except numbers 3 and 4 had baseline levels of Cu.

The August applications in White Slough resulted in essentially the same pattern of Cu movement and dissipation (Fig. 52).

Generally, for all sites, the greatest difference in Cu levels between the top and bottom of the water column occurred within the treated site in the first 6 hours after applications. Thereafter, and at the off-site stations, the Cu distribution was more uniform vertically. This is most likely caused by the relatively poor mixing of water on the slack and first tidal flows after applications and because the injections at made into to the upper 12 to 24 in. of water. By the time the second tidal flow in under way (e.g. 9 h posttreatment), the water column has sufficiently exchanged to mix the Cu and create a fairly uniform distribution.

3. Uptake of Copper in Egeria.

As part of the June and August sampling protocols, intact apical tips of egeria were removed at the stations used for water sampling 24 h posttreatment as well as prior to Komeen applications. In order to estimate how much copper was associated with simply surface adsorption (as opposed to absorption into the egeria tissue), samples were split into two groups. One subsample was digested per above-described methods without rinsing; the other subsample was subjected to a 30 sec rinse in 0.2N HCL. The brief acid-rinse removes copper that has become adsorbed to surface carbonates and other charged materials as well as silt on the egeria leaf surface. For this reason, plants not subjected to the rinse may have higher apparent Cu levels since the sample contains not only copper taken up into the tissue, but also copper that was adsorbed to the surface of the plant.

Results of the June applications are presented in Figure 53, which shows that plants from most stations had ca. 350 to 400 ppm Cu 24 h posttreatment. The data also show that many of the un-rinsed plants had high Cu levels than those given the HCL rinse. Perhaps as much as 25 to 50% of the Cu associated with the plants was actually adsorbed to the surface materials. These data also show that the plants located at stations outside the immediate treatment site took up copper to about the same levels as plants in the site.

The copper uptake in egeria from the August applications showed a similar pattern, but several samples had much higher Cu levels than plants from the June application (Fig. 54). The data from the water samples indicate that the exposure to Cu was slightly higher in some stations during the June applications, but it appears that other factors may have caused the higher uptake in the August treatments. The higher uptake may be primarily due to a combination of better growing conditions at this time (e.g. warmer water, longer days providing more light).

From previous laboratory and field studies, we have established that tissue levels of Cu from about 250 to 400 ppmw result in defoliation and necrosis. Most plants exhibited Cu levels above 200 ppm, with some nearly up to 2,000 ppm (e.g. Fig. 54, Owl Harbor station #5). Therefore, Komeen as applied in these sites would be expected to result in excellent efficacy not only directly in the sites treated, but also in adjacent areas from 500 to 1000 ft. away.

E. Water Quality Assessments.

Datasondes placed in some of the mechanical-harvest and herbicide-treatment sites were used to assess immediate effects of these activities on temperature, dissolved oxygen, pH and conductivity. Results for the harvests are shown in Figures 55-57 for the fall, 1997. None of these variables changed significantly post-harvest at any of the sites. Temperature was extremely uniform at ca. 17C in Sandmound Slough and Owl Harbor, and ca. 15C at White Slough. DO and pH showed very little change (ca. 7 -8 ppm DO; pH 7 to 7.5).

The Komeen application at Owl Harbor in June had no effect on pH or conductivity, and no apparent affect on DO or temperature (Fig. 58). DO fluctuated between 4.5 and 6 and showed a typical late-afternoon maximum. Temperature varied between 22 and 25.5 C also showing a late-afternoon maximum.

Figures 59-61 show the same water quality variables recorded at the Diquat sites in June. No appreciable changes occurred with the exception of a drop in pH and slight rise in DO at White Slough from ca. midnight through the next morning (Fig. 59).

Summary and Conclusions:

1. Dye Studies. The dissipation and movement of Rhodamine WT provided a good approximation of specific tidal-flow directions and approximate dilution rates in the sites used. The dye also provided a basis for establishing fixed sampling stations to monitor the herbicides used. This is important because the physical and bathymetic characteristics of the sloughs and lesser channels do not always suggest the actual magnitude nor directions of water flows on each tidal cycle. Finally, since dye movement also can help predict movement of materials such as plant fragments, use of the dye may provide important information on the probability of dispersal patterns following harvesting and cutting operations. It is clear from the studies on fragment viability that there is a high probability for dispersal of egeria during and immediately following harvesting operations.

2. Fragment Production and Viability. Most fragments produced by the harvest operations used here were short (<20 cm) and therefore capable of drifting into uninfested areas, small marinas, coves and sheltered bends in the Delta. Nearly 100% of collected fragments were capable of producing numerous lateral shoots and roots whether maintained in strictly aqueous conditions (Delta water with no sediment), or lodged on top of Delta sediments in outdoor cultures. There were no significant differences in ability of fragments to re-grow regardless of fragment sizes ranging from 9 to 23 cm, which comprise the bulk of sizes collected after harvest operations. Fragments re-grew equally well in spring, mid or late summer.

3. Herbicide Dissipation.

a. Sonar. Fluridone, the active ingredient in Sonar, persisted only as long as repeated applications were continued. The high dilution rates of most sites therefore will necessitate using frequent, split applications whether the pelleted or liquid formulation is used. The only site showing significant, long-term symptoms was Big Break Marina, a site that had the lowest wash-out rate. From the samples in the other sites, it is clear that higher rates will be needed to compensate for high wash-out rates, and that a more uniform application of pelleted material is needed. The current product is a 5% loaded formulation. If a 1% loaded formulation were available, a much more uniform distribution of the active ingredient could be obtained (roughly a five-fold increase in particle per unit area). These applications were made in June, July and August. However, it is clear from the mode of action of fluridone, that earlier (spring) applications may provide better uptake and efficacy. Likewise, integrating harvesting or use of contact herbicides such as Komeen or other products, may also provide more optimal conditions for improving the efficacy of Sonar. Given the long-term exposure required and the likely conflicts between irrigation needs in spring, it would be advisable to determine if fall/winter applications of Sonar in certain areas might provide efficacy. Finally, as a means to lower application costs, it would be useful to develop automated injection systems for Sonar (4AS), and Komeen or other efficacious contact herbicides.

b. Komeen. The patterns of copper movement and dissipation are consistent with studies conducted previously in the Delta. Applications to the 3 to 5 acre plots resulted in efficacious concentrations of Cu for approximately 6 to 9 h in the plot and also to adjacent areas within 500 to 1000 ft. of the site. Movement and dissipation is primarily due to tidal flows but is also influenced by net river flows. The tidal action also tends to mix the water vertically so that by the second tidal movement (ca. 9 hours), Cu is relatively uniformly distributed in the water column. This has the advantage of providing more consistent exposure to the target weed, egeria. The applications resulted in egeria tissue levels of Cu ranging from 200 to nearly 2000 ppm, with most falling the area of 400 ppm. These levels have been shown to produce defoliation and necrosis of egeria in previous studies.

4. Water Quality. None of the variables measured showed changes that could be attributable to the immediate effects of the harvests or applications of herbicides. Temperatures varied slightly between some sites and between the three different harvest/herbicide applications dates as would be expected. Furthermore, at no time during the pre- or entire posttreatmeent sampling of water in the herbicide sites were there ever observed stressed, floating or dead fish or other animals, or animals exhibiting unusual behavior.

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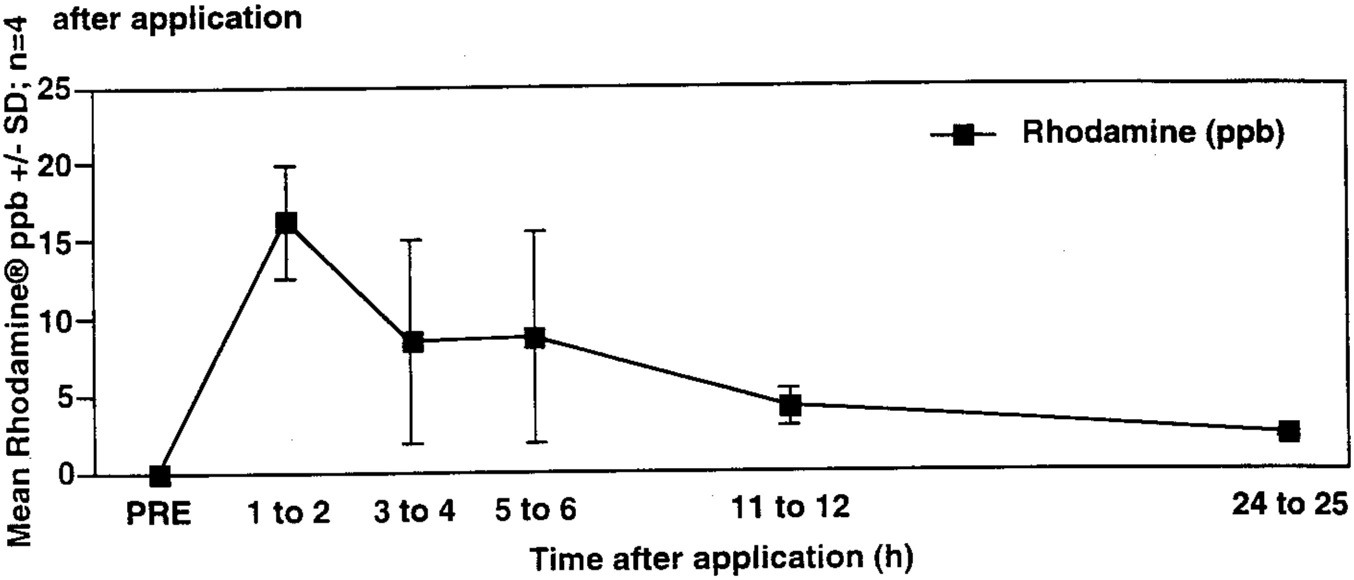
Figure 1A. Rhodamine® dye dissipation in the Komeen® treatment site 25 h after application



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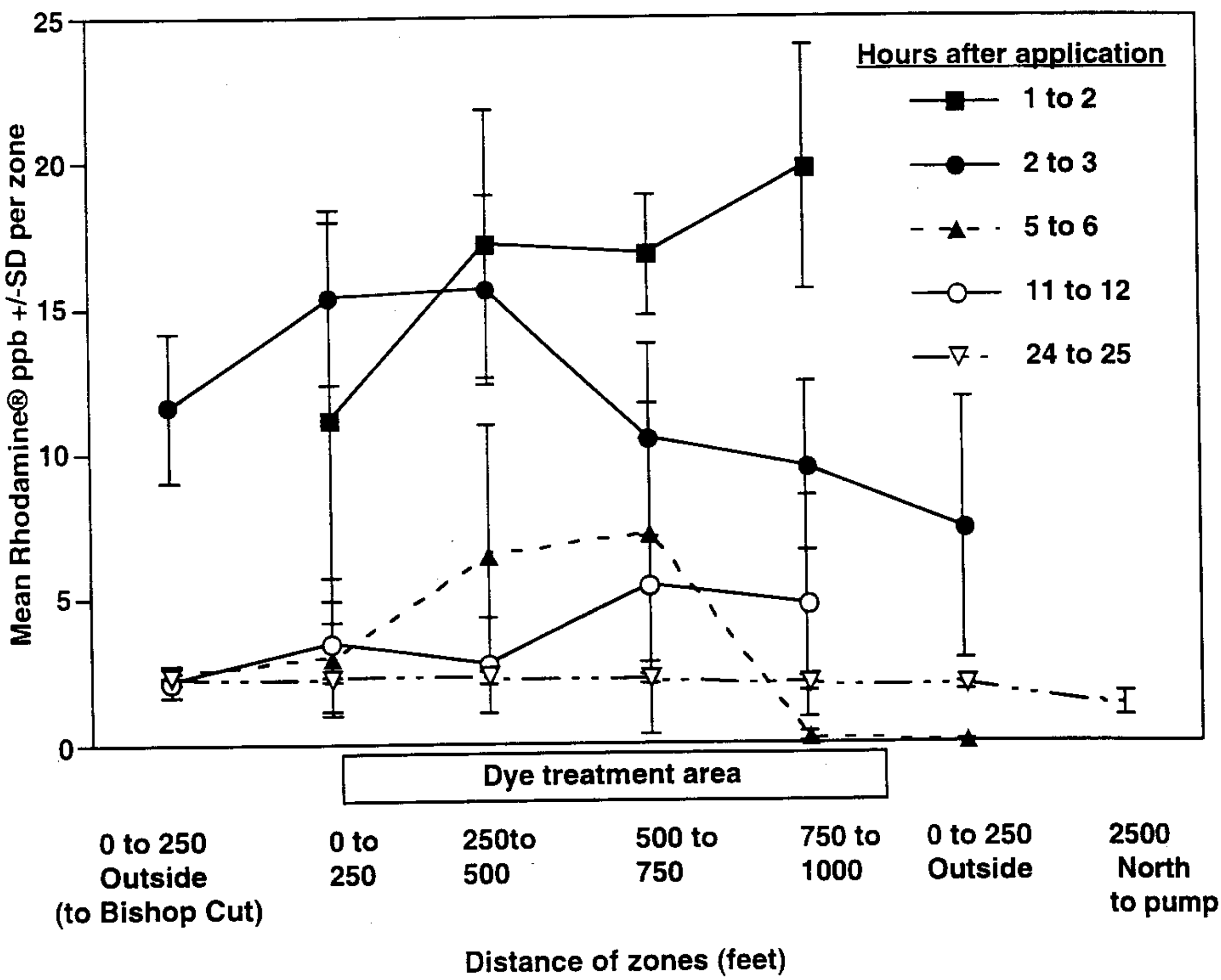

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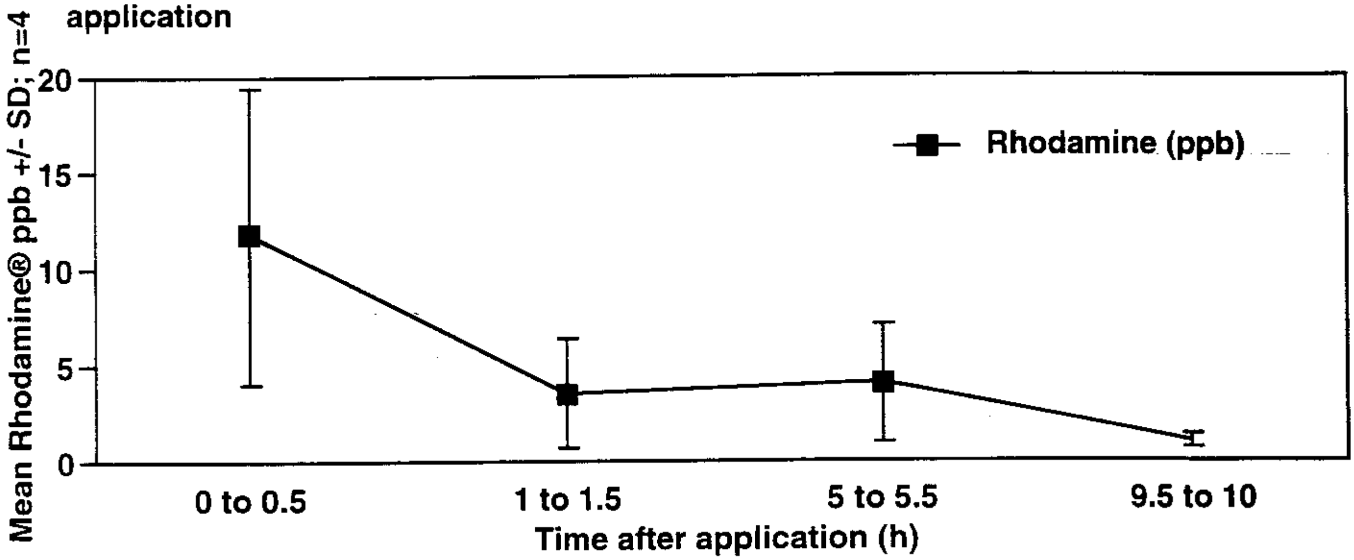

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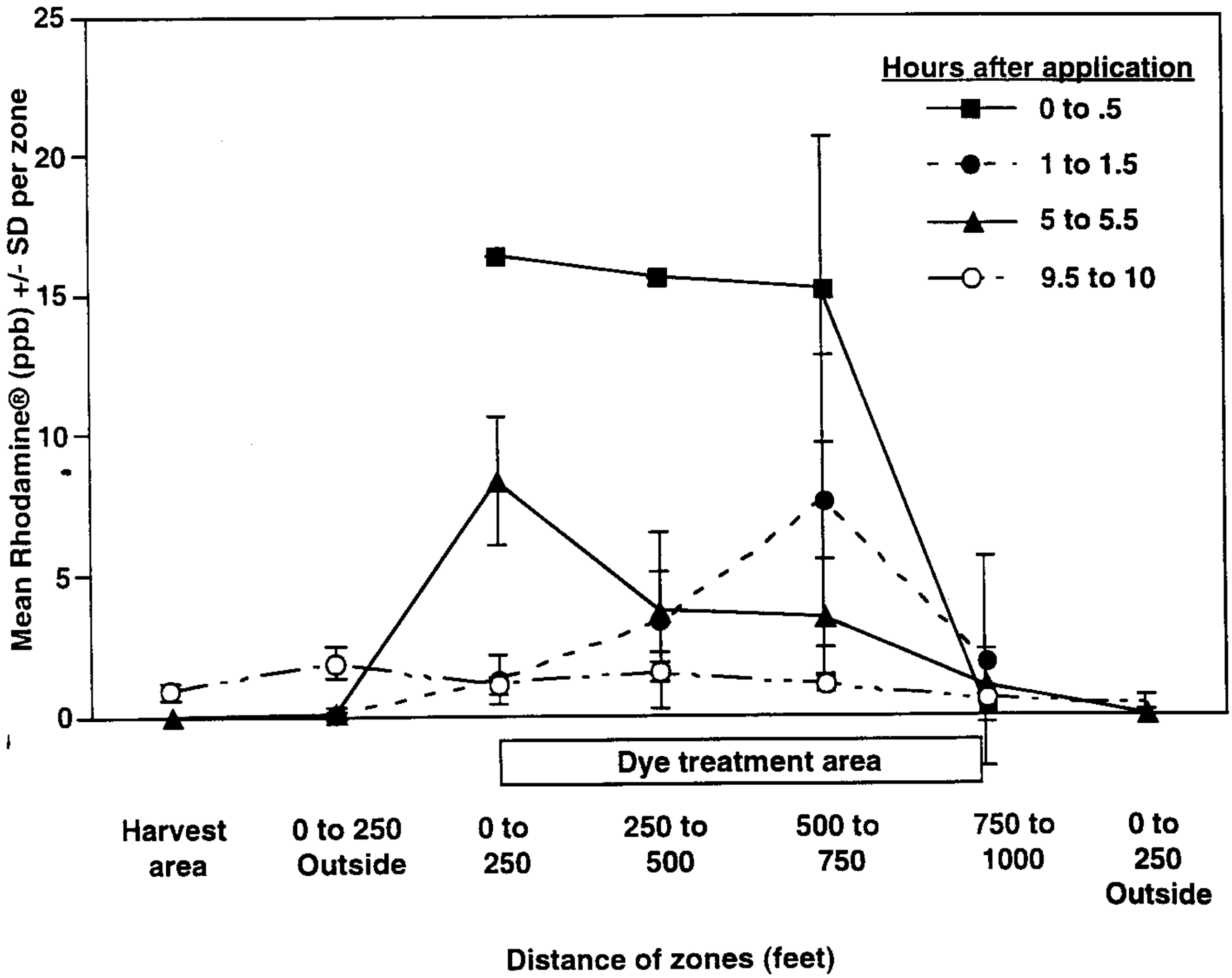

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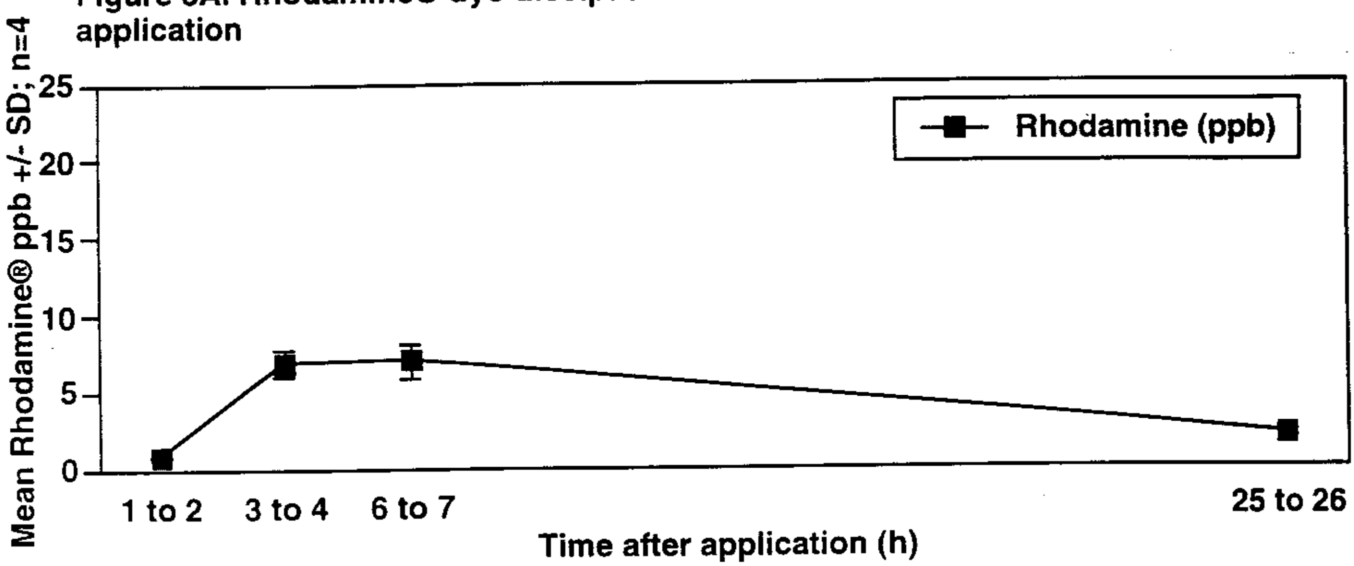

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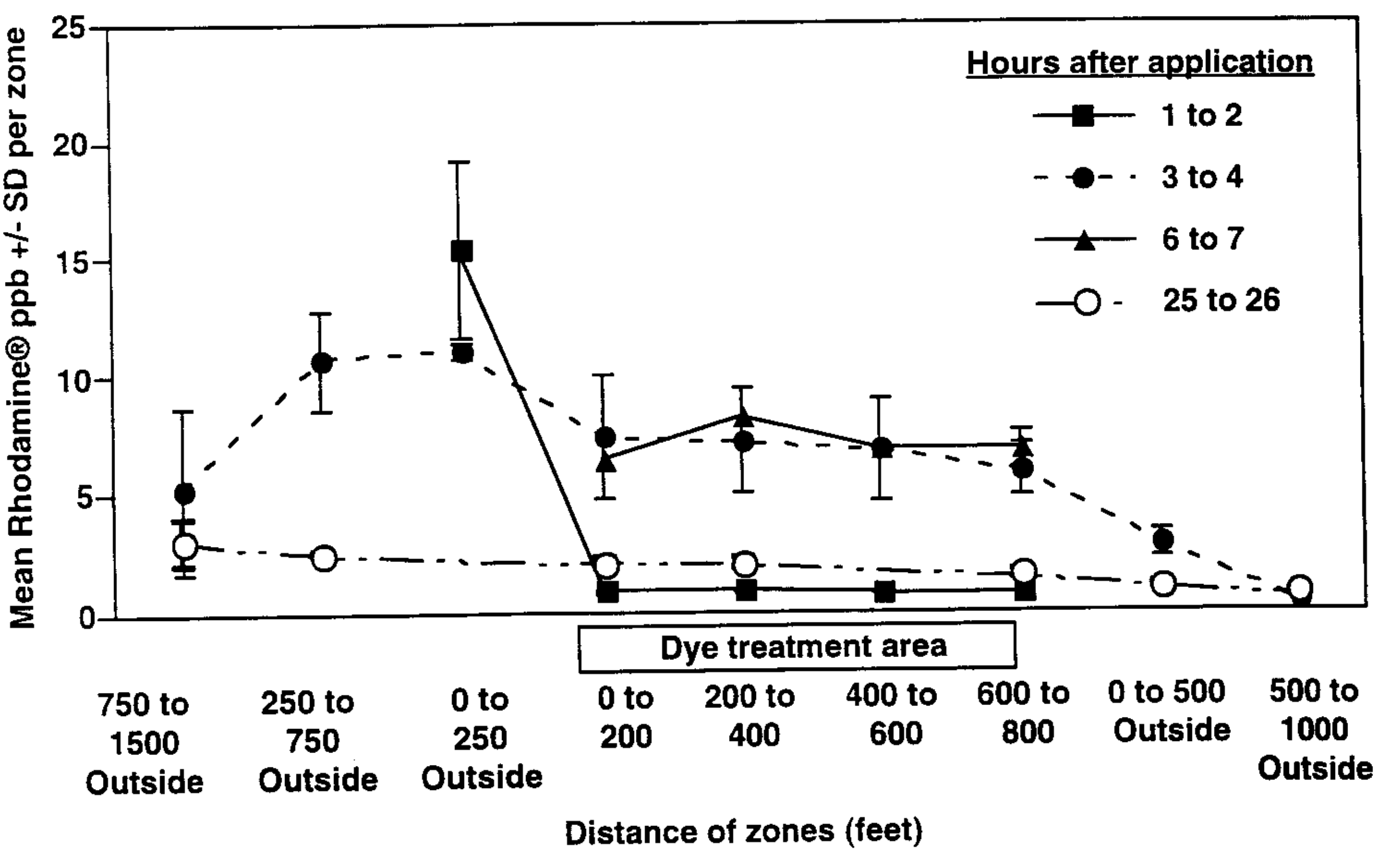

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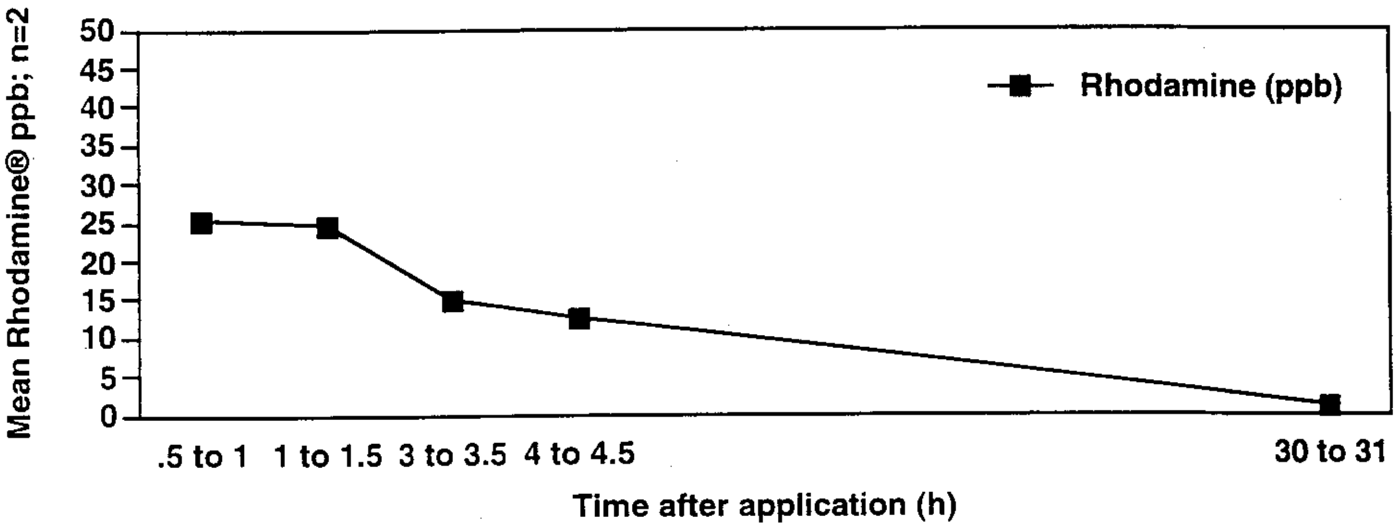

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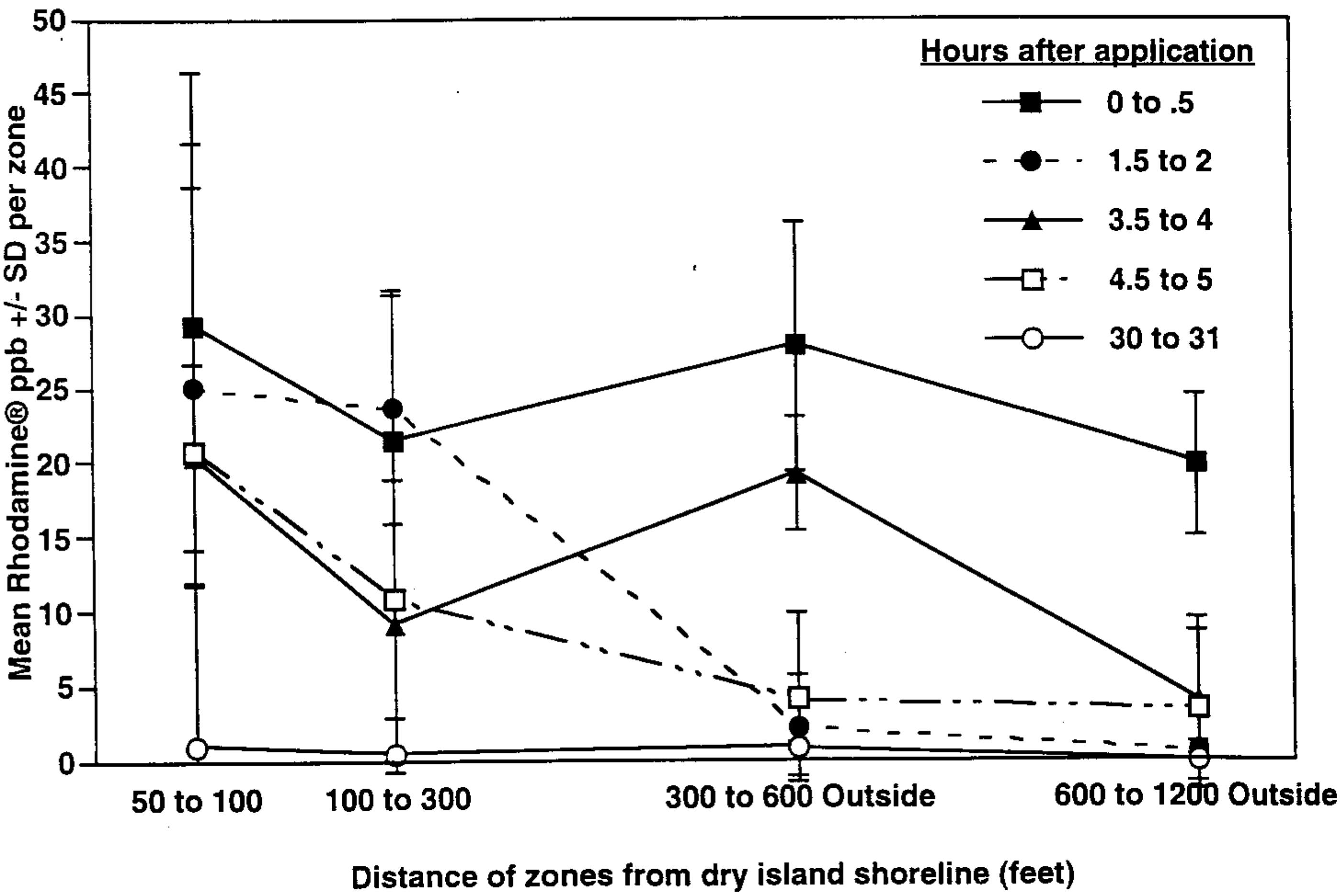

