The effects on invertebrates of Spartina control by the herbicide dalapon

Tucker, G. M.

THE EFFECTS ON INVERTEBRATES OF SPARTINA CONTROL BY THE HERBICIDE DALAPON.

BY

G.M.TUCKER

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ABSTRACT

Areas of *Spartina anglica* at Lindisfarne N.N.R have been controlled with the aquatic herbicide Dalapon in combination with a wetting agent (Agral), in order to reclaim areas of mud-flat, lost as potential feeding areas for bird and fish populations. A study was carried out on the longterm implications of this control programme, and showed that an increase in diversity and density of macroinvertebrates occurred in the sprayed areas of *Spartina*. The implications of this increase in potential food resource is discussed.

Investigation of the sediment in the sprayed areas showed significant variation in physico-chemical factors in comparison to the sediment in the *Spartina* sward and open mud-flat. This difference was accounted for by the tidal removal of fine sediment from the sprayed areas after the destruction of the *Spartina* sward. Many of the observed changes in macroinvertebrate density were considered to be caused by these changes in sediment character.

Field studies on the immediate effect of the Dalapon and Agral spray, found a significant absence of *Carcinus maenas* in areas after treatment. This was considered most likely to have been caused by the direct toxic effects of the spray. No other species of macroinvertebrate studied showed any detectable decline after spraying.

Laboratory studies were carried out on the mud-flat macroinvertebrates *Corophium volutator*, *Hydrobia ulvae* and *Nereis diversicolor*. The two former species showed toxic effects at concentrations of spray equal to 1/10 to 1/100 the working spray concentration. However, *Nereis diversicolor* only showed a mortality response between 1/10 and the equivalent of the working spray concentration. These results are discussed in relation to the likely concentrations encountered by the mud-flat invertebrates.

CHAPTER 1.

INTRODUCTION

The cord-grass *Spartina anglica* (C.E. Hubbard) has become a common plant of intertidal mud-flats around the British coast, over the last fifty years, having spread rapidly due to its ability to colonise unstable sediments of coarse or fine texture, and endure a wide range of salinities. This has been accelerated by the deliberate planting of *Spartina* in order to reclaim intertidal land. Associated with this increased abundance, has been the realisation that large swards of *Spartina* can provide substantial environmental problems. Ranwell (1964) showed that the presence of *Spartina* on intertidal mud-flats results in the accretion of fine sediments from the silt bearing tidal water. This material is deposited within the *Spartina* sward and on adjacent areas where the tidal flow is interrupted. Problems can then result from the fine sediments which can spoil nearby amenity beaches (Truscott 1982).

Millard (1976) showed that the *Spartina* sward supports a less diverse and lower density population of macroinvertebrates than the open mud-flat. This has implications regarding the food supply to the bird and fish populations utilizing this resource. At Lindisfarne it was found that many of the invertebrate components of shore birds diets are absent from the areas of *Spartina* (Millard 1976). Also direct observation of birds feeding in the area showed that birds may also avoid the *Spartina* for behavioural reasons rather than absence of food (Millard and Evans 1982). This reduction of feeding areas for wintering bird populations could have important consequences. Pienkowski (1981) showed that Grey Plovers (*Pluvialis squatorola*) and Ringed Plovers (*Charadrius hiaticula*) in winter needed to feed throughout all daylight hours and partly at night inorder to meet energy requirements. Clearly a complete uppershore sward of *Spartina* in a tidal area would restrict available feeding time, with potentially serious consequences in severe winter weather.

The presence of patches of *Spartina* may result in an increase in density of bird flocks using the remaining areas of open mud-flat. Higher densities of birds exploiting a given density of prey are likely to lead t to an increased mortality of waders (Evans and Dugan 1984). Furthermore the loss of *Zostera spp* and *Enteromorpha spp* due to competition from *Spartina* would threaten wildfowl populations dependant on these as food resources. Decreases in the use by birds of mud-flats with encroaching *Spartina* have been recorded by Corkhill (1982), and Davis and Moss (1982), confirming the potential adverse effects of a complete coastal band of inter-tidal *Spartina*.

In order to restrict the growth of *Spartina* and alleviate these problems, herbicidal control has been used at Lindisfarne National Nature Reserve and at Southport. Both treatments have used the herbicide Dalapon (sodium salt of dichloropropionic acid) together with a wetting agent. These treatments have generally been successful, with a 90 - 100% kill of *Spartina* at Lindisfarne (Corkhill 1982), and similar mortality at Southport (Truscott 1982).

Although the effects of the herbicide on *Spartina* have been monitored, very little is known about the more general ecological effects of the spraying and of the eventual destruction and removal (by tidal action) of the above ground *Spartina*. Although the statutory tests have been carried out in order to approve the use of Dalapon as a general aquatic herbicide, no published data exists as regards the potential toxicity of Dalapon in marine environments. The work of Tooby (1971), and Mckee and Wolff (1963) on the acute toxicity of Dalapon relates to freshwater species, and the generalization of these results to a marine situation is not valid. Similarly, Thiegs (1955) demonstrates that Dalapon breaksdown in warm organic soils within 2-4 weeks due to microbial activity, however the fate of Dalapon in waterlogged, anaerobic estuarine sediments is not known.

In my study I examined the side effects of treatment of *Spartina* with Dalapon on an intertidal mud-flat. Particular attention was paid to whether the destruction of the *Spartina* results in a higher density and diversity of macroinvertebrates. I also examined the potentially toxic effects of the spray on invertebrates, by field and laboratory tests.

In the work on the invertebrate fauna of *Spartina*, Millard (1976) suggested that the species excluded from the *Spartina* areas were absent due to the effect the plant had on the sediment, rather than any direct property of the plant itself. Therefore it was anticipated that any changes in invertebrate density after spraying occurred through changes in the sediment characteristics after the removal of the above ground plant material. With this in mind the potentially important sedimentary characteristics were measured and correlated with associated changes in the macroinvertebrate populations.

In this thesis I attempt to demonstrate that changes in macroinvertebrate populations do occur and are caused by sedimentary changes associated with the removal of the overlying *Spartina*.

CHAPTER 2.

STUDY AREA..

The Lindisfarne National Nature Reserve is situated on the Northumberland coast, approximately 80 km (50 miles) North of Newcastle-upon-Tyne. The reserve itself was designated in 1964 and its total area from Goswick Sands in the north to Budle Bay in the south (shown in Figure 1) is approximately 3,000 ha, of sand-dunes, saltmarsh, and inter-tidal mud-flat. The area is recognised as a Wetland of International Importance under the Ramsar Convention, because of the large numbers of waders and wildfowl that use the area in winter. Maximum winter counts of the commonest species have included 22,000 Dunlin (Calidris alpina), 6,700 Bar-Tailed Godwits (Limosa lapponica), 11,000 Knot (Calidris canutus), 1,300 Redshank (Tringa totanus), 27,000 Wigeon (Anas penelope) and 1,000 pale bellied Brent Geese (Branta bernicola) (Millard 1976). The importance of this site as a wintering area and potential staging post during migration, is further highlighted by the fact that the nearest estuaries are the Forth (100 km north) and the Tees (130 km south), both of which are under threat of reclaimation and subject to pollution. Therefore the encroachment of Spartina onto the mud-flats at Lindisfarne could pose a particularly serious threat to bird populations using the reserve, if it reduced their feeding areas. Figure 1 shows the extent of Spartina at

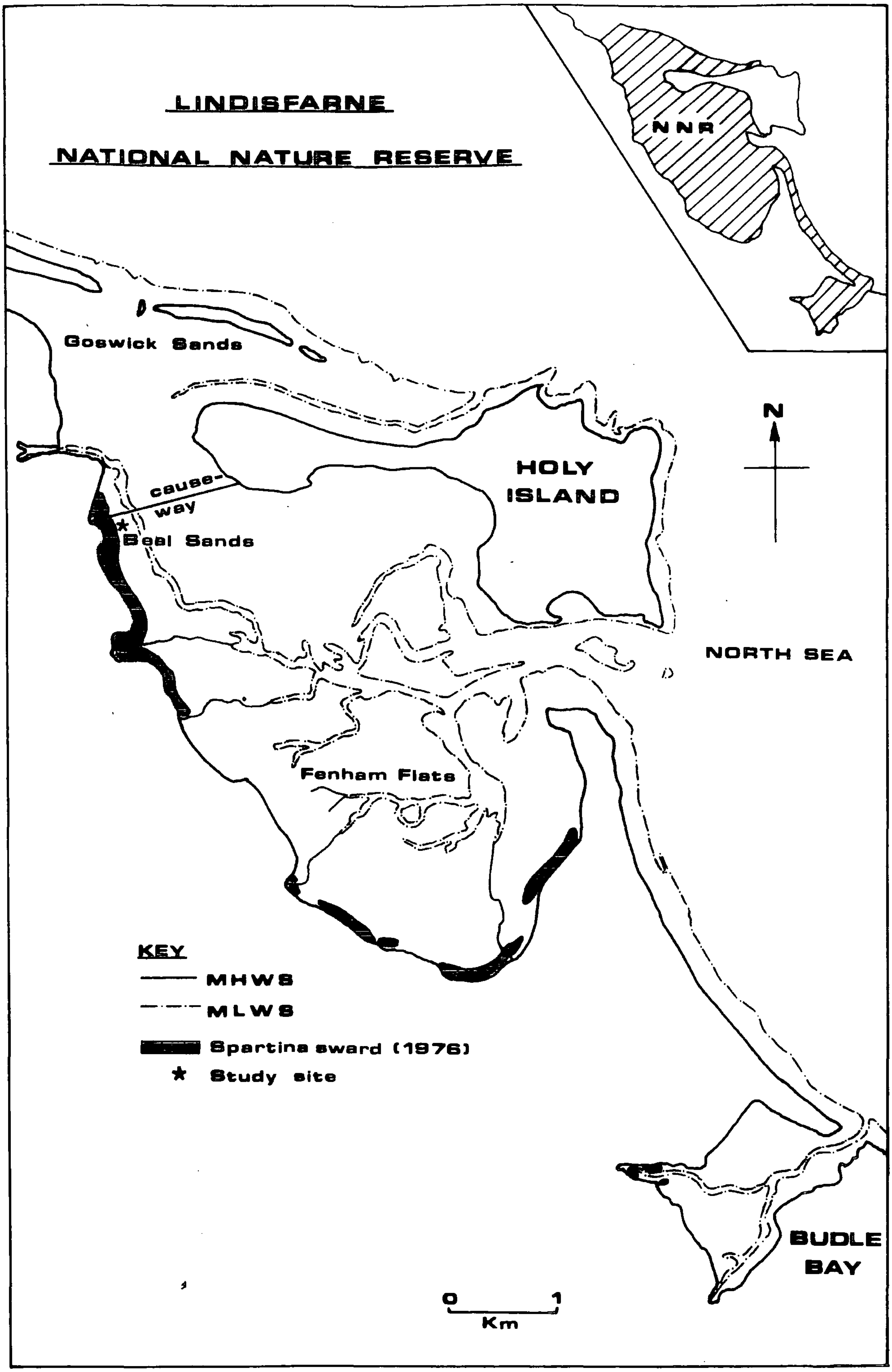


FIGURE 1. Adapted from Millard (1976)

Lindisfarne as estimated by Millard (1976).

Spraying of the *Spartina* sward was carried out by the Nature Conservancy Council in the area of Beal Sands for four succesive years, in order to reduce substantialy the encroachment of the *Spartina* onto the mud-flat. Spraying was carried out each year from the seaward edge of the sward in bands running parallel to the shore. This resulted in the pattern of treated areas shown in Figure 2. The pioneer zone of *Spartina* clumps at the seaward edge of the sprayed area was not succesfully treated and therefore remained intact. Areas of Beal Sands containing adjacent strips sprayed on four succesive years were chosen for my study. However, I was aware that the horizontal transect pattern of spraying might produce problems of interpretation of results, because the more recently sprayed areas lay at slightly higher tidal levels.

Figure 2 shows a sketch map of the site and the sampling locations, the details of which are described in the following chapter.

PLATE 1: The sprayed areas of the study site, looking South from the causeway. Unsprayed *Spartina* on the right.

PLATE 2: The sprayed areas to the North of the causeway. Unsprayed *Spartina* on the left.

CHAPTER 3.

FIELD STUDY OF THE LONG TERM EFFECTS OF TREATMENT OF SPARTINA WITH DALAPON.

(i)Methods.

To ascertain the long term effects of the treatment, an invertebrate sampling programme was carried out in a region that contained typical areas of open mud-flat, areas of *Spartina* that had been sprayed from one to four years ago and unsprayed *Spartina* sward. This region, which lies on either side of the landward end of the causeway is shown in Figure 1 and Plates 1 and 2.

(a) Preliminary Study. A preliminary study of the invertebrate distribution and abundance was carried out with the assistance of the zoology undergraduates at Durham University, by taking samples every 50 metres along a transect of the region, as shown in Figure 2. The method used for this study consisted of taking two samples, each of 100 cm^2 from the lower edge of the area of *Spartina* sprayed four years ago and two from the open mud immediately adjacent. The samples were then sieved on site using a 30 mesh per inch seive, the animals retained in 70% alcohol and later identified. The results of this study were then used for guidance in planning the complete macroinvertebrate survey.

(b) The Main Survey.

The sampling of all areas took place to the south of the causeway as shown in Figure 2. Ten sets of samples were taken from each of seven 10 m by 10 m areas. The characteristics of each of these sample areas are presented below in Table 1 and the positions are shown in Figure 2.

TABLE 1.

SAMPLE AREA LOCATIONS AND CHARACTERISTICS.

AREA CODE	SPARTINA STATUS	PERIOD SINCE SPRAYING	ADJACENT PRELIMINARY SITE
1	SWARD	UNSPRAYED	20
2	SPRAYED	1 YEAR	10
3	SPRAYED	2 YEARS	10
4	SPRAYED	3 YEARS	10
5	SPRAYED	4 YEARS	10
6	SPRAYED	4 YEARS	14
7	ABSENT *	UNSPRAYED	10

* A few isolated clumps of Spartina are present in this area.

Areas 2 - 7 provide a transect across the sprayed region and the adjacent open mud. The location of area 1 as a site of unsprayed Spartina was chosen due to the absence of a wide band of recently sprayed Spartina bordering the area. Also it appeared that the area was lower down the shore, and therefore comparisons between sprayed areas and this area would be more valid than a comparison with an area of Spartina further up the shore. Thus this area was considered to be more typical of the Spartina sward. It was also intended to take samples from the Spartina adjacent to area 2, however this was not possible in the available time. A set of replicate samples was also intended for each of the sprayed areas, however only one was taken (Area 6), due to the limitation of time. Random sampling was attempted within each area by the use of a grid system and the generation of random numbers to place each sample position on the grid. Each set of samples consisted of a 10 x 10 cm surface area, by 8 cm deep core for root material estimation, a 2.5 cm diameter core of approximately 5 cm length (collected and sealed in a glass tube) for water content analysis and a 100 cm^2 sample of the top 3 cm of sediment, all of which were taken as close together as possible.

The macroinvertebrates were extracted by a technique involving three stages, the first two of which were used by Dugan (1981). Firstly they were placed in a constant temperature room at 5 degrees C in plastic containers filled with enough fresh seawater to cover the samples. Over the next three days all the samples were regularly inspected and all visible animals were removed. This stage was succesful in extracting the majority of live Hydrobia ulvae and Owenia fusiformis. Following this stage the seawater was replaced with water of approximately double the concentration of sodium chloride (75 grams of NaCl per litre of tap water). Animals appearing over the following three days were then collected. These consisted mainly of Corophium volutator and Nereis diversicolor. The remaining macroinvertebrates (mainly Macoma balthica and Oligochaeta) were then extracted by sieving with a 20 mesh (1.00 mm) per inch sieve and a gentle jet of water to wash through the sediment. Due to the presence of root material (which made sieving very difficult and obscured the smaller animals) this stage took about 1/2 to 3/4 hour for each sample. Therefore it was this stage that constrained the number of samples that could be examined during the 3 1/2 months of this study.

All specimens extracted were retained, preserved in 70 % alcohol and identified. Root material was assessed by sieving each 0.001 m^2 core using a 30 mesh per inch sieve, and carefully extracting by hand all the visible root material. This was then weighed after air drying at 105 degrees C for 24 hours.

Water content of the sediment was estimated by the same technique used by Stopford (1951) and Millard (1976). An open ended glass tube of 2.5 cm diameter was screwed into the sediment to a depth of approximately 5 cms, and a bung was then used to seal the top. The tube was then carefully extracted and sealed at the bottom also with a bung. The contents were emptied in the laboratory onto a heat resistant dish and after intial weighing the mud sample was air dried at 105 degrees C for 48 hours, and then reweighed.

Median particle size was measured using approximately 100 grams of air dried sediment, taken from the 100 cm^2 by 3 cm deep sample. The sediment was broken up, and dry sieved for two minutes through a graded series of sieves using an Endicott mechanical siever. Sieves of 20, 30, 40, 60, 100 and 200 meshes per inch were used. The amount of sediment retained by each sieve was then weighed and converted into a percentage of the total weight of the complete sample, and then into accumulated percentages retained up to each mesh size. The mean phi value (phi=-log2 D , where D is the diameter of the grain size in millimeteres) was then calculated graphically as described by Holme and McIntyre (1971). Accumulated percentage was plotted against the mean phi value for each sieve size and the median phi estimated as that which corresponds to 50% of the accumulated sediment on the plotted curve. Although a measure of the sorting (ie the variability of grain size) of the sediment, by estimation of the phi interquartile deviation, would have been useful in the study, it was found that the mud particles were to small to allow this analysis to be completed accurately in the time available.

Carbon content was estimated from a one gram sub-sample of sediment. This was the material that passed through the 30 mesh per inch sieve in order to exclude large coal particles and root matter. Oxidisable organic carbon was then estimated by the Dichromate Oxidation technique of Gaudette, Flight, Tower and Folger (1974). The percentage oxidisable organic carbon component was then calculated according to the following equation,

% Organic Carbon = 10(1-T/S)(1(N)(0.003)100/W)

where

T = volume of 0.5 N Ferrous Ammonium Sulphate used in the

S = volume of 0.5 N Ferrous Ammonium Sulphate used in the blank titration

N = normality of Potassium Dichromate

W = weight of sediment sample

Redox potential and pH within the sediment were measured using a portable Phillips PW9411 pH meter with Orion redox potential and pH probes. Measurements were made at 2 cm depth, with readings taken after 60 seconds. All redox potential and pH measurements were made on the same day and within a period of two hours, to minimise variation due to temperature and tidal differences.

The tidal height of each sample, in relation to the mean high water mark, was measured using a surveyor's level and staff, to a comparative accuracy of one centimetre. However, the position of the M.H.W.M could only be estimated from the position of tidal debris, and therefore the absolute height of each sample could be subject to considerable error.
All measurements were taken on the same day and related to the estimated M.H.W.M debris.

Salinity was not measured in this study because it was considered unlikely that any significant differences would occur, due to the similar tidal heights of each area. This assumption is supported by Millard (1976) who found no significant difference in salinity over a greater tidal range than considered in this project.

(ii)Results of Faunal Survey.

(a) Preliminary Survey.

The preliminary study provided densities of Nereis diversicolor, Macoma balthica, Hydrobia ulvae and Corophium volutator in open mud and adjacent areas of sprayed Spartina the latter areas having been sprayed four years ago and now totally devoid of all above ground spartina. The results are shown in Figure 3. The graph clearly shows the patchy nature of the distribution of all four species. Only Corophium volutator show consistently higher densities (up to twenty times) in the open mud-flat sample sites than in the sprayed areas. The other three species did not show statistically significant differences between the two areas, although densities of Nereis diversicolor and Macoma balthica were generally higher in the open mud. However, the main information gained from this preliminary study was an appreciation of the variability in invertebrate density over the entire study site. This information was then utilised to plan the selection of sample areas, size of each sample unit and the number of samples needed from each site. Because of the patchiness of the fauna, it was considered most efficient to sample randomly within defined blocks, rather than completely at random, and that a minimum of ten 100 Cm^2 samples per block would provide data with the required level of precision.

(b) The Main Faunal Survey.

The faunal survey provided 15 species that could be positively identified, and a further three that were identified only to family. The following species were encountered.

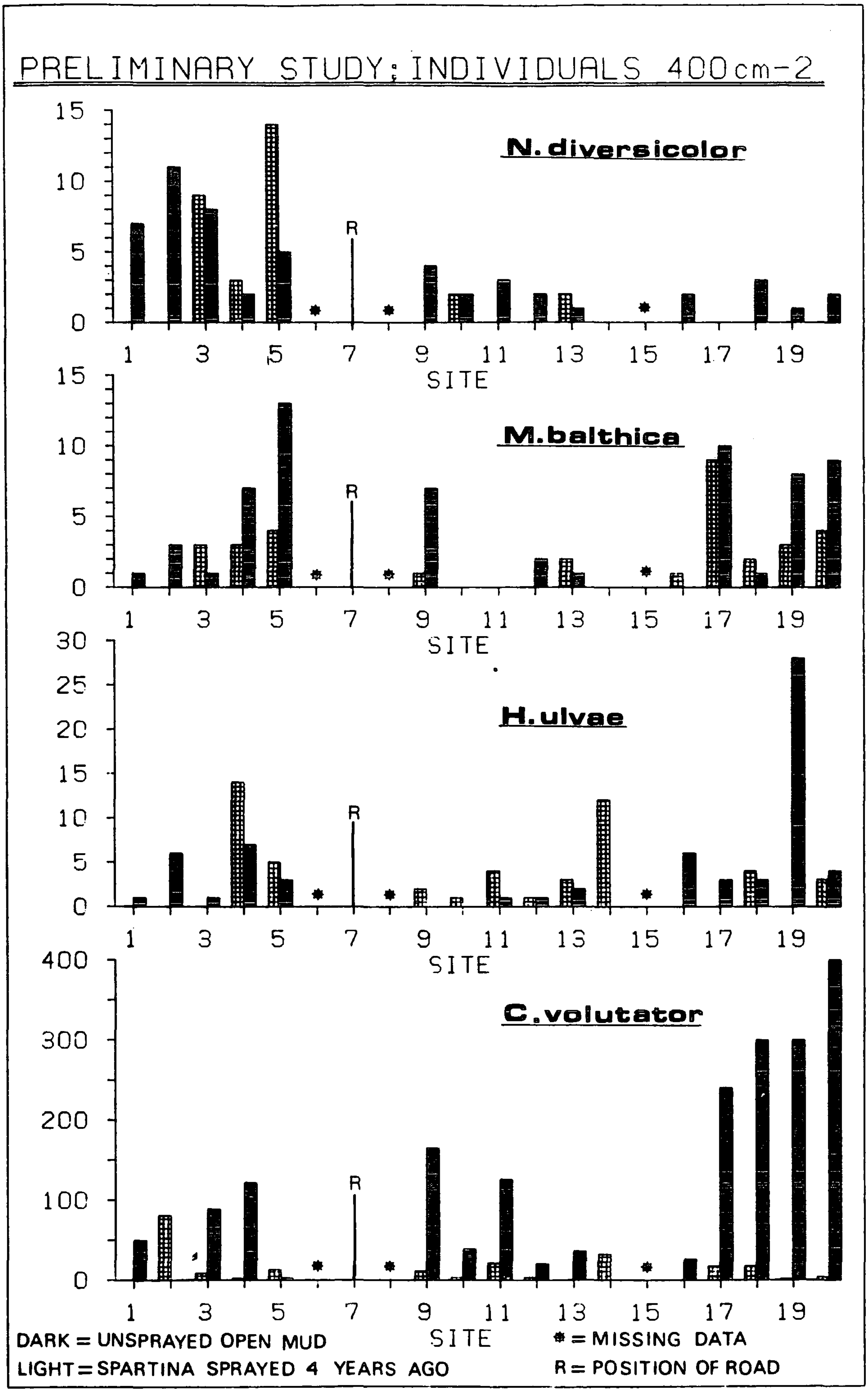

PRELIMINARY STUDY; INDIVIDUALS 400cm-2
N. diversicolor
M. balthica
H. ulvae
C. volutator
SITE
R
DARK = UNSPRAYED OPEN MUD
LIGHT = SPARTINA SPRAYED 4 YEARS AGO
* = MISSING DATA
R = POSITION OF ROAD

FIGURE 3

TABLE 2

LIST OF SPECIES IDENTIFIED IN THE FAUNAL SURVEY.