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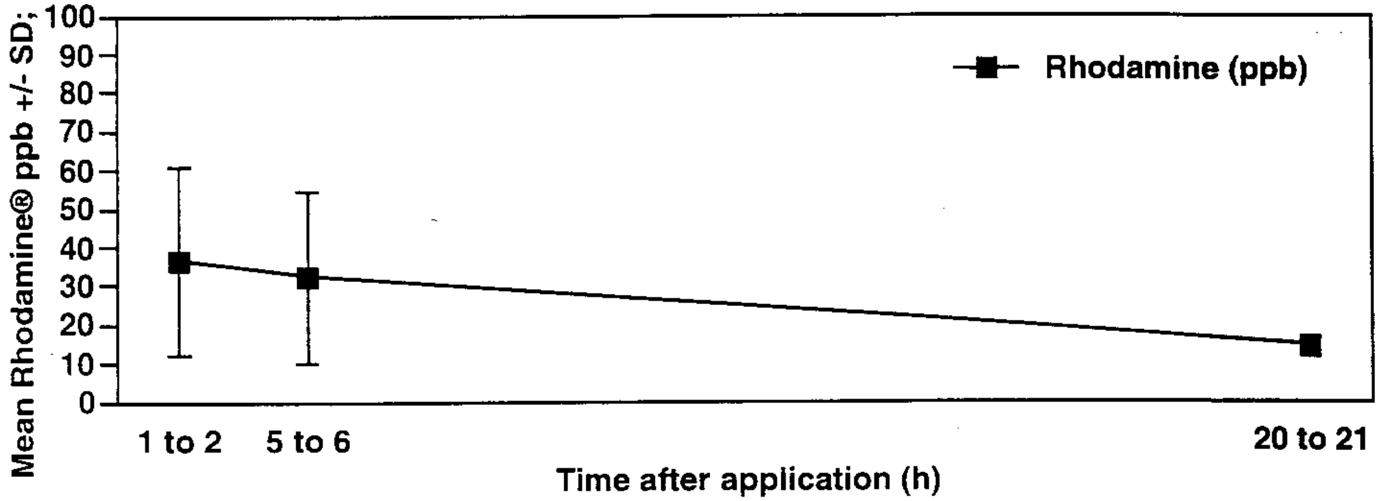

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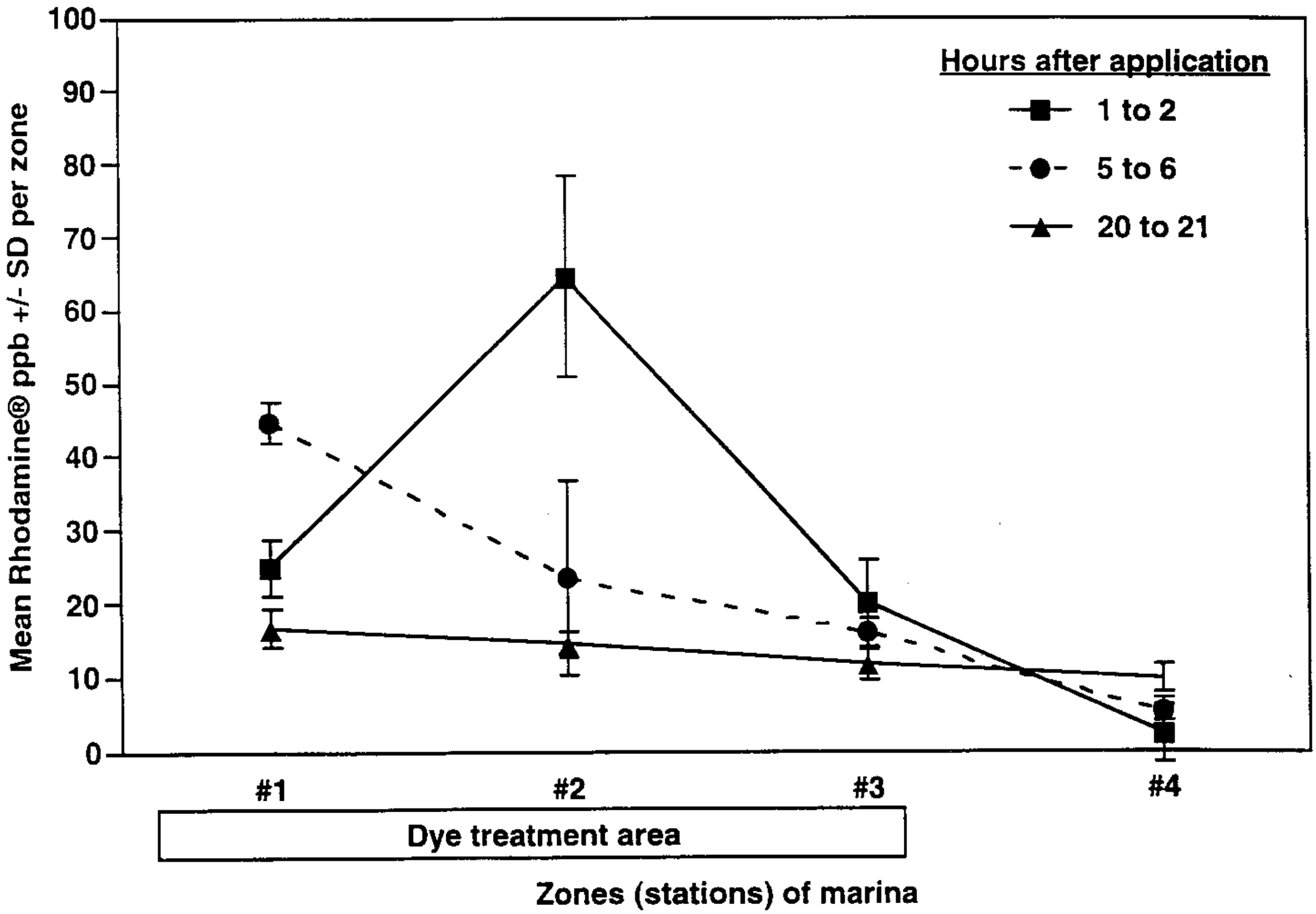

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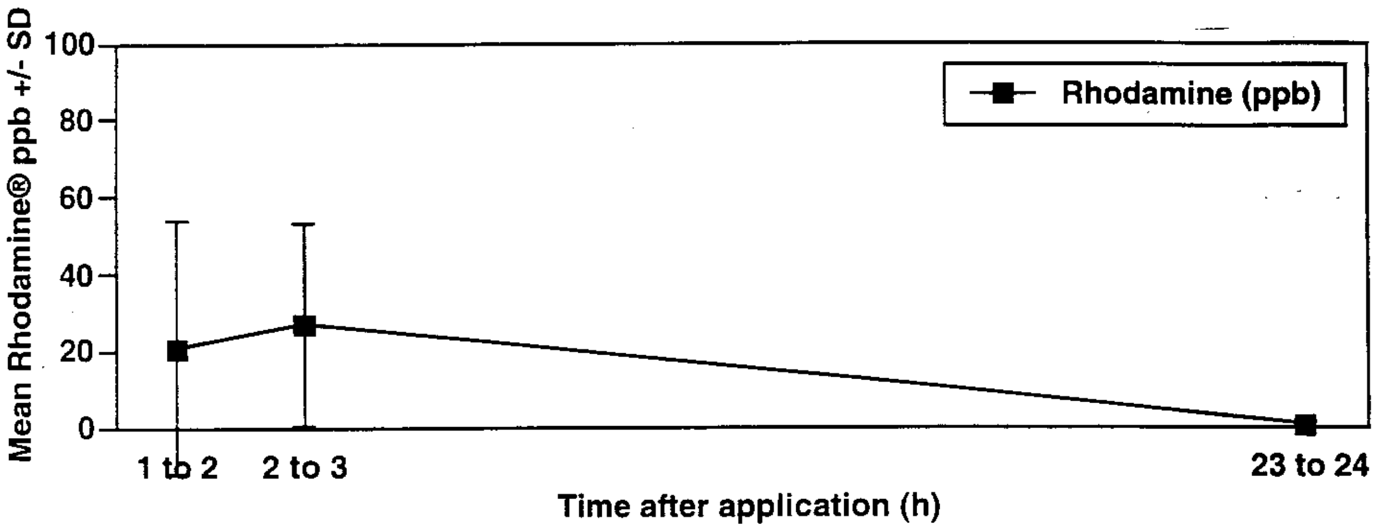

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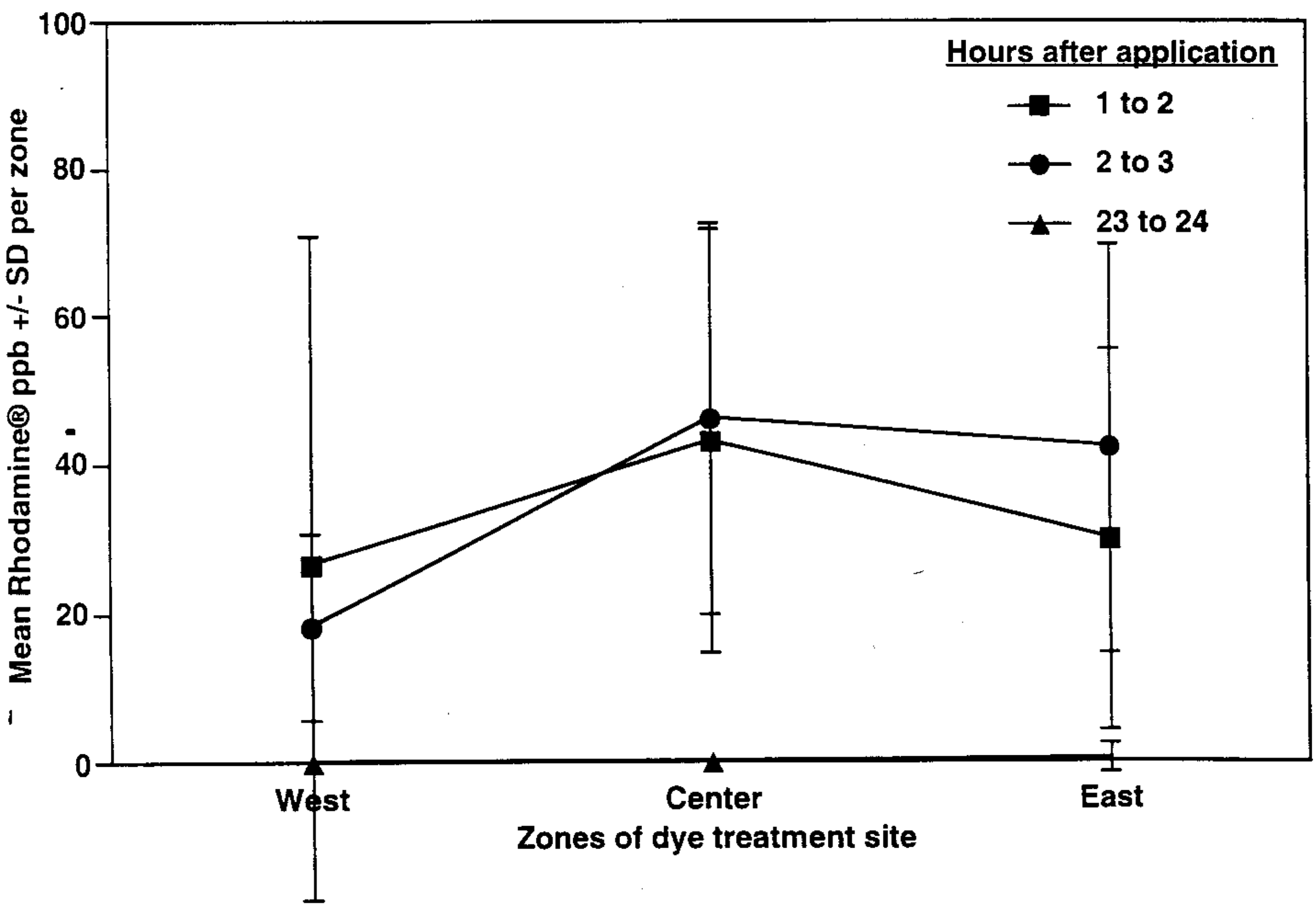

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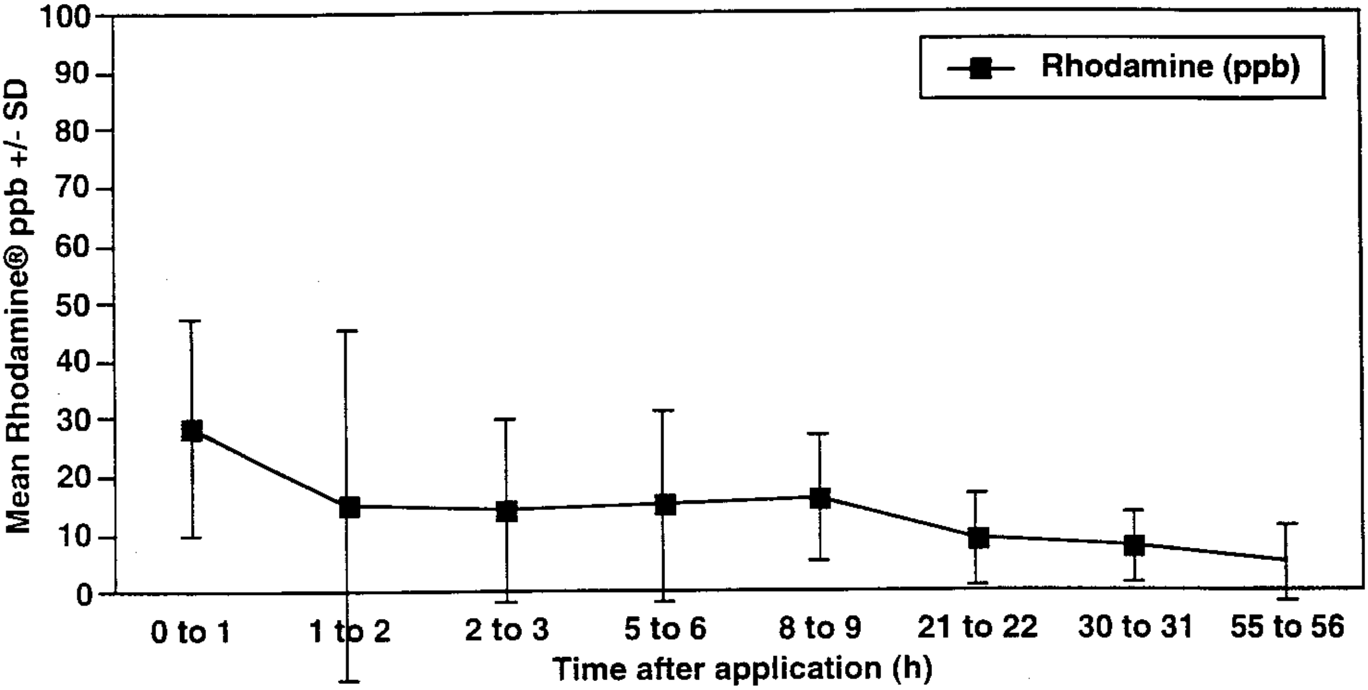

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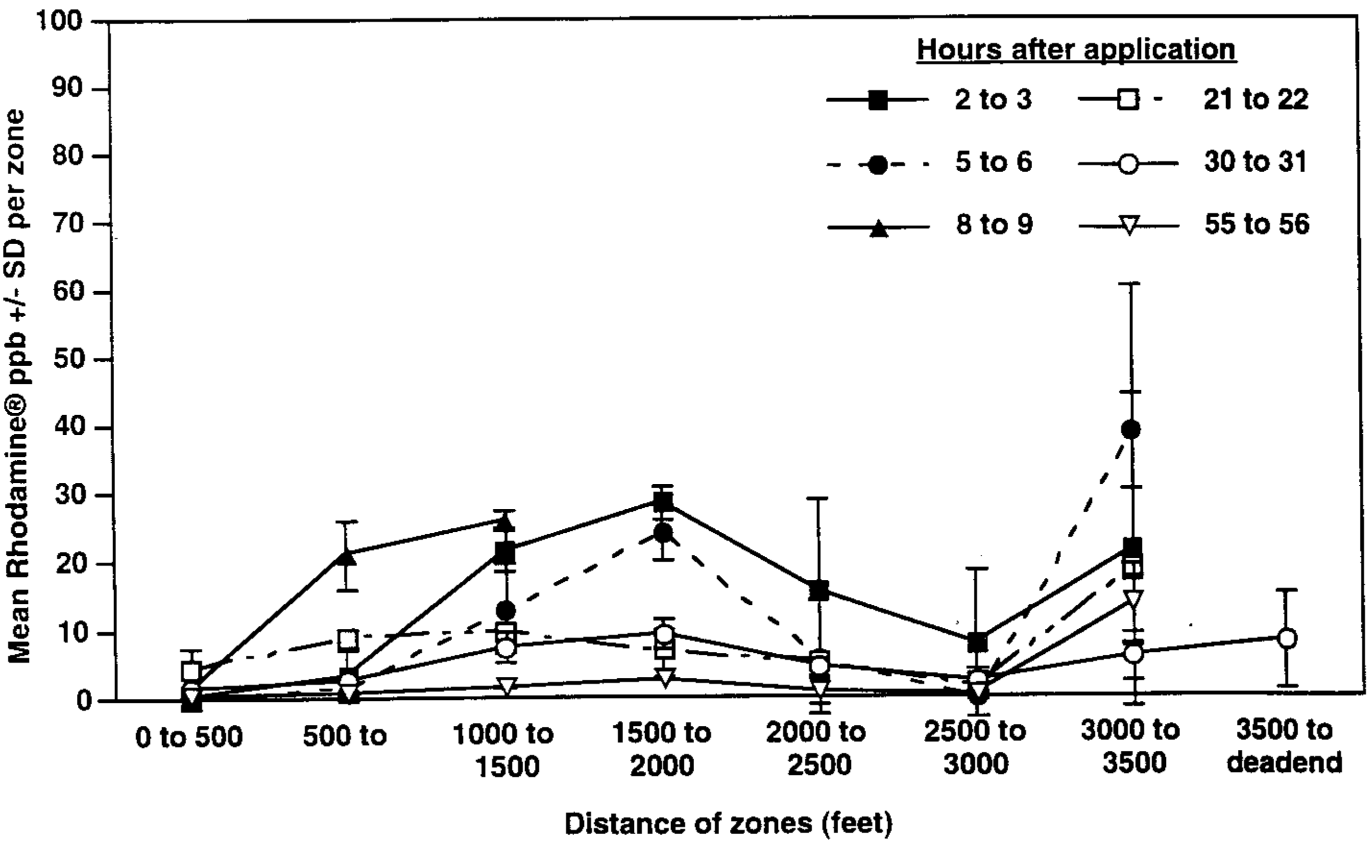

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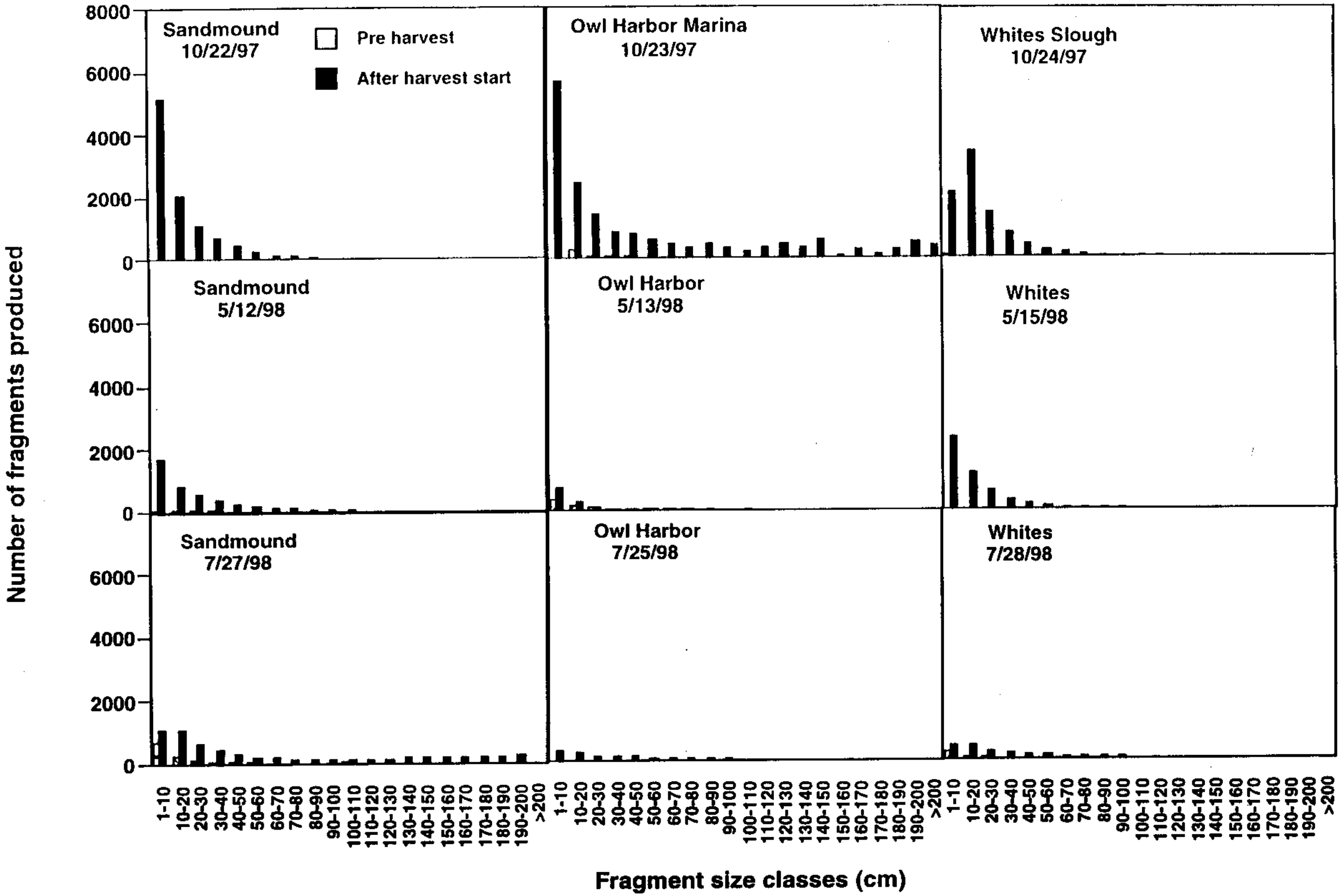

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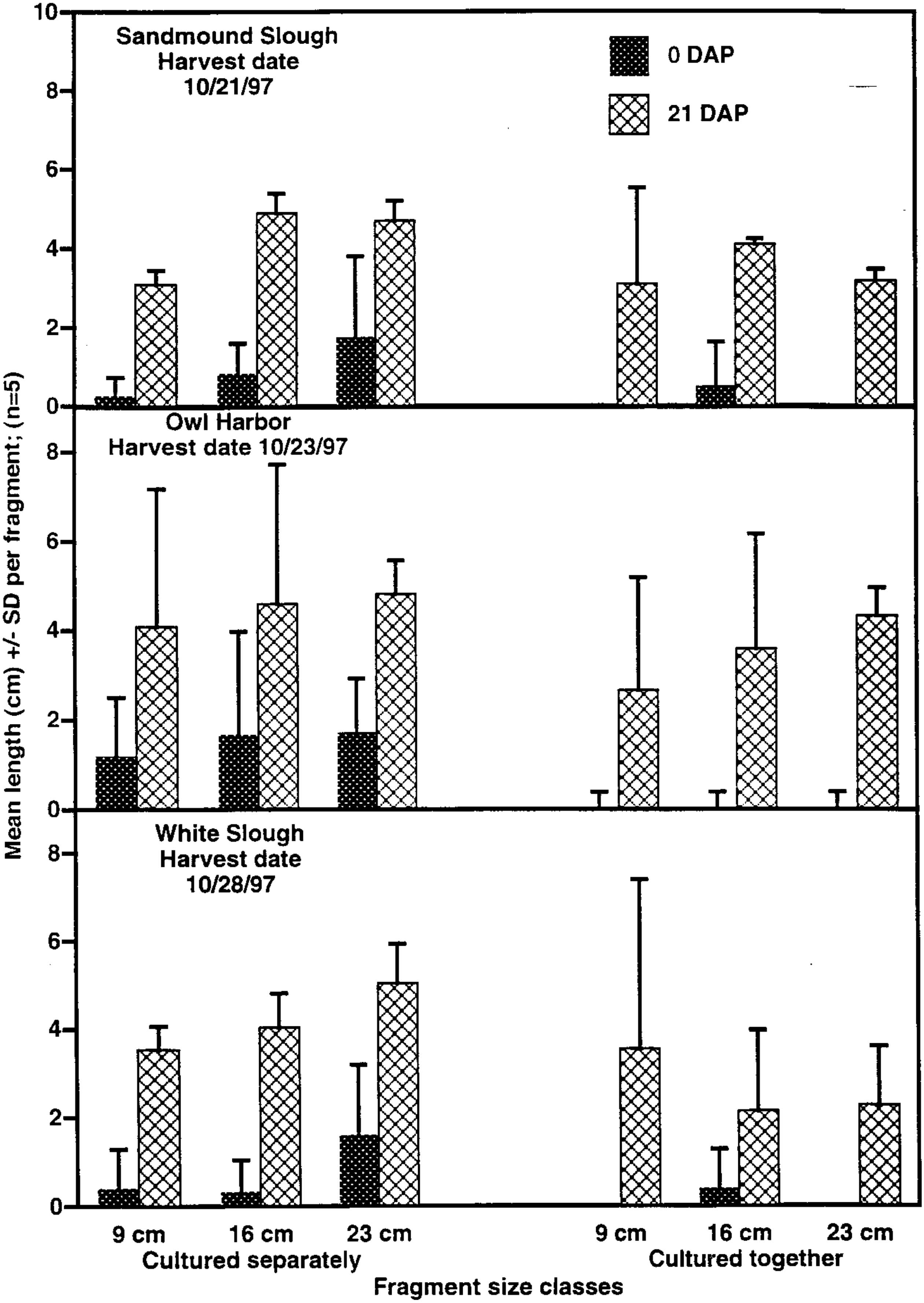

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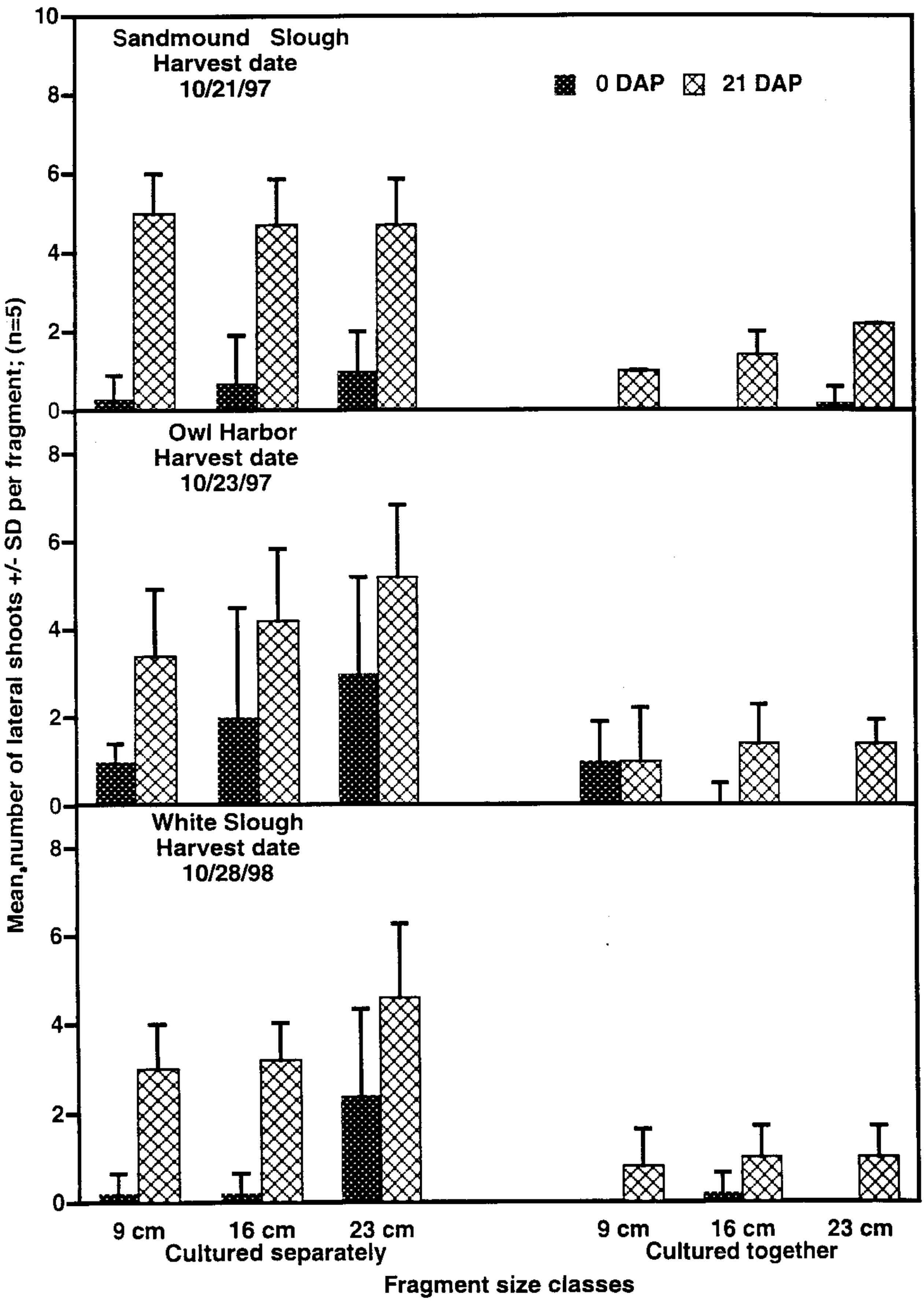

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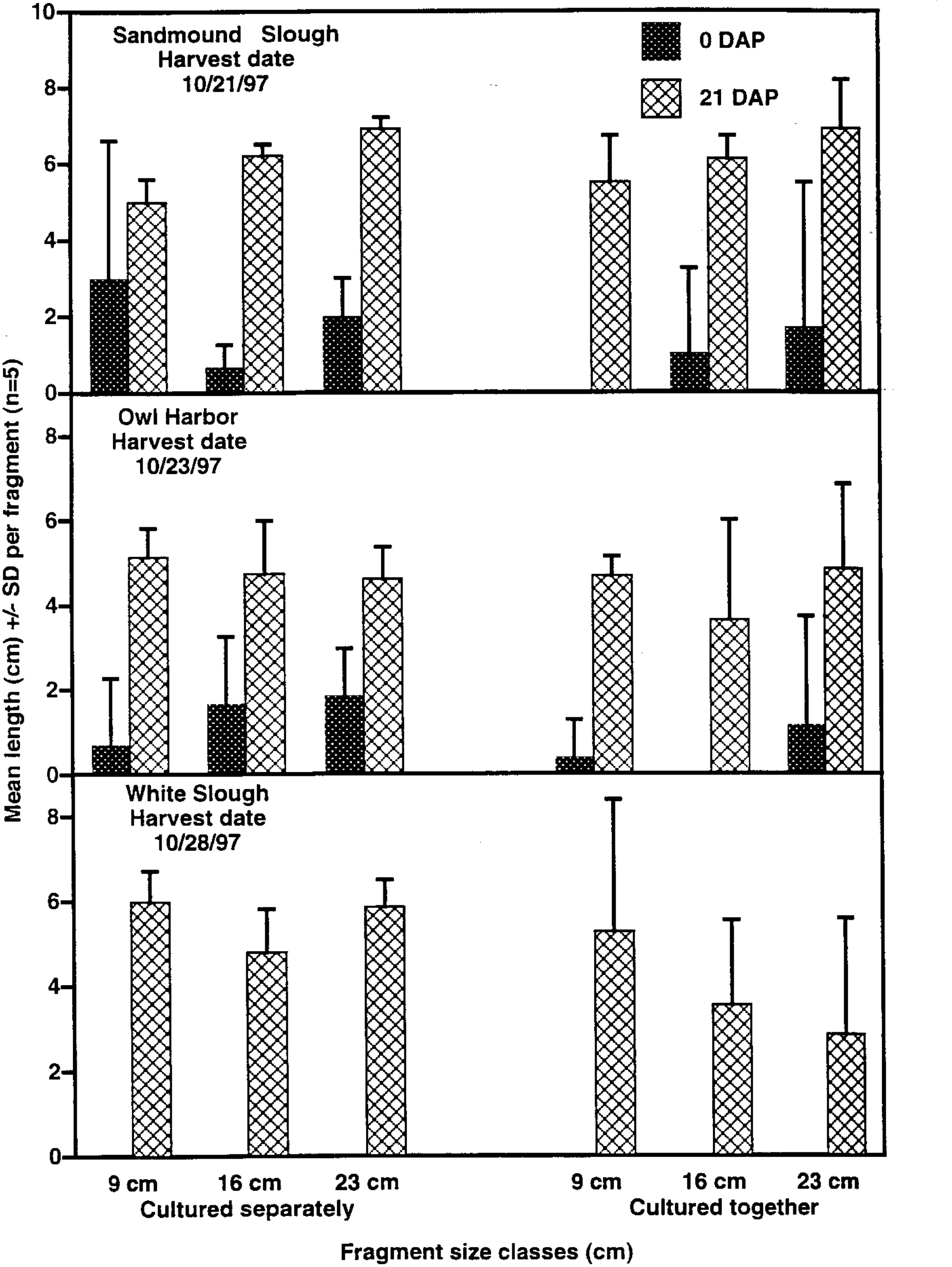