NEMERTINI	Tetrastemma melanocephalum	
ANNELIDA	Nereis diversicolor	
	Eteone longa	
	Scoloplos armiger	
	Arenicola marina	
	Ampharete balthica	
	Owenia fusiformis	
	Enchytraeus albidus	*
	Lumbricillus (species n.d.)	*
	Tubifex (species n.d.)	*
MOLLUSCA	Hydrobia ulvae	
	Littorina saxatilis spp	**
	Littorina littoralis	
	Macoma balthica	
ARTHROPODA; CRUSTACEA	Corophium volutator	
	Hyale nilssoni	
	Carcinus maenas	
INSECTA	Dolichopodidae (species n.d.)(Diptera)	***

* These are grouped and treated as Oligochaeta.

** The four distinct species described by Heller (1975) are not separated in this study and the species complex is refered to as L.saxatilis.

*** Probably all examples of Dipteran larvae encountered during the survey were members of this family.

Of the species listed above only ten species and one group (Oligochaeta) occured in suuficient numbers for consideration in the analysis of results. Counts of Enchytreaus albidus, Lumbricillus species and Tubifex species were summed as positive identification of these was possible only by examination of each individual under a microscope. Due to limited time individual identification proved impossible during this project.

Table 3 below shows the mean number of individuals occuring in each 100 Cm^2 sample, for each of the more commonly encountered species in each area. The standard errors of the mean for each value are also displayed in the table. Additionally as Areas 2-5 constituted the sprayed Spartina areas, the mean of the combined data from these four areas is also presented.

Although the oligochaeta are treated in the analysis as one group identification of specimens in each sample was carried out later by Dr.Standen. (the results are presented in Table 1 of the Appendix). Fragmentation of the specimens caused great difficulty in accurate assesment of numbers. Also, preservation in alcohol resulted in the loss of visibility of anatomical features frequently needed for specific identification and therefore only Enchytreaus albidus could be identified to species level.

TABLE 3.

MEAN SPECIES ABUNDANCE PER 100 Cm SAMPLE.

Standard errors of the mean are shown within brackets.

SPECIES	AREA MEAN							
	1	2	3	4	5	2-5	6	7
NEREIS		0.60 (0.22)	0.60 (0.27)	1.10 (0.35)	1.60 (0.65)	0.97 (0.21)	0.6 (0.34)	2.3 (0.75)
ARENICOLA		1.0 (0.26)	1.6 (0.58)	1.5 (0.45)	0.3 (0.21)	1.10 (0.21)	1.2 (0.36)	
ETEONE		0.3 (0.21)	0.2 (0.13)	0.4 (0.22)	0.3 (0.21)	0.3 (0.10)	0.1 (0.10)	
CAPITELLA		3.7 (1.66)	6.3 (3.10)	8.0 (4.24)	1.4 (0.88)	4.85 (1.40)		
OLIGOCHAETA	8.4 (1.56)	22.9 (8.55)	25.4 (5.70)	39.5 (11.2)	17.5 (8.10)	26.3 (43.4)	13.9 (3.6)	5.5 (2.35)
HYDROBIA	2.3 (0.77)	7.9 (2.27)	6.2 (1.86)	25.9 (8.18)	28.8 (6.91)	17.2 (3.12)	45.6 (10.5)	23.9 (5.71)
LITTORINA	0.9 (0.31)	0.6 (0.31)	0.2 (0.13)	0.3 (0.15)	0.2 (0.13)	0.32 (0.10)	4.2 (1.80)	0.6 (0.5)
MACOMA		0.4 (0.22)	0.4 (0.48)	1.4 (1.30)	4.9 (0.81)	1.80 (0.47)	12.9 (1.48)	1.5 (0.79)
COROPHIUM	0.1 (0.10)	38.3 (13.9)	16.4 (5.82)	6.5 (2.77)	9.8 (9.58)	17.7 (4.78)	12.6 (4.25)	
OWENIA		3.3 (1.31)	4.1 (1.72)	5.6 (1.40)	0.8 (0.51)	3.45 (0.69)		
CARCINUS	0.4 (0.16)	0.3 (0.21)	0.4 (0.22)		0.1 (0.1)	0.20 (0.82)		
DIPTERA			0.1 (0.10)	0.1 (0.10)	0.4 (0.27)	0.15 (0.08)	0.4 (0.22)	0.3 (0.15)

(N = 10 SAMPLES PER AREA)

Figures 4, 5 and 6 illustrate the variation in density between each sample area of the species in Table 2 with the addition of the Diptera. It is clear from these graphs that only in the case of Carcinus maenas was the density in the unsprayed Spartina sward comparable with that in the sprayed areas. The other species were absent from unsprayed Spartina, or only present at low densities. Also apparent are the generally higher densities in the sprayed areas when compared with the open . Only Nereis diversicolor occured at higher densities in the open mud. Hydrobia ulvae and Macoma balthica were present in similar densities in sprayed areas and on open mud, except on sprayed Area 6 which held markedly higher densities of both species than any other area. Arenicola marina, Capitella capitata, Corophium volutator, and Owenia fusiformis were completely absent from the open mud. For Corophium volutator this was surprising since in the preliminary survey (see Figure 3) this species was abundant in many parts of the open mud, and was present at preliminary sample site 10, corresponding with the main survey Area 7.

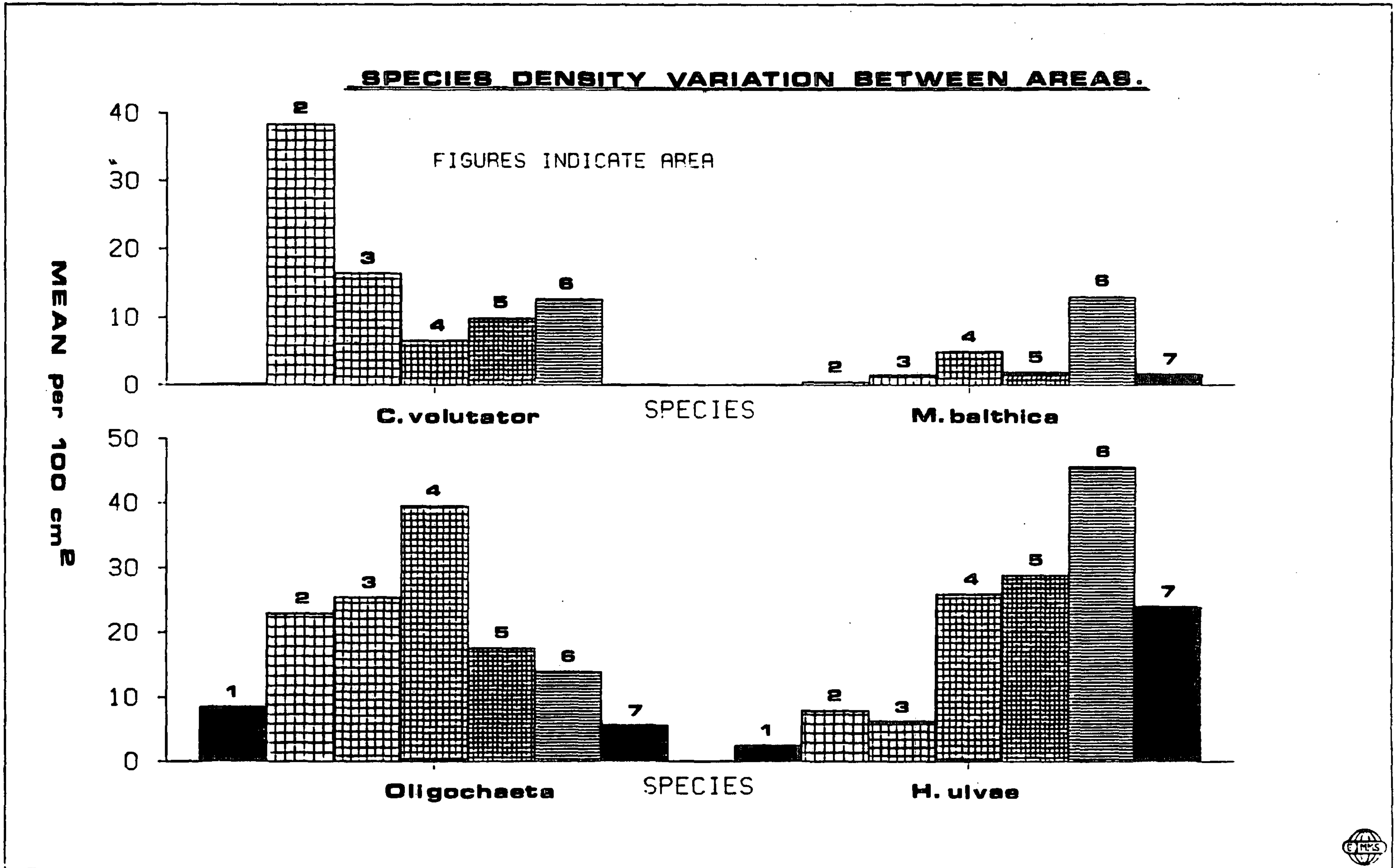

SPECIES DENSITY VARIATION BETWEEN AREAS.
FIGURES INDICATE AREA
MEAN per 100 cm2
C. volutator
M. balthica
SPECIES
Oligochaeta
H. ulvae
SPECIES

FIGURE 4

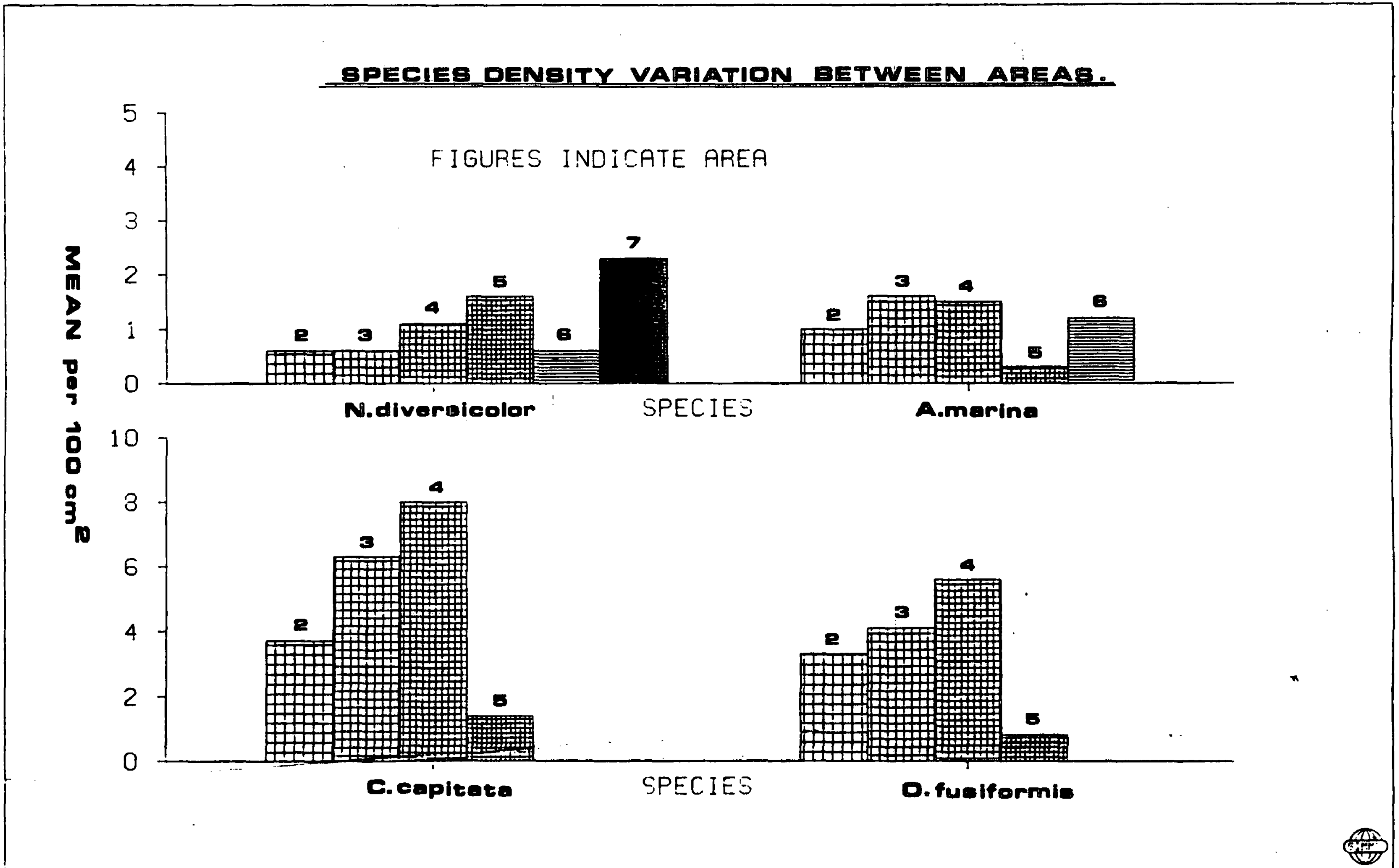

SPECIES DENSITY VARIATION BETWEEN AREAS.
FIGURES INDICATE AREA
MEAN per 100 cm2
SPECIES
N.diversicolor
A.marina
C.capitata
O.fusiformis
0
1
2
3
4
5
6
7
8
10

FIGURE 5

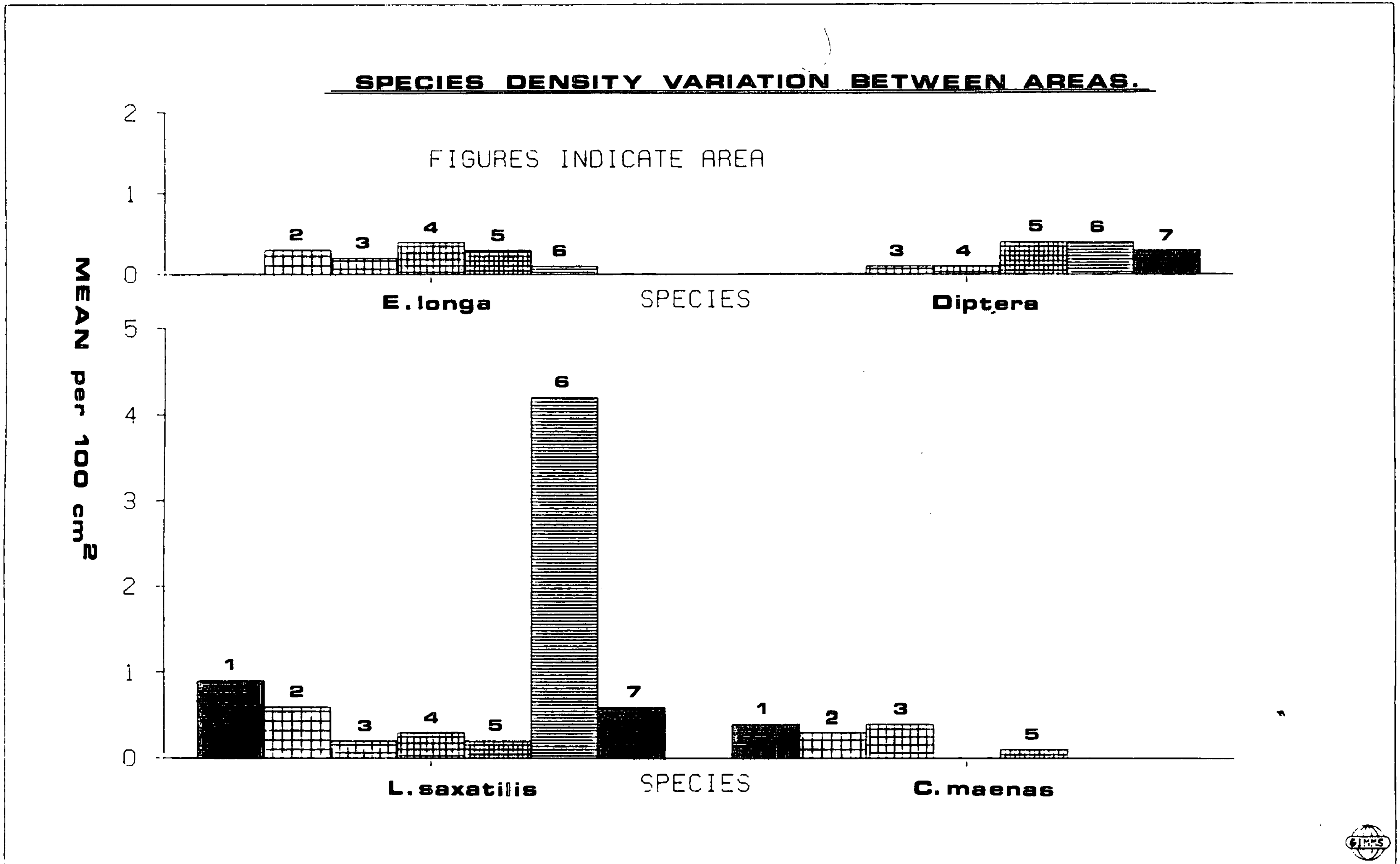

SPECIES DENSITY VARIATION BETWEEN AREAS.
FIGURES INDICATE AREA
MEAN per 100 cm2
0
1
2
3
4
5
6
7
E. longa
SPECIES
Diptera
L. saxatilis
C. maenas

FIGURE 6

In order to make comprehensive and quantitative comparisons of the variation of density in each species amongst areas, a thorough statistical analysis was carried out.

Firstly the raw data on densities were examined for normality in order to select appropriate statistical tests. Examination showed that in most cases the data was significantly skewed and the variance was proportional to the mean. Therefore the mean values with standard errors presented in Table 3 and the accompanying figures must be interpreted with caution. In order to overcome this problem several transformations were made. The transformation Z=Log10(X=1) (where X= density per 100 Cm sample) was found to reduce deviation from normality in the data in most cases, but there remained a significant number of exceptions. Only Hydrobia ulvae and Oligochaeta data was uniformly normal after transformation throughout all areas. Because of this problem it was decided to carry out all tests (including those on Hydrobia ulvae and Oligochaeta so as to simplify comparisons) using non-parametric statistics.

(c) Comparison Between Invertebrate Densities in Sprayed Areas and Unsprayed Areas.

The crucial question regarding the desirability and effects of spraying Spartina is whether or not there are significant differences between invertebrate densities in the sprayed areas and the unsprayed Spartina sward. To examine this the Spartina in the region, Area 1, and the sprayed Areas 2 , 3, 4 and 5 were tested by comparison of homogeneity of species density over all

5 areas, by non-parametric Kruskall-Wallis 1 way analysis of variance. The results of this test are shown in Table 4.

TABLE 4.

COMPARISION OF SPECIES ABUNDANCE BETWEEN CONTROL SPARTINA AND SPRAYED AREAS.

Kruskall-Wallis 1 Way ANOVA.

Area 1 Unsprayed control Spartina
Areas 2-5 Sprayed

SPECIES	AREA MEAN RANKS 1	2	3	4	5	CHI SQUARE	SIGNF
NEREIS DIVERSICOLOR	14.0	25.2	24.2	31.1	32.9	12.7	0.013
ARENICOLA MARINA	14.0	31.1	31.0	32.5	18.8	16.5	0.002
CAPITELLA CAPITATA	15.0	27.7	28.9	34.2	21.6	12.7	0.013
OLIGOCHAETA	16.7	26.4	30.5	34.7	19.1	10.8	0.028
HYDROBIA ULVAE	11.3	22.1	19.7	36.4	37.8	24.5	<0.000
LITTORINA SAXATILIS	31.7	27.5	22.0	24.2	22.0	4.8	0.306
MACOMA BALTHICA	13.0	18.8	27.4	41.0	27.3	24.3	<0.000
COROPHIUM VOLUTATOR	12.3	36.1	33.7	28.0	17.3	21.8	<0.000
OWENIA FUSIFORMIS	13.0	28.1	31.7	35.6	19.0	18.6	0.001
CARCINUS MAENAS	30.1	25.8	28.2	20.5	22.9	5.86	0.210

(Significance estimates are corrected for ties).

The results show that there are significant differences (probability of < 0.05) in distributions amongst areas of all species except Littorina saxatilis and Carcinus maenas the region. Within the region.

To examine whether these inhomogenous differences arise because of differences between the sprayed areas and the unsprayed *Spartina* sward, further tests were conducted between pairs of areas for the relevant species. The results of these Mann-Whitney U-Wicoxon Rank Sum tests are shown in the Appendix in Table 2.

Table 5 below includes a summary of significant differences for each species between the unsprayed Spartina and the sprayed areas.

TABLE 4.

SUMMARY OF THE TESTS OF SPECIES ABUNDANCE VARIATION BETWEEN UNSPRAYED SPARTINA SWARD AND SPRAYED AND OPEN MUD AREAS.

MANN-WHITNEY U -WILCOXON RANK SUM W TEST RESULTS.

Values of Significance; *= less than 0.05
**= less than 0.01
Compared area shows higher density if significance level shows + or lower density if - is indicated.

AREA	COMPARED AREA	NER	ARN	CAP	OLI	HYD	LIT	MAC	COR	OWN	CRC
1	2	+*	+**	+*					+**	+**	
1	3	+*	+**	+*	+*			+**	+**	+**	
1	4	+**	+**	+**	+**	+**		+**	+**	+**	
1	5	+**				+**		+**			
1	6		+**			+**		+**	+**		
5	6						+**	+**	+*		
1	7	+**				+**		+**			

Area 2= Sprayed 1 year previously
Area 3= " 2 years "
Area 4= " 3 years "
Area 5= " 4 years "
Area 6= " 4 years " (replicate area)
Area 7= Open mud-flat

These analyses confirm statistically the impression of higher numbers in sprayed areas indicated in the graphs, and clarifies the results of the analysis of variance given in Table 4. There are higher densities in each sprayed region for all species examined except *Littorina saxatillis* and *Carcinus maenas*. The greatest dissimilarity occurs between the *Spartina* area 1 and sprayed area 4. In this case there are higher densities of all species in the sprayed area. Area 5, sprayed one year earlier than Area 4, and so which might been expected to show similar differences from the *Spartina*, only shows higher densities of *Nereis diversicolor*, *Hydrobia ulvae* and *Macoma balthica*. Areas 2 and 3, sprayed more recently, have a greater number of species with significantly higher densities than in the *Spartina*.

These results and the distributions shown in Figures 4, 5, and 6 indicate that there are differences in the way the varoius species responded to the differing environmental factors in each area, in addition to the spraying treatment examined statistically so far. This will be investigated further with the measured physico-chemical variables.

To asses whether Area 6 (the replicate sample of an area sprayed four years ago) shows similar differences to unsprayed *Spartina* as Area 5 in the density of its fauna, a further Mann-Whitney U test was carried out for each species. It is also important to ask whether or not the two sample areas sprayed four years ago (Areas 5 and 6) differ significantly in the densities of their fauna, and therefore a similar test was also carried out between these areas. The results of both these tests are shown in the Appendix in Tables 3 and 4, and are summarised within Table 5 above.

Area 6 in common with Area 5 showed higher densities of

Hydrobia ulvae and Macoma balthica than the Spartina sward area. However, Area 6 also showed higher densities of Arenicola marina and Corophium volutator. In comparison with Area 5, Area 6 held higher densities of Macoma balthica, Corophium volutator and Littorina saxatilis. These differences may be attributable to physico-chemical dissimilarities between the areas and this will be investigated later.

Previous work by Millard (1976) showed that sediment in Spartina swards generally held lower densities and fewer species of macroinvertebrate than open mud, the only notable exceptions being Littorina saxatilis and Carcinus maenas which occured at higher densities within Spartina swards. Therefore the invertebrate densities in Spartna Area 1 with those of open mud (Area 7) were compared to assess whether similar differences occurred. The results of the Mann-Whitney U test are presented in Table 5 in the Appendix and are summarised in Table 5 above. Nereis diversicolor, Hydrobia ulvae and Macoma balthica all occured at higher densities in the open mud, as found by Millard (1976). However Arenicola marina and Corophium volutator were absent from both areas.

(d) Comparison Between Invertebrate Densities in Sprayed Areas and Open Mud.

Although it is apparent that areas that had been sprayed held higher densities of macroinvertebrates than the Spartina sward area, further investigation was carried out to compare the sprayed areas with the "original" undisturbed open mud (Area 7).

A Kruskall-Wallis 1 way analysis of variance was carried out to detect any significant inhomogeneity in density of any species throughout the region comprising of sprayed Areas 2, 3 , 4, 5 and 6, and the open mud Area 7. These results are presented below in Table 6.

TABLE 6.

COMPARISION OF SPECIES ABUNDANCE BETWEEN SPRAYED AREAS AND (UNSPRAYED) OPEN MUD.

Kruskall-Wallis 1 Way ANOVA.
Areas 2-5 Sprayed
Area 7 Unsprayed

SPECIES	AREA MEAN RANKS 2	3	4	5	6	CHI SQUARE	SIGNF
NEREIS DIVERSICOLOR	20.6	20.0	26.4	28.7	31.7	5.43	0.246
ARENICOLA MARINA	31.1	31.0	32.5	18.8	14.0	16.5	0.002
CAPITELLA CAPITATA	27.7	28.9	34.2	21.6	15.0	12.7	0.013
OLIGOCHAETA	27.4	31.3	35.1	21.5	12.0	15.4	0.004
HYDROBIA ULVAE	16.2	13.4	31.3	33.6	32.9	18.2	0.001
LITTORINA SAXATILIS	29.2	23.8	26.2	23.8	24.5	1.68	0.793
MACOMA BALTHICA	16.0	24.9	39.2	24.6	22.6	14.7	0.005
COROPHIUM VOLUTATOR	36.2	33.8	28.2	17.7	11.5	23.0	$<$0.000
OWENIA FUSIFORMIS	28.1	31.7	35.6	19.0	13.0	18.6	0.001
CARCINUS MAENAS	27.6	30.0	22.5	24.9	22.5	6.36	0.174

(Significance levels indicated are corrected for ties).

The analysis indicates that there is significant inhomogeneity in density amongst the areas for all species except Nereis diversicolor, Littorina saxatilis and Carcinus *maenas*. Further investigation by Mann-Whitney U tests between each sprayed area and the open mud area were carried out in order to examine whether any of the variation was due to differences between individual sprayed areas and the open mud. Table 6 in the Appendix presents the results of the tests between Area 7(open mud) and each of the sprayed Areas 2, 3, 4 and 5. Results of the comparison of Areas 7 and area 6 (replicate area sprayed four years ago) are also presented in the Appendix in Table 7.

Table 7 includes a summary of these tests.

TABLE 7.

SUMMARY OF THE TESTS OF SPECIES ABUNDANCE VARIATION BETWEEN SPRAYED AREAS AND OPEN MUD.

MANN-WHITNEY U -WILCOXON RANK SUM W TEST RESULTS.

Values of Significance; *= less than 0.05
**= less than 0.01
Compared area shows higher density if significance level shows + or lower density if - is indicated.

AREA	COMPARED AREA	NER	ARN	CAP	OLI	HYD	LIT	MAC	COR	OWN	CRC
7	6		+**		+*	+*	+**	+**	+**		
7	5										
7	4		+**	+**	+**			+**	+**	+**	
7	3		+**	+*	+**	-**			+**	+**	
7	2		+**	+*	+*	-*			+**	+**	

Area 2= sprayed 1 year previously
Area 3= " 2 years "
Area 4= " 3 years "
Area 5= " 4 years "
Area 6= " 4 years " (replicate area)
Area 7= Open mud-flat

Higher numbers of Arenicola marina, Capitella capitata, Oligochaeta, Owenia fusiformis and Corophium volutator occured in all the sprayed areas, except in Area 5 (and Area 6 in the case of Owenia fusiformis). As there is no significant difference between Area 5 and the adjacent open mud for any species, it appears that after four years the sprayed area has a similar macroinvertebrate population to the open mud. Area 6 however, seems to indicate otherwise, as this area (also sprayed four years ago) shows many differences in density with the open mud (area 7). In the case of Area 6 however, it must be emphasised that the sampled open mud area (Area 7) is not adjacent to Area 6 and therefore any interpretation of the difference between these two areas must be tentative.

Hydrobia ulvae was the only species to show a significantly higher density in the open mud than in any sprayed area.

The results of the statistical tests on species distribution in conjuction with the interpretation of Figures 4, 5 and 6 lead to the general conclusion that, firstly spraying of the Spartina sward has resulted in a substantial increase in diversity and density of macroinvertebrates. The exceptions are Littorina saxatilis and Carcinus maenas, as the distribution and density of these species is apparently unaffected by spraying, within the time scale studied. Secondly the resulting densities of Arenicola marina, Capitella capitata, Oligochaeta, Corophium volutator and Owenia fusiformis within the four year period after spraying are higher in the sprayed areas than in the adjacent open mud.

(e) Comparison of between Invertebrate Densities in Areas Sprayed in Different Years.

Earlier comparison of Area 5 with Area 6 showed that there may be significant differences in macroinveretebrate density between areas sprayed in different years. Therefore a further examination by a Kruskall-Wallis 1 way analysis of variance was carried out, in order to examine whether there are significant variations in density between areas of the entire region sprayed in different years. Table 8 presents the results of this analysis.

TABLE 8.

COMPARISION OF SPECIES ABUNDANCE BETWEEN AREAS SPRAYED IN DIFFERENT YEARS.

Kruskall-Wallis 1 Way ANOVA.

SPECIES	AREA MEAN RANKS 2	3	4	5	CHI SQUARE	SIGNF
NEREIS DIVERSICOLOR	17.7	17.2	22.6	24.4	3.19	0.363
ARENICOLA MARINA	22.1	23.0	24.0	12.8	6.66	0.083
CAPITELLA CAPITATA	20.2	21.4	25.2	15.5	4.25	0.236
OLIGOCHAETA	19.2	22.4	26.0	14.3	5.47	0.140
HYDROBIA ULVAE	14.6	12.5	26.7	28.1	14.3	0.002
LITTORINA SAXATILIS	23.3	18.9	20.8	18.9	1.62	0.655
MACOMA BALTHICA	12.3	19.4	31.0	19.3	14.1	0.003
COROPHIUM VOLUTATOR	27.2	24.3	19.2	11.2	11.0	0.012
OWENIA FUSIFORMIS	20.1	22.7	26.6	12.5	8.19	0.042
CARCINUS MAENAS	21.6	23.5	17.5	19.4	3.88	0.275

(Significance levels indicated are corrected for ties).

The significant variations in Hydrobia ulvae, Macoma balthica, Corophium volutator and Owenia fusiformis shown by the above analysis are further examined by a series of Mann-Whitney U tests, the results of which are presented in the Appendix in Table 8. Table 9 below illustrates the significant differences between pairs of areas by the test.

TABLE 9.

SUMMARY OF SIGNIFICANTLY DIFFERENT DENSITIES BETWEEN SPRAYED AREAS 1-5.

Levels of probability indicated are;
*=less than 0.05
**=less than 0.01

AREA	SPECIES	2	3	4	5
3	HYDROBIA ULVAE		--		
	MACOMA BALTHICA		--		
	COROPHIUM VOLUTATOR		--		
	OWENIA FUSSIFORMIS		--		
4	HYDROBIA ULVAE	*	**	--	
	MACOMA BALTHICA	**	*	--	
	COROPHIUM VOLUTATOR			--	
	OWENIA FUSSIFORMIS			--	
5	HYDROBIA ULVAE	*	**		--
	MACOMA BALTHICA			*	--
	COROPHIUM VOLUTATOR	*	**	*	--
	OWENIA FUSSIFORMIS		*	**	--
		2	3	4	5

There were no significant differences between Areas 2 and 3, and no apparent pattern to the variation found between the sprayed areas. To further investigate the factors that may influence variation in macroinvertebrate density throughout the study site a thorough survey of potentially important physico-chemical factors is required. The following section presents the results of such a survey.

Results of the Physico-Chemical Factor Survey.

(a) Comparison Between Physico-Chemical Factors in the *Spartina* Sward and the Sprayed Areas.

Table 10 below shows the mean value of the physico-chemical factors measured for each area.

Many of the mean values show a gradient negatively correlated to the area code, because this is related to the length of time since spraying (recovery time) and the height, such as seen in % carbon content of the sediment, median phi value (thus particle size increases towards area 7) % water content, root mass and height. These trends are seen in Figures 7 and 8, where the above factors are plotted against area code. Therefore the factors of height below M.H.W.M and recovery time are also plotted on each graph as they are intuitively thought to be the most likely factors influencing the other physico-chemical varaibles. Recovery time is given the value of 0 for the open mud (Area 7), although strictly speaking it has been available for colisation by macroinvertebrates for a longer time than can be measured by this study. However the physico-chemical factors in the open mud are considered to be in equilibrium rather than responding to a perturbation as in the sprayed areas. It is this time since the onset of change by spraying, and the ensuing Spartina destruction that the recovery time value attempts to describe.

TABLE 10.

MEAN VALUES OF PHYSICO-CHEMICAL CHARACTERS OF THE SAMPLE AREAS.

Standard errors of the mean are shown within brackets.

FACTOR	AREA MEAN 1	2	3	4	5	6	7
REDOX POTENTIAL	-48.0 (16.0)	-82.5 (23.0)	+11.1 (28.0)	-41.0 (40.0)	-121 (86.0)	80.8 (8.50)	-91.7 (7.50)
HEIGHT (Cms below H.W.M.)	-7.4 (1.1)	-12.7 (0.45)	-17.2 (1.1)	-18.2 (0.7)	-20.9 (0.57)	-15.7 (0.52)	-24.5 (0.92)
ROOT MASS (Grams)	1.93 (0.30)	1.37 (0.21)	1.00 (0.26)	0.929 (0.21)	0.778 (0.10)	0.706 (0.12)	0.215 (0.17)
% WATER	40.1 (1.7)	46.4 (0.74)	40.8 (2.5)	38.4 (1.6)	29.7 (1.0)	31.3 (1.0)	23.4 (0.6)
MEDIAN O VALUE	2.46 (0.13)	2.70 (0.09)	2.39 (0.19)	2.31 (0.14)	1.78 (0.04)	1.98 (0.09)	1.29 (0.03)
pH	8.20 (0.12)	7.97 (0.12)	8.00 (0.11)	8.22 (0.09)	8.42 (0.10)	8.17 (0.08)	8.74 (0.05)
% CARBON	0.852 (0.06)	0.948 (0.02)	0.779 (0.06)	0.657 (0.04)	0.617 (0.03)	0.485 (0.04)	0.387 (0.03)

(N = 10 SAMPLES PER AREA)

Figure 7 shows the gradients in particle size, % carbon content, and root mass. As this study attempts to ascertain whether or not any change in physico-chemical factors between the areas is due to the removal of the Spartina, a detailed examination of the relationship between the physico-chemical

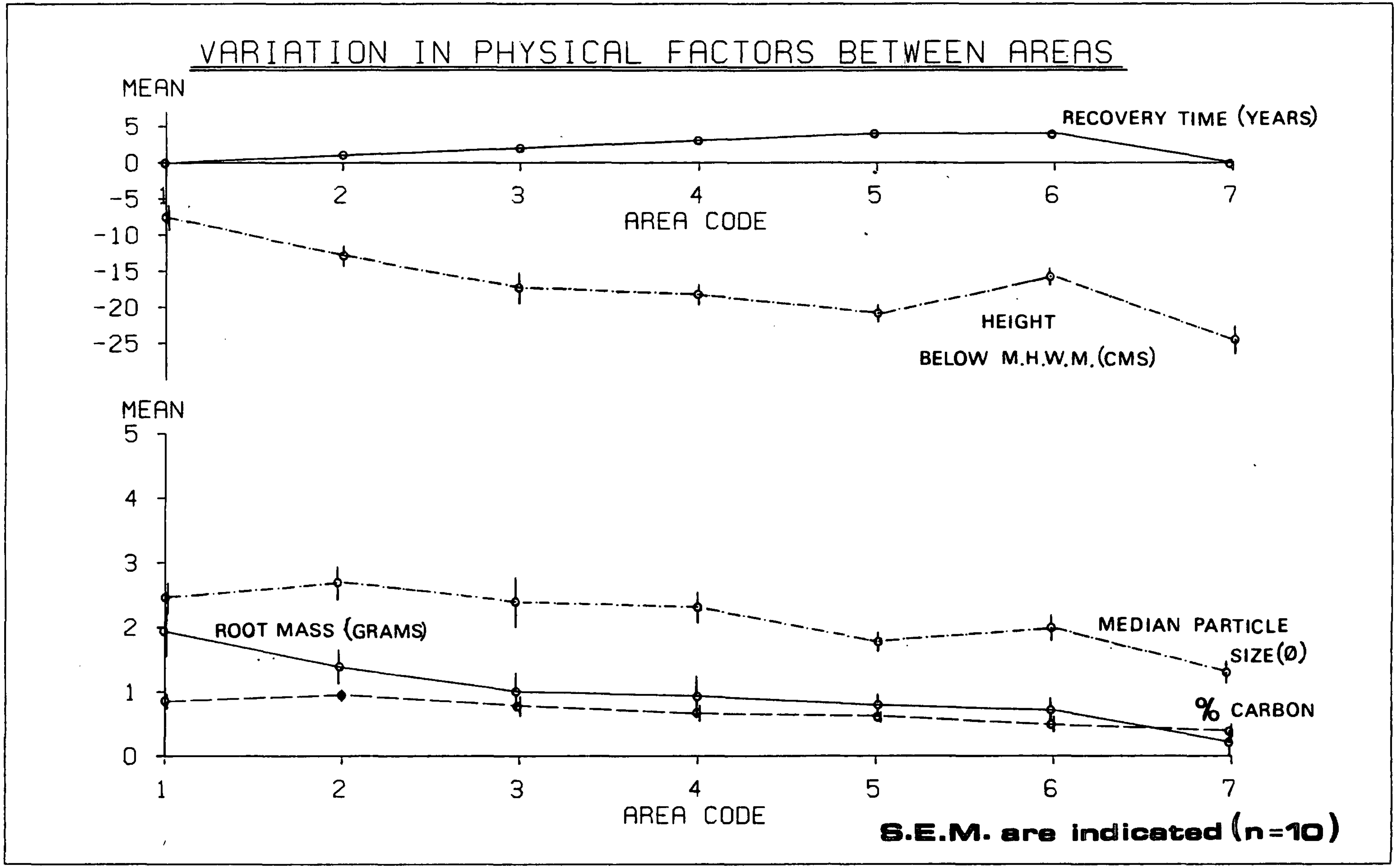
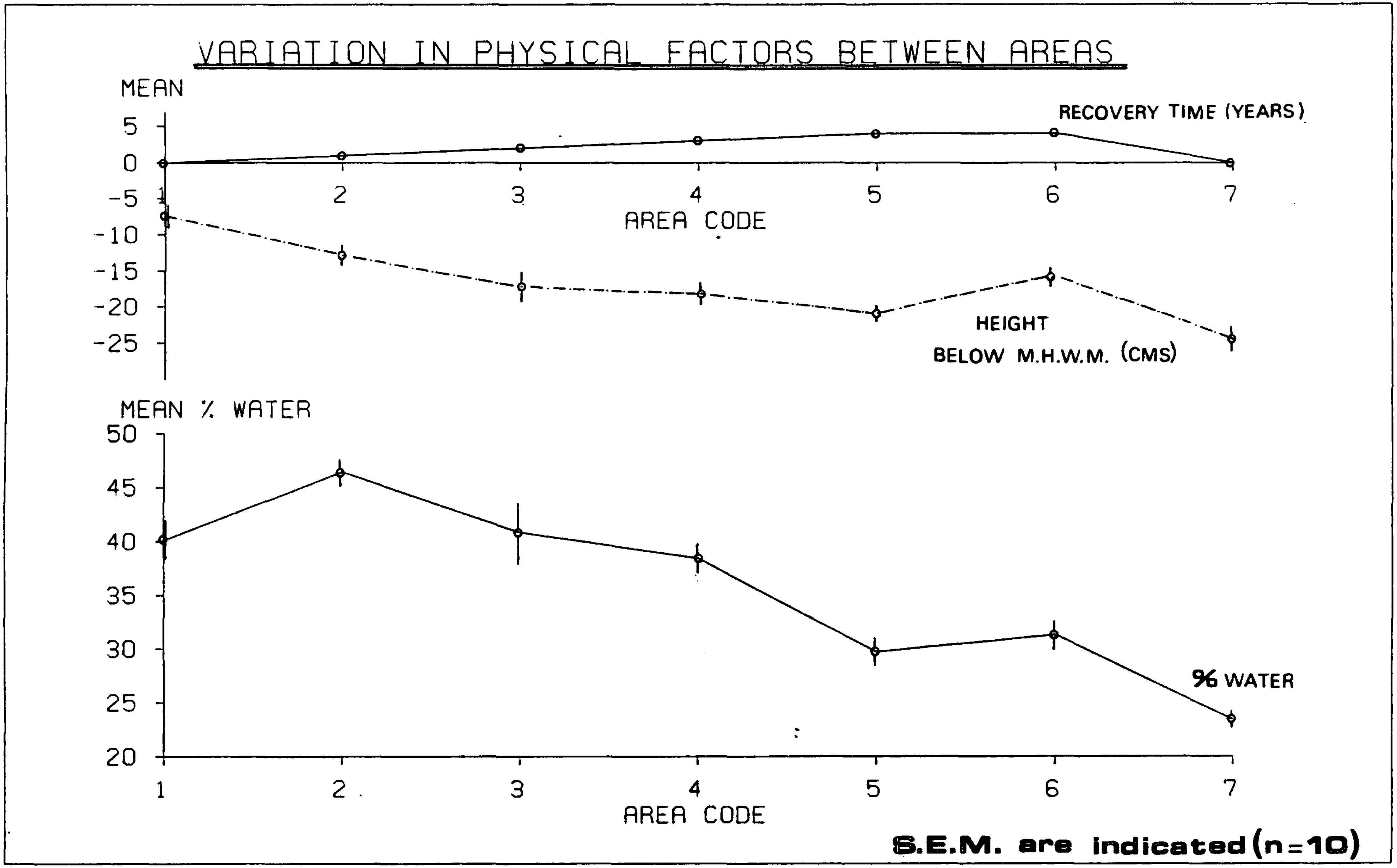

VARIATION IN PHYSICAL FACTORS BETWEEN AREAS
MEAN
5
0
-5
-10
-15
-20
-25
RECOVERY TIME (YEARS)
1
2
3
4
5
6
7
AREA CODE
HEIGHT
BELOW M.H.W.M.(CMS)
MEAN
5
4
3
2
1
0
ROOT MASS (GRAMS)
MEDIAN PARTICLE
SIZE(Ø)
% CARBON
1
2
3
4
5
6
7
AREA CODE
S.E.M. are indicated (n=10)

FIGURE 7



VARIATION IN PHYSICAL FACTORS BETWEEN AREAS
MEAN
5
0
-5
-10
-15
-20
-25
RECOVERY TIME (YEARS)
1
2
3
4
5
6
7
AREA CODE
HEIGHT
BELOW M.H.W.M. (CMS)
MEAN % WATER
50
45
40
35
30
25
20
% WATER
1
2
3
4
5
6
7
AREA CODE
S.E.M. are indicated (n=10)

FIGURE 8

factors and both the recovery time and height is required. The obvious deflection in the gradient of height is also shown in the gradient of median particle size at Area 6. This seems to indicate that there is a positive relationship with tidal height. However, the lower median phi value of particle size at Area 1 where height is greatest contradicts this simple hypothesis. A similar pattern is seen in Figure 8 with the % water content dropping with sites lower down the shore and with longer recovery times, and with a subsidary peak present at Area 6. Also Area 1 is markedly lower in % water content than Area 2, yet the height of Area 1 is greater. In Figure 9 %pH shows a gradient pattern which appears to be a mirror image of the % water gradient. In the case of pH Area 6 has a lower value than the surrounding areas and Area 1 has a higher pH than Area 2. As Area 5 and Area 6 have equal recovery times with other things being equal we would expect them to have similar values for their physico-chemical factors if recovery time is itself a major influencing factor.

Figure 10 presents graphically the mean values of redox potential as measured at each area. Although there appear to be differences between some areas (despite frequently high standard errors), there is no visible pattern or gradient to the values of this physical factor.