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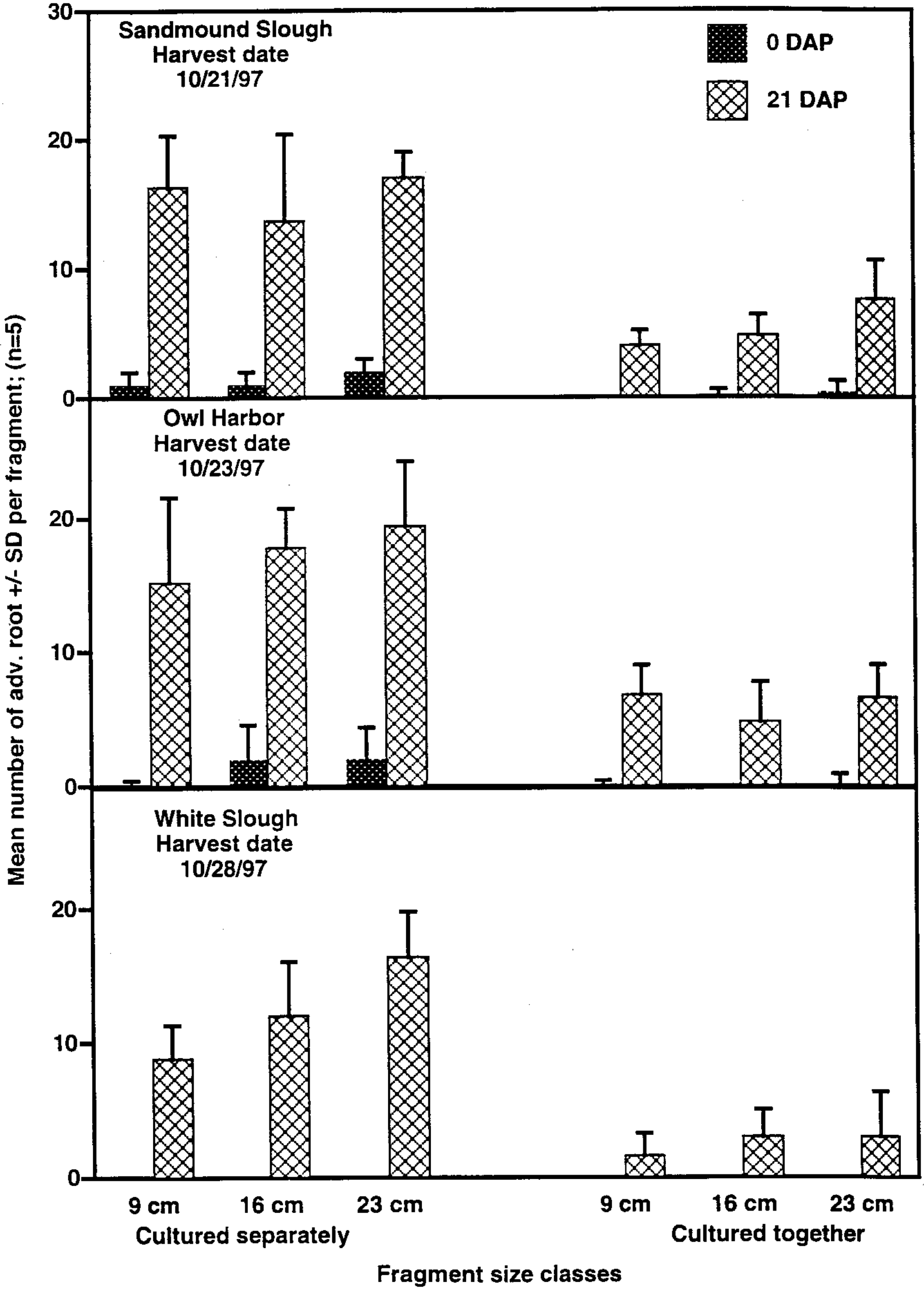

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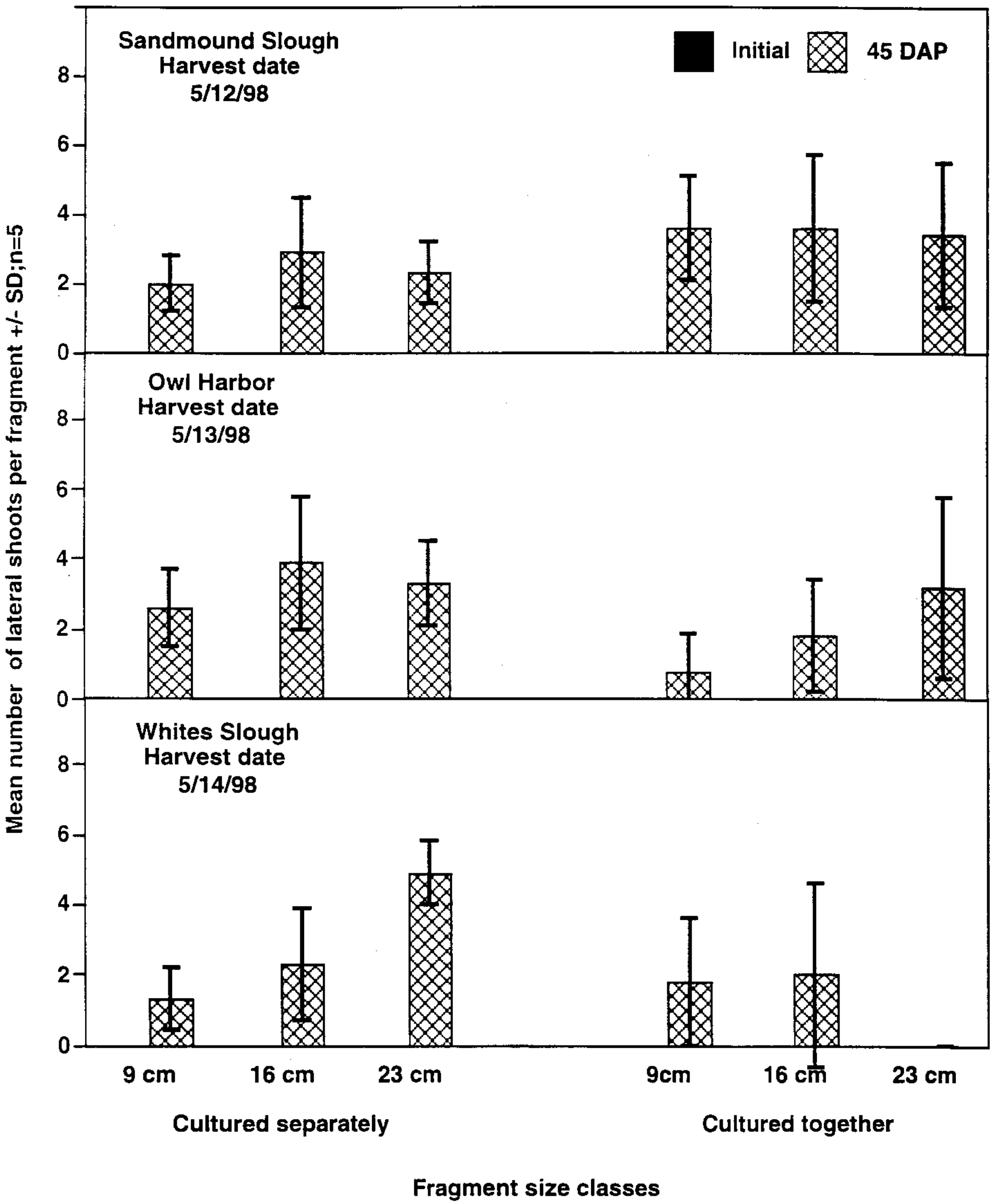

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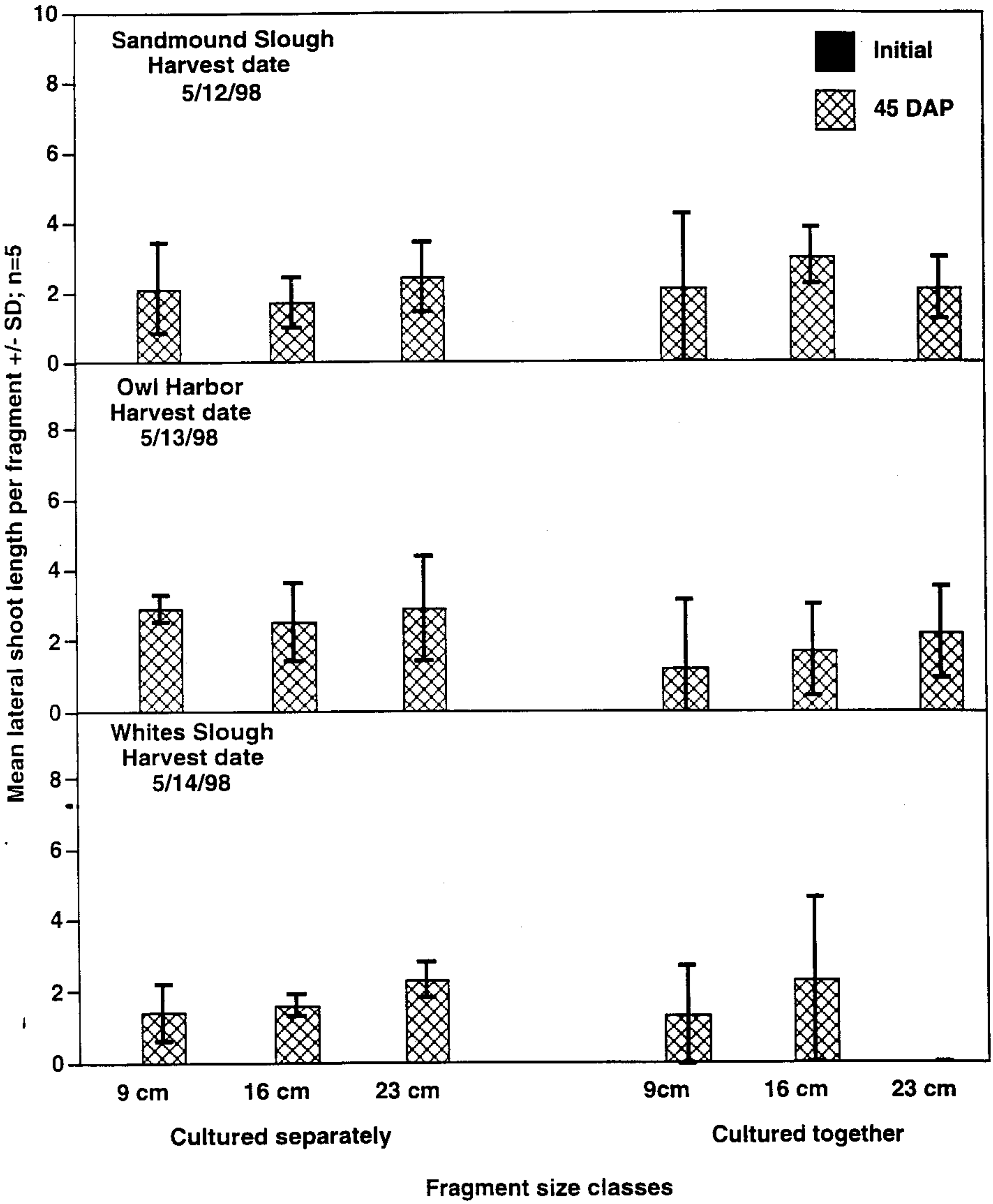

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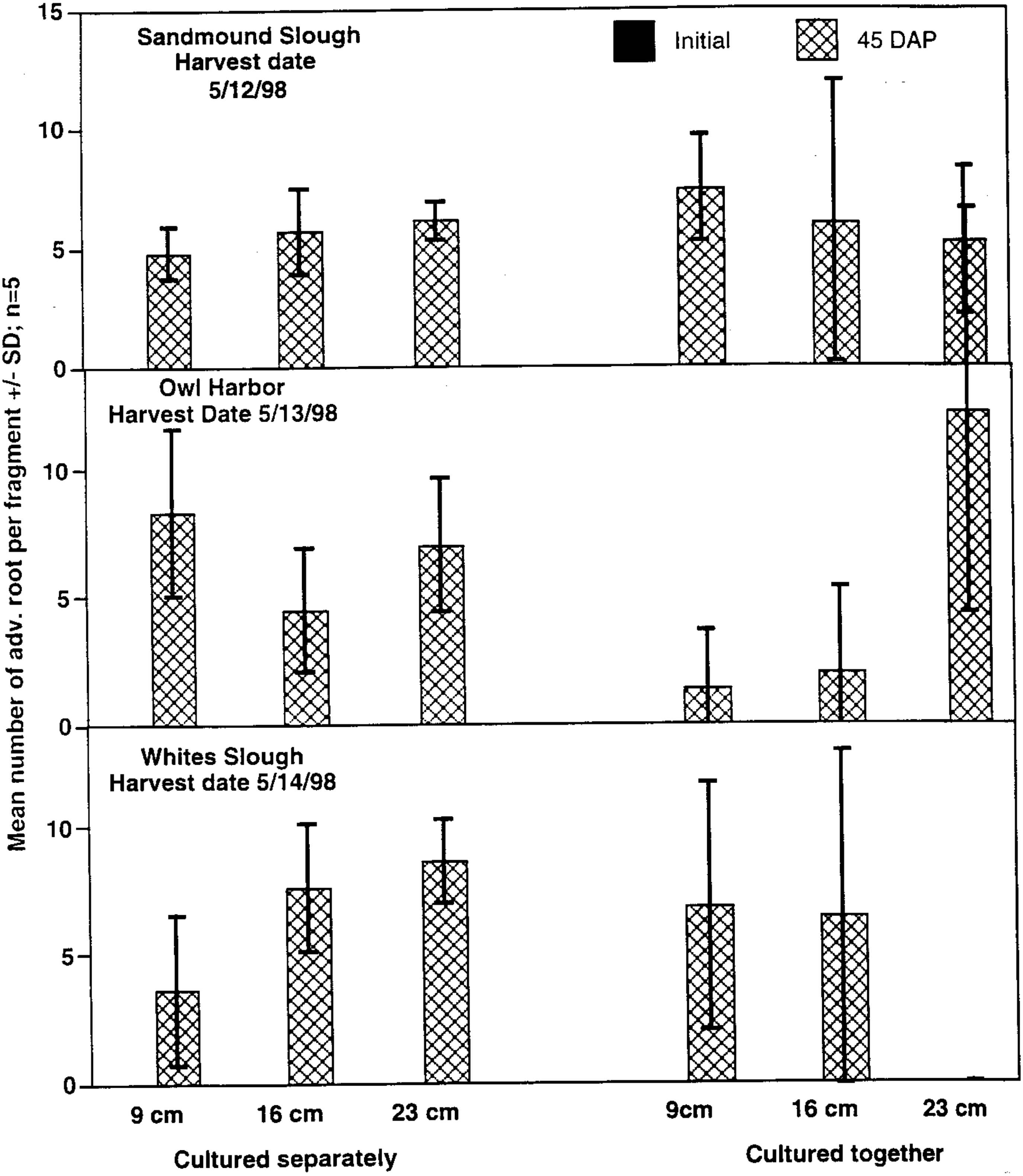

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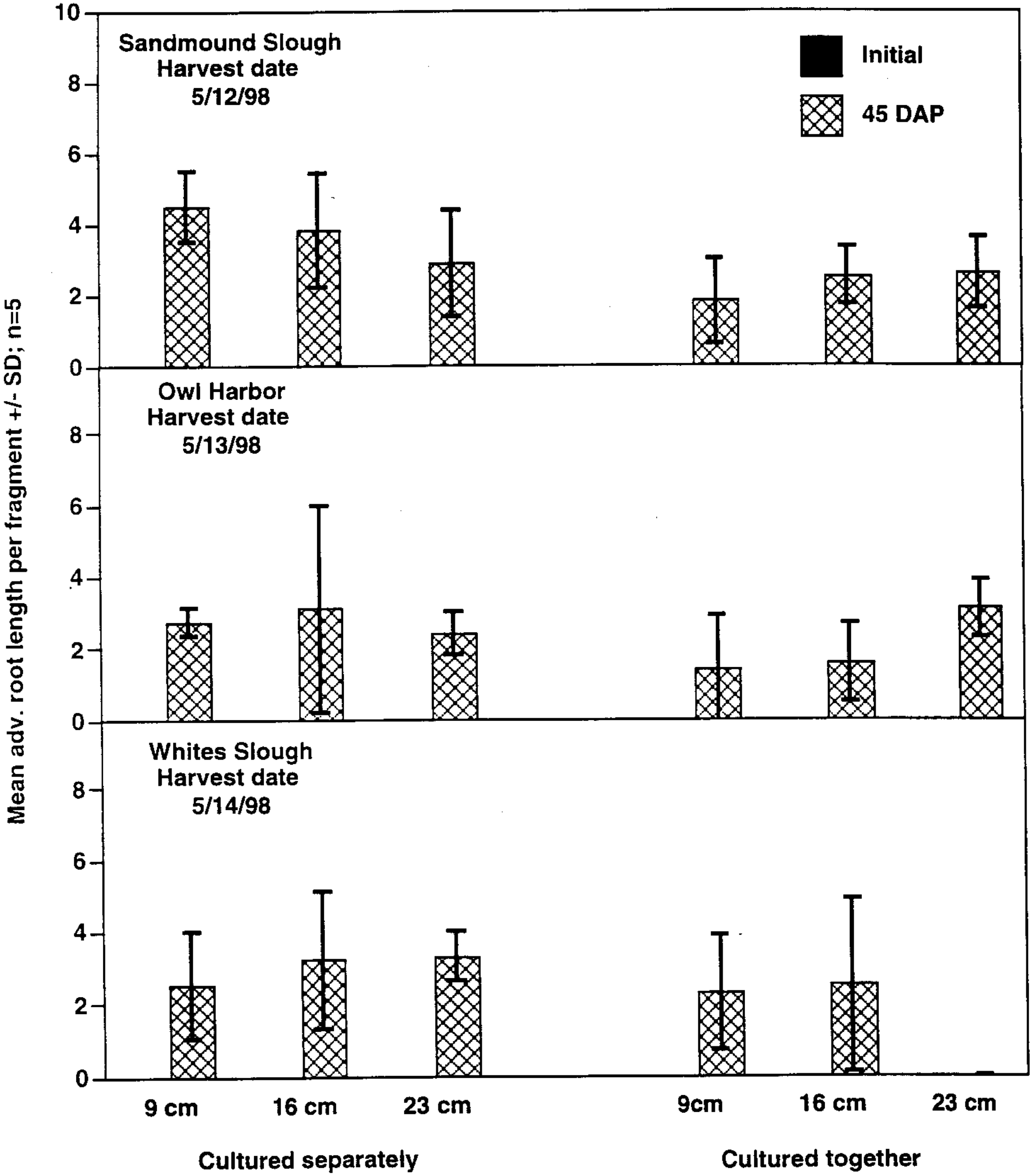

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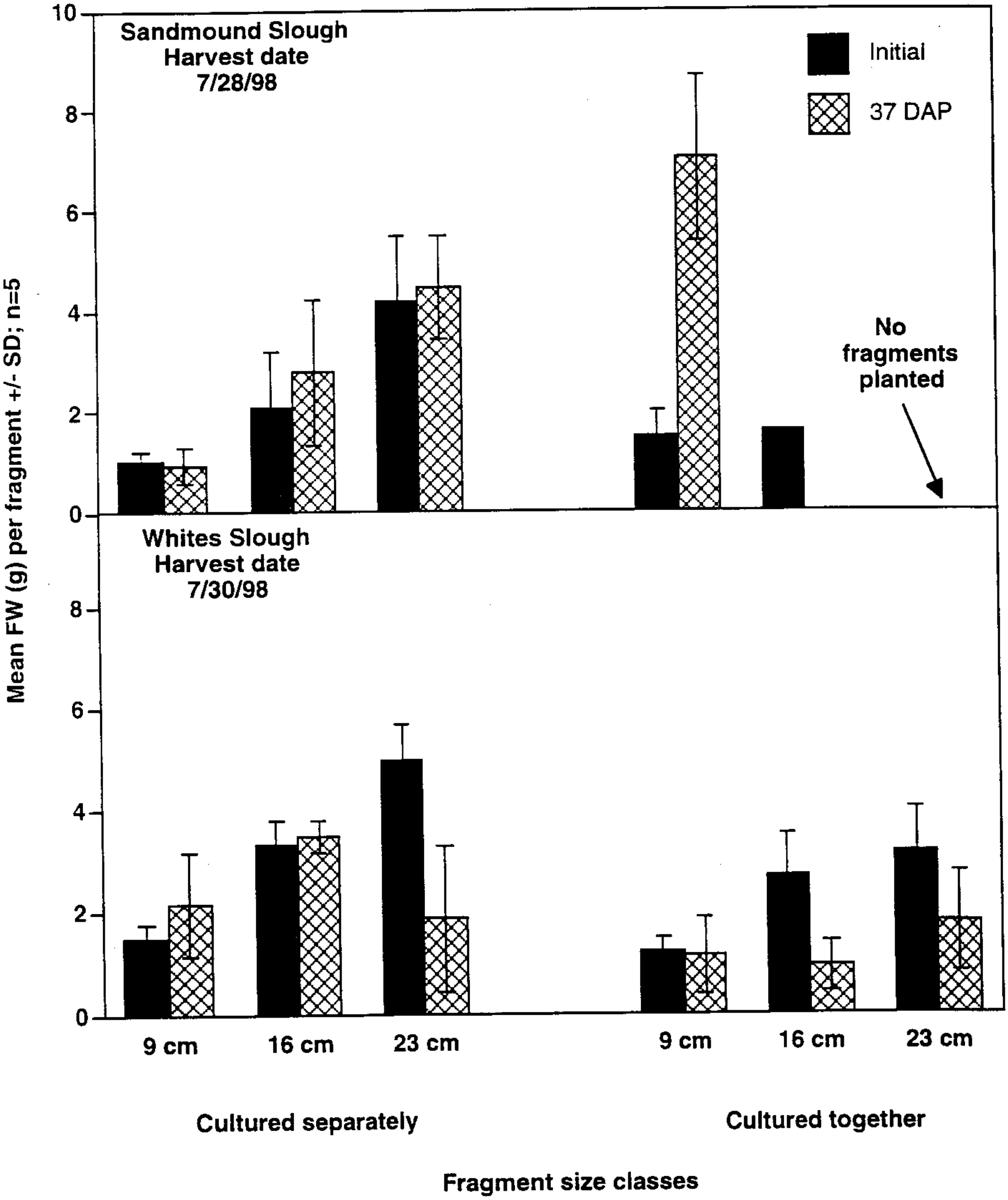

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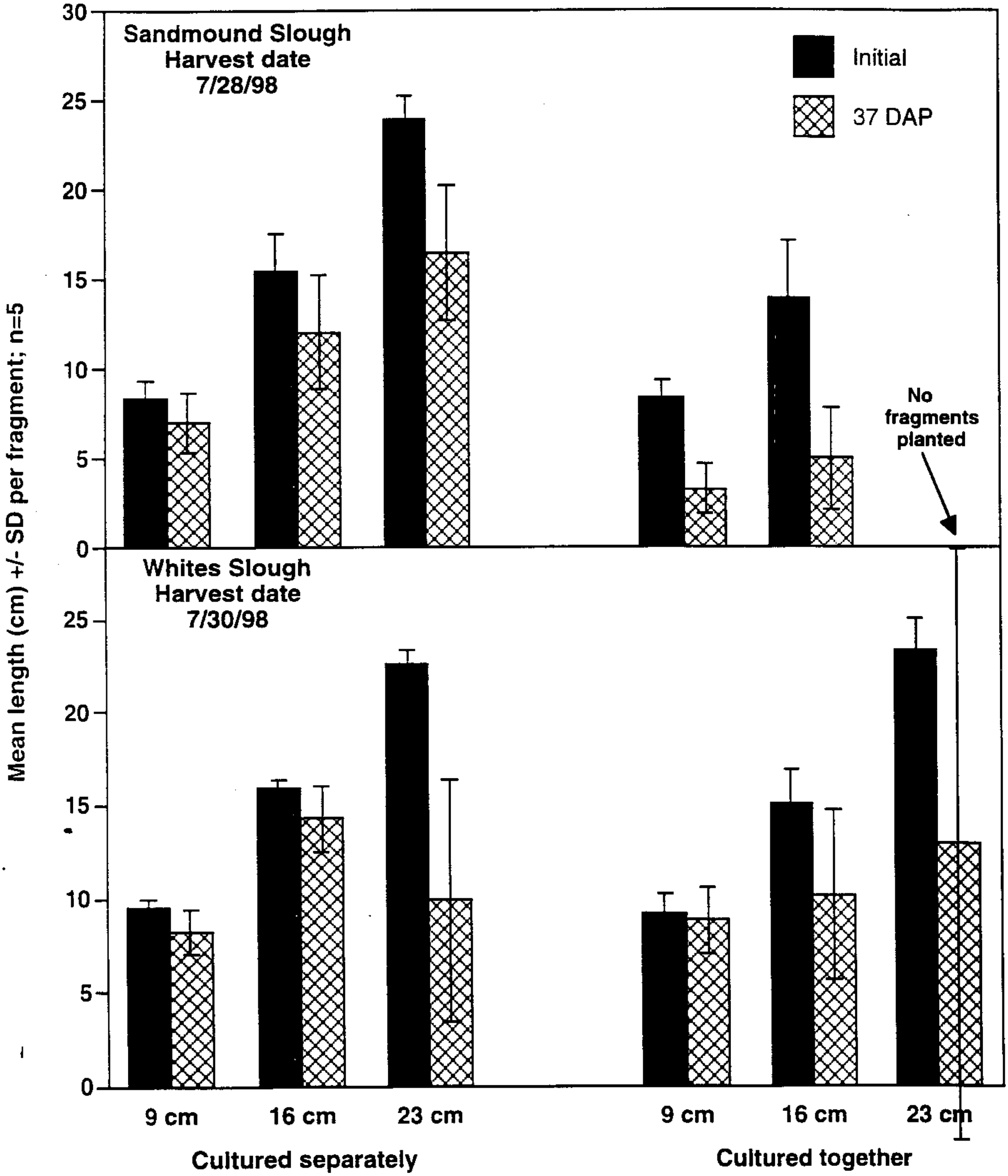

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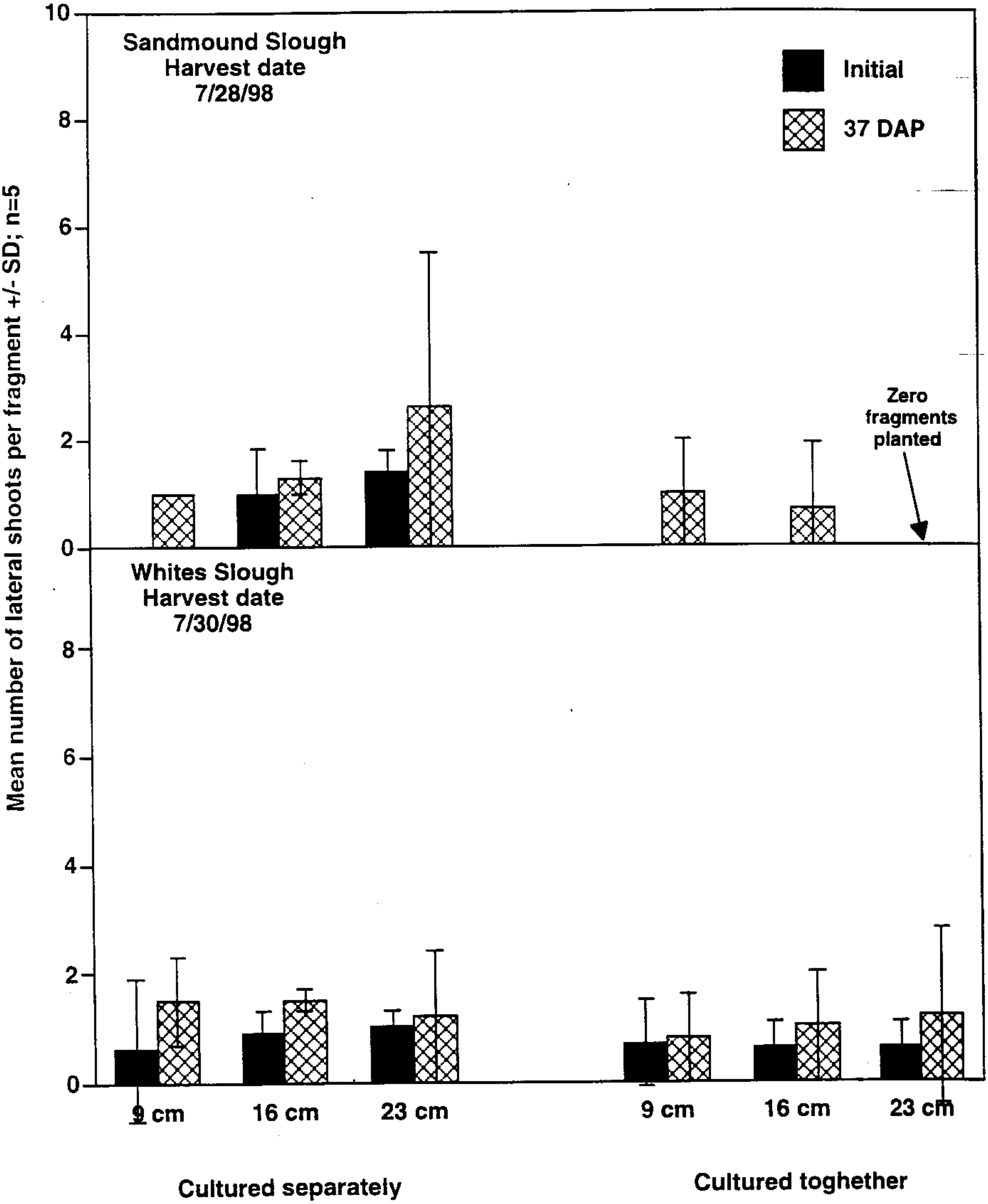