Generally it appears that a change occurs after spraying as seen in the gradient of most factors from Areas 2 to 5. This primary response gradient is then modified by the influence of height. In order to confirm statistically these tentative

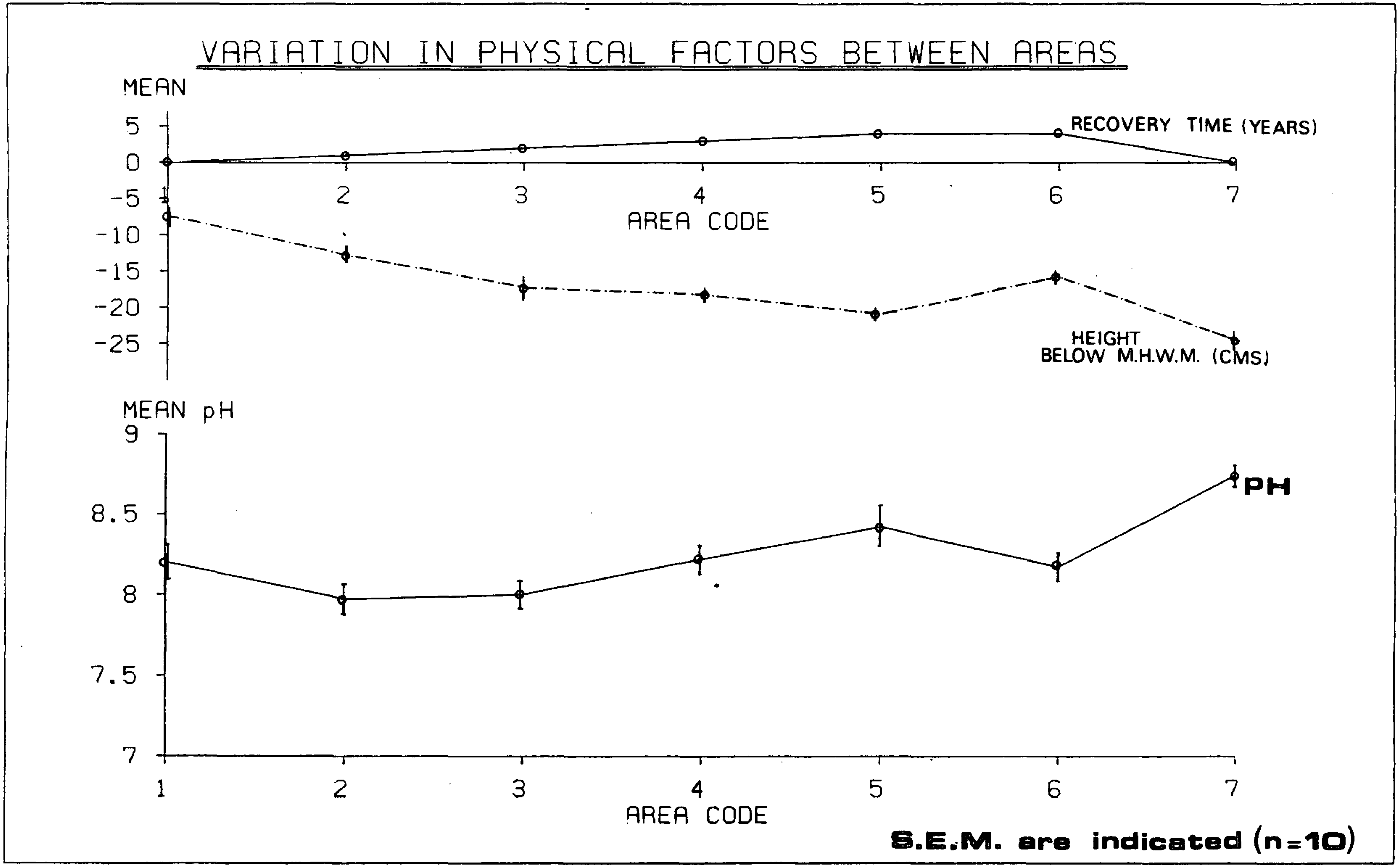

VARIATION IN PHYSICAL FACTORS BETWEEN AREAS
MEAN
5
0
-5
-10
-15
-20
-25
1
2
3
4
5
6
7
AREA CODE
RECOVERY TIME (YEARS)
HEIGHT
BELOW M.H.W.M. (CMS)
MEAN pH
9
8.5
8
7.5
7
PH
AREA CODE
S.E.M. are indicated (n=10)

FIGURE 9

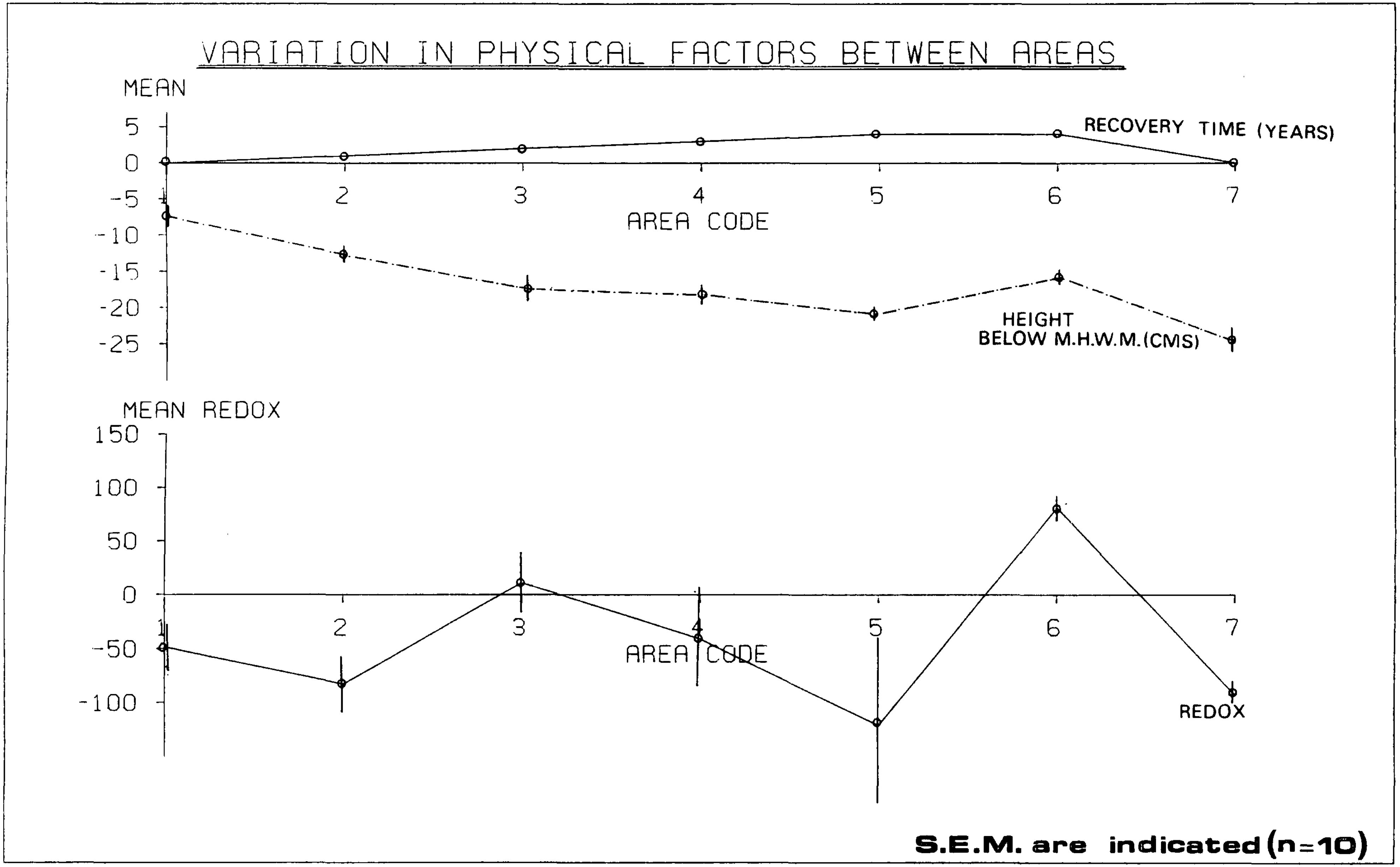

VARIATION IN PHYSICAL FACTORS BETWEEN AREAS
MEAN
5
0
-5
-10
-15
-20
-25
1
2
3
4
5
6
7
AREA CODE
RECOVERY TIME (YEARS)
HEIGHT
BELOW M.H.W.M.(CMS)
MEAN REDOX
150
100
50
0
-50
-100
AREA CODE
REDOX
S.E.M. are indicated (n=10)

FIGURE 10

conclusions, the data was examined for normality so that the validity of the statistical analysis could be ensured. However it was found that deviation from normality occurred, which could not be dealt with adequately by transformation. Therefore it was decided to use non-parametric statistical procedures for comparisons of the physico-chemical data values.

TABLE 11.

COMPARISION OF PHYSICO-CHEMICAL VARIABLES BETWEEN CONTROL SPARTINA AND SPRAYED AREAS.

Kruskall-Wallis 1 Way ANOVA.

Area 1 Unsprayed control Spartina
Areas 2-5 Sprayed

PHYSICO-CHEMICAL FACTOR	AREA MEAN RANKS 1	2	3	4	5	CHI SQUARE	SIGNF
REDOX POTENTIAL	27.9	21.8	34.7	27.3	15.7	9.57	0.048
HEIGHT	44.5	35.8	20.7	17.5	9.25	38.0	<0.000
ROOT MASS	36.9	29.8	21.6	19.7	19.4	11.1	0.026
% WATER CONTENT	26.8	41.0	28.8	23.3	7.60	27.2	<0.000
MEDIAN PARTICLE SIZE	29.0	40.0	26.6	24.0	7.75	25.5	<0.000
pH	27.9	18.2	19.2	27.9	34.2	8.35	0.074
% CARBON CONTENT	31.7	39.6	25.9	16.5	13.6	21.7	<0.000

(Significance levels are corrected for ties).

Although gradients in physico-chemical variables have been illustrated graphically a Kruskall-Wallis 1 way analysis of variance was carried out in order to test for significant variation in each of these factors amongst the areas. Table 11 presents the results of comparisons of physico-chemical factors between Areas 1, 2, 3, 4 and 5. These results confirm that there are significant differences between areas in all factors tested except for pH. To ascertain whether this variation occurs between the individual sprayed areas and the unsprayed *Spartina* (Area 1) a series of Mann-Whitney U tests were carried out between each sprayed area and Area 1. These results are presented in the Appendix in Table 9.

TABLE 12.

SUMMARY OF THE TESTS OF BIO-PHYSICAL FACTOR VARIATION BETWEEN SPARTINA SWARD AND SPRAYED AREAS.

MANN-WHITNEY U -WILCOXON RANK SUM W TEST RESULTS.

Values of Significance; *= less than 0.05
**= less than 0.01
Compared area shows higher value if significance level shows + or lower value if - is indicated.

AREA	COMPARED AREA	REDOX	HEIGHT	ROOT MASS	% WATER	MEDIAN 0	pH	% CARBON
1	2		-**		+*	+*		+*
1	3		-**	-*				
1	4		-**	-**				+*
1	5	-*	-**	-**	-*	-**		-*
1	6	+**	-**	-**	-**	-**		-**
1	7	-*	-**	-**	-**	-**	+**	-**
5	6	+**	+**					+*

Areas 2 - 5 = sprayed
Area 6 = replicate of area 5(sprayed 4 years ago)
Area 7 = open mud

Tests were also carried out comparing the unsprayed

Spartina (Area 1) with the replicate area sprayed four years ago (Area 6) and also with the open mud (Area 7). These results are also presented in the Appendix in Tables 10 and 11. A summary of all these tests is shown below in Table 12.

There were significant differences in height between all areas and the unsprayed *Spartina*. There are also significant differences in all factors between the *Spartina* area and Area 5. This is as expected from the interpretation of figures 5, 6 and 7. Area 6 shows the same dissimilarity from the *Spartina* sward as Area 5 except for the redox potential.

Generally it appears that areas recently sprayed have higher water and carbon content and a smaller median particle size than the unsprayed *Spartina* sward. However areas that have been sprayed three or four years ago have lower water and carbon contents, and larger median particle size.

It was anticipated that changes in sediment character would occur with increased recovery time due to movement of the finer sediments when these are nolonger protected by the *Spartina* sward, or restrained by root structures. These results indicate that root structure breakdown is signifiont in the second year after spraying.

The comparison of the *Spartina* sward area with the open mud shows that the former has higher water and carbon content and a smaller median prticle size. These findings are in agreement with the study by Millard (1976). However it must be noted that the height difference is significant (as it is with all areas compared with the *Spartina*) and this may have an influence on all physico-chemical factors.

The results of a comparison of Areas 5 and 6 are also shown in Table 9 and indicate that the replicate area sprayed four years ago (Area 6) is significantly higher. However the only other differences between the physico-chemical factors is seen in redox potential and carbon content. Neither of these appear to show correlation with height from the graphs of mean values, and therefore it is suggested that in this case height itself is not the major factor influenceing the value of other physico-chemical variables.

(b) Differences in Physico-Chemical Factors Between Sprayed Areas and the Open Mud.

In order to assess whether or not the sprayed areas are significantly different from the open mud regarding physico-chemical factors, further statistical analysis was carried out. Table 13 below shows the results of a Kruskall-Wallis 1 way analysis of variance between Areas 2, 3, 4, 5 and 7.

TABLE 13

COMPARISION OF PHYSICO-CHEMICAL VARIABLES BETWEEN UNSPRAYED OPEN MUD AND SPRAYED AREAS.

Kruskall-Wallis 1 Way ANOVA.

Area 7 Unsprayed open mud
Areas 2-5 Sprayed

PHYSICO-CHEMICAL FACTOR	AREA MEAN RANKS					CHI	
	2	3	4	5	6	SQUARE	SIGNF
REDOX POTENTIAL	23.7	36.3	28.6	16.4	22.3	10.5	0.033
HEIGHT	44.6	30.0	26.9	17.6	8.25	35.4	<0.000
ROOT MASS	35.5	27.9	26.9	27.2	9.90	16.7	0.002
% WATER CONTENT	42.1	33.1	29.6	16.8	5.75	38.4	<0.000
MEDIAN PARTICLE SIZE	42.1	31.7	30.4	17.6	5.50	37.8	<0.000
pH	15.4	16.4	23.8	30.1	41.7	22.2	<0.000
% CARBON CONTENT	42.9	32.0	24.2	22.0	6.30	34.3	<0.000

(Significance levels are corrected for ties).

These results clearly show that there is a significant difference in all factors within the region.

Therefore further analysis was carried out by comparing each sprayed area with the open mud (Area 7) by a Mann-Whitney U test. These results are shown in Table 12 in the Appendix, and are summarised below in Table 14.

TABLE 14.
SUMMARY OF THE TESTS OF PHYSICO-CHEMICAL FACTOR VARIATION BETWEEN OPEN MUD AND SPRAYED AREAS.

MANN-WHITNEY U -WILCOXON RANK SUM W TEST RESULTS.

Values of Significance; *= less than 0.05
**= less than 0.01
Compared area shows higher value if significance level shows + or lower value if - is indicated.

AREA	COMPARED AREA	REDOX	HEIGHT	ROOT MASS	% WATER	MEDIAN O	pH	% CARBON
7	6	+**	+**	+**	+**	+**	-**	+*
7	5		+**	+**	+**	+**	-*	+**
7	4		+**	+**	+**	+**	-**	+**
7	3	+*	+**	+**	+**	+**	-**	+**
7	2		+**	+**	+**	+**	-**	+**

Area 2-5= Sprayed areas.
Area 6= Replicate sample of an area sprayed four years ago.
Area 7= Open mud.

The open mud had significantly lower water and carbon content, and larger particle size than all the sprayed areas. Also pH was significantly lower in the open mud, whereas the redox potential is significantly higher in the open mud but only when compared with two of the sprayed areas. As *Spartina* is virtually absent from the open mud area sampled (a few small isolated clumps were encountered) it is expected that root mass should be significantly lower in Area 7, and this is confirmed in the results. Also confirmed by the results is the significance of the lower height of the mud-flat in the open mud (Area 7) to all other compared areas.

The results relating to the physico-chemical variables presented so far describe the general properties of these factors in each area. From these descriptions and their interpretation tentative proposals have been made regarding the central question; what are the physico-chemical changes brought about by spraying and the resulting *Spartina* sward destruction? In order to further examine and clarify these proposals the presence of correlations between the factors was studied by the production of a rank correlation matrix, the results of which are shown in Table 15 below.

TABLE 15.

CORRELATIONS BETWEEN PHYSICO-CHEMICAL FACTORS.

SPEARMAN RANKED CORRELATION COEFFFICIENTS (RHO).

SPECIES	RECOVERY TIME	REDOX	HEIGHT	ROOT MASS	% WATER	MEDIAN O	pH	% CARBON
RECOVERY TIME	XXXXX XXXXX	N=60	N=60	N=59	N=60	N=60	N=60	N=60
REDOX POTENTIAL	+.133 0.155	XXXXX XXXXX	N=69	N=70	N=70	N=70	N=70	N=70
HEIGHT	-.732 0.001	+.099 0.210	XXXXX XXXXX	N=69	N=69	N=69	N=69	N=698
ROOT MASS	-.456 0.001	-.074 0.272	+.650 0.001	XXXXX XXXXX	N=70	N=70	N=70	N=70
% WATER CONTENT	-.663 0.001	+.063 0.302	+.725 0.001	+.561 0.001	XXXXX XXXXX	N=70	N=70	N=70
MEDIAN O	-.639 0.001	+.011 0.464	+.769 0.001	+.496 0.001	+.859 0.001	XXXXX XXXXX	N=70	N=70
pH	+.187 0.076	-.548 0.001	-.335 0.002	-.158 0.096	-.463 0.001	-.481 0.001	XXXXX XXXXX	N=70
% CARBON CONTENT	-.680 0.001	-.205 0.045	+.717 0.001	+.566 0.001	+.815 0.001	+.837 0.001	-.352 0.001	XXXXX XXXXX

(Significance levels are based upon a one tailed test).

These results are based upon data from Areas 1 to 6. Area 7 is not included as the recovery time cannot be legitimately described in a way that can be used for the correlation study. Also the central question relates to the changes that occur through spraying and require before and after data, and therefore the open mud data is not neccessary for this comparison (and could complicate the issue in question).

A summary of the significant correlations between the variables is presented below in Table 16.

TABLE 16.

SUMMARY OF SIGNIFICANT CORRELATIONS
BETWEEN PHYSICO-CHEMICAL FACTORS.

SPEARMAN RANKED CORRELATION COEFFICIENTS (RHO)

Values of Significance; *= less than 0.05
**= less than 0.01
+ or - signs indicate positive or negative correlation relationships respectively.

SPECIES	RECOVERY TIME	REDOX	HEIGHT	ROOT MASS	% WATER	MEDIAN 0	pH	% CARBON
RECOVERY	XXXXX	N=60	N=60	N=59	N=60	N=60	N=60	N=60
REDOX		XXXXX	N=69	N=70	N=70	N=70	N=70	N=70
HEIGHT	-**		XXXXX	N=69	N=69	N=69	N=69	N=698
ROOT MASS	-**		+**	XXXXX	N=70	N=70	N=70	N=70
% WATER	-**		+**	+**	XXXXX	N=70	N=70	N=70
MEDIAN 0	-**		+**	+**	+**	XXXXX	N=70	N=70
pH		-**	-**		-**	-**	XXXXX	N=70
% CARBON	-**	-*	+**	+**	+**	+**	-**	XXXXX

(Significance levels are based upon a one tailed test).

The correlation coefffiicients suggest that height has the greatest effect on the root mass, water content, carbon content and median particle phi value (with correlation coefficients of +0.650, +0.725, +0.717 and +0.769 respectively). Therefore many of the variables are themselves interelated, either through direct causal relationships or through their similar correlations to height. For example carbon content is highly positively correlated with median particle phi value (correlation coefficient = +0.837). However pH is negatively correlated to median particle phi value (correlation coefficient =-0.481) but not to height (or recovery time) and therefore it appears that there is likely to be an interrelationship with particle size. Similarily redox potential is only correlated to pH (rho =-0.548) with no other significant relationship with any other physico-chemical variable.

The most important relationship from the point of view of this study is the correlation between height and recovery time, assuming that these factors are most likely to influence the physico-chemical character of each area. Due to the basic pattern of spraying, where each treated band of Spartina is higher on the shore than the area treated the previous year, it was anticipated that the factors of height and recovery time would be highly correlated. This is statistically confirmed by the correlation coefficient between the two factors (rho =-0.732). This provides the great problem of separating the two factors as potential causal factors on other bio-physiacl characters. Although from the above results the factor of height has the largest correlation with root mass, carbon content, water content and particle size, much of this effect could be due to the effect of spraying combined with time as described by the recovery time value. The larger rank correlation coeffecients of height do however, suggest that in this case height is the dominant factor. However, it should be noted that the rank correlation coefficient analysis is based upon a simple model, and earlier interpretation of Figures 7 and

8 suggest that such a response model may be simplistic.

The number of significant correlations between the physico-chemical factors studied indicate the presence of an extremely complex system. The identification of the causal factors in this system is clearly impossible from the observations of this study alone. However the results presented will be discussed later in the light of previous studies in order to try and identify the most likely cause of the observed differences in the physico-chemical characteristics of the studied areas.

Interelatioships between the Fauna and Bio-Physical Factors.

The identification of possible factors causing the observed differènce in macroinvertebrate distribution and density is the main purpose of the physico-chemical survey. The possible relationships were investigated by the production of a rank correlation matrix (based upon the same subset of data as the physico-chemical correlation matrix), which is presented below in Table 17.

TABLE 17.

CORRELATIONS OF SPECIES DENSITY WITH PHYSICO-CHEMICAL FACTORS.

SPEARMAN RANKED CORRELATION COEFFICIENTS (RHO)

SPECIES	RECOVERY TIME	REDOX	HEIGHT	ROOT MASS	% WATER	MEDIAN 0	pH	% CARBON
SAMPLE SIZE	60	70	69	70	70	70	70	70
NEREIS	+.311	-.308	-.333	-.103	-.213	-.189	+.304	-.086
	0.015	0.009	0.005	0.396	0.077	0.117	0.011	0.478
ARENICOLA	+.132	+.342	+.044	+.036	+.224	+.202	-.535	+.036
	0.316	0.004	0.721	0.768	0.062	0.093	0.001	0.829
CAPITELLA	-.021	-.105	-.049	-.036	+.340	+.376	-.357	+.318
	0.874	0.387	0.752	0.766	0.004	0.001	0.002	0.007
OLIGOCHAETA	+.027	+.022	+.234	+.280	+.363	+.380	-.144	+.356
	0.840	0.856	0.053	0.019	0.002	0.001	0.233	0.002
HYDROBIA	+.732	-.034	-.450	-.357	-.448	-.421	+.141	-.462
	0.001	0.782	0.001	0.002	0.001	0.001	0.244	0.001
LITTORINA	+.094	+.187	+.231	+.089	-.013	+.073	-.232	-.081
	0.476	0.121	0.056	0.462	0.914	0.549	0.054	0.503
MACOMA	+.655	+.347	-.264	-.294	-.257	-.241	-.084	-.437
	0.001	0.003	0.026	0.014	0.031	0.045	0.487	0.001
COROPHIUM	+.056	+.279	+.253	+.165	+.361	+.436	-.401	+.254
	0.670	0.019	0.036	0.173	0.002	0.001	0.001	0.034
OWENIA	-.065	-.035	+.047	+.100	+.394	+.435	-.330	+.324
	0.623	0.776	0.703	0.408	0.001	0.001	0.005	0.006
CARCINUS	-.329	+.031	+.257	+.158	+.219	+.202	-.358	+.268
	0.010	0.799	0.033	0.190	0.068	0.094	0.002	0.025

(Significance levels are based upon a two tailed test)

The number of significant relationships indicated by the rank correlation analysis presents a complicated picture, with no immediately obvious factor controlling all macroinvertebrate distribution and density. However, although different species react in different ways to the various physico-chemical characteristics of each area, groups of species with similar reponses can be identified. The significant correlations shown in Table 17 are summarised below in Table 18. The species are regrouped according to their response to the physico-chemical variables.