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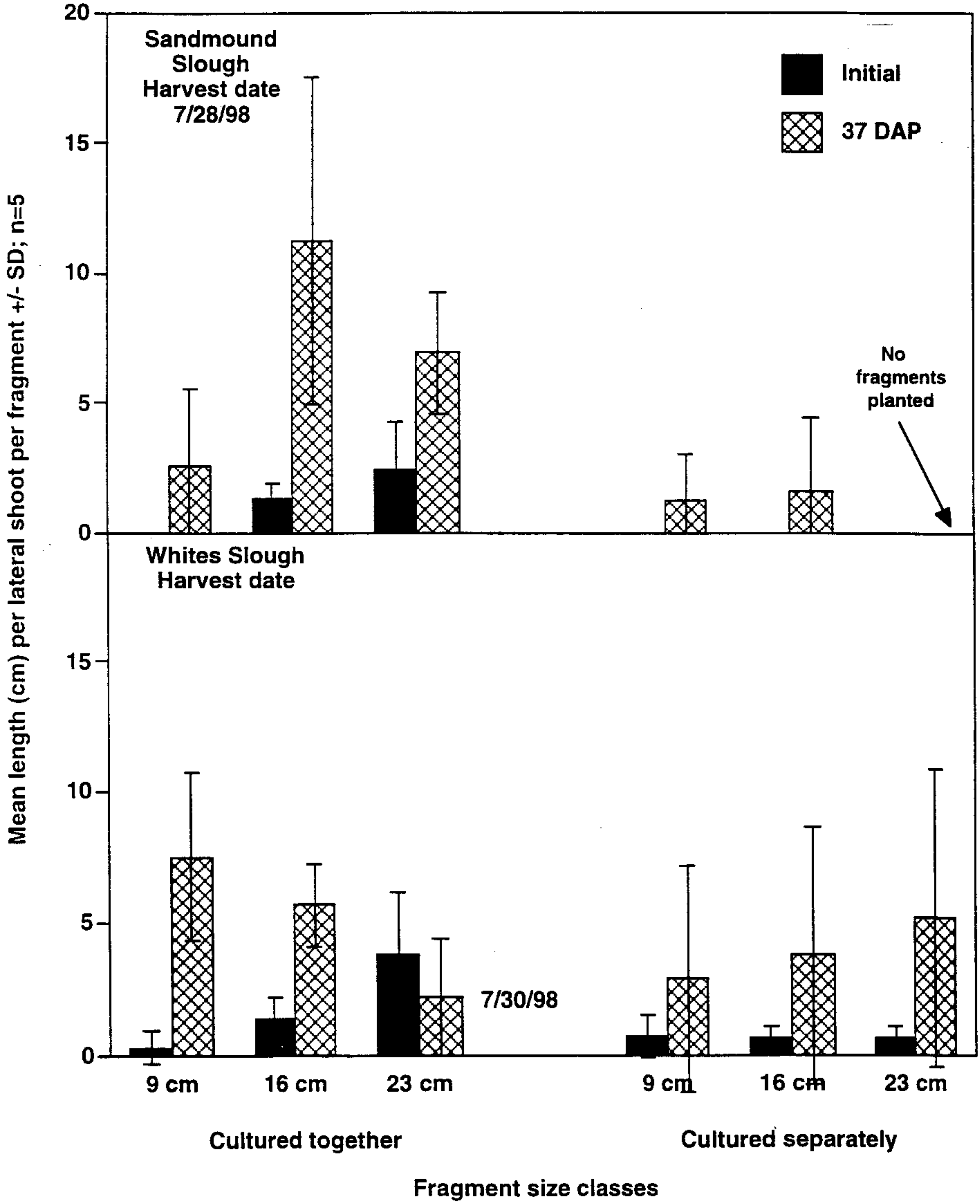

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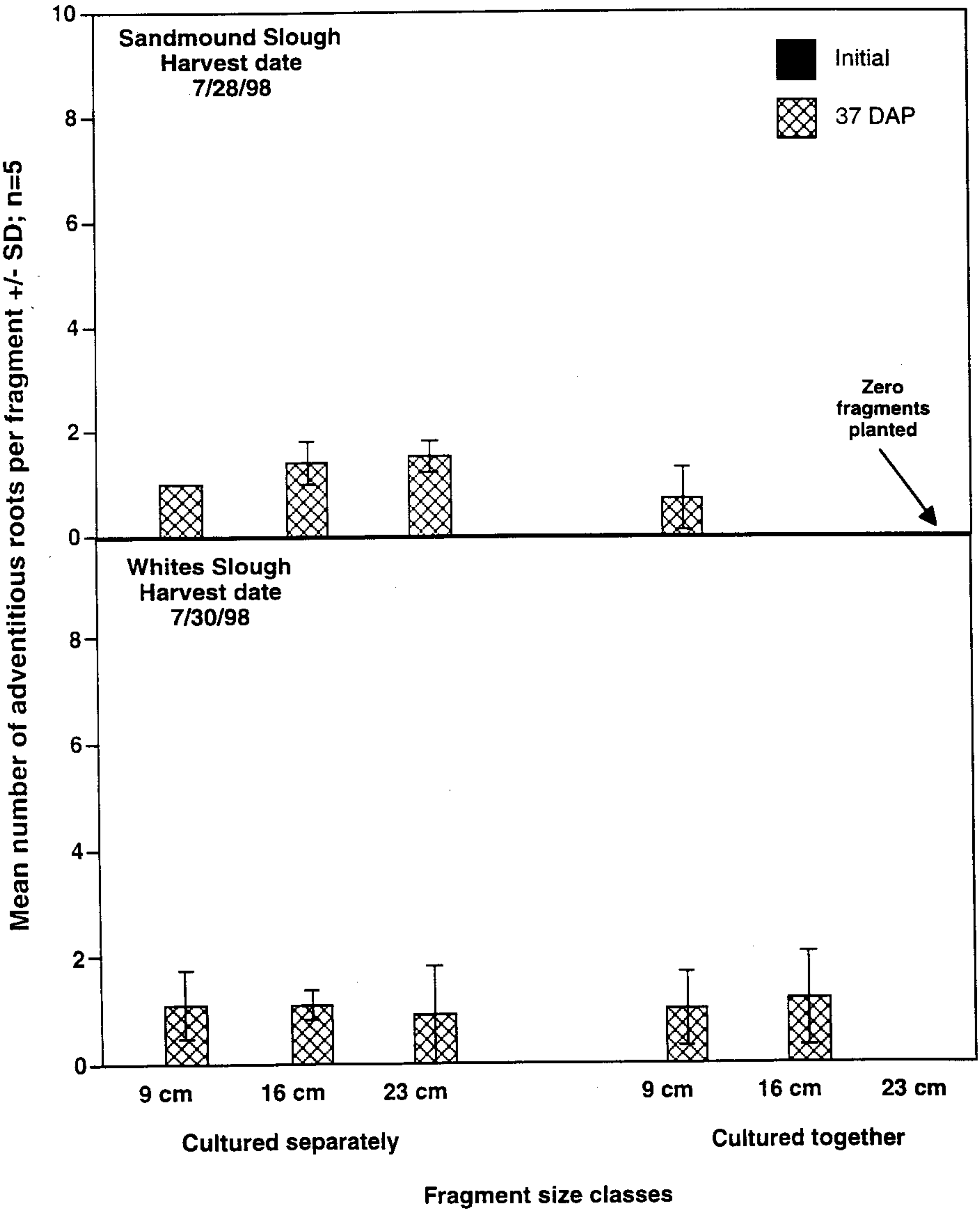

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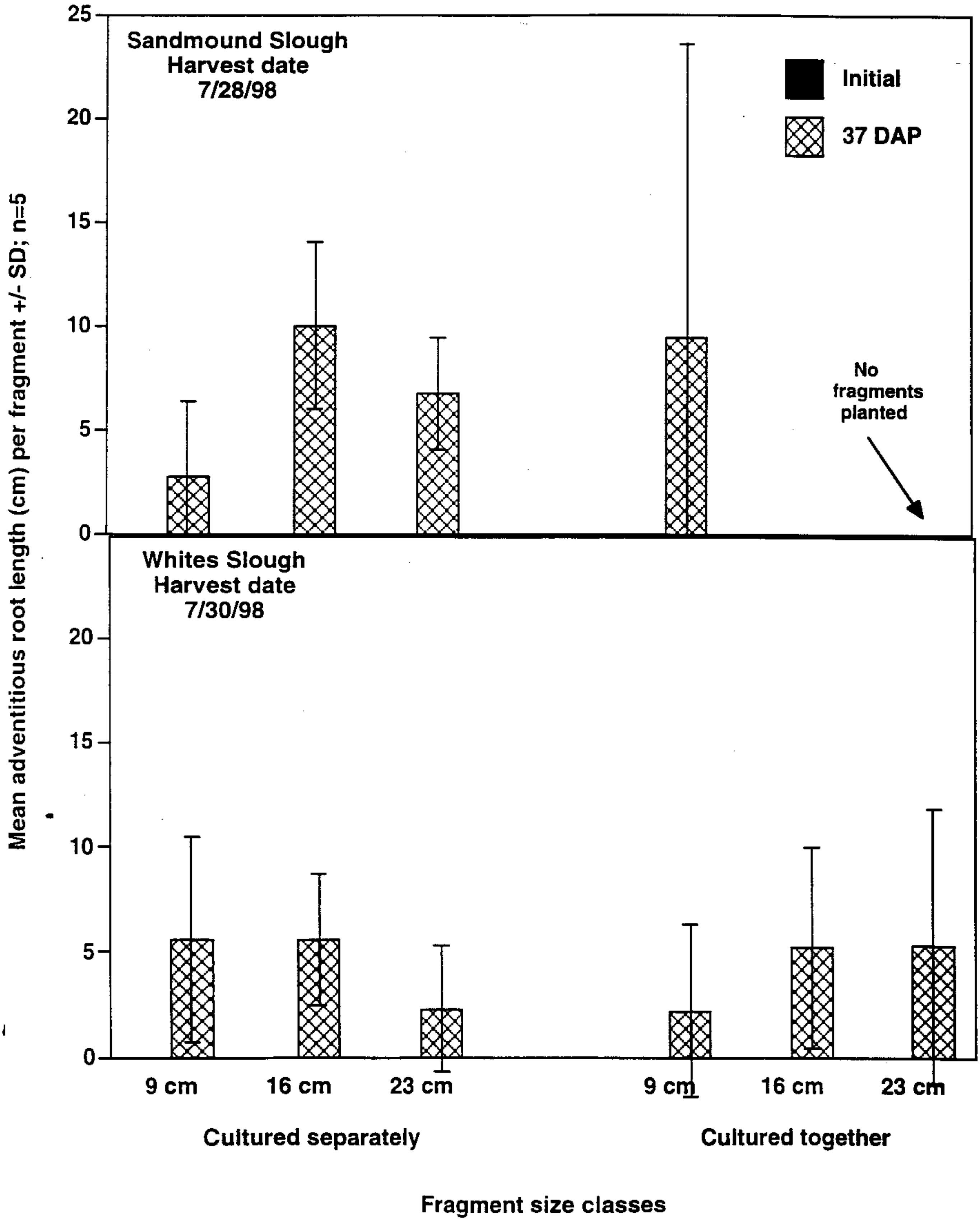

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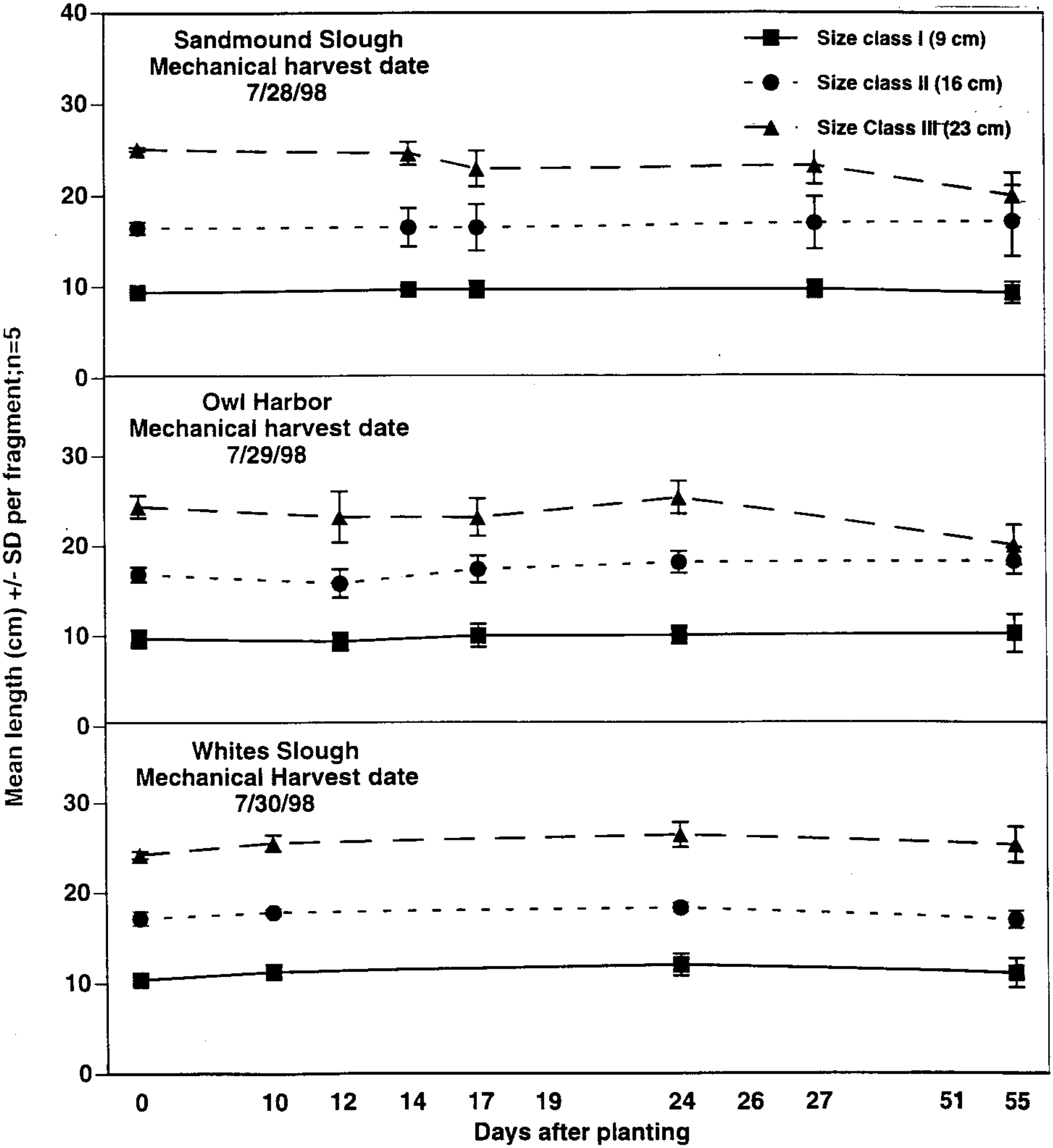

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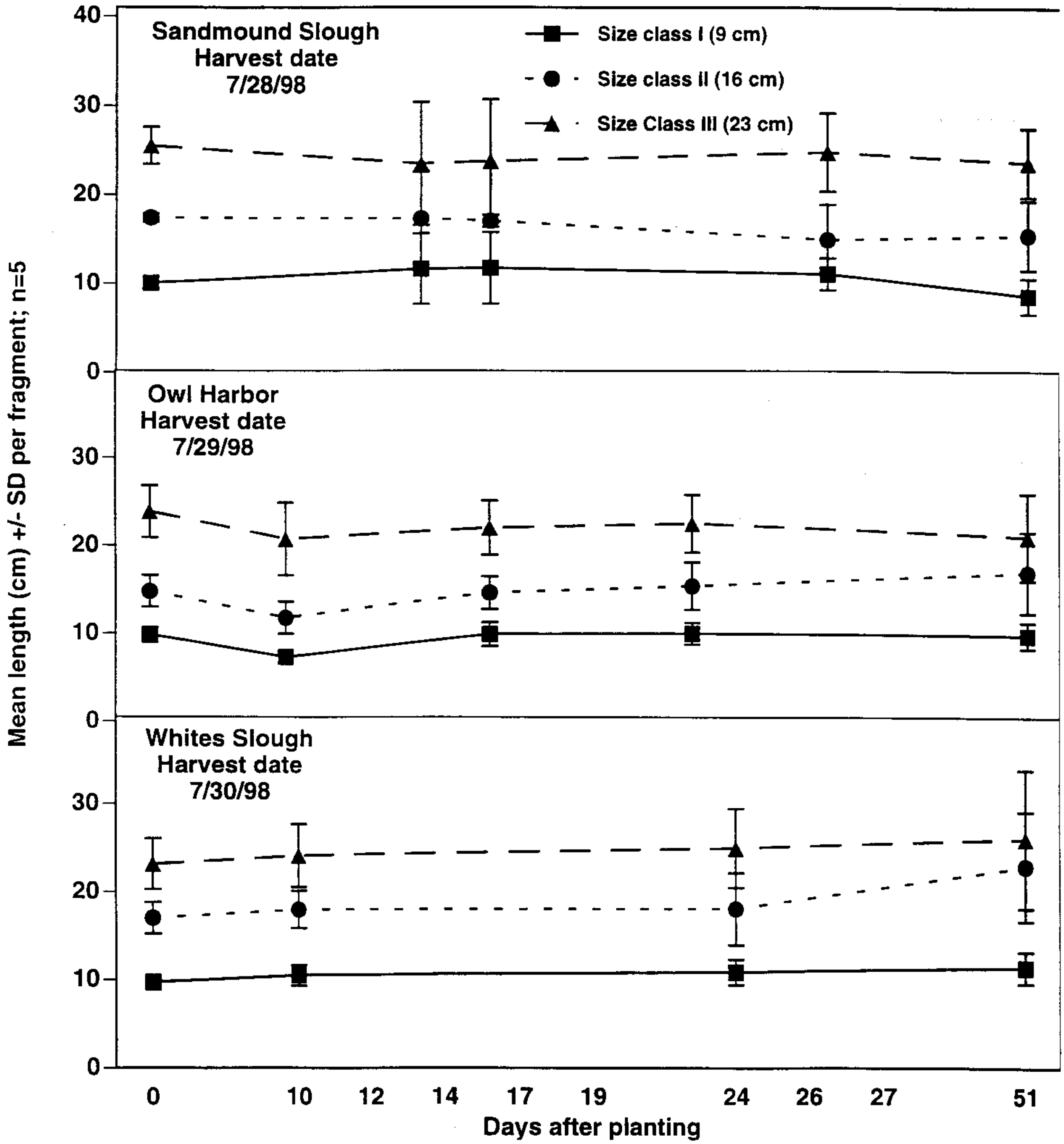

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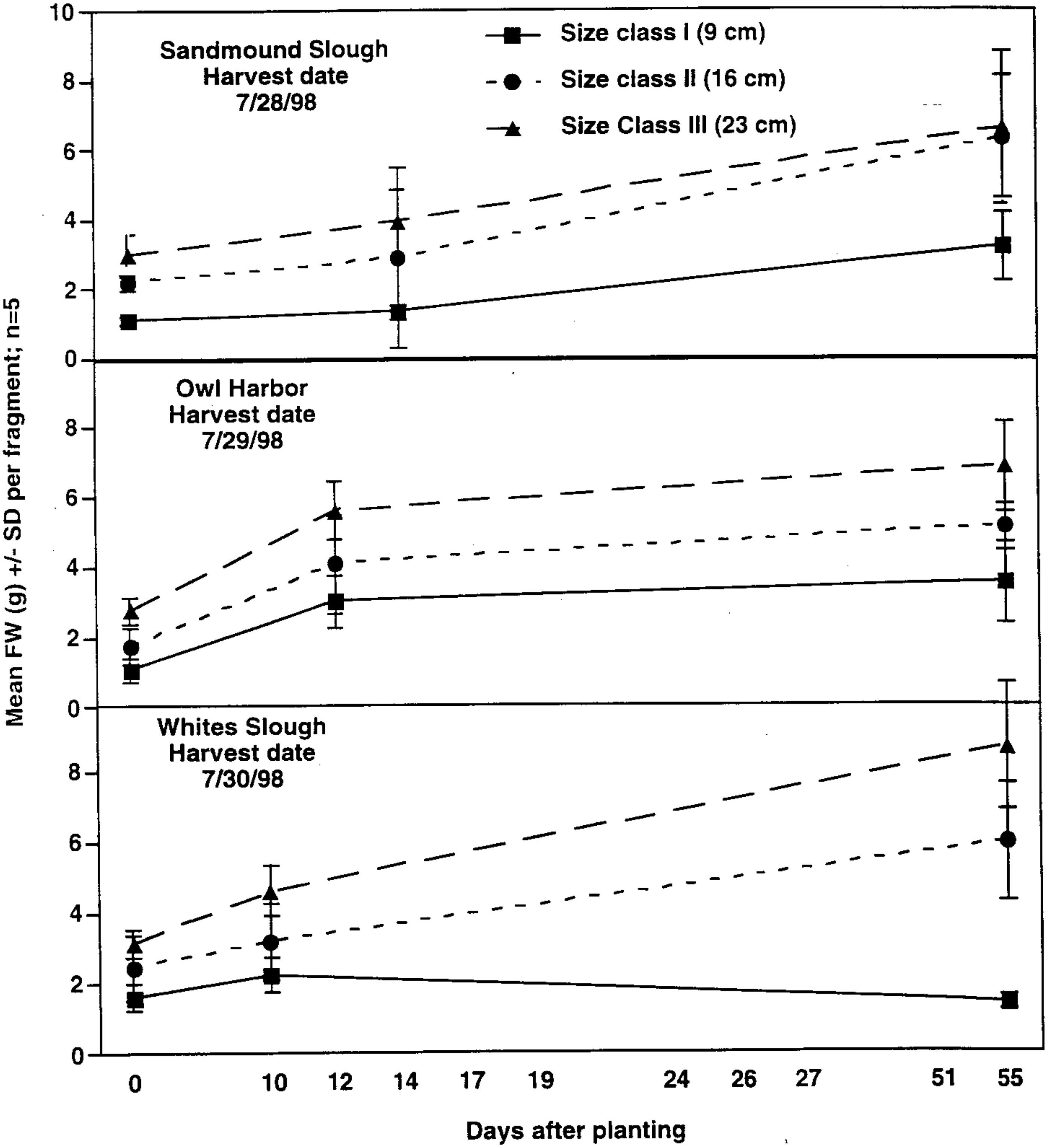

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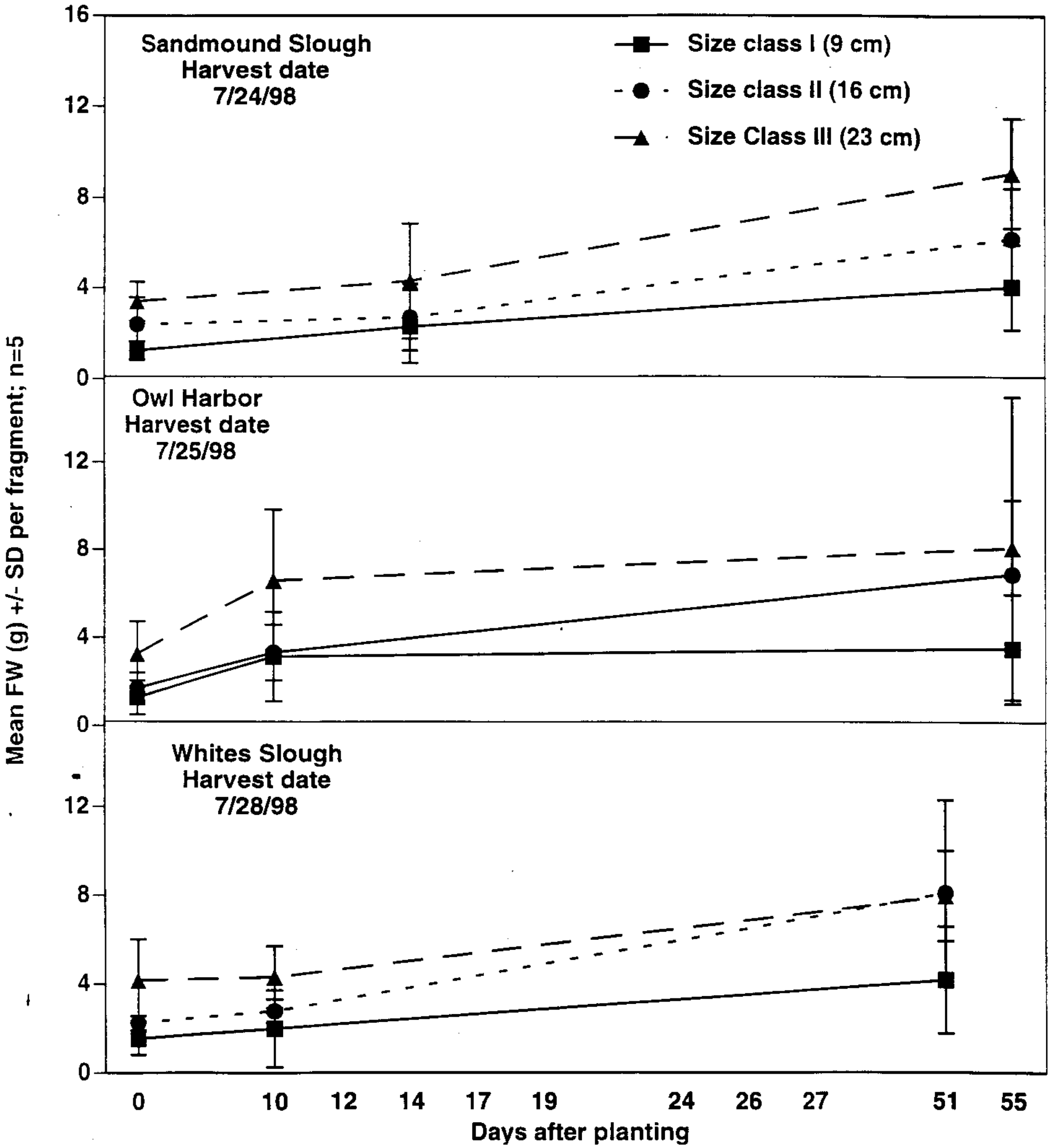

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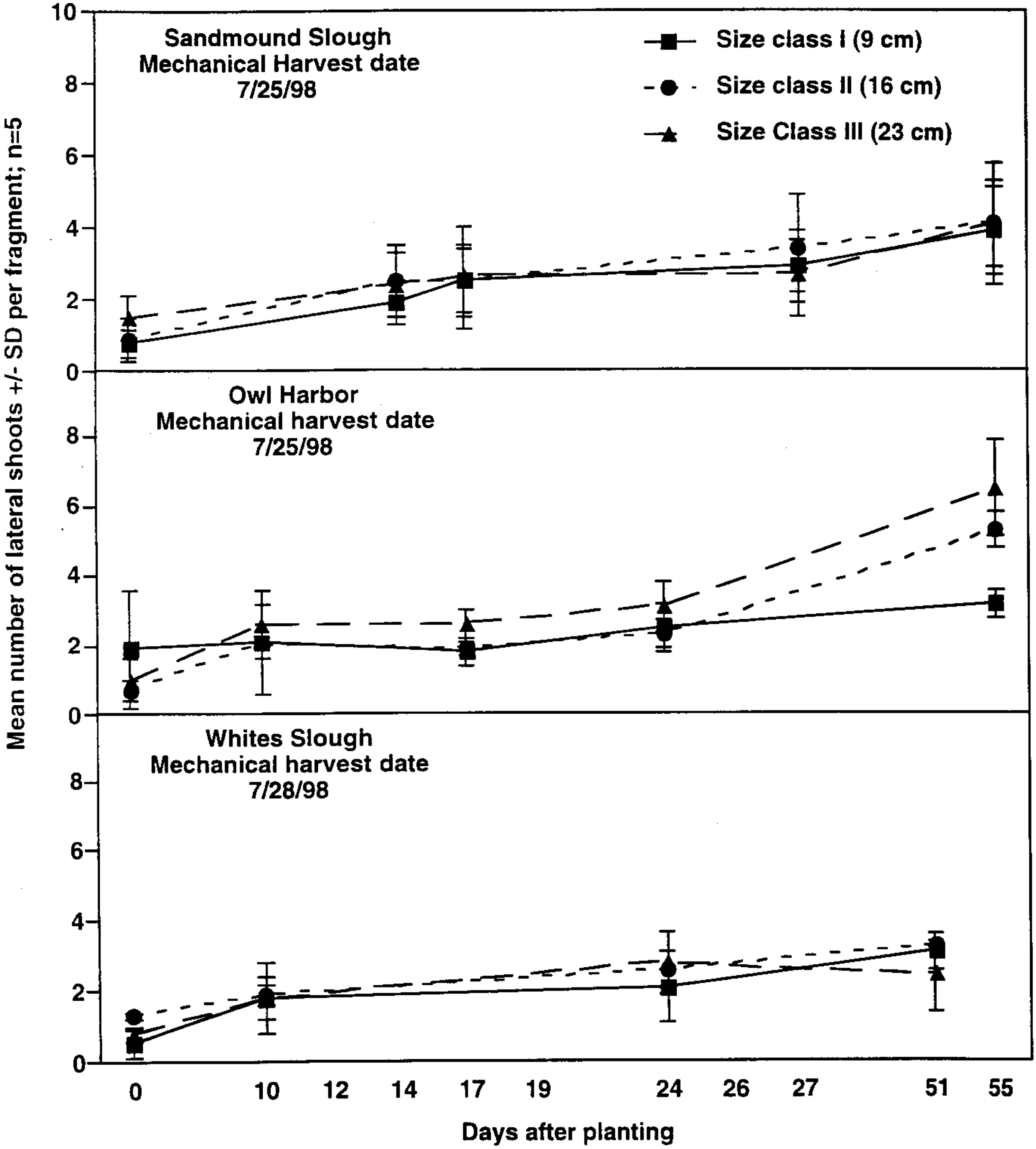

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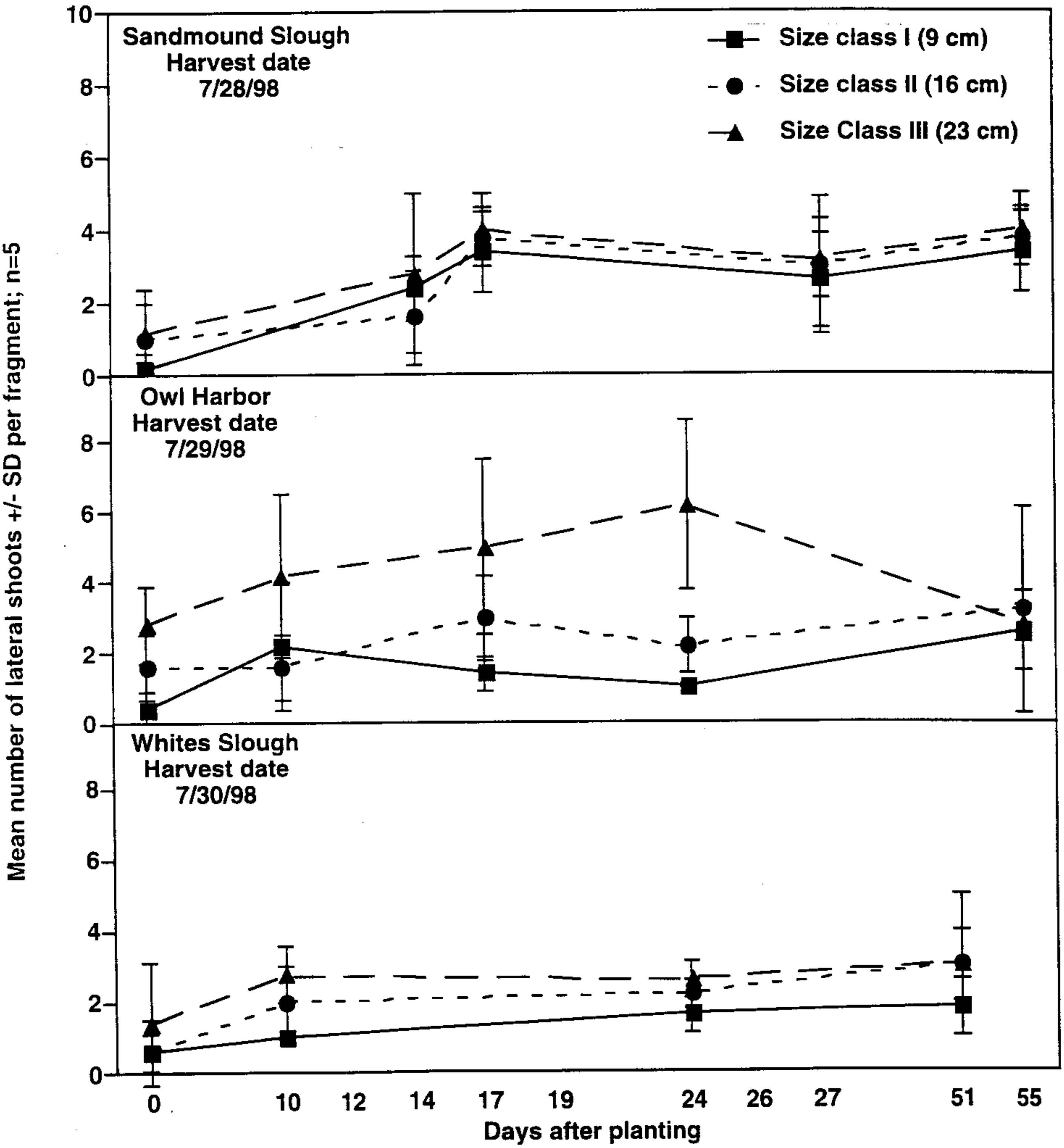