TABLE 18.

SUMMARY OF CORRELATIONS OF SPECIES DENSITY WITH PHYSICO-CHEMICAL FACTORS

SPEARMAN RANKED CORRELATION COEFFICIENTS (RHO).

Values of Significance; *= less than 0.05
**= less than 0.01
+ or - signs indicate positive or negative correlation relationships respectively.

SPECIES	RECOVERY TIME	REDOX	HEIGHT	ROOT MASS	% WATER	MEDIAN 0	pH	% CARBON
SAMPLE SIZE	60	70	69	70	70	70	70	70
NEREIS	+*	-**	-**				+*	
HYDROBIA	+**		-**	-**	-**	-**		-**
MACOMA	+**	+**	-*	-*	-*	-*		-**
OLIGOCHAETA				+*	+**	+**		+**
COROPHIUM		+*	+*		+**	+**	-**	+*
OWENIA					+**	+**	-**	+**
CAPITELLA					+**	+**	-**	+**
CARCINUS	-*		+*				-**	+*
ARENICOLA		+**					-**	
LITTORINA								

(Significance estimates are based upon a two tailed test)

The group of species comprisng of Hydrobia ulvae, Macoma balthica and Nereis diversicolor show highest correlations with recovery time and height. Due to the colinearity shown between height and recovery time it is expected that both should be significant, however, in the case of Hydrobia ulvae and Macoma balthica the correlation coefficients with recovery time (rho = +0.732 and +0.655 respectively) are markedly higher than the correlations with height (rho = -0.450 and -0.264 respectively), which suggests that in these cases recovery time is the predominant factor influencing density. In the case of Nereis diversicolor the correlation coefficients of height and recovery time are close in value (rho = -0.333 and +0.311 respectively) and therefore the relative importance of the two as causal factors is unclear.

The species Capitela capitata, Corophium volutator, Owenia fusiformis and the group comprised of the Oligochaeta all show increased density with increased median particle phi value (ie a decrease in the median particle diameter of the sediment). Associated with this correlation are significant but lower correlations indicating higher densities with higher water and carbon content, lower pH and in the case of Corophium volutator higher redox potential.

Carcinus maenas shows highest correlation with pH (rho = -0.358). Also the correlations with height and recovery time are also high (rho = +0.257 and -0.329 respectively). However as pH is correlated to height (rho = -0.535) the relative roles of pH and height are unclear.

In the case of Arenicola marina density is most highly correlated to pH (rho = -0.535), and secondly to redox potential (rho = +0.342).

As Log10(x+1) transformations of the abundance data of Hydrobia ulvae and Oligochaeta gave normal distributions, multiple regression analysis could be legitimately carried out with these species as dependant variables, in order to further examine the effects of changes in physico-chemical conditions. Thus a stepwise multiple regression procedure (spss release 7 "new regression") was used to investigate the effects of the independant variables of recovery time, redox potential, height, root mass, % water component, median particle size, pH and % carbon content. Transformations were used to improve the normality of the indépendant variable data. The transformation of Log10(x+1) was used for root mass, median particle phi value and pH. Transformations of Log10(100-x) were used for the % water content and % carbon content data. Values for recovery time, height and redox potential were not transformed. However there remained examples of deviance from normality in some of the transformed data. Also the variables of recovery time and height are not normaly distributed but show a linear gradient. However, the robustness of the technique was considered sufficient to deal with the independant variable data problem, when coupled with careful interpretation of the regression equation and its associated residual varainces.

The multiple regression procedure found no significant factors that control the density of Oligochaeta. However, a significant result was obtained from the _Hydrobia ulvae_ data. This showed that the only significant factor influencing the density of this species was recovery time. The recovery time factor had the folowing characters;

R = 0.719 Standard Error = 0.384

R Squared =0.516

F = 60.9 significance of F =0.0000

No other factor produced a significant contribution to the regression equation, therefore the final parameters of the equation are presented below.

Recovery time B = 0.0218 Standard Error = 0.0028

Constant B =0.4077 Standard Error = 0.0921

Regresson of hydrobia ulvae density with recovery time.

Hydrobia Density (y) = 0.4077 + 0.0218 (rt)

Analysis of standardized residuals by a normal probability plot of observed against expected showed that the regression equation was a good fit and lacked any visible interdependance or trend.

Although the problem of colinearity presents a problem in interpretation of the multiple regression results, the fact that height nolonger showed a significant correlation with _Hydrobia_ density after the inclusion of recovery time into the regression equation, indicates that in this species the effect of recovery time is the most likely factor influencing density.

As the presence of root material in the sediment has been found to be significant in relation to densities of Oligochaeta, Hydrobia ulvae and Macoma balthica, an investigation of the rate of breakdown of dead root material was carried out. The rate of decomposition was assessed by a linear regression analysis of root mass plotted against recovery time. Figure 11 shows the resulting regression line. The correlation coefficient of the line is -0.527 (R Squared = 0.272). The following regression equation shows, the breakdown of the root matter is slow.

Regression Equation of Root Mass plotted against Time

Root Mass (y) = 1.74 -(0.268(Recovery Time))

The estimated period for complete breakdown of root matter based on the above equation is approximately 6.5 years. However it should be noted that the rate of breakdown is unlikely to be of a linear type response, due to decrease in bacterial activity after 'palatble' components of the root material are exhausted. Therefore the time taken for complete breakdown is likely to be longer than estimated from the above equation.

ROOT MATERIAL DECOMPOSITION WITH TIME.

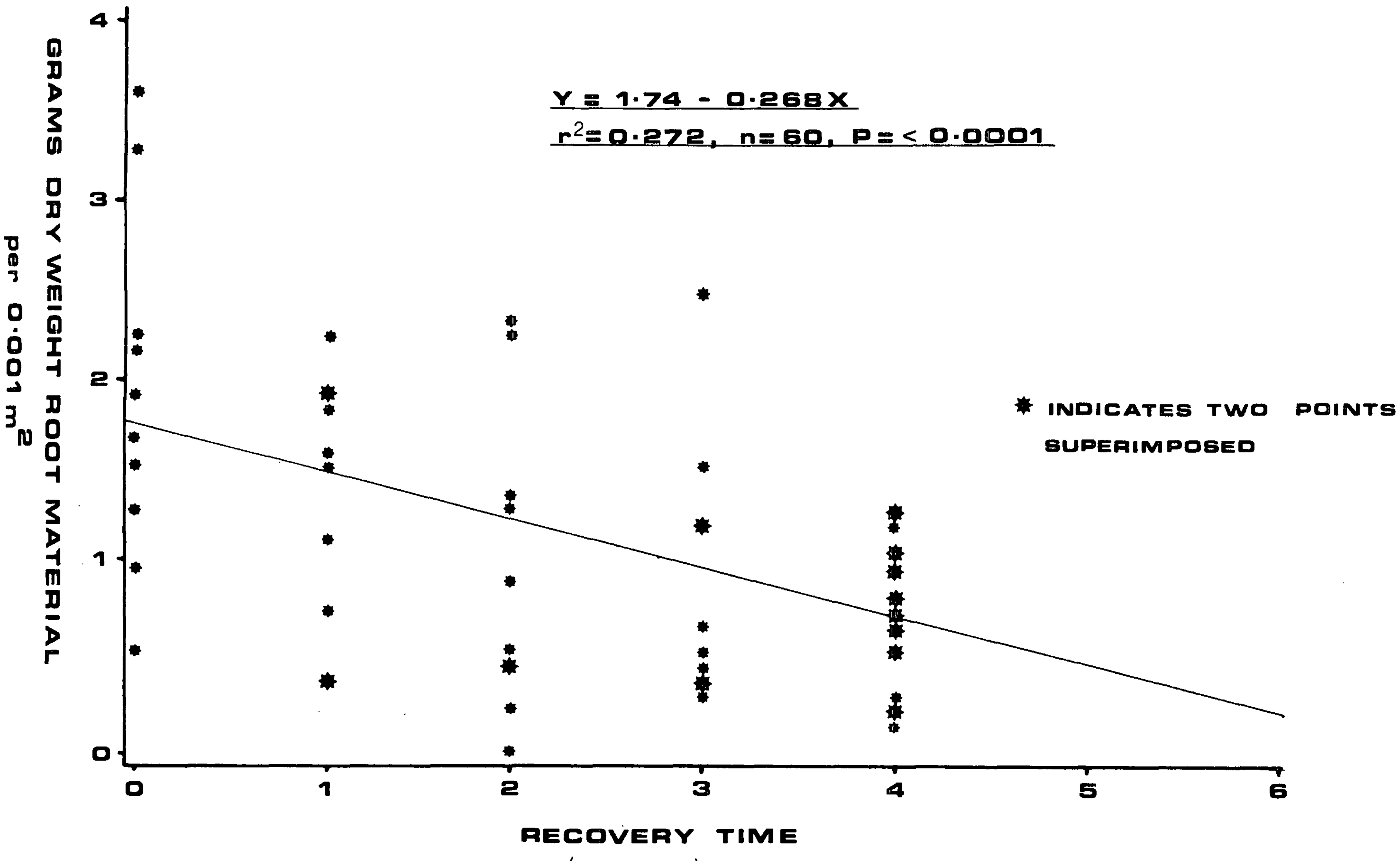


FIGURE 11

The results of the physico-chemical factor survey and interpretation of correlations with observed macroinvertebrate densities, show that generally there are significant differences between the physico-chemical characteristics of the areas, and these differences can be frequently correlated with the densities of the species present. The outcome of these results and their consequences will be discussed later.

CHAPTER 4.

FIELD STUDY OF THE IMMEDIATE EFFECTS OF DALAPON TREATMENT OF *SPARTINA ANGLICA*.

(i) Methods.

The control area used for the investigation of the immediate effects of Dalapon treatment consisted of the 10 metre by 10 metre plot of the *Spartina* sward Area 1. The baseline populations before spraying are therefore those used in the longterm study of spraying effects, and comprise samples 001 to 010. Shortly after Area 1 was sampled in order to describe the original macroinvertebrate population of the *Spartina* sward the area was sprayed once with Dalapon administered in the normal way, as described by Corkhill (1982). The concentration of Dalapon used was approximately 55 grams per litre, in conjunction with approximately 1 ml of the wetting agent Agral with every 20 litres of solution. These concentrations are now the standard spraying concentrations used at Lindisfarne, and in field working conditions amount to 25 kilograms of Dalapon per 100 gallons of water. This quantity is then used to treat 1 Acre of *Spartina* sward. Spraying was carried out at low tide, during a neap tide period, from an Agrocat eight wheel, low ground pressure vehicle fitted with agricultural spraying equipment.

Seven days after spraying the macroinvertebrate fauna was resampled using the standard techniques described in Chapter 3. Spraying was carried out again one month later (and under similar conditions) on an unsprayed area immediately adjacent (and at the same height) to Area 1. One day after the administration of the spray, the macroinvertebrate fauna was sampled. Although this area had not been sampled prior to spraying it is assumed that the macroinvertebrate populations of this area are not significantly different from Area 1.

(ii)Results

The mean numbers of the macroinvertebrate species found in each set of ten samples taken before spraying, 1 day after and 7 days after spraying are shown below in Table 19.

It is apparent from these results that the density of most species does not drop after spraying as would be expected if the spray had toxic effects. Only *Carcinus maenas* was completely absent from both sprayed areas. Since the population density was very low before spraying, the significance of this change is not clear. Figure 12 shows the mean values of the four most abundant species encountered in the samples. Clearly these results suggest that there was no decrease in density after spraying as in all cases the mean densities were higher in the sprayed areas than in the control areas.

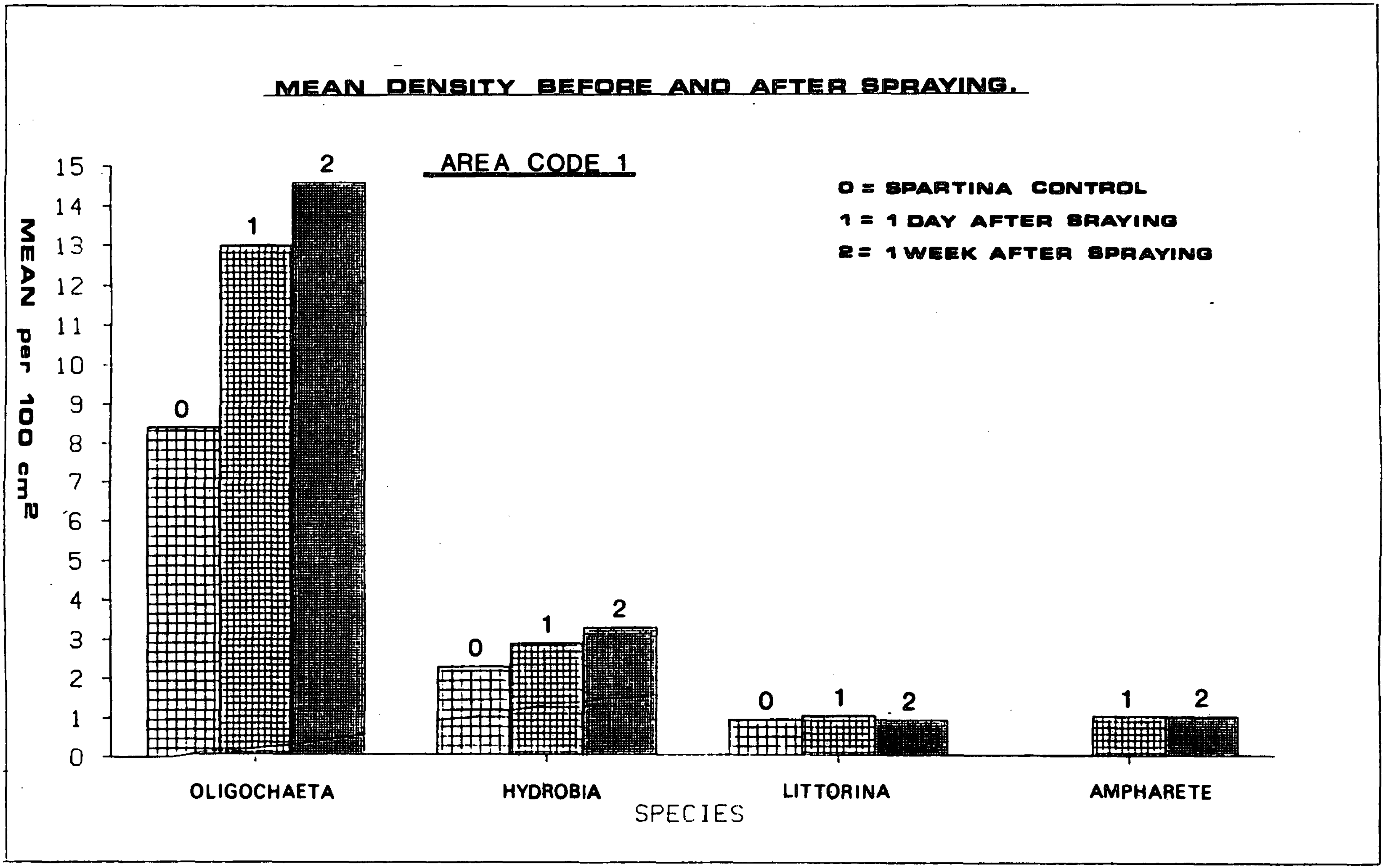

MEAN DENSITY BEFORE AND AFTER SPRAYING.
AREA CODE 1
0 = SPARTINA CONTROL
1 = 1 DAY AFTER SRAYING
2= 1 WEEK AFTER SPRAYING
MEAN per 100 cm2
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
OLIGOCHAETA
HYDROBIA
LITTORINA
AMPHARETE
SPECIES

FIGURE 12

TABLE 19.

MEAN DENSITY PER 100 Cm SAMPLE IN SPRAYED AND UNSPRAYED SPARTINA SWARD.

Standard errors of the mean are shown within brackets.

SPECIES	UNSPRAYED SPARTINA	1 DAY AFTER SPRAYING	7 DAYS AFTER SPRAYING
CAPITELLA		0.40 (0.40)	
OLIGOCHAETA	8.00 (1.56)	13.0 (4.23)	14.6 (2.97)
HYDROBIA	2.30 (0.77)	2.90 (1.02)	3.30 (0.76)
LITTORINA	0.90 (0.31)	1.00 (0.33)	0.90 (0.18)
MACOMA		0.20 (0.13)	
COROPHIUM	0.10 (0.10)		
OWENIA		0.30 (0.30)	
CARCINUS	0.40 (0.16)		

In order to test for any significant response to the spraying of the *Spartina* a Kruskall-Wallis 1 way analysis of variance was carried out on all species. The results are shown in Table 20.

TABLE 20.

COMPARISION OF SPECIES DENSITY VARIATION WITHIN SPRAYED AND UNSPRAYED SPARTINA.

Kruskall-Wallis 1 Way ANOVA.

	MEAN RANKS				
	UNSPRAYED	SPRAYED			
SPECIES	CONTROL SPARTINA	1 DAY AFTER	7 DAYS AFTER	CHI SQUARE	SIGNF
CAPITELA CAPITATAOR	15.0	16.5	15.0	2.00	0.368
OLIGOCHAETA	12.6	15.2	18.6	2.39	0.303
HYDROBIA ULVAE	14.1	14.4	18.0	1.25	0.536
LITTORINA SAXATILIS	15.1	15.7	15.7	0.03	0.938
MACOMA BALTHICA	13.5	16.5	16.5	2.23	0.328
COROPHIUM VOLUTATOR	16.5	15.0	15.0	2.00	0.368
OWENIA FUSIFORMIS	15.0	16.5	15.0	2.00	0.368
CARCINUS MAENAS	19.5	13.5	13.5	8.93	0.012

(Significance levels are corrected for ties).

All the species tested, with the exception of *Carcinus maenas* showed no significant difference in densities between any of the sampled areas, either before or after spraying. However *Carcinus maenas* showed a significant decrease (corrected significance level of 0.012) in abundance. Therefore it must be concluded that the effect of the Dalapon and Agral spray is either total mortality of the exposed population or total dispersal. As regards the remaining species, there is no evidence from this study of any deleterious effects on the populations of these invertebrates from the treatment of *Spartina* by Dalapon and Agral, in the concentrations used.

CHAPTER 5

LABORATORY STUDY OF THE TOXIC EFFECTS OF DALAPON AND AGRAL.

(i) Methods of acute toxicity estimation (long exposure time).

Four species of mud-flat macroinvertebrates, *Corophium volutator*, *Nereis diversicolor*, *Hydrobia ulvae* and *Macoma balthica* were used for a preliminary study of direct toxicity of Dalapon with Agral. However it proved impossible to distinguish live from dead *Macoma balthica*, so further tests were carried out on the remaining three species. Specimens were collected at low tide from Lindisfarne (under permit from the N.C.C), by sieving on site, and transportation to Durham in fresh seawater filled containers. Upon arrival at the laboratory the required number of each species for each test was placed in tanks containing two litres of freshly collected seawater. The tanks were kept at a constant temperature of 10° C, with the water in each individually aerated. For each species, 4 groups were tested, each consisting of 50 individuals of *Corophium volutator* and *Hydrobia ulvae*, and 10 individuals of *Nereis diversicolor*. The lower number of individuals used in the case of *Nereis diversicolor* arose from difficulties in obtaining large numbers of live specimens. An acclimatisation period of twelve hours was given before any experiments were carried out.

Each group was then exposed to concentrations of either Dalapon, Dalapon and Agral, or only Agral, within the concentration range of 1/1000 to the equivalent of the standard spraying concentration at Lindisfarne. These tests (and the concentrations of Dalapon and Agral used) are outlined in the following table.

TABLE 21.

CONCENTRATIONS OF DALAPON AND AGRAL USED IN TOXICITY TESTS.

DALAPON; Sodium Salt of 2,2-Dichloropropionic Acid

AGRAL; 90% Alkly Phenol Ethylene Oxide Condensate (ICI 1984)

GROUP (TANK)	WORKING CONCENTRATION	DALAPON PER LITRE	AGRAL PER LITRE
A*	1	55.0 GMS	0.05 ML
B	1/10	5.50 GMS	0.005 ML
C	1/100	0.55 GMS	0.0005 ML
D	1/1000	0.055 GMS	0.00005 ML
E	CONTROL	-	-

* THIS CONCENTRATION WAS ONLY USED IN THE TEST FOR DALAPON WITH AGRAL.

Experiments were carried out in the order

(i) Dalapon and Agral

(ii) Dalapon only

(iii) Agral only

The chemicals were introduced by dissolving the appropriate amounts in 500 ml of seawater and then adding this to the tank. With a final volume of 2.5 litres in each tank, solutions contained the required concentration of herbicide and agral.

The number of animals alive was ascertained by tactile stimulation, at periods of 24, 48 and 72 hours after intial exposure to the chemicals.

(ii) Results.

The responses of each species to the various concentrations of herbicide and wetting agent encountered for each time period, are shown in Figures 13 to 20. Each figure includes an upper graph of % mortality against log10 of the concentration for each exposure time period, and a lower graph of % mortality against exposure time for each concentration. For Nereis diversicolor responses consisted of complete mortality or no mortality within 72 hours. The plot of % mortality against log10 concentration is based upon the logistic model interpretation of toxicological test data for the establishment of LC50 estimates. The log10 concentration values of -3, -2, -1 and 0 refer to respectively to 1/1000, 1/100, 1/10 and 1 X the concentration used in field operations. From these results it can be seen that, at most, only two points on the graph lie between 0 and 100% mortality and therefore LC50 values cannot be estimated validly. However the tests can be used to define the upper and lower concentrations of Dalapon and Agral between which the LC50 lies. For each species the concentration range of such estimated LC50's are presented in the following tables.

TABLE 22.

ACUTE TOXICITY EFFECTS OF DALAPON.

LC50 RANGE IN RELATION TO SPRAYING CONCENTRATION

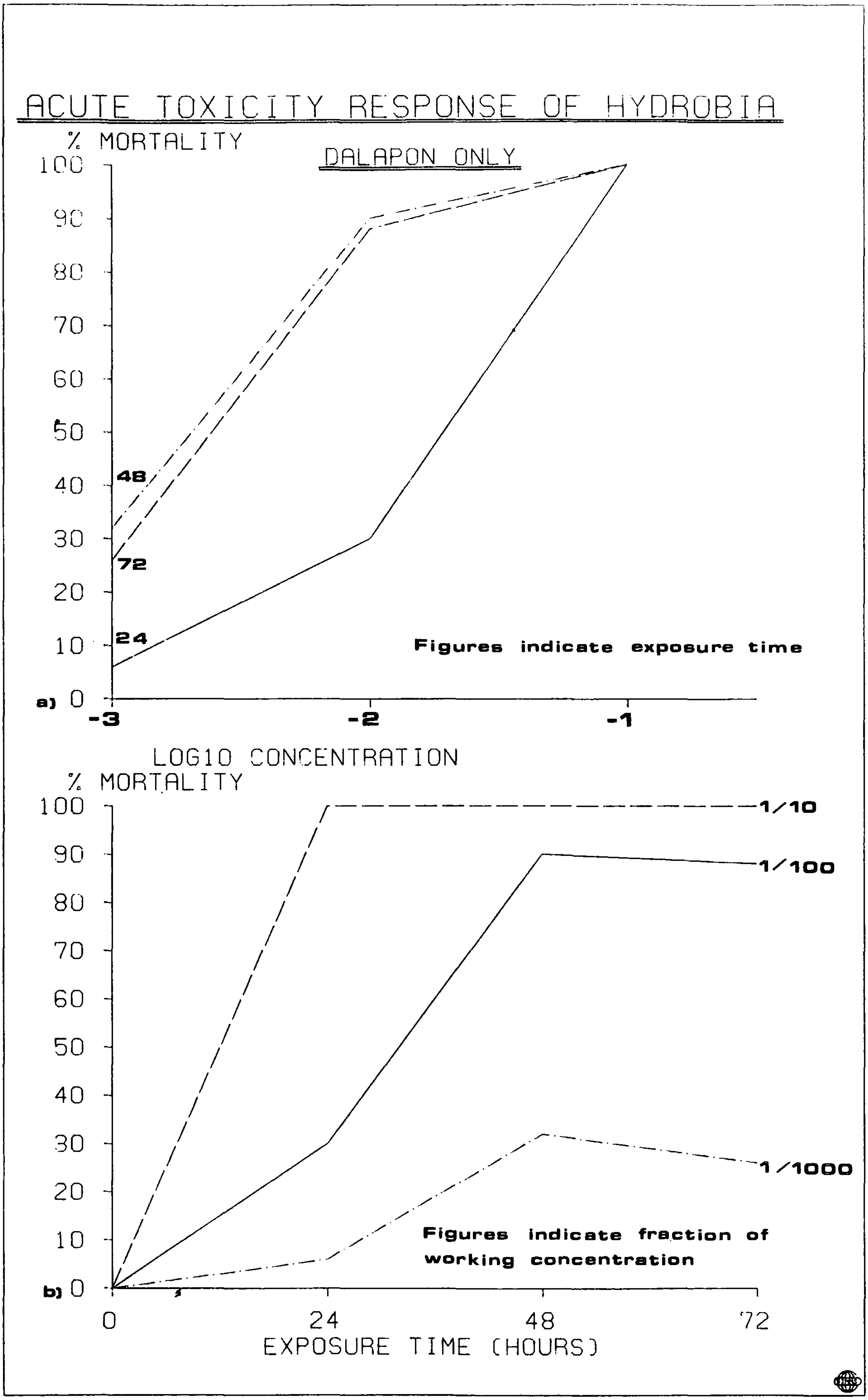
SPECIES	N	24 HOURS LC50	48 HOURS LC50	72 HOURS LC50
COROPHIUM	50	› 1/10	› 1/10	= 1/10
HYDROBIA	50	1/100-1/10	1/100-1/10	1/100-1/10
NEREIS	10	› 1/10*	› 1/10*	› 1/10*

* NO MORTALITY WAS RECORDED

SPRAYING CONCENTRATIONS: DALAPON = 55 GRAMS PER LITRE

The results in Table 22 show that for Dalapon only the LC50 estimate lay between 1/100 and 1/10 the normal working concentration for Corophium volutator and Hydrobia ulvae. However the concentration range spanning the LC50 is lower with increased exposure time in both cases. Nereis diversicolor showed no mortality at 1/10 working concentration.

As shown by Table 23 below and Figures 15 and 16, a similar range of Agral concentration spanned the LC50 values for Hydrobia ulvae and Corophium volutator, namely 1/10 to 1/100 the working concentrations.



ACUTE TOXICITY RESPONSE OF HYDROBIA
% MORTALITY
DALAPON ONLY
100
90
80
70
60
50
40
30
20
10
0
48
72
24
Figures indicate exposure time
a)
-3
-2
-1
LOG10 CONCENTRATION
% MORTALITY
100
90
80
70
60
50
40
30
20
10
0
1/10
1/100
1/1000
Figures indicate fraction of
working concentration
b)
0
24
48
72
EXPOSURE TIME (HOURS)

FIGURE 13

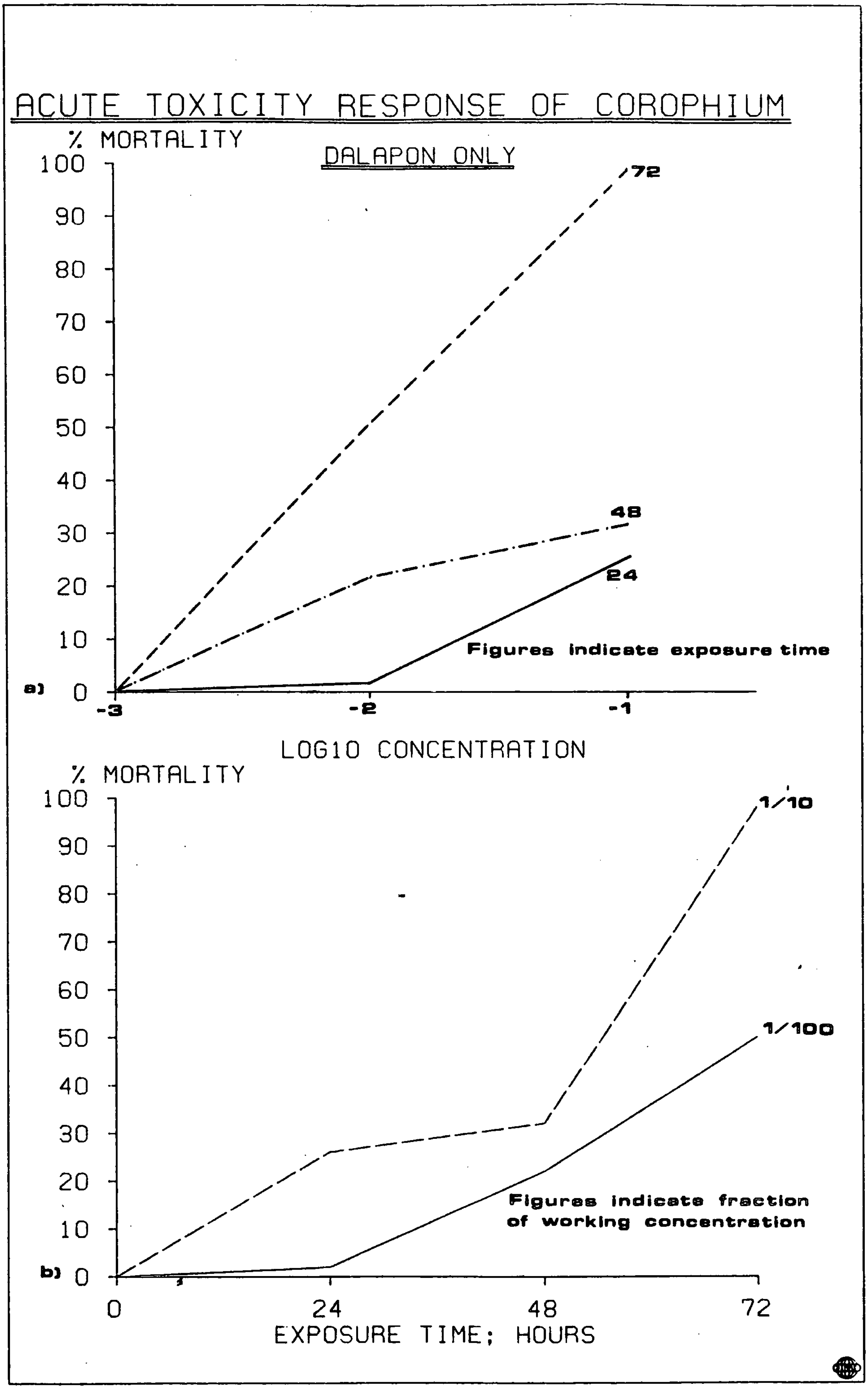

ACUTE TOXICITY RESPONSE OF COROPHIUM
% MORTALITY
DALAPON ONLY
100
90
80
70
60
50
40
30
20
10
0
72
48
24
Figures indicate exposure time
a)
-3
-2
-1
LOG10 CONCENTRATION
% MORTALITY
100
90
80
70
60
50
40
30
20
10
0
1/10
1/100
Figures indicate fraction of working concentration
b)
0
24
48
72
EXPOSURE TIME; HOURS

FIGURE 14

TABLE 23.

ACUTE TOXICITY EFFECTS OF AGRAL.

LC50 RANGE IN RELATION TO CONCENTRATION.

SPECIES	N	24 HOURS LC50	48 HOURS LC50	72 HOURS LC50
COROPHIUM	50	1/100-1/10	1/100-1/10	1/100-1/10
HYDROBIA	50	1/100-1/10	1/100-1/10	1/100-1/10
NEREIS	10	› 1/10*	› 1/10*	› 1/10*

* NO MORTALITY WAS RECORDED

SPRAYING CONCENTRATIONS: AGRAL = 0.05 ML PER LITRE

However *Corophium volutator* showed a response to a lower concentration of Agral than with Dalapon. In both species there was no measurable increase in mortality with increased exposure time to agral, as indicated by the levelling off in Figure 16(b) of the mortality of *Hydrobia ulvae* and the obvious decrease in slope in Figure 17(b) of the mortality of *Corophium volutator*. As in the previous test, no mortality of *Nereis diversicolor* occured even at the highest concentration tested (1/10 working concentration).

The test using Dalapon with Agral relates more closely to the field situation, where both chemicals are used in the treatment of *Spartina*. The previous experiments with Dalapon and Agral in isolation showed that both have toxic effects. Table 24 below shows the results of use of both these chemicals in combination.

TABLE 24.

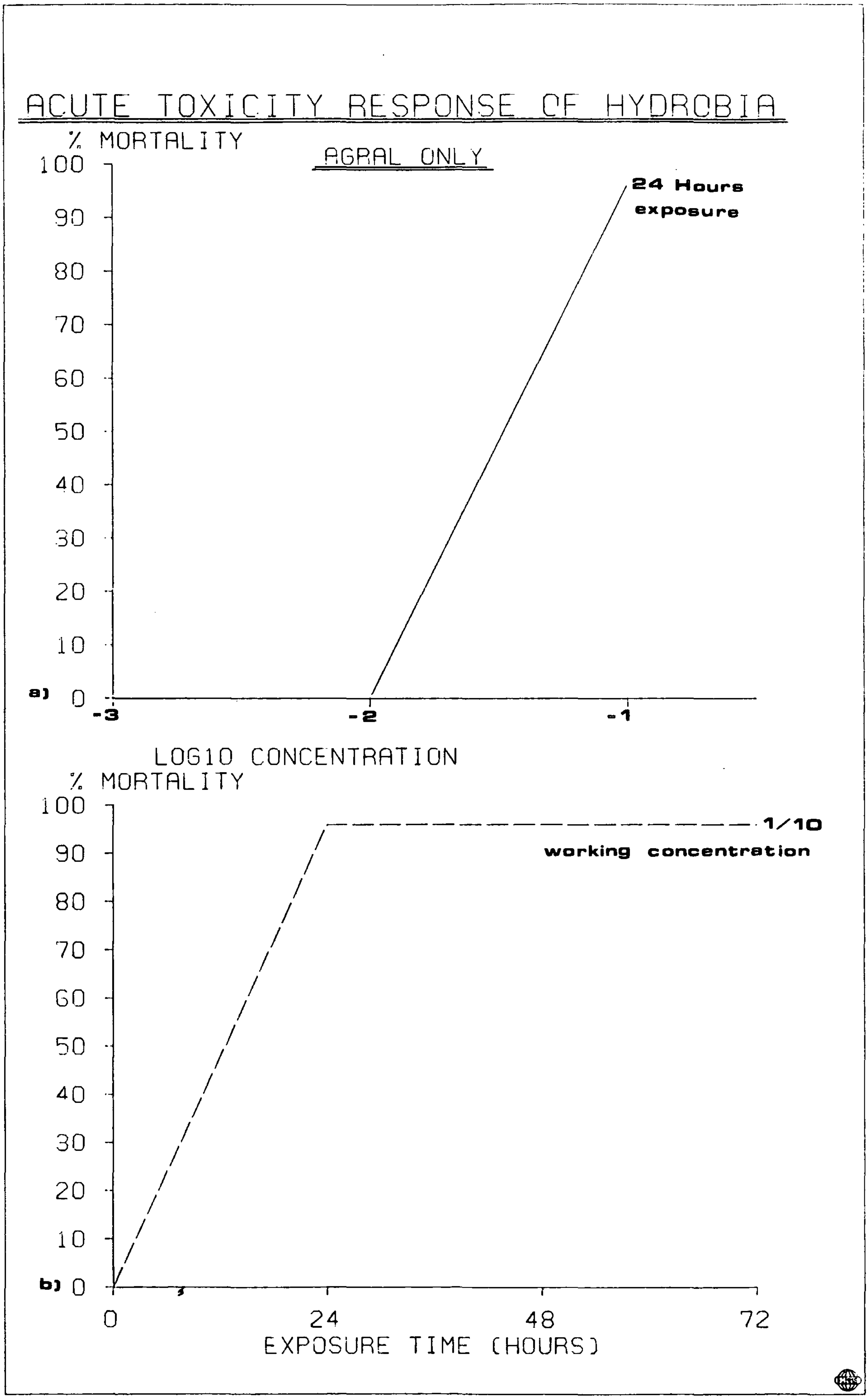

ACUTE TOXICITY RESPONSE OF HYDROBIA
% MORTALITY
AGRAL ONLY
24 Hours exposure
a)
100
90
80
70
60
50
40
30
20
10
0
-3
-2
-1
LOG10 CONCENTRATION
% MORTALITY
1/10 working concentration
b)
0
24
48
72
EXPOSURE TIME (HOURS)

FIGURE 15

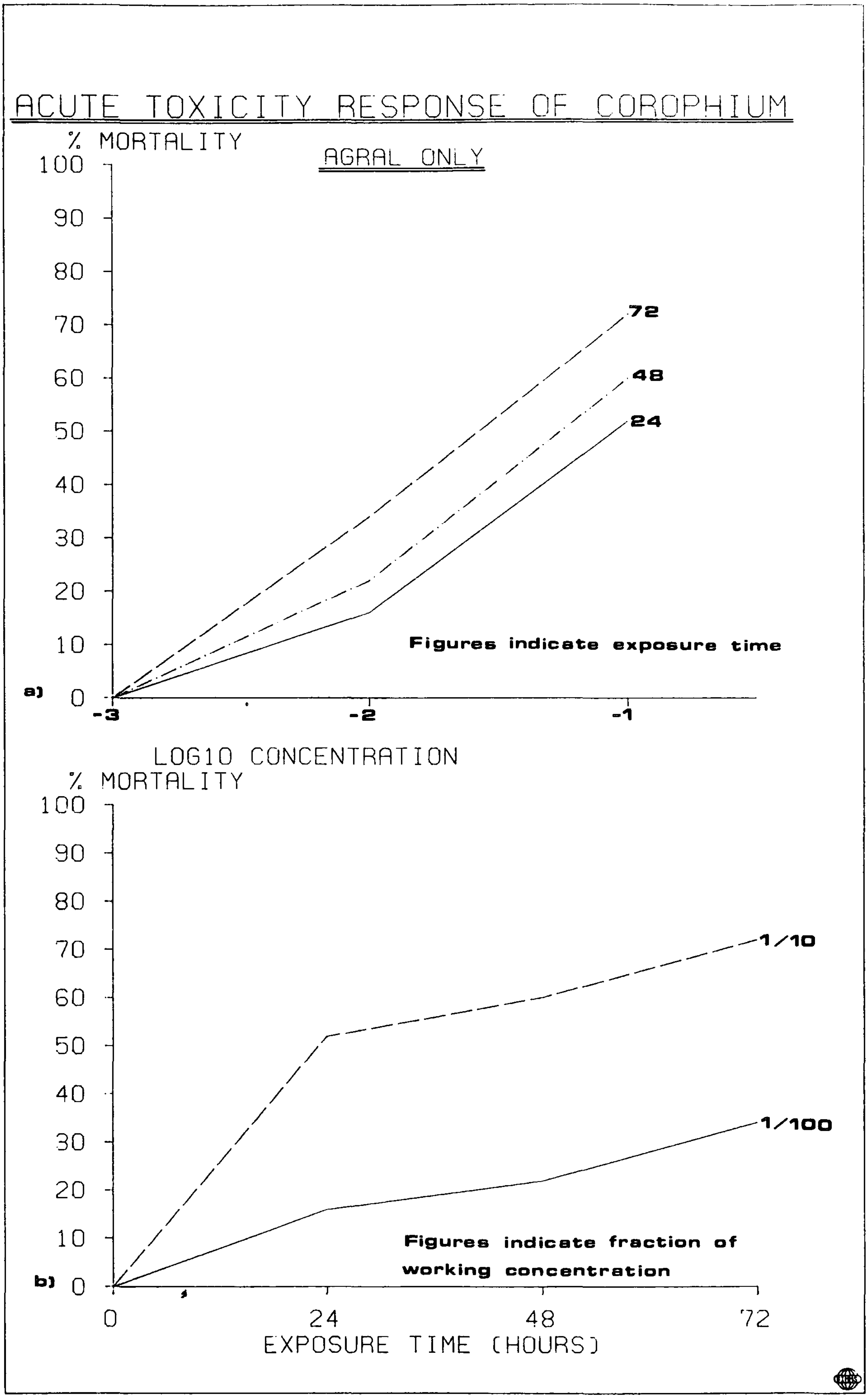

ACUTE TOXICITY RESPONSE OF COROPHIUM
% MORTALITY
AGRAL ONLY
100
90
80
70
60
50
40
30
20
10
0
72
48
24
Figures indicate exposure time
a)
-3
-2
-1
LOG10 CONCENTRATION
% MORTALITY
100
90
80
70
60
50
40
30
20
10
0
1/10
1/100
Figures indicate fraction of
working concentration
b)
0
24
48
72
EXPOSURE TIME (HOURS)

FIGURE 16

ACUTE TOXICITY EFFECTS OF DALAPON WITH AGRAL.

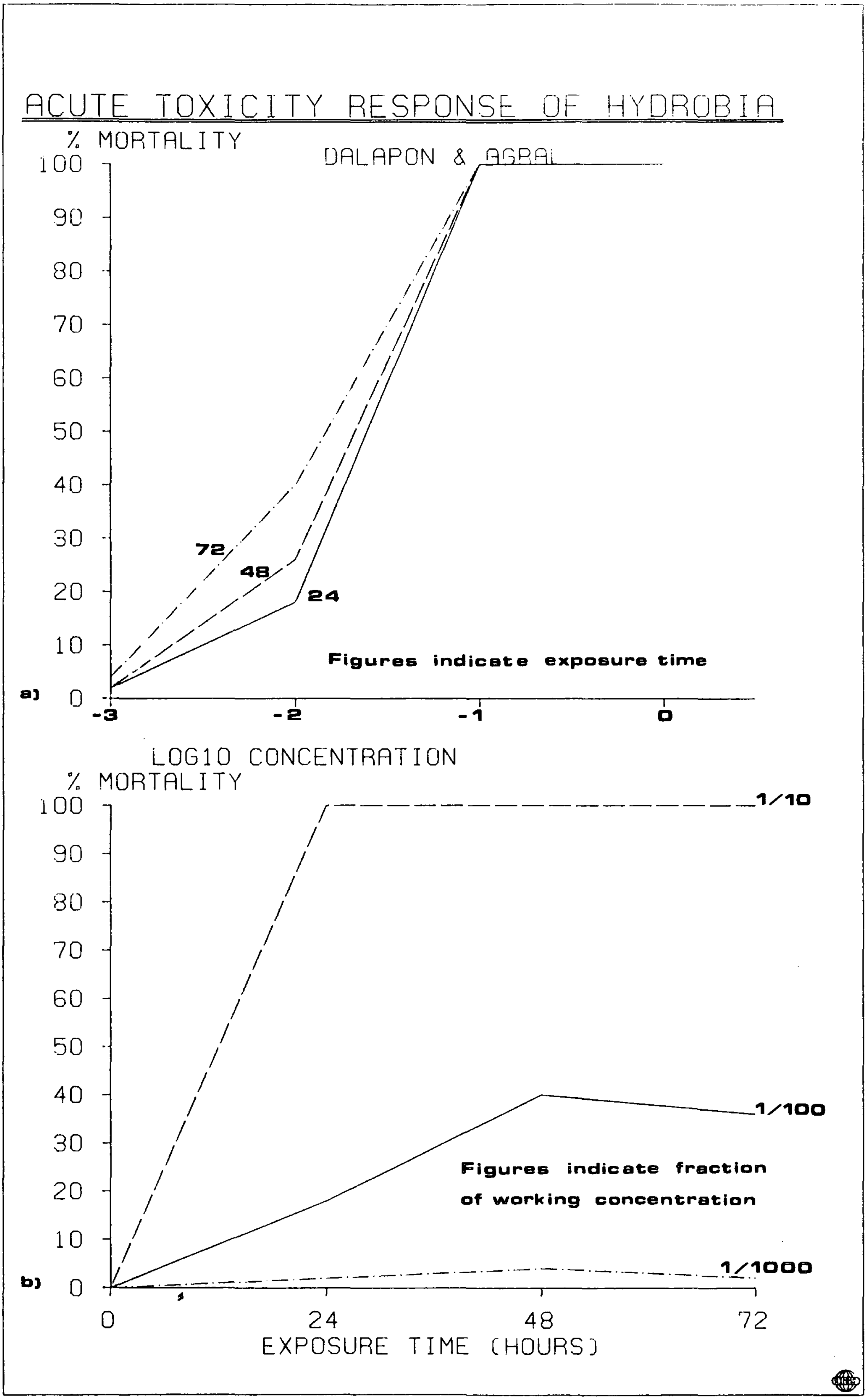
LC50 RANGE IN RELATION TO SPRAYING CONCENTRATION.

SPECIES	N	24 HOURS LC50	48 HOURS LC50	72 HOURS LC50
COROPHIUM	50	1/100-1/10	1/100-1/10	1/100-1/10
HYDROBIA	50	1/100-1/10	1/100-1/10	1/100-1/10
NEREIS	10	1/10-1	1/10-1	1/10-1

SPRAYING CONCENTRATIONS: DALAPON = 55 GRAMS PER LITRE
AGRAL = 0.05 ML PER LITRE

The combined effects resulted in mortality at concentrations similar to those shown by either chemical alone. Nereis diversicolor showed a response in this experiment unlike in previous tests with Dalapon and Agral alone, because the highest concentration of the mixture used was equal to the working concentraion of the spray, rather than 1/10 of the working concentration as in the previous tests. Figures 17 and 18 show that there is no increased mortality with exposure times longer than 24 hours. The drop in the estimated mortality level of Hydrobia ulvae occured because some apparently dead animals had only been immobilised and later recovered. These results show that there are no appreciable synergistic effects between Dalapon and Agral.