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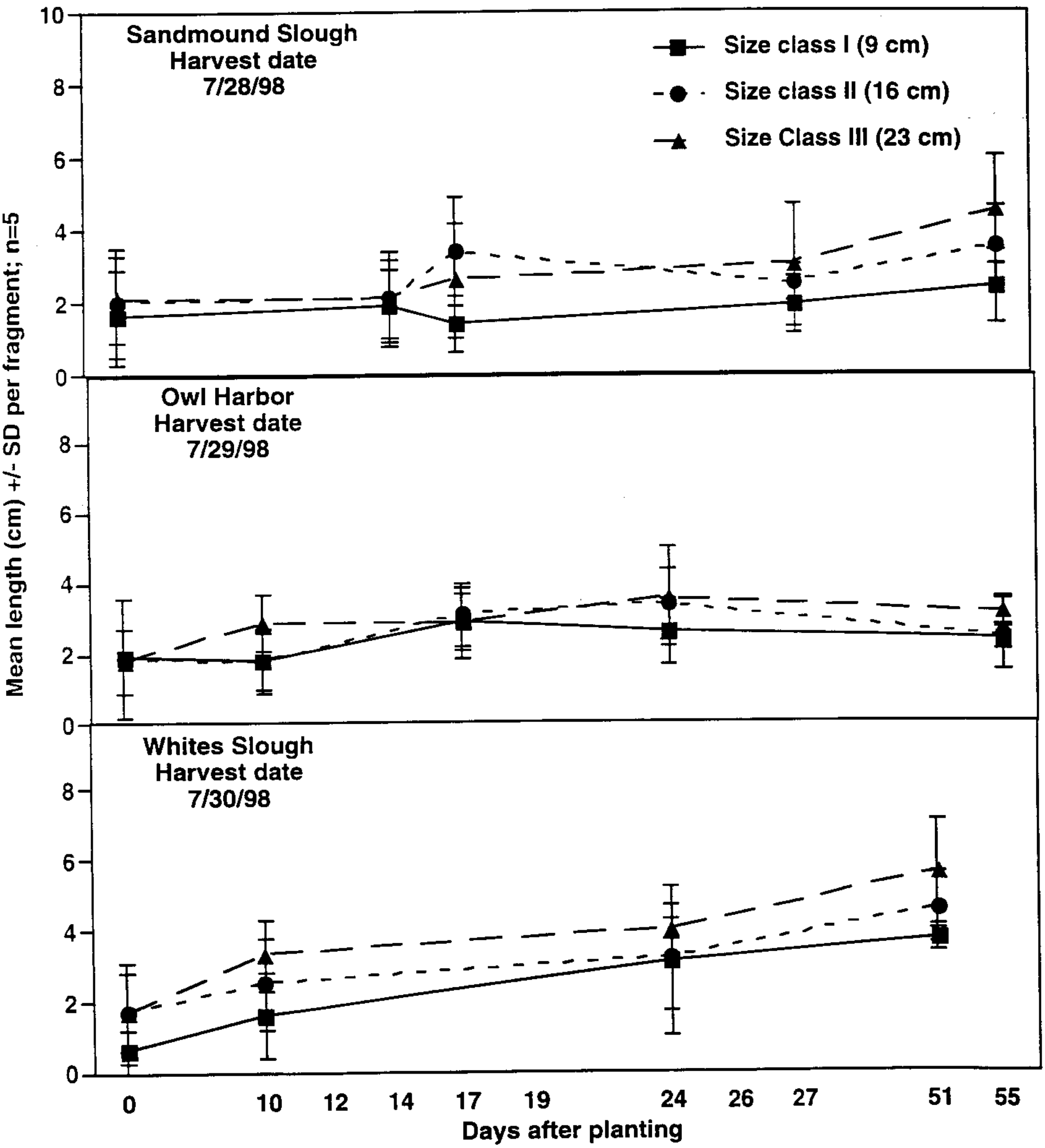

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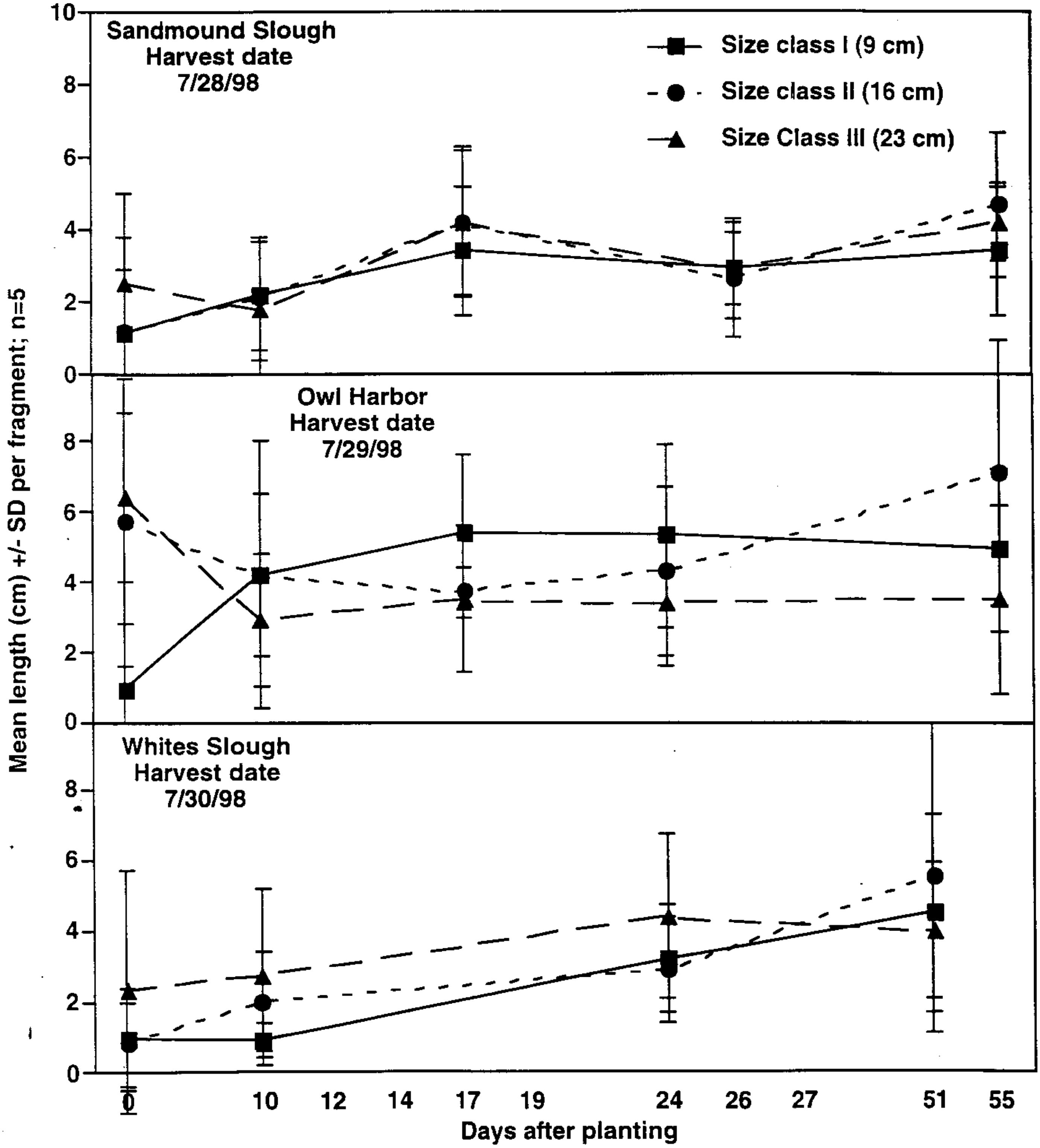

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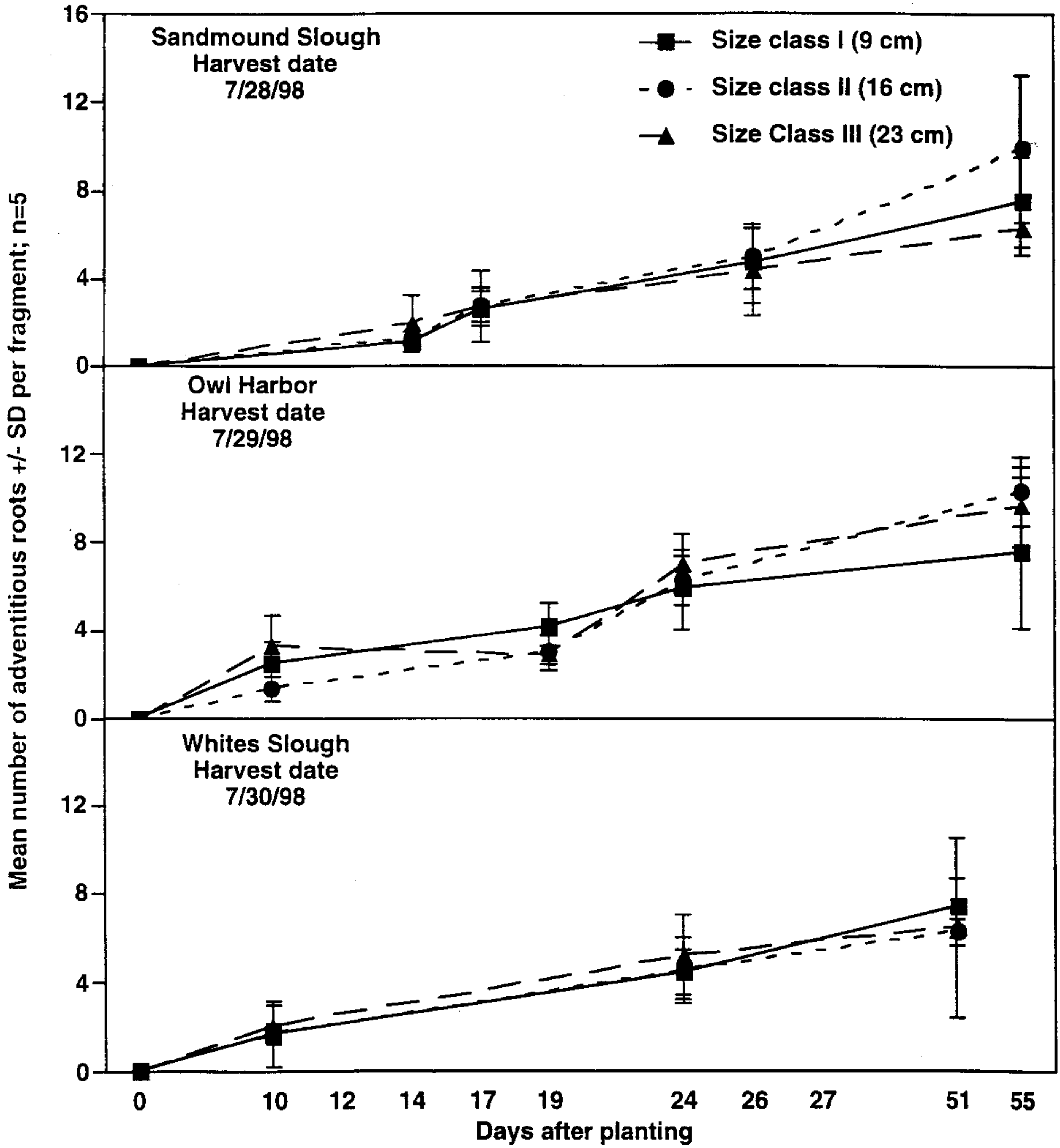

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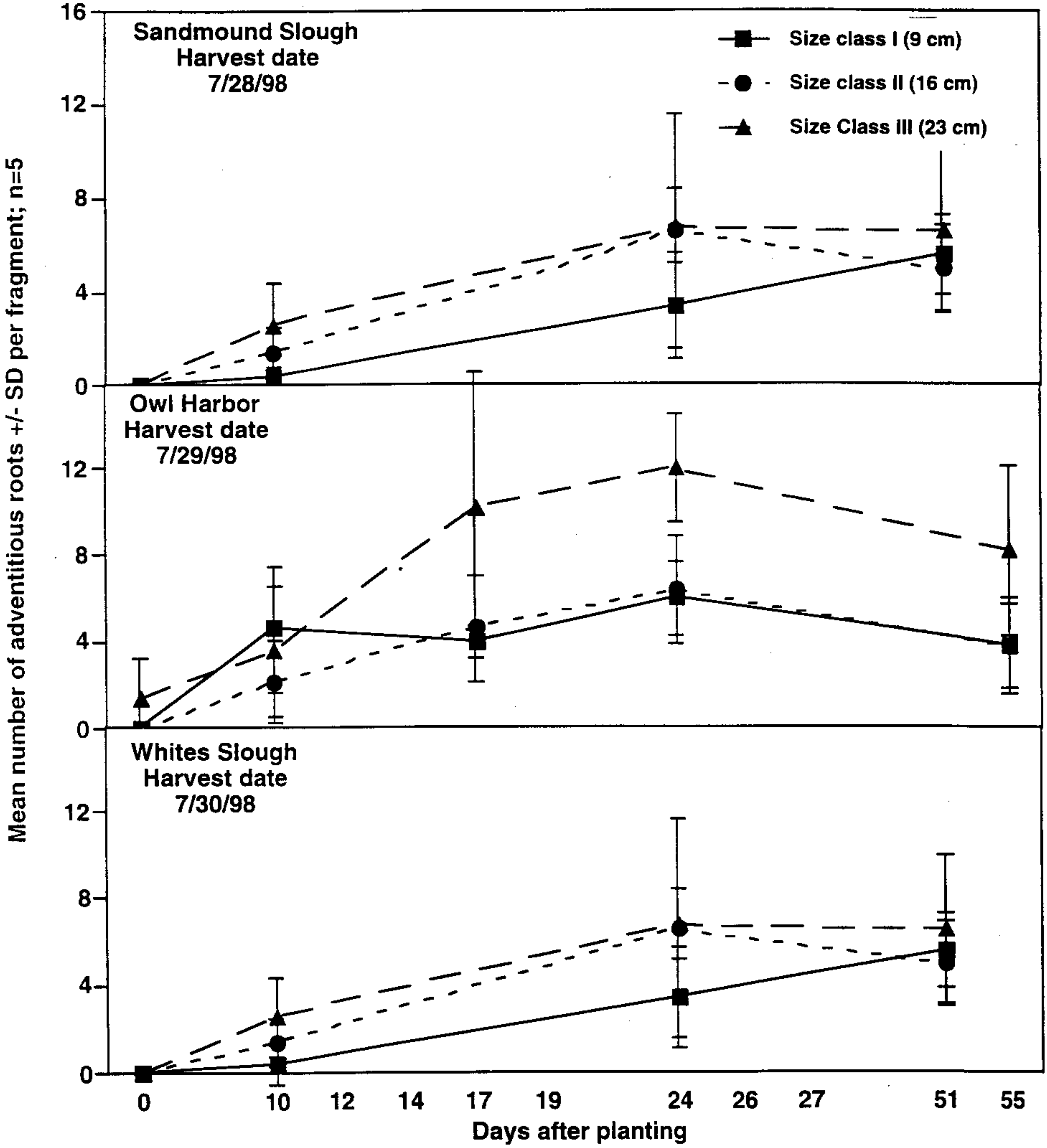

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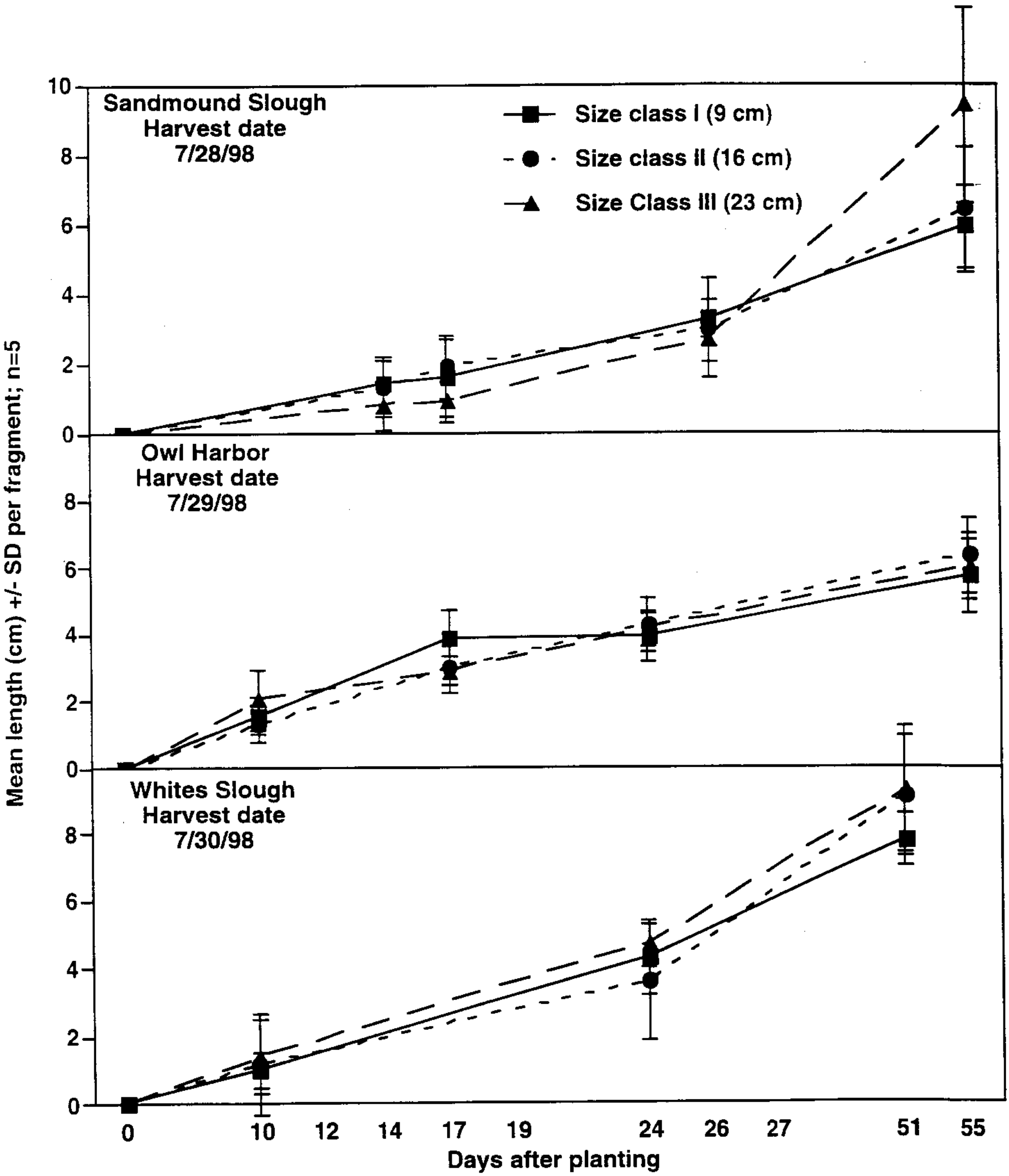

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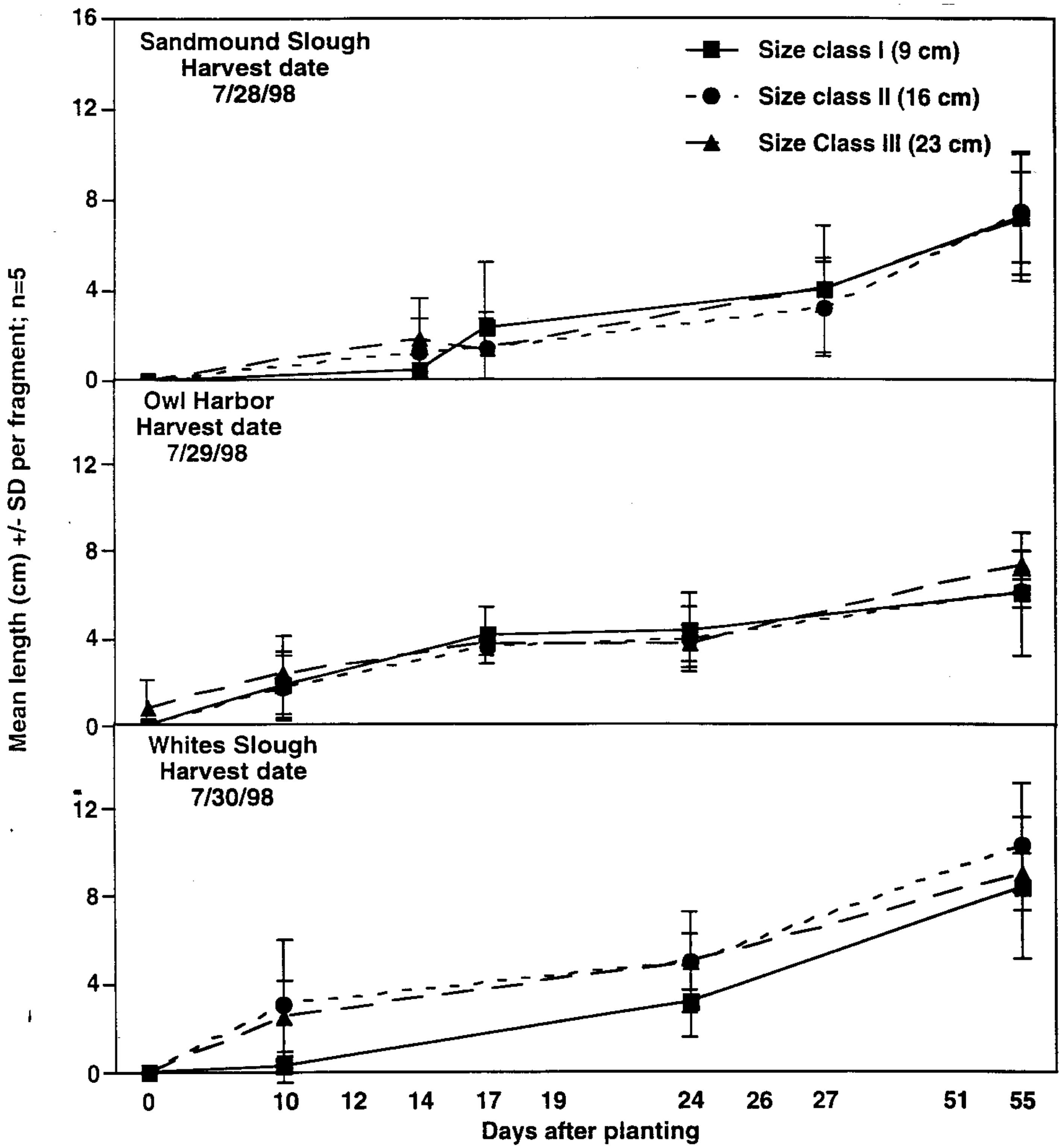

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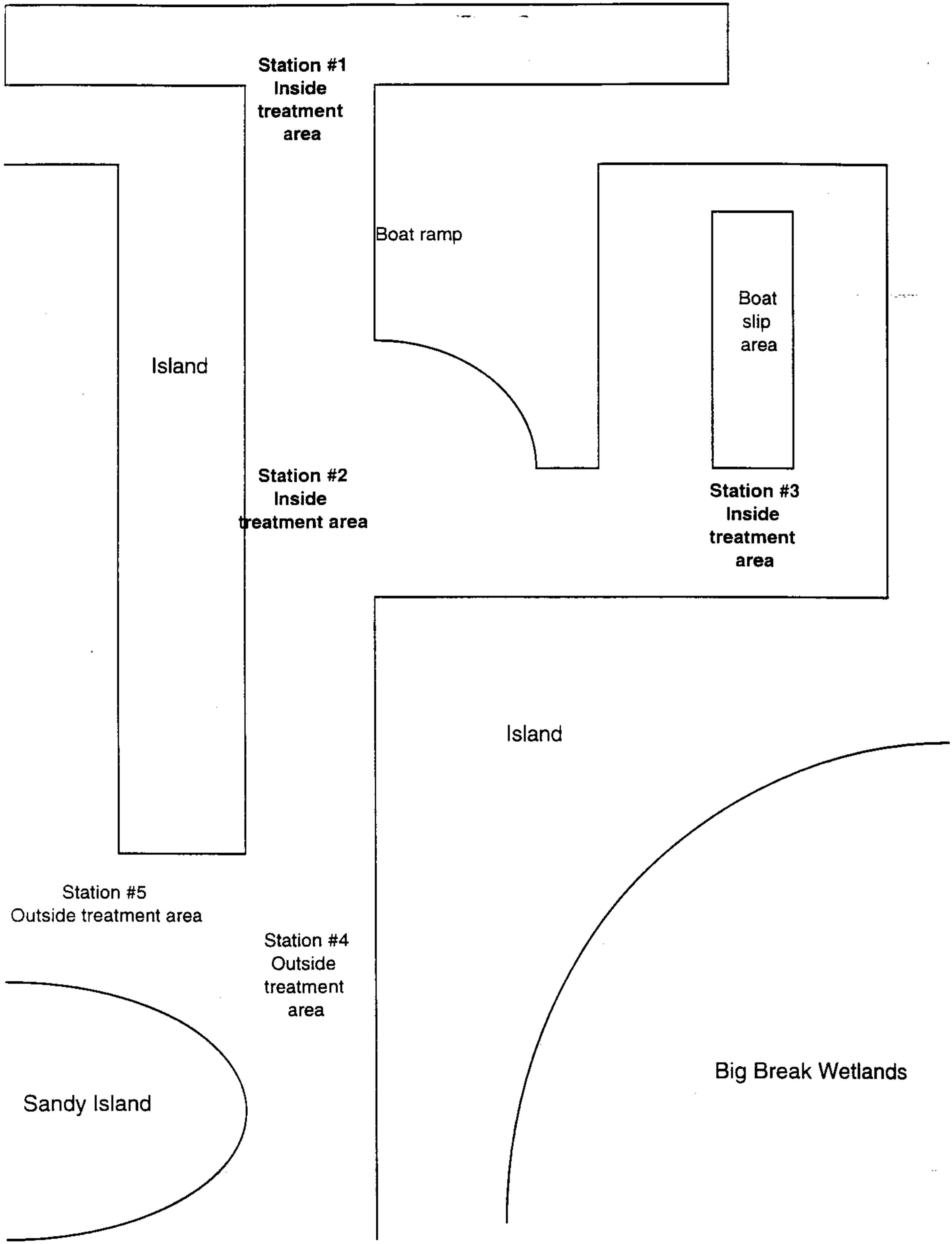

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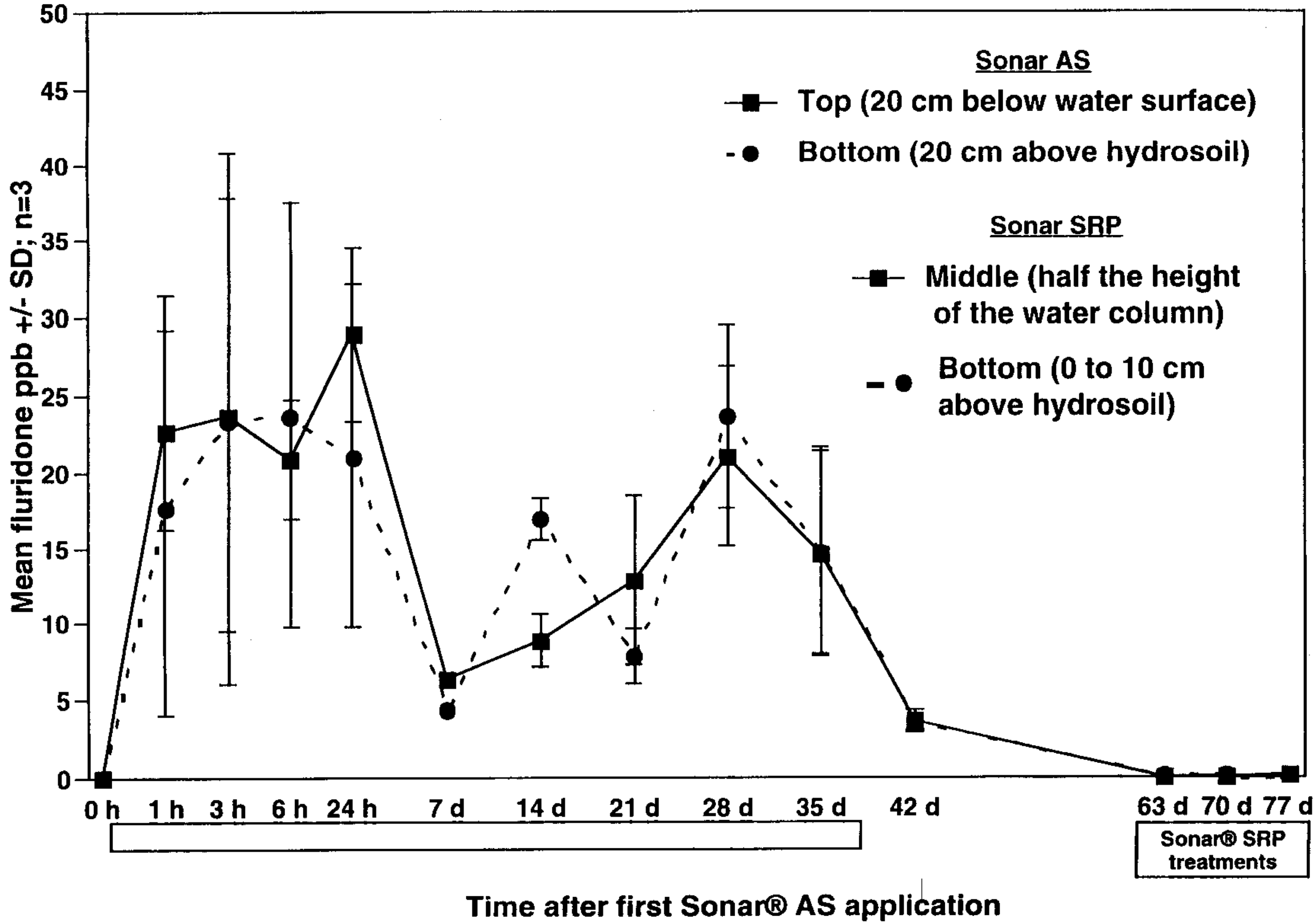