Longterm exposure tests can indicate only a broad range of concentrations over which mortality may occur. However they showed that mortality of Corophium volutator and Hydrobia ulvae occured at concentrations of 1/10 to 1/100 the working



ACUTE TOXICITY RESPONSE OF HYDROBIA
% MORTALITY
DALAPON & AGRAL
100
90
80
70
60
50
40
30
20
10
0
72
48
24
Figures indicate exposure time
a)
-3
-2
-1
0
LOG10 CONCENTRATION
% MORTALITY
1/10
1/100
Figures indicate fraction
of working concentration
1/1000
b)
0
24
48
72
EXPOSURE TIME (HOURS)

FIGURE 17

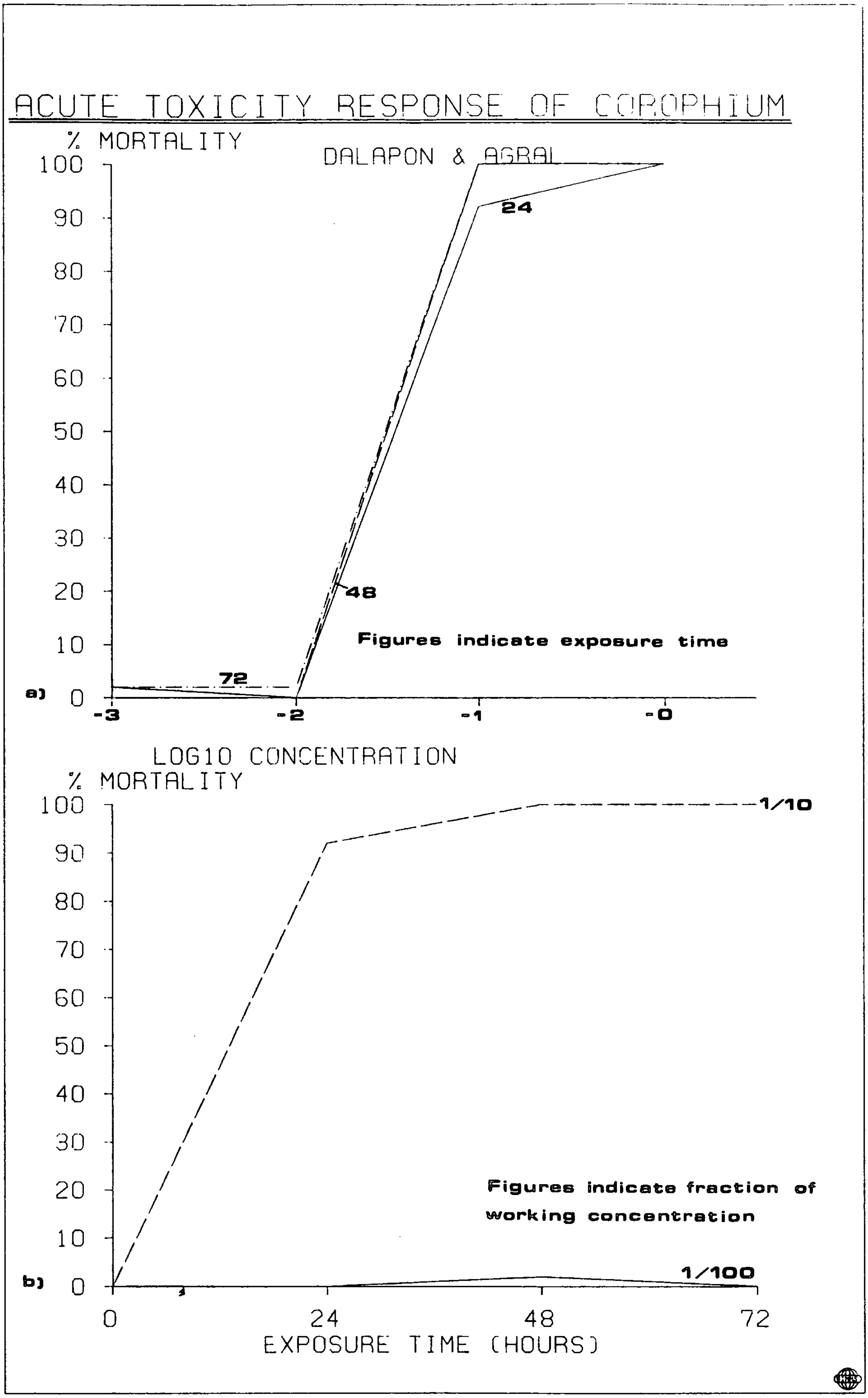

ACUTE TOXICITY RESPONSE OF COROPHIUM
% MORTALITY
DALAPON & AGRAL
100
90
80
70
60
50
40
30
20
10
0
24
48
72
Figures indicate exposure time
a)
-3
-2
-1
-0
LOG10 CONCENTRATION
% MORTALITY
1/10
Figures indicate fraction of working concentration
1/100
b)
0
24
48
72
EXPOSURE TIME (HOURS)

FIGURE 18

concentration, and between 1/10 and the equivalent of the working concentration in Nereis diversicolor. As all control tests (in the absence of Dalapon and Agral) showed mortalities of less than 1%, it must therefore be concluded that the spray does have a potentially toxic effect.

The long term tests are based upon standard toxicological techniques. However, in studying the effects of spraying Spartina at Lindisfarne a more realistic approach should incorporate a shorter exposure time, because the action of the tide limits the period for spray to remain on the mud surface to six hours, after which it is dispersed by the rising tide. Therefore further tests were carried out to take this factor into account.

(iii) Methods of acute toxicity estimation (six hour exposure).

Experiments were carried out in the same way as in the earlier tests, except that the response to Dalapon and Agral combined was assessed, using concentrations of 1/100, 1/10 and the equivalent of working concentrations (55 gms dalapon per litre, 0.05 ml Agral per litre). The animals were exposed to these concentrations of Dalapon and Agral for only six hours, and then transferred to clean tanks with 2.5 litres of fresh seawater. The numbers of individuals alive was then noted at 24, 48 and 72 hours after transfer. In this experiment the numbers of individuals of each species in each tank, were 20 Corophium volutator, 50 Hydrobia ulvae and 15 Nereis diversicolor.

(iv)Results

the results of this six hour exposure test are shown in Figures 19 and 20. The range of concentrations spanning the LC50 are shown below.

TABLE 25.

ACUTE TOXICITY EFFECTS OF 6 HOURS EXPOSURE TO DALAPON WITH AGRAL.

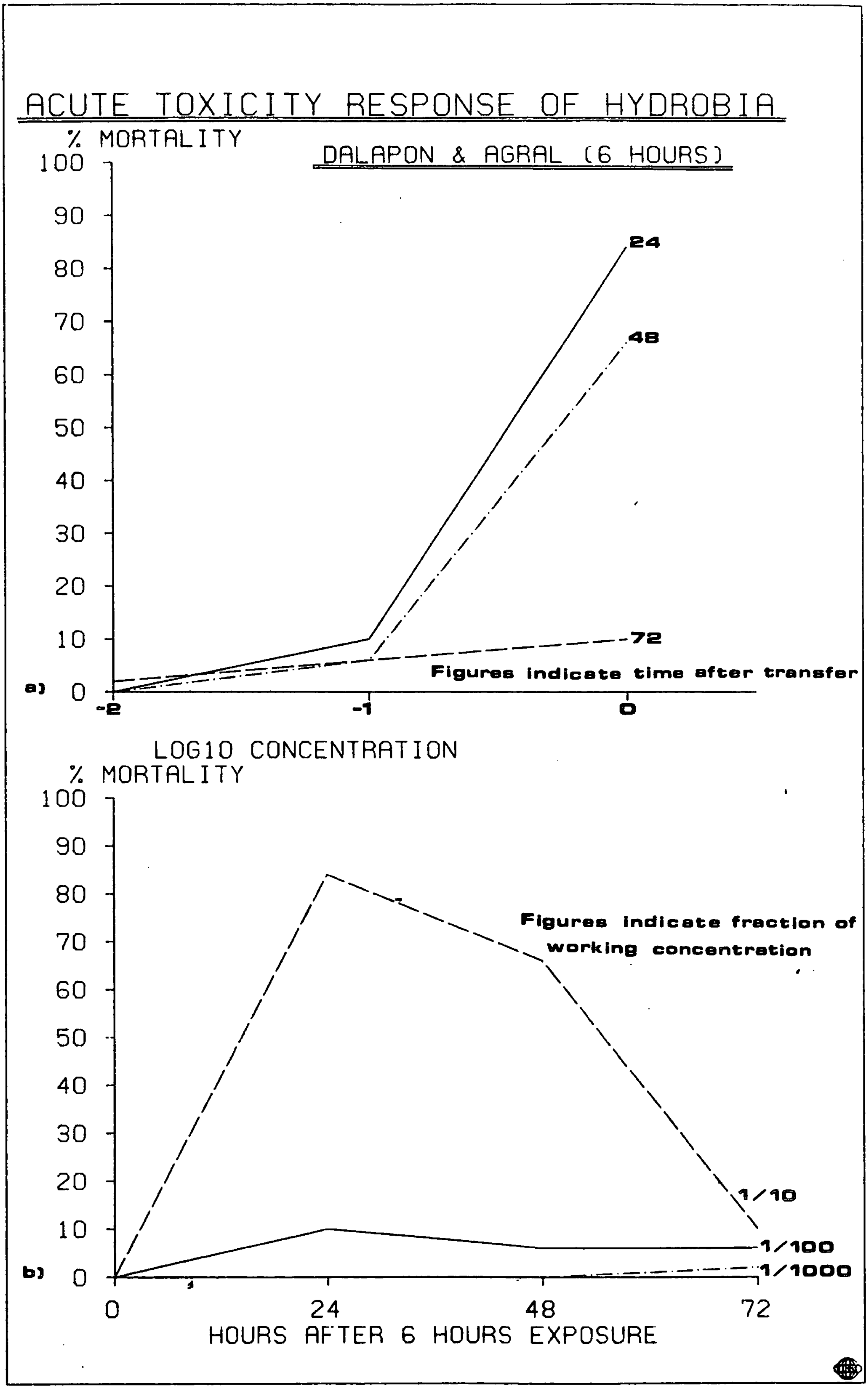
LC50 RANGE IN RELATION TO SPRAYING CONCENTRATION.

SPECIES	N	24 HOURS LC50	48 HOURS LC50	72 HOURS LC50
COROPHIUM	20	1/100-1/10	1/100-1/10	1/100-1/10
HYDROBIA	50	1/10-1	1/10-1	> 1
NEREIS	15	1/10-1	1/10-1	1/10-1

SPRAYING CONCENTRATIONS: DALAPON = 55 GRAMS PER LITRE
AGRAL = 0.05 ML PER LITRE

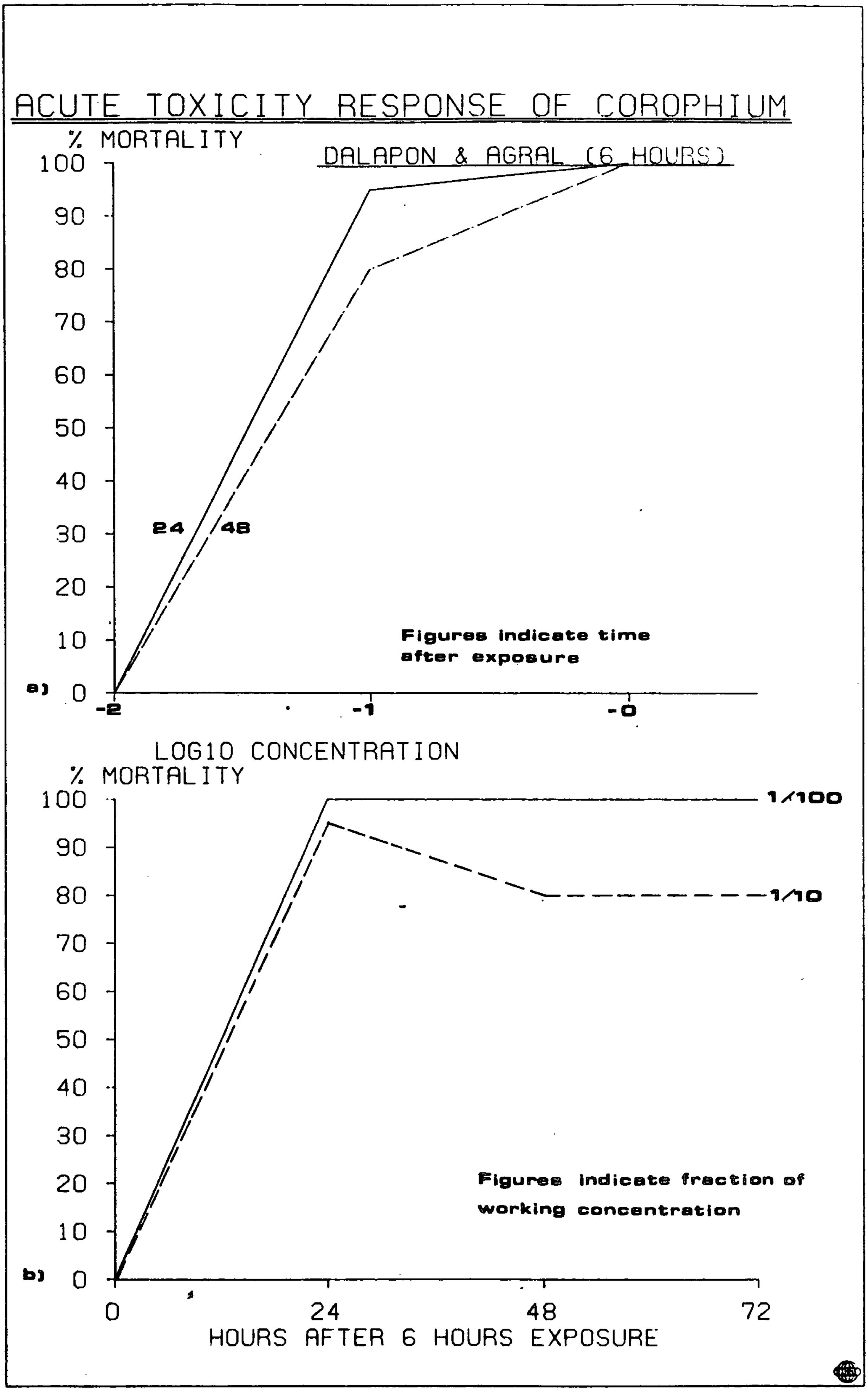
For *Corophium volutator* and *Nereis diversicolor* the results are comparable to the previous longterm exposure tests. However, in *Hydrobia ulvae* the concentration range spanning the LC50 was higher than found in the long exposure tests. Also, as Figure 19 clearly shows, the number of *Hydrobia ulvae* recorded as dead fell to only 10% 72 hours after transfer to seawater. A similar phenomenom was seen in *Corophium volutator* at a concentration of 1/100 the working concentration of spray. Therefore it appears that a short exposure to spray may only result in immbolisation from which recovery is possible.

These results have important implications for the assessment of the toxic effects of Dalapon and Agral in field



ACUTE TOXICITY RESPONSE OF HYDROBIA
% MORTALITY
DALAPON & AGRAL (6 HOURS)
100
90
80
70
60
50
40
30
20
10
0
24
48
72
Figures indicate time after transfer
a)
-2
-1
0
LOG10 CONCENTRATION
% MORTALITY
100
90
80
70
60
50
40
30
20
10
0
Figures indicate fraction of working concentration
1/10
1/100
1/1000
b)
0
24
48
72
HOURS AFTER 6 HOURS EXPOSURE

FIGURE 19



ACUTE TOXICITY RESPONSE OF COROPHIUM
% MORTALITY
DALAPON & AGRAL (6 HOURS)
100
90
80
70
60
50
40
30
20
10
0
24
48
Figures indicate time after exposure
a)
-2
-1
-0
LOG10 CONCENTRATION
% MORTALITY
100
90
80
70
60
50
40
30
20
10
0
1/100
1/10
Figures indicate fraction of working concentration
b)
0
24
48
72
HOURS AFTER 6 HOURS EXPOSURE

FIGURE 20

situations. Firstly the spray may have only an immobilising effect (even if encountered in concentrations of near working level) before dispersal by the rising tide. Secondly, the method of determination of dead _Hydrobia ulvae_ and _Corophium volutator_ (particularily the former) proved unreliable, and therefore the results of the long term exposure tests must be viewed with caution.

The implications of all the toxicological results will be discussed later.

CHAPTER 6

DISCUSSION

(i)Long term effects on macroinvertebrate populations.

The results of the survey agree with the general conclusion by Millard (1976) that *Spartina* sward at Lindisfarne supports a less diverse and less dense macroinvertebrate population than areas of open mud at the same tidal level. In this study only *Littorina saxatillis* and *Carcinus maenas* were of equal abundance outside and within the *Spartina*, all other species showed a higher density outside the *Spartina*. However Millard (1976) also found some areas of *Spartina* sward had higher densities of macroinvertebrates than adjacent areas of open mud-flat. This was attributed to the presence of atypically coares sediments in these areas of *Spartina*. No such area was found in this study and it is suggested that the results of this faunal survey reprsent typical populations found in the *Spartina* sward of Beal Sands.

Although an ideal study would have sampled areas prior to spraying this was not possible, and therefore all comparisons are based upon the assumption that the sprayed areas were not significantly different from each other before spraying in either macroinvertebrate population or sediment character from the area of *Spartina* sward used as the control. If this assumption is accepted then the macroinvertebrate populations found in the sprayed areas show that the spraying treatment and associated changes over four years has increased both the diversity and the density of the mud-flat fauna. Furthermore Arenicola marina, Capitella capitata, Littorina saxatillis, Macoma balthica and Owenia fusiformis populations were denser in the sprayed areas than in the open mud-flat. Therefore the aim of controlling Spartina in order to reclaim potential feeding areas for birds has been fullfilled, both because prey are available and the open nature of the habitat have been restored.

Although this study did not examine the effect of spraying on Zostera spp it was encouraging to note from casual observation that Zostera was growing abundantly in suitable wet sites, within the area sprayed four years ago.

This study anticipated that any changes in macroinvertebrate populations after spraying would be due to sediment characteristics. This is supported by the correlations between the densities of each species and the measured physico-chemical factors. However a general relationship between the sediment changes and macroinvertebrate population densities as a whole could not be made as it was found that the species responded in different ways.

Due to the associated change in height with recovery time the significant positive correlation of recovery time with density of Hydrobia ulvae, Nereis diversicolor and Macoma balthica is also reflected in the associated negative correlation with height. However the higher correlation coefficients with recovery time from Hydrobia ulvae and Macoma balthica indicate that the recovery time is the most likely causal factor. Also the height change from the lowest to the highest sample in relation to tidal height only amounts to approximately 19 centimetres, and it is unlikely that this degree of vertical difference could directly account for the observed variation in inveretebrate densities. Also these species distributions are well within the tidal range described by Wolff (1973), Millard (1976) and Howcroft (1983). Similarly it is suggested that the positive correlation with height below M.H.W.M shown in Corophium volutator and Carcinus maenas are not a direct causal relationship, but occur through an associated relationship with the other physico-chemical factors, such as particle size.

The Oligochaeta, Corophium volutator, Owenis fussiformis and Capitella capitata show marked increases in density with a decrease in particle size. Similar results for Oligochaetes and Corophium volutator were found by Brady (1943), Stopford (1951), Brinkhurst (1964), Meadows (1964) and Millard (1976) in studies of mud-flat invertebrates in relation to physico-chemical variables. Also Capitella capitata was found by Tietjen (1969) and Warren (1977) to occur only in muddy areas. These observed increases in macroinvertebrate densities associated with fine sediments is considered to be caused by the increased presence of bacteria as food (Newell 1965). However this view is considered to be simplistic by Christian and Wetzel (1978), who state that the increased carbon content associated with fine sediments does not neccesarily indicate an increase in food availability.

Newell (1965) found that both Hydrobia ulvae and Macoma balthica occured in the finest sediments he sampled, but this was not confirmed by Millard (1976), or by this study, as Hydrobia ulvae showed a significant negative correlation with a decrease in particle size. Millard (1976) showed by laboratory experiments that above 50 % silt content the density of Hydrobia ulave was inversely proportional to particle size. As most of the sample sites in the study by Millard (1976) at Lindisfarne had a silt content of over 50 %, this difference (ie the nature of the substrate) is considered the most likely explanation for the observed decrease in density of Hydrobia ulvae in areas where the sediment is finest, and therefore available food content would be expected to be highest.

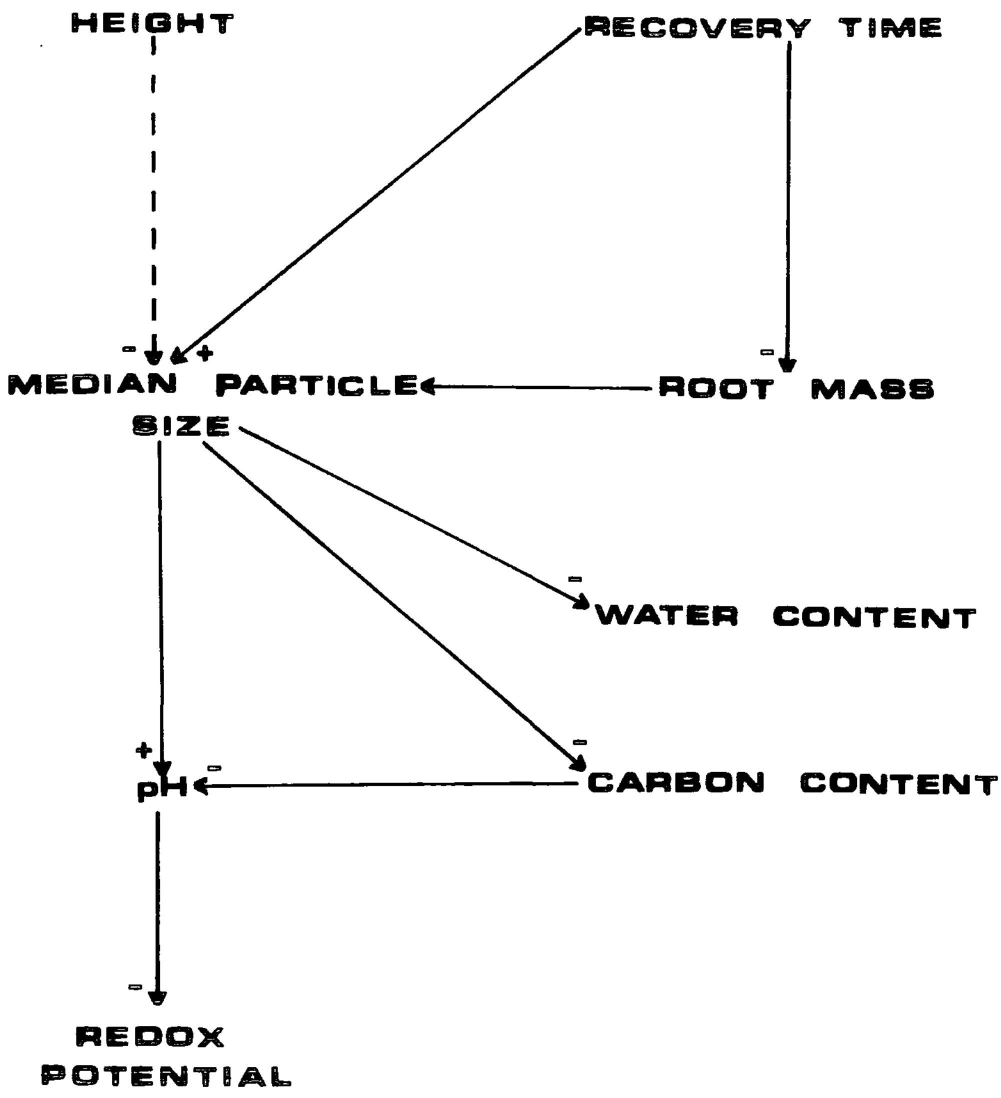
Millard (1976) considered that Macoma balthica was absent from Spartina sward due to either the lower redox potential, or difficulty in burrowing because of high water content in the sediment or the presence of root material. I found that Macoma balthica density was negatively correlated with carbon content, in contradication to the study by Newell (1965), but in agreement with the negative correlation with water content found by Millard (1976). Also I found that root mass was negatively correlated with Macoma balthica density and it is suggested that this is likely to be an important factor limiting this species.