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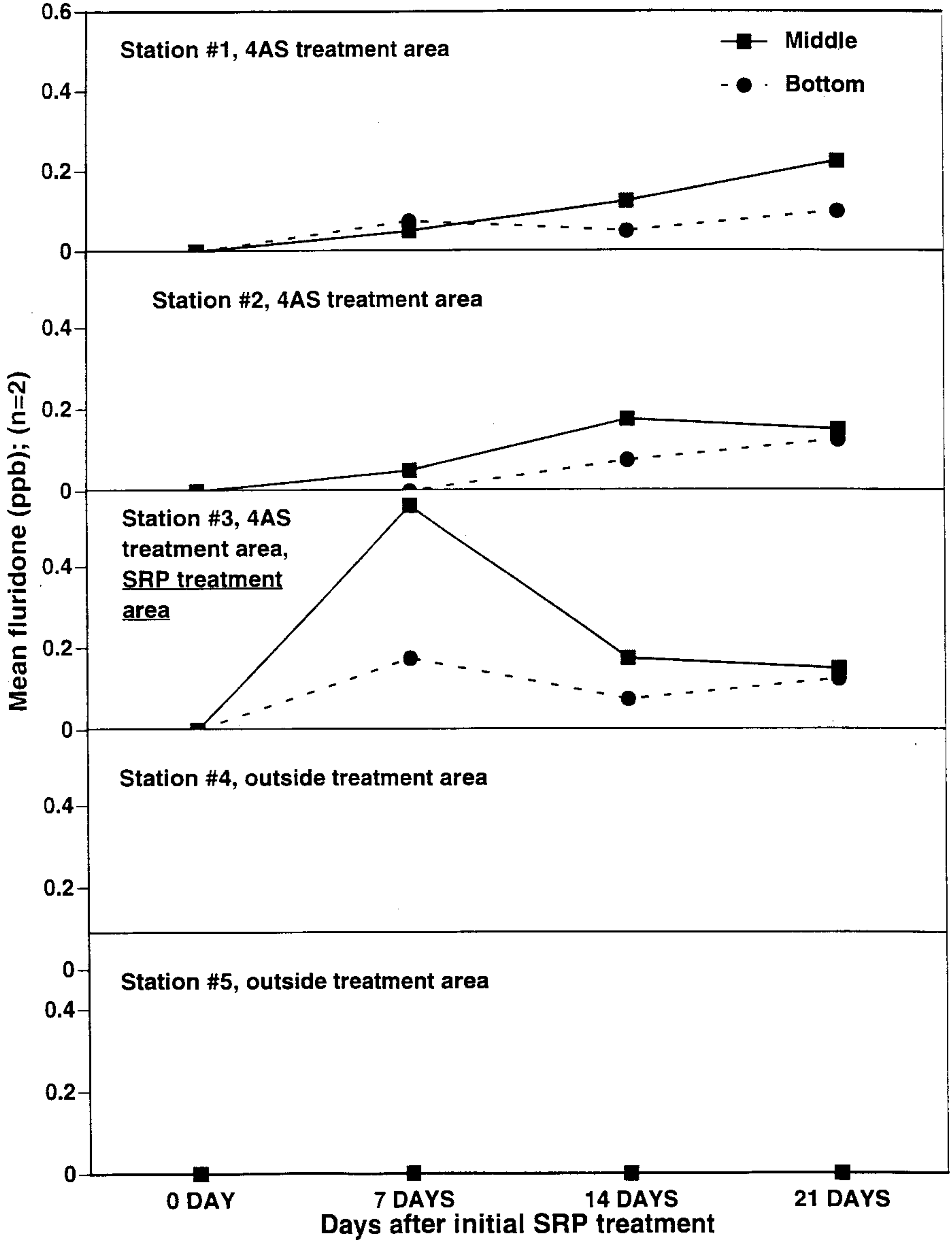

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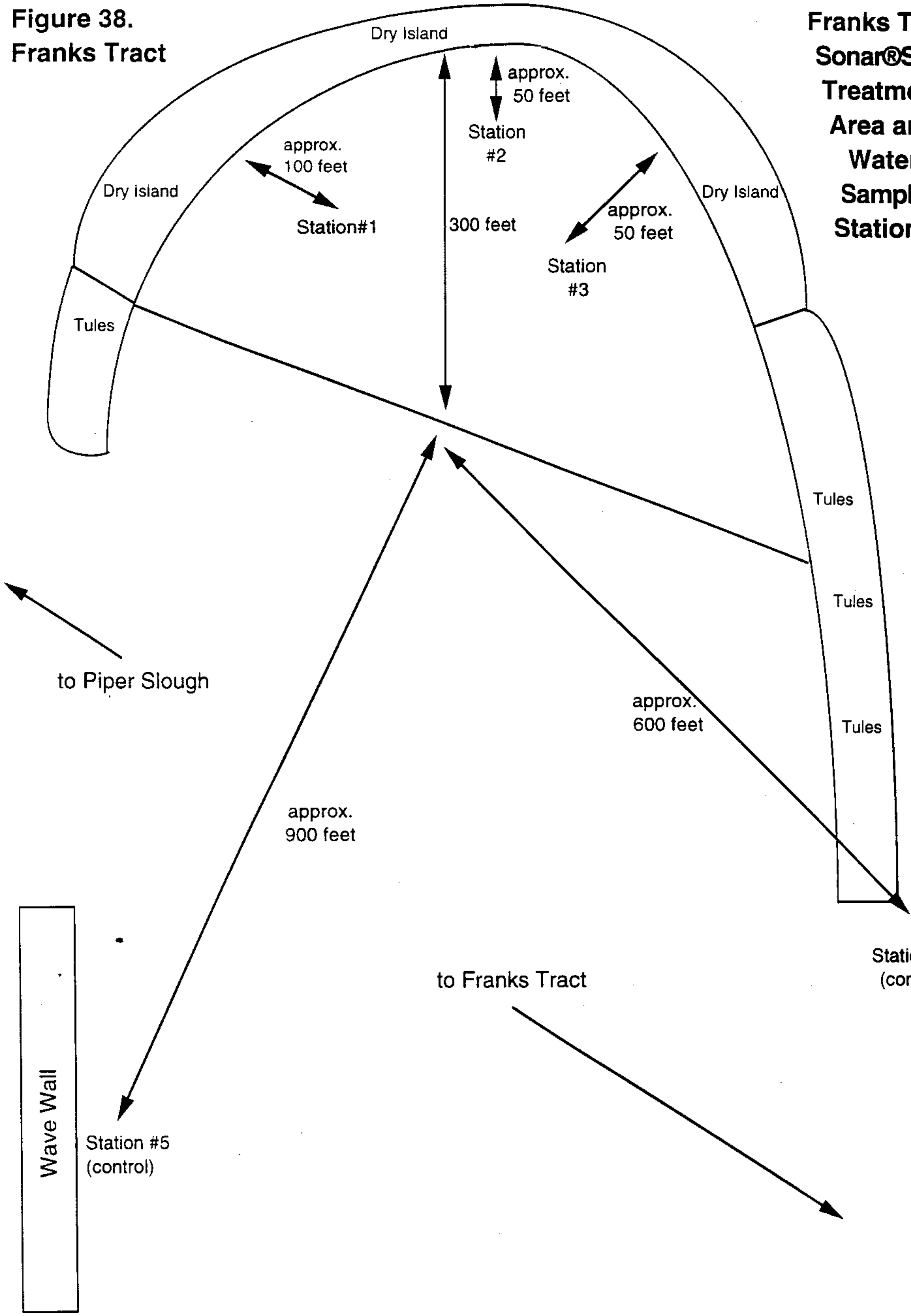

Franks Tract Sonar®SRP Treatment Area and Water Sample Stations

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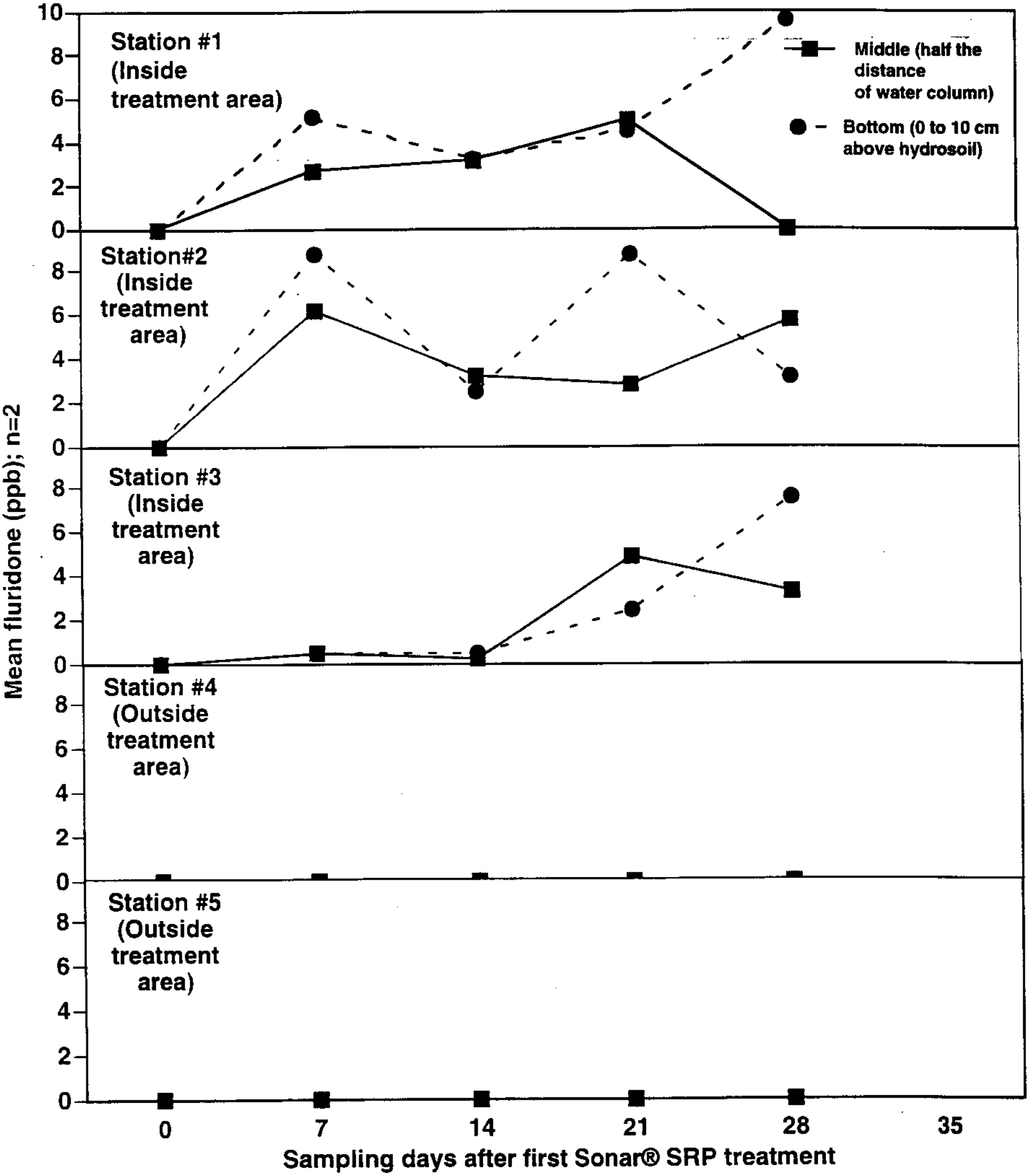

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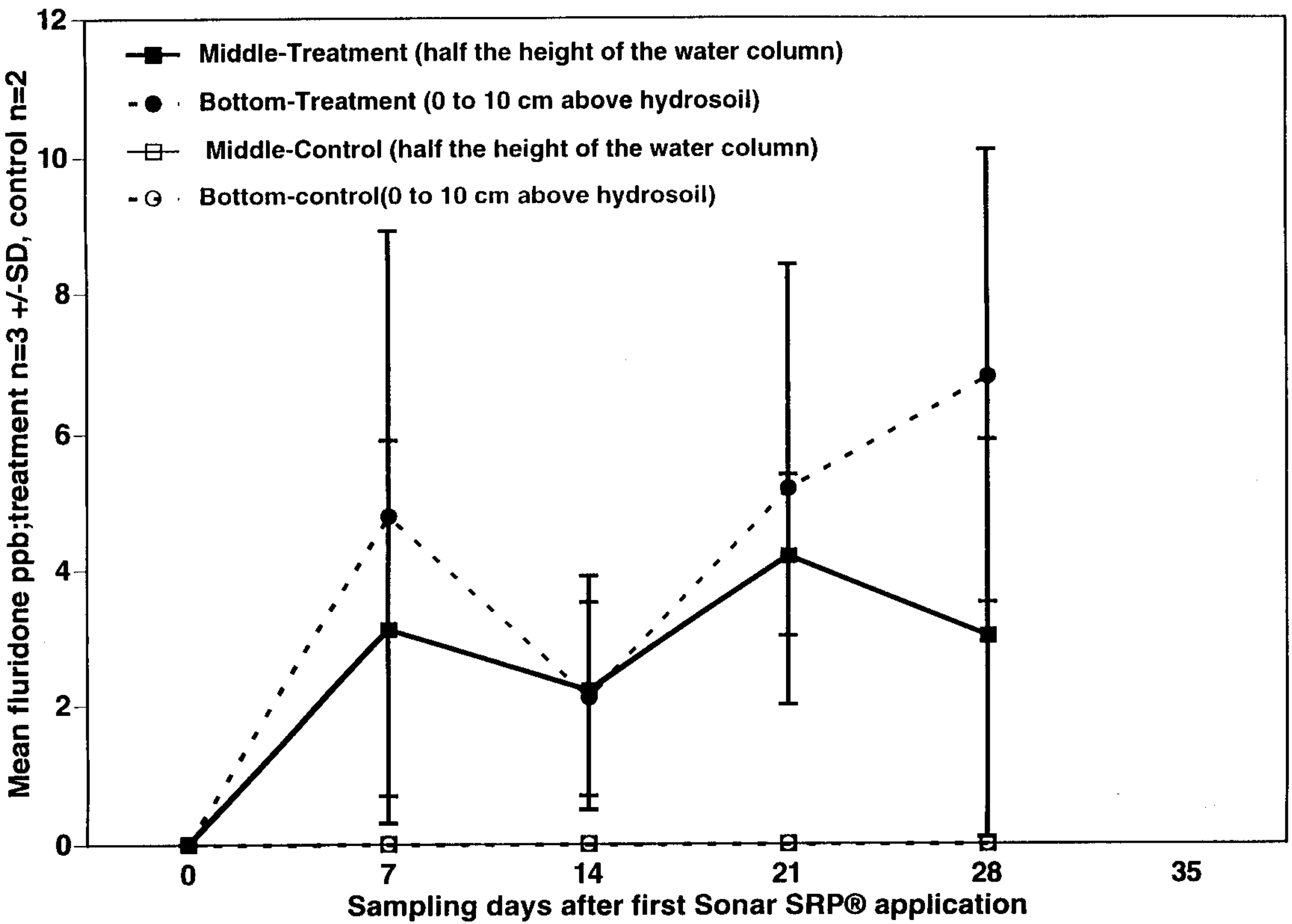

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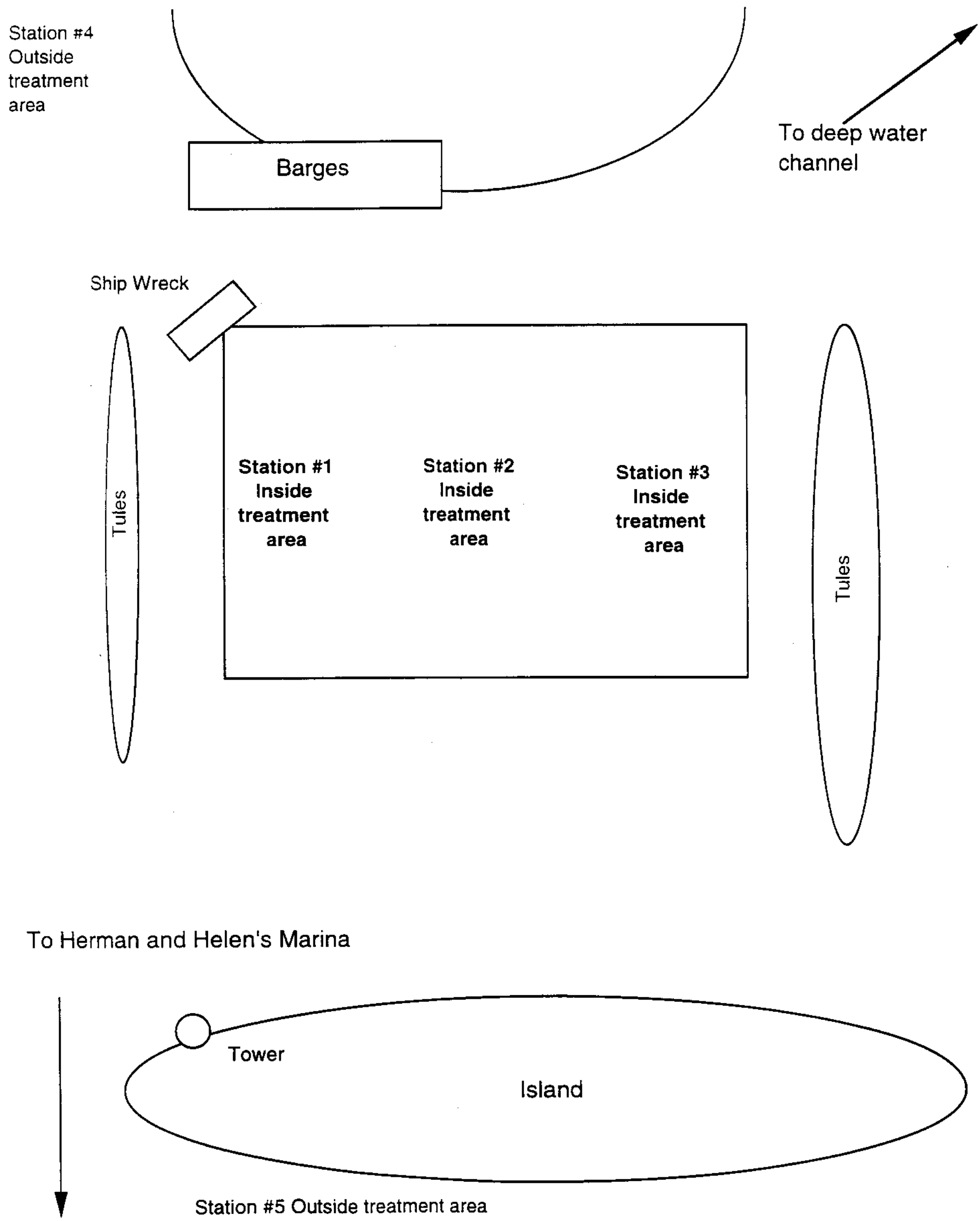

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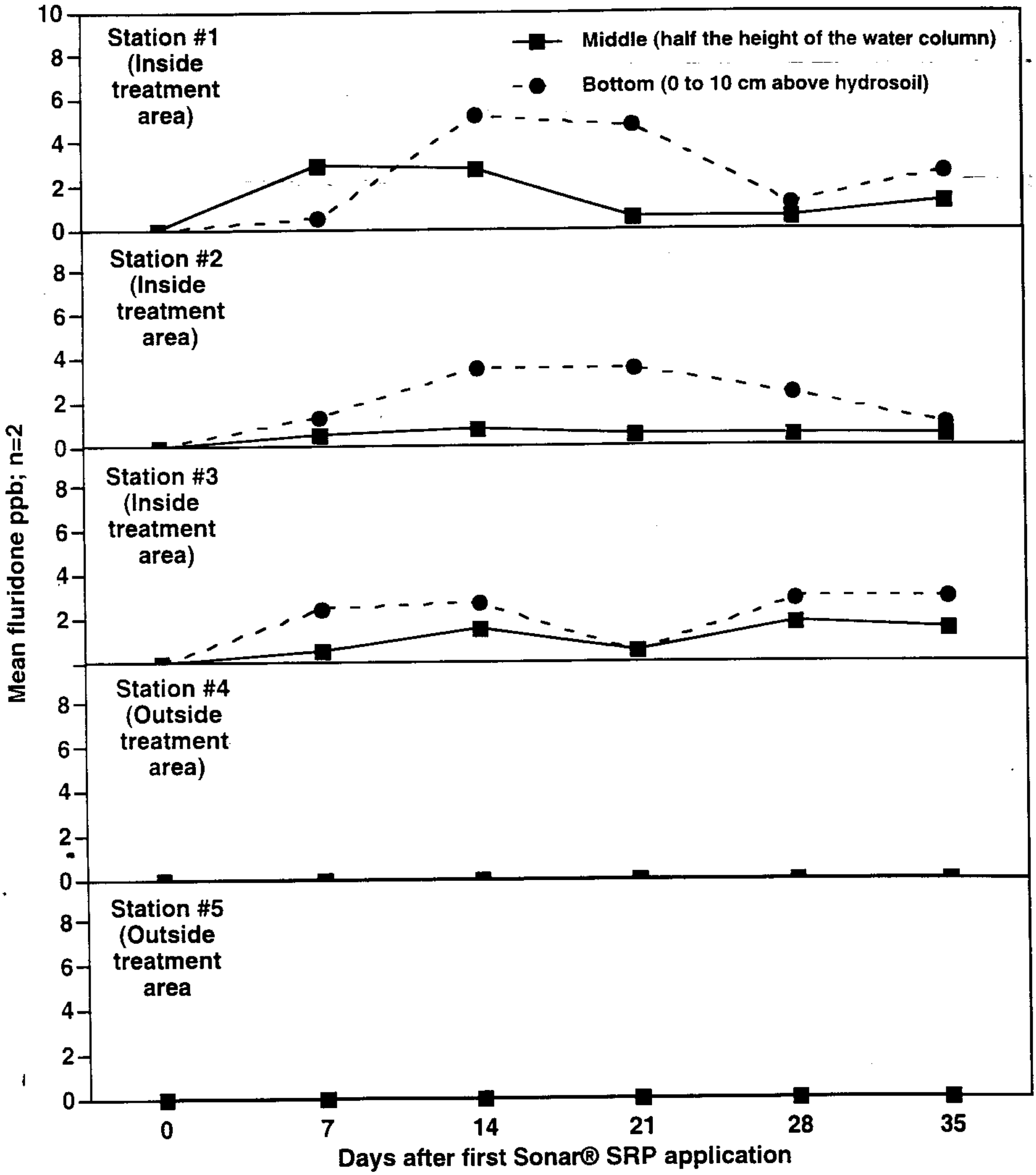


Figure 43. Fluridone dissipation in treatment and control areas of Venice Island after Sonar® SRP applications two times per week for six weeks. First application was 6/19/98.

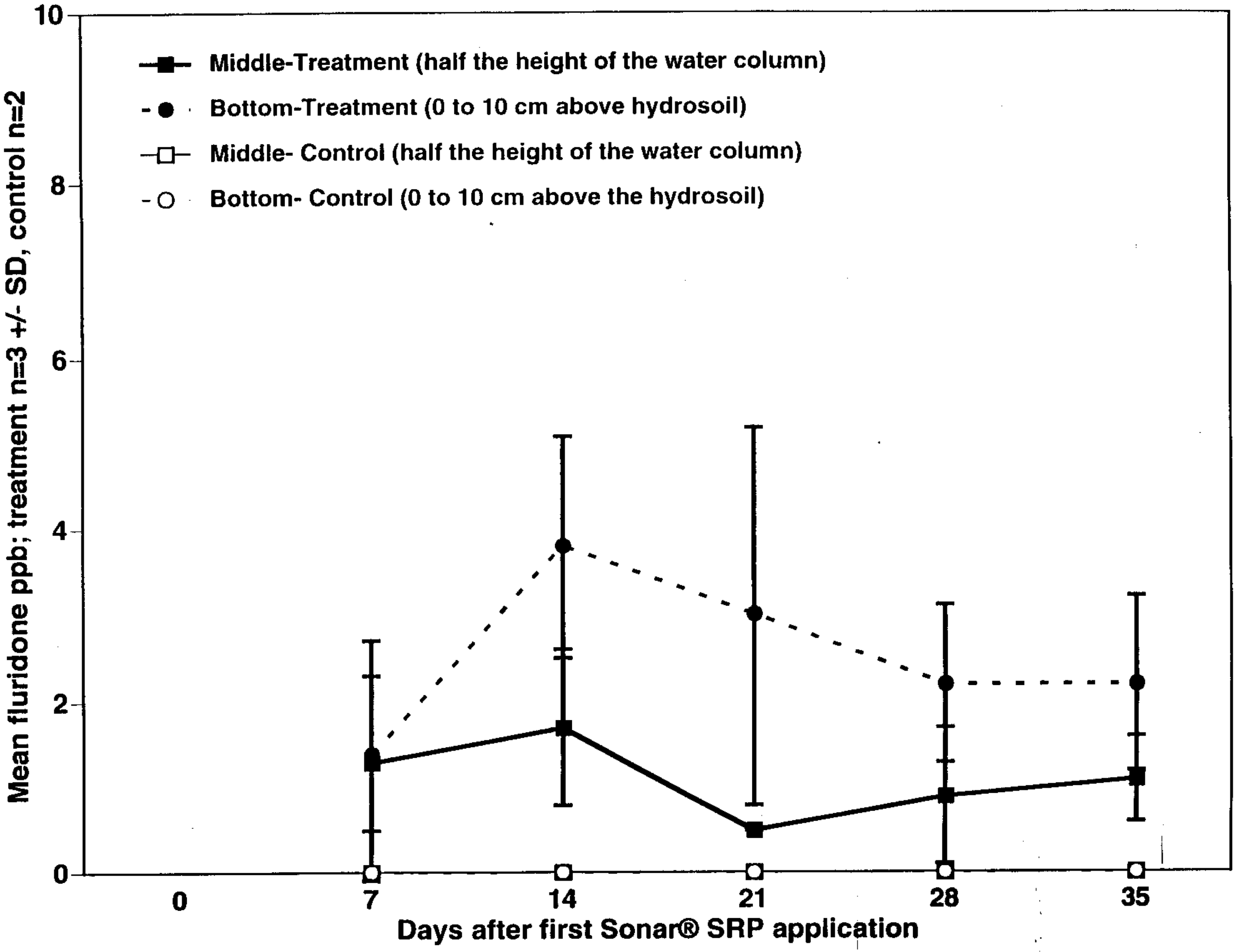
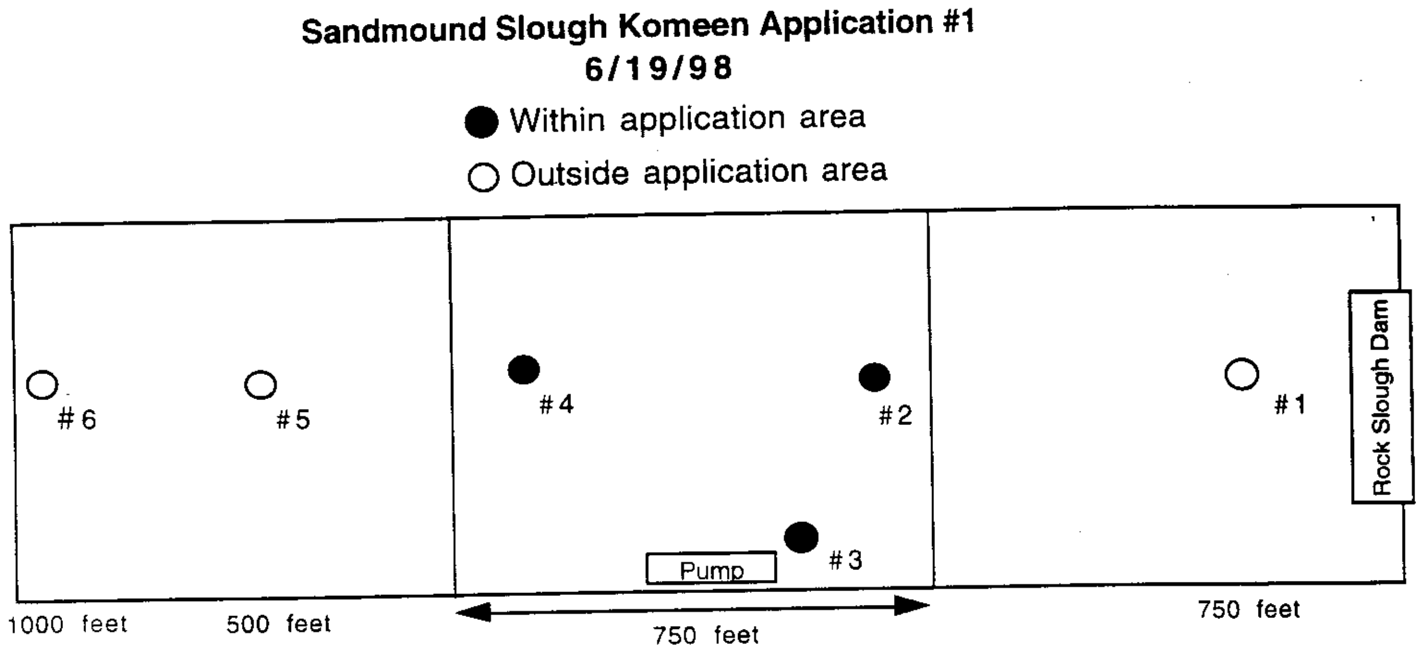


Figure 44. Water sample collection stations in Sandmound Slough for Cu samples after Komeen® applications



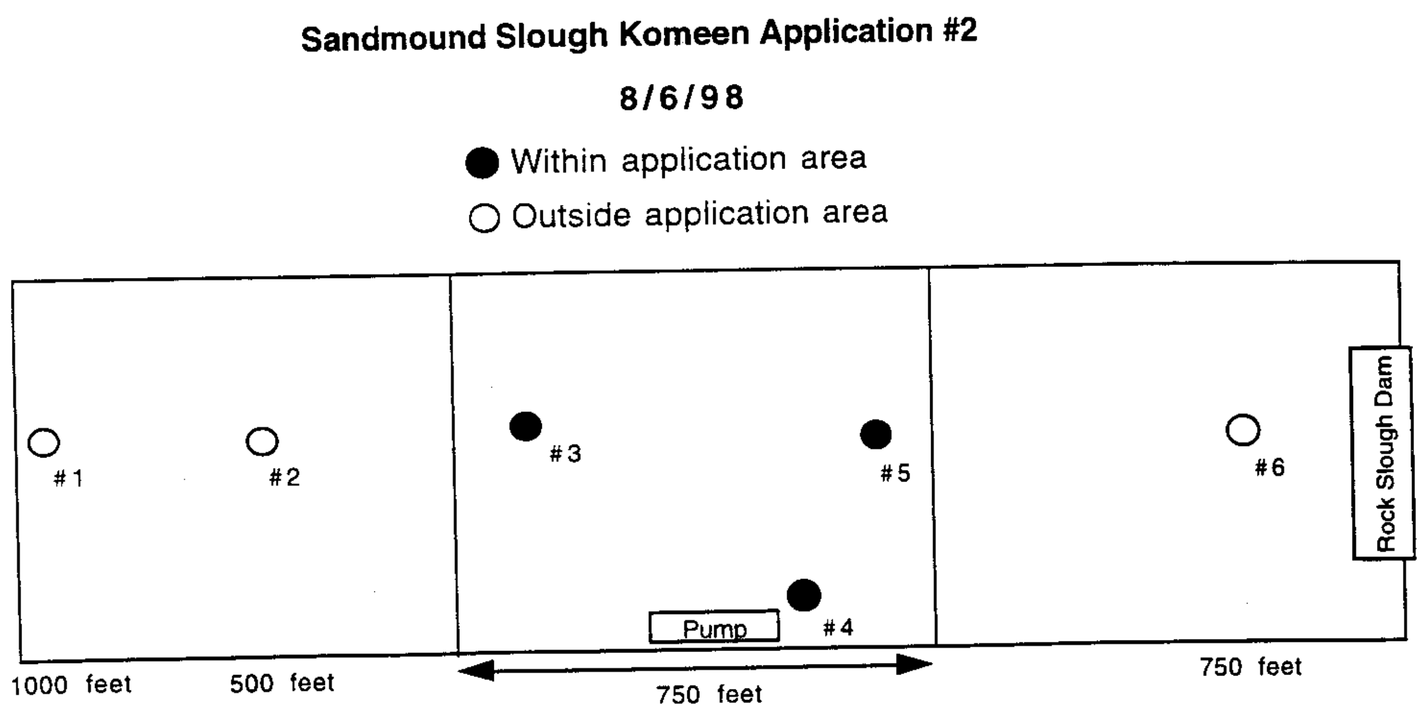


Figure 45. Total Cu dissipation at Sandmound Slough 24 hours after Komeen® application on 6/19/98