Longbottom (1970) found that the distribution of Arenicola marina was related to the sediment particle size due to its effect on burrowing ability, and the amount of orgnic matter present as food. However, no such relationship was found in this study.

Although the results of the correlation between physical variables could not clearly differentiate between the changes caused by the removal of Spartina and original zonation of sediment characteristics by tidal height, the greater degree of correlation with recovery time and the similarity between the two areas sprayed four years ago, indicated that recovery time was probably the main influencing factor. This question could only be answered by the examining a larger number of samples taken over a much wider area, but unfortuneately this was not possible in the 14 weeks available fieldwork for this study which was designed around an existing programme of Spartina control. However I suggest that the most plausible explanation of my findings is that removal of the Spartina sward by herbicide treatment causes an active change in the character of the sediment, the first change being the removal of fine sediment by tidal action. The degree of the change is affected mainly by time since the removal of the plant material, and it is expected that changes in sediment will cease once the physico-chemical factors are in equlibrium with the prevailing environment.

This study found many correlations between the physico-chemical factors which are suggested to have been caused by the change in sediment particle size. The following figure summarises the interrelationships found in the study that are thought to be causal, based on the observed correlationships of

INTERRELATIONSHIPS BETWEEN PHYSICO-CHEMICAL FACTORS



ONLY IDENTIFIED CORRELATIONS THAT ARE THOUGHT TO BE CAUSAL ARE INDICATED.

FIGURE 21

this study, and the previous work by Jansson (1967), Mayer (1973), Newell (1965) and Fenchel and Riedl (1970).

Although redox potential measurements did not show any clear relationship to any other factor except pH, it is noteworthy that the destruction of Spartina did not result in a detectable decrease in redox potential due to a large increase in microbial activity, a phenomena reported in freshwater situations by Newbold (1975, 1976) and Robson and Barrett (1977). The absence of such an effect is probably due to the removal of the decaying above ground Spartina by tidal action, and the slow rate of decay of the root matter in an orginally anaerobic sediment.

The increased diversity and density of the fauna caused by the removal of Spartina results in an increased availability in food for fish and bird populations using the mud-flats. Also the removal of the above ground plant material makes the prey resource available for flock feeding birds that might otherwise avoid the area, for behavioural reasons. However the presence of clumped Spartina lower on the shore may still deter such birds, and therefore observations are needed to examine this point. I could not study shorebird utilisation of the sprayed areas systematically but casual observations showed that Ringed Plovers (Charadrius hiaticula), Golden Plovers (Pluvalis apricaria) and Dunlin (Calidris alpina) frequently used the sprayed area for feeding. Further studies on the utilisation of the sprayed areas would assess the value of spraying as a means of increasing feeding areas.

(ii) toxicity effects of Dalapon and Agral.

The field test on the immediate effects of Dalapon and Agral spary found no detectable effect in the species present with the exception of Carcinus maenas. Although the disturbance factor of the spraying from the vehicle must be considered as a possible cause of the absence immediately after spraying, it is considered unlikely to have been responsible, especially as all samples were taken from areas other than in the vehicle tracks, where such species as Carcinus maenas would have been crushed.

Although the laboratory studies can only be considered as a preliminary investigation into the toxic effects of Dalapon and Agral, the results indicate a potential toxic effect of the spray. However these results must be considered in relation to the likely concentrations of spray actually encountered by the animals. In the field situation 100 gallons (454 litres) of spray is used to cover one acre (4047 sq metres) of Spartina. This equals an application of 0.112 litres of spray per sq metre. Therefore, if the top 2 cm of sediment consists of 50 % by volume water, then this sediment layer will hold approximately 10 litres of water per sq metre. The resulting concentration of spray encountered by the animals living in this surface layer would then be 0.011 times the working concentration. This is on the lower limit of concentration which led to dectectable toxic effects found in the laboratory tests for Corophium volutator and Hydrobia ulvae, and substantially lower than the lower limit of toxic effects for

Nereis divericolor. Although these calculations are simplistic I considered that the estimateof the concentration encountered is a maximum, as most spray will be intercepted by the *Spartina* sward, and dilution by water overlying the sediment is not considered. Therefore it is suggested that the spray is unlikely to have toxic effects in the field situation on those species studied. This is particularily true in the case of *Hydrobia ulvae*, which was able to recover after short periods of exposure to the spray, as seen in the laboratory tests. However it must be emphasized that the tests are only preliminary and do not consider the effects of the substrate on the chemicals, the possible accumulation in faunal components and the rate of breakdown of Dalapon and Agral in marine situations. Nor do the tests assess the effect on many components of the *Spartina* fauna, such as *Littorina saxatillis*, *Carcinus maenas* and *Oligochaetes*. It is suggested that further research should be carried out to investigate the mode of action of Dalapon and Agral on a wider variety of marine invertebrates.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS.

1) The destruction of *Spartina anglica* by treatment with the herbicide Dalapon, results in an increase in diversity and density of the macroinvertebrate population.

2) Although conclusive evidence could not be obtained the observed changes in the macroinvertebrate population is considered to have resulted from the sediment changes caused by the removal of the *Spartina*.

3) Field tests on the toxic effects of Dalapon and Agral in normal spraying concentrations and conditions failed to detect any affect in the majority of species. However the observed absence of *Carcinus maenas* after spraying may have been caused by the toxic effects of the spray. Further research is required to confirm this.

4) Laboratory studies on *Corophium volutator*. *Hydrobia ulvae* and *Nereis diversicolor* suggest that toxic effects are unlikely in field situations, at the present concentrations of chemicals used. However, higher concentrations of Dalapon should not be used. Further research is required into the toxicity, breakdown rate and possibility of bio-accumulation of Dalapon and Agral.

5) No obvious long term deterimental environmental effects were detected and therefore it is considered that the treatment of *Spartina* by Dalapon and Agral at present concentrations is an efficient and suitable method of control.

APPENDIX

APPENDIX TABLE 1.

OLIGOCHAETA IDENTIFIED FROM THE MAIN INVERTEBRATE SURVEY.

MEAN NUNBERS PER SITE WITH STANDARD ERRORS OF THE MEAN IN BRACKETS

SAMPLE AREA	ENCHYTRAEUS ALBIDUS	LUMBRICILUSS SPECIES	TUBIFEX SPECIES
1	4.3 (1.4)	0.3 (0.2)	0.9 (0.4)
2	0.7 (0.2)	6.0 (2.3)	2.0 (2.0)
3	9.9 (1.9)	0.1 (0.1)	2.4 (0.9)
4	18.4 (5.9)	0.3 (0.2)	0.7 (0.7)
5	9.9 (1.9)	0.1 (0.1)	2.4 (0.9)
6	3.8 (1.0)	1.5 (0.4)	6.2 (3.6)
7	2.5 (1.6)	0.2 (0.2)	1.8 (1.1)

APPENDIX TABLE 2.

COMPARISION OF SPECIES ABUNDANCE BETWEEN CONTROL SPARTINA AND SPRAYED AREAS.

Mann-Whitney U-Wilcoxon Rank Sum W Test.

Area 1 Unsprayed control Spartina
Areas 2-5 Sprayed

SPECIES	AREA CODE	COMPARED AREA CODE	Z	2 TAILED SIGNIFICANCE
NEREIS DIVERSICOLOR	1	2	-2.50	0.012
ARENICOLA MARINA	1	2	-3.51	0.000
CAPITELLA CAPITATA	1	2	-2.48	0.013
OLIGOCHAETA	1	2	-1.67	0.095
HYDROBIA ULVAE	1	2	-1.90	0.568
MACOMA BALTHICA	1	2	-1.82	0.068
COROPHIUM VOLUTATOR	1	2	-3.23	0.001
OWENIA FUSSIFORMIS	1	2	-2.75	0.005
NEREIS DIVERSICOLOR	1	3	-2.16	0.030
ARENICOLA MARINA	1	3	-2.80	0.005
CAPITELLA CAPITATA	1	3	-2.48	0.013
OLIGOCHAETA	1	3	-2.35	0.019
HYDROBIA ULVAE	1	3	-1.68	0.091
MACOMA BALTHICA	1	3	-2.80	0.005
COROPHIUM VOLUTATOR	1	3	-3.59	0.000
OWENIA FUSSIFORMIS	1	3	-3.42	0.000
NEREIS DIVERSICOLOR	1	4	-3.14	0.002
ARENICOLA MARINA	1	4	-3.11	0.002
CAPITELLA CAPITATA	1	4	-3.42	0.001

OLIGOCHAETA	1	4	-2.80	0.005
HYDROBIA ULVAE	1	4	-3.60	0.000
MACOMA BALTHICA	1	4	-4.04	0.000
COROPHIUM VOLUTATOR	1	4	-3.15	0.002
OWENIA FUSSIFORMIS	1	4	-3.41	0.001
NEREIS DIVERSICOLOR	1	5	-3.12	0.002
ARENICOLA MARINA	1	5	-1.45	0.147
CAPITELLA CAPITATA	1	5	-1.82	0.068
OLIGOCHAETA	1	5	-1.52	0.879
HYDROBIA ULVAE	1	5	-3.56	0.000
MACOMA BALTHICA	1	5	-2.80	0.005
COROPHIUM VOLUTATOR	1	5	-1.14	0.255
OWENIA FUSSIFORMIS	1	5	-1.82	0.068

(Significance estimates are corrected for ties).

APPENDIX TABLE 3.

COMPARISION OF SPECIES ABUNDANCE BETWEEN CONTROL AREA OF UNSPRAYED SPARTINA AND AREA 6 (REPLICATE AREA SPRAYED FOUR YEARS AGO).

Mann-Whitney U-Wilcoxon Rank Sum W Test.

SPECIES	AREA CODE	COMPARED AREA CODE	Z	2 TAILED SIGNIFICANCE
NEREIS DIVERSICOLOR	1	6	-1.82	0.068
ARENICOLA MARINA	1	6	-2.81	0.005
CAPITELLA CAPITATA	1	6	0.00	1.000
OLIGOCHAETA	1	6	-1.02	0.306
HYDROBIA ULVAE	1	6	-3.79	0.000
LITTORINA SAXATILIS	1	6	-1.95	0.051
MACOMA BALTHICA	1	6	-4.04	0.000
COROPHIUM VOLUTATOR	1	6	-3.15	0.002
OWENIA FUSSIFORMIS	1	6	0.00	1.000
CARCINUS MAENAS	1	6	-2.18	0.029

(Significance estimates are corrected for ties).

APPENDIX TABLE 4.

COMPARISION OF SPECIES ABUNDANCE BETWEEN AREAS SPRAYED FOUR YEARS AGO.

Mann-Whitney U-Wilcoxon Rank Sum W Test.

SPECIES	AREA CODE	COMPARED AREA CODE	Z	2 TAILED SIGNIFICANCE
NEREIS DIVERSICOLOR	5	6	-1.51	0.132
ARENICOLA MARINA	5	6	-1.94	0.052
CAPITELLA CAPITATA	5	6	-1.82	0.068
OLIGOCHAETA	5	6	-0.53	0.596
HYDROBIA ULVAE	5	6	-1.44	0.151
LITTORINA SAXATILIS	5	6	-3.28	0.001
MACOMA BALTHICA	5	6	-3.65	0.000
COROPHIUM VOLUTATOR	5	6	-2.15	0.032
OWENIA FUSSIFORMIS	5	6	-1.82	0.068
CARCINUS MAENAS	5	6	-1.00	0.317

(Significance estimates are corrected for ties).

APPENDIX TABLE 5.

COMPARISION OF SPECIES ABUNDANCE BETWEEN CONTROL AREA OF UNSPRAYED SPARTINA AND UNSPRAYED OPEN MUD.

Mann-Whitney U-Wilcoxon Rank Sum W Test.

SPECIES	AREA CODE	COMPARED AREA CODE	Z	2 TAILED SIGNIFICANCE
NEREIS DIVERSICOLOR	1	7	-4.49	0.000
ARENICOLA MARINA	1	7	0.00	1.000
CAPITELLA CAPITATA	1	7	-0.645	0.519
OLIGOCHAETA	1	7	-1.93	0.053

HYDROBIA ULVAE	1	7	-4.41	0.000
LITTORINA SAXATILIS	1	7	-1.73	0.084
MACOMA BALTHICA	1	7	-2.84	0.004
COROPHIUM VOLUTATOR	1	7	-0.64	0.518
OWENIA FUSSIFORMIS	1	7	-0.64	0.518
CARCINUS MAENAS	1	7	-1.35	0.176

(Significance estimates are corrected for ties).

APPENDIX TABLE 6.

COMPARISION OF SPECIES ABUNDANCE BETWEEN SPRAYED AREAS AND UNSPRAYED OPEN MUD

Mann-Whitney U-Wilcoxon Rank Sum W Test.

Area 7 Unsprayed Open Mud
Areas 2-5 Sprayed

SPECIES	AREA CODE	COMPARED AREA CODE	Z	2 TAILED SIGNIFICANCE
ARENICOLA MARINA	7	2	-3.51	0.000
CAPITELLA CAPITATA	7	2	-2.48	0.013
OLIGOCHAETA	7	2	-2.46	0.014
HYDROBIA ULVAE	7	2	-2.57	0.010
MACOMA BALTHICA	7	2	-1.11	0.265
COROPHIUM VOLUTATOR	7	2	-3.42	0.001
OWENIA FUSSIFORMIS	7	2	-2.80	0.005
ARENICOLA MARINA	7	3	-2.80	0.005
CAPITEELA CAPITATA	7	3	-2.48	0.013
OLIGOCHAETA	7	3	-2.92	0.003
HYDROBIA ULVAE	7	3	-3.11	0.002
MACOMA BALTHICA	7	3	-0.44	0.661
COROPHIUM VOLUTATOR	7	3	-3.72	0.000
OWENIA FUSSIFORMIS	7	3	-3.42	0.000
ARENICOLA MARINA	7	4	-3.11	0.002
CAPITELLA CAPITATA	7	4	-3.42	0.001
OLIGOCHAETA	7	4	-3.11	0.002
HYDROBIA ULVAE	7	4	-0.26	0.791
MACOMA BALTHICA	7	4	-2.49	0.013

COROPHIUM VOLUTATOR	7	4	-3.42	0.001
OWENIA FUSSIFORMIS	7	4	-3.41	0.001

ARENICOLA MARINA	7	5	-1.45	0.147
CAPITELLA CAPITATA	7	5	-1.82	0.068
OLIGOCHAETA	7	5	-1.71	0.087
HYDROBIA ULVAE	7	5	-0.34	0.073
MACOMA BALTHICA	7	5	-0.28	0.780
COROPHIUM VOLUTATOR	7	5	-1.82	0.068
OWENIA FUSSIFORMIS	7	5	-1.82	0.068

(Significance estimates are corrected for ties).

APPENDIX TABLE 7.

COMPARISION OF SPECIES ABUNDANCE BETWEEN UNSPRAYED AREA OF OPEN MUD AND AREA 6 (REPLICATE AREA SPRAYED FOUR YEARS AGO).

Mann-Whitney U-Wilcoxon Rank Sum W Test.

SPECIES	AREA CODE	COMPARED AREA CODE	Z	2 TAILED SIGNIFICANCE
NEREIS DIVERSICOLOR	7	6	-1.90	0.057
ARENICOLA MARINA	7	6	-2.81	0.005
CAPITELLA CAPITATA	7	6	0.00	1.000
OLIGOCHAETA	7	6	-2.01	0.044
HYDROBIA ULVAE	7	6	-2.08	0.037
LITTORINA SAXATILIS	7	6	-2.82	0.005
MACOMA BALTHICA	7	6	-3.70	0.000
COROPHIUM VOLUTATOR	7	6	-3.42	0.001
OWENIA FUSSIFORMIS	7	6	0.00	1.000
CARCINUS MAENAS	7	6	0.00	1.000

(Significance estimates are corrected for ties).

APPENDIX 8.

TEST OF VARIATION IN DENSITY BETWEEN ALL AREA PAIRS OF THE SPRAYED SPARTINA AREAS 1-5.

MANN-WHITNEY U -WILCOXON RANK SUM W TEST RESULTS.

		HYDROBIA		MACOMA		COROPHIUM		OWENIA	
AREA	AREA	Z	SIGNF	Z	SIGNF	Z	SIGNF	Z	SIGNF
2	3	-0.42	0.676	-1.62	0.105	-0.90	0.363	-0.58	0.564
2	4	-2.31	0.021	-3.50	0.000	-1.82	0.068	-1.15	0.250
2	5	-2.53	0.011	-1.46	0.144	-2.46	0.014	-1.53	0.125
3	4	-2.73	0.006	-2.37	0.018	-1.18	0.239	-0.99	0.322
3	5	-2.92	0.003	-0.04	0.969	-2.70	0.007	-2.23	0.026
4	5	-0.34	0.734	-2.22	0.026	-2.15	0.032	-2.62	0.009

(Significance estimates are corrected for ties).

APPENDIX TABLE 9.

COMPARISION OF BIO-PHYSICAL FACTORS BETWEEN CONTROL SPARTINA AND SPRAYED AREAS.

Mann-Whitney U-Wilcoxon Rank Sum W Test.

Area 1 Unsprayed control Spartina
Areas 2-5 Sprayed

BIO-PHYSICAL FACTOR	AREA CODE	COMPARED AREA CODE	Z	2 TAILED SIGNIFICANCE
REDOX POTENTIAL	1	2	-0.94	0.344
HEIGHT	1	2	-3.40	0.001
ROOT MASS	1	2	-0.62	0.597
% WATER CONTENT	1	2	-2.29	0.022
MEDIAN PARTICLE SIZE	1	2	-2.35	0.018
% CARBON CONTENT	1	2	-1.98	0.047
REDOX POTENTIAL	1	3	-1.36	0.174
HEIGHT	1	3	-4.24	0.000
ROOT MASS	1	3	-2.07	0.039
% WATER CONTENT	1	3	-0.11	0.912
MEDIAN PARTICLE SIZE	1	3	0.00	1.000
% CARBON CONTENT	1	3	-0.57	0.567
REDOX POTENTIAL	1	4	-0.11	0.910
HEIGHT	1	4	-4.41	0.000
ROOT MASS	1	4	-2.62	0.009
% WATER CONTENT	1	4	-1.91	0.056
MEDIAN PARTICLE SIZE	1	4	-0.90	0.367
% CARBON CONTENT	1	4	-2.02	0.043
REDOX POTENTIAL	1	5	-2.12	0.034

HEIGHT	1	5	-4.41	0.000
ROOT MASS	1	5	-3.32	0.001
% WATER CONTENT	1	5	-4.18	0.000
MEDIAN PARTICLE SIZE	1	5	-4.20	0.000
CARBON CONTENT	1	5	-2.46	0.014

(Significance estimates are corrected for ties).

APPENDIX TABLE 10.

COMPARISION OF BIO-PHYSICAL FACTORS BETWEEN CONTROL AREAS OF UNSPRAYED SPARTINA AND AREA 6 (REPLICATE AREA SPRAYED FOUR YEARS AGO).

Mann-Whitney U-Wilcoxon Rank Sum W Test.

BIO-PHYSICAL FACTOR	AREA CODE	COMPARED AREA CODE	Z	2 TAILED SIGNIFICANCE
REDOX POTENTIAL	1	6	-2.42	0.015
HEIGHT	1	6	-4.41	0.000
ROOT MASS	1	6	-3.86	0.000
% WATER CONTENT	1	6	-4.40	0.000
MEDIAN PARTICLE SIZE	1	6	-4.40	0.000
pH	1	6	-3.40	0.007
% CARBON CONTENT	1	6	-4.22	0.000

(Significance estimates are corrected for ties).

APPENDIX TABLE 11.

COMPARISION OF BIO-PHYSICAL VARIABLES BETWEEN CONTROL AREA OF UNSPRAYED SPARTINA AND UNSPRAYED OPEN MUD.

Mann-Whitney U-Wilcoxon Rank Sum W Test.

BIO-PHYSICAL FACTOR	AREA CODE	COMPARED AREA CODE	Z	2 TAILED SIGNIFICANCE
REDOX POTENTIAL	1	7	-2.42	0.015
HEIGHT	1	7	-4.41	0.000
ROOT MASS	1	7	-3.86	0.001
% WATER CONTENT	1	7	-4.40	0.000
MEDIAN PARTICLE SIZE	1	7	-4.40	0.000
pH	1	7	-3.40	0.001
% CARBON CONTENT	1	7	-4.22	0.000

(Significance estimates are corrected for ties).

APPENDIX TABLE 12.

COMPARISION OF BIO-PHYSICAL FACTORS BETWEEN SPRAYED AREAS AND UNSPRAYED OPEN MUD

Mann-Whitney U-Wilcoxon Rank Sum W Test.

Area 7 Unsprayed Open Mud
Areas 2-5 Sprayed

BIO-PHYSICAL FACTOR	AREA CODE	COMPARED AREA CODE	Z	2 TAILED SIGNIFICANCE
REDOX POTENTIAL	7	2	-0.53	0.597
HEIGHT	7	2	-3.80	0.000
ROOT MASS	7	2	-3.28	0.001
% WATER CONTENT	7	2	-3.78	0.000
MEDIAN PARTICLE SIZE	7	2	-3.78	0.000
pH	7	2	-3.78	0.000
% CARBON CONTENT	7	2	-3.78	0.000
REDOX POTENTIAL	7	3	-2.57	0.010
HEIGHT	7	3	-3.38	0.001
ROOT MASS	7	3	-2.85	0.004
% WATER CONTENT	7	3	-3.78	0.000
MEDIAN PARTICLE SIZE	7	3	-3.78	0.000
pH	7	3	-3.55	0.000
% CARBON CONTENT	7	3	-3.70	0.000
REDOX POTENTIAL	7	4	-0.91	0.364
HEIGHT	7	4	-3.34	0.000
ROOT MASS	7	4	-3.04	0.002
% WATER CONTENT	7	4	-3.78	0.000
MEDIAN PARTICLE SIZE	7	4	-3.78	0.000

pH	7	4	-3.40	0.001
% CARBON CONTENT	7	4	-3.40	0.001
REDOX POTENTIAL	7	5	-1.58	0.112
HEIGHT	7	5	-2.61	0.009
ROOT MASS	7	5	-3.04	0.002
% WATER CONTENT	7	5	-3.59	0.000
MEDIAN PARTICLE SIZE	7	5	-3.78	0.000
pH	7	5	-2.00	0.0451
% CARBON CONTENT	7	5	-3.48	0.000

(Significance estimates are corrected for ties).

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