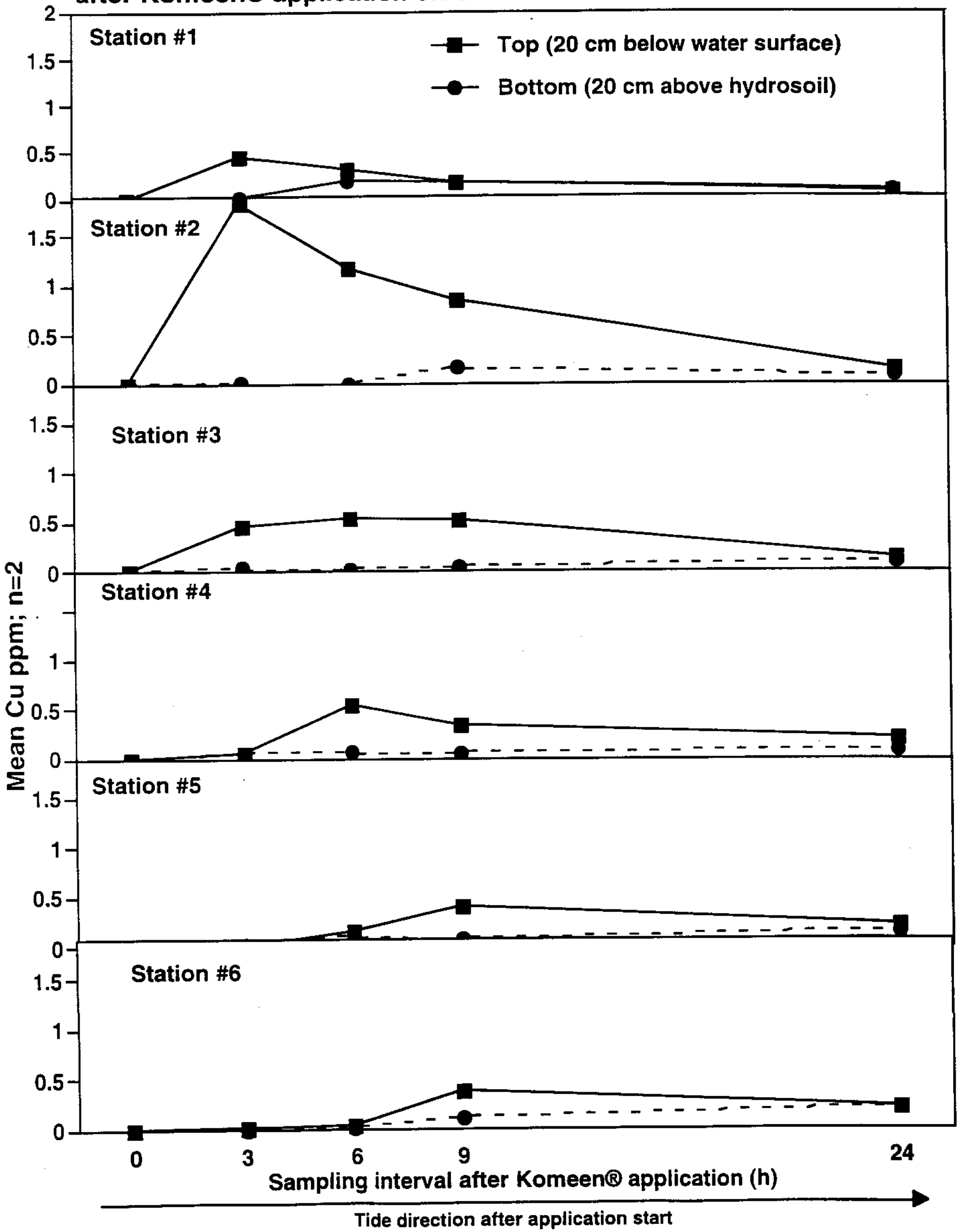


Figure 46. Total Cu dissipation at Sandmound Slough 24 hours after Komeen® application on 8/6/98

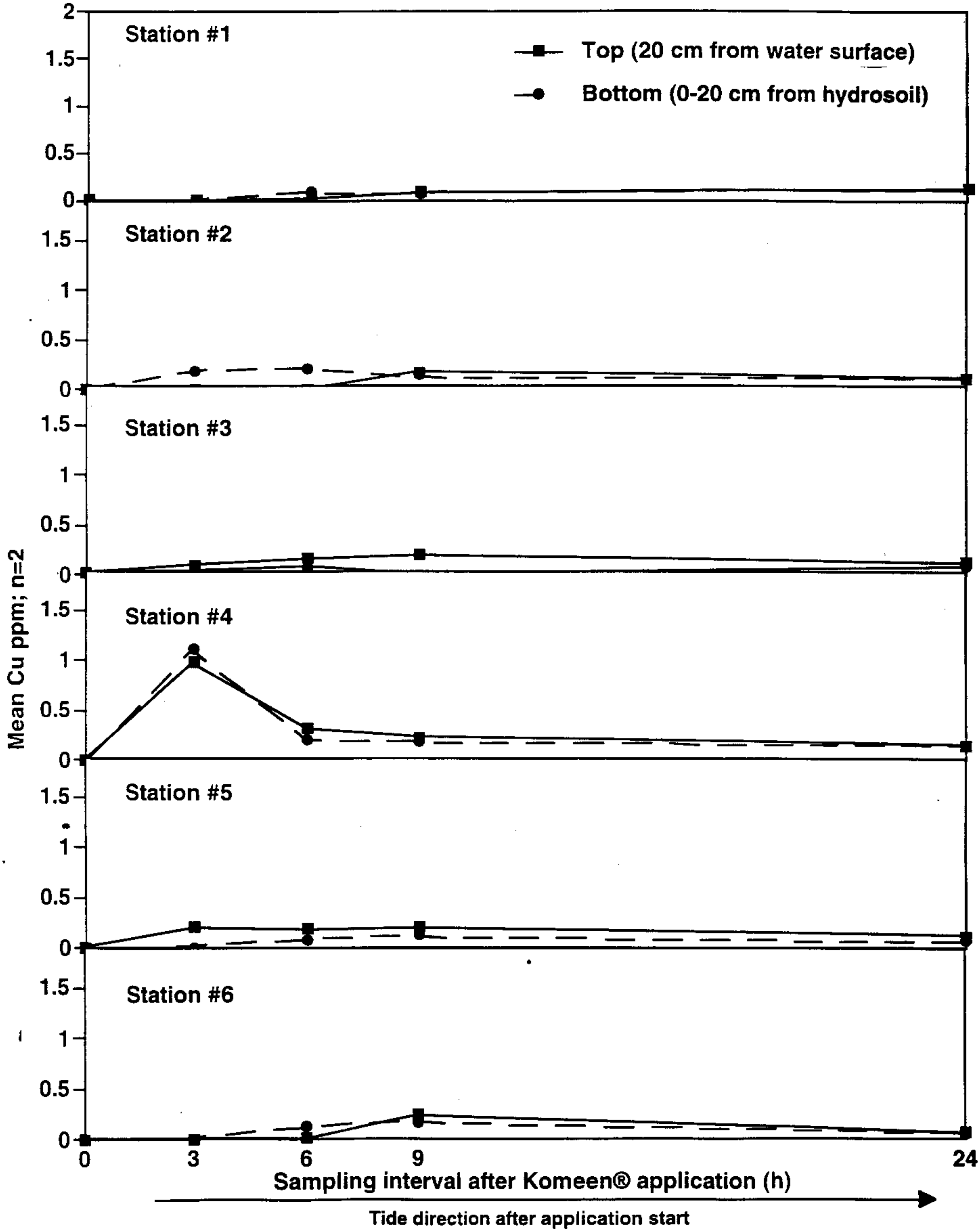
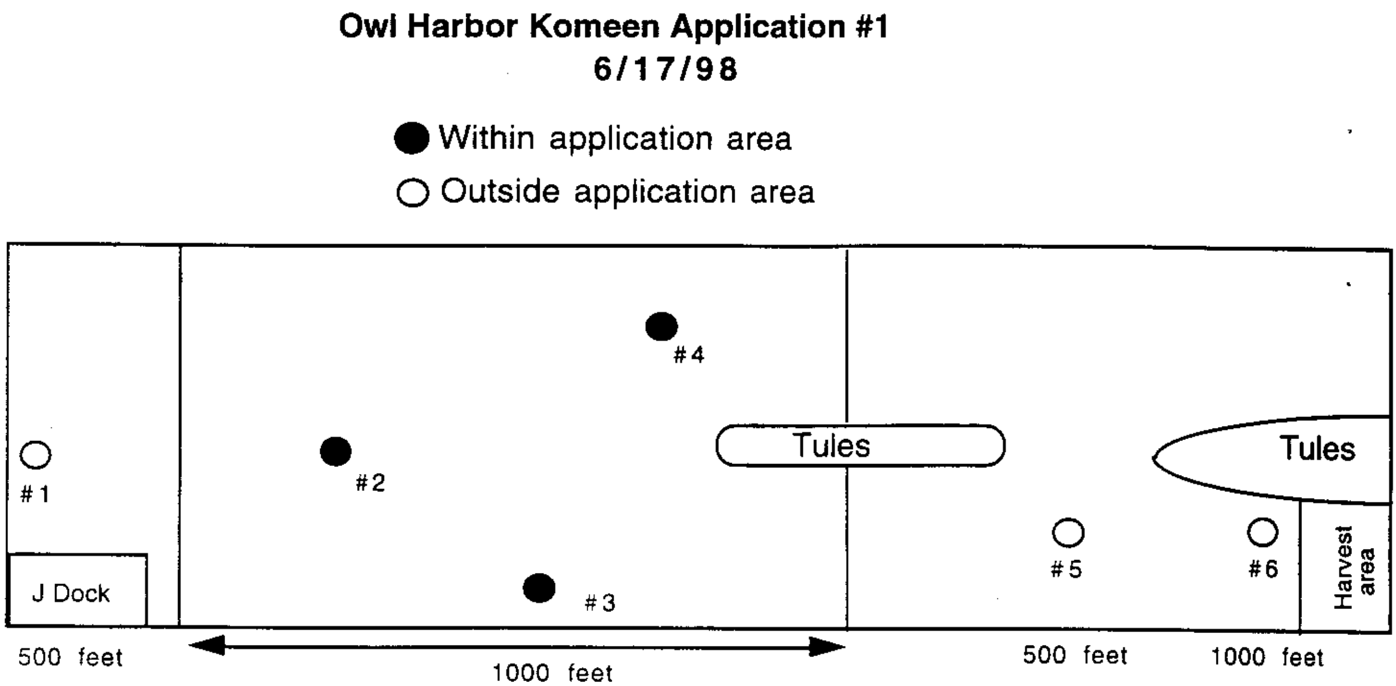


Figure 47. Water sample collection stations in Owl Harbor for Cu samples after Komeen® applications



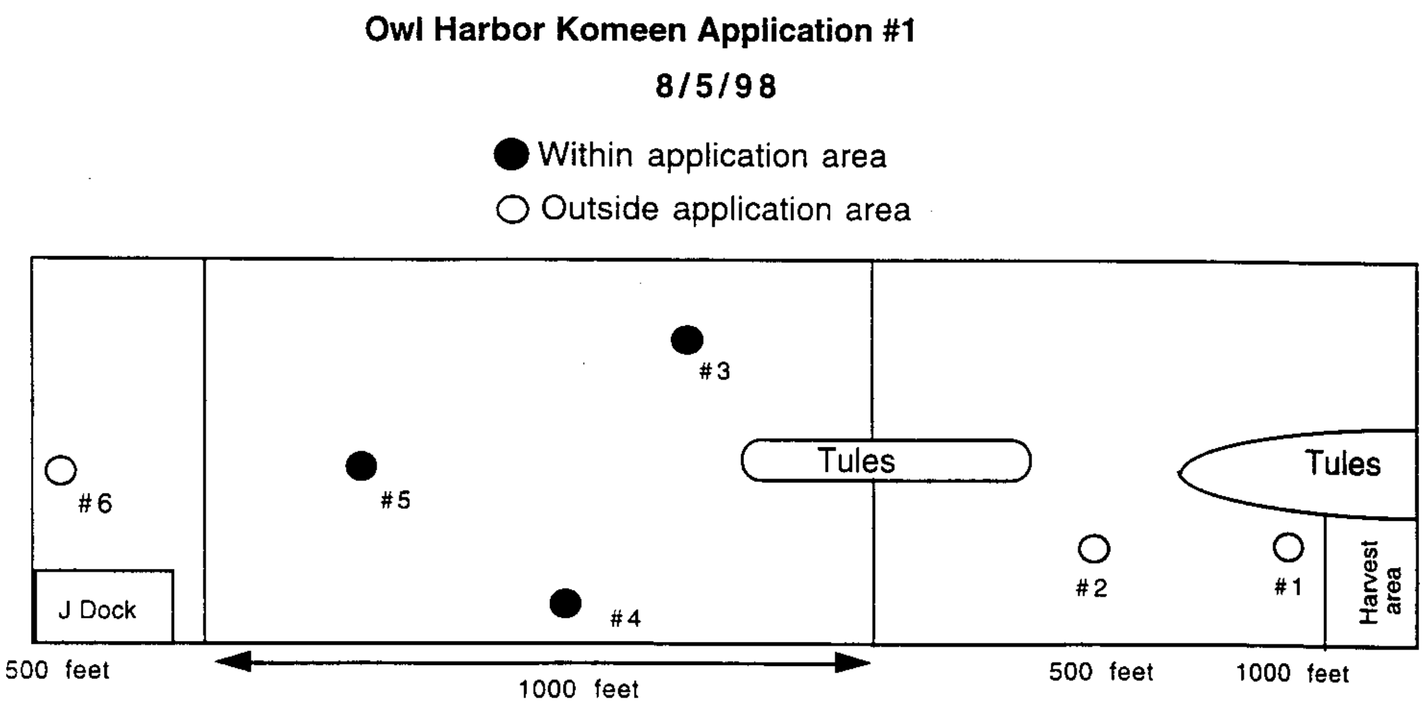


Figure 48. Total Cu dissipation at Owl harbor 24 hours after Komeen® application on 6/17/98

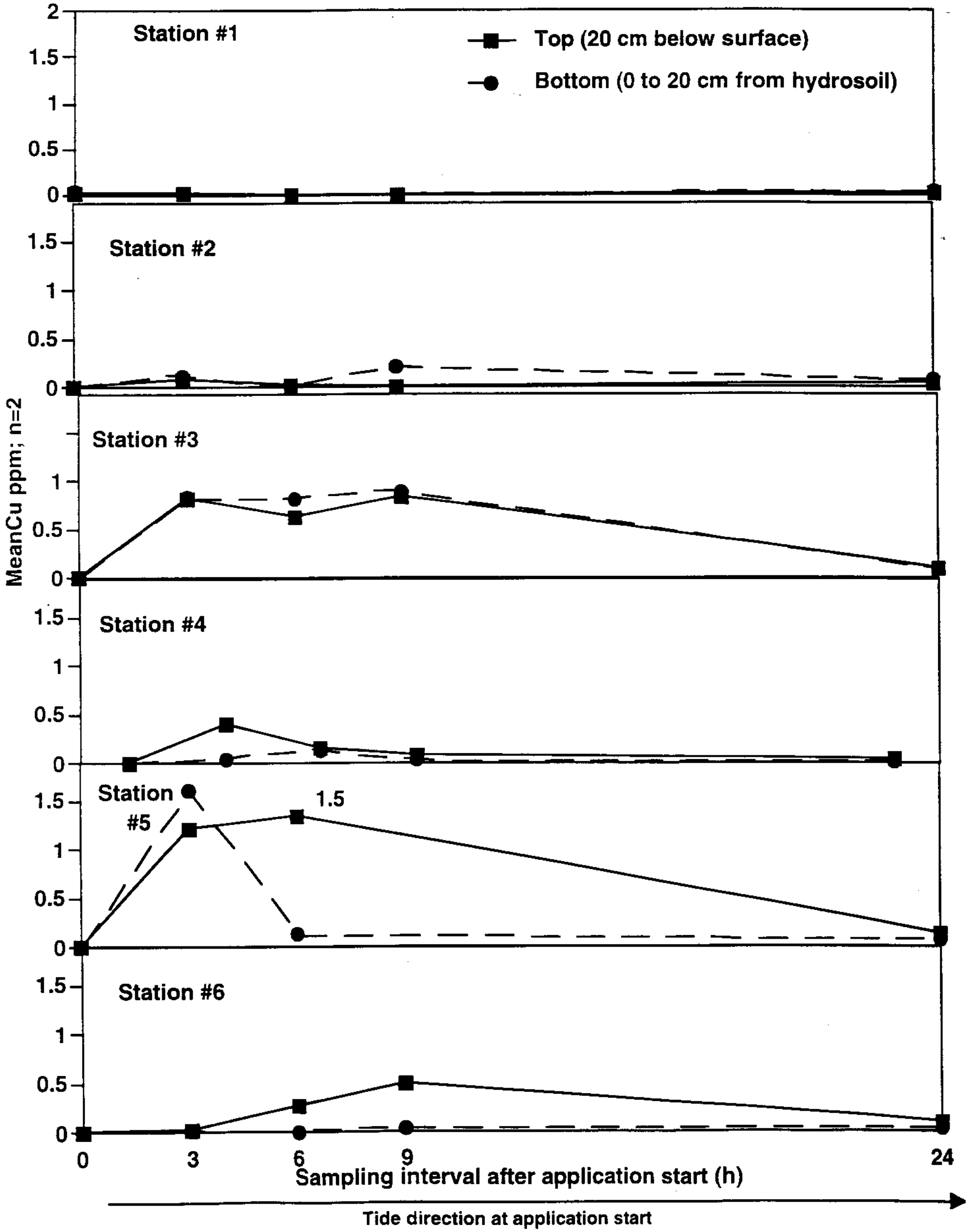


Figure 49. Total Cu dissipation at Owl Harbor 24 hours after Komeen® application on 8/5/98

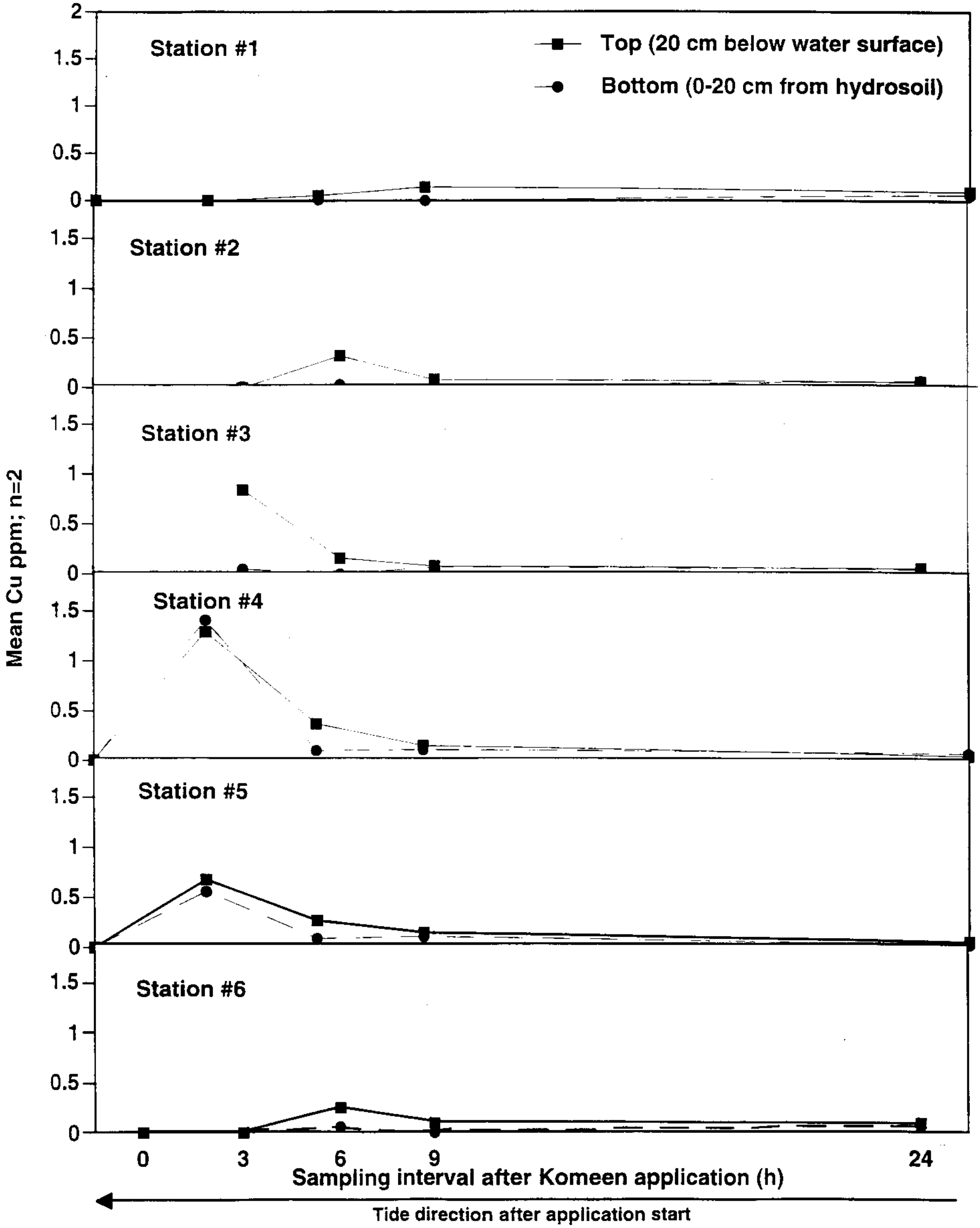
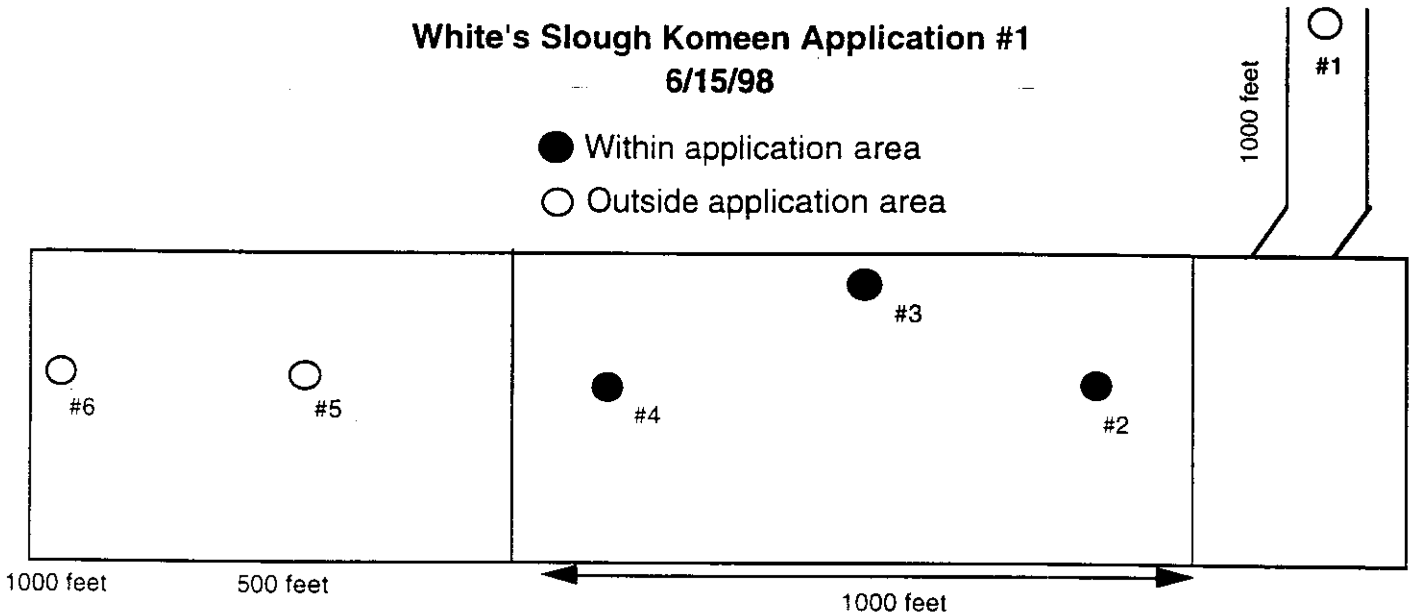


Figure 50. Water sample collection stations in Whites Slough for Cu samples after Komeen® applications



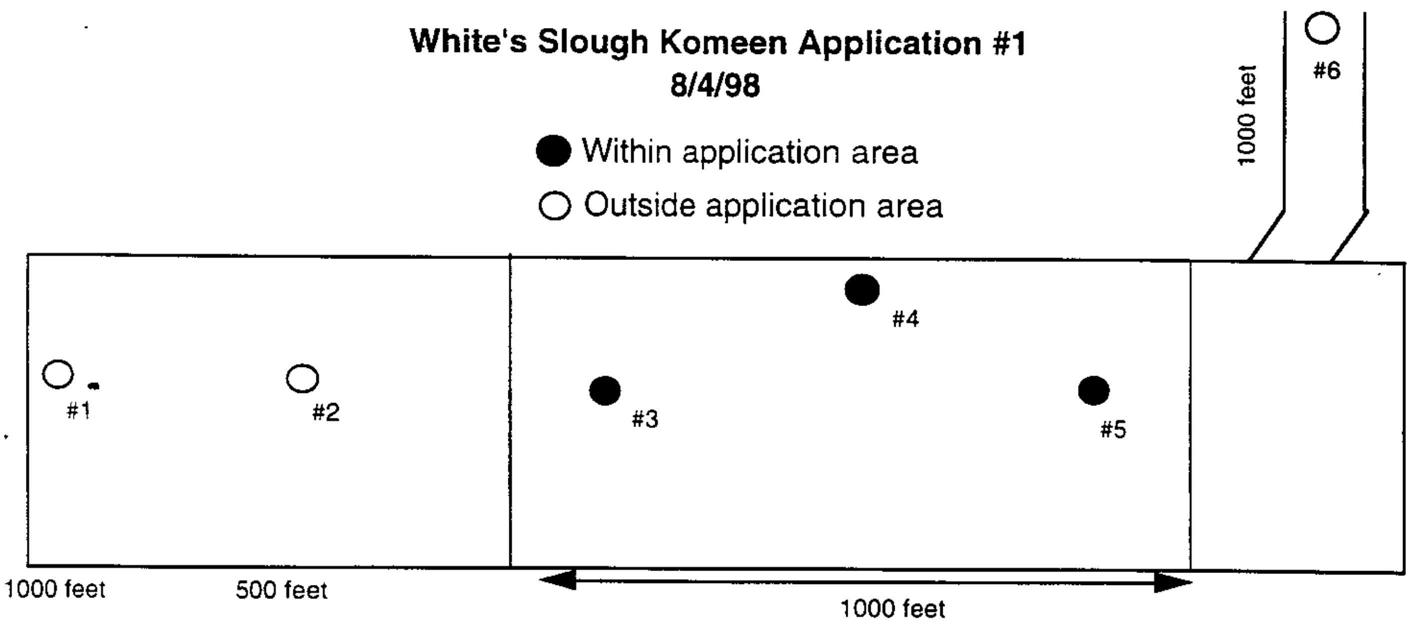


Figure 51. Total Cu dissipation in Whites Slough after Komeen® application on 6/15/98

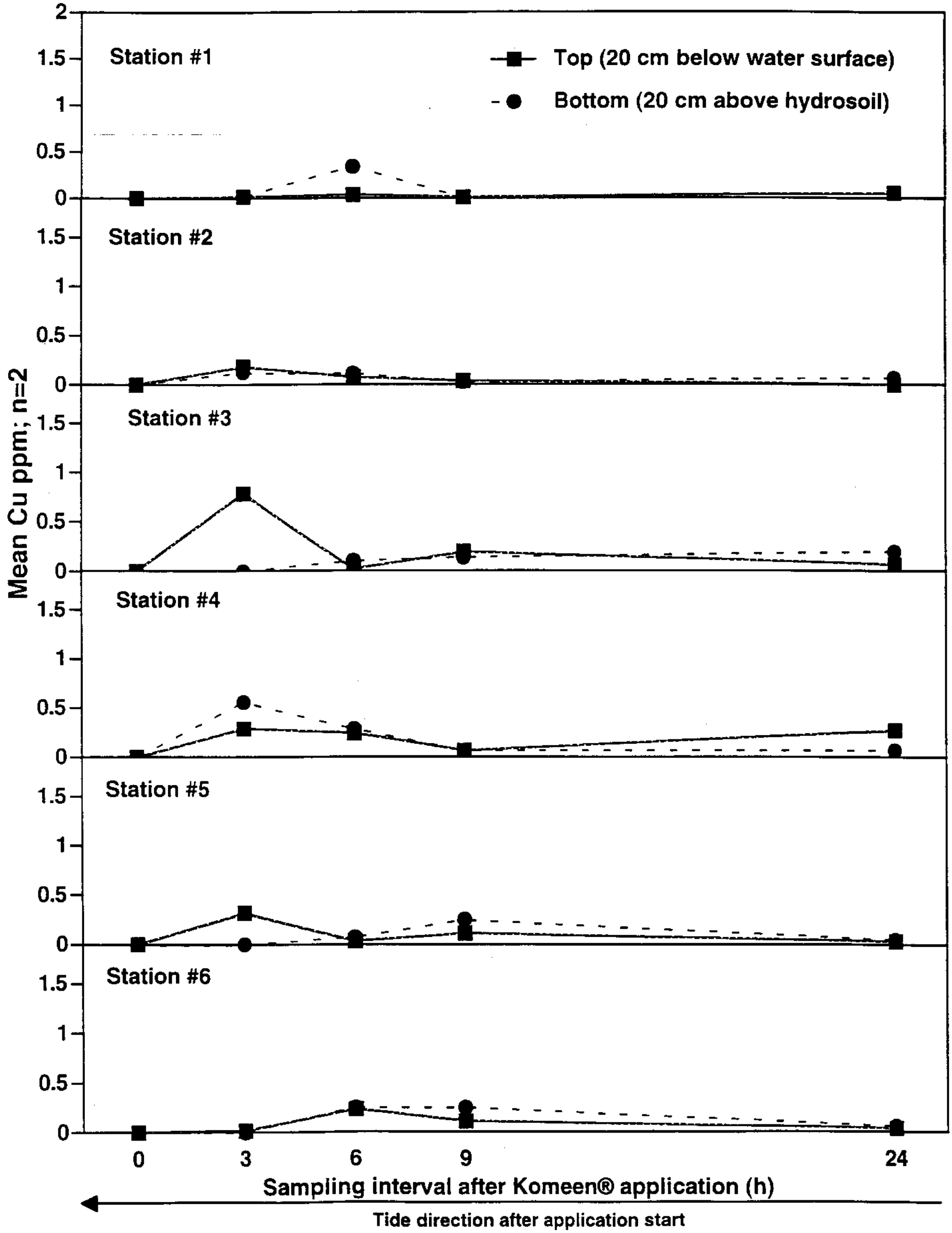


Figure 52. Total Cu dissipation in Whites Slough after Komeen® application on 8/4/98

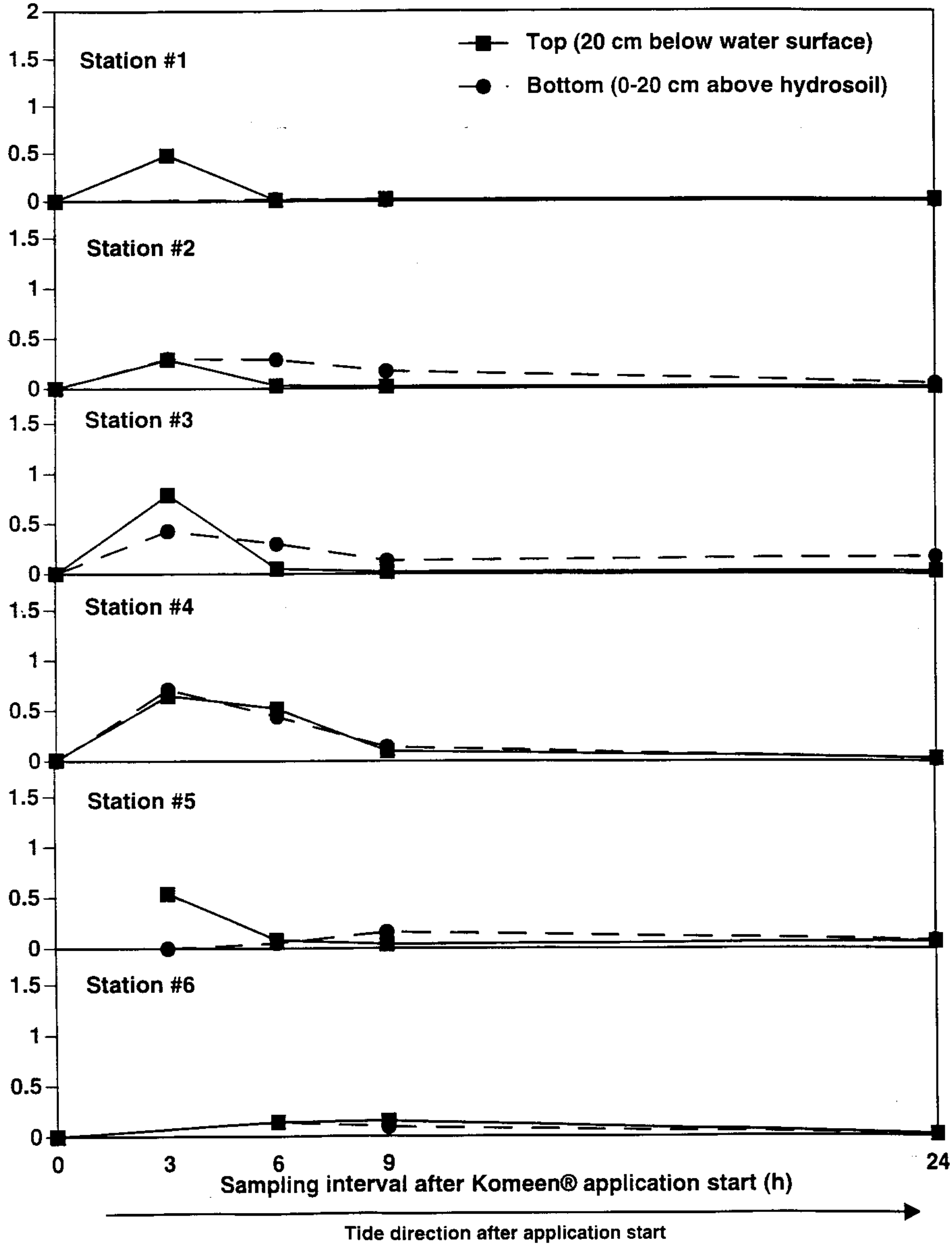


Figure 53. *Egeria densa* shoot uptake of Cu 24 hours after a Komeen® application in June 1998

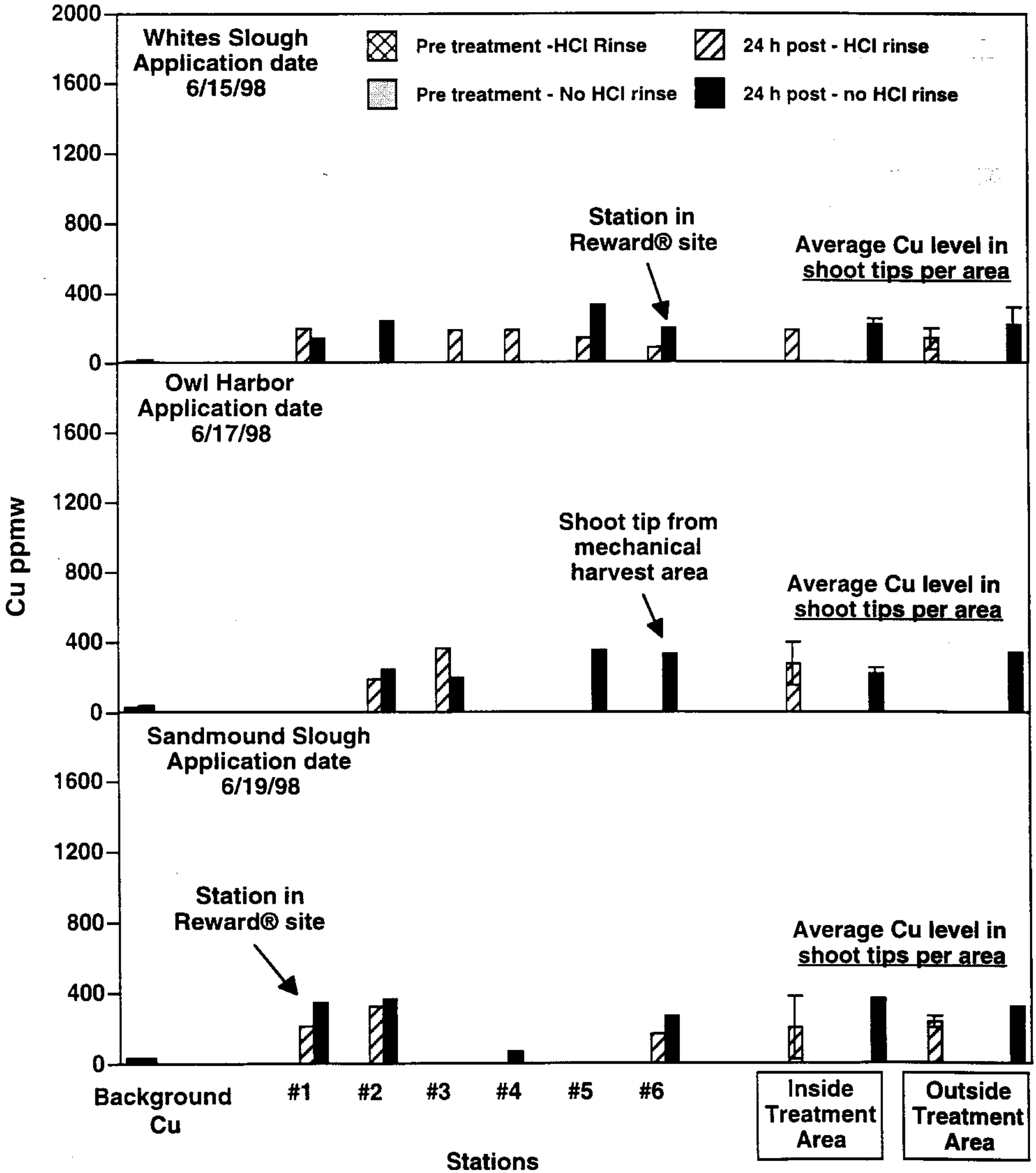


Figure 54. *Egeria densa* shoot uptake of Cu 24 hours after a Komeen application in August 1998

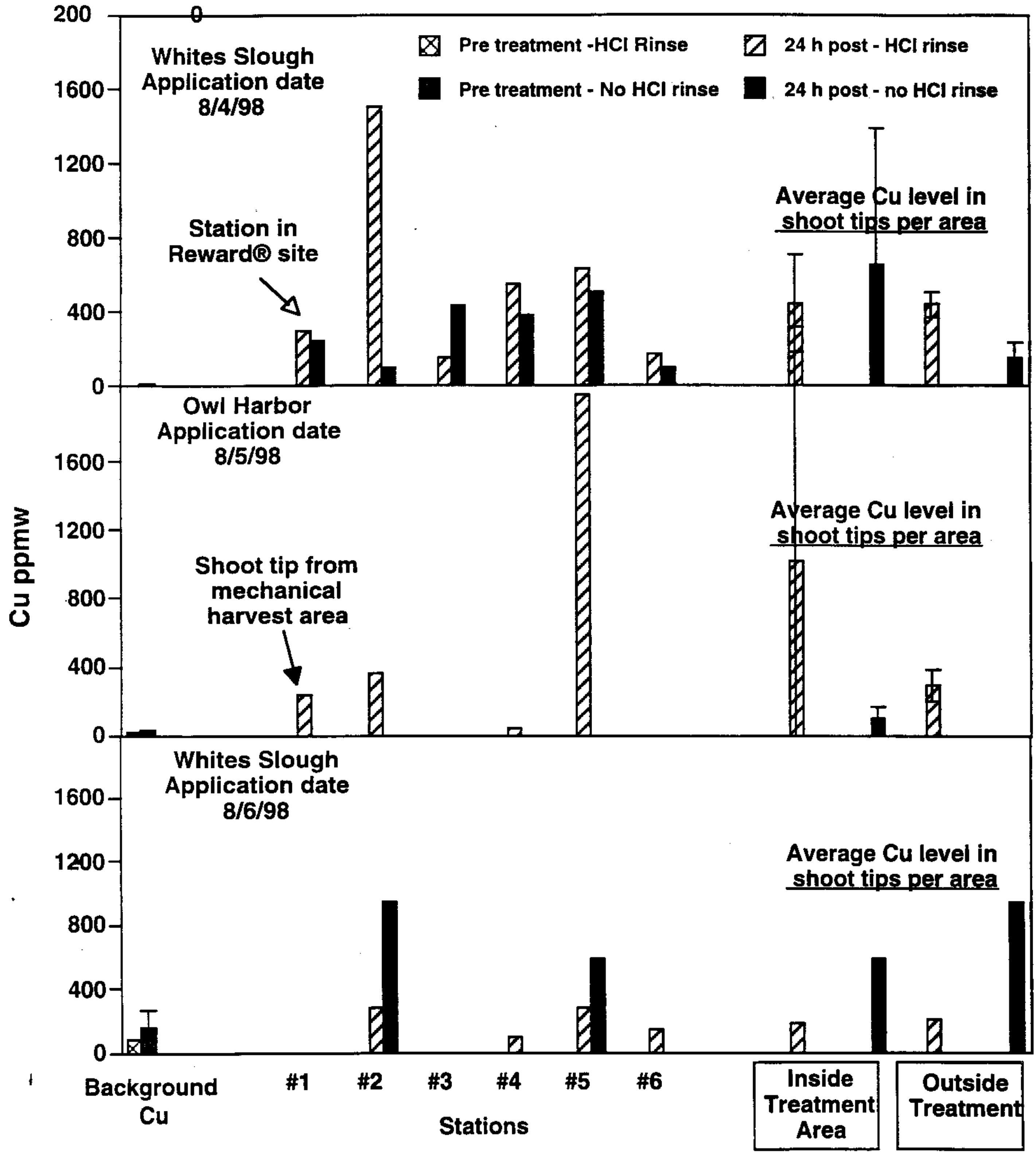


Figure 55. Water quality parameters during a mechanical harvest in Sandmound Slough on 10/21/97.

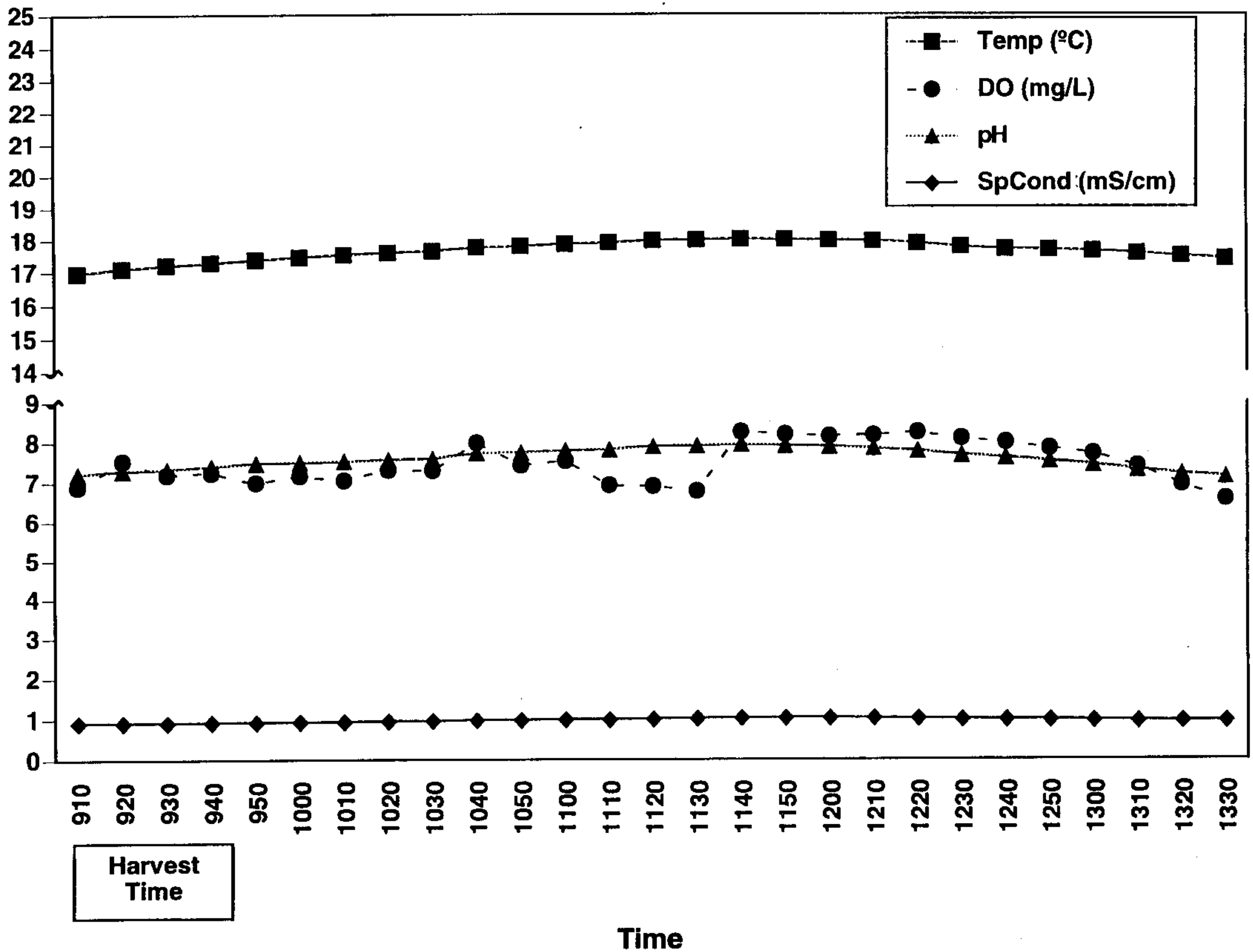


Figure 56. Water quality parameters during a mechanical harvest in Owl Harbor on 10/23/97.

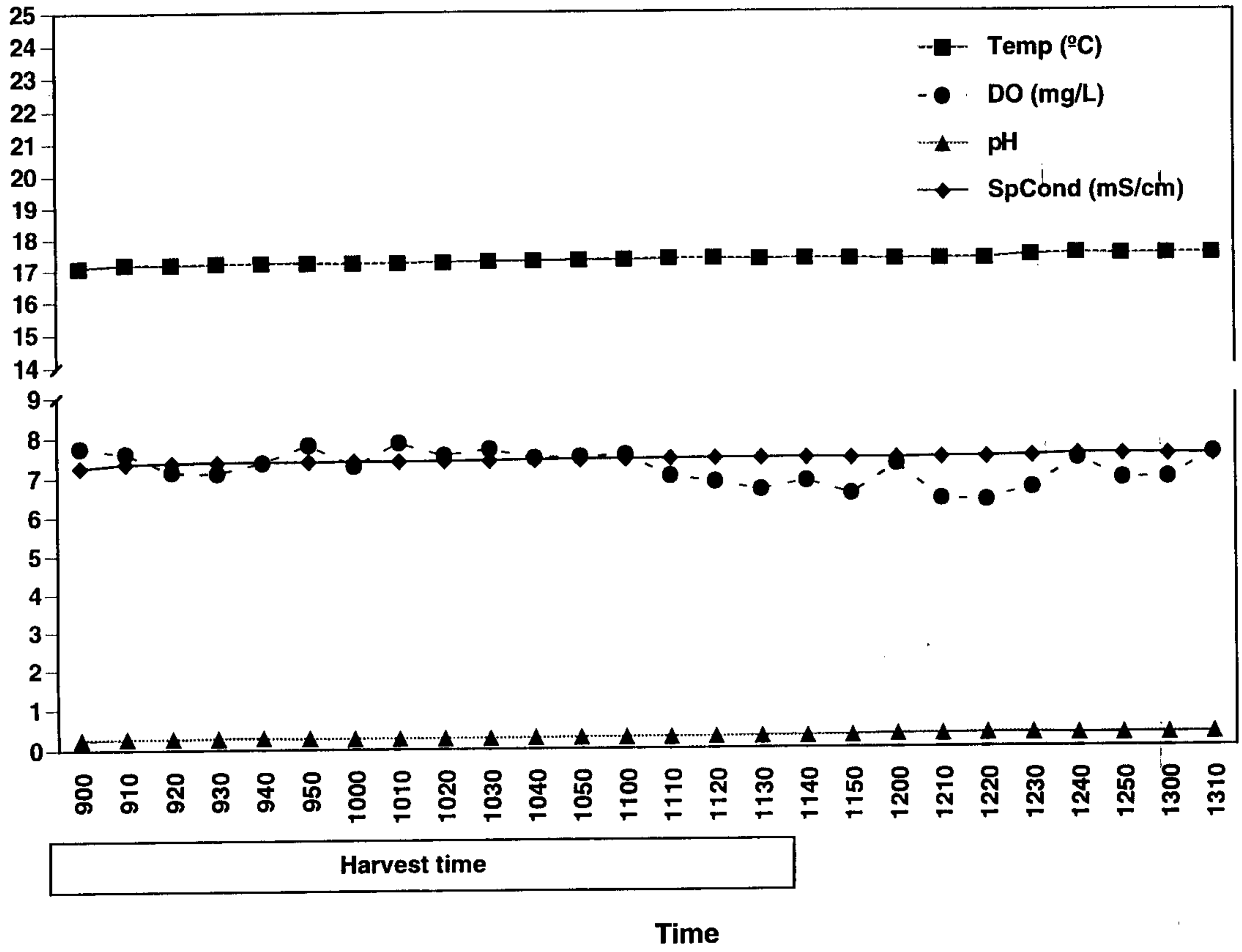


Figure 57. Water quality parameters during a mechanical harvest in Whites Slough on 10/28/97.

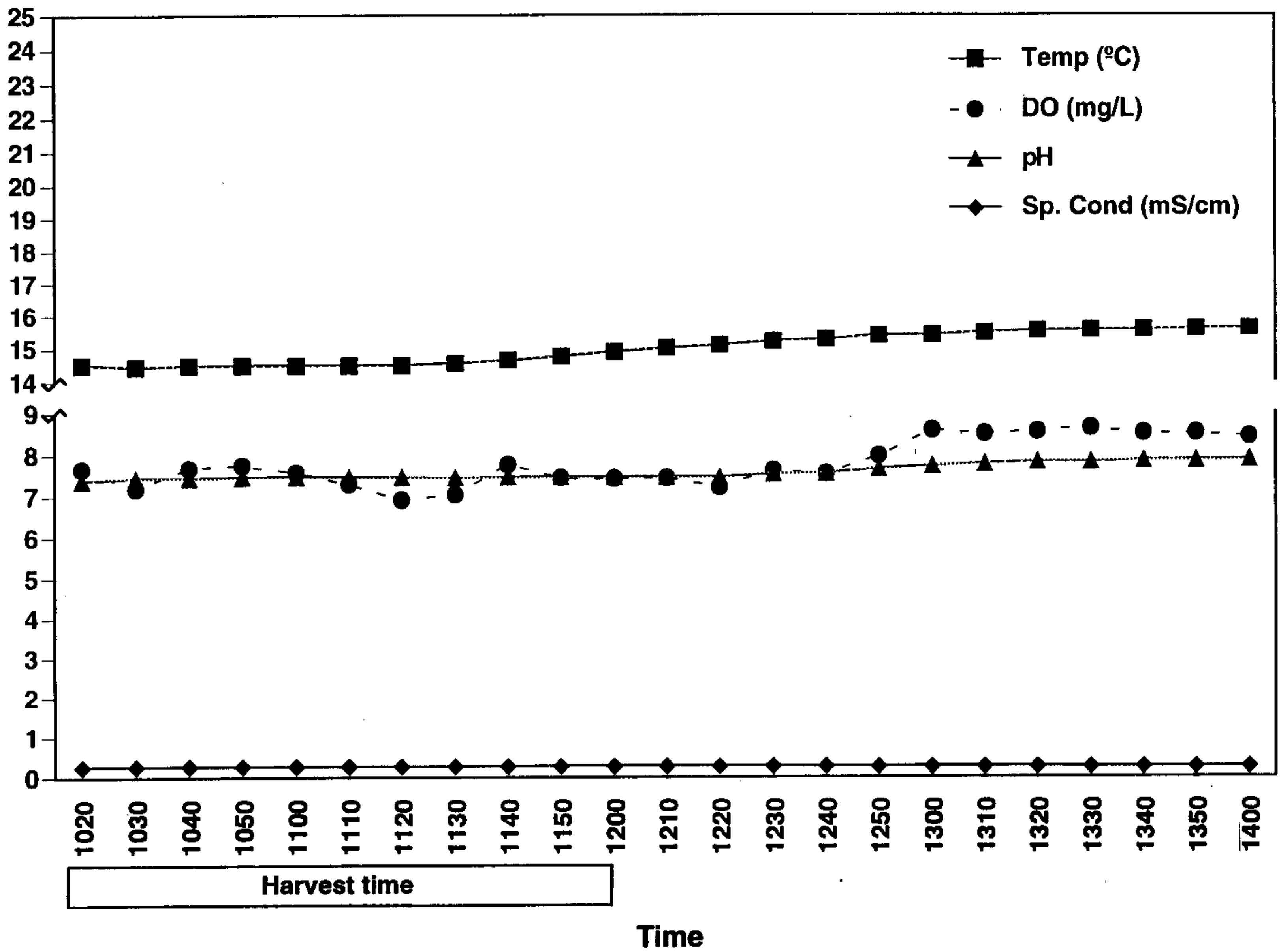


Figure 58. Water quality parameters at Whites after a Komeen® (copper complex) application on 6/15/98.

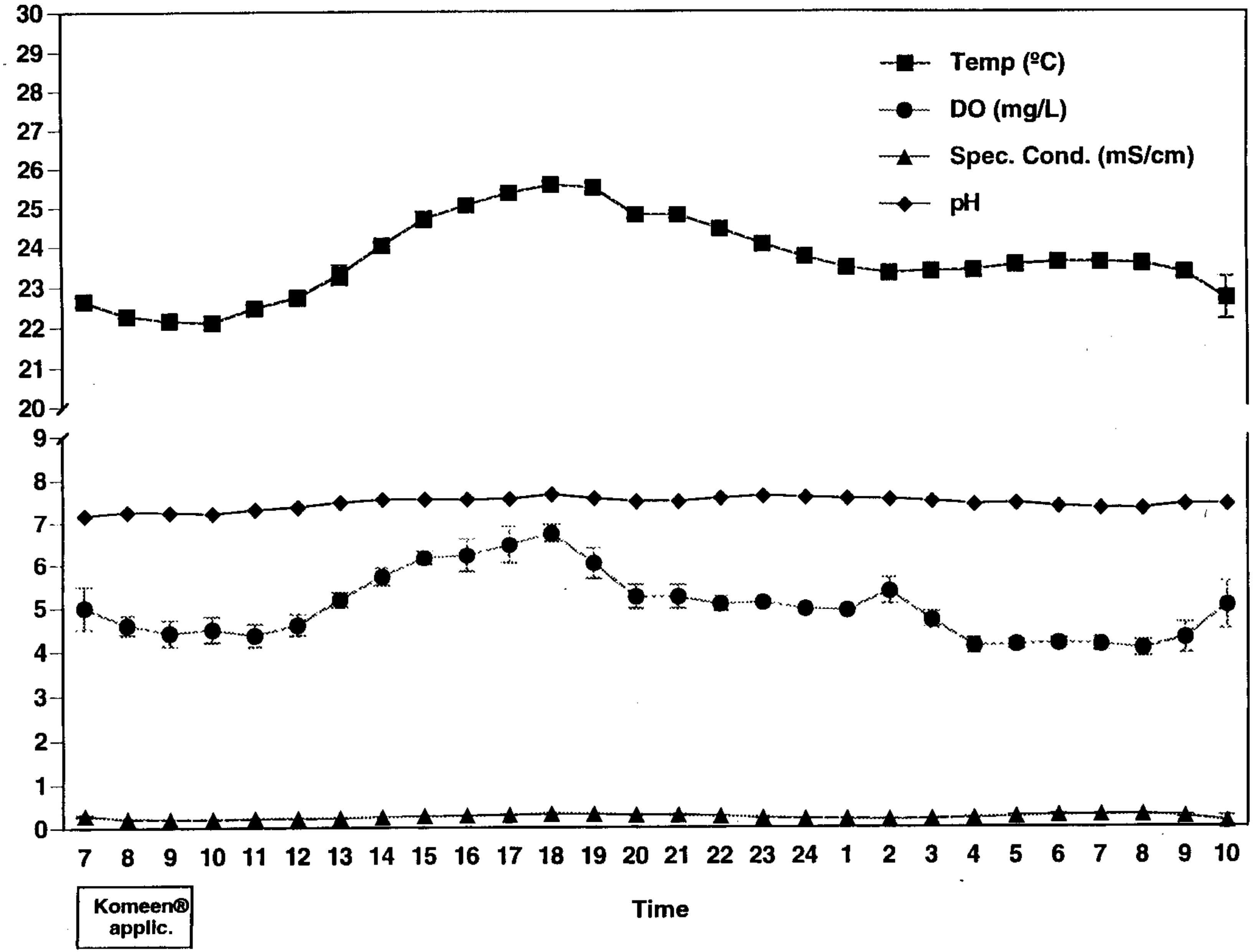


Figure 59. Water quality parameters at Whites Slough after a Reward® (Diquat) application on 6/1/98

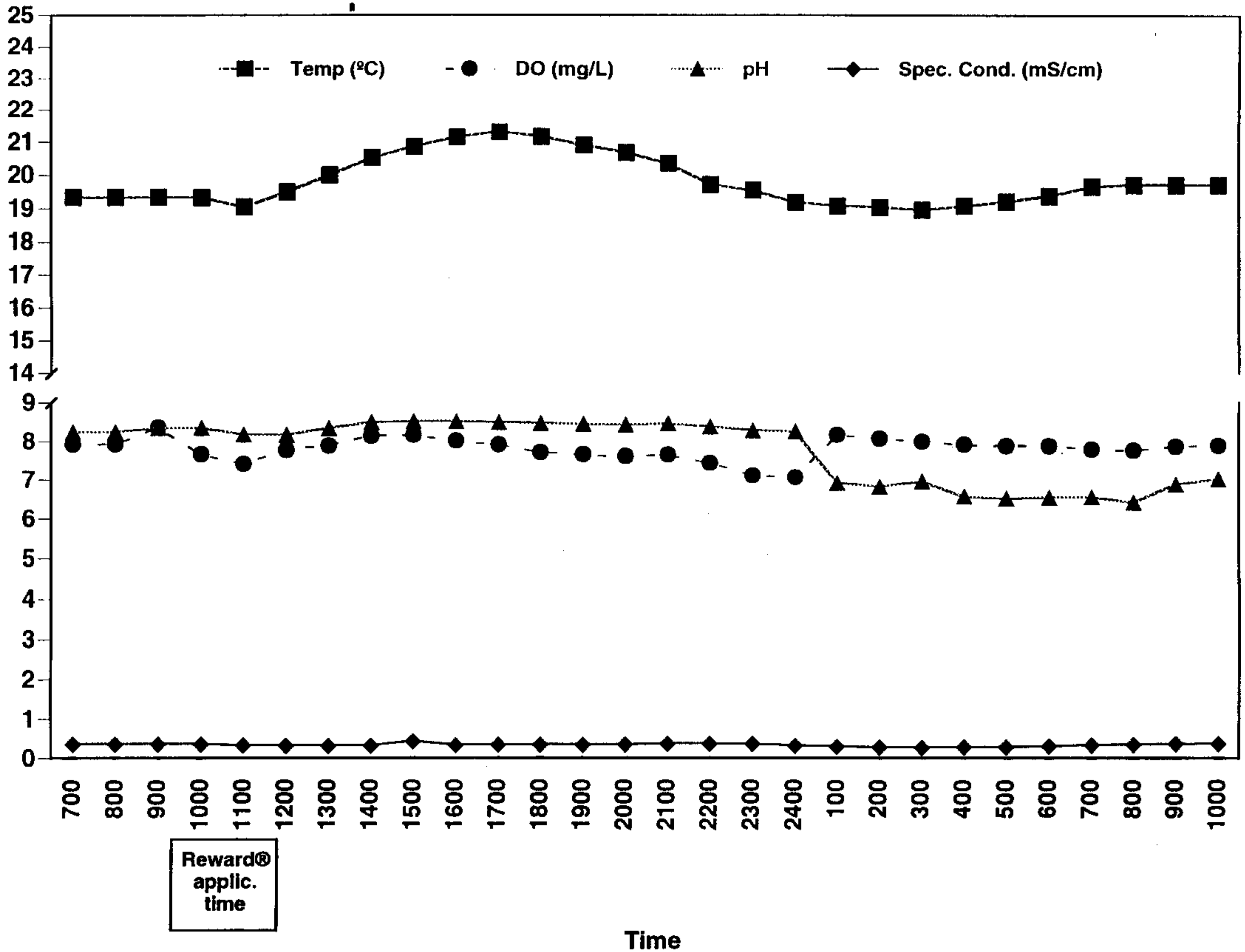


Figure 60. Water quality parameters at Owl Harbor after a Reward® (Diquat) application on 6/3/98.

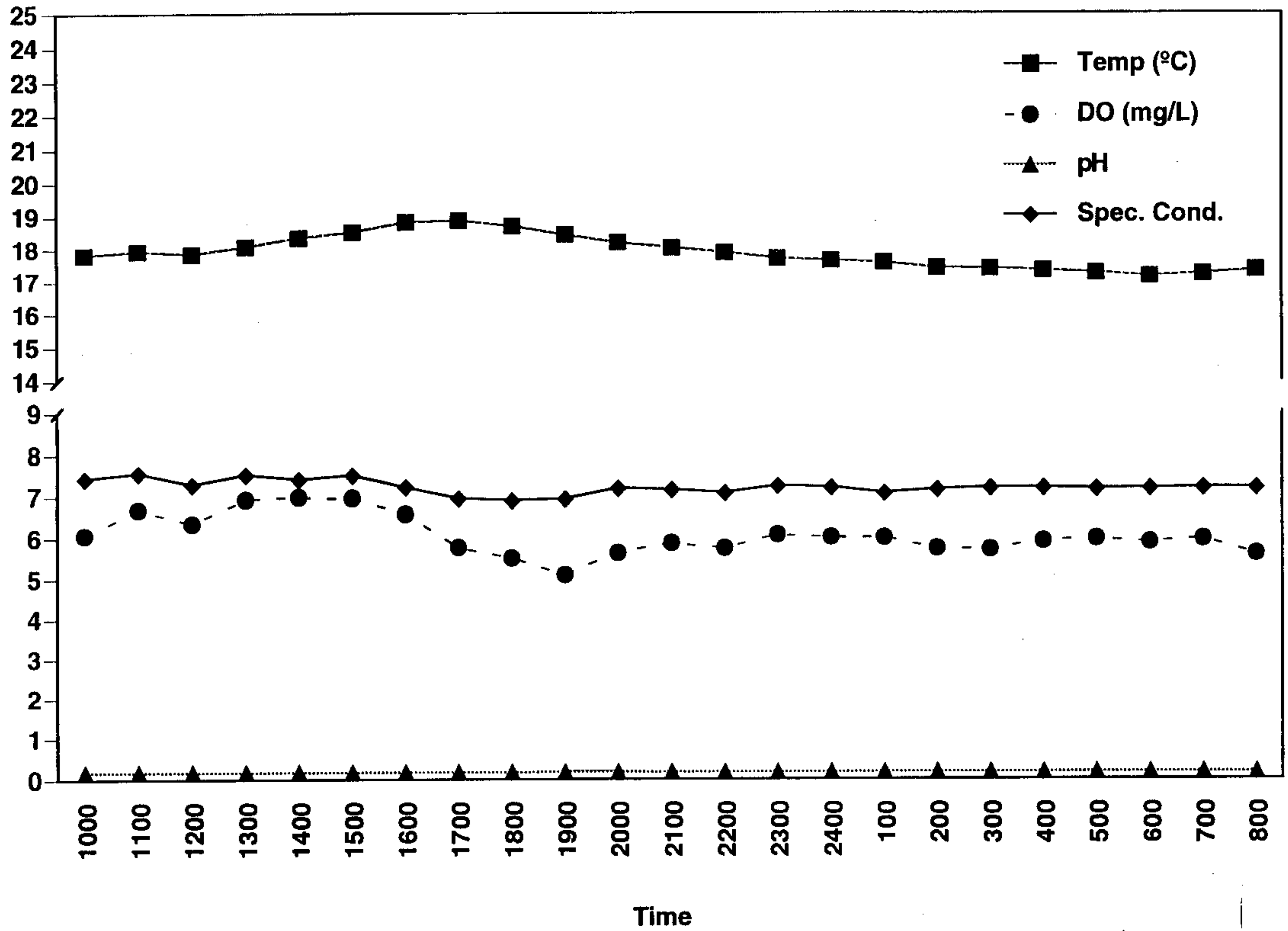


Figure 61. Water quality parameters at Sandmound Slough after a Reward® (Diquat) application on 6/5/98